Journal of Applied and Advanced Research 2016, 1(3): 1–7
doi.: 10.21839/jaar.2016.v1i3.26
http://www.phoenixpub.org/journals/index.php/jaar

**Research Article – Plant Stress Physiology**

# Propiconazole and Salicylic acid alleviate effect of drought stress in sorghum (*Sorghum bicolor* L. Moench) through biochemical and some physiological characters

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## Abstract

In this study, the protective role of propiconazole (PCZ) and salicylic acid (SA) in relation to biochemical content and some physiological parameters were investigated in drought stressed *Sorghum bicolor* plants. The pot culture experiment was carried out on Botanical garden, Deportment of Botany, Annamalai University. A 30 Days After Sowing (DAS) plants were subjected to 3, 6, and 9 Days Interval Drought (DID) stress and drought with PCZ at 1 mM and drought with SA at 1 mM at 30, 40, and 50 DAS. The plants irrigated regularly in alternate day interval were kept as control. The plant samples were collected on 60, 70, and 80 DAS from all the treatments. The biochemical contents like starch and sucrose, then physiological parameter like Relative Water Content (RWC) and Electrolyte Leakage (EL) were analysed. Under the drought stress reduced starch and RWC was observed then, sucrose and EL content were increased. The drought with growth regulator treated plants starch and RWC was reduced then, sucrose and EL was increased but it was lower than that of control. Together, our findings demonstrate that, PCZ and SA is an efficient growth regulator with diversified roles that contribute to its potential alleviating effect against drought stress.

*Key words:* Drought, propiconazole, salicylic acid, *Sorghum bicolor*

## Introduction

Stress is an altered physiological condition caused by factors that tend to disrupt the equilibrium. Plants are frequently exposed to many stresses such as drought and low temperature, salt, flooding and heat, which severely affect plant growth and food productivity. Water, comprising 80% - 90% of the biomass of plants, is the central molecule in all the physiological processes of plants by being the major medium for transporting metabolites and nutrients. Drought is the most severe abiotic stress factor limiting plant growth and crop production (Rohbakhsh, 2013). Water deficit is considered as a major environmental factor affecting many aspects of plant physiology and biochemistry (Charles *et al.*, 1994). The responses of plants to water deficit are observed in forms of phenological responses, morphological changes, physiological alterations, and biochemical adaptations, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses (Duan *et al.,* 2007).

Drought tolerance is not an easily quantifiable plant attribute, because it is a combination of complex physiological, morphological and molecular traits. Physiological traits affected by drought can be correlated with the rate of CO2 assimilation [Guo *et al.,* 2008], PSII photochemical activity [Jedmowski *et al.,* 2013], leaf water potential (stomatal conductance, transpiration rate, relative water content—RWC) [Jedmowski *et al.,* 2013; Bencze *et al.,* 2011] and plasma membrane integrity [Babu *et al.,* 2004]

Received: 21-07-2016; Accepted 25-08-2016; Published Online: 03-09-2016

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Sorghum (*Sorghum bicolor* L. Moench) is a multipurpose crop belongs to the family Poaceae. Sorghum is a genus of grasses with about 30 species, and there are several types of sorghum, including grain sorghums, grass sorghums (for pasture and hay), sweet sorghums (for syrups), and Broomcorn. 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.,* 2002).

Plant growth regulators (PGRs) have a particularly interesting role in modern agriculture. In Greece and others European countries the PGRs are commonly used on food crops (melon, pepper, celery etc.) in order to improve and accelerate plant productivity (Arivalagan and Somasundaram, 2015). 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.,* 2014).

Propiconazole is one of the most important triazole fungicides which have been used successfully in plant protection since early 1970s. Triazole compounds are the chemicals belongs to a class of compounds known as ergosterol biosynthesis inhibitors and are used as fungicides as wells as plant growth regulators (Fletcher *et al.,* 2000). Triazole plays a role in the morphological and physiological changes associated with triazole-treatment in various plants, which includes the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, increased antioxidant potentials and enhancement in alkaloid production (Jaleel *et al.,*2009).

Salicylic acid or ortho-hydroxy benzoic acid is ubiquitously distributed in the whole plant kingdom. Salicylic acid is an endogenous plant growth regulator of phenolic nature that possesses an aromatic ring with a hydroxyl group or its functional derivative. SA is a potential signalling water-soluble non-enzymatic antioxidant; this material plays a crucial role in regulating various physiological processes such as growth, plant development, photosynthesis, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance [Arfan *et al.,* 2007]. SA is a tool to increase plant tolerance against the adverse effect of biotic and abiotic stresses [Bosch *et al.,* 2007]. The present study was aimed to evaluate the biochemical contents and some physiological parameters of *Sorghum bicolor* under drought and drought with PCZ and SA treatments.

## 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.

### *Physiological parameters*

### *Relative Water Content*

Relative water content (RWC) was determined by the method of Mata and Lamattina (2001). RWC was calculated according to the formula, RWC (%) = (FW − DW)/(TW − DW) × 100. where: fresh weight (FW) was measured at the end of the drought period, and dry weight (DW) was obtained after drying the samples at 80°C for 48 h. Turgor weight (TW) was determined by subjecting leaves to rehydration for 2 h after drought treatment.

*Electrolyte leakage*

Electrolyte leakage (EL) was measured as described by Lutts *et al.* (1996) with few modifications. Plant material (0.3 g) was washed with deionized water, placed in tubes with 15 ml of deionized water and incubated for 2 h at 25°C. Subsequently, the electrical conductivity of the solution (L1) was determined. Samples were then autoclaved at 120°C for 20 min and the final conductivity (L2) was measured after equilibration at 25°C. The EL was calculated using the following formula: EL (%) = (L1/L2) × 100.

*Estimation of Starch Content*

Starch content was estimated following the method of Clegg, (1956). Ten ml of cold anthrone reagent was added with 1 ml of perchloric acid (PCA) extract and it was diluted with 5 ml of deionised water. The test tube was heated for 10 minutes at 100˚C in a boiling water bath. The test tube was cooled rapidly and the absorbance was read at 630 nm in a Spectrophotometer (U-2001–Hitachi). Starch content was calculated by multiplying glucose equivalents with the conversion factor 0.9.

*Estimation of Sucrose Content*

Sucrose content was estimated by the method of Bernt and Bergmayer (1970). For estimating sucrose, 1 ml of invertase (prepared by dissolving 250 units of yeast invertase in 500 ml of 0.2M sodium acetate buffer = pH 5.0) was added to 1ml of sugar extract and incubated at 37°C for 1h and, thereafter, the reaction was stopped by keeping the tubes in boiling water bath for 10 min. Under these conditions, sucrose was completely hydrolyzed. Glucose was determined by the glucose oxidase and peroxidase reaction (sigma) (Gascon and Lampen, 1968) before and after invertase hydrolysis and the difference between these values was taken as the actual amount of sucrose in the sample.

## Results and Discussion

The relative water content in the leaves decreased gradually with the increasing drought stress. Whereas the subsequent treatment of PCZ and SA significantly increased and also overcome the toxic effects generated by drought stress. But it was lower than that of control plants. Reduction of plant biomass and leaf RWC are very common effects of drought stress as studied in many researches (Amirjani and Mahdiyeh, 2013) in Wheat. Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the relative water content (RWC) in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta (2006) that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. Increasing of leaf relative water content in paclobutrazol treatment may attribute to decreasing leaf area. Paclobutrazol reduces evapotranspiration and decreases plant moisture stress by enhancing the relative water content of leaf area and develops resistance in the plants against biotic and abiotic stresses (Yadav *et al.,* 2005 and Parvin *et al.,* 2015). However, the exogenous application of SA may regulate stomatal openings and reduce transpirational water loss under drought conditions enabling the plants to maintain turgor, carry on photosynthesis and be productive under water deficit conditions. The similar observation was noted by (Rao *et al.,* 2012) in maize.

A significant increase was observed in electrolyte leakage with increasing the drought stress and age of the plants. The highest increase in electrolyte leakage was observed in 80 DAS 9 DID stressed plants. In addition, application of PCZ and SA reduced the electrolyte lekage (Fig -2). The remarkable decrease in electrolyte leakage is used as indicator of reduction of membrane damage, increased membrane stability and tolerance of plants (Orabi and Mekki, 2008) EL is an index, which can quantify the damage conceived by plant cell membrane. Its relative conductivity can be

**Table 1.** Effects on Starch content in root and shoot of the *S. bicolor* under drought with PCZ and SA treatments.

| Starch content (Root) | Treatments | | Days After Sowing | | |
|---|---|---|---|---|---|
| | | | 60 | 70 | 80 |
| | | Control | 6.03 ± .07 | 7.39 ± .05 | 8.76 ± .05 |
| | 3 DID | Drought | 4.93 ± .05 | 5.26 ± .06 | 6.67 ± .07 |
| | | Drought+PCZ | 5.24 ± .07 | 5.96 ± .09 | 7.09 ± .09 |
| | | Drought+SA | 5.46 ± .07 | 6.27 ± .05 | 7.18 ± .03 |
| | 6DID | Drought | 3.86 ± .03 | 4.97 ± .05 | 5.89 ± .04 |
| | | Drought+PCZ | 4.18 ± .07 | 5.39 ± .05 | 6.11 ± .08 |
| | | Drought+SA | 4.28 ± .08 | 5.55 ± .05 | 6.44 ± .05 |
| | 9 DID | Drought | 3.23 ± .08 | 4.54 ± .07 | 4.87 ± .06 |
| | | Drought+PCZ | 3.86 ± .06 | 4.98 ± .06 | 5.20 ± .04 |
| | | Drought+SA | 4.05 ± .05 | 5.04 ± .09 | 5.41 ± .02 |
| **Starch content (Shoot)** | | Control | 7.86 ± .04 | 9.64 ± .06 | 10.46±.07 |
| | 3 DID | Drought | 5.88 ± .07 | 7.95 ± .06 | 8.49 ± .04 |
| | | Drought+PCZ | 6.78 ± .07 | 8.26 ± .05 | 8.86 ± .07 |
| | | Drought+SA | 6.95 ± .05 | 8.74 ± .02 | 8.94 ± .08 |
| | 6DID | Drought | 4.99 ± .07 | 7.79 ± .06 | 7.99 ± .06 |
| | | Drought+PCZ | 5.67 ± .08 | 7.98 ± .05 | 8.19 ± .04 |
| | | Drought+SA | 5.97 ± .01 | 8.19 ± .03 | 8.44 ± .06 |
| | 9 DID | Drought | 4.20 ± .06 | 5.49 ± .06 | 6.53 ± .07 |
| | | Drought+PCZ | 4.88 ± .04 | 6.83 ± .09 | 7.22 ± .05 |
| | | Drought+SA | 5.13 ± .06 | 7.19 ± .04 | 7.33 ± .08 |

Data are mean ± standard error at P<0.05 level of probability, PCZ: Propiconazole, DID: Days interval drought, SA: Salicylic acid, DAS: Days after sowing

**Table 2.** Effects on Sucrose content in root and shoot of the *S. bicolor* under drought with PCZ and SA treatments.

| Sucrose content (Root) | Treatments | | Days After Sowing | | |
|---|---|---|---|---|---|
| | | | 60 | 70 | 80 |
| | | Control | 4.86 ± .07 | 6.50 ± .17 | 8.66 ± .07 |
| | 3 DID | Drought | 7.20 ± .07 | 9.62 ± .21 | 9.62 ± .21 |
| | | Drought+PCZ | 5.83 ± .03 | 8.61 ± .05 | 9.89 ± .04 |
| | | Drought+SA | 6.01 ± .08 | 8.71 ± .07 | 10.16 ± .12 |
| | 6DID | Drought | 9.46 ± .26 | 10.90 ± .05 | 12.85 ± .01 |
| | | Drought+PCZ | 8.54 ± .11 | 9.50 ± .07 | 11.57 ± .38 |
| | | Drought+SA | 8.79 ± .09 | 9.71 ± .07 | 12.03 ± .08 |
| | 9 DID | Drought | 10.92± .19 | 12.95 ±.14 | 14.30 ± .49 |
| | | Drought+PCZ | 9.94 ± .09 | 11.33 ± .56 | 13.77 ± .04 |
| | | Drought+SA | 10.20 ± .05 | 12.17 ± .06 | 13.95 ± .06 |
| **Sucrose content (Shoot)** | | Control | 5.92 ±.06 | 7.67 ±.20 | 10.14 ± .19 |
| | 3 DID | Drought | 7.87 ± .35 | 9.89 ± .15 | 12.50 ± .42 |
| | | Drought+PCZ | 6.68 ± .15 | 8.77 ± .04 | 11.37 ± .14 |
| | | Drought+SA | 6.94 ± .09 | 8.95 ± .04 | 11.84 ± .21 |
| | 6DID | Drought | 9.90 ±.04 | 11.92 ± .07 | 13.90 ± .04 |
| | | Drought+PCZ | 8.30 ± .36 | 10.73 ± .11 | 12.78 ± .06 |
| | | Drought+SA | 8.73 ± .09 | 10.98 ± .29 | 13.01 ± .06 |
| | 9 DID | Drought | 11.18 ± .06 | 13.52 ± .09 | 15.78 ± .12 |
| | | Drought+PCZ | 9.58 ± .51 | 12.13 ± .22 | 14.38 ± .11 |
| | | Drought+SA | 10.10 ± .07 | 12.79 ± .19 | 14.64 ± .06 |

Data are mean ± standard error at P<0.05 level of probability, PCZ: Propiconazole, DID: Days interval drought, SA: Salicylic acid, DAS: Days after sowing

**Fig.1** Effect of drought with PCZ and SA treatments on Relative water content of sorghum plants. Values are given as mean ± SD of five samples in each group. .

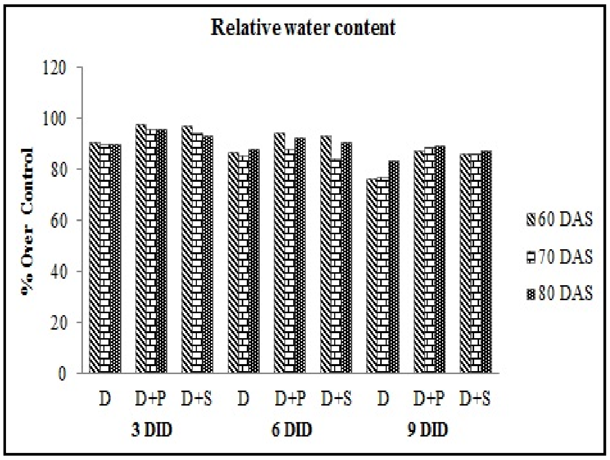
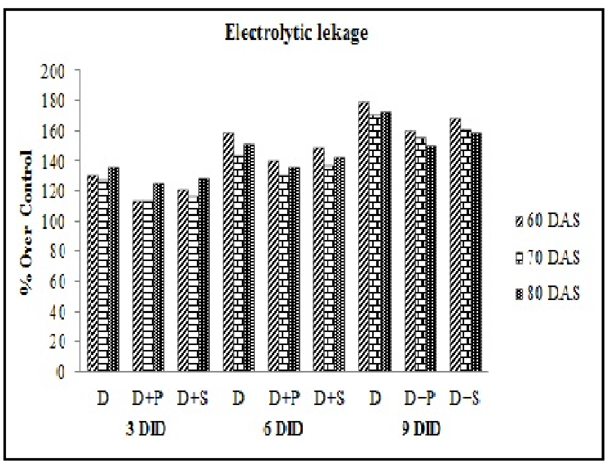



**Fig.2** Effect of drought with PCZ and SA treatments on Electrolytic leakage in sorghum plants. Values are given as mean ± SD of five samples in each group.




used to evaluate the damage on structure and function of cell membranes under stresses (Kordi *et al.,* 2013). The growth regulating treatments reduced the EL when compared to drought stressed plants. But it was higher than that of control. Triazoles altered the sterol biosynthesis and changed the composition of sterol in the plasma membrane. The changes in the sterol composition may induce changes in the cell membrane that may be reflected in increased membrane stability (Gomathinayagam *et al.* 2007). Kabiri *et al.* (2012) mentioned that pre-treatment with SA was evidenced by a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to control plants.

Drought stress reduced the starch content in all parts of the sorghum plants when compared control on various DID (3, 6 and 9 DID). Drought stress with PCZ and SA treatments lead to an enhancement of starch content when compared to drought stressed plants, But it was lower than that of well-watered plants. Starch may play an important role in accumulation of soluble sugars in cells. Under the water stress condition starch depletion in grapevine leaves were noted by Patakas and Noitsakis (2001) in response to drought stress. In this study, the concentration of soluble sugars increased at the same time as a decrease in the starch concentration was observed. It means that the raised soluble sugar fraction was accompanied by a sharp decrease in starch fraction as the drought stress increased. Similar result was also observed in maize (Nayer and Reza 2008). The exogenous application of triazole on drought treated sorghum plant will be increased the starch content when compared to drought stressed plants. The similar report observed on *Dioscorea rotundata* (Jaleel *et al*. 2007).

Drought stress induced higher accumulation of sucrose content was observed in root and shoot tissue of the sorghum plant when compared to control (Fig. 2). Thus sucrose seems to play a key role in the integration of plant growth and seems to be part of a wider mechanism for balancing carbon acquisition and allocation within and between organs [Shao *et al*., (2005) 131–135]. When plants were subjected to stress and the stimulation of sugar accumulation was proportional to osmotic adjustment (Yong *et al.*, 2006). The increased levels in sucrose content may be the cause of conversion of starch into sucrose. The exogenous application of PCZ and SA treatments to increase the sucrose content. But it was lower than that of drought stressed plants. The reduced amount of carbohydrate availability leads to a reduced development of other sink organs of the plant (Schussler et al. 1984). The triazole compounds induced a raise in ABA content in many plants and this increased ABA might have induced the sucrose level in sorghum plants. This result is in accordance with the previous reports of

(Jaleel *et al.,* 2007) in yam plants under triazole treatment. Similar results were found in SA treated sunflower plants (El-Tayeb *et al*., 2006).

**Acknowledgements**

The authors are thankful to Dr. V. Venkadasalu, Professor and Head, Department of Botany, Annamalai University, for providing laboratory facilities for the study. The authors wish to thank UGC-SAP by awarding (BSR Research Fellowship in Science). UGC Letter No. F.25-1/2013-2014(BSR)/ 7-11/2007(BSR), Dated 03.11.2014 for the financial support for this research work.

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