COMBINED EFFECTS OF PHOSPHORUS FERTILIZATION AND SOIL WATER DEFICIT ON LEAF DEVELOPMENT AND AMINO ACIDS CONCENTRATIONS IN TOW SOYBEAN (*GLYCINE MAX* L. MERR) CULTIVARS

EFECTUL COMBINAT AL FERTILIZĂRII CU FOSFOR ŞI DEFICITULUI DE APĂ DIN SOL ASUPRA DEZVOLTĂRII FRUNZELOR ŞI CONCENTRAȚIEI DE AMINOACIZI LA DOUĂ CULTIVARE DE SOIA (*GLYCINE MAX* L. MERR)

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Abstract. *The effects of phosphorus application (KH_2PO_4) levels 0 mg P/kg (P0), 10 mg (P10), 20 mg (P20) and 100 mg P/kg (P100)on leaves development and amino acids concentrations in two cultivars of soybean under two soil moisture regimes: control as normal irrigation, 70% of water holding capacity (WHC) and moderate stress (35% WHC) were studied. Plants were subjected to water deficit conditions at flowering stage for two weeks. Leaves area and amino acids concentrations in leaves were measured in relation to P nutrition. Plant performance of Licurici was better than Zodiac under P deficiency (P0) and low P (P10) supply. Leaves growth of both soybean cultivars was significantly reduced when the soil moisture content was decreased from 70% to 35% WHC. The highest leaf area and dry matter accumulation by leaves was found in the treatment with 100 mg kg^{-1} of P application. The same trend of P supplementation influence was denoted in plants subjected to drought stress, but to a lesser extent than in well-watered plants. Total soluble amino acids concentrations in leaves rose under temporary drought irrespective of P nutrition levels. Cultivar Zodiac displayed higher response in amino acids accumulation at suboptimal moisture regime. Hence, the adequate P fertilization improved the ability of soybean plants to tolerate drought stress.*
Key words: amino acids, *Glycine max.*, leaf area, moisture, phosphorus

Rezumat. *S-au studiat efectele aplicării diferitor doze cu fosfor 0 mg P/kg (P0), 10 mg (P10), 20 mg (P20) şi 100 mg P/kg (P100), asupra dezvoltării frunzelor şi a conținutului de aminoacizi liberi la două cultivare de soia cultivate în două regime de umiditate a solului: martor, irigare la 70% CTA şi stresul hidric moderat (35% CTA). Plantele au fost supuse deficitului de apă la faza de înflorire pentru două săptămâni. S-a determinat suprafața foliară şi conținutul de aminoacizi liberi în funcție de nivelul de nutriție cu P. Creşterea plantelor cultivarului Licurici a fost mai bună decât a cultivarului Zodiac în condițiile deficitului de P (P0) şi asigurării joase cu nutrient (P10). Dezvoltarea frunzelor la ambele cultivare s-a redus semnificativ când nivelul de umiditate din sol s-a micşorat de la 70% CTA la 35% CTA. Cea mai mare suprafață şi greutate de substanțe uscate acumulate în frunze s-a stabilit la aplicarea 100 mg kg^{-1} de P.*

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Aceeaşi influență a suplimentării plantelor cu P s-a depistat şi în condiții de secetă, dar la un nivel mai inferior comparativ cu plantele irigate normal. Concentrațiile de aminoacizi solubili din frunze s-a majorat sub acțiunea secetei temporare indiferent de nivelul de nutriție cu P. Cultivarul Zodiac a manifestat răspuns mai înalt la nivel de acumulare a aminoacizilor în condiții insuficiente de umiditate. Deci, s-a stabilit că fertilizarea adecvată cu P a îmbunătățit toleranța plantelor de soia la secetă.

Cuvinte cheie: aminoacizi, fosfor, *Glycine max.*, umiditate, suprafața foliară

INTRODUCTION

Phosphate (Pi) deficiency is a major agricultural production constraint in many soils involving up to 70% of global arable surface (Vance *et al.*, 2003). Phosphate is a component of nucleic acids and cellular membranes, and essential for a large range of metabolic processes (Raghothama, 1999; Vance *et al.,* 2003). Phosphate deficiency decreases plant growth and photosynthesis and thus biomass accumulation and yield. It was reported that insufficient P nutrition can affect cell division in growing tissues and restricts expansion growth of plant organs (Chiera and Rufty, 2002; Radin and Eidenbock, 1984).

Soybean is an important component of the agro-system due to its capacity to produce significant quantities of oil and protein-rich seed and to improve soil quality. However, its growth and productivity is very low due to many environmental constraints, particularly insufficient phosphorus nutrition and drought. According to research of Desclaux *et al.* (2000) soybean is very sensitive to water shortage during the stage of reproductive development. Water deficit often causes nutrient deficiency, particularly phosphorus (Haefele *et al.,* 2006). There is a general agreement that the ability of crops to cope with drought can be enhanced by adequate P nutrition (Gutierrez-Boem and Thomas, 1999). Studies by Burman *et al.* (2009) have demonstrated the beneficial impact of P application in reducing plant water stress in moth bean and cluster bean. Likewise, positive effects of P application on plant performance under stress environments have been reported for soybean (Devi and Sinclair, 2013). Under field conditions, crops are routinely exposed to a range of stress factors; therefore, understanding of the interaction between environmental factors such as P nutrition and soil moisture is critical for finite resources management of the sustainable agriculture. The goal of this study was to determine the effect of P application levels on leaf area development and amino acids contents in two soybean cultivars during exposure to water deficit conditions of soil.

MATERIAL AND METHOD

The pot experiment was conducted in a greenhouse 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 levels, two soil water regimes (control and low water supply) and two soybean (*Glycine max. L.*) cultivars namely Zodiac and Licurici. The P fertilizer was KH_2PO_4, and net P levels were 0, 10, 20 and 100 mg kg^{-1} of soil as P0, P10, P20 and P100, respectively. The growth medium consisted of a soil-sand mixture 3:1 by volume fertilized with macro- and microelements. All pots with P application received potassium (K) as KCl to equivalent potassium level. The content of available phosphorus was 4,4 mg kg^{-1} (CAL) (and 11,5 mg Olsen method), pH ($CaCl_2$) 7,74, total N - 0,04% and C - 1,42%. Seeds were treated with *Bradyrhizobium japonicum* at sowing time. Two soil water treatments, well-watered and temporary drought, commenced at the initial flowering stage (lasting for two weeks). Mean night temperature ranged within 18-20 ºC and mean day temperature varied 26-28 ºC. Relative humidity varied between 60-65%. The pots were placed on tables and rotated every second day for random distribution in a greenhouse. At harvest, 6 weeks after sowing, plants were separated into leaves, stem plus petioles, roots and nodules and leaf area was determined. Leaf area was determined by leaf meter (LiCor model 3000). The dry weight of plant materials was determined after drying in an oven at 70 °C until constant weight was obtained. The procedures for the total free amino acids contents in plant tissues were described in detail by Yemm and Cocking (1955). Data of experiments were averaged and the respective standard error (SE) was calculated. To determine the significance of the means differences, least significant differences (LSD) were estimated at 5% probability level.

RESULTS AND DISCUSSIONS

There is a general consensus about the importance of leaf area for plant growth and yield (Russel *et al.,* 1989). Many investigations have documented that leaf development is more susceptible to environmental stresses, particularly to P deficiency and drought than stems and roots. Since water shortage is more dangerous at flowering stage in grain legumes grown under rain-fed conditions, the present pot experiment focused at investigating the effects of different rates of P application on dry matter production of leaves and their area development as well as amino acids contents of plants subjected to low soil moisture regime at the beginning of flower bud initiation stage. The soil water regime influenced the leaf growth significantly and leaf parameters declined in the dry conditions compared to the wet soil treatment.

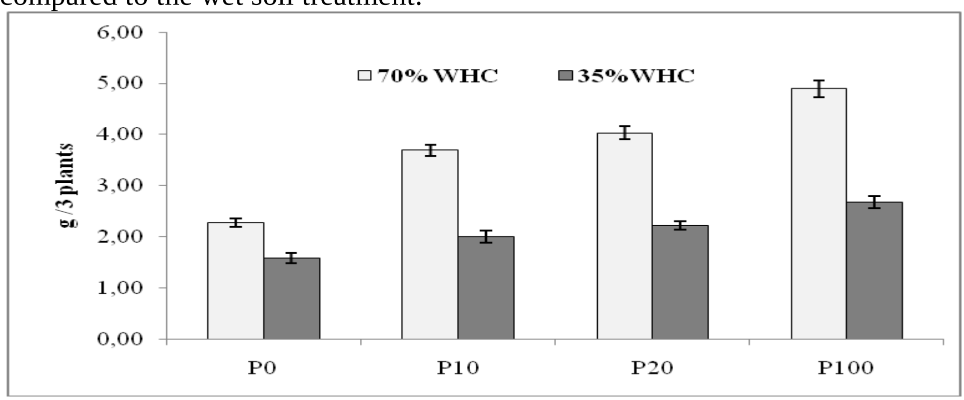

Fig. 1 Effect of phosphorus supply and soil water regime on leaves dry matter accumulation (g/3 plants) of cv Zodiac soybean plants. Bars indicate standard error of the mean. P<0.05

Experimental results revealed that leaves growth of cvs Zodiac and Licurici was affected significantly ($p<0.05$) by P fertilization (fig. 1 and fig. 2). The plant dry weight and total leaf area followed similar trend across the treatments at harvest. It was established that the leaves dry matter (DM) of two soybean cultivars was significantly reduced by combination of low P supply and water deficit. The soybean cultivars displayed differences in the responses of leaf growth to P application at two water regimes. Leaves growth of Zodiac had more pronounced responses to supplemental P nutrition than Licurici.

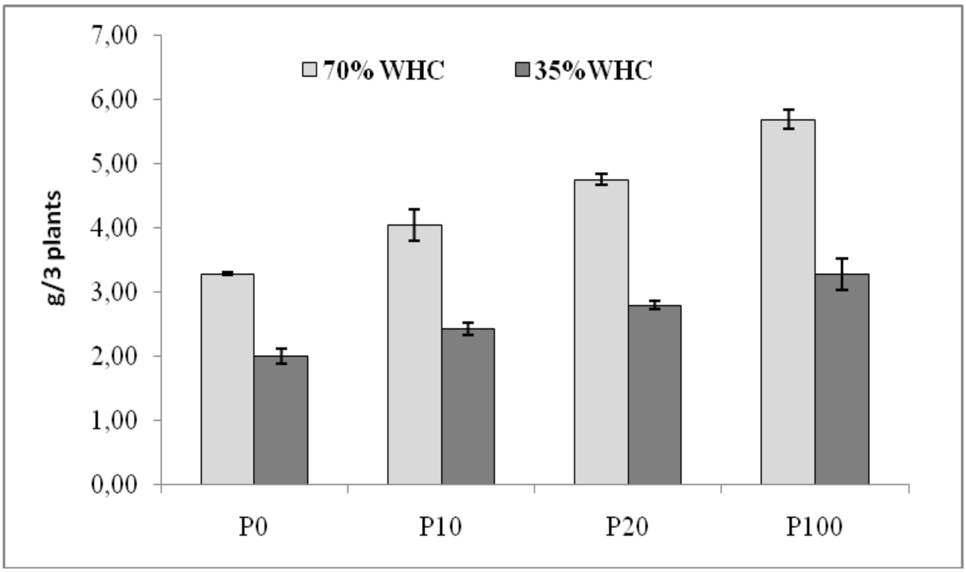

Fig. 2 Effect of phosphorus supply and soil water regime on leaves dry matter accumulation (g/3 plants) of cv Licurici soybean plants. Bars indicate standard error of the mean. $P<0.05$

The most pronounced difference between cultivars was established at insufficient P nutrition (P0). Radin and Eidenbock, (1984) reported that phosphate deficiency limited cell expansion by reducing hydraulic conductance inside plants, which may lead to reduced plant size and leaf area expansion. Under P insufficiency, the reduction in DM with cv. Zodiac (114%) was greater as compared to that with Licurici (73.2%) under normal water conditions and adequate nutrient (P100) supply. Increasing P supply promoted plant growth and the difference between Zodiac and Licurici was reduced. Shen *et al.* (2013) also stated that appropriately manipulating P supply can enhance plant growth, nutrient uptake and the ability to resist various stresses, including water deficit.

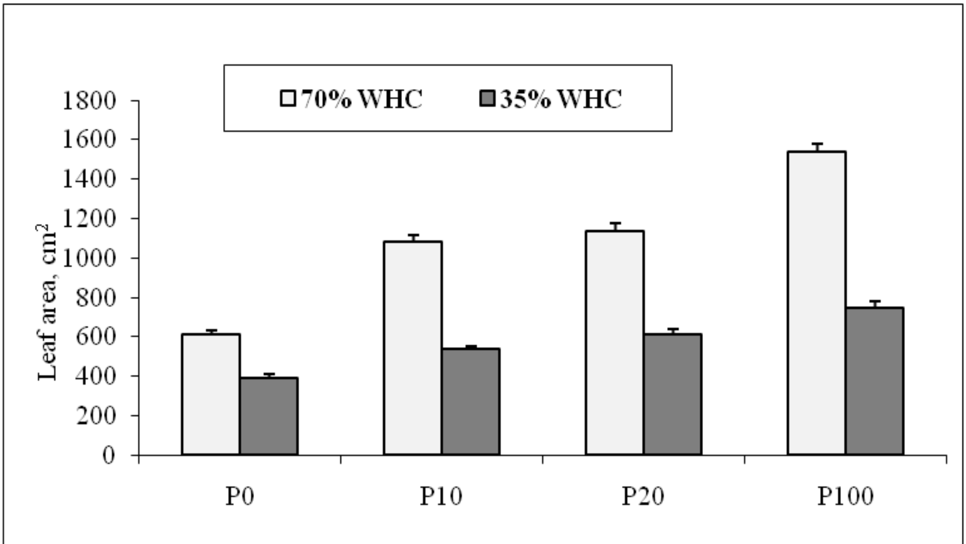

Fig. 3 Interactions of P addition and soil water regime on surface area (cm^2/3 plants) of cv Zodiac soybean plants. Bars indicate standard error of the mean

The significant response of plants to the low level of P application indicates that soil P was very scarce and, also, that soybean plants have a high requirement for fertilization. Our results are consistent with the data of Vadez *et al*., (1999) which confirmed the sensitivity of *Phaseolus vulgaris* to P deficiency. It is necessary to note that the influence of P fertilization on this morphological trait was more pronounced under sufficient moisture level. The application of P at different rates increased leaf area by 21.8-65.7% in Licurici (fig. 4) and by 36-90% in Zodiac (fig. 3) under drought conditions. The lowest leaf area was observed under low P nutrition (P0) and Zodiac had a lower value by 150% and 90,4% under well watered and stress treatments respectively compared to high P treatment (P100). Therefore, the maximum leaf area under optimal and low soil moisture level was observed in treatment with the application of 100 mg of P per kg of soil. We consider that the greater susceptibility of cv. Zodiac to P deficiency than Licurici is partly due to higher vulnerability of leaf growth to insufficient P supply. Studies (Guterez-Boem and Thomas, 1999) have demonstrated that P insufficient supply reduced leaf area and the number of leaves as well as the relative leaf appearance rate.

It was established that the irrigation of plants to optimal soil moisture (70% WHC) increased the mean leaf area by 69% and dry matter accumulation by 64% of Licurici in treatment without fertilization compared with the ones grown at low moisture level (35% WHC). In cv Zodiac, the increases of these two parameters due to normal water supply were 55.8% and 44.3%, respectively compared to plants subjected to drought.

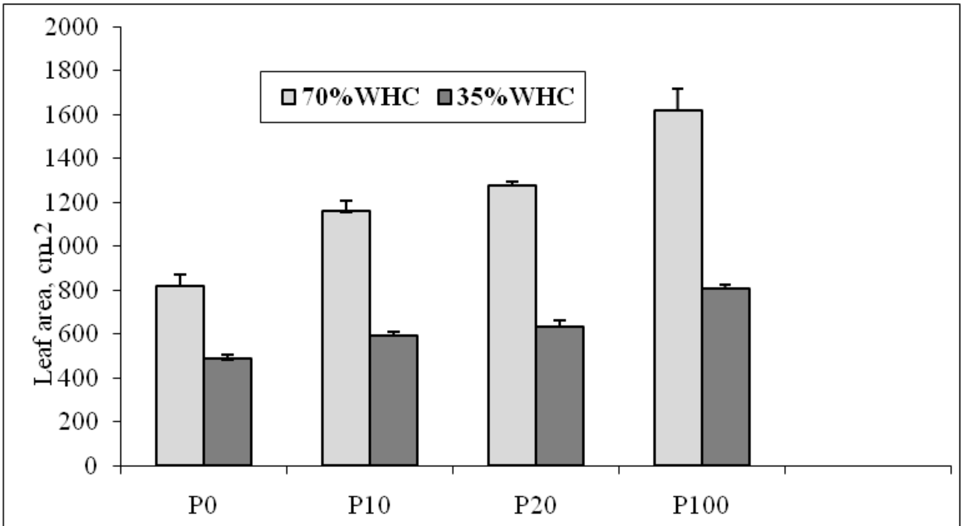

Fig. 4 Interactions of P addition and soil water regime on surface area (cm^2/3 plants) of cv Licurici soybean plants. Bars indicate standard error of the mean.

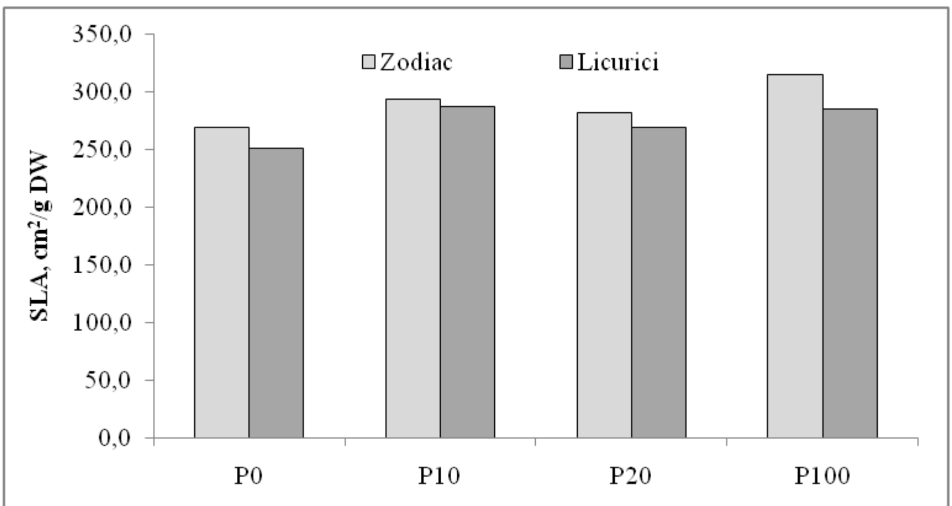

Fig. 5 Effect of P supply on specific leaf area (SLA, $cm^2\ g^{-1}$), of soybean plants under normal water supply

Therefore, experimental data clearly demonstrated that P supplemental nutrition helps plants overcome water deficit stress probably by increasing the hydraulic conductance of water (Singh and Sale, 2000). The combination of these two constraints resulted in a greater reduction in total leaf area than in plant dry mass. So our data is consistent with the results of other researchers (Gutierrez-

Boem and Thomas, 1999). The data presented in figure 5 shows that specific leaf area (SLA) did not change significantly in relation to P treatments under normal water supply. The higher P supply had only a tendency to improve this parameter. The same trend was observed in plants subjected to temporary drought (fig. 6). Under insufficient water regime Licurici displayed lower values than Zodiac in treatment with adequate phosphorus nutrition. Compared with those under well-watered condition (70% WHCC) treatments of drought stress (35% WHC) exhibited the lowest SLA.

Biochemical studies have shown that plants under environmental stresses accumulate a number of metabolites, termed compatible solutes because they do not interfere with metabolically reactions. The majority of such metabolites include carbohydrates and nitrogen-containing compounds, particularly amino acids.

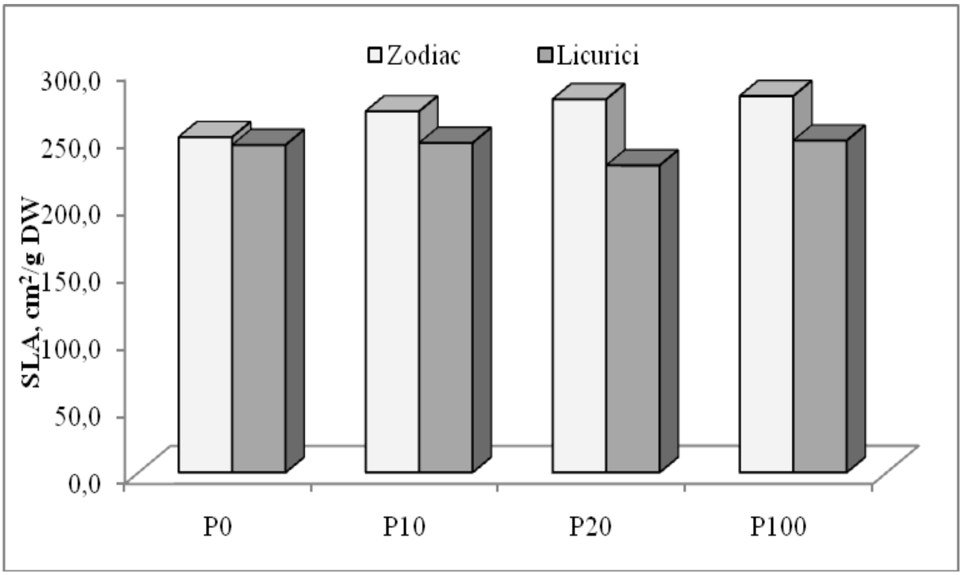

Fig. 6 Effect of P supply on specific leaf area of soybean plants under water deficit conditions (35% WHC)

Water stress and/or P treatments caused significant changes in the content of free amino acids in the two soybean genotypes (fig. 7 and fig. 8). In this study, the concentration of free amino acids decreased in direct proportion with the levels of P application only under limited moisture conditions. Also, plants subjected to drought stress allocated more amino acids in their leaves than well-watered counterparts (fig. 7).

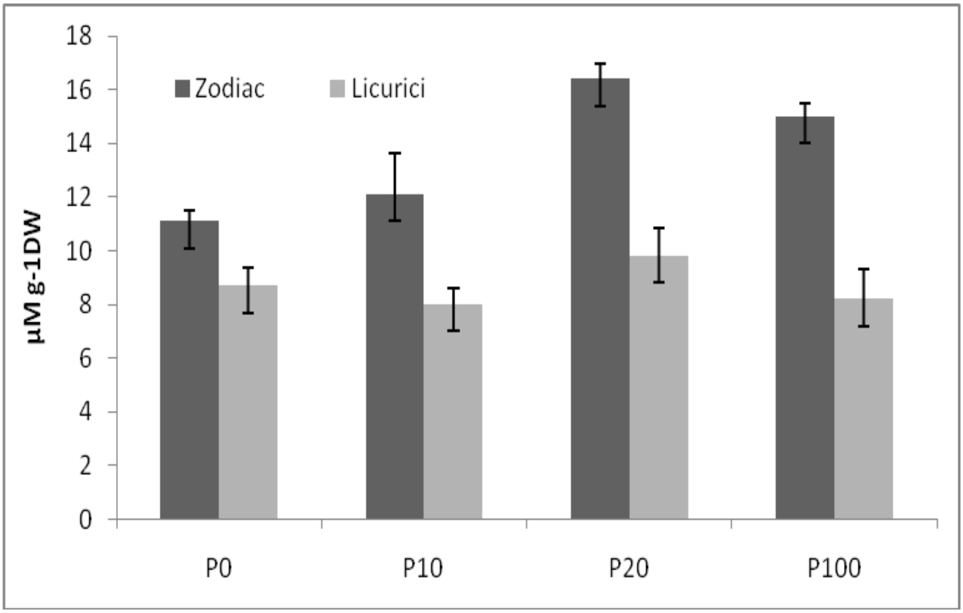

Fig. 7 The effect of phosphorus supply on the concentrations of total free amino acids in leaves of soybean cultivars at the flowering stage under normal soil moisture (70% WHC). Data are means of four replicates ± SE

Several explanations for the accumulation of free amino acids under stress have been suggested. These effects could be due to stimulated synthesis, impaired protein synthesis and/or enhanced protein degradation. This accumulation may play an important role in the osmotic adjustment of plants under stress as has been reported by Khavari-Nejad *et al*. (2008) and by Schubert *et al.* (1995). The P supplemental nutrition induced decreases of this physiological parameter in leaves in both cultivars under water stress conditions. Experimental data has shown that the application of P at high level (P100) decreased their concentration in leaves approximately by 2 times in plants of Licurici and Zodiac subjected to water deficit. We suggest that adequate P nutrition provided better conditions for nitrogen assimilation inclusive protein synthesis. Nevertheless, under drought stress the accumulation of these N-compounds in leaves was more evident in Zodiac than in Licurici, especially under insufficient nutrient supply. Hence, it was observed that supplemental P nutrition of plants subjected to drought decreased amino acids concentration in leaves and the lowest level (12-15 µM g^{-1} DW) was observed in treatment with adequate P nutrition (fig. 8). No effect of P treatment was apparent on amino acid concentrations in leaves tissues of Licurici plants provided with moderate (P20) and high rate (P100) fertilization under suboptimal water regime. Altogether, adequate P nutrition increased leaf area development which in turn may improve plant photosynthesis and stress tolerance (Devi and Sinclair, 2013). The mechanisms of plant stress tolerance involve a number of metabolic changes of cellular compounds, gene expression and protein modification.

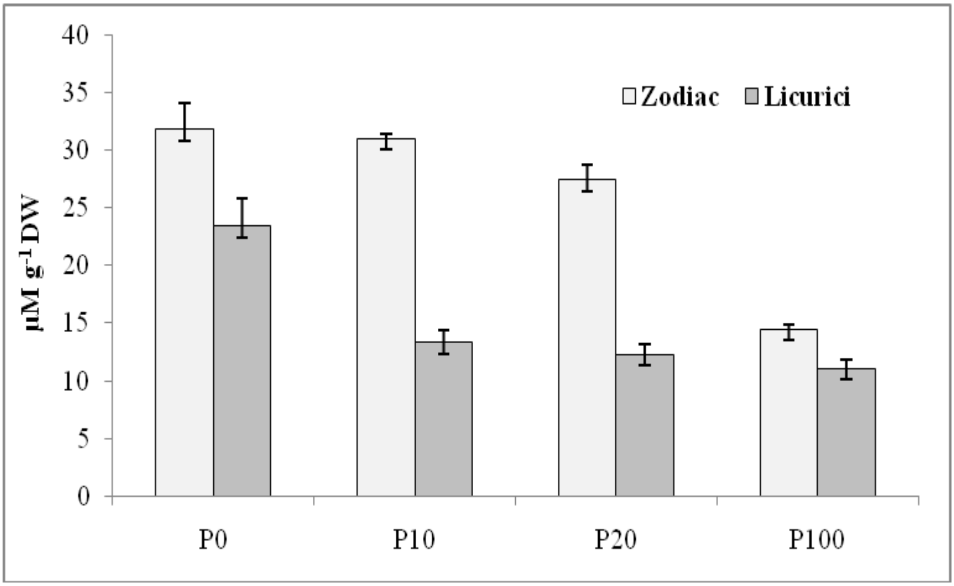

Fig. 8 The effect of phosphorus supply on concentrations of total free amino acids in leaves of soybean cultivars at the flowering stage under water deficit conditions (35% WHC). Data are means of four replicates ± SE

Understanding the regulation of these processes will be essential to developing crops with higher tolerance to multiple stresses in particular to drought and nutrient deficiency which necessitates further investigations of the complex responses and biochemical pathways of plants exposed to unfavorable conditions.

CONCLUSIONS

The phosphorus fertilization and soil moisture regime have significant influence on leaf development and amino acids content in two soybean cultivars.

Increased P rates increased the leaf area of both species, however, cv Zodiac displayed higher responses and it seems to be more susceptible to low P conditions than Licurici cultivar mainly due to poor leaf area development and higher free amino acids concentration.

Specific leaf area was not significantly affected by P treatment at flowering stage. Field studies are required to confirm the potential utility of P supplementation under suboptimal moisture conditions.

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