

**ORIGINAL ARTICLE****Effect of Foliar Application of Ascorbic Acid on Morphological Characteristics of Black Gram (*Vigna Mungo* L.) Under Saline Environment**Preety<sup>1</sup>, Deepti Narwal<sup>1</sup>, Anju Devi<sup>1</sup>, and Kirpa Ram<sup>1\*</sup><sup>1</sup>Department of Botany, Baba Mastnath University, Rohtak-124021

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**ABSTRACT**

Black gram is very significant worldwide due to its high nutritional value in the human diet but it is also acknowledged for being sensitive to salt stress and stress improved by the foliar application of ascorbic acid. Within this framework, the current investigation set out to assess the effects of foliar-applied ascorbic acid (0.5mM, 1.0mM, 1.5mM, and 2 mM) on parameters related to growth in two genotypes of *Vigna mungo* (PU-19 and UH 80-9) at different NaCl induced salinity levels (100 and 150 mM) during the early growth stage. Induced sodium chloride dominate salinity hampers the plant height and root length, number of root nodule, no. of flower per plant, fresh and dry weight of leaves, root and nodule whereas enhanced using ascorbic acid. The combination of 100 mM NaCl and 2.0 mM ascorbic acid was shown to be the ideal level of ascorbic acid, leading to improved growth properties in saline circumstances. Mean plant height with application of ascorbic acid was 43.96cm and 31.63cm, root length was 14.81 and 14.76 cm, number of root nodule 29.05 and 24.24, number of flowers 10.70 and 9.42 was observed in the genotype UH 80-9 and PU-19 respectively, whereas leaf fresh and dry weight was 7.78g and 2.34g (UH 80-9) and 5.64g and 2.31g (PU-19), root fresh and dry weight was 11.43g and 2.64g (UH 80-9) and 5.23g and 1.94g (PU-19) whereas root nodule fresh and dry weight was 5.38g and 3.95g (UH 80-9) and 4.46g and 3.40g (PU-19). By conducting this study, valuable insights can be gained into the relationship between every morphological traits in the PU-19 and UH 80-9 cultivars of black gram. The findings will contribute to our understanding of the growth dynamics and potential yield of these genotypes, providing valuable information for future breeding and cultivation practices.

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

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**INTRODUCTION**

One of the main pulse crops, black gram (*Vigna mungo* L.), is grown on 4.02 million hectares and produces 547 kg ha<sup>-1</sup> on average in Andhra Pradesh, Maharashtra, Madhya Pradesh, Tamil Nadu, and Uttar Pradesh [1]. Stress negatively affects the development and growth of a variety of staple food crops, which lowers productivity [2]. Crop morpho-physio-biochemical characteristics are negatively impacted by these non-biological variables, which cause yield losses of about 50% [3]. Salt stress has a deleterious impact on the morphology and metabolic processes. Salinity hinders agricultural plant germination, growth, development of the photosynthetic apparatus, stomatal conductance, morphology, metabolic processes and yield [4]. High concentrations of salinity in the growth medium have a negative impact on several aspects of the plant, including biomass, mineral ion accumulation, PSII reactions [5], and biochemical damage from reactive oxygen species (ROS) production, which ultimately results in poor plant growth and metabolic damage [6]. As a low molecular weight antioxidant, ascorbic acid helps plants grow and become more resilient to stress by scavenging reactive oxygen species [7]. In general, salinity prevents cell division, lipid metabolism, photosynthesis, nucleic acid synthesis, seed germination, vegetative growth, and lipid metabolism [8]. Naeem *et al.* [9] found that tomato plants exposed to salt stress had considerably lower heights than plants not exposed to this stress. In saline environments, plant height,

root length, and dry weight all drastically decrease [10]. Elevated NaCl levels lead to a decrease in yield, biomass, and leaf area, according to Zorb *et al.* [11]. Salinity stress significantly reduced plant height, leaf number, shoot and root weight, and overall biomass, according to Mukhtar *et al.* [12]. Ascorbic acid can accelerate plant growth and increase its resistance to stress, and proteins containing hydroxyproline need to be produced [13]. The negative effects of salt stress are lessened by the exogenous application of ascorbic acid, which increases the endogenous production of ascorbic acid within plant cells [14].

## MATERIAL AND METHODS

### Plant material and study location

Two cultivars PU-19 and UH 80-9 of black gram (*Vigna mungo* L.) were obtained from CCS Haryana Agricultural University, Hisar (IND). The seeds were grown in a pot house with a complete randomized design, three replications, and watered (maintained pots in field conditions) during the Kharif seasons of 2021–22 and 2021–22.

### Treatments

Eleven salinity condition with application of ascorbic acid *viz.*, control [no NaCl or ascorbic acid (T0)], 100 mM NaCl (T1), 100mM NaCl with 0.5 mM ascorbic acid (T2), 100mM NaCl with 1.0 mM ascorbic acid (T3) and 100mM NaCl with 1.5 mM ascorbic acid (T4), 100mM NaCl with 2.0 mM ascorbic acid (T5), [no NaCl or ascorbic acid (T6)], 150 mM NaCl (T7), 150mM NaCl with 0.5 mM ascorbic acid (T8), 150mM NaCl with 1.0 mM ascorbic acid (T9) and 150mM NaCl with 1.5 mM ascorbic acid (T10) and 150mM NaCl with 2.0 mM ascorbic acid (T11), were examined during the investigation. Following seed sowing, 150 mM of NaCl solution was added to the soil as a single shock to initiate the NaCl treatment. Ten days after sowing (DAS), or when new leaves started to appear, ascorbic acid was sprayed on the leaves.

### Morphological data measurement

Each potted plant was carefully taken out. Using a meter scale, the plant height (in centimetres) was measured from the base to the top of the main stem. Then split into shoots and roots to measure root length. Morphological characteristics, such as plant height and root length were measured after harvest. The biomass of the shoots, roots and nodule was measured after they were cleaned with distilled water and gently dried on a paper towel. Once the material had been dried for 72 hours at 70°C, the dry weight (DW) was determined. Five randomly chosen plants from each treatment had their flowers counted, and the average number of flowers per plant was calculated.

## RESULT AND DISCUSSION

**Growth:** Under normal conditions, salinity stress and salinity with ascorbic acid, the phenotypic features of black gram plants, such as root length, plant height, total fresh weight, and total dry weight, were measured. The following is a summary of how various plant treatments affect these characteristics, with table providing further information.

**Root length (cm):** Roots are typically the initial plant components impacted by elevated salt concentration in the rhizosphere. The results of the genotypes that were examined for the reduction of root length during salt stress were shown in Table 1. When administered at doses of 100 mM and 150 mM of NaCl, ascorbic acid treatment lengthens the roots. The control environment showed the maximum root length, while the 150mM NaCl application showed the minimum, which is improved by the ascorbic acid applications of 0.5mM (14.23%), 1.0 mM (31.18%), 1.5mM (45.53%) and 2.0mM (72.24%) application in genotype UH-80-9 whereas 22.54, 35.60, 48.58 and 55.12 % respectively for genotype PU-19. These results are consistent with those of other researchers, like Li *et al.* [29], who found that when salinity levels increased, common bean seed root growth decreased.

### Plant Height (cm):

The plant height of black gram was considerably lowered by salinity stress. Table 1 displayed the results for the investigated genotype reduction in plant height under conditions of salt stress. Ascorbic acid administration increases plant height when applied under concentrations of 100 mM and 150 mM of NaCl. Maximum plant height was observed in control environment and minimum was observed in the 150mM NaCl application which is improved by the 0.5mM (19.50%), 1.0 mM (32.89%), 1.5mM (51.34%) and 2.0mM (72.04%) application in genotype UH-80-9 whereas 12.77, 19.23, 31.43 and 51.29 % respectively for genotype PU-19 of ascorbic acid.

**Table 1: Influence of ascorbic acid on root length and plant height of black gram genotypes under salinity stress condition.**

Treatment	Root Length (cm)		Plant height (cm)	
	UH-80-9	PU-19	UH-80-9	PU-19
T0	21.22±0.401	18.90±0.484	55.67±0.633	40.85±0.324
T1	14.86±0.324	12.86±0.140	39.86±0.767	27.75±0.244
T2	15.92±0.149	13.99±0.080	41.33±0.797	30.58±0.286
T3	16.87±0.359	15.31±0.110	43.44±0.746	33.45±0.592
T4	17.44±0.083	16.22±0.229	46.21±0.505	35.45±0.369
T5	22.00±0.182	17.34±0.359	55.90±0.755	38.54±0.702
T6	10.96±0.250	10.25±0.095	27.97±0.395	22.46±0.131
T7	12.52±0.047	12.56±0.033	33.41±0.122	25.33±0.196
T8	14.45±0.345	13.90±0.291	37.17±0.776	26.78±0.432
T9	15.92±0.191	15.23±0.119	42.33±0.330	29.52±0.016
T10	18.89±0.099	15.90±0.312	48.12±0.729	33.98±0.797
<b>Factors</b>	<b>C.D.</b>	<b>SE(m)</b>	<b>C.D.</b>	<b>SE(m)</b>
<b>Genotype (G)</b>	0.214	0.075	0.469	0.164
<b>Treatments (T)</b>	0.505	0.177	1.101	0.385
<b>Interaction (G×T)</b>	0.010	0.250	1.557	0.544

When tomato plants are subjected to salt stress as compared to when they are not. Naeem *et al.* [9] found a statistically significant reduction in plant height. By encouraging cell proliferation and expansion, ascorbic acid application has been shown to increase the height of sweet pepper plants [15]. According to Nagda *et al.* [16], this growth-promoting effect is caused by enhanced antioxidant activity in plant cells, which promotes cell division and expansion. Gomaa *et al.* [17] also supported these results by showing that soybean plants cultivated in saline circumstances grew noticeably taller at increasing ascorbic acid concentrations, ranging from 500 to 1500 ppm. They also underlined how ascorbic acid increases plants' ability to withstand abiotic stressors, which in turn promotes vegetative development.

**Number of Root nodule:**

Table 2 presented the findings of the study on the black gram genotype reduction in number of root nodule under salt stress.

At a certain salinity threshold, nodule formation is entirely inhibited. The control environment had the highest number of root nodules, while the 150mM NaCl application had the lowest number. For the genotype UH-80-9, the improvements were 0.5mM (36.01%), 1.0mM (42.31%), 1.5mM (48.78%), and 2.0mM (74.89%), while for the genotype PU-19, they were 24.28, 41.33, 45.98%, and 58.79%, respectively in comparison to 150Mm NaCl.

The findings clearly demonstrate that Ascorbic acid has a positive impact on the number of root nodules per plant. Several experiments have demonstrated that salinity leads to a reduction in the quantity, size, and weight of nodules in plants such as chickpea, cowpea, mung bean, and soybean [9, 18] .

**Table 2: Impact of ascorbic acid on Number of root nodule and number of flowers per plant of black gram genotypes under salinity stress condition.**

Treatment	Number of Root nodule		No. of flower per plant	
	UH-80-9	PU-19	UH-80-9	PU-19
T0	37.75±0.642	32.29±0.729	16.12±0.009	13.56±0.920
T1	26.43±0.520	21.96±0.149	8.12±0.182	7.16±0.175
T2	26.88±0.491	23.90±0.172	9.71±0.131	7.88±0.042
T3	23.61±0.553	25.56±0.104	11.03±0.036	11.03±0.271
T4	30.96±0.661	27.70±0.172	11.99±0.125	11.23±0.005
T5	37.94±0.452	30.37±0.253	15.13±0.348	15.16±0.244
T6	18.88±0.401	15.46±0.057	6.12±0.030	5.13±0.098
T7	25.68±0.255	19.20±0.330	7.10±0.089	6.13±0.036
T8	26.87±0.559	21.85±0.467	7.98±0.158	8.12±0.140
T9	28.09±0.042	22.57±0.291	10.68±0.167	9.55±0.203
T10	33.02±0.119	24.55±0.458	13.12±0.199	10.58±0.244
<b>Factors</b>	<b>C.D.</b>	<b>SE(m)</b>	<b>C.D.</b>	<b>SE(m)</b>
<b>Genotype (G)</b>	0.355	0.124	0.142	0.050
<b>Treatments (T)</b>	0.833	0.291	0.332	0.116
<b>Interaction (G×T)</b>	1.179	0.412	0.470	0.164

**No. of flower per plant:**

The data clearly demonstrate that salt stress has a significant effect on the number of flowers during the entire sampling process (Table 2). The 150 mM NaCl application indicated the lowest no. of flowers per plant, while the genotype UH-80-9 showed the maximum number at 0.5 mM (16.01%), 1.0 mM (30.39%), 1.5 mM (74.50%), and 2.0 mM (114.37%) of ascorbic acid. The values were 9.53, 12.72, 38.43, and 73.12 % for the genotype PU-19, respectively. It is clear from the result that salt stress reduced the number of flowers per plant, but foliar application of ascorbic acid mitigates the negative impact of salinity. Previous study has also cleared that the number of flowers on the salt-stressed plants increased when ascorbic acid was applied externally [16]. According to studies, levels of Ascorbic acid are essential for controlling processes like flowering and senescence through intricately coordinated regulation of gene expression mediated by phytohormones [19]. The detrimental impacts of environmental stress on plants can be minimized by increasing intrinsic levels of Ascorbic acid through genetic engineering or exogenous application [20, 21, 12].

**Table 3: Impact of ascorbic acid on FW& DW of leaf of black gram genotypes under salinity stress condition.**

Treatment	FW of leaf (g)		DW of leaf (g)	
	UH-80-9	PU-19	UH-80-9	PU-19
T0	10.88±0.092	7.78±0.015	4.43±0.059	3.82±0.000
T1	6.64±0.050	5.12±0.128	1.61±0.015	1.84±0.000
T2	7.79±0.012	5.45±0.021	1.71±0.026	2.19±0.000
T3	8.23±0.110	6.15±0.113	2.13±0.036	2.76±0.000
T4	8.47±0.042	6.56±0.151	2.67±0.012	2.89±0.000
T5	10.65±0.050	7.78±0.026	4.10±0.059	3.6±0.001
T6	5.12±0.113	4.19±0.033	1.21±0.026	1.17±0.000
T7	5.49±0.078	4.33±0.057	1.32±0.026	1.28±0.000
T8	6.10±0.026	4.78±0.078	1.48±0.036	1.67±0.000
T9	7.95±0.137	4.98±0.074	1.96±0.036	1.89±0.000
T10	8.69±0.033	5.23±0.033	2.92±0.015	2.33±0.000
	<b>C.D.</b>	<b>SE(m)</b>	<b>C.D.</b>	<b>SE(m)</b>
<b>Genotype (G)</b>	0.068	0.024	0.120	0.008
<b>Treatments (T)</b>	0.160	0.056	0.050	0.018
<b>Interaction (G×T)</b>	0.226	0.079	0.071	0.025

**Fresh and dry weight of leaf:**

There was a noticeable variation in the black gram genotype growth under varying salt treatment conditions. Table 3 displayed the results of the genotypes evaluated for the reduction of mean FW and DW of leaves under conditions of salt stress. Under 100 mM and 150 mM concentrations of NaCl, ascorbic acid administration improves the FW and DW of the leaf. The 150 mM NaCl application produced the lowest FW, for the genotype UH-80-9, the improvements were 0.5mM (7.23%), 1.0mM (19.14%), 1.5mM (55.27%), and 2.0mM (69.72%), while for the genotype PU-19, they were 3.34, 14.08, 18.85%, and 24.76%, respectively in comparison to 150Mm NaCl. The 150 mM NaCl application showed the lowest DW of leaf, while the genotype UH-80-9 showed the highest concentration at 0.5 mM (9.09%), 1.0 mM (22.31%), 1.5 mM (61.98%), and 2.0 mM (141.32%) of ascorbic acid. For the genotype PU-19, the values were 9.40, 42.73, 61.53, and 99.14%, respectively. In this study, the growth characteristics of *Vigna mungo* were negatively impacted by salt stress. The current study found that giving black gram plants exogenous Ascorbic acid supplementation significantly increased fresh and dry weight of the plant. Bybordi, [22] states that an increase in leaf area, fresh and dry weight confirms the positive growth effects of ascorbic acid and increases seed yield.

**Table 4: Impact of ascorbic acid on FW and DW of root of black gram genotypes under salinity stress condition.**

Treatment	FW of root(g)		DW of root(g)	
	UH-80-9	PU-19	UH-80-9	PU-19
T0	11.10±0.008	9.68±0.250	4.23±0.054	3.12±0.021
T1	6.01±0.137	3.13±0.057	1.67±0.036	1.38±0.003
T2	7.28±0.062	4.12±0.050	1.99±0.026	1.58±0.006
T3	10.76±0.250	4.89±0.047	2.76±0.012	2.16±0.036
T4	16.75±0.297	6.24±0.074	3.01±0.062	2.34±0.021
T5	22.55±0.036	7.28±0.131	4.86±0.116	2.98±0.042
T6	3.12±0.018	1.95±0.155	1.15±0.000	1.01±0.021
T7	5.67±0.045	2.89±0.068	1.23±0.026	1.22±0.015
T8	7.28±0.083	3.56±0.089	1.99±0.038	1.65±0.006
T9	8.29±0.068	4.12±0.089	2.14±0.015	1.79±0.006
T10	11.85±0.030	5.33±0.040	3.76±0.062	2.12±0.042
<b>Factors</b>	<b>C.D.</b>	<b>SE(m)</b>	<b>C.D.</b>	<b>SE(m)</b>
<b>Genotype (G)</b>	0.105	0.037	0.034	0.012
<b>Treatments (T)</b>	0.247	0.086	0.081	0.028
<b>Interaction (G×T)</b>	0.350	0.122	0.114	0.040

**Fresh and dry weight of Root:**

Salinity treatment caused a drop in black gram biomass production (Table 4.). When the *Vigna mungo* genotype were stressed with NaCl, the fresh weight of the root decreased by 71.89% and 79.85%, in UH-80-9 and PU-19 respectively at 150mM NaCl concentration that was ameliorated by the application of ascorbic acid at 0.5 mM (81.73%), 1.0 mM (133.33%), 1.5 mM (165.70%), and 2.0 mM (280.44%) for genotype UH-80-9, and 48.20, 82.56, 112.28, and 173.33%, respectively, for genotype PU-19.

Similarly dry weight of root was reduced by 73.52% and 67.62%, in UH-80-9 and PU-19 respectively at 150mM NaCl concentration which is improved by ascorbic acid by 0.5mM (6.95%), 1.0 mM (73.04%), 1.5mM (86.08%) and 2.0mM (226.95%) application in genotype UH-80-9 whereas 20.79, 63.37, 77.23 and 109.90 % respectively for genotype PU-19.

Velmani *et al.* [23] found that when salt concentration increases, the presence of NaCl reduces root fresh and dry weight relative to the control. This conclusion is supported by Shakeel and Mansoor [24] and Wang *et al.* [14], who observed a significant salinity-related retardation of root dry weight. Application of ascorbic acid significantly increased seedling length, fresh weight (FW) and dry weight (DW) especially under NaCl stress [20].

**Table 5: Impact of ascorbic acid on FW and DW of nodule of black gram genotypes under salinity stress condition.**

Treatment	FW of nodule(g)		DW of nodule(g)	
	UH-80-9	PU-19	UH-80-9	PU-19
T0	8.00±0.071	7.12±0.083	5.69±0.074	4.89±0.057
T1	4.14±0.071	3.78±0.054	3.35±0.057	2.69±0.015
T2	4.98±0.059	4.01±0.021	3.69±0.042	2.99±0.015
T3	5.48±0.015	4.89±0.047	4.27±0.071	3.59±0.021
T4	6.09±0.062	5.31±0.110	4.78±0.078	3.76±0.036
T5	7.72±0.140	6.47±0.140	5.17±0.086	4.66±0.047
T6	3.19±0.062	3.03±0.047	2.39±0.009	1.86±0.047
T7	3.78±0.030	3.24±0.042	2.64±0.015	2.09±0.036
T8	4.11±0.078	3.53±0.033	2.82±0.062	2.21±0.047
T9	5.29±0.083	4.52±0.062	3.89±0.033	2.39±0.030
T10	6.50±0.078	5.84±0.018	5.09±0.068	3.23±0.026
<b>Factors</b>	<b>C.D.</b>	<b>SE(m)</b>	<b>C.D.</b>	<b>SE(m)</b>
<b>Genotype (G)</b>	0.062	0.022	0.043	0.015
<b>Treatments (T)</b>	0.146	0.051	0.100	0.035
<b>Interaction (G×T)</b>	0.206	0.072	0.142	0.050

**Fresh and dry weight of Nodule:**

There was a noticeable drop in nodule FW and DW during salt stress (Table 5). Plants with 150 mM NaCl had the lowest FW of nodule which was increased by the application of ascorbic acid at 0.5 mM (18.49%),

1.0 mM (28.89%), 1.5 mM (65.83%), and 2.0 mM (106.72%) for genotype UH-80-9, and 20.79, 63.37, 77.23, and 109.90%, respectively, for genotype PU-19. Similarly dry weight of nodule was reduced at 150mM NaCl concentration which is improved by ascorbic acid by 0.5mM (10.46%), 1.0 mM (17.99%), 1.5mM (65.52%) and 2.0mM (112.97%) application in genotype UH-80-9 whereas 12.36, 18.81, 28.49 and 73.65 % respectively for genotype PU-19. Fresh and dry weight was increased with Ascorbic acid treatment. Higher fresh and dry weight was observed in 2mM Ascorbic acid treated plants. Salinity has been shown to promote a reduction in both fresh and dry weight [25]. Hasan *et al.* [26] observed similar declines in black gram and mung beans. Farooq *et al.* [27] observed similar outcomes in cowpea. Under salinity stress, El-Tohamy *et al.* [28] discovered the same outcomes when Ascorbic acid was sprayed foliarly on *Solanum melongena* L. Following AA treatments, Brassica plant biomass production increased as well.

## CONCLUSION

The induced sodium chloride-dominated salinity negatively impacted various growth parameters in the PU-19 and UH 80-9 cultivars of black gram, including plant height, root length, number of root nodules, number of flowers per plant, as well as fresh and dry weight of leaves, roots, and nodules. Notably, the mean plant height, root length, number of root nodules, and number of flowers per plant showed considerable improvements with the application of ascorbic acid. Specifically, the genotype UH 80-9 exhibited perform better than PU-19 in overall morphological traits examination. Furthermore, the addition of ascorbic acid positively influenced the fresh and dry weight of leaves, roots, and nodules in both genotypes. The combination of 100 mM NaCl and 2.0 mM ascorbic acid was found to be the optimal level of ascorbic acid, resulting in improved growth properties under saline conditions. By conducting this study, valuable insights have been gained into the relationship between various morphological traits in the PU-19 and UH 80-9 cultivars of black gram. The findings contribute to our understanding the growth dynamics, providing suitable traits for future breeding and cultivation practices to overcome salinity. The application of ascorbic acid as a potential approach to mitigate the negative effects of salinity on black gram growth warrants further exploration and potential implementation in agricultural practices.

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