IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Effect Of Salt Stress On Biochemical And Yield Parameters Of Black Gram Under Foliar Application Of Ascorbic Acid

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ABSTRACT

To investigate the impact of ascorbic acid on water-soluble carbohydrates, protein content, leghaemoglobin levels, grain weight per pod, and the pods per plant under varying saline circumstances (100 mM and 150 mM NaCl), the black gram genotypes PU-19 and UH 80-9 were evaluated. Ascorbic acid (AsA) at concentrations of 0.5 mM, 1.0 mM, 1.5 mM, and 2 mM was applied externally to the genotypes in a controlled trial. Eleven treatments, each replicated three times, were used in this complete randomized design. Under salt stress, the amount of protein, leghaemoglobin, water-soluble carbohydrates, grain weight per pod, and number of pods on each plant all reduced; however, foliar ascorbic acid administration increased these levels. Ascorbic acid treatment resulted in a mean water-soluble carbohydrate content of 29.51 mg/g DW for UH 80-9 and 23.48 mg/g DW for PU-19. Leghaemoglobin values for UH 80-9 and PU-19 were 1.68 µg/ml and 1.35 µg/ml, respectively, while protein content was measured at 12.18 mg/g FW and 8.93 mg/g FW. In addition, UH 80-9 and PU-19 had average grain weights per pod of 3.54 g and 2.67 g, respectively, and mean number of pods per plant was 7.76 and 8.71, respectively. It was found that the optimal ascorbic acid concentration in saline conditions was 2.0 mM in combination with 100 mM NaCl. This resulted in improved yield and biochemical characteristics. The outcomes demonstrated that topical application of AsA effectively mitigated the harmful impact of salinity, particularly in black gram genotypes that are more susceptible to salt stress.

Keyword: *Vigna mungo,* Black gram, salt stress, Ascorbic acid,

Introduction:

Worldwide, millions of hectares of land are too saline to support an economic crop yield, and more land is rendered unproductive every year due to salt buildup. Although some salts are required for plant nourishment and are found naturally in soil, too much salt in the soil or water can damage plants and disturb ecosystems. Salt stress is now recognized as a significant obstacle to the production of food worldwide (Chourasia *et al*., 2021). Research has shown that salinity adversely affects the soil's physical and chemical characteristics, development, physiological traits, and plants' absorption of ions and water (Vimal *et al*., 2019; Prittesh *et al*., 2020). As a result, crop yield is decreased. Elevated levels of both ions sodium and chlorine in plants' cells are caused by salinity, disrupting plant processes and leading to substantial physiological imbalances (Jha *et al*.,2019). Salinity impairs the integrity of biological membranes and results in oxidative damage, which reduces agricultural productivity (Klein *et al*., 2018). Often referred to as urad, black gram is a diploid (n = 2x = 22) annual summer pulse crop that self-pollinates and has a short season (80–95 days). India is the country of origin and the main supplier to that nation for both production and consumption. The primary factor driving its production are its dry beans. Reddy *et al*. (2022) list it as one of the most important legume crops, growing quickly and adapting to a variety of agroclimatic conditions to thrive in all three seasons. Stressors both biotic and abiotic harm black gram yield, which has essentially stopped growing for the last 20 years (Pandey and Chakraborty, 2015).

Salinity rises the amount of reactive species of oxygen and results in oxidative stress in plant cells. Plants go through many morphological and biochemical processes to adapt to salinity stress. Reactive species of oxygen must be detoxified through antioxidants in order to avoid becoming dangerous radicals. Antioxidant enzymes either process free radicals (ROS) or catalyze related events to lessen their harmful effects (Noctor *et al*., 2018). The sharp rise in the overall number of soluble sugars is ascribed to the rising high temperatures, which is consistent with findings from other research that suggested dryness (Naresh *et al.,* 2013). Furthermore, it was discovered that chickpea under salt stress accumulated soluble carbohydrates (Gurdev, 2015). The The entire amount of soluble sugar in *Triticum aestivum* foliage exhibited an increase in conditions of soil impacted by salinity. This increase was further enhanced through foliar and priming applications of ascorbic acid (AsA). (El-Hawary *et al*.,2023). Salt stress resulted in a lower protein content in black gram, a species that is susceptible to salt (Altuntas *et a*l., 2020). According to Ahmad *et al.* (2016), decreased protein levels may be caused by high salt concentrations that cause an osmotic imbalance. Ascorbic acid (AsA) applied exogenously reportedly improves potatoe's protein content (Sajid *et al*., 2012) and chickpeas (Beltagi *et al*., 2008). Leghemoglobin is a molecule found in the root nodules of leguminous crops, that is essential to the nitrogen fixation process that occurs in these plants. Salinity impacted nodule initiation in mungbean, reducing their number, weight, and nitrogen-fixing efficiency, leading to an apparent decrease in leghaemoglobin concentration as nodules aged due to irreversible oxidation (Balasubramanian *et al*., 1974). Shahid *et al*. (2014) stated that saltinity decreased pea plant yield parameters such pod length and quantity of pods; comparable yield losses were noted in Vigna species (Sehrawat *et al*., 2015). Due to its important functions in stress tolerance, Ascorbic acid has a favorable effect on yield. Under salt stress

circumstances, Ascorbic acid is well recognized to lessen oxidative damage and boost plant growth and yield, as previously addressed by (Hafez *et al.,* 2016), (El-Hawary, *et al.,* 2019). The total number of grains per plant decreases when it experiences salinity stress; however, ascorbic acid treatment greatly increases seed production in these circumstances (Nagda *et al.,*2017).

2. Materials and Method

2.1 Plant material and study location

Vigna mungo cultivars UH 80-9 and PU-19 were obtained from CCS Haryana Agricultural University, Hisar (IND). During the Kharif seasons of 2021–22 and 2022–23, the seeds were cultivated in a pot house with a complete randomized design, three replications, and watering (maintain pots in field conditions).

2.3 Treatments

During the investigation, eleven salinity conditions with the application of ascorbic acid were examined: control (no ascorbic acid or NaCl), 100 mM NaCl (T1), 100 mM NaCl with 0.5 mM ascorbic acid (T2), 100 mM NaCl with 1.0 mM ascorbic acid (T3) and 100 mM NaCl with 1.5 mM ascorbic acid (T4), 100 mM NaCl with 2.0 mM ascorbic acid (T5), [no ascorbic acid or ascorbic acid (T6)], 150 mM NaCl (T7), 150 mM NaCl with 0.5 mM ascorbic acid (T8), 150 mM NaCl with 1.0 mM ascorbic acid (T9), 150 mM NaCl with 1.5 mM ascorbic acid (T10), and 150 mM NaCl with 2.0 mM ascorbic acid (T11). After the seeds were sown, the soil was treated with 150 mM of NaCl solution to initiate the NaCl treatment. Ascorbic acid was applied on the leaves ten days after sowing (DAS), or when new leaves began to appear.

2.4 Biochemical data measurement

Water-soluble carbohydrates: Using the methodology of Yemm and Willis (1954), water-soluble carbohydrates were determined. After finely powdering the plant tissue, 80% ethanol was used to extract it. The extract was then treated with anthrone reagent, which interacts with the carbohydrates in the extract to give a green to blue-green color. Spectrophotometrically measured at 620 nm by a glucose standard curve and reported as mg/g of dry weight (DW).

Protein content: The Bradford test, which Bradford first reported in 1976, was utilized to ascertain the protein concentration. Using this technique, plant tissue extracts are infused with a Brilliant Blue Coomassie dye, which links to the proteins and alters color. Using a spectrophotometer, the absorbance of the resultant blue complex was determined at 595 nm. By comparing the absorbance results to a standard curve created using known amounts of bovine serum albumin (BSA), protein concentrations were found.

Leghaemoglobin (LHb) content: The Hartree (1955) approach was utilized to ascertain the concentration of leuhemoglobin (LHb). To extract the hemoglobin, fresh root nodules were homogenized and centrifuged. After that, the supernatant was combined with pyridine and a reducing agent to create a stable complex, which was then detected at 556 nm using spectrophotometry. Through a comparison of the absorbance with a reference curve, the content of hemoglobin was determined.

Grain weights per Pod: Ten Mature pods were chosen and the grains were carefully separated, in order to calculate the grain weight per pod. After that, a precision balance was used to weigh the grains from each pod. By dividing the total grain weight by pod's number, the mean grain weight per pod was determined.

Number of pods per Plant: Ten mature plants were selected, and each number of pods per plant was calculated manually. This allowed us to calculate the number of pods per plant. Each plant's total number of pods was recorded and the mean number of pods per plant was determined by averaging the numbers from ten plants.

3. Result and Discussion:

3.1 Biochemical and yield parameters: Water-soluble carbohydrates, protein content, and leghemoglobin were among the biochemical and yield characteristics of black gram plants that were examined under normal conditions, salt stress, and salinity with ascorbic acid. The impact of different plant treatments on these attributes is summarized here, with a table presenting further details.

3.1.1 Water-soluble carbohydrates: For the genotypes UH-80-9 and PU-19, the mean levels of water-soluble carbohydrates were 38.79 µg/g DW and 43.23 µg/g DW, accordingly. The effect of salinity on water soluble carbohydrates (measured in µg/g DW) for each genotype is listed in Table 1. The value increased by 11.53% to 35.99 mg/g DW at 100 mM NaCl and by 26.43% to 40.8 mg/g DW at 150 mM NaCl. The particular increases were 13.51% at T2, 16.67% at T3, 20.02% at T4, 26.43% at T5, 40.38% at T7, 41.28% at T8, 44.62% at T9, and 49.58% at T10.Treatment with ascorbic acid, however, significantly reduced these declines. With varying ascorbic acid (AsA) treatments, both water-soluble carbohydrates increased when subjected to salt stress with 100 mM and 150 mM NaCl. Under salt stress, chickpeas were found to acquire soluble carbohydrates (Gurdev, 2015). In soil that was impacted by salt, the total soluble sugar content raised in *Triticum aestivum* foliage. Ascorbic acid (AA) treatments applied topically and as a priming agent further enhanced this rise. (El-Hawary and others, 2023)

3.1.2 Protein content (mg/g FW): Table 1 displays the impact of salt stress on the protein content (mg/g FW) of each genotype. Under control conditions, genotypes UH-80-9 and PU-19 had protein contents of 13.50±0.211 mg/g DW and 9.90±0.131 mg/g FW, respectively.The control group's mean protein level was 11.70 mg/g FW for the black gram genotype. The resulting concentration decreased to 10.41 mg/g FW (a reduction of 11.03%) at 100 mM NaCl and to 9.37 mg/g FW (a reduction of 19.91%) at 150 mM NaCl. The specific declines mentioned below were observed: The following were recorded: 9.23% at T2, 7.01% at T3, 5.38% at T4, 3.05% at T5, 16.41% at T7, 14.02% at T8, 8.38% at T9, and 4.06% at T10. Black gram, a species that is sensitive to salt, had a lower protein content because of salinity (Altuntas *et al.*, 2020). Foliar administration of ascorbic acid had shown to increase protein levels of chickpeas (Beltagi *et al.*, 2008) and potatoes (Sajid *et al.*, 2012).

Table 1: Impact of ascorbic acid on Water Soluble Carbohydrate (mg/g DW) and Protein content (mg/g FW) of black gram genotypes under salinity stress condition.

Treatment	Water Soluble Carbohydrate (mg/g DW)			Protein content (mg/g FW)		
	UH-80-9	PU-19	Mean(G)	UH-80-9	PU-19	Mean(G)
T0	32.39±0.341	32.15±0.571	32.27	13.50±0.211	9.90±0.131	11.70
T1	34.21±3.342	37.77±0.312	35.99	11.93±0.155	8.89±0.000	10.41
T2	35.09±3.408	38.17±0.437	36.63	12.13±0.107	9.11±0.151	10.62
T3	36.17±3.141	39.13±0.550	37.65	12.44±0.182	9.32±0.175	10.88
T4	37.22±2.900	40.24±0.125	38.73	12.67±0.303	9.47±0.167	11.07
T5	39.50±3.162	42.11±0.568	40.80	13.10±0.223	9.82±0.179	11.46
T6	40.05±6.726	46.31±0.482	43.18	11.24±0.286	7.50±0.057	9.37
T7	41.77±7.309	48.83±0.255	45.30	11.39±0.137	8.17±0.140	9.78
T8	42.04±6.851	49.13±0.333	45.59	11.69±0.262	8.43±0.187	10.06
T9	42.86±7.066	50.49±0.815	46.67	12.27±0.021	9.17±0.119	10.72
T10	45.37±6.907	51.17±1.145	48.27	12.82±0.259	9.63±0.080	11.225
Mean	38.79	43.23		12.18	8.93	
	C.D.	SE(m)		C.D.	SE(m)	
Genotype (G)	0.384	0.134		0.154	0.054	
Treatments (T)	0.901	0.315		0.361	0.126	
Intreaction (G×T)	1.274	0.445		0.100	0.178	

3.1.3 Leg HB(µg/ml):

Table 2 displays the reduction in Leg HB (µg/ml) for both genotypes under salt stress. In the control group, the mean Leg HB for the black gram genotype was 1.99 µg/ml. This finding decreased to 1.35 µg/ml (a 32.16% reduction) at 100 mM NaCl and to 1.03 µg/ml (a 48.24% reduction) at 150 mM NaCl. Among the notable decreases were the following: The percentages are as follows: 33.67% at T2, 23.12% at T3, 15.08% at T4, -8.04% at T5, 40.70% at T8, 26.13% at T9, and 7.04% at T10. However, ascorbic acid administration significantly reversed these declines. Leghaemoglobin concentration significantly decreased as nodules matured due to irreversible oxidation as a result of salinity's impact on nodule initiation in mungbean, which also reduced the nodules' weight, number and leghaemoglobin concentration (Balasubramanian *et al.*, 1974).

Table 2: Impact of ascorbic acid on Leg HB(µg/ml) of Black gram genotypes under salinity stress condition.

Treatment	Leg HB(µg/ml)		
	UH-80-9	**PU-19**	**Mean(G)**
T0	2.37±0.057	1.61±0.000	1.99
T1	1.36±0.012	1.34±0.026	1.35
T2	1.42±0.009	1.21±0.021	1.32
T3	1.72±0.036	1.33±0.033	1.53
T4	1.96±0.009	1.42±0.000	1.69
T5	2.60±0.030	1.70±0.026	2.15
T6	0.98±0.006	1.08±0.000	1.03
T7	1.02±0.015	1.19±0.021	1.11
T8	1.13±0.021	1.22±0.021	1.18
T9	1.65±0.012	1.29±0.006	1.47
T10	2.19±0.057	1.50±0.030	1.85
Mean(T)	1.68	1.35	
	C.D.	**SE(m)**	
Genotype (G)	0.022	0.008	
Treatments (T)	0.052	0.018	
Intreaction (G×T)	0.073	0.026	

3.1.4 Grain weight per pod:

The decline in grain per pod for both genotypes under salt stress can be observed in Table 3.

Under the control condition, the grain per pod values for genotypes PU-19 and UH-80-9 were 6.12±0.036 and 6.12±0.036, respectively. Both genotypes showed a reduction in grain per pod under 100 mM and 150 mM NaCl conditions when treated with AsA. At 100 mM NaCl and 150 mM NaCl, the mean grain per pod decreased to 3.78 and 2.66, indicating declines of 37.21% and 55.81%, respectively. 30.56% at T2, 20.76% at T3, 16.45% at T4, 5.48% at T5, 50.00% at T7, 40.86% at T8, 26.58% at T9, and 6.64% at T10 were the reductions. In many studies, such as those on cowpea by Manaf (2016) and Mini *et al.* (2019) stated that under salinity, yield, and yield-related traits significantly decrease. According to Shahid *et al.* (2014), salt stress lowered the length and number of pods produced by pea plants; similar yield losses were observed in Vigna species (Sehrawat *et al*., 2015). AsA had a favorable effect on the entire number of seeds on each plant, but induced salinity lessened the overall number of seeds on each plant (Nagda *et al*., 2017).

Table 3: Impact of ascorbic acid on Grain Weight/Pod(g) and No. of Pods /plant of Black gram genotypes under salinity stress conditions.

Treatment	Grain Weight/Pod(g)			No. of Pods /plant		
	UH-80-9	PU-19	Mean(T)	UH-80-9	PU-19	Mean(G)
T0	0.57±0.003	0.68±0.015	0.62	11.75±0.191	12.55±0.151	12.15
T1	0.21±0.006	0.23±0.003	0.22	6.32±0.134	7.36±.107	6.84
T2	0.28±0.006	0.26±0.007	0.27	7.95±0.182	7.79±0.172	7.87
T3	0.32±0.002	0.31±0.003	0.32	8.19±0.110	9.18±0.104	8.69
T4	0.38±0.007	0.38±0.001	0.38	8.89±0.172	9.65±0.137	9.27
T5	0.42±0.009	0.49±0.004	0.46	10.15±0.259	11.73±0.128	10.94
T6	0.17±0.003	0.18±0.006	0.17	4.78±0.113	5.67±0.137	5.23
T7	0.20±0.005	0.24±0.003	0.22	5.11±0.074	6.10±0.089	5.61
T8	0.24±0.003	0.28±0.001	0.26	5.49±0.026	6.78±0.021	6.14
T9	0.27±0.002	0.30±0.006	0.28	7.17±0.151	8.11±0.006	7.64
T10	0.30±0.004	0.30±0.003	0.30	9.56±0.098	10.87±0.232	10.22
Mean	3.54	2.67		7.76	8.71	
	C.D.	SE(m)		C.D.	SE(m)	
Genotype (G)	0.005	0.002		0.285	0.106	
Treatments (T)	0.011	0.004		0.102	0.043	
Interaction (G×T)	0.016	0.004		0.404	0.141	

3.1.5 Number of pods per plant: Table 3 reveals how both genotypes' pod counts dropped during salt stress. Under the control setting, the pod number per plant for genotypes PU-19 and UH-80-9 was 12.55±0.151 and 11.75±0.191, respectively. Under 100 mM and 150 mM NaCl conditions, treatments with AsA at concentrations showed a decreasing trend in the number of pods per plant for both genotypes. This decreased to 6.84 at 100 mM NaCl and even lower to 5.24 at 150 mM NaCl, according to the data, signifying a 43.70% and 56.95% decline, respectively. The following results, however, show how much the ascorbic acid treatment reversed this decline: 35.23% at T2, 28.48% at T3, and 23.70% at T4. T5: 9.96%, T7: 53.83%, T8: 49.47%, T9: 37.12%, and T10: 15.88%. On the number of pods counts per plant NaCl has unfavorable effects and AsA has favorable ones. The total number of pods per plant decreased substantially at various phases of growth (Nagda *et al*.,2017)

4.0 Conclusion:

According to recent studies, black gram under salt stress has improved biochemical yield characteristics when exposed to ascorbic acid at a threshold value of 2.0 mM. In order to precisely understand the mechanisms involved with ascorbic acid at the cellular and molecular levels in the alleviation of salinity effects on the one hand and environmental stresses on the other, work with ascorbic acid under salinity and other environmental stresses may be expanded under controlled conditions, in addition to its function as a signaling molecule.

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