THE EFFECT OF PHOSPHORUS AND IRON ON PLANT GROWTH AND NUTRIENT STATUS OF TWO SOYBEAN (GLYCINE MAX. L.) CULTIVARS UNDER SUBOPTIMAL WATER REGIME OF SOIL

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Abstract

Phosphorus (P) and iron (Fe) deficiencies are common in many soils. A pot experiment was conducted to investigate the effects of P and Fe application on the biomass production and nutrient status of two soybean (*Glycine max*. L. Merr) cultivars under water stress conditions. The soil experiment consisted of two P and Fe levels as sufficient and low supply. Control plants were grown at 70% water holding capacity (WHC) while their counterparts were subjected to water stress (35% WHC) at initial flowering stage for two weeks. Considerable variability was observed in leaves, roots, dry mass accumulation among the soybean cultivars (Zodiac, Licurici) at both P and Fe levels in relation to water regimes. The results showed that drought significantly reduced biomass production irrespective of nutrient supply but its adverse effect was more pronounced at low nutrient supply. Leaf development and nodules growth were the most sensitive to water deficit and insufficient nutrient supply. Combined application of P and Fe increased dry matter production and nutrient acquisition for both soybean cultivars. Concentrations of Fe in leaves differed significantly among cultivars at both sufficient and insufficient mineral nutrition. Phosphorus application increased its contents in all vegetative organs, but decreased Fe allocation to the leaves of Licurici. In soil culture conditions our results demonstrated that there was a positive effect of P and Fe adequate nutrition on plant growth and nutrient status. Hence, it was revealed that sufficient P and Fe supply partially alleviates the drought negative effect on performance of soybean plants.

Key words: iron, phosphorus, soybean, water stress

The major objective of sustainable agriculture is to obtain high quantity and quality of yield crops. Accomplishment of this desiderate has economic and ecological significance. The crops are subjected to different kinds of environmental stresses during the vegetation period. Among them the water deficit and low fertility of soil are common for many agricultural regions. These abiotic factors affect considerably the plant productivity (Marschner, H. 1995). Nutrition can significantly influence plant response to water conditions. Although these constraints persist simultaneously under field conditions, unfortunately a little is known about their combined influence on plant growth and nutrient status, in particular on soybean. Therefore, there is need to elaborate technological procedures based on physiological processes directed to attenuate negative impact of stresses factors. It was established that drought can modify the pattern of mineral uptake by affecting plant growth and physiological activity of roots as well as the mobility of nutrients in the soil (Fageria, N.K., Baligar, V.C., Clark, R.B. 2002). However, nowadays the deficiency of several macro and micronutrients is leading to either stagnate or decline in yield of the legumes. The use of high yielding varieties and use of unilateral phosphorus fertilizers might have depleted the available iron in soils. The application of P and Fe increases the available status of these nutrients thereby the crop yield.

The plant ability to acquire nutrients under drought conditions might have an important role in tolerance to drought (Payne, W.A., M.C. Drew, L.R., Hossner, R.J., Lascano, A.B. Onken, and C.W. Wendt. 1992; Rizhsky, L., Liang H.J., Shuman, J., Shulaev, V., Davletova S, Mittler, R., 2004; Samarah, N., Mullen, R., Cianzio, S., 2004). Phosphorus application inhibits the translocation of iron and also its precipitation at higher level of phosphorus (Moustaoui, D., Verloo, M. and Pauvels, J. 1991). Thus, decreasing trend in uptake of iron with higher levels of P was mainly attributed to the inhibition of translocation of micronutrient from roots to shoots. Likewise, it was demonstrated that application of phosphorus reduces the stress effect of water deficit on soybean productivity (Jian J., G. Wang, X., Liu, X. Pan, S., J. Herbert, 2005). Legumes in comparison

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with cereals have higher demand for P nutrition and that's why the application of P fertilizer alone could provoke imbalance of nutrients status, in particular of micronutrients, and thereby can affects the realization of the genetic potential of crops. From this point of view it appears that their combined application might modify the metabolism of plants in another manner in comparison with the impact only one of stress factor (Ladouceur A., S. Tozawa, S. Alam, S. Kamei and S. Kawai, 2006; Rizhsky, L., Liang H.J., Shuman, J., Shulaev, V., Davletova S, Mittler, R, 2004).

The symbiotic system of legumes is very sensitive to iron deficiency (Slatni,T., A.Kroma, S., Aydi, C. H. Gouia., C. Abdelly, 2008). Therefore, the insufficient supply of this micronutrient markedly affects plant growth and nodules functioning. Our previous investigation (Rotaru, V., Sinclair, T., 2009) conducted out with sand culture have shown that iron application stimulated nitrogen fixation of soybean plants. Administration of phosphorus under arid conditions enhanced water use efficiency (Payne, W.A., M.C. Drew, L.R., Hossner, R.J., Lascano, A.B. Onken, and C.W. Wendt, 1992) and tolerance to drought (Singh, D.K. and P.W.G. Sale, 2000). Analyze of literature revealed that there is scarce information about the effect of P and Fe on nutrients uptake in relation to soil water regime. So the interactions of different elements are quite important as these could be synergistic, antagonistic or merely additive. Keeping this in view an experiment was conducted to study the combined effect of phosphorus and iron on plant growth and nutrient (P, Fe) status of two soybean cultivars under suboptimal water regime of soil.

MATERIAL AND METHOD

A pot experiment was conducted out of soil with low available concentration of phosphates and iron (9 mg P and 5,4 mg Fe mg kg^{-1} soil). Two soybeans (*Glycine max. L)* cultivars namely Licurici and Zodiac were tested. Four nutrient treatments and two water regimes were investigated. A basal dose of 50 mg N per kg soil in the form of (Ca $(NO_3)_2x4H_2O$ was applied to all the pots. The quantity of P (KH_2PO_4) for soil experiment was 100 mg P kg^{-1} soil as sufficient supply (P100) and without P application as insufficient supply (P0). Iron was applied as Fe-EDTA at 5 mg Fe kg^{-1} soil as sufficient supply (Fe5) and without micronutrient application (Fe0). All treatments were replicated four times and their abbreviated names are listed in figure 1. Six seeds, inoculated with *Bradyrhizobium japonicum*, were sown in each pot and the seedlings were thinned to three per pot. Until flowering the plants received adequate water supply - 70% water holding capacity (WHC). At the onset of flower buds the two soil moisture treatments were performed by maintaining pots at normal level of moisture (control) and other set of plants was subjected to 35% WHC as water stress. Drought period lasted two week. Both control and water stressed plants were harvested at beginning of pod set up. The leaves, stems, roots and nodules were dried separately in an oven with air circulation at 75°C for 48 hours and the dry weights were recorded. Total phosphorus content was determined by the colorimetric molybdenum-blue method (Murthy and Rely, 1962). Iron was measured in plant tissue samples which were ashed overnight at 500 °C in a furnace and iron analysis by atomic absorption spectrophotometer.

RESULTS AND DISCUSSIONS

A summary parameter of metabolic activity of plants at the whole level is considerate the rate of dry matter accumulation. The experimental results of dry mass production and distribution within plant parts are presented in figure 1. They demonstrated that supplemental P nutrition separately as well as in combination with iron stimulated plant growth and development irrespective of soil moisture conditions. The cultivars differed (P=0.05) in total dry mass and leaves fraction, but less in root system fraction. Nutrients deficit have been reduced the plant growth of both genotypes, but Licurici was more susceptible to low fertility of soil. The lowest values of biomass production have been registered in treatment of insufficient nutrient supply of both nutrients in particular under suboptimal moisture level. The high response of soybean plants to P fertilization demonstrated that soil used in the experiment had low level of mobile phosphates and on the other hand legume responded significantly to P nutrition. The similar results have been obtained in other species of legumes (Garg, B. K., Burman, U., Kathju, S., 2004). A vigorous plant growth was observed in treatment of combined application of phosphorus and iron ($P_{100}Fe_5$).

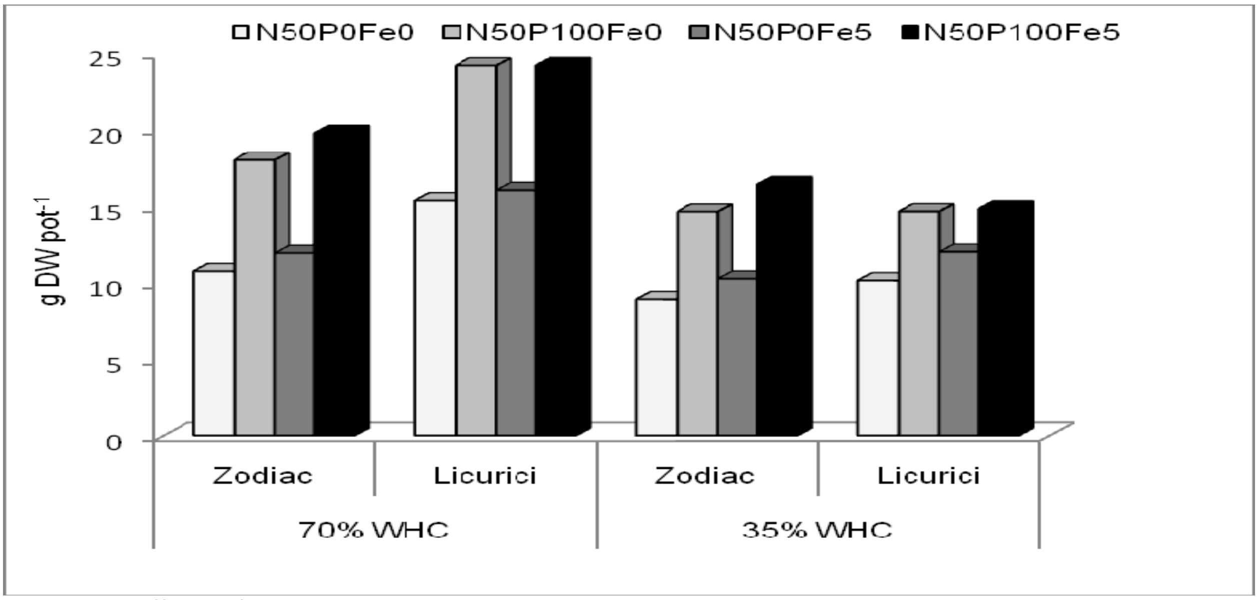

Figure 1 **Effects of P and Fe supply on dry matter accumulation in plants in relation to soil water regime**

Water regime significantly influenced total dry mass and leaf growth. Licurici produced greater dry matter than Zodiac under normal moisture regime (*fig. 1*). The examination of results in Zodiac has been stated that alone application P increased the plant production by 40% under normal water conditions. The same effect was observed in case of water deficit. Zodiac overcame Licurici regarding the amount of dry matter production only under water stress conditions.

Fredeen and their collaborators (Freeden, A.L., I.M. Rao, and N. Terry, 1989) have been demonstrated that leaves of soybean are more sensitive to P deficit in comparison with other vegetative organs. In the present study it was observed that drought affected substantial the formation of photosynthetic system indifferent to level of mineral nutrition. In general, the low water regime of soil and P supply influenced significantly leaves development and less root system (*tab. 1*). Under limited water conditions insufficient supply of P and Fe reduced leaves growth by 47% in comparison to treatment with application of both nutrients (*tab. 1*). Likewise, the sensitivity of leaves to water stress was markedly greater in treatment without nutrients fertilization. It was observed that the fraction of leaves of Licurici was reduced significantly by water shortage. Supplemental P nutrition of this cultivar increased dry matter accumulation of plants by 30,8% under optimal moisture level.

Some researches demonstrated that legumes have good responses to iron nutrition (Slatni,T., A.Kroma, S., Aydi, C. H. Gouia., C. Abdelly, 2008). The data of this study have been shown that iron effect was more pronounced in treatment where micronutrient was administrated with mineral nitrogen than with phosphorus. The reaction of genotypes to iron supplemental nutrition was the same under normal water conditions but there was a difference under scarce moisture environment. The increases of biomass in treatment with micronutrient application together with P were smaller irrespective of soil water regime. It is necessary to be noted that iron supply with nitrogen (without of P) did not change the number of pods irrespective of soil moisture level. However, it was observed an increase of pods dry mass because of iron application with macronutrients (N, P). Under such environmental conditions a higher number and mass of pods in Zodiac where due to adequate nutrition of Fe and the number of pods increased by 19,5% under optimal moisture level (70% WHC) and by 29,5% in drought conditions (the data are not shown). So, it was demonstrated that iron had a pronounced influence on development of reproductive organs, particularly under suboptimal water supply. Drying the soil from 70% WHC to 35% WHC led to significant decrease in nodulation in both cultivars. It must be emphasized that alleviation of iron nutrition increased nodules growth. Our results are consistent with those revealed in *phaseolus vulgaris* (Slatni,T., A.Kroma, S., Aydi, C. H. Gouia., C. Abdelly, 2008). The effect of iron application led to increase assimilates production in particular to plants subjected to water stress conditions.

The level of soil moisture and mineral nutrition influenced markedly the content and uptake of phosphorus. The determination of nutrient status in leaves, stems, roots and nodules revealed that the lowest concentration of the element was observed in treatment without fertilizers application (fig. 2).

Table 1
The influence of phosphorus and iron on leaves and roots growth under water stress conditions (g DW pot^{-1})

Treatments	Leaves		Roots	
	Zodiac	Licurici	Zodiac	Licurici
N0.05P0Fe0	2.59±0.23	3.87±0.28	2.60±0.20	2.71±0.15
N0.05P100	4.15±0.45	5.52±0.46	3.78±0.18	3.07±0.27
N0.05P0Fe5	2.83±0.22	4.18±0.15	2.71±0.14	2.50±0.12
N0.05P100Fe5	4.76±0.35	4.63±0.36	3.63±0.24	3.15±0.29

The contents of P in plat tissues decreased evidently under limited water conditions of soil. It must be noted that the levels of nutrient content in vegetative plant parts of Zodiac was superior than in Licurici (fig. 2, 3). Application of phosphorus increased concentration of nutrient in all organs of plants exception the nodules. This effect was more pronounced in leaves of Licurici. As regard Zodiac, it was observed an increase only in roots under optimal conditions. Iron application together with nitrogen fertilizer reduced the concentration of P in stems of Zodiac and there were not modifications of this physiological parameter in stems of Licurici. The combined application of Fe with NP decreased P levels in leaves and stems of Zodiac by 11,2% and 32,2%, respectively. Nevertheless, it was observed a trend of decreasing of P content in roots by Fe fertilization by 11,1% under normal water conditions.

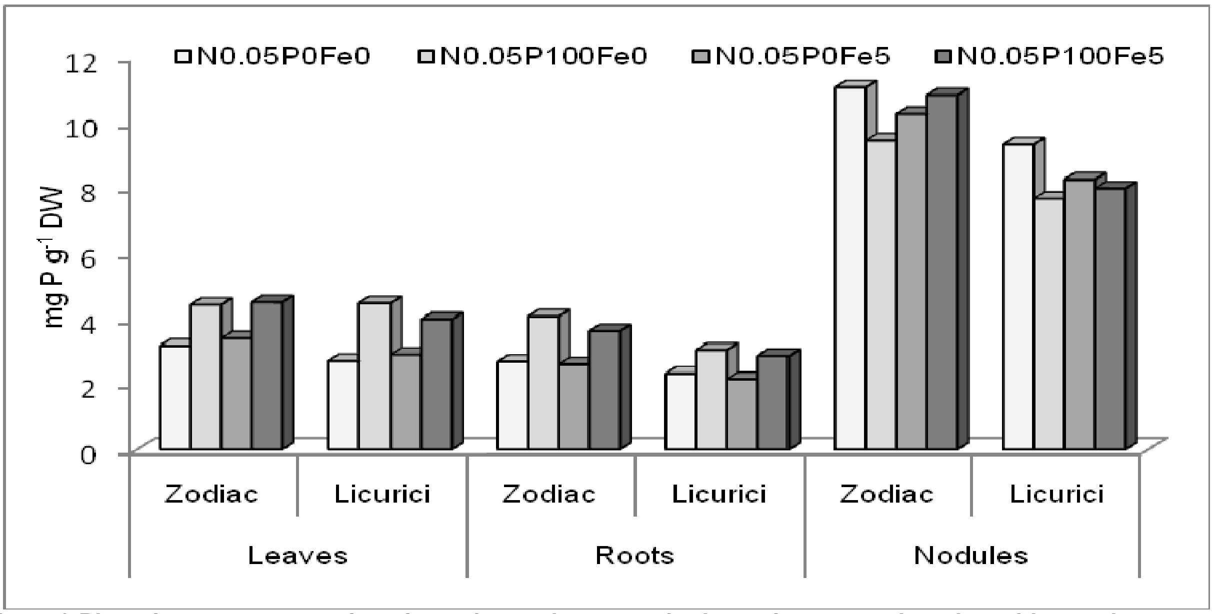

Figure 2 **Phosphorus concentrations in soybean plant parts in dependence on mineral nutrition under normal moisture of soil**

In treatment with combined application of phosphorus and iron there were not changes in nodules of Zodiac, but they induced the decline in P status of nodules in Licurici. Comparative analysis revealed that Zodiac overcame Licurici as regard P concentration in leaves, nodules, roots. Under low level of water (35% WHC) the content of P decreased (*fig. 3*). The value of nutrient status in stems was less under drought conditions and equivalent only 64% of amount observed in control plants. Also the concentration of P changed in roots from 2,66 mg per g DW in treatment without P application to 4,32 mg per g DW in treatment with P supply (*fig. 2*). Iron application inhibited phosphorus uptake in plant tissues of Zodiac. Experimental results revealed that P deficiency decreased nutrient content in leaves by 26,6% in Zodiac, at the same time this decrease was more evidently in Licurici (by 37,1%).

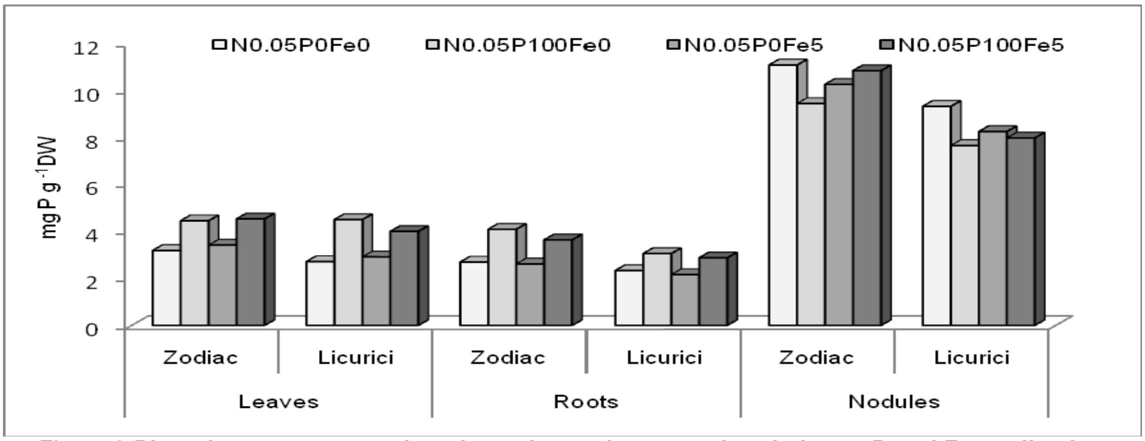

Figure 3 **Phosphorus concentrations in soybean plant parts in relation to P and Fe application under water stress conditions**

So genotype plays an essential role in nutrient acquisition under vulnerable phosphorus environment. Also, the accumulation of P in roots responds positive to additional of P where the level of P concentration increased by 38,4% in both cultivars. Therefore a better translocation of P to the shoots in Zodiac under low water regime of soil could provide a better assimilation of CO_2 that was demonstrated by higher photosynthetic activity (Fredeen et al., 1989). Application of micronutrient together with nitrogen increased concentration of P in leaves and roots in the same manner whereas application of iron with macronutrients (N, P) decreased P concentration in leaves by 16,2% in Licurici and only by 7,8% in Zodiac. The same dependence was established at roots level where the reductions were by 29,3% in Licurici and 7% in Zodiac. Thereby, the antagonist effect between these elements was stated more pronounced in Licurici, than in Zodiac. The level of soil moisture influenced the nutrient status of plants. For example it was observed that water deficit (35% WHC) increased nutrient level in treatment without fertilization by 36% and 24% in Zodiac and Licurici, respectively.

The estimations of ratios between concentrations of P and Fe in plants ascertained different values through treatments. Phosphorus fertilization contributed to increase this ratio at both cultivars, but more evidently in Zodiac. Iron supplemental nutrition did not modify this parameter in leaves of plants grown at optimal water conditions. Under stress conditions it decreased in treatment with Fe in combination with P. It was observed, that drought increased this value in treatments without fertilizers application, but it had the same value in conditions of balanced application of phosphorus and iron (data are not shown). Determination of this ratio in roots also revealed changes in relation to nutrient application. So the administration of P alone or in combination with Fe contributed to increase the parameter in Zodiac and did not change in Licurici of control plants. The evaluation of this ratio in treatment NPFe make up higher value in Zodiac, but decreased by 33% in Licurici. Abiotic factors affected also the concentrations of iron in plant parts. Phosphorus fertilization separately or in combination with iron changed the Fe contents in vegetative organs *(fig. 4).*

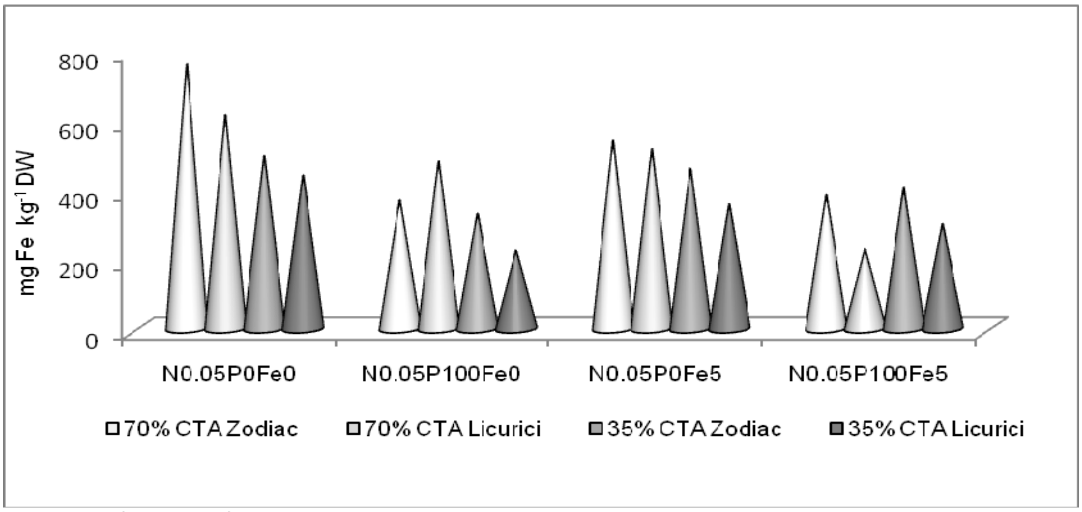

Figure 4 **The influence of P and Fe application on iron contents in leaves in relation to soil water regime**

The highest value was recorded in leaves in treatment without application of P and it was associated with reduction of biomass accumulation in leaves. Application of P separately under optimal conditions of water diminished the content of iron in leaves by 51% and 21,5% in Zodiac and Licurici, respectively (*fig. 4*). The same trend was observed in plants subjected to water stress where the decreases were by 34% and 49%. The results can be explained due to the effect of biological dilution of micronutrient as well as by depleting uptake by additional phosphorus. Supplemental iron nutrition together with mineral nitrogen (N50 mg kg^{-1} soil) did not contribute to any micronutrient accumulation in leaves. It must be mentioned that P fertilization inhibited the uptake of iron in roots in Zodiac and there was not significantly changes in roots of Licurici. Thereby, the reaction of both cultivars to application of P and Fe under unfavorable environment conditions takes a stereotypical character and differences were as a rule of quantitative order. This result suggests that an adequate nutrition of phosphorus and iron can increase soybean tolerance to drought stress compared with nutrient deficiency treatment and this finding is in agreement to some reports in literature.

CONCLUSIONS

Two soybean cultivars had different response to combined application of phosphorus and iron in relation to soil moisture regime.

Concentration of phosphorus and iron differed significantly among genotypes and phosphorus application increased its uptake in shoots evidently in Zodiac.

Phosphorus and iron deficiencies in the soil conducted to disorder of nutrient status in plant tissues irrespective of soil moisture level.

Balanced fertilization with P and Fe provide a vigorous growth of soybean plants both in well-watered and in stress moisture conditions, and iron supplemental nutrition increased number and mass of pods at the reproductive stage.

The control of water stress may be regulated by leaf more than root, because no significant effect of stress factor was found on the root fraction.

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