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Evaluation of Selected Soybean Genotypes (*GLYCINE MAX* L.) by Physiological Responses during Water Deficit

Hodnotenie vybraných genotypov sóje fazuľovej (*GLYCINE MAX* L.) na fyziologické reakcie v období deficitu vody

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Abstract

During the experiments were monitored the following varieties: Impala (South African Republic), Maverick (USA), 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. Mentioned genetic resources were provided for our research purposes by the Plant Production Research Center (PRRC) Piešťany, Gene Bank of the Slovak Republic. Seeding of the particular soy genotypes was made into the containers whilst 50% of seed corn from each genotype was before the sowing inoculated by the usage of Nitrazon inoculant (supplier: Agrokomp, spol. s.r.o., Modra). 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 in RWC plants, proline content, osmotic potential, SPAD, stress index as well as an amount of nodules on the roots by the all monitored varieties. According to an evaluation of the mentioned indicators more significant proline accumulation was confirmed by the genotype Maverick especially by the variant exposed to water deficit (3,25 μmol*g^{-1} FW according to the calculations on 100% RWC**)** without the inoculant Nitrazon use and inoculating variant 2.99 μmol*g^{-1} FW according to calculations on 100% RWC. Variety MAVERICK had got the best reaction to water stress and even more noticeable resistance to the stress was monitored in the variant with Nitrazon application in the foregoing seed treatment of soybean seeds. The opposite response to the inoculation was monitored by IMPALA genotype when RWC had dropped to 41.77% in the comparison with the variant without inoculation where RWC had dropped to 61.86%.

Keywords: chlorophyll, drought stress, inoculation, nodulation, proline, soybean, *Glycine max* L., stress index

Detailed Abstract

Pôda, zrážky a teploty sú hlavnými činiteľmi prostredia pre pestovanie strukovín. Produktivita sóje fazuľovej v rôznych agroekologických podmienkach realizácie produkčného potenciálu veľmi závisí na adaptačných schopnostiach pestovaných odrôd, čo podstatne ovplyvňuje úrodovú stabilitu sóje fazuľovej v daných podmienkach. Cieľ a výsledky nášho pokusu majú prispieť k rozšíreniu poznania podmienok realizácie produkčného potenciálu, ktorý je základným predpokladom cieľavedomého ovplyvňovania rastu a vývoja sóje fazuľovej pre priaznivé úrodotvorné a technologické parametre. Adaptácia rastlín na nepriaznivé abiotické vplyvy je bezprostredne spojená so zmenou fyziologických procesov, ktoré odpovedajú na stresy zmenami kvantitatívnych a kvalitatívnych ukazovateľov. V experimentálnom období 2012 boli sledované fyziologické reakcie vybraných genetických zdrojov sóje rôzneho pôvodu: Impala (ZAF), Maverick (USA), Rankoshi No.1h (JPN). Uvedené genetické zdroje nám na výskumné účely poskytlo CVRV Piešťany, Génová banka SR. Výsev jednotlivých genotypov sóje sa realizoval do nádob, pričom 50% osiva z každého genotypu bolo pred sejbou inokulované použitím inokulantu Nitrazon (dodávateľ: Agrokomp, spol. s r.o., Modra). Vo fenofáze kvitnutia bol simulovaný vodný stres pozastavením zálievky a zabránením akéhokoľvek prístupu zrážok. Počas postupnej dehydratácie bol sledovaný obsah vody v listoch (RWC), obsah prolínu, osmotický potenciál, SPAD, stresový index ako aj počet hrčiek na koreňoch pri všetkých genetických zdrojoch sóje. Pri hodnotení uvedených ukazovateľov sa výraznejšia akumulácia prolínu potvrdila pri genotype Maverick pri variante vystavenom vodnému deficitu (3,25 μmol*g^{-1} ČH v prepočte na 100 % RWC**)** bez použitia inokulantu Nitrazon a inokulovaný variant 2,99 μmol*g^{-1} ČH v prepočte na 100 % RWC. Uvedený genotyp reagoval aj výraznejším pokles omosmotického potenciálu (-3,75 MPa, resp. -2,32), ktorý súvisí s deficitom vody. Genotyp bez inokulácie reagoval výraznejšie na vodný stres, s čím súvisí aj hodnota stresového indexu pre daný variant (1,45), v porovnaní s inokulovaným variantom (0,36).

Opačný efekt na inokuláciu bol pozorovaný pri genotype IMPALA (Juhoafrická republika), kedy RWC pokleslo na 41,77 % (obsah prolínu 3,22 μmol.g^{-1}ČH v prepočte na 100 % RWC) v porovnaní s variantom bez inokulácie, kde RWC pokleslo na 61,86 % (obsah prolínu 2,60 μmol*g^{-1} ČH v prepočte na 100 % RWC). Uvedené konštatovanie súvisí taktiež s hodnotou stresového indexu 1,76 pre inokulovaný variant, kedy bol genotyp viac citlivý v porovnaní s neinokulovaným variantom (0,86).

Na základe získaných poznatkov možno konštatovať, že deficit vody negatívne ovplyvnil sledované fyziologické parametre, pričom najlepšie na vodný deficit reagoval genotyp Maverick (USA) s inokulovaným variantom, kde bol zároveň pozorovaný aj najvyšší počet a hmotnosť hrčiek na koreňoch rastlín (v priemere o 26%).

Kľúčové slová: chlorofyl, sucho, inokulácia, nodulácia,prolín, sója fazuľová, *Glycine max* L., stresový index

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 (Racz, 2003).

A choice of appropriate variety and rationally chosen nutrition and fertilization connected with a seed coat inoculation represent the essential steps of successful soy growing. It has got a high level of transpiration coefficient and for one gram of dry basis production it is needed 600 – 1,000 g of water. According to the calculations soybean needs for germinating 120 – 140 % of water per a seed weight (Javor, 2001; Lahola,1990; Špaldoň,1982). 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 (Švihra, Rodriguez, 1993).

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).

Javor (2001) mention the optimal rainfall during the period from the blooming growth stage to the ripening growth stage is 300 mm but it should be equally divided.

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 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. 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 coat is biological rationalization of the nutrient by fixed atmospheric nitrogen. This activity has a great economic and ecological value. It radically reduces a need of mineral nitrogen fertilizers and increases soy production economy including the subsequent crop plants. Soybean important role is enlarged by the fact that this nitrogen is acceptable only for soy and not for the weeds (Dashti, 1997; Downie, 1998). A number of inoculation preparations containing active nodule-creating bacteria in high concentration is 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 innovative inoculant which is called Nitrazon is producing (Fecák, 2008). Main qualities of this inoculant are more equal seed coat coverage and higher infertility. It is applied in a ration 1 kg per ha primarily by mixing with the seed corn directly in a storage bin of sowing machine. A benefit of foregoing seed inoculation of seed corn is harvest increase by 13-25 % but also increasing of some qualitative indicators, such as nitrogen substances and oil content.

Material and Methods

At the Department of Plant Physiology, Slovak University of Agriculture in Nitra, during the experimental period were monitored various genetic resources of soybean (*Glycine max L.*). Within the experiment, focused on the water stress, there were used genetic resources of different origin: Impala (South African Republic), Maverick (USA), Rankoshi No.1h (Japan), which were provided by Plant Production Research Center Piešťany, Gene Bank of Slovak Republic.

Characteristics of Used NITRAZON Inoculant during the Sowing

This vaccine is produced in Czech Republic and prepared from the roots of nodule-creating 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 $x10^9$. 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 Agrokomp, spol. s.r.o., Modra.

Relative Water Content in the Leaves (RWC, %)

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 (Olšovská, 2001). 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:

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

where FW is a Fresh Weight,

DW – Dry Weight,

SW – Saturated Weight.

Osmotic Potential (Ψ_S, MPa)

A psychometric 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 psychometric chamber, it was left for 15 seconds to defrost by the temperature of the surrounding.

Free Proline Determination [μmol . g^{-1} FW]

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).

The following step is the measurement of free proline content according to the equation:

$$Pro = \frac{A * B * 5}{115,5 * C} \quad [\mu mol * g^{-1} FW],$$

where A is proline content subtracted from the calibration curve [μg*mol^{-1} FW],

B is used ml of toluene,

C is a weight of sample in g.

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 (1992).

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

$$S.I. (x) = \frac{1 - [x(S_1)/x(K_1)]}{1 - [x(S)/x(K)]}, \text{ where}$$

$x(S_1)$ is a value of parameter by the concrete stress variant;

$x(K_1)$ 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.

A relative quantity, defined this way, enables us to synoptically compare the sensitivity of the concrete genotypes in the various signs (parameters).

Relation between the Chlorophyll Content and Nitrogen Concentration (SPAD)

Chlorophyll meter SPAD – 502 (Minolta, Japan) measuring of the nitrogen in the leaves. The method is based on the direct measuring of chlorophyll a + b in the leaves. Portable device SPAD – 502 works on a measuring method of light transmission through the leaves in two wave lengths 650 and 940 nm. The device will automatically calculate an average of these two data and indicate the so called SPAD number.

Portable chlorophyll meter SPAD – 502 can be used in ecophysiological research of measuring in situ on the different kinds of plants thanks to its non-destructive determination way of concentration in leaves and undemanding manipulation.

Determination of Nodule-creating Bacteria Number on the Soy 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). Afterwards the photo documentation was made.

Results and Discussion

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

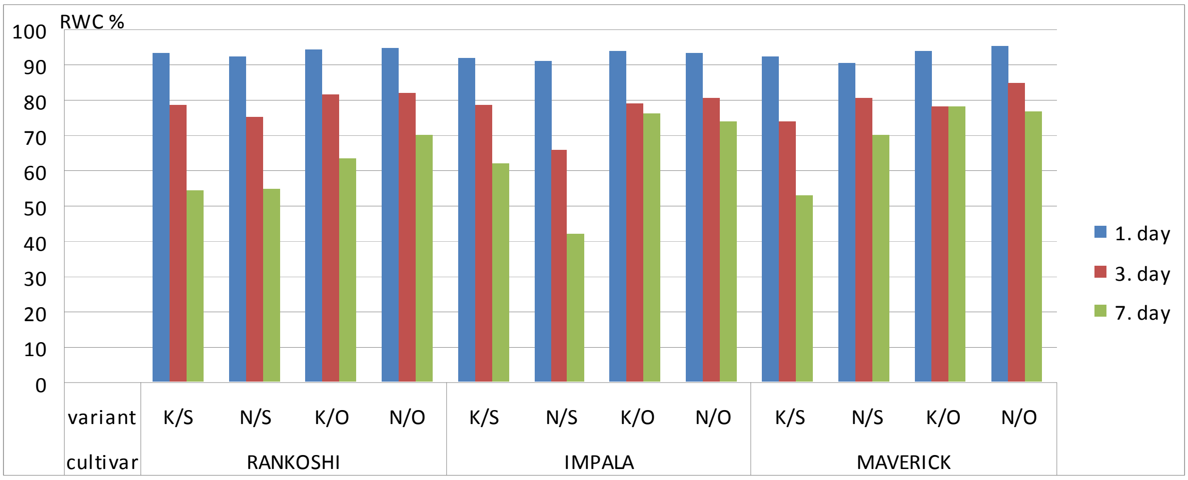
Because it occurs very often that only the concrete leaf is monitored and the root system and also generative organs, which normally react directly or indirectly to the stress, are forgotten.

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. It protects the biomembranes against a harm and enzyme system at the level of thylakoid and mitochondrion membranes (Blum, 1999).

Therefore the proline accumulation is almost a universal plant reaction to the water deficit (Hare, 1999). Its content can be conditioned by the genotype and it seems 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 (USA) genotype, in the variant 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).

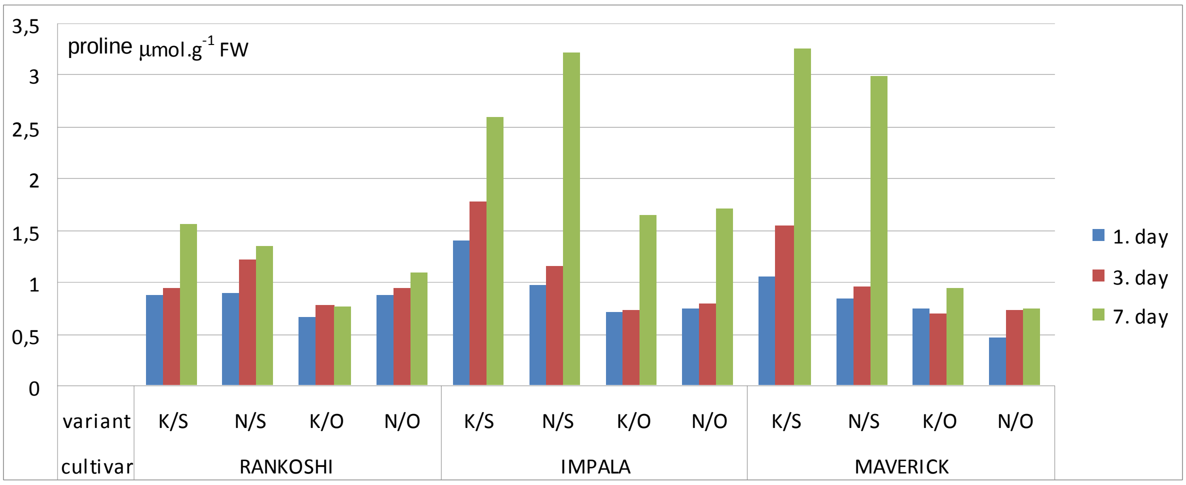


K/S control and water stress, N/S Nitrazon and water stress, K/O control variant, N/O Nitrazon variant

Figure 1: Relative water content in soybean genotypes leaves depending on dehydration period and seed inoculation

Obrázok 1: Relatívny obsah v listoch genotypov sóje fazuľovej v závislosti od doby dehydratácie a inokulácie semien

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 variant 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 variant 2.99 μmol*g^{-1} FW according to the calculation on 100 % RWC.

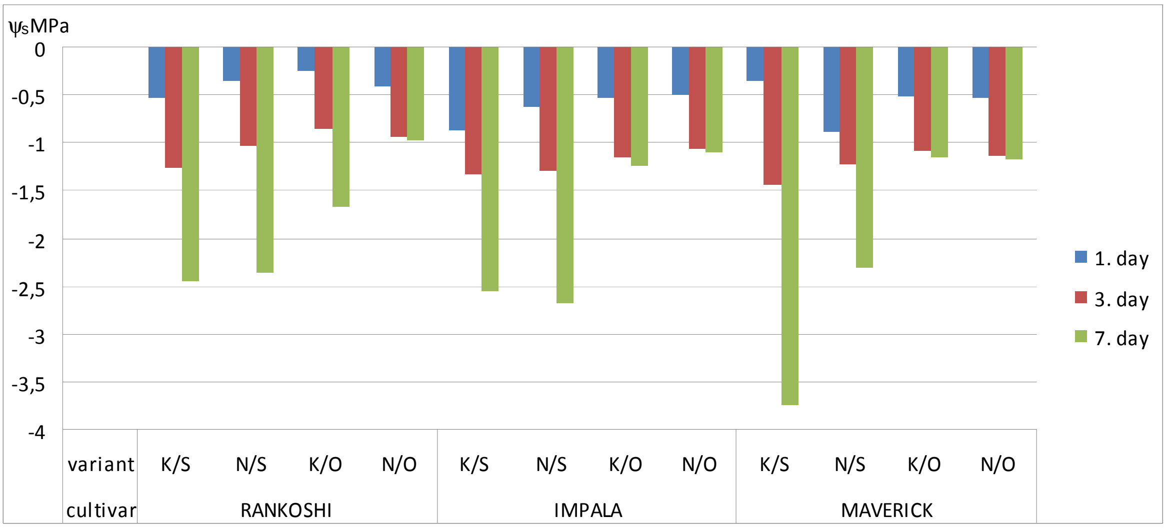


K/S control and water stress, N/S Nitrazon and water stress, K/O control variant, N/O Nitrazon variant

Figure 2: Proline content in soybean genotypes leaves depending on dehydration period and seeds inoculation

Obrázok 2: Obsah prolínu v listoch genotypov sóje fazuľovej v závislosti od doby dehydratácie a inokulácie semien

By the mentioned genotype, the more considerable drop of the osmotic potential (- 3,75MPa, or -2,32MPa) occurred which relates with the water deficit (Figure 3).



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

Figure 3: Osmotic potential in soybean genotypes leaves depending on dehydration period and seeds inoculation.

Obrázok 3: Osmotický potenciál v listoch genotypov sóje fazuľovej v závislosti od doby dehydratácie a inokulácie semien.

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

Table 1: Stress index (S.I) based on the relative water content in leaves of soybean plants induced by the water deficit.

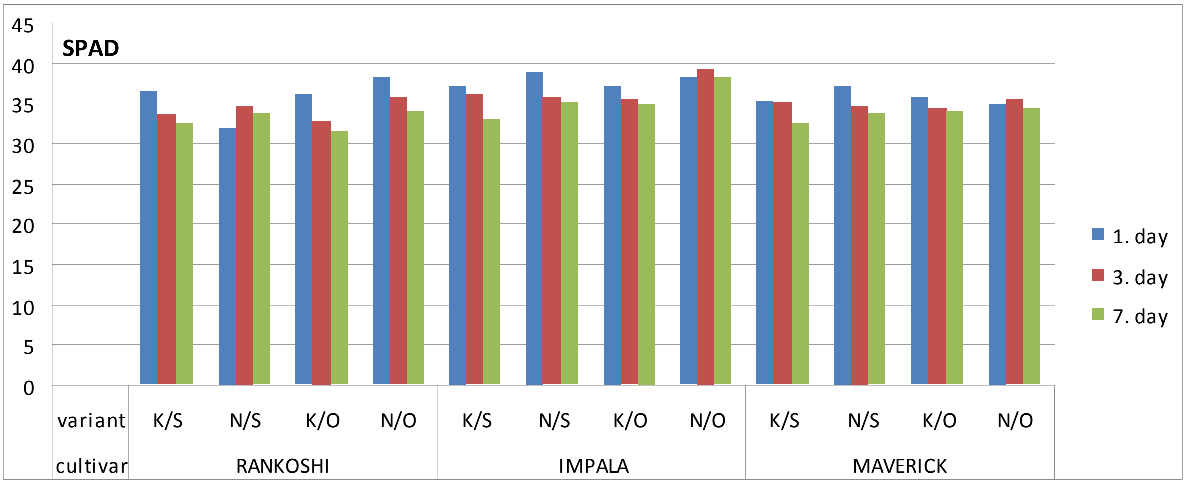
Tabuľka 1: Stresový index rastlín sóje indukovaný vodným deficitom

Soybean plants/ cultivar	KS/KO	NS/NO
RANKOSHI	**0,64**	**0,88**
IMPALA	**0,86**	**1,76**
MAVERICK	**1,45**	**0,36**

RWC relative water content
K/S control and water stress, N/S Nitrazon and water stress,
K/O control variant, N/O Nitrazon variant

Genotype IMPALA (South African Republic) was negatively reacting on the inoculation, when RWC dropped to 41,77 % (proline content 3.22 $\mu mol*g^{-1}$ 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 $\mu mol*g^{-1}$ 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).

Within the value measuring of SPAD – number which method is based on the measuring principle of chlorophyll content and nitrogen concentration was not monitored any noticeable effect of the inoculant usage in all observed soybean genotypes. Data about the concentration (SPAD value) in the specific variants from the experiment are included into the Figure 4.



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

Figure 4: Chlorophyll concentration (SPAD value) in soybean plants
Obrázok 4: Koncentrácia chlorofylu (SPAD hodnoty) v rastlinách sóje

The highest SPAD level was measured in IMPALA genotype, where this value was reaching the average 39.45 (Reddy, 2000). Vice versa, the lowest SPAD value was measured in RANKOSHI genotype 30.5. Thompson (1996) observed strong correlations of Chl content with SPAD readings and areal leaf mass (ALM) in soybean, and suggested that a portable SPAD Chl meter may be used to select for genotypes differing in ALM. Facile and rapid assessments of soybean N status by Chl meter would make this a very attractive method to screen large plant populations for genotypic differences in leaf N content as well.

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.

Growth and Number of Nodule-Creating Bacteria on the Soy 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, Figure 5).

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

Tabuľka 2: Počet vytvorených hrčiek na koreňoch rastlín sóje fazuľovej bez inokulácie a po inokulácii Nitrazonom

Amount of created nodules on the roots						
Soybean/	1. day		3. day		7. day	
cultivar	control	Nitrazon	control	Nitrazon	control	Nitrazon
RANKOSHI	1	14	4	16	6	19
IMPALA	1	12	3	13	4	14
MAVERICK	1	15	5	15	8	26

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

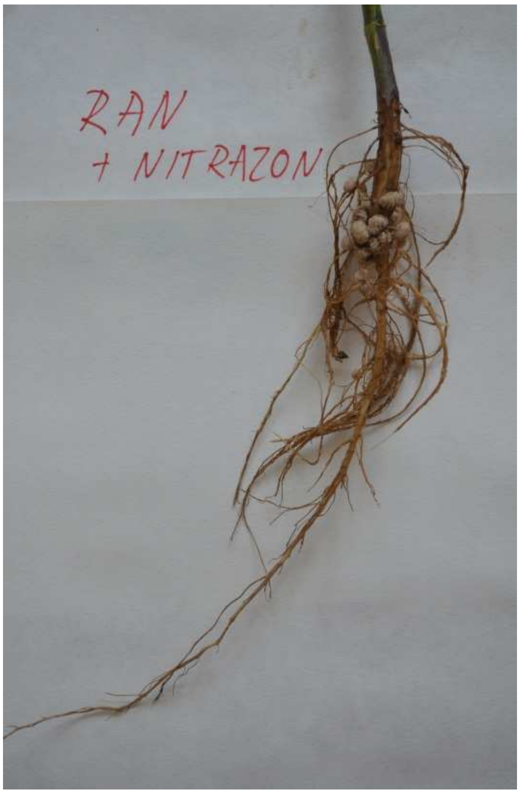


Figure 5: The soybean plant´s roots dependently on treatment and cultivar

Obrázok 5: Tvorba a počet hrčkotvorných baktérií na koreňoch rastlín sóje u všetkých sledovaných genotypov

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 family widespread all over the world. It comprises from 3 subfamilies – *Papilionoideae, Mimosoideae and Caesalpinioideae* to which belong more than 750 genus and 14,000 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).

Outer sign of the symbiosis is a creating of unique plant organs – nodules in which takes place the conversion of atmospheric nitrogen on ammonia form utilizing by the plants. Bacteria are attacking not only the main roots but also the roots of 2nd and 3rd rows which symptoms to the infection are shown as jellifying in the contact points and they penetrate to the root where they are procreating. The infection can be observed already during the third day when the roots become curly. Later the bacteria are releasing from the infected fibres to cytoplasm of host cells and in the fifth day they are “unhiding” by creating of nitrogen fixing nodules which reach maturity on the 12th day (Fischer, 1992).

During a reaction on flavonoids, which are excreting by the legume roots, bacteria produce specific lipo-chito-oligosaccharide signal of molecules – creating of outgrowths – nodules which activate the symbiosis in the legume roots. Primary aim of rhizobia as well as the nodules is the creating of root hair. When the legume root hair interacts with the bacteria, they start to turn around the bacteria and this way they can catch them in the root hair pocket. From this pocket, the plant gets infection through the fibre, starts to grow and places the bacteria into root cortex where the cell division takes part for a start of nodule-creating process. When they are placed in this nodule, bacteroids are detached from the bacteria and they start to fix atmospheric nitrogen (Downie, 1998; Nadelhoffer, 1994).

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 soy bean: content of inorganic nitrogen in soil - seed corn inoculation – starting nitrogen dose. It is justifiable to assume the required amount of nitrogen to ensure soy 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.

Conclusion

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 physiological criteria of resistance against water stress in soy 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 soy bean 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.

Croppers in the Slovak Republic have not still fully appreciated an importance of soy bean. It is only a loss for our croppers that a process of widening its sowing areas and with this connected building of manufacturing capacity for soy bean are presently very slow.

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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