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WATER STRESS TOLERANCE IN FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) INOCULATED WITH *BACILLUS POLYMYXA*, A PHOSPHATE-SOLUBILIZING BACTERIUM

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The effect of *Bacillus polymyxa,* a phosphate solubilizing bacterium (PSB) on phenological and biochemical parameters of *Trigonella foenum-graecum* L. (fenugreek) was studied under water stress. Water stress treatment in fenugreek plants with and without bacterial inoculum was given by withholding water supply for 3 and 5d in pot culture. The regularly irrigated plants of the two groups, i.e. with and without bacterial inoculum were taken as control. Water stress decreased relative water content, amount of chlorophyll, protein content and dry matter of plants. PSB mitigated the effect of water stress on fenugreek. The amount of chl a, chl b, total chlorophyll and protein contents was higher in stressed plants inoculated with PSB as compared with the stressed plants without inoculum. The water status in inoculated plants was always found higher than that of non-inoculated plants in control, stress and recovery treatments. The recovery in stressed plants with bacterial inoculum was faster as compared with that of plants without bacterial inoculum. PSB enhanced photosynthetic rate by increasing amount of photosynthetic pigments in PSB treated plants.

Key words: *Trigonella foenum-graecum, Bacillus polymyxa, phosphate, chlorophyll*, protein.

The environmental factors such as nutrient supply, light, wind, temperature and drought etc. cause water deficits in plants (Allen 1995, Ingram and Bartels 1996). Water stress affects nutrient use characteristics (Papakosta and Gagianas 1991, Latiri-Souki *et al.* 1998, Timsina *et al.* 2001), photosynthesis and other metabolic activities at different levels (Elstner 1982). Over all growth and productivity of plants were adversely affected in both short and prolonged period of water stress (Osmond 1994, Smirnoff 1993, Duan *et al.* 2006, Zgallai *et al.* 2006).

Recent discoveries on the associated growth of plants by particular micro-organism in the rhizosphere of certain plants mainly in the tropics have received the attention of many investigators all over the world. Phosphate solubilizing bacterium (PSB) is one of them which particularly studied for their efficient role in making phosphorus available to plants. Phosphorus is one of the most important plant nutrient required for the plant growth (Chen *et al.* 2006, Martinez and Martinez 2007). It is essential for food synthesis, flower formation, fruit and seed setting. Use of micro-organisms as biofertilizers to promote growth of plants is well documented but its effect related to water stress tolerance is scanty. Present study is an integrated approach of PSB and water stress tolerance in fenugreek (*Trigonella foenum-graecum* L.), which is an important spice, vegetable and medicinal crop.

MATERIALS AND METHODS

The seeds of *Trigonella foenum-graecum* L. (fenugreek) var. PD-1 were procured from the Agricultural Institute Naini, Allahabad and were divided into two sets. One set was inoculated with phosphobacterium (*Bacillus polymyxa*) by immersing the seed in liquid culture medium. Another set was not inoculated at all. Both the sets were sown in November, 2006 in fire clay

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pots (30 cm depth and 30 cm dia) filled with well manured sandy loam soil (15 kg/pot) in replicates, in the Department of Botany, University of Allahabad, Allahabad (24°47´ and 50°47´ N latitude; 81°91´ and 82°21´ E longitude; 78m above sea level). The pots were irrigated as and when required. Each set was further divided in two subsets. One subset of each set was regularly irrigated and the other one was subjected to water stress treatment by withholding the supply of water for 3 and 5d at vegetative stage after 21 DAS and at fruiting stages. The stressed plants were irrigated after 24h. The growth parameters and biochemical constituents were recorded in each treatment.

Relative Water Content

For the measurement of relative water content (RWC) leaf samples were cut into discs, weighed for fresh weight (FW) and were immediately floated on distilled water at 25°C in the dark. After 24 h the turgid weight (TW) of discs was measured and they were dried in oven at 80±C for 48 h before taking dry weight. The RWC was calculated according to Bars and Weatherly (1962).

$$RWC (\%) = (FW-DW)/(TW-DW) \times 100$$

Chlorophyll and Protein assays

Chlorophyll from leaves of experimental plants was extracted with 80% acetone. The amount of chlorophyll was quantified following they method of Arnon (1949). Protein content was determined following the method of Lowry *et al.* (1951). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

Drought index (Dc) was estimated on the basis of yield using the following relationship (Huang and Zhao 2001)

$$Dc = Y_D/Y_N$$

Where YD is the average grain yield under water stress condition and YN is the average grain yield under adequate water supply.

RESULTS

Effect of mild (3d) and prolonged (5d) water stress on RWC, photosynthetic pigments, protein content, fresh and dry weight was recorded in control and stressed fenugreek plants with and without bacterium. There was a significant alteration in biochemical and growth parameters caused by water deficit, which was more pronounced in plants without bacterium (Table 1 and 2).

Relative Water Content

The plants inoculated with PSB plants exhibited highest level of relative water content (RWC) in comparison to non-inoculated plants under control condition. Although water deficit caused a remarkable decrease in RWC in all plants which was more visible in plants with 5d stress where 32% decrease was recorded in vegetative stage and 15% in fruiting stage of non-inoculated plants, while the inoculated plants exhibited only 26 and 12% decrease in RWC at vegetative and fruiting stages respectively as a result of prolonged water stress (5d) treatment. Recovery rate was better in inoculated plants under both mild and prolonged water stress, whereas non-inoculated stressed plants showed good recovery results only when rewatered after mild water stress.

Photosynthetic pigments

Photosynthetic pigments (chlorophyll a and chlorophyll b) were measured in inoculated and non-inoculated plants under the two water stress and recovery treatments at vegetative and fruiting stage. The highest amount of total chlorophyll was recorded in +B plants under control, which was slightly decreased under 3d stress, however a negligible increase was

Table 1. Effect of water stress on PSB inoculated and non-inoculated fenugreek at vegetative stage

Treatment		RWC (%)	Chlorophyll (mg/gFW)				Weight/Plant (g)	
			Chl a	Chl b	Chl a+b	Chl a/b	FW	DW
Control	-B	92.44 ± 1.66	5.06 ± 0.05	3.54 ± 0.05	8.59 ± 0.07	1.29 ± 0.05	2.53 ± 0.44	0.43 ± 0.04
	+B	96.51 ± 0.55	6.04 ± 0.06	3.87 ± 0.10	9.91 ± 0.06	1.56 ± 0.05	3.18 ± 0.21	0.52 ± 0.16
3dS	-B	82.05 ± 2.00	2.77 ± 0.26	1.55 ± 0.07	4.32 ± 0.22	6.93 ± 1.37	2.11 ± 0.10	0.38 ± 0.10
	+B	87.18 ± 2.61	4.77 ± 0.11	1.77 ± 0.22	6.54 ± 0.18	2.73 ± 0.39	3.07 ± 0.64	0.46 ± 0.03
3dSR	-B	90.81 ± 5.06	4.77 ± 0.20	3.71 ± 0.11	8.48 ± 0.27	1.43 ± 0.03	2.64 ± 0.39	0.49 ± 0.06
	+B	95.40 ± 2.44	5.82 ± 0.12	3.24 ± 0.11	9.06 ± 0.12	1.80 ± 0.08	3.21 ± 0.43	0.53 ± 0.04
Control	-B	91.49 ± 1.61	5.92 ± 0.10	3.71 ± 0.20	9.63 ± 0.21	1.60 ± 0.10	2.17 ± 0.14	0.36 ± 0.02
	+B	95.73 ± 2.18	6.76 ± 0.25	3.74 ± 0.05	10.51 ± 0.24	1.81 ± 0.07	3.05 ±0-.19	0.54 ± 0.05
5dS	-B	62.76 ± 3.00	4.76 ± 0.19	2.95 ± 0.17	7.62 ± 0.32	1.58 ± 0.08	1.64 ± 0.33	0.33 ± 0.05
	+B	71.07 ± 2.36	6.88 ± 0.11	3.71 ± 0.20	8.59 ± 0.17	1.86 ± 0.12	2.88 ± 0.85	0.43 ± 0.04
5dSR	-B	86.52 ± 3.33	5.39 ± 0.10	3.50 ± 0.20	8.89 ± 0.27	1.54 ± 0.08	2.37 ± 0.33	0.56 ± 0.02
	+B	88.11 ± 1.88	6.62 ± 0.15	3.60 ± 0.30	9.22 ± 0.23	1.85 ± 0.19	3.32 ± 0.33	0.56 ± 0.02

-B=non-inoculated, +B=PSB inoculated fenugreek; S=stress; SR=stress recovery; FW=fresh weight; DW=dry weight.

Table 2. Effect of water stress on PSB inoculated and non-inoculated fenugreek at fruiting stage

Treatment		RWC (%)	Chlorophyll (mg/gFW)			Weight/Plant (g)		
			Chl a	Chl b	Chl a+b	Chl a/b	FW	DW
Control	-B	91.10 ± 7.95	4.49 ± 0.43	2.39 ± 0.39	6.88 ± 0.82	1.89 ± 0.14	25.05 ± 4.37	3.67 ± 0.50
	+B	93.10 ± 7.71	5.47 ± 0.48	3.32 ± 0.42	8.79 ± 0.89	1.65 ± 0.08	36.07 ± 5.14	4.46 ± 0.49
3dS	-B	81.49 ±10.79	4.81 ± 0.56	2.34 ± 0.42	5.16 ± 0.97	2.07 ± 0.15	20.61 ± 2.35	2.73 ± 0.45
	+B	89.32 ±16.01	5.41 ± 0.44	2.65 ± 0.41	7.06 ± 0.83	2.06 ± 0.18	22.11 ± 2.52	4.15 ± 0.27
3dSR	-B	87.75 ± 8.01	5.64 ± 0.40	3.46 ± 0.50	7.09 ± 0.89	1.64 ± 0.12	19.39 ± 6.50	3.15 ± 0.16
	+B	92.61 ±11.78	6.56 ± 0.43	3.84 ± 0.50	9.40 ± 0.92	1.72 ± 0.12	31.03 ± 3.75	4.26 ± 0.12
Control	-B	92.81 ± 7.01	3.68 ± 0.52	2.60 ± 0.40	6.28 ± 0.91	1.42 ± 0.05	26.13 ± 4.21	5.13 ± 0.14
	+B	95.18 ± 5.48	4.36 ± 0.49	3.12 ± 0.28	7.48 ± 0.77	1.39 ± 0.04	36.70 ± 4.67	5.37 ± 0.38
5dS	-B	79.30 ±10.71	3.36 ± 0.47	1.53 ± 0.43	4.89 ± 0.89	2.26 ± 0.39	17.58 ± 4.98	2.50 ± 0.40
	+B	84.57 ±10.30	4.82 ± 0.47	1.50 ± 0.43	6.32 ± 0.87	1.95 ± 0.16	21.92 ± 5.32	3.18 ± 0.24
5dSR	-B	87.82 ± 9.16	4.53 ± 0.45	1.58 ± 0.40	6.11 ± 0.85	1.76 ± 0.10	19.27 ± 5.07	3.91 ± 0.52
	+B	90.65 ± 8.17	5.43 ± 1.03	2.20 ± 0.31	7.33 ± 1.27	1.60 ± 0.24	30.13 ± 4.54	5.30 ± 0.26

observed under 5d stress at both the growth stages. Non-inoculated plants exhibited decrease in total chlorophyll under both stress treatments. Inoculated plants were recorded better recovery. Chlorophyll a was least affected under water stress in both groups of plants for, i.e. inoculated and non-inoculated.

Protein content

The main effect of water stress was recorded at vegetative stage. Non-inoculated plants exhibited a lesser amount of protein in comparison to inoculated plants in the two growth stages. Under mild water stress treatment the non-inoculated plants showed a gradual decrease in protein content which was not recovered at vegetative stage, while a slight increase was recorded in recovery at fruiting stage. PSB inoculated plants exhibited less decrease under water stress treatments. High recovery rate was observed at both the stages (Figure 1).

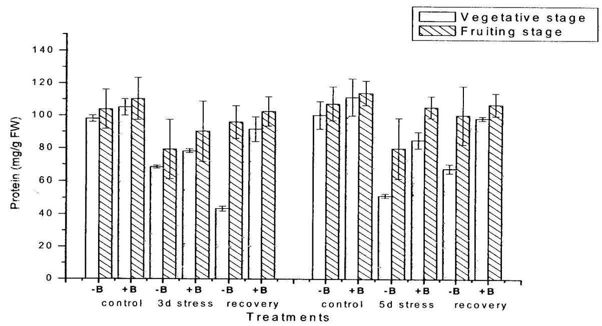

Figure 1. Effect of water stress on protein contents of PSB inoculated and non-inoculated fenugreek.

Fresh and dry weight

The fresh (FW) and dry weight (DW) of plants under control and stress with and without bacterium varied significantly. Though the FW and DW of plants in vegetative stage under 3d stress were decreased, but the recovery was faster in inoculated and non-inoculated plants with maximum recovery rate in +B plants. The similar trends were observed at fruiting stage.

Yield

Total number of pods per plant and total count of seeds per plant was recorded. +B plants recorded higher productivity because the number of pods and seeds were higher in inoculated plants under normal irrigation. Water deficit decreased the yield of the plants which was more pronounced in 5d stress, while inoculated plants under stress were more productive and had more yield than non-inoculated plants under stress (Table 3).

Table 3. Effect of water stress on yield of PSB inoculated and non-inoculated fenugreek

Treatment		Number of Pods/plant	g FW/ Pod	Number of seeds/pod	Number of seeds/plant
Control	-B	7.57	0.54	16.12	122.6
	+B	10.4	0.76	13.38	139.2
3dS	-B	8.2	0.49	13.41	110.1
	+B	9.6	0.69	14.27	137.3
5dS	-B	7.8	0.47	13.07	102.5
	+B	9.5	0.59	13.68	130.4

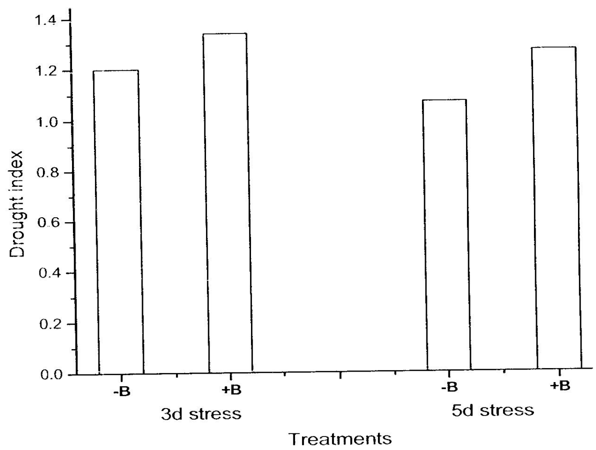

Figure 2. Drought index of PSB inoculated and non-inoculated fenugreek.

Drought index

The drought index (Dc) of stressed plants with and without bacterium was calculated. Dc of stressed bacterial plants was higher as compared to that of stressed plants without bacterial inoculation (Figure 2).

DISCUSSION

More reduction in growth, biochemical parameters and yield was observed in water-sensitive variety under water stress condition as compared with that of water stress tolerant one (Chandrasekhar *et al.* 2000). Jing and Huang (2002) reported that RWC was decreased under water deficit. Our results were also in consonance with this result. The inoculated plants under stress exhibited less decrease in RWC in comparison to non-inoculated plants under stress condition. This may be explained by the fact that phosphorus helps in root elongation. Hence more water absorbing area of the plant is exposed and it helps in absorbing water from deeper soil (Liang and Chen 1996, Zhang and Zhand 2001). This improved the water status in the plant. Dry weight attributed overall growth and development of plants, was decreased under water deficit. Decreased DW by drought has also been reported by Sanchez-Blanco *et al.* (2002). The water stress affected the growth of the plant adversely (Meyer and Boyer 1981, Sanchez-Blanco *et al.* 2002). Bacterium inoculated plants were least affected as evinced by drought index. Drought index was higher for inoculated plant than that of the non-inoculated one which reflected the tolerance of former due to bacterial inoculum. Water stress impaired the metabolic activities and caused inhibition of photosynthetic rate (Duan *et al.* 2006, Zgallai 2006). The photosynthetic pigments were reduced under stress while high availability of phosphorus (P), supplied by PSB in inoculated plant, caused betterment in the amount of pigments. This might be due to the positive role of P which helped inhibition of degradation of pigments under stress or caused betterment in water use efficiency of plant and ultimately improved the amount of pigments. High P caused stomatal opening (Radin 1984) and facilitated plants to accumulate/synthesize more protein in comparison to non-inoculated one. Accumulation of ABA caused by deficiency of P is directly proportional to degree of water stress. This results in stomatal closure and low photosynthetic rate (Singh *et al*. 2006). P fertility influenced water use efficiency, which was mediated through increased photosynthetic efficiency and contributing in osmotic adjustment in stressed plants (Ackerson 1981). Inhibition of photosynthesis and disturbances in metabolic activities due to water deficit resulted in decreased biosynthesis of protein (Malan *et al.* 1990, Bowler *et al.* 1992) or its degradation (Pacifice and Davies 1990). We can conclude that the phosphate solubilizing bacterium helped plants to improve the water status. It mitigated the effects of water stress by increasing the chlorophyll and protein contents of stressed plants as compared with non-bacterial stressed plants.

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