COMBINED EFFECTS OF DROUGHT AND PHOSPHOROUS DEFICIENCY ON PHYSIOLOGICAL AND BIOCHEMICAL INDICES OF SOYBEAN PLANTS

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Abstract

An outdoor pot experiment was carried out to determine the combined effect of water stress and phosphorous (P) deficiency in soil on physiological and biochemical indices of soybean (*Glycine max* (L.) Merr.). The soil used was collected at a depth of 10 -30 cm of a long-term field experiment with a suboptimal P supply (Pdl 30 mg/kg soil). It was classified as loamy sand. Water treatments included two levels of water supply - 70% (control) and 30 % (drought) of soil water holding capacity and were applied during eight weeks. The results revealed that water deficit induced inhibition of growth processes, accompanied by a significant reduction of the plant height and shoot biomass, as well as P, K, Ca, Mg, and N uptake by plants. We also found a decrease in the activities of nitrate reductase, glutamine synthetase, and glycolate oxidase in leaves of the stressed plants. On the other hand, proline was accumulated in the leaves under water and P deficiency. Similarly, the activities of acid phosphate (AcP), alkaline phosphatise (AlP), and phosphodiesterase (PDE) in leaves were higher under P and water stress conditions. The average values of AcP for both water treatments were about 4 times higher than those of AlP and PDE. These data highlighted the important role of enzymes for plants under stress conditions.

Key words: soybean, water stress, phosphorous, nitrate reductase, glutamine synthetase, glycolate oxidase, phosphatases

Soybean (*Glycine max* (L.) Merr.), representative of the *Leguminaceae* family, like other legume crops, represents a very significant agricultural plant as a source of important nutritional compounds. This crop is cultivated extensively worldwide in tropical, subtropical, and temperate areas.

Plants, including soybean, can be and usually are affected by diverse environment stresses, including water and nutrient deficiencies. Extensive investigations on diverse responses of plants to drought impacts revealed that water deprivation limits plant growth, photosynthesis and yield [Lawlor D.W., Cornic G., 2002, Yordanov I. et al., 2003]. Yield of soybean (*Glycine max* (L.) Merr.) has been demonstrated to be affected considerably by the drought stress [Manavalan L.P**.** et.al.2009]. Another important factor that constraints plant growth is phosphorous (P) insufficiency, due to the low availability of this element to the root systems [Fairhust T. et al. 1999]. It is considered, that P fertilization is the major mineral nutrient yield determinant among legume crops [Chaudhary M.I. et al., 2008]. However, legume crops vary widely in the ability to take up and use P during element deficiency. More P is translocated and distributed into leaves in soybean than in mungbean and mashbean.

The conditions of soil limited water contents led to the aggravation of the nutrient migration processes in soil, to the formation of strong P compounds and to the reduction of P uptake by some plants [Brandt C**.,** 2011]. Effects of both factors – water deprivation and P deficiency on soybean growth and developments were studied largely as separate factors. Only some data are reported [Gutierrez-Boem F. H., Thomas, G. W., 1999] concerning the effects of phosphorus nutrition and water stress on soybean growth parameters. The authors observed some interactions between P deficiency and water stress.

The objective of the present investigation was to study the combined effects of phosphorous and water deficiency on some physiological and biochemical indices of soybean plants.

MATERIAL AND METHODS

Plant material and growth conditions. An outdoor pot experiment was performed with soybean (*Glycine max* L. Merr.) plants grown

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under green house control conditions of the University of Rostock, Germany. Mitscherlich pots were filled with 6 kg of air dried and sieved soil. The soil used was collected at a depth of 10-30 cm of an arable long-term field experiment (Pdl 30 mg/kg soil). It was classified as loamy sand. Before sowing, basic nutrients, except P, were mixed thoroughly with soil (per pot: 1.3 g NH4NO3, 1.4 g MgSO4+7H2O, 1.2 g KCl). The same number - 8 seeds of soybean were sown in each pot. Before BBCH 13, irrigation was done with distilled water for all pots according to crop demand. Later, two levels of irrigation were established. The drought treatments were irrigated according to 30 % water holding capacity (WHC). The well watered control treatments were irrigated according to 70 % WHC. All plants were harvested after eight weeks of growth. The collected leaf samples were frozen and stored at –70 °C in liquid nitrogen until use. The other plant material was oven-dried at 65 °C, weighted and milled.

The shoot P, Mg and K concentrations were measured after dry ashing and digestion in 20 % hydrochloric acid. For P determination the vanadad-molybdate method [Page et al. (1982) was used. Mg contents in the filtrate were determined 4 photometrically. Total K content was analysed using flame spectrometry. The Kjeldahl method was used to analyse the N concentration.

Assay of glutamine synthetase (GS) and glycolate oxidase (GO) activities. GS activity was measured using the ADP-dependent transferase assay according to [Shapiro B.M., Stadtman E.R., 1970] method. GO activity was determined by the method [Kolesnikov P.A., 1962].

***in vivo* Nitrate reductase (NR) activity** was estimated according to [Brunetti N., 1976]

Assay of acid phosphatase (AcP), alkaline phosphatase (AlP), phosphodiesterase (PDE) activities and proline content in the plant leaves were determined using the methods described in [Brandt C**.,** 2011].

RESULTS AND DISCUSSION

The yield increased significantly with higher soil humidity (tab.1). The yield increase for 70% WHC was by 2.5 times higher in comparison to the 30 % WHC treatment. This difference in yield correlated with a significant increase of nutrient uptake. The P uptake of soybean in the better watered pots was by about 3.7 times higher than in the 30 % WHC treatment. Higher nutrient uptakes under sufficient water conditions were also shown for shoot K, Ca, Mg and N. Particularly soybean absorbed a high amount of nutrients, as has already been described in earlier studies of our group [Bachmann S., Eichler-Loebermann B., 2010). The application of both stress factors, such as water stress and P deprivation, decreased P uptake more than N uptake in soybean (tab.1) In the experiment with subjecting of soybean plants only to P deficiency, the decrease of P accumulation was observed to be higher than N accumulation [Chaudhary M.I. et al., 2008]. The water stress reduced P absorption and P concentration of soybean stems under field conditions [Gutierrez-Boem F. H., Thomas G. W., 1999]. Among the factors that plants develop in their adaptation to diverse environment stresses, an important role is attributed to formation of osmolytes, including proline [Chen T.H.H., Murata N., 2002]. Determination of free proline formation in soybean leaves demonstrated an accumulation of this amino acid under combined treatments of water stress and phosphorous deficiency in soil (fig. 1A). An increase in free proline accumulation in plants under water deficit and phosphorous nutrition was recently observed by our group in other C3 plants, like oilseed rape and rye [Brandt C. et al., 2011].

Table 2

Activity of nitrate reductase (NR), glutamine synthetase (GS) and glycolate oxidase (GO) in soybean leaves under water and phosphorous deprivation

Soil humidity level; % of water holding capacity (WHC)	NR; µg NO_2/ hour/ g FW	GS;µM γGHA/miin/ G FW	GO; mM Glyoxilic acid/ /min /g FW
70	17,36 (1,58)*	7,22 (0,37)*	0,197 (0,003)*
30	7,20 (1,018)*	5,88 (0,248)*	0,184 (0,006)*
γGHA (gamma glutamyll hydroxamic acid). **STDEV** is given in parentheses, n=4			

Table 1

Effects of irrigation and phosphorous deficiency on soybean biomass production (g/pot); P, K, Ca, Mg and N uptake (mg/pot) in the pot experiment (n=4)

Irrigation	Plant height (cm)/pot	Fresh weight (g)/pot	Shoot P uptake	Shoot K uptake	Shoot Ca uptake	Shoot Mg uptake	Shoot N uptake
70 % WHC	54,55(3,16)*	94 (6,73)*	34.5 a A	324 a A	313.9 c A	96.8 c A	618.8 b A
30 % WHC	40,13 (5,68)*	30,95(2,55)*	9.3 a B	126 a B	124.3 c B	36.8 c B	262.9 a B
Different small letters indicate significant differences between the crops (p ≤ 0.05), different capital letters indicate significant differences between the irrigation treatments. Duncans multiple range test after one way analysis of variance. **STDEV** is given in parentheses							

Soybean plants, like other C_3 crops, exhibit photorespiration and losses of about 30% of CO_2 assimilated in photosynthesis. The key enzyme of photorespiration is glycolate oxidase (GO), localized in peroxisomes, that catalyzes the oxidation of glycolate, as it is formed rapidly during photosynthesis in chloroplasts. It is known that N and C metabolisms in plants have some points of intersection, including photorespiration [Oliveira I., 2002]. Investigations [Rachmilevitch S. et al., 2004] demonstrated that photorespiration determines NO^{3-} assimilation in plants. Nitrate is converted to NH^{4+} by nitrate reductase (NR). The ammonia ions formed are assimilated in reaction, catalyzed by glutamine synthetase (GS). Our results on GO, NR and GS from soybean leaves in relation to soil P and water deficiency are presented in table 2. It can be observed that all three enzyme activities were reduced when grown under P deprivation and water stress (30 % WHC) conditions. But the more drastic response to drought and P deficiency was observed in the NR activity inactivation (tab.2), while the GO activity only slightly declined in the stressed leaves. The results on the NR and GS activities (tab.2) correlate with the data on fresh weight and N uptake in soybean stems (tab.1). The evolution of N assimilation and synthesis processes in plants require energy.

water stress with respect to P deficiency in soil provoked an increase of all three leaf enzymes under study – acid phosphatise (AcP), alkaline phosphatise (AlP) and phosphodiesterase (PDE). The activity of AcP in soybean leaves under both factors has been shown to be much higher (by about 3.5 times), than AlP and PDE. The activity of AcP was proposed to be utilized as a test for the P status in plants, as a P-deficiency indicator [Besford R.T., 1980]. It is considered that acid phosphatase activity in plants participate in the internal Pi-remobilization from cell sites less metabolically active to sites more metabolically active [Schachtman D.P. et al., 1998]. An enhancing AcP activity was observed in plants suffered of P deficiency and in young developing plant organs [Duff S.M.G. et al., 1994]. These authors supposed that AcP hydrolyzes P organic compounds from older tissues with formation of inorganic Pi and subsequently translocation of the latter to plant apical growing parts.

Some scientists observed correlations between the activity of AcP and P concentrations in organs of *Phaseolus vulgaris* L. and *Vigna unguiculata* L Walp [Fernandez D.S., Ascencio J., 1994], But these authors studied only different P doses and did investigate water stress and did not estimate P uptake. As it can be observed, our data on P uptake by plant shoots (tab. 1) and AcP activity in leaves

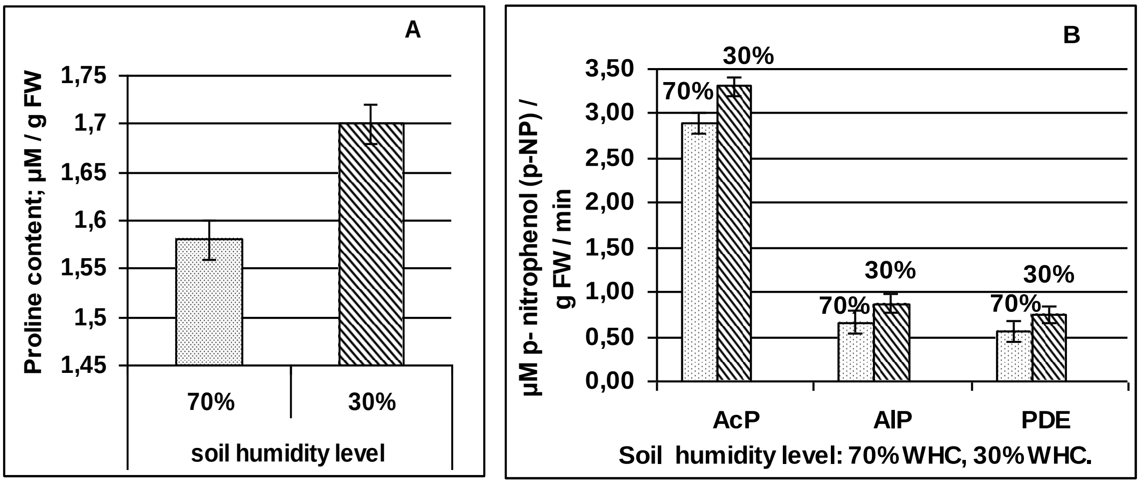

Figure 1. **Proline accumulation and phosphatase activities in soybean leaves in relation to soil water level and P insufficiency. AcP- acid phosphatise; AlP – alkaline phosphatise; PDE – phosphodiesterase.**

P compounds, that represent the main cell energy sources, are involved in a large number of metabolic events in plants. Phosphatases represent the main class of enzymes implicated in P uptake, utilization and internal remobilization of Pi [Duff S.M.G., et al., 1994].

The enzyme activities of phosphatises were measured under the combined effect of water and P deprivation. The results (fig. 2B) show that

(fig. 2B) do not correlate. Inhibition of P uptake in soybean stems by water stress (30% WHC) and P deficiency (tab.1) provokes an increase of AcP, AlP and PDE in leaves under these conditions (fig.2B). These findings indicate that under the impact of the investigated factors, i.e. water and P deprivation, some remobilizations in internal P compounds take place with involvement of phosphatase activities.

CONCLUSION

The results on soybean yield parameters and assimilation of main nutrient elements show that water stress accompanied by P deficiency in soil induced inhibition of growth processes, significantly declining plant height and shoot biomass, as well as P, K, Ca, Mg and N uptake by plants. Reduction in growth indices under water and P deficiency evolved through the decreasing of leaf enzyme activities of nitrate reductase, glutamine synthetase and glycolate oxidase.

On the other hand, our study demonstrates that to cope with water stress and P deficiency conditions, soybean plants involved enhancement of proline accumulation and catabolism of internal phosphorous esters by inducing the activities of acid phosphate (AcP), alkaline phosphatase (AlP) and phosphodiesterase (PDE) in leaves. It should be noted that the average values of AcP, for both water treatments, were by about four times higher than those of AlP and PDE, the fact that indicates the imposed contribution of AcP in P metabolism.

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