

The Screening of the Analytical Biochemistry Researches Involved In Plant Response to Stress Conditions

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“Every species of plant is a law unto itself. Henry A. Gleason, The Individualistic Concept of the Plant Association, Bulletin of the Torrey Botanical Club, 1926, 53, 26”. Considering that the global population is expected to increase, the removal of the causes that lead to the decrease of the vegetable production is vital. One of the main causes that diminish the quality of vegetables is represented by the abiotic stress factors. The analytical biochemistry researches were usually focused on the support of the reduction activity of these factors (improvement) [1], by studying the biochemical processes involved in the plant reaction at stress conditions, the development of methods and selection criteria with high efficiency in order to identify genetic differences [2], to improve the resistance to low and high temperatures, drought, humidity excess, salinity and soil acidity. In order to achieve this goal, it is required the analytical biochemistry researches to obtain conclusive information regarding the fundamental processes of the plant life by:

- study of the physiological and biochemical processes involved in the plant response to stress conditions
- studies regarding the growth and development of the plants, mineral nutrition, productivity
- studies regarding the plants quality
- use of the mathematical models in agriculture
- Estimation by teledetection of the applied agrotechnical measures efficiency.

Resistance at Low Temperature

The analytical biochemistry researches that were made in this field, took into account the elucidation of some theoretical problems, intended to enrich the knowledge regarding the plant defense mechanisms against low negative temperatures and the concern to find new methods of investigation, cheaper, faster and effective, necessary for the characterization of the new genotypes, in terms of resistance to winter. The temperature and the duration of sunshine during quenching are decisive in this regard and maintaining (keeping) the degree of the resistance is determined by the evolution of the temperatures, during the winter in relation to the genetic constitution of the plant [3]. It was emphasized that the resistance capacity at frost of the young leaves is higher than the mature leaves [4] and the sensitive of the root system is higher compared to the strain [5]. Using histo-cyto-biochemical methods, it was revealed that the sensitive of the growth peak is higher than the fundamental embryonic tissue, indicating the increased potential for recovery of the plants characteristic of this area [6]. In order to explain the resistance mechanisms to frost, it has initiated a series of biochemical researches on the dynamics of the hydrosoluble substances, amino acid (proline), phytohormones (abscisic acid (ABA)) cellular enzymatic processes (concentration of sugars, ascorbic acid, catalase activity, the peroxidase, etc.).

The results revealed the importance of the decreased metabolism during the winter, which corresponds to a deep state of sleep [7]. It was established especially in leaves, the role and the importance

of the accumulation of the specific metabolites (proline) and the phytohormones, and the direct relationship with the degree of frost resistance [8]. The cold resistance plants synthesize faster and in larger quantities, proline during the quenching process compared to the sensitive plants; the abscisic acid has a mediator role in starting the metabolic processes that lead to the increase of the plant resistance to cold [9]. The study of the correlations between the osmotic potential and the proline content, on the one hand, and the dry substances accumulation during the quenching process of the plants, on the other hand, has shown a negative relationship between these parameters: the decrease of the osmotic potential is beneficial for the increase of the plants resistance to frost [10].

Another cause of the injuries produced by cold is the formation of oxygen free radicals (ROS). The plants have specific enzymes (catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), glutathione S-transferase (GST)), known as antioxidant enzymes, in order to annihilate the negative effect of ROS. Besides the destructive effect produced by ROS in plants also, these have a critical role in the transduction signal. So, it is important to maintain a balance between formed ROS and the removed/annihilated radicals. The resistant plants to oxidative stress generated by ROS, are resistant to frost, drought and salinity. The temperatures decreased to below 0°C leads to extracellular freezing. Cell membrane forms a barrier against the growing of the ice crystals, causing water movement outside the cell (ice has a lower chemical potential compared to the water). The stress generated by the cellular dehydration caused by the frost is severe, and plant cells will lose most part of the active water (osmotic point of view). Frost tolerance must include the tolerance to severe dehydration, induced by freezing.

Cell membrane is recognized as the primary site of injury by frost, so that the measuring method of its damage has been improved for rapid identification of the plants with high stability of cell membrane at low temperatures [11]. The large thermic fluctuations from day to night cause disturbances in the growth and the development of the plants, with a negative action on the male generative organs, which arise before the fertilization, disturbing the formation and filling processes of the grain (if the action of the temperature fluctuations occurs after fertilization).

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Resistance to Heat (heat stress) and Drought (hydric stress)

The lack of water in soil manifests its stressful action on plants which are in the phase of formation of straw. The drought effects are irreversible and influence the growth processes, photosynthesis, differentiation and formation of generative organs; leading to a significant decrease of the production [12].

The drought resistant plants are characterized by a low rate of dehydration of leaves with a high percentage of chlorophyll, ascorbic acid, total reducing sugars, dry substance, bound water [13], reduced deterioration of the photosynthetic apparatus and tilacoidal membranes [14], low cuticular transpiration [15]. Thus:

- In the early stages of vegetation, photosynthetic apparatus is less affected by moderate drought conditions, when water reserves from soil, although reduced, allow the triggering of the adaptation mechanisms and avoid the tissue dehydration.
- In severe drought conditions, the photosynthetic function decreases significantly, mostly by the deterioration of the photosynthetic apparatus and tilacoidal membranes; only in accentuated conditions of soil and plants, dehydration can be observed with differentiated reactions of plants.
- In the early stages of vegetation, the high temperature represents a stronger stress factor than the lack of water, with direct and immediate effect on the photosynthetic apparatus; the heat determines the degradation of chlorophylls in the chlorophyll antenna, especially of chlorophyll "a" and photo inhibition of the photosynthetic apparatus.
- The changes from the internal membrane of the chloroplast, under the influence of drought and high temperatures, can be measured by analysis of the chlorophyll fluorescence in the primary leaf.
- The fluorescence indicators of the chlorophyll; the quenching index was correlated with the plant response under natural conditions of drought and heat, the dryness degree of the foliar apparatus and the biomass at harvest and production.
- The high temperature and the osmotic stress as the unfavourable environmental factors that cause major damages of the cell membrane, the ions efflux from cells and the tissues death.
- The conductometric assay applied to the seedlings and the mature plants revealed the role of the cell membrane stability in the plant growth, the formation of the fertile brothers (plant tillering), the grain filling in order to achieve high grain production under hydric and thermal stress (overheat) conditions.

As in the case of the frost stress and in drought conditions, there can be the oxidative degradation of products at cellular level that leads to "oxidative stress". The hydric and temperature stress increase the production of H_2O_2 . The studies regarding the plant resistance at drought show the induction of the protection of enzymatic systems against the oxidative stress, at cellular level [16].

The correlation between proline accumulation and drought resistance was estimated, based on the production in stress conditions and is difficult to determine because of a big number of biochemical, physiological and differentiation stages. The proline accumulation can have an advantage at the cellular level in the short term, but it is not a unique criterion for selection and practically usable.

The drought tolerance is a complex phenomenon and requires multiple investigations (e.g. the quantification of such indicators as cuticular transpiration, proline content, electrical resistance of the leaf and cell membrane stability, which complement each other and show different resistance mechanisms). The tolerance to high temperatures (heat) can be quantified by association with the cellular components (closely related to the cell membranes). The cell membrane damage by the heat can be estimated by conductometric measurements of the electrolyte leakage from cells.

The analytical biochemistry contribution in order to identify the plants with high oleic acid were then used to obtain different types of plants with high levels of oleic acid ("high oleic") [17]. Abscisic acid (ABA) and glutathione reductase (GR) are two compounds with implications in plant resistance to drought. It made researches regarding the effects of hydric stress manifested as humidity excess from soil to plants, which emphasized the negative role of some factors that overlap to the humidity excess (e.g. the high temperature of the air and the water that stagnates on the soil surface, the temperature fluctuations, the low brightness, the inadequate level of the plants quenching).

Resistance to Salinity, Acidity, Loss and Illness

The understanding of the resistance/tolerance mechanisms to salinity is important, both for improved plant and the wild ones, which often offers larger amplitude of the tolerance to this type of stress. The salinity interferes with protein synthesis inhibition, phenomenon evidenced by incorporation of amino acids in proteins. This inhibition is characterized by an increased concentration of free amino acids, especially proline [18].

The analytical biochemistry researches that were made in order to identify some aspects of the plant biochemistry, in which the hydric and aluminium stress have acted separately or combined, have determined that: plants have a wide range in the resistance to drought, but are relatively similar regarding the tolerance at high content of aluminium ions. The hydric stress reduced the accumulation of biomass when the both factors acted alone and together, the high content of aluminium (Al^{3+}) in acid soil affects a higher proportion of the root system than the aerial root system, resulting to a lowering of the weight and volume of roots but also a superficial rooting and an increase of plant susceptibility to drought. The reduction of biomass accumulation compared to the aerial root system suggests that the first reaction of response to soil acidity is the inhibition of the growth of roots.

The reduction of the leaf area and the chlorophyll content manifested progressively with plant age and the occurrence of the nutrition disturbances (similar symptoms to those produced by phosphorus deficiency indicating the absorption inhibition, and the use of phosphorus in the presence of aluminium). The increase of the aluminium concentration with the increase of the action time of this stress factor caused a reduction of the relative rate of the root system growth. The citric acid exudation is a specific response of plants to acid stress [19]. The plant response to soil acidity was the growth inhibition of the main root leading to apparent changes of photosynthesis, but also to the increasing of peroxidase enzyme activity. Plants have internal detoxification mechanisms resulting in the increased activity of peroxidase, an enzyme which acts as garbage collector of the cells.

"In a very real sense, we're all made out of sunlight.

Sunlight radiating heat, visible light, and ultraviolet light is the source of almost all life on Earth. Everything you see alive around you is there because a plant somewhere was able to capture sunlight and

store it. All animals live from these plants, whether directly (as with herbivores) or indirectly (as with carnivores, which eat the herbivores). This is true of mammals, insects, birds, amphibians, reptiles, and bacteria...everything living. Every life-form on the surface of this planet is here because a plant was able to gather sunlight and store it, and something else was able to eat that plant and take that sunlight energy in, to power its body” -Thom Hartmann.

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