THE EFFECTS OF PHOSPHORUS APPLICATION ON SOYBEAN PLANTS UNDER SUBOPTIMAL MOISTURE CONDITIONS

Vladimir ROTARU [1]

[1] **Institute of Genetics and Plant Physiology Moldovan Academy of Sciences, Chişinău, Republic of Moldova**

Abstract

A greenhouse experiment was conducted on soil to evaluate response of phsophorus (P) application in a factorial combination on growth and ureides contents of soybean (*Glycine mx L.*) cultivar Licurici. Soybean plants were grown in a soil very low in available P. The water treatments were 70% water holding capacity (WHC) as normal level and 35% WHC as stress drought. The all plants were grown till 4 weeks at normal water regime (70% WHC). Drought was imposed 4 weeks after sowing by withholding water from pots until 35% of soil water holding capacity was achieved. Suboptimal moisture of soil was imposed for 2 weeks. P application significantly increased dry weights of plants and leaf area irrespective of soil moisture level. Nodulation was the most susceptible to both abiotic factors P deficiency and water shortage. Plants at P deficiency and low levels of P supply had lower growth rates, higher ureides and amino acids concentrations in nodules and roots than plants grown with high (100 mg P kg^{-1} soil) P. The same trend of P influence was denoted in plants subjected to drought stress (35% WHC). P application much more raised DM and decreased level of ureides in roots under well-watered regime. Hence, adequate P nutrition maintained greater DM and activity of symbiotic system and attenuated the adverse effect of drought.

Key words: *Glycine max.,* phosphorus, ureides, water regime

Legumes plant plays an essential role in sustainability of agricultural systems. Their role is increasing during conversion of traditional agriculture to organic one. Legumes, in particular soybeans *(Glycine max. L.)* plants are considered as major sources of vegetable proteins and oils. Besides their economic importance they have also an ecological significance, diminishing atrophic pressure on the environment. Likewise, it is established that the main products of biological nitrogen fixation of tropical legumes are ureides. These compounds are transported outside of nodules through xylem to above ground plant parts. According to literature data N_2 fixation is very sensitive process to environmental stress factors (Sinclair T.R., and collab., 1995, Streeter, J.G., 2003). Nevertheless it was established that nitrogen fixation is severely affected by nutrients deficiency, particularly by low availability of phosphorus. Phosphorus deficiency is wide spread in many agricultural regions and it causes substantial economic losses (Sinclair, T.R., 2002). Some investigations have been demonstrated that is a strong relationship between the rates of nitrogen assimilation and plant productivity. Legumes have a high phosphorus requirement for growth and also for nodulation and N fixation (Israel, D.W., 1987). Sinclair and Serraj, 1995 showed that legumes species producing ureides are more sensitive to drought than amide producers. Ureides concentrations in the tissues have been used widely in assessment of the nutritional status of tropical grain legumes.

The higher P concentration in nodules demonstrates the higher demand of legumes for P nutrition. Some studies have established that improving plant nutrition with P contributed to increase plant tolerance to drought. It was observed that phosphorus fertilization stimulates root growth (Singh, D.K., Sale, P.W.G., 2000), photosynthesis (Freeden, A.L. and collab., 1989) and increase hydraulic conductibility of roots (Radin, J.W., M.P. Eidenbock, 1984). Although it is known the beneficial effect of P supply on crops there is scarce scientific information about interaction of P and water soil regime on physiological responses of soybean plants. In particular, there is not elucidated the effect of P application on ureides distribution in plant parts under water stress conditions.

The objective of the present study was to evaluate the influence of P supply on growth, biomass accumulation and ureides concentrations in reproductive stage (flowering) of soybean plants in relation to soil moisture regime. The identification of such morphological and physiological particularities of plant adaptation to unfavorable conditions could offer useful information for elaboration of legumes breeding program legumes in view to enhance efficiency of non-renewable resources.

MATERIAL AND METHOD

A pot experiment was conducted in a glasshouse with P deficiency soil. Treatments included the factorial combination of four P levels and two soil water regimes (control and water stress). There were four P application rates namely as 0, 10, 20 and 100 mg P kg^{-1} soil which were termed as P deficiency (P0), low phosphorus (P10), moderate low P (P20), and higher P (P100). All pots with P application received potassium (K) as KCl to equivalent potassium level. Each pot was filled with 6 kg soil of P deficient soil that was sieved before. The content of available phosphorus was 4,4 mg kg^{-1} (CAL method). Soybean seeds (cultivar Licurici) were treated with *Bradyrhizobium japonicum* at sowing time and after emergence plants were thinned to three per pot. The water treatments were 70% water holding capacity (WHC) as normal level and 35% WHC as stress drought. Pots were arranged in a complete randomized design with 4 replications per treatment. The all plants were grown till 4 weeks at normal water regime (70% WHC). Sub optimal moisture of soil was imposed for 2 weeks after 4 weeks of growth. At harvest plants were separated into leaves, stem plus petioles, roots and nodules and leaf area was determined. The soil was washed off the roots and the nodules separated and counted. Plant tissues were dried at 70 C to constant weights and weighed. The leaf area of a plant was measured with a leaf area meter (Li-3000). Ureides analysis was performed by standard method (People, M.B. et collab. 1989).

RESULTS AND DISCUSSIONS

The results of growth and development characters are presented in figures 1 and 2. It was established that abiotic factors affected significantly the rate of biomass accumulation. Phosphorus deficiency (treatment without P application) reduced substantial the plant productivity and its amount was only 58% and 53% of control plants, grown at sufficient P level (100 mg P kg^{-1} soil).

The best growth was recorded in variant with application of moderate dose of P. The mean effects of P fertilization showed that were significant responses to P supply just at low nutrient dose (P10). This result indicates that soil P was very low and soybean plants had a high response to P application. In our study there were wide differences in plant biomass production regardless water supply. The response to P supplemental nutrition was the greatest between 80 and 100 mg P kg^{-1} soil. The influence of fertilizer on soybean plants was affected by water soil regime (*fig. 1*). Drought diminished the positive effect of phosphorus on plant development. Similar effects were observed in other species of crops such as *Trifolium repens L.* (Singh, D.K., Sale, P.W.G., 2000), *Triticum aestivum* (He, Z.Q., Z.G. and collab., 2002). Therefore, legumes fertilization with phosphorus is justified as in normal moisture of soil as well as in water stress conditions. In some studies it was shown that the P deficit has different effects on plant organs (Gutierrez-Boem, F., Thomas, G.W., 1999). Leaves development are very sensitive to low P supply. Insufficient plant nutrition with phosphorus affected more pronounced leaves growth than roots.

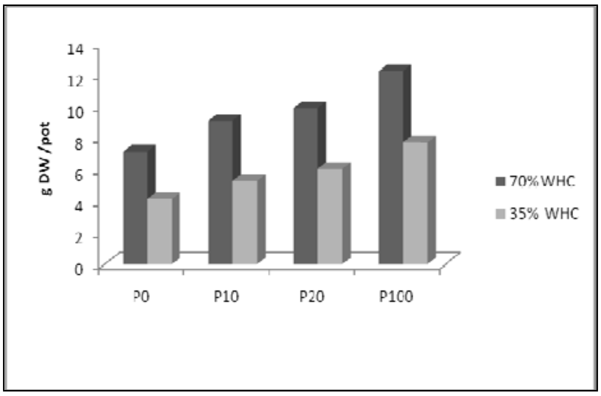

Figure 1 **Biomass acumulation by soybean plants in relation to P supply and water regime**

It is necessary to note that plant productivity correlated positive with leaf area data, which provide on one hand symbiotic system with carbohydrates, on the other hand they are considered strong sinks of nitrogen compounds, include the products of biological nitrogen fixation. In this respect it is important to maintain a good growth of leaves indifferent of environmental conditions. Our experimental data demonstrated that application of moderate P level stimulated the development of assimilation canopy in both moisture conditions optimal and stress (*fig.2*). But P deficiency reduced this parameter by 40% in comparison with treatment with adequate phosphorus nutrition.

Leaf area development had the same trend as biomass accumulation. Evaluation of leaf area of plants grown under stress conditions revealed that leaves growth was decreased by insufficient P nutrition. So, our data were in agreement with the results of other researchers (Israel, D.W., 1987, Singh, D.K., Sale, P.W.G., 2000).

In this connection it must be emphasized that P application under sub optimal water level attenuated the negative impact of drought on canopy development. Besides leaves, P fertilization also improved roots growth of soybean plants regardless water supply level. In this case P supply stimulated the utilization rate of assimilates in root system. These alterations lead to increase phosphorus acquisition and as well as other nutrients, in particular nitrogen that was well demonstrated in our previous study (Rotaru, V., and collab., 2006). The formation a vigorously root system provide a higher tolerance to unfavorable conditions.

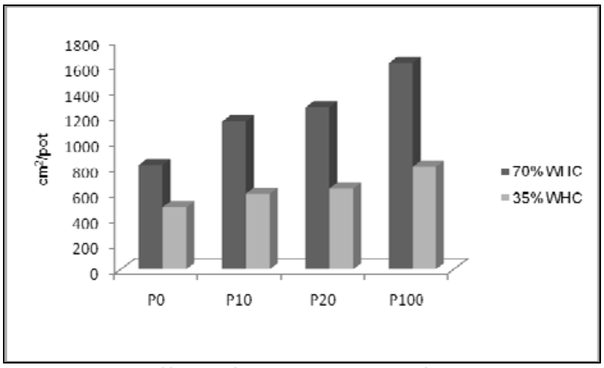

Figure 2 **Effect of P supply on leaf area under suboptimal soil moisture**

In this connection it must be emphasized that P application under sub optimal water level attenuated the negative impact of drought on canopy development. Besides leaves, P fertilization also improved roots growth of soybean plants regardless water supply level. In this case P supply stimulated the utilization rate of assimilates in root system. These alterations lead to increase phosphorus acquisition and as well as other nutrients, in particular nitrogen that was well demonstrated in our previous study (Rotaru, V., and collab., 2006). The formation a vigorously root system provide a higher tolerance to unfavorable conditions.

On average there were larger differences between treatments in nodulation capacity as well as between water soil regimes. The best nodulation was established in treatment P100 under well-watered conditions. Drought significantly reduced the beneficial effect of P application on nodulation (*fig.3*).

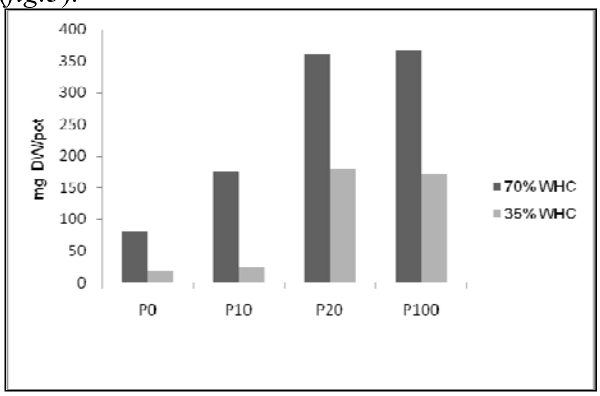

Figure 3 **Effect of P fertilization on nodules growth of soybean in relation to soil water regime**

In treatment with P deficiency dry weights of nodules were reduced more 8 times under water stress and 2 times under normal water regime. However, the nodule dry weight did also responded to low P supply as plant growth. This suggested that nodulation is sensitive to P nutrition. Under low soil P conditions plants form fewer and smaller nodules. Low sensitivity of nodulation to environmental stress factors was observed in other species (Singh, D.K., Sale, P.W.G., 2000). The poorest nodulation was revealed in treatment without P supply under water stress conditions. In Israel's (Israel, D.W., 1987) experiments it was shown that nodules initiation has a high demand in P nutrition. Hence, the results of present study confirmed the importance of plant nutrition with phosphorus under suboptimal moisture of soil. Therefore, the nodulation process responded significantly to supplemental phosphorus nutrition in both water regimes. The analysis of literature data revealed a relationship between dry weights of nodules and ureides concentrations in vegetal tissues. Maintaining the rate of nitrogen assimilation at high level improve tolerance of plants to stress abiotic factors. Supplemental mineral nutrition of plants had an important role not only in nodules growth but also in stimulation of physiological activity of symbiotic system.

It is well documented that the synthesis of ureides, the major nitrogen compounds, is localized in nodules. Ureides in soybean plants constitute more 80% of total nitrogen that are allocated from nodules to above ground plant parts and they are involved in further process of ureides catabolism (McClure, P.R., D.W. Israel, 1979.

That's way the formation a strong symbiotic system contributes to better nutrition of plants with nitrogen, which is necessary to obtain an acceptable yields and quality.

There have been confirmed that drought severely inhibits nitrogenous activity (Streeter, J.G., 2003), N_2 fixation and nodulation (Minguez, M.I., F. Sau, 1989). From this point of view nodulated plants are more susceptible to shortage of water than legumes based on mineral nitrogen nutrition (Minguez, M.I., F. Sau, 1989). Our experimental results established that P deficiency induced the accumulation of ureides in roots and nodules. Nevertheless, drought increased the adverse effect of P deficiency on ureides metabolism as well as on their distribution within plants parts. Accumulation of these metabolites in nodules triggered feedback mechanism of N_2 fixation (Serraj, R., V. Vadez, T.R. Sincalir, 2001). So there were significant differences in ureides concentrations between nutrient treatments. Experimental data showed that the lowest concentration of these compounds in nodules and roots were observed in adequate P supply. This result confirmed that P is involved in the process of ureides catabolism as well as in their elimination from nodules. Elimination of these compounds from nodules stimulates activity of NG and in consequence there are favorable physiological conditions for better increases the sinks capacity, stimulating flux of ureides to shoots. In addition, improving mineral nutrition increases the hydraulic conductivity of roots, associated with more water and nutrients acquisition.

Hence, the experimental results of this study proved that P fertilization stimulates ureides production and utilization and also induce their transport to the shoots. According to our results and from literature data it emergences a need to continue the research focused on identification of soybean genotypes with high capacity of adaptation to unfavorable environmental conditions.

Table 1

Ureides concentrations in soybean plant parts in response to P fertilization and soil moisture conditions ($\mu M\ g^{-1}$ m.u.)

Treatments	Leaves		Roots		Nodules	
	Control	Drought	Control	Drought	Control	Drought
P0	7,82	9,70	3,86	5,81	9,47	21,71
P10	9,47	14,63	4,24	5,35	23,60	33,13
P20	10,66	16,18	2,29	4,79	36,76	45,04
P100	17,56	15,78	1,36	3,04	43,72	47,51

CONCLUSIONS

Large differences were found in all the parameters (growth, leaf area, and nodulation and ureides contents) in plants in relation of P fertilization and water soil regime.

Nodulation was very sensitive to both environmental constraints P deficit and drought and the poorest nodulation was found in treatment without P supply under water stress.

-Application of P fertilizer could offset the impact of drought stress during the reproductive period, resulting in less growth losses and maintaining functionality of systems Glycine max-Bradyrhizobium japonicum at adequate level.

BIBLIOGRAPHY

Freeden, A.L., Rao, I.M. and Terry, N., 1989 - *Influence of phosphorus nutrition on growth and carbon*

Gutierrez-Boem, F., Thomas, G.W., 1999 - Phosphorus *nutrition and water deficits in field–grown soybeans*. Plant and Soil, v. 207, p. 91-95.

He, Z.Q., Zhu, Z.G., Smith, S.E., and Smith, F.A., 2002 - *Interactions between soil moisture content and phosphorus supply on spring wheat plants grown in pot culture*. J.Plant Nutrition, v. 25, 4, p. 913-925.

Israel, D.W., 1987 - *Investigation of the role of phosphorus in symbiotic dinitrogen fixation.* Plant Physiol. v. 84, p. 294-300.

McClure, P.R. and Israel, D.W., 1979 - *Transport of nitrogen in the xylem of soybean plants.* Plant Physiol. v. 64, p. 411-416.

Minguez, M.I., Sau, F., 1989 - *Responses of nitrate-fed and nitrogen-fixing soybeans to progressive water stress*. J.Exp.Bot. v. 40, p .497-502.

People, M.B. et,al. 1989 - *Methods for evaluating Nitrogen Fixation by nodulated legumes in the Field.* Australia 76p.

Radin, J.W., and Eidenbock, M.P., 1984 - *Hydraulic conductance as a factor limiting leaf expansion of phosphorus–deficit cotton plants*. Plant Physiol. v. 75, p. 372-377.

Rotaru, V., Gojineţchi, O., Toma S, 2006 - *Influenţa nutriţiei minerale asupra activităţii NR şi distribuirii biomasei în organele plantelor de soia în condiţii de secetă.* Buletinul AŞ a RM. v. 3, Chişinău, p. 51-58.

Serraj, R., V. Vadez, T.R. Sincalir, 2001 - *Feedback regulation of symbiotic N_2 fixation under drought stress*. Agron. v. 21, p. 621-626.

Sinclair T.R., Serraj R., 1995 – Dinitrogen fixation sensitivity to drought among grain legume species. Nature, 378, p. 344.

Sinclair, T.R. and V. Vadez, 2002 - *Physiological traits for crop yield improvement in low N and P environments.* Plant and Soil, v. 245, p. 1-15.

Singh, D.K. and Sale, P.W.G., 2000 - *Growth and potentially conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency*. Agronomy J., v. 92, p. 868-874.

Streeter, J.G., 2003 - *Effects of drought on nitrogen fixation in soybean root nodules.* Plant, Cell and Envir. v. 26, p. 1199-1204.