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Plant Functioning Under Environmental Stress

Edited by

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PREFACE

Maciej T. Grzesiak

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In the popularization of knowledge on plant stress physiology, the important role belongs to scientific conferences aiming at presenting the current state of the research, sharing opinions and provide the possibly to initiate new scientific projects. International Conferences *„Plant Functioning Under Environmental Stress"* are organized by the *Franciszek Górski* Institute of Plant Physiology, Polish Academy of Sciences in Cracow under auspices of Polish Botanical Society and Committee of Agronomic Sciences, Polish Academy of Sciences, under patronage of professor Jarosław Gowin, Minister of Sciences and Higher Education and in co-operation with University of Alberta in Edmonton, Slovak Agricultural University in Nitra, Plant Protection Institute, Hungarian Academy of Sciences, Warsaw University of Life Sciences - SGGW in Warsaw, Pedagogical University in Cracow and Agricultural University in Cracow. This year, the conference has about 180 registered participants including about 50 from abroad.

The research on environmental stress has aroused the interest of plant scientists and provide an opportunity for us to study physiological, biochemical and molecular. The scientific and economic importance of different stresses and its impact on crop production provides a certain urgent motivation and need of funding support for research projects. Now we have real opportunity to solve significant problems, unlike in previous years, at physiological, biochemical and molecular levels. Investigations in the plants stress biology are the multidisciplinary, covering aspects of plant physiology, ecology, biochemistry, biophysics, genetics and breeding. They provide information on physiological processes that are disturbed as a result of the influence of the imposed stresses factor. Higher plants, as sessile organisms, during evolution developed defence mechanisms in order to cope with unfavourable conditions and to function under environmental stresses. Physiological basis for plant reaction to environmental stress factors, are despite of this, still the object of researchers interest. Nevertheless, we are far from fully understanding the mechanisms of plant defence against stresses as yet. The modern methodological approach and the applied techniques allow research at various levels of biological organization beginning with field, through single plants, organs, cells and organelles down to the molecular level. The progress in analytical methods allows the investigation of the successive stages of signal transductions or the role of secondary metabolites taking part both in plant defence reactions against stresses and of those responsible for generating stress at the cellular level. The results of these complementary investigations, important from the academic point of view, also provide highly valuable and applicable information, which may be used in agricultural and environmental biotechnology including genetic engineering, selection, and breeding as well as in agronomy.

On a global scale, 90% of all agriculturally used land are influenced by abiotic or biotic stress and also within the areas that are said to be stress-free, are observed short and of various intensity deviations from the optimum. However, we are still far away from the full and comprehensive knowledge of mechanisms underlying plant acclimatization and adaptation to stress factors. This is due to the complexity of both the reception and transduction of signals, and reactions of plant on various levels of biological organisation, magnified by a multi-gene control of plant responses to stressors, together with the fact, that in many cases there are two or more stress factors appearing together or sequentially. The greater challenge, however, seems to be the proper utilization of the gained knowledge in practice. Further substantial progress in yielding, both quantitatively and qualitatively, obtained through selection, classical breeding, transgenesis in case of genetically modified organisms or through specially designed agro -technologies, may be effective only if the scientific achievements of physiological, biochemical and molecular bases of plant reactions and tolerance to environmental stress factors are utilized.

Plants, similarly to all other living organisms, exist under the influence of the surrounding environment, which in many cases provides conditions far or extremely far from optimal for their growth, development and yielding. Higher plants, as sessile, cannot change their place of existence. Therefore, through evolution, they have developed combinations of molecular, biochemical, physiological, anatomical, morphological, and behavioural features and processes enabling them to adapt to and survive in unfavourable environmental conditions. The processes of acclimatization and adaptation to the changing over time, types and intensity of stressful factors are affected accordingly to the emergence of new threats. The problems of famine in many countries, the losses in food production resulting from drought, floods, too low or too high air temperatures, environmental pollution connected with the industrialization, urbanization, chemicalization of agriculture and plant diseases, have become problems of world-wide importance, and they will recur on a global scale because the climate is more and more changeable. The tendency of the climate to warm-up, which has been confirmed by meteorologists, as a result of the "greenhouse effect" does not necessarily imply that there will be no more severe winters.

Professor Jacob Levitt classified the response of plants into two categories – avoidance or tolerance. In agronomy management we almost always employ the avoidance responses but now biotechnology improve may be able to exploit the alternative strategy of stress tolerance with less adverse environmental impact. Our present knowledge and biotechnology improve may be able to exploit the alternative strategy of tolerance with less adverse environmental impact. Complication arise because response are very stress specific and because in nature, stresses rarely occur individually, but often are composites. Discussion on our previous conferences has pointed out common problems to stress – the action of ABA, injury to cell membranes, reaction with oxygen may be considered as General Adaptation Syndrome (GAS), according to Hans Selye`s terminology. The existence of common physiological responses has practical role in improve stress tolerance of plants using biotechnology methods because it implies that are genes that are active during stress periods. Genes controlling membrane fluidity and transport processes are also likely candidates. Admittedly those genes do not function at the primary site of stress damage, because these sites differ among stresses, but apparently code for secondary defence system. It is current hope that manipulation of those defence systems that are components of GAS may lead to the development of multiple stress resistance in new cultivars.

After this optimistic look at the future, I would like to close of my preface to conference with a sobering quotation used by professor Peter Steponkus from Cornell University, to remind us of our own limitation:

Rothchild`s rule
"To every problem, however complicated, there is a simply elegant solution, which one will find if one looks hard enough. This solution will turn out to be wrong ".

In spite of this it seems to be necessary also to remind the postulate put forward by professor of Jacob Levitt in plenary lecture at the conference in East Lansing in 1988 on "Stress interaction –back to the future". In this lecture Levitt postulates that in order to attain future progress in stress biology it is to reconsider the older, often forgotten research, and make an attempt to the synthetic survey of the present knowledge.

Finally, organizers would like to avail ourselves of this opportunity to express our gratitude to Professor Edward Gwóźdź of Adam Mickiewicz University in Poznan, to whom – on occasion of his 80th birthday – we dedicated this year conference. Professor Gwóźdź participated in previous conferences, giving plenary lecture and actively participating in discussion. Organizers and all participants wish Professor E. Gwóźdź good health and all the best on his birthday.

Organizers wish also to express their gratitude to our invited speakers for their inspiring lectures that provided us with the most recent achievements in the field of plant sciences. We would like to express our profound gratitude to His Magnificence, Rector Kazimierz Karolczak and the senate of the Pedagogical University of Cracow for their support in organising this conference.

Organizers would like to thank for the financial support and for providing some souvenirs to the Polish Botanical Society, Committee of Agronomic Sciences Polish Academy of Sciences, Kraków Municipality, Polish Academy of Arts and Sciences, Jagiellonian University, Ministry of Science and Higher Education, Ministry of Agriculture and Rural Development, Ministry of the Environment, Ministry of Culture and National Heritage, National Museum in Kraków and publishers: Springer „Biały Kruk", Social Publishing Institute "ZNAK", Scientific Papers Authors and Publishers Society UNIVERSITAS and sponsors: GEOMOR-TECHNIK Ltd., Krakow Waterworks, Force-A, and PP Systems.

On behalf of the conference organisers, we would like to warmly welcome all our guests and wish them productive sessions and a pleasant stay in Cracow.

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PROFESSOR EDWARD A. GWÓŹDŹ - A NESTOR OF RESEARCH ON PLANT STRESS BIOLOGY OF POZNAŃ

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Professor Edward Antoni Gwóźdź was born in 1938 in Siemianowice Śląskie at Upper Silesia where he finished the education at ground and secondary school. In 1958 he begun the study on the Faculty of Biology and Earth Sciences at the Adam Mickiewicz University. He graduated from the Faculty of Biology and Earth Science, where he achieved further scientific degrees. He obtained the Master of Science degree in 1963 and PhD degree in 1972. He became a habilitated doctor in plant physiology in 1979 and a full professor in 1991. In the years 1973-1974 he worked as a post-doc at University of Calgary (Canada).

Professor Edward Gwóźdź performed many functions in the field of science organization at Faculty of Biology, A. Mickiewicz University in Poznań as well as many national institutions. In the years 1978 – 1991 he was organizer and head of Isotopic Laboratory. In 1990 he contributed to the establishment of Department of Plant Ecophysiology, which he managed until he retired in 2009. Between 1985 and 1990 he was the deputy-dean of Faculty of Biology, and in the years 1990 – 1996 he was organizer and the head of Ph.D. Study. During 1992 - 1994 he was a member of the Expert Committee at the Ministry of National Education and between 2001 - 2002 he was a member of the Evaluation Team of the University Accreditation Committee. Between 2012 and 2014 he was an Expert at the National Science Center. For many years (1999 - 2015) he was a member of the Committee of Physiology, Genetics and Plant Breeding of the Polish Academy of Sciences. Moreover Professor Gwóźdź was (or still is) a member of editorial boards in such journals as *Acta Physiologiae Plantarum, Dendrobiology* and *Acta Societatis Botanicorum Poloniae* as well as the member of Polish Botanical Society and Federation of European Societies of Plant Biology. In 1997 Professor organized the international conference „Molecular Biology of Plants under Environmental Stress" that gathered outstanding scientists from around the world.

Professor Edward Gwóźdź research and scientific achievements concern several thematic areas. His interests related to the phytohormonal regulation of morphogenesis contributed to the preparation of a master's thesis titled "Effect of indolylacetic acid and kinetin on growth and organogenesis of fragments of chicory root grown *in vitro*" (1963) and then to PhD thesis titled "The role of auxins in RNA metabolism of regenerating fragments of chicory roots grown *in vitro*" (1972). In this work he used, as the firs in Poland, radioactive precursors to analyze the metabolism of RNA in tissue culture. The main findings of this work indicated that auxin determined the type of growth and organogenesis of the callus tissue and this was tightly connected with mRNA induction and polyribosome formation. In 1973 professor Gwóźdź joined the team of dr Derek J. Bewley at the University of Calgary where for 2 years conducted investigations on the protein synthesis in resurrection moss *Tortula ruralis.* The main achievement was to show that slow desiccation of the moss is of great importance for the ribosomal run-off and faster resumption of protein synthesis on rehydration. On return prof Gwóźdź went back to the molecular aspects of hormonal regulation of growth and development, which in 1979 resulted in a habilitation work: "The role of cytokinins in protein synthesis". In the next decade the main work was focused on the aspects of storage protein biosynthesis in developing lupin seeds. From 1990 after the organization of the Department of Plant Ecophysiology professor Gwóźdź started with investigations on stress physiology with main attention to heavy metals as well as salinity and heat shock. In 1999 Professor and his co-worker discovered the accumulation of a pathogenesis like protein PR-10 in lupin roots exposed to heavy metals, which allowed further experiments on its organellar and subcellular localization. Professor Gwóźdź is also interested in plant signaling and defense strategies, including the role of hydrogen peroxide, nitric oxide, specific stress proteins and antioxidant enzymes. Research in this field led to the completion of the five doctoral dissertations, in which the professor was a promoter. The high quality of the research conducted at Department of Plant Ecophysiology was possible thanks to the funds obtained by the Professor from various sources, such as the Scientific Research Committee and the National Science Center as well as due to his ability to cooperate with scientists from other scientific institutions.

Professor Gwóźdź is author of about 60 scientific papers, review articles, co-authors of handbooks, with the number of citation amounting to 1000 (according to Web of Science). Professor was a visiting professor or lectures at University of Cambridge (UK) , Milan (Italy), Louvain-la-Nueve (Belgium) and Nitra (Slovakia). He was also frequently invited as a lectures (22) or Scientific Session Organizer to different national and international Conferences.

Professor Edward Gwóźdź has been supervisor of 6 PhD thesis as well the reviewer of 30 PhD thesis, 10 habilitation and 12 professorship procedures.
For his scientific and organization achievements Professor Gwóźdź was awarded by Golden Cross of Merit (1985), medal of National Education Commissions (2000), twice by Ministry of Science and Higher Education (in 1980 and 2004), by the Rector of Warsaw University of Life Sciences (2002) and many times by Rector of A. Mickiewicz University.

After Professor retired he still actively participates in scientific life of Faculty of Biology, especially in the field of popularizing science, giving lectures and preparing an article on plant intelligence.

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PLANT RESISTANCE TO ABIOTIC STRESS FACTORS. WHAT DOES IT MEAN TODAY?

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Abiotic stresses include excessive or deficit level of physical and chemical environmental factors that determine plants growth and those which are never beneficial for plants. They influence plants performance leading to unfavourable structural and functional changes that adversely affect growth and development. Plants almost never find the optimal environmental conditions essential for growth and development. It is predicted that due to climate change unfavourable growth conditions in many parts of the world will become a big problem. Since the beginning of this century the number of publication focused on plant responses to stressful environment have increased several fold (Cramer et. al. 2011).

Being sessile organisms plants have to adjust to the existing conditions. Varied defence strategies responsible for plants resistance to environmental stresses have been developed during evolution. Resistance to stress factors is an important feature for plants growth, survival and reproduction under unfavourable environmental conditions. It is a complex trait which depends on genetic potential and includes gene expression, enzymes activation\inactivation, synthesis of specific compounds, changes in hormones balance as well as changes in growth of aboveground and underground organs, and flowering. The cost of defence responses triggering affects biomass accumulation and yielding. Energy and nutrients are relocated from growth and biomass production to the processes responsible for resistance (Parsch and Sonnewald 2015).

Resistance to stress can be either biological or agricultural resistance. Biological resistance includes plant defence strategy which means the ability to survive, recover and reproduce under adverse conditions. Agricultural resistance means the ability to maintain stable and good quality yields under stress conditions and that is what farmers expect. Therefore, the definition of resistance depends on the established criteria and needs.

Negative environmental factors affect the state of stress (strains) in plant tissues which creates signals triggering resistance responses including the avoidance and/or tolerance mechanisms (Blum 2015). Unfortunately, the term ‘stress resistance’ is often used in the literature alternatively with ‘stress tolerance’. This presentation attempts to discuss the differences between these two terms on the basis of the results of experimental research focused on plants responses to abiotic stresses playing an important role in crop production. This issue is important in clarifying the relationship between the effect of stress factors on plants and their resistance assessed on the basis of well-defined criteria.

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THE EFFECT OF SENESCENCE OR JUVENILITY OF PLANTS ON THEIR RESISTANCE/TOLERANCE TO PATHOGENS

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It has been known since a long time that senescence or juvenility of plant tissues has strong effect on their reactions to pathogen attacks. Generally necrotrophic pathogens prefer senescent, while biotrophic pathogens prefer juvenile tissues. Accordingly, senescent tobacco leaves were more susceptible to the necrotroph *Alternaria alternata*, but less to the biotroph powdery mildew. The elevated tolerance of juvenile tobacco leaves to necrotrophs was mainly due to their higher tolerance to toxins, cell-wall degrading enzymes, to reactive oxygen species and to the more fluid cell membrane composition. Recently we found that cytokinin overproducing paraquat tolerant (PT) tobaccos are more tolerant, while NahG salicylic acid deficient tobaccos are more sensitive not only to necrotrophic pathogens, but also to the reactive oxygen H_2O_2 than their respective controls (Gullner et al. 2017). In addition, we showed that Arabidopsis NAP-related proteins (NRPs) can induce age-related pathogen resistance (Barna et al. 2018). On the other hand, treatments of Arabidopsis or tobacco plants with senescence inhibiting two cytokinin hormones benzyl adenine or kinetin, differently effect plant reactions to drought stress, to virus, bacteria or fungi, in addition to changes in their microarray gene expressions profiles.

The possible mechanisms of plant tolerance to necrotrophic and biotrophic pathogens will be discussed.

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CHLOROPHYLL *A* FLUORESCENCE: PAST–PRESENT–FUTURE

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Chlorophyll *a* fluorescence is a reliable, non- invasive and widely applied technique addressed to study the photosynthetic efficiency of any photosynthesizing organisms. We followed the development of chlorophyll fluorescence researches during the period of 1947-2018. A comprehensive network analysis on the available bibliometric data, collected from Web of Science Core Collection database, was applied. We observed a sharp increase in the number and diversity of chlorophyll *a* fluorescence publications after the 90-ties and a vigorous development of this discipline during the last ten years. The increase of these publications number matched with the increase of research areas and institutions involved, and was triggered by the accumulation of knowledge and technological advancement, especially modern fluorometry analysis. The network analyses of keywords and research areas confirmed that, chlorophyll fluorescence researches has been shifted to a modern, multidisciplinary and highly collaborative discipline involving a 'core' disciplines such as plant science, environmental sciences, agronomy, food science and industry. However, the promising areas of its application are: biochemistry and molecular biology, remote sensing, management of big data, and artificial intelligence.

THE IMPACT OF CADMIUM ON SOYBEAN - SIGNALING, RESPONSE AND RECOVERY

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Contamination of the environment with cadmium is a serious problem in many parts of the world. This highly mobile element is readily absorbed by plants leading to disturbances in mineral homeostasis, cell ultrastructure, cell division and photosynthesis, which results in hampered growth and yield. Moreover, cadmium accumulated in tissues of crop plants can enter human food chain leading to serious disorders including carcinogenesis (Gallego et al. 2012, Tran and Popova 2013).

Presented lecture is a summary of research carried out at the Department of Plant Ecophysiology at Adam Mickiewicz University in Poznań, dedicated to cadmium impact on important crop species, soybean. The studies confirmed that this metal is rapidly uptaken by plants and that roots are the main site of its accumulation. Exposure to cadmium led to significant inhibition of growth and induction of oxidative stress (Chmielowska-Bąk et al. 2017). The early response to metal included activation of signaling associated genes engaged in ethylene metabolism, nitric oxide generation, mitogen-activated protein kinase cascades and regulation of other genes expression (transcription factors). The observed stimulation of genes expression was accompanied by increased ethylene biosynthesis and nirate reductase activity (Chmielowska-Bąk et al. 2013). Cadmium treatment also induced changes in ribonucleic acids – formation of 8-hydroxyguanosine (8-OHG) and abasic sites (Chmielowska-Bąk et al. 2018). Despite the toxic effect of this metal, plants were able to efficiently recover from stress. After transfer to optimal conditions they restored the growth and showed no differences in cell viability or photosynthesis efficiency in relation to the control (data unpublished).

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THE QUALITY CONTROL MECHANISMS ON PLANT PEROXISOMES

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Peroxisomes are single membrane-bound organelles that are ubiquitously found in eukaryotic cells. Plant peroxisomes are involved in wide range of cellular processes such as photorespiration, biosynthesis of phytohormones and metabolism of fatty acids (Beevers, 1979). These metabolic processes are essential for plant life and controlled to suit the cellular state; dramatically different functions are carried out during plant responses to environmental and developmental changes (Hu, 2012). This flexible transformation is referred to as the functional transition of peroxisomes in which newly synthesized enzymes are transported into peroxisomes, while obsolete enzymes disappear. Recent our studies revealed that the functional transition of peroxisomes is supported by two quality control systems; one is the repair and degradation of proteins inside peroxisomes by LON protease, and the other is whole peroxisome degradation via autophagy (Shibata, 2013; Goto-Yamada, 2014). Autophagic degradation of peroxisomes (pexophagy) is important to eliminate damaged peroxisomes. Because various peroxisomal oxidases generate H_2O_2 via their enzymatic reactions, plant peroxisomes are constantly exposed to H_2O_2 and gradually damaged. These oxidized peroxisomes are selectively recognized by ATG8, an autophagosome receptor, and degraded (Shibata et al. 2013). Moreover, loss of LON protease results in acceleration of pexophagy, indicating that the biological importance of a balanced control between Lon protease and autophagy for attaining overall quality control of peroxisomes.

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PLANT RESPONSES TO THE ENVIRONMENT: PERCEPTION, COMMUNICATION, MEMORY

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Because plants are rooted in one place and can not move to avoid unfavourable biotic and abiotic environmental factors and this one fact is of fundamental importance in understanding their unique strategies for growth and survival. Plants are very sensitive to environmental stimuli and can undergo changes in physiology and development that acclimate them to their particular surroundings. Plant growth responses occur following several mechanical stimuli as touch, wind or sound. Plants do not passively exist in their environment, but they actively responding to changes and signals from the environment. Plants have mechanisms to measure time in that they can produce flowers at the right time of year and even determine night from day. Light is an important environmental cue to which plants are especially sensitive and different light quality are needed for specific responses. For a plant to persist and reproduce, it must constantly cope with the changing physical characteristics of their habitats, in which they are often exposed to multiple abiotic and biotic stresses and such combined stress treatments induce an unique pattern of gene expression. Plants emit volatile compounds that can function as communication method to neighboring plants, insects and pathogens. Plants release the volatiles in response to shoot or root damage by herbivores and can even attract their enemies to kill them. The volatile phytohormones methyl salicylate and methyl jasmonate serve as important signaling molecules that enhance the resistance of the receiver. Plants have innate constitutive defense mechanisms, however, under unfavourable conditions they evolved a kind of immunization, which is called *priming.* a physiological state, which involves prior exposure to a biotic or abiotic stress factor making plant more resistant to future stress. The primed state involves metabolite mobilization, hormonal recruitment as well as some epigenetic changes, which involve modification of DNA activity by methylation, histone modification and/or chromatin remodelling without alteration of DNA sequence. The epigenetic changes lead to some kind of stress memory, which involves the storage of information on the stress cue and can be transferred to next generation. The ability of plants to learn and memorize previous experience in order to optimize fitness allows effective acclimation to environmental stresses and can be considered as a specific form of intelligence.

THE ROLE OF HORMONE SIGNALING IN PLANT RESPONSES TO SOIL DRYING: FOCUS ON GIBBERELLIN

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Insufficient soil moisture is a serious limitation to yield for major crops globally, causing economic losses and risks to food security. Furthermore, the effects of drought are predicted to become more serious in many areas with the changing climate. Soil drying imposes a combination of stresses: as well as limiting the availability of water and nutrients, lack of moisture causes soils to become stronger, thereby imposing a mechanical restriction to root growth. The relative contributions of these stresses depend on the degree of drying and the nature of the soil, with some soils increasing substantially in strength after a relatively low level of drying. We are interested in the signaling pathways that mediate the effects of soil -born stresses on plant growth and have focussed particularly on the involvement of the gibberellin (GA) growth hormones, which we have investigated in *Arabidopsis thaliana* and in the crop species *Triticum aestivum* (bread wheat). In both species, we have determined changes in expression of genes involved in GA metabolism and signal transduction in response to water restriction by quantitative RT-PCR and RNA sequencing. Plants have been shown to respond to abiotic stresses by promoting GA inactivation through up-regulation of GA 2-oxidase (*GA2ox*) genes, with different members of this gene family responding to different stresses. In Arabidopsis leaves, drought promoted expression of *AtGA2ox1* as well as down-regulating specific GA-biosynthesis genes. In wheat, we have preliminary data indicating that expression of *TaGA2ox3* and *TaGA2ox6* is up-regulated in leaves during soil drying, but down-regulated in roots, consistent with a redistribution of GA content and growth in response to the stress. This is in contrast to the ABA-biosynthetic *NCED* genes, expression of which is strongly up-regulated by drought in both leaves and roots. Gibberellins act by promoting degradation of DELLA proteins, which are growth suppressers that regulate gene expression in partnership with transcription factors. In leaves, drought resulted in up-regulation of *RGL3*, one of the five Arabidopsis *DELLA* genes, and of the wheat *DELLA* gene *RHT-1*. It has been shown recently for tomato that DELLA reduces stomatal aperture by enhancing ABA signaling (Nir et al. Plant Cell 29: 3186-3197, 2017). We have investigated the effect of soil strength in the absence of other stresses in wheat seedlings using weighted sand columns with normal water and nutrient availability. Strong soil reduced root and leaf length as well as the number of tillers (Coelho Filho et al., Plant Soil 371: 81-94, 2013). Leaf length could be recovered by treatment with GA_3, while root length and tiller number were further reduced by this treatment. We have preliminary data from rice indicating that the effect of soil strength on tiller number is mediated, at least in part, by strigolactone signaling. For future research we have assembled a range of wheat lines with mutations in GA and other hormone signaling pathways and will determine their peformance in dry soil. The work should indicate the contribution of different hormone pathways to stress responses and suggest targets in breeding for improved stress tolerance.

MODULATION OF STOMATAL RESPONSE BY ELEVATED ATMOSPHERIC CO_2 CONCENTRATION IN PLANTS UNDER DROUGHT STRESS

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Plants respond to drought stress by adapting different strategies that may help them to survive and grow. Buffering the leaf water status by adjusting the width of stomatal opening to regulate the rate of passage of water vapour from the plant to the atmosphere is one of the several adaptive mechanisms to drought. Elevated atmospheric CO2 concentration ($e[CO_2]$) also reduces stomatal conductance (g_s) through stomatal closure, but the mechanisms by which $e[CO_2]$ and soil water deficits induce stomatal closure can be different. When plants are grown under $e[CO_2]$, they display different strategies for regulating g_s. One of them is through coordinated short-term physiological responses (change in aperture) and long-term morphological adjustments (change in stomatal density) to regulate water use efficiency. On the other hand, g_s decreases in response to soil drying mainly due to partial stomatal closure induced by root-to-shoot chemical signaling (mainly xylem-borne abscisic acid, ABA) at moderate water stress and by the decrease in leaf turgor under severe water stress. The stomatal density can also be modified by prolonged soil water deficits. It is believed that drought stress has a stronger impact on g_s than $e[CO_2]$, and when plants are grown under combined drought stress and $e[CO_2]$, the water deficits induce a reduction in g_s that often override the reduction caused by $e[CO_2]$. In addition, $e[CO_2]$ has a direct effect on attenuating the damage of drought stress by reducing g_s and transpiration rate. Then the important questions to answer will be whether and how crop plants grown under $e[CO_2]$ can regulate stomatal behavior and water use when experiencing drought stresses. The ability of crop plants to tolerate drought under $e[CO_2]$ is largely dependent on the effectiveness of stomatal control over transpiration. The sensitivity of g_s to ABA signaling during soil drying could be modified by $e[CO_2]$; yet to date, there is no consensus regarding the modulation of $e[CO_2]$ on the response of g_s to soil water deficits. Also, it remains largely unknown about the significance of hydraulic and chemical signals in controlling g_s of drought-stressed plants grown under $e[CO_2]$. Therefore, there is a need to examine how $e[CO_2]$ modulates g_s response to soil drying in different crop species, and what are the underlying bio-physiological mechanisms regulating stomatal aperture of plants grown in a future drier and CO_2-enriched climate.

ENVIRONMENTAL STRESS: AVOIDANCE, ADAPTATION AND ACCLIMATION BY PLANTS IN THE DYNAMICS OF VEGETATION ISLANDS ON SAND PLAINS

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The biological stress concept distinguishes eustress and distress with hardening and exhaustion, respectively. Strong stress by single stress factors on the one side and low stress with high affluence on the other side both lead to low biodiversity, whereas multi-factorial medium stress elicits high biodiversity. The answers of plants are avoidance, short term acclimation and long term adaptation. They may be functionally combined.

Examples chosen are three sand plains. Two of them are in the tropics, i.e. the sand dune restingas at the Atlantic coast of Brazil and salinas at the Caribbean coast of Venezuela. The third one is Pustynia Błędowska in the North-West of Kraków, Poland. It is fascinating that the sand plain of the Błędowska Desert presents a landscape physiognomy identical to the two tropical habitats. Vegetation islands are started on the sand plains by individual pioneer plants which function as nurse plants supporting the establishment of other species as well as soil micro-biota. Although developing vegetation islands may also be dying off again, in successions denser vegetation can develop from them. A typical nurse plant in the Brazilian restingas is *Clusia hilariana* Schltdl. In the Polish Błędowska Desert *Salix repens* ssp. *arenaria* (L.) Hiit. plays such a role. Reactions of plants may combine answers of avoidance, acclimation and adaptation controlling water relations at the level of morphological and anatomical features of roots and leaves, stomatal regulation, photochemistry and biochemistry of photosynthesis.

The performance of the plants in vegetation islands of sand plains follows the biological stress concept.

COMPARTMENT SPECIFIC DISTRIBUTION OF GLUTATHIONE AND ASCORBATE AND THEIR DYNAMIC CHANGES IN PLANTS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Ascorbate and glutathione are multifunctional metabolites in plants playing essential roles in plant development, growth and stress defense. Ascorbate and glutathione are major antioxidants. They are involved in the detoxification of reactive oxygen species (ROS), redox signaling, in the modulation of defense gene expression and they are important for the regulation of enzymatic activities. For these reasons, levels of glutathione and ascorbate are often used as stress markers in plants (Noctor and Foyer 1998, Tausz et al. 2004, Noctor 2006, Foyer and Noctor 2009, Miller et al. 2010, Szarka et al. 2012).

In our studies, we focused our interest on the dynamic compartment specific changes of glutathione and precursors as well as ascorbate, to gain thorough knowledge about the subcellular distribution of these antioxidants in plants and on the importance of these antioxidants in certain cell compartments during stress situations.

Different agricultural plants (*Cucurbita, Nicotiana and wheat*), as well as *Arabidopsis* were used as model plants under different environmental conditions in order to detect and quantify subcellular glutathione and ascorbate. For this purpose beside other techniques, an immunogold cytohistochemical approach with computer-supported transmission electron microscopy approach was developed and adapted to different plant material.

These studies and methods can now be used for the development of new defense strategies for agricultural use in the future, and can protect farmers from possible crop losses induced by environmental stress situations in the future.

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MOLECULAR MECHANISMS OF MAIZE RESPONSE TO COLD

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The most important factor limiting maize adaptation to the conditions prevalent at higher latitudes is its cold-sensitivity manifested by strong retardation of growth and development at temperatures below 17ºC, severe injuries below 8ºC, and even death below 4ºC. The mechanism of maize cold-sensitivity has been studied for several years. Studies have concentrated mostly on stress physiology and genetics (Leipner and Stamp, 2009) and demonstrated the importance of the damage to the photosynthetic apparatus enhancing production of reactive oxygen species (Foyer et al. 2002); reduced activity of enzymes of the dark phase of photosynthesis (Leipner and Stamp, 2009) and compromised phloem loading (Bilska and Sowiński, 2010), together affecting the carbohydrate status of the leaf (Sowiński et al. 1999; Marocco et al. 2005). Additional contributors to cold-response of maize are alteration of secondary metabolism (Christie et al. 1994); modification of the cell wall (Sobkowiak et al. 2016; Bilska-Kos et al. 2016); and water deficit (Janowiak and Markowski 1994).

A large volume of genetic information related to cold-stress responses in plants has been accumulated over the past several years. Nevertheless, data concerning low-temperature-induced genes in cold-resistant and frost-tolerant plants such as *Arabidopsis*, alfalfa, barley, or wheat are largely available (Ahmed et al., 2015). In general, their functionality is related to plant hardening or acclimation resulting in increased frost tolerance (Foyer et al., 2002). In maize, however, the process of acclimation is only poorly articulated and is limited to a small increase in the tolerance towards chilling in the range of 5-10ºC after a period of growth at 10-15ºC (Verheul et al., 1996) Additionally, the regulatory mechanism that engage the CBF-type transcription factors in cold-resistant species is not fully operational in tropical grasses, like maize (Tondelli et al., 2011). Fortunately, due to application of high-throughput methods, recent years have brought a plentiful molecular data on maize response to cold. The speech will be focused on presenting and systematization of these data with the aim to indicate the key responses.

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INDUCTION OF COLD TOLERANCE BY PRIMING AND STRESS MEMORY IN CROPS

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Cold is one of the major environmental factors limiting plant growth and development. The increased climatic variability and more frequent episodes of extreme conditions also result in plants being exposed to not only one single cold event but also multiple abiotic stresses at different periods. Although the abiotic stresses occurring at different stages result in a higher risk of injury, earlier stress events may prime the plant to protect it against later stresses. A large body of evidence has shown that a previous exposure to different types of stress can affect the subsequent responses and eventually prepare the plants to more quickly or actively respond to future stresses. Cold stress tolerance in plants involves diverse and multiple physiological and molecular mechanisms. Priming and stress memory are key processes, by which plant may increase the tolerance to subsequent drought events. Stress memory involves multiple modifications at physiological, proteomic, transcriptional levels and epigenetic mechanisms. We summarized recent advancements in physiological, biochemical, and molecular studies related to drought priming and its effect on drought tolerance in plants. The mechanisms of drought stress memory and the possible priming induced cross-tolerance to other abiotic stresses are discussed. As one of main focuses in plant-abiotic stress research, studies on plant drought priming and stress memory is still rare. To date, most of results on plant drought priming were obtained in controlled lab experiments, which might be different from the natural conditions. Thus, in future studies, a combination of experiments from controlled lab evaluations with observations and simulation under field conditions should be performed. Collectively, to further understanding the processes and mechanisms of priming effects, eco-physiologists and molecular biologists should work together to reveal the complete regulation network at different levels and scales, such that management strategies could be developed to sustain crop productivity under future climate changes scenarios.

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BRASSICACEAE PLANTS SPECIFICALLY DEVELOPED CHEMICAL DEFENSE SYSTEM BASED ON ER BODIES

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Plants as sessile organisms are exposed to a large and diverse array of herbivores and pests. To cope with this, plants produce defensive molecules include small chemicals derived from the secondary metabolites. In Brassicaceae plants, glucosinolates constitute one of the important secondary metabolites with defensive function. Glucosinolates are sulfur-containing glucosides, and enzymatically deglycosilated to produce defensive isothiocyanates, which are known as mustard oil. The substrates (glucosinolates) and enzymes are stored separately and feeding damage by insect comes to contact them each other to produce isothiocyantes. This defense system is known as mustard-oil-bomb. In Brassicaceae plants, specific subfamily of β-glucosideases (BGLUs), namely thioglucoside glucosidase (TGG) had been thought to be the enzyme that deglycosilates glucosinolates. However, recent findings indicated that several other BGLUs can deglycosilate glucosinolates (Nakano, 2017). Here, I show endoplasmic reticulum (ER) bodies accumulate such BGLUs, PYK10/BGLU23 and BGLU21 in *Arabidopsis thaliana* seedlings, and responsible for defense against herbivore attack. ER bodies are ~10 μm fusiform structures that are derived from ER. ER bodies accumulate in epidermal cell of seedlings and roots. The seedlings of an ER body deficient mutant, *nai1*, and an ER-body BGLU deficient mutant *pyk10 bglu21* are sensitive by woodlouse attack. The production of isothiocyantes seems lower in the *pyk10 bglu21* mutant. Since ER bodies are observed in various Brassicaceae species, these findings suggest that Brassicaceae developed ER bodies as a chemical defense system.

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ROLE OF AQUAPORINS IN PLANT RESPONSES TO ROOT HYPOXIA

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Root hypoxia frequently occurs in plants as a result of flooding and soil compaction. Responses of plants to root hypoxia entail the signals originating in roots and shoots. Root oxygen deprivation rapidly affects water relations by inhibiting water transport. The effects of root hypoxia often appear as plant wilting and the plants attempt to restore water balance by reducing transpiration rates through stomatal closure. The processes that contribute to the inhibition of root hydraulic conductance involve changes in root morphology and alteration of the aquaporin-mediated root water transport. Recent studies have also demonstrated that aquaporins are involved in the transport of gases such as carbon dioxide and oxygen, which may have major consequences to plant tolerance of hypoxic conditions (Uehlein et al. 2003, Zwiazek et al. 2018). The function of aquaporins is affected by the acidification of the cytoplasm and depletion of ATP that is required for aquaporin phosphorylation and membrane functions. Cytoplasmic pH, phosphorylation, and intracellular Ca^{2+} concentration that are affected by oxygen deprivation directly control aquaporin gating (Maurel et al. 2015). There are a number of structural determinants that are essential for pore conformational changes in aquaporins, to highlight the underlying mechanisms triggered by oxygen deprivation stress. Gene expression of aquaporins is also modified in hypoxic plants, which may constitute an important, yet little explored, mechanism of hypoxia tolerance. In addition to water and gas transport, aquaporins may contribute to hypoxia tolerance by transporting H_2O_2 and lactic acid. These complex responses may involve ethylene, abscisic acid, and possibly other hormonal factors and signalling molecules in ways that remain to be elucidated.

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INFLUENCE OF ENVIRONMENTAL FACTORS ON SEED YIELD OF COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH)

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Common buckwheat (*Fagopyrum esculentum* Moench) belongs to the *Polygonaceae*. It has many advantages e.g. can protect ground against soil erosion and has ability to absorb nitrogen and phosphorus from the soil. In addition, the plant is characterized by resistance to pests and plant diseases, as well as the low sensitivity to soil conditions. Common buckwheat has extensive economic use, it is cultivated for seeds production or fodder for animals. Its seeds do not contain gluten and have high dietary signification. Due to the increase of demand, it is necessary to examine buckwheat productivity. The aim of our study was to investigate the factors limiting seed production.

Buckwheat is sensitive to drought, ground frost and high temperatures which may cause flower and embryo abortions. Moreover, buckwheat forms dimorphic flowers with different length of pistils and stamens – Pin and Thrum type showing self-incompatibility. Buckwheat blooming period takes from 30 to 60 days. One plant produces between 500 to 2000 flowers, however, only a few percent of them produce seeds (Ruszkowska and Ruszkowski, 1981). Frequently, even if the flowers are pollinated, flowers, young embryos and fruits degenerate, probably for trophic reasons.

Among the reasons for the low seed set is: (1) sensitivity to heat and drought stress, (2) insufficient fertilization, (3) embryo abortion, (4) self-incompatibility, (5) lack of assimilates occurring in aging plants (Slawinska and Obendorf, 2001).

The studies of common buckwheat have shown that the cultivation at a temperature below 25°C can improve the forming seeds up to 40% (Slawinska and Obendorf, 2001). The results of our experiment have shown that used bio-stimulants increase seed production (Słomka et al., 2017). Thus far, there is no self-ending buckwheat form available. The current studies relate to the impact of the thermal and trophic stresses on the embryological development and seed yield of buckwheat. We would like to determine if obtaining of the self-ending form of this plant species will solve the problem of low seed set. This research project is financed by the National Science Centre (2017/25/B/NZ9/00148).

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GENETIC CONTROL OF RESISTANCE TO GOLDEN POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS* IN POTATO PLANTS *SOLANUM PHUREJA*

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Golden potato cyst nematode (GPCN) is an important pathogen of potatoes, tomatoes, and other plants in the family *Solanaceae*. Nowadays GPCN is found worldwide and it appears to be the one of the most deleterious pathogens for potato. Protection against GPCN is complicated because the eggs of nematodes can remain viable in the soil for more than 30 years. In addition, most chemical nematicides are not efficient, thus the protection is mostly obtained through the introduction of the resistance genes. The majority of known R genes in plants belong to the NBS-LRR family. These receptors recognize specific molecular patterns and can induce programmed cell death at the site of a pathogen invasion. Thus, we focused our analysis on this type of R-genes.

In this study we analyzed the resistant cultivar of diploid potato *Solanum phureja* to reveal new resistance genes through comparison of root transcriptomes of resistant and susceptible genotypes.

For RNA-seq, total RNA was extracted from root samples collected in time points 0, 24 and 72 hours after inoculation with GPCN. Sample preparation was carried out by colleagues from Vavilov Institute of Plant Genetic Resources and All Russian Research Institute for Plant Protection (Saint Petersburg, Russia) according to [1]. Sequencing was performed on Illumina NextSeq 500 platform. FastQC and Prinseq tools were used to assess sequences quality and filter the libraries. STAR and TopHat were used to map the filtered libraries to the reference genome. Search for the differentially expressed genes was performed using Cufflinks pipeline and EdgeR package for R. Lists of differentially expressed genes (DEGs) were further analyzed with Biomart and the databases AgriGO, KEGG, and PlantCyc. De novo transcriptome assembling was carried out with Trinity software. Prediction of NBS-LRR genes was based on their typical domain structure.

Analysis of *S. phureja* transcriptomic data revealed differential expression of a number of genes. Candidate resistance genes expressed in *S. phureja* resistant cultivar were predicted for further analysis of segregating populations.

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THE INFLUENCE OF LEAD ON GENERATION OF SIGNALLING MOLECULES AND ACCUMULATION OF FLAVONOIDS IN PEA SEEDLINGS IN RESPONSE TO PEA APHID INFESTATION

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An important goal from the point of view of ecology is the study the interactions between abiotic and biotic stress at different levels of biological organization. Plants exposure to one stress alter metabolic status which can influence the intensity or ability to adaptively respond to the subsequent stress. The aim of this study was to investigate the effect of an abiotic factor, i.e. lead at various concentrations (low inducing the metabolic status of the plants, causing the hormesis effect, and high causing the toxic effect), on the generation of signalling molecules in pea seedlings (*Pisum sativum* L. cv. Cysterski) and next during the cross-talk of lead and infestation by the pea aphid (*Acyrthosiphon pisum* Harris). The second objective was to determine the level of flavonoids in response to the impact of the above-mentioned stressors. Additionally, within the second goal was performed total quantitative analysis of metabolites in the roots and leaves of pea seedlings. The third objective was to determine the levels of semiquinone radicals, which may to a certain degree limit cell penetration by insect. Results of our research revealed a significant accumulation of signaling molecules in the roots and leaves of pea seedlings growing on lead and next during infestation by pea aphid. Increased generation of these molecules strongly enhanced the biosynthesis of flavonoids. Our results show also a significant accumulation of flavonoid and isoflavonoid glycosides, in response to lead alone and to the cross-talk of lead and the phytophage. Higher levels of these metabolites were recorded in the roots than in the leaves. The level of glycosides was also higher in the organs of pea seedlings growing with a high lead concentration in comparison to seedlings cultured with a low lead concentration. An important defence line of pea seedlings growing on lead-supplemented medium and next during the cross-talk of lead and *A. pisum* was a high generation of semiquinone radicals. Moreover, our research highlighted lead-induced hormetic growth response. These studies showed that the low concentration of lead stimulated slightly growth, especially the shoots of pea seedlings. It was proposed to consider hormesis as an adaptive response to stress. This research provided insights into the cross-talk between the abiotic (lead) and biotic factor (aphid) on the level of the generation of signalling molecules and their role in the induction of flavonoid biosynthesis.

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THE EXPRESSION OF GENES CODING THE CRUCIAL ENZYMES OF THE CALVIN CYCLE UNDER WATER DEFICIT AND FURTHER REWATERING IN THE *LOLIUM-FESTUCA* SPECIES AND HYBRIDS

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The expected climate changes will be the major challenge for the world economy in the future, especially for the agricultural sector. Drought periods are among the main reasons responsible for ecological damage, land desertification as well as soil erosion, potentially limiting crop production. Photosynthesis is, in turn, among the primary processes to be affected by drought (Kosmala et al. 2012, Perlikowski et al. 2016a,b). This important physiological process can be limited by a decreased CO_2 availability caused by the stomata closing – stomatal limitations and/or by alterations in the cellular metabolism, including the Calvin cycle efficiency – non-stomatal limitations (Perlikowski et al. 2016a). *Lolium multiflorum* (Italian ryegrass) is forage grass species with a high forage quality, but a relatively low tolerance to biotic and abiotic environmental stresses. Contrary, *Festuca arundinacea* (tall fescue) is characterized by a high level of abiotic stress tolerance, particularly tolerance to water deficit (Kosmala et al. 2012). The hybridization of both species enables the assembly of their complementary characters within a single genotype (Perlikowski et al. 2016ab; Augustyniak et al. 2018).

In this work, which is a part of the comprehensive project, the effects of water deficit and further rewatering conditions on the expression of genes coding the crucial enzymes of the Calvin cycle (phosphoglycerate kinase, glyceraldehyde 3-phosphate dehydrogenase, and fructose-1,6-bisphosphate aldolase) in the introgression forms of *L. multiflorum/F. arundinacea* and in the genotypes of *F. arundinacea*, were analyzed. Within each group of plants the individuals with distinct levels of tolerance to water deficit, were used. The gene expression profiling followed the measurements of physiological parameters (relative water content, electrolyte leakage, and chlorophyll fluorescence), and reactive oxygen species accumulation. The performance of the Calvin cycle under stress conditions and the involvement of the antioxidant system in the tolerance to water deficit in forage grasses, are discussed.

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HORMONAL CHANGES RELATED TO THE INDUCTION OF MICROSPORE EMBRYOGENESIS IN WINTER TRITICALE (×*TRITICOSECALE* WITTM.)

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Microspore embryogenesis (ME) is a unique process of cellular reprogramming where microspores (immature pollen grains) abandon their gametophytic developmental program in response to a stress treatment and produce androgenic structures, which develop and regenerate to haploid/doubled haploid plants. It has been proved that the effectiveness of ME could be significantly modified by many environmental factors influencing physiological state and condition of the donor plant. One of the most important factors determining ME effectiveness is the endogenous level of two key plant growth regulators (PGRs), i.e. auxins (Auxs) and cytokinins (CKs). In the present study, the hypothesis that modification of hormonal signaling may influence ME effectiveness in isolated microspore cultures of winter triticale (×*Triticosecale* Wittm.) was verified.

Two DH lines of triticale previously described by Żur et al. (2014) as 'responsive'(DH28) and 'recalcitrant'(DH19) with respect to ME induction were studied. For the last 4 days before microspore isolation p-chlorophenoxyisobutyric acid (PCIB) – inhibitor of Auxs, and 2.4-dichlorophenoxyacetic acid (2.4-D) – synthetic Aux, both at two concentrations: 5 and 12.5 μM were applied. The microspores were isolated according to the protocol established by Żur et al. (2009). Endogenous Auxs and CKs were purified by multiStageTips and analysed by UHPLC-MS/MS method (Pěnčík et al. 2009, Svačinová et al. 2012).

The studied DH lines varied significantly in terms of ME efficiency, which was associated with significantly different content of endogenous PGRs. Microspores of DH28 were characterized by higher amount of Auxs compared to DH19. They also contained high amount of 6-benzylaminopurine (BAP) but lacked one of the inactive forms –*trans*-zeatin N7-glucoside (tZ7G). In contrast, in microspores of DH19, no BAP and only low content of tZ7G were detected. In cold-treated microspores of both DH lines, the level of *cis*-zeatin (cZ) was similar. Its amount increased two times in DH28 after pretreatment with 12.5 μM PCIB. Generally, in DH28 this treatment resulted in increased accumulation of the majority of analysed CKs, and this effect was associated with increased frequency of green plants regeneration by approx. 35% compared to control.

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EFFECT OF SELENIUM ON THE RESISTANCE OF LETTUCE (*LACTUCA SATIVA* L.) TO BIOTIC STRESS

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Abstract
Lettuce (*Lactuca sativa* L.) is an important dietary component and its biofortification in selenium (Se) may have health-promoting effects for humans. Selenium fertilization may change plant resistance to abiotic and biotic stresses. The aim of this study was to determine the effects of Se (IV) has on lettuce resistance to biotic stress induced by *Botrytis cinerea* (gray mold). The results have been discussed in relation to: (1) the potential use of Se for supplementation of crops to counteract Se deficiency in diet and (2) to the effect of Se-enrichment on the susceptibility of plants to pathogens.

Keywords: *Lactuca sativa* L., *Botrytis cinerea* Pers., selenium, stress, biofortification

Introduction

Selenium (Se) is an essential trace element for humans, often lacking in human diet. As Se is incorporated into the food chain mainly through crop plants, biofortification of crops with Se appears to be a relatively cost-effective and safe way to reduce Se deficiency in human diet. Moreover, Se applied at low doses has been shown to increase the tolerance of crop plants to abiotic stress (Hawrylak-Nowak 2015). This effect is attributed to Se-induced up-regulation of the antioxidant defense systems. In Se hyperaccumulators, Se can protect plants against herbivores and fungal pathogens (El Mehdawi and Pilon-Smits, 2012). In non-accumulators, however, the role of Se in protecting plants from pathogens is not well recognized and both positive and negative effects have been reported (Pilon-Smits et al, 2017).

Lettuce (*Lactuca sativa*) is an important dietary component and its fortification in Se may have positive effects in long-term human health. We studied the effect of Se (IV) on resistance of lettuce to biotic stress caused by *Botyrtis cinerea* Pers. (causing gray mold disease). The development of gray mold in lettuce plants treated with Se was assessed. The concentration of chlorophyll and carotenoids, H_2O_2 content as well as the activities of antioxidant enzymes: ascorbate peroxidase (APX) and catalase (CAT) were also determined due to their importance for plant defense response to pathogens (Kuźniak and Skłodowska, 2005; Chojak-Koźniewska et al, 2017). The obtained results will broaden the knowledge on the impact of Se on the plants resistance to infectious diseases and may be important for the development of strategies for supplementation of crops to counteract Se deficiency in diet.

Materials and Methods

Lettuce plants were grown in soil, in a growth chamber at photoperiod 16 h/8 h (light/darkness), at 23°C. After three weeks of growth the plants were divided into three groups, two from which were sprayed with 2μM and 10μM Se (IV) solution and the control plants were treated with distilled water. Seven days later all plants were inoculated with *B. cinerea* spore suspension (1×10^6 spores ml^{-1}) prepared as described by Kuźniak et al (2010). Lettuce leaves were harvested at the point of inoculation (T0), 2 days (T2) and 5 days (T5) after inoculation. We determined: the leaf dry weight, protein content (Bradford, 1976), chlorophyll and carotenoids concentrations (Wellburn, 1994) as well as the activities of ascorbate

peroxidase (APX; Nakano and Asada, 1991) and catalase (CAT; Dhindsa et al, 1981). Leaf extracts for the determination of APX and CAT activities were prepared according to Kuźniak et al (1999). Hydrogen peroxide was detected histochemically by 3,3'-diaminobenzidine (DAB) leaf staining according to Thordal-Christensen et al (2009). The effect of Se (IV) on *B. cinerea* growth in *in vitro* culture was examined as descried by Fourie and Holz (1998). The data are the mean values ± SD from 3-6 independent experiments (n = 3-6).

Results and discussion

There is an increasing number of evidence showing that low concentrations of Se may increase plant tolerance to environmental stresses (Hawrylak-Nowak, 2015). Foliar Se application (T0) slightly increased leaf dry weight and protein content (Figs. 1, 5C). After inoculation, no negative effects on lettuce growth and the photosynthetic pigments contents were observed (Figs. 1, 4). However, in Se-treated plants the severity of gray mold disease symptoms caused by *B. cinerea* was reduced (Fig. 2A). The less severe disease symptoms could be the effect of pathogen growth inhibition as Se reduced the proliferation of *B. cinerea* mycelium *in vitro* (Fig. 2B). Histochemical detection of H_2O_2 (Fig. 3.) showed less H_2O_2 spots in 10 µM Se (IV)-treated plants than in the control which confirmed the antioxidant effect of Se on plants after infection. This was suggested to be due to its stimulating effect on the plant antioxidant system (Hawrylak-Nowak, 2015). We observed higher activities of APX (Fig. 4A) and CAT (Fig. 4B) in 10 µM Se (IV)-treated lettuce plants at T0. Thereafter, no significant changes in the antioxidant enzymes activities were found. Considering that oxidative stress is induced by pathogens and promotes the growth of necrotrophic fungi, like *B. cinerea* (Govrin and Levine, 2000), the antioxidant effect of Se (IV) may have a vital role in restricting the development of gray mold disease.

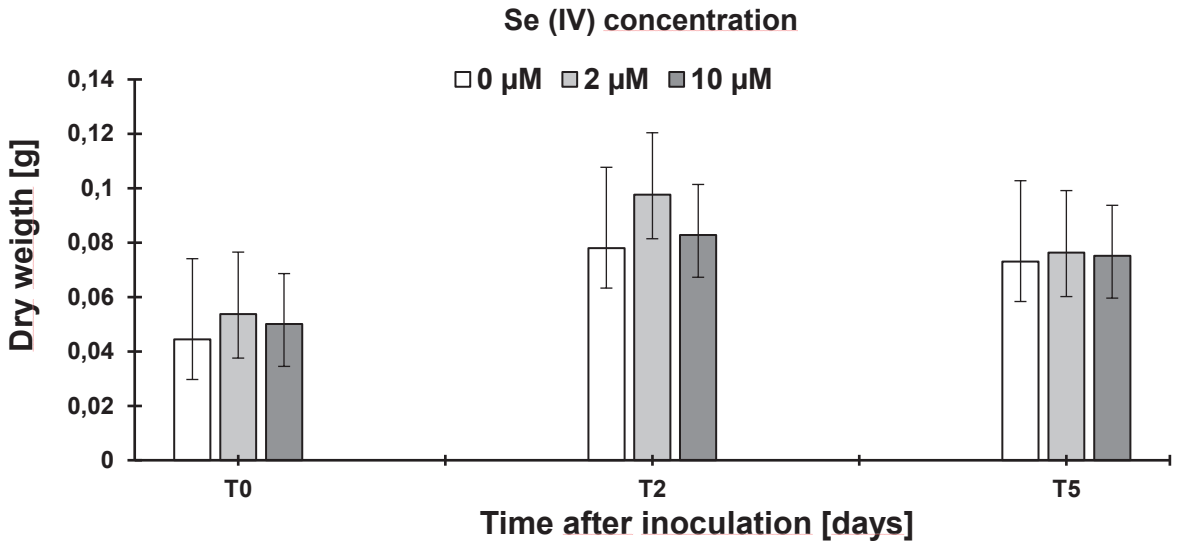

Fig. 1. Biomass [g] of lettuce leaves treated with Se (IV) and infected with *B. cinerea*.

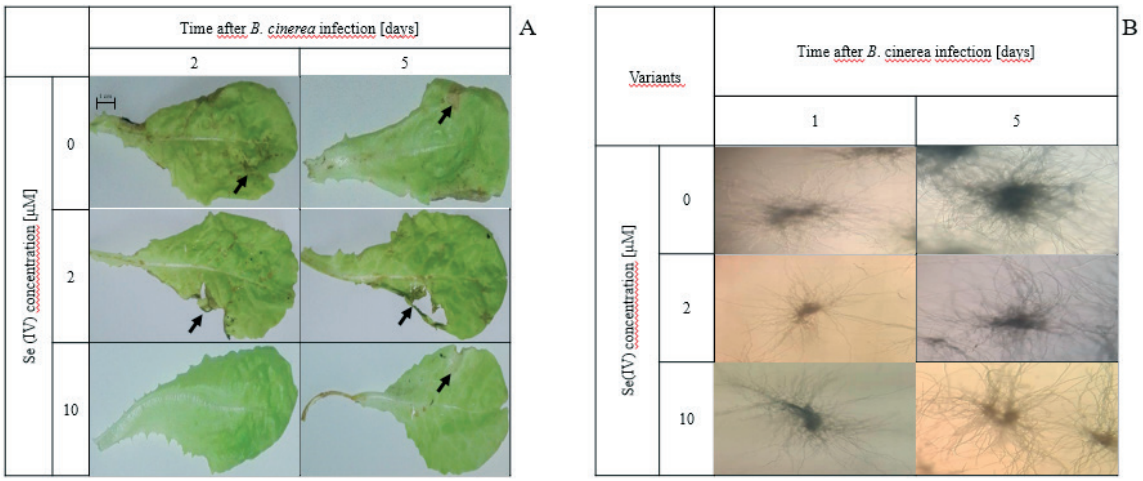

Fig. 2. . *B. cinerea* infection symptoms on lettuce leaves (A) and effect of Se (IV) on *B. cinerea* growth *in vitro (B).* The pictures show representative leaves and similar results were routinely obtained in all experiments. Arrows indicate symptom sites on leaves.

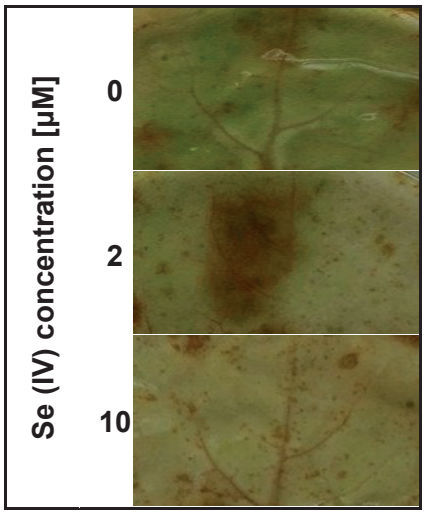

Fig. 3. Histochemical detection of H_2O_2 in Se (IV)-treated lettuce leaves. Control and Se (IV)-treated leaves two days after *B. cinerea* inoculation.

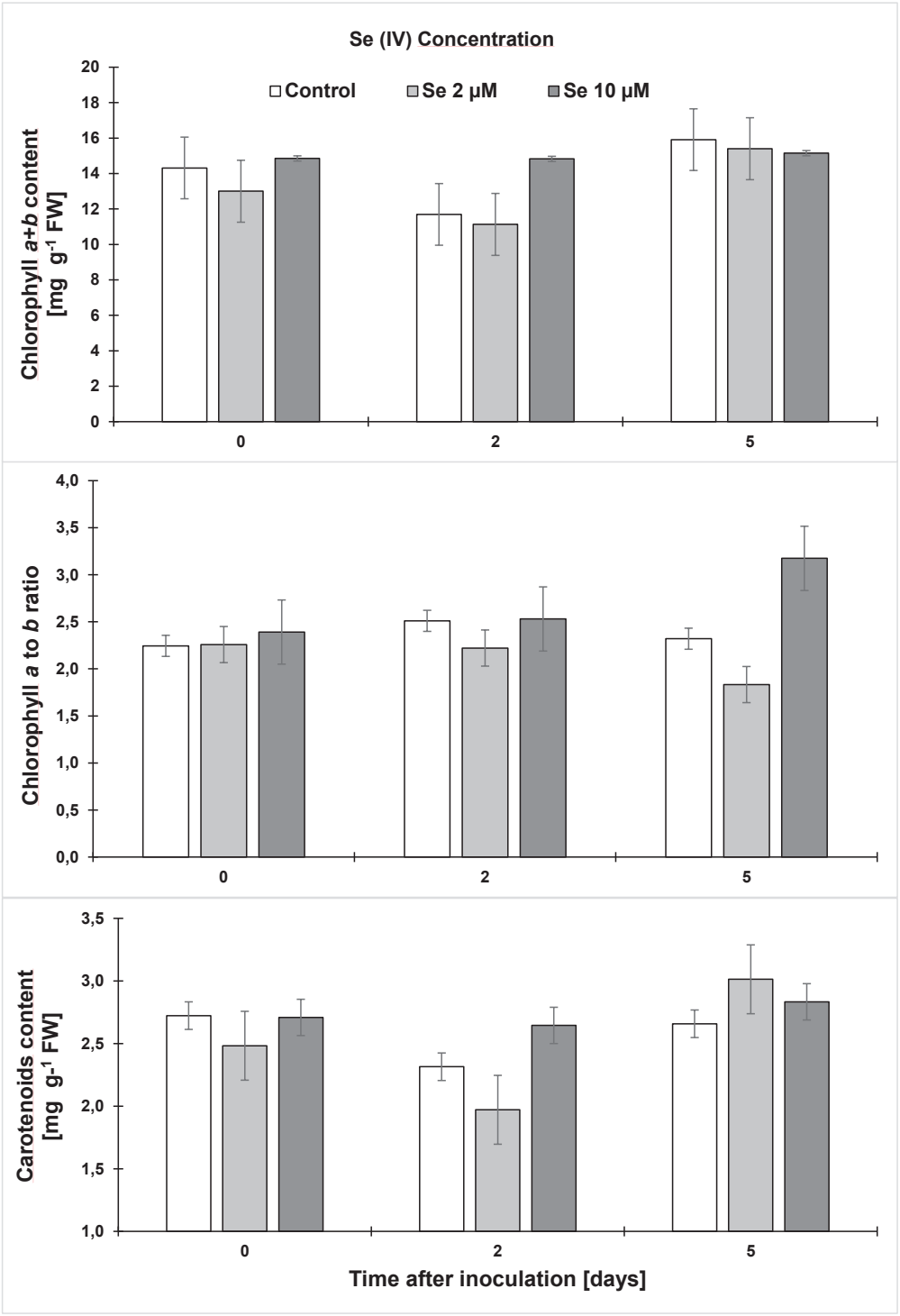

Fig. 4 Total chlorophyll content (A), chlorophyll *a* to *b* ratio (B) and carotenoid content (C) in Se (IV)-treated lettuce leaves after *B. cinerea* infection.

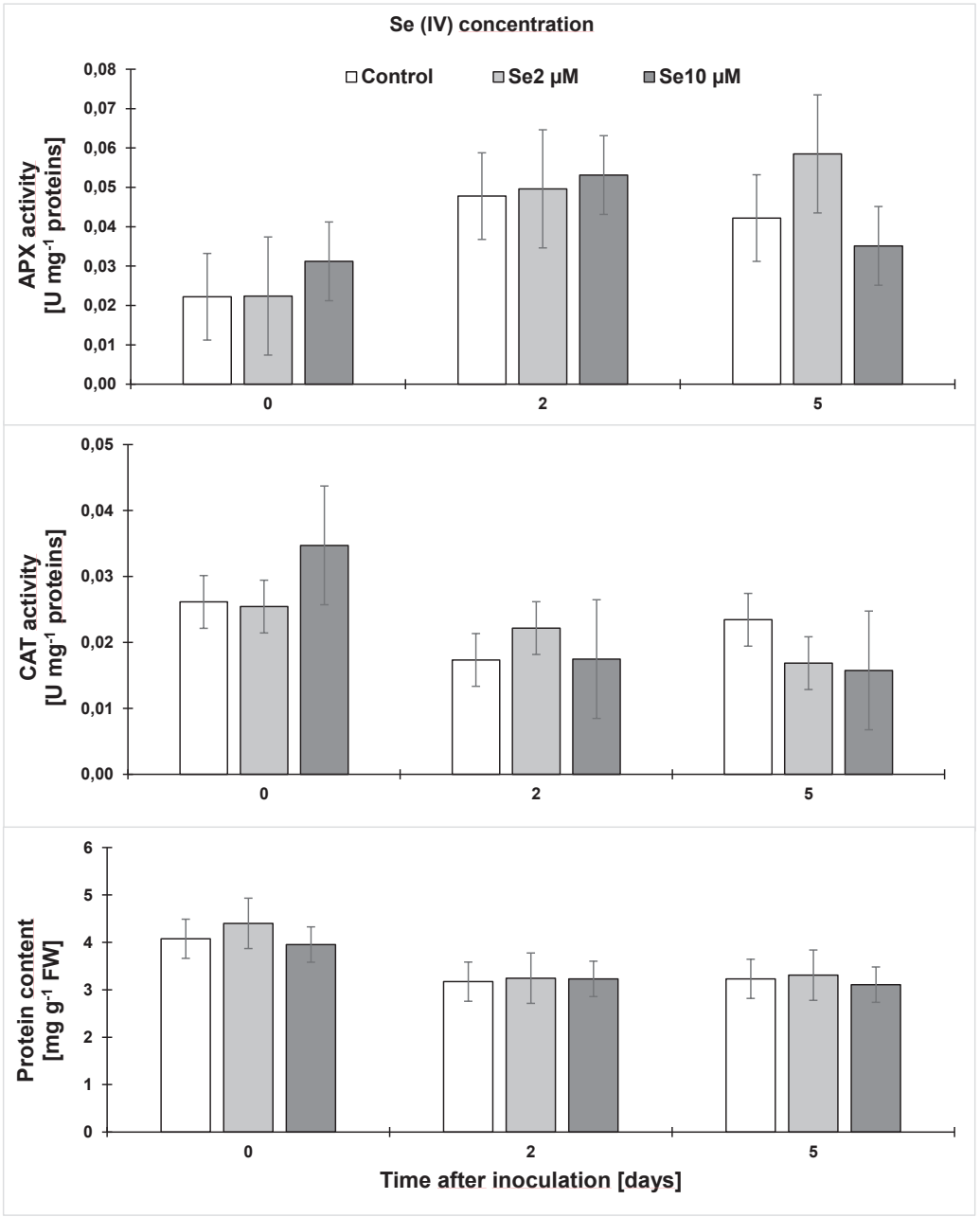

Fig. 5. APX activity (A), CAT activity (B) and protein content (C) in Se (IV)-treated lettuce leaves after *B. cinerea* infection.

Conclusions

Se (IV) applied at low doses has favored lettuce growth. In plants treated with 10 μM Se, the gray mold disease symptoms occurred later and were less intense than in the non-treated control plants. This effect could be related to the inhibition of *B. cinerea* growth and changed H_2O_2/antioxidant enzymes balance favoring the antioxidant defense.

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INDUCTION OF OXIDATIVE STRESS AS CANAVANINE INDIRECT MODE OF ACTION

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Canavanine (CAN) is a non-protein amino acid, a structural analog of arginine (Arg), thus it exhibits high toxicity toward plant and animals (Rosenthal 2001). CAN commonly occurs in legume plants, mostly in seeds e.g. of *Canavalia ensiformis* (L.) DC., but also in leaves of *Sutherlandia frutescens* (L.) R.Br. Seeds and sprouts of alfalfa (*Medicago sativa* L.) are rich source of CAN. Its harmful effect in living organisms, mostly herbivores, but also in plants is due to CAN incorporation into proteins in the place of Arg, leading to formation of abnormal proteins. This reaction is documented as primary mode of action of CAN (Staszek et al. 2017).

The purpose of the work was to study indirect effects of CAN supplementation in plants. Experiments were done using young seedlings of tomato (*Solanum lycopersicum* L.) treated with 10 or 50 μM CAN for 3 days. CAN inhibited elongation growth of tomato roots in concentration dependent manner. Restriction in growth of roots resulting from seedlings' supplementation with CAN correlated with induction of oxidative stress, demonstrated as over-production of ROS (Krasuska et al. 2016). It was accompanied by stimulation of cellular antioxidant system. Accumulation of antioxidant molecules and alterations of activity of ROS scavenging enzymes (catalase, peroxidases, glutathione peroxidase and glutathione reductase) were detected. Moreover, high level of protein carbonyl groups was observed, suggesting oxidative damage of the tissue.

We may conclude that secondary mode of action of CAN in plant cells involves disturbances in ROS production and metabolism.

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INVOLVEMENT OF REACTIVE OXYGEN SPECIES (ROS), REACTIVE NITROGEN SPECIES (RNS) AND VOLATILE ORGANIC COMPOUNDS (VOC) IN INDUCTION OF CUCUMBER RESISTANCE BY *TRICHODERMA ATROVIRIDE* TRS25 AGAINST DISEASE CAUSED BY *RHIZOCTONIA SOLANI*

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Fungi of the *Trichoderma* species are biological control agents (BCA) which promote plant growth as well as increase their resistance to pathogens. Their beneficial influence on plants depends on *Trichoderma* strain, plant species, pathogen, and soil-climate conditions. In plants, they are able to activate Induced Systemic Resistance (ISR), rarely Systemic Acquired Resistance (SAR), and according to the results of the latest studies, mixed ISR/SAR type of resistance called *Trichoderma*-Induced Systemic Resistance (TISR). The mechanism of defense responses induction and the type of resistance induced in plants by *Trichoderma* are issues which still arouse much controversy (Harman, 2012, Vinale, 2008).

Rhizoctonia solani, a necrotrophic fungus is emphasized as the pathogen against which natural plant protection methods are in great demand (Stodart, 2007). Results of the latest research as well as own preliminary studies on *T. atroviride* have shown that the strain TRS25 belonging to them has the potential to reduce the development of disease symptoms caused by this pathogen. Therefore, the main objective of the present studies was to find out the basis of systemic defense responses and resistance of cucumber plants (*C. sativus* L.) to this pathogenic fungus, induced by new Polish *Trichoderma* strain *T. atroviride* TRS25.

The studies showed the ability of the tested *Trichoderma* strain to stimulate plant growth and to induce biochemical, molecular and structural responses that enhance natural protection against *R. solani*. As compared to the control plants and the plants inoculated with *R. solani*, cultivated in the medium without *Trichoderma* spores, the plants which were grown in the medium with TRS25 spores were less susceptible to the pathogen attack and were characterized by the increase in the content of hydrogen peroxide (H_2O_2), nitrogen oxide (NO), s-nitrosothiols (SNO) and decrease in lipid peroxides (TBARS) content. The increase of reactive oxygen and nitrogen species was accompanied by increases in activity of defense enzymes e.g. guaiacol and syringaldazine peroxidase (GPX, SPX), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO). Moreover, the studies showed that volatiles: ethylhexyl salicylate (EHS), β-cyclocitral and fatty acid derivatives e.g. Z-3-hexanal, Z-3-hexenol and E-2-hexenal might be involved in plant resistance induction. These compounds have not been considered in plant resistance induced by *Trichoderma* against *R. solani* so far. We put forward a hypothesis that, together with methyl salicylate (MeSA) and H_2O_2 or NO, these compounds enhance expression of genes of defence-related proteins including *PR1* and *PR5* genes – *SAR* markers and *PR4* genes characteristic of ISR resistance, allowing to induce in plants the TISR.

TRS25 strain which seem to induce cucumber resistance to *R. solani* may be considered for further studies aimed at production of new biopreparations for use in the sustainable cultivation of plants, being an alternative to chemical plant protection products.

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Microscopic analyses were obtained in Laboratory of Microscopic Imaging and Specialized Biological Techniques in Faculty of Biology and Environmental Protection at University of Lodz.

INTERACTION BETWEEN STRESS HORMONES AND THE EXPRESSION LEVEL OF TWO *ARABIDOPSIS PP2-LIKE* GENES DOWN-REGULATED DURING *HETERODERA SCHACHTII* INFESTATION

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Phytohormones are known to play the major role in many physiological processes as well as during plant response to biotic and abiotic stresses. Among them, the stress hormones: abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) are known to play the most important roles in mediating plant defence response against pathogens and abiotic stresses. An identification of sophisticated connections between genes, phytohormones and other factors involved in plantpathogen interaction are of particular importance in plant studies and agrobiotechnology. At*PP2A3-like* and At*PP2A8-like* genes encode proteins with PP2-like domain. Phloem Protein 2 (PP2) is one of the phloem lectins. The function of PP2s is not clear. However, several studies suggest their putative role in short and long-distance transport in plants. The purpose of the experiment was to examine, whether the stress hormones affect the expression of At*PP2A3like* and At*PP2A8like* genes.

A. thaliana Columbia (Col-0) wild type (WT) seedlings were inoculated with parasitic beet cyst nematode (*H. schachtii*) infective juveniles (J2) and root tissue were collected from both infected and noninfected plants at 5. and 15. day after inoculation (dai) for RNA isolation. The transcript accumulation level of *PP2-like* genes was analyzed using Real-Time PCR technique. The *pp2a3-like* and *pp2a8-like* knock-down insertion mutants were used for nematode susceptibility assays. Seedlings growing *in vitro* were inoculated with *H. schachtii* J2 and infection sites and females were counted at 5. and 15. dai, respectively. The 15dayold WT seedlings were treated with one of the phytohormone ABA, SA, JA and methyl jasmonate (meJA) solutions and the roots were harvested after 24 hours of treatment. For the gene expression analysis the Real-Time PCR technique was used. Additionally, the *PP2-like* genes expression was examined in roots of the stress hormone signaling mutants: *abi1*, *coi1* and *pad4*. The roots were harvested from 15-day old seedlings for RNA extraction and the gene expression level was analyzed using RT-PCR technique. Mutant lines were not treated with phytohormones.

At*PP2A3-like* and At*PP2A8-like* genes were found to be down-regulated in cells modified by the *H. schachtii*. *pp2a3-like* and *pp2a8-like* mutants showed reduced susceptibility to nematode infection, indicating that the studied putative phloem protein genes are required for the optimal nematode development. The significant difference in transcript accumulation level of At*PP2-like* genes between mock and ABA-treated roots was found. In JAtreated and meJatreated roots only At*PP2A3-like* gene showed significant changes in transcript accumulation level. SA treatment did not trigger any significant changes in the expression level of both genes. The *abi1* and *pad4* mutants showed decrease in At*PP2A3-like* transcript accumulation level as compared to the WT plants, whereas no differences in the selected gene expression between the *coi1* mutant and WT plants were observed. There were no significant changes in At*PP2A8-like* transcript accumulation level in any of examined mutant lines. This knowledge might be useful for further investigation of signal pathways during plant-nematode interaction.

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PHYSIOLOGICAL AND BIOCHEMICAL GROUNDS OF SWEET BRIAR ROSE (*ROSA RUBIGINOSA* L.) EXPANSION INTO DRYLANDS

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Rosa rubiginosa belongs to the group of wild growing roses, is native to Europe and common throughout the continent (Zimmermann et al. 2010, 2011). The species was introduced to South America, Australia and New Zealand, where it adapted to the new environments and colonized such areas as dry steppes.

The purpose of this study was to disclose physiological and biochemical basis for the expansion of sweet briar rose (*Rosa rubiginosa* L.) into drylands. So far, this species has not been studied in this context. We assume that sweet briar rose has an effective adaptive mechanism enabling its survival in a dry environment. The study involved Polish populations of *Rosa rubiginosa*. We analyzed the activity of their photosynthetic apparatus by measuring chlorophyll fluorescence, chlorophyll and carotenoids content, plant water status, carbohydrates and phenolics level.

Depending on soil water status, we observed differences in the activity of photosynthetic apparatus, osmotic potential and the content of assimilation pigments, carbohydrates and phenolics. These results support the conclusion that soil water content induces protection mechanisms in sweet briar rose. We intend to expand our studies on *Rosa rubiginosa* acclimatization in drylands.

THE ELECTRICAL POTENTIAL OF *NICOTIANA BENTHAMIANA* AFFECTED BY MICROWAVE EXPOSURE

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Abstract

The exposure of plants to microwave electromagnetic radiation has increased over recent years as a consequence of the ongoing extensive deployment of wireless communication networks. To date, however, exposure to microwaves has not been considered a prominent abiotic stress factor for plants. Nevertheless, exposure to microwaves has been shown to have various effects on the morphology and physiology of plants. On the basis of findings such as accumulated H_2O_2, altered photosynthetic pigment contents, altered electrical properties, and retarded growth, exposure to microwaves can be considered a source of stress for plants. Of the oscillating electrical and magnetic fields characterizing microwaves, electrical fields can be considered the most influential, as they promote the dielectric activity of charged ions (K^+, Ca^{2+}, and Cl^-) and dipolar molecules (H_2O) within plants. However, to date, the mechanisms underlying the effects of microwaves on plants have not been conclusively ascertained. In this study, we investigated the effects of microwave exposure on the electrical signaling of mature wild-type Nicotiana benthamiana plants. Specifically, we recorded burn injury-induced electrical potentials in plants under conditions of microwave and non-microwave exposure. A comparison of the amplitudes of the electrical potentials revealed that these were significantly reduced in response to microwave exposure. The results of this study indicate that exposure of plants to microwaves influence the electrical signaling in plants. In present-day environments, microwaves have become a ubiquitous phenomenon and their effects on plants are readily apparent. We accordingly suggest that microwaves be considered a crucial environmental factor in plant research.

The environmental presence of microwaves (electromagnetic wavelength frequencies in the range 3×10^2–3×10^6 MHz) has become a ubiquitous feature in almost all parts of the world. In recent years, microwave activity has increased markedly concomitant with the worldwide expansion of mobile communication technologies (Senavirathna and Asaeda, 2014a). Together with other wireless communication technologies such as wireless broadband access and wireless networking, the presence of microwave in urban environments has increased significantly. Not only in modern cities but also in comparatively less developed cities, the presence of microwaves has increased in parallel with reductions in technological- and hardware-related costs. Further, wireless networks have become a vital feature in our homes, most of which are connected to the internet via broadcasted broadband internet networks. In addition to this already extensive deployment, to address the increasing demand for data, service providers have further extended the range of utilized frequencies (smaller wavelengths), with the latest 5G technology utilizing a wide spectrum in the frequency band above 6 GHz (Imran and Zoha, 2014).

The exposure guidelines for microwaves have been formulated based on the dielectric heating of biological tissues (thermal effect) (ICNIRP, 2009). Dielectric heating is caused by the vibration of charged ions and dipolar molecules in biological tissues upon exposure to the oscillating electrical fields of electromagnetic waves. The rapid vibration of ions and dipolar molecules generates heat because of the collisions. With increasing wave frequency and power density (electrical field strength), the vibration becomes more intense and heating increases. However, other than the thermal effect, there is currently little evidence indicating whether microwaves have adverse effects on biological material, even under the current exposure guidelines (Pirogova et al., 2009).

To date, the influence of microwaves on the biological tissue has mainly been studied in the context of human exposure. Little attention, however, has been focused on the potential effect of microwave radiation on plants, even though some studies have provided evidence for such effects (Senavirathna and Asaeda, 2014a; Vian et al., 2016). The reported effect of microwaves on plants mainly relate to changes in morphology (growth and germination rates) and physiology (photosynthetic pigments, oxidative stress, antioxidant and scavenging enzymes, and electrical signaling). In addition to these effects, the increased expression of stress-related genes has also been reported in several studies (Roux et al., 2006; Roux et al., 2008; Vian et al., 2006). Growing concern regarding the effects of mobile technology-associated microwaves on flora and fauna have recently been discussed in dedicated forums (Erich et al., 2018), and these have highlighted the importance of recognizing electromagnetic waves, including the microwaves, as prominent environmental factors.

The present study was conducted in order to determine the effect of microwave electromagnetic radiation (2.01 GHz) on tobacco (*Nicotiana benthamiana*) plants, and is a follow-up investigation to our previous study, titled "Microwave radiation alters burn injury-evoked electric potential in *N. benthamiana*," which examined the effects 2.45 GHz wavelengths (Senavirathna and Asaeda, 2018). Furthermore, we conducted the present study with the aim of raising awareness among plant scientists and to highlight the importance of microwave radiation as an environmental factor.

In this study, we used wild-type *N. benthamiana* plants growing in peat pallets (36-mm diameter, Jiffy 7 Peat Pellets). The seeds of *N. benthamiana* were sown directly into peat pellets and grown under an 18/8 h photoperiod providing 90–95 μmol m^{-2} s^{-1} photosynthetically active radiation. Plant nutrients were provided in the form of 0.01% commercial concentrated growth solution (Hyponex, Osaka, Japan) for young plants and then with 0.05% Hyponex solution for mature plants. Plants were grown under these conditions until they attained a height of approximately 15 cm and produced at least six fully unfolded leaves. All the plants were grown at 23- 25°C in a temperature-controlled room. The experiments were conducted in an anechoic chamber, the side walls of which were layered with ferried Electromagnetic radiation absorbing flat foam and the base covered with spiked foam (Senavirathna et al., 2014b). Within the anechoic chamber, light and temperature conditions were maintained at levels identical to those under which the plants had been grown. The anechoic chamber was equipped with a top-mounted exhaust fan to disperse excess heat and to enhance air circulation.

Plants were subjected to wounding stress by exposing the tips of leaves for 3 s to a flame produced by a general purpose liquid petroleum kitchen lighter. Exposure to the flame caused damage to approximately 35% of the leaf blade. The electrical potential generated in response to the burn injury was recorded in the plant stem by inserting a <20 μm Ag/AgCl gel-stabilized glass reading electrode (Unisense RD25; Unisense, Denmark), approximately 7–10 cm below the top of the plant. The reference electrode was inserted into the substrate. The generated electrical potentials were recorded into a computer at a 1 Hz sampling rate via an mV meter (pH/mV- Meter; Unisense, Aarhus N, Denmark), with 0.001 mV sensitivity (Figure 1). Plants were subjected to a continuous wave of 2.01 GHz, 2.0–2.1 Wm^{-2} power density (measured at the plant top), microwave exposure. The burn injury was applied to the first three (from top) fully unfolded leaves at three timepoints: pre- microwave (before microwave exposure), on-microwave (during microwave exposure), and post-microwave (after microwave exposure). The pre-microwave burn injury was applied to the first leaf (topmost) without microwave transmission and recorded the propagation of electrical potentials. After recording the pre- microwave exposure electrical potential, the plants were exposed to microwaves and recorded the propagation of electrical potentials in the second intact leaf with the burn injury. Following the termination of microwave transmission, recorded the post-microwave burn injury-triggered electrical potentials of the third leaf. The method used in the present study was the same as that used in our previous study, in which plants were subjected to 2.45 GHz wavelengths (Senavirathna and Asaeda, 2018).

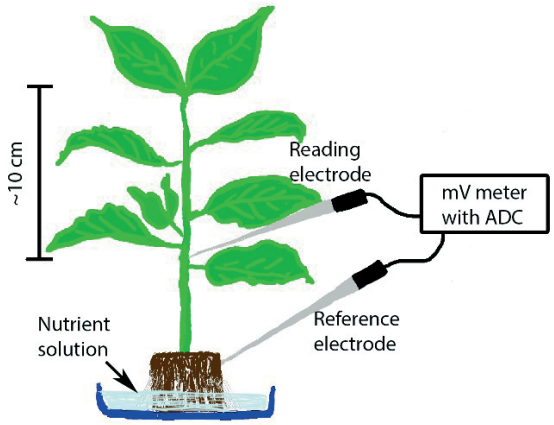

Figure 1. A schematic representation of the recording of electrical potentials in a *Nicotiana benthamiana* plant. The reading electrode was inserted approximately 10 cm below the top-end of the plant. The plant substrate was maintained in a 0.05% Hyponex solution (5 mm depth) to ensure a steady moisture supply.

Results and Discussion

The burn injury-evoked electrical potentials in *N. benthamiana* showed an inverted dumbbell shape, which represents the pattern of depolarization and repolarization. The magnitude of depolarization is plant specific; however, all signals exhibit unique characteristics during the depolarization phase. When the wound is intact, the depolarization reaches the reading electrode at an approximate propagation speed of 15 cm min^{-1}. Depolarization imparts a unique characteristic to the signal, as at a random point, the depolarization ceases and repolarization commences for a short duration (30–150 s). Thereafter, the depolarization continues until reaching the minimum electrical potential. Even under microwave exposure, this spike was observed (Figure 2).

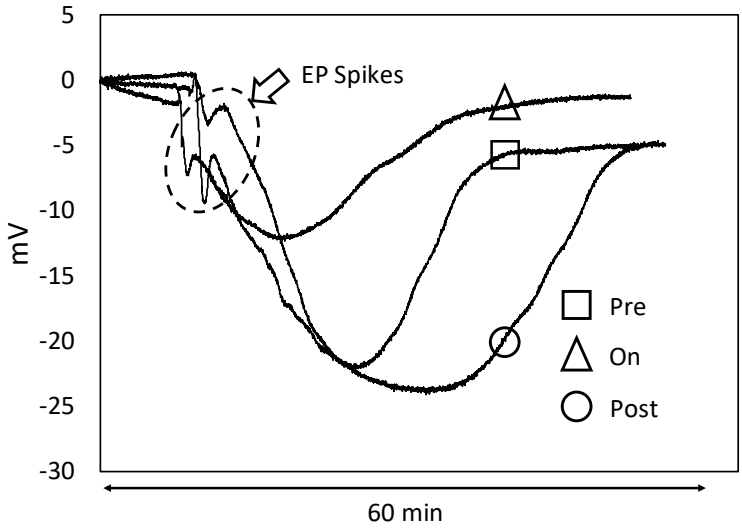

Figure 2. A representative graph of a burn injury-evoked electrical potential in *Nicotiana benthamiana* during pre-microwave (Pre), on-microwave (On), and post-microwave (Post) exposure. The dashed circle encloses the unique electric potential spike observed in signals.

With regards to the amplitudes (the minimum electrical potential reached during the depolarization phase) of the signals, we observed that microwave exposure caused a significant reduction in amplitude (t-test $P < 0.05$; Figure 3). During the post-microwave period, the amplitude recovered to a level that was almost the same as the pre-microwave amplitude. The average amplitude difference between the pre -microwave and on-microwave exposure periods and the on-microwave and post-microwave exposure periods was 55%.

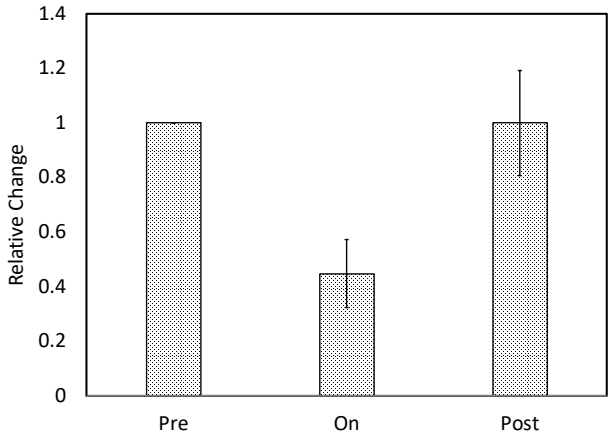

Figure 3. The relative difference in electrical potential amplitude of on-microwave (On) and post-microwave (Post) periods compared with the pre-microwave (Pre) period. The error bars represent standard deviations.

Consistent with the findings of our previous study, conducted using a different frequency (2.45 GHz), we found in the present study that exposure to microwave radiation influences the pattern of wounding -induced electrical potentials (Senavirathna and Asaeda, 2018). However, although the findings of these two studies are consistent, the magnitudes of the effects are not identical. By taking measurements from three different leaves at three different stages of the study (pre, on, and post microwave exposure), we observed resulting electrical potentials with different magnitudes, even in control studies, which can vary by up to 13.6% between the first two leaves and by 23% between the first and third leaves (Senavirathna and Asaeda, 2018). However, in response to microwave exposure, the electrical potential amplitude was reduced by 55%, which represents a significant effect. Consistent with these findings, previous studies on rapid fluctuations in plant resting potentials and the nanometric fluctuations observed in *Myriophyllum aquaticum* stems (which might be related to plant electrical potentials) have also revealed a reduction in potential amplitude upon microwave exposure (Senavirathna et al., 2014a; Senavirathna and Asaeda, 2014b).

Propagation of an electrical potential along the stem of a plant is required for intracellular and extracellular ion exchange (K^+, Ca^{2+}, and Cl^-). The dielectric activity induced in these ions and dipolar molecules (e.g., H_2O) might tend to influence the propagation of electrical potentials by disturbing ion exchange mechanisms. However, it is important to investigate the influence of reduced electrical potential amplitudes on information delivery to the destination (the unique electrical potential spikes detected in signals remained unchanged, and it is currently not known whether these are encoded signals or a different phenomenon).

In present-day environments, and particularly in urban areas, electromagnetic radiation in the 2.0–2.5 GHz range has become increasingly all-pervasive, and in the near future the range is likely to be extended up to 6 GHz. The influence of microwaves on plant physiology and morphology has been demonstrated in many studies (Senavirathna and Asaeda, 2014a; Vian et al., 2016), and it is indisputable that plants are subjected to microwave exposure throughout their life cycles, although the influences may vary according to factors such as plant species, growth stage, exposure frequency, microwave power density, and wave polarity (Halgamuge et al., 2015; Roux et al., 2006; Senavirathna and Asaeda, 2017; Sharma et al., 2009). We accordingly believe that greater attention should be paid to microwaves as an influential environmental factor in plant studies.

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EFFECT OF FOLIAR FERTILIZERS AND PLANT GROWTH REGULATORS IN REDUCING STRESS IN SUNFLOWER PLANTS UNDER CONDITIONS OF CLIMATE CHANGE IN THE FOREST -STEPPE OF UKRAINE

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Abstract
In the context of global climate change in Ukraine, there have recently been prolonged dry periods, alternating with brief torrential rainfall, and sharp fluctuations in day and night temperatures. Due to such climatic changes, there is an urgent need to develop ways to reduce biotic stress on plants. Ukraine has retained its place as leading producer of sunflower seeds globally for the past 10 years. A recent report in 2018 indicate that, Ukraine presently (2017−2018) ranks first in sunflower production globally with a 28.2 % share of entire world sunflower output of 46.11 million metric tons (United States Department of Agriculture - USDA, 2018). The yield levels have also increased significantly and are among the highest in the world. At the present stage of agricultural development, an important reserve is the application of foliar fertilizers containing trace elements (micronutrients), which is the key to obtaining high and sustainable yields and increase resistance to unfavourable environmental conditions. Therefore, this study investigates the effect of foliar fertilizers in reducing stress in sunflower plants under conditions of climate change in the Forest-Steppe of Ukraine. A two-year (2016 and 2017) field experiments were undertaken at the training and practical center of Sumy National Agrarian University. Experiments were organised on black soil, typical for coarse-medium loam. On May 12 and May 20, seeds of high oleic sunflower hybrid (PR64H32) were sown and correspondingly harvested on September 14 and September 27 in 2016 and 2017. The experiments were established in Randomized Complete Block Design (RCBD) with three replications. Seeds were sown at a plant density of 60,000 plants/ha with 4 rows in each plot. An inter row gap of 70 cm was retained. Initial fertilizer was applied to the soil during sowing at the rate of $N_{60}P_{60}K_{60}$. An estimate of the chlorophyll content was also ascertained with SPAD-502 plus chlorophyll meter (Spectrum Technologies, 2011). Manual harvesting was done at maturity by harvesting two inner rows in each plot. Mass of Seeds and 1000-Seed Weight were then determined. Spraying of crops prior to flowering (Sol Bor + Basfoliar 6-12-6; Wuxal Bio Aminoplant + Wuxal Boron; Spectrum Askorist + Spectrum B + Mo) caused a yield increase of 7.5−9.3%. These formulations contain: N, P_2O_5, K_2O, MgO, Mn, Cu, Fe, B, Zn, Mo, K, Amino acids, Seaweed extract of *Ascophyllum nodosum.* We also studied the influence of foliar fertilizers and plant growth regulators on chlorophyll concentration and the ratio of chlorophyll “a” and “b”, which suggests a reduction in stress of plants under the conditions of the years investigated. This research provides evidence of the positive effect of foliar application of fertilizers and plant growth regulators on productivity of sunflower plants. Nonetheless, the success of this agro-activity is contingent on the present weather conditions and therefore necessitates further study.

Key words: chlorophyll concentration, foliar fertilizer, leaf surface area, plant growth regulator, SPAD-502, stress, sunflower productivity,

Introduction

In the context of global climate change in Ukraine, there have recently been prolonged dry periods, alternating with brief torrential rainfall, and sharp fluctuations in day and night temperatures. Due to such climatic changes, there is an urgent need to develop ways to reduce biotic stress on plants. Ukraine has retained its place as leading producer of sunflower seeds globally for the past 10 years. A recent report in 2018 indicate that, Ukraine presently (2017−2018) ranks first in sunflower production globally with a 28.2 % share of entire world sunflower output of 46.11 million metric tons (United States Department of Agriculture - USDA, 2018). The yield levels have also increased significantly and are among the highest in the world. At the present stage of agricultural development, an important reserve is the application of foliar fertilizers containing trace elements (micronutrients), which is the key to obtaining high and sustainable yields and increase resistance to unfavourable environmental conditions.

An important component in the production of plants is the development of the assimilation surface, in particular the leaf surface area and the chlorophyll content in them. The leaf surface area could determine the photosynthetic capacity and hence the productivity of plants as sunlight for photosynthesis is absorbed by the leaf surface. A larger surface area allows for more plant pigments to absorb light energy, enabling a more productive photosynthesis. Also, leaf area growth determines light interception and is an important parameter in determining plant productivity (Gifford et al., 1984; Koester et al., 2014).

Chlorophylls are the most important photosynthetic pigments in plants (Silla et al., 2010). Leaf chlorophyll concentration also is an important parameter that is frequently measured as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content, or general plant health (Ling et al. 2011; Neto et al., 2017).

Foliar fertilization of crops provides a valuable supplement to the application of nutrients via the soil and under certain circumstances, foliar fertilization is more economic and effective (Fageria et al., 2009). Of significance, this mode of applying fertilizers ensures immediate uptake and translocation of nutrients to various plant organs via the leaf tissues and thus enables rapid correction of nutrient deficiencies (Fageria et al., 2009). The agricultural practice that is successfully employed to eliminate the negative effects of stressful situation on crop productivity is the application of plant growth regulators (PGRs) (Calvo et al., 2014).

Therefore, this study investigates the effect of foliar fertilizers and plant growth regulators in reducing stress in sunflower plants under conditions of climate change in the Forest-Steppe of Ukraine. We also investigated the influence of foliar fertilizers and plant growth regulators on chlorophyll concentration and the ratio of chlorophyll “a” and “b”.

Materials and Methods

A two-year (2016 and 2017) field experiments were undertaken at the training and practical center of Sumy National Agrarian University. Experiments were organised on black soil, typical for coarse-medium loam. On May 12 and May 20, seeds of high oleic sunflower hybrid (PR64H32) were sown and correspondingly harvested on September 14 and September 27 in 2016 and 2017. The experiments were established in Randomized Complete Block Design (RCBD) with three replications. Seeds were sown at a plant density of 60,000 plants/ha with 4 rows in each plot. An inter row gap of 70 cm was retained. Initial fertilizer was applied to the soil during sowing at the rate of $N_{60}P_{60}K_{60}$. An estimate of the chlorophyll content was also ascertained with SPAD-502 plus chlorophyll meter (Spectrum Technologies, 2011). The same samples were immediately transferred to the laboratory where the chlorophyll content was determined by the classical method using the ULAB-102 Spectrophotometer. Manual harvesting was done at maturity by harvesting two inner rows in each plot. Number of seeds and Mass of Seeds

were then determined. Data were subjected to statistical ANOVA followed by Duncan's multiple range test at 5% level of probability with the use of Statistica 8 software (StatSoft. Inc.).

Results and Discussions

Weather conditions for the vegetative period of 2016 and 2017 are shown (Figure 1). The growing season in 2016 was characterized by excessive rainfall in certain months. The humidification level was characterized by excessive rain in May (109.4 mm), June (71.4 mm) and August (62.7 mm). Respectively, the figures were higher than the average long-term (perennial) data by 61.5 mm, 8.2 mm and 24.4 mm. There was insufficient rainfall in July (40.3 mm) and this was lower by 20.4 mm (Figure 1a). For the year 2017, the vegetation period had insufficient rainfall compared to 2016. In May, June, July and August, the rainfall recorded were respectively, 39.4 mm, 41.2 mm, 29.9 mm and 7.2 mm.

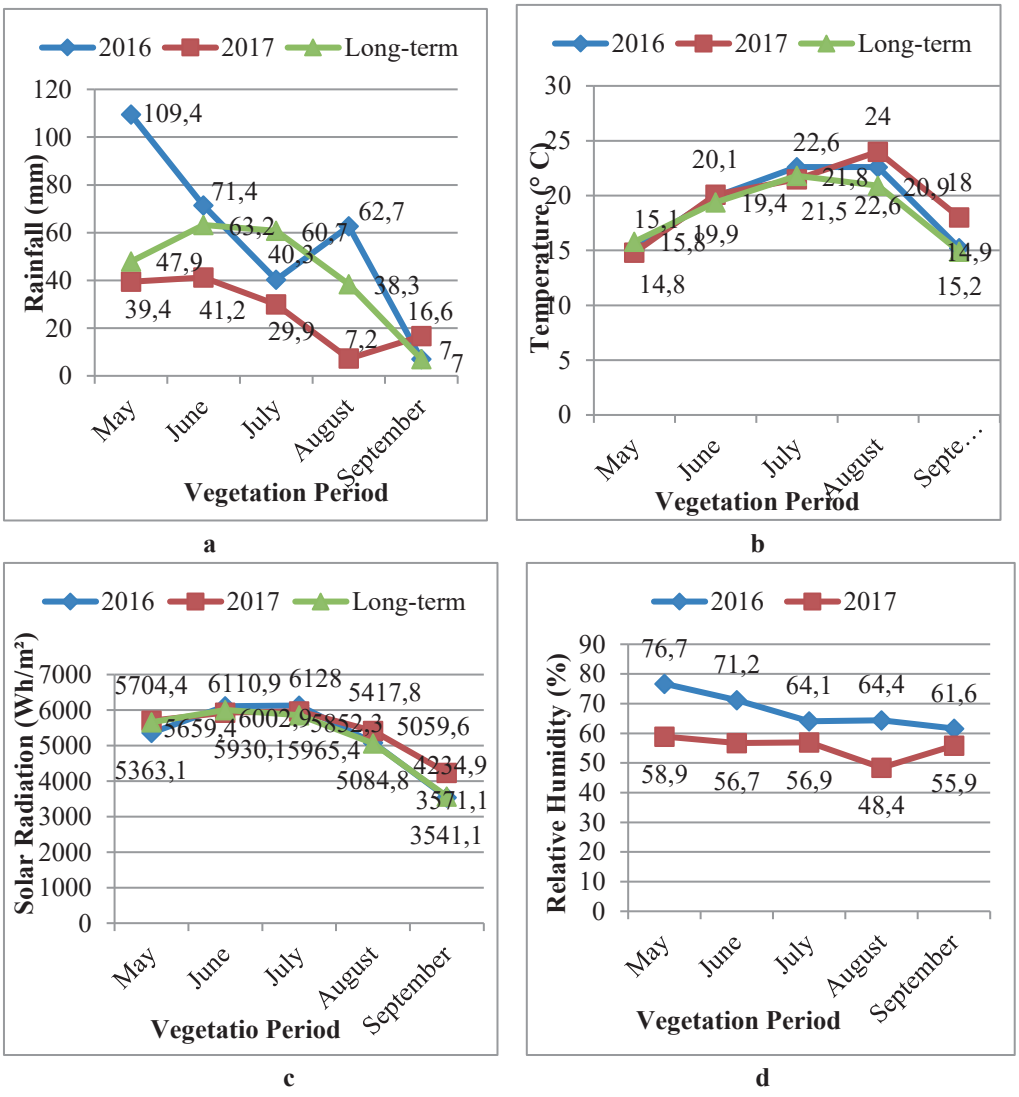

Figure 1. Weather conditions for the vegetative period of 2016 and 2017: a–Rainfall, b–Temperature, c–Solar Radiation, d–Relative Humidity.

There were no considerable difference in temperature between the two years during the vegetative season except for August and September, where there were considerable higher temperatures in 2017. The following average temperatures were recorded in the months of the vegetative period of 2016: May (15.1° C); June (19.9 ° C); July (22.6 ° C); August (22.6 ° C); September (15.2 ° C).The temperature for each month of the growing season exceeded the average perennial temperature, except for May, which generated a lower (negative) temperature. Specifically, the following temperature deviations were respectively obtained: May (-0.7 ° C); June (0.5 ° C); July (0.8 ° C), August (1.7 ° C); September (0.3 ° C) (Figure1b).

Spraying of crops prior to flowering (Sol Bor + Basfoliar 6-12-6; Wuxal Bio Aminoplant + Wuxal Boron) significantlyn increased the leaf surface area to 0.60 and 0.64 m^2 compared to control (0.52 m^2) (Table 1). However, application of Spectrum Askorist + Spectrum B + Mo did not show a significant effect. These formulations contain: N, P_2O_5, K_2O, MgO, Mn, Cu, Fe, B, Zn, Mo, K, Amino acids, Seaweed extract of *Ascophyllum nodosum.*

Table 1. Productivity of sunflower hybrid PR64H32 depending on foliar fertilizers and plant growth regulators (average for 2016-2017).

Foliar fertilizers and plant growth regulators	Leaf surface area, m^2	Chlorophyll concentration „a" and „b", mg/g fresh weight	Chlorophyll content SPAD value	Number of seeds per head, pieces.	Mass of seeds per head, g
Control	0.52	1.59	41.00	1174.5	75.0
Sol Bor + Basfoliar 6-12-6	0.60	1.71	43.35	1191.0	80.6
Wuxal Bio Aminoplant + Wuxal Boron	0.64	1.82	44.20	1216, 9	81.9
Spectrum Askorist + Spectrum B + Mo	0.57	1.67	42.83	1187.50	76.1
Duncan test	**0.06**	**0.05**	**2.15**	**16.5**	**4.8**

We also studied the influence of foliar fertilizers and plant growth regulators on chlorophyll concentration and the ratio of chlorophyll "a" and "b", which suggests a reduction in stress of plants under the conditions of the years investigated (Table 1). Maximal chlorophyll concentrations using ULAB-102 spectrophotometer were 1.82 and 1.71 mg/g respectively for Sol Bor + Basfoliar 6-12-6 and Wuxal Bio Aminoplant + Wuxal Boron compared to control (1.59). The higher chlorophyll content was determined with the SPAD-502 plus in the same variant of foliar application of fertilizers and plant growth regulators (43.35-44.20 SPAD Value).

Our regression analysis with a correlation coefficient (r=0.55) shows a positive linear proportional relationship between actual chlorophyll concentration and SPAD value (Figure 2). It should be noted that the chlorophyll content determined plus was higher in the year 2017 than 2016.

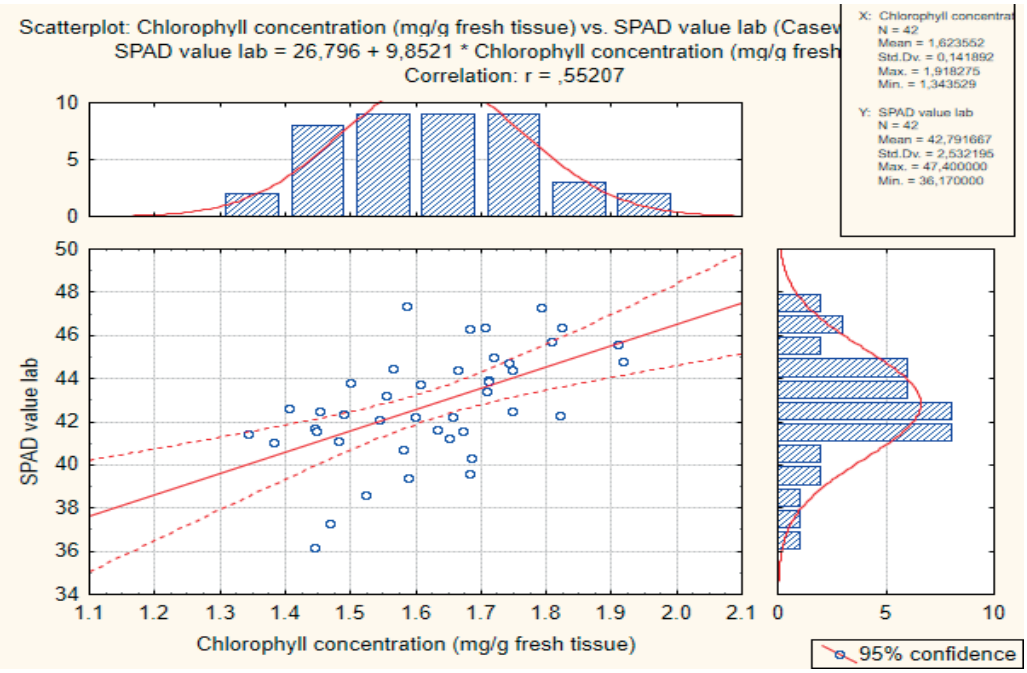

Figure 2. Correlation of chlorophyll concentration (mg/g fresh weight) and SPAD Value

The conducted regression analysis allowed creating a line that can be used to construct a calibration graph. This approach (development) can greatly simplify the determination of the chlorophyll content and allows for rapid analysis in the field.

The main elements of the structure of the sunflower crop are the number and weight of seeds formed per head on one plant. The results of the analysis revealed differences between these indicators based on foliar fertilizers and plant growth regulators sprayed (Table 1). Significantly higher performance was characterized by plants grown under the application of Wuxal Bio Aminoplant + Wuxal Boron and Sol Bor + Basfoliar 6-12-6. Thus, the above variants on the same plant on average produced 1191-1216.9 pcs, with a total seed weight of 80.6-81.9 g, which is 7.5-9.3% more than control. It has been observed that application of Plant growth regulators stimulates photosynthetic performance and antioxidative defense metabolism, in addition to water, light and mineral use efficiency, as well uptake of mineral nutrition. Ultimately, these plant responses reportedly minimize the negative effects of environmental stresses on crop productivity (Rhodes et al., 1999; Oosterhuis and Robertson, 2000; Djanaguiraman et al., 2004; Kovár and Černý, 2012).

Conclusion

This research provides evidence of the positive effect of foliar application of fertilizers and plant growth regulators (Wuxal Bio Aminoplant + Wuxal Boron and Sol Bor + Basfoliar 6-12-6) on productivity of sunflower plants. Nonetheless, the success of this agro-activity is contingent on the present weather conditions to further verify the application of foliar fertilizers and plant growth regulators in reducing the negative impacts of environmental stresses on productivity of sunflower.

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THE EFFECT OF SOIL MOISTURE ON ANATOMICAL STRUCTURE AND SILICON CONTENT IN *PHRAGMITES AUSTRALIS* LEAF

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Abstract. The results of comparative the anatomical structure and silicon presence of *Phragmites australis* air-aquatic and terrestrial ecotype leaves presented. The light-optical microscopy and X-ray analysis were used for leaves investigations. It was found that the reduced soil moisture (51-55%), on which plants grow the terrestrial ecotype, causes the certain changes in the structure of the leaves compared with the plants of the air-water ecotype growing in the water. It is established that the size and thickness of the leaf blade, epidermis structure and the cell size is an adaptive-plastic signs, which vary depending on the conditions of water supply. The comparative analysis of silicon presence, its content in leaf epidermis of *Ph. australis* two ecotypes showed the images of silicon quantitative distribution in epidermal cells and revealed the depending silicon content on the cell type and on environmental conditions of plant growth. The studies encourage concept that the phenotypical plasticity of plants is support for adaptation of plant to change of environment.

Key words: water deficit, leaf anatomy, silicon, *Phragmites australis*

Introduction

It is known that the growth of plants depends on surrounding conditions, including soil moisture, the decrease of which leads to moderate drought, which significantly affects the growth and reproduction of plants (Packer et al., 2017). In the process of evolution, wild plants approached the mechanisms for adapting to such changes in combination of molecular, biochemical, physiological and anatomical and morphological levels (Grzesiak, 2013). One of these plants is a cane, which serves as a model plant system to study the mechanisms of adaptation to changes in water status of the soil. It is known that the cane can grow in rather contrasting conditions of ground water supply: on the banks of rivers and lakes, as well as on terrestrial conditions, even in the mountains (Liang Chen et al., 2013). The researchers found that plants that grew in the mountains and in arid soils with reduced humidity occurs optimization of metabolic components (sugars and proline), change of photosynthetic rate and leaf gas exchange, and also energy reserves with the change of the structure of the epidermis, reduction of transpiration and minimization of water losses (Koson, 2006; Kerstein, 2006; Bacze-Kwinta, Koziel, 2010; de Micco, Aronne, 2012; Manivannan, Ahn, 2015). In addition, it is known that one of the chemical elements, namely, silicon (Si), has a significant effect on the intensification of photosynthesis (Soares et al. 2012) and indirectly involves in the inhibition of cuticle transpiration (Perry and Lu 1992). Taking into account the data that the leaves is the main organ regulating the water balance of the plant (Kerstein, 1996; 2006), the aim of our work was to investigate of the leaf structural plasticity and participation of Si ions in the phenotypical plasticity of *Phragmites australis* air-aquatic and terrestrial ecotypes grown in the zone of Kiev (Ukraine).

Material and methods

Plant material

Phragmites australis (Cav.) Trin. ex Steud, leaves of two ecotypes were used to the study. Air-water and terrestrial plants were harvested on June 2017 at the vegetative stage. Plants of water ecotype grew in water along the shore (at a depth to 50 cm) of Venetian Strait (the left bank of the Dnepr River (Kiev, Ukraine); terrestrial plants grew near 12-15 meter from the strait in a sandy soil. Photosynthetic photon

fluency rate (PPFR) was measured by means of the light Meter LI-250 (USA, LI-COR) on the adaxial surface of studied leaves. PPFR was equal to 1340 μmol quantum.$m^{-2} \cdot s^{-1}$ on the leaf upper surface of air-water plant. The PPFR was equal to 790 μmol quantum.$m^{-2} \cdot s^{-1}$ was equal on the leaf upper surface of terrestrial plants. The standard biochemical method was used to determine of the relative water content of in the soil. This method based on drying the soil specimens in a thermostat at a temperature of 105 ° C to constant weight (**Ermakov, 1982**). The relative humidity of the soil on which the air-water plants grew was 77.4 ± 2.1%, while the soil humidity on which the dry plants grew was lower and was 53.6 ± 1.9, accordingly. The water temperature in the Venetian Strait was +19° C in June. .

To determine the relative water content in the leaf blades, the standard biochemical method used which based on drying the samples in a thermostat in a desiccator at a temperature of 95 ° C to an unchanged weight (Ermakov, 1982). We used the middle part of 12 leaves from six air-water and 12 leaves from six terrestrial plants to determine the relative content of water in the leaves, Data were compared by using the Student's t-test.

Microscopy
The fixation of leaf samples carried out in field conditions, on shore. Middle part of the third leaves from 4 air-aquatic and from 4 terrestrial plants fixed at noon in the solution of 3% paraformaldehyde and 1% glutaraldehyde (1:1, v) in 0.05 M phosphate buffer, pH 7.2 for 24 h at +22°C, washed in the identical buffer, dehydrated in graduated ethanol series then acetone, and impregnate with epon/araldite resin. The sections of 10-12 μm thickness stained 0.1% toluidine blue and analyzed with Axioscope (Germany) the light-optical microscope. Besides, the fixed leaf samples prepared for scanning electron microcopy to the protocol by Kordyum et al. (2003) and analyzed with JSM-35 scanning electron microscope. To determine the linear sizes of leaves, three leaves of eight plants used. Linear cell sizes determined in 30-40 cells of the epidermis and in 50-60 mesophyll cells from each sample. To determine the relative water content in the leaves, the standard biochemical method used which based on drying the samples in a thermostat in a desiccator at a temperature of 95 ° C to an unchanged weight (**Ermakov, 1982**). Data were compared by using the Student's t-test

The energy dispersive X-ray micro analytical studies carried out under standardized conditions according to **Talbot and White (2013**). Leaf segments of 7x7 mm cut from middle part of lamina from seven plants of *Ph. australis* air-water and seven terrestrial plants. The samples after the freeze-drying pasted on the stub and coated with gold for the study by scanning electron microscope JSM 6060 LA (Japan) fitted with EX-S4175GMU (JEOL) energy disperses X-ray micro analyzer with ZAF system for quantitative analysis of elements (%/mass) in samples.

Statistical analysis
The dimensions of cell size were measured for 30-40 epidermis cells and for 50-60 mesophyll cells from each leaflet. For statistical processing we selected four air-water and four terrestrial plants; we took the first and second leaves from each plant. Values of cytological studies are expressed including the standard error (±SE).

Three measures of quantitative analysis of elements (%/mass) per the sample carried out for each epidermal cell. The results mean values of three results (± SE) were calculated by expressing the net counts for silicon element in a given cell as a percentage of the cell total mass.

Results
Anatomical structure.

Air-aquatic ecotype. Mature leaves of *Ph. australis* had linear, lancelet shape (**Fig. 1 a**). On the transverse sections of the leaves, the upper surface of the plate is almost equal; the lower is wavy with the formation of butt in the vein zone (**Fig. 1 b**). The structure of the leaf is an isolateral. The intercellular space in the mesophyll is poorly developed (**Fig. 1c**). The dimensions of cells are presented in Table. On the cross section, the shape of the epidermal cells is oval, mesophyll cells are almost rounded, occasionally elongated - in the zone of vein The thickness of the leaf blade is uneven: in the zone of veins, it is wide and ranges from 300 to 600 μm, in the narrow zone, where the veins are absent it ranges from 200 to 380 μm. The 11-12 layers of the mesophyll cells are in the vein zone, in the narrow zone – 4-6 layers. The middle number of chloroplasts per cut of mesophyll cell was 5.82 ± 0.40. The group of six-seven bulliform cells is characteristic for leaf adaxial surface (**Fig. 1b**). These cells are almost transparent on the cut, narrowed on the outside (on the periclinal side and extended - in the contact area with the mesophyll.

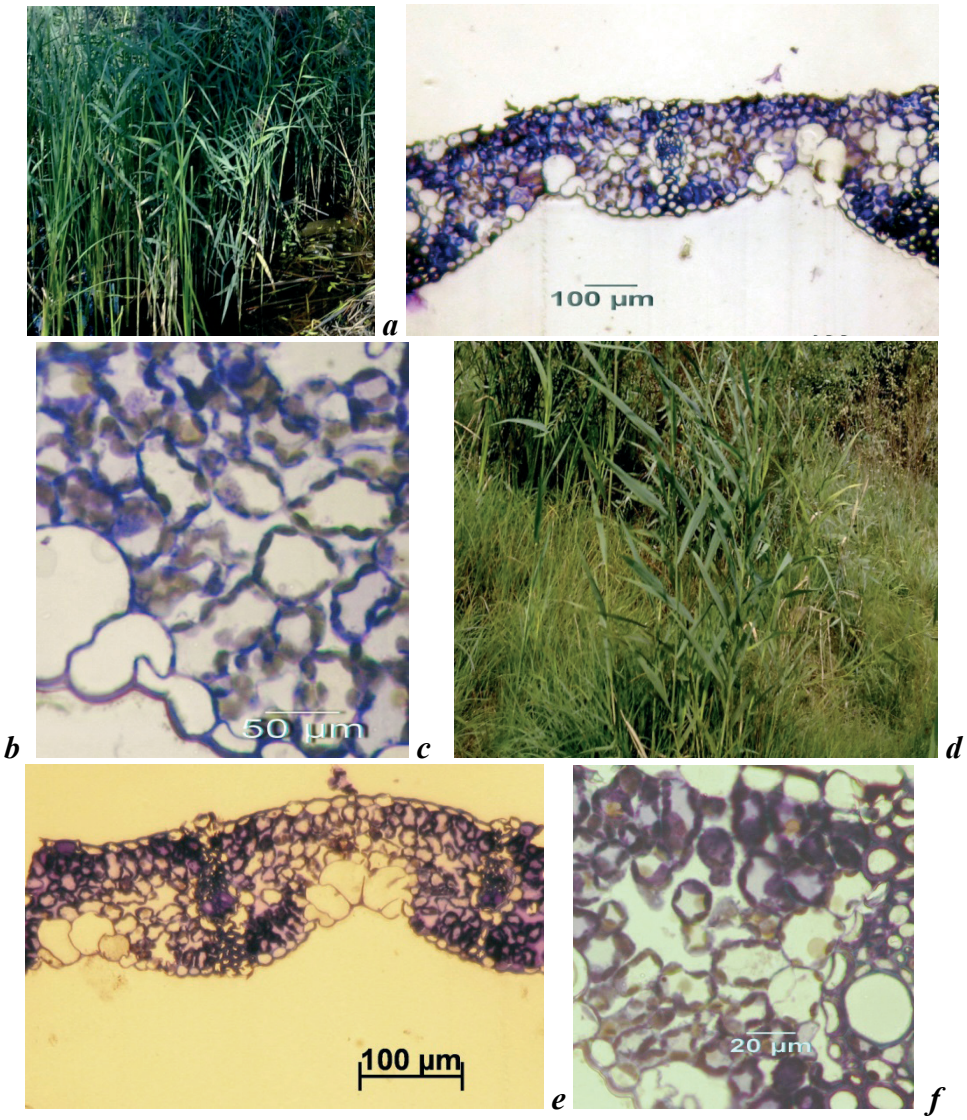

Fig. 1. The general view of *Phragmites australis* air-aquatic (a) and terrestrial (d) ecotype; the cross sections of air-aquatic leaf (Fig. 1b, c) and terrestrial leaf (Fig. 1e, f).

Table. The size of cells in *Phragmites australis* of air-aquatic and terrestrial plants

Parameter	Air-aquatic plant	Terrestrial plant
Average size of leaf blade (cm):		
long axis	51.3 ±7	71.3 ±8.9
short axis	1.3±0.7	3.0 ±0.2
Thickness of the leaf in the vessel passage area (μm)*	350 ± 43	200 ±23
Average size of adaxial epidermal cell (μm)		
long axis (height)	33 ± 1.4	11 ± 1.7
short axis (width)	63 ± 2.1	19 ± 1.3
Average size of mesophyll cell (μm)		
long axis	56 ± 4.3	20 ± 1.7
short axis	59 ± 2.7	17 ± 0.9
Average size of abaxial epidermal cell (μm)		
long axis (height)	38 ± 2.1	11 ± 0.7
short axis (width)	52 ± 1.7	14 ± 1.1

* - the table shows the data for the thickness of the leaf blade in the area of the second vessel from the edge of the leaf blade.

<u>*Terrestrial ecotype*</u>. The shape of mature leaves of terrestrial *Ph. australis* was like (**Fig. 1 c**) to that of air-aquatic ecotype. The structure of the leaf was also an isolateral (**Fig. 1e, f**). However, the size of long and short axis of leaf blade was more in comparison with that of air-water plants (**Table**). The thickness of the leaf blade was reduced due to the decreased size of the epidermis and mesophyll cells (**Table).** The thickness of the leaf lamina is uneven: in the zone of veins, it was 200 to 230 μm, in the narrow zone, where the veins are absent it ranges from 150 to 170 μm. The 8-10 layers of the mesophyll cells are in the vein zone, in the narrow zone – 4 layers. The group of four bulliform cells is situated in abaxial epidermis. The average amount of chloroplasts per mesophyll cell was 6.3 ± 0.28.

Ultrastructure of leaf surface and X-ray analysis.

<u>*Air-water plants.*</u> Two structural-distinct zones of adaxial leaf surfaces can to see with scanning electron microscopy: stomata zone with trichomes and almost smooth convex vault (over veins) between the stomata zones (**Fig. 2 a**). It is significant the presence of small trichomes on surface of the convex vault of abaxial surface (**Fig. 2 b**). .The density of stomata was 910±77 (number per mm^2 area) on adaxial surface, on abaxial surface was 1410±109, accordingly

X-ray analysis revealed the presence of silicon elements in stomata cells, trichoma's base, in cells around stomata and in convex vaults (**Fig. 2 e, f**). The content of silicon in the abaxial surface of *Ph. australis* air-aquatic ecotype differs from that on adaxial surface cells (**Fig. 3**).

Terrestrial plants. Similarly, ultrastructure of epidermis surfaces in terrestrial leaves of *Ph. australis* has shown the presence of two zones (**Fig 2. c, d**). The density of stomata on adaxial surface was 450 ± 39 and on the abaxial surface was 699 ± 53 numbers per mm^2 area. The surface of adaxial surface covered with the looked like trachoma's and with small needle-like wax structures. Abaxial epidermis, are also covered with trichomes and wax; the clear borders of the cells surrounding the stomata are not visible. In the vault clearly visible protrusions of elongated cells, which are situated along the length of leaf, and are characterized by slightly wavy or even smooth anticlinal walls, on the surface of such cells are short, hook-shaped trachoma's.

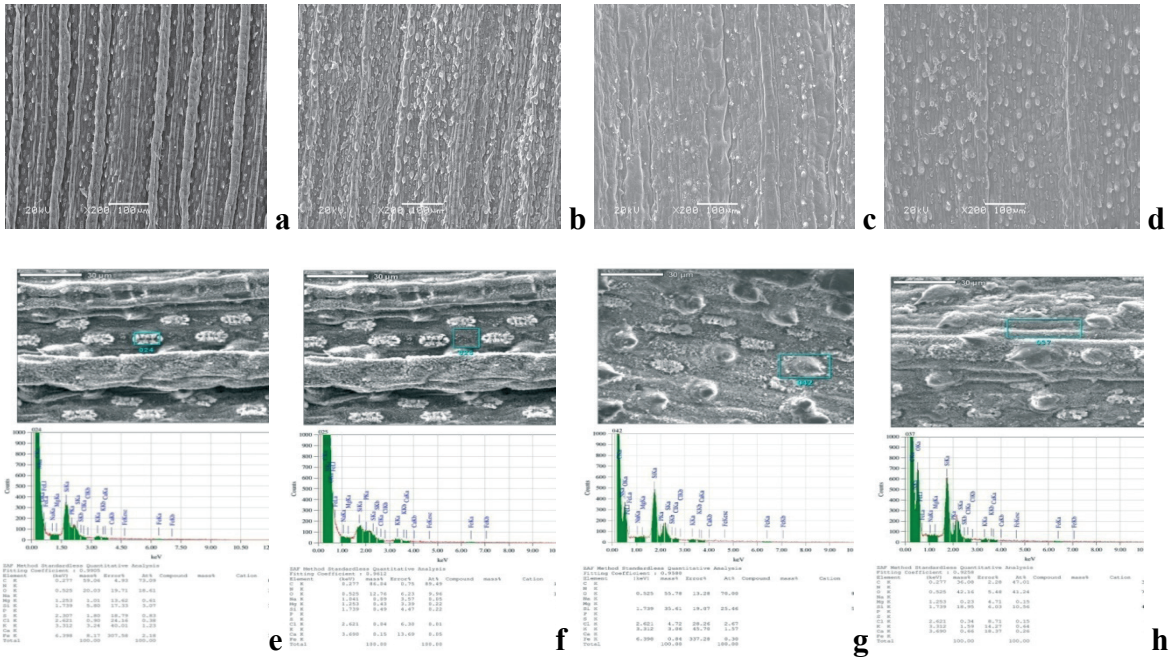

Fig. 2. The structure of the adaxial (a, c) and abaxial surfaces (b, d) of leaves *Phragmites australis* air-water (a, b) and terrestrial (c, d) plants. Figures e, f, g and h shows micrographs of different epidermal cells with spectra of elements including of silicon ions measured by X-ray technique of air-water (e, f) and terrestrial (g, h) plants at the vegetative stage of growth. On the upper part of each figure (e-h), outlined in the green square the cell or part of epidermis surface that was scanned by X-ray method. The bottom of each figure is the histogram of the content of chemical elements, including silicon; the axes: Y-axis showed as counts per second (cps), notably impulses eV per second; and X-axis showed energy in keV (kilo-electron volts)

X-ray analysis leaf surfaces of *Ph. australis* terrestrial ecotype revealed the presence of silicon elements in analogical types of cells (stomata, trichomes, in cells around stomata and in convex vaults) (**Fig. 3**). The content of silicon in the abaxial surface of terrestrial ecotype was more than that in the adaxial epidermis.

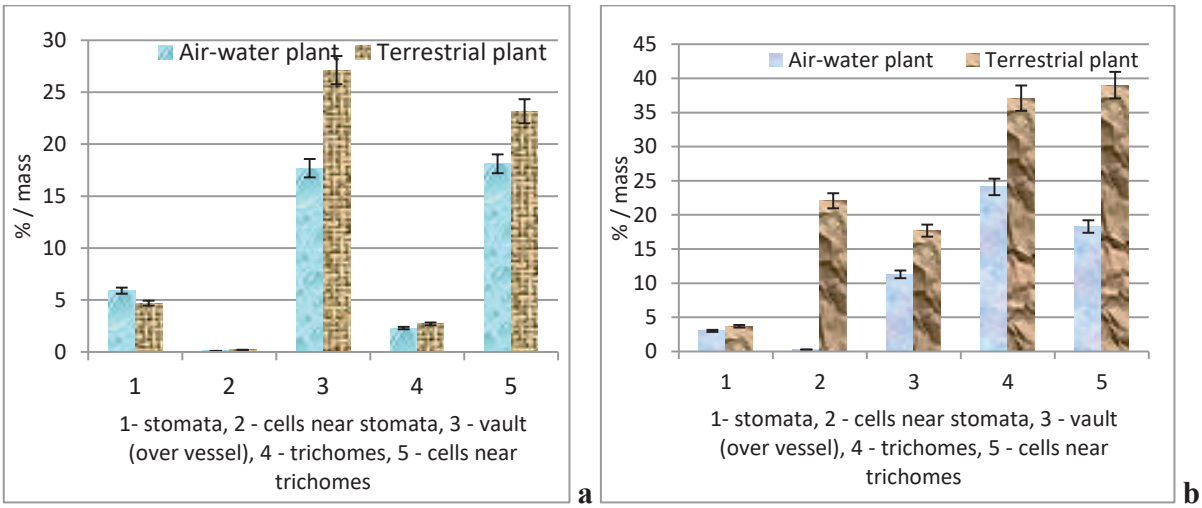

Fig. 3. Silicon content (%/ mass) in the cells of leaf adaxial (a) and abaxial (b) epidermis *Phragmites austra*lis air-water and terrestrial ecotype

Discussion

Thus, we have established that the leaves of *Ph. australis*, which grew in the water, differed in size from the leaves of terrestrial plants of this species. The larger sizes of the length and width of the leaf blades of plants growing at a certain distance from the shore are obviously due to the increase in the rate of division, or their earlier development, and possibly the optimal conditions for the growth of *Ph australis*. We do not exclude the differences in the rate of passage of the cell cycle in the leaves of investigated plants, just as this indicator is quite sensitive to the water content in the plant and in the soil. It is known that in conditions of small soil drought (-2.2 MPa), plant cell cultures inhibited inhibition of the S-phase of the cell cycle (**Setter, Flannigan, 2001**); whereas in the case of prolonged water deficiency, the relative rate of fusion of *Helianthus annuus* leaf cells decreased by 39% by blocking the passage of the cell cycle (**Granier, Tardie, 1999**). We also do not rule out that the plants of the air-water and terrestrial ecotype that we studied have a different ploidy, which affects morphological features, in particular, the size of the stems and leaves and also the height of the stems. According to the literature, octoploid, hexaploid and decapoid cane plants (**Pauca-Comanescu et al., 1999**) differ morphological signs. It is shown that the smaller the ploidy provoked the smaller the size of the cells (**Hansen et al., 2007**).

The obtained results have shown that leaf stomata density differed significantly between *P. australis* air -water and terrestrial plants. Air-water plants had approximately twice more the stomata density on both leaf surfaces. It is known that stomata density may be influenced by environmental factors such as light intensity, water availability, and CO_2 concentrations (**Hetherington, Woodward 2003**), one can assume that those parameters were different in the place of *Ph. australis* plants. Besides it is established the decrease of water potential in leaves of drought stressed agriculture's plants (**Grzesiak et al., 2008**). Taking into-account and the experimental results, we suggest that decrease density of stomata in epidermis of *Ph. australis* terrestrial ecotype is the sign of tolerance of plants to decrease moisture soil in nature.

The increase of trichomes number and wax in epidermis of terrestrial plants of *Ph. australis* can to explain the next. Trichomes and wax, as a plant protective barrier against ultraviolet, irradiation, pathogen attacks, and excessive transpiration, play a key role in the development of plants (Werker 2000). Trichomes may be formed to the accumulation and secretion of some alkaloids and the latter can strengthen the role of resistance in biotic stress and under the condition of extreme high a low temperature (Yamasaki and Murakami 2014). Besides, the trichomes can absorb UV radiation and reduce the damage by UV-B to photochemical activity and prevent stomatal closure. It is known that wax, which is formed on the outer side of the epidermis cells, inhibits transpiration and reflects ultraviolet rays of light (**Kerstiens 1996; 2006**). Taking the data of literature and the received our results, we can to suggest that an increased number of trichomes and wax in leaf epidermis of *Ph. australis* terrestrial plant helps this species to adapt to the soil with low humidity.

We revealed the silicon presence in leaf epidermal cells of *Ph. australis* air-water and terrestrial plants. It is known that silicon content across plants varies between about 0.1% to more than 10 % on a dry weight basis (Epstein 1994). This ion is a bioactive element associated with effects on mechanical and physiological properties of plants. We are observed with X-ray analysis silicon ions in all cell types of leaf epidermis regardless of *Ph. australis* both ecotypes, but its smallest content was in the stomata and cells surrounding the stomata. It is known that the evaporation of water in plants promotes xylem sap condensation and contributes to the formation of solute sediments, including silica. However, even though most of the water evaporates from mesophyll cells and passes through stomata to the atmosphere, silicification of the guard cells occurs during of plant growth and advances with age (Motomura et al. 2004). Taking into account the above and the results of our research with X-ray method, we can suggest that this localization and increased content of silicon in leaves of terrestrial plant can to optimize the water balance of plants and thus increase their resistance to decrease of soil humidity.

Conclusion

The comparative light-optical and X-ray analysis of the leaves of *Phragmites australis* of the air-water and terrestrial ecotypes showed that the essential reduction of the soil moisture, on which the growing terrestrial plants, leads to changes in the size of the leaves, the decrease of stomata density, the increase in the content of trichomes and wax, and also in increasing the content silicon in the epidermis of terrestrial plants. The question of the cellular mechanisms of the variability of the morpho-anatomical indicators of the cane that we studied needs of further study.

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WHEAT RESPONSE TO CADMIUM UNDER NORMAL, LOW AND HIGH TEMPERATURES

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Abstract. The aim of this study was to investigate the effects of cadmium (100 μM) in combination with low and high hardening temperatures (4 and 37 °C) on wheat (*Triticum aestivum* L.). According to the results of cadmium accumulation in wheat leaves, the low temperature leads to decrease in Cd content, whereas Cd accumulation under the high temperature was higher but the additive effect was not observed. The combination of Cd with low or high temperature resulted in less biomass accumulation compare with Cd impact under normal temperature. Although, all stress conditions led to an increase in antioxidant enzymes activity, proline content along with decrease in H_2O_2 content. We can supposed, that due to activation of antioxidant system as an one of general protective mechanisms of plant's tolerance it promote the plants survive under stress conditions.

Key words: adaptation, antioxidant enzymes, cadmium, cold, heat, wheat

Introduction

Plants in their natural environment are often subjected to various abiotic stress factors such as extreme temperatures, salinity, heavy metals, etc., and thus have evolved a multitude of defense mechanisms to increase their tolerance (Nakabayashi and Saito 2015).

Between all stresses the contamination of agriculture soils with heavy metals is a serious threat to crop production worldwide (Rizwan et al., 2016). Among heavy metals, Cd is highly toxic to plant and animals even at low concentrations (He et al., 2017). The Cd-contaminated food is the main source of Cd entry to humans via food chain (Dai et al., 2012). Wheat production in the world is about 650 million tons per year, thus wheat-derived products are the major source of Cd intake by humans (Lopez-Luna et al., 2016; Rizwan et al., 2016). Cd toxicity decreased the uptake and translocation of essential elements, affected the growth and plant development and caused overproduction of reactive oxygen species that lead to oxidative stress in plants (Ci et al., 2009).

Adaptation of plants to stresses, including heavy metals is linked to mobilization of antioxidant defense system (Waśkiewicz et al., 2014). This system includes the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and non-enzymatic antioxidant such as glutathione, ascorbic acid, carotenoids, and proline (Blokhina et al., 2003). Previously was shown the enhancement in antioxidant enzymes activity under cadmium treatment (Ci et al., 2009; Chen et al., 2010; Poghosyan et al., 2014). However, to date there are no studies available on the role of antioxidant system under the combined effect of different stress factors. Moreover, the molecular and metabolic responses of plants to a combination of cadmium and low or high temperatures cannot be directly extrapolated from plant responses to each of these individual stresses. In this case, it can be expected that the response to a combined stress of heavy metal and low or high temperatures may differ

from the response of the plants to each of these stressors alone. For this reason, the importance of focusing the researches on the response of plants to a combination of different forms of abiotic stress has been emphasized.

Material and methods

Wheat seedlings were cultivated on nutrient solution (pH 6.2–6.4), in a growth chamber with 14-h photoperiod, a photosynthetic photon flux density (PPFD) of 180 μmol $m^{-2} s^{-1}$, a temperature of 22 °C and relative humidity of 60–70%. Seven-days-old seedlings were exposed to cadmium sulphate (100 μM) under normal (22°C), low (4 °C) or high (37 °C) temperature for 7 days.

The Cd content was determined using an atomic absorption spectrophotometer A7000 (Shimadzu, Japan). Fresh mass of wheat shoots and roots were measured, and then dry mass measured after drying the shoots and roots to a constant weight at 80 °C.

Proline analysis was performed according to Bates et al. (1973).

SOD activity was assayed by the ability of the enzyme to inhibit photochemical reduction of nitroblue tetrazolium (NBT) to formazan according to Beauchamp and Fridovich (1971). CAT activity was determined by disappearance of H_2O_2 (ε = 39.6 $M^{-1}cm^{-1}$) in min at 240 nm (Aebi 1984). The assay of quaiacol-dependent peroxidase (G-POD) activity was based on the increase in optical density caused by the oxidation of guaiacol to tetraguaiacol in the presence of H_2O_2 (Maehly and Chance 1954).

Gene expression was analyzed by real-time PCR method. Total RNA was extracted using TRizol reagent (Evrogen, Russia) as instructed by the manufacturer. The purity of RNA samples and their concentrations were determined spectrophotometrically (SmartSpecPlus, Bio-Rad, USA): samples with A260/A280 ratios within 1.8-2.0 were used for further analysis. One μg total RNA was reverse-transcribed using MMLV RT kit (Evrogen, Russia) following the supplier's recommendations. Quantitative real-time PCR was performed using the iCycler iQ Real-time PCR Detection System (Bio-Rad, USA). PCRs were performed using the SYBR Green PCR kit (Evrogen, Russia). The PCR conditions consisted of denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 40 s and extension at 72 °C for 45 s. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using software with the iCycler iQ Real-time PCR Detection System. To minimize sample variations, mRNA expression of the target gene was normalized relative to the expression of the housekeeping gene actin. The mRNA levels of target gene (*P5CS*) were quantified in comparison to the control by ΔΔCt (Livak and Schmittgen 2001). The following primers were designed (using Primer Design program) for gene-specific transcript amplification: *P5CS* (AB193551), forward (fw) – 5′-GGAGACAAGTCCCGTGTTGGTAG-3′

and reverse (rv) – 5′-GCAGCAACAGCCATTTCACGGAC-3′;

actin gene was used as the control (AJ579382), fw – 5′-GGGACCTCACGGATAATCTAATG-3′ and rv – 5′-AACCTCCACTGAGAACAACATTAC-3′.

All experiments were performed at least three times. Data were subjected to analysis of variance (ANOVA). Data are presented as the mean values ± standard error. The research was carried out using the equipment of the Core Facility of the Karelian Research Centre of the Russian Academy of Sciences.

Results and discussion

According to the results of Cd accumulation in wheat leaves, it was shown that its content in wheat leaves was higher at 22 °C compared with 4 and 37 °C. The effect of Cd with 4 °C led to reduction of Cd accumulation in leaves on 7th day of experiment, it was 4-fold less (15 mg kg^{-1} DM) as compared with Cd at normal temperature (90 mg kg^{-1} DM) treated plants. It might be a result of direct negative influence of low temperature on nutrition transport in plants that caused in reduction in Cd concentration in leaves. However combination of Cd and 37 °C did not show the additive effect on Cd accumulation (50 mg kg^{-1} DM) compare with effect of Cd at 22 °C. We can supposed, that in this case in plants defense mechanisms activated, that prevented to higher accumulation of Cd in leaves. Previously, we have noted cadmium accumulation in roots of plants that were treated with cadmium sulphate (100 μM) for 1 h and that the cadmium accumulation increased in the leaves during the next 7 day. Cd content in wheat roots was higher than in leaves (Repkina et al. 2015). This is typical for wheat as well as for some other plants species which mainly accumulate heavy metals in root for preventing their entry into the aboveground parts (Kovacs and Szemmelveisz 2017).

The results of combined effects of Cd and low or high temperatures on dry mass (DM) of wheat shoots demonstrated a decrease in DM under all types of stresses (Tabl.). Cd under normal temperature caused a negative effect on DM of shoots but not so significant as in combination with low or high temperatures. On 7th day the most negative effect on DM was observed under combination of Cd with high temperature (37 °C) compare with low temperature (4 °C). Considering that 4 °C led to less Cd content in wheat leaves and the inhibition of growth in response to the low positive temperatures is one of the prerequisites for the adaptation of winter cereals to cold (Klimov 2009). We can suggest that in combination Cd with 4 °C, the temperature has dominant effect that resulted in decrease in DM and reaction of wheat seedlings similar with influence 4 °C alone. While, the high temperature 37 °C can enhance the Cd toxicity, cause to its higher accumulation in leaves compare with low temperature.

Table. Effect of Cd (100 μM) combination with normal (22 °C), low (4 °C) or high (37 °C) temperatures on wheat shoot dry mass (mg)

Treatment	Exposure, days			
	0	1	3	7
Cd + 22 °C	13,7±0,5e	17,4±0,4cd	20,8±0,6b	29,2±0,8a
Cd + 4 °C	13,7±0,5e	14,0±0,6e	15,8±0,4d	19,6±0,5b
Cd + 37 °C	13,7±0,5e	15,5±0,9d	16,3±0,6d	18,3±0,6c

Different stressors directly or indirect lead to an increase in generation of reactive oxygen species (ROS) (Mittler, 2002). Plants possess an antioxidant system that helps mitigate oxidative stress. This system includes the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and non-enzymatic antioxidant such as glutathione, ascorbic acid, carotenoids, and proline (Mittler 2017).

SODs are the key enzymes catalyzing dismutation of superoxide radical into hydrogen peroxide and oxygen (Perry et al., 2010; Dinakar et al., 2012). In our experiment SOD activity increased under all stress conditions. On 7th day its significant increase was observed at Cd treatment under normal temperature (Fig. 1). The combination of Cd with low and high temperature also led to increase in SOD activity but less than under normal temperature.

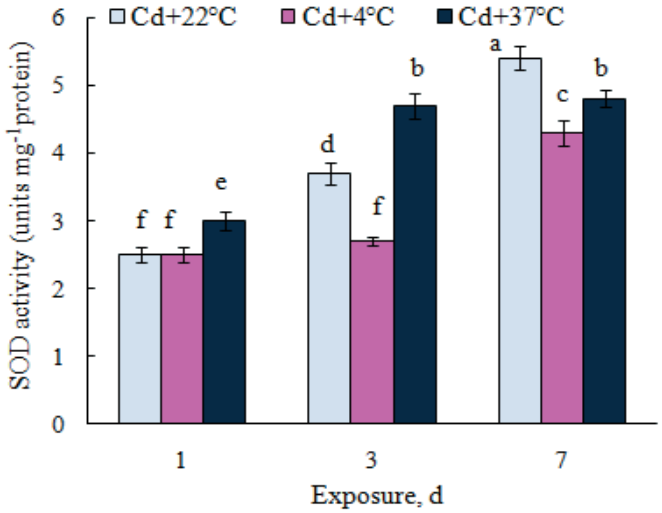

Figure 1: Effect of Cd (100 μM) combination with normal (22 °C), low (4 °C) or high (37 °C) temperatures on SOD activity. Initial level is 2,1±0,1f units mg^{-1}protein.

Other antioxidant enzymes such as G-POD and CAT catalyze the decomposition of hydrogen peroxide (Carmody et al., 2016). The activity of these enzymes in wheat also increased under all type of stressors but not unambiguous as SOD activity. Particularly, G-POD activities significantly increase in Cd-treated seedlings under normal temperature on 7th day (data not shown). Less increase was observed at Cd in combination with 4 °C and slightly changes in G-POD activity were found at 37 °C. Moreover, on 7th day CAT activity decreased under Cd treatment with low temperature but at normal and high temperatures CAT activity increased. Perhaps, under Cd in combination high temperature plants need in more intensive activization of antioxidant enzymes for prevention of damage. For evidence of effective work of antioxidant enzymes we can suggest the results of reduction in H_2O_2 content in wheat leaves on 7th day of experiment (data not shown).

However, all stress conditions lead to proline accumulation in wheat leaves. It was found that under cadmium impact at normal temperature the proline content slightly increased (Fig.2).

Although at combination of cadmium with cold and heat proline accumulation dramatically increased. However, in case of Cd combination with 37 °C the significant increase in proline content can be a result of protein degradation, while at 4 °C and its combination with Cd caused in proline accumulation as a key osmoprotector and low-molecular-weight antioxidant. Moreover, Cd under normal and low temperature increase in *P5CS* gene expression, encoding the enzyme of proline synthesis whereas accumulation of *P5CS* gene transcripts was not found under combination of Cd with 37 °C (data not shown).

In conclusion it can be supposed that under combine effects of cadmium and unfavorable temperatures, the plants reaction can be different from Cd treatment alone. Therefore, their combination should be regarded as a new state of abiotic stress in plants. Due to the influence of temperature on the transport of nutrients, it is able to modifying the rate of cadmium intake into wheat plants. Through the activation of the general mechanisms of tolerance, in particular the activation of antioxidant enzymes, the plant realizes a protective cascade reactions directed at the survival of plants under these conditions.

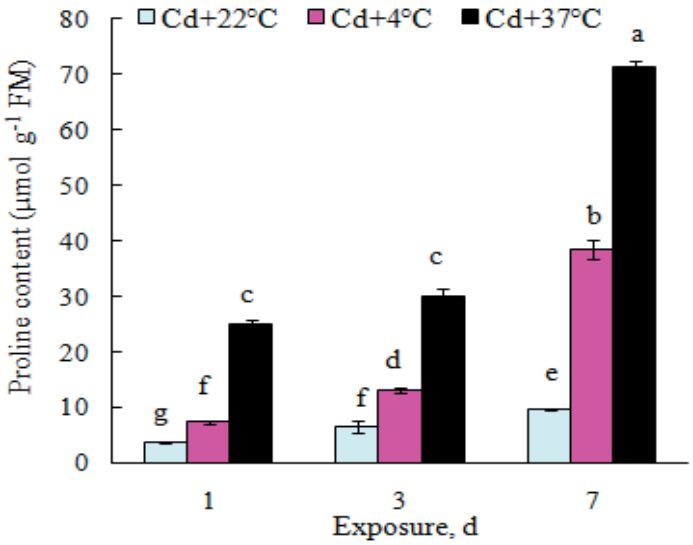

Figure 2: Effect of Cd (100 µM) combination with normal (22 °C), low (4 °C) or high (37 °C) temperatures on proline content. Initial level is 2,96±0,10g µmol g^{-1}FM.

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SEED PRIMING AS A STRATEGY TO OVERCOME ABIOTIC STRESSES DURING GERMINATION

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Seed priming is an old empirical technique used in modern time to alleviate stress response during seed germination and seedling establishment. It is addressed mainly to crop plants, where the quality of dry seeds is of special importance for agriculture because of economical point of view. Seeds are a starting material for crop production and crucial for achieving a good harvest. Seeds quality includes seedling performance parameters such as total emergence, the rate and uniformity of emergence, emergence under suboptimal conditions and seed longevity. To improve the uniformity of seedling emergence and to impart higher stress tolerance of plants, priming treatments may be applied (Côme et al., 1998; Soeda et al., 2005). Priming is a presowing treatment which involves a controlled hydration of seeds sufficient to permit pregerminative metabolic events to take place, but insufficient to allow radicle emergence. These seeds can be dried back to their original moisture content, which allows for convenient storage of such seeds and subsequent distribution to the grower. Since certain germination-related processes are initiated, priming generally causes faster germination and emergence, especially under adverse conditions. Common priming techniques include osmopriming (soaking seed in osmotic solutions such as PEG or in salt solutions), hydropriming (soaking seed in water), matricpriming (treating seed with a solid matrix), thermopriming (treating seed with low or high temperatures) and hormopriming (treating seed with plant growth hormones). To prevent radicle protrusion, water uptake must be either limited by imbibition in an osmotic solution instead of water (osmopriming) or by restricting the period of water imbibition and drying the seeds prior to radicle protrusion. During priming only a subset of events occurs, compared to germination *sensu stricto* (Bray, 1995). As a consequence of these processes seeds do not lose desiccation tolerance and their germination potential is stimulated. The priming process, in addition to the increase in the quality of seeds, improves the tolerance of seeds and seedlings to stress conditions (Jisha et al., 2013; Ibrahim, 2016; Wojtyla et al., 2016). Therefore, the beneficial effects of priming may be more evident during germination or seedling/plant growth under unfavorable rather than favorable conditions.

In our study two priming techniques were applied: osmopriming and hydropriming; both of them base on partial hydration of seeds necessary for dormancy break and metabolism reactivation. *Brassica napus* was chosen as a model to determine some aspects of the molecular mechanisms associated with seed priming and post-priming germination. We studied the impact of seed priming on germination under salt, drought and heavy metals stresses. As germination tests in all of analyzed situations showed incre-

ase in speed and efficiency for *Brassica napus*, the difference between seedlings grown from primed and unprimed seeds were less clearly outlined in the situation when stress factors were absent, however stress exposure revealed some physiological adjustment involved in improved stress resistance.

Conclusions from the results of our study and from literature suggest several mechanisms underlying the increased tolerance to abiotic stresses of germinating primed seeds. Among them we outline regulation of gene expression and protein accumulation. Our transcriptomic and proteomic analysis showed that mostly the same functional categories were affected at the transcriptomic and proteomic levels during osmopriming and germination of primed seeds. However, each of phases triggered distinct specific array of physiological and biochemical processes (Kubala et al. 2015a). The improvement of germination by priming was associated with an increase in protein synthesis potential, post-translational processing capacity and targeted proteolysis. Higher expression of genes and proteins involved in water transport and cell wall modification was also linked to the advanced germination of primed seeds. Moreover, improved germination of primed seeds was associated with higher expression of genes and enhanced activities of antioxidative enzymes such as ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) in primed seeds (Kubala et al. 2013). The management of oxidative stress could be one of the mechanisms of increased tolerance of primed seeds of *Brassica napus* to salt stress. The second mechanism postulated by us is proline accumulation (Kubala et al. 2015b). Our results revealed proline accumulation, which could be a result of hydrogen peroxide-induced *P5CSA* expression and P5CS activity. Other mechanisms, which could play an important role in improved germination of primed seeds, are activation of DNA repair mechanisms prior to seed germination and cell cycle regulation as well as mitochondrial biogenesis and cytoskeleton reorganization (Lutts et al. 2016).

Other analysis show enhanced tolerance of germinating primed seeds also to water deficit and presence of heavy metals such as lead and cadmium. Besides germination tests some physiological response were observed at seedling stage in response to salt and drought. The difference in seedlings grown in favorable and unfavorable condition were slightly visible, however stress application to plants enabled difference in water management, photosynthetic rate and biomass accumulation.

Our observation as well as data published by other groups provide a quite comprehensive look at stress response and activation of tolerance mechanisms in primed seeds prior to germination. Such situation seems to reflect plant priming, hardening and stress pre-exposure to enhance stress response and stress tolerance. The initial exposure to osmotic stress or even partial hydration of seeds during priming results in greater stress tolerance during post-priming germination, a feature which could be likely linked to a 'priming memory'. If so, the priming process, which is artificially initiated to sustain stress conditions, by its similarity to a natural stress and processes that plants undergo in the field, should rise the question, whether 'priming-induced' stress tolerance can be transferred to the descendants for improving tolerance to stress recurring in the next generation.

The difference observed in way and strength of response by primed and unprimed seeds may lead to suppose or assumption, that pre-sowing seed priming could be a valuable, effective and not costly technique applied to counteract stress effects at germination stage. However, the exact mechanism is rather multidirectional and molecular basis is difficult to read. The aforementioned processes are supposed to be the heart of understanding the regulatory function of seed priming.

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THE MOST IMPORTANT MOLECULAR MARKERS RELATED TO SOMATIC EMBRYOGENESIS (SE) WITH THE SPECIAL FOCUS ON B-1,3-GLUCANASES AND CHITINASES

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Plant cells posses the capacity for totipotent growth and development under specific conditions e.g. in *in vitro* culture (Horstman et al. 2017 and citations included therein). Somatic embryogenesis (SE) is one of asexual processes, where somatic or vegetative cells are induced to form embryos that can develop into a new organism. *In vitro* SE potential makes this technique an advantageous tool for plant propagation with applications in both industrial and life science sectors (Chawla 2002). Often the application of SE is the only way to propagate the plant of interest on a commercial scale (Guan et al. 2016). SE can also be used in basic research in the field of plant biology, where cytological, physiological and molecular aspects are under in-depth investigation (Dong and Dunstan 2000, Stasolla et al. 2002).

The first mention of SE is from the fifties of the 20th century, when Steward et al. (1958) and Reinert (1959) reported embryo-like structures formation in *Daucus carota* cultures. Those two papers started a rapid increase in the amount of similar studies published on different species (George et al. 2008) e.g. citrus (Kochba and Spiegel-Roy 1977, Tisserat and Murashige 1977, Gavish et al. 1991, 1992), Coffea sp. (Nakamura et al. 1992), *Macleaya cordata* (Kohlenbach 1965), Medicago sp. (McKersie and Bowley 1993), *Ranunculus sceleratus* (Konar and Nataraja 1969, Konar et al. 1972), *Zea mays* (Emons and Kieft 1991), *Pseudotsuga menziesii* and *Picea glauca* (Durzan 1980). Although the phenomenon of embryo formation in those cultures has been widely studied at different levels of plant development regulation, the mechanisms responsible for acquiring embryogenic potential was not completely recognized until recently (Quiroz-Figueroa et al. 2006).

In the literature, various SE-defined concepts are available (Boyer et al. 1993, Overvoorde and Grimes 1994, Raghavan 2000, Zhao et al. 2008). The differences depend on the plant species (or even genotype) investigated and protocols used for the analysis of SE at the induction and regenerative phases (Kielly and Bowley 1992, Joshi et al. 2009).

In general, the choice between the types of SE-inducing stress treatments, explant types (e.g. hypocotyls, cotyledons, leaves, nodals, mature zygotic embryos, protoplasts), medium composition, growth regulators (auxin, cytokinin, GA_3) and physical parameters of culture (pH, concentration of dissolved oxygen) determine both the success of SE and the pathway of somatic embryo development – direct (Leljak-Levanic et al. 2004, Yamamoto et al. 2005) or indirect through callus formation (Somleva et al. 2000, Thibaud-Nissen et al. 2003, Sharma et al. 2008, Feher 2015). Overall, the presence of auxin, most often 2,4-dichlorophenoxyacetic acid (2,4-D), seems to be required to initiate SE in the majority of explants of crop species such as cereals and legumes (Pasternak et al. 2002).

SE induction coincides with the expression of a number of markers (Horstman et al. 2017), including genes (Quiroz-Figueroa et al. 2006, Wickramasuriya and Dunwell 2015) and proteins (Boyer et al. 1993) induced by stress. Among them, *BABYBOOM* (*BBM*) gene and others like *LEAFY COTYLEDON* (*LEC1* and *LEC2*) and *SOMATIC EMBRYOGENESIS RECEPTOR KINASE* (*SERK*) are postulated to be involved in the acquisition of embryogenic competence by somatic cells (Kulinski-Lukaszek et al. 2012).

BBM is a member of the AINTEGUMENTA-LIKE (AIL) clade of AP2/ERF TFs which was initially identified as a marker for the induction of haploid embryo development from *Brassica napus* immature pollen grains. Ectopic expression of *BBM* in Arabidopsis and Brassica led to spontaneous formation of somatic embryos and cotyledon-like structures in seedlings without exogenous hormone application (Boutilier et al. 2002).

Ectopic expression of *LEC1* and *LEC2* induces somatic embryo formation in cotyledons and leaves of Arabidopsis seedlings (Lotan et al. 1998, Stone et al. 2001). Later, it was found that *L1L/NUCLEAR FACTOR Y* subunit B6 (NF-YB6) and three other NF-Y subunits, A1, 5 and 9, involved in embryo development, drought resistance, and ABA perception (Kwong et al. 2003, Warpeha et al. 2007, Li et al. 2008) also induce spontaneous SE in seedlings when overexpressed (Mu et al. 2013).

Among the genes that have been identified during SE, only *SERK* was confirmed to be a specific marker distinguishing individual embryo-forming cells in carrot suspension cultures (Schmidt et al. 1997). The *SERK* was found to be expressed during pro-embryogenic mass formation up to the globular stage of somatic embryo. It could also be detected in zygotic embryos up to the early globular stage, but not in unpollinated flowers or any other tissue (Schmidt et al. 1997).

Plant chitinases and β-1,3-glucanases, commonly referred to as pathogenesis-related (PR) proteins, are mainly studied in the context of defense responses to various biotic (Moravcikova et al. 2004, 2007, Żur et al. 2013) and/or abiotic (Meszaros et al. 2013, Gregorova et al. 2015, Maglovski et al. 2017) stresses. However, their role in plant growth and development processes was also proved (Leubner-Metzger and Meins 1999, Kasprzewska 2003, Michalko et al. 2017). These enzymes have been well characterized in different plant species such as pea (*P. sativum*; Petruzzelli et al. 1999), barley (*H. vulgare*; Ballance et al. 1976), maize (*Z. mays*; Cordero et al. 1994) and wheat (*T. aestivum*; Caruso et al. 1999).

Plant chitinases are enzymes which hydrolyse β-1,4-N-acetyl-D glucosamine (GlcNAc) linkages (Cohen-Kupiec and Chet 1998). Although the true substrate for chitinases in plants is unknown, these enzymes are thought to decompose non-specific arabinogalactan proteins (AGPs) (Kasprzewska 2003), which are involved in biological processes such as somatic or microspore embryogenesis (Pereira et al. 2016). Plant chitinases are transcriptionally regulated during zygotic (Hodge et al. 1996, Krishnaveni et al. 1999) and somatic embryogenesis (Horstman 2017). The experiments performed on *Sorghum*, *Pinus sylvestris* and *Eucalyptus pilularis* revealed that chitinases expression precede zygotic embryo development (Hodge et al. 1996, Krishnaveni et al. 1999). The important role of chitinases in embryo formation was also proved in the experiment with *D. carota* thermo-sensitive mutant *ts11* (De Jong et al. 1992; van Hengel et al. 1998). Interestingly, the addition of 32 kDa acidic endochitinase (extracted from *in vitro* cultures of wild type *D. carota*) to culture medium unblocked the development of somatic embryos of *ts11*, arrested at the embryo' globular stage at a non-permissive temperature (De Jong et al. 1992). A crucial role of acidic endochitinases was also confirmed in SE of *Dactylis glomerata* (Tchorbadjiva and Pantchev 2006), *Cichorium, Picea glauca,* and *Picea abies (*Baldan et al. 1997, Dong and Dunstan 1997, 2000). These experiments showed that acidic endochitinases could act as extracellular signal molecules in SE regulation. Moreover, the *Norway spruce Chia4-Pa1* gene encoding a typical basic class IV chitinase was described as the gene controlling somatic embryo transition (Wiweger et al. 2003).

Plant β-1,3-glucanases are glycoside hydrolases which hydrolyse 1,3-beta-D-glucosid linkages of plant (1→3)-beta-D-glucan, referred to as callose (Pirselova and Matusikova 2013). Callose is a component of cell walls or cell wall-associated structures at different stages of cell/tissue growth and differentiation (Tao et al. 2012, Liu et al. 2015). β-1,3-glucanases were found to be regulated developmentally e.g. during microsporogenesis (Bucciaglia and Smith 1994, Neuhaus 1999), flowering (Neale et al. 1990) or seed germination (Vögeli-Lange et al. 1994). The activity of these enzymes regulates the degradation of the callose wall surrounding the microspore tetrads, rich in β-1,3-glucan (Bucciaglia and Smith 1994). The difluoromethylarginine treatment inhibiting callose deposition implicated β-1,3-glucanases involvement in SE. An extracellular β-1,3-glucanase of a size of 37.7 kDa was identified as an enzyme playing a role in SE-signalling. It was hypothesized that β-1,3-glucanases accumulated in the culture media may be responsible for the degradation of the callose in the cell wall surrounding the embryogenic cells (Helleboid et al. 2000).

During the process of somatic embryogenesis the accumulation of various proteins has been demonstrated, among which the activity of two major PR proteins: β-1,3-glucanases and chitinases on transcriptional and protein levels seems to be very important for proper somatic embryo development. We can consider them to some extent as the biochemical marker of SE.

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Plant-stress conferences in Cracow aiming at the popularization of the knowledge of plant stress physiology, presenting state-of-the-art research, and providing the possibly to exchange opinions and ideas as well as initiate new scientific projects.

International Conferences „Plant Functioning Under Environmental Stress" are organized by the F. Górski Institute of Plant Physiology of the Polish Academy of Sciences in Cracow under auspices of the Polish Botanical Society and Committee of Physiology, Genetic and Breeding of Plant, Polish Academy of Sciences in co-operation with University of Alberta, Slovak Agricultural University in Nitra, Plant Protection Institute, Hungarian Academy of Sciences, Pedagogical University in Cracow, University of Life Sciences (SGGW) in Warsaw, and Agricultural University in Cracow.

The conference in 2015 had 150 participants including 37 from abroad (Australia, Canada, Denmark, England, Germany, Hungary, Iran, Russia, Slovakia, Turkey, Ukraine). Six plenary lectures were delivered, and 18 oral and 90 poster presentations were given (abstracts are available at the APP website at www.springer.com).

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