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EFFECTS OF DIFFERENT PHOSPHORUS LEVELS ON UREIDES CONTENTS AND NODULATION OF SOYBEAN (*Glycine max L.*) IN RELATION TO SOIL MOISTURE REGIME

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Abstract

Soybean is a legume crop and it may fix efficiently atmospheric nitrogen (N) if an adequate amount of phosphorus (P) is present in the soil. The main product of nitrogen fixation in soybean root nodules is ureides. A greenhouse experiment was conducted on a low soil P silly clay soil- sand mixture to evaluate the effect of P application and water regimes in a factorial combination on ureides contents and nodulation of soybean (Glycine max L. cv Zodiac). Soybean plants were grown in a soil very low in available P. Seeds were inoculated with bacteria Bradyrhizobium japonicum at sowing. The P application significantly increased ureides concentration and dry weights of nodules irrespective of water soil regime. In general, low amounts of ureides in nodules were found in watered plants, but those levels were significantly increased in stressed plants. Maximum dry weight of nodules and plants was obtained at 100 mg P kg^{-1} soil under normal water regime of soil (70% WHC). The same trend of P influence was denoted in plants subjected to drought stress (35% WHC) but its effect was reduced by insufficiency water supply. Hence, the adequate P supply raised DM accumulation and chlorophyll contents but decreased level of ureides in roots of soybean plants. This research indicates that the ureides concentrations in plant tissues are more sensitive to P deficiency and drought than chlorophyll contents in leaves. The obtained results demonstrated that the adequate P nutrition of soybean significantly increases nodulation and ureides production in comparison to unfertilized treatment.

Key words: *drought, nodulation, phosphorus, soybean, ureides.*

INTRODUCTION

Legumes are an important component of the agroeosystems in many countries due to their capacity to produce significant quantities of protein-rich seed and to improve soil quality by inclusion of organic matter and biological nitrogen fixation (Vance et al., 2003, Vance, 2001). They are grown in a range of environments and nutrient deficiency and water supply are considered the major ecological factors limiting growth and yield. Worldwide, at least 45% of the total agricultural land area, some 5,8 billion hectares suffers from phosphorus deficiency (Batjes, 1997). Therefore, P deficiency is a common problem in many soils and it is considered a major constraint for the production of legumes also in the Republic of Moldova (Andrieş, 2007). Phosphate fertilizer application remains the most effective way to increase crop productivity in soils with low levels of plant-available phosphates. Soil moisture plays a critical role in both nodule formation and N2 fixation. Low soil moisture during the early stages of the plant growth decreases nodule formation (Gan et al., 2008), and low moisture during late vegetative to early flowering period decrease efficiency of N2 fixation. In general, drought stress strongly inhibits plant growth and development, but the application of suitable fertilizers can alleviate these effects (Zhu et al., 2009).

Although P deficiency and drought are extremely important in cropping systems, however, in majority of cases they have been studied separately. It is well documented that the ureides allantoin and allantoate are major forms of nitrogen transported from root nodules to shoots in tropical legume. Assimilation of atmosphere nitrogen has a higher demand of P nutrition because nitrogen fixation required considerable sources of energy.

Ureides synthesized in the nodules are transported to the shoots where they should be degraded and their N content re-assimilated.

The activity of symbiotic system *Glycine max-Bradyrhizobium japonicum* is estimated by ureides production and partitioning within soybean parts. The objective of this study was to evaluate the effects of different levels of fertilizer P on ureides partitioning between leaves, roots and nodules as well as on nodule growth in relation to water soil regime in soybean.

MATERIALS AND METHODS

A pot experiment was conducted in a glasshouse at the Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics Department, University of Kassel, Germany. Treatments included the factorial combination of four P fertilization levels, two soil water regimes (control and water stress) and a soybean (*Glycine max.* L. Merr) cultivar namely Zodiac. 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) and 11,5 mg kg^{-1} by Olsen method (Olsen and Sommers, 1982), pH ($CaCl_2$) 7,74, total N - 0,04% and C - 1,42. At 5 days after emergence plants were thinned to three ones per pot.

The water treatments were 70% water holding capacity (WHC) as normal level and 35% WHC as insufficient moisture. The all plants were grown during 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. Suboptimal moisture of soil was imposed for 2 weeks. Normal and low water supplies were maintained by weighing the pots every day and on the basis of weight loss, re-watering them to corresponding weights. Mean night temperature ranged from 18-20 C and mean day temperature varied 26-28 C. Relative humidity varied between 60-65%. The pots were placed on tables and rotated every 2 days for random distribution in a greenhouse. Chlorophyll content readings were taken with a handheld dual wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). The instrument stored and automatically averaged these readings to generate one reading per plot (Richardson et al., 2001).

Ureides in plant tissues were determined by basic hydrolysis of allantoin to allantoic acid, acid hydrolysis of allantoic acid to glyxoylate and urea, and spectrophotometrically determination of glyoxylate after its reaction with phenylhydrazine and ferric cyanide (Vogels and Van der Drift, 1970).

Data in figures represent the average value of the results of chemical analysis of plants in three replications. The experimental results were analyzed statistically, determining significant differences at the level of P = 0,05. In figures are presented means of 3 replicates ± standard deviations. The STATISTICA (version 7) package was used for statistical analysis.

RESULTS AND DISCUSSIONS

The ureides have been identified as the export products of N_2 fixation of many tropical legumes in particular soybean (McClure and Israel, 1979). Although the effects of P nutrition on early growth, nodule activity of soybean plants are known (Sa and Israel, 1995), information is not available in the literature on the pattern of ureides-N allocation within the soybean plant in relation to soil moisture regime. In this experiment ureides concentration and partitioning within plants of soybean were altered by both abiotic factors water regime and P supply. Ureides content in leaves and nodules are shown in figures 1 and 2. It was observed a low concentration of ureides in leaves and nodules of soybean under low P supply (treatment without fertilization). These trends were revealed in both water regimes. Adequate P nutrition (100 mg P kg^{-1} soil) stimulated the production of ureides in nodules which was associated with decreasing of these compounds in roots. These metabolites of nitrogen fixation in leaves changed not so much under normal water regime. However, the supplemental P nutrition increased ureides level in leaves. Probably, improvement of mineral nutrition stimulated canopy development and in consequence increased demand for nitrogen compounds to maintain good growth. Similar trends were observed in plants of *Phaseolus vulgaris* (Vadez et al., 1999).

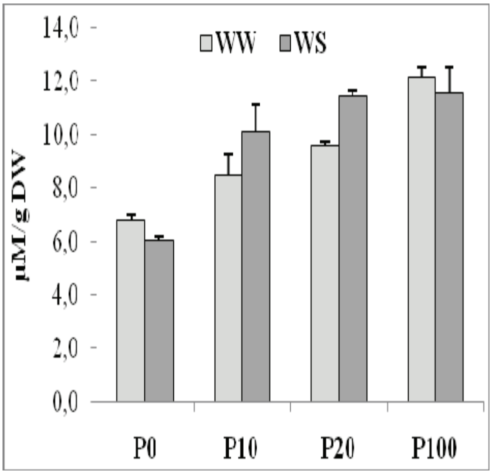

Figure 1. Effect of phosphorus application on the ureides concentrations (µM/g DW) in leaves of soybean in relation to soil water regime

Our study also showed that the negative effect of moisture stress on nodule efficiency was reduced when the plants were supplied with P fertilizer. Decreased ureides N concentrations in leaves tissues demonstrate, as has been reported previously (Israel, 1987), that P low supply imposed some degree of N deficiency on symbiotically soybean plants. Soybean plants are sensitive to soil moisture regime. The experimental data have shown that water deficit induced accumulation of these N metabolites in tissues irrespective of P supply. In particular, under water stress conditions there was a higher accumulation of ureides in nodules in comparison to well-watered plants (Figure 2).
Increased concentrations of ureides were observed in the leaves and nodules when increasing the level of P in the soil (Figure 1 and 2). The opposite effect of phosphorus supply was observed in the roots. The increase of P dose decreased the ureides concentrations in soybean roots. Probably, the improvement of phosphorus nutrition facilitated the transport of metabolites in the shoots. In general, dry weights of roots were higher under P supplemental nutrition than in control treatment (data are not shown).
The proportion of coarse roots increased under fertilization of phosphorus; therefore this in turn could increase the translocation of ureides

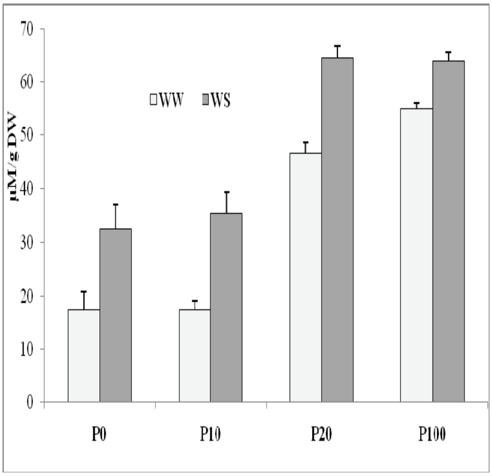

Figure 2. Effect of phosphorus application on the ureides concentrations (µM/g DW) in nodules of soybean in relation to soil water regime

The lowest P level was clearly limiting the capacity of N2 fixation by nodules. Likewise, it is necessary to note that the low soil moisture had a negative effect on the translocation of N compounds from nodules to aboveground parts in the forms of ureides. Reduction in symbiosis efficiency due to low water regime and P deficiency has also been reported by other researchers (Gan et al., 2008). Sa and Israel (1995) observed a decreased flux of ureides and other N constituents in the xylem sap of P-deficient soybean plants grown hydroponically. It is known that grain legumes transporting fixed N as amides, such as chickpea, faba bean and lupine are less sensitive to water stress than those transporting ureides, such as soybean. Hence, low soil moisture restricted ureides translocation out of the nodules. The experimental data are consistent with the observations reported in *Medicago sativa* cultivated under P insufficiency (Suleiman et al., 2013). However, the P application increased this physiological trait under normal water regime. Such findings were observed in common bean (Vadez et al., 1999) and in lupine (Shulze et al., 2006).

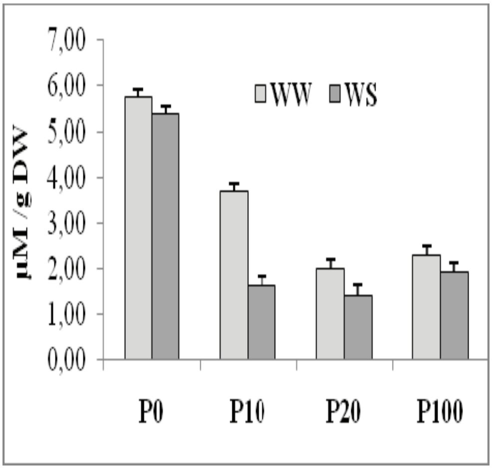

Figure 3. Effect of phosphorus application on the ureides concentrations (µM/g DW) in roots of soybean in relation to soil water regime

The fertilization with P in moderate and high rates decreased significantly ureides concentrations in roots (Figure 3).

The development of N-fixing nodules on legumes roots upon invasion of *Bradyrhizobium* bacteria is subjected to regulation by environmental factors. In particular, the extent of nodulation is restricted by water deficit and low P supply.

There is a realtionship between ureides production and nodule growth. Changes in ureides concentration paralleled the change in nodule mass produced by increasing P levels in the medium (Figure 4).

It is well documented that P supplementation had significant impact on nodulation of legumes. However, little is known about the effect of P on nodules developmnet in relation to soil moisture conditions.

Our results for nodule dry weight are presented in figure 4. The P supplementation had great influence on nodule dry weight. There was a significant interaction ($P < 0.05$) among abiotic factors and location in nodule dry weight. Highest mean nodule dry weight was recorded in treatments with higher levels of P fertilization compared to non treated control in both water soil regimes.

Also, there was found a large difference in nodule dry weight between watered and stressed plants irrespective of P nutrition level, but P supplementation attenuated the negative influence of drought (Figure 4). Therefore, the results of this study were shown that with application of phosphorus up to 100 mg kg^{-1} soil dry weight of nodules per plant was increased. So it was stated that the P application had beneficial impact on nodules formation and plant biomass in soybean. The experimental results demonstrated that the nodulation was improved by P supply at both soil moisture levels, but its effect was more pronounced under not limiting water regime. This effect suggests that P limited nodule formation and growth whereas shoot and root growth were less affected (data are not presented). Drought conditions had a very negative impact on nodulation and their growth (Figure 4). Therefore, we conclude that inhibitory impact of water deficit on nodulation could be partially reversed by increased P availability as suggested by a previous report (Tsai el al., 1993).

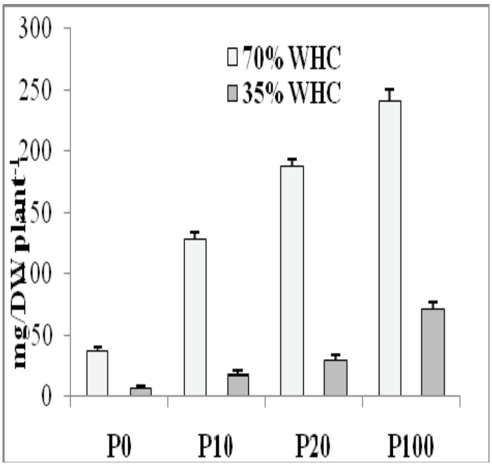

Figure 4. Mean nodules dry weight (g) of soybean in relation of P supply and moisture of soil

The growth and activity of nodules depends on assimilates supply. Chlorophyll maintenance and consequently photosynthesis durability in stressful conditions are among physiological indicators of stress resistance (Zhang et al., 2006). The photosynthetic intensity is determined by chlorophyll concentration in leaves and has significant effects on nodules supply with resources.

Phosphorus supply affect the leaf chlorophyll contents, which influences the leguminous plant to manufacture its own food through photosynthesis process, which ultimately increases yields and uptake of important nutrients in different soybean plant tissues (Imsande, 1988). Phosphorus plays a very important function in almost every plant process that involves energy transfer. High-energy phosphate, detained as a part of the chemical structures of adenosine diphosphate (ADP) and adenosine triphosphate (ATP), is the source of energy that drives the huge number of chemical reactions within the plant.

Results presented in figure 5 shows significant effect of phosphorus supplementation on the leaf chlorophyll content for glasshouse experiment. The experimental results demonstrated an increase of the chlorophyll value in treatments with higher dose of P compared with unfertilized control.

The lowest SPAD value was obtained at P0 treatment. Based on obtained data, there was a positive correlation between number of nodules per plant and SPAD value.

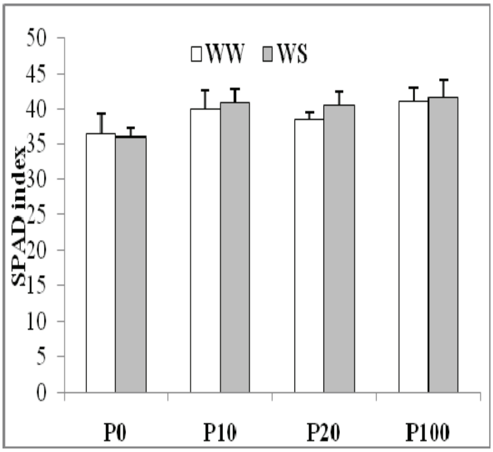

Figure 5. The influence of P supplementation on SPAD values of soybean leaves in relation to soil water regimes (5 days after dry soil imposition)

The results of leaf chlorophyll analysis showed that P fertilizer significantly increased the total chlorophyll contents relative to the control treatment at 5 and 10 days after water deficit imposition in the green house experiment (Figure 5 and 6).

The application of P fertilizers generally improved total chlorophyll content in soybean leaves relative to the control treatment for both soil moisture levels.

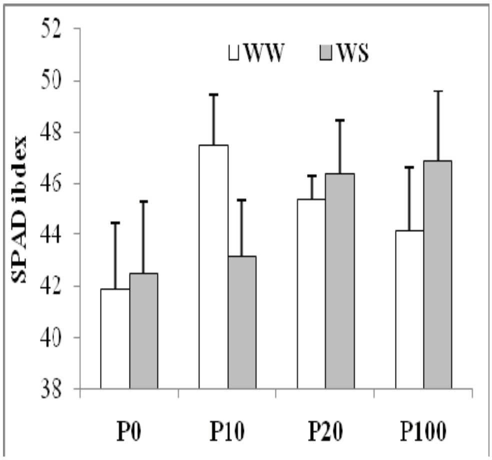

Figure 6. The influence of P supplementation on SPAD values of soybean leaves in relation to soil water regimes (10 days after dry soil imposition)

Hence, the experiment results revealed the positive role played by P fertilizer on chlorophyll contents of the soybeans as it significantly increased total chlorophyll content by 14 % and 6 % for the reading taken after one week after subjected plants to low water supply (35% WHC). It seems that P could partially counteract the detrimental effect of P deficiency and drought on photosynthetic activity of leaves which in turn affect the N_2-fixation capacity of soybean.

In addition, experimental results of this study confirm that the metabolites production of the N_2-fixation process is the stage in the nitrogen assimilatory pathway of nitrogen fixing plants that is sensitive to phosphorus deficiency as well as to water deficit.

Therefore, the fertilization with phosphorus of soybean plants grown at a low accessible phosphates increased nodulation, photosynthetic pigments concentrations and ureides metabolism compared to unfertilized control plants.

CONCLUSIONS

The present study indicated that the P application reduced adverse effects of low water regime on ureides production and nodulation of soybean. Greater nodules growth under low water supply could play an important role in acquisition of N from atmosphere. Results showed that there were significant growth and ureides partitioning responses to P supply in relation to soil moisture level. In general, nodule growth was enhanced by supplemental phosphorus nutrition under optimal soil moisture conditions, demonstrating the requirement of adequate soil moisture for better growth.

However, it should be noted that these results came from greenhouse investigations; therefore, it would be necessary to carry out further experiments under field conditions which would be quite important research for sustainable crop production under unfavorable environmental conditions.

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