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Physiological responses of genotypes soybean to simulated drought stress

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The objective of this research was to investigate possible genetic variation in the sensitivity of soybean genotypes for nitrogen fixation rates in response to soil drying. To assess resistance to drought in different soybean genotypes were selected appropriate physiological parameters such as relative water content, osmotic potential stress index and formed nodules on the roots of plants. In the growing season 2014 an experiment with four genetic resources of soybean was launched. Sowing of Maverick (USA), Drina (HRV), Nigra (SVK) and Polanka (CZK) genotypes was carried out in the containers of 15 l capacity. Water stress had a negative impact on the physiological parameters. By comparing the Relative Water Content, the decrease was more significant at the end of dehydration, which was monitored in Maverick (41.59%) and Drina (38.24%) genotypes using the Nitrazon inoculants and water stress effect. Inoculated stressed Nigra and Polanka genotypes have kept higher water content till the end of dehydration period. Also the proline accumulation was monitored during the water stress, whilst higher content of free proline reached of Maverick. More remarkable decrease of osmotic potential was again registered in a foreign Drina and Maverick genotypes in the inoculated variations. Nigra and Polanka genotypes responses not so significant in the given conditions. Under conditions of water deficit were just more resistant genotypes originating from Slovakia (Nigra) and Czech Republic (Polanka). In all studied genotypes was observed positive effect of inoculation in dry conditions compared with uninoculated variants.

Keywords: soybean, drought, stress, inoculation of Nitrazon

1 Introduction

Drought is one of the most serious world-wide problems for agriculture. Water deficit results in various physiological and biochemical changes in plants (Farooq et al. 2009).

Among the plant oils, soybean has special features. It has a wide and varied range of applications. Soybean oil is one of the main components of edible oil market. And to feed of many people is consumed especially as margarine and hydrogenated fats. Soybean is a valuable and strategic plant with 35 to 45 percent and 18 to 22 percent of the oil in the seed. In addition to the various uses, vegetable oils in the diet, in the industry is also made of it, various materials. Soybean oil contains unsaturated fatty acids such as oleic acid, linoleic acid, linolenic acid and this unsaturated fatty acids, are very important in terms of supplying vitamin and maintaining health of humans. The use of bacteria at planting soybeans instead of nitrogen fertilizer adds to its importance. Nitrogen fixation by *Rhizobium* bacteria by reducing the use of nitrogenous fertilizers and related costs prevent from nitrate pollution resulted from fertilizer. Although biological nitrogen fixation systems is a long-term process in agricultural, so as a sustainable source of energy is important in agriculture. And has reduced from the use of needed nitrogen fertilizers for food production in recent decades. Soil microbiology experts always try to focus on the isolation and identification of *Rhizobium* strains of soybean symbiotic that have appropriate efficiency in Nitrogen fixation. While agricultural scientists are seeking to find the ideal potential growth in terms of potential of soybean performance yield and growth of various types (Medium, early and late) in different environmental conditions and consistent, with the change in factors (Sancholi, 2015).

Soybean is characterized by an ability to fix atmospheric nitrogen by means of nodule-creating bacteria which belong to the species *Bradyrhizobium japonicum*. These bacteria can be found only in soybean. In the conditions where a presence of free rhizobia in the soil is not expected, the inoculation (bacterization) of seeds are carried out. For the symbiotic effect use during the legume growing is necessary to inoculate the seed coat by the appropriate kind of rhizobia and mainly on the soils, where the particular legume has not been grown for a longer period of time (Malhorta et al., 2004). An advantage of the bud plants is longer phase of utilization of assimilation surface of leaves and air nitrogen fixing.

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It reflects in a production of bigger legume and seed amount on the plant, as well as in the total higher production per hectare (Downie, 1998).

And soybean is among the top 10 of the most widely grown crops, with a total production of over 260 million tonnes in 2012 (Ku et al., 2013) . Greenhouse and field studies showed that drought stress led to significant reduction in seed yield (24~50%) from distinct locations and time (Frederick et al., 2001; Sadeghipour et al., 2012). Soybeans are more flexible than corn in adapting to periods of moisture stress.

Therefore the aim of this study was to follow the physiological responses of soybean genotypes during increasing drought conditios. In dry conditions it was also observed effect of inoculation with selected soybean genotypes. Provenance of selected genotypes was different due to the impact of climate change and selected genotypes using vaccine were tested for practical needs.

2 Material and methods

At the Department of Plant Physiology, Slovak University of Agriculture in Nitra, during the experimental period 2014 were monitored various genetic resources of soybean (*Glycine max* L.). Seeding of the particular soybean genotypes was made into 10 containers (15 l) whilst 50% of seed soybean from each genotype was before the sowing inoculated by the usage of Nitrazon inoculant. Within the experiment, focused on the water stress, there were used genetic resources of different origin: Maverick (USA), Drina (HRV), Nigra (SVK) and Polanka (CZK), which were provided by Plant Production Research Center Piešťany, Gene Bank of Slovak Republic.

At the beginning of the growth phase R1 and R2 – flowering, water stress was simulated by suspending the irrigation and preventing any moisture to occur. The above mentioned physiological parameters (Relative Water Content, Osmotic Potential, Stress index) were during water stress period evaluated on 2nd, 6th and 9th day (the end of dehydration) on the inoculated variants and also on the variants without inoculation comparing to the control fully hydrated plants and variants with dehydration. Also was determined of Nodule-creating Bacteria Number on the soybean plants.

2.1 Characteristics of Used Nitrazon Inoculant

This vaccine was provided for our research purposes by Agrokomp, spol. s r.o., Modra. Mentioned vaccine is also produced in Czech Republic and prepared from the roots of nodulecreating bacteria selected in Plant Production Research Centre in Prague. It is made from the selected bacteria separately for particular species of crop plants belonging to legume family (Fabaceae) and has got a high level of living bacteria – even 3.08 ×109. The vaccine directly increases protein content in the growing crop plants, thrives to the yield growth and better microbial activity of soil. Its use is not difficult.

2.2 Relative Water Content (RWC) in the Leaves

During the gradual dehydration *RWC* in % was monitored which showed us the water content in leaf tissue relatively to the maximum water content in the tissue after saturation. Leaf segment was left saturating by distilled water for 4 hours with temperature 4 °C. After saturation the sample was drying for 12 hours by temperature 100 °C for a determination of dry basis weight.

RWC value was calculated according to an equation:

$$RWC = \frac{FW - DW}{SW - DW} \cdot 100, \quad (\%)$$

where:
FW – is a Fresh Weight
DW – is a Dry Weight
SW – is a Saturated Weight

2.3 Stress Index

Stress index serves us for a comparison of measured parameters changes between the control and stress for variety collection as relative change evaluation (decline or incline) of parameter against the control (edited by Fischer, 1978).

Relation for the stress index (*S.I.*) calculation for a sign "*x*" can be solved as following:

$$S.I.(x) = \frac{1 - [x(S1)/x(K1)]}{1 - [x(S)/x(K)]} \cdot 100$$

where:
$x(S1)$ – is a value of parameter by the concrete stress variant
$x(K1)$ – is a value of monitored parameter by the particular control variant
$x(S)$ – represents the average parameter value of the stressed plants for the whole testing collection
$x(K)$ – represents the average parameter value by the controls. Stress index, calculated this way for the gene collection has got the mean value equal one and it is agreed that:
S.I. = 1 → stress effect at the average level of the whole collection
S.I. >1 → more noticeable effect than the average – genotype is in the monitored sign sensitive
S.I. <1 → effect weaker than the average – genotype is in the particular sign less sensitive (more tolerant)
S.I. = 0 → no stress effect on the monitored sign at the given genotype

For the signs, which are developing during the stress, the average stress index value is calculated for the monitored period.

2.4 Osmotic Potential

A psychrometric method (WESCOR, LOGAN, UTAH, USA) was used for Ψ_S measuring. For Ψ_S determination of leaf tissue a disc with 5 mm diameter was taken away from a middle part of the leaf and the disc was placed into the aluminium foil and left in liquid nitrogen till the Ψ_S measuring. Before the sample was put into the psychrometric chamber, it was left for 15 seconds to defrost by the temperature of the surrounding.

2.5 Determination of Nodule-creating Bacteria Number on the Soybean Plants

After the plants were taken away from the containers, the number of nodules on the particular plant roots was determined. The nodules were distinguished also according to the size and colour. Active nodules have got pink colour and their green colour leads to a loss of activity. Brown and black nodules are dead (Patterson, 1983).

These parameters were evaluated during water stress period evaluated on 2nd, 6th and 9th day. The end of dehydration was on 9th day.

The statical analysis was carried out in Unistat 5.1 programme using ANOVA and LSD methods with a 95% level of probability.

3 Results and discussion

Relative water content (*RWC*) is an important indicator for WDT – plant water deficit tolerance (Virginia, 2012). Under the given circumstances it was therefore intended to monitor *RWC* decrease in selected soybean genotypes. *RWC* was therefore used as a covariate to adjust the genotypic values of π and ge in order to facilitate comparison at a consistent plant water status of 70% *RWC* (James, 2008). When comparing *RWC* values a more significant decrease at the end of dehydration was registered in foreign genotypes Maverick (41.59%) and Drina (38.24%) using Nitrazon inoculant and the effect of water stress. Inoculated stressed genotypes Nigra (SVK) and Polanka (CZK) maintained higher water content at the end dehydration (64.12% and 60.05%). In controlled watered variants with inoculation *RWC* content (Figure 1) in foreign genotypes was as follows: 80.78% (Maverick) a 80.38% (Drina) compared to the inland genotypes, which maintained higher levels of *RWC* (Nigra 90.46%, Polanka 88.01%).

Soybean (*Glycine max* (L.) Merr.) seedlings osmoregulate when the supply of water is limited around the roots. The osmoregulation involves solute accumulation (osmotic adjustment) by the elongating region of the hypocotyls (Rasaei et al., 2013) Within the ongoing water stress it was necessary to monitor another important indicator– increasing of osmotic potential values (Figure 2). More significant decrease was again recorded in foreign Drina (-3.34 MPa) a Maverick (-2.39 MPa) genotype in inoculated,

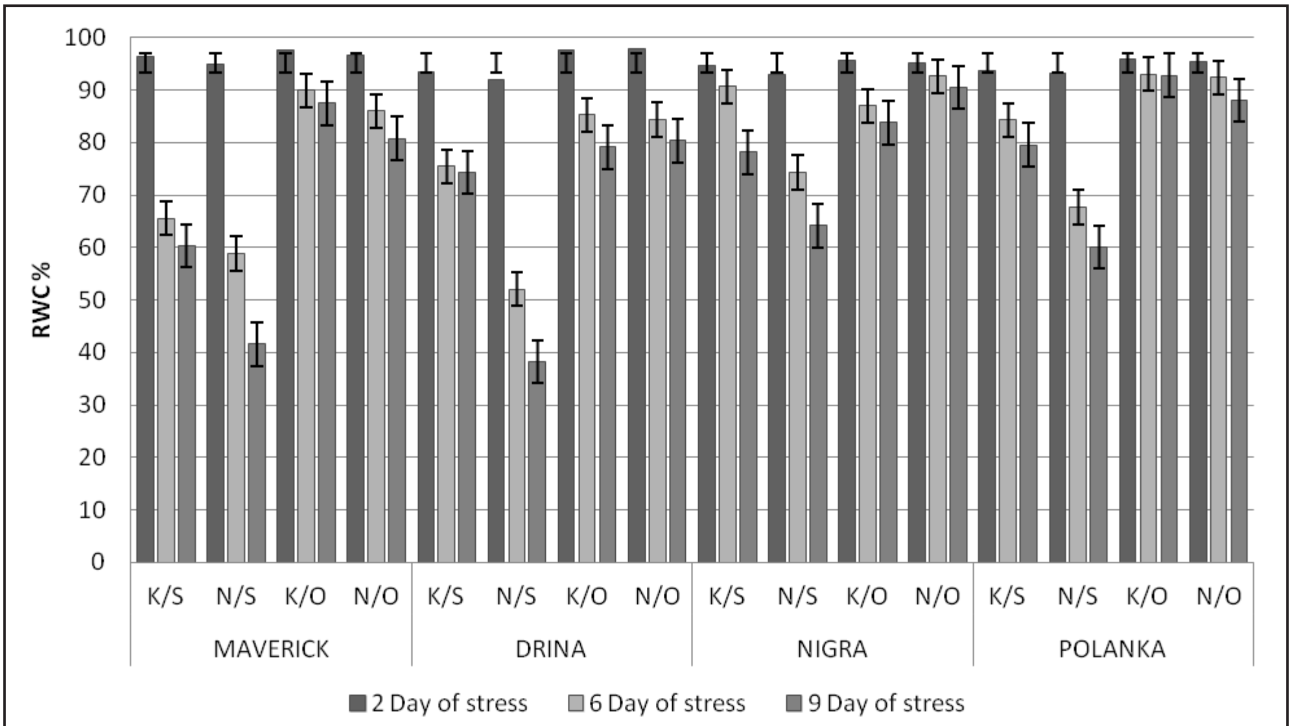

Figure 1 Relative water content in soybean genotypes leaves depending on dehydration period and seed inoculation (%): K/S control and water stress, N/S Nitrazon and water stress, K/O control variant, N/O Nitrazon variant)

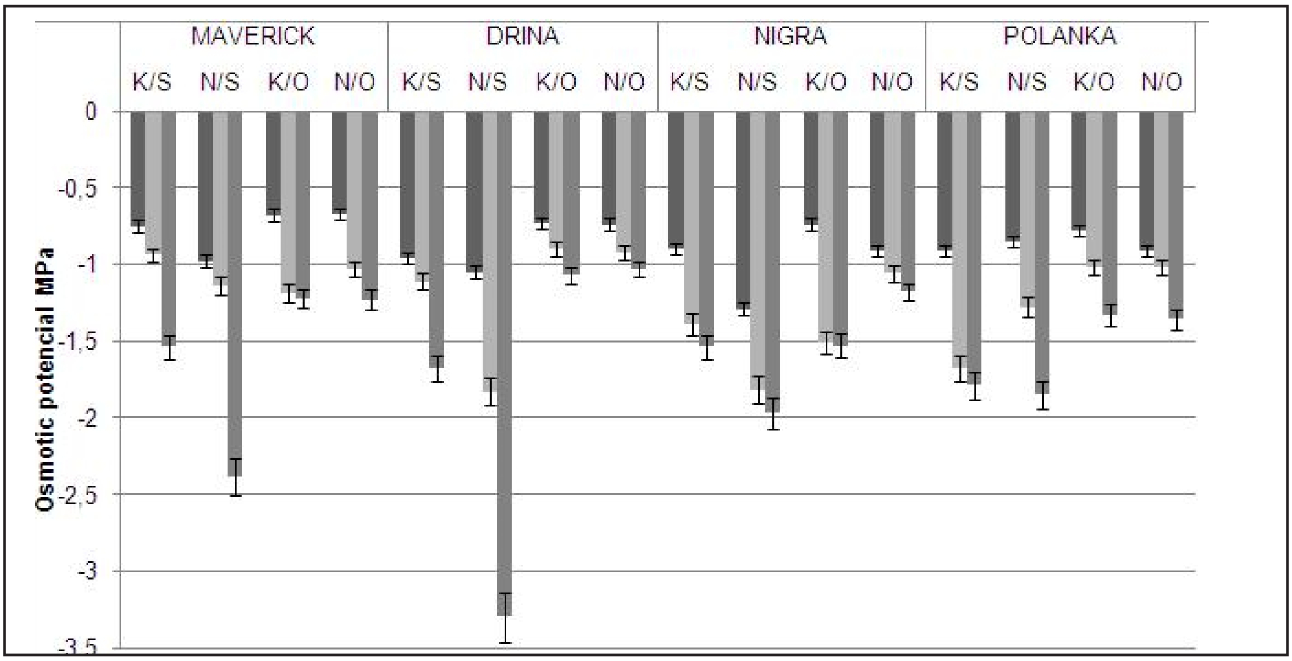

Figure 2 Osmotic potential in soybean genotypes leaves depending on dehydration period and seeds inoculation (MPa): K/S control and water stress, N/S Nitrazon and water stress, K/O control variant, N/O Nitrazon variant

variants, exposed to the water stress. Genotypes less Nigra (-1.97 MPa) and Polanka (-1.85 MPa) responded less remarkable in the given conditions. Osmotic potential decresed less significant on the dehydrated variants (Maverick -1.54 MPa, Drina -1.68 MPa, Nigra -1.54 MPa a Polanka -1.79 MPa) in the conditions without inoculation, what partially indicates their smaller tolerance to the drought. Large differences in midday leaf water potential were observed in rainfed legume crops, with chickpea and lentil reaching -3.3 MPa while pea and faba bean reached -2.0 MPa, but only soybean maintained photosynthesis below -3.0 MPa (Leport et al., 2003; Candráková et al., 2006). In plants of soybean, determination of leaf water potential was useful in describing the simulated drought stress, but was not very suitable for discriminating tolerant genotypes (Riccardi et al., 2001), suggesting that water

Table 1 Stress index (*S.I.*) based on the relative water content in leaves of soybean plant induced on dehydration period

STRESS INDEX (*S. I.*)		
Soybean plants/genotype	Relative Water Content in the leaves	
	Control and Water stress/ Control Variant	Nitrazon and Water Stress/ Nitrazon Variant
POLANKA	0.93 + 0.05	0.80 + 0.04
NIGRA	0.87 + 0.04	0.73 + 0.03
MAVERICK	2.07 + 0.17	1.23 + 0.10
DRINA	1.40 + 0.13	1.30 + 0.12

Table 2 Amount of created nodules on the roots of soybean plants without inoculation and after Nitrazon inoculation

Amount of created nodules on the roots						
Soybean/genotype	2nd day		6th day		9th day	
	control	Nitrazon	control	Nitrazon	control	Nitrazon
DRI DRINA	1±0.3	22±0.85	4±0.4	29±1.09	6±0.89	37±1.41
NIG NIGRA	1±0.5	14±0.33	3±0.3	25±1.01	5±1.06	34±2.20
MAV MAVERICK	1±0.3	15±1.24	5±0.2	21±1.13	6±0.94	32±1.75
POL POLANKA	1±0.3	10±1.24	3±1.24	16±1.24	5±1.24	28±1.24

Figure 3 Roots of soybean genotypes DRINA control, after Nitrazon inoculation and during water stress

potential was not the defining feature of the tolerance. Water retention in the morning and water content in leaves in the afternoon were potentially useful for screening drought tolerance in soybean.

Stress index serves us for a comparison of measured parameters changes between the control and stress for variety collection as relative change evaluation of parameter against the control. A relative quantity, defined this way, enables us to synoptically compare the sensitivity of the concrete genotypes in the various parameters. Genotype Maverick without inoculation reacted more markedly to the water stress, what is expressed also by the value of stress index for the given variant (2.07), in a comparison with the inoculated (1.23). The both variants were showing the same, i.e. weaker effect on the water stress. The value of stress variant by the genotype Drina was by the variant without inoculation 1.30, by the inoculating variant 1.40, what means low sensitivity to the water deficit (Table 1). Genotype Nigra (SVK) and Polanka (CZK) were more tolerantly to the water stress (0.73 Nigra and 0.80 Polanka). By all genotypes was monitored possitive affect on the water stress, there were used soybean genotypes with Nitrazon inoculant.

After the plants were taken from the soil and later washed under the water flow we ascertained the number and a state of health of the nodules on the plant roots. The healthy nodules had in a point of the cut red colour (98% in all varieties), unhealthy had green-black or yellow-white colour (only 2%). In our experiments due to the effect of Nitrazon inoculant, the number and weight of nodules on the plant roots grew in average by 63% in Polanka genotype, 40% in Nigra genotype, 32% in Maverick and the least 23% in Drina genotype. This fact was also proved on a resistance strategy in the particular varieties against the stress caused by the water lack (37% in Drina genotype, 32% in Maverick genotype, 34% in Nigra genotype, 28% in Polanka genotype). Higher amount of nodules on the soy root can positively influence resistance of the plants exposed to the water deficit in soil. The results were consistent with the results of Sancholi et al. (2015). From 50 species of microorganisms (bacteria and cyanobacteria), which are able to synthesize the enzymes of nitrogenase and reduce air nitrogen on ammonia, acceptable form for the plants. Some plants had afterwards an ability of symbiotic cooperation with these microorganisms (Mirabella, 2000). Among the usual characteristics, we can find nodule-creating on the roots which is occurring approximately till the 3rd week since they were germinated. The legume creates these nodules on the roots as a reaction of an infection caused by nodule-creating bacteria. Bacteria usually live in soil or they have to be added during the process of sowing by the means of seed corn bacterization (vaccination, inoculation). One should remember a fact that nodule-creating bacteria are specific according to the variety.

Conclusions

From the point of view of ecophysiology, it is important to solve a question of climatic changes by the means of detailed cognition of physiological processes rules in the plant. Overall, the results of this study indicate that important genetic variations for sensitivity of nitrogen fixation to soil drying exist in soybean, and that the variation may be useful in physiology and breeding studies. Understanding of heterogeneity of genotype productive capabilities offers an assumption for a better characterization of new and prospective biological material in breeding practice. Negative effects of climate change can be mitigated through appropriate choice of plant species, improving water management and irrigation systems.

By comparing the Relative Water Content values, the decrease was more significant at the end of dehydration, which was monitored in Maverick (41.59%) and Drina (38.24%) genotypes using the Nitrazon inoculant and water stress effect. Inoculated stressed Nigra (SVK) and Polanka (Czech Republic) genotypes have kept a higher water content till the end of dehydration period (64.12% and 60.05%). More remarkable decrease of osmotic potential was again registered in a foreign Drina (-3.34 MPa) and Maverick (-2.39 MPa) genotype in the inoculated variations, exposed to the water stress. Nigra (-1.97 MPa) a Polanka (-1.85 MPa) genotypes respont not so significant in the given conditions. Among the evaluation of genotypes were more resistant genotypes derived just from Slovakia (Nigra) and Czech Republic (Polanka). By all genotypes was monitored possitive affect on the water stress, there were used soybean genotypes with Nitrazon inoculant.

Generally, we can conclude that inoculation on have important role on performance and components of seed and can increase that. As a result, with the goal of planting soybeans (an increase of quantity or quality) can choose the best cultivar or the best inoculated combination of genotypes.

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