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Correspondence Address:
GRĂDINA BOTANICĂ "ANASTASIE FĂTU"
Str. Dumbrava Roşie nr. 7-9, 700487 - IAŞI
http://www.botanica.uaic.ro
E-mail: gbot.is@uaic.ro

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CELEBRATING 165 YEARS OF COLLABORATION BETWEEN THE *ANASTASIE FĂTU* BOTANICAL GARDEN AND THE NATURAL SCIENCES SCHOOL FROM IAŞI

Cătălin TĂNASE*[1], Ciprian Constantin BÎRSAN[2], Ana COJOCARIU[2], Cristiana Virginia PETRE[2], Constantin MARDARI[2]

[1] Faculty of Biology, Alexandru Ioan Cuza University of Iași, Carol I 20A, 700505 Iași – Romania
[2] "Anastasie Fătu" Botanical Garden, "Alexandru Ioan Cuza" University of Iași, 7-9 Dumbrava Roșie, 700487 Iași – Romania
* Corresponding author. E-mail: tanase@uaic.ro

Besides *ex situ* conservation of plant species, the mission of the Botanical Garden of "Alexandru Ioan Cuza" University of Iaşi is to offer the opportunity to learn about native and exotic plant species, and to promote new perspectives for ecological education. Since its foundation, the institution plays a very important role in the didactic activity related to plants research and conservation and offers excellent conditions for the student field practice for the Faculties of Biology, Geography-Geology, Agronomy, Horticulture, Medicine, Pharmacy, Architecture, etc. Along its 165 years existence, numerous personalities carried out didactic activities in the Botanical Garden while using it as a valuable educational resource, thus establishing a fruitful relationship with the academic community. This article presents the scientific and didactic activity (depending on the scientific field) related to the Botanical Garden of all those personalities as well as their contribution to the permanent development of the institution.

Botany

Botanical Garden – academic environment relationships were firstly established by the founder of this institution, **dr. Anastasie Fătu** (1816-1886), professor of Botany at the Faculty of Sciences from the University of Iași (since 1874), and member of the Romanian Academy (since 1871). He was also one of the founders of the Faculty of Medicine (1879), which, at that time was a part of the University of Iași. By founding the Botanical Garden, Professor Fătu tried and succeeded, among others, "…to encourage young students to learn botany…", and to offer a valuable educational resource for the didactic activity. Thus, the plant species "from Fătu's garden" were presented by Professor Dimitrie Brândză to the students attending the botany courses as living didactic material. He published the first catalog with the plants in the Botanical Garden (1871) including over 2,500 species of native and also of some exotic genera – *Enumeraţiunea specieloru de plante cultivate în Grădina Botanică din Iassy* (*Enumeration of the plant species cultivated in the Botanical Garden of Iasi*). In this work, he adopted the Latin nomenclature, presented a classification system in concordance with the taxonomical principles of that time, and also the plant names as they were known by Romanian people.

Moreover, Professor A. Fătu is the author of first university textbook of botany – *Elemente de botanică*: *Histologia*, *Organographia şi Phyisiologia vegetală* (*Botanic notions*: *histology*, *organography and plant physiology*), with the first part, published in Iași (1880), including aspects of cytology, histology, organography and plant physiology, and the second part comprising elements of plant taxonomy and plant geography, remaining as manuscript –

Taxonomia, *Phytographia şi Geographia Botanică* [TOMA, 2015; TĂNASE & al. 2016]. By synthetizing the whole international scientific literature of that time, this epochal work [POP, 1967] represented the main source of information, and significantly contributed to the educational process of students in the fields of natural sciences, medicine or agronomy at the end of the nineteenth century.

His remarkable didactic career was preceded by studies in Vienna and Paris, where he was granted two PhD degrees: in philosophy and law sciences (1841) as well as in medicine (1846). In turn, the great professor encouraged the young students to go and specialize abroad, at well-known universities, in various scientific domains [AIFTINCĂ, 2014], by founding, together with dr. Ion Ionescu de la Brad, the *Society for encouraging Romanian youth to study abroad* (1855). His scientific activity was internationally recognized, and, consequently Professor Fătu became member in *Silesian Society for science development* and *Society of Natural Sciences of Frankfurt*. Professor Fătu had also an active political activity, before the union of the Romanian Principalities, and after that. He was the president of the Assembly of Deputies (1868), and, in 1869 was elected senator. Besides the political activity, he had a significant contribution in organization of the medical and social assistance in Moldova, in elaborating, applying and organizing the public health domain. The scientific and cultural activity of dr. Anastasie Fătu was materialized in approximately 3000 pages published in 40 years of activity [ZAMFIR, 1987].

Contemporary with dr. Anastasie Fătu was the great Professor, physician and botanist **dr. Dimitrie Brandză** (1846-1895) who organized (1872) a second Botanical Garden in the courtyard of the Museum of the Society of Physicians and Naturalists in Iaşi. In this new location were planted species from the garden of Anastasie Fătu as well as species obtained from seeds collected by the botanist Iosif Szabó from different regions of the country; some of the initial trees are still existing today. The plants from Botanical Garden and herbarium sheets were used by Professor Brandză to illustrate his botany courses at the Faculty of Natural Sciences of the University of Iaşi, courses appreciated as solemn and of high academic level [TOMA, 2015]. In 1874 he was transferred to the Botany and Zoology department of the Sciences Faculty from Bucharest, but in the short time he spent in Iaşi he carried out a fruitful activity as professor, researcher, and medic. Starting with 1875 he coordinated the organization of the Botanical Garden of the University of Bucharest, institution bearing the name of the great Professor (since 1994). He founded the first Botanical Museum which harbored the Herbarium of Romanian Flora. He is also the founder of the Institute of Botany, inaugurated in 1892, with the first academic course of botany [ZANOSCHI & TOMA, 1995].

He studied medicine and natural sciences at the Sorbonne University in Paris, and had a remarkable scientific activity. His fundamental work – *Prodromul florei române sau Enumeraţiunea plantelor până azi cunoscute în Moldova şi Valachia* (*The Conspectus of Romanian flora or the Enumeration of the plant species known until today in Moldova and Wallachia*) – includes 2,100 plant species [BRANDZĂ, 1883], some of them described for the first time, and represents the first critical and synthetic analysis of Romanian flora. As a recognition of his contributions to the knowledge of Romanian flora, of his scientific and academic activity, in 1879 he was elected full member of the Romanian Academy. He had an active implication in evolution of the "Scientific Section" of the Academy, and between 1893 and 1895, he was elected vice-president of the same institution. In addition, some plant, fungi as well as a fossil, were named after the great researcher and Professor Dimitrie Brandză, by great scientific personalities as Henri-Ernest Baillon, Adrien Réne Franchet, Iuliu Prodan etc.

In 1876, **Professor dr. Cristea Buicliu** (1857-1916), tried to organize a third botanical garden around the University of Iaşi (currently, the University of Medicine and Pharmacy). He was a medicine doctor who studied in Paris and a prominent figure of the medical science in Romania, and, in this endeavor collaborated with dr. Anastasie Fătu. He was a university professor at the Faculty of Medicine from Bucharest at the department of internal medicine, and coordinated the medical clinic of the *Brâncovenesc* hospital.

Since 1895 until 1936, **Professor dr. Alexandru Popovici** (1866-1941) held the Botany courses at the Faculty of Sciences within the University of Iaşi, where he re-organized the Laboratory of Botany, the Herbarium, and the Library. Besides his prestigious academic activity, he was preoccupied by the foundation of a new university botanical garden, taking into consideration the importance of living plant collections in the academic education process. His initial intention was to create a new botanical garden in the vicinity of the Administrative Palace, (currently the Palace of Culture). Although the plans for this garden were designed (including the construction of a central greenhouse complex and a Botanical Institute) and despite the efforts of Professor Popovici, his idea was not materialized because of the lack of funds [MITITIUC & TONIUC, 2006]. Still, he was not discouraged, and surrounded by a small but enthusiastic team of collaborators, started the first plantings on a terrain situated near the University. He was the director of the Botanical Garden from the University Palace (currently *Titu Maiorescu* Dendrological Park), institution he organized and coordinated between 1921 and 1936. The garden included a greenhouse for tropical species which endures even today. The *Popovici Garden* [TOMA, 2015] represented for over 40 years the most valuable resource for the botanical academic education in the city of Iaşi.

For his researches focused on plant cytology, he was granted a PhD degree at the University of Bonn. For over 40 years he taught Botany and was one of the most active professors at the University of Iaşi. He was one of the founders of the publication *Annales scientifiques de l'Université de Jassy* (1900). The scientific activity of Professor Alexandru Popovici was preponderantly focused on mycology; he published over 500 species of fungi from the historical region of Moldova. As a recognition of his experience and the high value of his research, he was elected member in the Mycological Society of France and honorary member of the Romanian Academy [IACOB, 2011].

Closely working with Alexandru Popovici on organizing the botanical garden near the University Palace was head of works **Constantin Petrescu** (1879-1936), botanist and mycologist at the Faculty of Sciences within the University of Iași, directly involved in the plantation activities and in diversifying the plant collections. He was assigned to travel to various steppe habitats all across Romania to gather spontaneous plant species and to bring from the Botanical Garden of Cluj trees and shrubs for the new botanical garden [BURDUJA, 1979].

As a researcher, his main domains of interest were the flora and the micromycetes of Moldova and Dobrogea, but he also visited several locations in Muntenia and Oltenia, identifying and collecting numerous rare and unique plants and fungi, which were included in his personal Herbarium and used as teaching material for students. He showed tenacity and passion and decisively contributed to the enrichment of both botanical and mycological data regarding many rare plant species and host pathogen systems [BURDUJA & MIHAI, 1976-1977].

Between 1937 and 1958, the activities in Botanical Garden were coordinated by **Professor dr. Constantin Papp** (1896-1972). They were mainly focused on development of this institution through plant collections enrichment, through trades with similar institutions from the country and abroad, especially after the Second World War when the Garden was

almost entirely destroyed. In this regard, he elaborated a reconstruction plan, repaired some greenhouses and built a new one. He restored the outdoor plant collections and reorganized all of the garden's sections. Always, his Botany courses were illustrated with an impressive living botanic material from the Botanical Garden, institution he considered fundamental for teaching Biology at academic level.

In his impressive didactic and research career, Professor Constantin Papp was mainly preoccupied by the taxonomy of bryophytes and plant species as well as by the communities edified by these organisms. His remarkable researches started with his PhD dissertation focused on study the bryophyte species in Moldova (1926), and continued by the publication of the first identification keys for these organisms in Romania - *Flora pentru determinarea briofitelor din Moldova* (*Flora for the identification of bryophytes in Moldova*) in 1943, and the monograph on *Briofitele din România* (*The bryophytes of Romania*) in 1970, which remained for a long time the only reference book on these organisms in the country [MITITELU & TOMA, 1988]. He approached also studies on cormophyte taxonomy, among which, the most valuable is the *Monographie der Europäischen Arten der Gattung Melica* L. (*Monography of the European species of genus Melica L.*), published in 1933. His vast experience was also valued during the elaboration of the monumental botanic monograph *Flora R.S. România*, vol. 12 (*Flora R.S. Romania*, tome 12). For his contribution to the above mentioned scientific domains, he is considered the greatest Romanian bryologist, while two plant species were named in his honor [TOMA, 2015].

Professor dr. Constantin Burduja (1906-1983) continued the activity of his illustrious predecessor and mentor, and coordinated, as director, the activities of the Botanical Garden between 1958 and 1962. He was mainly preoccupied to extend the garden, to enrich the plant collections especially in the *Systematic* section, to *ex-situ* conserve some rare plant species from Eastern and Southeastern Romania and to intensify the international exchange with similar institutions [MITITELU & TOMA, 1983]. Professor Burduja can be considered as one of the founders of the current Botanical Garden, being designated by decision of the Rector of the University (1959) as the scientific coordinator of the new botanical garden which was to be established on the Copou Hill [TOMA & TONIUC, 1984].

He was a very exigent teacher, very documented, and for many decades he taught the *Botany*, *Pharmaceutical Botany*, *Geobotany*, *Plant Ecology* and *Plant Morphology* courses. The prodigious scientific activity of Professor Burduja was focused on floristic and phytosociological studies, chorology of some threatened species, history of botany in Romania, and nature conservation. He initiated the first researches on the morphology and anatomy of plants at the University of Iaşi, and was mentor for many students and collaborators who will become great personalities in this field [TOMA, 2015]. He was President of the Sub-commission of Nature Monuments and a significant contributor to *Flora Romaniae Exsiccata* and *Flora Moldaviae and Dobrogeae Exsiccata*.

Associate Professor dr. Constantin Dobrescu (1912-1990) coordinated the activity of the Botanic Garden for a short (1962-1963) but very important time, respectively the transfer of the institution from near the University to its current location. Distinguished teacher, meticulous connoisseur of flora and vegetation and very appreciated taxonomist, he contributed to the elaboration of the 12th volume of the *Flora R.S. România*, describing in detail 10 *Poaceae* taxa [MITITELU, 1984]. In addition, he described, for the first time, new species (e.g. *Asperula moldavica* Dobrescu), hybrids and 20 infrataxa [IACOB, 2011].

Besides the *Phytopathology* course, Professor Dobrescu held the academic courses of *Phytogeography* and *Botany* for whose illustration used plants from the Botanical Garden. He

published, in collaboration, impressive works as *Păşunile şi fâneţele din România* (*The pastures and hayfields from Romania*) in 1963. He was a member in the *Subcommittee for Nature Protection* and president of the Society of Biological Sciences - Iaşi Branch. He promoted the establishment of many natural reserves, including important plant areas for both species richness as well as rarity of the species perspectives. He was a valuable contributor to the *Flora Romaniae Exsiccata* (20 species) and *Flora Moldaviae et Dobrogeae Exsiccata* (34 species) [MITITELU, 1984].

Remarkable botanist and excellent teacher, ample and meticulously documented, **dr. Emilian Ţopa** (1900-1987) was invited and agreed (advised also by dr. Alexandru Borza) to coordinate the organization of the Botanical Garden on its current place [MORARIU, 1978-1980], in whose service he put all his previously accumulated experience as director of the botanical gardens from Cluj-Napoca (1952-1960) and Chernivtsi (1944). Thus, in collaboration with other botanists and architects, he elaborated the plan of the Botanical Garden and the scientific themes of the sections. After transferring the plants from the Garden near the University, he initiated activities for the stabilization of the eroded slopes by planting tree and shrub species in the most affected places, and increased the area of the institution from 30 hectares to 65 hectares. Under his coordination (1963-1970), were built: the greenhouses complex (7,100 m^2), the main entrance, the first alley system (3,000 m in length) and also some irrigation systems [ŞTEFUREAC, 1979]. He also put the bases of the *Herbarium*, the museum and the library of the Botanical Garden.

In addition to coordinating the above mentioned institutions, dr. Emilian Ţopa carried out an impressive activity as curator at the Museum of the Botanical Garden in Cluj-Napoca and also an important didactic activity in various universities from Bucharest, Chernivtsi, and Iaşi [MITITELU & LEOCOV, 1987]. His scientific contribution was focused on studies of plant taxonomy and ecology, phytosociology, ethnobotany and nature conservation. He was an expert in the most advanced research methods and analysis of vegetation, knowledge accumulated at Montpellier, under the guidance of dr. Josias Braun-Blanquet. He was the first author who investigated and described the halophilic vegetation in the country (e.g. *Halophilic vegetation in northern Romania in connection with the halophilic vegetation in the rest of the country*). The vegetation class of *Puccinellio-Salicornietea* Ţopa 1939 is still valid and widely recognized today. In his publications are highlighted 70 new species for Romania. He was one of the erudite who elaborated nine (of thirteen) volumes of the *Flora R.S. România*, by characterizing 207 species in 91 genera [MITITELU & LEOCOV, 1987]. He collected and contributed to *Flora Romaniae Exsiccata* (Cluj) and *Flora Moldaviae et Dobrogeae Exsiccata* (Iaşi) with an enormous quantity of herbarium material. Dr. Emilian Ţopa remains for eternity a symbol of erudition, the man who dedicated himself to science, education, organization and development of the Botanical Garden of Iaşi.

For establishing the plan and the general theme of the Botanical Garden from Copou Hill, Director Ţopa collaborated with botanist **Maria Lazăr** for the *Greenhouse Complex* (who also coordinated the *Herbarium* between 1968 and 1977), botanist **Elena Marin** for the *Ornamental* Section (who also coordinated the *Herbarium* between 1977-1984), dr. **Toader Chifu**, for the *Systematic* Section, agronomist engineer **Ioan Ostaciuc** for the *Rosarium* Section, agronomist engineer **Valeriu Movileanu** for the *Useful Plants* Section, agronomist engineer **dr. Ionel Lupu** for the *Dendrological* section and dr. **Corneliu Tăbăcaru** for the *Biological* Section [MITITIUC & TONIUC, 2006].

A close collaborator of Director Ţopa was, during 1965-1968 the botanist (at that time) **Professor dr. Toader Chifu** (born in 1936), who organized the *Systematic* Section of the

Botanical Garden according to the main phylogenetic systems. His great scientific and didactic career started as assistant professor at the Botany Department of the Faculty of Natural Sciences and Geography of the "Alexandru Ioan Cuza" University (1960-1965), continued at the Botanical Garden, at the Romanian Academy (Iaşi branch, 1968-1976) and as Head of the Collective for terrestrial ecology within the Center of Biological Research from Iaşi (1976-1980). Also he held *Botany* and *Plant Ecology* courses at the National Agronomical Institute from Alger (1976-1980). Returned in the country he connects the research and didactic activities, both at the Center of Biological Research and the Faculty of Biology. He is interested in mycology, mycocoenology, botany, nature conservation, and particularly phytosociology. The four volumes of *Diversitatea fitosociologică a vegetației României* [*The Phytosociological diversity of Romania's vegetation*] synthesize his great experience in vegetation classification and describe over 1,200 syntaxa from Romanian vegetation. He is member in the scientific board of numerous scientific journals, of many scientific societies from Romania or abroad, and mentor for numerous PhD students [MARDARI & TĂNASE, 2016].

One of his PhD students is **dr. Constantin Mardari**, who in 2003 became a biologist at the Botanical Garden of Iași and is presently coordinator of the *Romanian Flora and Vegetation* and *Moldavian Silvosteppe* sections. His researches focus on the ecology and chorology of certain plant species, on the syntaxonomy and structure analysis of several plant communities. He completed his PhD studies with the thesis *Diversitatea floristică și fitocenologică a ecosistemelor naturale din bazinul Neagra Broștenilor* (*Floristic and phytocoenological diversity of natural ecosystems from Neagra Broștenilor basin*), publicly defended in 2010. Dr. Mardari is also associated with the Faculty of Biology, involved in the specialized field practice for Biology and Ecology students. He also held the theoretical course and seminar for *The methodology for preparing the environmental impact studies*. As an active member of the Eurasian Dry Grassland Group, he is part of several international actions, with many relevant publications in the above mentioned scientific fields.

After a relatively short time as biologist (1998-2000) in the *Romanian Flora and vegetation* section of the Botanical Garden, **dr. Ciprian Mânzu** obtained, by contest, an academic position at the Faculty of Biology and currently he is Lecturer within the same institution. He published, in collaboration, reference books of plant taxonomy, floristics and phytosociology, such as *Flora ilustrată a plantelor vasculare din Estul României* (*Illustrated flora of vascular plants from Eastern Romania*) or *Flora și vegetația Moldovei* (*Flora and vegetation of Moldova*) in two volumes (2006), as well as numerous scientific articles in prestigious journals, focusing on flora, biodiversity conservation, plant and vegetation ecology, Natura 2000 habitats, climate change, glacial and post-glacial relict plant species. Currently he holds several academic courses and laboratories: *Plant systematics*, *Forest resources*, *Field methods in ecology*, *Habitats and species of Community Interest*, etc.

Between 1973 and 1990 **Professor dr. eng. Mandache Leocov** (1928-2018), an expert in agriculture, landscape architecture, exotic and native ornamental species, etc., coordinated and continued to organize the Botanical Garden. Thus, by taking into administration the so called "Petrescu plot", donated by the family of the great botanist Constantin Petrescu, he opened the access (through a wood gate carved with plant motifs, creation of Professor Dumitru Zaucă) in the Garden directly from the Exhibition Park [TĂNASE & OPREA, 2018]. He inaugurated the *Palmarium* greenhouse (1974) and organized the *Didactic-Experimental* section. Together with his colleagues, he put the bases of the *Rosarium* section, inaugurated it in 1979, started (in 1976) to organize the main exhibitions of the Botanical Garden (one displaying azaleas and camellias as floral arrangements in the hall of the administrative building,

and another presenting the chrysanthemums and bonsai collections) and continued to consolidate some problematic areas affected by landslides.

He continued the work of his predecessors to *Flora Moldaviae et Dobrogae Exsiccata*. Together with the architect V. Carmazin-Cacovschi he created the first model of the institution, highlighting each section. In his didactic activity he went through all the academic stages until full professor. He held the *Dendrology*, *Agriculture*, *Agro-phyto-technology* courses [MITITELU & COSTICĂ, 1993] in various academic institutions from Iasi. Besides the Botanical Garden, Professor Leocov organized many green spaces in Iaşi and was preoccupied by the protection of some monumental trees in the city (as Eminescu's linden). He edited the first editions of the *Guide of the Botanical Garden from Iaşi* volume (1985 and 1989), presenting the history of this institution and the personalities who was involve in its establishment and organized the national symposia dedicated to the anniversaries of the Botanical Garden in 1976, 1981, and 1986 [MITITELU & COSTICĂ, 1993]. He is remembered as the Professor who always presented Iaşi as a city of flowers and parks [TĂNASE & OPREA, 2018].

An important role in the development of the Botanical Garden had engineer **dr. Ionel Lupu**, coordinator of *Dendrarium* section between 1967 and 1998. He was directly involved in the elaboration of this section's general plan, in the organization and diversification of the woody plants collections, introducing and acclimatizing many indigenous and exotic species. He perfected his studies by defending, in 1980 his PhD thesis at the Agronomic Institute of Iași – *Flora și vegetația pădurilor dintre Siret, Moldova și Șomuzul Mare* (*The flora and vegetation of forests between Siret, Moldova and Șomuzul Mare*), under the supervision of professor dr. Mihai Răvăruț. Member of many associations dedicated to the conservation of woody species, he initiated several civil projects for the inventory, monitoring and protection of trees across the city of Iași, as well as trees plantations activities, implicating people from different social classes, pupils and students, children and adults, raising awareness towards the importance of forests preservation.

Another great name, who brought value to both the Botanical Garden and the Faculty of Biology, was **Professor dr. Dumitru Mititelu** (1929-2005). He was a member of the Scientific Committee of the Botanical Garden (1967-1975) and in 1988 was named honorary director of this institution. He graduated the Faculties of Natural Sciences, Philosophy and Agronomy, all from Iași and defended his PhD thesis – *Flora and vegetation of the Elan basin and hills* in 1973, under the supervision of Constantin Burduja [OPREA, 2019]. He started his career at the Agronomic Institute of Iași and after he transferred to the Faculty of Biology, where he held the *Plant systematics*, *General biology*, *Romanian phytocoenology and vegetation* courses. He also got to taught *Tropical systematics and sinecology* at the University of Kisangani (Republic of Zair, Africa).

With his studies focused especially the flora and vegetation of Moldova, he brought a new and modern approach to plant systematics, vegetation mapping and phytocoenology researches. He extensively published his investigations in more than 200 papers, as a single author or in collaboration with other specialists, revealing important data regarding, among many other aspects, over 800 rare species, from which 12 species were new for Romania, and 80 new for Moldova, 28 new for science plant communities and 2 subassociations, contributing at the *Flora Romaniae exsiccata*, *Flora Moldaviae et Dobrogae exsiccata* and *Flora districti Bacoviensis exsiccata*, with more than 80,000 Herbarium sheets [BARABAȘ, 1980-1993]. Professor dr. Mititelu's dedication and professionalism impressed many generations and made him a mentor for tens of students, from which several followed a teaching or research career.

After eight years at the Department of Botany of the Faculty of Biology, **Senior Researcher dr. Ion Sârbu** (born in 1933), joined the team of specialists from the Botanical Garden of Iași. He is a great personality of the Romanian Botany, a man passionate about the study of plants, perfectionist, full of patience and modesty, and of great moral integrity [TOMA, 2013]. For approximately 30 years (since 1975) he was the coordinator of the *Romanian Flora and Vegetation* section, and, for many years he served as Scientific Secretary of the Botanical Garden.

His prodigious research activity started in 1967 as curator of the *Herbarium of the Faculty of Biology*, and consists in numerous articles, books, and research projects, in fields of plant taxonomy, phytosociology, and nature conservation, activity for which he was granted many awards. He was actively involved in the development and implementation of the protected areas network "Natura 2000" in Romania. Reference books such as *Plante vasculare din România. Determinator ilustrat de teren* [*Vascular Plants of Romania. Illustrated Field Guide*] published in 2013 in collaboration with dr. Adrian Oprea and Professor dr. Nicolae Ștefan, synthesize his vast experience and is highly appreciated by both specialists, and people who are interested in discovering the Romanian flora. He is a valuable contributor to *Atlas Florae Europaeae* and *Euro+Med PlantBase*. He is also valued by many generations of biology students (but also students from the Environmental Engineering department of the Technical University) to whom he held academic courses and laboratories referring to forest resources, biodiversity of terrestrial and aquatic environments, botany, biogeography, etc. [OPREA, 2013].

Dr. Adrian Oprea, presently coordinator of the *Systematic* section, completed his studies under the supervision of professor dr. Mititelu, defending his PhD thesis entitled *Flora și vegetația din Câmpia Tecuciului și Bazinul Inferior al Siretului* (*Jud. Galați*) [*Flora and vegetation from Tecuci plain and Inferior Basin of Siret river (Galați County)*] in 1998, at the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași. Besides his involvement in the development and maintaining the natural patrimony of the section, he actively contributes to the better knowing of the flora and vegetation of certain regions of Romania, including natural protected areas, the identification and distribution of several vascular plants, proposal of new plant communities and sub-communities (for science or for Romania), restoring plant communities on tailings. He is member of the *Romanian Phytosociology Society* and the *International Dendrology Society*, associated with the Faulty of Biology, where he held the practical works and specialized field practice for the *Phytosociology* course, actively collaborating in his researches with academic personnel from many Universities from Romania and abroad.

Plant morphology and anatomy

Studies focused on the anatomic structure of plant species and also on histology, and plant physiology were published stating with the second half of the nineteenth century [IACOB, 2011] by great scientists who also had close relationships with the Botanical Garden of Iași. The first contributions in these scientific fields were published by Dimitrie Brândză (1867) and Alexandru Popovici (1893) and mainly represents the results of their doctoral theses. The first university manual of plant histology, organography, and plant physiology was elaborated by Anastasie Fătu (1877). Also, Constantin Papp carried out studies on the structure of the leaves of some medicinal plants (1942). Until 1948 the discipline of plant morphology was taught within the more broadly course of Botany. The botanist and phytosociologist, director of the Botanical Garden, Professor Constantin Burduja held the *Plant Morphology* course until 1971 (he is the founder of the first school of plant morphology and anatomy in Romania), and, since

then, for almost forty years, this academic course (besides plant cytology, plant embryology, evolutionary strategies in plant realm, and others) was held by **Academician Constantin Toma** (1935-2020).

Professor dr. Toma was also appointed Director of the Botanical Garden between 1970 and 1973, where he continued the organization of the garden's sections, enrichment of plant collections, the establishment of microhabitats with characteristic plant species, organization of scientific expeditions in order to collect plants, fruits and seeds from species in different regions of the country, and initiated fruitful collaborations with similar institutions from Romania and abroad.

Professor dr. Toma initiated the design and construction of the *Palmarium* greenhouse and edited two centurias of *Flora Moldaviae et Dobrogea Exsiccata*. He promoted the use of the Garden in teaching and education activities and had the initiative to organize and develop a laboratory of plant morphology and plant anatomy in this institution where he started the first studies on medicinal and ornamental species as well as studies of experimental and ecological anatomy [MITITIUC & TONIUC, 2006]. He was passionate about the history of the Botanical Garden and by the personalities who contributed to its foundation and development, and published appreciative articles about Anastasie Fătu, Dimitrie Brândză, Constantin Papp, Constantin Burduja and Constantin Dobrescu [TONIUC, 2010]. He continued to be present in the Botanical Garden all his life, and had unforgettable interventions especially on the occasion of flower exhibitions.

His exceptional didactic career extends on over sixty years, period when he developed at the Faculty of Biology of the "Alexandru Ioan Cuza" University the most valuable school of plant morpho-anatomy in the country [IVĂNESCU & ZAMFIRACHE, 2010], widely recognized. He was a Professor with great vocation, highly appreciated for his memorable academic lectures, coordinator for 28 doctoral theses. In his prolific scientific career he published over 500 scientific articles and reference books, as well as other hundreds of articles of popularization, on the history of biology, book reviews, etc. [IVĂNESCU & al. 2020] for which he was granted many distinctions and awards. His extensive and valuable contributions to the development of numerous research directions in the field of plant biology recommended him to become a member (1991) of the Romanian Academy in the Department of Biological Sciences, and honorary member of the Academy of Sciences of the Republic of Moldova (2011). He was the scientific secretary of the Romanian Academy – Iași Branch and president of the Sub-commission of Nature Monuments of Moldova within the same institution.

Besides the position of the director of the Botanical Garden, he was vice dean and dean of the Faculty of Biology, scientific secretary in the Senate of "Alexandru Ioan Cuza" University, director of the Institute of Biological Research from Iași, vice-president and honorary president of the National Society of Biological Sciences (since 2007), member of the National Council for Academic Evaluation and Accreditation (1994-2005), member of the Biology Commission of the National Council for Attestation of University Degrees, Diplomas and Certificates (1994-2005), member in the Council of the Romanian Academy Publishing House (1996-2003) [IVĂNESCU & al. 2020].

He was a Professor distinguished by the elegance of expression, precision and clarity of the language, emblematic by the attitude and scientific authority in the department, in the laboratory and in the field, respected, by collaborators, who shaped the professional destinies of most of the disciples who continues his activity [MITITELU, 1993; IVĂNESCU & ZAMFIRACHE, 2010], both at the Faculty of Biology and the Botanical Garden.

At the Botanical Garden he was surrounded by an enthusiastic team of young botanists and engineers, deeply involved both in their professional development and the development of the botanical garden. One of his collaborators, was botanist **dr. Corneliu Tăbăcaru**, coordinator of the *Biological* section between 1967-2004. He contributed to the elaboration of the organizational plan and the theme of this section, he was implicated in the introduction of new species within the existing collections, dedicating his time to research in the fields of plant morphology and embryogenesis. He graduated the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași, further specialized at the University of Bucharest and in 1984, under the coordination of Traian Ștefureac defended his PhD thesis entitled *Cercetări de morfologie ale mugurilor, embriogeneză și fenologie la nuc și vișin cu unele considerații taxonomice și importanța lor în pomicultură* (*Researches about buds morphology, embryogenesis and phenology in walnut and sour cherry with taxonomical considerations and their importance in tree culture*). Even after his transfer to the Botanical Garden of Iași, he continued the collaboration with the academic personnel from the University of Bucharest, carrying on studies and researches in the above mentioned fields.

Under the supervision and coordination of **Academician Constantin Toma**, many biologists from "Anastasie Fătu" Botanical Garden completed their studies and became specialists in their field.

An illustrious representative of the Plant Morphology and Anatomy School from Iași, and a remarkable researcher for over 30 years at the Botanical Garden was **dr. Rodica Rugină** (1940-2021). She coordinated the *Systematic* Section, and increased the dimensions of plant collections, with a special preoccupation for rare and threatened plant species and their *ex situ* conservation. She closely supervised the introduction and acclimatization of some protected plant species in the Botanical Garden while tried to increase the interest of visitors by creating an open-air amphitheater, a basin with aquatic plants and some fountains [ADUMITRESEI & IFRIM, 2020]. Before transferring to the Botanical Garden, dr. Rugină started her academic career in 1963 as assistant professor at the Faculty of Pharmacy and Faculty of Biology, in the field of plant morphology [TOMA, 2015]. She continued to collaborate with the specialists of the Plant Biology Department in studies focused on comparative, ecological and experimental plant anatomy. Reference books as *Plante ocrotite din România* (*Protected plant species from Romania*) and *Anatomia plantelor medicinale* (*Anatomy of medicinal plants*) highlights her main scientific preoccupations.

With a career of over 35 years, **dr. Angela Toniuc** coordinated the *World Flora* section (currently the *Phytogeographic* section) of the Botanical Garden. From the very beginning she was involved in the elaboration of the scientific theme of the section and the establishment of the plantation, followed by the care and enrichment of the plant collections. She put the bases of the bonsai collection, which now includes hundreds of specimens, and is probably the most valuable in the country. She also was responsible for the international seed exchange (1985-2001) and publication of the *Delectus Seminum et Sporarum* catalogue, distributed to hundreds of similar institutions from all continents. The valuable experience dr. Angela Toniuc determined her appointment as member in the scientific council of the Botanical Garden of Iași (and also of Galați Botanical Garden since 1990). The didactic activity, carried out between 1990 and 2000, as associated professor at the Faculty of Biology, was focused on disciplines as *Plant anatomy*, *Plant embryology* and *Ecological biogeography* [TOMA, 2014; IFRIM & ADUMITRESEI, 2019].

The scientific activity of dr. Angela Toniuc is related to plants morphology and anatomy, plants embryology, as well as plant taxonomy, floriculture and history of botany

[TOMA, 2014]. She published as single author or in collaboration, over 130 articles and books, including reference books as: the four volumes of *Flora lemnoasă spontană şi cultivată din România* (*The ligneous flora native and cultivated in Romania*] in collaboration with dr. Ion Sârbu, and professor Valeriu Zanoschi, or *Adaptarea la mediu în lumea plantelor* [*Adaptation to the environment in the plant world*). In *Grădina Botanică Anastasie Fătu. File de istorie* (*Anastasie Fătu Botanical Garden. History sheets*), together with Professor Mihai Mititiuc, she documented and presented the efforts of all who contributed, in time, to the foundation and development of this institution. All her collaborators appreciate that dr. Angela Toniuc is an example of seriousness, tenacity, erudition, and altruism.

Dr. Georgeta Teodorescu was the coordinator of the *Central and South America* subsections of the *World Flora* section (1970-1994) and the *Greenhouse Complex* (1988-2004), actively participating in the development of these sections, diversifying the plant collections and promoting them. She obtained her PhD title in 1998 with the thesis entitled *Cercetări privind morfologia și structura unor plante în condiții de parazitare* (*Researches regarding the morphology and structure of some plants in parasitic conditions*), under the supervision of Professor dr. Toma. Her major scientific interest included the morphological and histological study of angiosperms plantlets, structural modification induced by phytopathogenic fungi, the horticultural value of several plant collections, publishing various articles in collaboration with her mentor, Professor dr. Toma, or with colleagues from the Botanical Garden and the Faculty of Biology.

In 1994-2012, **dr. Violeta Tănăsescu** carried out scientific activities in numerous sections of the Botanical garden: she coordinated and elaborated the scientific thematic of the Memorial plants section (1994-2003), contributed to the enrichment of plant collection from Ornamental section (2000-2001) and the Useful Plants section (1997-2005), and coordinated the Greenhouses Complex (2005-2012). Her research activity was focused on the morphology and anatomy of medicinal, ornamental or rare and threatened plant species. Under the supervision of Academician Constantin Toma, she elaborated the PhD thesis - *Cercetări de morfo-biometrie și histo-anatomie ontogenetică, comparată și experimentală la diferite specii și soiuri de pomi fructiferi din familia Rosaceae* (*Researches of morpho-biometry and ontogenetic, comparative and experimental histo-anatomy of various species and varieties of fruit trees in Rosaceae family*). She was also preoccupied of the guidance in botanical gardens, especially of aspects for the improvement of the communication process to the persons with special needs, and of the modalities to use the botanical garden's patrimony in order to maximize the benefits for the same category of people.

Dr. Camelia Ifrim, coordinator of the *World Flora* section between 2001 and 2009 and currently of the Greenhouse Complex, is a disciple of Professor dr. Toma, under whose supervision, in 2005, obtained her PhD title with the thesis – *Cercetări morfo-anatomice și fiziologice asupra organelor vegetative de la unele plante decorative ierbacee* (*Morpho-anatomical and physiological researches of vegetative organs from some herbaceous decorative plants*). Her domains of interest include morphological, histological and anatomical aspects of plant species with different bio-geographical origin, plant physiology, embryogenesis, seed dormancy and mechanisms to break it. As coordinator of the Greenhouse Complex she carries out specific activities such as: identification of exotic taxa, organizing and maintaining the plant collections and developing ways to present them to the public, involvement in the educational and cultural events organized by the Botanical Garden.

Although for a relatively short time in the Botanical Garden (2008-2010), **dr. Irina Elena Stănescu** brought an important contribution to the development of the institution

especially of the collections of the *Useful Plants section* (as coordinator), the international seed exchange and publication of the catalogue *Delectus Seminum et Sporarum*. Her scientific activity was focused on plants morphology and anatomy as well as plant taxonomy, particularly on carnivorous plant species, the subject of her doctoral thesis, *Cercetări citologice și histo-anatomice asupra unor specii de plante carnivore* (*Cytological and histo-anatomical research on certain carnivorous plant species*) coordinated by Academician Constantin Toma.

Dr. Lidia Adumitresei, presently coordinator of the *Biological* section completed her professional training with the doctoral studies under the supervision of Professor dr. Toma, defending her PhD thesis entitled *Cercetări morfo-anatomice și fiziologice asupra unor specii și soiuri de Rosa* L. (*Morpho-anatomical and physiological researches of several species and varieties of Rosa* L.), in 2011, at the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași. She is actively participating at the development and conservation of the natural patrimony of the *Biological* section, but also involved in research activities regarding the nomenclature and origin, stem and leaf morphology and anatomy of several rose species and varieties adapted to local conditions, the content of carbohydrates, assimilatory and anthocyanin pigments, volatile oils and enzymes involved in the respiratory process from several taxa belonging to *Rosa* genus. She is a member in various national and international societies and associations, such as: *Amicii Rozelor* Association from Romania, and International Hibiscus Society.

Mycology and phytopathology

The scientific activity in fields of phytopathology and mycology at the Faculty of Natural Sciences from Iași starts in the early twentieth century and is coordinated by Professor Alexandru Popovici, who, besides Botany, was preoccupied by diversification and development of new scientific domains. Detached from the discipline of Botany, the *Phytopathology* academic course was held by also by Professor Constantin Dobrescu and continued, for almost five decades by Professor dr. Mihai Mititiuc, while nowadays it is held by Professor dr. Cătălin Tănase (in this article were highlighted only the academic personalities who coordinated or worked in the Botanical Garden).

With a great managerial capacity, tenacity, and perseverance, **Professor dr. Mihai Mititiuc** (1937-2020) coordinated activities in the Botanical Garden during 1990-2007, in a period characterized by many social and economic problems. During this time, together with his colleagues, he managed to repair and modernize the greenhouses complex, and to inaugurate three new ones while preparing the project for a new aquarium greenhouse, founded the subsection for people with visual disabilities within the *Ornamental* section and supported the organization of annual flower exhibitions. He contributed to enrichment of plant collections and *ex situ* conservation of rare or endangered species by promoting national and international exchange of plant seeds and by organizing field expeditions in various regions of the country. In this direction, he was preoccupied to establish an alpine Botanical Garden at the Potoci research station. He edited the last two centurias of *Flora Moldaviae et Dobrogae Exsiccata*.

Concerned with honoring the memory and achievements of the predecessors, Professor Mititiuc ordered the installation of bronze busts that evoke great personalities involved in the development of the Botanical Garden, and organized scientific symposia with the occasions of celebrating the 140th, 145th, and 150th anniversary of the Botanical Garden. He edited the third edition of *Guide of the Botanical Garden* and reorganized the journal of the institution - *Buletinul Grădinii Botanice Iaşi* (*Bulletin on the Botanical Garden from Iaşi*). He was also involved in organizing 12 editions of the National Symposium of Mycology (1995-2007) as

well as Second National Congress of Biology (1992). He was the president of the Mycological Society of Romania and of the Association of Botanical Gardens from Romania.

The didactic activity of Professor dr. Mititiuc was mainly carried out in the fields of *Phytopathology* and *Mycology*, disciplines for which he organized the Laboratory of Mycology and Phytopathology within the Faculty of Biology (1993). He also held the academic course of *Biogeography*, and in the early stages of his career, laboratories of *Plant morphology and anatomy*, *Biology of plant pathogens*, *Plants Protection*, etc. As PhD coordinator, he contributed to the formation of many young specialists in the field of mycology and phytopathology [TĂNASE & al. 2020]. He had a prolific scientific activity, materialized in identification and publication of numerous micromycetes species, new for science, of their taxonomy, ecology and biology. Moreover he studied and published the micromycete species identified in the Botanical Garden [MITITIUC, 1973; MITITIUC, 1979; MITITIUC 1982; MITIUC 1984a; MITIUC 1984b; MITITIUC 1985; MITITIUC, 1986a; MITITIUC, 1986b; MITITIUC, 1987a; MITITIUC, 1987b; MITITIUC, 1989; MITITIUC, 1991; MITITIUC, 1992a; MITITIUC, 1992b; MITITIUC, 1993]. His PhD thesis, coordinated by Professor dr. Olga Săvulescu, was focused on fungal diversity from important natural reserves - *Contribuţii la cunoaşterea micromicetelor şi macromicetelor din rezervaţiile naturale Ponoare şi Frumoasa - Suceava* (*Contributions to the knowledge of micromycetes and macromycetes from the nature reserves Ponoare and Frumoasa - Suceava*). He also published reference books in the fields of phytopathology [TĂNASE, 2012], such as *Biologia paraziţilor vegetali* (1980) (*Biology of plants parasites*), *Ciuperci parazite pe arborii şi arbuştii din pădurile noastre* (1997) (*Parasitic fungi on the trees and shrubs from Romanian forests*) or *Bolile şi dăunatorii plantelor medicinale şi aromatice* (2000) (*Diseases and pests of medicinal and aromatic plants*). He also had the initiative to organize the scientific communication sessions of the Faculty of Biology in close collaboration with the specialists from the Botanical Garden. He remains a symbol of tenacity, continuous effort and devotion to scientific knowledge and to the Botanical Garden.

He had an important contribution to the scientific perfecting of the employees from the Garden, by sending them in training stages at other institutions in the country or abroad and many of his students continued their professional development by enrolling at the doctoral school from the Faculty of Biology.

One of his students, **dr. Profira Vidrașcu**, was coordinator of the *Ornamental* Section – *Chrysanthemum collection*, closely involved in the diversification of the chrysanthemum collection, culture methods and phytopathology, developing new varieties with decorative value. Over the years, she published articles and books covering many aspects regarding the culture technology for chrysanthemums and completed her university studies with the PhD thesis entitled *Cercetări asupra taxonomiei, fitopatologiei și bazele ecofiziologice ale tehnologiei de cultură a unor specii și soiuri de crizanteme din colecția Grădinii Botanice din Iași* [*Researches regarding the taxonomy, phytopathology and ecophysiological basis of the culture technology for several species and varieties of chrysanthemums from the collection of the Botanical Garden of Iași*], which she publicly defended in 1995, at the Faculty of Biology.

Between 2001-2004 botanist **dr. Cristina Pricop-Ciocoiu** coordinated activities in the Ornamental section of the Botanical Garden. Besides decorative plant species, her research activity was also focused on the parasitic micromycetes on cultivated tree species in parks and gardens, within *Uredinales* and *Erysiphales* taxonomical orders. For the thesis entitled *Ciuperci parazite pe plante lemnoase ornamentale din spaţiile verzi urbane ale Moldovei* (*Parasitic fungi on ornamental woody plants in the urban green spaces of Moldova*), coordinated by Professor Mititiuc, she was granted a PhD diploma in the field of Mycology.

Another student of Professor dr. Mititiuc, **dr. Vasilică Claudiu Chinan** coordinated the current maintenance activities in the *Romanian Flora and vegetation* section of the Botanical Garden (2002-2004) and carried out mycological research activities. Currently, he is Lecturer at the Faculty of Biology, where he teaches the *Plant taxonomy* courses and laboratories for *Mycology* and *Phytopathology*. He published, in collaboration, reference books in fields of fungi taxonomy – *Macomicete din Romania* (*Macromycetes from Romania*), and fungi diversity – *Parcul Național Călimani. Studii ecologice și de biodiversitate, Mycobiota* (*Călimani National Park. Ecological and biodiversity studies. Mycobiota*), as well as numerous scientific articles in prestigious journals from the country and abroad, highlighting new fungal species for Romania which he discovered.

Dr. Ana Cojocariu was the coordinator of the *Useful Plants* section (2005-2008; 2010-2015) and currently coordinates the activity within the *Ornamental* section – the *Chrysanthemum collection*. She specialized in mycology, in 2007 defending her PhD thesis entitled *Macromicete implicate în degradarea lemnului din construcții la monumentele istorice din Moldova* (*Macromycetes involved in the degradation of construction wood from historical monuments in Moldova*), having as main interests macromycetes taxonomy and ecology, fungal mechanisms of wood degradation, fungal succession and participating, as a member of the Romanian Mycological Society and the European Mycological Association, at scientific and popularization activities regarding fungi and their importance in natural and anthropic environments. She was also involved in the teaching activities at the Faculty of Biology, by holding the laboratory works for the *Phytopathology* and *Biology of vegetal parasites* courses. The nature of the specific activities carried out within the section she coordinates gave her the opportunity to maintain and diversify a unique plant collection, to improve the culture methods, and to develop, from the existing taxa, new varieties of chrysanthemums, very well adapted to the local conditions, resistant to pathogens and with special decorative value.

After the short period (2003-2004) in which the biologist Constantin Ciocoiu coordinated the activities in the *Dendrarium* section, since 2005 **Dr. Ciprian Bîrsan** took over these tasks and is involved in the conservation and preservation of the natural patrimony of the Botanical Garden, in the development of the living plants and fungi collections, participating at botanical expeditions in different regions of Romania and gathering material (seeds, cuttings, fruiting bodies) for research or educational purposes. He completed his studies with the PhD, which he defended in 2008 with the thesis *Diversitatea taxonomică și ecologică a macromicetelor din masivul Giumalău (Carpații Orientali)* (*Taxonomical and ecological diversity of macromycetes from the Giumalău Massif* (*Eastern Carpathians*)) at the Faculty of Biology. His main domains of interest include the taxonomy, ecology and chorology of macromycetes, monitoring natural habitats and ecosystems. He is also associated with the Faculty of Biology, where he held the laboratory works for the *Mycology* course and collaborating with academic personnel in elaborating studies, books and articles in the related fields. He is a member of the Romanian Mycological Society, being involved in organizing and implementing scientific and educational activities.

In March, 2007, after the results validation of the contest for the position of manager, **Professor dr. Cătălin Tănase** was appointed Director of the Botanical Garden by the University's management. From the very beginning he was preoccupied to promote the Garden as an institution of education and culture, and to modernize the existing infrastructure. Professor Tănase was decisively involved in the foundation of the *Laboratory for micro-propagation and preservation of germplasm*, improving the conditions for seed conservation, and establishing the *Research Group for Micro-propagation and Preservation of Germplasm*

(GERMOBIOTECH). Under his management, almost all compartments of the greenhouses were rehabilitated: the exhibition greenhouse, the multiplier greenhouse, the greenhouse for the indoor culture of chrysanthemums, as well as the spaces for storing and conditioning the biological material, and for garden's staff. Together with the Rector of the University, Professor Vasile Işan, he inaugurated the new greenhouses, with an area of 1.100 m^2 (2013). He reorganized the *Ornamental* section, and reopened after 17 years, the garden's main entrance. Also, through various projects that Professor dr. Tănase coordinated, in the *Systematic* Section, the Auditorium for educational and cultural activities was completed, and a new pool harboring species and varieties of water lilies and other aquatic species was inaugurated (2016), the *Useful Plants* section was reorganized (2018) and a new subsection including *plant species named to honor the great scientific personalities* was presented to the public, the lighting system on the main alleys was modernized and a photovoltaic system was introduced the *Rosarium* section. He also modernized the Herbarium, while in the Biological section was started (2019) the organization of a Japanese garden.

Professor Tănase continues today the work of his predecessors, coordinating the specific activities of enrichment the living plant collections, organizing the traditional events: the Autumn Flowers (the *Chrysanthemum* collection increased during his mandate to 450 varieties, including new created varieties; he also started and increased the collections of decorative cabbage - 76 taxa, chilies - over 130 taxa, and decorative pumpkins - over 200 taxa), the Exotic Plants exhibitions, editing the journal of the Botanical Garden (named since 2007 *Journal of Plant Development*) and the Catalogue with the seed offer for exchange, promoting the institution's achievements and honoring all the predecessor who contributed to the establishment and development of the Garden. Also, honoring the memory of his predecessors, he installed the bust of Professor dr. Mandache Leocov in the Administrative building, a work of art realized by sculptor Constantin Crengăniş and donated by Professor Emil Gentimir. The exhibitions of fresh edible and toxic mushrooms represents a novelty element. Under his coordination the institution was accepted as member in the *International Network for Plant Exchanges* (2009) and of the *Association of Botanical Gardens of Coimbra Group Universities* (2011).

The academic career of Professor Tănase is focused on the fields of Mycology and Phytopathology, and since 2009, he coordinates doctoral theses in the field of Biology. His research activity focuses on taxonomy and ecology of fungi, conservation of fungal diversity, and the biocontrol and bioremediation of pollutants. His scientific activity is widely recognized and, as a consequence, he was granted many awards. For the thesis *Cercetări sistematice şi ecologice asupra micromicetelor din Masivul Rarău* (*Systematic and ecological researches on the micromycete species from Rarău Massif*) he received a PhD diploma in Botany domain. He authored numerous reference books, among which *Macromicete din România* (*Macromycete species from Romania*), *Concepte actuale în taxonomia ciupercilor* (*Current concepts in fungi taxonomy*) or *Fungi cu aplicaţii în agricultură, medicină şi patrimoniu* (*Fungi with applications in agriculture, medicine and heritage*) are highly valued by specialists. Due to his remarkable scientific contributions, in 2018 was elected corresponding member of the Romanian Academy. He is also member of prestigious scientific organizations as the Romanian Mycological Society (president since 2014); Association of the Botanical Gardens in Romania (vice president 2008-2016; president 2016-2018) or in the council of the European Mycology Association (since 2007). He organized a significant number of scientific events, among which, the 155th, 160th and 165th anniversaries of the institution (2011, 2016, and 2021) which were attended by many specialists from all botanical gardens in Romania and from many similar foreign institutions.

By creating the slogan "*Science and culture for nature*", Professor dr. Tănase defines the purpose of the "Anastasie Fătu" Botanical Garden, and reinforces its mission as an institution dedicated to *ex situ* conservation of plant species, education, recreation, and promotion of the cultural values.

A valuable and close collaborator of Professor dr. Tănase is **dr. Tiberius Balaeş**, who worked for five years (2013-2018) as a researcher in the Botanical Garden, in fields such as taxonomy and ecology of fungi, bioremediation of certain categories of pollutants, isolation in pure culture and selection of fungi, optimization of culture conditions, cryopreservation of fungal mycelium, etc. In 2013 he defended his PhD thesis entitled *Izolarea și selecția unor specii de macromicete cu rol în biodegradarea coloranților sintetici* (*Isolation and selection of some species of macromycetes involved in the biodegradation of synthetic dyes*). The results of his researches were published in prestigious national and international journals. His didactic talent and qualities, recommended him for an academic career and currently is head of works at the Faculty of Biology, "Alexandru Ioan Cuza" University from Iași, where he teaches courses and laboratories, both for the bachelor and master programs, for several disciplines such as: *Microbial bioconversions*, *Cryptogams systematics*, *Medical mycology*, *Strategies in biodiversity conservation*, *Principles of eco-tourism*, etc. and is responsible for the specialized filed practice for the Faculty's students.

Dr. Cristiana Virginia Petre, presently coordinator of the *Useful Plants* section, is involved in maintaining and diversifying the useful plants collections and is responsible with the educational activities organized within the Botanical Garden, alone or in partnership with different institutions: schools, universities, NGOs, being part in many biodiversity conservation actions as an active member of the Romanian Mycological Society and Romanian Ornithological Society. In 2018 she participated at the specific actions for reorganizing the *Useful Plant* section, information gathering and ways to present it to the public. Alongside with this activity, her domains of interest include fungal taxonomy and ecology, *in vitro* isolation of fungal species, biotechnological potential of fungi. She obtained the PhD title in 2016 with the thesis entitled *Studiul unor specii de basidiomicete lignicole cu rol în sinteza compușilor organici volatili* (The s*tudy of some lignicolous basidiomycetes species involved in the synthesis of volatile organic compounds*). She currently holds the laboratory works for the *Cryptogams Systematics* course at the Faculty of Biology.

For approximately 10 years, **dr. Ovidiu Copoț** carried out various research activities in the *Dendrarium* section of the Botanical Garden. His scientific researches were mainly focused on using the statistical methods for the analysis on diversity of macromycete species. For the thesis *Modelarea ecologică și spațială a unor specii de de macromicete în ecosisteme forestiere din Regiunea de Nord-Est (România) - Ecological and spatial modeling of some macromycete species in forest ecosystems of the North-East Region (Romania)* he was granted a PhD diploma. In his research activity he is focused on identifying the relevant abiotic and biotic factors influencing the richness of fungi species, the composition of fungal communities, and on statistical modelling of their potential spatial distribution.

The current team of the Botanical Garden express their admiration for the dedication, passion and competence of their great predecessors and tries to rise to the height of their achievements. Although there was a change between generations, caused by the retirement of some great specialists, the relatively young, but enthusiastic team of biologists and engineers continued the researches, the organization of the traditional events and, when needed, the didactic activities, collaborating every year with academic personnel from "Alexandru Ioan Cuza" University of Iași, "Ion Ionescu de la Brad" University of Life Sciences from Iași or

"Gigore T. Popa" University of Medicine and Pharmacy, in organizing the specialized field practice for the students. Today, that team includes 7 biologists: **dr. Lidia Adumitresei**, **dr. Ciprian Bîrsan**, **dr. Ana Cojocariu, dr. Camelia Ifrim**, **dr. Constantin Mardari**, **dr. Adrian Oprea**, **dr. Cristiana Virginia Petre** and 3 engineers: **Iuliana Gaţu** (*Phytogeographic* section), **Mihaela Mihalache** (*Rosarium* section) and **Mihaela Popa** (*Ornamental* section).

Conclusions

"Anastasie Fătu" Botanical Garden of Iași represents an important scientific, educational and cultural pole in Romania, the efforts of the founder, previous and present generations making it a valuable patrimony element. The dedication of people who participated at the establishment of this institution, their will and perseverance in overcoming every obstacle and their determination in offering the academic community, as well as the general public the opportunity to research and discover the world of plants are truly worth cherishing and celebrating.

The idea of organizing a botanical garden wasn't just a simple job requirement, but a soul driven mission, serving a greater purpose, that 165 years later still prevails. The 3 main objectives of any botanical garden: *ex situ* conservation of plant species, scientific research and educating people in the spirit of nature, were drawn since the beginning of this initiative, and permanently performed and improved.

The nature of the activity carried out at the Botanical Garden of Iași and the diverse and complex plant collections, offer vast opportunities for quality research within this institution, but also the opportunity of the employees to collaborate with academic personnel from the field of natural sciences: biology, agronomy, horticulture, pharmacy. In this way, the research of the biologists from the botanical garden alone or in collaboration cover various domains, starting with plant and fungal taxonomy, plant morphology, anatomy, physiology, embryology, biochemistry, phytocoenology, plant and fungal ecology and mapping, phytopathology, use of fungi in biotechnologies.

With every generation the value of "Anastasie Fătu" Botanical Garden of Iași increases, the fields of research expand, the species preservation techniques on medium and long term change with the development of technology, the culture methods improve, the plant collections diversify, and the manner of presenting them to the public shifts from the traditional approach to a more modern one, in order to meet both the plants requirements and the people's expectations.

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PRELIMINARY HISTOANATOMICAL STUDY OF *SAPRIA HIMALAYANA* GRIFF. F. *ALBOVINOSA* FLOWER BUDS AND ITS INTERSECTION WITH ITS HOST PLANT *TETRASTIGMA LAOTICUM* GAGNEP.

Adhityo WICAKSONO[1*], Hữu Đăng TRẦN[2,3**], Syarifah Haniera Sheikh KAMAL[4]

[1] Division of Biotechnology, Genbinesia Foundation, Gresik 61171, East Java – Indonesia.
[2] Southern Institute of Ecology, Vietnam Academy of Science and Technology, Ho Chi Minh City – Vietnam.
[3] Becamex Institute of Research and Development, Becamex IDC Corp., 08, Hung Vuong St., Hoa Phu Ward, Thu Dau Mot City, Binh Duong Province – Vietnam.
[4] Faculty of Applied Sciences, University Teknologi MARA, 40450 Shah Alam, Selangor – Malaysia.
* Corresponding authors. E-mail: adhityo.wicaksono@gmail.com; tranhuudang@gmail.com

Abstract: *Sapria*, a holoparasitic plant of Rafflesiaceae is still understudied in term of anatomical, even more in histoanatomical study. This study aimed to perform preliminary observation on *Sapria himalayana* Griff. f. *albovinosa* morphology on its early and late stage of flower bud development inside its host, *Tetrastigma laoticum* Gagnep. (Vitaceae). The results show the progression from the early flower bud as it starts to grow in the host vascular cambium area towards proximal direction to reach the host xylem and distal direction to reach the host phloem and to reach the next stage of the life cycle, into the late flower bud where differentiation occurs. In late flower bud, some primordial of the organs are visible as the flower bud grows larger distally towards the periderm. This development pattern is similar to the progression of development in the previous studies in *Rafflesia* and *Rhizanthes*, where endophytic growth was observable in both proximal (xylem area) and distal (peridermal). Future comparative study is encouraged, especially to compare between *Sapria* species and between different stages of growth. Although, an efficient method and less invasive way of sampling is encouraged to prevent decline of *Sapria* species in the wild.

Keywords: anatomy, flower, holoparasite, parasitic plant, Rafflesiaceae.

Introduction

Sapria is one of holoparasitic plant in Rafflesiaceae. Its distribution is restricted to the South China, East India, Myanmar, Vietnam, and Thailand region [AHMAD & al. 2020]. Just like two other genera of Rafflesiaceae, *Rafflesia* and *Rhizanthes*, *Sapria* is also a parasite of *Tetrastigma* (Vitaceae) liana. So far, *Sapria* consisted only by four species, *Sapria himalayana* Griff., *S. myanmarensis* Nob. Tanaka, Nagam., Tagane & M. M. Aung, *S. poilanei* Gagnep., and *S. ram* Bänziger & B. Hansen. All grown in specific *Tetrastigma* species as well, such as *S. himalayana* with *T. obovatum* (Laws.) Gagnep., *T. laoticum* Gagnep., and *T. cruciatum* Craib & Gagnep. [ELLIOTT, 1992], *S. poilanei* with *T. laoticum*, *S. ram* with *T. harmandii* Planch. [BÄNZIGER & HANSEN, 1997]. Only *S. myanmarensis* has undocumented *Tetrastigma* species.

Some papers refer *Sapria*, specifically *S. himalayana* to also grow in other Vitaceae, *Vitis* sp. [HUANG & GILBERT, 2004, *cit*. WEAVER, 105]. However, since no study has reported which species of *Vitis*, the speculation remained dubious, especially since some *Tetrastigma* species were once considered as genus *Vitis* until Jules Émile Planchon excluded the genus in 1887 [RAHAYU & al. 2018].

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Previous anatomical-histological study has been done in Rafflesiaceae. In *Rafflesia*, the study was by NIKOLOV & al. (2014a, 2014b), MURSIDAWATI & al. (2019, 2020), MURSIDAWATI & WICAKSONO (2020), and KAMAL & al. (2021). In the same literature by NIKOLOV & al. (2014a, 2014b), there are also anatomical study on *Rhizanthes*, and only in few parts on *Sapria* as endophyte.

Therefore, no study until now has reveal the anatomical development of *Sapria*. All three genera are known as root parasites of *Tetrastigma*, but only *Raffesia* is known for its aerial form grown in the aerial/erected root of *Tetrastigma* liana, as alternative form to most found terrestrial form [MURSIDAWATI & al. 2021]. Both *Rhizanthes* and *Sapria* until now, only known to grow in terrestrial form.

This preliminary study aim is to reveal the general, semi-microscopic parasitic-host profile of *S. himalayana* Griff. f. *albovinosa* on its host *T. laoticum* on the early bud and later bud. This profile will provide first, preliminary information about *Sapria* and *Tetrastigma* parasite-host relationship. From this study, in the future, the profile between all Rafflesiaceae genera can be made.

Material and methods

Plant preparation

Host tissue is *Tetrastigma laoticum* Gagnep. infected with *S. himalayana* Griff. f. *albovinosa* (Figure 1). The specimen was taken in March 2019 at Lang Biang Plateau, Vietnam, specifically at Tuyền Lâm Lake, Cam Ly and Nam Ban Protection Forest (similar to the sampling area of the previous study, TRẦN & al. 2018). The plant materials were sent to Ho Chi Minh City Medicine and Pharmacy University, Faculty of Pharmacy for the staining and microscopy sample preparations.

Staining and microscopy analysis

The staining method applies carmine alum and iodine green, aimed to stain the lignified tissue in green while the rest part of tissue in red [LOCQUIN & LANGERON, 2013]. The samples were prepared by slicing manually by hand with razor for approximately 200 µm thick. Then, the samples were soaked in sodium hipochlorite/commercial bleach (NaHClO) for 15 minutes or until the samples were completely discolored, and cleansed with tapwater for 4 times to remove the bleach. To initiate the staining, the samples were soaked in acetic acid 10% for 5 minutes and rinsed, before soaked in carmine alum dye and iodine green for 15 minutes. The samples were then cleansed from the dye in tap water for 2 times and preserved in glycerol 50% until observation under light microscope.

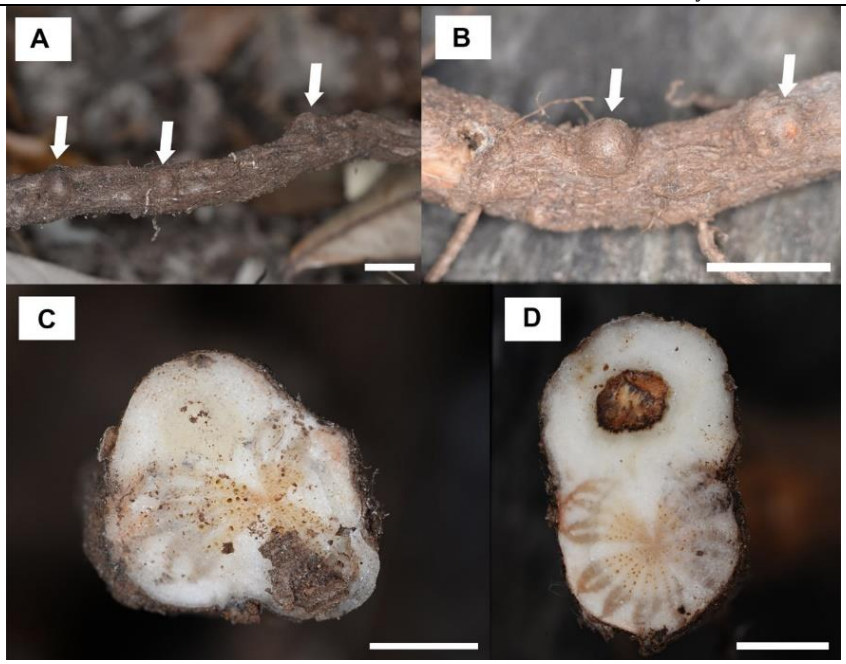

Figure 1. Root of *T. laoticum* with *S. himalayana* f. *albovinosa* buds at swollen stage (white arrows) (A) and zoomed root area with buds (B). Cross-sectioned root with the early stage of bud (C) and late stage (D). Scale bars = 1 cm (A, B), 0.5 cm (C, D). Photo by Trần Hữu Đăng.

Results

Early bud

At early bud stage (Figure 2), the flower bud (or *Sapria* parasitic tissue, SPT) morphologically resembles an inverted teardrop figure. The proximal part of the flower bud is located between the host secondary xylem vessels, and the distal part is located between the bent host secondary phloem with the farthest distal tip in contact with the host cortex layer. The neighboring phloem bundles of the host have been disfigured by the flower bud distal area growth as it appears to be laterally flattened (Figure 2; PAP). The neighboring xylem bundles of the host, however, only partially disfigured in the proximity area to the flower bud proximal area (Figure 2; PAX).

As comparison, closer to the *T. laoticum* root cross-sectional core, the xylem appears to be unchanged as the rest of xylem bundles located farther away from the *Sapria* flower bud growth. The parasitic tissue non-affected (normal) root vascular bundle of *T. laoticum* has developed through secondary growth, with ramified secondary xylem area of the vascular bundle (Figure 2; SX) at the proximal point close to the pith (Figure 2; Pi), bordered with vascular cambium (Figure 2; VC) before the phloem, and re-merged in the secondary phloem area of the vascular bundle (Figure 2; SP) close to the cortex layer of the root (Figure 2; Pe/Cx).

The root with secondary growth has its original cortex layer compressed outwards to the epidermis layer, forming a periderm/cork layer, while the new cortex tissue developed from meristematic pericycle tissue along with the vascular cambium [BECK, 2010; GAMBETTA &

al. 2013], hence the labeling of cortex in this figure. Although, the thin layer of the periderm is consistent to NIKOLOV & al. (2014a) study, which found rhytidome in *Rafflesia*-infected *Tetrastigma* only, not *Sapria* and *Rhizanthes*, where the phellogen is originated from outer cortex cells.

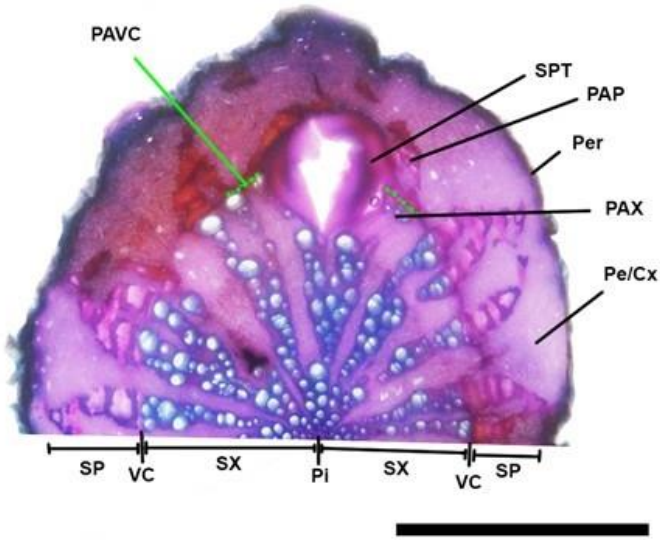

Figure 2. Halved *T. laoticum* root cross-sectional view with early/pre-cupular *S. himalayana* f. *albovinosa* bud (5 month-old), stained with carmine alum-iodine green staining, under light microscope. Pe/Cx – Pericyle/Root Cortex, PAX – Parasite-affected xylem, PAP – Parasite-affected phloem, PACV – Parasite-affected vascular cambium (marked with green dotted lines), Per – Root periderm, SPT – *Sapria* parasitic tissue, SP – Secondary phloem of host, VC – Host vascular cambium, SX – Secondary xylem of host, Pi – Pith (tiny area in the middle of the root cross-section). Scale bar = 0.5 cm. Magnification: 10 × 10. Photo by Nguyễn Thị Ngọc Hương, brightness-contrast-saturation was enhanced by Adhityo Wicaksono.

Late bud

At later stage, the flower bud growth initiates the pre-cupula/swollen stage (analogous to *Rafflesia* life cycle staging by SUSATYA, 2020), where it exhibits gall-like growth that pushes the cortex and periderm area further outwards, swelling the appearance (hence the stage name). At this stage, the bud emergence is already visible by eyes (see Figure 1A and B, white arrows). Despite the flower sex is not yet determined, the possible ovary region (if the flower develops into female or bisexual flower) is already visible in the cross-section (Figure 3; PriOv). The rest of the vegetative structure primordials are also already developed, e.g., primordial central chamber (Figure 3; PriCh) and primordial perigone lobes and bracts (Figure 3; PriPer).

In early development of the late flower bud (Figure 3A), the central chamber already possesses the streaks that is closely resembles the mature flower [TRẦN & al. 2018], while the perigone-bract primordial remains undifferentiated. In later development of the bud (Figure 3B), central chamber enlarges, other vegetative structures like the central column (Figure 3; PriCC) is now visible, and the primordial perigone-bract layers has formed a protective layer surrounding the central chamber of the flower (and the flower undeveloped diaphragm). These

additional parts will develop into the flower organ of *Sapria*. The number of affected host vascular bundles cannot be seen clearly, however the pattern of the parasitic tissue growth shows the similar trend as in the early bud (Figure 2). The parasite-affected tissue (Figure 3; PAT) has pushed the host vascular bundles further laterally and grows deeply into the host secondary xylem area.

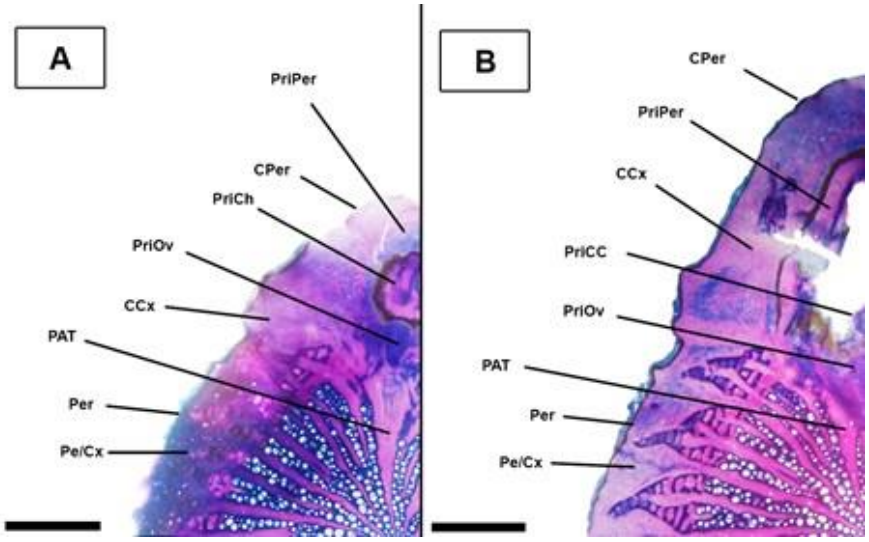

Figure 3. Quarter of *T. laoticum* root cross-section with *S. himalayana* f. *albovinosa* bud in late stage of pre-cupula stage, stained with carmine alum-iodine green staining, under light microscope. At 5 month-old (A) and 7 month-old (B). Pe/Cx - Pericyle/Root Cortex, Per – Root periderm, PAT – Parasite-affected tissue, CCx – Cupular cortex, PriOv – Primordial ovary (if the flower is female), PriCC – Primordial central column, PriCh – Primordial central chamber, PriPer – Primordial perigone lobes-bracts, CPer – Cupular periderm. Scale bars = 0.25 cm. Magnification: 10 × 10. Photo by Nguyễn Thị Ngọc Hương, brightness-contrast-saturation was enhanced by Adhityo Wicaksono.

Discussions

Sapria tissue growth and comparison across Rafflesiaceae

In general, during swollen stage or pre-cupula stage (analogous to *Rafflesia* life stage description by SUSATYA & al. 2020), the parasitic tissue of *Sapria* appears morphologically like an inverted teardrop. This morphology resembles to the floral development stage in the rest of Rafflesiaceae genera, based on observation in *Rafflesia patma* Blume [MURSIDAWATI & WICAKSONO, 2020], *R. azlanii* Latiff & M. Wong [KAMAL & al. 2021], *Rhizanthes lowii* (Becc.) Harms [NIKOLOV & al. 2014b]. The parasitic tissue (Figure 2 and 3; PAT) is centered in the vascular cambium tissue layer of the host root. The proximal region of the parasitic tissue appears to grow towards the core of the root where xylem vessels are abundant, presumably to obtain water and minerals from the host xylem, while the distal region starts to grow and swell towards the surface (direction of the root periderm) and reach the host phloem for nutrition.

This type of movement also can be seen in *Rafflesia* species [MURSIDAWATI & WICAKSONO, 2020; KAMAL & al. 2021] and *Rhizanthes* [NIKOLOV & al. 2014a] where the

parasite tissue grows proximally towards the host root xylem area with the conic-shaped tissue in the host-parasitic attachment/haustorial region.

In the early bud stage (Figure 2), the penetration of the parasitic tissue goes between two xylem area of the vascular bundles, altering the growth of the adjacent vascular bundles, while the remaining vascular bundles continues to grow normally. This is consistent to the *Rafflesia* study in KAMAL & al. (2021), where the early stage of *R. azlanii* bud with size of 2.7 cm, and *R. patma* in MURSIDAWATI & WICAKSONO (2020) only penetrated to single area of host vascular bundles. Assumptively, the number of damaged host vascular bundles in *Rafflesia*-infected *Tetrastigma* is increased by the age of flower bud growth as the parasitic tissue is growing larger and larger, compressing more vascular tissue of the host [MURSIDAWATI & WICAKSONO, 2020]. One vascular bundle is damaged during the cupule stage, two vascular bundles in cupule-bract transition (CBT) stage and three host vascular bundles ruptured by *Rafflesia* in the bract stage for *R. azlanii* and *R. cantleyi* [KAMAL & al. 2021]. In the case of multiple bud formation (as in Figure 1A and B; for *Rafflesia* in MURSIDAWATI & WICAKSONO, 2020), the multiple buds are most likely to grow in the same orientation. This "behavior" is believed to be a strategy to minimize the damage of the host, preventing the death of the host that will ultimately also killing the parasite [MURSIDAWATI & WICAKSONO, 2020].

In initial growth, the primordial tissues develop into the primordial ovary area, followed by the chamber, and perigone lobe-bract primordial area (as shown in Figure 2). In later growth, the floral central chamber expands, the central column matured to then develop the anther/stigmatic region in mature flower, and the laminations by perigone lobes and bracts primordial is becoming visible. This growth pattern resembles the *Rafflesia* at 3-months-old stage, which also considered as early bud growth. The growing meristematic tissue was composed of three regions namely proximal region, middle and distal region, where the proximal region consisted of elongated cells that interference with the host tissues, followed by the middle area, which the location of ovary flower developed at the later stage and distal region where the bracts and perigone lobes developed [MURSIDAWATI & WICAKSONO, 2020].

Host-parasite intersection pattern across Rafflesiaceae

Despite the great resemblances across Rafflesiaceae for the stage in floral accessory growth pattern, the host-parasite intersection area or haustorium in all three genera are slightly different. *Rafflesia* endophyte is distributed in the host vascular cambium area close to the host phloem [NIKOLOV & al. 2014a; MURSIDAWATI & al. 2019], *Rhizanthes* (based on *Rh. lowii*) is distributed from the host phloem, host vascular cambium, and even between the host xylem vessels [NIKOLOV & al. 2014a], and for *Sapria*, the information is still limited as in the study by NIKOLOV & al. (2014a), *Sapria* endophyte was found in the vascular cambium layer of the host. The newest update, however, revealing the growth activity of *Rafflesia* endophyte in the xylem as well [BASCOS & al. 2021], which is similar to *Rhizanthes* [NIKOLOV & al. 2014a]. The reason and mechanisms behind Rafflesiaceae endophytic growth towards the host xylem area require further investigation.

Even in the flower bud stage, the haustorial growth pattern in three genera appears to be distinctive. The proximal meristematic growth in *Rafflesia* appears to be restricted, as the haustorium intersection is found only in the vascular cambium layer and albeit slightly close to the xylem area [MURSIDAWATI & al. 2019; KAMAL & al. 2021].

In *Rhizanthes* (based on *Rh. lowii*), the proximal meristematic growth is visible and the haustorium intersection reaches the xylem bundles area [NIKOLOV & al. 2014a]. Based on this

study, *Sapria* appears to exhibit the same pattern with the other Rafflesiaceae member genera. There should be some differences in morphological adaptations of the haustorium, especially since *Sapria* is the only Rafflesiaceae that infects *Vitis*, not only *Tetrastigma* [HUANG & GILBERT, 2003], although no clarification of which *Vitis* species infected by *Sapria* until now. Hypothetically, due to growth in small *Tetrastigma* root area but possess relatively large (host cross-sectional diameter to the flower size), *Rhizanthes* and *Sapria* requires greater anchorage to the host.

Comparatively, *Rafflesia* is often found in larger root area and even in aerial root with large diameter. As *Rafflesia* flower size is also massive [NAIS, 2001], shallower haustorium possibly reduces mechanical damage to the host, thus preventing further stress in the host that will potentially kill it, endangering the parasite.

Future prospects

This study has some limitations, including sample variations and details. More species can be aimed for future sampling, as well as multiple organ sampling during different stage of development or flower organs during blooming/anthesis period. Multiple organ histoanatomical study can be used to understand the different features in the flower or even endophyte inside *Tetrastigma* in a species or across multiple species. This similar concept of study has been done initially by NANTAWAN & SUVIT (2002), and later by MURSIDAWATI & al. (2020) in *Rafflesia*. Moreover, the haustorial attachment differences between all three genera can also be modelled in biomechanics study.

However, this sampling for histoanatomical analysis, requires strategic and optimized sampling, especially due to the rarity of *Sapria* (i.e., *S. himalayana* is considered "Vulnerable", *S. poilaineі* and *S. ram* is considered "Endangered" under IUCN Red List; CHAMCHUMROON & al. 2017). Despite the rest of the species are not considered endangered, Rafflesiaceae, especially based on *Rafflesia*, has complex yet mysterious life cycle that implies if the number in the wild is reduced, the recovery process will take a long period of time [WICAKSONO & al. 2020].

Based on this condition, propagation study of *Sapria*, as in *Rafflesia*, should be greatly encouraged. Also, potential future studies require efficient sampling method with to optimize the amount of data with only small amount of sample and development of less invasive method of analysis to reduce the chance of population disruption during progression of the study.

Conclusions

Sapria has not became a subject of anatomical study, compared to other Rafflesiaceae members, *Rafflesia* and *Rhizanthes*. This study provides brief preliminary overview on *Sapria* histoanatomy, using *S. himalayana* f. *albovinosa* samples, stained using carmine alum-iodine green. The results show the progression of *Sapria* growth from the young, inverted-teardrop-like early flower bud, to the late flower bud that already developed some primordial organs, including flower chamber, central column, and perigone lobe-bract complex.

In term of haustorial intersection, *Sapria* is similar to all Rafflesiaceae genera, *Rhizanthes* and *Rafflesia*, as the proximal growth reaches the xylem area of the host. Although further investigation is required in anatomical samples of all Rafflesiaceae genera in the future to confirm this statement. From the functionality aspect, this deeper anchorage facilitates the large flower growth in small roots, although biomechanical study is required to confirm this hypothesis. Future study to obtain higher details on each organ during different stage of growth

or across different *Sapria* species is encouraged with strategic way of sampling and optimized way to obtain the data.

Notes on contributors

Adhityo WICAKSONO – is a researcher in Generasi Biologi Indonesia (Genbinesia) Foundation as Head of Biotechnology Department in 2018 until now. His main research is on plant science, focused on Rafflesiaceae since 2017 and plant molecular biology. He is also a PhD student in Åbo Akademi University, Finland.

Hữu Đăng TRẦN – is a researcher at Southern Institute of Ecology and Becamex Institute of Research and Development and have experience in most of the forests in Southern Vietnam and few in Northern Vietnam. His conducted research on taxonomy, including characterization of *Sapria himalayana* morphology identification. He and his team acquired the samples in this study.

Syarifah Haniera Sheikh KAMAL – is postgraduate student (MSc) with a special interest in plant anatomy and conservation. She carried out the anatomical study on Rafflesia species with the host, Tetrastigma and morphology, growth and mortality rate of Rafflesia species.

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IDENTIFICATION OF SEX IN *ZAMIA INERMIS* USING ISSR MARKERS

Lourdes Georgina IGLESIAS-ANDREU[1*], Enrique FAVIÁN-VEGA[1]

[1] Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana, Avenida de las Culturas Veracruzanas 101, Colonia Emiliano Zapata, C.P. 91090, Xalapa, Veracruz – Mexico.
* Corresponding author. E-mail: liglesias@uv.mx

Abstract: *Zamia inermis* is a dioecious cycad endemic to the State of Veracruz that is on the risk extinction. Sex-specific markers are important in understanding the mechanism of sex determination. However, since little is known about the mechanism of sex determination in *Z. inermis*, we proposed looking for Inter-simple Sequence Repeats (ISSR) markers that could be linked to sexual expression in this species. Using DNA bulk samples of male and female genotypes and 6 ISSR primers, a female marker (~867 bp) was identified with primer ISSR-18, which was present in 66% of the DNA mixtures of the female genotypes analyzed. The results of the Principal Coordinate Analysis performed revealed a tendency for clustering of genotypes of the same sex.

Keywords: conservation, cycad, dioecious, molecular markers, sex.

Introduction

Cycads are a type of gymnosperm that can be found in tropical and subtropical areas of the world. There are 54 species of cycads in Mexico, divided into three genera (*Ceratozamia* Brongn., 26 species, *Zamia* L., 15 species and *Dioon* Lindl., 13 species). MARTÍNEZ-DOMÍNGUEZ & al. (2018) found that 89 percent of them are endemic and provide 17% of the diversity.

Zamia inermis Vovides, J. D. Rees & Vázq. Torres (Zamiaceae, Cycadales), is an endemic species of Veracruz, Mexico, which is stated as a critically endangered species on the International Union for Conservation of Nature's Red List (IUCN, 2011) and as an endangered species on SEMARNAT's NOM-059-SEMARNAT-2010 [SEMARNAT, 2010]. This is mainly due to the absence of their natural pollinator to low recruitment rates, illegal trade, and land use change [OCTAVIO-AGUILAR & al. 2017; IGLESIAS-ANDREU & al. 2017]. Like all cycad species, *Zamia inermis* is dioecious and it is difficult to determine the sex of seedlings during the early stages of development; additionally, reproductive events are rare, with low and sporadic seed production, from which only a small proportion of seedlings are obtained [OCTAVIO-AGUILAR & al. 2017]. The morphological evaluation of cycad reproductive structures has been used to determine sex [SÁNCHEZ-TINOCO & al. 1990; SÁNCHEZ-TINOCO & al. 1993; BALDO-ROMERO & al. 2013]. Other molecular markers, such as Random Amplified Polymorphic DNA (RAPD marker) [WILLIAMS & al. 1990] and Amplified Fragment Length Polymorphism (AFLP marker) [VOS & al. 1995], have lately gained popularity.

Although some molecular markers have been successfully employed for sex identification in cycad species such as *Encephalartos natalensis* R. A. Dyer & Verdoorn. [PRAKASH & VAN STADEN, 2006], and *Ceratozamia mexicana* Brongn. [IGLESIAS-

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ANDREU & al. 2010], it should be highlighted that markers based on Simple Sequence Repeats (ISSR) [ZIETKIEWICZ & al. 1994] have been highly efficient for distinguishing the sexes of some cycad species, like *Cycas circinalis* L. [GANGOPADHYAY & al. 2007] and *Cycas tanqingii* D. Y. Wang [JING & al. 2007], and *Ceratozamia mexicana* Brongn. [SÁNCHEZ-COELLO & al. 2018] due to their simplicity, abundance, reproducibility, low cost, and speed of analysis. However, no information about its use for this purpose has been found in *Z. inermis* to date. To contribute to the identification of sex in this species, we propose to develop a genetic sex marker that allows us to gender as well as sex ratio determination at any stage in the life cycle and, eventually, the understanding of sex ratio dynamics in this unique natural population.

Material and methods

Study site and plant material. The only natural population of *Zamia inermis* is observed in the Sierra de Manuel Diaz, close to the town of Mozomboa (municipality of Actopan), in the central part of Mexico's Veracruz State [VOVIDES, 1983], between 19°32'54.90" north latitude and 96°27'29.80" west longitude [VOVIDES, 1983]. This population, which is distributed across about 2.5 km^2 of seasonally dry and scattered grassland, is extremely small. This population has an approximate altitude ranging from 200 to 300 m.a.s.l. This locality is characterized by an AW_1 climate with two climatic subtypes according to the Koppen climate classification system modified by GARCÍA (1988). This type of climate is characterized by being hot and sub-humid, with an intermediate level of humidity, between AW_0 and AW_2, with rainfall in summer, and a mean annual temperature of the coldest month higher than 18 °C and the hottest month higher than 22 °C.

Sample collection was conducted in the 9.7 km^2 area located on two hills in central Veracruz [OCTAVIO-AGUILAR & al. 2017] from adult individuals sexually identified based on their reproductive structures. To conduct this study, 2-4 leaflets were collected from each of the 36 individuals (18 males and 18 females) in four 20 m × 20 m plots. With the aid of a GPS (Garmin eTrex Legend, Olathe, Kansas, USA), all the individuals were georeferenced. Each leaf sample was individually labeled and transported in a cooler to the "Instituto de Biotecnología y Ecología Aplicada" (INBIOTECA), from "Universidad Veracruzana", for molecular studies.

Genomic DNA isolation. The genomic DNA from the collected individuals of *Z. inermis* was extracted using the STEWART & VIA (1993) procedure, which depends on the use of CTAB (Cetyltrimethylammonium bromide, Sigma Aldrich). A fluorometer (Qubit 2.0, Invitrogen, USA) has been used to assess the purity of the DNA once it has been correctly obtained. Each sample's DNA stock solution was diluted to a concentration of 50 ngL^{-1} for PCR amplification. The Bulk Segregant Analysis (BSA) method was used to establish six DNA bulk samples. The bulk DNA samples came from three individuals (one for each sex) [MICHELMORE & al. 1991]. This method was used to screen the individuals of known sex to rapidly detect markers linked to any genomic region that may be sex-linked. A potential sex-linked marker was one that was present in the sex bulks analyzed but not in the alternate sex bulk.

ISSR markers. Twenty-five ISSR primers were selected (UBC, University of British Columbia, Vancouver, Canada) (Table 1).

Table 1. List of ISSR primers, their sequences and annealing temperature

No.	ISSR primer	*Sequence of nucleotides (5'-3')	Annealing temperature (°C)
1	UBC-809	AGAGAGAGAGAGAGAGG	52
2	UBC-818	CACACACACACACACAG	52
3	UBC-827	ACACACACACACACACG	52
4	UBC-829	TGTGTGTGTGTGTGTGC	52
5	UBC-848	CACACACACACACACARG	55
6	ISSR-01	GTAGTAGATAGATA	40
7	ISSR-02	GTAGTAGATAGATARG	45
8	ISSR-03	GTAGTAGATAGATARY	45
9	**ISSR-04**	**GACAGACAGACAGACA**	48
10	ISSR-05	GACAGACAGACAGACARG	55
11	ISSR-06	GACAGACAGACAGACARY	55
12	ISSR-07	GACACGACACGACACGACAC	55
13	ISSR-08	ACTGACTGACTGACTG	48
14	ISSR-09	ACTGACTGACTGACTGRG	55
15	ISSR-10	ACTGACTGACTGACTGRY	55
16	ISSR-11	GATAGATAGATA	35
17	ISSR-12	GTAGTAGATAGATARG	37
18	ISSR-13	YRGATAGATAGATA	37
19	ISSR-14	GACAGACAGACAGACA	40
20	ISSR-15	GACAGACAGACARG	45
21	ISSR-16	YRGACAGACAGACA	40
22	ISSR-17	GACACGACAC	35
23	**ISSR-18**	**ACTGACTGACTG**	40
24	ISSR-19	ACTGACTGACTG	45
25	**ISSR-20**	**YRACTGACTGACTG**	40

*R, purine (A or G), Y, pyrimidine (C or T). The primers selected are indicated in bold. UBC: University of British Columbia, Canada.

PCR reactions were performed in duplicate, using a reaction mix in a 25 µL volume containing: 5X PCR buffer, 25 mM $MgCl_2$, 5 mM dNTPs, 25 pM primers, 1.5U of Taq DNA polymerase, and 50 ng of DNA. Amplifications were carried out in a thermal cycler (AXIGEN®, Foster City, California, USA) under the following thermal conditions: a 7-minute denaturation step at 94 °C, 35 cycles of 30 seconds at 94 °C and 45 seconds at various alignment temperatures (37-55 °C), depending on the primer, and a 10-minute final extension at 72 °C. Positive and negative controls were included in all cases. Electrophoretic separations were performed at 100 V for 80 min on 2% (w/v) agarose gels in 1X TAE buffer. A 100 bp molecular weight marker (Bioline brand, Memphys, Tennessee, USA) was used to evaluate 5 µL of each

of the amplified products. Afterwards, the gels were stained with ethidium bromide (10 $mgmL^{-1}$) and photographed using a photo-documenter (UVP, Upland, California, USA).

Analysis of data. Based on the type of primer and their size in base pairs (bp), bands that exhibited appropriate resolution and reproducibility in the obtained band profiles were recorded in a primary data matrix (values of 1: presence and 0: absence of the band). The latter was determined using a 100-bp DNA molecular size marker (Bioline brand) as a reference. Using the Paleontological Statistics (PAST) software, the data matrix was analyzed using Principal Coordinate Analysis (PCA) to identify the grouping patterns among the genotypes evaluated according to their sex [HAMMER & al. 2001].

Results and discussions

According to the results, only 6 of the 25 ISSR primers examined allowed for the detection of repeatable and reproducible band profiles. The ISSR protocol was correctly developed on this basis, enabling the detection of 56 and 59 bands, respectively, for the DNA bulk male and female samples evaluated. ISSR-18 was one of the primers in this analysis that had the best resolution and repeatability. In this study, the number of bands detected ranged from 7 to 14 in DNA bulk male samples and from 6 to 15 in DNA bulk female samples, with an average of nine ISSR bands in both sex expressions (Table 2).

Table 2. Sequence of ISSR primers and number of bands detected by sex

ISSR Primers	*Sequence of nucleotides (5'-3')	No. of bands / sex	
		Male	Females
UBC-848	CACACACACACACACARG	7	7
ISSR-04	GACAGACAGACAGACA	14	13
ISSR-05	GACAGACAGACAGACARG	8	6
ISSR-06	GACAGACAGACAGACARY	10	15
ISSR-18	ACTGACTGACTG	9	10
ISSR-20	YR ACTGACTGACTG	7	8
Total		**56**	**59**

*R, purine (A or G), Y, pyrimidine (C or T). Y: C/T; R: A/G. UBC: University of British Columbia, Canada.

The presence of an 867 bp band was detected with the ISSR-18 primer in four of the six DNA bulk samples of males and females examined; that is, it was present in 66% of the DNA samples of female individuals (Figure 1) evaluated.

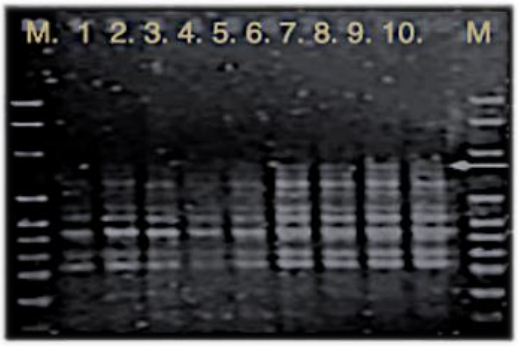

Figure 1. ISSR band profiles. The arrow indicates the 867 bp band detected with the ISSR-18 primer in DNA bulk female samples. Lanes 3-8: DNA bulk male samples (1-5); lanes 6-10: DNA bulk female samples (from left to right); M: 100-bp ladder.

According to the results of principal coordinate analysis (PCA), the first two principal coordinates explained 72.17% of the total accumulated variance (Figure 2). DNA samples from sexually differentiated individuals were spatially distributed within the first two coordinates (Figure 2). Group I, located in the lower left part of the graph, explained 51.4% of the total variance. This group consisted of five bulk DNA samples, of which four corresponded to female bulk DNA samples. Group II, located at the top of the graph, explained 20.87% of the total accumulated variance (Figure 2). This group was composed of five DNA bulk samples, corresponding to one female DNA bulk sample and four male DNA bulk samples (Figure 2). It was not possible to observe in this study a clear grouping of individuals by sex, using Principal Coordinates Analysis (PCoA) due to the atypical spatial distribution presented by two of the bulk DNA samples evaluated (3M: bulk male DNA sample, and 6H: bulk female DNA sample) (Figure 2).

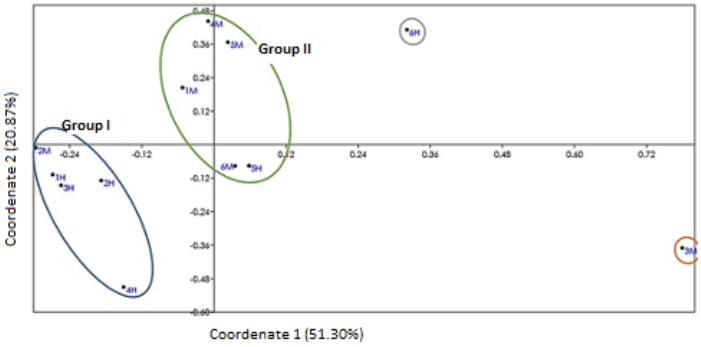

Figure 2. Principal Coordinates Analysis (PCoA) of DNA bulk samples of sexually differentiated individuals in *Z. inermis*. Group I (4 DNA bulk female samples), Group II (4 DNA bulk male samples). H = female, M = male.

Even though dioecy is a common trait among gymnosperms, accounting for roughly 65 percent of all currently identified species [WALAS & al. 2018], the search for sex-co-segregating molecular markers in this group of plants has been limited [ROY & al. 2012]. Sex-

linked bands, as is widely known, occur rarely and not always in the same way. As is known, sex-linked bands usually occur very infrequently and not always consistently. As a result, very little is known regarding cycads, although they will presumably be the first dioecious seed-bearing lineage and occupy a major evolutionary position among land plants.

In *Zamia fischeri*, two RAPD markers were reported to be linked with each sex, as well as a male gender-specific band which showed some homology with a microsatellite sequence from *Araucaria angustifolia* (Bertol.) Kuntze [ROY & al. 2012], potentially useful for early male sex identification. Sex-specific RAPD markers have been identified in *Cycas circinalis* [GANGOPADHYAY & al. 2007] and *C. mexicana* [IGLESIAS-ANDREU & al. 2017].

It was interesting to find that the ISSR marker detected was associated with the female genotype even though, according to AINSWORTH (2000), most molecular markers are associated with the male sex. Bands are linked with the female sex in other cycad species, like *Encephalitis natalensis*. PRAKASH & VAN STADEN (2006) found RAPD markers specific to the female method analysis, and a RAPD marker specific to the female gender in *Cycas tanqingii*, which was later converted to a "Sequence Characterized Amplified Regions" (SCAR marker) for use in male and female identification prior to flowering [JING & al. 2007]. SCAR markers have the advantage of becoming more reliable and enabling the identification of a single locus [JIANG & SINK, 1997].

The ISSR marker found to be associated with female sex in this study is only a starting point to contribute to sex identification in *Z. inermis*. However, it is necessary to validate its usefulness, for which future studies will be developed to detect a SCAR marker like those found in other gymnosperms [JING & al. 2007; LIAO & al. 2009; SÁNCHEZ-COELLO & al. 2018].

Conclusions

The results demonstrated the ISSR marker linked with female sex found in *Z. inermis* is a suitable starting point to start looking for other genetic and epigenetic molecular markers to improve early sex identification in this significant genetic resource. We can't rule out the possibility that sex expression in this species is influenced by epigenetic factors (gene expression changes without changes in the nucleotide sequence), so it'd be advisable to evaluate the usefulness of markers such as Methylation-Sensitive Amplification Length Polymorphism (ms-AFLP) in elucidating the role of possible epigenetic factors in sex determination.

Notes on contributors

Lourdes Georgina IGLESIAS-ANDREU is a researcher at the Institute of Biotechnology and Applied Ecology of the "Universidad Veracruzana" Mexico, and a member of Mexico's National System of Researchers (SNI II) with expertise in plant genetics and biotechnology.

Enrique FAVIAN-VEGA – he has a PhD in Ecology and Biotechnology, and his work has focused on the conservation biology of Mexican cycads.

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SUPRA-PHYSIOLOGICAL LEVELS OF GIBBERELLINS / DELLAS ALTER THE PATTERNING, MORPHOLOGY AND ABUNDANCE OF ROOT HAIRS IN ROOT TIPS OF *ARABIDOPSIS THALIANA* SEEDLINGS

Iva MCCARTHY-SUÁREZ

Hormone Signalling and Plant Plasticity Group. Instituto de Biología Celular y Molecular de Plantas (CSIC-Universidad Politécnica de Valencia). C/ Ingeniero Fausto Elio s/n (46022), Valencia – Spain.
E-mail: ivmcsua@upvnet.upv.es

Abstract: In spite of the role of gibberellins/DELLAs in leaf hair production, no investigations have assessed their function in the production of root hairs. To this aim, the effects of supra-physiological levels of GAs/DELLAs on the gene expression patterning of the root hair (CPC) and non-hair (GL2, EGL3 and WER) epidermal cell fate markers, and on the distribution, morphology and abundance of root hairs, were studied in root tips of 5-day-old *A. thaliana* seedlings. Results showed that excessive GAs/DELLAs misarranged the *CPC*, *GL2*, *EGL3* and *WER* gene expression patterning and the location, shape and frequency of root hairs. However, when the gai-1 (GA-insensitive-1) DELLA mutant protein was specifically over-expressed at the root epidermis, no changes in the patterning or abundance of root hairs occurred. Thus, results suggest that, in *A. thaliana* seedlings, the GAs/DELLAs might regulate the patterning, morphology and abundance of root hairs from the sub-epidermal tissues of the root.

Keywords: DELLAs, Gibberellins, root hair morphology, root hair number, root hair patterning.

Introduction

The epidermal cell organization in roots of *A. thaliana* seedlings, consisting of single rows of hair-bearing cells (Trichoblasts, which lay over the cleft between two cortical cells) alternating with double rows of hairless cells (Atrichoblasts, which lay over just one cortical cell) has been shown to be genetically determined by a complex network of transcription factors and positional signals, such as CAPRICE (CPC), GLABRA2 (GL2), WEREWOLF (WER) and ENHANCER OF GLABRA3 (EGL3), and regulated by auxin, ethylene (ET), abscisic acid (ABA), nitric oxide (NO), brassinosteroids (BRs), cytokinins (CKs) and strigolactones (SLs)) [SILVERMAN & al. 1998; CAO & al. 1999; VAN HENGEL & al. 2004; LOMBARDO & al. 2006; KAPPUSAMY & al. 2009; SCHIEFELBEIN & al. 2009; NIU & al. 2011; SALAZAR-HENAO & al. 2016]. These hormones, in turn, seem to act downstream of the *GL2* gene network, permitting root cells to have fate plasticity, i.e., ability to change to the alternative differentiation route at a relatively late state, as it is not cell lineage, but position, and sometimes even a position-independent mechanism, what seems to continuously determine cell fate [GRIERSON & SCHIEFELBEIN, 2002; SCHIEFELBEIN & al. 2009; YU & al. 2017]. Moreover, these hormones mediate the changes in the root hair patterning associated to the plant responses to soil stress without altering the expression of the WER and GL2 epidermal cell fate markers [SCHMIDT & al. 2000; YANG & al. 2007; MARTÍN-REJANO & al. 2011].

Given that the GAs/DELLAs have a role in trichome (leaf hair) production in *A. thaliana* [CHIEN & SUSSEX, 1996; TRAW & BERGELSON, 2003] and participate in

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microtubule (MT) cytoskeleton organization [LOCASCIO & al. 2013], which is essential for the growth of trichomes and root hairs and for establishing the identity and shape of root cells [BAO & al. 2001], and because there are no reports concerning the hypothetical implication of GAs/DELLAs in the root hair patterning, this study aimed to investigate the effect of excessive levels of these hormones on the distribution and abundance of root hairs in seedlings of *A. thaliana*. In addition, because changes in the levels of auxin, ET, ABA, NO, BRs and SLs have been correlated to alterations in root hair morphology in response to nutritional stresses, such as low availability of P, B or Fe in the soil (longer and branched root hairs) [SCHMIDT & al. 2000; YANG & al. 2007; MARTÍN-REJANO & al. 2011], this work also aimed to determine whether the GAs/DELLAs might have a role in regulating the morphology of root hairs in seedlings of *A. thaliana*. To this aim, the spatial expression of the GUS or GFP-fused transcripts of the root hair (CPC) and non-hair (GL2, EGL3, WER) epidermal cell fate markers, as well as the arrangement, shape and density of root hairs, were studied in *A. thaliana* seedlings grown for 5 days under (or harbouring) excessive levels of GAs/DELLAs. Finally, to locate the tissue from which these hormones might hypothetically affect the patterning of root hairs, the root hair distribution was studied in 5-day-old mutant seedlings resulting from expressing the *gai-1* DELLA dominant allele in different tissues of the root (UAS (GAL4-UPSTREAM ACTIVATION SEQUENCE) expression directed system lines; Dr. JIM HASSELHOFF'S laboratory). Results of this study suggested that the GAs/DELLAs might be involved in regulating the patterning, morphology and abundance of root hairs in *A. thaliana* seedlings.

Material and methods

Plant material and growth conditions

Arabidopsis thaliana Col (0) seeds were sterilized (70% Ethanol (v/v) and 0.01% Triton X-100 (v/v)), sown on half-strength MS medium plates (0.8% (w/v) agar and 1% (w/v) sucrose), stratified for 3-4 days (4 ºC, darkness), germinated, and grown vertically (22 ºC; 5-7 days) under continuous white light (Percival growth chamber E-30B) (http://www.percival-scientific.com) as described by LEE & SCHIEFELBEIN (1999).

Hormone and chemical treatments

Stock solutions of paclobutrazol (PAC, 10 mM in acetone 100% (v/v)), GA_4 (1 mM in 100% ethanol (v/v)) or GA_3 (50 mM in 100% ethanol (v/v)) were conveniently diluted and added to MS agar medium or water (in the case of liquid incubation experiments) to obtain a final concentration of 0.5 µM PAC, 1 µM GA_4 and 30 µM GA_3.

Mutant lines

The spatial patterning of gene expression of the hair (CPC) and non-hair (GL2, EGL3, WER) epidermal cell fate markers in roots of *A. thaliana* seedlings was studied by using their GUS or GFP-fused promoter lines (*CPCpro::GUS*, *GL2pro::GUS*, *EGL3pro::GUS* and *WERpro::GFP*) as well as those derived from crossing lines harbouring constitutively excessive levels of GAs/DELLAs with the *GL2pro::GUS* line (*GID1b ox* x *GL2pro::GUS*, *gai-1* x *GL2pro::GUS*, *HSp::gai-1* x *GL2pro::GUS*, *pGAI::gai-1:GR* x *GL2pro::GUS* and *SCR::gai-1:GR* x *GL2pro::GUS* (L*er* x *GL2pro::GUS* background)). The effect of transient increases in the levels of the gai-1 dominant DELLA on the root hair distribution in *A. thaliana* seedlings was examined by using the heat-shock inducible *HSp::gai-1* (which over-expresses the gai-1 DELLA upon heat shock) and dexamethasone (DEXA)-inducible *pGAI::gai-1:GR* and *SCR::gai-1:GR* (with glucocorticoid-binding domain) mutant lines. The *HSp::gai-1* mutant seedlings were grown at 37 ºC for 4 h (heat shock) and then at 22 ºC for 2 h (recovery period),

whereas the *pGAI::gai-1:GR* and *SCR::gai-1:GR* mutant seedlings were incubated in 0.1, 0.2, 0.5, 1.2 or 10 µM DEXA for a minimum of 6 h. The root hair distribution was also studied in mutants with excessive levels of GAs/DELLAs (*gai-1*, *GAI-ox* (GAI-over-expressing), *QD (quadruple DELLA mutant)*, *5X (quintuple DELLA mutant)*, GID1b-*ox* (which over-expresses the GA receptor GID1b (GIBBERELLIN INSENSITIVE DWARF1), in mutants over-expressing gai-1 in different tissues of the root (*ML1::gai-1* (epidermis) and UAS expression directed system (GAL4-UPSTREAM ACTIVATION SEQUENCE) mutants: *UAS::gai-1* x C24 (control, background); *UAS::gai-1* x J0951 (epidermis of the meristematic zone (MZ)); *UAS::gai-1* x J2812 (MZ epidermis and cortex); *UAS::gai-1* x N9142 (cortex of the elongation zone (EZ)); *UAS::gai-1* x M0018 (MZ cortex and endodermis); *UAS::gai-1* x J0571 (MZ cortex and endodermis); *UAS::gai-1* x Q2393 (all tissues but the endodermis); *UAS::gai-1* x Q2500 (MZ endodermis/pericycle); *UAS::gai-1* x J0121 (EZ pericycle); *UAS::gai-1* x J0631 (all tissues of the EZ); *UAS::gai-1* x J3281 (vessels)), and in the *wer*, *cpc* and *35S::CPC* (cauliflower mosaic virus 35S promoter) mutants.

GUS activity assay

GUS (β-glucuronidase) staining of the *GL2pro::GUS*, *CPCpro::GUS* and *EGL3pro::GUS* reporter lines was performed as described by FRIGERIO & al. (2006), but using 8 mM instead of 2 mM potassium ferro/ferricyanide and incubating the seedlings (15 min to 2 h) in the reaction mixture at 4 ºC instead of 37 ºC.

Microscopy

The patterning of the hair/non-hair epidermal cell types in roots of *A. thaliana* seedlings was studied by staining the roots with 0.67 mg/ml propidium iodide, by observing the root tips under a Nickon Eclipse E6000 microscope, and by calculating the percentage of hairs/non-hairs at the Trichoblast/Atrichoblast positions (Dr. BENEDICTE DESVOYES' method). The patterning of *GL2pro::GUS* expression in cross sections of root tips was studied on ultra-thin sections of plastic resin-embedded roots as previously described at Dr. SCHIEFELBEIN Protocols (http://www.mcdb.lsa.umich.edu/labs/schiefel/protocols.html). Seedlings were included in 1% agarose in 0.1M sodium phosphate buffer, pH 6.8, and stained for GUS activity. Root-containing blocks were then cut, fixed with 4% para-formaldehyde in PBS, dehydrated in ethanol series (15%, 30%, 50%, 75%, 95% and 100%, 1 h each), kept in 100% ethanol overnight, incubated in Technovit ® 7100 infiltration solution for 2 days, inserted in gelatine capsules, and embedded for 9 days in Technovit® 7100 plastic resin (Heraeus Kultzer, Germany). Ultramicrotome (Ultracut E, Reichert Jung, Germany) cross sections of resin-embedded roots were then stained with 0.06% (w/v) toluidine blue and observed under a Nikon Eclipse E600 microscope. The *WERpro::GFP* expression was visualized by using a Leica Confocal Microscope (excitation: 488 nm; detection: 500-530 nm band-path filter for GFP).

Results

Excessive levels of GAs/DELLAs altered the root hair patterning in seedlings of *A. thaliana*

To assess whether the GAs/DELLAs might have a role in the root hair patterning of *A. thaliana* seedlings, the spatial gene expression of the root hair (CPC) and non-hair (GL2, EGL3, WER) epidermal cell fate markers was studied in seedlings of the *GL2pro::GUS*, *CPCpro::GUS*, *EGL3pro::GUS* and *WERpro::GFP* transgenic lines grown for 5 days under supra-physiological levels of GAs/DELLAs (Figure 1A). Results showed that growth under

excessive levels of GAs/DELLAs altered the normal patterning of gene expression of the root hair/non-hair epidermal cell fate markers (Figure 1A). This was confirmed by analysing the spatial expression of *GL2* in the *GID1b-ox* (which over-expresses the GA receptor GID*1b*), *gai-1, HSp::gai-1* (which over-expresses the gai-1 DELLA upon exposure to heat (37 ºC, 4 h)), and DEXA-inducible *pGAI::gai-1:GR* and *SCR::gai-1:GR* mutants (Figure 1A). Moreover, the alteration of the *GL2pro::GUS* expression pattern under excessive levels of GAs/DELLAs was corroborated in ultra-thin sections of resin-embedded roots (Figure 1B).

An analysis of the distribution of the root hair and non-hair cells relative to their position over the cortex cells showed that excessive levels of GAs/DELLAs impaired the correct positioning of the root hair/non-hair cells (Tables 1 and 2), giving rise to ectopic root hairs (at the Atrichoblast position) and ectopic root non-hairs (at the Trichoblast position) (Figures 2A and 2B). Interestingly, treatment with GA_4 (1 µM) reduced the percentage of ectopic root hair cells in the hairy mutant *35S::CPC*, whereas treatment with PAC (0.5 µM) slightly decreased the percentage of ectopic root non-hair cells in the bald mutant *cpc* (Table 2). In accordance with these changes, growing *A. thaliana* seedlings under supra-physiological levels of GAs/DELLAs for 5 days altered the arrangement of root hairs in root tips, giving rise to ectopic root hairs (in a non-hair row), ectopic root non-hairs (in a hair row) and adjacent root hair rows (Figures 3A and 3B). This was confirmed in the *gai-1*, *QD*, *5X*, GID*1b-ox*, *pGAI::gai-1:GR* and *SCR::gai-1:GR* mutants (Figures 3A and 3B).

To ascertain from which particular tissue of the root the GAs/DELLAs might be affecting the root hair patterning, the positioning of the root hair/non-hair cells over the root cortex cells and the distribution of root hairs were studied in *A. thaliana* transgenic seedlings expressing the *gai-1* DELLA allele in different tissues of the root (Figures 2B, 3A and 3B; Table 2). Results showed that the root hair distribution changed when gai-1 was over-expressed at the cortex, endodermis or pericycle of the meristematic (MZ) or elongation (EZ) zones of the root (J2812 >> *gai-1*, M0018 >> *gai-1*, Q2500 >> *gai-1*, J0121 >> *gai-1*, Q2393 >> *gai-1* and J0631 >> *gai-1* lines), but not when *gai-1* was over-expressed at the root epidermis (J0951 >> *gai-1* and *ML1::gai-1* lines) (Figure 3A). In fact, the gene expression pattern of *GL2* did not change when gai-1 was over-expressed at the epidermis (*ML1::gai-1* line) (Figure 1). Moreover, ectopic hairs, ectopic non-hairs and adjacent hair rows appeared when gai-1 was over-expressed at the cortex (J2812 >> *gai-1* and N9142 >> *gai-1* lines), endodermis (M0018 >> *gai-1* and J0571 >> *gai-1* lines) or pericycle (Q2500 >> *gai-1* and J0121 >> *gai-1* lines) of the root, or in all root tissues but the endodermis (Q2393 >> *gai-1* line) (Figures 2B, 3A and 3B). However, when *gai-1* was over-expressed in the root vessels (J3281 >> *gai-1* line), the growth of the root and the production of root hairs stopped (Figure 3A).

Excessive levels of GAs/DELLAs altered the morphology, length and abundance of root hairs in root tips of *A. thaliana* seedlings

Excessive levels of GAs/DELLAs also modified the morphology of Trichoblasts and root hairs in root tips of *A. thaliana* seedlings, frequently giving rise to two-haired cells, two-tipped hairs and branched hairs (Figure 4). In addition, excessive levels of GAs/DELLAs altered the length and density of root hairs. Whereas high levels of DELLAs increased the length and number of hairs near the root tip, high levels of GAs had the opposite effect (Figures 5A and 5B; Table 3). Moreover, root hair abundance in root tips of *A. thaliana* seedlings increased when gai-1 was over-expressed at the cortex (J2812 >> *gai-1*), endodermis (M0018 >> *gai-1*) or pericycle (Q2500 >> *gai-1* and J0121 >> *gai-1*) of the root, but not when gai-1 was over-expressed at the epidermis of the root MZ (J0951 >> *gai-1*) or the cortex of the root EZ (N9142

>> *gai-1*) (Table 3). Also, treatment of the bald mutant *cpc* with PAC slightly increased the root hair frequency (and length) near the root tip, whereas treatment of the hairy mutants *wer* and *35S::CPC* with GA_4 reduced it (Figure 5B; Table 3).

High levels of GAs/DELLAs also altered the abundance of root hairs in the radial dimension of the root tips (Tables 4 and 5). The number of root hairs per root cross section, calculated as the summary of root hairs at the Trichoblast and Atrichoblast positions (or the summary of root hairs and ectopic root hairs per root cross section) increased under excessive DELLAs (PAC, *gai-1*) but decreased in the *5X* mutant (Table 5). On the other hand, the number of root non-hairs per root cross section, calculated as the summary of root non-hairs at the Atrichoblast and Trichoblast positions (or the summary of root non-hairs and ectopic root non-hairs per root cross section), decreased under excessive DELLAs, but experienced an enhancement in the *5X* mutant (Table 5). Thus, the estimated abundance of root hairs in the radial dimension of the root tips seemed to increase under excessive DELLAs, but to decrease under excessive GAs.

Table 1. Distribution of the root hair and non-hair cells at the Trichoblast/Atrichoblast positions in root tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Numbers in parenthesis refer to the number of cells analyzed. At least 15-20 roots were used per treatment.

	Trichoblast position		**Atrichoblast position**	
	Hair Cell (%)	**Non-Hair cell (%)**	**Hair Cell (%)**	**Non-Hair cell (%)**
Col (0) (MS)	97.5 ± 0.7 (73)	2.5 ± 0.7 (2)	0 ± 0 (0)	100 ± 0 (75)
PAC (0.5 µM)	77.4 ± 5.7 (109)	22.6 ± 5.7 (32)	36.7 ± 8.2 (80)	63.3 ± 8.2 (138)
GA_4 (1 µM)	81 ± 2.7 (83)	19 ± 2.7 (20)	12.5 ± 3.5 (5)	87.5 ± 3.5 (35)
PAC (0.5 µM) + GA_4 (1 µM)	94 ± 4.2 (71)	6 ± 4.2 (5)	5 ± 2.8 (4)	95 ± 2.8 (71)
L*er*	95.8 ± 2.2 (167)	4.2 ± 2.2 (7)	4.5 ± 3.5 (6)	95.5 ± 3.5 (120)
gai-1	82.7 ± 4.5 (75)	17.3 ± 4.5 (16)	40.4 ± 5 (55)	59.6 ± 5 (81)
QD	78.8 ± 4.5 (126)	21.2 ± 4.5 (34)	24 ± 4.9 (38)	76 ± 4.9 (122)
***pGAI::gai-1:GR* (MS)**	93.5 ± 2.1 (41)	6.5 ± 2.1 (3)	25 ± 7.1 (10)	75 ± 7.1 (30)
***pGAI::gai-1:GR* (10 µM DEXA)**	78 ± 2.8 (38)	22 ± 2.8 (11)	50.5 ± 6.4 (22)	49.5 ± 6.4 (22)
***SCR::gai-1:GR* (MS)**	83.8 ± 3.3 (36)	16.2 ± 3.3 (7)	35 ± 6.2 (13)	65 ± 6.2 (25)
***SCR::gai-1:GR* (0.1 µM DEXA)**	67 ± 12.7 (30)	33 ± 12.7 (15)	15 ± 7.1 (6)	85 ± 7.1 (34)

Table 2. Percentage of ectopic root hair/non-hair cells at the Trichoblast/Atrichoblast positions in root tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. *Seedlings analyzed at 48 h after a heat-shock experiment (37 ºC, 4 h). *GL2pro::GUS* (22 ºC): control seedlings grown at 22 ºC for 4 h. *GL2pro::GUS* (37 ºC): control seedlings grown at 37 ºC for 4 h (ectopic root hair cells might have appeared due to heat stress). *HSp::gai-1* x *GL2pro::GUS* (37 ºC): inducible *gai-1* mutant seedlings grown at 37 ºC for 4 h. The number of ectopic root hair and non-hair cells from a single experiment is shown in parenthesis.

	Trichoblast position		Atrichoblast position	
	Nº epidermal cells examined	% Ectopic root non-hair cells	Nº epidermal cells examined	% Ectopic root hair cells
L*er*	41	2 (1)	30	7 (2)
5X	20	35 (7)	20	0 (0)
GAI-ox	15	7 (1)	15	53 (8)
GL2pro::GUS (22ºC)*	30	0 (0)	29	0 (0)
GL2pro::GUS (37ºC)*	30	7 (2)	30	30 (9)
Hsp::gai-1 x GL2pro::GUS (37ºC)*	29	28 (8)	27	44 (12)
wer	28	32 (9)	36	50 (18)
***wer* (1 µM GA_4)**	30	20 (6)	29	72 (21)
cpc	28	64 (18)	28	21 (6)
***cpc* (0.5 µM PAC)**	28	54 (15)	28	4 (1)
35S::CPC	30	23 (7)	30	60 (18)
***35S::CPC* (1 µM GA_4)**	30	13 (4)	29	17 (5)
***SCR::gai-1:GR* (MS)**	21	19 (4)	20	30 (6)
***SCR::gai-1:GR* (0.2 µM DEXA)**	19	21 (4)	19	26 (5)
***SCR::gai-1:GR* (0.5 µM DEXA)**	20	30 (6)	18	22 (4)
***SCR::gai-1:GR* (1.2 µM DEXA)**	10	20 (2)	10	40 (4)
UAS::gai 1 x C24 (control)	50	0 (0)	44	0 (0)
ML1::gai-1	41	2 (1)	30	3 (1)
UAS::gai-1 x J0951	60	0 (0)	60	0 (0)
UAS::gai-1 x J2812	30	10 (3)	30	50 (15)
UAS::gai-1 x Q2393	9	22 (2)	16	63 (10)

Table 3. Length and abundance of root hairs in root tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Analyses of hair length and abundance were performed on micrographs of root tips of *A. thaliana* seedlings (4X). (*) Seedlings analyzed at 24h and 48h after a heat-shock experiment (37 ºC, 4 h). Analyses of hair abundance performed at 31.5X (lens; Control, PAC and GA_4), 3.2X (lens; L*er*, *gai-1* and *QD*) or 4X (microscope; other mutants and *UAS::gai-1* lines).

	Hairs analyzed	Hair Length (µm)	Roots examined	Nº Root Hairs per field
Col (0) (MS)	69	209 ± 121 *(100%)*	17	38 ± 8 *(100 %)*
Col (0) (0.5 µM PAC)	94	270 ± 128 *(129 %)*	19	54 ± 12 *(142 %)*
Col (0) (1 µM GA_4)	37	178 ± 93 *(85 %)*	18	31 ± 8 *(82 %)*
Ler	45	201 ± 99 *(100 %)*	5	43 ± 7 *(100 %)*
gai-1	120	397 ± 186 *(198 %)*	3	56 ± 1 *(130 %)*
QD	25	139 ± 85 *(69 %)*	3	24 ± 5 *(56 %)*
GID1b-ox	14	80 ± 25 *(40 %)*	6	28 ± 8 *(88 %)*
***GID1b-ox* (30 µM GA_3)**	10	64 ± 35 *(32 %)*	3	18 ± 6 *(57 %)*
***Hsp::gai-1* (22 ºC) at 24h (*)**	6	55 ± 14 *(100 %)*	1	18 ± 0 *(100 %)*
***Hsp::gai-1* (37 ºC) at 24h (*)**	11	405 ± 208 *(201 %)*	2	32 ± 4 *(178 %)*
***Hsp::gai-1* (37 ºC) at 48h (*)**	23	411 ± 165 *(204 %)*	3	83 ± 31 *(459 %)*
***pGAI::gai-1:GR* (MS, 30h)**	40	270 ± 118 *(100 %)*	4	56 ± 7 *(100 %)*
***pGAI::gai-1:GR* (0.5 µM DEXA, 30h)**	57	314 ± 177 *(116 %)*	8	79 ± 17 *(142 %)*
***SCR::gai-1:GR* (MS, 3d)**	30	245 ± 87 *(100 %)*	3	49 ± 20 *(100 %)*
***SCR::gai-1:GR* (0.5 µM DEXA, 3d)**	35	507 ± 173 *(207 %)*	5	76 ± 31 *(154 %)*
***wer* (MS)**	24	192 ± 88 *(100 %)*	3	91 ± 12 *(100 %)*
***wer* (0.5 µM PAC)**	8	243 ± 134 *(127 %)*	3	125 ± 29 *(137 %)*
***wer* (1 µM GA_4)**	6	133 ± 23 *(70 %)*	3	70 ± 5 *(77 %)*
***cpc* (MS)**	7	104 ± 29 *(100%)*	3	17 ± 1 *(100 %)*
***cpc* (0.5 µM PAC)**	9	213 ± 92 *(204 %)*	3	18 ± 2 *(106 %)*
***cpc* (1 µM GA_4)**	8	88 ± 51 *(85 %)*	7	11 ± 3 *(65 %)*
***UAS::gai-1 x C24* (control)**	20	161 ± 105 *(100 %)*	2	48 ± 23 *(100 %)*
UAS::gai-1 x J0951	34	240 ± 118 *(149 %)*	3	49 ± 13 *(101 %)*
UAS::gai-1 x J2812	59	243 ± 118 *(151 %)*	9	77 ± 26 *(161 %)*
UAS::gai-1 x J0571	25	586 ± 273 *(364 %)*	2	60 ± 11 *(125 %)*
UAS::gai-1 x M0018	90	685 ± 195 *(425 %)*	10	92 ± 26 *(192 %)*
UAS::gai-1 x Q2500	37	680 ± 189 *(422 %)*	2	96 ± 12 *(200 %)*
UAS::gai-1 x Q2393	48	272 ± 146 *(169 %)*	4	67 ± 26 *(140 %)*
UAS:gai-1 x N9142	21	195 ± 97 *(121 %)*	2	30 ± 1 *(63 %)*
UAS::gai-1 x J0121	47	233 ± 120 *(145 %)*	5	67 ± 13 *(140 %)*
UAS::gai-1 x J0631	8	386 ± 129 *(240 %)*	2	96 ± 15 *(200 %)*

Table 4. Percentage and estimated number of epidermal cells at the Trichoblast/Atrichoblast positions per root cross section in 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Analyses performed on micrographs of cross sections of resin-embedded roots (40X).

	Nº root cross sections examined	% Epidermal Cells at Trichoblast position	% Epidermal Cells at Atrichoblast position	Average number of epidermal cells per root cross section	Predicted Nº of epidermal cells at the Trichoblast position per root radial section	Predicted Nº of epidermal cells at the Atrichoblast position per root radial section
Control	19	35.5 ± 0.8	64.5 ± 0.8	23 ± 1	8 *(100 %)*	15 *(100 %)*
PAC (0.5 µM)	25	29.8 ± 2	70.2 ± 2	27 ± 2	8 *(100 %)*	19 *(127 %)*
GA_4 (1 µM)	20	36 ± 3	64 ± 3	23 ± 2	8 *(100 %)*	15 *(100 %)*
Ler	20	39.1 ± 3.8	60.9 ± 3.8	21 ± 2	8 *(100 %)*	13 *(100 %)*
gai-1	19	34.9 ± 1	65.1 ± 0.9	23 ± 1	8 *(100 %)*	15 *(115 %)*
QD	31	40.8 ± 6.2	59.2 ± 6.2	23 ± 1	9 *(113 %)*	14 *(108 %)*
5X	22	41.5 ± 2.4	58.5 ± 2.4	20 ± 3	8 *(100 %)*	12 *(92 %)*

Table 5. Estimated number of root hairs and root non-hairs per root cross section in root tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Calculations were made by considering the data of Table 1 (distribution of hair and non-hair cells at the Trichoblast/Atrichoblast positions), Table 2 (percentage of ectopic root hair/non-hair cells at the Atrichoblast/Trichoblast positions) and Table 4 (average number of epidermal cells per root cross section, estimated number of epidermal cells at the Trichoblast position per root cross section, and estimated number of epidermal cells at the Atrichoblast position per root cross section). Estimated number of root hairs per root cross section = [hairs at the Trichoblast position + hairs at the Atrichoblast position]. Estimated number of root non-hairs per root cross section = [non-hairs at the Atrichoblast position + non-hairs at the Trichoblast position].

	Trichoblast position		Atrichoblast position			
	Hairs per root cross section	Non-hairs per root cross section	Hairs per root cross section	Non-hairs per root cross section	Estimated Nº of Root Hairs per root cross section	Estimated Nº of Non-root hairs per root cross section
Control	8	0	0	15	8 (100 %)	15 (100 %)
PAC (0.5 µM)	6	2	7	12	13 (163 %)	14 (93 %)
GA_4 (1 µM)	6	2	2	13	8 (100 %)	15 (100 %)
L*er*	8	0	1	12	9 (100 %)	12 (100 %)
gai-1	7	1	6	9	13 (144 %)	10 (83 %)
QD	7	2	3	11	10 (111 %)	13 (108 %)
5X	5	3	0	12	5 (56 %)	15 (125 %)

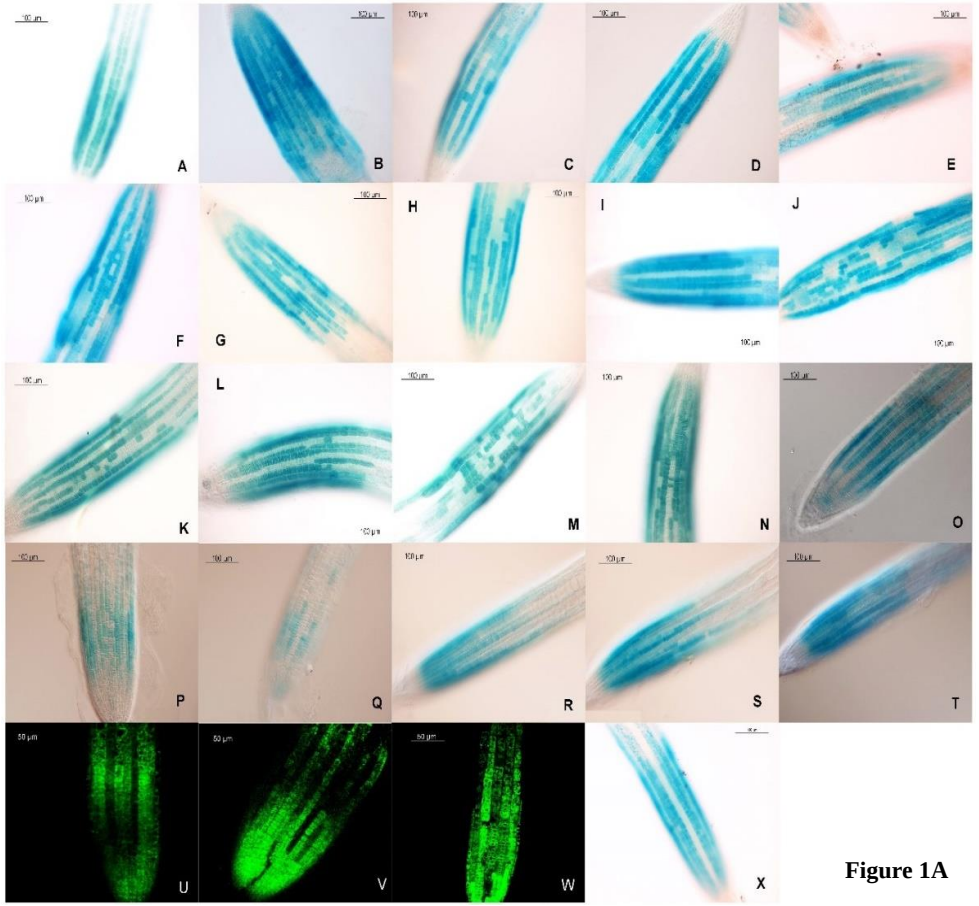

Figure 1A

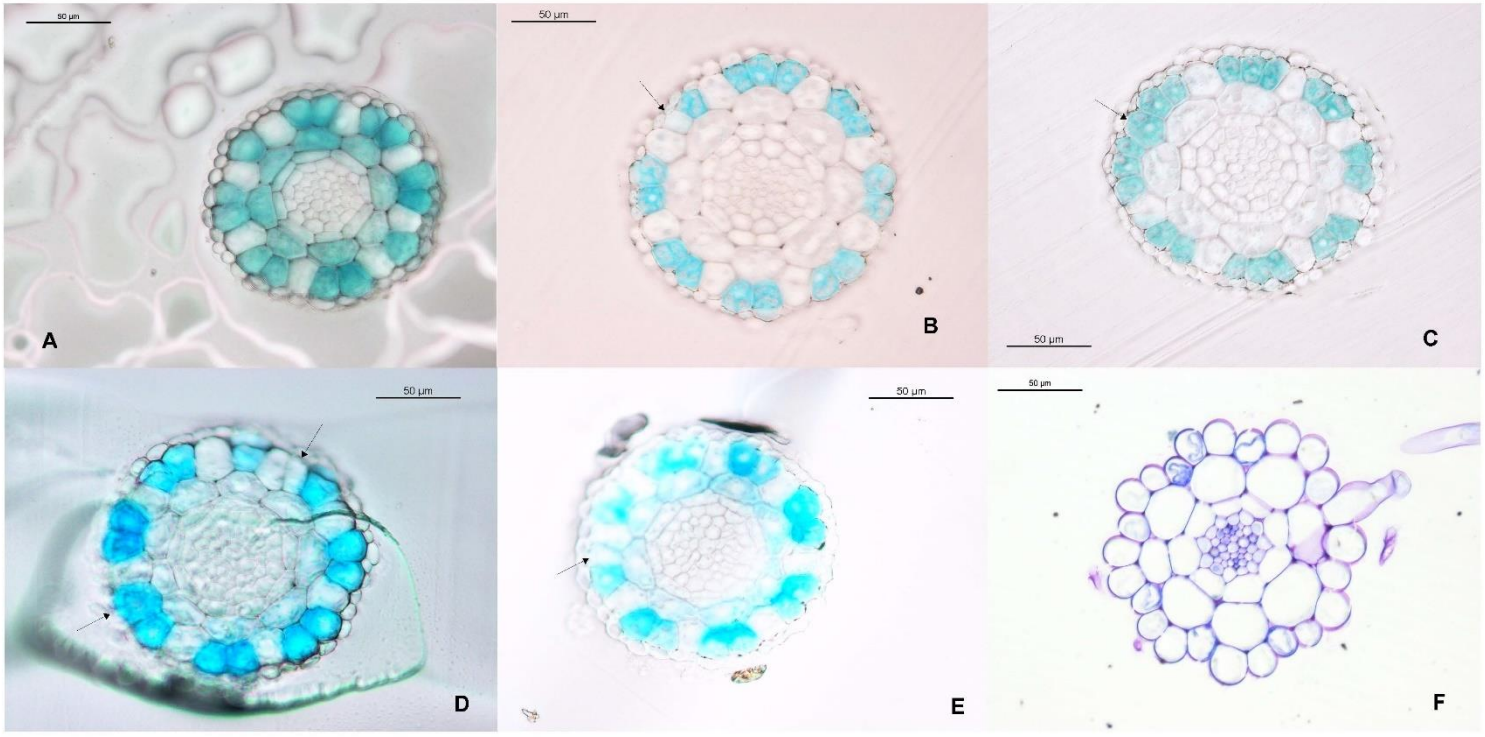

Figure 1B

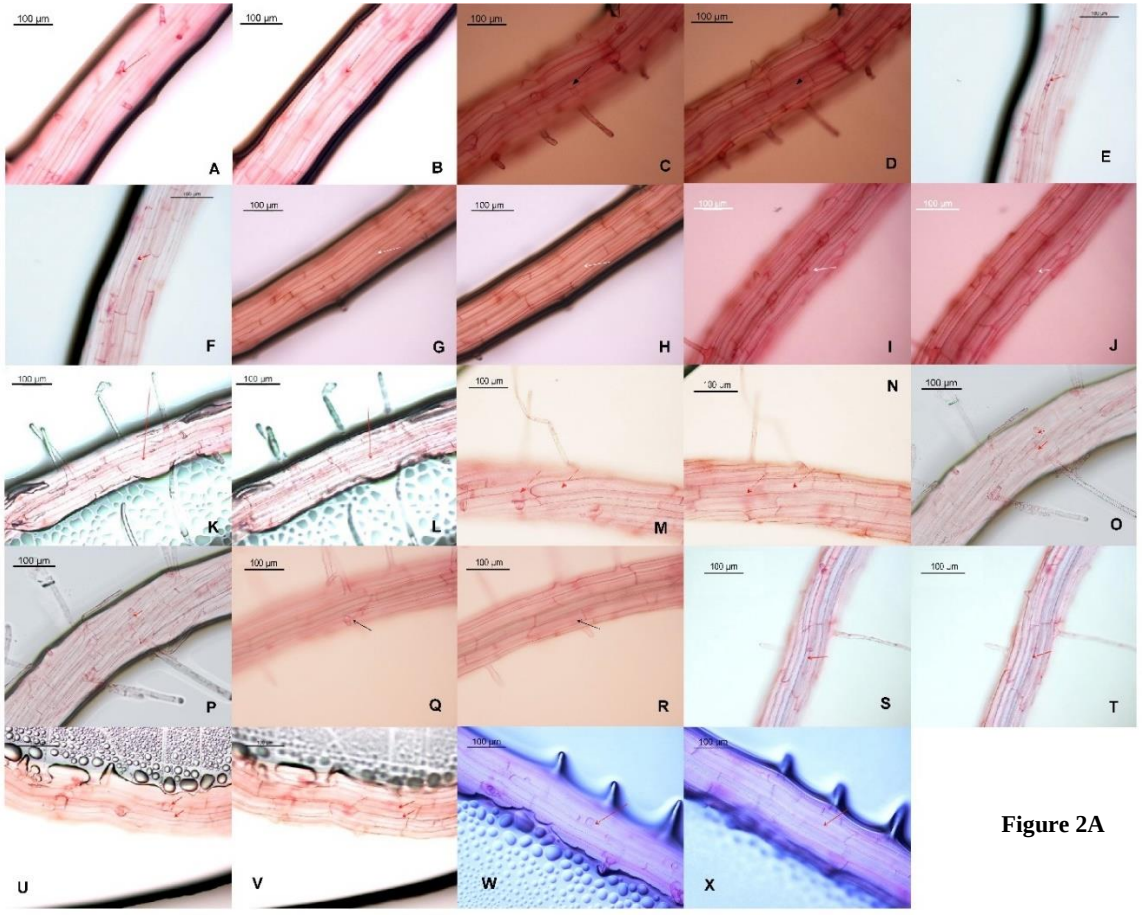

Figure 2A

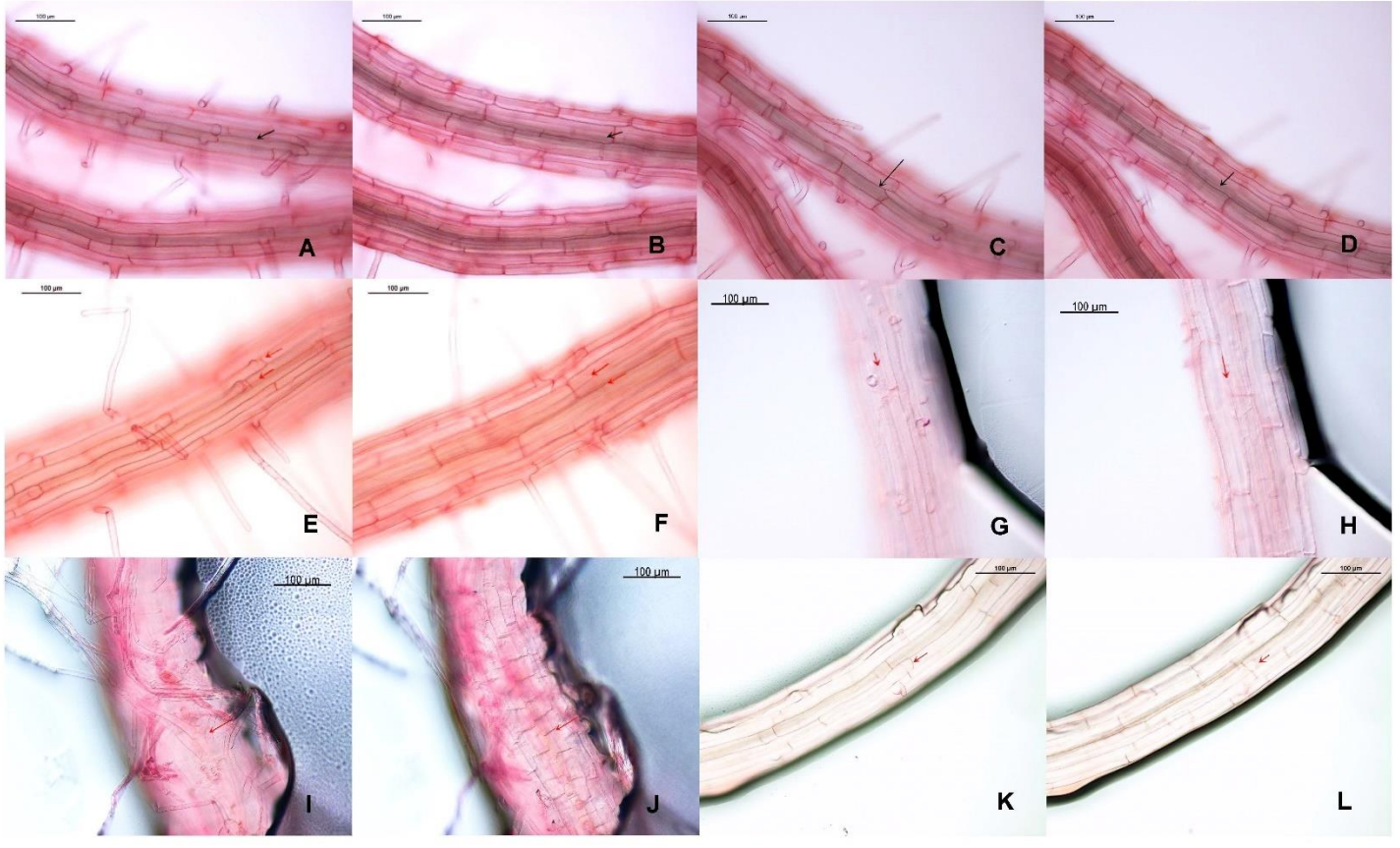

Figure 2B

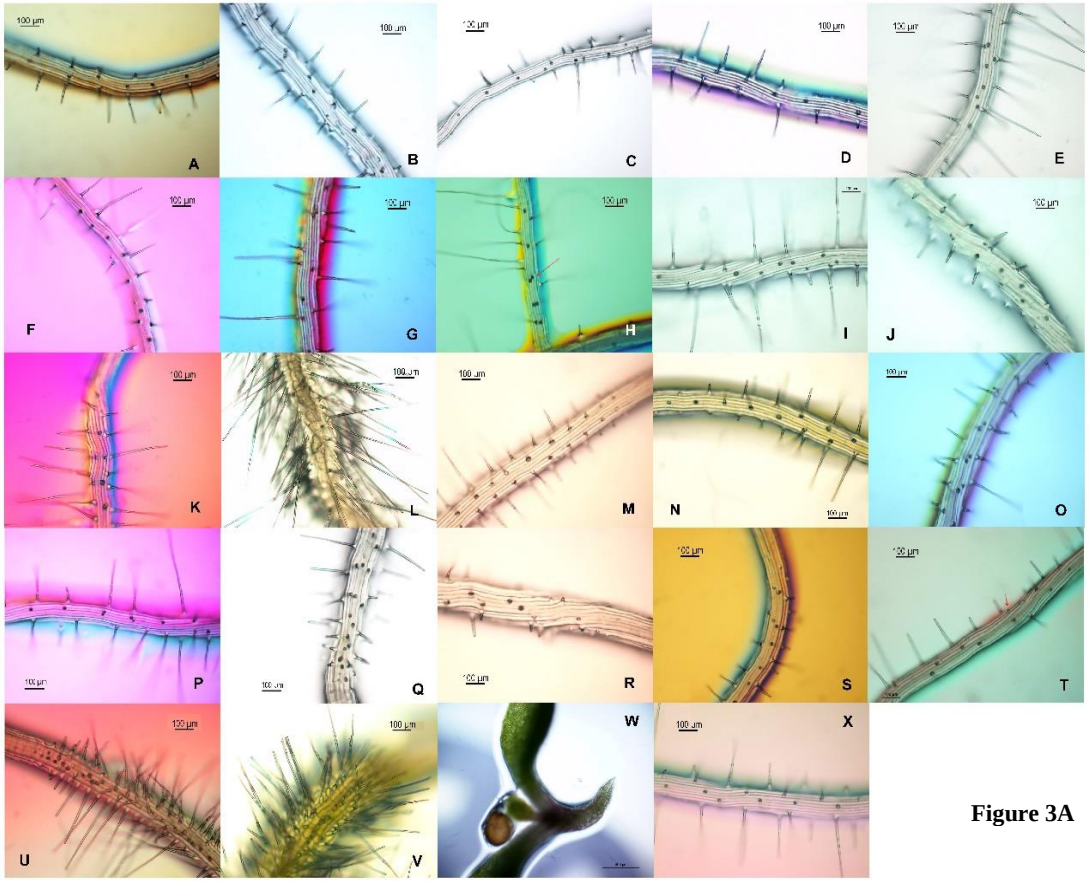

Figure 3A

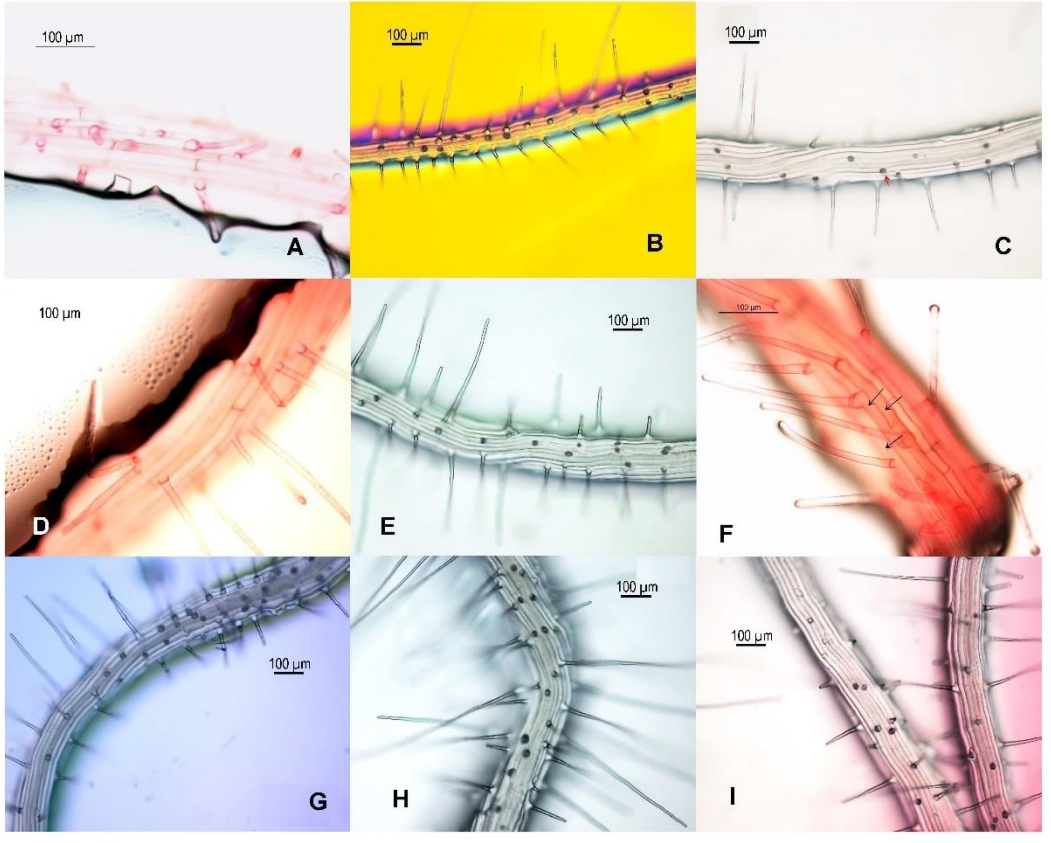

Figure 3B

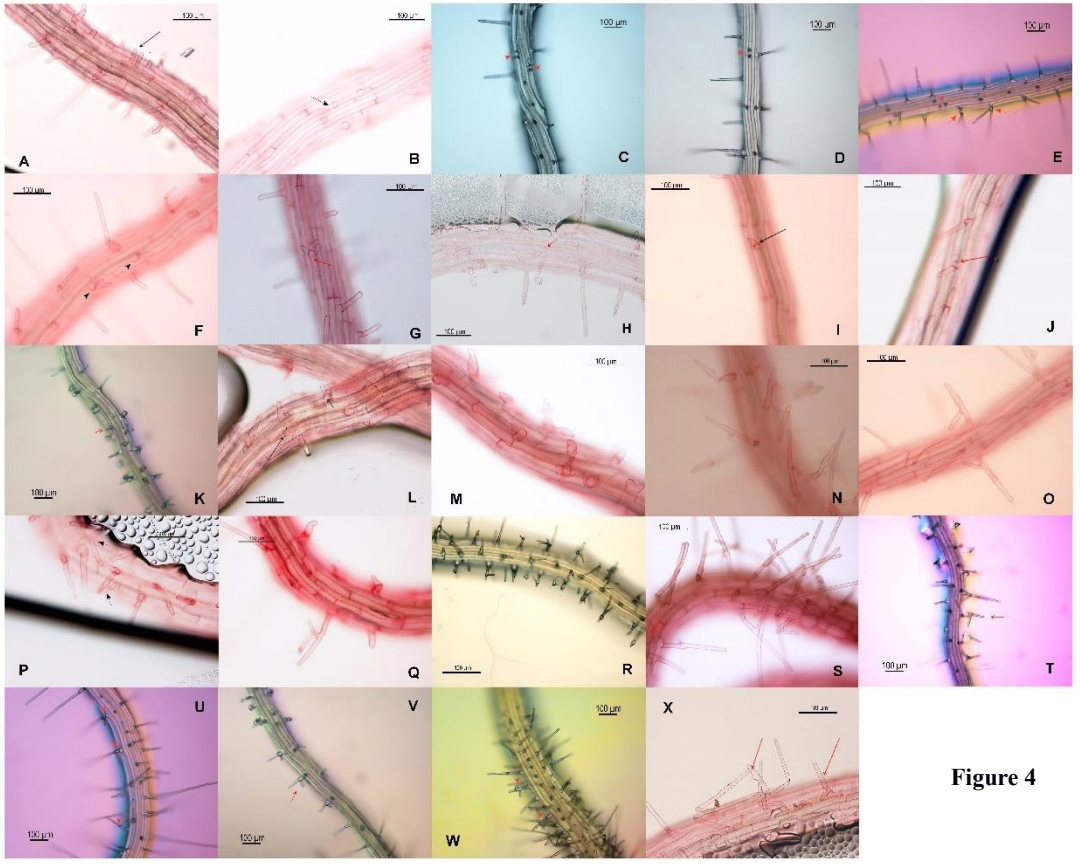

Figure 4

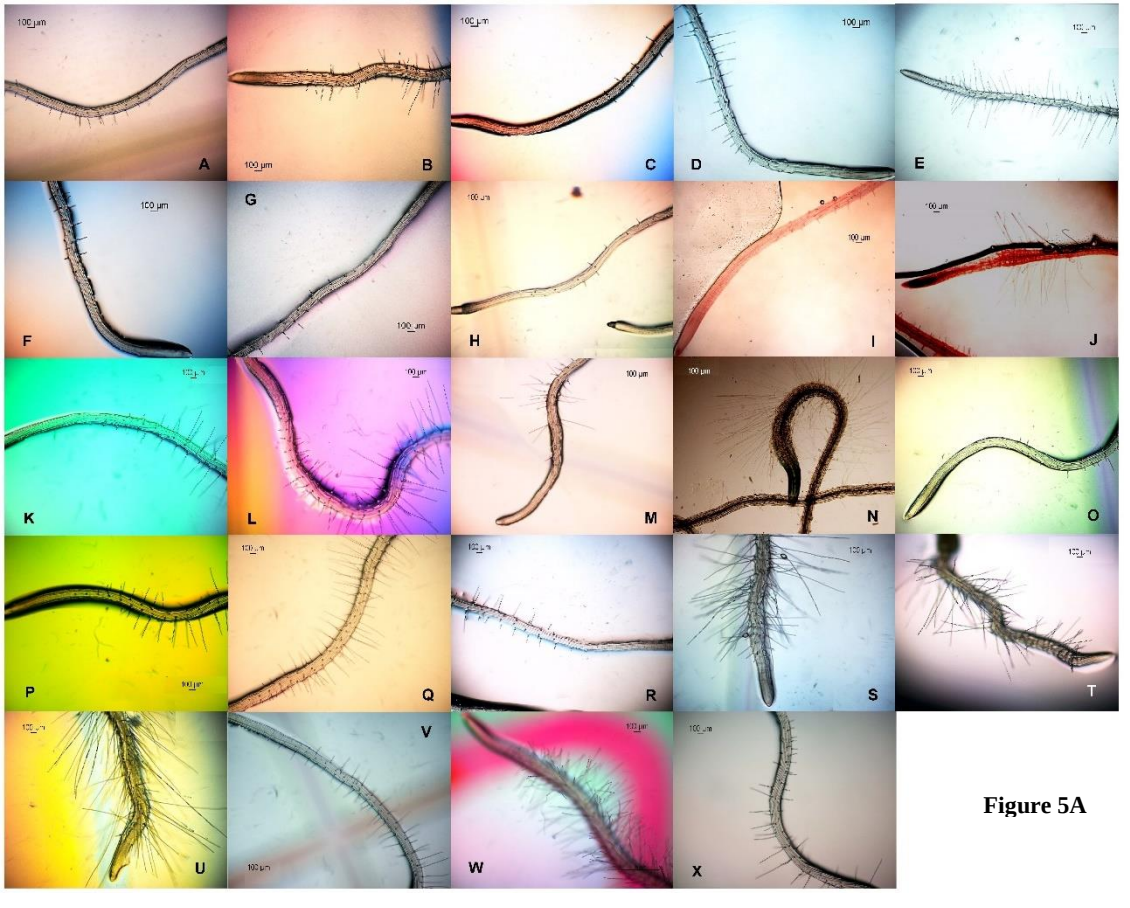

Figure 5A

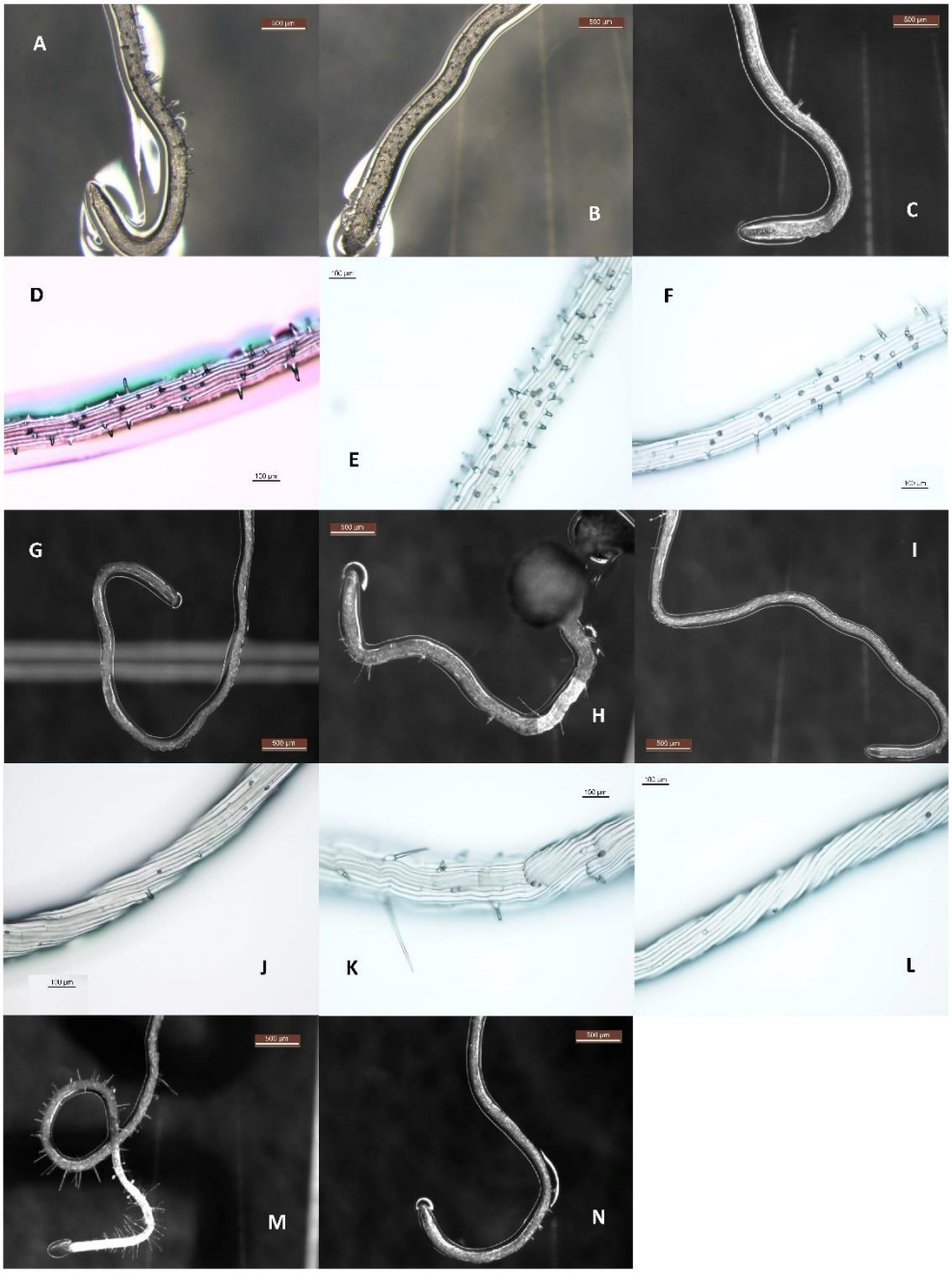

Figure 5B

Figure legends

Figure 1A. Spatial gene expression of the root hair (*CPC) and non-hair (GL2, *EGL3, WER) epidermal cell fate markers in 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. A) GL2pro::GUS (MS), 20X; B) GL2pro::GUS (0.5 μM PAC), 0X; C) GL2pro::GUS (1 μM GA4), 20X; D) GL2pro::GUS (30 μM GA3), 20X; E) Ler x GL2pro::GUS (MS), 20X; F) gai-1 x GL2pro::GUS (MS), 20X; G) GID1b-ox x GL2pro::GUS (24 h in H2O; liquid incubation experiment; leaky line), 20X; H) GID1b-ox x GL2pro::GUS (24h in 1 μM GA4; liquid incubation experiment), 20X; I) HSp::gai-1 x GL2pro::GUS (22 ºC, 4 h), 20X; J) HSp::gai-1 x GL2pro::GUS (37 ºC, 4 h), 20X; K) pGAI::gai-1:GR x GL2pro::GUS (24 h in MS; leaky line), 20X; L) pGAI::gai-1:GR x GL2pro::GUS (24h in 10 μM DEXA), 20X; M) SCR::gai-1:GR x GL2pro::GUS (24h in MS; leaky line), 20X; N) SCR::gai-1:GR x GL2pro::GUS (24h in 10 μM DEXA), 20X; O) CPCpro::GUS (MS), 20X; P) CPCpro::GUS (0.5 μM PAC), 20X; Q) CPCpro::GUS (1 μM GA4), 20X; R) EGL3pro::GUS (MS), 20X; S) EGL3pro::GUS (0.5 μM PAC), 20X; T) EGL3pro::GUS (1 μM GA4), 20X; U) WERpro::GFP (MS), 40X; V) WERpro::GFP (0.5 μM PAC), 40X; W) WERpro::GFP (1 μM GA4), 40X; X) ML1::gai-1 x GL2pro::GUS, 20X. In control seedlings, GL2 is expressed in root non-hair (Atrichoblast) cells. *CPC protein is expressed in root non-hair cells, but migrates to root hair cells. *EGL3 protein is expressed in root hair cells, but migrates to root non-hair cells. The scale bar represents 100 μm (20X) or 50 μm (40X).

Figure 1B. Spatial gene expression of the root non-hair epidermal cell fate marker GL2 in cross sections of resin-embedded roots of *A. thaliana* seedlings grown for 5 days under excessive levels of GAs/DELLAs. A) GL2pro::GUS (MS); B) GL2pro::GUS (0.5 μM PAC): Lack of GL2 expression in an Atrichoblast cell; C) GL2pro::GUS (0.5 μM PAC): Ectopic expression of GL2 in a Trichoblast cell; D) GL2pro::GUS (1 μM GA4): Ectopic expression of GL2 in a Trichoblast cell and lack of GL2 expression in an Atrichoblast cell; E) GL2pro::GUS (1 μM GA4): Lack of GL2 expression in an Atrichoblast cell; F) Ectopic root hair cell in gai-1. Magnification: 40X. The scale bar represents 50 μm.

Figure 2A. Ectopic root hairs and ectopic root non-hairs in root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. A) Col(0) (MS) correct hair (epidermis); B) Col(0) (MS), correct hair (cortex); C) Col(0) (0.5 μM PAC) ectopic hair (epidermis); D) Col(0) (0.5 μM PAC) ectopic hair (cortex); E) Col(0) (1 μM GA4) ectopic hair (epidermis); F) Col(0) (1 μM GA4) ectopic hair (cortex); G) Col(0) (MS) correct non-hair (epidermis); H) Col(0) (MS) correct non-hair (cortex); I) Col(0) (0.5 μM PAC) ectopic non-hair (epidermis); J) Col(0) (0.5 μM PAC) ectopic non-hair (cortex); K) Col(0) (1 μM GA4) ectopic non-hair (epidermis); L) Col(0) (1 μM GA4) ectopic non-hair (cortex); M) Ler, correct hair and non-hair (epidermis); N) Ler, correct hair and non-hair (cortex); O) gai-1, ectopic hair (epidermis); P) gai-1, ectopic hair (cortex); Q) QD, ectopic hair (epidermis); R) QD, ectopic hair (cortex); S) 5X, ectopic non-hair (epidermis); T) 5X, ectopic non-hair (cortex); U) pGAI::gai-1:GR (10 μM DEXA), ectopic non-hair and ectopic hair (epidermis); V) pGAI::gai-1:GR (10 μM DEXA), ectopic non-hair and ectopic hair (cortex); W) HSp::gai-1, 2d after heat shock (37 ºC, 4 h), ectopic hair (epidermis); X) HSp::gai-1, 2d after heat-shock (37 ºC, 4 h), ectopic hair (cortex). Magnification: 20X. The scale bar represents 100 μm. Propidium iodide staining.

Figure 2B. Ectopic hairs and non-hairs in root tips of 5-day-old A. thaliana seedlings over-expressing the gai-1 DELLA in different tissues of the root. A) UAS::gai-1 x J2812, ectopic hairs (epidermis); B) UAS::gai-1 x J2812, ectopic hairs (cortex); C) UAS::gai-1 x J2812 (ectopic non-hair, epidermis); D) UAS::gai-1 x J2812 (ectopic non-hair, cortex); E) UAS::gai-1 x Q2393 (ectopic hair, epidermis); F) UAS::gai-1 x Q2393 (ectopic hair, cortex); G) UAS::gai-1 x Q2393 (ectopic non-hair, epidermis); H) UAS::gai-1 x Q2393 (ectopic non-hair, cortex); I) UAS::gai-1 x Q2500 (ectopic hair, epidermis); J) UAS::gai-1 x Q2500 (ectopic hair, cortex); K) UAS::gai-1 x J0121 (ectopic non-hair, epidermis); L) UAS::gai-1 x J0121 (ectopic non-hair, cortex). Magnification: 20X. The scale bar represents 100 μm. Propidium iodide staining.

Figure 3A. Arrangement of root hairs in root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. A) Col(0) (MS), 10X; B) Col(0) (0.5 μM PAC), 10X; C)

Col(0) (30 µM GA3), 10X; D) Ler, 10X; E) gai-1, 10X; F) QD, 10X; G) 5X, 10X; H) GID1b-ox (MS, leaky line), lateral root, 10X; I) pGAI::gai-1:GR (30h in MS; leaky line), 10X; J) pGAI::gai-1:GR (30h in 10 µM DEXA), 10X; K) SCR::gai-1:GR (72h in MS; leaky line), 10X; L) SCR::gai-1:GR (48h in 10 µM DEXA), 10X; M) UAS::gai-1 x C24, 10X; N) UAS::gai-1 x J0951, 10X; O) UAS::gai-1 x J2812, 10X; P) UAS::gai-1 x N9142, 10X; Q) UAS::gai-1 x M0018, 10X; R) UAS::gai-1 x Q2500, 10X; S) UAS::gai-1 x Q2393, 10X; T) UAS::gai-1 x J0121, 10X; U) UAS::gai-1 x J0631, 10X; V) UAS::gai-1 x J0571, 10X; W) UAS::gai-1 x J3281, 4X; X) ML1::gai-1, 10X. The scale bar represents 100 µm (10X) or 500 µm (4X).

Figure 3B. Adjacent hair rows in root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. A) Col(0) (0.5 µM PAC), 20X; B) gai-1 (lateral root), 10X; C) QD, 10X; D) HSp::gai-1, 2 days after heat shock (37 ºC, 4 h), 20X; E) pGAI::gai-1:GR (MS, leaky line), 10X; F) SCR::gai-1:GR (MS, leaky line), 20X; G) UAS::gai-1 x J2812, 10X; H) UAS::gai-1 x M0018, 10X; I) UAS::gai-1 x Q2393, 10X. The scale bar represents 100 µm.

Figure 4. Morphology of Trichoblasts and root hairs in root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. A) Two-haired cell in PAC (0.5 µM), 20X; B) Cell with two hair bulges in PAC (0.5 µM), 20X; C) Two-haired cells in gai-1, 10X; D) Two-haired cells in QD, 10X; E) Two-haired cells and branched hairs in UAS::gai-1 x Q2393, 10X; F) Two-tipped hairs in PAC (0.5 µM), 20X; G) Two-tipped hairs in GA4 (1 µM), 20X; H) Two-tipped hairs in gai-1, 20X; I) Two-tipped hairs in QD, 20X; J) Two-tipped hairs in pGAI::gai-1:GR (10 µM DEXA), 20X; K) Two-tipped hairs in UAS::gai-1 x J0121, 10X; L) Two-tipped and branched hairs in PAC (0.5 µM), 20X; M) Branched hairs in PAC (0.5 µM), 20X; N) Branched hairs in gai-1, 20X; O) Branched hairs in QD, 20X; P) Branched hairs in pGAI::gai-1:GR (10 µM DEXA), 20X; Q) Branched hairs in SCR::gai-1:GR (MS; leaky line), 20X; R) Branched hairs in UAS::gai-1 x J0951, 20X; S) Branched hairs in UAS::gai-1 x J2812, 20X; T) Branched hairs in UAS::gai-1 x N9142, 10X; U) Branched hairs in UAS::gai-1 x Q2393, 10X; V) Branched hairs in UAS::gai-1 x J0121, 10X; W) Branched hairs in UAS::gai-1 x J0631, 10X; X) Branched hairs in ML1::gai-1, 20X. The scale bar represents 100 µm.

Figure 5A. Length and abundance of root hairs in root tips of 5-day-old A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. A) Col(0) (MS); B) Col(0) (0.5 µM PAC); C) Col(0) (1 µM GA4); D) Ler; E) gai-1; F) QD; G) 5X; H) GID1b-ox (MS; leaky line); I) HSp::gai-1 (24h at 22 ºC); J) HSp::gai-1 (24h after heat-shock (37 ºC, 4 h)); K) pGAI::gai-1:GR (30h in MS; leaky line); L) pGAI::gai-1:GR (30h in 10 µM DEXA); M) SCR::gai-1:GR (24h in MS; leaky line); N) SCR::gai-1:GR (24h in 10 µM DEXA); O) UAS::gai-1 x C24; P) UAS::gai-1 x J0951; Q) UAS::gai-1 x J2812; R) UAS::gai-1 x N9142; S) UAS::gai-1 x M0018; T) UAS::gai-1 x J0571; U) UAS::gai-1 x Q2500; V) UAS::gai-1 x Q2393; W) UAS::gai-1 x J0631; X) UAS::gai-1 x J0121. Magnification: 4X. The scale bar represents 100 µm.

Figure 5B. Length and abundance of root hairs in root tips of wer, cpc and 35S::CPC mutant seedlings grown for 5 days under excessive levels of GAs/DELLAs. A) wer mutant (MS), lens; B) wer mutant (0.5 µM PAC), lens; C) wer mutant (1 µM GA4), lens; D) wer mutant (MS), microscope; E) wer mutant (0.5 µM PAC), microscope; F) wer mutant (1 µM GA4), microscope; G) cpc mutant (MS), lens; H) cpc mutant (0.5 µM PAC), lens; I) cpc mutant (1 µM GA4), lens; J) cpc mutant (MS), microscope; K) cpc mutant (0.5 µM PAC), microscope; L) cpc mutant (1 µM GA4), microscope; M) 35S::CPC mutant (MS), lens; N) 35S::CPC mutant (1 µM GA4), lens. Magnification: 2.5 X (lens) or 10X (microscope). The scale bar represents 500 µm (lens) or 100 µm (microscope).

Discussion

The GAs/DELLAs might regulate the root hair patterning in *A. thaliana* seedlings

Whereas the role of GAs/DELLAs in the production and distribution of leaf hairs has been well studied [TELFER & al. 1997; PERAZZA & al. 1998], their hypothetical function in the determination and arrangement of root hairs has not been examined up to date. To this aim,

the effects of high levels of GAs/DELLAs on the spatial gene expression of the hair (CPC) and non-hair (GL2, WER and EGL3) markers of root epidermal cell fate, as well as on the distribution of root hairs, were analysed in seedlings of *A. thaliana*. Results showed that excessive levels of GAs/DELLAs impaired the spatial gene expression of the root hair/non-hair epidermal cell fate markers and disarranged the normal distribution of root hairs, what suggested that the GAs/DELLAs might be involved in regulating the root hair patterning in seedlings of *A. thaliana.* In fact, stable or inducible mutants with low (*gai-1, HSp::gai-1*, *pGAI::gai-1:GR*, *SCR::gai-1:GR*) or high (*QD, 5X, GID1b-ox*) levels of GAs showed not only a random expression of *GL2* at the MZ and EZ of the root, known as the cell fate-decision zones [PERNAS & al. 2010], but also a disarrangement of the root hairs. Because neither the spatial expression of *GL2* nor the distribution of root hairs suffered changes when the gai-1 DELLA was over-expressed at the root epidermis (*ML1::gai-1* x *GL2pro::GUS, ML1::gai-1* and *UAS::gai-1* x J0951 transgenic lines), it was concluded that the GAs/DELLAs do not seem to affect the root hair patterning in *A. thaliana* seedlings by acting on this root cell layer, but on tissues placed underneath. In fact, over-expression of gai-1 at the cortex, endodermis or pericycle of the root MZ altered the root hair patterning.

Interestingly, expressing *CPC* at the stele rescues the phenotype of the hairless mutant *cpc*, what suggests that epidermal cell differentiation might be controlled from the internal tissues of the root [RISHMAWI & al. 2014]. Therefore, the results of this study suggest that, as it was previously reported for auxins, ET, ABA, NO, BRs and SLs [SCHIEFELBEIN, 2003], the GAs/DELLAs might regulate the root hair patterning in seedlings of *A. thaliana* independently from the gene network for the specification of root epidermal cell fate, although confirmatory studies might be required.

The reason why excessive levels of GAs/DELLAs disarranged the root hair patterning in seedlings of *A. thaliana* might have been, in part, related to their effects on the cytoskeleton of MT. The MT cytoskeleton, consisting in polymers of α and β tubulin, is essential for the appropriate distribution of positional signals during development [SCHIEFELBEIN, 2003]. Also, the orientation of MT participates in the determination of epidermal cell fate [PIETRA, 2014]. Thus, MT lay randomly in Trichoblasts but transversally in Atrichoblasts [DUGARDEYN & VAN DER STRAETEN, 2008]. Hormone-induced reorganization of MT is also necessary for root hair initiation [BAO & al. 2001; SCHIEFELBEIN, 2003]. Interestingly, the GAs/DELLAs regulate MT organization by interacting with prefoldin, a protein required for the folding of tubulin [LOCASCIO & al. 2013]. As a result of this interaction, MT are organized in the presence of GAs, like in root or mesocotyl epidermal cells, and disorganized in the presence of DELLAs [PERAZZA & al. 1998; BOUQUIN & al. 2003; LOCASCIO & al. 2013]. On the other hand, mutants impaired in MT assembly have an altered root hair patterning **[**BOUQUIN & al. 2003]. The *lue1* mutant, which lacks a MT-severing and cell wall (CW) biosynthesis-related katanin protein, and whose MT are disorganized, is allelic to ectopic root hair 1 (*erh1*) and has an altered root hair patterning [BOUQUIN & al. 2003; WEBB & al. 2002]. In addition, *lue1* presents an inappropriate regulation of the GA biosynthesis-related AtGA*20ox* activity and responds to GAs [SCHNEIDER & al. 1997; BOUQUIN & al. 2003].

Ectopic root hairs have also been described in TUA6/AS transgenic lines under-expressing α-tubulin genes, in plants treated with MT polymerization-inhibiting drugs or with trichostatin A (TSA, a histone deacetylase (HDA) inhibitor), during the inducible expression of MT-interacting phospholipase-D (PLD) activity, as well as in mutants of MT severing/reorganization-related proteins, such as HDA, COBRA, SABRE and katanin p60 [SCHIEFELBEIN & al. 1997; BAO & al. 2001; BOUQUIN & al. 2003; SEDBROOK, 2004;

WANG, 2005; XU & al. 2005; LI & al. 2006, 2015; CHEN & al. 2015; PIETRA & al. 2015]. In fact, the katanin complex is required for the specification of root epidermal cells [WEBB & al. 2002]. In addition, the katanin P60-related alteration of MT organisation affects the composition and deposition of the CW [SEDBROOK, 2004]. Histone deacetylation also participates in cellular patterning, because TSA-induced histone acetylation modifies GL2, WER and CPC expression and localization and induces ectopic root hairs [XU & al. 2005; CUI & BENFEY, 2009]. Lack of SABRE function equally destabilizes the expression of cell fate markers, including WER and GL2 [PIETRA & al. 2015]. In addition, a delocalized expression of GL2 has been documented for the *jkd* (jackdaw) and *scm* (scrambled) mutants [HASSAN & al. 2010; PIETRA, 2014].

Therefore, the MT participate in cell identity specification [WEBB & al. 2002]. Cell identity, in turn, mediates the root responses to abiotic stress [DINNENY & al. 2008]. Thus, ectopic root hairs and non-hairs have been described in *A. thaliana* seedlings exposed to gamma irradiation, Cd or As, and during P deficiency, although without quantitative changes in the *WER* and *GL2* expression [MA & al. 2001; NAGATA & al. 2004; YANG & al. 2007; BAHMANI & al. 2016]. Moreover, stress down-regulates actin and tubulin gene expression [SÁNCHEZ-CALDERÓN & al. 2013]. In turn, a reduced expression of the α-tubulin gene results in MT disassembly, with MT laying in an aberrant way, and in their reorganization [BAO & al. 2001].

Consequently, the root hair patterning responds to environmental signals [SALAZAR-HENAO & al. 2016]. For instance, the photoperiod and thermoperiod control the root hair patterning in tomato [TSAI & al. 2004]. Interestingly, the GAs participate in thermotolerance [ALONSO-RAMÍREZ & al. 2009]. Thus, the results of this study suggest that the GAs/DELLAs might regulate, in part, the root hair patterning in *A. thaliana* seedlings by altering MT organization. In root cells, excessive levels of DELLAs might disorganize the cytoskeleton of MT, thereby impairing the link between positional signals and cell fate, whereas excessive levels of GAs might stabilize it.

Results of this study also point at a possible role for the DELLAs in regulating the root hair patterning in response to nutritional deficiencies. The random disposition of root hairs under excessive levels of DELLAs might favour the foraging of scarce or non-mobile minerals in deficient soils. Thus, altering the root hair patterning by modulating the levels of GAs/DELLAs might constitute a mechanism used by plants for increasing the possibilities of acquiring non-available minerals, such as P or Fe, in deficient soils. In fact, plant deficiencies in P, B or Fe disarrange the root hair patterning and induce ectopic root hairs [SCHMIDT & al. 2000; PÉRET & al. 2011; JANES & al. 2018]. Moreover, low availability of P increases the levels of DELLAs and reduces the levels of GAs in roots [JIANG & al. 2007].

Results of this study also suggest that the GAs/DELLAs might affect the root hair patterning in *A. thaliana* seedlings by acting not at the epidermis, where the gene network for the root hair/non-hair epidermal cell fate operates, but at tissues placed underneath (cortex, endodermis and pericycle). However, confirmatory studies are still needed to uncover why the epidermal expression of *gai-1* did not modify the root hair patterning in *A. thaliana* seedlings, in spite that the DELLAs promote the disorganization of MT in root epidermal cells. Moreover, the fact that only one DELLA (gai-1) was over-expressed in this study, and that expression of *gai-1* at the epidermis (*ML1::gai-1*, J0951 >> *gai-1*) induced longer and branched root hairs, suggests that the effects of the GAs/DELLAs on the root epidermal cells and/or the root hair patterning in seedlings of *A. thaliana* might be different depending on the particular concentration at which these hormones might be present.

The GAs/DELLAs might regulate the shape, length and abundance of root hairs in root tips of *A. thaliana* seedlings

Supra-physiological levels of GAs/DELLAs in *A. thaliana* seedlings also induced two-haired root epidermal cells, two-tipped root hairs and branched root hairs. Multiple hairs per root epidermal cell, two-tipped root hairs and branched root hairs have also been reported in the SUPERCENTIPEDE (*scn1*) mutant, with supernumerary root hair initiation sites, in TUA6/AS *A. thaliana* transgenic lines under-expressing α-tubulin genes, in root hair defective 3, 4 and 6 (*rhd3*, *rhd4*, *rhd6*) and *PLD* mutants, in plants treated with MT-depolymerizing oryzalin, MT-disorganizing 1-butanol (a PLD-inhibitor) or MT-stabilizing Taxol, in ROP2 (proteins controlling MT organization) over-expressing plants, and in plants subjected to Fe or NO_3^- deficiency [SCHIEFELBEIN & SOMERVILLE, 1990; SCHIEFELBEIN & al. 1993; MASUCCI & SCHIEFELBEIN, 1994; GILROY & JONES, 2000; SCHMIDT & al. 2000; BAO & al. 2001; FOREMAN & DOLAN, 2001; GRIERSON & SCHIEFELBEIN, 2002; JONES & al. 2002; GARDINER & al. 2003; MÜLLER & SCHMIDT, 2004; CAROL & DOLAN, 2006; ISHIDA & al. 2008; SHIN & al. 2011; PIETRA, 2014]. Interestingly, hormone-induced reorganization of MT is required for the morphogenesis of root hairs [BAO & al. 2001; SCHIEFELBEIN, 2003]. In turn, the phenotype of root hair branching, due to changes in actin distribution and dynamics, has been related to the induction of genes for GA biosynthesis and CW modification, and reported during legume-rhizobium symbiosis (i.e., soybean infected with *Bradyrhyzobium japonicum*), in plants treated with MT-inhibiting drugs, and in mutants of genes necessary for a correct growth of root hairs, such as *TIP1* (involved in the biosynthesis of CW components and probably in the arrangement of actin filaments) and *RHD3* [SCHIEFELBEIN & SOMERVILLE, 1990; SCHIEFELBEIN & al. 1993; BAO & al. 2001; SALAZAR-HENAO & al. 2016].

The disruption of MT also affects trichome branching [GILROY & JONES, 2000], as actin regulates the shape and growth of trichomes [RODRÍGUEZ-SERRANO & al. 2014]. In addition, the GAs promote trichome branching and influence CW growth [TELFER & al. 1997; PERAZZA & al. 1998]. Thus, the *spy5* mutant (with high levels of GAs and which also displays ectopic root hairs) has over-branched trichomes [PERAZZA & al. 1998; MUTANWAD & al. 2020]. On the other hand, during trichome development, the number of branches and the level of endo-reduplication, which is induced by GAs, are closely related [PERAZZA & al. 1998; KONDOROSI & al. 2001].

In this study, excessive levels of DELLAs in *A. thaliana* seedlings also induced longer root hairs near the root tip. Interestingly, nutrient availability prevents root hair elongation [TSAI & al. 2004], whereas deficiencies in P, B or Mg induce root hair elongation, being the higher levels of DELLAs the mediators of the extra-elongation of root hairs [PÉRET & al. 2011; LIU & al. 2018]. Elongated root hairs have also been described in plants exposed to gamma irradiation, Cd or As, as well as in polyploids [NAGATA & al. 2004; SETTER & al. 2015; BAHMANI & al. 2016; SALAZAR-HENAO & al. 2016]. Conversely, shorter root hairs have been reported in mutants of the *TIP1*, *PLDζ1-PLDζ2*, and *RSL4* (a component of GAs signalling) genes [SCHIEFELBEIN & al. 1993; LI & al. 2006; PÉRET & al. 2011]. Moreover, the GAs are necessary for root hair elongation, as the *ga* 1-3 mutant (deficient in GAs) produces shorter root hairs [PÉRET & al. 2011]. However, the GAs might act at a later stage of root hair development, as apparently, in this study, high levels of GAs did not stimulate root hair elongation near the root tip as much as the high levels of DELLAs did. Therefore, the changes induced, in this study, by excessive levels of GAs/DELLAs on the shape and length of root hairs

in seedlings of *A. thaliana* might have been related to the effect of these hormones on the MT cytoskeleton and/or the CW biosynthesis of the root epidermal cells.

Regarding root hair abundance, it is known that nutrient availability inhibits root hair production [TSAI & al. 2004]. Excess of Na^+ reduces root hair abundance [DINNENY & al. 2008], whereas deficiencies in P, Fe or B increase the frequency of root hairs, mainly by inducing ectopic root hair cells [SCHIEFELBEIN, 2003; MARTÍN-REJANO & al. 2011; PÉRET & al. 2011; SHIN & al. 2011; SALAZAR-HENAO & al. 2016; JANES & al. 2018]. An increased density of root hairs has also been reported in ROP2 over-expressing plants, in *arm* (*ctl1*; cellulose biosynthesis-related) and *sabre* mutants, in plants exposed to Cd, V or As, and in polyploids [JONES & al. 2002; PIETRA, 2014; LIN & al. 2015; BAHMANI & al. 2016; SALAZAR-HENAO & al. 2016]. Interestingly, the levels of GAs determine trichome number [PERAZZA & al. 1998]. In turn, HDA19 controls the response of the root hair density to low P [CHEN & al. 2015].

Because of the GAs/DELLAs are involved in plant stress responses [ALONSO-RAMÍREZ & al. 2009], the results of this study suggest that these hormones might have a role in regulating the response of the root hair abundance to nutrient availability. In fact, in this study, root hairs near the root tip were denser and longer under excessive DELLAs, but scarcer and shorter under excessive GAs. With this respect, it is known that root hairs grow closer to the root MZ under mechanic stress or B deficiency [OKAMOTO & al. 2008; MARTÍN-REJANO & al. 2011]. Also, the abundance and length of root hairs respond to environmental signals [SALAZAR-HENAO & al. 2016]. Light signalling, for instance, influences root hair length [GRIERSON & SCHIEFELBEIN, 2002]. In turn, the photo-period conditions affect the biosynthesis and/or sensibility of GAs [TELFER & al. 1997].

As PLD inhibitors break the organization of MT, which is essential for the correct directionality, elongation and morphology of root hairs [GARDINER & al. 2003], then, the morphological alterations of root hairs observed in this study point to a possible impairment, by excessive levels of GAs/DELLAs, of the actin microfilaments, the cytoskeleton of MT, and the ROP GTPase proteins. In fact, hair cell morphogenesis requires α-tubulin and Rho-like GTPase activity, which, in turn, interacts with katanin P60 to promote MT ordering [FOREMAN & DOLAN, 2001; LIN & al. 2013]. Moreover, the SABRE protein (involved in MT organisation and the stabilization of epidermal patterning factors) acts upstream of ROPs [PIETRA, 2014].

Conclusions

The results from this study suggest that the GAs/DELLAs might regulate the patterning, shape and abundance of root hairs in root tips of *A. thaliana* seedlings, and that they might do it by acting from the sub-epidermal tissues of the root. In fact, growth of *A. thaliana* seedlings under supra-physiological levels of GAs/DELLAs altered the distribution, morphology and frequency of root hairs.

Notes on contributor

Iva MCCARTHY-SUÁREZ – is a postdoctoral researcher in plant biology with special interest in the mechanism of action of plant hormones, senescence and environmental stress.

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SUPRA-PHYSIOLOGICAL LEVELS OF GIBBERELLINS / DELLAS MODIFY ROOT CELL SIZE / NUMBER AND ROOT ARCHITECTURE IN ROOT TIPS OF *ARABIDOPSIS THALIANA* SEEDLINGS. CONNECTIONS TO THE ROOT HAIR PATTERNING AND ABUNDANCE

Iva MCCARTHY-SUÁREZ

Hormone Signalling and Plant Plasticity Group. Instituto de Biología Celular y Molecular de Plantas (IBMCP-UPV-CSIC). Ingeniero Fausto Elio s/n (46022), Valencia – Spain.
E-mail: ivmcsua@upvnet.upv.es

Abstract: A previous study showed that excessive GAs/DELLAs altered the arrangement, shape and frequency of hairs in root tips of *A. thaliana* seedlings by acting from the root sub-epidermal tissues. The present study showed that excessive GAs/DELLAs also modified root tip cell size/number in *A. thaliana* seedlings. While excessive DELLAs shortened and widened the root epidermal, cortical, endodermal and pericycle cells, excessive GAs, excepting the epidermal cells, generally narrowed them. However, no root cell size changes occurred when gai-1 was over-expressed at the root epidermis. Also, excessive DELLAs induced extra cells at the root epidermis, cortex, endodermis and pericycle and lateral root outgrowth, whereas excessive GAs induced extra cells at the root cortex and pericycle. Thus, excessive GAs/DELLAs might modify root tip cell size/number by acting from the sub-epidermal tissues of the root. This, in turn, might impact on the root hair patterning & abundance and the root architecture.

Keywords: DELLAs, Gibberellins, lateral roots, root architecture, root cell number, root cell size.

Introduction

A previous study showed that supra-physiological levels of GAs/DELLAs altered the patterning, the morphology and the abundance of root hairs in *A. thaliana* seedlings, and that they did it by possibly acting from the sub-epidermal tissues of the root [MCCARTHY-SUÁREZ, unpublished manuscript]. In fact, the GAs/DELLAs have a role in the production of trichomes (leaf hairs) in *A. thaliana* [CHIEN & SUSSEX, 1996; TRAW & BERGELSON, 2003] and participate in the organization of the cytoskeleton of microtubules (MT) [LOCASCIO & al. 2013], which is essential for trichome and root hair growth, for establishing root cell identity and shape, and for plant cell expansion and division [BAO & al. 2001].

Because auxin, ethylene, abscisic acid, nitric oxide, brassinosteroids, cytokinins and strigolactones regulate the root hair patterning [CAO & al. 1999; VAN HENGEL & al. 2004; LOMBARDO & al. 2006; KAPPUSAMY & al. 2009; NIU & al. 2011], and because changes in the levels of these phytohormones are correlated to alterations in the root cell size/number and the root architecture in response to nutritional stresses, such as low availability of P, B or Fe in the soil (e.g. swelling of root cortex cells, induction of extra cells at the root cortex, and production of lateral roots (LR)) [SCHMIDT & al. 2000; YANG & al. 2007; MARTÍN-REJANO & al. 2011], this study wanted to determine whether supra-physiological levels of GAs/DELLAs might also have an effect on the size/number of root cells and on the production of LR in root tips of *A. thaliana* seedlings. To this aim, the size and/or number of root tip cells

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were examined in Col (0) seedlings grown for 5 days under excessive levels of GAs/DELLAs, as well as in GAs (*QD, 5X*, GID*1b*-ox) or DELLAs (*gai*-1, *HSp::gai-1*, *pGAI::gai-1:GR*, *SCR::gai-1:GR*)-overproducing mutants. Moreover, the size of root tip cells was examined in 5-day-old mutant seedlings resulting from expressing the *gai-1* (GA-insensitive) DELLA allele in different tissues of the root (UAS (GAL4-UPSTREAM ACTIVATION SEQUENCE) expression directed system lines; Dr. JIM HASSELHOFF'S laboratory). On the other hand, the presence of LR was also analysed in root tips of *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Results of this study suggested that the GAs/DELLAs might have a role in regulating the size, number and organization of root cells, as well as the root architecture, in root tips of *A. thaliana* seedlings.

Material and methods

Plant Material and Growth Conditions

Arabidopsis thaliana Col (0) seeds were sterilized (70% Ethanol (v/v) and 0.01% Triton X-100 (v/v)), sown on half-strength MS medium plates (0.8% (w/v) agar and 1% (w/v) sucrose), stratified for 3-4 days (4 ºC, darkness), germinated, and grown vertically (22 ºC; 5-7 days) under continuous white light (Percival growth chamber E-30B) (http://www.percival-scientific.com) as described by LEE & SCHIEFELBEIN (1999).

Hormone and Chemical treatments

Stock solutions of paclobutrazol (PAC, 10 mM in acetone 100% (v/v)), GA_4 (1 mM in 100% ethanol (v/v)) or GA_3 (50 mM in 100% ethanol (v/v)) were conveniently diluted and added to MS agar medium or water (in the case of liquid incubation experiments) to obtain a final concentration of 0.5 µM PAC, 1 µM GA_4 and 30 µM GA_3.

Mutant Lines

In a previous study [MCCARTHY-SUÁREZ, unpublished manuscript], the spatial gene expression of the root non-hair epidermal cell fate marker GL2 was studied in root tips of *A. thaliana* seedlings by using the *GL2pro::GUS* mutant line as well as those derived from crossing lines harbouring constitutively excessive levels of GAs/DELLAs with the *GL2pro::GUS* line (L*er* x *GL2pro::GUS* background). In the present study, the effect of transient increases in the levels of expression of the *gai*-1 (GA-insensitive-1) DELLA alelle on the size and number of root tip cells in *A. thaliana* seedlings was examined by using the *gai*-1 mutant lines of heat-shock inducible *HSp::gai-1* (which over-expresses gai-1 upon heat shock) and of dexamethasone (DEXA)-inducible *pGAI::gai-1:GR* and *SCR::gai-1:GR* (with glucocorticoid-binding domain). The *HSp::gai-1* mutant seedlings were grown at 37 ºC for 4 h (heat-shock) and then at 22 ºC for 2 h (recovery period), whereas the *pGAI::gai-1:GR* and *SCR::gai-1:GR* mutant seedlings were incubated in 0.2, 1.2 or 10 µM DEXA for a minimum of 6h. Root cell size and number was also studied in mutants with excessive levels of GAs/DELLAs (*gai*-1, *QD (quadruple DELLA mutant)*, *5X (quintuple DELLA mutant)* and GID1b-*ox* (which over-expresses the GA receptor GID1b (GIBBERELLIN INSENSITIVE DWARF1)), in mutants over-expressing gai-1 in different tissues of the root (*ML1::gai-1* (epidermis) and UAS expression directed system (GAL4-UPSTREAM ACTIVATION SEQUENCE) lines: *UAS::gai-1* x C24 (control, background); *UAS::gai*-1 x J0951 (epidermis of the meristematic zone (MZ)); *UAS::gai*-1 x J2812 (MZ epidermis and cortex); *UAS::gai-1* x N9142 (cortex of elongation zone (EZ)); *UAS::gai-1* x M0018 (MZ cortex and endodermis);

UAS::gai-1 x J0571 (MZ cortex and endodermis); *UAS::gai-1* x Q2393 (all tissues but the endodermis); *UAS::gai-1* x Q2500 (MZ endodermis/pericycle); *UAS::gai-1* x J0121 (EZ pericycle); *UAS::gai-1* x J0631 (all tissues of the EZ); *UAS::gai-1* x J3281 (vessels)), and in the *35S::CPC x GL2pro::GUS* and *scm* (scrambled) x *GL2pro::GUS* mutants.

GUS activity assay

GUS (β-glucuronidase) staining of the *GL2pro::GUS* reporter line was performed as described by FRIGERIO & al. (2006), but using 8 mM instead of 2 mM potassium ferro/ferricyanide and incubating the seedlings (15 min to 2 h) in the reaction mixture at 4 ºC instead of 37 ºC.

Microscopy

Cell organization at the root tip was studied on ultra-thin cross sections of plastic resin-embedded roots as described by Dr. SCHIEFELBEIN Protocols (http://www.mcdb.lsa.umich.edu/labs/schiefel/protocols.html). Seedlings were included in 1% agarose in 0.1 M sodium phosphate buffer, pH 6.8, and stained for GUS activity. Root-containing blocks were then cut, fixed with 4% para-formaldehyde in PBS, dehydrated in ethanol series (15%, 30%, 50%, 75%, 95% and 100%, 1 h each), kept in 100% ethanol overnight, incubated in Technovit ® 7100 infiltration solution for 2 days, inserted in gelatine capsules, and embedded for 9 days in Technovit ® 7100 plastic resin (Heraeus Kultzer, Germany). Ultramicrotome (Ultracut E, Reichert Jung, Germany) cross sections of resin-embedded roots were then stained with 0.06% (w/v) toluidine blue and observed under a Nikon Eclipse E600 microscope. The GFP expression in HASELHOFF's crossed lines was visualized by using a Leica Confocal Microscope (Excitation: 488 nm; Detection: 500-530 nm band-path filter for GFP).

Results

Excessive levels of GAs/DELLAs modified the size and number of root tip cells in seedlings of *A. thaliana*

Apart from altering the patterning, morphology and abundance of root hairs [MCCARTHY-SUÁREZ, unpublished manuscript], high levels of GAs/DELLAs also modified the size and number of root tip cells in seedlings of *A. thaliana* (Figures 1-3; Tables 1-3). While excessive levels of GAs, excepting the epidermal cells, usually narrowed the root cells, excessive levels of DELLAs frequently widened, shortened and twisted the root epidermal, cortical, endodermal and pericycle cells, resulting in wider roots (Figures 1-3; Tables 1-3). Moreover, in the *HSp::gai-1* and *SCR::gai-1:GR* mutants, the widening and shortening of the root epidermal, cortical, endodermal and pericycle cells observed at 24 h after heat-shock (37 ºC, 4 h) or after growth in DEXA (10 μM), respectively (Figure 3), was accompanied, as previously reported [MCCARTHY-SUÁREZ, unpublished manuscript], by an alteration in the spatial expression of *GL2* and in the distribution of root hairs. Similar changes were observed when gai-1 was over-expressed at the subepidermal tissues of the root (Figures 1, 2 and 4; Table 1). However, when gai-1 was over-expressed at the root epidermis, no apparent changes occurred in the size of the root epidermal, cortical, endodermal or pericyclic cells (Figures 1, 2 and 4; Table 1), in the patterning of *GL2* gene expression or in the root hair distribution [MCCARTHY-SUÁREZ, unpublished manuscript], what suggested that the changes in root cell size that took place when gai-1 was over-expressed at the sub-epidermal tissues of the root might have been

connected to the alterations in the root hair patterning induced by excessive levels of GAs/DELLAs.

Growth of *A. thaliana* seedlings for 5 days under excessive levels of GAs, in contrast, caused the narrowing of the root cortical cells, an effect that was corroborated in the *QD, 5X* and GID1b *ox* mutants (Figure 2; Table 1). Nevertheless, excessive levels of GAs also seemed to slightly increase the relative width of the root epidermal cells (Figures 1, 5 and 6). Frequently, under GA_3 (30 µM) or GA_4 (1 µM), changes of cell fate at the root epidermis coincided with changes in the width of the epidermal cells and/or with changes in the size of the root cortical and endodermal cells (Figure 3; Tables 1 and 2).

In fact, estimations of tissue depth in root tips of *A. thaliana* seedlings uncovered the swelling of the root epidermal, cortical, endodermal and pericyclic cells under high levels of DELLAs (PAC) and their slight thinning under high levels of GAs (*5X* mutant) (Table 4). Moreover, growth of seedlings of the *scm* x *GL2pro::GUS* mutant in PAC (0.5 µM) for 5d caused the radial swelling of the epidermal, cortical and endodermal and pericyclic cells of the root tip (Figure 6). On the other hand, in seedlings of the *35S::CPC* x *GL2pro::GUS* mutant, changes of cell fate at the root epidermis were accompanied by changes in the width of the underlying root epidermal and cortical cells (Figure 3).

Interestingly, cell size changes in root tips under excessive levels of GAs/DELLAs were often accompanied by the presence of multinucleated cells at the epidermis, cortex, endodermis and pericycle of the root (Figure 6).

Excessive levels of GAs/DELLAs also modified the radial cell organization in root tips of *A. thaliana* seedlings (Figure 5). Treatments with PAC (5d, 7d) frequently increased the number of cells at the epidermis, cortex, endodermis and pericycle of the root (Figures 5 and 6; Table 5) and induced anticlinal/diagonal cell divisions at the root epidermis (T-clones) (Figure 3) as well as periclinal cell divisions at the root cortex and endodermis (middle cortex (MC)) (Figure 6). Furthermore, growth of the *scm* x *GL2pro::GUS* mutant for 5 days in PAC induced the proliferation of the root epidermal, cortical and endodermal cells and the formation of a MC (Figure 6). Treatments with excessive levels of GAs, in turn, sometimes increased the number of cortical and pericycle cells, but not of epidermal cells, in the radial dimension of the root, and induced epidermal T-clones and a MC (Figures 3, 5 and 6; Table 5). Moreover, the number of root epidermal cells decreased in the *5X* mutant (Figure 5; Table 5), which, in part, might have explained the lower abundance of root hairs per root radial section in this mutant [MCCARTHY-SUÁREZ, unpublished manuscript]. In fact, in root cross sections, frequently only one epidermal cell was seen at the Atrichoblast (non-hair) position under high levels of GAs, whereas up to four cells could be seen under high levels of DELLAs (Figure 5), in tune with the reduced number of epidermal cells in the *5X* mutant and the increased number of epidermal cells under PAC (Table 5). Furthermore, given that root non-hair cells lay over just one cortical cell, then, the observed increase in the width of the cortex cells under high levels of DELLAs (Figures 2, 4 and 5; Table 1) might have accounted for the higher percentage of epidermal cells at the Atrichoblast position, as well as the lower percentage of epidermal cells at the Trichoblast position, detected per root radial section under this treatment [MCCARTHY-SUÁREZ, unpublished manuscript]. Conversely, the decrease in the width of the cortex cells seen under high levels of GAs (*5X* mutant) (Figure 2; Table 1) might have explained the lower percentage of epidermal cells at the Atrichoblast position, and the higher percentage of epidermal cells at the Trichoblast position, found per root radial section under this treatment [MCCARTHY-SUÁREZ, unpublished manuscript]. Nevertheless, considering that the average number of epidermal cells per root radial section increased under high levels of DELLAs (PAC,

gai-1) and decreased under high levels of GAs (*5X* mutant) [MCCARTHY-SUÁREZ, unpublished manuscript], then, the predicted number of epidermal cells at the Trichoblast position did not change under excessive levels of GAs/DELLAs in 5 day-old *A. thaliana* seedlings [MCCARTHY-SUÁREZ, unpublished manuscript].

The root diameter at the MZ, in addition, increased by 40% under excessive levels of DELLAs (Table 3), in tune with the increased number of cells at the root epidermis, cortex, endodermis and pericycle (Figure 5; Table 5) and the wider and/or deeper cells at the root cortex, endodermis and pericycle (Figures 5 and 6; Tables 1 and 4). Conversely, under excessive levels of GAs, the root diameter decreased (Table 3), in accordance with the lower number of cells at the root epidermis (*5X* mutant) (Table 5), and the narrower and shallower cells at the root cortex and endodermis (Figures 2, 5 and 6; Tables 1 and 4). Nevertheless, at the MZ-EZ transition zone, the root also seemed to swell under excessive levels of GAs, and there was variability of cell sizes [MCCARTHY-SUÁREZ, unpublished manuscript] (Figure 6), maybe due to the swelling of the epidermal cells and to the deepening of the pericycle cells (Figure 1; Table 4). In fact, GA_3 treatments increase the ratio of (xylem/whole root) area [WANG & al. 2015].

Excessive levels of GAs / DELLAs modified the outgrowth of lateral roots in root tips of *A. thaliana* seedlings

Excessive levels of DELLAs also induced the outburst of LR near the root tip in *A. thaliana* seedlings, whereas excessive levels of GAs inhibited it (Figure 7).

Table 1. Average length and width of root cortical cells in root tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Analyses performed on micrographs of root cortex cells, 20X. (*) At 48h after a 4h-heat-shock experiment.

	Root cortical cell			
	Nº Cells analysed	**Length (µm)**	**Nº Cells analysed**	**Width (µm)**
Col (0) (MS)	25	171 ± 28 *(100 %)*	66	31 ± 3 *(100 %)*
PAC (0.5 µM)	23	153 ± 34 *(89 %)*	60	43 ± 3 *(142 %)*
GA_4 (1 µM)	26	209 ± 52 *(122 %)*	26	27 ± 2 *(87 %)*
PAC (0.5 µM) + GA_4 (1 µM)	24	173 ± 49 *(101 %)*	28	19 ± 2 *(61 %)*
Ler	28	188 ± 29 *(100 %)*	18	30 ± 4 *(100 %)*
ML1::gai-1	12	171 ± 33 *(91 %)*	28	30 ± 3 *(97 %)*
gai-1	71	138 ± 41 *(73 %)*	72	37 ± 7 *(123 %)*
QD	33	180 ± 34 *(96 %)*	53	27 ± 3 *(90 %)*
5X	15	187 ± 34 *(99 %)*	30	27 ± 4 *(90 %)*
GID1b-ox	23	210 ± 54 *(112%)*	34	24 ± 4 *(80 %)*
***Hsp::gai-1x GL2pro::GUS* (22 ºC, 4 h)**	48	184 ± 39 *(100 %)*	47	27 ± 4 *(100 %)*
***Hsp::gai-1 x GL2pro::GUS* (37 ºC, 4 h)**	38	158 ± 52 *(86 %)*	33	31 ± 4 *(115 %)*
Hsp::gai-1 x GL2pro::GUS* (22 ºC) 48 h	23	195 ± 43 *(100 %)*	34	29 ± 3 *(100%)*
Hsp::gai-1 x GL2pro::GUS* (37 ºC) 48 h	36	61 ± 19 *(31 %)*	44	46 ± 9 *(159 %)*
***pGAI::gai-1:GR* (MS)**	20	180 ± 56 *(100 %)*	20	34 ± 4 *(100 %)*
***pGAI::gai-1:GR* (10 µM DEXA)**	29	129 ± 31 *(72 %)*	29	46 ± 5 *(135 %)*
***SCR::gai-1:GR* (MS)**	77	140 ± 51 *(100 %)*	77	34 ± 5 *(100%)*
***SCR::gai-1:GR* (10 µM DEXA)**	64	70 ± 32 *(50 %)*	63	53 ± 10 *(156 %)*
***UAS::gai-1 x C24* (control)**	46	154 ± 49 *(100 %)*	52	28 ± 4 *(100 %)*
***UAS::gai-1 x J0951* (epidermis)**	54	155 ± 42 *(101%)*	72	31 ± 4 *(111 %)*
***UAS::gai-1 x J2812* (epi + cortex)**	79	116 ± 48 *(75 %)*	91	35 ± 6 *(125 %)*

UAS::gai-1 x J0571 **(cortex + endo)**	74	71 ± 18 *(46 %)*	74	48 ± 8 *(171 %)*
UAS::gai-1 x M0018 **(cortex + endo)**	49	89 ± 31 *(58 %)*	46	46 ± 9 *(164 %)*
UAS::gai-1 x Q2500 **(MZ endo/pericycle)**	79	51 ± 13 *(33 %)*	85	41 ± 8 *(146 %)*
UAS::gai-1 x Q2393 **(all but endo)**	23	174 ± 59 *(113 %)*	32	32 ± 5 *(114 %)*
UAS::gai-1 x J0631 **(elong. tissues)**	73	46 ± 8 *(30 %)*	67	37 ± 6 *(132 %)*
UAS::gai-1 x J0121 **(EZ pericycle)**	16	177 ± 45 *(115 %)*	19	27 ± 3 *(96 %)*

Table 2. Cortical and endodermal cell area in cross sections of root tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Measurements of transversal cell length and width were performed on micrographs of cross sections of resin-embedded roots (40X). Area was calculated by multiplying cell length (µm) by cell width (µm).

	Nº cross sections analysed	**Cortical cell area (µm²)**	**Nº cross sections analysed**	**Endodermal cell area (µm²)**
Col (0) (MS)	84	507 ± 7 *(100 %)*	82	169 ± 3 *(100 %)*
PAC (0.5 µM)	105	737 ± 17 *(145 %)*	82	215 ± 7 (*127* %)
GA_4 (1 µM)	98	375 ± 13 *(74 %)*	81	157 ± 5 *(93 %)*
Ler	8	509 ± 9 *(100 %)*	24	172 ± 4 *(100 %)*
gai-1	100	464 ± 5 *(91 %)*	89	186 ± 4 *(108 %)*
QD	16	264 ± 6 *(52 %)*	23	138 ± 4 *(80 %)*
5X	7	270 ± 2 *(53 %)*	51	143 ± 3 *(83 %)*

Table 3. Average width of the root MZ in root tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Measurements of root width were performed on micrographs of whole root tips (10X, longitudinal view) or cross sections of resin-embedded roots (40X).

		Root width (µm)		
	Nº roots analysed	**Whole root tips**	**Nº cross sections analysed**	**Root cross sections**
Col (0) (MS)	12	115 ± 4 *(100 %)*	84	144 ± 10 *(100 %)*
PAC (0.5 µM)	19	161 ± 12 (*140* %)	107	164 ± 14 (*114* %)
GA_4 (1 µM)	19	99 ± 12 (*86* %)	96	128 ± 18 *(89 %)*
L*er*	10	122 ± 5 *(100 %)*	8	145 ± 4 *(100 %)*
gai-1	18	125 ± 18 *(102 %)*	100	148 ± 13 (*102* %)
QD	16	107 ± 18 (*88* %)	41	129 ± 7 (*89* %)
5X	5	111 ± 0 (*91* %)	50	132 ± 8 *(91 %)*

Table 4. Estimated tissue depth in roots tips of 5-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. Measurements of tissue diameter (µm) were performed on micrographs of cross sections of resin-embedded roots (MZ to early EZ; 40X). Estimated epidermal depth = [Root diameter (data from table 3) – (Cortex-Endodermis-Pericycle-Vessels diameter)]/2. Estimated cortical depth = [(Cortex-Endodermis-Pericycle-Vessels diameter) – (Endodermis-Pericycle-Vessels diameter)]/2. Estimated endodermal depth = [(Endodermis-Pericycle-Vessels diameter) – (Pericycle-Vessels diameter)]/2. Estimated pericycle depth = [(Pericycle-Vessels diameter) – (Vessels diameter)]/2. Endo: Endodermis; Peri: Pericycle. Number of cross sections analysed: Control (27-29), PAC (46-49), GA_4 (74-78), L*er* (21-22), *gai-1* (36), *QD* (34-36), *5X* (47-48).

	Col (0) (MS)	**PAC (0.5 µM)**	**GA_4 (1 µM)**	**L*er***	***gai-1***	***QD***	***5X***
Cortex-Endo-Peri-Vessels diameter	95 ± 6 *(100%)*	110 ± 11 *(116 %)*	83 ± 12 *(87 %)*	90 ± 7 *(100 %)*	91 ± 5 *(101 %)*	81 ± 5 *(90 %)*	82 ± 4 *(91 %)*
Endo-Peri-Vessels diameter	59 ± 6 *(100 %)*	70 ± 7 *(119 %)*	58 ± 7 *(98 %)*	60 ± 4 *(100 %)*	59 ± 3 *(98 %)*	59 ± 6 *(98 %)*	55 ± 2 *(92 %)*
Peri-Vessels diameter	45 ± 5 *(100 %)*	54 ± 5 *(120 %)*	45 ± 6 *(100 %)*	45 ± 3 *(100 %)*	44 ± 2 *(98 %)*	45 ± 5 *(100 %)*	40 ± 2 *(89 %)*
Vessels diameter	35 ± 3 *(100 %)*	40 ± 3 *(114 %)*	32 ± 5 *(91 %)*	33 ± 3 *(100 %)*	34 ± 2 *(103 %)*	33 ± 4 *(100 %)*	30 ± 2 *(91 %)*
Estimated Epidermal depth	25 *(100 %)*	27 *(108 %)*	22 *(88 %)*	27 *(100 %)*	28 *(104%)*	24 *(89 %)*	25 *(93 %)*
Estimated Cortex depth	18 *(100 %)*	20 *(111 %)*	13 *(72 %)*	15 *(100 %)*	16 *(107 %)*	11 *(73 %)*	13 *(87 %)*
Estimated Endodermis depth	7 *(100 %)*	8 *(114 %)*	6 *(86 %)*	8 *(100 %)*	7 *(88 %)*	7 *(88 %)*	7 *(88 %)*
Estimated Pericycle depth	5 *(100 %)*	7 *(140 %)*	6 *(120 %)*	6 *(100 %)*	5 *(83 %)*	6 *(100 %)*	5 *(83 %)*

Table 5. Radial cell organization in roots of 5 or 7-day-old *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLA. Analyses performed on micrographs of cross sections of resin embedded-roots (40X).

	Nº root cross sections analysed	**Epidermis**	**Cortex**	**Endodermis**	**Pericycle**
Control (5d)	75	20–25	8	8–10	13–15
PAC (5d)	56	25–34	8–9	9–13	14–18
GA_4 (5d)	56	17–25	8–9	8–10	12–16
Control (7d)	14	24–27	8	8–10	14–15
PAC (7d)	18	26–33	8–10	11–13	14–18
GA_4 (7d)	45	23–25	8–9	8–10	14–16
L*er* (5d)	13	18–24	8	8	13–14
***gai-1* (5d)**	57	19–25	8	8–9	12–14
***QD* (5d)**	24	22–24	8–11	8–11	14–18
***5X* (5d)**	54	18–21	8–9	7–8	12–16

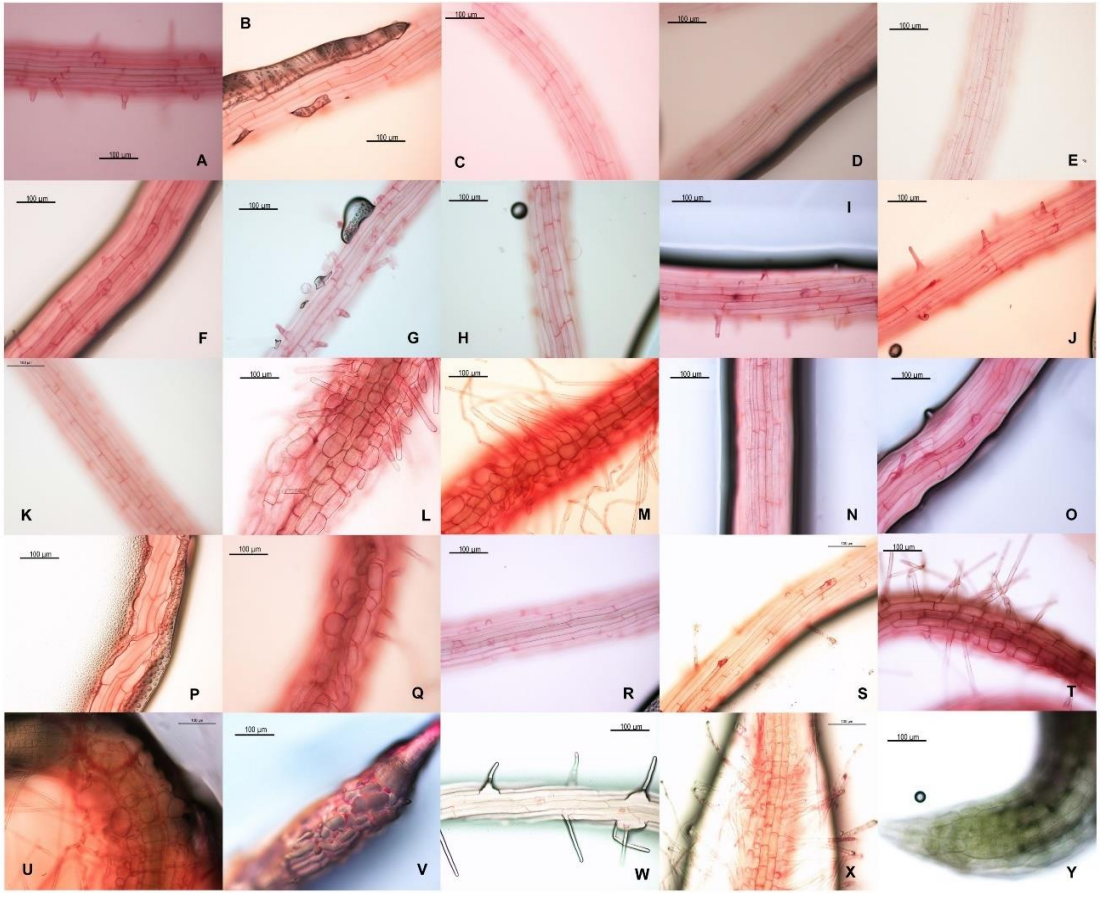

Figure 1

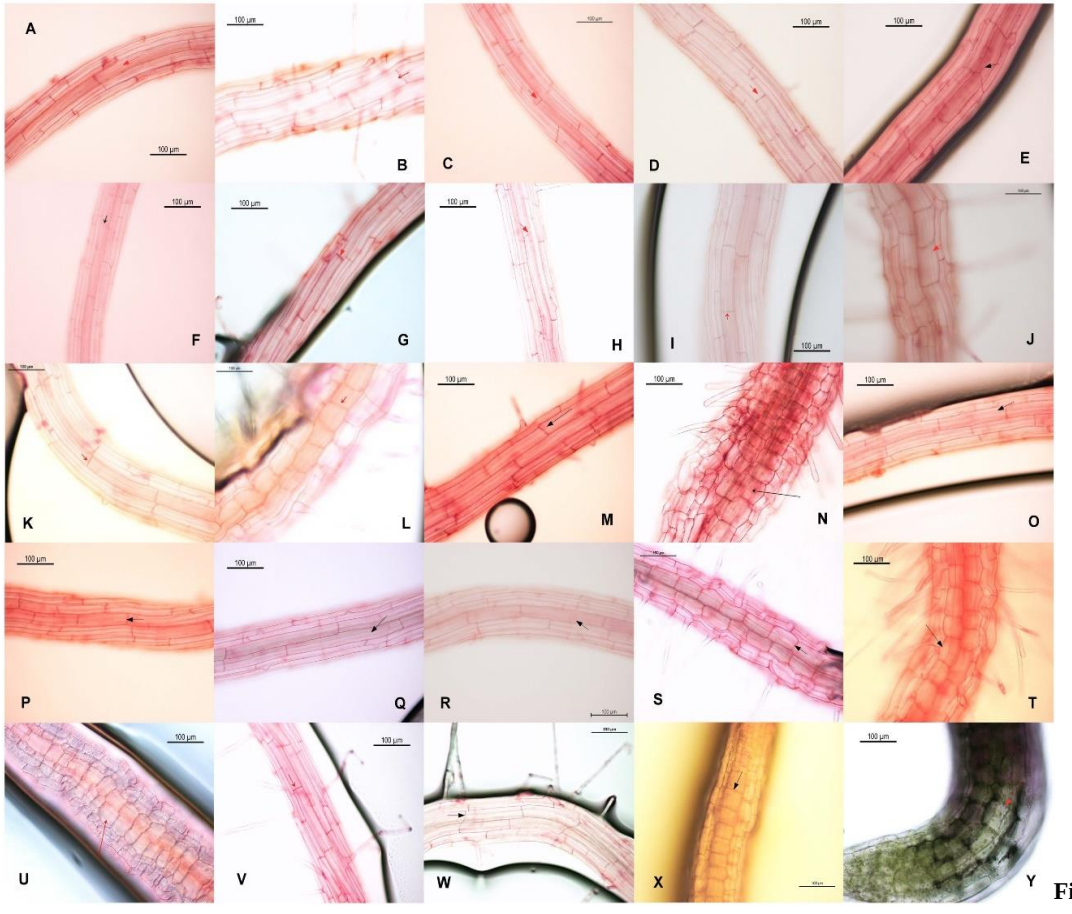

Figure 2

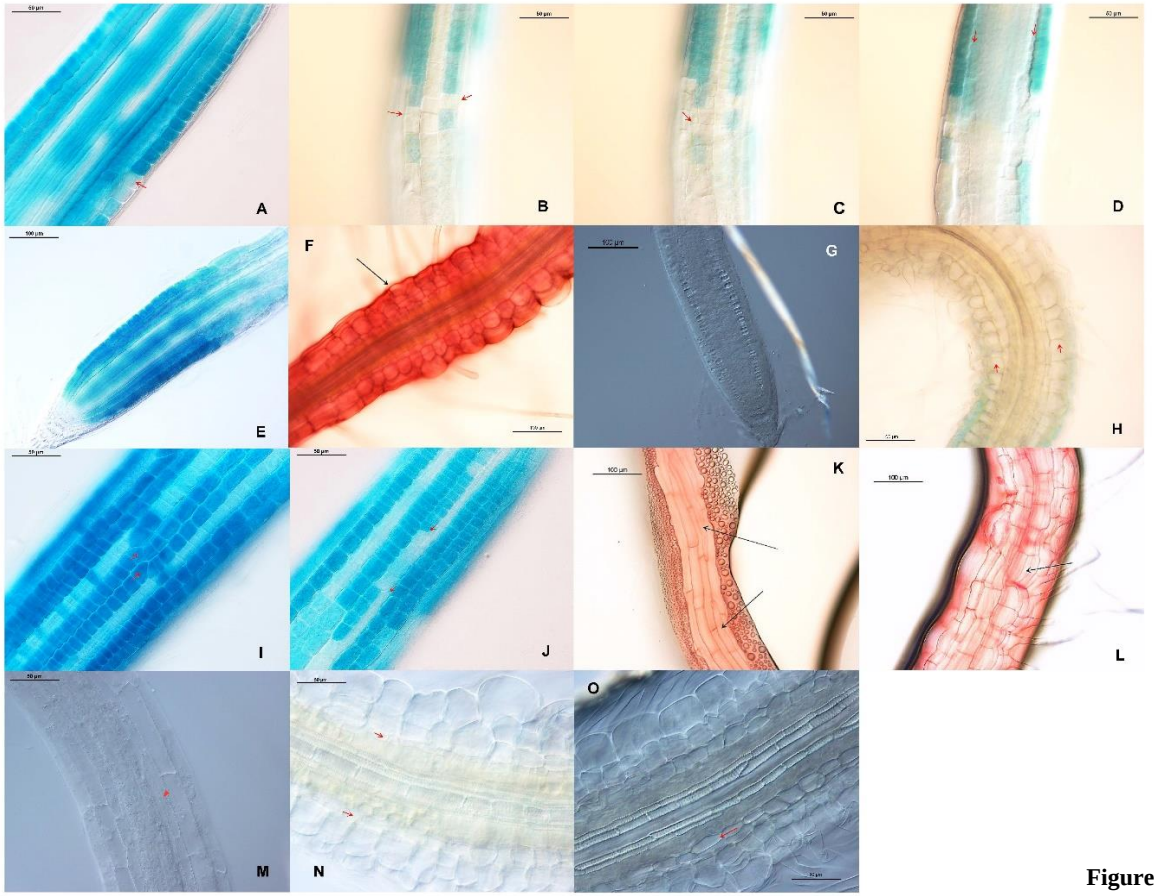

Figure 3

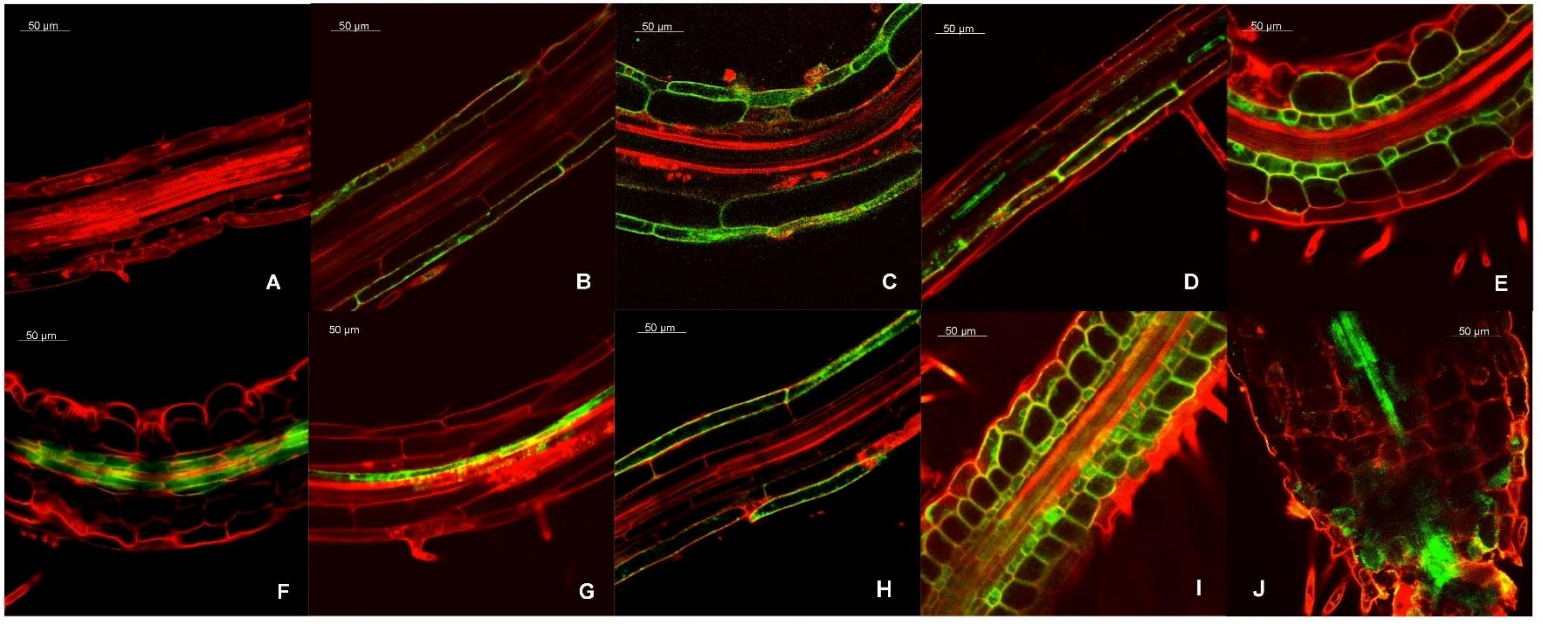
50 µm
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Figure 4

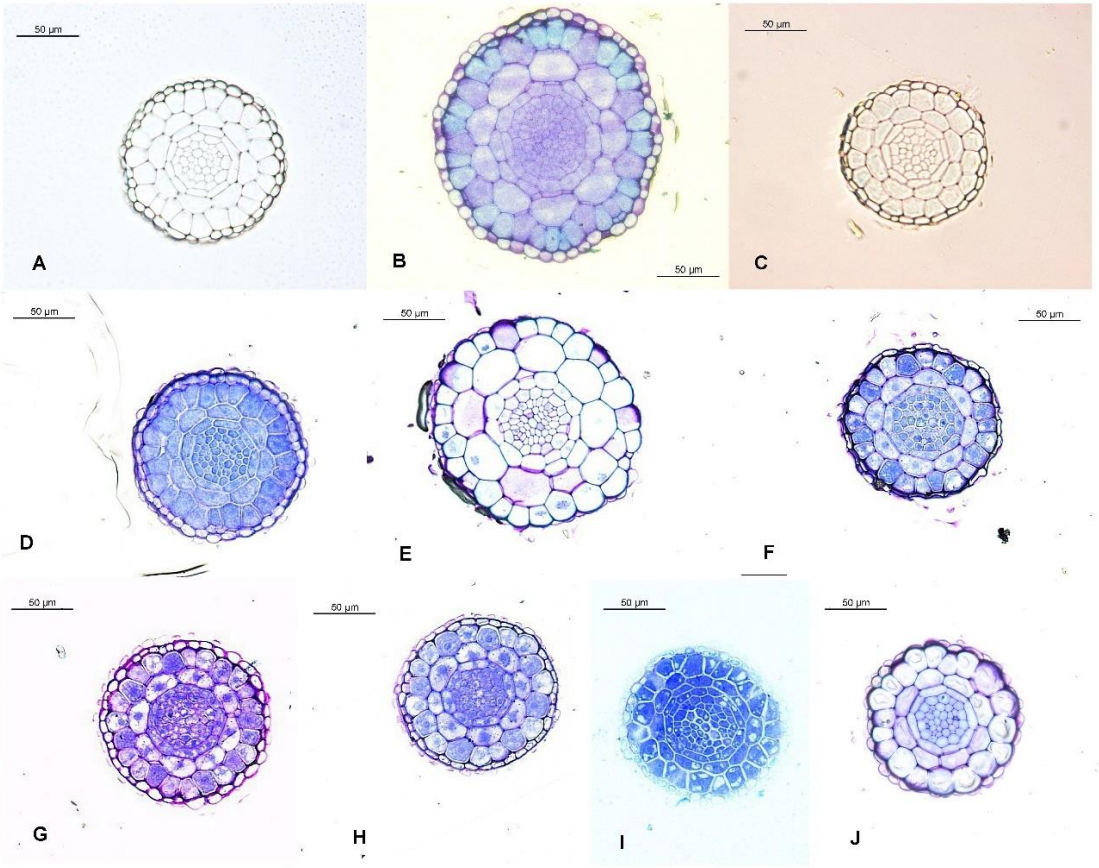

Figure 5

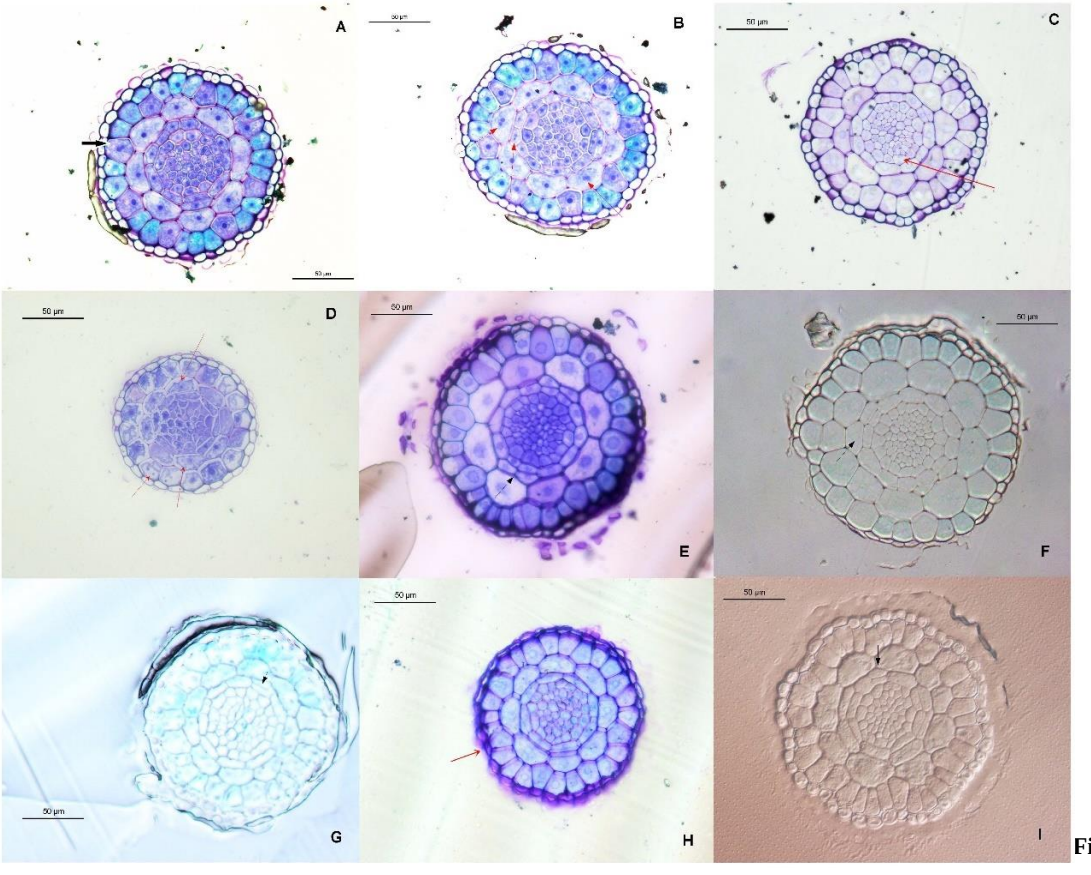

Figure 6


500 µm
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I

Figure 7

Figure legends

Figure 1. Excessive levels of GAs/DELLAs altered the size of root epidermal cells in root tips of 5-day-old *A. thaliana* seedlings. **A)** Col(0) (MS); **B)** Col(0) (0.5 µM PAC); **C)** Col(0) (30 µM GA3); **D)** Col (0) (1 µM GA4); **E)** L*er*; **F)** *gai-1*; **G)** *QD*; **H)** *5X*; **I)** GID1b-*ox* (MS); **J)** *HSp::gai-1* (22 ºC, 4 h); **K)** *HSp::gai-1* (immediately after heat shock (37 ºC, 4 h); **L)** *HSp::gai-1* (24 h after heat-shock (37 ºC, 4 h); **M)** *HSp::gai-1* (48 h after heat-shock (37 ºC, 4 h); **N)** *pGAI::gai-1:GR* (MS); **O)** *pGAI::gai-1:GR* (10 µM DEXA); **P)** *SCR::gai-1:GR* (MS, leaky line); **Q)** *SCR::gai-1:GR* (10 µM DEXA); **R)** *UAS::gai-1* x C24; **S)** *UAS::gai-1* x J0951; **T)** *UAS::gai-1* x J2812; **U)** *UAS::gai-1* x J0571; **V)** *UAS::gai-1* x Q2500; **W)** *UAS::gai-1* x J0121; **X)** *UAS::gai-1* x J0631; **Y)** *UAS::gai-1* x J3281. Magnification: 20X. The scale bar represents 100 µm. Propidium iodide staining.

Figure 2. Excessive levels of GAs/DELLAs altered the size of root cortical cells in root tips of 5-day-old *A. thaliana* seedlings. **A)** Col(0) (MS); **B)** Col(0) (0.5 µM PAC); **C)** Col(0) (1 µM GA4); **D)** L*er*; **E)** *gai-1*; **F)** *QD*; **G)** *5X*; **H)** GID*1b-ox;* **I)** *pGAI::gai-1:GR* (3d in MS); **J)** *pGAI::gai-1:GR* (3d in 10 µM DEXA); **K)** *SCR::gai-1:GR* (3d in MS); **L)** *SCR::gai-1:GR* (3d in 10 µM DEXA); **M)** *HSp::gai-1* x *GL2pro::GUS* (24h after 22 ºC for 4 h); **N)** *HSp::gai-1* x *GL2pro::GUS* (24h after heat-shock (37 ºC for 4 h)); **O)** *GL2pro::GUS* (heat-shock control) (24 h after 22 ºC for 4 h); **P)** *GL2pro::GUS* (heat-shock control) (24h after heat shock (37 ºC for 4 h)); **Q)** *UAS::gai-1* x C24; **R)** *ML1::gai-1*; **S)** *UAS::gai-1* x J2812; **T)** *UAS::gai-1* x M0018; **U)** *UAS::gai-1* x Q2500; **V)** *UAS::gai-1* x N9142; **W)** *UAS::gai-1* x J0121; **X)** *UAS::gai-1* x 0631; **Y)** *UAS::gai-1* x J3281. Magnification: 20X. The scale bar represents 100 µm. Propidium iodide staining.

Figure 3. Excessive levels of GAs/DELLAs induced cell size changes and T-clones at the epidermis, cortex and endodermis of root tips of 5-day-old *A. thaliana* seedlings. **A)** *GL2pro::GUS* (30 µM GA3) (all layers): expansion of an epidermal cell and narrowing of a cortical cell coincide with a change in epidermal cell fate, 40X; **B)** *35S::CPC* x *GL2pro::GUS* (epidermis): A change in epidermal cell size coincides with a change in epidermal cell fate, 40X; **C)** *35S::CPC* x *GL2pro::GUS* (cortex): A change in the width of a cortical cell coincides with a change in epidermal cell fate, 40X; **D)** *35S::CPC* x *GL2pro::GUS* (all layers): epidermis and cortex vary in cell size, 40X; **E)** *HSp::gai-1* x *GL2pro::GUS* (48 h after 22 ºC for 4 h) (all layers), 20X; **F)** *HSp::gai-1* x *GL2pro::GUS* (48h after heat-shock (37 ºC, 4 h) (all layers): swelling of the root epidermal, cortical, endodermal and pericyclic cells, 20X; **G)** *SCR::gai- 1:GR* (MS) (all layers), 20X; **H)** *SCR::gai-1:GR* x *GL2pro::GUS* (24 h in 10 µM DEXA) (all layers): swelling of the root cortex, endodermis and pericycle, 20X; **I)** Epidermal T-clones in PAC (0.5 µM), 40X; **J)** Epidermal T-clones in GA3 (30 µM), 40X; **K)** *SCR::gai-1:GR* (0.2 µM DEXA): epidermal T-clones, 20X; **L)** *SCR::gai-1:GR* (1.2 µM DEXA): periclinal cell division at the root cortex, 20X; **M)** *SCR::gai-1:GR* (MS) (all layers): arrow on endodermis, 40X; **N)** *SCR::gai-1:GR* (10 µM DEXA) (all layers): periclinal cell divisions at the endodermis, 40X; **O)** *SCR::gai-1:GR* (10 µM DEXA) (all layers): periclinal cell divisions at the endodermis, 40X. The scale bar represents 100 µm (20X) or 50 µm (40X). Propidium iodide or GUS staining.

Figure 4. Over-expression of gai-1 in different tissues of the root modified the cell size in root tips of 5-day-old *A. thaliana seedlings.* **A)** *UAS::gai-1* x C24 (Background); **B)** *UAS::gai-1* x J0951 (epidermis of the MZ); **C)** *UAS::gai-1* x J2812 (epidermis and cortex of the MZ); **D)** *UAS::gai-1* x N9142 (cortex of EZ); **E)** *UAS::gai-1* x J0571 (cortex and endodermis of the MZ); **F)** *UAS::gai-1* x Q2500 (endodermis/pericycle of MZ); **G)** *UAS::gai-1* x J0121 (pericycle of EZ); **H)** *UAS::gai-1* x Q2393 (all tissues but the endodermis); **I)** *UAS::gai-1* x J0631 (elongating tissues); **J)** *UAS::gai-1* x J3281 (vessels). Magnification: 40X. The scale bar represents 50 µm. Propidium iodide staining.

Figure 5. Radial cell organization in root tips of 5 or 7 day-old *A. thaliana seedlings* grown under (or harbouring) excessive levels of GAs/DELLAs. **A)** Col(0) (MS, 5d); **B)** Col(0) (0.5 µM PAC, 5d); **C)** Col(0) (1 µM GA4, 5d); **D)** Col(0) (MS, 7d); **E)** Col(0) (0.5 µM PAC, 7d); **F)** Col(0) (1 µM GA4, 7d); **G)** L*er* (5d); **H)** *gai*-1 (5d); **I)** *QD* (5d); **J)** *5X* (5d). Magnification: 40X. The scale bar represents 50 µm. Toluidine blue staining.

Figure 6. Excessive levels of GAs/DELLAs induced multinucleated cells, a middle cortex (MC) and extra cortical cells in root tips of *A. thaliana seedlings.* **A)** PAC (0.5 µM, 5d): Epidermal multinucleated cell; **B)** PAC (0.5 µM, 5d): Cortical and endodermal multinucleated cells; **C)** PAC (0.5 µM, 5d): Pericyclic multinucleated cell; **D)** GA4 (1 µM, 5d): Epidermal and cortical multinucleated cells; **E)** PAC (0.5 µM, 5d): MC; **F)** PAC (0.5 µM, 7d): MC (arrow) and 10 cortical cells; **G)** GA4 (1 µM, 5d): MC (arrow) and 9 cortical cells; **H)** *scm* x *GL2pro::GUS* (MS, 5d); **I)** *scm* x *GL2pro::GUS* (0.5 µM PAC, 5d): MC (arrow) and 9 cortical cells. Magnification: 40X. The scale bar represents 50 µm. Toluidine blue staining.

Figure 7. Over-expression of gai-1 induced an early outburst of lateral roots in root tips of *A. thaliana* seedlings. **A)** *SCR::gai-1:GR* (MS, 5d) (Leaky line), 4X; **B)** *SCR::gai-1:GR* (10 µM DEXA, 5d), 4X; **C)** *UAS::gai-1* x C24 (control, 5d), 4X; **D)** *UAS::gai-1* x J2812 (5d), 20X; **E)** *UAS::gai-1* x M0018 (5d), 4X; **F)** *UAS::gai-1* x J0571 (5d), 4X; **G)** *UAS::gai-1* x Q2500 (5d), 4X; **H)** *UAS::gai-1* x N9142 (5d), 20X; **I)** *UAS::gai-1* x J3281 (8d) (aborted primary root), 4X. The scale bar represents 100 µm (20X) or 500 µm (4X).

Discussion

The GAs/DELLAs might regulate the size and number of root tip cells in seedlings of *A. thaliana*.

Root cell size: Connections to the root hair patterning and abundance

Apart from altering the patterning, morphology and abundance of root hairs [MCCARTHY-SUÁREZ, unpublished manuscript], results of the present study suggest that excessive levels of GAs/DELLAs also modified the size of root tip cells in seedlings of *A. thaliana*. While excessive levels of DELLAs frequently shortened and widened the epidermal, cortical, endodermal and pericyclic cells of the root, what resulted in wider root tips, excessive levels of GAs, with the exception of the epidermal cells, often narrowed them. Thus, because root hair cells are shorter than the root non-hair cells [SALAZAR-HENAO & al. 2016], then, the inhibition of epidermal cell elongation that occurred when gai-1 was over-expressed in tissues placed underneath the MZ epidermis (J2812 >> *gai-1*, J0571 >> *gai-1*, M0018 >> *gai-1*, Q2500 >> *gai-1* or Q2393 >> *gai-1* lines) or in all tissues of the EZ (J0631 >> *gai-1* line) might have contributed, in part, to the appearance of ectopic root hairs, and, therefore, to the higher density of root hairs observed near the root tip under excessive levels of DELLAs [MCCARTHY-SUÁREZ, unpublished manuscript]. In fact, *Arabidopsis* increases root hair density by decreasing the length of root epidermal cells, as shown under P deficiency [JIANG & al. 2007; PÉRET & al. 2011; SALAZAR-HENAO & al. 2016; JANES & al. 2018]. Alternatively, given that GAs promote the elongation of root epidermal cells, accumulate at the endodermis of the root EZ, and affect the expansion of root EZ cells by destabilizing DELLAs and inducing expansin genes [UBEDA-TOMÁS & al. 2008, 2009; GOU & al. 2010; BAHIN & al. 2011; SHANI & al. 2013], then, the extra elongation of root epidermal cells which is known to occur under high levels of GAs [BAND & al. 2012] might have contributed, in turn, to the lower density of root hairs that was observed at the root tip under this treatment [MCCARTHY-

SUÁREZ, unpublished manuscript]. In fact, in WT, cell expansion at the root EZ is strictly polar and is not accompanied by an increase in the root diameter [BAO & al. 2001].

Interestingly, epidermal patterning genes instruct epidermal cell size [LÖFKE & al. 2013]. In turn, variations in the expression of HDA 19 (histone deacetylase 19), which controls epidermal cell elongation, affect root cell elongation and, thus, root hair density [CHEN & al. 2015; SALAZAR-HENAO & al. 2016]. In this study, however, neither the patterning or abundance of root hairs [MCCARTHY-SUÁREZ, unpublished manuscript] nor the epidermal cell length seemed to suffer any alterations when *gai-1* was over-expressed at the root epidermis (Figure 4), what suggests that the changes of cell size induced by excessive levels of GAs/DELLAs in roots of *A. thaliana* seedlings might have been orchestrated from the sub-epidermal tissues of the root. Furthermore, WILD & al. (2016) showed that expressing *gai-1* at the root epidermis did not affect the root length.

Results of this study also seem to point at a link between cell size and cell fate, because expressing *gai-1* at the elongating tissues of the root (J0631 >> *gai-1*) caused the shortening and widening of the majority of the root cells (Figures 1, 2 and 4) along with a hairy phenotype similar to that of the *wer* mutant [MCCARTHY-SUÁREZ, unpublished manuscript]. In fact, the DELLAs inhibit the elongation of root EZ cells and primary root [ALONSO-RAMÍREZ & al. 2009; UBEDA-TOMÁS & al. 2009; LEE & al. 2012] and down-regulate PIF4, a phytochrome-interacting factor which induces cell elongation genes [ACHARD & GENSCHIK, 2009]. Deficiencies in B, P or Fe, which increase the levels of DELLAs at the root MZ and induce ectopic root hairs, also reduce the primary root length [MARTÍN-REJANO & al. 2011; PÉRET & al. 2011; WILD & al. 2016]. Furthermore, it has been suggested that production of ectopic root hairs in ectopic root hair 2 (*erh2)* occurs at late stages of root development, correlated with cell expansion. However, it has also been suggested the independence of root hair initiation from cell expansion, as *erh3* acts as soon as cell fate specification [SCHNEIDER & al. 1997]. Particularly, ERH3 is required for the stable fixation of positional signals at the cell wall (CW) for cell fate specification. Moreover, ERH3 codifies a MT-severing p60 katanin protein and has a role in CW biosynthesis [WEBB & al. 2002]. On the other hand, given that the expression of cell identity markers is altered in *erh3*, it has been suggested that MT are directly active in the specification of root cell identity, and that MT disruption in *erh3* results in the development of defective identities [WEBB & al. 2002]. In fact, in several animal systems, MT are involved in the specification of cell identity and polarity [WEBB & al. 2002].

Shortening and radial expansion of root cortical and endodermal cells has also been reported in the *cobra, pom-1* (both cellulose deficient), *shoebox* (GA biosynthesis-impaired), *dgl1* (GA-insensitive), TUA6/AS (α-tubulin-deficient), *erh, sabre, PLD* (phospholipase D), *wer, scm* and *jkd* mutants, in plants treated with umbelliferone (a cellulose biosynthesis inhibitor), MT-breaking drugs or 1-butanol (an inhibitor of PLD), and in plants stressed by salinity, gamma irradiation or mineral deficiency (Fe, P) [JANKAY & MULLER, 1976; SCHIEFELBEIN & al. 1997; SCHNEIDER & al. 1997; BAO & al. 2001; MA & al. 2001; SCHERES & al. 2002; GARDINER & al. 2003; NAGATA & al. 2004; KOMORISONO & al. 2005; WELCH & al. 2007; DINNENY & al. 2008; PIETRA & al. 2015; JANES & al. 2018]. Cortical cell expansion as a result of excessive DELLAs has also been described by BENFEY & al. [1993]. Interestingly, PAC induces the expression of expansin genes at the root cortex (At4g21280 (+2,002); Arex data), being *EXPANSIN 7* a specific marker of root hair cells [OHASHI & al. 2003; GENDRE & al. 2019]. In addition, it has been suggested that the DELLA GAI might have a role in the expansion of endodermal cells in *Arabidopsis* primary roots, and that the expansion of endodermal cells determines the elongation of whole roots [UBEDA-

TOMÁS & al. 2009; ZHANG & al. 2014). On the other hand, the GAs control the size of the root apical meristem (RAM) in *Arabidopsis* by affecting cortical cell expansion [NELISSEN & al. 2012; FONOUNI-FARDE & al. 2019]. In fact, for different accessions of *Arabidopsis*, there is a correlation between cortex cell length and the length of the root MZ [ZHANG & al. 2014]. Furthermore, when gai-1 is over-expressed at the root endodermis (the most important tissue for GA-dependent root growth), the cessation of anisotropic cell growth expands radially the cortical cells and causes the outward protrusion of epidermal cells [UBEDA-TOMÁS & al. 2008]. With this regard, it is known that the endodermis of the EZ regulates nutrient uptake [PÉRET & al. 2011; SHANI & al. 2013; CUI, 2015], whereas the cortex participates in the root response to P deficiency [SHIN & al. 2005].

Therefore, the shortening and widening of the root epidermal, cortical, endodermal and pericycle cells induced by excessive levels of DELLAs in seedlings of *A. thaliana* might have explained the radial expansion of the root tips observed. Interestingly, treatment with PAC also increased the root diameter in carrot [WANG & al. 2015]. In fact, in this study, root tips became thinner under excessive GAs (Table 3), as previously reported in carrot and *Eucalyptus grandis* [WANG & al. 2015; LIU & al. 2018]. A wider root diameter has also been described in the *arm*, *sabre*, *cobra*, *erh-1*, *pom-pom1* and α-tubulin under-expressing mutants, and in plants exposed to 1-butanol, umbelliferone, gamma irradiation or P deficiency [JANKAY & MÜLLER, 1976; SCHNEIDER & al. 1997; BAO & al. 2001; MA & al. 2001; GARDINER & al. 2003; NAGATA & al. 2004; HERMANS & al. 2010; PIETRA, 2014]. Moreover, the reduction of the actin cytoskeleton induces the radial expansion of plant cells, making them shorter and wider, as the interphase MT determine the direction of plant cell elongation [BALUŠKA & al. 2001; BAO & al. 2001]. This means that the capacity of cells to elongate longitudinally depends on the orientation of the cytoskeletal MT [DUGARDEYN & VAN DER STRAETEN, 2008]. Thus, a reduced expression of the α-tubulin gene in *A. thaliana* seedlings results in an abnormal expansion of the root tip (MZ and EZ), with its diameter increasing dramatically at 8 days after germination [BAO & al. 2001]. De-polymerization of MT by oryzalin or 1-butanol also causes the swelling of the root MZ and EZ in *A. thaliana* seedlings, whereas MT stabilization by Taxol expands the root EZ and DZ [BAO & al. 2001; GARDINER & al. 2003].

Therefore, a connection exists between aberrant orientation of MT and reduced cell elongation, as MT regulate the oriented deposition of cellulose microfibrils that determines the direction of cell elongation [BURK & YE, 2002]. More specifically, MT are essential for anisotropic cell expansion because they direct the insertion of cellulose synthase in the CW and guide the orientation of cellulose microfibrils to a perpendicular position with respect to the growth axis, thereby restricting radial cell expansion [JANKAY & MULLER, 1976; LIN & al. 2013]. Interestingly, mutations in P60 katanin protein, essential for anisotropic cell growth, cause an inappropriate feedback regulation of the *DGL1* gen for GAs biosynthesis [KOMORISONO & al. 2005].

With this respect, a link has been proposed between aberrant orientation of MT, radial cell growth and alteration of the root hair patterning [BALUŠKA & al. 2001; BAO & al. 2001]. Thus, a low expression of the α-tubulin gene (TUA6/AS transgenic lines), mutations that inhibit MT polymerization or drugs that brake the actin MT produce aberrant microtubular structures, expand radially the root tip cells, especially at the epidermis and cortex of the MZ and EZ, and induce ectopic root hairs in 5 day-old *A. thaliana* seedlings [BAO & al. 2001; COLLINGS & al. 2006]. On the other hand, the *erh1* and *erh3* mutants, with an altered root hair patterning, exhibit disorganized MT and radially-enlarged layers of root cortex and endodermis, what suggests a connection between radial cell expansion and root hair initiation [SCHNEIDER &

al. 1997; BOUQUIN & al. 2003; MÜLLER & SCHMIDT, 2004; PIETRA & al. 2015]. Moreover, *erh2* is allelic to *pom-1*, a mutant with abnormally-expanded layers of root epidermis and cortex [SCHNEIDER & al. 1997; PIETRA & al. 2015]. In fact, not only cell length, but also cell width differs between Trichoblasts and Atrichoblasts [LÖFKE & al. 2013]. Deficiencies in Fe or P also induce the swelling of root cortical cells, along with ectopic root hairs [PIETRA & al. 2015]. Another clue about the link among MT, cell expansion and cell fate is illustrated by the mutants *cobra* and *sabre*, both with an abnormal cell expansion at the root tip and ectopic root hairs [SCHIEFELBEIN & al. 1997]. The mutation of the SABRE protein, involved in MT organization, causes an abnormal cell expansion at the root cortex [BENFEY & al. 1993], whereas the mutation of the COBRA protein, which is associated to the longitudinal CW of the rapidly-growing root EZ, entails a cellulose deficiency and causes the swelling of the root epidermis and cortex [SCHERES & al. 2002]. Interestingly, the GAs influence CW growth in mesocotyl epidermal cells [PERAZZA & al. 1998]. Therefore, under the experimental conditions of the present study, excessive levels of DELLAs might have impaired the biosynthesis, organization and/or homoeostasis of MT in root tip cells of *A. thaliana* seedlings, and this, in turn, might have caused the inhibition of cell elongation and the altered patterning of *GL2* gene expression and of root hairs at the MZ and EZ of the root. In fact, the DELLAS destabilize the MT, giving rise to non-polar cell growth [LOCASCIO & al. 2013].

In this respect, it is known that the levels of ploidy exert an important control over cell size, and that cell size and morphology are, in turn, linked to DNA content [KONDOROSI & al. 2001]. Moreover, in many tissues, cell elongation is associated to the endo-reduplication of the DNA (replication without mitosis that occurs before cell elongation, resulting in a logarithmic accumulation of genome copies in each nucleus) [SANZ & al. 2012]. Thus, the earliest morphological signs in trichome initiation are the induction of endo-reduplication and the increase in the nuclear and cellular size [PERAZZA & al. 1998]. With this regard, it is known that GAs induce endo-reduplication in a dose-dependent manner and regulate cyclin gene expression. In fact, trichomes in the *spy5* mutant have two times more DNA than WT trichomes [PERAZZA & al. 1998; KONDOROSI & al. 2001]. Moreover, in GA-deficient transgenic plants, the observed root swelling, due to MT disorganization, is associated to the induction and accumulation of cyclin CYC3;1 and CYCB1;1 proteins, because the DELLAs are involved in cell cycle progression [UBEDA-TOMÁS & al. 2009; GOU & al. 2010; SÁNCHEZ-CALDERÓN & al. 2013]. In addition, the halting degree of the cell cycle is related to the GA endogenous level [LI & al. 2015b]. Mutations in the α-tubulin gene and drugs that inhibit MT polymerization also induce multi-nucleated cells [BAO & al. 2001]. Interestingly, the control of endo-reduplication in trichomes participates in the regulation of epidermal patterning [PIETRA & al. 2015], whereas the *RHL* genes, related to endo-reduplication, affect root epidermal cell fate independently from the *GL2* gene network [GUO & al. 2009].

Given that stress reduces root cell length and, thus, root length [DINNENY & al. 2008], then, the morphological alterations that were observed in the root cells under excessive levels of DELLAs in 5-day-old *A. thaliana* seedlings might be in tune with the known role of these proteins as mediators of the plant Stress-Induced Morphogenic Responses (SIMR), which are characterized by changes in MT metabolism, CW flexibility and cell cycle progression [POTTERS & al. 2007]. Moreover, it is known that stress inhibits growth by reducing GA levels and promoting the stabilization and accumulation of DELLAs [ACHARD & GENSCHIK, 2009; ALONSO-RAMÍREZ & al. 2009], and that the DELLAs mediate the SIMR associated to P deficiency [JIANG & al. 2007]. From this, it might be hypothesized that an alteration of MT homoeostasis might have been implicated in the cell size changes that were observed in roots of

A. thaliana seedlings grown under (or harbouring) excessive levels of GAs/DELLAs. While excessive levels of DELLAs might have disorganized the MT cytoskeleton, excessive levels of GAs might have stabilized it, giving rise to the changes of cell size and cell fate observed at the MZ and EZ of the root.

Results also suggest that cell fate decisions at the root epidermis might be synchronized with the cell size changes at the inner tissues of the root, such as the cortex. Thus, another reason for the appearance of extra root hairs in *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs/DELLAs might have been the reduction of the ratios of cortical/epidermal cell length (e.g. under excessive DELLAs, which decrease the cortex cell length) and of cortical/epidermal cell width (e.g. under excessive GAs, which decrease the cortex cell width), as they might give rise to epidermal cells laying over two cortical intersections instead of one, and, hence, to two-haired epidermal cells. This would imply that not only the length and width of epidermal cells, but also the length and width of cortical cells might contribute to the number of hairs produced by the root, although additional studies are needed to confirm this hypothesis.

On the other hand, results of this study also point at the cortex, endodermis and pericycle as root tissues from which the GAs/DELLAs might influence the root hair patterning, because transgenic lines over-expressing gai-1 at these root tissues produced ectopic root hairs and non-hairs [MCCARTHY-SUÁREZ, unpublished manuscript]. In fact, it has been shown that blocking GAs signalling at the root endodermis induces morphological defects in the root epidermal cells [LÖFKE & al. 2013; PIETRA & al. 2015; JANES & al. 2018].

Root cell number: Connections to the root hair patterning and abundance

Excessive levels of GAs/DELLAs also altered the radial cell organization in root tips of seedlings of *A. thaliana*. While excessive levels of DELLAs frequently induced additional cells at the epidermis, cortex, endodermis and pericycle of the root, excessive levels of GAs sometimes induced extra cells at the root cortex and pericycle. With this respect, whether the cell proliferation at the root cortex-endodermis-pericycle under excessive levels of DELLAs was another reason for the observed disorganization in the root hair patterning [MCCARTHY-SUÁREZ, unpublished manuscript], it might be worth confirming in future experiments by using inhibitors and/or mutants of cell division.

Regarding the epidermis, interestingly, the predicted number of cells at the Atrichoblast position per root radial section increased under excessive DELLAs (PAC, *gai*-1), but decreased under excessive GAs (*5X* mutant) [MCCARTHY-SUÁREZ, unpublished manuscript]. Nevertheless, as the predicted number of cells at the Trichoblast position did not change, and the percentage of ectopic root hairs was higher under excessive DELLAs as compared to excessive GAs [MCCARTHY-SUÁREZ, unpublished manuscript], then, the higher abundance of root hairs seen under excessive DELLAs in comparison to excessive GAs was probably due to the induction of ectopic root hairs – and, thus, to the higher number of cells at the Atrichoblast position – and not because of the appearance of new Trichoblast positions, given that the number of cells at the Trichoblast position remained unchanged [MCCARTHY-SUÁREZ, unpublished manuscript].

In fact, *Arabidopsis* increases root hair density in the radial dimension by increasing the number of epidermal cells that differentiate into root hair cells [JANES & al. 2018]. An increased number of epidermal cells in the radial domain of the root has also been described under P deficiency and in *tip1* mutants [MA & al. 2001; GRIERSON & SCHIEFELBEIN, 2002; MÜLLER & SCHMIDT, 2004]. However, under P deficiency, the extra epidermal cells at the

Trichoblast position (up to 12) do not increase the abundance of root hairs in the radial axis as ectopic non-hairs also appear [MA & al. 2001; JANES & al. 2018]. Interestingly, a distorted radial patterning of root cells has also been described in mutants of WRKY75, a negative regulator of root hair formation [RISHMAWI & al. 2014].

Radial proliferation of the root cortex cells has also been reported under stress (e.g. P deficiency) as well as in *tip1*, *erh3* and *jkd* mutants, all with an altered root hair patterning [MA & al. 2001; MÜLLER & SCHMIDT, 2004; HASSAN & al. 2010; CUI, 2015; JANES & al. 2018]. Periclinal cell divisions (extra layers) of the root cortex have equally been described in mutants of JKD, which acts from the root cortex to specify the patterning of epidermal cell types [WELCH & al. 2007; LYER-PASCUZZI & BENFEY, 2008; HASSAN & al. 2010]. Interestingly, the GAs restrict the production of extra layers of cortex cells in *Medicago truncatula* roots, thereby generating thinner roots [FONOUNI-FARDE & al. 2019]. In contrast, PAC treatments, or mutations in components of GA signalling, increase the number of layers of root cortex cells, that is, they induce a premature middle cortex (MC) [PAQUETTE & BENFEY, 2005; CUI & BENFEY, 2009]. Moreover, the GAs suppress the MC formation that is proper of the root responses to stress, whereas the DELLAs promote it [CUI & BENFEY, 2009; FONOUNI-FARDE & al. 2019]. Thus, the formation of a MC, due to random and periclinal cell divisions at the root endodermis, and that later on will acquire identity of root cortex, has been described under P deficiency [CUI & BENFEY, 2009; JANES & al. 2018]. Although the production of a MC has also been reported in roots of 3-day-old WT *A. thaliana* seedlings, the presence of a premature MC in the *spy* mutant, with high levels of GAs, suggests that imbalances in GAs/DELLAs homoeostasis, which can be triggered by stress, might bring about the formation of a MC [CUI & BENFEY, 2009; CUI, 2015]. Maybe, this was the reason, in this study, for the presence of a MC in roots of *A. thaliana* seedlings grown under (or harbouring) excessive levels of GAs (1 µM GA_4 and *QD*) (Figures 5 and 6).

Because the PAC-inducible phenotype of MC is also present in the *scr* (scarecrow) and HDA mutants, as well as in trichostatin A (TSA)-treated plants, all producing ectopic root hairs [CUI & BENFEY, 2009], then, a possible link between MC formation (or ectopic cell proliferation at the cortex/endodermis) and alteration of the root hair patterning might be established. Whether this contributed to the disorganisation of the root hair patterning observed under excessive levels of DELLAs, where a cell proliferation was equally observed at the cortex/endodermis/pericycle of the root, it is not known, but might be worth studying in future experiments by using cell division inhibitors and/or mutants. In fact, the alteration of the root hair patterning in the *SCR::gai-1:GR* mutant after growth in DEXA [MCCARTHY-SUÁREZ, unpublished manuscript] was accompanied by random and periclinal cell divisions at the root endodermis (Figure 3). Moreover, because the root MZ and EZ constitute cell fate-decision zones in *Arabidopsis*, then, any changes in cell division at tissues placed underneath the epidermis of the MZ/EZ might bring about changes in epidermal cell fate. In fact, the proliferation of cortex cells is known to influence the root epidermal patterning [LÖFKE et al. 2013; PIETRA & al. 2015; JANES & al. 2018]. Interestingly, histone deacetylation, which affects the root hair patterning, has a role in the proliferation of root cortex cells [XU & al. 2005; LI & al. 2015a]. Increases in the number of root endodermal cells have also been reported in *erh* mutants, during P deficiency, and in *rhizobium*-infected plants [MÜLLER & SCHMIDT, 2004; MA & al. 2001; JANES & al. 2018]. The schizoriza (*scz*) mutant, in turn, has defects in the root radial patterning, with extra periclinal cell divisions that result in multiple layers of ground tissue (cortex and endodermis) [MYLONA & al. 2002]. Thus, the results of this study suggest that the alterations in the root hair patterning of *A. thaliana* seedlings grown under

excessive levels of GAs/DELLAs might also have been related to changes in the number of the cortical/endodermal/pericycle cells of the root.

Other possible cause, in this study, for the appearance of ectopic root hairs under excessive levels of GAs/DELLAs [MCCARTHY-SUÁREZ, unpublished manuscript] might have been the anticlinal, diagonal or asymmetric cell divisions (T-clones) frequently observed at the root epidermis, as they gave rise to changes in the *GL2pro::GUS* expression patterning and the size of daughter cells (Figure 3). These T-clones, in turn, might have been linked to alterations in the MT cytoskeleton, as MT are required for the correct positioning of cell division planes [SCHERES & BENFEY, 1999; BAO & al. 2001; RODRIGUEZ-SERRANO & al. 2014]. Moreover, a reduced expression of the α-tubulin gene impairs cell division and results in defects of tissue organization at the root tip [BAO & al. 2001]. Also, the regulation of asymmetric cell divisions in plants is necessary for the generation of cell diversity and patterns [PERNAS & al. 2010]. In fact, root hair cells are shorter than root non-hair cells, so that when an asymmetric cell division takes place at the epidermis of the MZ, the larger cell becomes the root non-hair cell [SALAZAR-HENAO & al. 2016]. With this respect, it is known that the GAs induce cell proliferation at the root MZ and promote the division of epidermal cells [UBEDA-TOMÁS & al. 2009; LEE & al. 2012]. Furthermore, the DELLAs inhibit root cell division in the longitudinal dimension when mediating the SIMR associated to P deficiency [JIANG & al. 2007; PÉRET & al. 2011]. Interestingly, in a dwarf GA-deficient mutant, the MT exhibit an oblique orientation [BOUQUIN & al. 2003]. Also, in the *erh3* mutants, which act in the same route as *cpc* and *rhd6* (root hair defective 6), but independently from WER, the CW are dis-aligned, diagonally orientated, and malformed, that is, the positioning of the cell plates and the CW is abnormal, what indicates that ERH3 participates in orienting the cell plates during cytokinesis [WEBB & al. 2002]. The cortex-associated SABRE protein is also involved in the orientation of cell division planes [PIETRA, 2014; PIETRA & al. 2013, 2015]. In addition, GL2 is involved in the production of T-clones, whereas WER regulates cell proliferation, what suggests that genes that regulate the cell specification also regulate the cell division planes [LEE & SCHIEFELBEIN, 1999; SCHERES & al. 2002].

Thus, these results suggest that changes in cell number induced by excessive levels of GAs/DELLAs at the epidermis, cortex, endodermis and pericycle of the root tip in *A. thaliana* seedlings might influence the root hair patterning. Moreover, changes in cell number at tissues placed underneath the root epidermis might bring changes of cell fate at the root epidermal cells.

In addition, results of this study suggest that excessive levels of DELLAs in roots of *A. thaliana* seedlings might impair the biosynthesis and/or the assembly of MT, as judged by the swelling of the root tip cells (Figures 1-4), the presence of multi-nucleated cells at the MZ (Figure 6), and the occurrence of ectopic root hairs, branched root hairs, and cells with multiple root hairs [MCCARTHY-SUÁREZ, unpublished manuscript]. In fact, a reduced expression of the α-tubulin gene results in the disassembly and aberrant reorganization of MT [BAO & al. 2001]. This means that the alteration of the root hair patterning in *A. thaliana* seedlings by excessive levels of DELLAs might have been correlated to their inhibitory effect on MT organisation. In fact, MT are essential to stablish root cell identity in *Arabidopsis* [WEBB & al. 2002].

The GAs/DELLAs might regulate the root architecture in *A. thaliana* seedlings

Excessive levels of DELLAs also promoted the outburst of LR near the root tip in *A. thaliana* seedlings, whereas excessive levels of GAs inhibited it. This means that any alteration in the levels of GAs/DELLAs might affect not only the root hair patterning, morphology and abundance, but also the root architecture. Thus, in seedlings of *A. thaliana*, physiologically-controlled levels of GAs/DELLAs might have a function in establishing a correct patterning and morphology of root hairs as well as in organising a proper root structure. In fact, supra-physiological levels of DELLAs mediate the root architecture changes that are associated to abiotic stress in plants (i.e., root elongation inhibition, root radial expansion, MC formation, pericycle cell proliferation and LR induction) [YIH & CLARK, 1965; JIANG & al. 2007; GOU & al. 2010; MARTÍN-REJANO & al. 2011; PÉRET & al. 2011; CUI, 2015; WILD & al. 2016]. For example, soil deficiencies of P, B, Fe or NO_3^- stimulate LR production, as nutrient concentration regulates LR production [YIH & CLARK, 1965; HERMANS & al. 2010; ZHANG & al. 2014]. Early production of LR has also been reported in the *erh1*, *jkd* and *arm* mutants, equally with a shortened primary root [SCHNEIDER & al. 1997; WELCH & al. 2007]. Interestingly, LR formation, which initiates at the pericycle, is correlated to an alteration in actin and tubulin expression [PASTERNAK & al. 2005; PÉRET & al. 2011; SÁNCHEZ-CALDERÓN & al. 2013]. Therefore, promoting LR outburst by increasing the local levels of DELLAs in roots might constitute a mechanism used by plants to increase the specific area of the root per mass unit, in a similar way as branched root hairs do.

Conclusions

As previously reported for other hormones, the results of this study, and of a previous one [MCCARTHY-SUÁREZ, unpublished manuscript] point to a possible role for the GAs/DELLAs in mediating the changes in the distribution, shape and frequency of root hairs, as well as in the configuration of the root, that take place in plants under situations of stress. Moreover, it is known that auxins, ET, ABA, BRs and SLs mediate these changes without altering the quantitative expression of *WER* and *GL2* [SCHIEFELBEIN, 2003; YANG & al. 2007; MARTÍN-REJANO & al. 2011]. This implies that, by regulating the elongation and/or division of root cells, as well as the production of LR, the GAs/DELLAs might contribute to mediating the changes in the patterning, morphology and abundance of root hairs, and the root architecture adaptations, that occur in plants under environmental stress conditions.

Notes on contributors

Iva MCCARTHY-SUÁREZ – is a postdoctoral researcher in plant biology with special interest in the mechanism of action of plant hormones, senescence and environmental stress.

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INTERCROPPING AND N FERTILIZATION EFFECTS ON *STRIGA* INFESTATION, SOIL C AND N AND GRAIN YIELD OF MAIZE IN THE SOUTHERN GUINEA SAVANNA OF NIGERIA

Moses Samuel BASSEY[1*], Joy Ekaette ETOPOBONG[1], Bigun Ishaku PONMAN[2], Sheriff Adam BADOM[1], Aliyu USMAN[1], Abubakar Kutigi MOHAMMED[1], Opeyemi Ruth IBRAHIM[3]

[1] National Cereals Research Institute, P.M.B. 8, Badeggi – Nigeria.
[2] Department of Agricultural Engineering, Federal Polytechnic Bida – Nigeria.
[3] National Biotechnology Development Agency, Ilori, Nigeria.
* Corresponding author. E-mail: mosessamuel36@yahoo.com

Abstract: Millions of hectares devoted to cereal production in Africa were affected by *Striga* infestation across locations and time. A study was conducted in 2012 and 2013 rainy seasons at the Teaching and Research Farms of Niger State College of Agriculture, Mokwa and the Teaching and Research Farms of Federal University of Technology, Minna, in the Southern Guinea Savanna ecology of Nigeria to determine cereal / legume intercropping and N fertilization effects on *Striga* infestation, Soil C and N and grain yield of maize. The treatments consisted of four inorganic N fertilizer levels (0, 60, 90, 120 kg ha^{-1}), alternate hill and same hill intercropping of *Aeschynomene histrix*. Intercropping maize with *A. histrix* has the potential of reducing *Striga* parasitism with about 33-47% with respect to *Striga* shoots per m^{-2} and *Striga* shoots per plot thus, enhancing maize grain yield. The use of herbaceous legumes in intercropping contributed about 58% SOC and 52-57% reduction in number of *S. hermonthica* due to application of N using urea, thereby helping to control *Striga* infestation. Intercropping maize with *A. histrix* improved the soil organic matter and hence, the physical, chemical and biological properties of the soil for good crop growth. Incorporation of the *A. histrix* residues substantially increased the soil N content. There was response to inorganic N fertilizer application, suggesting the need for N application to maize for optimum grain yield. Nitrogen rate of 60 kg ha^{-1} was optimum for maize yield in the study area.

Keywords: Grain yield, intercropping, legume, Maize, *Striga* infestation.

Introduction

Maize (*Zea mays* L.) ranks third globally after wheat and rice, provide 35% of food requirement in most countries and belongs to family Poaceae [BASSEY & al. 2019a]. More than 50% of the total maize production is being used as a food in developing countries [ARUN-KUMAR & al. 2008]. Nigeria's corn (maize) production in 2019 (October-September) is about 10.5 MMT, two percent less than 10.7 million metric tons in 2018 estimates [AATF, 2011]. The phenomenal increase in maize production in Nigeria over the past few years was attributed to increase in its utilization for various food items, livestock feed and industrial materials, as well as research activities [FAO, 2009].

Intercropping (IC) is an ancient multiple-cropping system that is popular with smallholder farmers in developing countries today, due to its higher land and nutrient use efficiency [LI & al. 2007], better economic returns [VAN ASTEN & al. 2011], and lower pest and disease incidence [ZHU & al. 2000] as compared to sole crops [HUANG & al. 2019]. Numerous studies have been conducted on cereal / legume IC systems based on field experiments

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in Africa, Asia, Europe and Latin America [YU & al. 2015; LI & al. 2016; MARTIN-GUAY & al. 2018]. These studies have shown that IC has yield advantages generated by the mechanisms of interspecific facilitation (or complementarity) and/or competitive production principles [ZHANG & LI, 2003]. Due to facilitation and complementarity between species, cereal/legume IC has been widely practiced and promoted for sustainable agriculture development [ZHANG & LI, 2003]. The use of intercropping of host crops with legume crops is to serve as trap crop. Leguminous trap- crops stimulate suicidal germination of *Striga* seeds and, therefore reduce the seed bank and improve soil fertility [SCHULTZ & al. 2003]. KOLO & LAWAL (2009) observed that sorghum interplanted with jointvetch (*A. histrix*) delayed *Striga* shoot emergence by about two weeks and reduced its density thus, increased the grain yield of the crop. DUGJE & al. (2003) found that interplanting sorghum and millet with groundnut, respectively, reduced *Striga* infestation compared with sole cropping. Improved soil fertility conditions especially N is likely to lead to reduced *Striga* infestation. The use of herbaceous legumes can contribute to soil N, thereby helping to control *Striga* infestation. The yields benefit have been attributed to increased soil N availability following the legume through biological N fixation [YUSUF & al. 2009a], mineralization of their residues and release of N from the breakdown of roots and nodules after harvest and higher soil organic carbon [YUSUF & al. 2009b].

Striga hermonthica (Delile) Benth. (family Orobanchaceae)-is a debilitating root parasite and possesses an ominous obstacle to the African continent that is struggling with food security as it affects the livelihood of more than 300 million people [BABIKER, 2007]. Prodigious seed production, prolonged viability of the seeds and the subterranean nature of the early stages of parasitism make the control of the parasite by conventional methods difficult if not impossible. The increasing incidence of *Striga* has been attributed to poor soil fertility and structure, low soil moisture, intensification of land use through continuous cultivation and an expansion of cereal production [BERHANE, 2016]. Many potentially successful approaches developed to control this weed include using resistant/tolerant varieties, sowing clean seeds that are not contaminated with *Striga* seeds, rotating cereal hosts with trap crops that induce abortive germination of *Striga* seeds, intercropping, applying organic and inorganic soil amendments such as fertilizer or manure, fumigating soil with ethylene, applying post emergence herbicides, push-pull technology and using biological control agents [ABDALLAH & al. 2015]. Based on some studies, the interaction of tied-ridging with N fertilizer and resistant varieties; cereal-legume intercropping and its interaction with N fertilizer revealed low *Striga* infestation. No single management option has been found effective across locations and time. AATF (2011) stated that *Striga* infests 40% of arable land in the African Savannah region and two – thirds of the 73 million hectares devoted to cereal production in Africa were affected by *Striga*. The hectarage of land under maize production infested by *Striga* was put at four million [ANON, 2011]. Nearly 100 m ha of the African Savanna is infested annually with the witchweed and more than half of African farmers recognize that *Striga* infestation is on the increase on their farms [EJETA, 2011]. Over US $1billion losses per year was estimated for *Striga* infested maize alone in Africa [AATF, 2011]; a major cause of food insecurity in the region. In Nigeria based on an average grain loss of 39% caused by *S. hermonthica* on sorghum, an estimated annual loss of US $93.6 million was incurred [AATF, 2011].

The control of *Striga* has proved exceptionally difficult. In Nigeria, the use of inorganic fertilizers to increase the N content in the soil is not feasible for the peasant farmers due to lack of resources, inaccessibility, low industrial technology and poor road network, among others. One alternative to inorganic fertilizer to increase soil N is by intercropping with herbaceous legumes especially *A. histrix* or similar ones. Not many studies on the use of *A. histrix* which grows widely in Niger State, Nigeria, in the southern Guinea savanna have been done. Hence,

the objectives of the study were to evaluate the effect of *A. histrix* on *Striga* infestation, soil organic carbon and nitrogen content, and maize yields.

Materials and Methods

Field experiments were conducted on a *Striga* infested field in 2012 and 2013 rainy seasons at the Teaching and Research Farms of Niger State College of Agriculture, Mokwa (09°18'N; 05°50'E) and the Teaching and Research Farms of Federal University of Technology, GidanKwano, Minna (9°31.860'N; 6°27.244'E; 254 m), situated in the Southern Guinea savanna agro ecological zone of Nigeria. Rainfall pattern of both sites is monomodal with the rainy season in Minna starting in April or May and ending in October, while that of Mokwa start in March or April and end in October or November. Monthly rainfall during the period of study at both sites are shown in Table 1. The soil of the Minna site is with loamy sand surface soil texture, slightly acidic, low organic carbon, N, and medium phosphorus. Selected soil physical and chemical properties of both sites before land preparation in 2012 are shown in Table 2. The two fields were heavily infested with *Striga hermonthica* which makes them to be sparingly cultivated with maize and sorghum over the years with no fertilizer.

Table 1. Monthly rainfall of the two experimental sites during the period of study

Months	Rainfall (mm)			
	Mokwa	Minna	Mokwa	Minna
	2012		2013	
January	0.0	0.0	0.0	0.0
February	58.0	1.5	0.0	0.0
March	0.0	0.0	20.1	0.0
April	88.0	258.0	43.4	34.2
May	235.4	140.4	165.0	204.5
June	123.0	67.3	174.0	96.5
July	268.2	194.7	346.0	333.1
August	132.0	160.4	423.2	376.9
September	111.4	301.8	487.0	337.2
October	38.0	100.3	76.3	158.0
November	0.0	0.0	12.1	0.0
December	0.0	0.0	0.0	0.0
Total annual rainfall	1054	1224.4	1747.1	1540.4

Source: College of Agriculture metrological station

Table 2. Some soil physical and chemical properties of both sites before planting in 2012

Properties	Values	
	Mokwa	**Minna**
Sand (g kg^{-1})	795	860
Silt (g kg^{-1})	116	93
Clay (g kg^{-1})	89	47
Textural class	Loamy sand	Loamy sand
pH (H_2O) (g kg^{-1})	6.7	6.8
Organic Carbon (g kg^{-1})	3.30	2.39
Total Nitrogen (g kg^{-1})	1.80	0.15
Available Phosphorus(mg kg^{-1})	18	12
Na+ (cmol kg^{-1})	0.09	0.23
K+ (cmol kg^{-1})	0.19	0.36
Mg++ (cmol kg^{-1})	0.98	1.65
Ca++ (cmol kg^{-1})	4.96	2.77
Exchangeable acidity(cmol kg^{-1})	0.11	0.04
ECEC (cmol kg^{-1})	6.32	5.05

Treatments and experimental design

The two sites had the same treatments, experimental designs and plot sizes. The treatments were four inorganic N fertilizer levels (0, 60, 90, 120 kg ha^{-1}), alternate hill and same hill intercropping of *A. histrix*. The treatments were laid out in a randomized complete block design with three replicates. There were 18 experimental plots, such that gross plot size was 8 m × 4 m (32 m^2) and the net plot size was 18 m^2, separated by 1 m alley. The number of ridges in the plot was five while the length of ridge was 8 m.

Both sites have the same crop establishment and management. The fields were manually cleared and ridged using hoe at 75 cm apart in 2012 and 2013. The maize variety, SUWAN 1, obtained from premier seeds, highly susceptible to *Striga* was manually planted at 3 seeds per hill, spaced 50 cm within rows. The seedlings were thinned to two plants per hill at two weeks after sowing (WAS) to give a plant population of 53, 3333 plants ha^{-1}. Basal application of 30 kg P ha^{-1} as single superphosphate and 30 kg K ha^{-1} as muriate of potash were carried out at 2 WAS after thinning. Inorganic N fertilizer as urea was split – applied to plots that were to receive N fertilizer. At 2 WAS, one-third of the N was applied, while the remaining two-third were applied at 6 WAS. Fertilizers were applied by side banding at about 5 cm away from the seedlings and at about 5 cm deep along the ridge. The first hoe – weeding was carried out at 3 WAS while the second weeding was at 5 WAS followed by careful hand-pulling of weeds other than *Striga*.

Data collection

The data collected from both sites were the same. The number of *Striga* shoots per maize plant was taken by counting each *Striga* shoot present per maize plant stand starting from 6 WAS. The number of *Striga* shoots flowering was taken by counting closely the number that flowered in each plot. The number of *Striga* shoots per meter squared was taken by counting closely the number of *Striga* present in each plot per m^2. Days to 50% *Striga* shoot flowering was carried out by counting the number of days from the day the first *Striga* shoot emerged to the day that 50% of

Striga shoots flowered. The *Striga* reaction score was taken on the scale of 0-9 using visual observation to measure mild, severe and very severe or death infestation of *Striga* on maize plant.

Ten maize plants from each of the net plot were randomly tagged for periodic observation at 3, 6 and 9 WAS. The following observations made were: The maize plant population was carried out by counting individual plants at 3, 6 and 9 WAS. This is also known as plant population count and expressed in hectare. The maize plant height was observed by tagging ten plants from the inner rows at random which were used throughout for taking the measurements. The plant height was measured using meter rule from the top of the uppermost leaf to the base of the plant at 3 and 6 WAS but from the base to the tip of the tassel at 9 WAS and expressed in centimeters. Days to 50% maize tasseling was taken through observation by counting the number of days from the sowing date to the day when about 50% of all the maize plants in each plot has tasseled and expressed in percentage. The average cob length of 10 harvested tagged maize plant from the inner row of each plot were taken and measured using meter rule and expressed in centimeters. The number of maize cobs from the inner rows of each plot was counted and estimated per hectare. This was done when the plant attains physiological maturity. The number of maize grain per cob was also obtained by weighing those harvested from the inner rows and shelled at harvest time. This was done by counting. 100 maize grain weights was taken from the ten harvested cobs from each plot, shelled and weighed using a weighing balance, expressed in grams. The maize grain yield analysis was carried out by harvesting maize ears in the two central rows leaving out the border plants at both ends (net plot of 18 m^2). These were shelled, air- dried and weighed. The grain yield was adjusted to 12% moisture content for each plot and weighed.

Soil samples were taken at harvest from each treatment plot and subjected to routine analyses. Particle size analysis was done by the hydrometer method [KLUTE, 1986], organic matter was determined by the procedure of Walkley and Black using the dichromate wet oxidation method [NELSON & SOMMERS, 1982]. Total N was determined by the micro – Kjeldahl digestion method [BREMNER & MULVANEY, 1982] and available P was by Bray – 1extraction followed by molybdenum blue colourimetry [BREMNER & MULVANEY, 1982]. Exchangeable K, Ca and Mg was extracted by EDTA titration method [THOMAS, 1982]. Soil pH was determined in 1:2 soil-water ratio using digital electronic pH meter.

Data analysis

The data collected were subjected to analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test at 5% level of probability. The statistical package used was Statistical Analysis System (SAS), version 9.2 (2002).

Results

The effect of intercropping and N fertilization on *Striga* shoot m^{-2} was significant (Table 3). In 2012 at Mokwa site, AH intercropping had the least *Striga* shoot at 9 WAS while the zero N application had the highest. However, the effect of 60-120 kg N ha^{-1} fertilizer application and SH intercropping on *Striga* shoot were similar. Also, at 12 WAS in Mokwa site, SH and AH intercropping had the least *Striga* shoot which were similar, and the effects of 60-120 kg N ha^{-1} fertilizer application and intercropping treatments on *Striga* shoot were not significantly different. In 2013, AH intercropping had the least *Striga* shoot at both sites at 9 WAS while zero N application had the highest. However, the effect of 0-90 kg N ha^{-1} application on *Striga* shoot were similar at both sites. In the same year at 12 WAS, at Minna site, AH had the least *Striga* shoot while zero N application had the highest. However, application of 0-120

kg N ha^{-1} fertilizer on *Striga* shoot were similar at Mokwa, while at Minna site, application of 60-120 kg ha^{-1} fertilizer and SH intercropping had similar effects on number of *Striga* hoots.

Table 3. Effect of intercropping and N fertilization on *Striga* shoot m^{-2} at 9 and 12 WAS in 2012 and 2013 cropping seasons

Treatment	*Striga* shoot m^{-2}							
	Mokwa	Minna	Mokwa	Minna	Mokwa	Minna	Mokwa	Minna
	9 WAS 2012		12 WAS 2012		9 WAS 2013		12 WAS 2013	
0 kg N ha^{-1}	3a	17a	6a	11a	6a	14a	12a	44a
60 kg N ha^{-1}	2ab	16a	5ab	11a	2ab	10a	11a	30b
90 kg N ha^{-1}	1b	11a	2ab	11a	2ab	8a	8ab	30b
120 kg N ha^{-1}	1b	9a	2ab	10a	1b	8a	8ab	29b
SH	1b	8a	1b	10a	1b	5b	6b	29b
AH	0c	8a	1b	8a	0c	0c	4b	22c
SE±	0.30	1.01	0.70	1.03	0.50	1.02	1.20	3.03

Means in the column with different letter(s) are significantly different from each other at P<0.05 using Duncan Multiple Range Test (DMRT). WAS – Weeks after planting, SH – Same hill, AH – Alternate hill, SE – Standard error.

The effect of intercropping and N fertilization on *Striga* shoot growing with maize was significant (Table 4). In 2012 at Mokwa site, SH and AH intercropping had the least number of *Striga* shoot growing with maize while zero N fertilizer had the highest number of shoots. However, the effect of 60-120 kg N ha^{-1}fertilizer application, and SH and AH intercropping on *Striga* shoot were similar. Zero N fertilization had the highest *Striga* shoot at 12 WAS while 60 to 120 kg N ha^{-1} and intercropping treatments had the lowest. In 2013, AH intercropping had the least *Striga* shoot at Mokwa site at 9 WAS which were similar to 120 kg N ha^{-1} and SH intercropping. In the same year, at 12 WAS, AH had the least *Striga* shoot at Minna site while zero N fertilizer had the highest number of shoots.

Table 4. Effect of intercropping and N fertilization on *Striga* shoots growing with maize plant at 9 and 12 WAS in 2012 and 2013 cropping seasons

Treatment	*Striga* shoot per maize plant							
	Mokwa	Minna	Mokwa	Minna	Mokwa	Minna	Mokwa	Minna
	9 WAS 2012		12 WAS 2012		9 WAS 2013		12 WAS 2013	
0 kg N ha^{-1}	7a	20a	15a	29a	3a	29a	4a	124a
60 kg N ha^{-1}	3ab	19a	3b	28a	2ab	27a	4a	93a
90 kg N ha^{-1}	2b	19a	2b	26a	2ab	25a	3a	74a
120 kg N ha^{-1}	1b	18a	1b	25a	1bc	22a	3a	66a
SH	0b	13a	1b	22a	1bc	22a	3a	60b
AH	0b	12a	1b	20a	0c	19a	3a	45c
SE±	0.81	3.00	1.88	2.01	0.33	2.03	0.25	7.04

Means in the column with different letter(s) are significantly different from each other at P<0.05 using Duncan Multiple Range Test (DMRT). WAS – Weeks after planting, SH – Same hill, AH – Alternate hill, SE – Standard error.

The effect of intercropping and N fertilization on Soil C and N was significant (Table 5). In 2012 at both sites, AH intercropping had the highest Soil C while zero N application had the lowest. However, the effects of SH intercropping and N fertilization treatments at Minna site, and 60-90 kg N ha^{-1} fertilizer application and AH intercropping were similar in Soil C. In the same manner, SH intercropping had the highest soil C at both sites in 2013 while zero N application had the lowest. Soil N was significantly higher in AH intercropping than the N fertilization treatments in 2012. However, the effects of SH intercropping and N fertilization treatments were similar in Soil N.

Table 5. Effect of intercropping and N fertilization on Soil C and N at physiological maturity of maize in 2012 and 2013 cropping seasons

Treatment	Soil C (g kg^{-1})				Soil N (g kg^{-1})			
	Mokwa	Minna	Mokwa	Minna	Mokwa	Minna	Mokwa	Minna
	2012		2013		2012		2013	
0 kg N ha^{-1}	3.18b	2.39b	5.80ab	3.21b	1.08b	0.15b	1.12a	0.12a
60 kg N ha^{-1}	3.73ab	2.41b	6.23ab	3.23b	1.05b	0.17b	1.16a	0.16a
90 kg N ha^{-1}	3.63ab	2.44b	5.13b	3.36b	1.08b	0.19b	1.12a	0.12a
120 kg N ha^{-1}	2.97b	2.45b	4.97b	3.37b	1.07b	0.18b	1.19a	0.19a
SH	3.05b	2.47b	6.67a	3.45a	1.11b	0.19b	1.12a	0.12a
AH	4.78a	2.53a	5.27b	3.39b	1.85a	0.24a	1.15a	0.15a
SE±	0.20	0.02	0.02	0.01	0.01	0.02	0.01	0.01

Means in the column with different letter(s) are significantly different from each other at P<0.05 using Duncan Multiple Range Test (DMRT). SH – Same hill, AH – Alternate hill, SE – Standard error.

The effect of intercropping and N fertilization on grain yield was significant (Table 6). In 2012 and 2013 at both sites, application of 60 kg N ha^{-1} produced the highest maize grain yield than the higher rates of N fertilization and intercropping treatments. However, in 2012 at Mokwa site, the effect of 60-120 kg N ha^{-1} fertilizer application and AH intercropping on maize grain yield were similar. In the same manner, at Minna site in 2013, the effect of 60 kg N ha^{-1} fertilizer application and AH intercropping on maize grain yield were similar.

Table 6. Effect of intercropping and N fertilization on Grain yield of maize in 2012 and 2013 cropping seasons

Treatment	Grain yield (kg ha^{-1})			
	Mokwa	Minna	Mokwa	Minna
	2012		2013	
0 kg N ha^{-1}	1306c	1207e	2222d	1804e
60 kg N ha^{-1}	1757a	1590a	3505a	3034a
90 kg N ha^{-1}	1628ab	1436b	3131bc	2567b
120 kg N ha^{-1}	1539ab	1442b	2587cd	1890d
SH	1412bc	1385c	2616c	2117c
AH	1552ab	1368d	3295b	3010ab
SE±	5.10	4.2	1.60	1.42

Means in the column with different letter(s) are significantly different from each other at P<0.05 using Duncan Multiple Range Test (DMRT). SH – Same hill, AH – Alternate hill, SE – Standard error.

Discussion

Striga shoots per m^{-2} and *Striga* shoot per maize plant were generally reduced by and varied between N fertilization and *A. histrix* intercropping with maize in this study. This clearly demonstrated that alternate plants of *A. histrix* could cause a reduction in *Striga* emergence, similar to application of N at 60-120 kg N ha^{-1}. Furthermore, same hill intercropping of *A. histrix* also produced a reduction in *Striga* shoots in this study. These might be attributed to *A. histrix* acting as a trap or catch crop and the shading effect from *A. histrix* canopy. In addition to shading out *Striga* in intercropping systems, the *A. histrix* has also shown to stimulate the germination of *Striga* without acting as host, just like cowpea and soybean. Our findings is in agreement with various studies that shown that intercropping cereals, mainly with legumes such as cowpea (*Vigna unguiculata*), peanut (*Arachis hypogaea*) and green gram (*Vigna radiata*) can reduce the number of *Striga* plants [CARSKY & al. 2000; BASSEY & al. 2019a]. Potentially, they might be acting as traps crops, stimulating suicidal *Striga* germination or the microclimate under the crop canopy may be altered and interfere with *Striga* germination and development [KUREH & al. 2000]. It is also hypothesized that nitrogen fixed by the legumes might interact with *Striga* growth, as increasing the amount of available nitrogen can reduce *Striga* densities [KUCHINDA & al. 2003; BASSEY & al. 2019b]. Our findings in this studies show that 52-57% reduction in number of *S. hermonthica* recorded was due to application of N using urea. This is because the nitrogenous compound fertilizer which contains urea considerably suppressed germination of *S. hermonthica* when applied during conditioning. It could also be because the germination of *S. hermonthica* seed is associated with the secretion of germination stimulants by host plants. The secretion ultimately depends upon the nutrient status of the soil. Our findings is in agreement with [BASSEY & al. 2019c], who reported 55-82% reduction in number and weight of *S. hermonthica* due to application of N using urea in Niger. BERHANE (2016) also reported that N fertilizers altered assimilate partitioning in favour of the ear and increased maize grain yield and reduced *Striga* count by 64%. Similarly, the study of ABDALLAH & al. (2015) conducted in North east Nigeria showed a reduction in *Striga* infestation and damage with the application of N fertilizer on maize varieties.

The soil organic carbon (SOC) and soil total nitrogen (STN) were increased by intercropping. The findings of this experiment indicated that *A. histrix* fixed about 5% SOC. This can be attributed to the high C/N ratio of the *A. histrix* residue which ensure a slow rate of mineralization of the residue, with consequent increase in SOC. There was also significant effect of intercropping and N fertilization on soil total nitrogen (STN), although there was a slight decrease across all the treatment. This scenario might be due to high STN in the organic form, which was not immediately available for crop use. The significant effects of intercropping and N fertilization on SOC and STN at physiological maturity might be due to dead leaves and roots added to the soil. The immobilization of N as a result of the high C/N ratio of the residues could be responsible for the high STN. Intercropping and N fertilization had positive effects on SOC and STN at physiological maturity of maize. Our finding was in agreement with those of CRICK (2007) and BASSEY & al. (2019b), who noted an appreciable increase in soil fertility in crop mixture, involving certain tropical legumes after cropping. They adduced the increase in soil fertility to the ability of legumes to fix large quantities of nitrogen into the soil. The inclusion of legumes in many crop mixtures had been reported to include improvement in N status of the soil through nitrogen fixation, its short lifespan, as well as its ability to cover the ground, with resultant decreases in the incidence of weed infestation and soil erosion [AYA, 2004; GERH, 2007]. The inclusion of certain tropical legumes in crop mixture has been reported to increase soil organic carbon, total nitrogen, available phosphorus and exchangeable potassium. In addition to the above, the inclusion of legumes in crop associations minimizes the risk

of crop failure and brings about higher total returns per unit area of land which allows larger financial gains for farmers [BEADER, 2004]. Significant effects of increasing maize planting density in a cowpea/maize mixture on soil nutrients and cowpea yield have been demonstrated by many studies [EZEMADU, 2007; VINE, 2007].

Maize grain yield was increased but varied between N fertilizer levels and *A. histrix* intercropping with maize in this study. The positive response (increase) observed in this study for grain yield due to N application and intercropping with *A. histrix* could probably be due to incorporation of residues resulting in high SOC and legume root system turnover. Increase in soil organic matter level might have resulted in increase in soil fertility, nutrient supply, porosity, permeability and thus, soil productivity [GRAY & MORANT, 2003; BASSEY & al. 2019b]. Our findings obtained are consistent with that of other workers in the same savanna agroecological zone of Nigeria [YUSUF & al. 2009a]. FRANKOW-LINDBERG & DAHLIN (2013) have suggested that a major part of the legume root system turnover occurs in the uppermost part of the soil profile. In a study in coastal lowland Kenya, SAHA (2015) observed the highest maize root length density in the top 30 cm of the soil profile. Therefore, intercropped maize is likely to benefit from the root system turnover of cowpea planted within the same row. Grain yield without inorganic N fertilizer was significantly lower than that of the other inorganic N levels. Similar response to inorganic N fertilizer has been reported in the study area by ADEBOYE & al. (2009) and AFOLABI & al. (2017). The high yield obtained in the study area might also be attributed to reduced temperature and moisture conservation effected by the overlapping maize and legume canopies. Nutrient uptake is known to increase with improved soil moisture. Maize intercropped with legume within the row probably responded to soil moisture conservation by increasing its nutrient uptake, leading to increased yields [TENEBE & PETU-IBIKUNLE, 2012].

Conclusion

From the results of this study, it can be concluded that intercropping maize with *A. histrix* has the potential of reducing *Striga* parasitism with respect to *Striga* shoots per m^{-2} and *Striga* shoots per plot thus, enhancing maize grain yield. The use of herbaceous legumes in intercropping contributed to soil N, thereby helping to control *Striga* infestation. *Striga* infestation is frequently associated with low soil fertility. Intercropping maize with *A. histrix* improved the soil organic matter and hence, the physical, chemical and biological properties of the soil for good crop growth. Incorporation of the *A. histrix* residues substantially increased the soil N content. There was response to inorganic N fertilizer application, suggesting the need for N application to maize for optimum grain yield. Nitrogen rate of 60 kg ha^{-1} was optimum for maize yield.

Notes on contributors

Moses Samuel BASSEY is a plant protectionist with special interest in weed science and agronomy. His focus is on the *Striga* management practices and Maize productivity. Joy Ekaette ETOPOBONG is a soil scientist with special interest in soil fertility. Bigun PONMAN Ishaku is a crop scientist with special interest in crop agronomy. Sheriff Adam BADOM is a research extension scientist with special interest in result dissemination. Aliyu USMAN is a crop scientist with special interest in crop agronomy. Abubakar Kutigi MOHAMMED is a plant breeder with special interest in crop improvement. Opeyemi Ruth IBRAHIM is a research scientist at National Biotechnology Development Agency.

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COMBINING ABILITY STUDIES ON YIELD AND YIELD COMPONENTS IN RICE GENOTYPES (*ORYZA SATIVA* L.)

Maiwa'azi Abu BARDE[1,2], Mohammed Sagir MOHAMMED[2], Muhyideen OYEKUNLE[2], Inuwa Shehu USMAN[2], Aondover SHAAHU[3*]

[1] National Agricultural Seeds Council, Abuja – Nigeria.
[2] Plant Science Department, Ahmadu Bello University, Zaria – Nigeria.
[3] National Cereals Research Institute, Badeggi, Niger State – Nigeria.
* Corresponding author. E-mail: iorpen001@gmail.com

Abstract: Nigeria has a wide range of arable rice growing environments including the inland valleys. Despite being the largest rice producer in West Africa, Nigeria is still a rice deficit nation. One of the major constraints to rice production in the inland valleys of Nigeria is iron (Fe) toxicity. The understanding of the genetic basis of Fe tolerance mechanisms can provide useful information for the breeding of tolerant varieties. Based on the aforementioned, the research was conducted to study the general and specific combining ability for yield and its components and to estimate the gene action for seed yield and its components. Eight rice varieties were planted and crossed using incomplete diallel mating design to generate 28 hybrids which were evaluated along with the eight parents and two checks (Alhaji Baba and Ewodufagi) at Edozhigi during the 2017/2018 dry season. Data were collected on grain yield, days to 50% flowering, number of tillers, number of leaves, plant height, panicle length, number of seeds per panicle, panicle exertion, number of effective tillers, 1000 grain weight, first and second iron toxicity scores were subjected to diallel analysis of variance (ANOVA) for both parents and hybrids, using Statistical Analysis System (SAS) software package (2002). The results from the study are summarized as follows GCA and SCA mean squares were significant for most measured traits under iron toxicity hotspot, indicating importance of additive and non-additive gene action for controlling the traits. There was preponderance of additive gene effects over non-additive gene effects for all the traits measured indicating that additive gene action was more important in the inheritance of the traits under iron toxicity hotspot. FARO 52, SUAKOKO 8, CK-21 and CK-43 were good general combiners for grain yield under iron toxicity hot spot conditions.

Keywords: gene action, general combining ability, grain yield, iron toxicity, rice, specific combining ability.

Introduction

Rice (*Oryza sativa* L.) belongs to the family of grass (Poaceae) [CHANDRASEKARAN & al. 2007] and it is one of the world's most important food crops with a total production of around 600 million tons, occupying 11% of the world's total arable land [GUIMARAES, 2009]. The crop supplies 2,808 calories/person/day, which represents 21% of the total calorie supply consumed by humans. Rice is an important annual crop in Nigeria and is one of the major staples.

Success of any plant breeding programme depends on the choice of appropriate genotypes as parents in the hybridization programme. The combining ability studies provide information, which helps in the selection of better parents for effective breeding programme. Combining ability analysis also provides information on additive and dominance variance. Its role is important to decide parents, crosses and appropriate breeding procedure to be followed to select desirable segregants [SALGOTRA & al. 2009].

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In Nigeria, lowland rice is cultivated on an estimated area of 3 million hectares out of which 1.8 million hectares are prone to iron toxicity [NCRI, 2012]. One of the areas where rice is commonly grown in Nigeria is Edozhigi village in Bida Local Government Area of Niger State. The people of this environment are predominantly lowland rice farmers. A survey conducted by the West African Rice Development Association (WARDA) in the area showed that average yield of lowland rice at Edozhigi seldom exceeds 1.5 tons ha^{-1} [NARTEH & SAHRAWAT, 1999]. Iron toxicity is a nutritional disorder associated with high level of ferrous iron concentration in the soil and is found mainly in waterlogged lowlands [CHERIF & al. 2009]. The response of rice to iron toxicity varies among different rice varieties. Some varieties have the mechanism to retain high iron levels in their roots or as oxides in the rhizosphere while other varieties are susceptible to iron toxicity, expressed in poor adaptability to iron toxicity stressed environment [MANDAL & al. 2004]. FUKUDA & al. (2012), reported that the surest way to counter iron toxicity is by using tolerant rice varieties. Genetic improvement of iron-toxicity tolerance implies the need of varietal screening to make good use of the existing diversity for iron toxicity tolerance. Since rice is a staple food in Nigeria, low production due to iron toxicity threatens the country's food security.

Several researches have been carried out on iron toxicity effects on rice production around the world. But in Nigeria, works on iron toxicity in rice have been based on mere selection. Therefore, adequate information has not been clearly understood about the constraint. Understanding of the genetic basis of iron tolerance mechanisms can provide useful information that will guide in designing strategic programme for the breeding of iron toxicity tolerant rice varieties. Consequently, knowing genetic bases of the materials formed the basis of this research. The objectives of the present study were to determine; Genetic variability of some agronomic traits for iron toxicity in rice: to study the general and specific combining ability for yield and its components and to estimate the gene action for seed yield and its components.

Materials and methods

Experimental site and climate

The research was conducted at the National Cereals Research Institute (NCRI) research field Edozhigi, Bida in Niger State from November 2017 to February 2018. NCRI research field Edozhigi is known to be an iron toxicity hot spot located in the Southern Guinea Savannah agro ecological region of Nigeria which has geographical bearing of latitude 09˚45' N, longitude 06˚07' E and altitude 50.57 m above the sea level (Table 1). It has bimodal rainfall pattern with an annual rainfall of about 900-1050 mm distributed between May and October. The average daily temperature is 35 °C with an annual mean of 27.4 °C and the mean relative humidity of about 52-73% [OGAH, 2013].

Table 1. Soil analysis of the study area: Edozhigi, Bida, Niger State, 2018

Parameters	0-15 cm value	15-30 cm value
Sand (%)	84.96	83.24
Silt (%)	10.56	8.28
Clay (%)	4.48	8.48
USDA Textural Class	**Sandy loam**	**Sandy loam**
pH (H_20)	4.64	4.65

Organic Carbon (%)	0.59	0.68
Organic Matter (%)	1.02	1.18
Total Nitrogen (%)	0.15	0.10
Available Phosphorus (mg/kg)	25.00	26.88
Exchangeable Bases (Cmol/kg)		
Calcium (Ca^{2+})	2.48	2.88
Magnesium (Mg^{2+})	4.63	5.90
Sodium (Na^{+})	0.17	0.13
Potassium (K^{+})	0.15	0.20
Exchangeable Acidity (Cmol/kg)	0.05	0.08
Cation Exchange Capacity (Cmol/kg)	7.48	9.19
Fe ($mgkg^{-1}$)	546	537

Experimental materials

The experimental materials comprised of eight genotypes of rice five obtained from the National Cereals Research Institute (NCRI) Badeggi, Niger State and three from West African Rice Centre, Ibadan. The description of the rice genotypes is presented in Table 2.

Development of F_1 population

The eight rice genotypes were mated using half diallel mating design method IV model I to generate 28 F_1 (Table 3). Seeds were planted in 128 buckets in four successions at an interval of one week (32 buckets in each succession) to synchronize flowering. Emasculation was carried out between 07.00 am and 09.00 am which involved direct removal of anthers before anthesis. In rice, anthers are enclosed in lemma and palea. Scissors was used to cut the un-matured caryopsis to expose the anthers and carefully removed with forceps without causing damage to the style and stigma. For the hybridization, flowers containing matured pollen grains from the male plants were shed on the emasculated panicles between 10am and 12noon. Pollinated flowers were covered with envelops sizeable enough to avoid contaminations and damages [MOHANAN, 2010].

Field evaluation of the F_1 genotypes and their parents

The eight parents, 28 F_1 hybrids and two checks making a total of 38 entries were evaluated at an iron toxicity spot in-situ at Edozhigi, Niger State. Plots were laid in a randomized complete block design with three replications. The genotypes were randomly planted with five stands per plot at 20 cm × 20 cm inter and intra row spacing respectively. All cultural practices for rice production were carried out in accordance with the recommendations of the NCRI, Badeggi.

Table 2. Description of the genetic materials used for the study

Genotype/variety	Source	Potential yield (kg/ha)	Plant height (cm)	Maturity days	Reaction to Iron Toxicity
FARO 44 (P_1)	NCRI	6442	115	95-105	S
FARO52 (P_2)	NCRI	6710	129	125-135	T
FARO 60(P_3)	NCRI	6754	120	100-115	S
FARO 57 (P_4)	NCRI	7954	124	120 -135	T
FARO61(P_5)	NCRI	6312	115	100-110	S
SUAKOKO 8 (P6)	WARDA	5500	141	115-120	T
CK- 43 (P_8)	WARDA	5000	75-80	80 – 90	T

Source: NCRI/WARDA 2017, S: Susceptible, T: Tolerant.

Table 3. Diallel method IV Model 1 adopted to develop the plant population used in the study

	P_1	**P_2**	**P_3**	**P_4**	**P_5**	**P_6**	**P_7**	**P_8**
P_1		P_1P_2	P_1P_3	P_1P_4	P_1P_5	P_1P_6	P_1P_7	P_1P_8
P_2			P_2P_3	P_2P_4	P_2P_5	P_2P_6	P_2P_7	P_2P_8
P_3				P_3P_4	P_3P_5	P_3P_6	P_3P_7	P_3P_8
P_4					P_4P_5	P_4P_6	P_4P_7	P_4P_8
P_5						P_5P_6	P_5P_7	P_5P_8
P_6							P_6P_7	P_6P_8
P_7								P_7P_8
P_8								

Key: P1: FARO 44, P2: FARO 52, P3: FARO 60, P4: FARO 61, P5: SUAKOKO 8, P6:FARO57 P7: CK-21, P8: CK-43.

Data were collected on the followings traits: Plant height at maturity (cm), Number of tillers, Number of leaves, Days to 50% flowering, Panicle length (cm), Number of seeds per panicle, Iron toxicity score: This was recorded on the scale 1 to 9, where 1.0 = highly resistance, 3.0 = resistance, 5.0 = moderately susceptible, 7.0 = susceptible and 9.0 = highly susceptible $IRRI_{SES,}$ (2001), Grain Yield after harvest (g), Number of effective tillers, One thousand (1000) grain weight (g) and Panicle Exertion.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA). Using general linear model procedure of Statistical Analysis System (SAS) package (2002). Significant difference between treatments means were compared using least significant difference (LSD) using linear model as shown below:

$$Y_{ijk} = \mu + \alpha_i + r_j + \ell_{ijk}$$

Where: Y_{ijk} is the observation in treatment i and block j and k,

μ is the overall mean, α_i is the effect of treatment, ℓ_{ijk} is the random error.

Results

Combining ability and gene action

The analysis of variance for general combining ability (GCA) and specific combining ability (SCA) of F_1 genotypes showed highly significant ($P<0.01$) differences for all traits measured (Table 4). The mean squares due to GCA and SCA were highly significant ($P<0.01$) for most of the traits except for the number of seeds per panicle and first iron toxicity score which were not significantly different.

Estimate of general combining ability effect for grain yield and other agronomic traits of rice under iron toxicity hotspot at edozhigi

A highly significant ($P<0.01$) positive GCA effects for grain yield were observed for FARO 52 (19.93). Highly significant ($p<0.01$) positive GCA effects for grain yield were observed for suakoko 8(8.67) and highly significant ($P<0.01$) negative GCA effects of first and second iron toxicity scores of (-0.73) and (-1.19), respectively, (Table 5). Highly

significant ($p<0.01$) positive GCA effects for days to 50% flowering were observed for FARO 57 (3.42) and Suakoko 8 (3.42) and highly significant negative GCA effects were observed for FARO 60 (-6.70) and CK-43 (-5.5).

Table 4. Analysis of variance for general combining ability (GCA) and specific combining ability (SCA) evaluated at iron toxicity condition at Edozhigi, in 2018

Source of variation	Replication	Genotype	GCA	SCA	Error
Df	2	37	7	27	70
Grain yield (kg/ha)	35.11	3614**	13046.07**	1112.49**	102.9422
Days to flowering	3.06	364**	515.99**	328.85**	54.8194
Number of tillers	36.05	126.41**	326.50**	84.21**	22.6187
Number of leaves	0.86	2.12**	7.10**	0.94**	0.42067
plant height (cm)	98.93	1707.60**	6077.39**	574.29**	140.711
panicle length (cm)	5.09	19.67**	39.94**	12.31**	2.05154
Panicle exertion (cm)	0.49	4.85**	15.43**	2.82**	0.87474
Number of effective tillers	20.47	181.14**	461.20**	111.23**	42.7898
Number of seed per panicle	24.62	6263.00**	12478.29**	3897.31ns	562.205
1000 grain weight (g)	0.06	26.11**	60.71**	14.44**	2.68021
1st iron score (ses)	2.13	4.84**	15.20**	2.21ns	1.75461
2nd iron score (ses)	2.59	10.30**	20.05**	1.73**	1.88636

** = Significant at 0.01 probability level; df = Degree of freedom

NB: Leaf bronzing score ranged from 1.0 to 9.0 in response to pulse stress according to IRRI (2001), Standard evaluation system (SES), 1.0 is considered highly resistance, 3.0 resistance, 5.0 moderately susceptible, 7.0 susceptible and 9.0 highly susceptible.

Table 5. Estimate of General combining ability and specific combining ability effects for grain yield and other agronomic traits of rice under irontoxicity condition at Edozhigi in 2018

Genotype	Grain yield	Days to 50% flowering	Number of tillers	Number of leaves	Plant height	Panicle length	Number of seeds per panicle	Panicle exertion	No. of effective tillers	1000 grain weight	1st iron score	2nd iron score
Parent												
Faro 44	-24.41**	2.46*	-4.56**	0.11	-16.13**	-1.63**	-31.14**	0.78**	-5.60**	-0.32	1.02**	1.65**
Faro 52	19.93**	1.84	0.08	-0.15	-4.58**	0.94**	3.91	-0.22	-0.12	-2.09**	-0.1	-0.19
Faro 60	-26.93**	-6.70**	-2.81**	0.06	-10.52**	-1.22**	-18.77**	1.03**	-2.81**	-0.69**	0.44*	0.73**
Faro 57	12.64**	3.42**	0.74	0.24**	-4.23*	0.65**	28.36**	-0.14	0.84	-0.19	0.31	0.81**
Faro 61	-27.38**	-2.12*	-3.49**	0.76**	-14.38**	-1.37**	-7.17*	0.45**	-4.38**	-1.18**	0.60**	0.81**
Suakoko 8	8.67**	3.42**	1.03	-0.90**	8.90**	1.43**	-1.82	-0.22	1.5	2.23**	-0.73**	-1.19**
CK-21	19.25**	3.26**	4.64**	-0.46**	22.51**	1.02**	30.82**	-0.97**	5.04**	0.22	1.23**	-1.52**
CK-43	18.23**	-5.58**	4.37**	0.34**	18.43**	0.18	-4.2	-0.71875**	5.53**	2.03**	-0.31	-1.10**
SE±	**1.23**	**0.98**	**0.58**	**0.09**	**1.72**	**0.20**	**3.24**	**0.13**	**0.85**	**0.21**	**0.18**	**0.19**
Faro 44 x Faro 52	-24.07**	-1.63	-1.26	0.1	-8.2	-2.01**	-10.89	0.47	-1.68	1.38*	1.02*	1.27*
Faro 44 x Faro 60	14.16**	-11.76**	0.7	-0.31	-4.79	1.01	-17.75*	1.22**	-3.32	-0.75	1.15*	0.35
Faro 44 x Faro 57	-3.77	8.79**	3.82	0.11	1.32	0.01	45.39**	-1.61**	3.56	0.78	-0.73	-0.4
Faro 44 x Faro 61	13.77**	3.66	-2.75	-0.32	0.6	-1.58**	-14.48	1.14**	-2.36	0.01	-0.35	0.27
Faro 44 x Suakoko 8	-8.51**	9.45**	-1.8	0.05	13.19**	0.49	2.97	-0.2	-0.7	0.03	-0.35	-1.06*
Faro 44 x CK- 21	-6.75*	-3.71	2.65	0.61*	2.05	0.84	19.20*	-0.78*	5.29*	-0.83	0.15	0.6
Faro 44 x CK- 43	-25.17**	-16.96**	4.94	-0.83	2.18	1.18	22.33	-0.5	5.87	0.35	-0.67	0.08
Faro 52 x Faro 60	-10.51**	0.2	1.73	-0.25	28.79**	2.10**	18.67*	-1.11**	3.46	-0.94	-1.73**	-1.81**
Faro 52 x Faro 57	-0.08	-7.92**	-7.65	0.03	-16.03**	-2.13**	-72.99**	1.39**	-9.63**	1.20*	0.73	1.44**

Table 5. (cont.)

Genotype	Grain yield	Days to 50% flowering	Number of tillers	Number of leaves	Plant height	Panicle length	Number of seeds per pan	Panicle exersion	No. of effective tillers	1000 grain weight	1st iron score	2nd iron score
Faro 52 x Faro 61	-2.74	-5.05	2.68	0.25	5.52	2.12**	85.67**	-1.20**	5.62*	3.61**	-0.56	0.1
Faro 52 x Suakoko 8	2.55	9.41**	9.83	0.11	3.11	0.59	53.25**	-0.53	11.15**	-2.57**	0.1	-0.56
Faro 52 x CK- 21	9.24**	-11.4	-0.66	0.07	-8.83	-1.33*	-53.12**	0.22	-5.39*	-2.56**	-0.73*	-0.9*
Faro 52 x CK- 43	-19.03**	-6.92	-9.95	-1.29**	0.59	0.22	3.31	1.83**	-10.91*	-4.22**	2.21*	2.25*
Faro 60 x Faro 57	-8.92**	-14.05**	3.27	0.56*	2.84	-0.54	1.22	0.80*	3.7	1.83**	-0.15	1.19*
Faro 60 x Faro 61	18.10**	17.49**	-3.63	0.17	-3.61	-3.59**	-35.19**	0.89*	-5.81*	-0.22	1.56**	1.19*
Faro 60 x Suakoko 8	-7.99*	-14.05**	3.45	-0.37	5.52	3.34**	23.66**	-0.45	7.91**	-0.96	-1.10*	-0.15
Faro 60 x CK-21	-12.26**	16.45**	-0.37	-0.01	-17.49**	0.03	14.42	-1.03**	0.84	2.45**	0.06	-1.15*
Faro 60 x CK- 43	-35.43*	-13.13**	-4.38	0.72	34.98**	6.20**	52.83**	-2.25**	4.53	-2.13*	-0.58	-2.17*
Faro 57 x Faro 61	-12.84**	10.37**	1.15	0.72**	-1.16	0.88	8.28	-0.61	2.6	-2.05**	-0.31	-1.56**
Faro 57 x Suakoko 8	-35.09**	-1.51	-3.63	-0.08	-4.51	-1.72**	-11.07	0.72	-4.74*	-1.43*	0.35	-0.9
Faro 57 x CK- 21	-2.80	-1.34	2.95	-0.19	17.89**	-0.77	-4.37	0.14	1.58	-0.35	0.19	0.77
Faro 57 x CK- 43	-36.33**	-11.33*	-16.03	0.63	32.41**	-1.6	-37.04*	0.58	-17.69**	-2.49*	1.29	1.25
Faro 61 x Suakoko 8	-9.28**	-6.63*	-0.13	0.13	-20.49**	-1.18	-25.74**	0.8	-3.32	-2.44**	0.73	1.10*
Faro 61 x CK- 21	-23.72**	1.87	-2.86	-0.51*	18.64**	2.31**	13.16	-0.45	4.47*	1.64**	-0.77	-0.56
Faro 61 x CK- 43	-32.97**	2.46	1.85	0.89*	6.26	0.85	92.43**	-0.83	2.09	-1.88	-0.42	-1.42
Suakoko 8 x CK-21	24.00**	-12.01**	-9.27	0.08	-6.91	-0.63	-28.79**	0.22	-10.54*	-0.47	0.56	0.77
Suakoko 8 x CK- 43	2.78	21.00**	3.39	-1.91**	2.27	0.52	-48.75**	0.5	0.5	0.99	0.92	-0.08
CK- 21 x CK- 43	-26.88**	-15.50**	20.34	0.67	2.08	-2.57*	16.22	-0.25	18.31**	-4.82**	-1.58	-0.42
SE±	3.28	2.63	2.92	0.24	4.59	0.54	8.67	0.35	2.28	0.57	0.48	0.52

The result showed highly significant positive GCA effects for CK-21 and CK-43 (4.64 and 4.37), respectively, and highly significant negative GCA effects were also observed for FARO 44 (-4.56), FARO 60 (-2.81) and FARO 61 (-3.49). Highly significant positive GCA effects were observed for number of leaves for FARO 57 (0.24), FARO 61 (0.076) and CK-43 (0.34) and highly significant negative GCA effects were recorded for Suakoko 8 (-0.90) and CK-21 (-0.34). A highly significant positive GCA effects were observed for plant height for Suakoko 8 (8.90), (CK-21 (22.51) and CK-43 (18.43) while a significant negative GCA effects were observed for FARO 44 (-16.13), FARO 60 (-10.52), FARO 52 (-4.85) and FARO 61 (-14.38). Highly significant positive GCA effects were observed for panicle length for FARO 52 (0.94), FARO 57 (0.65) and FARO 61 (-1.37) showed highly significant negative GCA effects. Highly significant positive GCA effects were for number of seeds per panicle for FARO 57 (28.36) and CK-21 (30.82), while FARO 44 (-31.14) and FARO 60 (-18.77) showed highly significant GCA negative effects. The significant positive GCA effects were observed for panicle exertion 61 (0.45) and FARO 60(1.03) while CK-21 (-0.97) and CK- 43 (-0.72) recorded highly significant negative GCA effects. On number of effective tillers, CK-21 (5.04) and CK-43 (5.53) recorded highly significant positive GCA effects while FARO 44 (-5.60), FARO 6O (-2.81) and FARO 61 (-4.38) showed highly significant negative GCA effects. On one thousand (1000) grain weight Suakoko 8 (2.23) and CK-43 (2.03) showed highly significant positive GCA effects whereas highly significant negative GCA effects were observed for FARO 52 (-2.09), FARO 60 (-0.69 and FARO 61 (-1.18). First iron toxicity score, showed highly significant positive GCA effects for FARO 44 (1.02) and FARO 61 (0.60) while highly significant negative GCA effects was observed for Suakoko 8 (-0.73) and CK-21 (-1.23). The second iron toxicity score showed highly significantly positive GCA effects for FARO 44 (1.65), FARO 60 (0.73), FARO 57 (0.81) and FARO 61 (0.81) while Suakoko 8, CK-21, and CK- 43 recorded highly significant negative GCA effects (-1.19), (-1.52), and (-1.10), respectively.

Estimate of specific combining ability (SCA) effects for grain yield and other Agronomic Traits of Rice under Iron Toxicity Hotspot at Edozhigi.

A highly significant ($P<0.01$) positive SCA effects for grain yield were observed for FARO 52 x CK-21 (9.24) and also showed high significant negative SCA effects for first and second iron toxicity scores (-0.73) and (-0.9), respectively, (Table 4). Highly significant ($p<0.01$) positive SCA effects were observed for days to 50% flowering in Suakoko 8 x CK-43 (21.00) and highly significant ($p<0.01$) negative SCA effects were observed for FARO 60 x FARO 57 (-14.05).

Highly significant positive SCA effects were observed for number of leaves for FARO 57 x FARO 61 (0.72) and highly significant ($p<0.01$) negative SCA effects were observed for Suakoko 8 x CK- 43 (-1.91). High significant ($P<0.05$) positive SCA effects for number of leaves for FARO 44 x CK-21 (0.61) while FARO 52 x CK-43 (-1.29) showed highly significant negative SCA effects for number of leaves. Highly significant positive SCA effects for plant height were observed for FARO 57 x CK-43 (32.41) and highly significant negative SCA effects for plant height were observed for FARO 61 x Suakoko 8 (-20.49).

Highly significant positive SCA effects were observed for panicle length for FARO 52 x FARO 60 (2.10) while highly significant negative SCA effects were observed for panicle length for FARO 44 x FARO 61 (-1.58). Highly significant positive SCA effects were observed for number of seeds per panicle for FARO 61 x CK-43 (92.43) and highly

significant negative SCA effects were observed for number of seeds per panicle for FARO 52 x FARO 57 (-72.99). Highly significant positive SCA effects were observed for panicle exertion for FARO 44 x FARO 60 (1.22), while highly significant, but negative SCA effects were expressed in FARO 44 x FARO 57 (-1.61). High significant positive SCA effects were observed for number of effective tillers for FARO 44 x CK-21 (5.29) and significant negative effects was observed in FARO 52 x CK- 21 (-5.39). While highly significant positive SCA effects were recorded for one thousand (1000) grain weight for FARO 52 x FARO 61 (3.61) and highly significant negative SCA effects were observed for FARO 52 x Suakoko 8 (-2.57). First iron toxicity score showed highly significant positive SCA effects in FARO 60 x FARO 61 (1.56) and highly significant negative SCA effects were observed for FARO 52 x 60 (-1.73). Second iron toxicity score showed highly significant positive SCA effects in FARO 52 x FARO 57 (1.44) and while highly significant negative SCA effects were observed in FARO 52 x FARO 60 (-1.81).

Variance component of GCA and SCA on iron toxicity and agronomic traits of rice F1 genotypes

The GCA variances were generally higher than the SCA variances for all the traits studied except for days to 50% flowering (9207.92), number of tillers (2357.88), panicle length (344.74) and number of seeds per panicle (109124.58), (Table 6). The highest GCA were observed in grain yield (91322.48) followed by number of seeds per panicle (87348.01) and plant height (42541.75) whereas the lowest were associated with number of leaves (49.75), panicle exertion (94.45) and 1st iron score (106.07). The number of seeds per panicle (109124.58) revealed the highest SCA followed by grain yield (31149.61) and plant height (16080.11) while the lowest were linked with number of leaves (26.26), panicle exertion (69.10) and first iron score (61.87). The GCA/SCA ratios ranged from 0.39 for days to 50% flowering to 2.93 for grain yield. The highest ratio was reported for grain yield (2.93) followed by 2nd iron score (2.90) and plant height (2.65) while the lowest were seen in days to 50% flowering (0.39) followed by number of seeds per panicle (0.80), number of tillers (0.97) and panicle length (0.81). The following traits: days to 50% flowering, number tillers, panicle length and number of seeds per panicle recorded GCA/SCA ratios less than one (1) less than unity.

Table 6. Variance component of GCA and SCA on iron toxicity agronomic traits of rice F_1s at Edozhigi in 2018

Source of variation	σ^2_{GCA}	σ^2_{SCA}	$\frac{\sigma^2_{GCA}}{\sigma^2_{SCA}}$
Grain yield (kg/ha)	91322.48	31149.61	2.93
Days to flowering	3611.95	9207.92	0.39
Number of tillers	2285.46	2357.88	0.97
Number of leaves	49.75	26.26	1.9
Plant height (cm)	42541.75	16080.11	2.65
Panicle length (cm)	279.56	344.74	0.81
Number of seeds per panicle	87348.01	109124.58	0.8
Panicle exertion (cm)	94.45	69.1	1.37

Number of effective tillers	3229.08	3114.52	1.04
1000 grain weight (g)	425	404.34	1.05
1st iron score (ses)	106.07	61.87	1.71
2nd iron score (ses)	264.76	91.32	2.9

NB: Leaf bronzing score ranged from 1.0 to 9.0 in response to pulse stress according to IRRI (2001), Standard evaluation system (SES), 1.0 is considered highly resistance, 3.0 resistance, 5.0 moderately susceptible, 7.0 susceptible and 9.0 highly susceptible.

Discussion

The analysis variance for gene action showed highly significant difference among the genotypes for all traits measured with both additive genes and non additive genes playing important roles in most of the traits. However, the SCA revealed the existence of highly significant differences for most of the traits except for number of seeds per panicle and first iron toxicity score for which the difference was not significant. The relatively greater importance of GCA than SCA implies that breeding progress could be effective using recurrent selection in improving yield and other traits in iron toxicity condition. This present study is in conformity with the results reported by ISMAILA (2012).

Combining ability refers to the ability of a parent to transmit desirable performance to its hybrid progenies or crosses. PAINKRA (2014) pointed out that combining ability of parents gives important information of the choice of parent in terms of expected performance of their progenies. The significance of GCA and SCA mean squares suggested the importance of both additive and non-additive variances for all the characters studied. The highly significant positive GCA effects observed among, SUAKOKO 8, CK-21, FARO 52, and CK-43 for grain yield and also highly significant negative GCA effects of first and second iron scores indicated that these parents are tolerant to iron toxicity and may be preferred for hybridization and selection programmes. The SCA effects revealed that for hybrid rice development, cross FARO 52 x CK-21 with highly significant positive SCA effects for grain yield and with high significant negative SCA effects of first and second iron toxicity scores could be better choice for most of the traits including yield under iron toxicity condition. This showed combining ability and gene effects revealing contribution of both additive and non additive gene effects playing important roles for all traits studied. This is in conformity with the results reported by PAINKRA (2014). Positive GCA effects are desirable for grain yield and number of effective tillers, but negative GCA effects are desirable for rice crop plant height because of lodging and days to 50% flowering because of earliness. In the present study, parents FARO 52 and FARO 57 were found to be good general combiners in the right direction for grain yield per plant, plant height and number of effective tillers. Similar findings were recorded by GNANASEKARAN & al. (2006), SHARMA (2006), ISMAILA & ECHEKWU (2015) whose research work reported rice genotypes with good GCA effects for plant height and number of tillers. The parent materials are potential varieties for future breeding programmes aimed at developing new hybrids tolerant to iron toxicity.

SCA effect is the index that determines the usefulness of a particular cross combination in the exploitation of heterosis. Shorter culm reduces the respiratory loss. On the other hand, tall stature facilitates light penetration. Thus an optimum plant height is however, desirable. Short statured plant height is a desirable trait in rice crop to avoid greater yield loss due to lodging. This is in line with CHANDRASEKARAN & al. (2007). Six

hybrids FARO 44 x SUAKOKO 8, FARO 52 x FARO60, FARO 60 x CK-43, FARO 57 x CK-21, FARO 57 x CK-43 and FARO 61 x CK-21 expressed positive SCA effects reflecting an increase in plant stature. FARO 52 x FARO 57, FARO 60 x CK-21 and FARO 61 x SUAKOKO 8 showed negative SCA fell in the category of average effects. The cross FARO 52 x FARO 57 exhibited the best showing negative SCA effects for plant height indicating that improvement in plant height in terms of intermediate or short stature can be achieved by exploitation of hybrid vigor in this cross combination.

Number of effective tillers per plant and increased panicle length are also desirable traits for increased grain yield per plant in rice. The cross combinations FARO 44 x FARO 60, FARO 44 x FARO 57, FARO 44 x CK-21, FARO 44 x CK-43, FARO 52 x FARO 60, FARO 52 x FARO 61, FARO 52 x SUAKOKO 8, FARO 60 x FARO 57, FARO 61 x CK-43, SUAKOKO 8 x CK-43 and CK-21 x CK-43 had positive SCA effects for number of effective tillers per plant. These results are in conformity with the reports of MEHLA & al. (2000), SHARMA & MANI (2005) and SHARMA (2006) who reported similar SCA estimates of number of tillers per plant. Grain yield is the ultimate objective of rice breeding programme. The cross combinations; FARO 44 x FARO 60, FARO 44 x FARO 61, FARO 52 x CK-21, FARO 60 x FARO 61 and SUAKOKO 8 x CK-21 had positive SCA effects for grain yield per plot indicating relative importance of non-additive gene effects for these traits. ISMAILA (2012) reported that non-additive gene effects were predominant for grain yield and its components. In the present study, it was observed that cross combinations which expressed high SCA effects for grain yield, invariably exhibited positive SCA effects for one or more yield related traits also. These results are in line with ISMAILA & ECHEKWU (2015), who reported good specific cross combinations in rice. None of the cross combinations were found to be good specific cross combinations for all the characters studied. Generally, in most of the cross combinations at least one general combiner was involved for all the traits under study along with grain yield. It also indicated both additive and none-additive types of gene action. While selecting the best specific combination for yield, it would be important to give due considerations to yield related traits. Leaf bronzing score is often recorded to screen plants for tolerance to ferrous iron toxicity [DEVI & al. 2016]. In the study, leaf symptom scoring was performed to study the rice genotypes' response to excess iron. The rice genotypes showed varying degrees of leaf bronzing in response to excess iron. Leaf bronzing score ranged from 1.0 to 9.0 in response to pulse stress according to IRRI (2001), Standard evaluation system (SES), 1.0 is considered highly resistance, 3.0 resistance, 5.0 moderately susceptible, 7.0 susceptible and 9.0 highly susceptible. Parents Suakoko 8, Ck-21 and Ck-43 with high significant positive GCA effects and with high significant negative GCA effects of iron scores can be utilized for hybridization programme for selection of superior combination in segregating progenies in iron toxicity condition as explained by MULLER & al. (2015). This study conforms to the results reported by DEVI & al. (2016), that such varieties are capable of oxidizing large amount of iron translocated to the shoots.

Gene action refers to the behavior or mode of expression of gene in a genetic population. The ratio of GCA to SCA (additive / non additive genes) ranged from 0.39 (days to 50% flowering) to 2.93 (grain yield). Among the twelve traits, four: days to 50% flowering, number of tillers, panicle length and number of seeds per panicle showed less than unity (lower than 1) and eight recorded more than unity (greater than 1). Lower than unity indicated higher importance of non additive genes in the expression of the traits or non additive component had a more significant role in the inheritance of days to 50% to flowering, number

of tillers, panicle length and number of seeds per panicle. Furthermore, GCA/SCA ratio in the hybrids lower than one (1) indicated that the non additive component of genetic variance made a larger contribution to the total genetic variance for grain yield than the additive one as reported by DRAGAN & al. (2000). Traits showing more than unity indicate preponderance of additive gene action in inheritance of these traits. Similar results were reported by PAINKRA (2014).

Conclusions

Presence of substantial additive gene effects suggests that reciprocal recurrent selection would be effective in improving populations and developing superior cultivar for iron toxicity soil. Based on mean performance of populations, their GCA effects, recurrent selection that exploit both GCA and SCA effects, would include Faro 57, Faro 52, CK-21, CK-43 and suakoko 8 for developing superior cultivars under iron toxicity conditions. Additive gene and non-additive gene actions were observed for the control of all measured traits including iron toxicity in the study, indicating the possibility of developing desirable genotypes that can be advanced for selection and production under iron toxicity, or used in rice breeding programmes.

Recommendations. The following recommendations are highlighted from the present study: the iron toxicity tolerant rice genotypes (FARO 52, SUAKOKO 8, CK-21 and CK-43) were identified as good combiners in the study which could be useful to rice breeders interested in breeding for iron toxicity tolerance and synthetic cultivars for iron toxicity prone regions in Nigeria.

The F_1 genotype identified in this study including FARO 52 x CK-21 with highly significant positive SCA effects for grain yield (9.24) and with high significant negative effects of first and second iron toxicity scores (-0.73) and (-0.90), respectively, should be tested extensively in more hot spot regions prone to iron toxicity for adoption and production to alleviate food security problems in Nigeria.

Notes on contributors

Maiwa'azi Abu BARDE is a plant breeder/seeds specialist, currently working on iron toxicity in rice. Works at National Agricultural Seeds Council, Jos, Nigeria.

Mohammed Sagir MOHAMMED, PhD in Plant Breeding. Postgraduate Coordinator and a Lecturer at Plant Science Department, Ahmadu Bello University Zaria, Nigeria.

Muhyideen OYEKUNLE, PhD in Plant Breeding/Maize Breeder, Institute for Agriculture Research, Plant Science Department, Ahmadu Bello University Zaria, Nigeria.

Inuwa Shehu USMAN, Professor of Plant Physiology, Head of Biofactory, Plant Science Department, Ahmadu Bello University Zaria, Nigeria.

Aondover SHAAHU is a PhD student/Researcher, currently working on soybean breeding and evaluation of advanced lines at National Cereals Research Institute, Badeggi, Nigeria.

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ORGANIC COMPOST CONTROL OF BLIGHT DISEASE OF OKRA (*ABELMOSCHUS ESCULENTUS*) AND TOMATO (*SOLANUM LYCOPERSICUM*) PLANTS

Habiba Maikudi MUHAMMED[1*], Usman Ibrahim HAMZA[1], Hadiza ISYAKU[1], Donadeen OLACHI[1]

[1] Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai – Niger State.
* Corresponding author. E-mail: habibamaliyu@gmail.com

Abstract: Organic composts are derived from plant and animal wastes as substrates in the control of blight disease of vegetable crops; tomato (*Solanum lycopersicum*) and okra (*Abelmoschus esculentus*) plants were evaluated. Cow dung, sawdust, rice husk, poultry droppings and goat dung were collected from the agricultural research farm Ibrahim Badamasi Babangida university Lapai, Niger State, Nigeria. The organic matter were allowed to decompose watered daily for twenty four days to foster the microbial activities of the composts. Three kilogram (3 kg) of sterilized topsoil was bulked with the compost at 50, 100, 150, 200 and 250 g and allowed to decomposed for the period of 10 days respectively. At 200 g and 250 g the suppression was significantly different ($P \leq 0.05$) than other lower quantities. Goat dung gave the highest suppression of 88.2% followed by rice husk (85.0%). At 250 g it was 99.0%, 93.7%, 93.7%, 84.0%, and 94.7% with poultry droppings, rice husk, cow dung, saw dust and goat dung respectively. Goat dung amended soil gave the best blight disease suppression (88.2%) in okra and poultry droppings gave the best in tomato (99.0%). A good combination of compost consisting of goat dung and poultry droppings is recommended for the suppression of blight disease in tomato and okra plants.

Keywords: blight disease, okra, organic compost, suppression, tomato.

Introduction

The term organic compost refers to farming practices that may be called agro ecological, sustainable, or ecological; utilize natural (non-synthetic) nutrient-cycling processes; exclude or rarely use synthetic pesticides; and sustain or regenerate soil quality. These practices may include cover crops, manures, compost, crop rotation, intercropping, and biological pest control [LÓPEZ-GONZÁLEZ & al. 2015].

BOT & BENITES (2013) highlighted the role of organic compost in sustaining the fertility of soil good for production of vegetables and binding the soil for best performance is obtained on well drained fertile soil with adequate organic matter content. Organic matter are very active and important component of the soil. It is the minerals reservoirs for crop production, it also protects the soil against erosion, supplies the cementing substance for desirable aggregate soil formation and loosen the soil for crop production.

According to MEHTA & al. (2014) many researches have enhanced the natural ability of compost to suppress diseases by enriching it with specific disease-fighting microorganisms. This amended compost can then be applied to crops infected by known diseases. Researches has shown that tailored compost significantly reduced or replaced the application of pesticides, fungicides and nematicides which could adversely affect water resources, food safety and workers safety [HOITINK & al. 1997]. The use of amended composts can also be more cost-effective than chemical soil treatments such as methyl bromide. Soil treated with compost

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retains irrigation water better, which lowers water costs. Over the years, farmers in Nigeria have employed the use of composted organic materials such as plant debris and animal manure to add nutrient to the soil in other to increase its fertility [MUHAMMAD, 1998]. Indiscriminate use of fungicides and pesticides in controlling seedling blight diseases has polluted both the environment and the plants. The mechanisms of biological control of plant pathogens by antagonistic bacteria and fungi have been the subjects of many studies in the past two decades [JANISIEWICZ & al. 2000; KILLANI & al. 2011]. Losses due to blight in an unsprayed fields vary enormously from 5-78% without the control of disease causing about 32 million dollars [OLANYA & al. 2009], and 85% loss in okra annually [AHMED & al. 2015].

Therefore, this study will focus on addressing the challenges of controlling seedling blight diseases of tomato (*Solanum lycopersicum* L., family Solanaceae) and okra (*Abelmoschus esculentus* (L.) Moench, family Malvaceae) crop productions. It will emphasize the use of organic control measures which are eco-friendly and cheap to obtain in order to bring about high yields of the crops.

Materials and methods

Experimental location

The experiment was conducted at the Botanical Garden, Department of Biological Sciences, Federal University of Technology, Minna, Niger State, Nigeria on Latitude 9º39'12.38"N and Longitude 6º31'24.79"E. The State is situated in latitudes 8º20' N and 11º30' N and longitude 3º30' E and 7º20' E which is in the Southern Guinea Savanna ecological zone of Nigeria.

Collection and preparation of organic composts

Animal and plant wastes were collected from the Agricultural Research farm of the Department of Agriculture, Ibrahim Badamasi Babangida University Lapai, Niger State. The composts were prepared from rice husks, cow dung, chicken droppings and sawdust by adopting the method of ABO & BADR (2001), LÓPEZ-GONZÁLEZ & al. (2015). Four (4) sterilized perforated labeled polythene bags were separately filled with 30 kg each of rice husks, cow dung, poultry droppings and saw dusts. These mixtures of organic wastes were allowed to decomposed, watered daily, and turned at an interval of two days for the period of 24 days.

In-vivo test of soil amended with the composts on blight disease

Modified method of SHEHU & al. (2008) was used. Three kilogram (3 kg) of sterilized topsoil was bulked thoroughly with compost at 50, 100, 150, 200 and 250 g per polypots of (20 × 30 cm). Amended soil was allowed to decompose for the period of 10 days. Three weeks old seedlings of tomato was transplanted into each polypots while okra seeds were sown directly into amended soil. Twenty one (21) days after sowing the seedlings were inoculated with 20 ml spore suspension containing 5×10^5 spores/ml of inoculum of fungi and bacteria. Control was prepared without amendment and three replicates were prepared. The seedlings were kept on benchtop at the screen house and were observed and evaluated for the symptoms of blight disease. To determine the influence of amendment on the disease development [COVENTRY & al. 2005]. The number of seedlings showing the symptoms in each treatment (y), was divided by the total number of plants in the treatment (z) multiply by 100.

Fungal and bacterial cultures

Aspergillus niger, *Microsporum gypseum*, *Fusarium oxysporum*, *Rhizopus nigricans* and *Penicillium citrinum* were previously isolated from blighted okra and tomato plants and the cultures were maintained on potato dextrose agar (PDA) and nutrient agar slants bottles at 37 ºC. [RAY & al. 2000; SWAIN & RAY, 2009].

Inoculation

Modified method of MUHAMMED & MUHAMMAD (2013) was used to inoculate five weeks old tomato and okra seedlings growing in oven-sterilized topsoil (0.5 cm) contained in 15 cm diameter poly pots were inoculated with the mycelia suspension of the bacterial and fungal isolates. The plants were then placed on top benches in screen house and observed for symptoms of the disease.

Statistical analysis (ANOVA) are performed following a completely randomized design to test the significant effects and means compared using the LSD test ($p \leq 0.05$) as outlined by DUNCAN (1955).

Results and discussion

In-vivo Control of Seedling blight Tomato and Okra using Some Organic Composts

The results of the percentage blight disease suppression on *A. esculentus* (Table 1) amended soil with different compost at different quantities per 3 kg show that as the quantity of amended increased, the disease suppressing also increased. At 200 g and 250 g the suppression was significantly different ($P \leq 0.05$) than other lower quantities. At 250 g it was 67.2% with poultry droppings and 85.0%, 83.6%, 88.2%, and 88.2% with rice husk, cow dung, saw dust and goat dung respectively. Goat dung gave the highest suppression of 88.2% followed by rice husk (85.0%). This trend was also followed by the suppression of the disease in tomato. At 250 g it was 99.0%, 93.7%, 93.7%, 84.0%, and 94.7% with poultry droppings, rice husk, cow dung, saw dust and goat dung respectively (Table 2).

Table 1. Percentage blight disease suppression on Okra grown on soil amended with organic composts

Quantity of organic material g/3 kg soil	Percentage disease suppression				
	Poultry droppings	Rice husks	Cow dung	Saw dusts	Goat dung
50	16.40^{a}	14.80^{a}	20.60^{a}	18.60^{a}	19.80^{a}
100	42.20^{b}	45.80^{b}	43.40^{b}	41.20^{b}	43.00^{b}
150	43.40^{b}	68.00^{c}	53.00^{c}	55.40^{c}	57.80^{c}
200	60.60^{c}	79.00^{d}	68.20^{c}	77.80^{d}	76.60^{d}
250	67.20^{d}	85.00^{e}	83.60^{d}	82.20^{e}	88.20^{e}

Values are mean ± Standard Error of Mean. Mean with the same superscript along row are not significantly different at $P \geq 0.05$.

Table 2. Percentage blight disease suppression on Tomato grown on soil amended with organic composts

Quantity of organic material g/3 kg soil	Percentage disease suppression				
	Poultry droppings	Rice husks	Cow dung	Saw dusts	Goat dung
50	19.00^{a}	19.00^{a}	23.00^{a}	17.70^{a}	21.00^{a}
100	51.33^{b}	51.70^{b}	49.70^{b}	52.00^{b}	53.30^{b}
150	81.33^{c}	70.00^{c}	68.30^{c}	68.00^{c}	71.00^{c}
200	89.00^{d}	83.30^{d}	79.70^{d}	79.70^{d}	82.00^{d}
250	99.00^{e}	93.70^{e}	93.70^{e}	93.70^{e}	94.70^{e}

Values are mean ± Standard Error of Mean. Mean with the same superscript along row are not significantly different at $P \geq 0.05$.

The control of seedling blight disease of tomato and okra have establishing that compost is capable of suppression. Similar report has been reported by [GARRETTE, 1975; CHEN & al. 1998; PHARAND & al. 2002] that compost suppress root rot diseases in vegetables. The findings in this study revealed that suppression increases as increase in the quantity of composts. This is in agreement with the reports of SHEHU & al. (2008) that 80% disease suppression was achieved with soil amended with composts to control onion disease. Out of all the organic composts used to amend the soil, poultry droppings and goat dung gave the highest suppression of the blight disease of tomato and okra probably because the percentage frequency of the soil borne microbes isolated from them were high. The findings has earlier been reported in the control of seedling blight diseases in vegetables by HULL (2002) and WESTPHAL & al. (2016).

Conclusions

The research have revealed that agricultural wastes such as rice husks, cow dung, chicken droppings and sawdusts used as organic material for amended soil are very good in suppression of blight disease in okra while animal droppings used to amend soil produces better result in suppressing blight diseases in tomato.

Notes on contributors

Habiba Maikudi MUHAMMED is a plant biologist with special interest in plant pathology and plant physiology. Her focuses in the plant diseases and management methods of vegetable crops.

Usman Ibrahim HAMZA is a botanist with special interest in plant mycology.

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Conflict of interests

The authors declare that there are no conflicts of interest related to this manuscript.

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EFFECTS OF COKE ON GERMINATION AND GROWTH OF *VIGNA RADIATA* (L.) R. WILCZEK, *VIGNA MUNGO* (L.) HEPPER AND *VIGNA UNGUICULATA* (L.) WALP.

Rasheeda JAMALI[1], Muhammad ZAFAR IQBAL[1], Muhammad SHAFIQ[1*]

[1] Department of Botany, University of Karachi, Karachi – Pakistan.
* Corresponding author. E-mail: shafiqeco@yahoo.com

Abstract: Coke production is one of the important sources of pollution from steel industries. The effects of coke (sludge of iron and steel) on seed germination and growth of *Vigna radiata* (L.) R. Wilczek, *Vigna mungo* (L.) Hepper and *Vigna unguiculata* (L.) Walp. were recorded. The seed germination percentage of *V. radiata* was increased with increase in coke concentration treatment at 25-50% as compared to control treatment in petri dish. Seedling length and root growth performance of *V. radiata* was also slightly increased with the increase in coke concentration treatment at 75 and 100% as compared to control. The seed germination percentage and root length of *V. mungo* was reduced with increase in coke concentration treatment at 25%. The obtained results showed more positive effects of coke on seed germination and seedling growth performance of *V. unguiculata* than *V. mungo* and *V. radiata*. Meanwhile, experiment showed decrease in seedling vigor index of *V. radiata* and *V. mungo* with the increase in concentration of coke at 100% as compared to control. A gradual increase in seedling vigor index for *V. unguiculata* with the increase in coke concentration was observed. The coke extracts treatment influence on the seedling tolerance indices of *V. radiata, V. mungo* and *V. unguiculata*. The better tolerance to coke treatment in all bean crops might be mainly due to some resistance potential ability of seedlings to coke concentration.

Keywords: Bean crop, coke, ecotoxicity, seedling vigor index, tolerance.

Introduction

Naturally found coal is converted to coke which is suitable for metallurgical industries [GHOSE, 2002]. Cokes wastewater has been considered the most toxic one to be treated before being discharged into the environments [LEE & PARK, 1998; ZHANG & al. 1998]. Steel industries generate various wastewaters during the manufacture and processing of iron [KIM & al. 2007]. Significant quantities of sludge and slag are generated as waste material or byproduct every day from steel industries. They usually contain considerable quantities of metals and other materials [DAS & al. 2007]. Steel is one of the most utilized and recycled materials within the global economy [ZHANG & al. 2009]. The iron and steel industry have generated significant amounts of hazardous waste and has emitted vast quantities of toxic pollutants into the atmosphere. Metal dusts, slag, carbon monoxide, nitrogen oxides, and ozone are examples of substances generated during the steel making process and coke oven emissions contain harmful substances like polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), benzene, particulate matter, and dioxins [ROSENFELD & FENG, 2011]. The production of coke is an integral component of the steel manufacturing process [WALSH & THORNLEY, 2012]. Industrial activities, including iron steel metallurgy, are major source of atmospheric heavy metals emissions like copper (Cu), zinc (Zn), nickel (Ni), lead (Pb), chromium (Cr) and cadmium (Cd) [BOLONIAZ & BULINSKI, 1984; GRITSAN & BABIY, 2000; KAMINSKI & LANDSBERGER, 2000; ADAMO & al. 2002; SALEMAA & al. 2001; VENDITTI & al. 2000;

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SHAFIQ & IQBAL, 2012; SHAFIQ & al. 2019]. These toxic airborne metals settle on soil surfaces and vegetation canopies [KADEM & al. 2004].

The iron and steel industry are a resource and energy intensive industry [MA & al. 2014] and an important source of high level of pollution and emissions [LIU & al. 2014]. There are few reports available on the effects of coke on germination and growth of plants. Coke oven emissions are known as human carcinogen, which is a complex mixture of polycyclic aromatic hydrocarbon [ZHAI & al. 2012]. Coke is used chiefly to smelt iron ore and other iron bearing materials in blast furnaces, acting both as a source of heat and as a chemical reducing agent, to produce pig iron, or hot metal [AVIDSYS, 2021]. Coking is one of the most important emission sources of (PAHs) polycyclic aromatic hydrocarbons (PAHs) [MU & al. 2014]. Coke oven emissions contain literally several thousand compounds, several of which are known carcinogens and/or cocarcinogens (including polycyclic organic matter from coal tar pitch volatiles, beta-naphthylamine, benzene, arsenic, beryllium, cadmium, chromate, lead, nickel sub sulfide, nitric oxide and sulfur dioxide [GRAHAM & HOLTGRAVE, 2014].

An external and internal factor, ranging from soil humidity, diseases, pests, competition, and pollutants to individual genes and plant age influence plant phenology [MENZEL, 1999]. The growth and physiological effects of coke on *Triticum aestivum* and *Deschampsia caespitosa* in a greenhouse study showed the stress symptoms such as reductions in transpiration (45-91%) and stomatal conductance rates (44-92%) in *T. aestivum*, biomass in *T. aestivum* (5-83%) and *D. caespitosa* (43-90%), photosynthetic pigments in *T. aestivum* (32-68%) and *D. caespitosa* (33-44%) and proline concentrations in *D. caespitosa* (77-97%) observed [NAKATA & al. 2011]. The impact of industrial effluent from 4 different surface treatment companies using seeds of the lettuce *Lactuca sativa* were measured for bioassay test. The results were used to compare the overall toxicity of the different effluents containing copper, nickel, zinc and aluminum. The germination tests confirmed that mixtures of metals have higher toxicity than the sum of their separate constituents. These biological tests are found cheap, easy to implement, reproducible and highlight the effects caused by effluent treated with the methods commonly applied in industry today [CHARLES & al. 2011]. Plant growth and development can be highly restricted by environmental stressors such as cadmium (Cd) pollution. The effects of environmentally realistic Cd exposure (5 and 10 μM) on the alternative respiratory chain in *Arabidopsis thaliana* using a kinetic exposure setup was reported [KEUNEN & al. 2013]. The effects of atmospheric PM-selected components (brake dust – BD, pellet ash – PA, road dust – RD, certified urban dust NIST1648a – NIST, soil dust – S, coke dust – C) affected the root morphology of *Arabidopsis thaliana* development due to inducive oxidative stress [PIACENTINI & al. 2019].

Vigna genus has wide distributions in tropical areas of the world along with many species likewise *Vigna radiata* (L.) R. Wilczek, *Vigna mungo* (L.) Hepper and *Vigna unguiculata* (L.) Walp. The behavior of 6 genotypes of cowpea (*Vigna unguiculata* (L.) Walp. and 5 genotypes of mung beans (*Vigna radiata* (L.) R. Wilczek), of Chinese origin, in comparison with the indigenous cowpea genotype, Aura 26 in the conditions of the sandy soils of SCDCPN Dăbuleni, between 2016-2018 in Romania was carried out in sandy soil. The physiology of the studied plant species revealed a better adaptability of the genotypes of cowpea as compared to mung beans. The obtained results at the harvest level showed the production of grains in the range of 670-2511.9 kg/ha in the cowpea genotypes and 1861.9-2209.5 kg/ha at the genotypes in the mung beans [DRĂGHICI & al. 2019].

The coke productions play an important role in steel industries. Coke release high concentration of toxic pollutants in the environment and can inhibit or decrease the germination and growth of plants. Many researchers have drawn their attention on the effects of coke on

plant growth. *Vigna radiata* (L.) R. Wilczek, *Vigna mungo* (L.) Hepper and *Vigna unguiculata* (L.) Walp. are an important legume crops and cultivated in larger area of Pakistan. Therefore, the objective of this study was to investigate the effects of coke on seed germination and seedling growth of crops by using different levels of coke and compared it with control.

Material and methods

The coke sample (sludge of iron and steel) was collected from Pakistan steel mill, Karachi, Pakistan. Pakistan steel mill is one of the enormous and gigantically expanded industrial complex in the country that is located at a distance of 40 km Southeast of Karachi at Bin Qasim near Port Muhammad Bin Qasim. It is one of the largest industrial complexes in Pakistan as well as in South Asia.

The composition of coke was high fixed carbon (80-85%), low ash (10-15% ash), low volatile matter (2% maximum) and low phosphorous (0.3% maximum). The seeds of *Vigna radiata* (L.) R. Wilczek, *Vigna mungo* (L.) Hepper and *Vigna unguiculata* (L.) Walp. were obtained for the experiment from the local market. The petri dishes and filter paper were sterilized in an autoclave to prevent microbial contamination. The seeds were imbibed for half an hour to break the dormancy of seed and then transferred into medium sized petri dishes on Whatman filter paper. In each petri dish ten seed were placed. Seed germination was tested on moist filter paper. 10 g coke was added in 100 ml distilled water and that mixture was kept for 24 hours and then filtered. This filtered solution was assumed to be the standard solution which was 100%. From this standard solution further dilution 25%, 50%, 75% and 100% were made in distilled water. Distilled water was used as control for the experiments. Each treatment had three replicates. Initially, seeds were treated with 5 ml of respective extract solution and later replaced with 2 ml of fresh extract solution on alternate days. Controls were maintained by moisting the filter paper with 5.0 ml distilled water. All petri dishes were kept in dark for seed germination and later on shifted in light (200 volts). Germination percentage was recorded daily. Seedlings were removed from the petri dishes after 10 days growth. Three best seedlings were selected from each Petri dish for mean values. The experimental design was completely randomized. Growth parameter in terms of root, shoot and seedling length were measured with the help of meter scale. The seedlings were dried in an oven at 80 °C for 24 hours until the seedlings were completely oven dried. The seedling vigor index was also determined according to BEWLY & BLACK (1982) and Tolerance indices of seedlings were determined with the help of following formula:

Tolerance Indices = Mean root length of coke extract / Mean root length of control without coke extract × 100

The data obtained were statistically analyzed on personnel computer using COSTAT version 3.

Results

The effects of different concentration (0, 25, 50, 75 and 100%) of coke extract on seed germination and seedling growth of three different bean crops viz. *Vigna radiata*, *Vigna mungo* and *Vigna unguiculata* were recorded (Table 1; Figures 1-2). Seed germination of *V. radiata* did not show any significant changes on seed germination percentage when treated with different concentration of coke as compared to control. Coke extract treatment at 25 and 50% increased the rate of seed germination in *V. radiata* as compared to control. Coke treatment at

25, 50, 75 and 100% showed 100%, 100%, 90 and 96.66% rate of seed germination in *V. radiata* as compared to control (96.66%). The coke treatment not showed any significant ($p<0.05$) sign of phytotoxicity on root, shoot, seedling growth and root / shoot ratio of *V. radiata* as compared to control treatment (Table 1).

The effects of different concentration of coke extract treatment on seed germination, root, shoot, seedling length, root / shoot ratio and seedling dry weight of *V. mungo* were observed (Table 1). Increasing concentration of coke at (25%) decreased seed germination percentage (90%) of *V. mungo* as compared to control. Low percentage of seed germination (96.66%) also found at 100% coke treatment as compared to control (100%). Coke treatment at all level showed no significant changes in root, shoot and seedling length and seedling dry weight of *V. mungo* as compared to control. Similar to seed germination, root, shoot and seedling length was decreased at 100% coke extract treatment as compared to control. It was observed that coke treatment at 25% showed significant ($p<0.05$) changes in root / shoot ratio of *V. mungo.*

A pronounced effect in seed germination percentage, root, shoot, seedling length, seedling dry weight and root / shoot ratio of *V. unguiculata* was found in response to different concentration of coke extract treatment as compared to control (Table 1). There was an increase in seed germination percentage of *V. unguiculata* with increasing coke extract treatment. Seed germination of *V. unguiculata* was significantly ($p<0.05$) increased in 25% to 100% coke extract treatment by 73.33%, 90%, 76.66% and 86.66%) as compared to control (40%), respectively. Coke extract treatment at all concentration did not show any significant effects in shoot growth of *V. unguiculata* as compared to control. Root growth of *V. unguiculata* was found significantly increased in coke extract treatment at 50% (9.36 cm) as compared to control (5.60 cm). A significant result on seedling dry weight of *V. unguiculata* by 25% concentration of coke treatment was observed. Root shoot ratio significantly affected by 25% coke treatment.

Table 1. Effects of different concentrations of coke extract (0, 25, 50, 75 and 100%) on seed germination, seedling growth and yield of some bean crops (*Vigna radiata*, *Vigna mungo* and *Vigna unguiculata*)

Crop species	Coke Treatments (%)	SG (%)	Root length (cm)	Shoot length (cm)	Seedling size (cm)	Root/shoot Ratio	Seedling dry weight (g)
Vigna radiata	00	96.66a±3.3	6.73a±1.6	18.66a±1.2	25.46a±2.6	0.35a±0.06	0.026ab±0.0
	25	100.00a±0.0	7.80a±2.0	17.76a±1.4	25.56a±3.3	0.42a±0.08	0.020a±0.0
	50	100.00a±0.0	5.40a±0.4	21.03a±0.7	26.460a±0.4	0.25a±0.02	0.036b ±0.0
	75	90.00a±5.8	8.36a±1.4	21.86a±1.8	30.30a±2.6	0.38a±0.06	0.026ab±0.0
	100	96.66a±3.3	7.10a±0.6	19.43a±1.3	26.53a±1.8	0.35a±0.02	0.016a±0.0
Vigna mungo	00	100.00a±0.0	7.85a±0.5	16.50a±2.5	24.30a±2.0	0.49ab±0.1	0.030a±0.000
	25	90.00a±5.8	6.70a±0.3	17.56a±1.2	24.26a±0.9	0.38a±0.08	0.023a±0.003
	50	100.00a±0.0	8.76a±0.8	15.70a±1.2	24.53a±1.9	0.55b±0.02	0.026a±0.003
	75	90.00a± 5.8	6.66a±1.1	17.96a±0.6	24.70a±1.7	0.36a±0.06	0.023a±0.003
	100	96.66a±3.3	7.66a±0.3	16.10a±0.4	23.83a±0.5	0.47ab±0.0	0.020a ±0.005
Vigna unguiculata	00	40.00a± 10.0	5.60ab±0.3	11.05a±0.3	16.60a±0.0	0.50ab±0.04	0.045a±0.01
	25	73.33b± 3.3	5.83ab±0.2	14.20a±1.2	20.06ab±1.2	0.41a±0.03	0.096b±0.00
	50	90.00b± 5.8	9.36c±0.6	14.13a±2.4	23.53b±2.8	0.68c±0.08	0.080ab±0.01
	75	76.66b± 6.7	4.50a±0.3	10.40a±1.1	14.96a±1.2	0.44a±0.05	0.066ab±0.01
	100	86.66b±8.8	6.46b±0.8	10.06a±1.0	16.53a±1.8	0.63bc±0.01	0.090b±0.01

Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at <0.05 level. ± Standard Error, SG= Seed germination

A positive increase and decrease in tolerance index in seedlings of bean crops to different concentrations of coke extract treatment was recorded (Figure 1). The lowest tolerance indices 84.84% and 80.35% in seeding of *V. mungo* and *V. unguiculata* was recorded with the coke treatment at 76% as compared to control. An increase in concentration of coke treatment at 100% showed better tolerance index 105.49% and 115.35% in seedlings of *V. radiata* and *V. unguiculata* as compared to control.

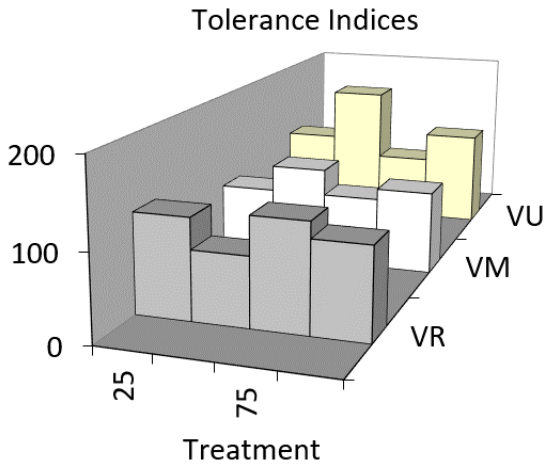

Figure 1. Tolerance indices of seedlings of bean crops (VR = *Vigna radiata*, VM = *Vigna mungo* and VU = *Vigna unguiculata*) at different concentration of coke extract 25, 50, 75 and 100%

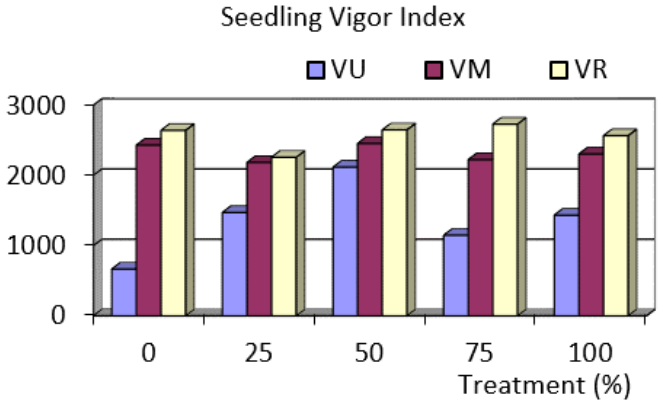

Figure 2. Seedling Vigor Index (S.V.I.) of seedlings of bean crops (VR = *Vigna radiata*, VM = *Vigna mungo* and VU = *Vigna unguiculata*) at different concentration of coke extract (0, 25, 50, 75 and 100%)

eedling vigor index (SVI) is the potential of seed germination and seedling size against the toxicity and tolerance of coke extract having pollutant. Results indicate a decreasing trend of SVI in seedlings obtained through different concentrations of coke treatment, when compared with control. It was found that the coke extract treatment affected on seedling vigor index of *V. radiata*, *V. mungo* and *V. unguiculata* (Figure 2). Seed germination and seedling growth tests

using different concentration of coke extract on crop bean showed different response of seedling vigor index. Max seedling vigor index (2641) was found in control seedlings of *V. radiata* was found. Low seedling vigor index (2564) was found in seedlings of *V. radiata* at 100% coke treatment. Reduced seedling vigor index possessed by *V. mungo* with increasing concentration of coke dust is probably due to less tolerance. Retardation in seedling length in *V. mungo* due to toxic concentrations of coke extract may be an important reason for reduction in seedling vigor index at 100% coke treatment.

Discussion

Coke is an important raw material in the iron and steel industry [BOND & al. 2013]. Present study revealed the toxic effects of coke on germination and growth of some bean crops species viz. *V. radiata*, *V. mungo* and *V. unguiculata* mainly due to high concentrations of coke extract treatment as compared to control. Comparative analysis of the obtained data using different growth parameters (seed germination, root, shoot and seedling length, root / shoot ratio, seedling dry weight, seedling vigor index and tolerance indices) of *V. radiata*, *V. mungo* and *V. unguiculata* can allow the validation on understanding the toxic nature of coke as a pollutant. Coke extract treatment produced positive and negative effects on the growth of selected bean crop species as compared to non coke extract treated plants. Germination and early seedling growth have been regarded as critical phases, which are greatly influenced by stressful conditions [SHAH & DUBEY, 1995]. Concerning the impact of coke dust pollution on seed germination percentage of *V. radiata*, the same trend detected in the reduction in seed germination of *V. mungo.* Whereas, coke treatment at all concentration increased the seed germination percentage of *V. unguiculata* which might be due to its tolerance to coke extract to some extent as compared to control. Reduction in plant height of *V. mungo* and *V. unguiculata* showed that the losses generally can be attributed to the coke extract treatment which contained toxic metals. Coke concentration added at higher concentrations (100%) produced significant ($p<0.05$) impact on seedling growth performance of *V. unguiculata* as compared to control. Similarly, Petro-coke treatment at the rate of 2 g petro coke m^{-2} d^{-1} for 65 d age of green gram (*Phaseolus aureus*) plants with respect to transpiration rate, mineral accumulation, and contents of ascorbic acid, protein, carbohydrate, pigments, root and shoot lengths, number of leaves, nodules, flowers, and pods were found reduce in Petro-coke-treated plants [PRASAD & RAO, 1981].

In present studies the seed germination and seedling growth elongation tests using as seedling vigor index for all bean crop showed variation in seedling indices value as compared to control. The study reported herein reveals that the reductions in seedling dry weight of *V. radiata*, *V. mungo* and *V. unguiculata* was due to reduction in root and shoot growth as the concentration of coke treatment increased in substrate as compared to control. Growth characteristics such as root shoot and seedling length decreased and treated seedlings of bean crops. The seedling vigor index of *V. radiata* (2564) and *V. mungo* (2303) was highly decreased with 100% coke treatment as compared to control 2640 and 2430, respectively. This effect was dose dependent, and was more significant at higher concentration as compared to control. The effect of steel factory effluent on seed germination and seedling, growth of *Phaseolus mungo* cv. T-9, showed that increasing concentration of effluent induced a gradual decrease in germination percentage [KUMAR, 2006].

Conclusion

Overall results showed that germination and growth of two *Vigna* species *V. radiata* and *V. mungo* was not significantly ($p<0.05$) affected by all levels of coke concentration treatment as compared to control. Coke treatment was found most toxic for *V. unguiculata* seedlings than *V. radiata* and *V. mungo*. The study of plant behavior in coke pollution allows the identification and selection of pollution indicating species. On the basis of this study, it could be concluded that seedling growth of *V. unguiculata* was found to be more affected than *V. radiata* and *V. mungo* by coke pollution, which might be due to the presence of different toxic pollutants in coke. *V. unguiculata* was found highly affected by coke treatment. *V. mungo* was found moderately affected, while, *V. radiata* was found less affected by coke application. It is suggested that the variation in the seedling growth parameter of bean crops might be used as tolerance indicator for pollution by coke. The coke treatment induced decrease in growth of *Vigna unguiculata* plants may be due to toxic pollutants present in the medium and their interference with seedling growth and reducing its growth rate which in turn affects the uptake of water and this influences growth of the entire plant resulting in biomass production. Furthermore, this study can be helpful in building a baseline data for future long term field studies essential for developing coke management guidelines.

Notes on contributors

Rasheeda JAMALI, MSc., is botanist and research scientist with special interest in plant ecology and impact of industrial pollution on plant growth.

Professor Dr. Muhammad Zafar IQBAL Ph.D. is ex chairman Department OF Botany, University OF Karahi, Pakistan. The area of research interest are plant ecology, sociology, dynamic ecology, climatology, soil science and environmental pollution. Dr. M. Z. Iqbal has published 200 scientific research papers in national and international scientific peer reviewed renowned scientific journals and supervised 58 research project/ thesis.

Dr. Muhammad SHAFIQ Ph.D. is a research scholar with area of research interest on the plant ecology/environmental pollution/heavy metal toxicity and tolerance in plants and seedling growth behavior of plants. Dr. M. Shafiq has published more than 110 scientific research papers in national and international scientific peer reviewed renowned scientific journals. He has also published two books on “Impact of Automobile Pollutants on Plants” and “Poison Land, “Vegetation of disturbed and polluted areas in Pakistan".

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NUTRITIONAL AND ANTI-NUTRITIONAL PROPERTIES OF THE SEEDS OF SIX SELECTED NIGERIAN CUCURBIT GERMPLASM

Ibrahim Umar KARAYE[1*], Muhammad HAYATU[2], Yahya MUSTAPHA[2], Lawan Abdu SANI[2]

[1] Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto – Nigeria.
[2] Department of Plant Biology, Bayero University, Kano – Nigeria.
* Corresponding author. E-mail: ikaraye09@yahoo.com

Abstract: Biochemical characterization of the seed extracts of the seeds of six selected Nigerian cucurbit germplasm was carried out using standard biochemical procedures. All treatments were replicated three times and the results presented as mean ± S.E. On proximate analysis, percentage moisture ranged from (3.83±0.10%) to (5.17±0.65%) with the highest in *Lagenaria breviflora* (5.17±0.65%) while the least value (3.83±0.10%) was obtained in *Cucurbita pepo*. Crude protein composition ranged from (32.66-0.11) to (35.94±2.89%) the highest (35.94±2.89%) in *Lagenaria breviflora* while the least (32.66-0.11%) was for *Cucurbita maxima*. Crude carbohydrate ranged from (27.33±0.20) to (36.66±0.2) with the highest (35.66±0.20) in *Citrullus lanatus* while the least (27.33±0.20) was for *Cucurbita pepo*. Crude lipid analysis showed the range of values (31.33±0.27%) to (24.50±0.29%) with the highest in *Cucumeropsis mannii* while *Citrullus lanatus* had (24.50±0.29%). Crude fiber contents ranged from (15.83±1.23%) to (5.68±0.87%) with the highest (15.83±1.23%) in *Cucurbita maxima* while *Lagenaria breviflora* had (5.83±0.07%). Ash content revealed the range of values (9.17±0.52) to (5.86±0.29%) with the highest (9.17±0.52%) in *Cucurbita maxima* while the least (5.86±0.29%) was in *Lagenaria breviflora*. Analysis of available energy (kcal.) revealed the range of values (555.97±2.45) to (500.90±2.21 kcal) with the highest in *Cucumeropsis mannii* while *Citrullus lanatus* had (500.90±2.21 kcal). With significant difference ($P \leq 0.05$) in the contents of crude carbohydrates, lipids, crude fiber and available energy in (kcal). Mineral analysis revealed nitrogen (N) composition with a range of values (3.11±0.38) to (5.75±0.97 g/100 g) with the highest in *Lagenaria breviflora* while the least (3.11±0.38) g/100 g was in *Citrullus lanatus*. Sodium (Na) analysis revealed the range of values (11.83±0.79) to (31.67±2.43) g/100 g with the highest in *Cucurbita pepo* (31.67±2.43) g/100 g while *Cucumeropsis mannii* had the least (11.83±0.79 g/100 g). Magnesium (Mg) analysis similarly revealed range of values (22.00±1.18) to (34.60±2.17) g/100 g with the highest in *Cucurbita maxima* (34.60±2.17) g/100 g while the least value (22.00±1.18 g/100 g) was in *Lagenaria siceraria*. Similarly, calcium (Ca) analysis revealed the presence of the valuable mineral with a range of values (10.83±1.32) to (36.67±2.76) g/100 g) with highest (36.67±2.67 g/100 g) in *Cucurbita maxima* while the least (10.83±1.32 g/100 g) was obtained in *Cucurbita maxima*. Contents of phosphorus (P) revealed the range of values (4.50±0.66) to (5.71±0.98) g/100 g with the highest (5.71±0.98 g/100 g) in *Lagenaria siceraria* while least (4.50±0.66) g/100 g was obtained in *Citrullus lanatus*. More so, analysis of potassium (K) revealed a range of values (1,266.67±3.67) to (2,266.70±3.69 g/100 g) with the highest (2,266.70±3.69) g/100 g in the seeds of *Cucurbita maxima* while the least (1,266.67±3.67 g/100 g) was obtained in *Citrullus lanatus*. With significant difference ($P \leq 0.05$) among the species in the contents of sodium, magnesium, calcium and potassium. Based on phytochemicals analyzed, tannin content was highest in the seeds of *Citrullus lanatus* with (29.66±2.26) while the least value (11.62±0.84) was obtained in *Cucurbita maxima*. Volatile oil analysis ranged from (25.47±1.90) in *Lagenaria siceraria* while the least (11.68±0.69) was obtained in *Cucurbita pepo*. There is significant difference ($P \leq 0.05$) in the contents of phenol between *Citrullus lanatus* and the other species. Also, similar trend have been found to occur in the composition of saponin glycosides, as well as the volatile oil. Based on these results, it can be asserted that seeds of the members of Cucurbitaceae are high in proximate, minerals and phytochemicals that could be harnessed as food by man as well as used in animal feed formulation strategies in addition to various vital applications of the phytochemicals evaluated in the seeds.

Keywords: Antinutritional, *Citrullus*, *Cucumeropsis*, *Cucurbita*, feed - formulation, *Lagenaria*, nutritional.

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Introduction

Cucurbits are among the many plant species endowed with various potentialities that if properly harnessed, could help in achieving food security and generate job opportunities to our teeming populace as evident in Latin America and Asia. It is a known fact that nutritional contents of plant species could be affected by a number of environmental factors in addition to other parameters. Thus, is highly imperative to provide reports on the profile of each of the plant in its own geographical area for people to appreciate so, in addition to creating awareness to the populace on the importance of utilizing the locally available plant resource as evident in developed and some developing countries in the world. Cucurbits refer to those plant species placed in the large family of Angiosperm, known as Cucurbitaceae. Members of the gourd family that includes hundreds of species of vines with coiled climbing tendrils characterized by production of the most unusual fruits in the world. Cucurbitaceae family is among the abundant crop domesticated and grown at wild in most tropics (especially in Nigeria). Members of Cucurbitaceae are known to be very useful, serving as food, ornamental purposes, utensils, fuel as well as medicinal purposes [ABASCAL & YARMELL, 2005]. In Nigeria, Cucurbitaceae is represented by 21 genera, and 41 species many of which are of considerable economic importance. Indigenous people of Nigeria traditionally utilize a wide range of these plant species as food and medicine. Archeological evidence has indicated that cucurbits were present in ancient and prehistoric cultures. *Lagenaria* for instance, was associated with man as early as 12, 000 BC in Peru. Archeological expeditions in the Oaxaca region of Mexico have reported *Cucurbita pepo* to be associated with man as early as 8500 BC and cultivated by 4050 BC [ESQUINAS-ALCAZAR & GULICK, 1983].

Similarly, written Chinese records describing the use of cultivated cucurbits have been found as from as early as 685 BC. American Indians cultivated squash in pre-Columbian times. Cucurbits are among the economically most important vegetable crops worldwide and are grown in both temperate and tropical regions [PITRAT & al. 1999; BISOGNIN, 2002].Despite their agronomic, cultural and culinary importance, there is scanty of information on these species from research and development and are often categorized as orphan crops [HAIM, 2007]. Members of Cucurbitaceae are known to be very useful, serving as food, ornamental purposes, utensils, fuel as well as medicinal purposes. In Nigeria, Cucurbitaceae is represented by 21 genera, many of which are of considerable economic importance. Indigenous people of Nigeria traditionally utilize a wide range of these plant species as food and medicine. Archeological evidence has indicated that cucurbits were present in ancient and prehistoric cultures. *Lagenaria* for instance, was associated with man as early as 12, 000 BC in Peru. Archeological expeditions in the Oaxaca region of Mexico have reported *Cucurbita pepo* to be associated with man as early as 8500 BC and cultivated by 4050 BC [ESQUINAS-ALCAZAR & GULICK, 1983]. Similarly, written Chinese records describing the use of cultivated cucurbits have been found as from as early as 685 BC. American Indians cultivated squash in pre-Columbian times.

Cucurbits in nutrition

The necessity to feed the rapidly expanding human population remains the major task to accomplish. In order to have a healthy population that can promote development, the relationship between food, nutrition and health must be reinforced [OBI & al. 2004]. This becomes imperative since large segments of the population especially in developing countries suffer from protein malnutrition and projections based on current trends indicate a widening gap between human population and protein supply [ABDELATIEF, 2011]. This explains why today,

food shortage remains a serious problem especially in Africa with about 36 million humans starving to death every year coupled with childhood malnutrition, which contribute to the global burden of disease [MURRAY & LOPEZ, 1997].

There is a need to develop other sources of concentrated plant proteins [VOSE, 1980] which ideally should be crops that are widely grown in tropical countries [ABDELATIEF, 2011]. The consumption of whole-plant foods slows digestion and allows better absorption, and a more favourable balance of essential nutrients per calorie, resulting in better management of cell growth, maintenance, and mitosis as well as better regulation of appetite and blood sugar. Many plant proteins usually in the form of protein extracts or seed flours are being investigated and tested for new products such as low cost fabricated foods that are nutritious, attractive and acceptable to consumers. Seeds of cucurbits have been reported to have nutritive and calorific values, which make them necessary in diets [HAMZA & al. 1995].

Botany of plants studied

The family Cucurbitaceae consists of members commonly known as gourds that include cucumbers, squashes, luffas and melons. Cucurbits are among the economically most important vegetable crops [LOUKOU & al. 2007]. The family is distributed around the tropics, where those with edible fruits were among the earliest cultivated plants in both the old and new worlds. It is a large family with about 125 extant genera and 960 species [JEFFREY, 1978]. The family is represented by 21 genera and 41 species in Nigeria [HUTCHINSON, 1954]. Melon, pumpkin and gourd seeds are reported to be rich in protein and could be useful in fortification of food products [ABIODUN & ADELEKE, 2010].

***Citrullus lanatus* (Thunb.) Matsum. & Nakai: the watermelon**

Watermelon, otherwise locally known as 'Kankana' in Hausa language is considered as a prominent member of the large family Cucurbitaceae. The plant is believed to have originated in southern Africa, particularly the Kalahari desert, where the plant is found growing wild with a high genetic diversity there, resulting in sweet, bland and bitter forms. There are a number of facts to prove that watermelon was indigenous to tropical Africa and increase in popularity of the species became widespread early in history and today; it is cultivated throughout the world The crop can be described as a deep-rooted with ability to survive in relatively dry conditions of the northern part of the country, the relatively dry conditions of the northern part of the Nigeria supports higher production yield of crop. In addition, better yield is obtained with irrigation in the region [ILELABOYE & PIKUDA, 2009]. The pulp of the fruit is highly cherished and eaten ripe. In some parts of the world as Nigeria and Middle East countries, the seeds are used for extracting cooking oil while in majority of the countries, its utility is restricted for additive purpose. The seeds are used as condiment, garnisher and thickener in soups; fat binder, flavourants and as snack in most parts of the world [EL-ADAWY & TAHA, 2001].

Watermelon is characterized by sweet and juicy fruit containing high content of lycopene [LIU & al. 2013]. The production of watermelon accounts for approximately 9.5% of total vegetable production in the world [FAO, 1973]. Watermelon fruit contains a variety of nutrients including fiber, vitamins, antioxidants and minerals, which are essential for human health. The commercial quality of watermelon fruits is determined by many factors such as fruit size and shape, rind color and thickness, flesh color and texture, sugar content, aroma, flavor and nutrient composition. The sweet, colored and juicy fruit makes it the model system for the study of sugar and carotenoids metabolism of non-climacteric fleshy fruit [GUO & al. 2011].

***Cucurbita maxima* Duchesne: the winter squash**

C. maxima its common name is winter squash or 'kabewa' in Hausa language. The species is believed to have originated from South America some 4,000 years ago. This cucurbit is a perennial creeping herb, that can grow up to 10 m long, unisexual with the calyx joined to the corolla while the male ones having the stamens connected into column. Hirsute stems, lobed. Its leaves are 20-30 centimeters wide. It generally grows at low altitudes in hot climates with heavy rainfall, but some varieties have been found above 2,200 meters. Pumpkin seeds are flat, dark green and some are enclosed in a yellow-white husk although some varieties of pumpkins produce seeds without shells. The seeds have a malleable, chewy texture and a subtly sweet, nutty flavor that are delicious and nutritious. The fruits are variable in size, color, shape and weight. The seeds of pumpkins are becoming popular in Africa for their medicinal properties and they are eaten as antihelmintic. They are characterized by moderately hard rind, with a thick, edible flesh below, and a central seed cavity. There are numerous seeds in the fruits [MUHAMMAD, 2004]. The seeds are plump, covered with a testa that serves a protectant around the seeds. The plants grow in vines that spread low across the ground with large leaves and yellow-orange flowers. Pumpkin seeds are rich with both essential and non-essential amino acids.

***Cucurbita pepo* L.: the pumpkins**

Cucurbita pepo L. is one of the oldest known cultivated species. The plant is native of Northern Mexico and southwestern and eastern USA. *C. pepo* is an annual creeping or climbing plant with 5-angled stems up to 15 m long. The shallow root system is branched, growing from a well-developed taproot. The stems are rugged and bristle, branching 6-24 cm long, usually rooting at the nodes. The plant bears tendrils at 90 degrees to the leaf insertion, which are coiled and 1-6 branched. On bushy plants, tendrils may be poorly developed. The leaves are simple, alternate, broadly ovate to deltoid, basally cordate, apically acute, palmately lobed with 5-7 lobes, marginally toothed, scabrous, palmately veined, 20-30 cm long, and 10-35 cm broad leaves with 5-25 cm petioles that are ovate-cordate to sub orbicular –cordate, with or without white spots on the surface and have three to five rounded or obtuse, apiculate lobules, the central one bigger than lateral ones.

Pumpkin is monoecious and bears solitary actinomorphic flowers (-10 cm across) that produce nectar. Male flowers are long and pedicellate and have a campanulate calyx that is 5 to 10 mm long and almost as wide 5-15 mm, 1-2 mm linear sepals and a tabular campanulate corolla that is rather broader towards the base, 6 to 12 cm long and yellow to pale orange. Flower has three stamens. Female flowers have thick peduncles, 3-5 cm long, an ovoid to elliptical, multilocular ovary, sepals that are occasionally foliaceous and a corolla is somewhat larger than that of the male flowers. They have a thickened style and three lobate stigmas. Fruits are variable in shape, color and size. It can be oval, cylindrical, flattened, globular, scalloped, fusiform, and or tapering curved or straight neck on one or both ends. Fruits are variable in size, color, shape and weight [MUHAMMAD, 2004]. They have a moderately hard rind, with a thick, edible flesh below, and a central seed cavity. There are numerous seeds in the fruit. Most seeds are plump and tan or soft white. They are all covered with a testa that serves as protectant around the seeds [ROBINSON & DECKER-WALTERS, 1997]. A pumpkin seed orange flesh is eaten for human consumption such as soup, purees, jams, and pies throughout the world [MUHAMMAD, 2004]. It has been domesticated in the New World for thousands of years. Some authors maintain that *Cucurbita pepo* is derived from *Cucurbita texana*, while others suggest that *C. texana* is merely feral *Cucurbita pepo*. They have a wide variety of uses, especially as a food source and for medical conditions. *Cucurbita pepo* seems more closely related to *Cucurbita fraterna*, though disagreements exist about the exact nature of that connection. It is considered a delicacy in

traditional local cuisines such as for pumpkin soup, potato salad. Pumpkin seed oil contains fatty acids, such as oleic acid and alpha-linolenic acid.

***Lagenaria breviflora* (Benth.) Roberty: the wild colocynth**

Lagenaria breviflora (Benth.) Roberty is another member of the family Cucurbitaceae. Common names wild colocynth (English) local names in Hausa include gojin jima; gunar jiímaà. *Lagenaria breviflora* is a perennial climber ascending to the forest canopy, occurring from Senegal to West Cameroons, and generally widespread in tropical Africa. The leaves very scabrid and sandpapery. The stem when broken has an unpleasant smell, and a decoction from it is said to be used in Nigeria for headache. The root is used in Tanganyika as a purgative and in Nigeria as a vermifuge. The fruits are dark green with creamy blotches, and are ovoid to 9 cm long. They are commonly used in Nigeria for depilating hides. The seeds are grey and 5 mm long by 3 mm wide. They are edible but similarly bitter, nutty-flavored, and rich in fat and protein. They are eaten whole or used as an oilseed. The fruits are cut up, put in water with lye of wood-ashes and in this hides are left to soak for one or two days. Alternatively the hides are stretched and the inner surface scraped clean, and then the fruit pulp is rubbed in followed by a free application of dry wood-ash. Depilating is done after the folded hide has been steeped for a further day in the lye of wood-ash. The fruit is perhaps also used in bating-bath to prepare skin to receive the tanning material [ADEDAPO & al. 2013].

***Lagenaria siceraria* (Molina) Standl.: the bottle gourd**

The origin of bottle gourd is acknowledged to be Africa, although archeological evidence has placed it in Peru around 12000 BC; in Thailand about 8000 BC, and in Zambia around 2000 BC [ESQUINAS-ALCAZAR & GULICK, 1983]. It travelled widely, perhaps due to the hard, dry skin of the mature fruits is impervious to water; they are capable of floating on salt water for the better of a year without any loss in seed viability. Tolerant a wide range of rainfall, it may be grown either on the ground or trellised [NG, 1993]. Calabash possesses simple leaves which are 400 mm long and 400 mm broad, oval shape and whitish seeds embedded in a spongy pulp, 7-20 mm long. They are widely grown in Northern part of Nigeria for excavation of domestic utensil and food containers. The fruits of these species contain vast number of seeds that have no commercial application in the locality they are produced [SOKOTO & al. 2013]. Phytogenetically, bottle gourd is close to many economically important cucurbit species including cucumber and melon that belong to the genus *Cucumis* as referred to as vegetable gourd of the family, Cucurbitaceae [WARRIER & al. 1995]. Bottle gourd is among the earliest cultivated plants used for medicinal and nutritional values [DAVIES &STEWART, 1990; DUKE, 1992; NEWEL & al. 1996; PIZZORNO & MURRAY, 1985; WERBACH, 1991].

Young fruits are used as cooked vegetables; the flesh is white, firm and has an excellent texture and mild taste. Young shoots and leaves can be cooked while the seeds can be used in soup preparations. They can be used as multi-purpose containers (bowls, boxes, water jugs, cups, and planters); utensils, ladles and pipes, musical instruments, floats for fishnets and rafts, or for ornamental purposes such as masks or native artifacts [NG, 1993].

***Cucumeropsis mannii* Naudin: the Egusi**

Cucumeropsis mannii is a member of the melon native to tropical Africa west of the East African Rift where it is grown for food and as a source of edible oil. *Cucumeropsis mannii* is a member of the Cucurbitaceae family. Its common names include egusi in Yoruba and agushi in Hausa. In English it is known as Mann's Cucumeropsis and white-seed melon. It produces climbing vines up to 4 meters long which are covered in stiff hairs. The heart-shaped or roughly palmate leaves are up to 12 centimeters long and 14 wide. It bears small yellow male and female flowers with petals under a centimeter in length. The fruit is egg-shaped or an elongated ovate

shape, up to about 19 centimeters long and 8 wide, and cream in color with green streaks. The fruit and white seeds are edible. The plant is grown more often for the seed oil than for the fruit. Vernacular names for this crop include egusi-itoo and white seed melon [OBUTE & al. 2007].

The crop is often referred to as "the real egusi" given its long history in West Africa, dating back 4,000 years [KORTSE & OLADIRAN, 2013]. This crop is primarily harvested for its large white seeds called egusi-itoo. The seeds are commonly processed into soups and oil products, and are also eaten individually as a snack. Benefits of *Cucumeropsis mannii* crops are many. The crop thrives in harsh climates and high yields are attainable in barren landscapes. Pests and disease are rarely a problem for farmers of this crop. Further benefits include increased soil quality through ground cover and suppression of weeds. This crop also holds significant nutritional value. Oil makes up 44% of the seed, where 30% is protein rich in essential amino acids. The seed is an excellent vegetable protein, and is ideal for battling nutritional debilitations. High in essential vitamins and minerals, egusi-itoo compliments the starch and grain diet of most Africans. Despite the crops obvious advantages, *Cucumeropsis mannii* remains an underutilized tool for nutritional intervention in Africa.

Material and methods

Samples collection and preparation

Fresh fruits of *Citrullus lanatus*, *Cucurbita pepo* and *Cucurbita maxima* were purchased at Kasuwan Daji market within the Sokoto municipal while freshly harvested seeds of *Cucumeropsis mannii* were purchased from Kasuwan Dankure market in Sokoto. Freshly harvested seeds of *Lagenaria siceraria* were purchased at Gummi market, Zamfara state while ripe and fully grown fruits of *Lagenaria breviflora* were obtained from roadsides in the outskirts of Sokoto town where the species was found growing as wild. All the six samples were taken to the Department of Biological Sciences Herbarium, Usmanu Danfodiyo University, Sokoto for authentication by a taxonomist where voucher specimens were deposited. Seeds were removed from the fruits by cutting the individual fruit longitudinally and scrapping out the seeds using cleaned knife. The seeds were removed from bad ones. The seeds were dried to a constant weight in an oven at 70 ºC, milled using mechanical blender, placed in six labeled air-tight containers and stored in desiccators prior to analysis.

Proximate composition analysis

Proximate composition (crude proteins, crude lipids, fibre, moisture and ash) of the seeds of the sampled cucurbits were determined using the methods of Association of Official Analytical Chemists (1990) while carbohydrate was determined by difference. The calorific values in kilo joule (k) were calculated by multiplying the crude fat, protein and carbohydrate by Atwater factors of (k) 37, 17, and 17 respectively.

Mineral composition analysis

The minerals were analyzed by first dry ashing the samples at 550 ºC in the muffle furnace. The filtered solutions were used to determine Na, K, Ca, Mg, P and N by means of atomic absorption spectrophotometer [AAS] [Buck Scientific Model-200A/210, Norwalk, Connecticut [06855] and phosphorus was determined calorimetrically by Spectronic 20 [Gallenkamp, UK] using the phosphovanado molybdate method [AOAC, 2008].

Quantitative phytochemical analysis

Qualitative Phytochemicals of the samples were determined following the methods of TREASE & EVANS (1978) and HARBONE (1998).

Data analysis

Treatments were replicated three times and the data obtained has been presented as means± S.E. of the means. Results obtained were subjected to one way Analysis of Variance [ANOVA]. Same superscripts means that there was no significant difference [P≤0.05] and where the superscripts differ, it means that there was a significant difference [P≤0.05].

Results and discussion

Proximate analysis

Table 1 presents results of proximate analysis of the seeds of Nigerian cucurbits germplasm. Moisture contents ranges from 3.83±0.10% -5.1±0.65% with the highest content found in *Lagenaria breviflora* while the least value of 3.83±0.10%was obtained for *Cucurbita pepo*, *Citrullus lanatus* had 4.83±0.23% while *Cucurbita maxima*, *Cucumeropsis mannii* 4.67±0.19%. This moisture content was in close agreement with the report of ACHU & al. (2005) who reported moisture content of 6.49±0.62% on seeds of *Cucumeropsis mannii* while *Cucurbita maxima* had 6.94±1.92%, *Lagenaria siceraria* with 6.09±0.20% and *Cucurbita moschata* with 8.21±1.47%. Similarly, result compares with those as reported on protein composition of the seeds of some cucurbits by KARAYE & al. (2013) who reported a range of 29.49.±0.87-33.48±0.87%. In another study by ACHU & al. (2005) (2005), range of crude protein content of *Cucurbita maxima* was 34.93±0.42% a range which is in close range with the obtained value in this study. In another study by JACOB & al. (2015), moisture content and crude protein were 7.10% and 30.65% respectively as reported. In same study, crude lipid was 49.05% higher than obtained in this study. Lower values of crude fibre and ash contents were 6.00% and 4.52% respectively than obtained in the current study. The result is as well in close agreement with that reported on *Cucumis sativus* with 28.68±2.38% while *Cucumeropsis mannii* had 40.49±2.75% a bit higher than obtained in the current study. In another study by JACOB & al. (2015), a closely related agreement on moisture and crude protein contents of 7.10% and 30.65% was reported on *Citrullus lanatus* seed extract. Crude lipid of the range of 24.50±0.29-31.33±0.34% obtained in this study was a bit lower than 38.00% as reported by ELINGE & al. (2012) on *Cucurbita pepo*; and 49.05% on *Citrullus lanatus* seed extract as reported by JACOB & al. (2015) on. The result obtained in this study is also lower than 44.85±4.03% and 49.05±2.48% as reported by ACHU & al. (2005) on the seed extracts of *Cucumeropsis mannii* and *C. maxima.* Crude carbohydrate content obtained in the current study ranges from 24.06±0.08-36.34±0.29% which tallies with the reported 27.88±0.42-33.60±0.42% by KARAYE & al. (2013) in another study on cucurbits seeds. Ash content reported by ACHU & al. (2005) *C. mannii* with 3.74±0.35% while that of *Cucurbita moschata* had 4.75±0.47%. Different values were reported by KARAYE & al. (2020) on Desert date (*Balanites aegyptiaca*) seed and fruit pulp extracts. These differences could be attributed to the species variability. This suggests the cucurbits seeds as rich sources of nutrients that could boost nutrients availability in the diets and their role in food and feed formulation strategies. Available energy (kilo calorie) obtained in this study was in agreements with that as reported by ELINGE & al. (2012) 564.00 kcal /100 g on *Cucurbita pepo*. Closely similar result was reported by GBOGOURI & al. (2011) 526.53 kcal in *Cucumis melo* and 572.00 kcal on *Citrullus lanatus* seeds respectively. Also, in agreement with the report of JACOB & al. (2015).This suggests the cucurbits seeds as veritable source of energy.

Table 1. Proximate analysis of the seeds six Nigerian Cucurbits Germplasm (Presented as % DW)

Species	Moisture	Protein	Carbo-hydrates	Lipid	Fibre	Ash	Energy (K/Cal.)
Citrullus lanatus	4.83±0.23[a]	34.94±0.18[a]	24.06±0.08[a]	24.50±0.29[a]	12.67± 0.16[a]	8.17±0.43[a]	500.90±2.32[a]
Cucurbita maxima	4.67±0.19[a]	32.66±0.11[a]	30.66±0.21[b]	28.27±0.20[b]	15.13±0.23[a]	9.17 ±0.52[a]	507.71±2.06[b]
Cucurbita pepo	3.83±0.10[a]	34.01 ±0.17[a]	31.16±0.20[b]	27.33±0.20[b]	14.70±0.19[a]	8.83±0.47[a]	506.64±2.24[b]
Cucumeropsis mannii	4.17±0.26[a]	33.10±0.16[a]	35.40± 0.51[c]	31.33±0.27[c]	13.87±0.20[a]	7.67±0.32[a]	555.97±2.21[c]
Lagenaria breviflora	5.17±0.65[a]	35.94±0.26[a]	36.34 ±0.29[d]	25.67±0.18[b]	5.83±0.07[b]	5.86±0.29[a]	520.15±2.10[d]
Lagenaria siceraria	4.33± 0.27[a]	33.10±0.08[a]	34.10 ±0.26[d]	27.17±0.20[b]	6.33±0.09[b]	6.17±0.36[a]	513.33±2.12[e]

Results presented as mean ± S.E. of the means. All treatment were replicated three times. Different superscripts indicate significant difference at $p \leq 0.05$.

Mineral analysis

Mineral analysis of the seeds of six selected cucurbits germplasm has been presented in Table 2. Results of the analysis of the seeds revealed a high proportion of the vital biochemicals necessary for healthy growth and development. The range of values obtained for Ca in this study was 10.83±1.32-34.67±2.76. This is in close agreement with results reported by JACOB & al. (2015) Mg as 20.46 mg/100 g. Also, in close agreement with 22.33 mg /100 g; and 23.67 mg/100 g as reported by KARAYE & al. (2020) on *Balanites aegyptiaca* seed extract. Magnesium is an activator of many enzyme systems and helps in the maintenance of electrical potential in nerve [ONIBON & al. 2007]. Similarly, calcium content obtained in this study (22.00±1.80) to (34.60±2.23) was in disagreement with 9.78 mg/ 100 g as reported by ELINGE & al. (2012) on seeds of pumpkin. Ca and Mg as reported on *C. mannii* were higher than obtained in this study. Calcium has been reported to help in the regulation of muscular constrictions, transmits nerve impulse and has been implicated for bone formation. Calcium intake especially from regular use of calcium supplements may be associated with increased risk of kidney stone. In another study by GBOGOURI & al. (2011), values reported for K and Na were 806.10±21.21 and 11.00±2.31 for *Cucumeropsis mannii* which were in close agreement to those obtained in this study. Sodium plays an important role in osmotic regulation of the body fluids and transmission of nerve impulses. Na/K ratio in the body is important because it helps in controlling high blood pressure. Na/K ratio of less than one is recommended [YUSUF & al. 2007]. Potassium content of the seeds obtained was higher than those reported for the fruit pulp and the seeds 383.33±12.47 and 256.33±10.47 for *Balanites aegyptiaca* by KARAYE & al. (2020). These differences could be attributed to species variability. It was reported that plant foods have high amount of potassium [HASSAN & al. 2006]. High amount of potassium in the body was reported to increase iron utilization [ADEYEYE, 2002].

Table 2. Results of mineral analysis of the seeds of six Nigerian Cucurbit Germplasm.
Results presented as g/100 g

Species	N	Na	Mg	Ca	P	K
Citrullus lanatus	3.11±0.38[a]	15.83±1.65[a]	22.33±2.03[a]	10.83±1.32[a]	4.50± 0.86[a]	1266.67± 3.67[a]
Cucurbita maxima	5.24±0.78[a]	20.83±2.12[b]	34.60±3.17[b]	36.67±2.76[b]	4.54±0.98[a]	2266.70± 5.69[b]
Cucurbita pepo	5.43±0.84[a]	31.67±2.43[c]	32.00±2.12[b]	28.00±2.05[b]	4.84±1.02[a]	1916.70± 3.76[b]
Cucumeropsis mannii	3.12±0.50[a]	11.83±0.79[d]	21.77±2.24[a]	11.77±0.98[c]	5.11±0.76[a]	1550.00±4.54[b]

Lagenaria breviflora	5.75±0.97[a]	24.17±2.32[d]	34.60±2.23[b]	36.67±2.74[b]	4.54±0.44[a]	2266.67±5.66[c]
Lagenaria siceraria	5.30±0.69[a]	21.67±2.21[b]	22.00±1.18[c]	31.33±1.98[b]	5.71±0.74[a]	1783.33±4.92[b]

Results have been presented as means ± Standard Error of the means. All treatments were replicated three times. Different superscripts indicate significant difference at $p \leq 0.05$.

Results of Quantitative Phytochemicals

Quantitative analysis of the seeds of six selected cucurbits germplasm has been presented in Table 3. Phytochemicals are plant compounds that have interest as a source of safer or more valuable substitutes than synthetically created antimicrobial agents. Phytochemical progress has been aided extremely by the development of rapid and accurate methods of screening plants for particular chemicals [ATIF & al. 2012]. From the results, flavonoid contents revealed the highest in *Lagenaria siceraria* (23.33±1.66) while the least was identified (12.67±0.84) in *Citrullus lanatus*. Terpenoid was highest in *Cucumeropsis mannii* (23.67±1.92) while the least (14.57±0.99) was obtained in *Cucurbita pepo*. Glycoside was evaluated with highest in *Lagenaria breviflora* (27.23±2.06) while the least was found in *Lagenaria siceraria* (14.57±1.03). Alkaloid was identified to be highest in *Cucurbita pepo* while the least (24.43±1.18) was identified in *Cucurbita maxima*. Saponin glycoside was evaluated and the highest was identified in *Citrullus lanatus* with (28.81±2.06) while the least (12.67±0.76) was identified in *Cucumeropsis mannii*. The values were higher than (3.20±0.40) and (2.60±0.35) as reported on seeds and dry pulp of *Balanites aegyptiaca* by KARAYE & al. (2020). Also, lower value (3.13 mg/100 g) was reported on dehulled seeds of *Luffa aegyptiaca*. These differences could be attributed to species variability. Tannin is an astringent, bitter plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids [REDDEN & al. 2005]. The term is applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3000 [MUZQUIN & al. 2000]. Saponin are secondary compounds that are generally known as non-volatile, surface active compounds which are widely distributed in nature, occurring primarily in the plant kingdom. Saponin molecules form soap-like foams when shaken with water. They are structurally diverse molecules that are chemically referred to as triterpene and steroid glycosides. They consist of non-polar aglycones coupled with one or more monosaccharide moieties. This combination of polar and non-polar structural elements in their molecules explains their soap-like behavior in aqueous solutions [GEMADEH & RATTA, 2014]. Saponin have found wide applications in beverages and confectionery as well as in cosmetics and pharmaceutical products. Due to the presence of a lipid-soluble aglycones and water soluble sugar chain (s) in their structure (amphiphilic nature), saponins are surface active compounds with detergent, wetting, emulsifying and foaming properties [SHANTHAKUMARI & al. 2008]. Saponins are attracting considerable interest as a result of their beneficial effects in humans. Recent evidence suggests that saponins possess hypocholesterolemic, immunostimulatory and anticarcinogenic properties. In addition, they reduce the risk of heart diseases in humans consuming a diet rich in legumes containing saponins. Saponin-rich foods are important in human diets to control plasma cholesterol, preventing peptic ulcer, osteoporosis and reduce the risk of heart disease. Saponins are used as adjuvants in viral and bacterial vaccine (e.g. Quillaja saponins) applications. In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones [LOEWUS, 2002]. Alkaloids are one of the largest groups of chemical compounds

synthesized by plant acids such as oxalic, malic, tartaric or citric acid. Alkaloids are small organic molecules, common to about 15 to 20 percent of all vascular plants, usually comprising several carbon rings with side chains, one or more of the carbon atoms being replaced by a nitrogen. They are synthesized by plants from amino acids. Decarboxylation of amino acids produces amine oxides to form aldehydes. The characteristic heterocyclic ring in alkaloids is formed from Mannich-type condensation from aldehyde and amine groups [FELIX & MELLO, 2000].

Table 3. Results of Quantitative Phytochemicals of the seeds of six cucurbits germplasm (Results presented as % DW)

Phytochemicals	*Citrullus lanatus*	*Cucurbita maxima*	*Cucurbita pepo*	*Cucumeropsis mannii*	*Lagenaria breviflora*	*Lagenaria siceraria*
Flavonoid	12.67±0.84^{a}	14.63±0.96^{a}	16.12±1.00^{a}	14.64±0.97^{a}	12.77±0.89^{a}	23.33±1.66^{b}
Terpenoid	17.16±1.11^{a}	16.23±1.02^{a}	14.57±0.99^{a}	23.67±1.92^{b}	16.77±1.00^{a}	21.17±1.37^{b}
Glycoside	23.66±1.16^{a}	24.53±1.28^{a}	15.62±1.10^{b}	23.67±1.17^{a}	27.23±2.06^{c}	14.57±1.03^{a}
Alkaloid	26.23±1.91^{a}	24.45±1.19^{a}	29.79±2.21^{a}	24.77±1.54^{a}	28.66±2.07^{a}	27.76±2.00^{a}
Saponin	28.81±2.06^{a}	19.23±1.05^{b}	14.38±0.97^{c}	12.67±0.76^{c}	13.68±0.73^{c}	15.23±0.99^{c}
Tannin	29.66±2.26^{a}	11.62±0.84^{b}	24.66±1.31^{a}	25.61±1.86^{a}	24.23±1.49^{a}	25.87±1.87^{a}
Phenol	28.61±2.21^{a}	12.53±0.86^{b}	24.43±1.41^{c}	11.68±0.81^{b}	24.66±1.51^{c}	29.62±2.23^{a}
Cardiac glycoside	26.23±1.87^{a}	24.47±1.28^{a}	25.62±1.89^{a}	24.27±1.26^{a}	21.67±1.10^{a}	22.23±1.21^{a}
Cyanide	11.67±0.89^{a}	12.82±0.92^{a}	29.62±2.45^{b}	11.86±0.84^{a}	24.47±1.48^{b}	25.67±1.93^{b}
Resin	12.23±0.91^{a}	11.66±0.82^{a}	24.23±1.24^{b}	21.87±1.16^{b}	11.66±0.86^{a}	24.47±1.90^{b}
Volatile oil	24.12±1.48^{a}	25.31±1.69^{a}	11.68±0.69^{b}	24.67±1.47^{a}	25.23±1.57^{b}	25.47±1.90^{b}

Results presented as means ± Standard error of three replications. Same superscripts indicated no significant difference in means at (P≤0.05), while where superscripts differ, it means there is significant difference (P≤0.05).

Conclusions

Based on the results of the current study, it can be said that members of the family Cucurbitaceae stand a veritable position for use as active ingredients in food especially to provide remedy for protein and crude lipid deficiencies facing a large segments of populations of the underdeveloped and the developing nations where a large section of the populace depend on starchy foods. This is in addition to providing succor for animal feed fortification strategies to help ameliorate the problem of high cost of fish and poultry feeds.

Notes on contributors

KARAYE Ibrahim Umar is a Plant Biologist, a PhD. Student with a special interest in the Biochemical Properties in the Dicot families.

HAYATU Muhammad is a Reader in Plant Biology with interest is General Physiology.

MUSTAPHA Yahya is a Professor in Plant Biology with special interest in Plant Physiology. SANI Lawan Abdu is a plant scientist with bias in general Plant Physiology and plant genetics.

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Authors declare that there is no existence of competing interest.

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RECENT ADVANCES IN POST-TRANSLATIONAL REGULATION OF PLANT DEFENSE RESPONSES BEYOND PHOSPHORYLATION

Tim XING[1*], Ziwei GUO[1]

[1] Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario – Canada.
* Corresponding author. E-mail: tim.xing@carleton.ca

Abstract: During plant-microbe interactions, plant immune signaling relies significantly on post-translational modifications (PTMs) to induce rapid downstream changes. Organization at protein level is extensively complex and various forms of PTMs of transcript products provide a unique system in maintaining such an organization. With current proteomic research some detailed mechanisms of these PTMs have started to be uncovered. Pathogens also take PTMs as a virulence strategy to overturn host immunity through the activities of their effector proteins. In this review, we will address the importance of PTMs other than phosphorylation in plant defense response.

Keywords: acetylation, glycosylation, plant defense, post-translational modification, SUMOylation, ubiquitination.

Introduction

Plants are under constant interaction with pathogens. Unlike mammals, which have highly specialized immune cells that can migrate to all parts of body, plants have evolved strategies that upon infections cells are regulated to achieve immunity responses [WITHERS & DONG, 2017]. There are two main types of molecular immunity response, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). Conserved microbial elicitors called pathogen-associated molecular patterns are recognized by pattern recognition receptors (PRRs) on the external surface of the plant cell [DODDS & RATHJEN, 2010; NEWMAN & al. 2013]. ETI can be initiated by cell surface perception or intracellular perception [XING & al. 2017]. PRRs can recognize PAMP and host-derived damage-associated molecular patterns (DAMPs) to activate immune response [XING & al. 2017]. Examples of these molecular patterns are pathogen cell wall components including lipopolysaccharides, peptidoglycan, chitin, and proteins including flagellin, EF-TU and host produced substances (DAMPs) including plant peptides (PEP), oligogalacturonides and extracellular ATP [TREMPEL & al. 2016]. The ligand recognition by PRR activates downstream signaling pathways and eventually trigger defense response such as transcription of defense-related genes, production of antimicrobial compounds, influx of Ca^{2+}, rapid burst of reactive oxygen species (ROS), and hypersensitive response (HR) [BIGEARD & al. 2015; TREMPEL & al. 2016]. Through evolution, pathogens have developed mechanisms to deliver pathogen effectors into plant cells to overcome plant PTI. As such, plants have developed *R* gene products to recognize pathogen effectors and activate ETI. ETI is significantly stronger than PTI and can lead to HR and massive transcriptional reprogramming [XING & al. 2017]. ETI also triggers systemic acquired resistance (SAR), which act away from the infection site to protect the whole plant [CUI & al. 2015; WITHERS & DONG, 2017].

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To ensure a rapid switch of signaling pathway and the correct activation of immune response, components of plant defense signaling pathways are under precise regulation through PTMs. PTM plays roles in both the activation and the inhibition of PTI and ETI [WITHERS & DONG, 2017]. Phosphorylation is one of the most commonly used and the best studied PTM in plant defense response [XING & LAROCHE, 2011; XING & al. 2017]. Besides phosphorylation, other PTMs also play critical roles. In some cases, they may not directly pass immune signals to downstream components but can take part in maintaining the normal function of signaling components or attenuating immune response [WITHERS & DONG, 2017]. In this review, the role of these other PTMs including ubiquitination, glycosylation, SUMOylation, and acetylation on plant immunity is discussed with the focus on some highly regulated defense components.

Ubiquitination regulates PTI through degradation of existing components

Remodeling of the plasma membrane (PM) protein composition is emerging as a key aspect regulating receptor signaling and mediating signal resolution in space and time. Receptor ubiquitination may regulate protein levels by modulating PRR traffic at different stages after endocytosis. Following internalization, cargoes go through a sorting process, which decides whether they will be recycled and returned to the PM, or transported to the vacuole for degradation [WITHERS & DONG, 2017]. In the secretory pathway, components of the endoplasmic reticulum (ER)-quality control ensure the proper accumulation and function of PRRs such as FLS2 receptors [WITHERS & DONG, 2017].

Upon flg22 perception, *Arabidopsis* BAK1 and PUB12/13 associate with FLS2. BAK1 phosphorylates PUB 12/13 to induce the polyubiquitination of cytosolic domain of FLS2, which leads to the degradation of FLS2 by 26S proteasome [LU & al. 2011]. PUB 12/13-deficient mutant display enhanced immune responses and ROS production upon flg22 perception [LU & al. 2011]. The recruitment of E3 ligases to FLS2 modulates PTI through the attenuation of immune signaling [LU & al. 2011]. CERK1 is a membrane localized receptor-like kinase that can activate PTI by sensing chitin and chitosan [YAMAGUCHI & al. 2017]. Similar to FLS2, PUB12-CERK1 interaction upon the recognition of chitin targeted CERK1 for degradation and attenuated immune response [YAMAGUCHI & al. 2017].

Interestingly, PRRs also regulate the activity of ubiquitin ligase and hence facilitate downstream immune response. In rice, PID2, a transmembrane lectin receptor-like kinase, confers resistance to *Magnaporthe oryzae* infection [CHEN & al. 2006]. OsPUB15, a rice U-box/ARM repeat protein, is reported to possess E3 ligase activity and interact with PID2 [WANG & al. 2015]. Upon PAMP recognition, activated PID2 proteins homo-dimerize to phosphorylate OsPUB15 to activate its E3 ligase function. Overexpression of OsPUB15 up-regulated expression of pathogenesis-related (PR) genes, increased ROS production, and enhanced resistance to rice blast [WANG & al. 2015]. Based on these findings, OsPUB15 acts as an inducer of PID2-triggered immunity and up-regulates PTI [WANG & al. 2015].

Ubiquitination of cytosolic components of PTI signaling pathway also contributes to the regulation of PTI. The regulation of BIK1 is explained by the modulation of non-activated and activated BIK1 pools [WANG & al. 2018]. E3 ubiquitin ligase PUB25 and PUB 26 negatively regulate PTI by targeting the degradation of non-activated BIK1 [WANG & al. 2018]. In this model, CPK28, heterotrimeric G proteins (AGG1, AGG2, XLG2, and AGB1), and PUB25/26 together regulate BIK1-induced downstream immune responses [WANG & al. 2018]. Before pattern recognition, BIK1 is not phosphorylated by FLS2 and is susceptible to PUB25/26 mediated degradation. Heterotrimeric G proteins associate with FLS2-BIK1 complex

and stabilize BIK1 by inhibiting PUB25/26 E3 ligase activity [LIANG & al. 2016]. Upon pattern recognition, BIK1 is phosphorylated by FLS2 and activated [WANG & al. 2018]. The perception of flg22 by FLS2 increases the phosphorylation of PUB25/ 26 by CPK28 and hence the E3 ligase activity of PUB25/26 [WANG & al. 2018]. However, PUB25/26 do not have ligase activity on activated BIK1 but non-FLS2 interacting BIK1 is rapidly removed by active PUB25/26 [WANG & al. 2018]. Ubiquitination of BIK1 modulates PTI by retaining necessary signaling and removing excess signaling component to prevent unnecessary immune response [WANG & al. 2018].

PUB4, a CERK1-interacting E3 ubiquitin ligase, has also been reported to regulate immunity by ubiquitinating BIK1 [DERKACHEVA & al. 2020]. *Arabidopsis Pub4* mutant display reduced ROS burst upon flg22 and elf8 treatment, suggesting that PUB4 positively regulates PTI [DERKACHEVA & al. 2020]. PUB4 associates with FLS2/EFR-BIK1 complex and targets the degradation of only non-activated BIK1 [DERKACHEVA & al. 2020]. The E3 ligase up-regulates downstream immune response and ROS burst and acts as a positive regulator of PTI by promoting the accumulation of activated BIK1 upon PAMP perception [DERKACHEVA & al. 2020]. The degradation of non-active BIK1 stabilizes the system in a resting state, which contributes to the activation of immunity in this case.

Glycosylation is required for the normal function of receptors of PTI

Protein glycosylation is required for ER/Golgi processing and trafficking of membrane proteins to plasma membrane [TREMPEL & al. 2016]. Oligosaccharyl transferase (OST) complex in the ER lumen is significantly involved in the regulation of asparagine-linked glycosylation (*N*-glycosylation) in ER [AEBI, 2013]. Tunicamycin (TM), an *N*-glycosylation inhibitor, weakens overall immune system and disturbs ER quality control (ERQC) [CHAKRABORTY & al. 2017]. *N*-glycosylation of PRR is required for the localization of PRR to PM and ligand binding of PRR [AEBI, 2013]. *STT3A* gene encodes a subunit of OST and is critical for *N*-glycosylation in ER [HÄWEKER & al. 2010]. *STT3A* loss-of-function mutant has showed that *N*-glycosylation plays critical function for the EFR ligand binding and translocation to PM [HÄWEKER & al. 2010; FARID & al. 2013]. A single loss of EFR^{N143} glycosylation site in the leucine-rich repeat (LRR) ectodomain impaired the receptor stability and ligand recognition but not its translocation from ER to PM [HÄWEKER al. 2010]. Unlike EFR, FLS2 is relatively insensitive to mutation of putative *N*-glycosylation sites [SUN & al. 2012]. However, this does not mean that flg22-trigger PAMP signaling is independent of *N*-glycosylation. Studies found that flg22 induced pathogenesis-related protein 1 (PR1) accumulation is lowered in *stt3a* [KANG & al. 2015] and FLS2 with octuple-PGS [putative *N*-linked glycosylation sites] mutations partially lost flg22 responsiveness [SUN & al. 2012]. In tomato Cf-9, all PGSs except PGS18 were shown to be *N*-glycosylated and all of the glycosylation sites were important for Cf-9 activity [VAN DER HOORN & al. 2005].

When wild type plant was treated with TM, the binding of ligand and translocation to PM of FLS2 was impaired [HÄWEKER & al. 2010]. Upon the perception of PAMP by PRR, extracellular calcium influxes into cytoplasm. As a result, under-glycosylation of PRR should display impaired calcium influx. *Arabidopsis cce2/cce3* mutants display reduced calcium elevations after treatment with MAMP or DAMP including flg22, elf18, chitin, LPS and AtPep1 [TREMPEL & al. 2016]. *CCE2/CCE3* encode α-1,3-mannosyltransferase, *ALG3*, and its activity in *alg3* mutant was reduced and PRRs of *cce2/cce3* were under-glycosylated but they were still localized to PM [TREMPEL & al. 2016]. This seems to indicate that *N*-glycosylation of PRRs is responsible for calcium elevation of PTI signaling [TREMPEL & al. 2016]. Similar

results were observed in *Arabidopsis cce1* mutant with a mutated *ALG12* [TREMPEL & al. 2020].

Chitosan oligosaccharide (COS), generated by enzymatic hydrolysis of chitosan, can have priming effect on plant-pathogen interaction by enhancing PR1 expression and activating salicylic acid (SA)- and/or jasmonic acid (JA)-dependent signaling pathway [JIA & al. 2018]. The increase of resistance of plant by COS involves glycosylation. *Stt3a* and *ManI* mutants are *N*-glycosylation impaired and are more susceptible to *Pst* DC3000 infection [JIA & al. 2018]. However, *Pst* DC3000 infection was rescued in *Stt3a* and *ManI* mutants by pretreatment with COS [JIA & al. 2018]. This indicates the under-glycosylation of immune signaling components is restored by COS. Also, COS-pretreated and *Pst* DC3000-infected plants showed differences in the accumulation of nucleotide sugar donors [JIA & al. 2018]. The finding indicates that *N*-glycosylation of plant innate immune response and COS induced resistance are regulated via partially non-overlapping pathways [JIA & al. 2020].

Defection in *N*-glycosylation was also shown to up-regulate some defense responses [CHAKRABORTY & al. 2017]. TM treatment induced PR1 expression independently of PTI but the increased PR1 did not enhance pathogen resistance, possibly offset by the negative effect of ER stress and the negative effect of under-glycosylation of plant defense pathways on the immunity. As PR1 is a PTI activation marker and a SAR (systemic acquired resistance) mediator, this study seems to indicate that inhibition of *N*-glycosylation could trigger some plant immune responses even though the pathway is unknown. [CHAKRABORTY & al. 2017].

SUMOylation affects protein-protein interaction

SUMOylation is similar to ubiquitination, where E1, E2, and E3 enzymes are involved. SUMOylation modulates protein stability, protein-protein interaction and protein subcellular localization, and is involved in plant defense mechanism [NIU & al. 2019]. An example is SCE1, an *Arabidopsis* SUMO E2 enzyme, which induces SUMO1/2 conjugation to suppress immune response [SKELLY & al. 2019]. Upon pathogen recognition, the increased nitric oxide (NO) level induced *S*-nitrosylation of SCE1 at Cys139, suppressed SCE1-mediated SUMOylation and hence up-regulated immune response [SKELLY & al. 2019].

As a master regulator of basal and SAR, NPR1 confers immunity through a transcriptional cascade including transcription activators (e.g. TGA3) and repressors (e.g. WRKY70) [FU & DONG 2013; SALEH & al. 2015]. SA accumulation promoted dephosphorylation of Ser55/Ser59 through an unknown mechanism and induced SUMOylation of NPR1, resulting in dissociation from WRKY70 and inactivation of this repressor [SALEH & al. 2015]. Modification of NPR1 by SUMO3 was required for its phosphorylation at Ser11/Ser15 to form a signal amplification loop to generate more activated NPR1 [SALEH & al. 2015]. This activated form of NPR1 interacted with the TGA3 transcription activator to induce *PR1* gene expression [SALEH & al. 2015]. Subsequently, the modified NPR1 was ubiquitinated and targeted for degradation by the 26S proteasome mediated by interaction with NPR3 to ensure the transient nature of the immune induction [SALEH & al. 2015].

SUMOylation on PRR is required for progression of PTI signaling pathway. FLS2, BAK1 and BIK1 form complex during flg22 perception [TANG & al. 2017]. SUMOylation of FLS2 promoted the dissociation of activated BIK1 from the complex to allow downstream BIK1 induced signaling [TANG & al. 2017]. Desi3a, one of the eight Desi type SUMO proteases, deSUMOylated FLS2 to down-regulate immune response [OROSA & al. 2018]. The amount of Desi3a was reduced when flg22 was present, suggesting that Desi3a take part in maintaining the system in the resting state in non-pathogenic conditions [OROSA & al. 2018].

Acetylation regulation in ETI

Acetylation of proteins are involved in plant defense mainly in two ways, the modification of histone to regulate gene expression, and the acetylation of non-histone proteins by pathogen type III effectors that function as acetyltransferase to alter plant immunity [SONG & WALLEY, 2016]. Several pathogen effector proteins encode acetyltransferase enzymes that directly acetylate host proteins (e.g. RRS1, RPM1, WRKY and cell skeleton proteins) and alter plant immunity [SONG & WALLEY, 2016]. Nucleotide binding leucine-rich repeat (NB-LRR) receptor protein was shown to interact with pathogen effector and the downstream immune response was down-regulated [LEE & al. 2015]. Bacterial effector PopP2 acetylated the C-terminal WRKY transcription factor domain of RRS1 as well as WRKY transcription factors and abolished DNA binding activity and suppressed immunity [TASSET & al. 2010; LE ROUX & al. 2015; SARRIS & al. 2015]. Another pathogen effector, HopZ3, was shown to inactivate RPM1 immune complex by acetylating its members including RIN4 and RIPK [LEE & al. 2015]. It was also reported that the acetylation of non-histone protein of maize by a plant-encoded histone deacetylase was involved in immune response [WALLEY & al. 2018].

Other PTMs in ETI

PTMs modulates the recognition of pathogen effectors at host-pathogen interface. Ubiquitination has been reported to up-regulate a virus induced ETI by targeting a protease and hence stabilize an R protein [LIM & al. 2018]. Virus can express suppressors that inhibit host RNA silencing to facilitate infection and these suppressors can be recognized as effectors by plant R proteins and trigger ETI [CHOI & al. 2004]. HRT, an R protein in *Arabidopsis*, can be activated *by* turnip crinkle virus (TCV) coat protein to trigger HR and resistance [LIM & al. 2018]. Double stranded RNA binding protein (DRB) 1 and 4 play a role in stabilizing HRT and are required for resistance to TCV [LIM & al. 2018]. TOP1, an E3 ligase, positively regulates TCV-induced ETI by negatively regulating proteases that target the degradation of DRB1 and DRB4 [LIM & al. 2018]. *S*-acylation also plays a role in ETI. RPS5 is responsible for immune response during *Pseudomonas syringae* infection [QI & al. 2012]. The activation of RPS5 was shown to require the cleavage of PBS1 protein kinase by AvrPphB, the effector of *Pseudomonas syringae* [QI & al. 2014]. The localization of PBS1 to PM and its cleavage both required N-terminal *S*-acylation on its Cys residues [QI & al. 2014].

SUMOylation modulates SA-dependent immune response and suppresses autoimmunity. SIZ1, a SUMO E3 ligase, regulates SA-dependent immune response by mediating SNC1 activity [GUO & al. 2017]. Overexpression of TOPLESS-RELATED 1 (TPR1), a SUPPRESSOR OF npr1-1, CONSTITUTIVE 1 (SNC1)-interacting protein, resulted in autoimmunity that reduced plant growth and development [NIU & al. 2019]. Loss of function of SIZ1, a SUMO E3 ligase, was shown to induce an autoimmune response, partially due to the elevated SNC1 levels [NIU & al. 2019]. SIZ1 physically interacted with TPR1 and facilitated its SUMO modification, with K282 and K721 residues in TPR1 as critical sites for SUMO attachment [NIU & al. 2019].

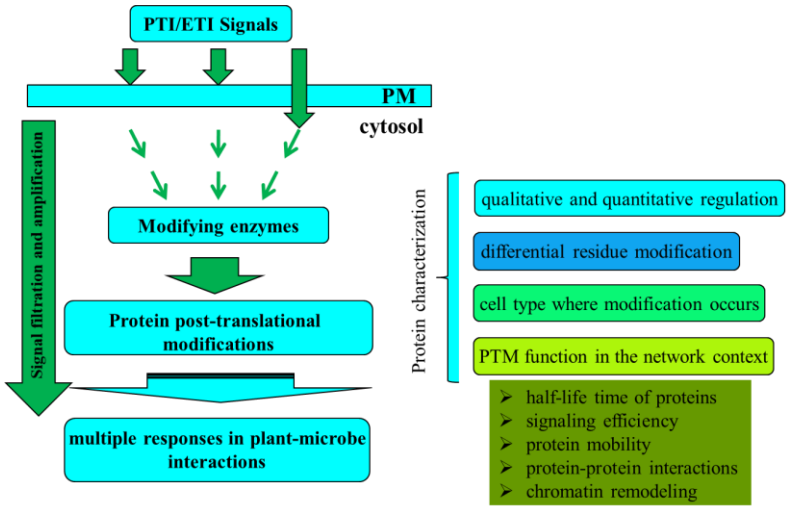

Figure 1. Post-translational modifications in plant defense mechanisms and challenges to research approaches

Conclusions

PTMs are used by plants to regulate immune response. Phosphorylation plays a critical role in defense signaling while other types of PTMs are increasingly shown to contribute to the regulation of plant-microbe interactions in various ways (Figure 1). Glycosylation works mainly in the preparation of the system by ensuring the production and translocation of the receptor. However, it is less involved during the modulation of signal transduction. This is probably because glycosylation is required for the maturing of protein and the process have to be done within ER and Golgi. The transportation of cytosolic signaling components into endomembrane system can be costly. On the other hand, ubiquitination is more dynamic and plays roles mainly in stabilizing the system rather than participating in signal transduction directly. The beauty of ubiquitination is that Ub can be attached to an individual protein precisely without affecting proteins in a complex. Taking this advantage, plant can finetune defense response accurately. For most of the time, a single immune signaling component is regulated by multiple other components and PTMs. This ensures the plant defense system in a resting state without pathogen and is turned on rapidly when a pathogen attacks. The components (e.g. BIK1) crosslink multiple pathways and are highly regulated. The advantage of PTM is that it does not involve transcription and many of them are reversible. This allows fast and accurate switch of physiology upon interaction with pathogen.

PTM study is much more challenging in the study of plant defense mechanisms as there is an additional interaction, i.e. the host plant and the microbe. A comprehensive understanding at PTM level with various forms of modifications is essential to uncover the mechanisms that govern this interaction and particularly the response by host plants. It is highly likely that detailed mechanisms of all types of PTMs remain to be elucidated. It is worth mentioning that some topics are covered by other recent reviews [WITHERS & DONG, 2017; DE VEGA & al. 2018; ZHANG & ZENG, 2020].

Notes on contributors

Tim XING is an associate professor and a plant molecular biologist with a special interest in cell signaling and plant-microbe interactions. He teaches plant physiology, molecular plant development, and cell signaling. Ziwei GUO is a graduate student.

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EFFECTS OF AFLATOXIN ON SOYBEAN

Gloria Chinasa NNAMANI[1], Andrew Shema GANA[2], Aondover SHAAHU[1*], Titus Saanmoiyol MSAAKPA[5], Prince Chisom LEMIBE[1], Maxwell Arinze NNAMANI[3], Emmanuel Obinna NWANYANWU[4]

[1] Soybean Research Programme, National Cereals Research Institute, P. M. B 8, Badeggi, Niger State – Nigeria.
[2] Plant Breeding and Seed Science, Federal University of Technology, Minna, Niger State – Nigeria.
[3] Federal Polytechnic, Bida, Niger State – Nigeria.
[4] Teesside University, Middlesbrough – United Kingdom.
[5] Plant Breeding and Seed Science, Federal University of Agriculture, Makurdi, Benue State – Nigeria.
[*] Corresponding author. E-mail: iorpen001@gmail.com

Abstract: Aflatoxin is widely known as human and animal carcinogen that can contaminate food and feed stuffs. It is also known as a major food quality problems all over the world. Aflatoxins are group of naturally occurring mycotoxins that are mainly produced by fungus *Aspergillus flavus* and *Aspergillus parasiticus.* There are seven (7) major groups of mycotoxins reported, which produced by different species of toxigenic fungal genus. The production of mycotoxins from these toxigenic fungi based on the surrounding intrinsic and extrinsic environments. These groups of mycotoxins that contaminates grains, foods and animal feeds are known as Aflatoxin, Trichothecene, Ochratoxins, Ergot alkaloid (Ergolin), Zearalenone, Patulin and Fumonisins. These mycotoxins could cause health hazards and death for human and animals by affecting the mammalian cells, causing some problems in normal cell function and a wide variety of clinical symptoms of diseases. These seven groups of mycotoxin are varied in their toxicity depending on the infection host i.e. human or animal, and also the immunity of human and animals. Soybean is an important food and nutritional security crop world widely. It is also promoted due to its potentiality in proteins for both adults as an infant weaning food.

Keywords: Aflatoxin, human, livestock, soybean, substrate.

Introduction

Aflatoxins (AF_3) are widely known as a group of highly toxic secondary metabolites produced mainly by the filamentous fungi known as *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxins are also the most studied and widely known mycotoxins [WILLIAMS & al. 2004]. And it was first discovered in the early 1960's in United Kingdom to describe the toxins associated with contaminated animal feed called peanut and the loss turkey in England. Aflatoxins probably grow on agricultural crops like cereals, grains, nuts and dried fruits. Human foods and animal feeds produced from fungi contaminated agricultural crops are highly hazardous to health for both human and animals. These kind of toxigenic fungi could grow under optimum conditions of temperature and humidity either during plant, or after crop harvesting, at crop storage time and during the processed food or feed products. [RICHARD, 2007]. The functions of mycotoxins are yet to be understood; perhaps they could function as an insecticide, and might play a role in fighting against the plant defence to the fungus in some means to complete their ecological role in nature. Mycotoxins are not

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detectable by the immune systems of human and animals and also they are non-pretentious in nature.

However, aflatoxin could accumulate through the food chain therefore causing a very serious health concern to both human and animals. Aflatoxins are highly mutagenic and carcinogenic in nature and as well it has been associated with various diseases, like aflatoxicosis. The contamination of aflatoxins is influenced by environmental factors like agronomic practices and geography. Aflatoxin B1 have been reported as the most common in food and also amongst the most potent genotoxic and carcinogenic aflatoxin, and it presence in food such as corn, milk and peanuts might enhance a person's risk of cancer of the liver. Contamination of aflatoxins is most acute and widespread in warm, humid climates of the tropical and subtropical regions of the world due to the fact that production of aflatoxins is optimal at relatively high temperatures [HUDLER, 1998].

Aflatoxin B1 is produced both by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M1 is the main metabolite of aflatoxin B1 in both human and animals which might be present in milk from animals been fed with animal's feed that are contaminated aflatoxin B1. Moreover, a report in LOPEZ-GARCIA & al. (1999) described that food and feeds contaminated with mycotoxins are unpredictable and unavoidable, which makes it a unique challenge for food and feeds safety. Food safety refers to minimizing the presence of those hazards, whether acute or chronic, that could make food dangerous to the health of those consuming it. The presence of mycotoxins in food totally makes the food products hazardous for human consumption, which eventually leads to the post-harvest loss and also becomes a problem to food security. The proliferation of different kinds of fungi in agricultural products causes low yield and consequential economic losses [ADEYEYE, 2016]. The different kind of fungi, their substrates and mycotoxins are shown in Table 1.

Table 1. Different kinds of fungi, their substrate and mycotoxins

Fungi	Substrate	Mycotoxins
Aspergillus flavus	Maize, groundnut, oilseed, cottonseed	Aflatoxin (carcinogenic to human)
Aspergillus parasiticus	Maize, groundnut, oilseed, cottonseed	
Aspergillus nomius	Maize, groundnut, oilseed, cottonseed	
Aspergillus ochraceus	Bakery Wheat	Ochratoxin (Potentially carcinogenic)
Aspergillus carbonarius	Grapes, Wine, Coffee	
Fusarium oxysporum	Wheat, Barley, Maize	Fumonisins (Hepatotoxic and Nephrotoxic)
Fusarium sp.	Wheat, Barley, Maize	T-2 Toxin
Penicillium verrucosum	Wheat, Barley, Maize	Ochratoxin (Potentially carcinogenic)
Claviceps purpurea	Rye	Ergot Alkaloid
Stachybotrys sp.	Hay	Satratoxin

Source: BENNETT & KLICH, 2003.

Health hazard effects of mycotoxin for human and animals

In JUAN-GARCIA & al. (2013), the general symptoms that surfaces after the intake of foods contaminated with mycotoxins or indirectly from consumption of meats or drinking milk from animals that have been fed feeds contaminated with mycotoxins are diarrhea, weakness, headache, fatigue, difficult concentration, light sensitivity, short memory, increase thirst, unusual skin sensations, morning stiffness, chronic cough, joint pain, shortness of

breath, increase urinary frequency, abdominal pain, bloating, blurred vision, sinus congestion, mood swings, sweats red eyes. These symptoms could as well leads to a serious health issue like cancer, acute pulmonary haemorrhage, kidney toxicity, immune suppression, depression, autism, chronic fatigue syndrome, neurotoxicity, birth defects and aplastic anaemia. These symptoms depends on the infected host i.e. human and animal and also the immune system of the host [SMITH & al. 1995].

Furthermore, aflatoxins are very poisonous and its grows in soil, decomposing vegetation, grains and hay of major found in hot, humid climates, colonizing mainly on the aerial parts of the plants [MARIN & al. 2013]. Mostly aflatoxins have similar structures and it have earned a significant attention due to their dangerous effects on human and animals likewise the international trade of foodstuffs. There are about 20 recognised kinds of aflatoxins are which are basically classified into aflatoxin B1 (AFB1), B2, G1, G2, M1 and M2 depending on thy structure, fluorescent characteristics and chromatography [EPHREM, 2015]. Below is Figure 1 showing the chemical structure of aflatoxin, B1, B2, G1, G2, M1 and M2.

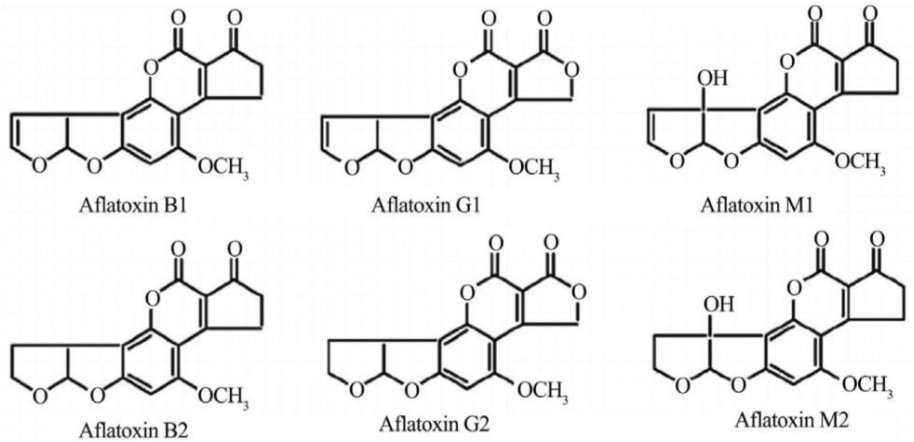

Figure 1. The chemical structure of aflatoxin, B1, B2, G1, G2, M1 and M2.
Source: MARIN & al. (2013)

Aflatoxin B1 is very high in toxicity and mainly metabolized by liver into AFB1-8, 9-exo-epoxide and 8, 9-endo-epoxide which normally binds to DNA to form 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-(AFB1-N7-Gua) and AFB1-N7-Gua can be converted to two secondary lesion which is an apurinic site in which more stable ring is opened. This shows that aflatoxins have an effect on amino acid metabolism. The main human cytochrome P450 (CYP) enzymes involved in aflatoxin metabolism are known as CYP3A4, 3A5, 3A7 and 1A2 respectively [MARIN & al. 2013].

As it was reported in JEF & al. (2015), drought and stress enhances the spread of aflatoxin in the field and also could be produced due to inadequate drying of crops that have been contaminated with aflatoxins before storage or crops been stored under humid environment. Due to aflatoxin firmness to severe processes of baking, roasting, cooking and extrusion, it's induces a tremendous problem in processed foods, for example bakery and

roasted nuts products and it could be found at the same time or alone, as well as co-occurring with other mycotoxins such as Ochratoxin A (OTA).

Ways of controlling mycotoxins

However, there four (4) ways in which the harmful effects of mycotoxin contamination food could be minimized:

1. By preventing contamination in the field through proper handling of crops;
2. Separation of contaminated materials from the healthy food commodity;
3. Reducing mycotoxin content in food and feed;
4. Proper treatment of exposed individuals.

A proper agronomic practices such as growing resistant crop varieties, soil tillage, crop rotation, insect control, chemical and biological control of plant diseases are major means in which mycotoxin contamination crops could be reduced in the field [EDWARDS, 2004]. In addition, a proper harvest and a good storage environment are very important in other to prevent fungal proliferation and accumulation of mycotoxin in harvest crops [JACOBSEN, 2014]. Food processing could initiate mycotoxins in food and feed raw material by physical removal, chemical transformation which could result in metabolites of lower or higher toxicity, release from masked or entrapped forms which might enhance bioavailability, enzymatic detoxification, and adsorption to solid surfaces.

Soybean as an important commodity for both human and livestocks

Soybean (*Glycine max* (L.) Merr.) are widely known as a leguminous crop, originated from Asia and was later introduced the USA in the year 1765 by Samuel Bowen [BRACHFELD & CHOATE, 2007]. Carl Linnaeus first introduced the genus name *Glycine* during his first edition of *General Plantarum.* The word glycine was derived from Greek work "glykys", which means sweet, and it refers to the sweetness of the pear-shaped edible tubers which was produced by Native American legume. In 1917, Merrill proposed the scientific name, *G. max* (L.) Merrill., which has known become the official name of this useful and live saving plant.

Soybean has been reported severally as a very good source of protein and dietary fiber and also the only vegetable with complete protein due to the fact that it has the capability to lower the bad cholesterol levels (LDI) in the body [HOFFMAN & FALVO, 2004]. The plantation of soybean in Nigeria commenced in the northern part of the country in year 1900s, and it has been fasten spread in almost the countries of the nation [SHURTLEFF & AOYAGI, 2007]. In DUGJE & al. (2009), the cultivation of soybean is usually in 6^{th} and 7^{th} month of the year (June/July), at the peak of the rainy seasons and harvested in 10^{th} and 11^{th} month of same year (October/November).

However, soybean was considered as an important crop in the year 1910s due to its use in crop rotation as a method of nitrogen fixation. The useful crop has played an important role in USA after the World War 1. The drought regions of the USA were able to regenerate their soil fertility and therefore enhanced their productivity that met with Government demands, due to the nitrogen fixation in soybean.

Soybean contain a high nutritional value of 40% protein and has best oil quality content of about 20% among other oilseed crops, depending on the genes and environmental factors. Soybean oil is unsaturated fatty acids, which is cholesterol free due to its high content of polyunsaturated fatty acid, and it is also digestible [HOFFMAN & FALVO, 2004]. It has been globally known as one of the most prominent crop. Soybean enhances soil fertility by

fixing the nitrogen in the soil which results in improving the crop yield for a better incomes. It is called the "miracle seed" because of its potentiality in supplying edible protein vegetable oil for human and animal consumption. Soybeans are recently the number one widely grown oilseed in the world. Soybean plant is known as an oilseed rather than pulse crop. About 85% of the global soybean crop is been proceed into soybean meal and vegetable oil. The demand for soybean is increasing tremendously to mirror the growth of global population for quality oil and protein content [OSHO, 2003]. The nutritional value of soybean (per 100 g) is shown in Table 2.

Table 2. Illustrating the nutritional value of soybean (per 100 g)

S/N	SOYBEAN NUTRITIONS	VALUE	S/N	SOYBEAN NUTRITIONS	VALUE
1	Energy	466 kcal	22	Fat	19.94 g
2	Carbohydrates	30.2 g	23	Saturated	2.89 g
3	Sugars	7.3 g	24	Monounsaturated	4.4 g
4	Protein	36.49 g	25	Polyunsaturated	11.26 g
5	Tryptophan	0.59 g	26	Water	8.54 g
6	Threonine	1.77 g	27	Vitamin A	1 mg
7	Isoleucine	1.97 g	28	Vitamin B6	0.4 mg
8	Leucine	3.31 g	29	Vitamin C	6.0 mg
9	Lysine	2.71 g	30	Vitamin K	47 mg
10	Methionine	0.55 g	31	Calcium	277 mg
11	Phenylalanine	2.12 g	32	Iron	15.7 mg
12	Tyrosine	1.54 g	33	Magnesium	280 mg
13	Valine	2.03 g	34	Phosphorus	704 mg
14	Arginine	3.15 g	35	Potassium	1797 mg
15	Histidine	1.1 g	36	Sodium	2 mg
16	Alanine	1.92 g	37	Zinc	4.9 mg
17	Aspartic acid	5.12 g	38	Phosphorus	704 mg
18	Glutamic acid	7.87 g	39	Potassium	1797 mg
19	Glycine	1.88 g	40	Sodium	2 mg
20	Proline	2.38 g	41	Zinc	4.9 mg
21	Serine	2.36 g			

Source: USDA National Nutrient Database (2004).

Furthermore, soybean is complete protein because it contains all nine (9) essential amino acids. The high content of sulphur containing amino acids servers as storage protein in soybean varieties, but their significant reduces ones their the crops encounter challenges in germination. It is a significant source of protein for some people, especially vegetarian, as it serves as meat to the vegetarians [RIZZO & BARONI, 2018]. Soybeans contain vitamins, A, B6, B12, C, and K. These chemical compounds cannot be synthesized in sufficient quantities by an organism and must be obtained from the diet.

However, aflatoxin disease is a not really a problem to soybean because of the little amount of zinc oxide of about 0.01 Mg/g, which is bound to phytic acid [IRVING, 1971]. A report in HOWELL (1968) shows that Aflatoxin producing strains of *Aspergillus flavus* can be grown on soybean seed in the laboratory, that the quantity of aflatoxins produced is much lower compared with other commodities. The occurrence of both *Aspergillus flavus* and aflatoxins in soybean is basically very low. The report also indicates that in the 1965 and 1966, *Aspergillus flavus* could not been isolated from more than three thousand and one hundred (3,100) samples of soybean seed examined. Another report in SHOTWELL & al. (1969), demonstrated that only 2 of 866 soybean samples contained aflatoxins. So many reasons have been proposed for the resistance of soybean to *Aspergillus flavus* such as

unfriendly moisture conditions for the fungus during the time of maturity of soybean, development of seeds in a closed pod, or it might be possible that the soybean seed comprised an inhibitor which could prevent fungus growth in soybean [HOWELL, 1968]. Soybean contains anti nutritional factors such as trypsin-chymotrypsin inhibitors. Although partially cooking of soybean reduces the proteinase inhibitor in soybean thereby producing a quality food [LIENER & TOMLINSON, 1981]. HENSARLING & al. (1983), recorded that the increased growth of *Aspergillus flavus* and *Aspergillus parasiticus* enhance toxin production, after the cooking of soybeans, soybean flour, or soybean-based medium, might indicate that heat-labile seed constituents such as proteinase inhibitors have an antifungal effect. However, this increase has rather been attributed to the availability of zinc, an essential trace element for aflatoxin synthesis and to the presence of phytate [MARSH & al. 1975]. Total aflatoxin production with non-autoclaved soybeans was only 0.34 mg per 100 g as compared to 6.85 mg per 100 g for autoclaved soybeans. A report in GUPTA & VENKITASUBRAMANIAN (1975), indicates that the total number of aflatoxin production with nonautoclaved soybeans was only 0.34 mg per 100 g as compared to 6.85 mg per 100 g for autoclaved soybeans.

Conclusion

Aspergillus flavus is among the dominating species colonizing the soybean seed surface. Exposition of soybeans to high temperature even for a short period promotes high production of aflatoxin diseases. Mycotoxins do not only pose health risk to human and livestock, but also impact global economy, and food safety. Since mycotoxins are ubiquitous and they can appear everywhere in every commodity thereby causing health and economic challenges, appropriate and environmentally friendly prevention and control strategies shall be given priority. Moreover the government shall consider the use of (biotechnological) molecular approaches to control mycotoxins [GIZACHEW & al. 2016]. Soybean varieties had acceptably low mycotoxin levels. Soybean has proven itself as an excellent nutrient source of protein, oil, and other small molecules. The demand for soybean consumption increased as expected in the past decade.

However, it can be promoted as a safe and nutritious food including for weaning, with good agricultural practices helping to ensure that contamination remains low. Given its importance as a major protein source in the human and livestock diet, there is a need to increase the cultivation and consumption of soybean in Nigeria. However, there is need for educating farmers on mycotoxin contamination in food and feeds to ensure better standards to safeguard the health of the consumers regarding these fungal metabolites. A larger surveillance on these toxins should be done world widely for a range of susceptible crops, as some weather is more conducive for mycotoxin production.

Notes on contributors

Gloria Chinasa NNAMANI is biotechnologist/Researcher, currently working on evaluation of soybean advanced lines.

Andrew Shema GANA is a professor of plant bleeding in the University of Technology, Minna.

Aondover SHAAHU is a PhD student/Researcher, currently working on soybean breeding and evaluation of advanced lines.

Titus Saanmoiyol MSAAKPA is an associate professor at Federal University of Agriculture, Makurdi, Benue State. His work focused on seed physiology.

Prince Chisom LEMIBE is an agronomist/Researcher, his work is focused on weeds evaluation.

Maxwell Arinze NNAMANI is a Computer Scientist and currently working on Microsoft designs.

Emmanuel Obinna NWANYANWU is a computer Engineer. His work is focused on hardware designs.

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CENTAUREA RUTHENICA LAM. (ASTERACEAE DUMORT.) IN THE FLORA OF THE REPUBLIC OF MOLDOVA

Pavel PÎNZARU[1,2]

[1] "Alexandru Ciubotaru" National Botanical Garden (Institute), Chișinău – Republic of Moldova.
[2] Tiraspol State University, Chișinău – Republic of Moldova.
E-mail: p_panzaru@yahoo.it

Abstract: The presence of *Centaurea ruthenica* Lam. in the flora of the Republic of Moldova was indicated by SCHMALHAUSEN (1886, 1897), near the village of Rascov, on the left bank of the Dniester River. This summer, the author has found this species near the village of Tipova, Rezina district, on the right bank of the Dniester River. This article presents the morphological description of the species, its biological and ecological features under the local conditions. It has been proposed to include *Centaurea ruthenica* Lam. in the Red Book of the Republic of Moldova, in the Critically Endangered (CR) category.

Key words: *Centaurea ruthenica* Lam., Asteraceae Dumort., morphological description, biological and ecological features, Republic of Moldova.

Introduction

Centaurea ruthenica Lam. [=*Rhaponticoides ruthenica* (Lam.) M. V. Agab. et Greuter] is a hemicryptophytic, xerophytic, heliophyte occurring in the Balkans, central Europe (Transylvania region in Romania), East Europe (southern part), the Caucasus, Western Siberia and Central Asia [TZVELEV, 1963; DOSTÁL, 1976; CZEREPANOV, 1994; DOBROCZAJEVA, 1999; PLANTARIUM, 2007-2021; AGHABABYAN, 2008; NEGREAN & DIHORU, 2009, SÂRBU & al. 2013; CWG, 2021], considered a rare Eurasian (continental) species, included in the Red Book of Romania, in the Critically Endangered (CR) category [NEGREAN & DIHORU, 2009], and some regions of Russia and Ukraine [PLANTARIUM, 2007-2021].

The presence of the species *Centaurea ruthenica* Lam. in the village of Rascov, on the left bank of the Dniester River was indicated by SCHMALHAUSEN (1886, 1897), in other bibliographical sources it is missing. In June, this year, it was found near the village of Țipova, Lalova commune, Rezina district.

This article presents the morphological description of the species, its biological and ecological features under the local conditions.

Materials and methods

The plants of *Centaurea ruthenica* Lam. were collected by the author on June 27, 2021, the exsiccatae are kept in the Herbarium of the "Alexandru Ciubotaru" National Botanical Garden (Institute), in the Herbarium of "Anastasie Fătu" Botanical Garden of Iași [IAGB 47715] and in the author's own Herbarium, donated to the State University of Tiraspol (Kishinev). Mature achenes were collected on July 27, 2021, which will be cultivated under *ex situ* conditions, for multiplication and reintroduction into characteristic natural habitats of the species.

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The species was determined according to the classical comparative-morphological method, using the illustrated guide for species determination "Vascular plants of Romania" [SÂRBU & al. 2013]. The evaluation and the determination of the conservation category was performed according to the criteria adopted by the International Union for Conservation of Nature [IUCN, 2001, 2003].

Results and discussions

As a result of the continuation of the floristic studies on the cliffs vegetation in the Republic of Moldova, the species *Centaurea ruthenica* Lam. was detected for the first time on the right bank of the Dniester River, near the village of Tipova, Rezina district.

Centaurea ruthenica Lam. 1785, Encycl. Méth. Bot. 1: 663; Schmalhausen, 1886, Fl. Jugo-Zap. Ross.: 329, id. 1897, Fl. Sred., Jug. Ross., Kryma, Sev. Kavkaza 2: 120; Tzvelev, 1963, Fl. URSS, 28:380; Dostál, 1976, Fl. Europ. 4: 263; Czerepanov, 1994, Fl. partis europ. URSS, 7: 270; Dobroczajeva, 1999, Opred. Vysch. Rast. Ukr., ed. 2: 355; Sârbu, Ștefan & Oprea, 2013, Pl. Vasc. România: 854. – *Rhaponticoides ruthenica* (Lam.) M. V. Agab. et Greuter, 2003, Willdenowia, 33: 61; Aghababyan, 2008, Caucas. fl. consp. 3 (1): 299. – **Centauree ruseasc**ă. Star thistle – Figure 1-3.

Figure 1. *Centaurea ruthenica* Lam., village of Țipova, Rezina district, 27.06.2021

Figure 2. *Centaurea ruthenica* Lam. – involucre, 27.07.2021

The plant is glabrous. Thickened rhizome, with 1-3 stems 100-120 (145) cm tall, simple or slightly branched at the top. The leaves are ovate-elongated, pinnatisect, with lanceolate segments, with toothed margins: the lower ones up to 25 cm long and up to 10 cm wide in the middle, with a 3-5 cm long petiole; the upper ones – gradually smaller, subsessile to sessile. The inflorescences are flower heads, grouped by 2-3, terminal, 5-6 cm in diameter. The involucre is 2-2.5 cm in diameter, the outer and middle involucral bracts – wide-ovate, green during flowering, without appendices, with scarious margin, the inner ones – lanceolate, membranous at the tip. The flowers are pale-yellow: the external ones – sterile, 37 mm long, with a 12 mm long tube, laciniae grouped by 5, long 25 mm and 1 mm wide; the internal flowers – fertile, smaller, 26 mm long, with 19 mm long tube, with 5 laciniae, 7 mm long, filiform. The stamens are slightly shorter than the laciniae of the corolla. The style is protruding, 6 mm longer than the corolla. The achenes are 6-7 mm long, slightly 4-edged, dark brown. The setae of the pappus are simple, unequal, up to 3-4 mm long, brown.

Figure 3. Achenes of *Centaurea ruthenica* Lam., 27.07.2021

Biological and ecological characteristics. Perennial hemicryptophyte, blooms in June-July (beginning), the dissemination occurs at the end of July – beginning of August. 2n = 30. The species is xerophytic, calcicole. In the Republic of Moldova, it occurs sporadically on rendzina soils, formed on limestone bedrock of Middle Sarmatian age. Altitude 140-143 m. It was found on east-facing slopes, with the inclination of 35°-40°. The general coverage of the herbaceous layer vary between 80 and 100%. In the vertical structure of the phytocoenoses, 3 layers are distinguished.

Figure 4. *Centaurea ruthenica* Lam. growing on a slope of the Dniester valley, village of Țipova, Rezina distict, 27.06.2021

Figure 5. The habitat of *Centaurea ruthenica* Lam. in the upper part of the cliff

The upper layer, 80-120 (145) cm high, consists of: *Agropyron cristatum* (L.) Gaertn., *Artemisia campestris* L., *Asparagus officinalis* L., *Asparagus verticillatus* L., *Bromus inermis* Leys., *Bupleurum falcatum* L., *Campanula rapunculoides* L., *Centaurea orientalis* L., *Centaurea ruthenica* Lam., *Elymus repens* (L.) Nevski, *Erysimum cuspidatum* (M. Bieb.) DC., *Hieracium virosum* Pall., *Isatis tinctoria* L., *Jurinea ledebourii* Bunge, *Melica transsilvanica* Schur, *Melilotus officinalis* (L.) Pall., *Scabiosa ochroleuca* L., *Serratula radiata* (Waldst. & Kit.) M. Bieb., *Sisymbrium loeselii* L., *Stipa capillata* L., *Tanacetum corymbosum* (L.) Sch.

Bip., *Thalictrum minus* L., *Valeriana collina* Wallr., *Verbascum phoeniceum* L., *Vincetoxicum hirundinaria* Medik.

The middle layer, 30-60 cm high, consists of: *Allium flavum* L., *Allium paniculatum* L., *Amygdalus nana* L., *Bromus squarrosus* L., *Camelina microcarpa* Andrz., *Cerinthe minor* L., *Euphorbia agraria* M. Bieb., *Festuca valesiaca* Schleich. ex Gaudin, *Lappula patula* (Lehm.) Gürke, *Marrubium peregrinum* L., *Medicago falcata* L., *Melampyrum arvense* L., *Nigella arvensis* L., *Odontarrhena muralis* (Waldst. & Kit.) Endl., *Poa compressa* L., *Papaver stevenianum* Mikheev, *Reseda lutea* L., *Salvia nemorosa* L., *Securigera varia* (L.) Lassen, *Sedum maximum* Suter, *Silene csereii* Baumg., *Stachis recta* L., *Veronica spicata* L., *Xeranthemum annuum* L.

In the lower layer, up to ± 20 cm, the following species grow: *Alyssum alyssoides* (L.) L., *Arenaria serpyllifolia* L., *Aurinia saxatilis* (L.) Desv., *Clinopodium acinos* (L.) Kuntze, *Iris pumila* L., *Poa bulbosa* L., *Sedum acre* L., *Teucrium chamaedrys* L., *Teucrium capitatum* L., *Veronica prostrata* L., *Vinca herbacea* Waldst. & Kit.

Quantitative aspect. It grows sporadically on an area of about 400 m^2, the only population detected consists of about 80 mature plants. Not all flower heads produce mature achenes, 1-5 (15) achenes reach maturity in one flower head.

Conservation status. Territorially protected in the "Țipova" Landscape Reserve.

Conservation measures. Inclusion in the List of species protected by the state and in the Red Book of the Republic of Moldova, in the Critically Endangered (CR) category; monitoring the condition of the existing population; *ex-situ* multiplication of the species and its repatriation into its characteristic natural habitats.

Local distribution. It has been found in the village of Țipova in Lalova commune, Rezina district, and its presence in Rașcov commune in Camenca district (probably extinct) was indicated by SCHMALHAUSEN (1886, 1897).

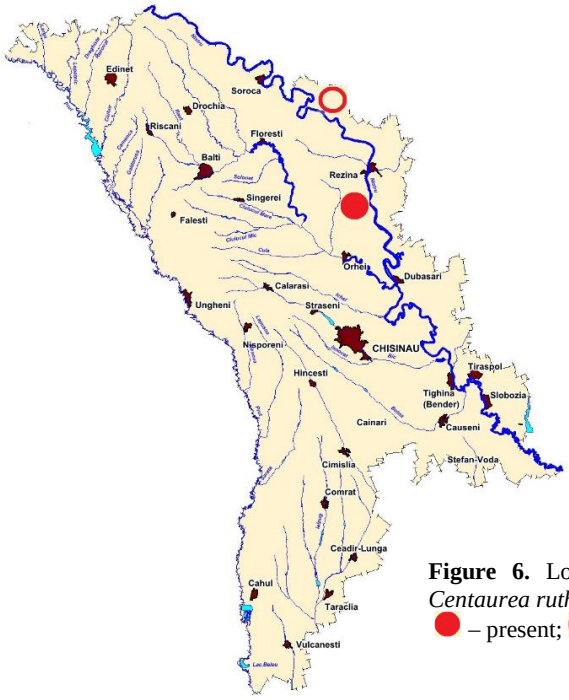

Figure 6. Localities of distribution of the species *Centaurea ruthenica* Lam. in the Republic of Moldova: ● – present; ○ – probably extinct.

Conclusions

Centaurea ruthenica Lam., under the climatic conditions of the Republic of Moldova, is a rare xerophytic species and we propose to include it in the *List of species protected by the state* and in the *Red Book of the Republic of Moldova*, in the Critically Endangered (CR) category.

We suggest to avoid collecting specimens of this species for herbaria from its natural habitat.

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THYMUS COLDEI PÎNZARU SP. NOVA (LAMIACEAE) IN THE FLORA OF THE REPUBLIC OF MOLDOVA

Pavel PÎNZARU[1,2]

[1] "Alexandru Ciubotaru" National Botanical Garden (Institute), Chișinău – Republic of Moldova.
[2] Tiraspol State University, Chișinău – Republic of Moldova.
E-mail: p_panzaru@yahoo.it

Abstract: The paper describes a new species for science – *Thymus coldei* Pînzaru sp. nova, occurring on Middle Sarmatian limestones, in the Republic of Moldova.

Key words: *Thymus*, Lamiaceae, new species, Republic of Moldova.

Introduction

Four species of the genus *Thymus* L. were identified in the cliff area of the Republic of Moldova: *T. pannonicus* All. [GHEIDEMAN, 1986], *T. marschallianus* Willd. [GHEIDEMAN, 1986; NEGRU, 2007], *T. moldavicus* Klokov et Des.-Shost. [GHEIDEMAN, 1986; NEGRU, 2007; PÎNZARU, 2020] and *T. roegneri* K. Koch [PÎNZARU & CANTEMIR, 2018; PÎNZARU, 2020], the last 2 species occur only on limestone slopes. The floristic research on species of the genus *Thymus* L. was continued by the author in order to describe them for the flora of Bessarabia.

This article highlights and describes a new species for science – *Thymus coldei* Pînzaru sp. nova, detected in the Middle Sarmatian limestone area in the Republic of Moldova.

Materials and methods

The floristic research was carried out by the author in the years 2003, 2005, 2009, 2015-2021 on the calcareous hills from the basins of the Dniester river and the Prut river. Twenty-one specimens of *Thymus coldei* Pînzaru sp. nova have been collected and botanized, the exsiccatae are stored in the Herbarium of the "Alexandru Ciubotaru" National Botanical Garden (Institute) in Kishinev [CHGB], in the Herbarium of "Anastasie Fătu" Botanical Garden of Iași [IAGB 47713] and in the Herbarium of the botanist Pavel Pînzaru at the Tiraspol State University (based in Kishinev) [CHUST-PP]:

- Glodeni district, Cobani commune, on rendzina soil, rich in calcareous gravel, of the Middle Sarmatian stage, 10 VI 2020, P. Pînzaru [CHUST-PP 2923];
- Florești district: Țîra village in Ghindești commune, on Middle Sarmatian limestones, on the left bank of the Răut river, 20 V 2015, P. Pînzaru [CHUST-PP 2916];
- Șoldănești district: Rogojenii Vechi village in Rogojeni commune, on Middle Sarmatian limestones, in the valley of the Răut river, 28 VII 2003, P. Pînzaru [*Paratype*: CHUST-PP 2923, 1894; CHGB 239638]; 21 V 2009, P. Pînzaru [CHUST-PP 2913; CHGB 239634];

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- Orhei district: Orhei city towards Păhărniceni commune, on rendzina soil, rich in limestone gravel of the Middle Sarmatian stage, on the left bank of Răut river, 12 V 2009, P. Pînzaru [CHUST-PP 2917]; Piatra commune, on Middle Sarmatian limestones, on the left bank of Răut river, 10 VI 2015, P. Pînzaru [CHUST-PP 2914, 2915; CHGB 239636]; Păhărniceni commune, on Middle Sarmatian limestones, on the left bank of Răut river, 12 V 2009, P. Pînzaru [CHUST-PP 2918], 10 VI 2015, P. Pînzaru [CHGB 239639];
- Dubăsari district: Molovata commune towards Marcăuţi commune, on Middle Sarmatian limestones, on the right bank of Dniester river, 20 V 2016, P. Pînzaru [CHUST-PP 1896; CHGB 239637];
- Kishinev municipality: Goian village in Ciorescu commune, on rendzina soil, rich in calcareous gravel, of the Middle Sarmatian stage, on the left bank of the Ichel river, 19 V 2018, P. Pînzaru [CHUST-PP 2919]; Făureşti village in Ciorescu commune, on Middle Sarmatian limestones, on the left bank of the Ichel river, 21 VI 2020, P. Pînzaru [CHGB 239635], 07 VI 2021, P. Pînzaru [CHUST-PP 2924]; Ciorescu commune, on Middle Sarmatian limestones, in the valley of the Ichel river, 08 VI 2021, P. Pînzaru [*Holotype*: CHGB 239640], 21 VI 2020, P. Pînzaru [*Isotype*: CHGB 239641], 08 VI 2021 P. Pînzaru [CHUST-PP 2920], Cricova town, Chişinău municipality, on rendzina soil, rich in calcareous gravel, of the Middle Sarmatian stage, in the valley of the Ichel river, 21 VI 2020, P. Pînzaru [CHUST-PP 2921].

Eleven exsiccatae of *Thymus moldavicus* Klokov & Des.-Shost., collected by the author from the districts of the Republic of Moldova, were examined as comparative material:

- Criuleni district, Maşcăuţi commune on the border with Morovaia village, on Middle Sarmatian limestones, on the left bank of the Răut river, 13 VII 2018, P. Pînzaru [CHGB 239643, 239644], 22 VI 2020, P. Pînzaru [CHUST-PP 869], 13 VII 2020, P. Pînzaru [CHUST-PP 1874]; Maşcăuţi commune, on Middle Sarmatian limestones, on the right bank of the Răut river, 23 V 2018, P. Pînzaru [CHUST-PP 871, 872];
- Orhei district, Butuceni village in Trebujeni commune, on Middle Sarmatian limestones, on the left bank of the Răut river, 23 VI 2020, P. Pînzaru [CHGB 239633; CHUST-PP 870]; Morovaia village in Trebujeni commune, on Middle Sarmatian limestones, on the left bank of the Răut river, 23 VI 2020, P. Pînzaru [CHGB 239642];
- Grigoriopol district, Taşlîc commune, on Middle Sarmatian limestones, on the right bank of the Taşlîc river, 21 VIII 2018, P. Pînzaru [CHGB 239645]; Butor commune, on Middle Sarmatian limestones, on the left bank of Dniester river, 21 VIII 2018, P. Pînzaru [CHGB 239646].

The identification of the plants was performed according to the classical comparative-morphological method, using the keys for identifying the species of the genus *Thymus* L. [JALAS, 1972; MENITSKY, 1978; GHEIDEMAN, 1986; DOBROCZAJEVA & al. 1999; NEGRU, 2007; SÂRBU & al. 2013; KNYASEV, 2015]. We examined the exsiccatae of the genus *Thymus* L. from the collection of the Herbarium of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Kishinev, and of the species *Thymus glabrescens* Willd. (species that shares some similar characteristics with the new species) from the collections of the Herbaria of the "Anastasie Fătu" Botanical Garden [IAGB] and of the "Alexandru Ioan Cuza" University of Iaşi [I].

The botanical illustration was drawn by the illustrator Petru Leca, in consultation with the author, based on the selected original material.

Results and discussions

As a result of the floristic research carried out on the species of the genus *Thymus* L. occurring in the cliff areas of the Republic of Moldova, a new species for science was identified – *Thymus coldei* Pînzaru sp. nova, described below. *Thymus coldei* plants, during the flowering stage, begin to develop repent stems, which continue to grow until autumn, forming serial clusters of leaves, which will produce flowering branches in the following year (Figure 1). The sterile repent stems, in some places, at the internodes, develop adventitious roots, favouring vegetative propagation. The fruiting branches die after dissemination (Figure 2).

Figure 1. *Thymus coldei* Pînzaru sp. nova, 08 VI 2021,
Ciorescu commune, Kishinev municipality

Thymus coldei Pînzaru sp. nova **– Coldea thyme –** Figure 1-4

Lemon-scented plants. Stems repent, long, with erect or ascending flowering branches towards the tips; during the flowering stage, the plant develops long, sterile stems, which take root. Flowering branches 2-7 (-12) cm tall, arranged in series; pubescent around: in the first half – evenly pubescent, in the second half (towards the top) on sides with short hairs, 0.5 mm, oriented downwards, and on the edges with twice longer, upright hairs. The lower leaves are lanceolate or slightly spatulate, 3-5 mm long, 1-3 mm wide, the middle ones – slightly longer than the upper ones, linear-lanceolate, 10 to 15 mm long and 1.5-2.5 mm wide, petiolate to subsessile, glabrous on both sides, at the base, with few cilia on margins. The inflorescences are usually heads, rarely with 1-2 whorls. The flowers are borne on ± 2 mm long peduncles. The bracts are ovate, ovate-elongated, as long as the calyx or shorter, with well-defined veins. The calyx is 3 mm long, on the ventral side densely pubescent, upright hairs. The upper teeth of the calyx are slightly elongated in shape, ciliated. The corolla is 6 mm long, pink, pubescent on the

outside, the upper lip is slightly emarginate, the lower lip – with 3 lobes almost equal in size, spotted at the base. There are 2 stamens under the upper lip and 2 exserted ones. The stigma is bifurcated, exserted. The seeds are rounded, ovoid, 0.7-0.8 mm long, brown.

Figure 2. *Thymus coldei* Pînzaru, 24 VIII 2021, Ciorescu commune, Kishinew municipality

H o l o t y p e: Republic of Moldova, Ciorescu commune, Kishinev municipality, on Middle Sarmatian limestones, on the left bank of the Ichel river, collected on 08 VI 2021, P. Pînzaru, determined on 10 VI 2021 [CHGB 239640].

I s o t y p e: Republic of Moldova, Ciorescu commune, Kishinev municipality, on Middle Sarmatian limestones, on the left bank of the Ichel river, collected on 21 V 2020, P. Pînzaru, determined on 10 VI 2021 [CHGB 239641].

P a r a t y p e: Republic of Moldova, Rogojenii Vechi village, Șoldănești district, on Middle Sarmatian limestones, in the valley of the Răut river, collected on 28 VII 2003, P. Pînzaru, determined on 10 VI 2021 [CHUST-PP 2923].

Figure 3. Holotype of *Thymus coldei* Pînzaru (CHGB 239640)

Figure 4. *Thymus coldei* Pînzaru – a. leaf; b, c. bracts; d. flower; e. calyx; f. segment of a flowering branch below the inflorescence

Affinity. The new species is similar to *Thymus moldavicus* Klokov et Des.-Shost, having repent, long stems, head inflorescences, bracts of different shape than the upper leaves, calyx with ciliated teeth, but it differs in the pubescence of the flowering branches – on the second half, on sides – with short, adpressed, downwards oriented hairs, and on edges – with twice longer, upright (not flowering branches equally pubescent all around, with short, downwards oriented hairs), the upper teeth of the calix are longer that wide (not upper teeth of

the calix of almost equal length and width), the plants smell like lemon and not otherwise. Both species grow on Sarmatian limestones, but separately one from another. Another species with similar habit is *Thymus glabrescens* Willd., but it differs from the species *T. coldei* in the shape of the leaves – they become larger from the base towards the tip of flowering branches, and the bracts are of the same shape as the leaves and do not have a lemon-like smell.

Biological and ecological peculiarities. Chamaephyte species, blooms in June-July, xerophyte, calcicole. It propagates by seeds and vegetatively. It is a melliferous, ornamental, spicy (used to prepare herbal tea) plant. It grows on bare limestones of the Middle Sarmatian stage or on rendzina soils, rich in calcareous gravel, it is characteristic of the vegetation in the alliance *Genisto tetragonae-Seselion peucedanifolii* P. Pânzaru 1997 (Figure 5). It grows at 50-110 m altitude.

Figure 5. *Thymus coldei* Pînzaru in its natural habitat, Ciorescu commune, 24 VIII 2021

Distribution in the districts of the Republic of Moldova (Figure 6): Glodeni (Cobani), Florești (Țîra), Șoldănești (Rogojenii Vechi), Orhei (Orhei, Piatra, Păhărniceni), Dubăsari (Molovata), Kishinev municipality (Ciorescu, Făureşti, Goian, Cricova).

Limiting factors. Specific habitat. Limestone extraction from open quarries and irrational collecting lead to a reduction in the areas of distribution of the species.

Conservation status. It is territorially protected within the area of the "Mâgla Rock" Geological and Paleontological Nature Monument, near Piatra commune, Orhei district.

Protection measures. Prohibition of limestone extraction from open quarries, the inclusion of the species in the *List of species protected by the state* and in the *Red Book of the Republic of Moldova*, in the vulnerable category (VU), the inclusion in the Network of Protected Areas of the canyon "Rogojenii Vechi" found near Rogojenii Vechi village, Şoldăneşti district, and the limestone hills in the valley of Ichel river (near Ciorescu commune and Făureşti and Goian villages).

The species has been named in honour of the corresponding member of the Romanian Academy Gheorghe COLDEA, Cluj-Napoca.

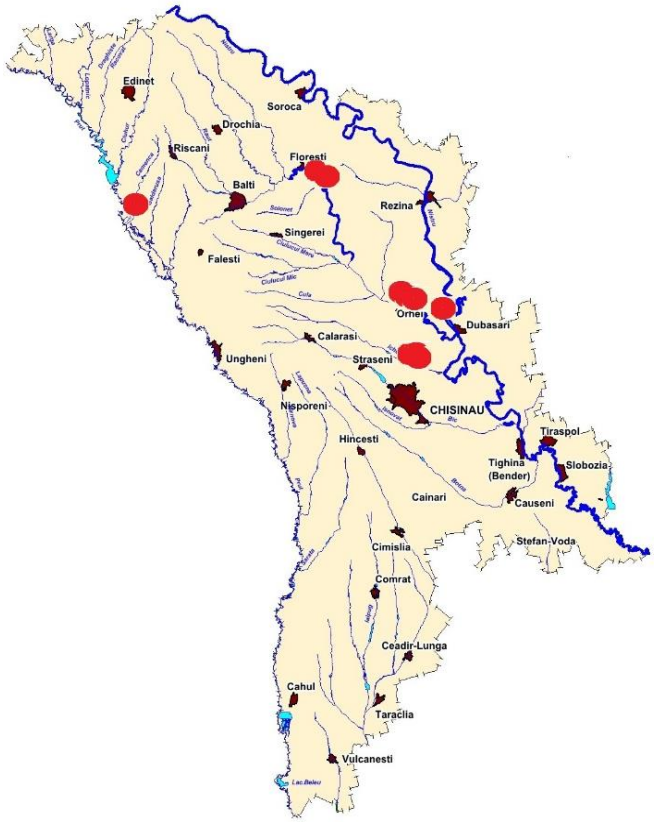

Figure 6. Locations of *Thymus coldei* Pînzaru in the Republic of Moldova

Conclusions

Thymus coldei Pînzaru is an endemic species of Middle Sarmatian limestones, xerophyte, vulnerable, therefore it is necessary to undertake protection measures, namely, to stop the extraction of limestone from open quarries in the valley of the Ichel river, in the sector Cricova – Goian, in Kishinev municipality.

The research was supported by the NARD through the project "Research and conservation of vascular flora and macromycobiota of the Republic of Moldova", 20.80009.7007.22 (contract Nr. 71/PS/2020).

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PROFESSOR PhD TOADER CHIFU AT THE 85TH ANNIVERSARY

On February 27, 2021, we celebrated the 85th anniversary of Professor PhD Toader CHIFU. He was born in Târnăuca, (currently in Ukraine) in 27th February 1936. He started primary school in 1941, in his native village, Crihăneşti (Târnăuca), but due to second word war he finished them in 1946 at the Şendriceni School of Applications in Dorohoi. He followed the secondary courses in Pomârla and attended, in the same locality, the courses of the Agricultural Technical High School, which he graduated in 1954. In 1960 he graduated the Faculty of Natural Sciences-Geography of the "Alexandru Ioan Cuza" University in Iaşi.

His academic career started as assistant professor at the Botany Department of the above mentioned institution (1960-1965). In the next three years he organized the Systematic Section of the Botanic Garden in Iaşi (1965-1968). From 1968 he was Scientific Researcher in the Geobotany Laboratory of the Romanian Academy (Iaşi Branch) and since 1976 he coordinated the Department of Terrestrial Ecology of the Biological Research Center in Iaşi. In 1976-1980 he held courses of Botany and Plant Ecology at the National Agronomical Institute from Alger. Since 1981 he held various courses of plant systematics, taxonomy, and ecology, productivity of ecosystems and others, and since 1995 he is full Professor at the Faculty of Biology.

His research activity is focused on mycology, myco-coenology, botany, phytosociology, and nature conservation and is highlighted by over 180 scientific articles and 11 monographies. For the thesis entitled *Floristic, ecologic-coenologic and economic research of the macromycetes from Neamțului Depression* he was awarded with a PhD diploma in Phytosociology-Mycology (1971). He elaborated important synthesis and monographies of Romanian vegetation as the *Phytosociological diversity of Romania's vegetation* (2014), describing over 1200 syntaxa, or *The floristic and phytosociological diversity of the vegetation in Ceahlău Massif* (2020). He attended and organized numerous scientific expeditions in the country and abroad, in Africa or Europe, and coordinated many research grants. Also he is member in scientific societies from Romania – *Romanian Phytosociological Society* or abroad – *Amicale Internationale de Phytosociologie*.

Professor Dr. Toader Chifu has a great academic and scientific career. He is a man with high moral and professional standards and mentor for numerous PhD students, specialists, collaborators and friends. All his accomplishments were possible due to tenacity, perseverance, and passion with which he approached the field of botany in general, but also due the understanding and support he received from his family. On behalf of all colleagues in the

"Anastasie Fătu" Botanic Garden of Iaşi, we wish Professor Toader Chifu long years of health, energy to continue his prolific scientific career and all the best for many years to come.

Happy Anniversary!

Constantin MARDARI
"Alexandru Ioan Cuza" University of Iaşi, "Anastasie Fătu" Botanic Garden
Cătălin TĂNASE
"Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology

Professor Alexandru POPOVICI (1866 – 1941)

The year 2021 marks 155 years since the birth of Alexandru Popovici and 80 years since his death. With this paper we want to commemorate the outstanding figure of Alexandru Popovici, professor and director of "Anastasie Fătu" Botanical Garden of Iași.

He was born on October 30th 1866 in the village of Cârniceni (Țigănași commune, Iași county). He attended the primary school in his native village, the "Ștefan cel Mare" gymnasium in Iași and the National College of Iași, which he graduated in 1885 [MITITIUC & TONIUC, 2006].

After that he left for the capital and started his university studies, at the Faculty of Sciences from the University of Bucharest, which he graduated in 1888. To complete his professional training, his mentor, Dimitrie Brândză send him to Germany, to enroll at Bonn University, where, under the supervision of the renowned professor Eduard Strasburger, he completed and defended, in 1893, his PhD thesis entitled *Ueber Struktur und Entwickelung eigenartiger Wandverdikungen in Samen und Fruchtschalen* / The structure and development of some particular thickenings of cell membranes in the seeds and pericarp of some angiosperm species [TOMA, 2015]. He followed the courses held by great botanists such as Noll ad Schenk and continued his studies at the University of Leipzig [PAPP, 1942].

He returned to Romania and continued his work as custodian and further as assistant at the Botanical Institute of Bucharest. In 1895 he becomes a substitute professor at the University of Iași, in 1900 associate professor and starting with 1904 he becomes titular professor at the prestigious higher education institution.

He was a dedicated professor who actively participated at the development of the Botany Laboratory, with specific features that allowed 40 students to simultaneously study practical Botany, the Herbarium with personal or bought sheets and the Library, with valuable Botany and Mycology volumes, including his own written courses (*Botanică Sistematică. Cryptogamia. 2. Bryophyta, Pteridophyta / Systematic Botany. Cryptogamia. 2. Bryophyta, Pteridophyta*, *Curs de Fanerogame* / *Phanerogams course*), necessary to study the fields of anatomy, physiology and systematics [PAPP, 1942]. Moreover, his domains of interest also included the study of mushrooms – mycology, his reference contributions to this field - *Contributions à la flore mycologique de la Roumanie* (*Contributions to the study of Romanian mycoflora*), being published between 1900 and 1910 in the Scientific Annals of the University of Iași, describing 29 species of myxomycetes and 204 genera with 440 species of ascomycetes and basidiomycetes.

His diligent and hard-working nature helped him in both his scientific and administrative activity, Alexandru Popovici being twice elected as Dean of the Faculty of Sciences [MITITELU & COSTICĂ, 1992].

He understood the fact that biology in general and botany in particular, must be learn in the middle of the nature, that plants aren't just a studying object, but a living organism, that have to be integrated in the environment [IFTIMOVICI, 1977] and it is necessary for the University of Iași to have a Botanical Garden similar with the one organized by *the great science loving professor dr. Fătu* [BURDUJA, 1979]. He strongly believed that *…a botanical garden*

has both an internal and external function. Internally, it shows a scientific, cultural and social functions and externally it brings prestige and honor to the people.

Being driven by this creed, he was closely involved in the establishment and development of the Botanical Garden of Iaşi. Therefore, in 1906 he was given the assignment to put together a plan for organizing the Botanical Garden in the near vicinity of the Palace of Culture. The planting activities begun, but unfortunately, due to the lack of founding, the idea of developing a botanical garden in that location failed to be accomplished.

Even in this situation, with an incredible tenacity, Alexandru Popovici addressed on many occasions the management of the University and the Ministry of Public Education, regarding the importance of a university botanical garden and asked to lead the planting activities on a terrain *empty and full of weeds*, in the vicinity of the University Palace. In 1921, he was granted permission and started organizing, with very few founds, a small botanical garden, with a surface of approximately 1 ha, that served the its purpose for over 40 years [TĂNASE, 2016].

In order to diversify the plant collections, he contacted the botanical gardens in Cluj-Napoca, Bucharest and Chernivtsi, requesting seeds, cuttings and plants from spontaneous or exotic species that he and his small team of collaborators and gardeners planted in the outdoor spaces and within the greenhouses [RESMERIȚĂ, 1982]. The plants were classified according to their ecology and geographical distribution in: flora of Iași county, flora of Ceahlău Massif, flora or Rarău Mountains, flora of Apuseni Mountains, collections with plant species from the steppe region, from maritime dunes, aquatic plants, halophytes and plant species that grow on rocky terrain, rare plants [BURDUJA, 1979].

Along with the development of the plant collections, in 1923 the first edition of the *Seed Catalogue* was published, as a mean to promote the botanical garden and to offer to other similar institutions plant material collected form the new botanical garden and from various location across Romania.

For all his merits, he was elected honorary member of the *Romanian Academy*, member of the *Physicians and naturalists Society of Iași* and member of the *Société Mycologique de France*. He dies on July 17th 1941, being buried at Eternitatea Cemetery in Iași.

The work Alexandru Popovici put into the development of the botanical garden never knew any limitations and overcame every obstacle (financial shortage, refusal from the authorities, fires), fact that underlines the devotion and desire to fulfill a lifetime dream.

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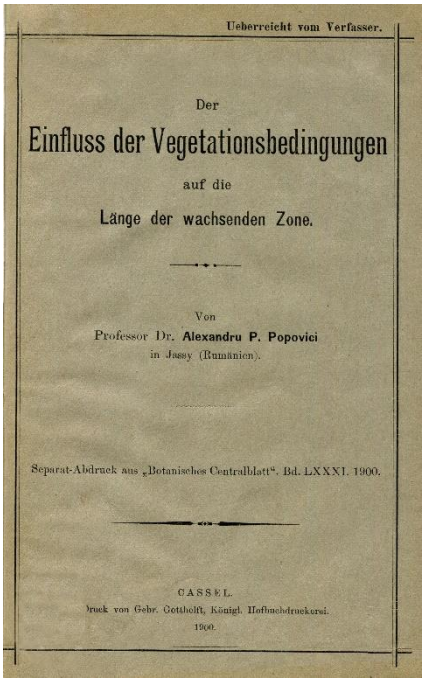
Cristiana Virginia PETRE
"Anastasie Fătu" Botanical Garden, "Alexandru Ioan Cuza" University of Iaşi
Cătălin TĂNASE
"Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology



Ueberreicht vom Verfasser.

Der

Einfluss der Vegetationsbedingungen

auf die

Länge der wachsenden Zone.

Von

Professor Dr. **Alexandru P. Popovici**

in Jassy (Rumänien).

Separat-Abdruck aus „Botanisches Centralblatt". Bd. LXXXI. 1900.

CASSEL.

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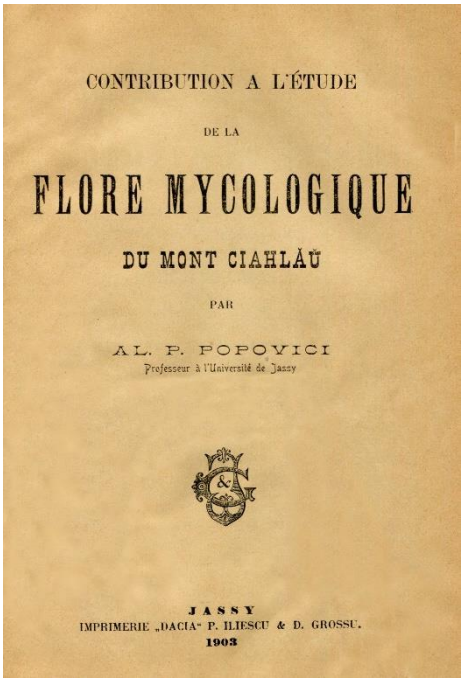


CONTRIBUTION A L'ÉTUDE

DE LA

FLORE MYCOLOGIQUE

DU MONT CIAHLĂU

PAR

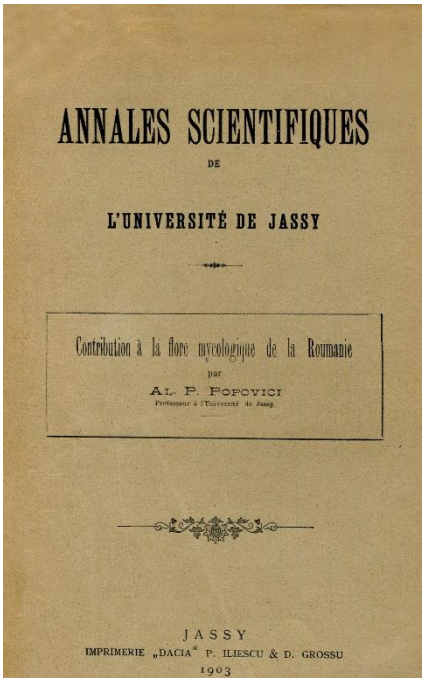
AL. P. POPOVICI

Professeur à l'Université de Jassy

JASSY

IMPRIMERIE „DACIA" P. ILIESCU & D. GROSSU.

1903



ANNALES SCIENTIFIQUES

DE

L'UNIVERSITÉ DE JASSY

Contribution à la flore mycologique de la Roumanie

par

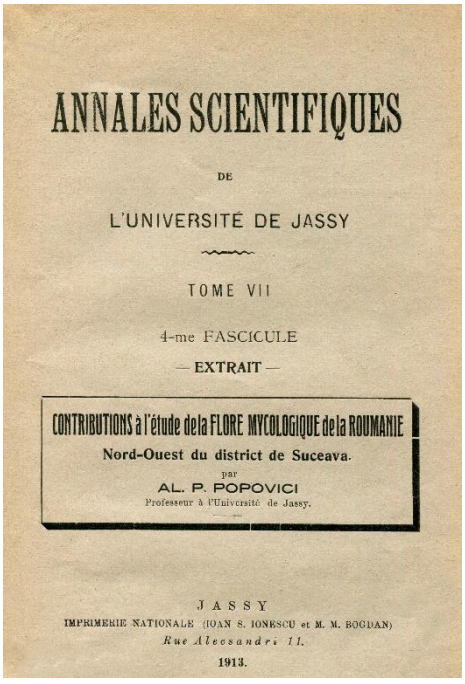
AL. P. POPOVICI

Professeur à l'Université de Jassy

JASSY

IMPRIMERIE „DACIA" P. ILIESCU & D. GROSSU

1903



ANNALES SCIENTIFIQUES

DE

L'UNIVERSITÉ DE JASSY

TOME VII

4-me FASCICULE

— EXTRAIT —

CONTRIBUTIONS à l'étude de la FLORE MYCOLOGIQUE de la ROUMANIE

Nord-Ouest du district de Suceava.

par

AL. P. POPOVICI

Professeur à l'Université de Jassy.

JASSY

IMPRIMERIE NATIONALE (IOAN S. IONESCU et M. M. BOGDAN)

Rue Alecsandri 11.

1913.

BOTANIST DR. RODICA RUGINĂ (1940 – 2021)

In the middle of the summer of 2021, on July the 2nd, Mrs. Rodica Rugină, one of the well-known botanists of the Botanical Garden "Anastasie Fătu" and Faculty of Biology in Iaşi, has passed away.

Mrs. Rodica Rugină was born on January, the 20th, 1940, in Clipiceşti (Vrancea county), in a family of teachers, Vasile and Paraschiva Surugiu. She attended her primary school studies in the native village, and the secondary and high-school classes in Focşani.

Between 1958 and 1963 she attended the courses of the Faculty of Biology-Geography (Biology-Botany section) of the "Alexandru Ioan Cuza" University in Iaşi.

As a proof of those necessary qualities for a future researcher (seriousness, permanent study, passion in research work, etc.), between 1963 and 1967 she worked as a university preparator at the *Pharmaceutical Botany* discipline of the Faculty of Pharmacy in Iaşi. Later on, she transferred to the Faculty of Biology-Geography of the "Alexandru Ioan Cuza" University, where she worked also as a university preparator at *Plant Morphology*, until 1978. She began her doctorate studies in 1971 with a PhD thesis called *Morphological and histo-anatomical research for normal weeds and those modified by herbicide treatment*, under the leadership of Professor Constantin Burduja. The thesis was defended in public in 1980, thus the passionate botanist obtaining the title of doctor in biology.

Following some restructurings within the Faculty of Biology-Geography, Mrs. Rodica Rugină becomes a researcher at the Botanical Garden "Anastasie Fătu" in Iaşi, where she coordinated the activity of the *Taxonomic Section*, collaborating permanently with members of the team of *Morphology and Plant Anatomy* of the Faculty of Biology.

In her scientific activity she has published over 90 scientific articles in various fields of research, such as: *Comparative Anatomy* (*Systematic*), *Ecological Anatomy* and *Experimental Anatomy*, with special attention paid to protected plant species and medicinal ones. Probably the most valuable work made after many years of studies is the one published in 1998, in the prestigious *Publishing House of the Romanian Academy*, together with Professor Dr. Constantin Toma, under the name: *Anatomy of Medicinal Plants*. *Atlas*. For her work, Mrs. Rodica Rugină was awarded the Emanoil Teodorescu Prize by the Romanian Academy, in 2000.

Mrs. Rodica Rugină has published, alone or in collaboration, some books of botanical interest, as they are: *Can the mysteries of life be solved*? (V. Rugină & R. Rugină, 1993), *Protected Plants in Romania* (R. Rugină & M. Mititiuc, 2003), *Volatile Oils and Aromatherapy* (R. Rugină & I. Boz, 2013), *The Adventure of Plants on the Globe* (R. Rugină, 2017). She made valuable contributions to the elaboration and publication of manuals for practical works for the discipline of *Plant Morphology and Anatomy*, but also for the work *Illustrated flora of vascular plants in Eastern Romania* (authors: I. Sârbu, N. Ştefan, L. Ivănescu & C Mânzu, vol. 1, 2001).

Most of the professional activity (over 35 years) of the researcher Rodica Rugină consisted in the coordination of the *Taxonomic Section* of the Botanical Garden in Iaşi, a section that represents the defining element in an institution of its kind; hence the difficult responsibility in conserving and enriching the collections of characteristic plant species. In order to face the

inherent difficulties, she was involved with passion and professionalism in all activities specific to the conservation of plant species *ex situ*, as: collecting of plant material directly from the wild habitats, obtaining specimens from species as diverse as possible (through appropriate horticultural techniques) from similar institutions in the country and abroad, the identification and reviewing the vascular plant species within the collections, the collection of plants for the publication *Flora Exsiccata of Moldova and Dobrogea*. She also paid a special attention to the rare plants collected from different areas of Romania, making great efforts to adapt and even naturalize them in the conditions of the city of Iași. Field work was also accompanied by laboratory work, most cultivated species being studied in a research project, conducted over 5 years (*Studies of conservation of the genofond of rare and protected plants in Romania, their introduction and acclimatization in the Botanical Garden Iaşi*).

In all her activity at the Botanical Garden she contributed to increasing the diversity of plant species in the *Taxonomic Section*, but also to bring elements that increase the interest of visitors, by creating an outdoor amphitheater, a small lake for aquatic plants and an artisanal fountain. To all this we must add the involvement in guiding visitors, but also students from different fields (biology, pharmacy, agronomy, geography, etc.).

The decades she dedicated to cultivate and studying plants were marked by the passion, seriousness, tenacity and critical spirit, which she approached with all the activities carried out.

For us, her younger colleagues, she remains a real model of high scientific professionalism!

Adrian OPREA
"Anastasie Fătu" Botanic Garden, "Alexandru Ioan Cuza" University of Iaşi

ZSIGMOND GYŐZŐ, ***Gombák. A magyar néphagyományban: etnomikológiai Tenulmányok*** **[*Mushrooms in Hungarian folk tradition*]**, 2020, Tortoma Publishing House, Baraolt, 366 p, ISBN 978-606-9716-00-7.

Professor Győző ZSIGMOND was born in Turda (Cluj county) on April 20th 1959. After he graduated "Mihai Viteazul" Highschool from Turda, he attended the Hungarian Language and Literature and French Language and Literature sections of the Faculty of Philology, "Babeș-Bolyai" University of Cluj-Napoca. In 1997 he defended hid PhD thesis in the field of popular mythology at Babeș-Bolyai" University of Cluj-Napoca.

Since 1992 he is a member of the Hungarology department of the Faculty of Foreign Languages and Literatures from the University of Bucharest, first as a lecturer (1992-1999), associated professor (1999-2004) and full professor (2004-present). In 2007 he was granted the position of PhD supervisor in the field of Philology.

His research domains are related to ethnography and folklore, ethnology, oral history, popular games and toys, popular customs and traditions (especially those regarding the manufacturing of artisanal objects from the tinder fungus (*Fomes fomentarius*), specific to Corund village, Harghita county), popular religion reflected in proverbs and sayings. Also he published studies of ethnoastronomy, referring to traditional knowledge and beliefs of the Hungarian people from Romania, studies included in his PhD research.

The ethnomycology investigations target the Carpathian basin, comprising folklore aspects from all Hungarian ethnographic regions, renewing and reforming the classic ethnomycology with numerous contributions from modern ethnomycology.

In his studies regarding the traditional naming, he focuses on specific toponyms and ethnotexts from three villages from Transylvania: Poiana-Turda and Mihai Viteazul from Cluj county and Dalnic From Covasna county.

He published over 100 studies and articles in national and international journals, in Hungarian, Romanian, French, German and English languages, and 10 books, at renowned publishing houses. Moreover he participated at many conferences, congresses, scientific colloquia, from Romania and abroad: France, Germany, Finland, Israel and Hungary. Between 2003 and 2013 he was chief of the Hungarology Department and between 2013 and 2014 he was director of institute.

In 1996 he wins a scholarship financed by the French Government, to research ethnology at universities and institutes from Aix-en-Provence and Paris. Between 1998 and 2003 he was granted an annual scholarship – *Domus Hungarica Scientiarum et Artium* offered by the Hungarian Academy of Sciences and in 2008, 2012, 2014, 2015 and 2017 he benefited from various monthly scholarships in the field of ethnomycology. He also researched ethnomycological aspects from the Carpathian-Danube basin, within a post-doctoral "Bolyai" grant, financed by the Hungarian Academy of Sciences.

As recognition of his scientific contributions, the *Hungarian Linguistic Society* (*Magyar Nyelvtudományi Társaság*) awarded him, in 1997, with the *Csűry Bálint* award and in 1998 he became corresponding member of the *Hungarian Ethnography Society* from Budapest.

In 2002, as guest professor, he taught at the University of Szeged (Hungary) the *Nature in popular culture / Ethnomycology and ethnoastronomy* course.

He is an active member of several scientific societies and organizations such as: *Researchers Corporation of the Hungarian Academy* (since December 2000), *Association Roumaine des Chercheurs Francophones en Sciences Humaines* (ARCHES), *Magyar Néprajzi Társaság* (*Hungarian Ethnography Society*, headquartered in Budapest, since 1997), *Romanian Association of French-Speaking Researchers in Humanistic Sciences* (headquartered in Bucharest, since 1997), *Kriza JÁNOS Ethnographical Society* (headquartered in Cluj-Napoca, since 1991), *Erdélyi Múzeum-Egyesület* (*Transylvanian Museum Society*), *Société Internationale d'Ethnologie et de Folklore*, president of *Kálmán LÁSZLÓ Mycological Society*, headquartered in Sfântu Gheorghe, Covasna county, since 1999. He is a founding member (1999), president (1991-1993 and 1995-1997), member of the managing committee (1998-2001) and member of the *Association for the preservation of Hungarian language in Transylvania*. In 2018 he organized at Bálványos Baths (Covasna county) the 36th edition of the *Journées Européennes du Cortinaire* Congress.

He was editor-in-chief of the *Hétpróba* journal (1990) and the *TIK-TAK* paper (1990-1991). Currently, together with Professor Ferenc Pál-Fám, is editor-in-chief of the *Moeszia* journal, which includes valuable researches in the field of mycology and also popularization articles regarding the activity of the *Kálmán LÁSZLÓ Mycological Society*.

His most recent publication, presented as a monography is *Gombák. a magyar néphagyományban*: *etnomikológiai Tenulmányok* [*Mushrooms in Hungarian folk tradition*], published in 2020 at Tortoma Publishing House, Baraolt.

Within this volume, the author, with an experience of over 25 years, gathered precious information regarding ethnomycology, especially Hungarian ethnomycology, focusing on aspects such as: the popular names of mushrooms, the use of mushrooms in popular medicine, mushrooms and mythology, mushrooms as food resource.

Using thorough and complex questionnaires, talking to local people and consulting various literature sources, he managed to create a very real profile of the *Mushrooms*, exactly as they appear in folklore and everyday life.

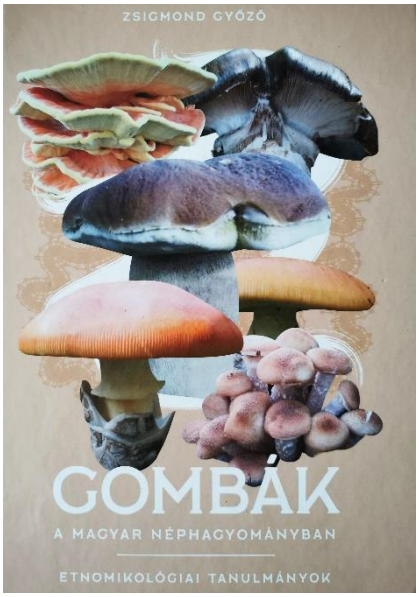
This volume is organized as a collection of 30 studies that present valuable data on many mushroom species, used over the years by the local Hungarian populations.

These studies are preceded by a brief *History of Hungarian Ethnomycology*, which sums up the most important figures that researched this field, with their valued contributions and published works.

The first study focuses on one species of Polypore, not yet identified (probably an *Albatrellus* species), only known by the Székely population, as an excellent edible mushroom.

The second study refers to the various uses of two widely known Polypores: the tinder fungus (*Fomes fomentarius*) and the birch polypore (*Piptoporus betulinus*). The tinder obtained from these fungi, processed in a very specific way, has multiple uses, starting from fire material, raw material for decorations, hats and slippers, bags and wallets, keychains and toys, these arts and crafts being an exclusive and unique local brand.

The following studies, present the importance of several fungal species in the regional folklore (including their various popular names, depending on the region), their ethno-pharmaceutical values, the history of mushroom gathering, the extensive use in the local cuisine, tales and myths, their connection with religious and profane beliefs, aspects regarding their ecology, their role as income source for the local populations: the chanterelle (*Cantharellus cibarius*), the peppery milkcap fungi (*Lactarius piperatus* and *Lactarius pergamenus*), the saffron milkcap (*Lactarius deliciosus*), the orange milkcap (*Lactarius deterrimus*), the wooly milkcap (*Lactarius torminosus*), the morels (*Morchella* sp.), the wrinkled caps (*Verpa* sp.), the penny bun (*Boletus edulis*), the summer cep (*Boletus reticulatus*), the slippery Jacks (*Suillus* sp.), the oyster mushroom (*Polyporus ostreatus*), the parasols (*Macrolepiota* sp.), the truffles (*Tuber* sp., *Elaphomyces* sp., *Choiromyces* sp.), the bird's nest fungus (*Cyathus olla*), the coral fungi (*Ramaria* sp.), the wood-ear (*Auricularia auricula-judae*), the funnel mushrooms (*Clitocybe* sp. and *Lepista* sp.), the chicken of the woods (*Laetiporus sulphureus*), the lacquered bracket fungus (*Ganoderma lucidum*), the velvet shank mushroom (*Flammulina velutipes*), the grey knight (*Tricholoma* terreum), the white domecap (*Lyophyllum connatum*), the St. John's mushroom (*Calocybe gambosa*), the dryad's saddle (*Polyporus squamosus*), the shield pinksgill mushroom (*Entoloma clypeatum*), the honey fungus (*Armillaria mellea*), the fairy ring mushroom (*Marasmius oreades*), puffballs (*Lycoperdon* sp.), several species belonging to Agaricaeae, Amanitaceae, and Russulaceae families, and many other fungal species.



Many of the presented species and the related by products, are illustrated in good quality pictures, which gives the reader a significant insight of mushrooms ecology and uses, local habits and activities.

The very well documented studies are followed by the questionnaire given to locals, the list of mushrooms that appear in the book, the index of Hungarian popular names for the mushrooms from the Carpathian basin (with 1165 names), the list of references, with over 600 titles, the list of people who provided valuable information and abstracts (in English language) for every study published within this volume.

The book represents an important tool in discovering and understanding Hungarian ethnomycology and not only, an impressive collection of valuable information that helps everybody who is interested, to explore the wide and exciting world of fungi, in very close contact to local history and language, occupations and practices, customs and traditions, beliefs and ways.

Cătălin TĂNASE
"Alexandru Ioan Cuza" University of Iasi, Faculty of Biology
Cristiana Virginia PETRE
"Anastasie Fătu" Botanical Garden, "Alexandru Ioan Cuza" University of Iași

JOURNAL OF PLANT DEVELOPMENT
GUIDE TO AUTHORS

AIMS AND SCOPE OF THE JOURNAL

Journal of Plant Development is the official scientific journal of the "Anastasie Fătu" Botanical Garden, which belongs to "Alexandru Ioan Cuza" University of IAŞI, ROMANIA. It was first published in 1979 (at that time as "Culegere de Studii şi Articole de Biologie"). The new series begins in 1993 under the name "Buletinul Grădinii Botanice Iaşi". From 2008 on, it has been published under its present name "**Journal of Plant Development**". It appears in one volume, with one or two issues per year.

Journal of Plant Development (JPD) is an international journal that acts as a medium for the exchange of ideas and provides publication (yearly) of articles in all areas of Plant Science and Botany (of all 'plant' groups in the traditional sense - including algae, cyanobacteria, fungi, myxomycetes). It covers topics in plant development field, as well as the plant ecology. The Journal also covers related fields such as: plant conservation, plant taxonomy, plant embryology, phytosociology, ecology, plant morpho-anatomy and histology, comparative and developmental morphology, physiology, ecophysiology, plant distribution, natural and artificial habitats, ornamental plants, pharmaceuticals uses of plants, plant molecular biology, plant cell, tissue and organ culture etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. All articles published in JPD are peer-reviewed.

TYPES OF MANUSCRIPTS AND LANGUAGE

The journal publishes original research articles, short communications and reviews in English. Journal of Plant Development also publishes book reviews and conference reports. Manuscripts may be of any length, but must be clearly and concisely written.

Three main *types* of manuscripts may be submitted:

Original research articles: should reports results of a substantial, completed and original work, and describe new and carefully confirmed findings. Experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short communications: are suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods or techniques. The style of main sections need not conform to that of original research articles. Short communications are 2 to 4 pages in length.

Review articles. Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Review articles are critical evaluations of material that has already been published. By organizing, integrating, and evaluating previously published material, the author considers the progress of current research toward clarifying a problem. A review article is a tutorial in that the author defines and clarifies a problem, summarizes previous investigations in order to inform about the state of current research, identifies relations, contradictions, gaps, and inconsistencies in the literature, suggests the next step or steps in solving the problem. Reviews should be concise and no longer than 14-16 printed pages. Reviews are also peer-reviewed.

OPEN ACCESS POLICY

Journal of Plant Development publishes fully open access articles, which means that all articles are available on the internet to all users immediately upon publication. Non-commercial use and distribution in any medium is permitted, without charge to the user or his/her institution. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles in this journal without asking prior permission from the publisher or the author. This is in accordance with the BOAI definition of open access.

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All contributions are subject to a double-blind reviewing process. Acceptance of papers is supervised by an international Editorial Board. Manuscripts considered suitable for peer review by the Editorial Board are sent to at least two referees (members of the Board or external). The journal gives referees a target of four weeks for the return of their reports. The review process takes between three weeks and two months.

PROOFS AND REPRINTS

Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage. Because JPD will be published freely online to attract a wide audience, authors will have free electronic access to the full text (in both HTML and PDF) of the article. Authors can freely download the PDF file from which they can print unlimited copies of their articles.

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(1) a cover letter, that should include the corresponding author's full name, address and telephone/fax numbers and should be in an e-mail message sent to the Editor. A Cover Letter is to be made upon submission, sending a revision or re-submission.

(2) a text file with the entire text, as an attachment, whose name should begin with the first author's surname.

(3) additional files for figures and tables.

Submission of a paper implies that it has not been published before (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors, and that, if accepted, will not be published elsewhere in the same form, in English or in any other language, without the written consent of the publisher.

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Journal of Plant Development have its own specific formatting. This defines how an article will look when it is published online or in print. In order to format your article ready for submission to JPD, **Word Template** are available on the website, ready for you to download and apply to your document. Read the instructions for the author/s, download and save the word template file to your computer and apply these styles to your paper as appropriate.

Authors should consult the **checklists** given here on **how to prepare the files**. Authors are expected to have their papers well checked for content and correctness in presentation of text and illustrations. Manuscripts not conforming to the guidelines will be returned to the author until satisfactory files are provided.

The corresponding author receives by e-mail an acknowledgment of receipt of the manuscript, mentioning the communicating editor and a manuscript reference number (Article ID). The manuscript number will be mailed to the corresponding author same day or within 72 hours. If you do not receive an acknowledgement you should inquire to be sure it was received.

Details on types of contributions

1. Original research articles

The papers will be published only in a foreign language (English), structured as follows: title, authors, affiliation of the authors, abstract, keywords, introduction, material and method, results & discussions, conclusions, acknowledgements, references, tables, figure captions.

Title should be a brief phrase describing the contents of the paper.

Authors names would not be abbreviated, capitals for surname (family name) and no capitals for first name (except initial letter). Each author name would be accompanied by a complete address, as a footnote on the first page. The affiliation should be provided in the following order: university (institution) name; faculty/department name; number and street name; city; country and email address. One of the authors should be designated as the corresponding author.

Abstract should be concise informative and completely self-explanatory, briefly present the topic, state the purpose of the research, indicate significant data, and point out major findings and conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. References should therefore be avoided, but if essential, they must be cited in full, without reference to the reference list. Non-standard or uncommon abbreviations should be avoided but, if essential, they should be defined at their first mention in the abstract itself.

Key Words: five to seven words, the most important ones, in alphabetical order, after someone could discover your paper on the internet engines. Key words should not repeat the title of the manuscript.

The **Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines. The introduction should conclude with a brief statement of the overall aim of the experiments and a comment about whether that aim was achieved.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely

without referring to the literature. Discussion and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The **Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusions should spell out the major findings of the work and may include some explanation of the significance of these conclusions and what further research should be done. Authors should include a general interpretation of the results in the context of current evidence, and not restricted to that which supports the findings of the present study.

Notes on contributors in maximum 65 words, provide short biographical notes on all contributors.

The **Acknowledgments** of people, grants, funds, etc. should be brief. People who contributed to the work but do not fit criteria for authorship should be listed in the Acknowledgments, along with their contributions. It is the authors' responsibility to ensure that anyone named in the acknowledgments agrees to being so named.

References should be listed at the end of the paper in alphabetical order. In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text. Authors are fully responsible for the accuracy of the references.

2. Short communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. Short Communications follow the same format as for the original research papers, with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion sections should be combined into a single section.

3. Review articles

Review articles are critical evaluations of material that has already been published. By organizing, integrating, and evaluating previously published material, the author considers the progress of current research toward clarifying a problem. Reviews should be concise and no longer than 14-16 printed pages. Reviews are also peer-reviewed.

4. Book reviews and conference reports

These types of contributions would not exceed an A4 format page.

5. Special Issues

Proposals for Special Issues of full research papers that focus on a specific topic or theme will be considered.

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☐	If the paper is a revised or resubmitted manuscript, the letter **explains** what changes have been made to the manuscript and where and why changes requested by the Editor and referees have not been carried out.

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	☐ All tables and figures are cited in the text and use 'Figure' or 'Table'.
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	☐ **Abbreviations** are used for units of measurement, molecular terminology, common statistical terms (e.g. ANOVA, *t*-test and *r*2), names of chemicals (e.g. ATP, Mes, Hepes, NaCl, O_2), and procedures (e.g. PCR, PAGE, RFLP). Other abbreviations are spelled out at first mention and all terms are written out in full when used to start a sentence.

	□	A list of **non-standard abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often in text. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.
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	□	The references are arranged alphabetically based on the last name of the first or sole author. Where the same sole author or same first author has two or more papers listed, these papers are grouped chronologically. Where such an author has more than one paper in the same year, these are ordered with single authored papers first followed by two-author papers (ordered first alphabetically based on the second author's surname, then by year), and then any three-or-more-author papers (in year order only). Letters 'a', 'b', 'c', etc., are added to the date of papers with the same authorship and year.
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	□	**Papers in periodicals**: the name of the author(s) would be given in capital letters. The Christian name(s) would be abbreviated. Before the last but one and the last author you must insert the sign "&". In the reference list you must mention all the authors of a certain paper. The year of a paper publication is put after the author (s). Title should be fully written. Between the year and the title we recommend to be inserted a point sign. Each periodical name is to be written in italics. A certain volume must be given in bolds. After it is placed the number of the periodical, inserted between the round brackets; next to it would be inserted the page numbers of the paper. BOSCAIU N. 2001. Ecotonul si importanta functiilor sale ecoprotective. *Ocrotirea Naturii.* **42**: 13-21. MEHREGAN I. & KADEREIT J. W. 2008. Taxonomic revision of *Cousinia* sect. *Cynaroideae* (*Asteraceae*, *Cardueae*). *Willdenowia.* **38**(2): 293-362.

	□	***Books*:** title should be fully written. The title of a book is written in italics. After the title, is placed the name of the town, the publishing house and the page numbers. BOSCAIU N. 1971. *Flora si vegetatia Muntilor Tarcu, Godeanu si Cernei*. Bucuresti: Edit. Acad. Române, 494 pp. HILLIER J. & COOMBES A. 2004. *The Hillier Manual of Trees & Shrubs*. Newton Abbot, Devon, England: David & Charles, 512 pp.
	□	**Chapters in books:** author(s), year, title, pages, a point sign, followed by "In": author (s) of the book, city, publishing house, number of pages. TUTIN T. G. 1996. *Helleborus* L. pp. 249-251. In: TUTIN T. G. & al. (eds). *Flora Europaea*. 2nd ed., 1993, reprinted 1996. Vol. **1**. *Psilotaceae to Platanaceae*. Cambridge: Cambridge University Press, XLVI, 581 pp.
	□	Article only available on-line with digital objet identifier (DOI): SONKÉ B., DJUIKOUO K. M. N. & ROBBRECHT E. 2008. *Calycosiphonia pentamera* sp. nov. (afrotropical Rubiaceae) from the 'Lower Guinea' area. *Nordic Journal of Botany*. http://doi.org/10.1111/j.0107-055X.2007.00141.x
	□	Thesis: NTORE S. 2004. Contribution à la connaissance systématique du genre afrotropical *Pauridiantha* (Rubiaceae). PhD Thesis, K. U. Leuven, Leuven, Belgium.

CHECKLIST FOR *ILLUSTRATIONS*

□	Photographs, drawings, maps, graphs, charts, plates and diagrams are all treated as 'Figures' and should be numbered consecutively in accordance with their appearance in the text.
□	The mentions at the drawings, figures, pictures and tables will be placed inside the round brackets – for instance (Figure 2), (Table 2).
□	All illustrations should be clearly marked with the figure number and the author's name.
□	Figures composed of several smaller figures are treated as one figure and the subfigures are labeled with capital letters (A, B, C, D, etc.). Scientific names in captions are not followed by authors.
□	Each table has a complete caption at the top and is Table 1, Table 2, etc. according to the order in which they are first mentioned in the text.
□	All the schemes, drawings, electron micrographs etc. would be accompanied by a scale bar.
□	Line diagrams are black and white. Use of colour in line diagrams is acceptable where this enhances clarity significantly.
□	Related graphics are grouped into a single figure whenever possible. Each part of a grouped figure is labelled A, B, C, etc. and not treated as separate figures. Labels are preferably placed in the upper left or lower left corner of the picture using.
□	Colour images are used where they enhance significantly the clarity of the scientific information and must be very clear, being accompanied by the explanations.
□	The diagrams should be made in Excel; pictures, ink drawings must be saved in JPG, JPEG, or BMP format, having a good resolution.
□	If figures or tables are not imported into your text, these would be placed on separate sheets, with indications of their inserting place in the text.
□	Table text is typed using Times New Roman. Capitals are avoided within table cells (exceptions: first letter of the heading of row or column, names of taxa requiring a capital, and abbreviations e.g. pH).
□	Tables must be cell-based. Do not provide tables as graphic objects.
□	Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

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