SIGNIFICANCE OF THE OSMOTIC ADJUSTMENT OF SOYBEAN (*GLYCINE MAX* L.) SELECTED GENOTYPES DURING INCREASING DROUGHT CONDITIONS

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Abstract: *In soybean [Glycine max (L.) Merr.], drought is the greatest threat to profitability and too often a crop with great promise ends up with only fair or poor yields because of dry weather. Nitrogen fixation activity by soybean (*Glycine max *(L.) Merr.) nodules has been shown to be especially sensitive to soil dehydration.. The aim of this paper is to characterize water stress in inoculated soybean genotypes (with Nitrazon inoculant) using some physiological characteristics. In the two growing seasons of experiments with three genetic resources of soybean (Drina, Maverick, Nigra) was launched. Drought stress was secured by an irrigation interruption for a 9-day period in the mentioned growth stage. The influence of water stress was tested on some physiological parameters - stomatal closure, relative water content in leaf (RWC), free proline content and osmotic adjustment. Drought had a negative impact on the tested parameters. The objective of this research was to investigate possible genetic variation in the sensitivity of soybean cultivars for nitrogen fixation rates in response to soil drying. During the water stress was monitored the proline accumulation. Proline serves during the osmotic stress as a mediator of osmotic adjustment, a sink of energy and a stress signal. The higher content of free proline reached genotype Maverick with a level of 16.95 μmol.g^{-1}Fresh Weight (according to the calculation on 100 % RWC) in dehydrated plants with the inoculant usage, what is related to the more notable RWC content decrease in the leaves. Among tested genotypes showed the higher capacity for osmotic adjustment during increasing drought conditions all genotypes with Nitrazon inoculation. The results indicate that physiological characteristics, such as relative water content, proline content and osmotic adjustment, are good indicators of water stress in soybean, proline content being a particularly reliable parameter corresponding to the actual water stress of plants.*

Key words*: drought, osmotic adjustment, soybean, proline*

INTRODUCTION

Drought is considered the major abiotic stress in many parts of the world and is responsible for heavy production losses in food legumes (MALHOTRA *ET AL.*, 2004). Some losses are due to intermittent drought during the vegetative phase while others are due to terminal drought during reproductive development (SERRAJ *ET AL.*, 2004). The severity of drought stress is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands of the atmosphere and moisture storing capacity of the soils. Therefore, characterization of the drought pattern of the target environment is an important step in designing strategies to alleviate drought stress (SUBBARAO *ET AL.*, 1995). Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where growing season is short and terminal drought stress predominates. Therefore, the success of increasing legume production in drier regions prone to terminal drought largely depends on the development of short season varieties that enable the crop to escape severe soil-water deficits. Avoidance is related to the maintenance o high tissue water potential and consists of mechanisms that both reduce water loss from plants, due to stomatal control of transpiration, and also maintain water uptake, through an extensive and prolific root system. Dehydration tolerance refers to the ability of the plants to withstand low tissue water potential. The effect of internal water status on plant functions provides clues concerning mechanisms of drought tolerance (POKLUDA *ET AL.*, 2010). Among the plant oils, soy has special features. It has a wide and varied range of applications. Soybean oil is one of the main components of edible oil market (SANCHOLI, 2015). Soybean as a moisture-demanding crop plant is reflecting a significant drop of seed crop during a season with the unbalanced rainfall and a lack of moisture. This crop drop is discouraging a plenty of croppers who have tried its growing. In spite of this disadvantage, it is undoubtedly a kind of crop with an important position in a seeding process which it gained thanks to a fact that it represents a foregoing crop plant as it leaves a huge amount of mineral nitrogen in a soil (RACZ, 2003). A choice of appropriate variety and rationally chosen nutrition and fertilization connected with a seed corn inoculation represent the essential steps of successful soy growing. Soybean is a moisture-demanding crop plant. It has got a high level of transpiration coefficient and for one gram of dry basis production it is needed 600 – 1000 g of water. According to the calculations soybean needs for germinating 120 – 140 % of water per a seed weight. There is a relatively high moisture demand and this is very often a negative cause why one cannot widen the soybean growing into the potential areas. In the areas with insufficient and inconveniently occurring rainfall is a soybean production very low despite the suitable temperature and water becomes a limiting factor of its growing (RODRIGUEZ, 1993). Plant water potential can be maintained by osmotic adjustment, brought about by the presence of sugars or other compatible solutes. Osmotic adjustment is the plants response to environmental changes related to any stress that could by perceived and results in the induction of dehydration, for example, drought (MIRANSARI *ET AL*. 2014, MIRANSARI, 2016).

MATERIAL AND METHODS

Plant material

In the growing seasons 2014 and 2015 an experiments with three genetic resources of soybean (*GLYCINE MAX* L.) was launched. The genotypes selection was undertaken according to their provenance. Seeds was provided by Gene Bank of the Slovak Republic in Piešťany. Sowing of Drina (HRV), Maverick (USA) and Nigra (SVK) genotypes was carried out in the containers. The sowing consists of several repetitions with Nitrazon inoculant and variants without inoculation, too. Plants of soybean were grown in 15L plastic pots under normal day-light conditions.

Nitrazon Inoculant (by Agrokomp, spol. s r.o., in Modra)

Nitrazon is prepared from stem of bacteria nodule, selected at the Crop Research Institute in Prague. Regarding the selected bacteria it is prepared separately for each crop species of the soybean family (*Fabaceae*). It has a high content of live bacteria. It directly contributes to increase of protein content in grown crops and is beneficial to yield increase and better microbial soil activity.

Figure 1 Genotypes Maverick, Nigra and Drina in the containers

Water stress

At the flowering was simulated water stress. During water stress period were evaluated physiological parameters on 2^{nd}, 4^{th}, 6^{th} and 9^{h} day (the end of dehydration) on the with the Nitrazon inoculation and also on the variants without inoculation comparing to the control fully hydrated plants and variants with dehydration.

Stomatal closure

Stomatal conductances of leaf were measured with a porometer Delta-T-Devices (Cambridge, England).

Free Proline Content

Free proline is being determined by (Bates, 1973) method in the leaf tissue. Approximately 0.5g of leaf FW with sulfosalycilic acid is homogenizing in a mortar with pestle. To this substrate it is being added ninhydrin acid and ice acetic acid. The solution is incubating in water bath by 100 ˚C for 1 hour. After this one hour the reactive mixture is being cooled in an ice bath, toluene is being added and the whole mixture is being stirred for 15-20 seconds. The coloration intensity is spectrometrically measured at 520 nm compared to the clean toluene. Proline concentration is determined from a calibration curve (made from L-proline with 0.1; 0.3; 0.5; 0.7; 1.0 $mol*dm^{-3}$ concentration).

Osmotic adjustment (OA)

For Ψ_S (Osmotic Potential in MPa) was used measuring a psychrometric method (Wescor, USA).

RWC (Relative Water Content in %) value was calculated as follow:

$$RWC = \frac{FW - DW}{SW - DW} * 100\ [\%],$$

where: FW is a Fresh Weight,
DW is a Dry Weight,
SW is a Saturated Weight.

By this method, OA was calculated as the difference in OP between nonstressed (a point measurement on the morning after last irrigation) and stressed leaves both calculated to well-watered state ($\Psi_{S\ 100}$) (Wilson *et al*., 1979). This RWC was chosen to assure sampling at wilting in all cultivars. The $\Psi_{S\ 100}$ was calculated as follows:

$$\Psi_{S100} = \Psi_S *[(RWC - B)/(100 - B)].$$

B- value of 18% was chosen for these calculations, as based on data for rice by Babu *et al.* (1999). A constant value of B was used for both stressed and nonstressed leaves of all cultivars since B did not change with cultivar or dehydration. A constant value of B has also been used by others.

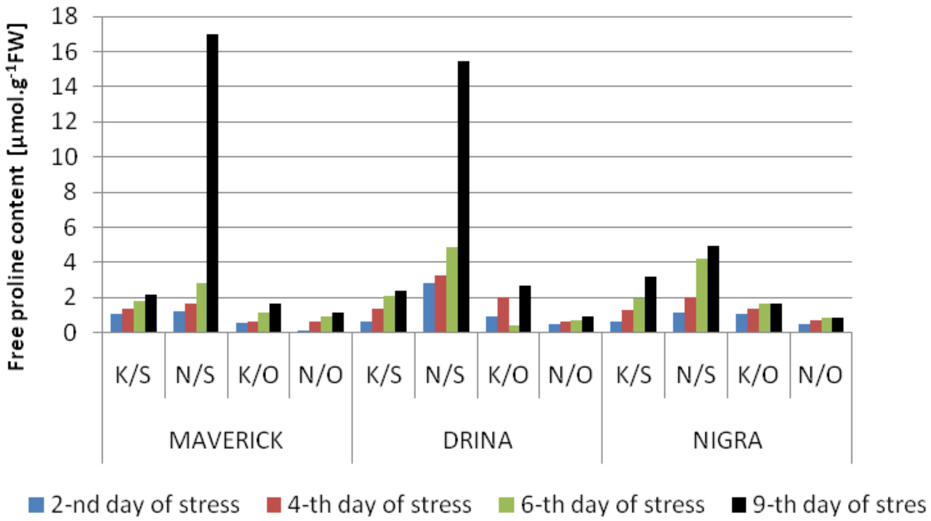
$$OA = \Psi_{S(100)\ stres} - \Psi_{S(100)\ kontrol}$$

RESULTS AND DISCUSSION

Drought stress is defined as a condition in which water available to plants is so low that it is unfavorable for the growth of a plant species. Plants will respond to conditions of drought stress through a number of physiological and developmental changes. Under stressful conditions, the stress factors, or the toxic molecules derived from the stress, attack the most sensitive molecules (primary targets) in cells to impair their function. The damaged targets recover either by repair or replacement via de novo biosynthesis (KRIVOSUDSKÁ, 2016).

The stomatal closure was one of the most rapid reactions induced by water deficit. Stomatal aperture is the dominant factor in the diffusion conductance of leaf surfaces. Stomatal closure during drying in all soybean genotypes was recorded on the 3rd day of stress.

Osmotic adjustment is a specific response to maintain water relations (turgor) under osmotic stress. A range of so-called osmotically active or compatible substances is involved, including soluble sugars, sugar alcohols, proline, $Ca^{2+,}$ K^{+}, and Cl^{-}. Many studies have also pointed to the role of proline as a defense mechanism during osmotic stress, which is perceived as the most limiting factor (JARECKI, 2016). Proline serves during the osmotic stress as a mediator of osmotic adjustment, a stabilizer of subcellular structures, a collector of free radicals, a sink of energy and a stress signal. Many studies showed the positive correlation between the proline accumulation and plant osmotolerance etc. and that the increasing proline level is a stress consequence (TOKIHIKO *ET AL.*, 1999). During water stress was therefore also monitored the proline accumulation, whilst the higher level of free proline was by the dehydrated plants with inoculant usage as follows: Maverick 16.95 $\mu mol.g^{-1}$FW and Drina 15.48 $\mu mol.g^{-1}$FW according to calculations to 100% of RWC. Inland stressed genotype, which kept higher RWC levels on the end of dehydration and therefore also in the accumulation of proline achieved lower values (Nigra 4.95 $\mu mol.g^{-1}$FW according to calculations to 100% of RWC). At which the genotypes without inoculation, exposed to the water stress, level of free proline in leaves was much lower (Figure 2).



N- Nitrazon, S - water stress, K - control, O - without Nitrazon

Figure 2 Free proline content in soybean genotypes leaves depending on dehydration period and seeds inoculation [$\mu mol.g^{-1}$Fresh Weight] for two years

Under water stress, plants naturally try to prevent loss of water by means of various mechanisms, such as pore closing (Chaves *et al*., 2003), synthesis of osmoprotectants–sugars, aminoacid proline and other substances (RAMANJULU *ET AL.*, 2002). It has been found out (CLAUSSEN, 2005) that proline content grows significantly as early as in 14 hours after water stress. The level of proline also depends on light intensity. A negative correlation of proline and leaf water content (LWC) was proved. The relative LWC decrease by 5 % doubled the increase of proline content. SHTEREVA *ET AL.* (2008) mention the possibility to use a ‘proline’ test as an instrument for finding soybean genotypes tolerant to water stress.

Accumulation the level of proline is independent of the water potential or turgor of cells, but not of the osmotic potential. Proline final level depends on the adaptation degree.

Reducing of the osmotic potential in the cell by accumulation of soluble substances is a defining feature of osmotic adjustment. It increases that way the tolerance to dehydration, what contributes to the prolongation of plants survival under conditions of several drought (SINCLAIR, 2000, HANDA, 1986). Therefore within the ongoing water stress it was necessary to monitor another important indicator – increasing of osmotic potential values.

We evaluated the osmotic adjustment capacity of three genotypes of soybean during a period of drought stress. Total osmotic adjustment increased with increasing severity of drought stress. Under conditions of drought stress, OA has been implicated in maintaining stomatal conductance, photosynthesis, leaf water volume, and growth. Soybean in the high stress showed a total osmotic adjustment of 1.29MPa genotype Maverick with the inoculated and too genotype Drina 1.03 MPa from two growing seasons (Table 1).

Table 1

Osmotic adjustment in soybean genotypes for two years

Osmotic adjustment (OA)			
Soybean plants/ genotype	YEAR		
	2014 Control/ Nitrazon	2015 Control/ Nitrazon	Average Control/ Nitrazon
Nigra	0.22 / 0.36	0.12 / 0.20	0.17 / 0.28
Drina	0.95 / 1.11	0.76 / 0.95	0.85/ 1.03
Maverick	1.05 / 1.20	0.98 / 1.38	1.02 / 1.29

CONCLUSIONS

The influence of water stress on some physiological parameters (stomatal closure, osmotic potential, free proline and osmotic adjustment) in leaves of three soybean genotypes (*Glycine max* L.) based on pot trials of Department of Plant Physiology of Slovak Agricultural University in Nitra, Slovak Republic, from two years (2014 -15) was observed. According measurements, which were done, genotype Maverick USA) showed bigger ability of osmotic adjustment under water stress, then others genotypes. By all genotypes with Nitrazon was notice positive influence on the osmotic adjustment. The results indicate a higher capacity of the genotypes for osmotic adjustment as a potential trait and are discussed with respect to OA as a trait of improved drought tolerance. The genetic variation of osmotic adjustment in a number of crop species has opened the way for the potential use of osmotic adjustment–related genes and molecular markers in breeding using marker-assisted selection to improve crop drought tolerance.

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