Pak. J. Bot., 52(3): 783-792, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-3(17)

PLANT GROWTH PROMOTING RHIZOBACTERIA IN COMBINATION WITH PLANT GROWTH REGULATORS ATTENUATE THE EFFECT OF DROUGHT STRESS

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Abstract

The present study evaluates the effects of plant growth hormones (PGR), salicylic acid (SA), abscisic acid (ABA) and plant growth promoting rhizobacteria (PGPRs) *Rhizobium pisi* (DSM 30132 strain) applied alone and in combination, on pea (*Pisum sativum* L.) cv. Florida plants under well-watered and drought stressed conditions. Prior to sowing seeds were soaked for 5h in broth culture (10^8 cfu/ml) of *Rhizobium pisi* and SA /ABA. Seeds were soaked for 6h in distilled water, ABA, SA solutions. Plants were subjected to drought stress on 21 days old seedlings by withholding the supply of water at two different time points; for 4d (TP_1) and for 8d (TP_2). Rhizosphere soil of abscisic acid treated plants exhibited higher retention of soil moisture at TP_1. Abscisic acid decreased the fresh and dry weight of plants under unstressed condition but increased the fresh weight as well as relative water content under drought stress. The response of *Rhizobium* and SA were at par. *Rhizobium* and SA ameliorated the adverse effects of drought stress more effectively than ABA. The *Rhizobium* inoculation reduced the stomatal conductance under unstressed condition but significantly increased stomatal conductance under drought stress at TP_2. SA alone and in combination with *Rhizobium* stimulated the stomatal conductance under unstressed condition. Under drought stress, at TP_1 all the treatments alone and in combination increased the relative water content (RWC) significantly over drought stressed plants. The FV/FM ratio was increased in SA treatment or in combination with SA, *Rhizobium* and ABA.

It is inferred from the data that *Rhizobium* alone or in association with SA may be used to mitigate drought induced inhibition on plant growth and biomass. At TP_1 the individual treatments of *Rhizobium*, ABA and SA exhibited better growth effect on pea plants. At TP_2, *Rhizobium* assisted SA and ABA to mitigate drought induced adverse effects over control. The combined application of PGPR and PGRs can be substantiated more effectively on crop plants under drought stressed condition. Furthermore, integrating these approaches in the cropping system can contribute to maintaining soil fertility status, with better economic returns for future use.

Key words: PGPRs, Salicylic acid, Abscisic acid, Abiotic stress, Pea

Introduction

Pea (*Pisum sativum* L.), a cool season food legume is a versatile crop cultivated worldwide (Mendler-Drienyovszki & Dobra´nszki, 2011; Nisar *et al*., 2008). The water requirements of pea is relatively high during growing season; the critical stages are the initial germination and the flowering. During the pod-filling phase the sensitivity of peas to drought stress is much less (Harrison, 2018). The drought stress induced during flowering stage reduces the number of pods per plant resulting in significant reduction in yield (Harrison, 2018).

Crop yield can be retained to a specific level by utilization of specific plant growth-promoting rhizobacteria (PGPR) that interact with crops (Glick 2012; Sandhya *et al*., 2010; Araus *et al.,* 2008), in the manifestation of suboptimal environments including; drought and high salinity (Glick, 2014). Recent studies revealed various nods of convergence between stress responsive hormonal and ROS mechanisms that lead to biotic and abiotic stresses (Sewelam *et al*., 2016; Glombitza *et al*., 2004). Plant growth regulators (PGRs) such as SA and ABA are considered as the principal phytohormones which accumulate in plants under drought stress environments. It is well described that SA plays pivotal role in plants against pathogenic attack. However, it is also involved in plant responses such as; regulation of growth, ripening, flowering, development and abiotic stresses respectively (Miura & Tada, 2014; Bandurska and Stroìnski, 2005; Munne-Bosch and Penuelas, 2003). Whereas, ABA has a fundamental importance under drought stress and increases 55 fold of the original. ABA interacts with SA signalling pathways in an intricate manner. The use of PGPR has been demonstrated as a solution for the sustainability of agro-ecosystem under stresses. These strains are responsible for alleviating the plant growth from biotic/abiotic stress responses.

Globally, the preceding climate changes are expected to have a considerable repercussion on precipitation, intensifying the drought stress. There is a dire need to improve drought tolerance in crops in order to enhance their growth and yield using a number of PGPRs and PGRs (Khan *et al*., 2019). Previous studies demonstrated the favourable effects of PGPRs and PGRs on wheat and maize crops alleviated drought stress (Khan *et al*., 2018; Mega *et al*., 2019; Kumar *et al*., 2019). However, literature is scanty on pea plants. The present study was aimed to assess the role of PGPR (*Rhizobium pisi*) and PGRs (SA and ABA) on the growth of pea under drought stress.

Materials and Methods

Plant material and growing conditions: The seeds of pea (*Pisum sativum* var. Pea-Florida) were sown in pots (14×12 cm^2) filled with sieved and autoclaved ED73 soil under in vitro conditions. Experiment was organized in completely randomize design, conducted in triplicates. Plants were grown in walk-in-chamber maintained at 16h photoperiod with temperature 24 ± 2°C (day/night), 65% relative humidity and light intensity of 100 µmol $m^{-2}s^{-1}$

(LI-COR LI-250A, serial No. Q 101421). Pea seeds were surface sterilized with 95% (v/v) ethanol followed by shaking in 5% (v/v) sodium hypochlorite with slight modification (addition of 50 µl of Tween 100) and subsequently washed thrice with autoclaved distilled water (Lindsey *et al*., 2017).

Exogenous application of SA and ABA: SA and ABA were used as PGRs. A stock solution of 10^{-6} M was prepared to conduct the experiment (Hadi *et al*., 2010). The seeds were soaked in aqueous solution of SA and ABA for 6h prior to sowing (Safari *et al*., 2018).

Preparation of *Rhizobium* inoculum: *Rhizobium pisi* DSM 30132 strain was used as PGPR. Broth cultures of *Rhizobium* were prepared by growing the *Rhizobium* in yeast extract mannitol (YEM) media for 3 days (10^8 cfu /ml and O.D ~ 1 at 660 nm).

Induction of drought stress: Drought stress was induced after three weeks of germination by withholding the supply of water followed by constant watering to maintain the moisture content of stressed plants at 40% (Pain *et al*., 2018). The experiment was performed with six replicates each for control and drought conditions. Treatment were: untreated control (C), inoculated with *Rhizobium pisi* (R), treated with salicylic acid (S), treated with abscisic acid (A), combined treatment of *Rhizobium* with salicylic acid (B), combined treatment of Rhizobium with abscisic acid (D) treated with both SA and ABA with PGPR (E).

Moisture content: Soil sample was taken at a uniform depth of 6 inches from the soil surface and its moisture content was determined by applying given formula (Valarmathi *et al*., 2019):

$$\text{Soil moisture (\%)} = \frac{\text{Weight of wet soil (g)-Weight of dry soil (g)}}{\text{Weight of dry soil (g)}} \text{ X } 100$$

Plant fresh, dry biomass and plant height: Fresh weight of seedling were measured. The seedlings were dried in an oven at 90°C till a constant weight was obtained. Plant height was measured from the base of the stem to the apex. Six biological replicates were made.

Stomatal conductance: Stomatal conductance estimates the rate of gas exchange (carbon dioxide uptake) and transpiration (water loss) though the leaf stomata as determined by the degree of stomatal aperture. Measurements were taken at 11:00 am. Stomatal conductance of three different leaves from each plant with three biological replicates was measured by a Porometer (AP-4, Delta T-Devices, Cambridge UK).

Stomatal Index: Leaves were randomly taken from the upper part of plant to remove the mesophyll. The adaxial surface of leaves were peeled off and stomata were observed under a light microscope (Leica DM1000, Meiji infinity 1, Canada) at 20x. The total number of stomata and other epidermal cells in the area of $1mm^2$ were counted. Stomatal Index (SI) was calculated according to Ogaya *et al*., (2011).

$$\text{SI (\%)} = \frac{\text{No. of stomata}}{\text{No. of stomata + No. of epidermal cells}} \text{ X } 100$$

Canopy temperature: To measure leaf temperature, an infrared thermal camera (calibrated) was used. Pots with plants were moved to the middle of the table, one day prior to the measurements. Infrared thermal snaps were taken such that plants were not moved from their position. Results regarding the change in temperature were calculated by FLIR Tools software, Version 5.2.

Relative water content (RWC) of leaves: Relative water content of leaves was measured at two time points after the periods of induction of water stress, following the method of Garcı´a-Mata and Lamattina (2001). Relative water content was calculated by the formula:

$$\text{Relative water content (RWC \%)} = \frac{\text{Fresh weight (FW)} - \text{Dry weight (DW)}}{\text{Turgid weight (TW)} - \text{Dry weight (DW)}} \times 100$$

Fresh weight (FW) was measured for each time point of drought period, and dry weight (DW) was obtained after drying the samples at 90°C for at least 72h. Turgor weight (TW) was determined by subjecting leaves to rehydration for 24h after drought treatments.

Chlorophyll content: Chlorophyll content of pea leaves were measured using chlorophyll meter (SPAD, Minolta). The different areas of a single leaf was measured (Koshy *et al*., 2018), and the biological replicates were used to determine chlorophyll content.

Chlorophyll fluorescence (PS II efficiency): Chlorophyll fluorescence was measured using a portable Chlorophyll Fluorimeter (MINI-PAM, Portable Chlorophyll Flourometer, Walz-Germany) after 10 min of dark adaptation. Chlorophyll fluorescence was estimated by the Fv/Fm ratio, which represented the maximum quantum yield of photosystem II. It was calculated as Fv/Fm = (Fm – Fo) / Fm, where Fm and Fo are maximal and minimal fluorescence of dark adopted leaves respectively and Fv is variable fluorescence (Jifon & Syvertsen, 2003).

Statistical analysis: The data was evaluated statistically using analysis of variance (ANOVA) technique for all performed attributes via completely randomized plots design. The comparison between the mean values of treatments were made by Least Significant Difference (LSD) to test significant differences at $p \leq 0.05$ using Statistix 8.1 (Gomez & Gomez, 1984). The data were graphically represented on Microsoft excel 2013.

Table 1. Soil moisture content (%) after sowing.

Treatments	0 d	5 d	10 d	15 d	20 d Induction of drought	TP1 d (after 4 days)	T.P2 d (after 8 days)
C	65 ± 0	64.91 ± 0.66	61.5 ± 0.39	64.41 ± 0.47	59 ± 0.79	49.16 ± 1.71	40 ± 0
R	65 ± 0	62.74 ± 0.58	62 ± 0.34	63.16 ± 0.69	59.16 ± 0.48	48.33 ± 1.72	40.1 ± 0.18
S	65 ± 0	63.33 ± 0.63	60.67 ± 0.45	60 ± 0.45	59.5 ± 0.49	46.33 ± 1.87	40 ± 0
A	65 ± 0	62 ± 0.51	61.83 ± 0.41	67.08 ± 0.6	65.16 ± 0.8	54.83 ± 1.24	42 ± 0.36
B	65 ± 0	61.33 ± 0.66	59.5 ± 0.46	61.83 ± 0.56	59.33 ± 0.88	47.5 ± 1.12	40.2 ± 0.17
D	65 ± 0	61.91 ± 0.5	60.5 ± 0.35	60 ± 0.59	59.92 ± 0.99	46.66 ± 1.42	39 ± 0.2
E	65 ± 0	64.83 ± 0.66	60.5 ± 0.49	59.66 ± 0.7	57.16 ± 0.96	49.83 ± 1.19	39.6 ± 0.35

Results

Moisture content: The drought was induced at 59% soil moisture even at this stage, the rhizosphere soil of ABA treated plants retained higher moisture content.at short term stress (TP_1), but at long term stress (TP_2) the ABA treatment (A) though having higher percentage of soil moisture than other treatments but the moisture content was dropped down to 42%. The indication of drought resulted in significance decrease in the moisture content of rhizosphere soil. The percent decrease was linear with the duration of drought stress (Table 1). A significant decrease in moisture content occurred in treatment S (SA), whereas a slight decrease was observed in treatment R (*Rhizobium pisi*) and treatment E (combined Rhizobium, ABA and SA) had no significant effects compared to control (C). Noteworthy, the least decrease was observed in treatment A (ABA) over **C** at TP_1. However, at TP_2 the decrease in moisture was non-significantly higher over C.

Seedling moisture content under stressed condition. Effect of different treatments on plant moisture content (values are the mean from six biological replicates mean ± SE (n=6) in days (d), Control with stress (C); *Rhizobium pisi* with stress (R); salicylic acid (SA) with stress (S); abscisic acid (ABA) with stress (A); *Rhizobium pisi* along with salicylic acid under stress (B); *Rhizobium pisi* with abscisic acid under stress (D); *Rhizobium pisi* with both PGRs (SA and ABA) under stress (E).

Plant fresh and dry biomass: Under unstressed condition fresh weight of the plant was not affected significantly at TP_1 or TP_2 except treatment B (inoculation of *Rhizobium* with SA), treatment A (ABA) and treatment E (*Rhizobium* combined with SA and ABA) which showed 43% significant increase in fresh biomass at TP_1 and 20% decrease in fresh weight at TP_2 whereas no significant effects were visible in treatments as compared to C (Fig. 1). Under drought stress at TP_1 except treatments D (*Rhizobium* with ABA) and E (combined treatment with Rhizobium, ABA and SA) which differed non-significantly, all the treatments showed increase over the C. The maximum increase was due to R > A > S > at TP_1 and TP_2.

Under unstressed condition the dry weight of the plants at TP_1 was significantly higher in R (*Rhizobium* alone), S (SA alone), B (*Rhizobium* combined with SA) treatments (Fig. 2). Whereas, treatments A (ABA alone), D (*Rhizobium* combined with ABA) and E (*Rhizobium* combined with SA and ABA) had no significant effect when compared with the C. Drought stress enhanced the dry biomass (15% to 16%) at TP_1 in treatments R (*Rhizobium* alone), S (SA alone) and B (Rhizobium with SA). While, treatments A (ABA alone), D (*Rhizobium* combined with ABA) and E (*Rhizobium* combined with SA and ABA) showed significant reduction over C (control). Significant increases of dry biomass were depicted in treatments, R, S and B (*Rhizobium* with SA) over C. Though, significant decreases were observed in A, D and E treatments at TP_2.

Plant height: At TP_1 under unstressed condition the height of the plants was not significantly affected in treatments R, S and B, whereas, treatments A and D showed decreases in comparison to C. At TP_2, R showed significant increase whereas A and E showed decreases over C.

Induction of drought stress indicated a significant increase in plant height in R > S treatments over control at TP_1 (Fig. 3). At TP_2 maximum increase in height was observed in treatment R (*Rhizobium*). But, the treatments S, B, and D displayed no significant difference over control. Though, A, and E treatments showed decreases over C.

Stomatal conductance (SC): Under unstressed condition the treatments showed significant increases in treatment B, A, S and D over C. Treatment R displayed decrease in stomatal conductance at TP_1 and treatment E had no significant effect (Fig. 4). At TP_2 the treatments S, A, B and E showed significantly higher SC over C. whereas, treatment R showed decrease and D had no significant effect at TP.

Under drought stress R and S have no significant effect whereas, A, B, D and E showed increases over control at TP_1. The maximum increase was due to A> D over C. At TP_2 all the treatments showed significant increases whereas B had no significant effect.

Stomatal Index (SI): Under unstressed condition at TP_1 treatments showed significant decreases in stomatal index (Fig. 5). At TP_2 the SI was not affected significantly in treatments A, D and E all other treatments showed significant decreases over C.

Under drought stress there was no significant difference in SI in the treatments over C except treatment B but at TP_2 the SI value was similar to C in all the treatments.

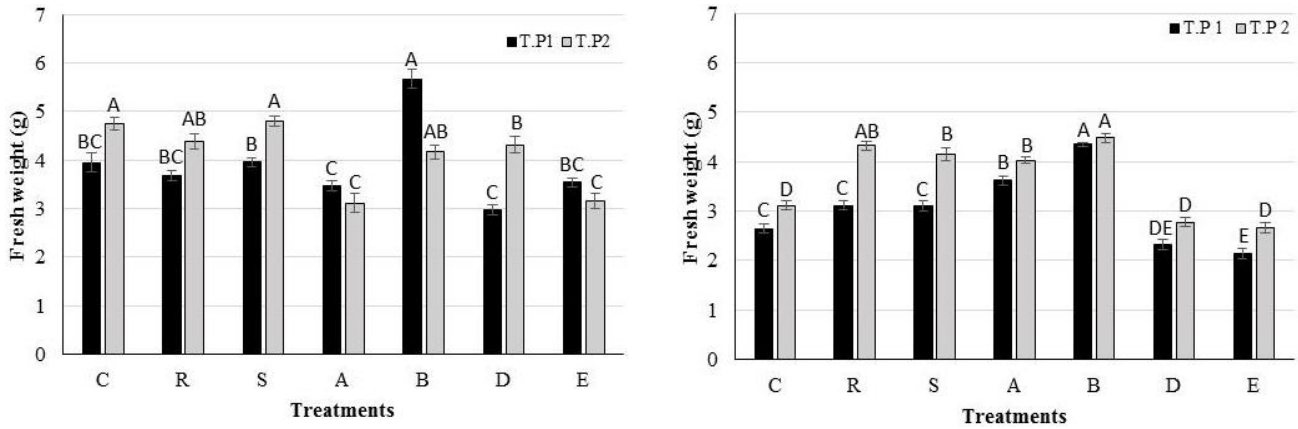

Fig. 1. Effect of different treatments on seedling fresh biomass (values are the mean from six biological replicates (mean ± SE (n=6), a: Seedling fresh biomass under un-stressed condition; b: Seedling fresh biomass under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
Uppercase alphabetic letters heading the bars exhibited significant differences within treatments, LSD significance difference test at $p \leq 0.05$). Time point 1= induction of 4 days of drought (TP1), Time point 2= induction of 8 days of drought stress ($T.P_2$).

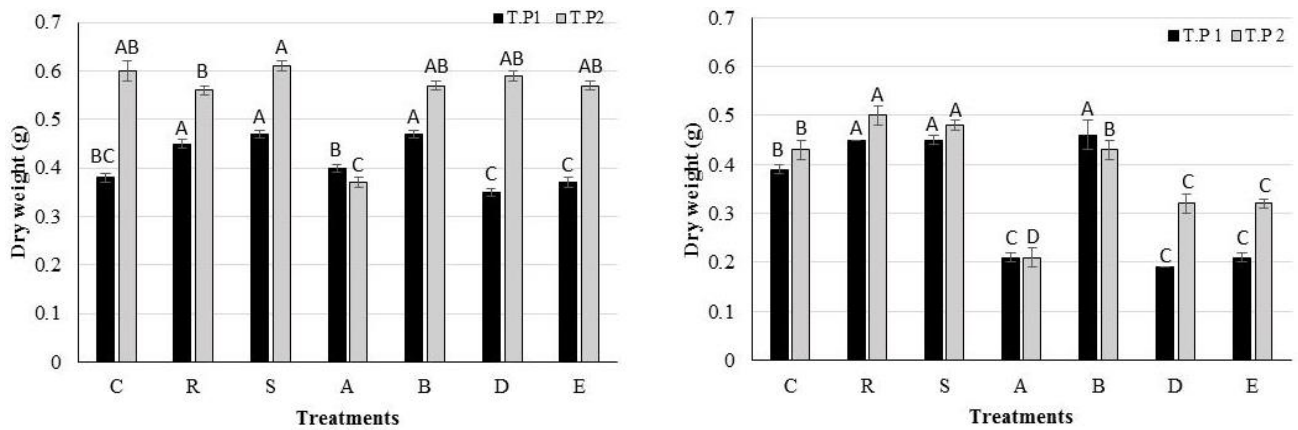

Fig. 2. Effects of different treatments on seedling dry biomass (values are the mean from six biological replicates (mean ± SE (n=6), a: Seedling dry biomass under un-stressed condition; b: Seedling dry biomass under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
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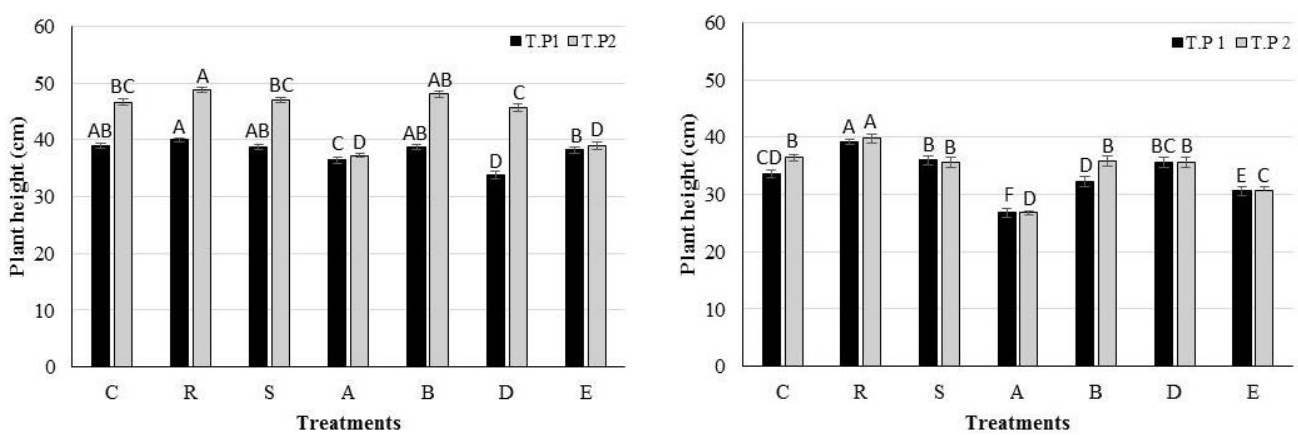

Fig. 3. Effect of different treatments on Seedling height (values are the mean from six biological replicates (mean ± SE (n=6), a: Seedling height under un-stressed condition; b: Seedling height under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
Uppercase alphabetic letters heading the bars exhibited significant differences within treatments, LSD significance difference test at $p \leq 0.05$). Time point 1= induction of 4 days of drought (TP1), Time point 2= induction of 8 days of drought stress ($T.P_2$).

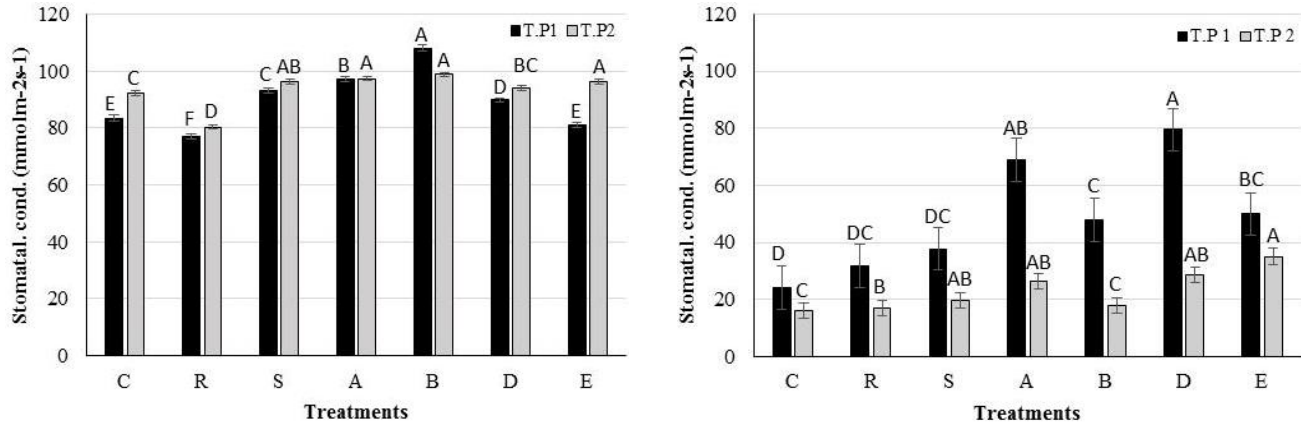

Fig. 4. Effect of different treatments on stomatal conductance (values are the mean from six biological replicates (mean ± SE (n=6), a: Stomatal conductance under un-stressed condition; b: Stomatal conductance under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
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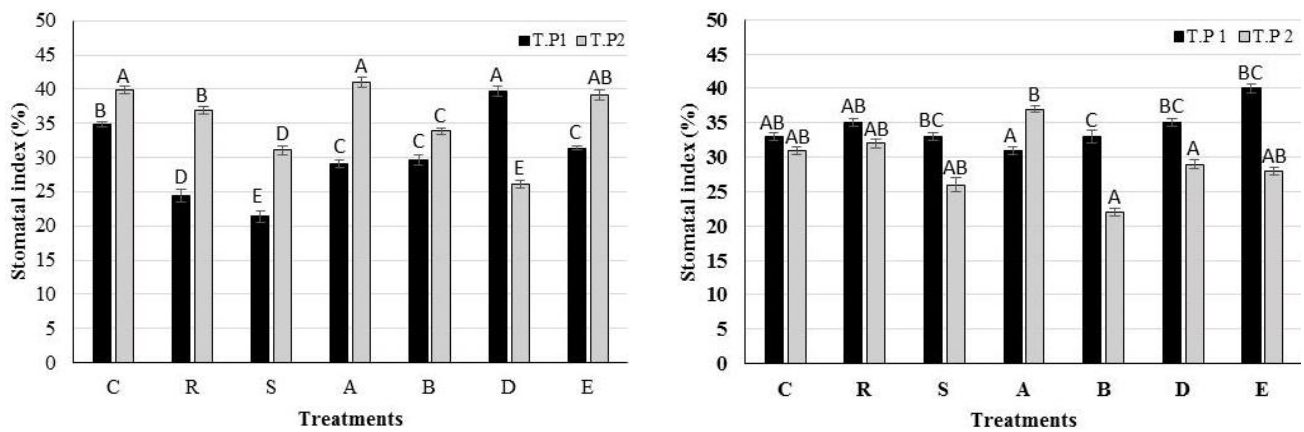

Fig. 5. Effect of different treatments on stomatal index (SI) (values are the mean from six biological replicates (mean ± SE (n=6), a: Stomatal index (SI) under un-stressed condition; b: Stomatal index (SI) under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
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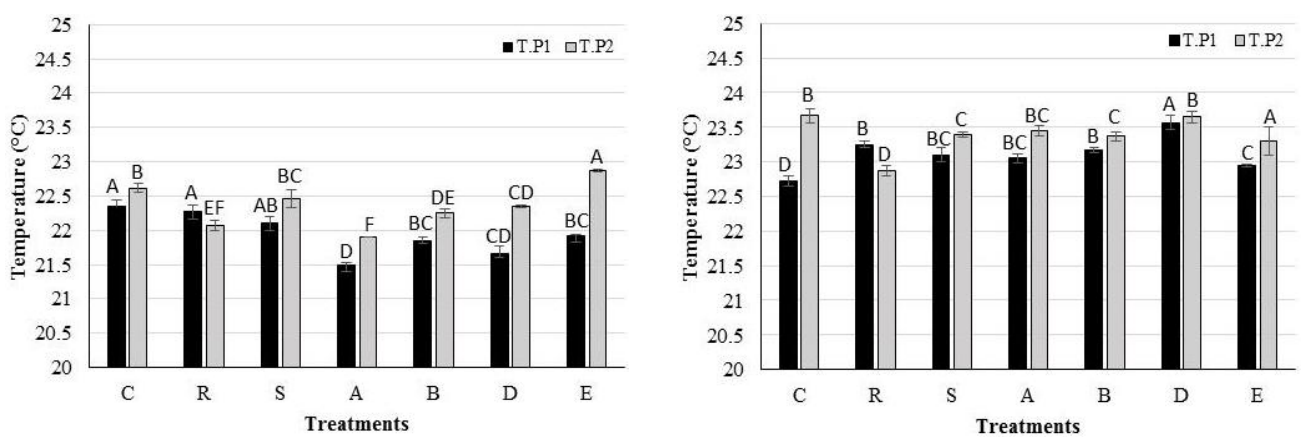

Fig. 6. Effect of different treatments on canopy temperature (values are the mean from six biological replicates (mean ± SE (n=6), a: Canopy temperature under un-stressed condition; b: Canopy temperature under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
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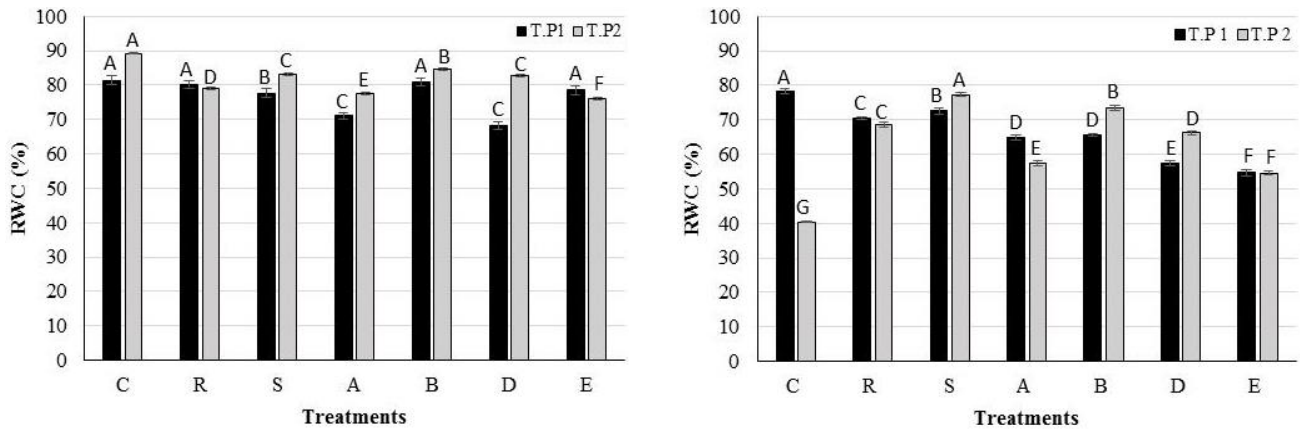

Fig. 7. Effect of different treatments on relative water content (RWC) (values are the mean from six biological replicates (mean ± SE (n=6), a: Relative water content under un-stressed condition; b: Relative water content under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
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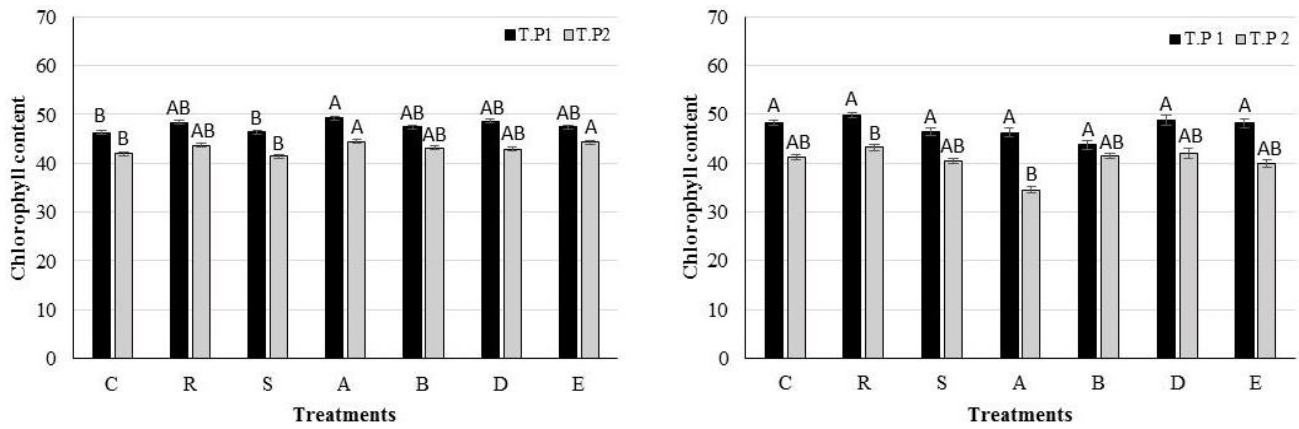

Fig. 8. Effect of different treatments on chlorophyll content (values are the mean from six biological replicates (mean ± SE (n=6), a: Chlorophyll content under un-stressed condition; b: Chlorophyll content under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
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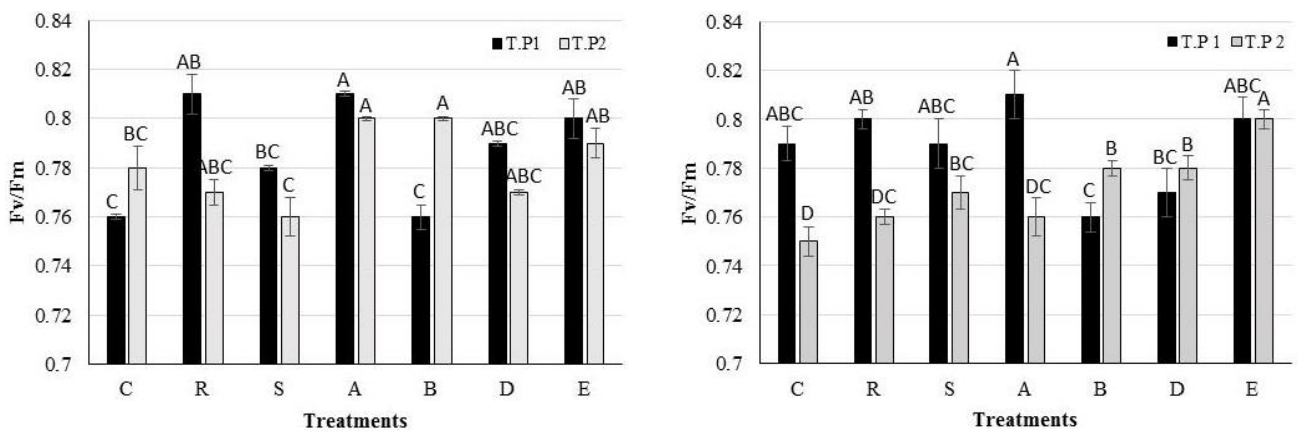

Fig. 9. Effect of different treatments on photosynthetic efficiency (PSII) (values are the mean from six biological replicates (mean ± SE (n=6), a: Photosynthetic efficiency (PS II) under un-stressed condition; b: Photosynthetic efficiency (PS II) under drought stressed condition. Untreated drought stressed Control (C); *Rhizobium pisi* (R); salicylic acid (SA) (S); abscisic acid (ABA) (A); *Rhizobium pisi* along with salicylic acid (B); *Rhizobium pisi* with abscisic acid (D); *Rhizobium pisi* with both PGRs (SA and ABA) (E)
Uppercase alphabetic letters heading the bars exhibited significant differences within treatments, LSD significance difference test at $p \leq 0.05$). Time point 1= induction of 4 days of drought (TP1), Time point 2= induction of 8 days of drought stress ($T.P_2$).

Canopy temperature: Under unstressed condition, the results revealed a decrease in canopy temperature in treatments A, B, D and E over C at TP_1 (Fig. 6). At TP_2 treatments E showed significant increase in canopy temperature over C (control), all other treatments showed no significant decreases over C (control). The maximum decrease in canopy temperature was in treatment A (ABA) at both TP_1 and TP_2 except treatment S which had no significant effect over C.

Under drought stress, at TP_1 all the treatments showed increases over C (Fig. 6). The maximum increase 3% over C was due to treatment D. At TP_2, except treatment A and treatment D which showed no significant affects in canopy temperature. There were slight decreases in canopy temperature maximum decrease in canopy temperature was noticed in treatment R.

Relative water content (RWC): Under unstressed condition, treatments A, D and S showed decrease in RWC, other treatments had no significant effect compared to C at TP_1 (Fig. 7). At TP_2 reassesses occurred in all the treatments, maximum was due to treatment E.

On induction of drought stress at TP_1, the RWC was decreased in all the treatments S, R, A, B, D compared to C (Fi. 7). The maximum decrease 30% was due to treatment E over C. At $T.P_2$ all the treatments increased the RWC significantly over control, 91 % was in treatments S > B.

Chlorophyll content: The results showed no significant effects of treatments on chlorophyll content either at TP_1 or TP_2 over C (Fig. 8). Under drought stress also treatments have no significant effect over C at TP_1 and at TP_2 (Fig. 8). The chlorophyll content decreased under drought stress.

Chlorophyll fluorescence (PS II efficiency): Under unstressed condition, no significant increase was recorded in treatments R, A and E over control at TP_1 (Fig. 9). But, at TP_2 the treatments A, and B effectively increased Fv/Fm over C.

On induction of drought stress at TP_1 no significant effect of treatments was observed in the Fv/Fm over C but, treatments S, B, D and E showed significant increases in Fv/Fm over C. The maximum increase was due to treatment E.

Discussion

The result revealed a distinct role of Rhizobium under drought stress which supercedef ABA in maintaining the water budget of the plant as evidenced by the RWC and fresh weight of the seedlings greater than the drought stressed treatment. Even under unstressed condition 15 days after sowing, the ABA treatment and *Rhizobium* inoculation maintained higher soil moisture content which demonstrates their ability in minimizing water loss in ABA treatment and hence the turgidly was better than the drought stress C (Ruggiero *et al*., 2017; Yang *et al*., 2016; Hussain *et al*., 2018; Staudinger *et al*., 2016). The maximum retention of soil moisture in ABA (A) treatment at TP1 may be attributed to the ABA enhanced water use efficiency of the plant which reduces the rate of transpiration by closing the stomata (Saradadevi *et al*., 2017). Earlier studies demonstrated the similar role of ABA (Aroca *et al*., 2006; Ngumbi & Kloepper, 2016) and *Rhizobium* (Grover *et al*., 2011; Figueiredo *et al*., 2008) on retention of soil moisture and water use efficiency. Noteworthy, the Rhizobium assistance to ABA at TP2 for improving RWC of leaves is demonstrated.

Fresh and dry weight and height of seedlings: Results demonstrated that *Rhizobium* was responsible for maintaining the turgidity of the plant in a much better way than ABA alone (Fig. 1). On the imposition of drought stress ABA not only alleviated the inhibitory effect of drought stress but also significantly increased the fresh weight over the C at TP_2. ABA acts as an inhibitory hormone under unstressed condition, but induce tolerance to drought stress by minimizing water loss. The maximum increase in the fresh weight of seedlings under drought stress was due to *Rhizobium* inoculation; SA, when used in combination with *Rhizobium* further, augmented the fresh weight over the C under drought stress. *Rhizobium* with ABA (D) or *Rhizobium* with ABA and SA (E) showed significant decreases in fresh weight under drought stress at both time points. Fresh weight is associated with water and nutrient uptake. This suggests that R action was suppressed by the ABA and the SA was unable to alleviate this inhibition (Miura & Tada 2014).

Notably, ABA showed maximum inhibition in dry weight at both time points which may be attributed to ABA inhibition of cell division and cell differentiation. Previous studies revealed similar role of ABA (Forni *et al.,* 2006; Aroca *et al*., 2006; Ngumbi & Kloepper, 2016) and *Rhizobium* (Grover *et al*., 2011; Figueiredo *et al*., 2008) on fresh biomass of seedlings which may be attributed to ABA-induced inhibition in the cell division and cell elongation (Takatsuka & Umeda, 2014; Melcher *et al*., 2010). Furthermore, the dry weight was significantly decreased in ABA treatments under stress even at TP_1 (Dhashnamurthi *et al*., 2013; Duan *et al*., 2007). The decrease in dry biomass demonstrates the growth inhibitory role of ABA. But under long term stress for 8d at TP2, ABA assisted the seedlings to withstand stress. The D and E treatments i.e. combined treatment of Rhizobium and Rhizobium, SA and ABA showed dry weight higher than ABA demonstrating the *Rhizobium* ability in the production of biomass, by augmenting cell division (Cohen *et al*., 2009).

The observed higher increase in the plant height in *Rhizobium* (R) or SA (S) treatment could be ascribed to *Rhizobium*-induced phytohormone production (El-Nasharty *et al*., 2019; Subramanium *et al*., 2015; Fahad *et al*., 2015; Nagata & Suzuki, 2014). ABA induced decrease in cell division may result in the observed reduction in plant height (Ferguson & Mathesius, 2014; Melcher *et al*., 2010).

Stomatal conductance and stomatal index: It was observed that water supply resulted in significantly higher stomatal conductance, net-photosynthesis, and transpiration rate (Mafakheri *et al*., 2010; deSouza *et al*., 2005). The ABA alone (A) and with *Rhizobium* (D) increased stomatal conductance at short term drought (TP_1). But, the value did not significantly differ at longer-term (TP_2) compared with Rhizobium treatment. The maintenance of higher RWC (%) of R treatment relative

to ABA having similar stomatal index indicates the efficiency of treatment R at TP_2 for maintaining the water budget of plant under drought stress.

Studies evaluated canopy temperature simulations as a function of soil water status (Webber *et al*., 2015). Canopy temperature is a useful trait used by breeders to select lines tolerant to environmental stresses (Pinto *et al*., 2010; Pinto & Reynolds, 2015). The canopy cooling appears to be associated with deeper roots in dry soils and greater root biomass (Pinto *et al*., 2010; Pinto & Reynolds, 2015). *Rhizobium* decreased the canopy temperature, possibly due to higher stomatal conductance and a hence higher rate of transpiration. The combination of ABA with R was unable to decrease the canopy temperature. This was evidenced by the observed decrease in RWC of the leaves of ABA treatment compared with S > R > B treatments under drought stress. Nevertheless, the combined treatments of R with ABA and SA or R with ABA have resulted in maximum Fv/Fm photosynthetic efficiency compared with other treatments.

Relative water content (RWC): Leaf relative water content (RWC) is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate (Lugojan & Ciulca, 2011). ABA treatment experiencing drought stress exhibited significantly higher RWC at TP_2. ABA has stomatal conductance much higher than the C facilitating the gaseous exchange. A significant increase (70%) in RWC was observed in *Rhizobium pisi* treatment. Exogenous application of SA significantly enhanced the relative water content of the leaves under drought-stressed conditions (Ahmad *et al*., 2017; Verma *et al*., 2017; Hayat *et al*., 2010). The role of rhizobia is pronounced in maintaining water balance in leaves, nutrient balance and hormonal adjustment under drought stress (Naveed *et al*., 2015). The exogenous application of SA significantly increased the RWC under drought stress, hence maintained the turgidity of leaves (Sharma *et al*., 2018; Shan & Wang, 2017). Results depicted that *Rhizobium* was more efficient in reducing the rate of transpiration as compared to ABA (Govindasamy *et al*., 2017; Fahad *et al*., 2017).

As the stomatal conductance at TP_2 under drought stress was reduced the dry weight of ABA treated plants were also reduced and the value was even lower than the C (Dhashnamurthi *et al*., 2013; Duan *et al*., 2007). Different strategies were adapted by Rhizobium which showed a significant increase in stomatal conductance over C at TP_2. However, it also showed higher RWC concomitant with the significant increase in fresh and dry weight at TP_2. Similar pattern of response was exhibited by SA.

Photosynthetic efficiency and chlorophyll content: The photosynthetic efficiency was significantly higher at TP_2 in treatments E > D > B > S demonstrating the synergistic role of Rhizobium with ABA and ABA and SA in augmenting photosynthetic efficiency under long term (TP2) drought stress.

Conclusion

It is inferred that *Rhizobium* inoculation may be more effective than that of ABA. The role of *Rhizobium* to mitigate drought stress supercedes that of SA and ABA but the combined treatment of *Rhizobium*, SA and R was found most efficient at TP_2 to ameliorate the inhibitory effects of drought stress on plant water status and photosynthetic efficiency. *Rhizobium* assisted ABA and SA in the induction of drought tolerance.

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(Received for publication 22 November 2018)