UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE OF CLUJ-NAPOCA
DOCTORAL SCHOOL IN AGRICULTURAL ENGINEERING SCIENCES

DOMAIN OF HABILITATION: HORTICULTURE

INVESTIGATION OF PHYSIOLOGICAL AND BIOCHEMICAL MARKERS OF PLANTS' TOLERANCE TO ENVIRONMENTAL STRESS FACTORS

ABSTRACT OF HABILITATION THESIS

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Cluj-Napoca
2024

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This habilitation thesis aims to present a synthetic overview of the personal scientific achievements of the candidate in the field of plant stress physiology, with special emphasis on physiological and biochemical parameters which reflect the functional acclimation of plants to adverse environmental conditions. These metabolic and developmental markers offer an insight into the intricate mechanisms of stress tolerance, revealing integrated adjustments of physiological processes and directed modulations of biochemical reactions which enable plants to survive during extreme growth conditions and to perform better under the influence of different abiotic stress factors. Several physiological changes induced by external stressors may be sensitive and early indicators of damages caused to various vital processes in the plants' organism, and of how different plant species and intraspecific varieties can cope with unfavorable environmental factors. In the present context of global climate changes and of the need for a controllable quantity and quality of crop production, this knowledge may offer specialists a valuable tool for an efficient selection of plants which perform better in the presence of specific environmental stress conditions (such as drought, flooding, extreme temperatures, high salinity of the soil water, excessive light and ultraviolet radiation, mineral nutrient deficiencies, air pollution, heavy metal and pesticide toxicity), as well as for an efficient breeding process toward a better stress tolerance of crop plants through an optimized exploitation of the self-protection mechanisms that may be activated in plants by specific signals. This stress tolerance of plants can be enhanced by specific priming and hardening procedures, thus the quantity and quality of crop production may be optimized under adverse growth conditions, in a cost-effective and environmental-friendly manner, avoiding the use of genetically modified plants and reducing the use of fertilizers and pesticides.

In this context, the main objective of the thesis is to present the relevance of those physiological and biochemical markers related to stress tolerance of the photosynthetic light use processes, of carbon dioxide assimilation, of the water economy of plants, of seed germination and of the antioxidative defense system, which during the research activities performed over twenty five years of academic career of the author since the attainment of the Ph. D. title proved to be useful tools in a precise characterization of the stress tolerance and of antistress defense characteristics of plants, especially with regard to high salinity, oxidative stress, low temperatures, high photon flux densities, heavy metal pollution and toxicity of pesticides and other organic xenobiotics. Many investigations dealt with mechanisms of salt tolerance, as under the circumstances of the present climate changes salt stress has emerged as a main constraint that limited crop production over even increasing agricultural areas, especially under incorrectly performed irrigation.

During the evolution of the scientific and academic career of the author, the first main research preoccupation was related to photoinhibition and photooxidative damage in the photosynthetic apparatus which ensures the photochemical conversion of light energy to fuel carbon dioxide assimilation, on which the entire primary production of plants relies. This topic enabled the development of skills in performing specific techniques of investigation of the light reactions of photosynthesis, such as the induced chlorophyll fluorescence, the thermoluminescence in frozen samples of isolated chloroplasts, or the polarographic determination of net oxygen production in the illuminated plant material. These methods proved to be also useful in studying the action mechanism of different environmental pollutants which possess action sites in the photosynthetic apparatus of the thylakoid membranes or in the metabolic pathways of carbon assimilation. In relation with the photooxidative processes induced by excessive photon flux densities, a further stage in the development of research activities was the investigation of over-production of reactive oxygen species in plants and of different aspects of antioxidative defense, implying the up-regulation of non-enzymatic antioxidants and the modulation of catalytic activities of several enzymatic antioxidants. This is how it became obvious that many abiotic stress factors share a common action mechanism by causing an internal oxidative stress, and thus inducing similar defense responses on which cross-tolerance to different stressors relies. A further research topic in plant stress physiology was the study of plant reactions to environmental pollutants such as heavy metals (e. g. cadmium, chromium, nickel, high concentrations of copper) and herbicides (e. g. diuron, methylviologen, gluphosinate), with emphasis on functional acclimation processes which enable a better survival with attenuated growth inhibition and only moderate losses in biomass production. Finally, studies concerning the mechanisms of salinity stress and salt tolerance in several crop plants (e. g. lettuce, garden rocket, canola, spinach, kidney bean, pea) and other species became the main topic of the research activities of the author, with special emphasis on how mild salt stress may enhance the biosynthesis of some health-promoting phytochemicals, and how priming treatments with natural bioactive compounds (such as S-methylmethyionine or vitamin U and triacontanol) may enhance salt tolerance of crop plants and contribute to a quantitative and qualitative improvement of crop production intended for human consumption.

The argumentation of the research achievements presented selectively in the thesis relies on the practical importance of offering new approaches to plant breeders and farmers through a thorough understanding of physiological adaptations of plants to those environmental changes which impact the quantity and quality of their production. Because several abiotic stress factors are hindering agricultural production globally, a cost-effective and environmental-friendly approach of enhanced plant tolerance towards the limiting growth conditions is at present a general food security issue. Original results concerning an accurate indication and characterization of how environmental stress factors affect different physiological processes and of how plants react with integrated adaptive changes to these external challenges were published by the author in more than forty research articles and book chapters, being also disseminated by participation at over eighty scientific conferences. New information of more general interest resulting from the investigations has been introduced in the content of several books written by the author, mainly in fields of plant physiology and ecophysiology. Research activities conducted in topics of abiotic stress in plants were supported by several research grants obtained by competition, the author of this thesis being director or partner coordinator of six of these grants (two of them being budgeted with the equivalent of more than ten thousand euros) and team member of thirteen of such research grants, covering a wide range of aspects of how environmental pollutants and other abiotic stress factors affect plant ontogenesis, metabolism and production in various habitats. These research projects also offered the possibility to gain experience in coordinating and managing research activities, as well as in working as part of research teams made up by academic personnel, students, postdoctoral fellows and senior researchers. Also, with the occasion of performing these research projects, the opportunities to establish and maintain fruitful co-operation with other research teams having related topics in several universities and research institutes have broadened the relations of our team with other professionals.

A better understanding of the mechanisms used by plants to cope with adverse environmental conditions opens possibilities for new, cost-effective and environmental-friendly crop management approaches, which rely on the own capacity of plants to efficiently react with coordinated metabolic and developmental adjustments and to enhance their stress tolerance. Knowing how physiological changes help plants to cope successfully with stress factors gives the opportunity to perform an efficient selection of plant species or cultivars which are less affected by specific stressors that occur frequently in their cultivation area, and to use this information in plant breeding towards an improved stress tolerance, without genetic transformation and avoiding the intensive use of pesticides and fertilizers. Furthermore, by revealing the intricate mechanisms of stress tolerance, which frequently share the same defensive changes in response to various external stressors, it becomes possible to exploit the benefits of cross-tolerance, when by obtaining tolerance to a certain stress factor the sensitivity to several other unfavorable physical or chemical factors is simultaneously decreased.

Considering that photosynthesis, water management, germination, growth dynamics and antioxidative defense are crucial determinants of plant survival and production in the presence of environmental stress factors, changes in the above-mentioned physiological processes were investigated in terms of sensitivity to different intensities of stressors and exposure times, as well as in terms of metabolic and developmental modulation during induction of defense mechanisms, during hardening and adaptive reprograming. Those functional and biochemical changes which occur specifically upon the action of an external stressor and are easily detectable, may represent early, sensitive and reliably markers of the tolerance of plants to abiotic stress factors, and may be applied in the selection and breeding of crop species and varieties to optimize plant production under specific local growth conditions where limiting environmental factors tend to impair growth and metabolism. This optimization means not only a higher yield under unfavorable conditions, but also an improved quality of the plant products in terms of content in health-promoting bioactive compounds.

Combination of chlorophyll fluorescence determinations with leaf gas exchange parameters (especially with net carbon dioxide assimilation rate) or with measurements of fluorescence of phenolic compounds synthesized as defense molecules, may offer a complex characterization of how different plants cope with environmental stressors, providing breeders with a reliable tool for identifying plant lines with better tolerance, which will perform a sustained production under adverse conditions of cultivation.

Based on the available data, the candidate’s team applied for the first time in Romania, more than twenty-five years ago, the method of induced chlorophyll fluorescence evaluation for revealing the influence of abiotic stress factors (namely excessive photon flux densities, herbicides, xenobiotic organic pollutants, heavy metals and high salt concentrations) on the photosynthetic performance of plants (Fodorpataki et al., 2003; Butiuc-Keul et al., 2004; Fodorpataki and Bartha, 2008; Bartha et al., 2010a; Fodorpataki et al., 2013; Fodorpataki et al., 2014; Tompa et al., 2020; Halmagyi et al., 2023). Until now, this team is still the one in the country that has demonstrated the usefulness of several chlorophyll fluorescence parameters, mainly the effective quantum yield of PSII and the vitality index of the photosynthetic apparatus reflected by the relative fluorescence decrease (Rfd), in evaluation of the influence of several pollutants and other environmental constraints on the light reaction of photosynthesis, and in the characterization of the degree of stress tolerance of different crop plant species (e. g. lettuce, spinach, garden rocket, canola) and cultivars (Bartha et al., 2010b; Fodorpataki et al, 2019; Tompa and Fodorpataki, 2021; Tompa et al., 2022).

These studies were among the first ones that demonstrated and documented the usefulness of the vitality index of photosynthetic apparatus in the investigation of stress tolerance of plants. A combination of the investigated markers of photosynthetic performance can be used for an efficient characterization of the influence of various environmental stress factors on crop plant productivity and for a scientifically supported selection and breeding for a better tolerance of unfavorable growth condition, in direct relation with optimization of plant yield in the context of current climatic trends and environmental pollution.

Many adverse environmental factors disturb plant metabolism by causing an overproduction of reactive oxygen species (ROS), which leads to oxidative stress. Oxidative stress is a common consequence of several external unfavorable conditions, such as drought, extreme temperatures, high salinity, excessive photon flux densities, intense UV-B radiation, heavy metals, some herbicides (e. g. methylviologen or paraquat), air-polluting gases (e. g. ozone in the troposphere, sulfur dioxide, nitrogen oxides, peroxyacetyl nitrate). This is why antioxidative defense is an important determinant of cross-tolerance to different stressors which have in common the intensified formation of ROS in the plant’s organism. Our findings offer, for example, an efficient solution for increasing the vitamin C content of leafy vegetables and other crop plants, thus improving the health-promoting nutritional quality of these plants for human consumption.

The results than concern the influence of various environmental stress factors on the dynamics of seed germination and seedling development may contribute to a proper selection of those germination parameters that enable a correct ranking of seed lots by vigor and offer a scientific support to take the best decisions regarding the planting potential of seed lots exposed to abiotic and biotic stressors.

It can be concluded that a better knowledge of how different physiological and biochemical markers are related with stress tolerance of different plants will enable specialists in agriculture and horticulture to select more efficiently crop plant species and varieties that cope more successfully with the adverse factors of their changing environments and to optimize the quantity and quality of crop production in a cost-effective and environmental-friendly way. We think that our results in this field have contributed to the development of this knowledge with direct practical implications in plant cultivation under continuously changing and challenging conditions.

The most important stress markers studied by us, along with the applied stress factors, the affected physiological processes and the plants used in the published experiments, are summarized in the following table.

Synthetic table with the main physiological and biochemical markers of plants' reactions to various environmental stress factors, studied by the author (original)

The physiological or biochemical marker	The environmental stress factor	The affected metabolic or ontogenetic process	The plant material used in studies
Stomatal conductance for water vapors, leaf transpiration rate	Moderate and pronounced hypersalinity of the water solution Salt stress	Regulation of gas exchange through leaf surfaces, acclimation to drought	*Lactuca sativa* *Bryophyllum daigremontianum*
Rate of net carbon dioxide uptake and fixation	Salinity stress	Primary biomass production, the ratio between Calvin cycle and photo-respiration	*Spinacia oleracea*
Water use efficiency during photosynthesis	Salt stress	Regulation of the equilibrium of leaf gas exchange (influx of CO_2 / transpiration)	*Brassica napus*
Net oxygen production	Nickel toxicity (15-150 µM) Hypoxia The herbicides glufosinate and methylviologen, cadmium toxicity (50 µM)	Water-splitting in photosynthesis, photorespiration and aerobic mitochondrial respiration	*Hydrilla verticillata* *Scenedesmus intermedius* *Scenedesmus opoliensis*
Hydraulic conductance of roots	Elevated salinity of soil water	Absorption and xylem transport of water and mineral nutrients	*Lactuca sativa*, several cultivars
Chlorophyll-*a* and/or chlorophyll-*b* content of leaves	Colonization of roots with mycorrhizal fungi on gleyic calcaric fluvisoil	Metabolism of porphyrins, harvesting of photosynthetically active light energy	*Echinacea purpurea*

	High salinity (250 mM NaCl)		*Spinacia oleracea*
Carotenoid pigment content	Moderate salt stress (100 mM NaCl)	Blue light harvesting for photosynthesis, photoprotection by heat dissipation upon light excess	*Eruca sativa,* *Lactuca sativa*
Chlorophyll-*a*/*b* ratio	Moderate and severe salt stress Cadmium toxicity (0.05-0.5 mM)	Extent of the light-harvesting pigment complexes around photosystems, acclimation to low and high light intensities	*Lactuca sativa*, various cultivars *Scenedesmus acuminatus*
Chlorophylls / carotenoids ratio	Chromium (6+) ions (5 µM) Moderate salt stress The herbicide diuron Dehydration / rehydration of leaves	Protection against photooxidative damages, photosynthetic acclimation to extreme light regimes	*Scenedesmus opoliensis* *Eruca sativa* *Lemna minor* *Xerophyta scabrida*
Final germination percentage	1 µM triacontanol (bioactive wax constituent)	Germination of seeds, seedling vegetative development	*Brassica napus, Spinacia oleracea*
Germination index and coefficient of velocity of germination	Allelopathic secretions of other plant species developing in the vicinity	Germination of seeds, growth of seedlings	*Brassica juncea*
Synchrony of germination	Low temperature and water shortage	Vitality and variability of seedling stands upon germination	*Brassica napus, Brassica juncea*
Peroxidation of unsaturated fatty acids in membrane lipids	The herbicide gluphosinate (10 µM) Prolonged salt stress	Redox homeostasis of plant cell compartments, integrity and selective permeability of biomembranes, antioxidative capacity	*Scenedesmus opoliensis* *Spinacia oleracea*

Free proline content	Short-term high salinity	Osmoregulation during acclimation to drought, defense against osmotic stress	*Lactuca sativa*
Vitamin C content, reduced ascorbate / oxidized dehydro-ascorbate ratio	High photon flux density (intense light) High salinity of the water solution	Protection against oxidative stress and photooxidation, annihilation of hydrogen peroxide	*Lactuca sativa* *Lemna minor*
Glutathione content, reduced to oxidized glutathione ratio	Long-lasting salt stress	Redox homeostasis, antioxidative defense, sequestration of heavy metals and organic xenobiotics	*Lactuca sativa, Lemna minor*
Phenoloid content	High salinity	Biosynthesis of secondary metabolites through the shikimate and malonate pathways, secondary antioxidative protection	*Eruca sativa, Lactuca sativa*
Ascorbate peroxidase activity	High photon flux density Salt stress The herbicide methylviologen	Antioxidative protection through elimination of hydrogen peroxide excess	*Lactuca sativa* *Lemna minor* *Scenedesmus opoliensis*
Potential quantum yield of photochemical reactions in photosystem II (Fv/Fm ratio)	Water-soluble heavy metals, organochlorinated pesticides, aromatic hydrocarbons Desiccation and rehydration of leaves	Photochemical conversion of the absorbed photon energy in the light reactions of photosynthesis	*Phaseolus vulgaris* *Xerophyta scabrida*
The Fv/Fo ratio of the induced chlorophyll fluorescence	Heavy metal stress and organic contaminants of water	Maximal light use efficiency in the photochemical reactions of PSII	*Phaseolus vulgaris*
Effective quantum efficiency of photosystem II (Φ_{PSII})	Salinity stress Excessive photon flux density	Photosynthetic conversion of light into chemical energy that will be used in carbon assimilation	*Spinacia oleracea* *Pisum sativum, Lactuca sativa*

Ground chlorophyll fluorescence in dark-adapted leaves (Fo)	Protonophores belonging to the acridones (organic xenobiotics) The herbicide glufosinate	The functional organization, the light-harvesting capacity and the resonative energy transfer efficacy of the photosynthetic pigment antennae	*Pisum sativum* *Scenedesmus opoliensis*
Induced maximal chlorophyll fluorescence (Fm)	Antifungal extract of celandine Excessive photon flux density (1200 μM m^{-2} s^{-1})	Photosynthetic electron transport rate on the acceptor side of PSII, redox state of quinone acceptors	*Tulipa gesneriana* *Pisum sativum*
Non-photochemical quenching of chlorophyll fluorescence	50 μM cadmium Combined toxicity of 5-50 μM of chromium(6+) and nickel(2+)	Protection against photoinhibition and photooxidative damage by thermal dissipation of the unused light energy in photosynthesis	*Tetradesmus obliquus* *Scenedesmus acuminatus*
Relative chlorophyll fluorescence decrease (Rfd) or the vitality index of the photosynthetic apparatus	Low temperature (5 °C) Copper toxicity (10-100 μM)	Overall functionality of the thylakoidal photosynthetic apparatus in the photochemical conversion of light energy	*Lactuca sativa* *Lemna minor*

With respect to the plans for professional evolution and career development of the candidate, it can be stated that during the last few years research topics concerning physiological and biochemical characterization of plants' tolerance to certain environmental stress factors have been combined with experiments that aimed to reveal the usefulness of priming treatments in the moderation of harmful effects of abiotic stressors, as well as with the study of how mild stresses and bioactive compounds with antistress action may enhance the production of health-promoting metabolites in crop plants. Another scientific preoccupation in the field of plant stress physiology refers to a better knowledge of the mechanisms of cross-tolerance upon the simultaneous action of different stress factors. And because phenotypic modifications occurring during priming, hardening, acclimation and conditioning of plants are often associated with epigenetic changes, a new and promising direction in continuing the investigation of plant stress responses may be the study of intricate processes that lead to the development of somatic, intergenerational and transgenerational stress memory. The professional evolution in the above-mentioned directions of the research activity has to be corroborated with the academic career development based on coordination of research teams, including students, involved in new research projects granted by national or international competition, in collaboration with other professionals from all round the world who have expertise and vast experience in these fields of direct practical interest for ensuring the global food security under the present climate challenges.

The personal capability of the candidate to organize and manage teaching activities relies on an experience of thirty-three years in the higher education, conducting lectures and laboratory activities mainly in plant physiology, ecophysiology of plants, structural botany, environmental stress physiology of plants. Being the manager of the master specializations in protection and utilization of plant resources and in medical biology at the Faculty of Biology of the Babeş-Bolyai University, conducting over fifty B. Sc. diploma works and M. Sc. dissertations, being the director or partner coordinator of six research projects (most of them in topics of abiotic stress reactions of plants and biomonitoring environmental pollution through plant physiological changes) and member of further thirteen research project teams, it became possible to gain a solid experience in managing research groups, in facilitating communication between specialists, in conducting and coordinating complex research activities. The dissemination of original results, which also ensures a national and international visibility of the professional achievements, was performed by participation to at least eighty scientific conferences, by editorial activities for several publication, by an active participation in the leadership of an academic committee, by affiliation to several scientific organizations, by publication of almost one hundred research and review articles, as well as by writing ten scientific books and textbooks, and three book chapters. Development of professional relations with other research teams dealing with plant stress physiology and related fields is also a priority of the candidate's academic career development.

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