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Original Paper

Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration

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Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation on the varieties Impala (Republic of South Africa), Maverick (US), Rankoshi No.1h (Japan) and their reactions to the water supply at the beginning of the growth stage R1 and R2 – blooming in an interaction with the Nitrazon inoculation of the seeds before a sowing were evaluated. Seeding of the particular soybean genotypes was made into the containers whilst 50 % of seed from each genotype was inoculated by the usage of Nitrazon inoculant before the sowing. Water stress was secured by an irrigation interruption for a 7-day period in the mentioned growth stage. This stress had a negative impact on the relative water content (% RWC) plants, proline content (μmol g^{-1} Fresh Weight), osmotic potential (MPa), stress index as well as an amount of nodules on the roots by the all evaluated varieties. According to an evaluation of the mentioned indicators, more significant proline accumulation was confirmed by the genotype Maverick especially by the treatment exposed to water deficit (3.25 μmol g^{-1} FW according to the calculations on 100 % RWC) without the inoculant Nitrazon use and inoculating treatment 2.99 μmol g^{-1} Fresh Weight according to calculations on 100 % RWC. Genotype Maverick had the best reaction to water stress and even more noticeable resistance to the stress was monitored in the treatment with Nitrazon application in the foregoing seed treatment of soybean seeds. The opposite response to the inoculation was monitored by Impala genotype.

Keywords: soybean, *Glycine max* (L.) Merr. genotypes, drought, inoculation, symbiotic bacteries

1. Introduction

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 (Temperly, 2006).

Soybean is particularly sensitive to the moisture lack during the blooming process (growth stages R1 and R2) and during the legume and seed growing process (growth stages R3 – R6) (Doss, 1974; Sionit, 1977). Mederski (1973) claims that water stress during the blooming process (growth stages R1 and R2) and legume growing process (growth stages R3 and R4) was noticed as a factor responsible for a flower and legume abortion however a seed size was reduced by the stress during the seed growing process (growth stages R5 and R6).

Soybean is characterised by an ability to fix atmospheric nitrogen by means of nodule-creating bacteria which belong to the specie *Bradyrhizobium japonicum* (Fisher, 1992). These bacteria can be found only in soy bean. In the conditions where a presence of free rhizobia in the soil is not expected, the inoculation (bacterization) of seed corn is carried out. For the symbiotic effect use during the legume growing is necessary to inoculate (bud) the seed corn 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. An advantage of the bud plants is longer phase of utilization of assimilation surface of leaves and air nitrogen fixing. 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).

The aim and purpose of the foregoing seed inoculation of the seed soybean is biological rationalization of the nutrient by fixed atmospheric nitrogen. It radically reduces a need of mineral nitrogen fertilizers and increases soybean production economy including the subsequent crop plants. Soy's important role is enlarged by the fact that this nitrogen is acceptable only for soybean and not for the weeds (Dashti, 1997; Downie, 1998). A number of inoculation preparations containing active nodule-creating bacteria in high concentration are used for the soy seed bacterization. Especially Rhizobia, imported from the Czech Republic, was widespread in our country, Slovak Republic. Its production was finished in 2008 and using the similar technology the

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innovative inoculant Nitrazon has been producing (Fecák, 2009). Main qualities of this inoculant are more equal seed soybean coverage and higher infertility. It is applied in a dose of 1 kg ha^{-1} primarily by mixing with the soya seed directly in a storage bin of sowing machine. A benefit of foregoing seed inoculation of seed is harvest increase by 13 – 25 % but also increasing of some qualitative indicators, such as nitrogen substances and oil content.

2. Material and methods

Within the experiment, focused on the water stress, there were used soybeans genotypes of different origin: Impala (Republic of South Africa), Maverick (US), Rankoshi No.1h (Japan), which were provided for the research purposes by Gene Bank of the Slovak Republic, Plant Production Research Center, Piešťany.

2.1 Characteristics of used NITRAZON inoculant during the sowing

This vaccine is produced in the Czech Republic and prepared from the roots of nodule-creating bacteria selected in Plant Production Research Centre in Prague. It was being verified for a long period in the experiments and practise in Czech and Slovak Republic.

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 3.08×10^9. 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. Mentioned vaccine was provided for our research purposes by enterprise Agrokomp, spol. s.r.o., Modra.

2.2 Relative water content in the leaves (RWC %)

During the gradual dehydration relative water content 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 (Olšovská, 2001). After saturation the sample was drying for 12 hours by temperature 100 °C for a determination of dry basis weight.

2.3 Osmotic potential (Ψ_S, MPa)

A psychometric method (Wescor Inc., Logan, Utah, US) 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 psychometric chamber, it was left for 15 seconds to defrost by the temperature of the surrounding.

2.4 Free proline determination (μmol g^{-1} FW)

Free proline is being determined by (Bates, 1973) method in the leaf tissue. Approximately 0.5 g of leaf fresh weight (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.

2.5 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 and Maurer (1978).

2.6 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 pink colour and their green colour leads to a loss of activity. Brown and black nodules are dead (Patterson, 1983). Afterwards the photo documentation was made.

3. Results and discussion

A plant reaction is an interaction result of differently age, functional specific organs between which are creating certain correlative or more precisely competitive relations.

It occurs very often that only the specific leaf is monitored and the root system and generative organs, which normally react directly or indirectly to the stress, are outside of consideration.

Only several types from the large complex of physiological criteria were tested in our experiment. RWC is a simple and meantime in the field conditions relatively easy findable indicator of water supply state in the plant.

According to many authors, a deciding factor is stress time period, its strength, and the duration. In the vegetation container experiment we were monitoring, except the physiological criteria, the stress effect in the particular phenophases also on a speed of assimilator's translocation in the plant and a progress of reproductive growth and especially, as it is recommended by (Schmitt, 2001; Pedersen, 2003; 2008), the reduction of certain harvest-creating elements.

Ability of osmotic adaptation to the various kinds of environmental stress is generally known by almost each organism. The important osmoprotective function is carried out by the proline amino acid (Blum, 1999).

Therefore the proline accumulation is almost a universal plant reaction to the water deficit (Hare, 1999). Proline content can be conditioned by the genotype and it seems that the proline plays specific roles in a protection of photosynthetic apparatus against the negative stress effects. More significant decline of the relative water content (RWC) was detected on the 7th day of dehydration in Maverick genotype, in the treatments exposed to the water deficit without the use of Nitrazon inoculant on 52.85 % and with the inoculant on 69.07 %, which decline under 70 % RWC frequently represents an encroachment to the metabolism of the fundamental physiological process and which leaves irreversible consequences also on the growth-productive processes (Figure 1).

In the stated period in our experiments at its end, when the proline (Figure 2) was more significantly accumulating by the Maverick genotype in the treatment exposed to the water deficit (3.25 μmol g^{-1} FW according to the calculation on 100 % RWC) without the use of Nitrazon inoculant and inoculated treatment 2.99 μmol g^{-1} FW according to the calculation on 100 % RWC.

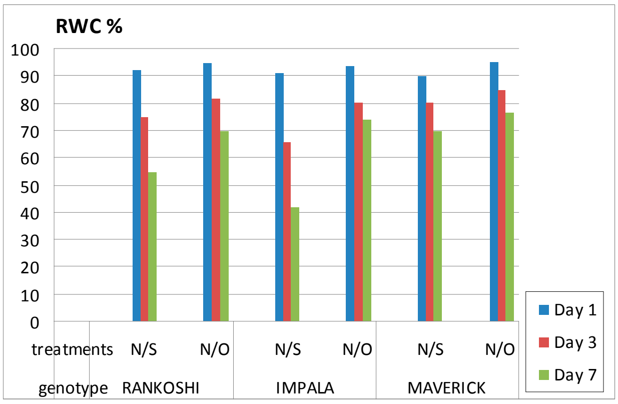

Figure 1 Relative water content in soybean genotypes leaves depending on dehydration period and seed inoculation
K/S control and water stress, N/S Nitrazon and water stress, K/O control treatment, N/O Nitrazon treatment

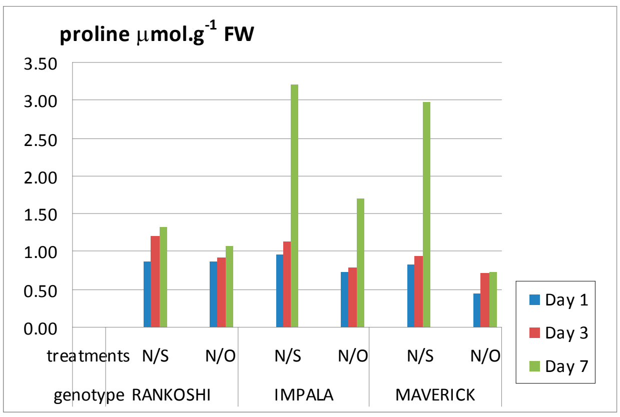

Figure 2 Proline content in soybean genotypes leaves depending on dehydration period and seeds inoculation
K/S control and water stress, N/S Nitrazon and water stress, K/O control treatment, N/O Nitrazon treatment

By the mentioned genotype, the more considerable drop of the osmotic potential (-3.75 MPa, or -2.32 MPa) occurred which relates with the water deficit.

Genotype without inoculation reacted more markedly to the water stress, what is expressed also by the value of stress index for the given treatment (1.45), in a comparison with the inoculated (0.36). Dealing with the Rankoshi genotype, it has to be said the proline was accumulating less obvious by the both treatments, exposed to the water stress (without inoculant 1.56 μmol g^{-1} FW according to the calculation on 100 % RWC, inoculated treatments 1.34 μmol g^{-1} FW according to the calculation on 100 % RWC) despite the fact that in this period, it means on day 7 of dehydration, was monitored more significant RWC drop (52.28 %. or 54.78 %). The both treatments were showing the same, i.e. weaker effect on the water stress. The value of stress treatments was by the treatments without inoculation 0.64, by the inoculating treatments 0.88 what means low sensitivity to the water deficit (Table 1).

Table 1 Stress index of soybean plants induced by the water deficit

Stress index (SI)		
Soybean plants/ genotype	RWC	
	KS/KO	NS/NO
RANKOSHI	0.64 ±0.03	0.88 ±0.04
IMPALA	0.86 ±0.05	1.76 ±0.07
MAVERICK	1.45 ±0.12	0.36 ±0.08

K/S control and water stress, N/S Nitrazon and water stress, K/O control treatment, N/O Nitrazon treatment

Genotype Impala was negatively reacting on the inoculation, when RWC dropped to 41.77 % (proline content 3.22 μmol g^{-1} FW according to the calculation on 100 % RWC) in the comparison with the variant without the inoculation, where RWC declined to 61.86 % (proline content 2.60 μmol g^{-1} FW according to the calculation on 100 % RWC). This claim is also connected with a value of stress index 1.76 for the inoculating variant, when the genotype was more sensitive compared to the non-inoculating variant (0.86). The value of osmotic potential became lower by the particular genotype without bigger differences in the controlled and inoculated variant (-2.56 MPa, or -2.69 MPa).

In a case, the stress has an effect on the unprepared plant very quickly, immediately with the growth suspension, i.e. without an involvement of the reactions at the level of the organism, the harm depends on a stability of all structures – from molecular, subcellular, to anatomic-morphological (Starling, 1998).

In the nature there is occurring the stress successive, gradual, long effecting with not high strength, in a day cycle often alternating with the relatively positive seasons for the growth and then a stability (resistance) of functions and adaptive processes, which are realizing mainly by the plant growth, have a fundamental importance (Barker, 2005). Functional resistance is extraordinary significant in the conditions of big daily and seasonal amplitudes of the factor (temperature, water supply). Problems of the resistance at the ecological level require an analyses of the system soil-climate-plants; organ-plant; harm-adaptation-resistance.

3.1 Growth and number of nodule-creating bacteria on the soybean plants

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 (Table 2).

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 26 % in Maverick variety, 18 % in Rankoshi variety and the least 13 % in Impala variety. This fact was also proved on a resistance strategy in the particular varieties against the stress caused by the water lack. Higher amount of nodules on the soy root can positively influence resistance of the plants exposed to the water deficit in soil.

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, 2004). 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 (Schlaman, 1998).

Fabaceae is one of the numerous families widespread all over the world. It comprises from 3 subfamilies – *Papilionoideae*, *Mimosoideae* and *Caesalpinioideae* to which belong more than 750 genus and 14000 species. In first two mentioned subfamilies the nodules are creating in more than 90 % of species and in *Caesalponioidae* there is the opposite case – in 70 % of species the nodules are not creating. *Rhizobium* interaction – plant is specific and commonly only the concrete rhizobia species can create the nodules on the limited number of genus or species of the host plants (Purcell, 2004).

If we would like to understand the interactions between bacteria and root hair, firstly we should identify the root hair itself, its development, growth, what takes part in the growth and what will happen to the hair root after an application of NF (Abendroth, 2005).

Nodulation factors (NF) represents the molecules synthesised and excreted by rhizobia during a reaction on plant flavonoids (Fischer, 1992).

It would be necessary to continue in a research of complex interaction of the elements in a system of nitrogen nutrition of soybean: content of inorganic nitrogen in soil – seed soybean inoculation-starting nitrogen dose. It is justifiable to assume the required amount of nitrogen to ensure soya nutrition will be gained by the fixation only in a case that the fixing apparatus is adequately working, it means effectual. Its effectiveness depends on several factors so on the outer, so on the inner environment.

4. Conclusions

Nitrogen fixation activity by soybean (*Glycine max* (L.) Merr.) nodules has been shown to be especially sensitive to soil dehydration. Specifically, nitrogen fixation rates have been found to decrease in response to soil dehydration preceding alterations in plant gas exchange rates. 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. Experiments with Maverick which induced localized soil drying around the nodules did not result in decreases in nitrogen fixation rates, but rather nitrogen fixation responded to drying of the entire rooting volume. The osmotic potential of nodules was found to decrease markedly upon soil drying. However, the decrease in nodule osmotic potential occurred after significant decreases in nitrogen fixation rates had already been observed.

On the ground of container, vegetative experiments in regulating conditions of environment, we have found out the RWC values of leaves and proline content could be used as

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	Day 1		Day 3		Day 7	
	control	Nitrazon	control	Nitrazon	control	Nitrazon
RANKOSHI	1 ±0.3	14 ±0.85	4 ±0.,4	16 ±1.09	6 ±0.89	19 ±1.41
IMPALA	1 ±0.5	12 ±0.33	3 ±0.3	13 ±1.01	4 ±1.06	14 ±2.20
MAVERICK	1 ±0.3	15 ±1.24	5 ±0.2	15 ±1.13	8 ±0.94	26 ±1.75

physiological criteria of resistance against water stress in soybean plants. The important factors for the legumes are time of stress occurring and relatively different reaction in the particular crop-producing elements on the stress.

A year of origin has got a significant influence on the crop of soybean seed and negatively affects the seed crop in the years with deficient rainfall and long periods of drought during the vegetative season as a consequence of global warming. Therefore, it is required from the future point of view the testing and right choice of appropriate biological material for the stress conditions and of course to test inoculant and nitrogen nutrition.

Growers in the Slovak Republic have not still fully appreciated an importance of soybean. 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. Process, experienced this way, can be objective and rationally used by agronomist and breeders.

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