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Research Article – Plant Physiology

Induction of drought stress tolerance by propiconazole and salicylic acid in *Sorghum bicolor* is mediated by enhanced osmoregulation, compatible solutes and biochemical accumulation

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Abstract

A pot-culture experiment was carried out to estimate the ameliorating effect of Propiconazole (PCZ) and Salicylic acid (SA) on drought stressed *Sorghum bicolor*. The plants were subjected to 3, 6 and 9 Day Interval Drought (DID) and drought with PCZ 1 mM and SA 1 mM alone from 30, 40 and 50 Days After Sowing (DAS). One day interval irrigation was kept as control. The plant samples were collected on 60, 70 and 80 DAS then separated into root and shoots for estimate the protein, proline, amino acid (AA), glycine betaine (GB) and total sugar content. Drought stress inhibited protein content then the proline, AA, GB and total sugar contents were increased when compared to control. Plants were treated drought with PCZ and SA these parameters to a larger extent when compared to drought stressed plants. The PCZ and SA treatments increased the protein content, but decreased the proline, AA, GB and total sugar contents when compared to drought stressed plants. From the results of this investigation, it can be concluded that the application of PCZ and SA caused a partial amelioration of the adverse effects of drought stress in sorghum plants.

Key words: Drought, Propiconazole, Salicylic acid, *Sorghum bicolor*, Osmoregulation, Compatible solutes.

Introduction

Stress is an altered physiological condition caused by factors that tend to disrupt the equilibrium. Strain is any physical and chemical change produced by a stress (Gaspar *et al.,* 2002). Stress being a constraint or highly unpredictable fluctuations imposed on regular metabolic patterns cause injury, disease or aberrant physiology. Plants are frequently exposed to many stresses such as drought, low temperature, salt, flooding, heat, oxidative stress and heavy metal toxicity while growing in nature. Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Water deficit and salt stresses are global issues to ensure survival of agricultural crops and sustainable food production (Jaleel *et al.,* 2007). In fact the drought stress indicated that proline accumulation can be the result of synthesis stimulation, inhibition of proline oxidation and/or alteration of proteins synthesis (Stewart *et al.,* 1977). Under drought, the maintenance of leaf turgor may also be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycinebetaine and other solutes in cytoplasm improving water uptake from drying soil. The process of accumulation of such solutes under drought stress is known as osmotic adjustment which strongly depends on the rate of plant water stress (Anjum *et al.,* 2011).

Sorghum (*Sorghum bicolor* L.) Moench is a multipurpose crop belongs to the family Poaceae. Sorghum is an important crop in Semi-Arid

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Tropics (SAT) and is the source of staple food crop for Africa, South Asia and Central America. It is one of the five major cultivated species the world because it has several economically important potential uses such as food (grain feed (grain and biomass), fuel (ethanol production), fibre (paper), fermentation (methane production) and fertilizer (utilization of organic by products). Sorghum is an important alternative for human and animal food, especially in regions of low water availability, in which seed is rich in protein, vitamins, carbohydrates and minerals (Carvalho *et al.,* 2000).

Plant growth regulators play an important role in the regulation of plant developmental processes and signalling networks as they are involved either directly or indirectly in a wide range of biotic and abiotic stress responses and tolerance in plants (Asgher *et al.,* 2015). SA is a endogenous growth regulator of phenolic compound synthesized throughout the plant kingdom via the phenylpropanoid pathway. SA is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline metabolism, production of glycine betaine, antioxidant defence system, and plant- water relations under stress conditions and thereby provides protection in plants against abiotic stresses (Miura and Tada 2014).

Propiconazole (1-[2-(2, 4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole) is a well-known triazole group of systematic fungicides, which have plant growth regulation properties (Fletcher and Arnold 1986). However, triazole-based fungicides induce a suite of morphological and physiological adaptations that allow plants to tolerate a broad range of environmental stresses including drought, herbicide treatment and elevated temperatures (Jaleel *et al.,* 2006).

Therefore the objectives of the present investigation was conducted to evaluate the osmotic maintenance, biochemical and compatible solute accumulation of *Sorghum bicolor* under individual and combined drought, drought with PBZ and drought with SA treatment.

Materials and methods

Plant cultivation and treatment induction

The sorghum variety CO-30 was obtained from Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India. The triazole compound PCZ obtained from Syngenta India ltd., Mumbai. The phenolic compound SA was purchased from Himedia India Ltd., Mumbai. The experimental part of this works was carried out in botanical Garden and Plant Growth Regulation Lab, Department of Botany, Annamalai University, Tamilnadu, India. The plants were raised from plastic pots. The plastic pots were filled with homogenous mixture of the garden soil containing red soil, and along with farm yard manure ratio of (1:1:1). The pots were arranged in Completely Randomized Block Design (CRBD).

The experimental seeds were surface sterilized with 0.2% Mercuric chloride solution for five minutes with frequent shaking and thoroughly washed with tap water. The plants were allowed to grow up to 30 days with regular water irrigation. After 30 days, well established plants were selected for treatments. The drought stress imposed on 3, 6 and 9DID from 30 to 60 DAS. The PCZ (1 mM) and SA (1 mM) treatments are given on 30, 40, and 50 DAS. The control plants were regularly irrigated with ground water. The plants were taken randomly 60, 70 and 80 DAS on 10 days interval.

Estimation of protein content

Extraction and estimation of the protein content was followed by the method of Lowry *et al.,* (1951). The 500 mg of plant material was ground with 10 ml of 20 % TCA. The homogenate was centrifuged at 800 rpm for 10 min. The supernatant was discarded and to the pellet, 5 ml of 0.1 N NaOH was added to solubilize the protein and the solution was centrifuged again at 800 rpm for 5 min. The supernatant was made up to 10 ml with 0.1 N NaOH and used for the estimation of protein content. To 1 ml of the extract was added with 5 ml of reagent 'c' than incubated at dark for 10 min. After 10 min 1 ml of Folin-ciocalteau reagent was added and it was incubated at dark for 30 min. The absorbance was read at 660 nm using a spectrophotometer.

Estimation of proline content

The Proline content was estimated by the method of Bates *et al.,* (1973). The plant material (0.5 g) was homogenized in 3 % aqueous sulfosalicylic acid and the homogenate was centrifuged. Then the homogenate was filtered through Whatmann No.1 filter paper. 2 ml of supernatant was taken in a test tube, and 2 ml of acid ninhydrin and 2 ml of glacial acetic acid was added to it and boiled at 100 ºC for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene, and the absorbance was read at 520 nm. The free proline was calculated, using L-proline as a standard.

Estimation of amino acids content

The amount of Amino acid content was estimated according to the method of Moore and Stein (1948). Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 80 % boiled ethanol. The extract was centrifuged at 800 rpm for 15 min. and the supernatant was made up to 10 ml with 80 % ethanol. In a 25 ml test tube, ethanol extract was taken and it was neutralized with 0.1 N NaOH using the methyl red indicator to which ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 min and then 5 ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm in a spectrophotometer.

Estimation of glycine betaine content

Glycine betaine content was estimated by the method of Grieve and Grattan (1983). Finely ground dry tissue was diluted with equal volume of 1 M H_2SO_4, made into aliquots of 0.5 ml in micro centrifuge tubes, cooled over ice for 1 h and to each of these tube, 0.2 ml of cold potassium iodide reagent was added. The reactants were gently stirred, stored at 4 ºC overnight and centrifuged at 10,000 rpm for 15 min at 4 ºC to get the precipitated per iodide crystals. The crystals were dissolved in 1,2-dichloroethane, and the absorbance was measured at 365 nm after 2 h. Glycine betaine was dissolved in 1 M H_2SO_4 and it was served as a standard

Estimation of total soluble sugar content

Total soluble sugar content was assayed as described by Nelson (1944). 0.5 gm of fresh plant material was homogenized in a mortar and pestle with 80 % ethanol. The extract was evaporated to dryness in a water bath. To the residue, 1 ml of distilled water and 1 ml of 6 N H_2SO_4 were added. The mixture was incubating in a water bath at 50 ºC for an hour. The solution was cooled and 1 N NaOH was added then made up to 10 ml of H_2O. To 1 ml of fresh copper reagent and 1 ml of extract were added. The mixture was heated mouth covered with a boiling water bath for 20 min then cooled and 1 ml of Arsenomolybdate reagent was added. The final volume was made up to 20 ml with distilled water. The resultant blue color was read at 520 nm in a spectrophotometer against the appropriate blank. The sugar content was expressed in milligram per gram dry weight.

Statistical analysis

Statistical analysis are performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± S.D. for seven samples in each group. P-values$\leq$0.05 was considered as significant.

Results and Discussion

Osmolytes have some important role in plant responses to water stress and resistance. Osmotic adjustment and turgor regulation are the well-illustrated functions of these compounds in plants and algae since their high solubility in water acts as a substitute for water molecules released from leaves. The primary function of compatible solutes is to prevent water loss to maintain cell turgor and to maintain the gradient for water uptake into the cell. This metabolite accumulation in cells leads to increase in the osmotic potential and finally resulted in higher water uptake capacity by roots and water saving in the cells.

The present study protein content has been reduced in root and shoots to a large extent in various DID (3, 6 and 9 DID) stressed *S. bicolor* plant when compared to well-watered plant (Figure 1). The combined effect of drought with PCZ and SA application it will be increased when compared to drought stressed plants, but it was higher than control. The reduction in the total soluble proteins showed in water stress condition is due to probable increase of the proteases enzyme activities, in which this proteases enzyme promotes the breakdown of the proteins and consequently decreases the protein amount presents in the plant under abiotic stress conditions (Debouba *et al.*, 2006).

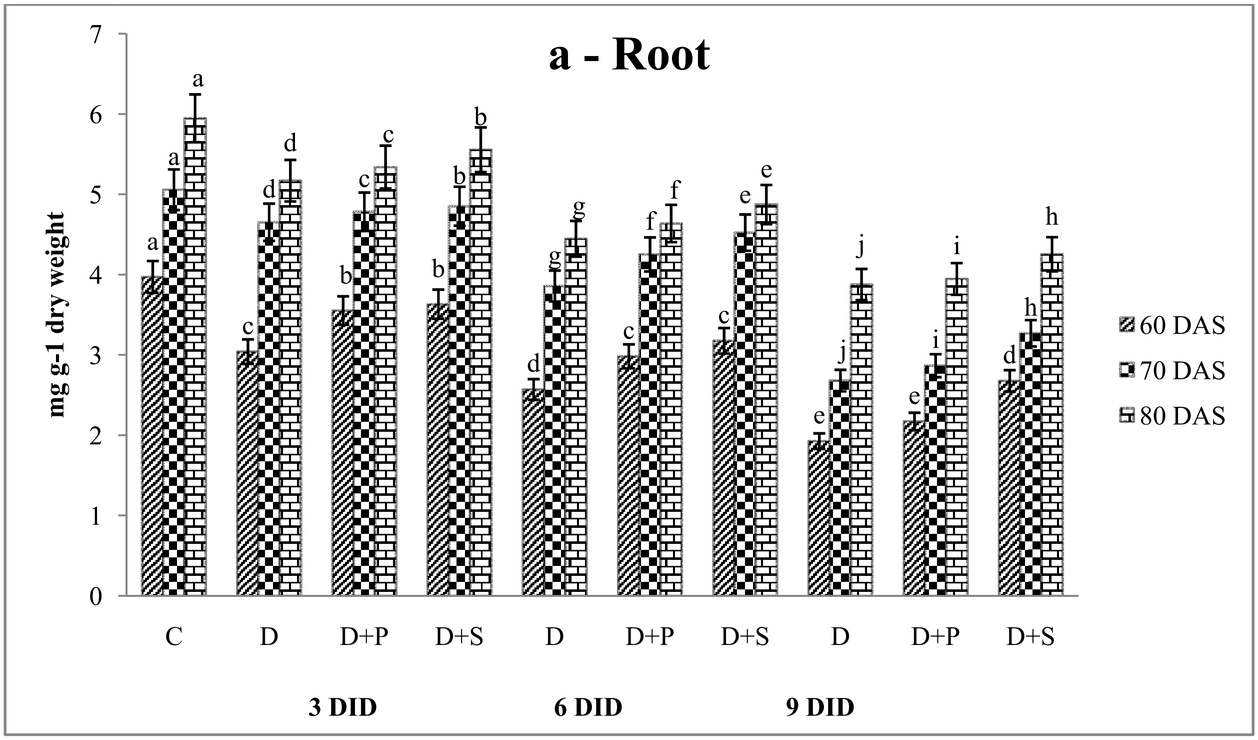
In inadequate conditions to the plant is active the pathway of proteins breakdown, because the plant use the proteins to the synthesis of carbon and nitrogen compounds as amino acids that might auxiliary the plant osmotic adjustment (Sankar *et al.,* 2007). Similar results on reduction in the proteins were found by (Shinde and Thakur 2015) in Pea plant. The SA was highly effective to increase the soluble protein content of sorghum plants. The results of present study are in agreement with (Khan *et al.,* 2012) who reported that exogenously applied SA enhanced soluble protein content in wheat seedlings under water stress conditions. Triazole with drought stress treatment resulted in increased Protein content in maize when compared to drought stress, but it was lower than that of control. The similar observation will be noted on Maize (Rajasekar *et al.,* 2015).

The compatible solute accumulates under water deficit plants. In the present investigation, an increase proline accumulation on 3, 6 and 9 DID stressed plants. The proline content reduced on combined effect of drought with PCZ and SA applied sorghum plants. The higher accumulation of proline content was observed in 9 DID, when compared to all other plants (Figure 2). The proline content mainly synthesized from glutamate, which is reduced to glutamate semialdehyde (GSA) by the enzyme pyrroline-5-carboxylate synthetase (P5CS), and spontaneously converted into pyrroline-5-carboxilate (P5C). These enzymes could be increased due to drought stress (Savoure 1995). The accumulation of proline content was response to abiotic stresses is widely reported and may play a role in plant stress adaptation within the cell (Shahbaz *et al.,* 2011; Sperdouli and Moustakas 2012). Depending on these a possible mechanism by which proline protects plant against abiotic stress, our results suggest that the increase of proline in stressed in Sorghum plants. The application of SA reduced the damaging effect of drought on plant growth and accelerated the restoration of growth process and reversed the effect of water deficit sorghum plant. The similar report was observed by (Fayez and Bazaid 2014) in barley. Accumulation of proline content was reduced on drought with triazole treated sorghum plant when compared to drought stressed plant. The same observation was noted by (Manivannan *et al.,* 2008) on *Helianthus annuus.*

The individual and combined treatments of drought with PCZ and SA enhanced the AA content in both root and shoot on all the sampling days of sorghum plants, when compared to well-watered plants (Figure 3). The accumulation of AA may be due to the increase showed in the free amino acids is due to high synthesis of amino acids from protein hydrolyses, in which the free amino acids are utilized by the plant to reduce the effects of the water deficit through organic solute accumulation and this way increased the water retention capacity (Sircelj *et al.,* 2005). Under water stress the free amino acids as proline and glycinebetaine are strongly influenced and consequently quickly accumulated (Nakamura *et al.,* 2001), as well as of secondary form occur the increase of aspartate, glutamate and alanine drought with SA treated plants AA content will be reduced when compared to drought stressed plants, but it was higher than that of control. The stimulation effect of SA on the biosynthesis of soluble proteins, may indicate that the possibility of their involvement in osmotic adjustment and consequently drought tolerance which contribute in reducing the injurious effects of water deficit and accelerating the restoration process during the period after action of stress, which might be a manifestation of the protective action of SA on sorghum plant. The reduction of proline and AA content was associated with increasing of soluble protein. The similar report was observed by (Azooz and Youssef 2010) in Hassawi wheat. Triazole treatment decreased AA content in plants under drought stress compared with the plants had received only water stress treatment in *Capsicum annuum* (Rabert *et al.,* 2013).

The plants exposure to drought stress increase GB content was observed in various DID (3, 6 and 9 DID) sorghum plant when compared to control. These study results suggest that the drought stress increased the amount of GB which might lead to the osmotic adjustment during the drought stress. The higher level of GB is accumulated in shoot tissue as compared to root tissue, which might be involved in improving photosynthesis. During drought stress, the accumulation of GB led to stabilization of the function of the thylakoid membranes, suppression of chlorophyll degradation (Zhao *et al.,* 2007). In addition, increase of GB was also involved in the protection

Fig. 1 Effect of drought with PCZ and SA treatments on protein content in (a) Root (b) Shoot of sorghum plants. Values are given as mean ± SD of five samples in each group. Bar values are significantly at ≤ 0.05 (DMRT).



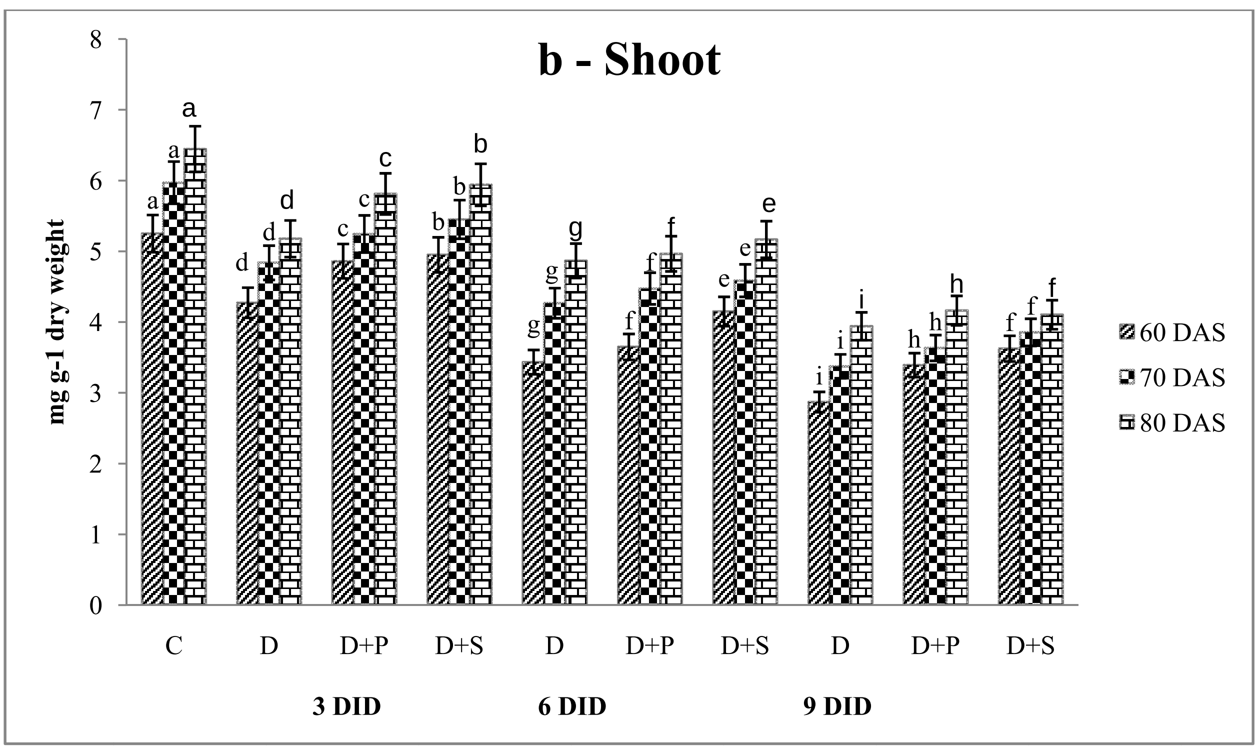
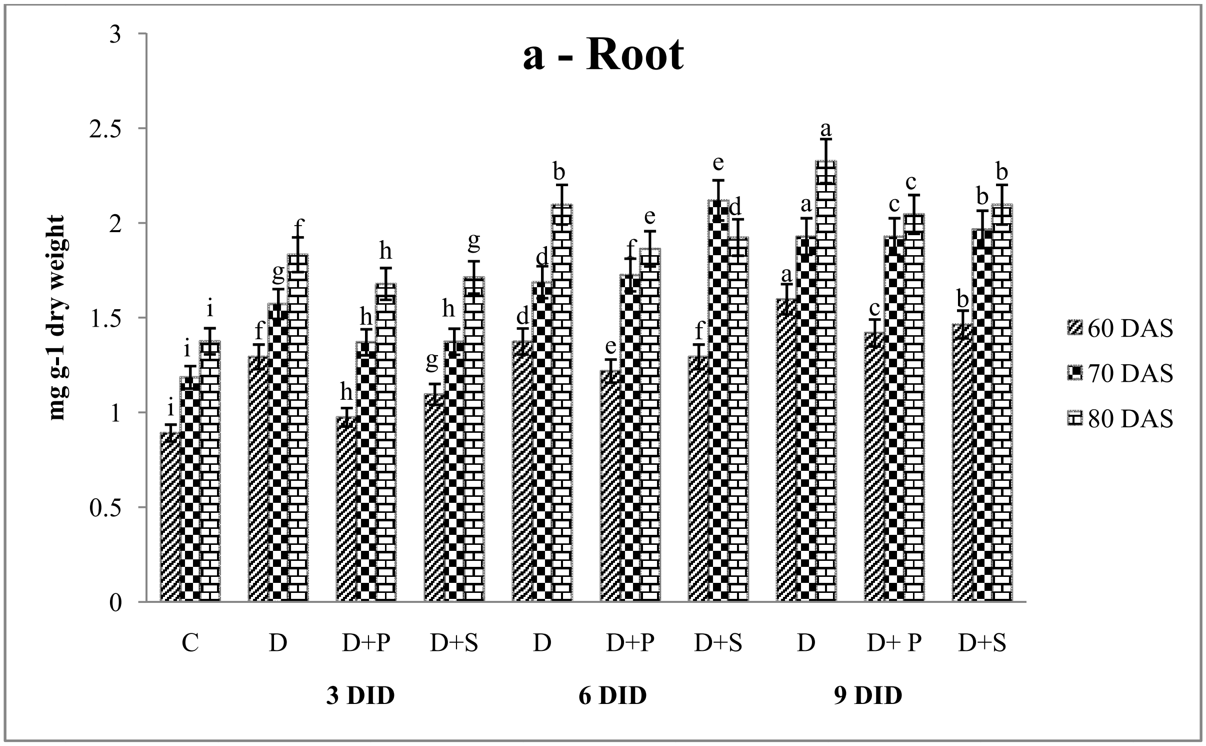


Fig. 2 Effect of drought with PCZ and SA treatments on proline content in (a) Root (b) Shoot of sorghum plants. Values are given as mean ± SD of five samples in each group. Bar values are significantly at ≤ 0.05 (DMRT).



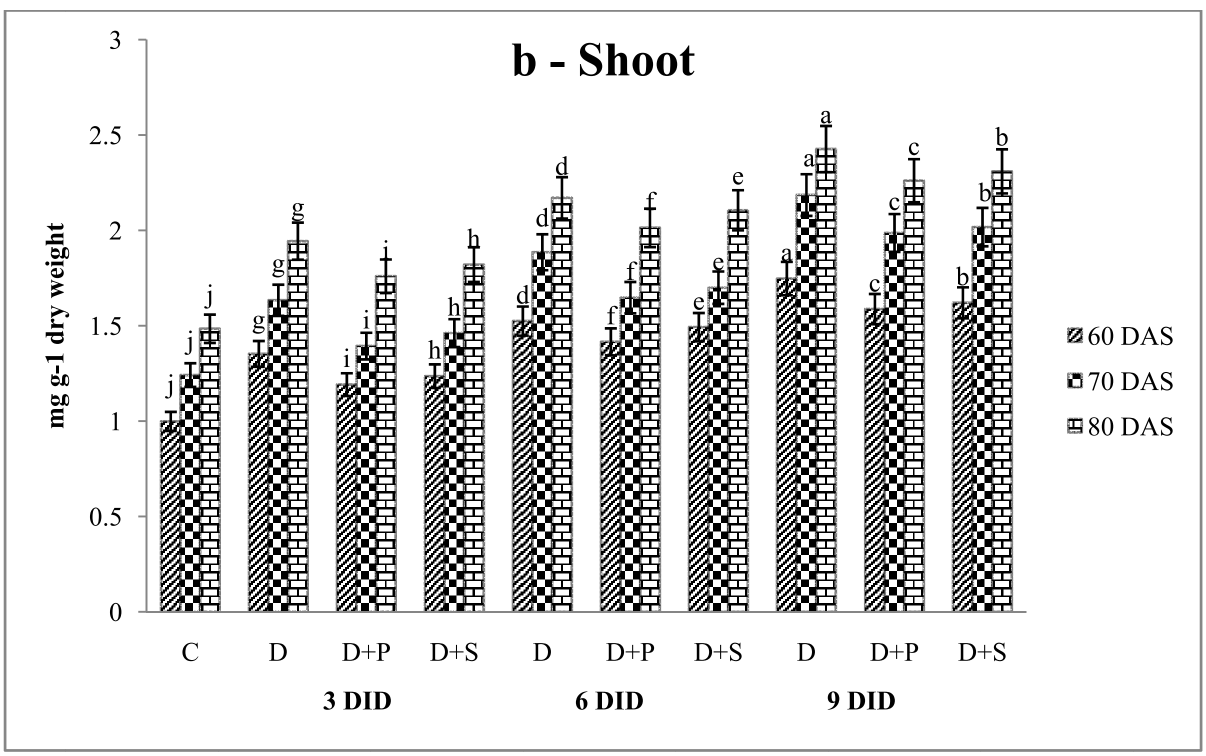


Fig. 3 Effect of drought with PCZ and SA treatments on amino acid content in (a) Root (b) Shoot of sorghum plants. Values are given as mean ± SD of five samples in each group. Bar values are significantly at ≤ 0.05 (DMRT).

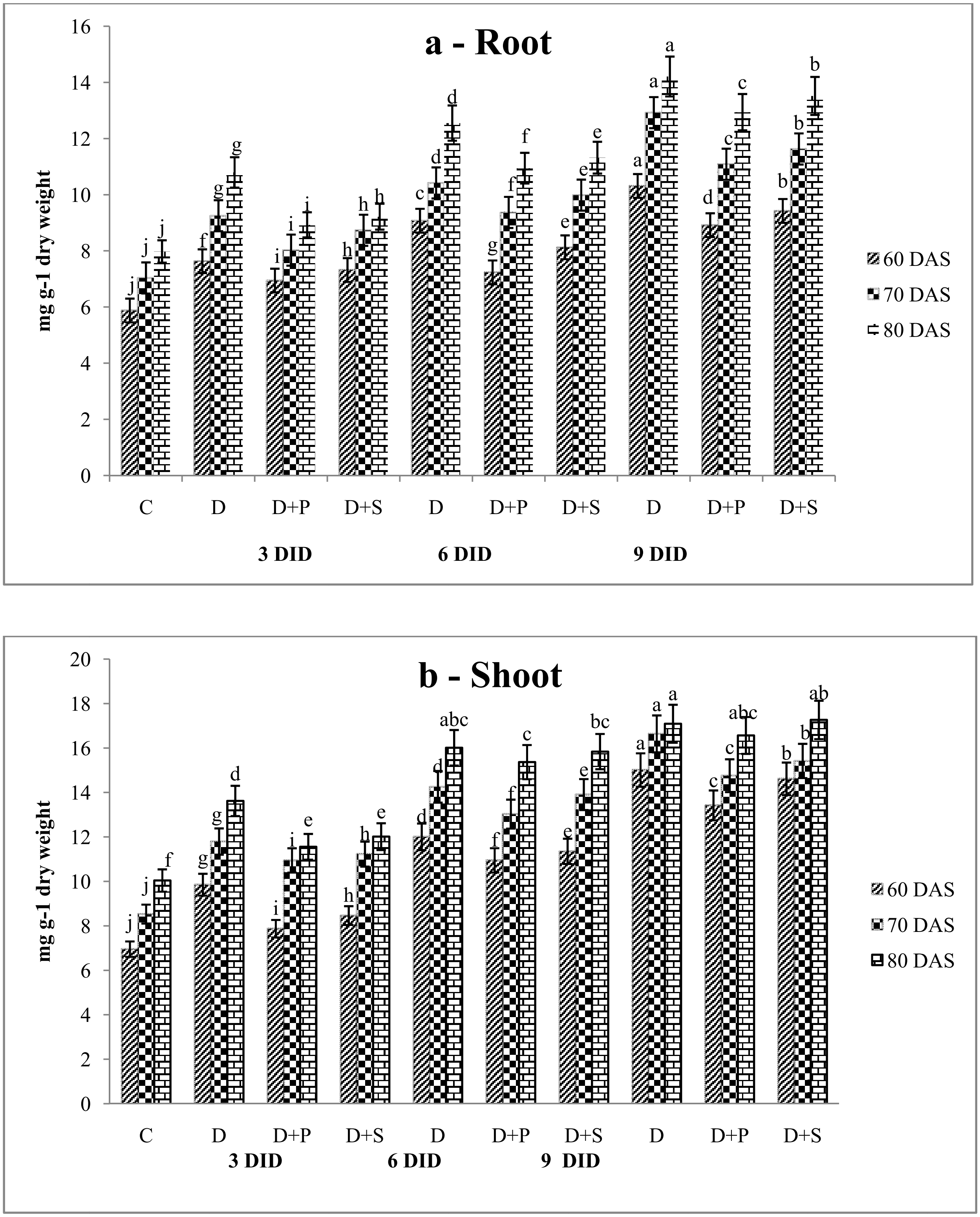
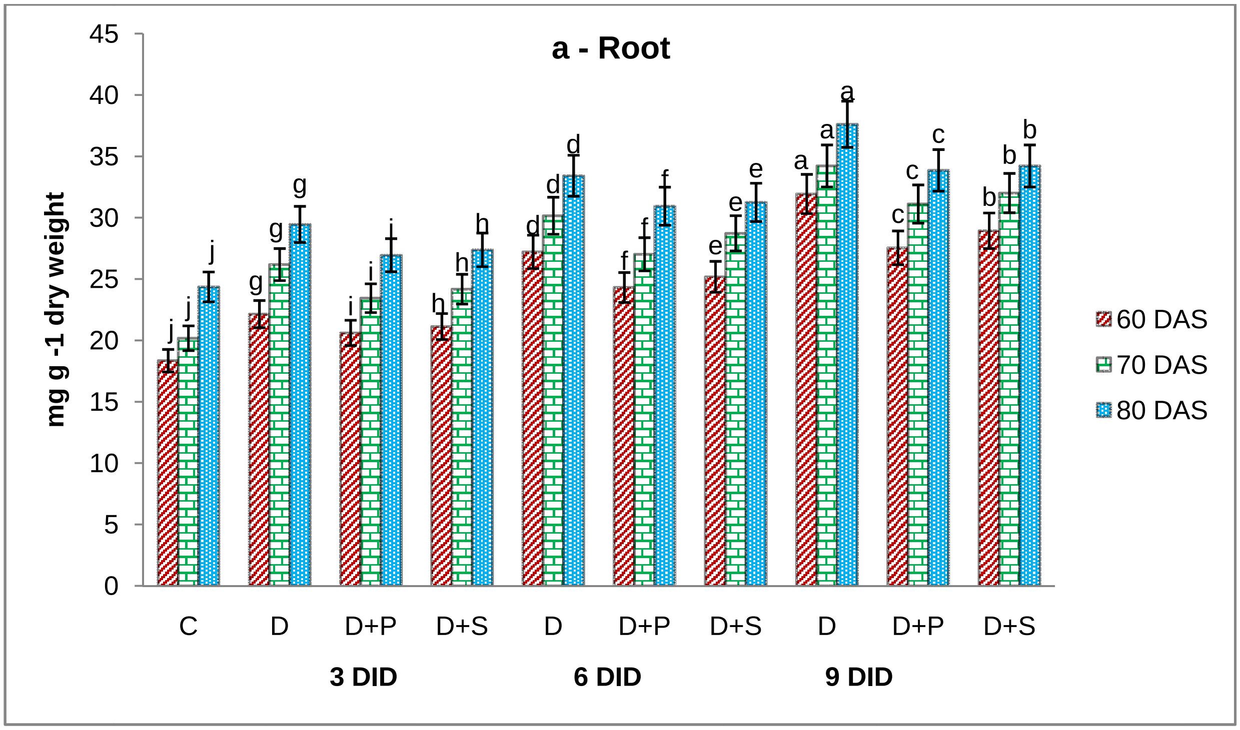


Fig. 4 Effect of drought with PCZ and SA treatments on glycine betaine content in (a) Root (b) Shoot of sorghum plants. Values are given as mean ± SD of five samples in each group. Bar values are significantly at ≤ 0.05 (DMRT).



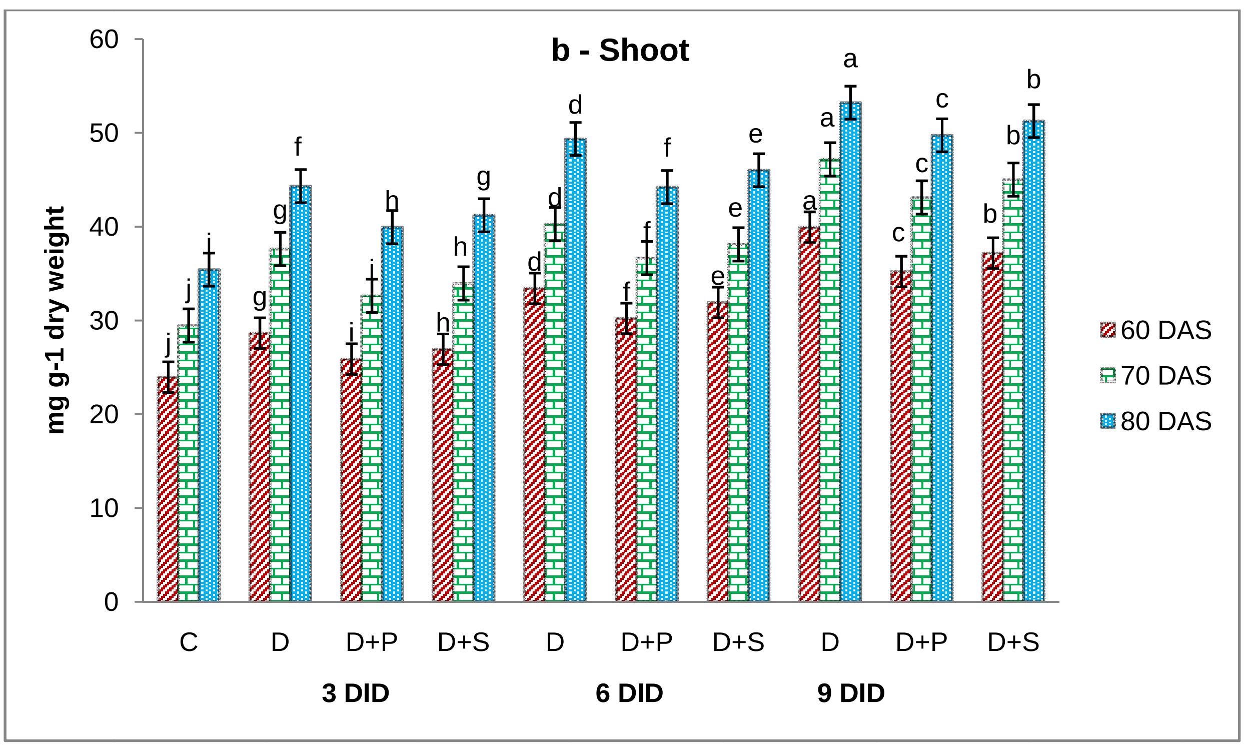
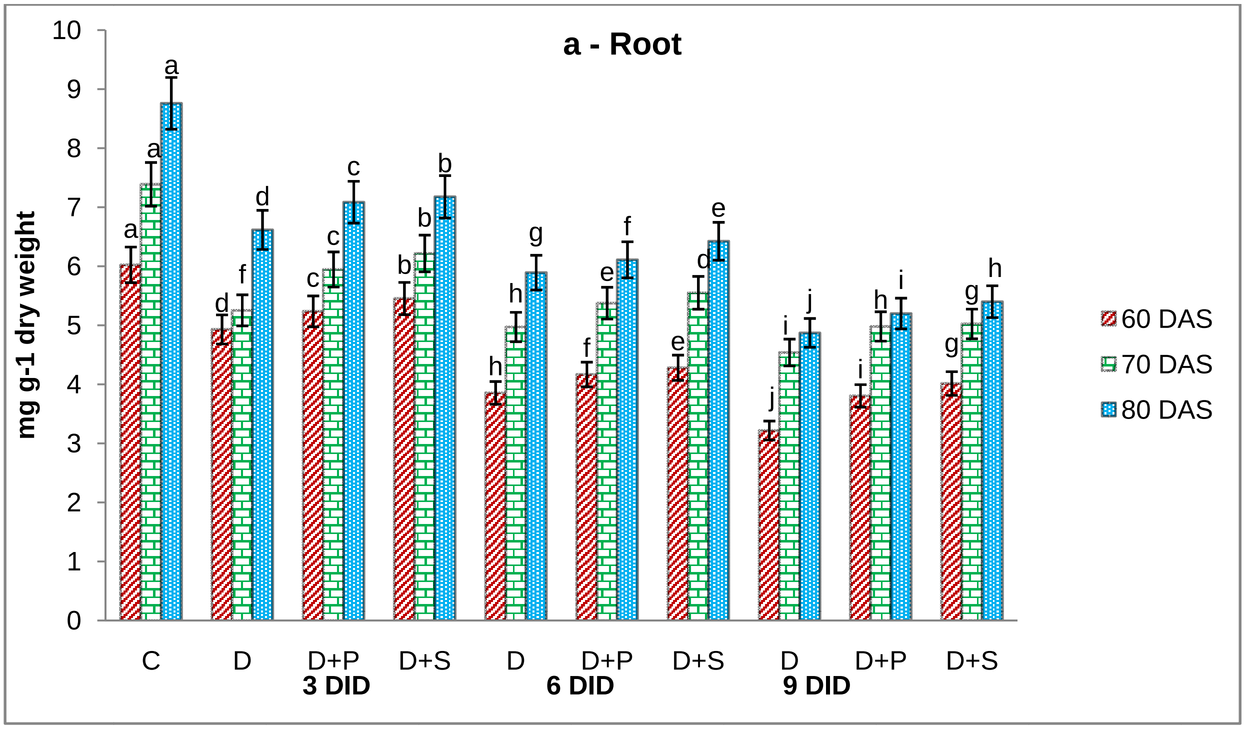
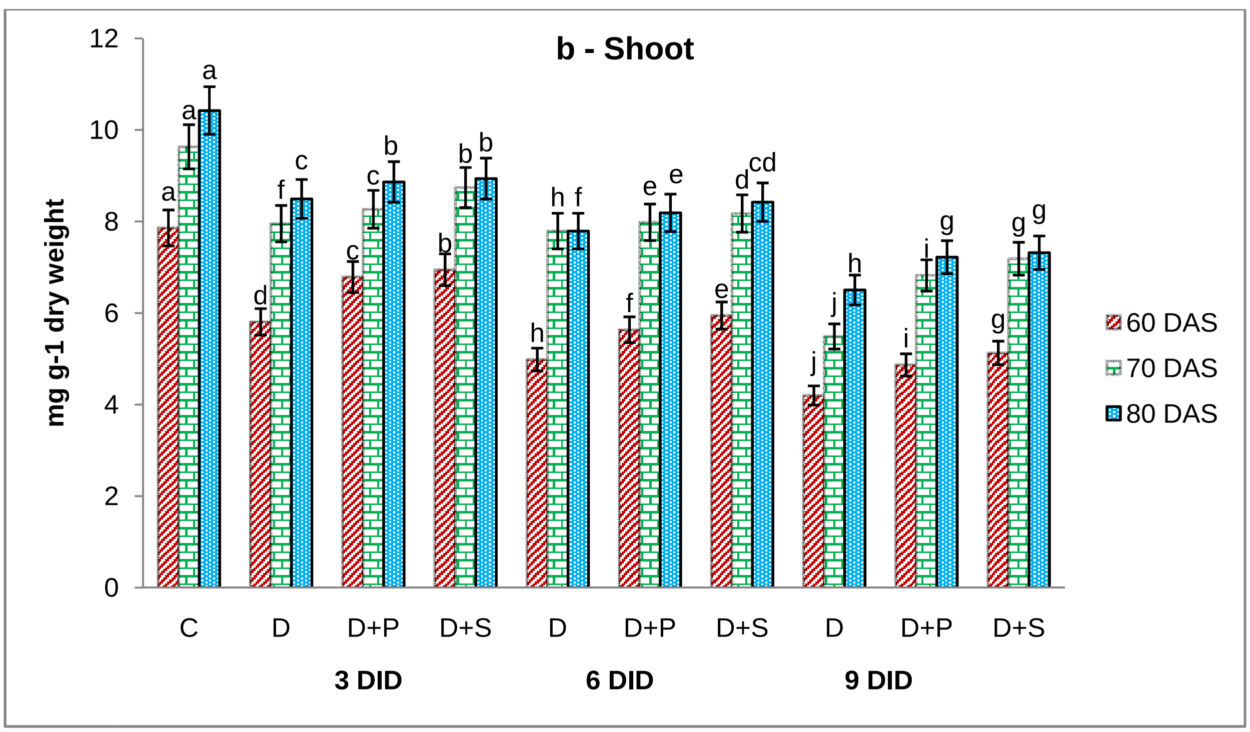


Fig. 5 Effect of drought with PCZ and SA treatments on total soluble sugar content in (a) Root (b) Shoot of sorghum plants. Values are given as mean ± SD of five samples in each group. Bar values are significantly at ≤ 0.05 (DMRT).





of the transcription and translation machinery under stress conditions (Cherian *et al.,* 2006). Aliphatic qac such as GB, stachydrine, homostachydrine, trigonelline have been found to accumulate in a large number of plants exposed to salt and water stress. The similar report was observed in (Manivannan *et al.,* 2007) in sun flower. Exogenous application of SA very effective in reducing the GB and adverse effect of drought stress in sorghum plants. The similar report was observed by (Sulian *et al.,* 2007) and suggest that GB may not only protect the integrity of the cell membrane from drought stress damage, but also be involved in transgenic cotton plants. During the drought stress with triazole treatments to decreased GB content, but it was higher than that of control. Similar results were observed in *Abelmoschus esculentus* (Rabert *et al.,* 2013a).

The total sugar content was increased in age of the plant, drought and drought with PCZ and SA treatments in all parts of the plants when compared to control. The drought with a combined effect PCZ and SA treatments were reduced the total sugar content when compared to drought stressed plants, but it was higher than that of control. The highest amount of total sugar accumulation was observed in 9 DID plants (Figure 6). The previous workers suggest that the sucrose and glucose either act as substrates for cellular respiration or osmolytes to maintain cell homeostasis while fructose is involved in the synthesis of secondary metabolites as well as erythrose-4-P, which acts as a substrate in lignin and phenolic compounds synthesis (Rosa *et al.,* 2009). Even though all this suggest that under stress conditions the metabolism of soluble sugars is a dynamic process simultaneously involving degrading and synthetic reactions, our study show the drought results in an increase in total sugars accumulation in all the parts of *S. bicolor*. This finding agrees with the suggested role of soluble sugars as signalling molecules under stress (Mutava *et al.,* 2015). Application of growth regulators to retarded the accumulation of sugar content in sorghum plant. The similar report was observed by soy bean (Al-Hakimi 2006) in SA application and (Rajasekar *et al.,* 2015a) in application of triazole.

Finally it can be indicated that the drought stressed plants under the application of PCZ and SA may influence the ability to maintain a balance between the compatible solute accumulation and osmatic maintenance leading to partial amelioration of oxidative stresses.

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