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An investigation on the impact of water stress on black gram seedlings

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Abstract
An investigation was carried out to evaluate the effect of drought stress on black gram with special reference to plant growth and metabolism. Relative water contents along with some biochemical indices associated with drought stress in terms of proline contents, sugar contents, chlorophylls were analyzed. Results showed that chlorophyll contents significantly decreased after 10th day of water stress of the black gram seedlings. On the other hand, the proline content showed an increase trend after the drought stress induction in both the shoot and roots of the test seedlings.

Keywords: water stress, plant growth, proline, black gram

Introduction

Black gram is one of the most nutritious pulses in tropical countries especially in India. The green pods are eaten as vegetables and seeds are the cheapest source of dietary protein for human beings. Optimum water supply is a primary reason for enhancing the crop production along with the physiological processes in plants which directly alters morphological features, growth and metabolism of plants. Otherwise, retardation of growth at the vegetative stages followed by subsequent flowering and fruiting stages is found in plants. Chaves (1991) [1] observed that water deficit led to decreased in photosynthetic carbon assimilation in plants. Increased water deficit condition also has several negative impacts on plant photochemical and biochemical processes leads to various adaptative mechanisms [2]. Several genes are actively engaged to overcome various abiotic stresses such as drought, salinity and extreme temperature etc. [3]. The drought-induced black gram plants showed retarded growth on both vegetative and reproductive stages [4]. It was also found that the electrolyte leakage and MDA content were increased at 60% and 20% of severe PEG treatments on black gram [5]. In the present investigation, water stress condition was imposed to black gram seedlings and some physiological and biochemical parameters were assessed to evaluate the effect of plant growth and metabolism of the plant.

Material and Methods

Plant material: Black gram (*Vigna mungo* L.)

Collection, Growth and Maintenance: Black gram seeds were collected from local market of Darjeeling, West Bengal, India. The seeds were surface sterilized with 0.1% (w/v) $HgCl_2$, where the seeds were first washed with $HgCl_2$ for 3-4 minutes and then washed thrice with sterile distilled water each time keeping the water for 3 minutes. 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 farmyard manure in the proportion of 2:1 ratio. 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 black gram was subjected 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 3rd, 5th, 7th and 10th day of drought stress along with the sampling of the well-watered control plants.

Determination of Leaf Relative water content (LRWC): Fresh leaf disk was immersed in10ml of distilled water for 24 hours at room temperature. After 24 hours they were taken out from the water and excess water of the surface was removed using blotting paper and then was weighed to determine turgid weight. After taking the weight, the leaf disks were dried at 80 °C for 24 hours and then the dry weight was taken. Leaf relative water content was calculated using the following formula [6]:

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

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

Extraction and Estimation of Chlorophyll: Chlorophyll was extracted according to the method of Harborne (1973) by homogenising 1g of leaf tissue in 80% acetone was repeatedly added from the top till the residue became completely colourless. The filtrate was collected and the total volume was measured.

Estimation of chlorophyll was done by measuring the OD values at 663nm and 645nm respectively in a UV visible spectrophotometer against a blank of 80% acetone and calculated using formula as given by Arnon (1949).

Total chlorophyll= $(20.2A_{645}+8.02A_{663})$ mg g^{-1}fresh weight
Chlorophyll a= $(12.7A_{663}-A_{645})$ mg g^{-1}fresh weight
Chlorophyll b = $(22.9A_{645}-4.68A_{663})$ mg g^{-1}fresh weight

Extraction and Estimation of Total Sugar: Total sugar was extracted by the method given Harborne (1973). 0.5 g of each of leaf tissue was extracted in 10 ml of 95% ethanol and alcoholic fraction was vaporized on boiling on water

bath. The aqua's fraction was redissolved in distilled water and volume made upto 5 ml which was then centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and volume was made upto 5ml with distilled water.

Estimation of total soluble sugar was done by Anthrone reagent method given by Plummer (2004). In 1ml of test solution added with 4ml Anthrone reagent (0.2% Anthrone in concentrated H_2SO_4) the reaction mixture was mix thoroughly and incubated in boiling water for 10 minutes then cooled under running tap water and observed in UV visible spectrophotometer at wave length of 620 nm against proper blank and quantified by using a standard curve of glucose.

Extraction and Estimation of proline: Extraction of proline from the leaves and root was done by the method of Bates *et al*., (1973). 0.5g of plant tissue was homogenized in 10mL of 3% sulfosalicylic acid and filtered through a Whatman No. 1 filter paper. The supernatant was collected for estimation.

To 1ml of extract, 3ml of distilled water and 1ml of ninhydrin solution (1g ninhydrin + 10ml acetone + 15ml distilled water) was added. The reaction mixture was kept on a boiling water bath for 30 minutes and then cooled at room temperature. The reaction mixture was transferred in separating funnel and 5 ml of toluene was added and mixed vigorously. The lower coloured layer was taken and the OD values were measured at 520nm in UV visible spectrophotometer against a blank and quantified from a standard curve of proline.

Results and Tables

Table 1: Influence of water stress on percentage of relative water contents (mg/g fresh weight) of black gram seedlings.

Percentage (%) of relative water contents after days							
3rd day		5th day		7th day		10th day	
Control	Stress	Control	Stress	Control	Stress	Control	Stress
87.80	79.92	89.95	76.20	90.00	72.46	95.10	70.30

Table 2: Influence of water stress on seedling height index of black gram.

Percentage (%) of seedling height index after days				
	3rd day	5th day	7th day	10th day
Shoot	86.53	75.14	72.81	68.92
Root	73.66	71.35	70.11	65.04

Table 3: Influence of water stress on chlorophyll contents (mg/g fresh weight) of black gram leaves.

Chlorophyll (Chl.) contents of stress-induced plants								
	3rd day		5th day		7th day		10th day	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Total Chl.	0.42	0.41	0.45	0.33	O.47	0.17	0.49	0.10
Chl. A	0.23	0.26	0.25	0.18	0.24	0.07	0.21	0.06
Chl. B	0.21	0.25	0.22	0.16	0.24	0.07	0.21	0.04

Table 4: Influence of water stress on sugar contents (shoot & root) of black gram plants.

Sugar contents (mg/g fresh weight) after water stressed plants								
	3rd day		5th day		7th day		10th 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
Root	5.26	4.08	9.40	7.80	9.21	6.63	5.20	4.36

Table 5: Influence of water stress on proline contents (shoot & root) of black gram plants.

Proline contents (mg/g fresh weight) after water stressed plants								
	3rd day		5th day		7th day		10th 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
Root	6.46	7.09	10.11	14.06	11.87	14.47	12.50	14.90

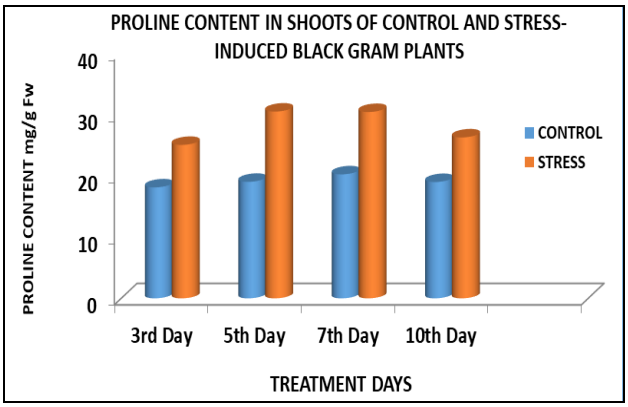

Fig 1

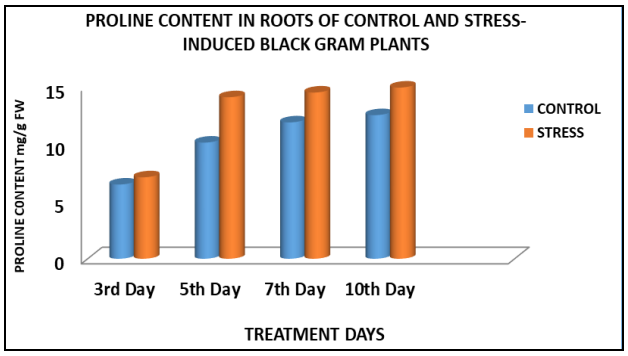

Fig 2

Conclusion

One week old black gram seedlings were subjected to drought stress by withholding water for 3rd, 5th, 7th and 10th day after which biochemical assays were performed on the treated as well as the control plants. The height index of both shoots and root showed gradual decrease along with the increase in the number of stress induction days. Control plants showed a height index of 86.53% and 73.66% of shoot and root respectively whereas the plants of 10 days under stress showed a height index of 68.92% and 65.04% of shoot and root respectively. Relative water content was seen to decrease gradually during the treatment days when compared with the control. Chlorophyll contents were seen to decrease during drought stress when compared to control. The reduced chlorophyll content under drought conditions results in poor light harvesting by plants. Increasing energy absorption by photosynthetic apparatus may trigger production of reactive oxygen species. To avoid this situation, plants may degrade absorbing pigments [11]. Similarly, total sugar contents in both root and shoot of the seedling was seen to decrease in the treated plants when compared to the control. Similar results were seen in studies performed by Bangar *et al.*, 2019 [12]. But unlike the other biochemical assays the proline content in both the shoots as well as in the roots showed an increase during the drought stress period than compared to the control plants of the same day. Significant increase in proline content was seen on the

5th, 7th and 10th day of stress. Proline accumulation during water stress also acts as a compatible solute thus regulating water loss from plant cell [13]. It also helps in supplying energy for survival and growth of plants [14]. Overall results showed that drought stress on one week old seedlings of black gram does show oxidative damage to some extent on the seedlings leading to decrease in most of the biochemical parameters

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