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EFFECT OF PROLINE AND ABSCISIC ACID ON THE GROWTH AND PHYSIOLOGICAL PERFORMANCE OF FABA BEAN UNDER WATER STRESS

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Abstract

Water stress is a serious threat to the agricultural production. Therefore, the present experiment was aimed to study the effect of abscisic acid (ABA) and/or proline (Pro) on the performance of faba bean under water stress condition. Under water deficit condition, all parameters [plant height and root length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, total soluble carbohydrates (TSC), chlorophyll (Chl) *a* and *b*, and activity of peroxidase (POD), and catalase (CAT)] of faba beans were strongly depressed, except malondialdehyde (MDA) and Pro contents. On the other hand, application of ABA and Pro alone as well as in combination improved all growth characteristics by improving Pro, TSC, photosynthetic pigments, CAT and POD. These results indicate that application of ABA and Pro together was more effective, and helped the plant to restore the altered physiological process induced by water stress.

Introduction

Water stress is one of the major constraints limiting the production of crop worldwide. Arid and semi arid areas cover four-tenths of the world's land and constantly increasing due to drought stress that affects agricultural land. These problems are accentuated by other abiotic stresses as well as the global warming (Blum, 2011). Today, it has become important task to combat drought stress worldwide. Under abiotic stresses such as drought, salinity and heavy metal toxicity, plant growth and many physiological processes are dramatically affected by the over-production of reactive oxygen species (ROS) that are responsible for oxidative damage of cell (Monneveux *et al.,* 2006; Siddiqui *et al.,* 2008; Azhar *et al.,* 2011; Siddiqui *et al.,* 2011; Moosavi 2012; Siddiqui *et al.,* 2012). Water stress inhibits the photosynthesis by altering the activity of ribulose-1,5-bisphosphate carboxylase and stomatal conductance (Cornic, 2000; Parry *et al.,* 2002). Also, respiration, translocation, ion uptake, carbohydrates, nutrient assimilation and growth promoters are disturbed under stress (Jaleel *et al.,* 2008; Farooq *et al.,* 2008). It is a fact that tolerance of plant to abiotic stresses is very complex, because various molecular, biochemical and physiological mechanisms are involved in plant growth and development (Razmjoo *et al.,* 2008).

Proline (Pro) is a universal osmoprotectant and acts as an osmolyte and antioxidant, as well as a source of energy, reducing equivalent, N and carbon and is a proteinogenic amino acid (Kuznetsov & Shevyakova, 1999, Matysik *et al*., 2002; Szabados & Savouré, 2009; Ali *et al*., 2011). To fight against abiotic stress, plant has developed mechanisms, such as osmotic adjustment that is usually accomplished by uptake of inorganic ions, as well as the accumulation of compatible solutes (osmoprotectants) such as Pro and glycinebetaine. Under stress, Pro accumulated in many plants and acts as a signaling molecule and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados & Savouré, 2009). However, the regulation and function of Pro accumulation are not yet completely understood (Szabados & Savouré, 2009). Abscisic acid (ABA) is an important phytohormone that plays a key role in plant signaling system which helps the plant to perform function normally under water and temperature stress conditions (Finkelstein *et al.,* 2002; Hussain *et al.,* 2010). Therefore, aim of the present experiment was to study the effect of Pro and abscisic acid (ABA) on the growth and physiological characteristics of *Vicea faba* L., under stress. We studied the ameliorating effect of Pro and/or ABA on the content of chlorophyll (Chl), total soluble carbohydrates (TSC) and Pro, and antioxidant enzymes activity under water stress.

Materials and Methods

Plant growth and experiment design: To achieve the objective mentioned in the introduction, under water stress, the responses of faba bean (*Vicia faba* L.) cv. TARA to Pro and ABA application were investigated by conducting a greenhouse pot experiment at the Department of Botany and Microbiology, King Saud University, Riyadh, KSA. Seeds were obtained from the local market of Riyadh. Before sowing, seeds were surface sterilized with ethyl alcohol then vigorously rinsed with double distilled water (DDW). Seeds were sown in pot (6 inch diameter) filled with perlite. The pots were arranged in a simple randomized design with a single factor and four replicates, and each pot irrigated with DDW lightly before sowing to maintain equal moisture content on the surface of perlite. The treatments as given follows (i) 100 DDW + 0 mM Pro + 0 mM ABA (T_1), (ii) 60 DDW + 0 mM Pro + 0 mM ABA (T_2), (iii) 30 DDW + 0 mM Pro + 0 mM ABA (T_3), (iv) 100 DDW + 20 mM Pro + 0 mM ABA (T_4), (v) 60 DDW + 20 mM Pro + 0 mM ABA (T_5), (vi) 30 DDW + 20 mM Pro + 0 mM ABA, (T_6), (vii) 100 DDW + 0 mM Pro + 0.1 mM ABA (T_7), (viii) 60 DDW + 0 mM Pro + 0.1 mM ABA (T_8), (ix) 30 DDW + 0 mM Pro + 0.1 mM ABA (T_9), (x) 100 DDW + 20 mM Pro + 0.1 mM ABA (T_{10}), (xi) 60 DDW + 20 mM Pro + 0.1 mM ABA (T_{11}) and (xii) 30 DDW + 20 mM Pro + 0.1 mM ABA (T_{12}). Treatments were applied at after every 2 days with Raukura's nutrient solution (Smith *et al*., 1983) containing Pro and ABA. The concentration of Pro and ABA were selected for the

study on the basis of earlier experiment (Ali *et al.,* 2007; Chen *et al.,* 2011). The plants were sampled at 30 days, after sowing to assess their growth characteristics (plant height plant^{-1}, root length plant^{-1}, root fresh weight (FW) plant^{-1}, shoot FW plant^{-1}, root dry weight (DW) plant^{-1}, shoot DW plant^{-1}, leaf number and area leaf^{-1} (LA) and physio-biochemical attributes (total soluble carbohydrates (TSC), Chlorophyll (Chl) *a* and Chl *b*, proline (Pro) and malondialdehyde (MDA) content, and activity of peroxidase (POD) and catalase (CAT).

Measurement of growth characteristics: The shoot and root length of plant were measured by using a meter scale after removal from the pots. The plants were then placed in an oven run at 60°C for 48 h. These dried plants were weighed to record the plant DW. LA was measured using a LI-3000 Portable Leaf Area Meter (LI-COR, Lincoln, NE, USA).

Determination of physiological and biochemical parameters: The youngest fully expanded leaves were extracted with 80% acetone and the absorbance was read spectrophotometrically. The Chl was determined by using the formula of Arnon (1949).

Assay of Pro concentration was determined spectrophotometrically by adopting the ninhydrin method of Bates *et al*., (1973). We first homogenized 300 mg fresh leaf samples in sulphosalicylic acid. To the extract, 2 mL each of ninhydrin and glacial acetic acid were added. The samples were heated at 100°C. The mixture was extracted with toluene and the free toluene was quantified spectrophotometrically at 528 nm using L-proline as a standard.

Determination of MDA concentration was determined according to the method of Heath & Packer (1968). Leaves were weighed and the homogenates, containing 10% trichloroacetic acid, 0.65% 2-thiobarbituric acid were heated at 95°C for 60 min, then cooled to room temperature and centrifuged at 10,000×g for 10 min. The absorbance of the supernatant was read at 532 and 600 nm against a reagent blank.

Determination of antioxidant enzymes activity

Preparation of enzyme extracts: A crude enzyme extract was prepared by homogenizing 500mg of leaf tissue in extraction buffer containing 0.5% Triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000×g for 20 min at 4°C. The supernatant was used for the enzymatic assays. For APX, extraction buffer was supplemented with 2 mM ascorbate.

POD (E.C. 1.11.1.7) activity was determined by the method of Chance & Maehly (1955). Five milliliters of the assay mixture for the peroxidase activity comprised: phosphate buffer (pH 6.8), 50 M of pyrogallol, 50 mM of H_2O_2, and 1 ml of the 20 times-diluted enzyme extract. This was incubated for 5 min at 25°C after which the reaction was stopped by adding 0.5 mL of 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by taking the absorbancy at 420 nm.

CAT (EC 1.11.1.6) activity was measured as described by Aebi (1984). The decomposition of H_2O_2 was monitored by the decrease in absorbance at 240 nm. For the assay, a 50 mM phosphate buffer (pH 7.8) and 10 mM H_2O_2 was used.

Statistical analysis

The data were analyzed statistically with SPSS-11 statistical software (SPSS Inc., Chicago, IL, USA). Mean was statistically compared by Duncan's multiple range test (DMRT) at P>0.05%.

Results

All growth traits (root length, shoot length, root FW, shoot FW, root DW, shoot DW, leaf number and LA) of faba bean decreased significantly with increasing water stress (Figs. 1A-D and 2A-D). However, exogenous application of Pro and ABA to the plants lowered the inhibitory effect of water stress by improving all growth parameters. Application of Pro gave maximum value in comparison to ABA for all growth attributes. The combined application of Pro and ABA was found to be more effective in alleviating the adverse effect of water stress on root length, shoot length, root FW, shoot FW, root FW, root DW, leaf number and LA. Under non-stress condition, the combined application of Pro and ABA significantly increased all growth characteristics.

Under water stress conditions, the concentration of MDA increased with increasing water stress treatments. However, application of Pro and ABA alone as well as in combination decreased the concentration of MDA in leaf of faba bean plants (Fig. 3A). Pro fed plants exhibited lower content of MDA than the plant exposed with ABA, but plants treated with combined application of Pro and ABA showed highest decrease in MDA concentration in leaf under water stress.

It is evident from Figure 3B that the concentration of TSC decreased with increasing water stress. However, the content of Pro increased with increasing water stress at certain level (Fig. 3C). Exogenous application of Pro and ABA individually on stressed or non-stressed plants enhanced significantly both Pro and TSC content in leaf of faba bean. However, the combined application of Pro and ABA proved more effective than alone application of Pro and ABA to improve the accumulation of TSC and Pro.

In the present study, results show that under non-stress condition, Pro and ABA-fed plants exhibited maximum Chl *a* and Chl *b* concentrations in comparison to the controls (Figs. 4A, B). The concentration of Chl *a* and Chl *b* decreased with increasing water stress. However, application of Pro and ABA alone was found to be effective in improving the content of Chl *a* and Chl *b*, but Pro application individually had better alleviating adverse effect than ABA alone. The efficiency of the combined Pro and ABA-treated plants was better in alleviating the adverse effect of water stress than alone for these traits.

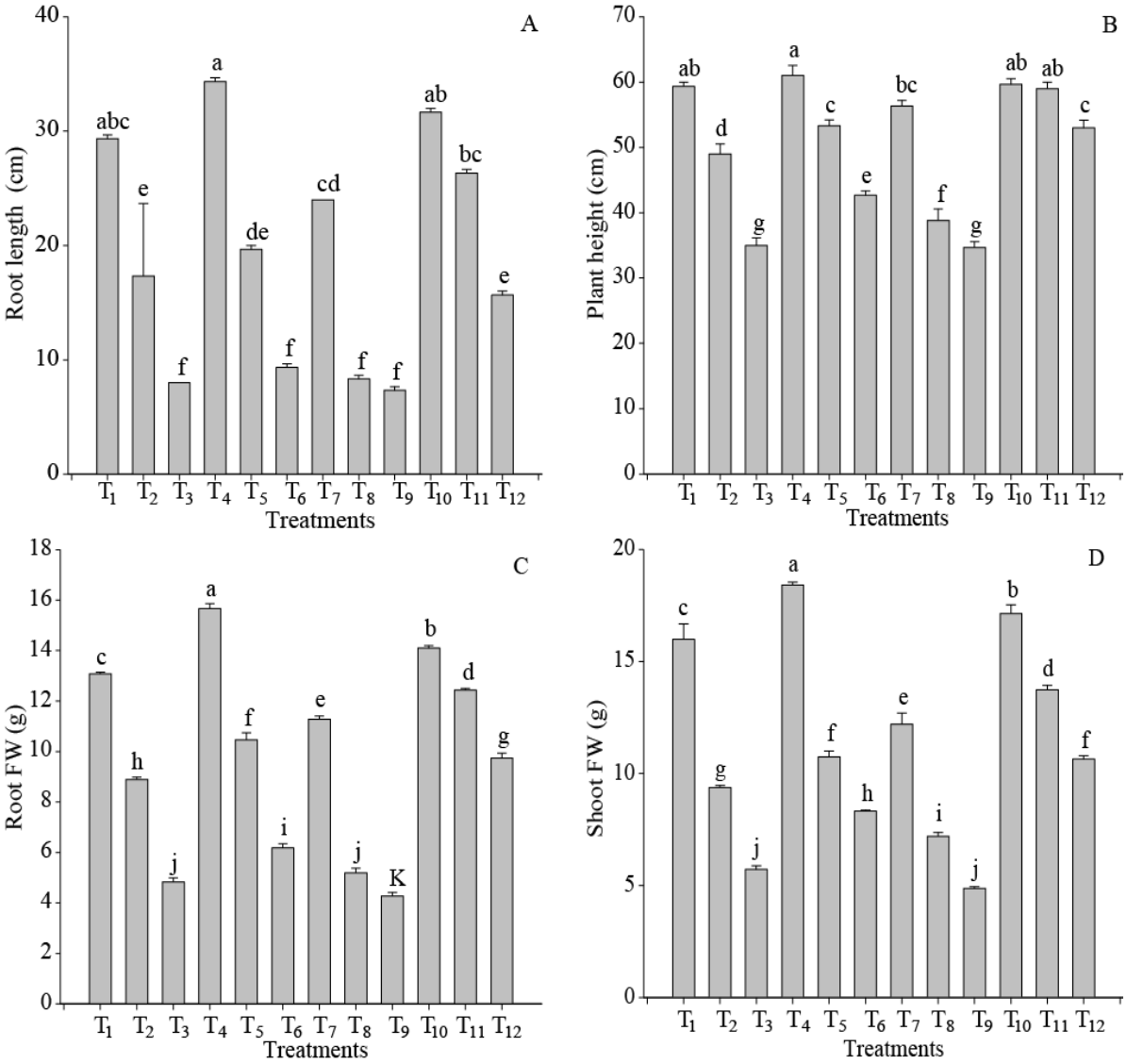

Fig. 1. Effect of ABA and Pro on root length plant^{-1} (A), plant height plant^{-1} (B), root fresh weight plant^{-1} (C) and shoot fresh weight plant^{-1} (D) of faba bean plants. Bars with the same letter do not differ at the P<0.05 level of significance (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

The activity of antioxidant enzymes (CAT and POD) was depressed by water stress (Figs. 5A, B). Application of Pro and ABA alone as well as in combination induced the activity of both enzymes under water stress, but highest activity of the CAT and POD was recorded in plants fed with Pro alone. However, the application of Pro and ABA together was found to be more potential in mitigating the adverse effect of water stress by increasing activity of antioxidant enzymes as compared with Pro and ABA alone.

Discussion

In the present experiment, plant growth parameters i.e., root length, shoot length, root FW, shoot FW, root DW, shoot DW, leaf number and LA decreased with increasing water stress (Figs. 1 A-D and 2 A-D). A decrease in plant growth might be due to inhibition of cell enlargement and cell division, and reduction of various plant growth metabolisms (Yordanov *et al.,* 2003; Farooq *et al.,* 2008). However, application of Pro and ABA enhance all growth trails by alleviating the adverse effect of low water availability. Application of Pro with ABA was found to be more effective in comparison to alone application of Pro and ABA. This result strengthens the findings of Ali *et al.,* 2007, who reported that exogenous application of Pro improved plant growth of maize under water stress condition. Interestingly, in the present study, application of Pro applied with ABA was found to be effective in alleviating the adverse effect of water stress by improving growth characteristics of plant. Also, application of ABA and Pro may be involved directly or indirectly in the growth process, hence, the enhanced values for various growth parameters of treated plants.

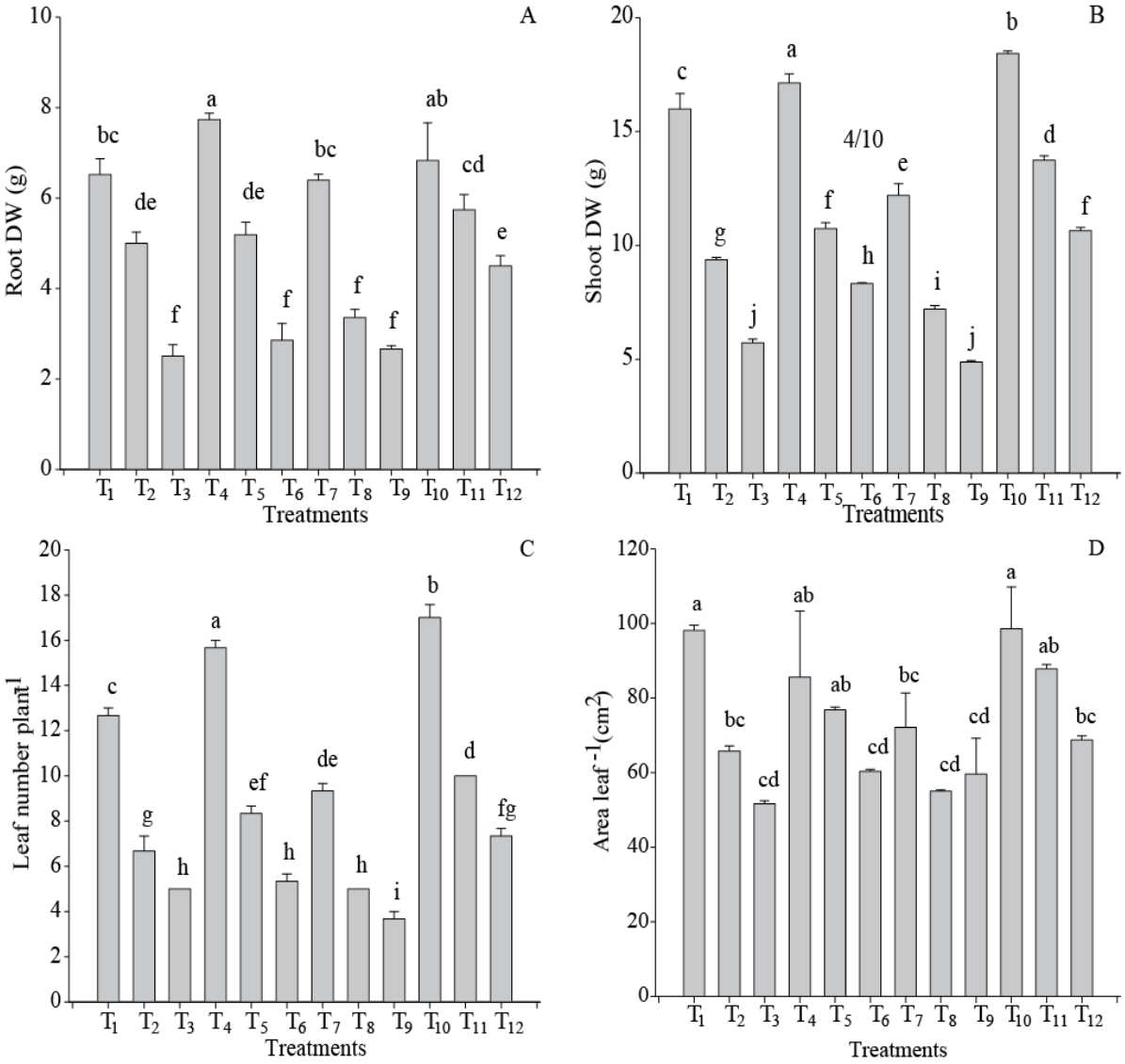

Fig. 2. Effect of ABA and Pro on root dry weight plant^{-1} (A), shoot dry weight plant^{-1} (B), leaf number plant^{-1} (C) and area leaf^{-1} (D) of faba bean plants. Bars with the same letter do not differ at the P<0.05 level of significance (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

The increase in MDA content in the plants grown in water deficit condition (Fig. 3A) indicates that water stress may be responsible for increasing lipid peroxidation leading to cell damage. This result is in accordance with previous findings of other workers (Sairam *et al.,* 2000; Moussa & Abdel-Aziz, 2008). However, application of ABA and Pro alone as well as in combination significantly reduced the accumulation of MDA in leaf of plant grown under water deficit condition (Fig. 3A). It may be due to the accumulation of Pro which has an adaptive significance, as it lowers the generation of free radicals and thus reduces the lipid peroxidation linked to the membrane deterioration under water stress (Siddiqui *et al.,* 2012; Ali *et al.,* 2012; Siddiqui *et al*., 2013). In plants under stress, high content of TSC and Pro was recorded when they treated with individual as well as in combination of Pro and ABA (Figs. 3 B &C). Stewart (1980) demonstrated that ABA induces the synthesis of Pro by stimulation of glutamic acid in barley plants. The improved-Pro accumulation with the application of ABA and /or Pro may be responsible for the membrane protection and pressure potential stabilization (Santarius, 1992) which seems to be more effective to improve the tolerance of plants to water stress. Pro, a universal osmoprotectant, acts as an antioxidant and a source of energy (Matysik *et al.,* 2002), and regulates gene expression for osmotic adjustment (Iyer & Caplan, 1998). TSCs are the major soluble constituents that help plant cells in maintaining osmotic balance and also provide rapid growing cells with energy and with the carbon skeletons required to synthesize organic compounds (Taiz & Zeiger, 2010). This work indicates that improvement of tolerance is associated with increases in the concentrations of Pro and TSC caused by the addition of both ABA and Pro to the growth medium.

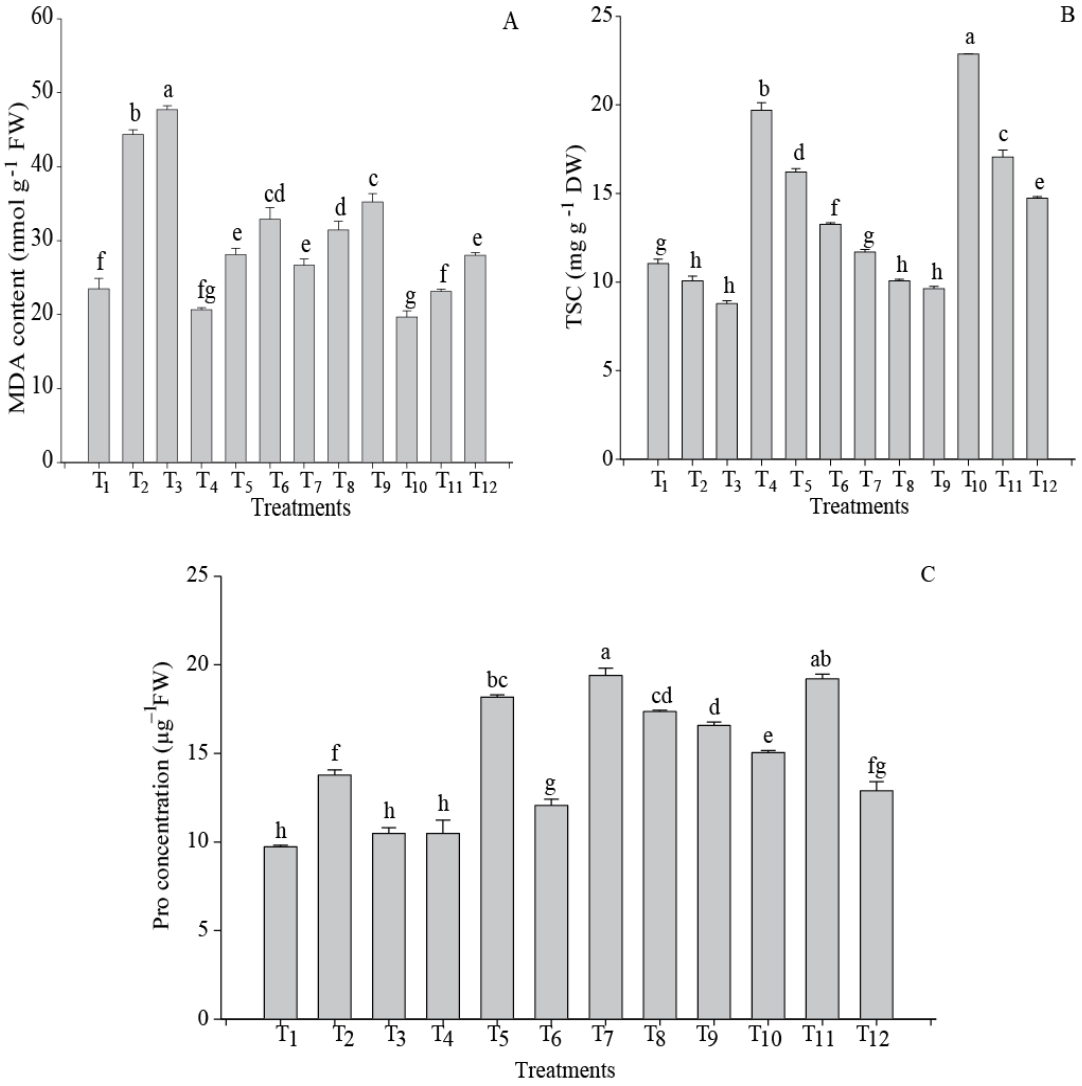

Fig. 3. Effect of ABA and Pro on the content of MDA (A), TSC (B) and Pro (C) in faba bean plants. Bars with the same letter do not differ at the $p<0.05$ level of significance (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

The present study also revealed that water stress caused a significant reduction of Chls synthesis (Fig 4 A and B). These results strongly agree with the findings of Ullaah *et al*., (2012). This may be due to the inhibition of photosynthetic electron transport chain (Mohanty *et al.,* 1989) and to lipid peroxidation and hydrogen peroxide accumulation, which were both significantly affected by stress. The inhibition of photosynthetic pigments may be due to the reduced photosynthetic capacity during water stress which indicates photo inhibition (Osonubi & Davies, 1980) or direct effects of dehydration on photosynthetic processes (Kaiser, 1987). Interestingly, it is very clear that application of ABA and Pro alone as well as in combination significantly reduced the inhibitory effect of water stress by improving both photosynthetic pigments and antioxidant enzymes (CAT and POD) (Figs. 4A & B and 5A & B). The increase in TSC content may be responsible for providing carbon skeleton and improvement of photosynthetic pigments that lead to the better dry mater production (Figs. 2A & B) (Al-Whaibi *et al.,* 2012). Both antioxidant enzymes significantly decreased with increasing water deficit (Figs. 5A & B). However, in plants under stress, high activity of both enzymes was recorded when they were treated with ABA and Pro individually as well as in combination. The enhanced activity of antioxidative enzymes might be due to the role of ABA in the accumulation of TSC in the plant. Thus, we may postulate that application of ABA with Pro in the present study was more effective to enhance the plant tolerance to water stress.

Conclusion

We conclude that application of ABA and Pro alone enhanced the concentration of Chl *a, b*, Pro, TSC and the activity of antioxidant enzymes resulted in an increase in plant growth parameters. However, an increase in photosynthetic pigments and TSC in ABA + Pro treated plants revealed that the combined treatment was very effective to alleviate the inhibitory effect of water stress by improving antioxidant system and growth traits of plants.

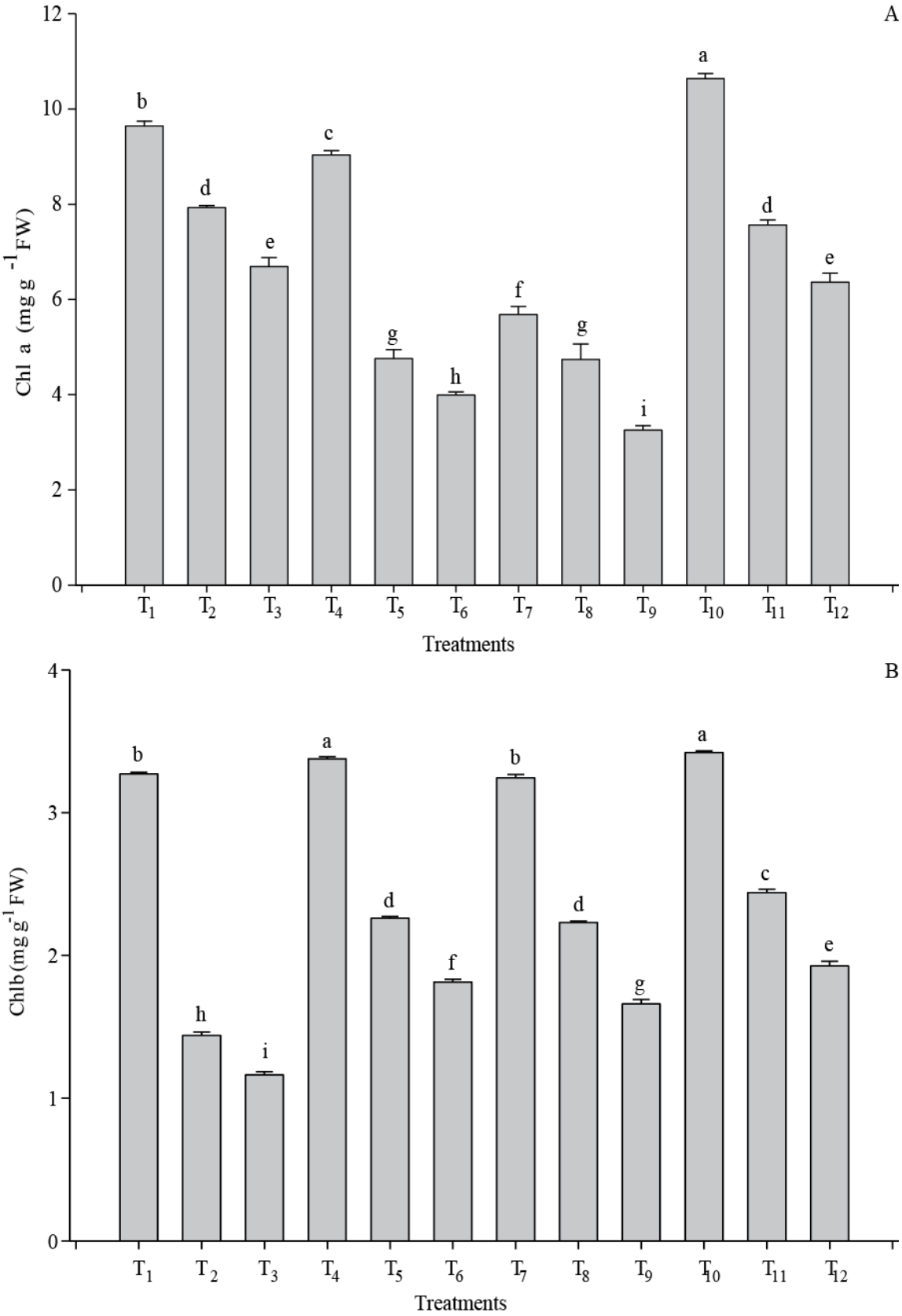

Fig. 4. Effect of ABA and Pro on the content of Chl *a* (A) and Chl *b* in faba bean plants. Bars with the same letter do not differ at the P<0.05 level of significance (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

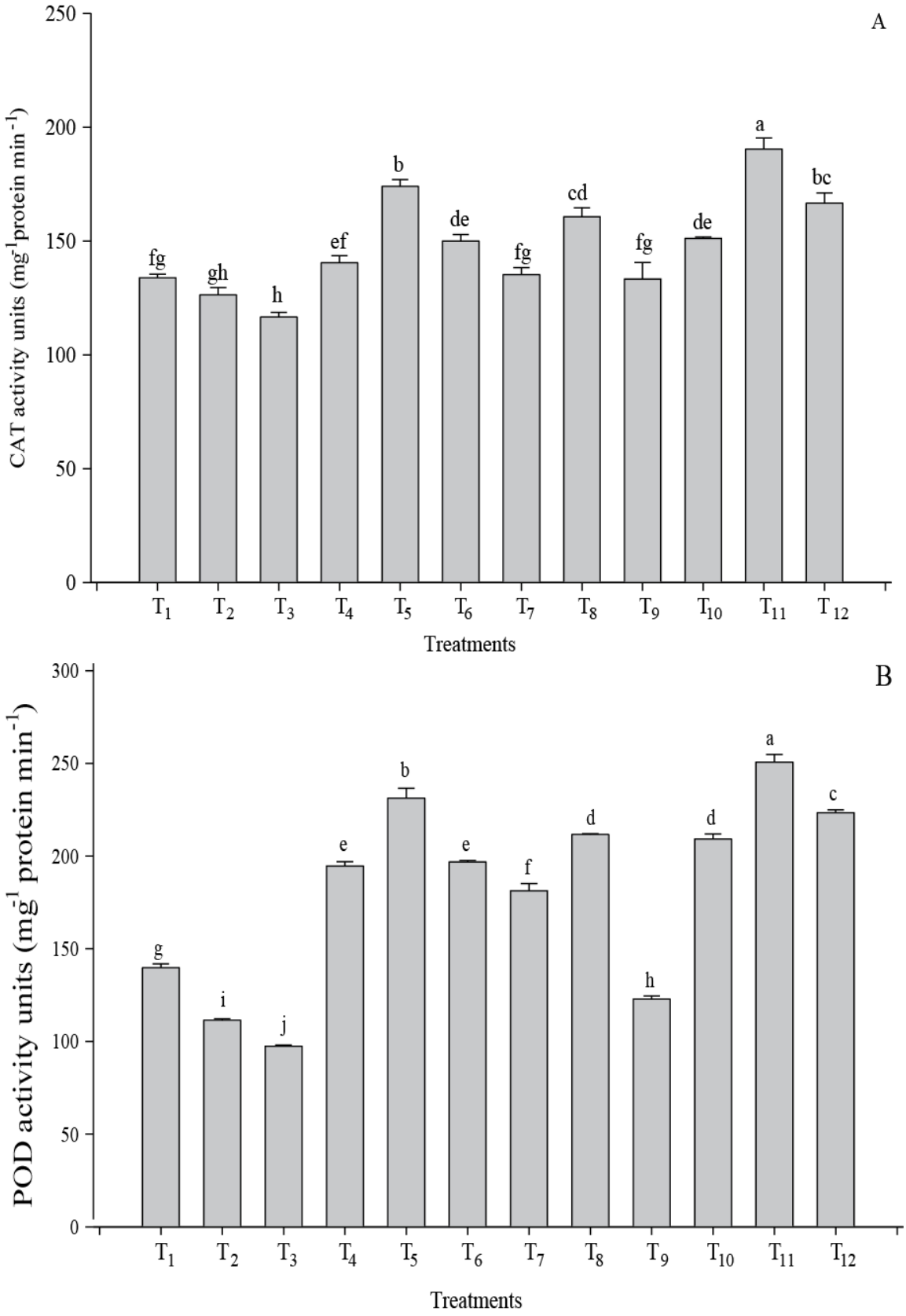

Fig. 5. Effect of ABA and Pro on the activity of CAT (A) and POD (B) in faba bean plants. Bars with the same letter do not differ at the $P<0.05$ level of significance (Duncan's multiple-range test). Average of four determinations are presented with bars indicating SE.

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