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Assessment of drought-induced modulation of Mung bean at early stage of development

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Abstract

In spite of the various biotic and abiotic stresses in the crop field, water deficit condition is a serious challenging situation for crops across the globe which adversely affects the development, growth and production yield of the plants. In the present study, one week old seedling of mung bean (*Vigna radiata* L.) kept in pot culture was subjected to drought stress. The test plants were then sampled on the 2nd, 4th, 6th and 8th day along with the control plants. Various physiological and biochemical parameters were analysed on the test plants along with the well-watered control treatment. Relative water content is seen to decrease during the period of treatment to drought stress. Chlorophyll contents in the leaves of mung bean was seen to decrease in the stressed plants when compared to the control. On the other hand, the proline contents showed an increase during the drought stress in both the leaves and the roots of the test plant. But the sugar contents showed a slightly different trend during the treatment days, where the sugar contents decreased on the 4th day of drought stress but again there was a slight increase on the 6^{th} and 8^{th} day of the treatments.

Key words : Drought stress, mung bean, chlorophyll, proline.

Pulses are very important when it comes to meeting the protein requirement of human food habit. Mung bean is a legume belonging to Fabaceae family that currently is cultivated in different regions, for its edible seeds and sprouts and have large role in nutrition at developing countries[8]. Drought means a situation that is created in the field crops due to a period without significant rainfall. Drought leads to a stressed condition in the crop plants which brings about various changes like physiological and biochemical impairment, pigment status along with phenophages of the plants[6]. Mung bean response to drought stress resulting in membrane damage, protein contents but increased proline content[2,13]. Drought is considered to be an extensive climatic event which most of the time limits growth of mung bean plants. Water stress-induced changes in morphological, physiological and pigment composition alters biomass yield of higher plants[5,12,16].

Yield status of mung bean is highly dependent on a surplus supply of water, thus it's a crucial factor leads to high yield of the plants[9]. It is also observed that the plant is affected morphologically as well as biochemically and physiologically by drought condition[11]. Water supply is required at vegetative growth stages for pod development. Water stress that persists at the reproductive stage have immense effects on grain yield of the crop plants[18]. Present investigation was carried out to analyse the effect of water deficit condition on mung bean seedlings.

Plant material: Mung bean (*Vigna radiata* L.)

Collection, growth and maintenance:

Fully viable mung bean seeds were surface sterilized with 0.1% (w/v) $HgCl_2$, where the seeds were first washed with $HgCl_2$ for 2-3 minutes and then washed properly using distilled water. Then the thoroughly washed seeds were transferred into autoclaved Petri plates in aseptic condition. Fully viable seeds are then allowed to germinate under optimum conditions and transferred to pots containing soil mixed with manure. Plants were watered regularly twice daily-morning and evening till the time of treatment. The healthy seedlings are used as bioassay material for experimental purposes.

Induction of water stress :

One week old plants of mung bean was introduced to drought stress by completely withholding water in the pots of the test plants, and then the sampling of the plants was performed on the 2nd, 4th, 6th and 8th day of drought stress along with the sampling of the well-watered control plants.

Determination of Leaf Relative water content (LRWC) :

Fresh leaf disks were immersed in10 ml of distilled water for 24 hours at room temperature. After 24 hours they were acted out from the water and adhering surface water was soaked using blotting paper and then turgid weight was determined. After taking the weight, drying process was done at 80 °C for 24 hours and then the dry weight was taken. The relative water content of leaf was calculated using the following formula[4].

$$LRWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Where: FW= Fresh weight; DW= Dry weight; TW= Turgid weight.

Extraction and estimation of chlorophyll :

Extraction of chlorophyll was done following the method of Harborne[7] where 80% acetone was added to 1 g of leaf tissues. After proper filtration, chlorophyll estimation was done following the methods and formula by Arnon[1].

Extraction and estimation of Total Sugar:

Extraction of sugar contents was done following the method of Harborne[7]. Sugar Extraction from leaf tissues (0.5 g) was done using 10 ml of 95% ethanol and alcohol vaporization was completed using boiling water bath. The aqua's fraction was redissolved in distilled water and the volume made upto 5 ml followed by centrifuge at 5000 rpm for 10 minutes. The supernatant was collected and volume was made upto 5ml with distilled water.

Estimation of soluble sugar contents was done using anthrone reagent and following the method adopted by Plummer[15]. 4 ml anthrone reagent (0.2% anthrone in concentrated H_2SO_4) was taken and mix with 1ml of test solution. After proper mixing followed by incubation in boiling water for 10 minutes and after that cooled under running tap water and OD values were taken using UV-VIS spectrophotometer at wave length of 620 nm. Quantitative estimation was done using a standard curve of glucose.

Table-1. Influence of water stress on percentage of relative water contents (mg/g fresh weight) of mung bean seedlings

Percentage (%) of relative water contents after days							
2nd day		4th day		6th day		8th day	
Control	Stress	Control	Stress	Control	Stress	Control	Stress
90.24	88.57	87.40	85.22	81.69	78.41	80.36	73.50
LSD 0.86 (P=0.05)		1.13		1.97		4.66	

Table-2. Influence of water stress on chlorophyll contents (mg/g fresh weight) of mung bean leaves.

Chlorophyll (Chl.) contents of stress-induced plants								
	2nd day		4th day		6th day		8th day	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Total Chl.	0.42	0.40	0.45	0.30	O.47	0.17	0.49	0.10
LSD (P=0.05)	NS		0.09		0.16		0.22	
Chl. A	0.23	0.20	0.25	0.18	0.24	0.07	0.21	0.06
LSD (P=0.05)	NS		NS		0.13		0.12	
Chl. B	0.21	0.18	0.22	0.17	0.24	0.07	0.21	0.04
LSD (P=0.05)	NS		NS		0.15		0.16	

NS=Not significant

Table-3. Influence of water stress on sugar contents (shoot & root) of mung bean plants

Sugar contents (mg/g fresh weight) after water stressed plants								
	2nd day		4th day		6th day		8th day	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Shoot	24.31	15.86	42.18	32.60	38.68	28.10	41.85	11.66
LSD (P=0.05)	1.54		3.10		2.77		1.10	
Root	5.56	4.08	9.40	7.80	9.21	6.63	6.20	4.36
LSD (P=0.05)	0.21		0.35		0.58		0.41	

Table-4. Influence of water stress on proline contents (shoot & root) of mung bean plants

Proline contents (mg/g fresh weight) after water stressed plants								
	2nd day		4th day		6th day		8th day	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Shoot	18.12	25.06	19.03	30.50	20.25	30.43	19.00	26.28
LSD (P=0.05)	1.35		1.88		1.94		1.63	
Root	5.46	7.09	10.11	14.06	11.87	14.47	12.50	15.90
LSD (P=0.05)	0.52		0.94		1.09		1.11	

Proline contents extraction and estimation from leaves and roots :

From leaves and roots, proline content was extracted and estimated following the method of Bates *et al.*[3]. Sulfosalicylic acid (3%) was used in homogenising of 0.5 g of plant tissue and volume made upto 10ml. After proper filtration, the supernatant was taken for estimation.

Quantitative estimation was done from the standard curve of proline. 1 ml ninhydrin solution was added to the 1 ml plant extract followed by addition of 3 ml distilled water. The ninhydrin solution was prepared by taking 1 g ninhydrin powder to 10 ml acetone followed by adding of 15 ml distilled water. After 30 minutes of boiling, 5 ml toluene was added the reaction mixture after proper cooling and transferred to separating funnel. The OD values at 520 nm was taken from the lower coloured layer of the separating funnel.

Statistical analysis of the data was done in terms of least significant difference

PHOTOGRAPHS

Fig. 1. 6 days old mung bean seedlings (Control)

Fig. 2. 6 days old mung bean seedlings (Drought-induced)

Fig. 3. 6 days old mung bean seedlings Control (left) and Drought-induced (right)

(LSD) which was calculated at 95% confidence limits and as per the method of Panse and Sukhatme[14].

Results shows that the relative water content in the drought-induced mung bean seedlings (fig. 2) is gradually decreased with the increase of the treatment days (table-1). In case of chlorophyll contents, it is found to be lesser than the control treatments (fig. 1) upto 8 days of the seedlings (table-2). Sugar contents of the stressed plants also significantly decreased over the control mung bean plants (table-3). But the proline contents were gradually increased in the drought-induced plants (table 4) which is an important marker of drought stress in both the leaves and the roots of the treated plants when compared to the control plants (fig. 3).

Drought-induced morphological, physiological and biochemical changes was found in many varieties of mung bean seeds and also shows drought sensitivity at various stages of development[10]. Drought stress restricted plant growth and development which led to a significant reduction in the yield of legume crops[19]. Drought stress affects mung bean at various phenophages leads to yield impairment. Enhanced drought situation may prevail in the coming decades, thus climate-smart mung bean cultivars is required to overcome the drought conditions in terms of yield attributes[17]. This investigation was also carried out to evaluate the biochemical and physiological expression of mung bean seedlings with respect to drought stress. The experimental results clearly revealed that, there are crucial effects of drought in the mung bean seedlings of certain parameters leads to physiological, biochemical along with the morphological changes of the plant.

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