Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 3308-3312
Received: 10-03-2019
Accepted: 12-04-2019

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Physiological studies for yield enhancement in finger millet under drought condition

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Abstract
An experiment was conducted to study the effect of nutrients and plant growth regulators in finger millet under drought condition in pot culture. The treatments comprised of Absolute control, Seed treatment with Brassinosteroid (0.1 ppm), Foliar spray of All 19:19:19 (1%), Urea (0.5%) + KCl (1%), Brassinosteroid (0.3 ppm), Salicylic acid (100 ppm), Chlormequat chloride (200 ppm) along with Control in ragi CO15 were followed after imposing stress during vegetative stage. The experiment was laid out in factorial completely randomized design with three replications. The physiological and biochemical traits *viz.,* gas exchange parameters, relative water content, chlorophyll index, chlorophyll fluorescence, proline content, catalase activity and yield traits were recorded. Foliar spray of 0.3 ppm Brassinosteroid showed significant effect on gas exchange parameters, chlorophyll fluorescence, proline content and yield traits. Chlorophyll index and relative water content was found to be higher in 100 ppm salicylic acid as foliar spray. Hence, foliar spray of Brassinosteroid (0.3 ppm) and Salicylic acid (100 ppm) were found to be effective for improving the physiological and biochemical aspects which exhibited better yield and yield attributes under drought condition in finger millet.

Keywords: Finger millet, Brassinosteroid, salicylic acid, foliar spray and yield

Introduction
Finger millet (*Eleusine coracana* (L.) Gaertn) is an important minor millet crop which is known as ragi, African millet and bird's foot millet. Finger millet, which is a native of Ethiopia gains importance for subsistence agriculture in the dry areas of India, Africa and Srilanka. It is cultivated in diverse and adverse environments, mostly in the dry semi arid to sub humid drought prone ecosystem. It is well adapted to very poor and marginal uplands (AICSMIP, 2009) [2]. India ranks third in both area (1.29 million ha) and production (2.19 million tonnes) after sorghum and pearl millet. Finger millet, being a C_4 crop, satisfies all the criteria that it has high suitability for both irrigated and rainfed marginal lands with high nutritive value. Finger millet has manifold nutritional benefits; it has thirty times more calcium than rice (Millet Network of India-Deccan Development Society-FIAN, 2009) [14]. It is regarded as a coarse grain because of its fibrous, tough layer and gluten free character in wheat. It contains large proportion of carbohydrate (74%) and thus provides bulk energy in diets.

The lower productivity in finger millet due to some factors, such as marginal and poor soils or inadequate moisture and poor management practices. Among these factors, one of the important reason for poor yield is inadequate moisture availability. Robin *et al.* (2003) [18] reported that drought is the most prevalent abiotic constraint that causes for widespread yield reduction in millets. Nutrients and PGRs to improve physiological efficiency of finger millet as one of the management options to exploit higher yield potential under drought condition. Anjum *et al.* (2011) [4] stated that foliar spray of Brassinosteroid (0.1mg l^{-1}) exhibited more positive response on gas exchange in maize under water stress condition. Afshari *et al.* (2013) [1] reported that the application of salicylic acid at 300μM enhanced the photosynthetic rate and transpiration rate. It has been reported that Rao *et al.* (2012) [17] recorded that application of 100 ppm salicylic acid (SA) maintained highest relative water content in maize. Sivakumar *et al.* (2002) [21] stated that the foliar application of Brassinosteriod @ 0.1 ppm increased the yield of pearl millet. Mady, (2009) [13] stated that the highest yield was obtained with foliar spray of salicylic acid (50 ppm) with vitamin E (200 ppm) in tomato. With this basis, the present investigation was focused on physiological, biochemical and yield attributes in finger millet by foliar application of nutrients and PGRs under drought condition.

Materials and Methods

The pot culture experiment was conducted at Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The experiment was conducted on variety CO15, comprising of eight treatments *viz*., T_1- Absolute control (Irrigated), T_2- seed treatment with Brassinosteroid (0.1 ppm), T_3- foliar spray of All 19:19:19 (1%), T_4-Urea (0.5%)+ KCl (1%), T_5- Salicylic acid (100 ppm), T_6- Brassinosteroid (0.3 ppm),T_7- Chlormequat chloride (200 ppm) and T_8- Drought Control. Uniform irrigation was given to all treatments. The experiment was laid out in factorial completely randomized design (FCRD) with three replications. Foliar application of nutrients and plant growth regulators applied after stress imposed at vegetative stage. Water was withheld for 8 days *i.e.*, from 35^{th} to 42^{th} day during vegetative stage and foliar spray was given at 38^{th} day. Soil moisture content was observed by using moisture meter (Data Devices Theta Probe) daily and rewatering was done, when the soil moisture reached 50% reduction.

The physiological and biochemical parameters were recorded at each day after foliar spray (DAF) continuously upto 4 days. Gas exchange parameters *viz*., photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), leaf temperature (°C) transpiration rate (mmol H_2O m^{-2} s^{-1}) were recorded in fully expanded leaf (3^{rd} leaf from top) using an advanced portable photosynthesis system (LI-6400 XT, LicorInc, Nebraska, USA). Chlorophyll index was recorded using chlorophyll meter (SPAD 502) designed by the Soil Plant Analytical Development (SPAD) section, Minolta, Japan. The chlorophyll fluorescence (F_v/F_m ratio) was measured using the fluorescence meter (opti-sciences OS5p). Relative water content (RWC) was assessed according to Barrs and Weatherly method (1962) [6] and expressed as per cent. The proline content was measured by using the method of Bates *et al.* (1973) [7] and expressed as mg g^{-1}fresh weight. The catalase activity was determined by measuring the rate of reduction of hydrogen peroxide (H_2O_2) as per the method of Samantary, (2002) [20] and expressed as Enzyme Unit (EU) where one enzyme unit was the amount of CAT enzyme that decomposes 1.0 mmol H_2O_2 min^{-1} g^{-1} of tissue on leaf fresh weight.

The yield parameters *viz*., number of productive tillers, ear head weight, ear head length, ear head per plant, 1000 grain weight and grain yield were recorded at harvest stage. The data were analyzed for all the attributes by using SPSS-16 and mean were separated by Duncan's multiple range test at 0.05 probability level.

Results and Discussion

Physiological and biochemical parameters

Gas exchange

Photosynthetic rate, stomatal conductance and transpiration rate were reduced under drought condition. Franca *et al*. (2000) [10] reported that reduction in photosynthetic rate is a common response of plants to water deficit stress. This response could be attributed to either stomata closure or metabolic impairment.

In the present study, foliar application of 0.3 ppm Brassinosteroid application showed highest photosynthetic rate, stomatal conductance and transpiration rate which was on par with 100 ppm Salicylic acid treatment followed by 200 ppm Chlormequat chloride (Fig.1a to 1c). Lowest leaf temperature was recorded in Brassinosteroid treatment (Fig.1d). Brassinosteroid induced the rate of increase in photosynthesis which could be improving the leaf water status as indicated by increased water potential under drought condition (Sairam 1994) [19]. Both Brassinosteroid and Salicylic acid application can improve the carbon assimilation by Rubisco activity and water use efficiency of leaves as a result of their involvement in the plasma membrane permeability under stressful conditions. (Afshari *et al*. 2013 [1] and Noreen and Ashraf, 2008 [15]).

Chlorophyll index, fluorescence, relative water content

The maximum chlorophyll index recorded in absolute control. Imposing of drought to the plants decreased the value of SPAD. Among the treatments, Chlorophyll index was maximum in foliar spray of Salicylic acid followed by Brassinosteroid application over control (Fig. 2a). This was due to increase in photosynthetic efficiency as reflected by increasing in both chl a, chl b and carotenoids content in the leaves. These results are in agreement with those obtained by Amin *et al*. (2008) [3].

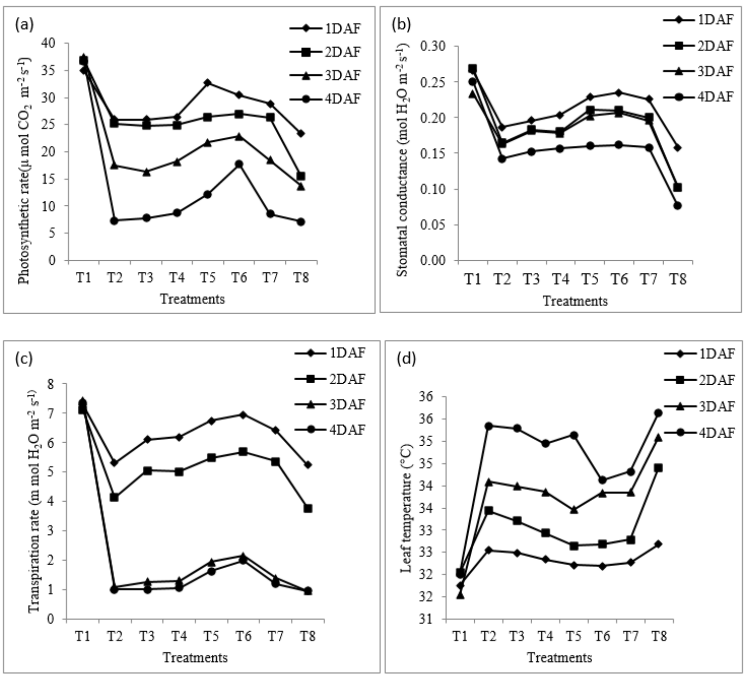

Fig 1: Effect of nutrients and plant growth regulators on (a) photosynthetic rate, (b) stomatal conductance, (c) transpiration rate, (d) leaf temperature in finger millet under drought condition (DAF - Days after foliar spray).

The SPAD value in the plants treated with Salicylic acid was increased which may be another reason for the increase in photosynthesis was reported by Hayat *et al*. (2008) [12]. Chlorophyll fluorescence (Fv/Fm ratio) was higher in absolute control. Among the treatments, maximum Fv/Fm ratio was observed in 0.3 ppm Brassinosteroid foliar spray which was on par with 100 ppm Salicylic acid application (Fig. 2b). Relative water content was significantly reduced under drought condition. It was improved by foliar application of Salicylic acid (100 ppm) which was on pair with foliar application of Brassinosteroid (0.3 ppm) followed by foliar spray of Chlormequat chloride @ 200 ppm (Fig. 2c). This might be due to the salicylic acid regulates the stomatal openings and reduces transpirational water loss under drought conditions enabling the plants to maintain turgor and photosynthesis under water deficit conditions. These findings were in accordance with Hayat *et al*., (2008) [12] and Rao *et al*. (2012) [17]. Further, Yuan *et al*. (2010) [23] and Anjum *et al*. (2011) [4] reported that application of Brassinosteroid may be reduced the water loss and ameliorate the drought stress.

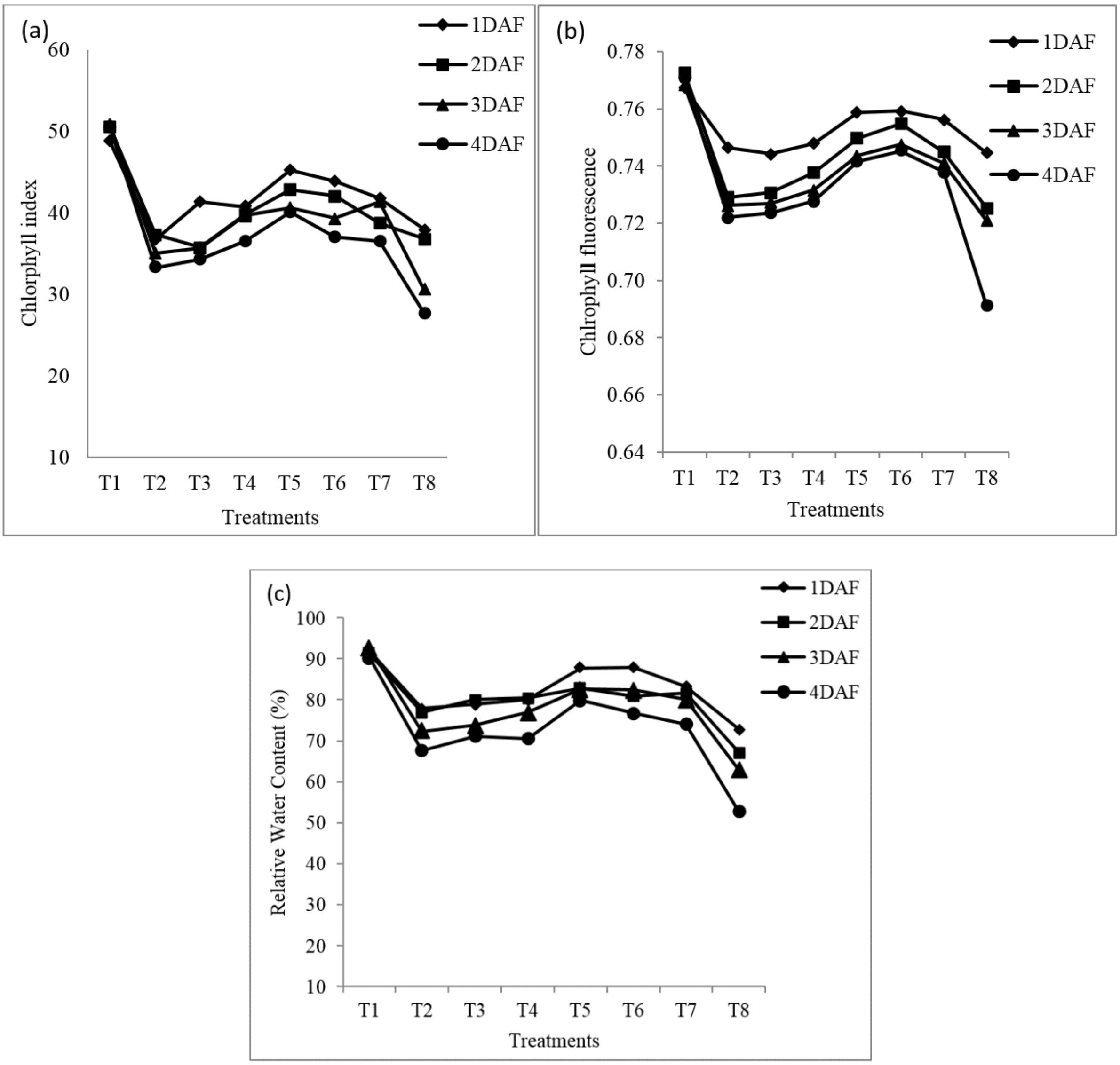

Fig 2: Effect of nutrients and plant growth regulators on (a) chlorophyll index (b) chlorophyll fluorescence and (c) relative water content in finger millet under drought condition (DAF- Days after foliar spray)

Proline and Catalase

The content of proline gradually increased in plants as the drought levels increased. Proline content was significantly higher in foliar application of nutrients and plant growth regulators. Foliar spray of 0.3 ppm Brassinosteroid registered the maximum proline content which was on par with 100 ppm Salicylic acid followed by 200 ppm Chlormequat chloride application (Fig. 3a). Increased proline content by Brassinosteroid application was also observed in tomata due to induced expressing of proline biosynthetic genes (Ozdemir *et al*., 2004) [16]. Proline accumulation under stress condition influences protein salvation and maintains membrane integrity, preserves the quaternary structure of complex proteins under dehydration stress and reduces oxidation of lipid membrane or photo inhibition (Demiral and Türkan, 2004) [9]. Therefore, a role of Brassinosteroid in the accumulation of proline as an important component of protective reactions in ragi in response to drought. Salicylic acid induces abscisic acid mediated protective reactions of plants to water deficit by increasing proline accumulation. This was accordance with the findings of Yoshiba *et al*. (1995) [22].

Drought led to significant modulation of antioxidant defense in leaves of ragi. Catalase activity was increased by the application of Brassinosteroid under water deficit condition which was on par with foliar spray of chlormequat chloride and salicylic acid (Fig. 3b). Brassinosteroid application regulates the transcription and translation level which led to enhanced activities of enzymatic antioxidants (Anjum *et al*., 2011) [4]. Brassinosteroid can ameliorate the adverse effect of drought stress via decreasing the oxidative damage of plant memebranes by induction of antioxidant defense system as reported by Behnamnia *et al*. (2009) [8]. The foliar spray of Salicylic acid had involved in antioxidant defense mechanisms (Hayat *et al*., 2008) [12] which contributed to the protection of their photosynthetic machineries against damages caused by reactive oxygen species during water deficit condition as reported by Anosheh *et al*. (2012) [5].

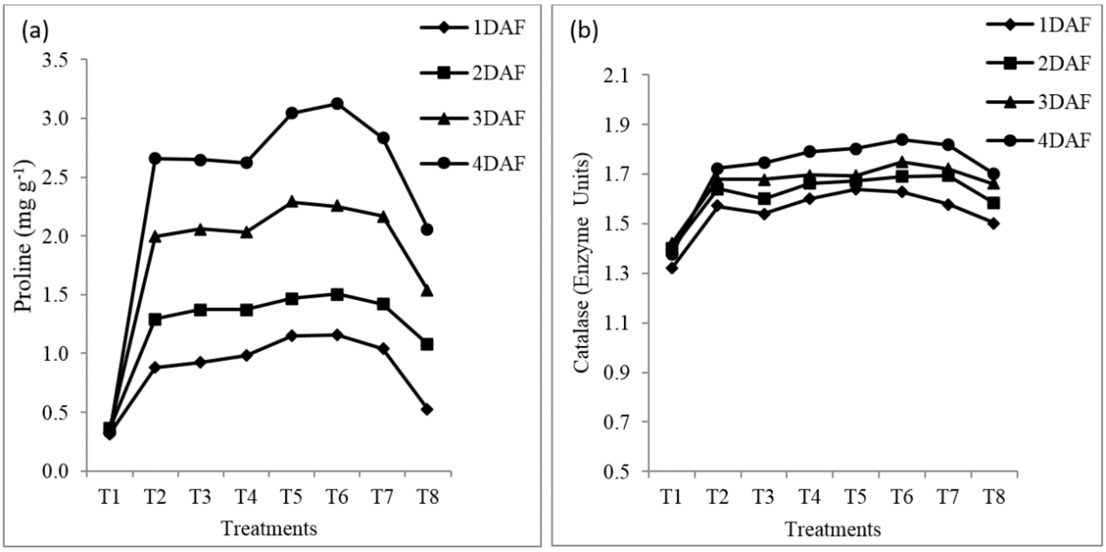

Fig 3: Effect of nutrients and plant growth regulators on (a) proline content and (b) catalase activity in finger millet under drought condition (DAF- Days after foliar spray)

Yield and yield traits

The yield traits were significantly influenced by the application of different nutrients and plant growth regulators under drought condition (Table 1). The number of productive tillers were higher in Absolute control (8.50) which was on par with Brassinosteroid treatment (7.83) followed by Salicylic acid (7.67). The number of ear heads per plant was recorded highest in Absolute control (9.67). Among the treatments, Brassinosteroid recorded highest number of ear head per plant (8) which was on par with Chlormequat chloride (7.83) and Salicylic acid treatment (7.67). Similar findings were recorded with Sairam (1994) [19]. The ear head length was highest in Brassinosteroid treatment which was on par with Salicylic acid, Chlormequat chloride, urea + KCl and All 19:19:19.

Ear head weight per plant recorded lowest in drought control. Among the treatments, highest ear head weight was observed in Brassinosteroid as foliar spray which was on par with Salicylic acid and Chlormequat chloride. Similar trend was recorded in 1000 grain weight. The grain yield per plant recorded maximum in absolute control (22.03g) and lowest in drought control (14.08g). Among the treatments, Brassinosteroid recorded highest grain yield (19.12g) which was on par with Salicylic acid (18.98g) followed by Chlormequat chloride (18.83g) and Urea + KCl application (18.52g). This might be due to induced increase in translocation of more photo assimilates towards grain. These findings are agreement with Gomes (2011) [11] and hence, Brassinosteroid and salicylic acid can ameliorate the drought by active translocation and partioning efficiency from source to sink.

Table 1: Effect of nutrients and plant growth regulators on yield attributes in finger millet under drought condition

Treatments	No. of productive tillers per plant	No. of ear heads per plant	Ear head length(cm)	Ear head weight (g)	1000 grain weight(g)	Grain yield per plant (g)
T_1 - Absolute control	8.50	9.67	7.67	2.83	2.53	22.03
T_2 - Seed treatment with Brassinosteroid (0.1ppm)	6.67	6.73	6.40	2.10	2.03	16.14
T_3 - All 19:19:19 (1%)	7.00	6.83	6.60	2.30	2.07	16.25
T_4 - Urea (0.5%) + KCl (1%)	7.33	7.00	6.67	2.40	2.10	18.52
T_5 - Salicylic acid (100ppm)	7.67	7.67	6.93	2.53	2.23	18.98
T_6 – Brassinosteroid (0.3ppm)	7.83	8.00	7.00	2.63	2.33	19.12
T_7 - Chlormequat chloride (200ppm)	7.67	7.83	6.80	2.50	2.27	18.83
T_8 - Control	5.67	6.37	6.23	1.83	1.73	14.08
Mean	7.25	7.65	6.79	2.39	2.16	17.99
SEd	0.35	0.34	0.24	0.09	0.08	0.34
CD(P=0.05)	0.75	0.73	0.52	0.20	0.17	0.72

Conclusion

The growth and development of finger millet gets affected by duration and intensity of drought. In the present study, the effect of nutrients and plant growth regulators were used to study the physiological, biochemical and yield characters. Among the treatments, foliar spray of 0.3 ppm Brassinosteroid and 100 ppm Salicylic acid showed better performance under drought. Hence, the foliar spray of 0.3

ppm Brassinosteroid or 100 ppm Salicylic acid can be used to improve the yield of finger millet under drought condition.

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