Physiological and Molecular Aspects of Sugar Beet Tolerance to Drought

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Definition?

A period of dry weather, enough long to be injurious to crops.
Amount and distribution of precipitation required for development of sugar beet

- Required amount of precipitation for successful production is 600 mm per year.
- During winter around 230 mm and during the vegetation (from April to October) approximately 370 mm of precipitate.
- Water requirement of plant, during the period of vegetation, depends on precipitation.
• The water loss due to evaporation is most intensive from June to August when the temperatures are high and the air is dry. The average potential ET for period of 30 years is 576 mm (528 and 625 mm due to weather conditions).

• Approximately 10-20% of total water requirement of sugar beet is fulfilled from the soil water reserves and the rest is obtained by precipitation and irrigation.

• The amount of water lost by transpiration is 392 mm in average (198-542 mm).

• The average precipitation during vegetation (Apr-Sept) is 359 mm (138-521).
• Amount and distribution of precipitation, in combination with the light and amount of heat mostly determine quality and yield of sugar beet.

• On the territory of Serbia, it is common that the lack of soil water, typical for summer months, sometimes occurs during moderately rained years.

• Lack of soil moisture outcomes 100-200 mm per year, but rarely exceeds 300 mm per year.

• Another problem is that a very small percentage of irrigation-suitable agricultural land is intensively irrigated.
The impact of water deficiency on sugar beet production

• Water shortage during vegetation is frequent and significant issue in agricultural production.

• Possible solution to this problem is selection of genotypes which do not show decreased yield under economically acceptable level, in the presence of water shortage.

• Great challenge in the process of genotype selection is to choose the convenient plant idiotype for the present agroecological conditions.

• Water deficiency has complex impact on plant physiology.
Impact on plant physiological processes

- First indicators of water deficiency in plants are the loss of turgor pressure and stomatal closure.
- Photosynthesis is also highly dependable on plant water supply.
- Disruption of water flow causes decrease in water content in assimilation tissue which leads to photosynthetic depression.
- Soil moisture, as well as relative air humidity determines photosynthetic intensity.
- A decrease in chloroplast size, an increase in stomatal density and disruption of tilacoid membrane structure, were reported as consequences of water deficit.
- Besides decrease in tissue water content, water shortage may cause synthesis of specific compounds in the roots, during the early growth phase.
- Roots are very significant sensors of soil changes (not only in terms of water, but also texture changes), which alert the aboveground tissues by “chemical drought signals” which are transported to leaves.
- These signals mostly refer to plant hormones such as abscisic acid (ABA).
Sugar beet tolerance to water deficiency

• Adaptation of plant metabolism on stress conditions is species specific.
• Plants more tolerant to drought have longer root system with bigger absorptive area, better developed photosynthetic parenhyma, thicker cuticle, smaller leaf area and number of stomata per leaf area and higher density of conductive elements.
• They also possess highly expandable protoplasm, higher content of bound water and osmolytes, enhanced accumulation of ABA, free proline and alanine.
• The following indicators point out to higher phenotypic tolerance of sugar beet to water shortage: more shiny leaves, higher turgor pressure of petiole and more sensitive leaves to expansion.
• Stress occurrence during early stages of growth and development may adversely affect sugar beet root growth which may result in yield loss by 46%.

• In addition, later stress occurrence may cause decreased leaf area and also number of leaves and by that, the efficiency in light usage becomes decreased.

• Water deficiency significantly increases concentrations of potassium and sodium which disturb sugar extraction from roots.

• Plant response to water stress can partially be explained by disorders in mineral nutrition.
• Water deficiency actually may retard or even stop ion assimilation which results in perturbation in ion ratios in specific tissues.

• This trend is manifested through ion deficiency symptoms in plants.

• The adverse effect of water stress in later phenophases is less pronounced, since plants already developed root system and canopy which completely covers the soil.

• Well-developed root system increases efficiency in water extraction and usage, which results in higher tolerance to water deficiency.

• First signs of water stress are usually seen on leaves.

• Minor drop in leaf water potential may cause significant decrease of total leaf area and the low water potential enhances emergence of new leaves and accelerates senescence of old leaves.
• Drought stress results in stomatal closure, limits the transpiration which increases leaf temperature.

• Both lower stomatal density and heat stress decrease photosynthetic outcome.

• Sugar beet leaves have higher number of smaller stomata on their abaxial side.

• Higher density and smaller size of stomata is a form of adaptation to drought, because it allows plants to be more efficient in regulation of water transport and transpiration.

• Varieties more efficient in tolerating lack of water are proven to have decreased stomatal density (70-150 stomata/mm²).

• During drought, when negative turgor pressure in guard cells generates, small epidermal cells with tightened cell walls increase plant resistance towards water stress.

• Response of sugar beet genotypes to drought may also be affected by percentage of adaxial and abaxial epidermis and palisade tissue thickness.
Chemical response of sugar beet to drought stress

• Plants also osmotically adapt to drought.
• Exposure to water deficiency results in accumulation of osmolytes such as betaine, proline and fructans. These substances often accumulate in form of compatible solutes in plants (compounds which do not take part in chemical reactions in plants, but affect cell water potential), which generate expression of genes encoding relevant enzymes.
• Osmolyte production, as well as change in osmotic pressure, may increase sugar beet tolerance to abiotic stress.
• Proline and glycine betaine help the preservation of cell, which makes them suitable for further investigation with purpose of increase stress tolerance of many species including sugar beet. They are involved in maintenance of cell turgor and osmotic balance but also in protection of cell structure from stress.
• However, it still remains unclear whether the plants, which accumulate osmolytes, better tolerate lack of water or not.
Proline accumulation

• Free proline is a key metabolite which accumulates in sugar beet exposed to drought.

• Changes of the free proline concentrations in tissues is an indicator of another kinds of stress such as temperature, environmental pollution, and misbalanced nutrition.

• The same factors may affect glucose accumulation and yield. In some cases stress conditions may increase sugar beet root quality and potential of recovery if plants were not highly damaged by water deficiency.
• Higher nitrogen supply also increases proline content, may increases leaf area index (LAI) and drought stress impact.

• Positive and significant correlation among proline and glucose content in sugar beet root indicates the relationship between the response to stress, carbohydrates and proline and glucose accumulation ratio.

• Presence of compounds such as proline and glucose adversely affect sugar crystallization and lead to the formation of colored components, thus reducing industrial quality of beet roots.
• Proline accumulated in sugar beet root, as a nitrogen compound, reduces the quality of roots. Both, the stress and an excess of nitrogen lead to the mobilization of accumulated carbohydrates which are the source of energy essential for adaptation to the stress conditions.

• Moreover, chemicals containing nitrogen (e.g. proline), reduce the yield of sucrose, and the quality of the roots.

• The importance of the accumulation of proline in osmotic adjustment is still debatable and varies from species to species.
• The highest proline accumulation is observed at the end of beet root growth.
• Correlation between drought and proline content suggests, however, that alteration in proline concentration is useful stress indicator in sugar beet.
• Proline may act as a signal molecule which alters mitochondrial function, affects cell division and gene expression.
• This role of proline may be very significant for plant recovery when favorable conditions are regained.
The use of plant biotechnology to increase tolerance to water deficiency

• Basic need for sustainable food production directed research programs towards improving traits of crops despite the size and complexity of their genome.

• Plant biotechnology is a process in which the use of molecular and cytological techniques help to increase the productivity of the plants, to improve the quality of plant products, to prevent the damage caused under the influence of various biotic and abiotic stresses.
• Plant breeding relying on the employment of molecular markers (MAS - Marker Assisted Selection) is one of promising techniques to improve crop resilience.

• A prerequisite for the success of MAS is defining the genes which regulate traits of interest and to test relationships between potential markers and those traits.

• Only when this link is defined, i.e. when the marker is physically located in the vicinity of or even within the gene of interest, it is possible to use it efficiently in breeding.

• In sugar beet, development of breeding programs aimed to increase drought tolerance is further complicated by the fact that several types of abiotic stresses often occur at the same time during the growing season, and approach which involves a manipulation of a group of genes for tolerance to drought seems necessary to solve this complex problem.
• In an era of rapid progress in the identification and characterization of complete segments of plant genome, proteins, transcripts, metabolites, as well as their interactions in a biological system, new discoveries will lead to better understanding and possibly to manipulation of physiological responses to water deficit.

• Evaluation of the relative contribution of genes conferring tolerance to the dehydration and elimination of those which do not affect the tolerance to stress is a major challenge.

• Although the yield is the basic goal of the breeders, it is very difficult to accurately predict the possibility of water utilization and identify candidate genes for further cloning.
• With the rapid development of genomic technology and the suitable statistical methods, there is an increased interest in the use of mapping strategies for the identification of genes encoding quantitative traits which have agricultural or evolutionary significance.

• Another major challenge is how to apply knowledge to improve crop tolerance to stress conditions.

• There is a problem between high yield and tolerance to stress since very often genotypes with higher stress tolerance have lower yield under optimal conditions.

http://www.telegraph.co.uk/foodanddrink/10145478/Germaine-Greer-Britain-doesnt-need-beet-sugar.html
• On the cellular level plant adaptation to stress includes regulation of the beginning of protein synthesis, an increase in antioxidant level, transient increase of the concentrations of ABA, the reduction of the energy consumption ways, as well as accumulation of the solution, and protective protein.

• All of these changes at the cellular level are of great importance for the maintenance of homeostasis after ion imbalance caused by abiotic stress.

• The deficit of water causes the synthesis and accumulation of ABA in plant cells and the genes corresponding to this has been defined.
• In order to achieve a combination of high yield and tolerance to stress in one variety, it is necessary to establish a connection of development of individual characteristics and mutual reactions which can be achieved only through cooperation among molecular biologists, physiologists and breeders.

• It is necessary to assess relationship between different morphological, anatomical, physiological and biochemical traits of sugar beet tissues in different phases of their growth and development during different periods of water shortage, in order to categorize genotypes with respect to their tolerance to drought.
Sugar beet experiment

Plant material

The study involved eleven genotypes (marked from 1 to 11) of sugar beet (*Beta vulgaris* ssp. *vulgaris*, L.) differing in levels of drought tolerance, according to observation test conducted in the field.

According to this test, genotypes were divided into 3 groups:

1) sensitive genotypes: 2, 5, 6 and 8,
2) moderately tolerant: 3, 7, 9, 11
3) tolerant: 1, 4 and 10.
Experiment was conducted in three stages:

1) Under semi-controlled conditions in greenhouse
2) In *in vitro* conditions of tissue culture
3) Gene expression analyses of water regime responsible genes in leaves (plants from the greenhouse experiment)
1) Experiment under semi-controlled conditions in greenhouse

- Sugar beet seeds were sown in growth substrate Potgrond H (Klasmmann), mixed with river sand (17.5:1) in plastic pots (31x37x13 cm). A single pot contained 12 plants. During 90-day period soil moisture was kept at 80% field capacity. Plant watering was conducted on the basis of evapo-transpiration. When the plants were at the 6-12 leaves stage they were exposed to water deficit by cessation of watering, while control plants were watered. Five days later, molecular and physiological analyses were done.

- % of dry matter
- activity of photosynthetic apparatus was assessed by monitoring of $F_0$ (initial), $F_m$ (maximal), $F_v$ (variable), $F_v/F_m$ using plant stress meter (PSM, BioMonitor S.C.I. AB)
- free proline concentration was measured in the both: *in vitro* and *in vivo* conditions.
- concentration of chloroplast pigments was determined spectrophotometrically.
- leaf area (LA) was measured by automatic leaf area meter LI-3000 (LI-COR, USA).
2) Experiment in tissue culture

• In this experiment MS basic substrate was used with 0.3 mg/l BA (benzyladenine) and 0.01 mg/l GA₃ (gibberellic acid). In order to obtain sufficient number of axillary shoots (64), equal in size, subcultivation was done every three weeks. Lack of water was caused by addition of polyethylene glycol to the substrate.
• Obtained shoots were set on a substrate for micropropagation with 0%, 3% and 5% of polyethylene glycol (PEG 6000, Duchefa, Netherlands).
• Plants were cultivated on this substrate for four weeks and afterwards fresh weight of shoots, as well as dry matter and free proline content were determined.
• The temperature during the experiment in air conditioning chamber was 21-23 °C, with photoperiod of 16 hours of light and 8 hours of dark.
3) Gene expression analyses of water regime responsible genes in leaves (plants grew in the first experiment)

• The changes in gene expression were analyzed in the leaves of the sugar beet plants grown in the greenhouse experiment. Candidate genes were selected from the previous studies. For thirteen candidate genes which are considered to be involved in osmotic and salt stress responses, primers were constructed and used to screen for polymorphisms at the DNA and gene expression levels. Ten selected candidate genes were homologous probes (BI543470, BI096135, AW697770, BI543640, BG932913, BI096146, BQ060651, BF011094, BI096078, BF011254), and heterologous probes from maize (X15290), alfalfa (BI543243) and carrot (BI073246). Samples for DNA/RNA analysis (leaves) were taken 5 days after the last watering (experiment 1) and used for DNA/mRNA extractions. mRNA was used to synthesize cDNA, and this cDNA was template in further PCR reactions.
Results

Climatic conditions in our region suggest the need for research which has the potential to enhance selection of genotypes more tolerant to drought.

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1) Experiment under semi-controlled conditions in greenhouse

• Sugar beet genotypes in semi controlled conditions showed different reactions to 5-day water deficiency. As expected, decline in turgor was observed in all genotypes. Number of leaves was significantly different between treatments and respective controls. Concentrations of photosynthetic pigments and leaf area varied between genotypes and standard normal distribution wasn't observed here. Therefore, the data were subjected to Johnson's data transformation which proved to be very effective. This procedure allowed assessing differences in concentrations of photosynthetic pigments between different genotypes.
Figure 1. Genotype separation on the basis of pigment concentration and leaf area for variables normalized according to Johnson's data transformation (jlarea-leaf area; jcar-carotenoids; jca-chlorophyll a; jcb-chlorophyll b; jcab-chlorophyll a+b)
- Secession in water supply caused water loss from plant tissues within both sensitive and tolerant genotypes.

**Figure 2.** The separation of the sugar beet genotypes based on experiments in greenhouse with a highlighting treatment (control, drought) for a variable normalized by Johnson's transformation (jrwc-relative water content; jpcdw-dry weight; jdwproli-free proline; jpcdwleaf-leaf dry matter; jpcdwstem-stem dry matter; jpcdwroot-root dry matter)
Figure 3. Effects of drought stress on growth traits and proline production of greenhouse grown plants of sugar beet genotypes (1 to 11) from three classes of visually field-assessed drought tolerance (DT). Observed values of three replicates (circles, ten plants each), average genotype positions (numbered grey lines) and class means with 95% confidence intervals (crossbars).

Plants subjected to stress conditions had in average:
- 3 leaves less
- 4% higher % of DM
- 7 times higher proline content
Figure 4. Maximal (Fm) and variable (Fv) chlorophyll fluorescence and their ratio (Fv/Fm) in sugar beet genotypes grouped according to their field-assessed drought tolerance (Ctrl-control; Drought; DT-drought tolerance)
• Plant development may be inhibited in different ways in field conditions. It may be affected by interactions among drought and other ecological stresses, precipitation and temperature availability as well as interactions with different microorganisms.

• On the contrary, semi-controlled conditions may only eliminate interference of other factors with plant development.

• Therefore, it is necessary to compare results obtained in the greenhouse with those obtained in the field.
2) Experiment in tissue culture (*In vitro*)

**Figure 5.** PEG effect on growth traits and free proline concentration of plants cultivated in in tissue culture (*Putnik-Delić et al., 2013*)
Proline accumulation under stress conditions increased under treatments in both experiments. In tissue culture, it was six times increased and in greenhouse sixteen times with respect to corresponding controls.

**Figure 6.** Water deficit effect on dry weight, water content and free proline concentration in greenhouse and in tissue culture experiment

3) Analyses of changes in expression of genes involved in reactions to water stress (plants from greenhouse experiment)

Changes in the expression of 13 candidates genes in 11 different sugar beet genotypes were followed in leaves of plants grown in the greenhouse. Expression pattern corresponding to BI543243 differed in plants exposed to drought in comparison with corresponding controls in genotypes 1, 10 and 11. Therefore it may serve to develop molecular marker useful to differentiate genotypes with respect to drought.

**Figure 7.** Expression pattern of gene corresponding to BI543243 in sugar beet leaves (c, template cDNA deriving from control plants; d, - template cDNA deriving from plants exposed to drought). Amplification on genomic DNA served as additional control (g). M, 100 bp DNA ladder size marker.
Conclusion

Tolerance to drought is very complex. Experiments in three different environments (tissue culture, greenhouse and field) with eleven genotypes, revealed that it is not easy to find single criteria for classification with respect to drought tolerance.

However, the results suggest that free proline accumulation may be used as a reliable parameter.

Therefore, similar fast tests, conducted with young plants and possibly aided by the use of molecular markers, can be useful for estimation of breeding material with respect to tolerance to water deficiency, which will significantly enhance sugar beet breeding for expected future changes in climate.
Thank you for your attention!

Acknowledgement

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