

Effect of drought stress on biochemical parameters in common bean, *Phaseolus vulgaris* L genotypes

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ABSTRACT

Experiment was conducted to assess drought tolerance in two common bean genotypes by inducing stress at flower initiation stage on the basis of inherent stress tolerance characters viz total chlorophyll content, chlorophyll stability index, nitrate reductase activity and total free proline content. Foliar spray of salicylic acid (SA), boric acid (BA) and CoCl_2 was also applied to check whether they enhance drought tolerance by showing their impact on these parameters. Seeds of fifty common bean genotypes were evaluated for drought tolerance in the laboratory with two PEG-6000 osmotic potential levels (-0.3 MPa and -0.5 MPa). Less reduction in germination percentage was observed in KR 60 and KRC 5 genotypes. Pot trials revealed that drought stress at flower initiation stage significantly reduced total chlorophyll content, chlorophyll stability index, nitrate reductase activity whereas total free proline content activity and drought susceptibility index were significantly enhanced. Foliar application of BA, SA and CoCl_2 effectively ameliorated the negative effects of drought stress in both genotypes. Reduction in drought susceptibility index was also observed under these treatments as compared to stressed non-treated plants. SA 150 ppm and BA 0.1 per cent were found to be most effective and surpassed other treatments.

Keywords: Common bean; chlorophyll; nitrate reductase activity; proline; foliar spray; drought tolerance

INTRODUCTION

Common bean, *Phaseolus vulgaris* L (Fabaceae) is an important food legume cropped for human consumption worldwide. Due to current climate change scenario about 60 per cent of common bean production in developing countries occurs under conditions of erratic drought stress (Zhang et al 2006). Drought stress reduces plant growth by affecting various physiological and biochemical processes such as photosynthesis, respiration, translocation, ion uptake, carbohydrate and nutrient metabolism (Farooq et al 2012). Therefore it becomes very important to understand the harmful impact of drought stress on plants and take measures to counteract these effects. Jayakumar et al (2013) evaluated that photosynthetic pigments viz chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents had beneficial value at 50 mg/kg cobalt level in the soil as compared to control. Sharaf et al (2009) found that application of boric acid

increased the contents of chlorophyll 'a', 'b' and total chlorophyll. Farouk and Osman (2011) reported that salicylic acid activated the synthesis of carotenoids which protect chlorophyll from oxidation and finally increase chlorophyll content in common bean.

MATERIAL and METHODS

Preliminary studies were conducted in the laboratory conditions for screening of seeds of fifty common bean genotypes procured from Mountain Agricultural Research and Extension Centre, Sangla, Kinnaur, HP. Seeds were surface-sterilized with mercuric chloride (0.1%) and treated with two levels of osmotic potential (-0.3 and -0.5 MPa) by polyethylene glycol-6000 and germinated in 12 cm diameter glass Petri dish at $25 \pm 2^\circ\text{C}$ in seed germinator. Observations on germination percentage were recorded when control showed 100 per cent germination (data not given). Two most well performing common

bean genotypes viz KR 60 and KRC 5 were selected for further study in 30 cm diameter pots in completely randomized block design (CRD) with four replications. The pots were prepared by mixing soil, vermicompost and sand in the ratio of 3:2:1 along with addition of N, P and K in the ratio of 20:60:0 as per recommended practice. Since the soil was rich in potassium therefore potassium was omitted. Foliar spray of salicylic acid, boric acid and cobalt chloride was given to genotypes KR-60 and KRC-5 when water was withheld ie ten days prior to flower initiation stage so that stress could be induced at this particular stage. Basis of selecting doses of chemicals used for spray was collected from literature and best performing doses were selected. Samples were taken for analysis after 30 days of stress imposition. After slight wilting symptoms 300 ml of water was given at fixed interval; control/unstressed plants were watered regularly in the net house that was covered from the top and hand weeding was done regularly. The treatments used were control, stress, stress + boric acid (BA) (0.01%), stress + BA (0.1%), stress + salicylic acid (SA) (150 ppm), stress + SA (300 ppm), stress + CoCl_2 (10 ppm) and stress + CoCl_2 (15 ppm). Chlorophyll content was calculated by the method given by Hiscox and Israelstam (1979), chlorophyll stability index (%) by Murthy and Majumdar (1962), nitrate reductase activity by Nicholus and Nason (1957), total free proline content by Bates et al (1973) and drought susceptibility index by Fischer and Maurer (1978). After inducing stress by withholding water, moisture depletion was measured by gravimetric method given by Hansen et al (1962) after 15 and 30 days after stress imposition and at temporary wilting stage in both the genotypes.

RESULTS and DISCUSSION

The results of present study revealed that water stress at flower initiation stage decreased the total chlorophyll content in the leaves of common bean (Table 1). Highest photosynthetic pigment content was recorded under control whereas salicylic acid and boric acid treatments also enhanced total chlorophyll under stress significantly. Water stress reduced chlorophyll 'a', chlorophyll 'b' and total chlorophyll content but SA activated the synthesis of carotenoids which protect chlorophyll from oxidation and finally increase chlorophyll content in common bean (Farouk and Osman 2011).

Chlorophyll stability index (CSI) is an indicator of stress tolerance capacity of plants and reflects the

performance of chloroplast under stress (Koleyoreas 1958). CSI is used to measure the integrity of the membrane (Murthy and Majumdar 1962). CSI was enhanced by SA and BA in both genotypes. Highest CSI was recorded in control plants while maximum reduction in CSI was present in unsprayed stressed plants. Tolerant and moderately tolerant cultivars and hybrids showed a lesser reduction (6 and 12%) in CSI at 50 per cent available soil moisture than control (Surendar et al 2013). Differential response of genotypes towards different treatments was observed. In KR 60 and KRC 5 minimum reduction in CSI was recorded under SA (150 ppm) (Table 1).

There were significant differences for nitrate reductase activity among the genotypes under various moisture regimes (Bahadur et al 2006). Foliar application of SA, BA and CoCl_2 enhanced the nitrate reductase activity in both common bean genotypes (KR 60 and KRC 5) (Table 2). Minimum reduction (3.07%) was observed under treatment SA (150 ppm) in KRC 5 genotype and KR 60 minimum reduction (11.64%). Akhtar et al (2013) reported that nitrate reductase activity (NRA) was reduced by stress in bean cultivars however foliar application of SA was effective in improving the NR activity.

Water stress at flower initiation stage significantly increased proline content in leaves of common bean plants as compared to unstressed plants (Table 2). More accumulation of proline in beans under drought was an adaptation for tolerating dryness which in turn helped the plant to survive and reproduce under drought conditions (Zadehbagheri et al 2012). Interaction between water stress and foliar treatments revealed that maximum accumulation of proline was present in unsprayed stressed plants. SA (150 ppm) treatment showed maximum increase in KR 60 (50.33%) and in KRC 5 (24.71%) in stressed plants over control. The exogenous application of SA creates effective mechanism that protects the plant from injuring effects and induces more drought tolerance in the plant. Amin et al (2009) showed that salicylic acid application accumulated more proline in the leaves. Farjam et al (2014) reported that salicylic acid (SA) enhanced drought tolerance in plants by a significant increase in proline concentration.

Soil moisture content was significantly reduced under the drought stress in both genotypes. At 30 days after stress imposition CoCl_2 (10 ppm) showed minimum reduction in KR 60 (8.06%) and KRC 5

Table 1. Effect of foliar spray on total chlorophyll content and chlorophyll stability index in two *Phaseolus vulgaris* L genotypes after 30 days of stress imposition

Treatment	Total chlorophyll content ($\mu\text{g/g FW}$)				Chlorophyll stability index (%) FW			
	KR 60	RP (%)	KRC 5	RP (%)	KR 60	RP (%)	KRC 5	RP (%)
Control	11.75	-	11.98	-	47.03	-	57.58	-
Stress	9.8	16.59	10.59	11.60	40.02	14.90	53.43	7.20
Stress + BA (0.01%)	9.89	15.84	10.68	10.85	42.40	9.83	53.40	7.24
Stress + BA (0.1%)	10.9	7.24	11.28	5.84	43.73	7.01	54.93	4.59
Stress + SA (150 ppm)	11.02	6.22	11.35	5.25	44.10	6.21	55.43	3.73
Stress + SA (300 ppm)	10.05	14.48	10.98	8.34	42.25	10.14	53.69	6.74
Stress + CoCl_2 (10 ppm)	9.82	16.44	10.71	10.60	42.30	10.04	53.75	6.64
Stress + CoCl_2 (15 ppm)	9.9	15.76	10.62	11.35	40.70	13.44	53.93	6.33
CD	0.39		0.14		0.73		0.60	

RP= Reduction percentage

Table 2. Effect of foliar spray on nitrate reductase activity and total free proline content in two *Phaseolus vulgaris* L genotypes after 30 days of stress imposition

Treatment	Nitrate reductase activity ($\mu\text{M NO}_3$ – reduced/g fresh weight/h)				Total free proline content ($\mu\text{g/g DW}$)			
	KR 60	RP (%)	KRC 5	RP (%)	KR 60	PI (%)	KRC 5	PI (%)
Control	3.95	-	2.93	-	176.04	-	178.33	-
Stress	2.85	27.84	2.41	17.74	268.68	52.62	218.58	22.57
Stress + BA (0.01%)	2.97	24.81	2.63	10.28	226.25	28.53	203.00	13.83
Stress + BA (0.1%)	3.38	14.43	2.69	8.19	217.75	23.70	209.00	17.20
Stress + SA (150 ppm)	3.49	11.64	2.84	3.07	264.62	50.33	222.40	24.71
Stress + SA (300 ppm)	3.05	22.78	2.63	10.23	229.52	30.39	203.30	14.00
Stress + CoCl_2 (10 ppm)	3.08	21.02	2.68	8.53	234.90	33.44	202.40	13.50
Stress + CoCl_2 (15 ppm)	2.98	24.55	2.64	9.89	220.35	25.18	201.60	13.05
CD	0.17		0.04		1.7		1.3	

RP= Reduction percentage, PI= Per cent increase

(10.71%) in stressed plants over control. At wilting stage SA (300 ppm) treatment displayed minimum reduction (10.25%) in soil moisture content in KRC 5 common bean genotype under stress over control. CoCl_2 (10 ppm) treatment also showed the lesser reduction in KRC 5 (11.49%) and KR 60 (13.89%) genotypes in stressed plants over control. In KR 60 and KRC 5 all treatments were significant with control at 45 and 60 DAS and wilting stage. Both genotypes showed minimum reduction in moisture content at SA (300 ppm) and CoCl_2 (10 ppm) after 15 and 30 days after stress imposition and temporary wilting stage and

were found to be effective in maintaining soil moisture content.

BA (0.1%) and SA (150 ppm) were found to be very effective among all the foliar treatments in lowering drought susceptibility index (DSI) in both the genotypes (Fig 1). Line diagram depicts that KRC 5 seems to be tolerant whereas KR 60 moderately tolerant genotype. The study showed that foliar application of boric acid, salicylic acid and cobalt chloride can counteract the deleterious effects of drought stress on common bean genotypes and are

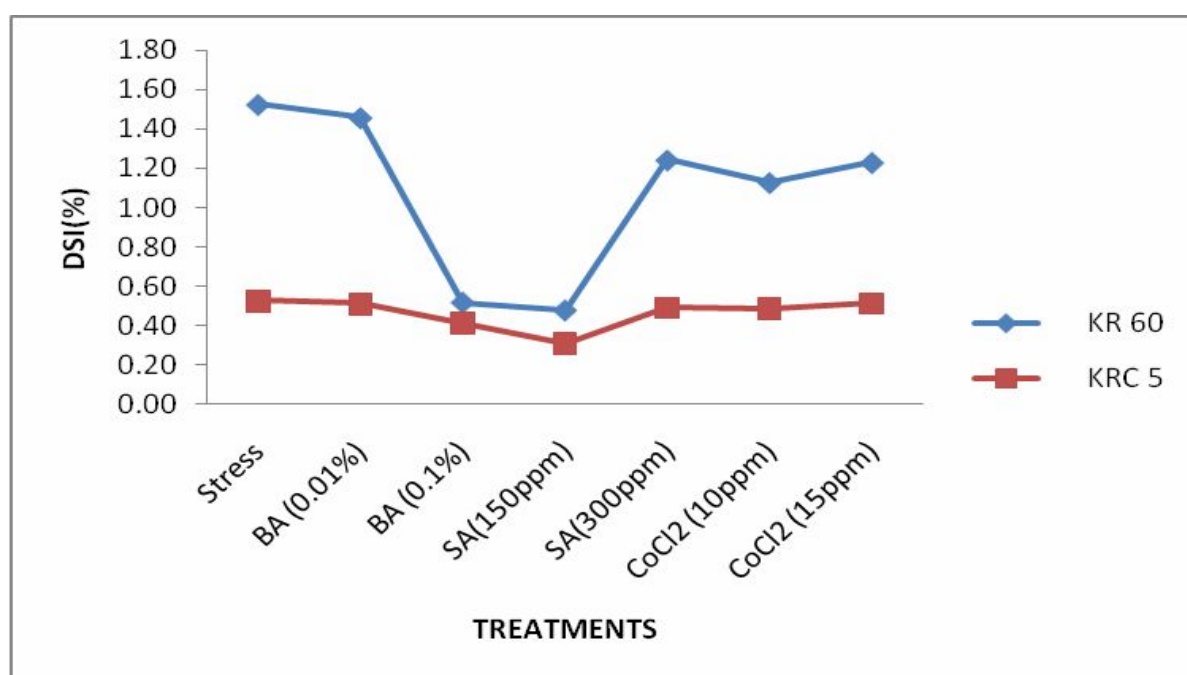


Fig 1. Effect of SA, BA and CoCl_2 on drought susceptibility index (%) in two common bean genotypes under stress at flower initiation stage

effective in maintaining water balance however boric acid and salicylic acid were found to be more effective treatments in alleviating drought stress. Overall study revealed that the presence of these treatments can ameliorate the stress injury and KRC 5 performed better as compared to KR 60 genotype for foliar treatments.

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