Journal of Ecobiotechnology 2015, 7: 17-23
http://scienceflora.org/journals/index.php/jebt/

Effect of propiconazole and salicylic acid on the growth and photosynthetic pigments in *Sorghum bicolor* (L.) Moench. under drought condition

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Received: 06.08.2015
Revised: 03.09.2015
Accepted: 05.09.2015
Published: 05.09.2015

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ABSTRACT

The present investigations were carried out to estimate the morphological, pigments variation and ameliorating effect of propiconazole (PCZ) and salicylic acid (SA) on drought stress in sorghum (*Sorghum bicolor* L. Moench.) Var. CO-30. A 30 days after sowing (DAS), the plants were subjected to 3, 6, and 9 days interval drought 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 root length, shoot length, fresh weight, dry weight, total leaf area, and photosynthetic pigment estimation was carried out in each treatment. Individual and combined drought stress with PCZ and SA treatments increased the root length when compared to control. The PCZ and SA treatments mitigated the drought stress by increasing the morphological and pigment contents and there by the way for overcoming drought stress in *S. bicolar* plants.

KEY WORDS: Drought, growth, photosynthetic pigments, propiconazole, salicylic acid, *Sorghum bicolor*

INTRODUCTION

Water is essential for living organisms and its play a very important role in building plants metabolism. Water availability and quality can be a limiting factor in plant growth (Arıkan Ceylan *et al*., 2012). Drought is one of the abiotic stresses, and it affects the physio-morphological characteristics, biochemical constituents and yield productivity of a plant. Drought impacts include growth, yield, membrane integrity, pigment content, osmotic adjustment, water relations, and photosynthetic activity (Benjamin and Nielsen, 2006).

Sorghum (*Sorghum bicolor* L. Moench.) is a multipurpose crop belongs to the family Poaceae. Sorghum is one of the five major cultivated species in the world because it has several economically important potential uses such as food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production), and fertilizer (utilization of organic by products). It is an important alternative for human and animal food, especially in regions of low water availability, in which seed is rich in proteins, vitamins, carbohydrates, and minerals (Carvalho *et al*., 2002). The propiconazole (PCZ) is a triazole group of systematic fungicide. The triazole compounds, such as pacloputrazole and PCZ, have a growth regulating properties. It induced many morphological and metabolic changes such as reduction in shoot elongation, stimulating of rooting, inhibiting gibberellins biosynthesis, increased chlorophyll content change in carbohydrate status and increased cytokinin synthesis (Fletcher and Hofstra, 1988). The triazole treatments to change the morphological and physiological effects in plants include inhibition of plant growth, elongated chloroplast and increased the photosynthetic pigment contents, thicker leaf tissue, increased root and shoot ratio (Jaleel *et al*., 2008).

Salicylic acid (SA) is a phenolic compound synthesized throughout the plant kingdom via the phenylpropanoid pathway. SA plays an important role in abiotic stress tolerance, and considerable interests have been focused on SA due to its ability to induce a protective effect under stress. SA significantly alleviated growth inhibition induced by drought and manifested by less decreased fresh and dry mass, plant height, root length, chlorophyll and many physiological roles (Kang *et al*., 2013).

MATERIALS AND METHODS

Plant Cultivation

The sorghum variety CO-30 was obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The triazole compound probiconazole obtained from Syngenta India ltd., Mumbai. The phenolic compound SA was purchased from Himedia India Ltd., Mumbai. The experimental part of this work was carried out in Botanical Garden and Water Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu, India. The plants were raised in plastic bags. The plastic bags filled with homogenous mixture of the garden soil containing red soil, sand and farmyard manure (1:1:1). The pots were arranged in Completely Randomized Block Design.

Experimental Design

The experimental seeds were surface sterilized with 0.2% mercuric chloride solution for 5 min 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 given on 3 days interval drought (DID), 6 DID and 9 DID from 30 DAS 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 collected at randomly 60, 70 and 80 DAS at 10 days interval.

Growth Parameters

The plant height was measured from the soil level to the tip of the shoot and expressed in cm. The plant root length was measured from the point of first cotyledonary node to the tip of root and expressed in centimeter. The total leaf area of the plants was measured by Kemp constant method the values are expressed in cm^2 per plant. After washing the plants in the tap water, fresh weight was determined by using an electronic balance (Model-XK3190-A7M) and the values were expressed in grams. After taking fresh weight, the plants were dried at 60°C in hot air oven for 48 h. After drying, the dry weight was measured and the values were expressed in grams.

Photosynthetic Pigments

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of (Arnon, 1949). Carotenoid content was estimated using the formula by (Kirk and Allen, 1965) and expressed in milligrams per gram fresh weight. Xanthophyll was extracted and estimated by the method of (Neogy *et al.*, 2001).

Statistical Analysis

Statistically significance was assessed at the $P < 0.05$ level using one-way ANOVA and means were separated by Duncan's multiple range test ($P < 0.05$) with the help of SPSS 16.0 software package.

RESULTS

Effect of PCZ and SA on Shoot length of Sorghum under Drought Stress

The drought stress was decreased the shoot length 95.50, 91.47 and 84.37 cm at various DID respectively on 80 DAS (Table 1). When compared to drought plant, drought with PCZ and SA increased the shoot length, and it was 108.27 cm and 115.20 cm, respectively, on 80 DAS.

Effect of PCZ and SA on Root length of Sorghum under Drought Stress

Table 2 shows that drought stress and drought with PCZ and SA treated plant root length were increased when compared to control plant, and it was 33.73 cm at 80 DAS. The highest root length was recorded in 42.30, 48.43, and 44.80 cm in drought, drought with PCZ and SA treatment respectively on 9 DID sorghum plants at 80 DAS.

Effect of PCZ and SA on Fresh and Dry Weight of Sorghum under Different DID

In whole control and treatment plants, the fresh weight was increased with the age of the plant (Table 3). The control plant fresh weight 143.70 g was noted at 80 DAS. Drought stress decrease plant fresh weight when compared to control plant and it was 122.23, 95.90, and 75.83 g on significantly on various DID. Drought with PCZ and SA increased 129.83 and 132.43 g significantly at 80 DAS. Drought stress caused the accumulation on reduction of dry weight and it was 56.30, 41.50 and 30.10 g significantly at various DID. When compared to drought plant drought with PCZ and SA treatment of sorghum plant dry weight was 61.63 and 63.83 g significantly increased at 80 DAS (Table 4).

Effect of PCZ and SA on Total Leaf Area of Sorghum under Different DID

From the data of the present investigation it was clear that the drought stress decreased the total leaf area of the sorghum plant (Figure 1). The total leaf area 217.60 cm^2/p was observed in control plant. In drought treated plant the total leaf area was significantly decreased when compared to control plant and it was 202.40, 179.47, and

Table 1: Effect of Shoot length in sorghum plant under drought with PCZ and SA treatments

DAS	Control	3 DID			6 DID			9 DID		
		Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA
60	95.70 ± 0.26^{a}	84.37 ± 0.73^{d}	87.43 ± 0.72^{c}	93.10 ± 0.95^{b}	80.23 ± 1.26^{e}	84.23 ± 0.59^{d}	87.03 ± 0.45^{c}	75.47 ± 0.55^{f}	80.23 ± 0.56^{e}	84.63 ± 0.50^{d}
70	108.43 ± 0.6^{a}	91.33 ± 0.43^{cd}	102.10 ± 1.08^{b}	106.73 ± 0.81^{a}	83.63 ± 0.64^{f}	88.47 ± 0.58^{e}	92.67 ± 0.63^{c}	79.27 ± 0.52^{g}	83.97 ± 0.92^{f}	89.40 ± 0.40^{de}
80	120.93 ± 0.8^{a}	95.50 ± 0.31^{e}	108.27 ± 0.71^{c}	115.20 ± 0.76^{b}	91.47 ± 0.55^{f}	95.50 ± 0.72^{e}	99.40 ± 1.04^{d}	84.37 ± 1.00^{g}	90.40 ± 0.66^{f}	95.17 ± 1.57^{e}

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: Effect of root length in *S. bicolar* under drought with PCZ and SA treatments

DAS	Control	3 DID			6 DID			9 DID		
		Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA
60	29.34 ± 0.37^{h}	30.47 ± 0.50^{h}	36.30 ± 0.67^{cd}	32.50 ± 0.70^{g}	33.40 ± 0.79^{ef}	38.50 ± 0.55^{b}	34.90 ± 0.46^{de}	37.47 ± 0.55^{bc}	41.73 ± 1.19^{a}	38.6 ± 0.74^{b}
70	32.03 ± 0.03^{g}	33.50 ± 0.35^{g}	39.80 ± 0.61^{c}	36.43 ± 0.58^{de}	35.50 ± 0.66^{f}	42.47 ± 0.41^{b}	37.73 ± 0.33^{d}	41.17 ± 0.74^{bc}	46.00 ± 1.27^{a}	41.43 ± 0.35^{bc}
80	35.73 ± 0.38^{e}	36.87 ± 0.26^{e}	42.57 ± 0.65^{c}	39.47 ± 0.75^{d}	39.67 ± 0.78^{d}	45.93 ± 0.85^{b}	40.10 ± 0.64^{d}	42.30 ± 0.53^{c}	48.43 ± 0.27^{a}	44.80 ± 0.64^{b}

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

Table 3: Effect of Fresh weight in *S. bicolar* under drought with PCZ and SA treatments

DAS	Control	3 DID			6 DID			9 DID		
		Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA
60	107.20 ± 1.04^{a}	92.90 ± 1.32^{d}	98.23 ± 0.52^{c}	101.60 ± 1.30^{b}	69.43 ± 0.43^{g}	75.87 ± 0.83^{f}	79.60 ± 0.93^{e}	47.20 ± 1.47^{i}	55.33 ± 2.08^{h}	57.53 ± 1.68^{h}
70	130.73 ± 0.48^{a}	108.60 ± 1.04^{c}	114.70 ± 0.50^{b}	118.63 ± 0.75^{b}	82.17 ± 1.58^{e}	85.17 ± 2.50^{e}	95.17 ± 1.16^{d}	61.13 ± 0.98^{g}	72.27 ± 1.94^{f}	74.53 ± 3.00^{f}
80	143.70 ± 0.74^{a}	122.23 ± 0.44^{c}	129.83 ± 0.32^{b}	132.43 ± 0.87^{b}	95.90 ± 1.19^{e}	99.03 ± 1.32^{de}	102.00 ± 0.72^{d}	75.83 ± 1.07^{g}	78.50 ± 2.15^{g}	83.33 ± 1.16^{f}

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

Table 4: Effect of dry weight in sorghum plant under drought with PCZ and SA treatments

DAS	Control	3 DID			6 DID			9 DID		
		Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA	Drought	Drought+PCZ	Drought+SA
60	50.97 ± 0.22^{a}	41.13 ± 0.43^{c}	45.67 ± 0.70^{b}	44.50 ± 1.74^{b}	30.97 ± 0.59^{e}	34.33 ± 0.43^{d}	35.43 ± 0.49^{d}	20.53 ± 0.45^{h}	23.93 ± 0.44^{g}	26.40 ± 0.40^{f}
70	58.53 ± 0.79^{a}	50.43 ± 0.65^{c}	54.20 ± 1.19^{b}	54.90 ± 1.42^{b}	36.23 ± 0.61^{e}	$39.07\pm0\ 0.09^{d}$	39.38 ± 0.70^{d}	24.17 ± 0.58^{g}	28.37 ± 0.65^{f}	30.13 ± 0.23^{f}
80	61.90 ± 0.96^{a}	56.30 ± 0.55^{b}	61.63 ± 0.67^{a}	63.83 ± 0.81^{a}	41.50 ± 0.49^{d}	45.90 ± 0.78^{c}	47.17 ± 0.94^{c}	30.10 ± 1.11^{f}	32.00 ± 0.86^{ef}	33.60 ± 1.61^{e}

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

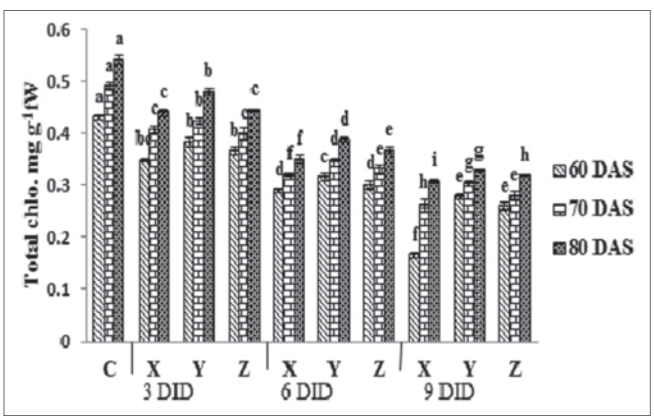

Figure 1: Effect of total chlorophyll in *Sorghum bicolor* under drought with propiconazole (PCZ) and salicylic acid (SA) treatments, C: Control; X: Drought; Y: Drought + PCZ; Z: Drought + SA

159.77 cm^2/p at various DID plant. The total leaf area was increased in drought stress with PCZ and SA treatments when compared to drought plant and it was 208.43 and 210.60 cm^2/p respectively on 80 DAS plant.

Effect of Chlorophyll Content in Drought with PCZ and SA Treated Plants

A significant reduction was observed on chlorophyll content on drought stress treatment when compared to control (Figures 2 and 3). Drought with PCZ and SA treated plant increased the chlorophyll "a" content when compared to drought stress plant and it was 0.295 and 0.285 mg/g f.w. in, respectively. The highest total chlorophyll content 0.540 mg/g f.w. was recorded in control plant. In drought treated plant the chlorophyll content was decreased 0.439, 0.349 and 0.307 mg/g f.w. when compared to control plant at various DID on 80 DAS. Drought stress with PCZ and SA increased the total chlorophyll content and it was 0.478 and 0.443 mg/g f.w., respectively at 80 DAS (Figure 4).

Effect of Carotenoid Content in Drought with PCZ and SA Treated Sorghum Plants

The carotenoid content increased with age of the plant. The carotenoid content and it was recorded 0.222 mg/g f.w.

in control plant. In drought plant, the carotenoid content was randomly decreased at various DID. Drought stress with PCZ and SA increased the carotenoid content and it was 0.177 and 0.171 mg/g f.w. respectively in 80 DAS (Figure 5).

Effect of Xanthophyll Content in Drought with PCZ and SA Treatment on Sorghum

The xanthophyll content increased with age in sorghum plant of leaves (Figure 6). The maximum xanthophyll content 0.227 mg/g f.w. was found in control plant. The drought stress decreased the xanthophyll content when compared to control and it was 0.198, 0.179 and 0.139 mg/g f.w. at various DID on 80 DAS. Drought stress with PCZ and SA treated plant increased the xanthophyll content, and it was 0.215 and 0.204 mg/g f.w. respectively on 80 DAS sorghum plant.

DISCUSSION

The main objectives of the present investigation to assess the effect of drought and drought with treatments like PCZ and SA in *S. bicolor*. The results obtained on growth parameters such as root and shoot length, total leaf area, fresh and dry weight and photosynthetic pigment contents were discussed. The shoot length of Sorghum plant was reduced when compared to control plant. The similar results were obtained in *Setaria italica* by (Paul Ajithkumar and Panneerselvam, 2013). Under the drought condition PCZ and SA treatments were increased the shoot length of Sorghum plant when compared to control. The similar report was noted by (Mohamadi and Rajaei, 2013; Yildirim *et al.*, 2008) in Olive and cucumber. This study reveals that the drought stress caused impaired mitosis, cell elongation and expansion inhibit the growth of Sorghum plant. The SA treated sorghum to amelioration of plant height under water stress may be related to improve mitosis and cell elongation.

The drought stress was increased the root length of Sorghum plant when compared to control. Under the drought stress an increased root growth was reported in *Catharanthus roseus* (Jaleel *et al.*, 2008). The drought

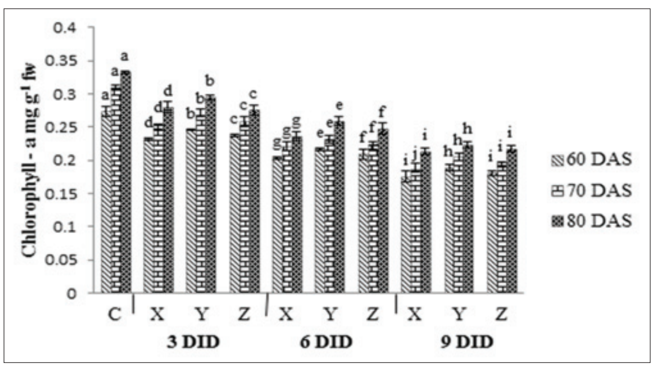

Figure 2: Effect of drought with propiconazole (PCZ) and salicylic acid (SA) treatments on Chlorophyll–a in *Sorghum bicolor* C: Control; X: Drought; Y: Drought + PCZ; Z: Drought + SA

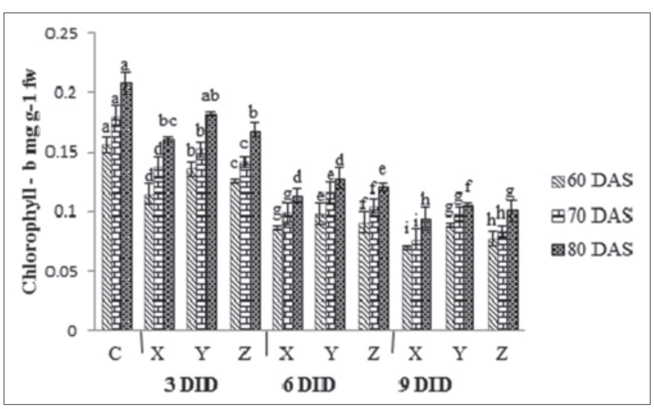

Figure 3: Effect of drought with propiconazole (PCZ) and salicylic acid (SA) treatments on Chlorophyll–b in *Sorghum bicolor* C: Control; X: Drought; Y: Drought + PCZ; Z: Drought + SA

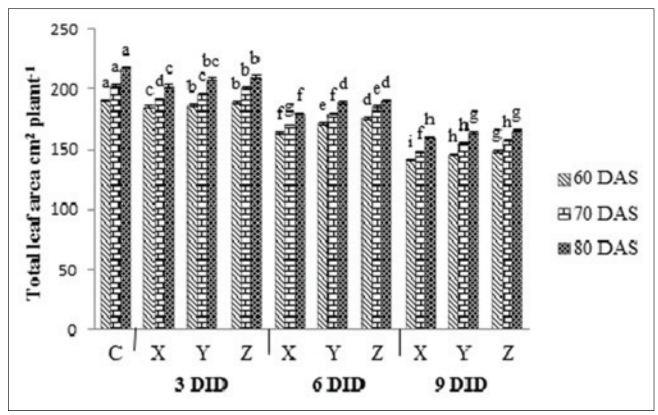

Figure 4: Effect of drought with propiconazole (PCZ) and salicylic acid (SA) treatments on total leaf area in *Sorghum bicolo*r C: Control; X: Drought; Y: Drought + PCZ; Z: Drought + SA

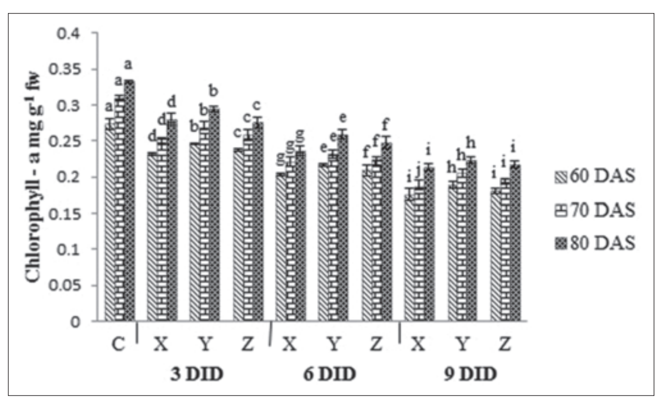

Figure 5: Effect of drought with propiconazole (PCZ) and salicylic acid (SA) treatments on Carotenoid content in *Sorghum bicolor* C: Control; X: Drought; Y: Drought + PCZ; Z: Drought + SA

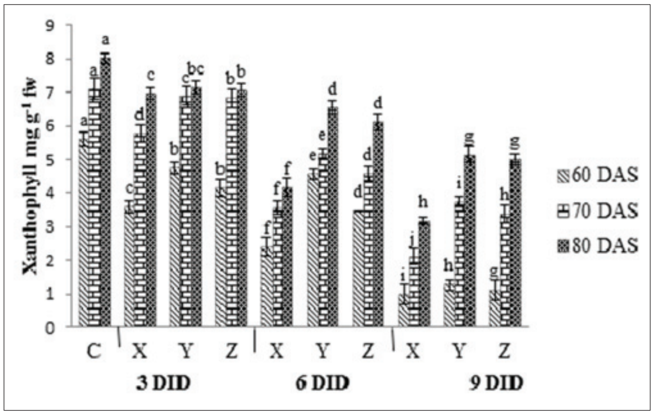

Figure 6: Effect of drought with propiconazole (PCZ) and salicylic acid (SA) treatments on Xanthophyll content in *Sorghum bicolor* C: Control; X: Drought; Y: Drought + PCZ; Z: Drought + SA

with PCZ and SA treatment was also increased the root length when compared to drought stressed plants. These results are in good agreement with reported by other investigator (Shah *et al*., 2002) in corn. The root length was increased in drought stressed conditions are able to access deeper water from soil and the increased root length was an adaptive response. The triazole compound act on plant hormones and it increase the cytokinin content leads to an increase in cell division and the root length. SA and its precursor benzoic acid have been reported to induce significant effects on various biochemical aspects in plants. The exogenous application of SA significantly increased the length of root and shoot in sorghum plant.

The drought stress cause reduction of fresh and dry weight of the Sorghum plant. The similar report has already been reported (Bhatt and Srinivasa-Rao, 2005). The drought with PCZ and SA treatment increased fresh and dry weight of the drought treated plant. The earlier workers are reported on under abiotic stress condition the triazole and SA are increased the biomass of the plants by (Manivannan *et al*., 2008; Baghizadeh *et al*., 2009; Lee *et al*., 2014) on *Vigna unguiculata* (L.) Walp, *Hibiscus esculentus* (L), and tobacco. The drought stress inhibit shoot growth and increase the transpiration so high amount of water evaporated as a result fresh and dry weight will be decreased. The fresh and dry weight of the sorghum plant increased root fresh weight has contributed to a larger extent to the increase of whole plant fresh and dry weight of the PCZ treatments. The enhancing effects of SA on photosynthetic pigments could be attributed to its stimulating effects on RUBISCO activity in photosynthesis. SA induced the synthesis of protein kinase, which plays an important role in regulating cell division, elongation, differentiation, and morphogenesis.

Water deficit stress reduced the leaf area when compared to control. The leaf growth was more sensitive to water stress in maize (Nayyar and Gupta, 2006). Similar results were observed under drought stress in (Yin *et al*., 2005; Blouin *et al*., 2007) on maize. Triazole treatment to the drought stressed plants resulted in increased leaf area in sorghum plants when compared to drought stressed plants, but it was lower than that of control. Triazole treatment increased the leaf area in olive (Thakur *et al*., 1998). The SA are improved the total leaf area in sorghum. Similar to our results, increasing of leaf area under treatment with SA has been reported by (Mathur and Vyas, 2007; Hayat *et al*., 2005) in pearl millet and wheat.

The chl-a, chl-b and total chlorophyll content of the drought treated Sorghum plant was decreased when compared to control plant. The similar report is observed in *Paulownia imperialis* (Astorga and Melendez, 2010; Oraki *et al*., 2012; Ebrahimia *et al*., 2014) under drought stress. The PCZ and SA treatments are increasing the chlorophyll content. Similar results were reported by (Kishorekumar *et al*., 2008) in *Solenostemon rotundifoliu* and (Arfan *et al*., 2007) in Wheat. The concept of photosynthesis consisting of two light reactions photosystem I and photosystem II, has been widely accepted. Both chlorophylls a and b primarily absorb red and blue light, the colors are most effective in photosynthesis. In land plants, the light-harvesting antennae around photosystem II contain the majority of chlorophyll b. Drought stresses causes not only a substantial damage to photosynthetic pigments, but it also leads to deterioration to thylakoid membrane. The photosynthetic pigments Chl-a, Chl-b and total chlorophyll declined with increasing drought stress. In severe drought stress condition chlorophyllase and peroxidase enzymes increased as a result the chlorophyll content are decreased. Triazole compound produce more mesophyll cells per unit leaf area and also increase the thickness of leaf and increase the chlorophyll content. The chlorophyll pigments play a key role in light capturing for photosynthesis and it's forced a direct impact on the intensity of photosynthesis.

The carotenoid content was decreased under drought condition in sorghum plant. The reduced carotenoid content was reported in soybean (Zhang *et al*., 2007) under drought stress. Triazoles and SA treated sorghum plants increased the carotenoid content when compared to drought stressed plants. The same result agreement on triazole treatment in *Raphanus sativus* (Sankari *et al*., 2006) and SA (Türkyılmaz *et al*., 2005) on *Phaseolus vulgaris* L. Carotenoids participate in energy dissipation and can aid plant resistance against drought stress. In addition, carotenoids have a critical role as photoprotective compounds by quenching the triplet state of chlorophyll molecules and singlet oxygen derived from excess light energy, thus limiting membrane damage. This area of carotenoid function has been reviewed extensively elsewhere (Tas and Tas, 2007).

Drought stress decreased the xanthophyll content when compared to control. Similar results were observed in tomato (Still and Pill, 2004). The drought with PCZ and SA treated sorghum leaves showed increased xanthophyll content at all stages of growth. The same results have been reported by PCZ (Gomathinayagam *et al*., 2008) in the leaves of Topica and SA treated *Solanum trilobatum* plants (Nivedithadevi *et al*., 2012).

CONCLUSION

The drought stress affected the growth, biomass accumulation and the reduction of photosynthetic pigments. The drought with PCZ and SA treatments are increased the biomass of Sorghum plant than compared to drought treated plants. All the photosynthetic pigments are more sensitive to water stress. The drought with growth regulator treatments are caused increase the photosynthetic pigment contents.

ACKNOWLEDGMENTS

The authors acknowledge UGC-SAP, BSR for providing the financial support and Professor Dr. K. Arumugam, Head of the Department of Botany, Annamalai University for providing laboratory and facilities for carrying out this research.

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