PROLINE CONTENTS IN TWO SOYBEAN CULTIVARS IN RELATION TO NUTRIENT SUPPLY UNDER LOW SOIL MOISTURE REGIME

CONŢINUTUL DE PROLINĂ A DOUĂ CULTIVARE DE SOIA SUB INFLUENŢA FERTILIZĂRII, ÎN CONDIŢII DE UMIDITATE SCĂZUTĂ A SOLULUI

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Abstract. *Proline accumulation is a common physiological response in many plants in response to a wide range of biotic and abiotic stresses. Response of free proline accumulation in two soybean (Glycine max.L.) cultivars to phosphorus (P) and iron (Fe) application under suboptimal water regime of soil at the critical flowering stage was studied in a pot experiment. P and Fe were applied at rate 100 mg and 5 mg per kg of soil, respectively. Plants were subjected to low water regime for 2 weeks at flowering stage. Plant dry matter accumulation of both cultivars increased with increasing P level regardless of soil water regimes. After two weeks of water stress (35% WHC- water holding capacity) dry mater production was significantly reduced whereas concentrations of free proline was increased in leaves and roots of both cultivars in treatment without fertilization. Cultivar Zodiac maintained higher level of proline accumulation than Licurici. Recovery upon re-watering was evidently in fertilized-plants than unfertilized plants. Cultivar Zodiac had a higher root/plant ratio of dry matter than Licurici under low nutrient and water environment. We suggest that application of nutrients could partially attenuate the adverse effect of drought on soybean productivity.*

Key words: *Glycine max.* L., iron, phsophorus, proline, water stress**.**

Rezumat. *Acumularea prolinei este considerată ca o reacţie fiziologică de răspuns a plantei la factorii abiotici nefavorabili. În condiţiile casei de vegetaţie s-au efectuat experienţe cu două cultivare de soia unde s-a examinat acţiunea aplicării fosforului (P) şi fierului (Fe) asupra modificărilor conţinutului de prolină în organele plantelor, în dependenţă de regimul de umiditate a solului. Plantele de Glycine max., L. au fost cultivate pe sol cernoziom carbonatat asigurat insuficient cu fosfaţi mobili. Fosforul şi fierul s-au aplicat în doze de 100 mg şi 5 mg per kg de sol. După două săptămâni de stres hidric (35% din CTA – capacitatea de apă a solului) acumularea substanţei uscate s-a redus semnificativ şi a fost asociată cu creşterea concentraţiei de prolină în frunze şi rădăcini la ambele cultivare, indiferent de nivelul nutriţiei minerale. Cultivarul Zodiac a manifestat o capacitate mai mare de acumulare a prolinei comparativ cu cultivarul Licurici. Reirigarea plantelor stresate a diminuat concentraţia prolinei. Plantele cultivarului Zodiac au înregistrat valori mai mari a raportului rădăcini/plantă, în condiţii vulnerabile de nutrienţi şi umiditatea solului. Aşadar, fertilizarea plantelor de soia poate parţial să reducă efectul advers al secetei asupra productivităţii.*

Cuvinte cheie: *Glycine max.* L., fier, fosfor, prolină, stres hidric.

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INTRODUCTION

Abiotic stresses are believed to cause major problems in agriculture by reducing crop growth and productivity. Phosphorus (P) deficiency and drought are major environmental abiotic factors restricting plant growth and development in many regions of the world, and while the two stresses often occur simultaneously, little is known about how their combination impacts crops (Rizhsky L. et al., 2004). Phosphorus fertilizer application remains the most effective way to increase crop productivity in soils with low levels of available phosphates (Burman U. et al., 2009). Application of large amounts of P fertilizers is also likely to cause a reduction in the bioavailability of micronutrients as well as concentrations in plant tissues. Phosphorus has been shown to have interactive effects with the uptake of iron (Fe) (Raeini-Sarjaz M., Barthakur N., 1995).

Soybean (*Glycine max., L.*) is the most widely grown leguminous crop providing large amounts of protein and oil for the human diet and animal husbandry. This crop is sensitive to environmental stress situations, such as phosphorus and water deficiency (Sinclair T., Vadez V., 2002; Lakshmi P. et al., 2009). For a legume crop such as soybean, the role of phosphorus and iron nutrition is very critical (O'Hara G. et al., 1988; Gunawrdena S. et al., 1993). In addition to supplying a nutrient for plant growth, P application could improve drought tolerance of crops to increase productivity under water stress environment (Burman U. et al., 2009). Accumulation of compatible solutes is one of the adaptive strategies of plants in response to abiotic environmental stresses. Accumulation of these solutes like proline, glycine betaine and sucrose contributes to osmotic adjustment, prevention of protein denaturation, preservation of enzyme structure and activity and protection of membranes from damage by reactive oxygen species (Hare et al., 1999). Many studies have focused on the interactions of P and Fe on nutrient absorption by higher plants, but little attention has been paid to their interactive impacts on the physiological metabolism in particular on proline contents in plants.

The objective of the present experiment was to investigate the effect of P and Fe supply on plant growth and proline concentrations under suboptimal water regime in two soybean cultivars differing in potential of productivity.

MATERIAL AND METHOD

The pot experiment was conducted in a glasshouse under controlled conditions with cernoziom carbonate, characterized by low of plant-available phosphates. Treatments included the factorial combination of two P levels, two soil water regimes (control and water stress) and two soybeans (*Glycine max*. L.Merr.) cultivars classified as low productivity (Zodiac) and high productivity (Licurici). Phosphorus was supplied as KH_2PO_4 at 0 and 100 mg per kg of soil (P0 and P100, respectively). Iron was supplied as Fe-EDTA at 0 and 5 mg per kg of soil (Fe0 and Fe5, respectively). All the treatments had four replicates. Each replication was the average of three plants per pot. Seeds of soybean were treated with *Bradyrhizobium japonicum* at sowing time.). At flowering stage of plant development half of pots were brought to 70% WHC and the other half to 35% WHC as suboptimal moisture level. Normal and low water

supplies were maintained by weighing the pots and on the basis of weight loss, re-watering them to corresponding weights. Suboptimal moisture of soil was imposed for 2 weeks. Free proline content in leaves and roots was determined according to the methodology of Bates B. et al., (1973), and expressed as µg proline g^{-1} fresh matter.

RESULTS AND DISCUSSIONS

One of important characteristics of plant metabolism under stress environment might be attributed to an accumulation of free proline which play an essential role in osmoregulation and osmotolerance as well as protection of proteins to overcome adverse responses from water stress (Ashraf M., Foolad M., 2007). The impact of nutrients supply and soil moisture regime on proline contents of soybean organs is presented in Figures 1 - 3.

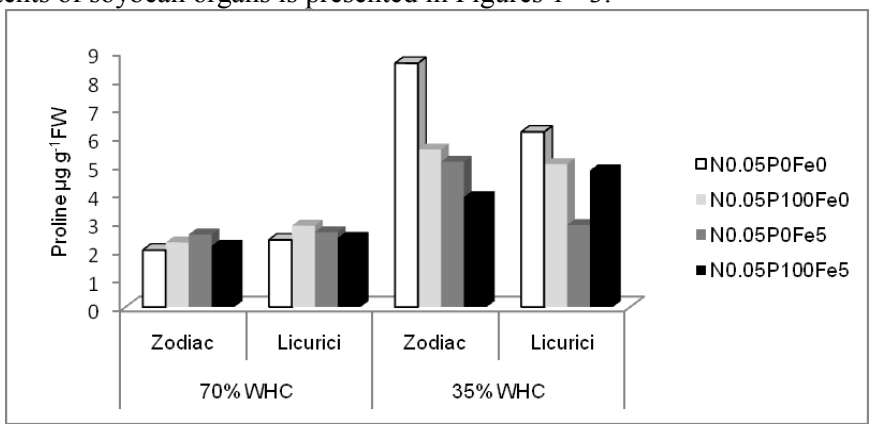

Fig. 1 - Proline concentrations in leaves of soybean cultivars in relation to nutrient supply under well-watered and drought conditions

The results have been shown that concentration of free proline was obviously increased under unfavorable moisture conditions. Proline accumulation in plant tissue under stressful conditions has been suggested to be result of a decrease in proline degradation, increase in proline biosynthesis, a decrease in protein synthesis or proline utilization and increased hydrolysis of proteins (Hare P. et al., 1999). Thus, both cultivars had higher proline accumulation during water-stressed than under non water-stressed conditions, but they differed in their response to added P. In this respect cultivar Zodiac overcame Licurici and these trends were consistent for both leaves and roots. Such a drought tolerance of cultivar can posses a good capacity to osmotic regulation under water stress. Phosphorus application increased its concentration in leaves by 13,5% in Zodiac and by 21,2% in Licurici in normal water soil regime. The alleviation of water stress by means of P application was confirmed for cluster bean and wheat species (Burman U. et al., 2009; Gutierrez-Boem F., Thomas G., 1998). The increase of free proline concentrations in cultivar Zodiac was superior than in Licurici under limited water conditions. Likewise, iron supplemental nutrition increased this

physiological parameter by average 21,6% and 17,5% in leaves of Zodiac and Licurici, respectively.

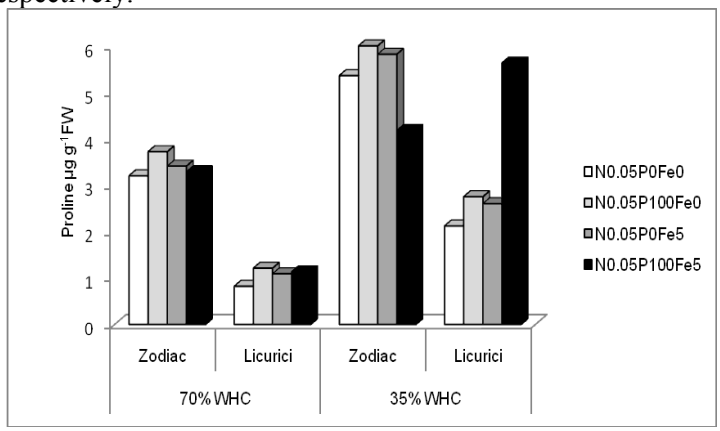

Fig. 2 - Proline concentrations in roots of soybean cultivars in relation to nutrient supply and soil water regime

Abiotic factors affected also the pattern of proline allocation to the roots (fig. 2). Under limited water supply in treatment without nutrient fertilization cultivar Zodiac accumulated greater free proline in roots approximately by 3 fold than Licurici. Experimental results revealed that iron addition in combination with P decreased its concentration in roots of Zodiac and did not affect its contents in Licurici. It was well documented that levels of soluble osmotic nitrides in plant tissues might be responsible for maintaining better water status and maintain the growth at higher level (Hare P. et al., 1999). Two days after plants were relieved of water stress proline accumulation in leaves decreased extensively, while proline accumulation in Licurici leaves decreased to the level for non water-stressed plants (data are not presented). Several investigators had reported similar observations. Therefore, high proline accumulation in leaves of water-stressed plants might be an adaptive response to drought.

Phosphorus deficiency as well water deficit is one of the important abiotic stresses and substantially affects productivity of crops. In this study plant growth was evaluated by measuring dry matter (DM) accumulation. Nutrient deficiency as well as shortage of water significantly decreased dry mass production in both cultivars (table 1). The application of nutrients separately or in combination increased the plant growth of both control and water stress plants. There was observed a cultivar difference in this regard. The reduction of plant growth was decreased by drought more evidently in Licurici than in Zodiac. According to registered data the differences between cultivars in term of dry matter production were negligible in treatment with sufficient P and Fe supply compared to treatment P0Fe0. Likewise, the iron supplemental nutrition had some beneficial influence on plant development in scarce water environment but at lower extent than application of phosphorus.

Table 1

Effects of phosphorus and iron application on dry matter (DM) of two soybean cultivars under suboptimal soil moisture conditions, g/pot

Treatments	70% WHC				35% WHC			
	Zodiac		Licurici		Zodiac		Licurici	
	DM	R/P*	DM	R/P	DM	R/P	DM	R/P
N0.05P0Fe0	10,72±0,13	0,24	14,75±0,19	0,24	8,64±0,09	0,29	10,12±0,35	0,27
N0.05P100Fe	15,99±0,28	0,22	23,11±0,14	0,21	13,40±0.16	0,26	14,63±0,06	0,21
N0.05P0Fe5	11,89±0,11	0,24	16,10±0,22	0,25	8,90±0,14	0,27	11,52±0,27	0,21
N0.05P100Fe	17,97±0,11	0,21	23,69±0,3	0,19	13,84±0,15	0,22	14,82±0,16	0,21

R/P* - root/plant ratio

There was not any antagonism interaction between P and Fe on plant development. The positive impacts of P fertilization on plant growth have also been demonstrated in a range of crops such as wheat (*Triticum aestivum L.*), cluster bean (*Cyamopsis tetragonoloba L.*) and others species. Some researchers revealed that P nutrition contributes to improvement of growth and yields of crops in low water conditions (Gutierrez-Boem and Thomas, 1998, Burman et la., 2009). Phosphorus and iron supply had a relatively smaller effect on root weight ratio (i.e., root weight to total plant weight) of the soybean cultivars (table 1). The root/plant DM ratio was higher in the P0Fe0 plants as compared to those supplied with adequate nutrition P100Fe5, this trend being, however, more pronounced in Zodiac than in Licurici. The trait values were relatively less affected by soil moisture regime. The difference in root/plant ratio between cultivars under normal water regime was very small. But this parameter slightly increased under low nutrient supply in plants subjected to water stress conditions. Thus, it was found that application of phosphorus separately or in combination with iron could obviously reduced the negative effect of drought on plant growth and increased osmolyte proline accumulation, which could exhibited its some anti-drought roles to protect cells and physiological machinery at whole plant level under water stress conditions. Having been drowning from pot experiments these conclusions must await validation under field conditions.

CONCLUSIONS

1. Phosphorus deficiency combined with low water regime of soil increased the concentration of proline in leaves and there were genotype difference in capacity to accumulate proline. The response of Licurici cultivar to water stress was more pronounced than of Zodiac.

2. Nutrient-deficit plants had a low plant biomass. Drought has less detrimental effects on soybean genotypes in treatment with balanced nutrition of phosphorus and iron. Root/plant ratio of dry matter decreased in response to phosphorus and iron supply.

3. The significant effects of interactions between nutrients and soil moisture regimes on both proline accumulation and plant growth indicate that balanced mineral nutrition enhances the ability of the plants to cope with a mild water stress.

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