RESEARCH ARTICLE
Legume Research- An International Journal, Volume 47 Issue 12: 2068-2076 (December 2024)

Citric Acid Alleviated Salt Stress by Modulating Photosynthetic Pigments, Plant Water Status, Yield and Nutritional Quality of Black gram [*Vigna mungo* (L.) Hepper]

Md. Aktaru Hossain[1], Mst. Smrity Khatun[1], Mottaleb Hosen[1], Zakariya Ibne Sayed[1], Md. Rafiqul Islam[2], Md. Kaium Chowdhury[3], Muhammad Aamir Iqbal[4], Ibrahim Al-Ashkar[5], Zeki Erden[6], Cagdas Can Toprak[6], Ayman El Sabagh[6,7], Mohammad Sohidul Islam[1]

10.18805/LRF-820

ABSTRACT

Background: Salt stress (SS) has seriously threatened the productivity of pulses including black gram in modern input-intensive farming systems which necessitates finding biologically viable, pro-farmer and environmentally friendly SS ameliorating strategies.
Methods: An experiment was conducted to assess three levels of both SS (0, 50 and 100 mM NaCl) and citric acid (CA, 0, 50 and 100 µM) applied as a foliar spray to ameliorate the deleterious effects of SS on black gram (cv. BARI Mash-3). The response variables included plant growth traits like plant height (PH), leaf number (BLPP) and root dry weight (RDW) along with chlorophyll contents (chl a, chl b and tchl), plant water status (relative water content RWC and water retention capacity WRC), grain yield (GY), stover yield (SY), biological yield (BY)) and harvest index (HI) along with nitrogen (N) and protein (P) content of black gram.
Result: The results revealed that CA (100 µM) remained unmatched by increasing PH (76.25%), NLPP (37.52%), RDW (83.67%), Chl a (17.80%), Chl b (11.59%), tChl (15.51%), RWC (9.81%) and WRC (26.64%) under highest level of induced SS. The same treatment also surpassed the rest of the doses in terms of grains number per pod (23.89%), 100 grains weight (59.74%), GY (82.86%), SY (59.66%), BY (64.94%) and HI. Moreover, CA accumulated N and P content (29.9%) in the grain under SS conditions. These results indicated that application CA alleviated the adverse effects of SS by triggering the growth, yield and nutritional quality which might be developed as a potent strategy to cope with the declining productivity of black gram in saline environment.

Key words: Grain legumes, Mash, Organic acid, Salt stress, Water status.

INTRODUCTION

Grain legumes are a vital source of protein, a wide range of minerals and numerous vitamins which are essential for robust human health (Iqbal *et al.,* 2019). These are the cheapest source of protein and hence referred to as poor man's meat (Abbas *et al.,* 2021; Iqbal *et al.,* 2018). Among pulses, black gram [*Vigna mungo* (L.) Hepper] has attained strategic pertinence in ensuring the food and nutritional security of a rapidly increasing population (Iqbal *et al.,* 2024; Ramamoorthy and Ariraman, 2023). Various traits including self-pollination, well-grown root network, drought tolerance, short life cycle (Hema *et al.,* 2024) and unprecedented adaptability under varying agro-climatic conditions favor its cultivation (Sadiq *et al.,* 2023). Despite significant genetic improvement of field crops using genetic engineering approaches (Li and Iqbal, 2024), legumes yield has remained stagnant owing to frequent incidence of abiotic stresses (soil salinity, heat stress, drought, *etc.*) (Iqbal and Iqbal, 2015) and biotic stresses (Arthanari, 2024).

Recently, soil salinity (SS) has emerged as one of the most challenging abiotic stresses for sustainable production of field crops including black gram (Ahmad *et al.,* 2023). The SS directly imposes physiological disruptions of vital metabolic processes like photosynthesis in crop plants, which eventually causes stunted growth

[1]Department of Agronomy, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.
[2]Department of Agronomy, Regional Agricultural Research Station (Bangladesh Agricultural Research Institute), Ishurdi, Pabna, Bangladesh.
[3]Department of Agricultural Extension, Agricultural Training Institute, Gaibandha 5700, Bangladesh.
[4]Department of Chemical Engineering, Louisiana Tech University, Ruston LA 71270, United States.
[5]Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia.
[6]Department of Field Crops, Faculty of Agriculture, Siirt University, Turkey.
[7]Department of Agronomy, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Shaikh 33516, Egypt.

Corresponding Author: Muhammad Aamir Iqbal, Department of Chemical Engineering, Louisiana Tech University, Ruston LA 71270, United States. Email: muhammadaamir.iqbal@fulbrightmail.org

How to cite this article: Hossain, M.A., Khatun, M.S., Hosen, M., Sayed, Z.I., Islam, M.R., Chowdhury, M.K., Iqbal, M.A., Al-Ashkar, I., Erden, Z., Toprak, C.C., Sabagh, A.E. and Islam, M.S. (2024). Citric Acid Alleviated Salt Stress by Modulating Photosynthetic Pigments, Plant Water Status, Yield and Nutritional Quality of Black gram [*Vigna mungo* (L.) Hepper]. Legume Research. 47(12): 2068-2076. doi: 10.18805/LRF-820.

Submitted: 07-06-2024 **Accepted:** 22-07-2024 **Online:** 09-08-2024

and even crop failure becomes imminent (EL-Sabagh *et al*., 2021). The saline conditions led to the imposition of ionic imbalance and osmotic stress which ultimately induces oxidative stress (Sagar *et al.,* 2023; Yasir *et al.,* 2021). Although, black gram is reported to be a moderately saline-tolerant crop, however, its productivity suffers under SS, which necessitates conducting fresh studies to find out biologically viable SS mitigation strategies. Citric acid (CA) is a 6-carbon tricarboxylic acid compound that is primarily synthesized by acetyl-CoA and oxaloacetate using citrate synthase catalyzed condensation process (Khatun *et al*., 2019). Moreover, CA modulated the physiological response of common bean plants (El-Tohamy *et al*., 2013). Likewise, CA applied in conjunction with ascorbic acid and salicylic acid ameliorated the adverse effects of SS in maize by improving photosynthesis rates and osmoregulation along with reduced biosynthesis of reactive oxygen species (ROS) (El-Hawary and Nashed, 2019).

Although, SS tends to hamper black gram productivity, however, very few research studies have previously aimed to mitigate the deleterious effects of SS on black gram by foliar application organic acids like CA. There have been noticeable research gaps pertaining to the optimization of CA doses for boosting yield attributes, grain yield and quality of black gram. Therefore, this study was undertaken to assess the adverse effects of different levels of salinity on the growth, physiology, nutritional status and yield of black gram and to sort out the most superior dose of foliar applied CA for boosting the yield and nutritional quality of black gram sustainably in an eco-friendly manner.

MATERIALS AND METHODS

An experiment was carried out in shade-house conditions at Department of Agronomy, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh (25°37′N latitude and 88°39′E longitude and the elevation was 37.5 m) during 2022. A two factorial experiment was carried out entailing three levels of salt stress *viz*. S_0 = 0 mM, S_1 = 50 mM and S_2 = 100 mM NaCl and three levels of citric acid (CA) *viz*., CA_0 = 0 μM, CA_1 = 50 μM and CA_2= 100 μM applied as sodium nitroprusside (SNP) for black gram (cv. BARI Mash-3). The experiment was laid out with completely randomized design (CRD) in factorial arrangement with three replications (three additional replications for recording response variables involving destructive protocols such as root and shoot length and weight, *etc*).

For filling pots, soil was thoroughly mixed and pots were filled with 10 kg soil. Nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) were applied as urea, triple super phosphate (TSP), muriate of potash (MOP) and gypsum, respectively, at the rate of 45, 90, 40 and 50 kg ha^{-1}, respectively, as a basal dose at the time of pots preparation. The seeds were treated with Bavistin-200 (0.3%) and sown on 6 March 2022 in 2-3 cm depth, wherein 20 seeds were sown per pot.

For measuring grain yield (GY), the grains were separated from each plant manually and sun-dried. The grain and stover stover yields (StY) were converted into yield (g) $plant^{-1}$. The biological yield (BY) of black gram was estimated by using the Eq 1 as described by Iqbal *et al.* (2019).

$$BY\ (g\ plant^{-1}) = GY + StY \quad ...(1)$$

The harvest index (HI) was determined by using the Eq. 2 as described by Iqbal *et al.* (2019).

$$HI\ (\%) = \frac{GY}{StY} \times 100 \quad ...(2)$$

Photosynthetic pigments measurements

From each treatment, third trifoliate leaves of black gram were separated carefully for determining the chlorophyll content (Witham *et al*., 1986). Fresh weight of leaves (0.10 g) was taken, then leaves were kept immersed in acetone (10 ml) for 48 hours in dark condition at room temperature. For estimating the chlorophyll a, b and total chlorophyll of black gram, the absorbance of the supernatant was noted using an UV-visible spectrophotometer (663 nm wavelength). Thereafter, their contents were measured (mg g^{-1}) using Eq 3-5 as described by Ahmad *et al.* (2023).

$$\text{Chlorophyll a} = \frac{[12.7(D663) - 2.69(D645)] \times V}{(1000 \times W)} \quad ...(3)$$

$$\text{Chlorophyll b} = \frac{[22.9(D645) - 4.68(D663)] \times V}{(1000 \times W)} \quad ...(4)$$

$$\text{Total chlorophyll} = \frac{[20.2(D645) + 8.02(D663)] \times V}{(1000 \times W)} \quad ...(5)$$

Where,

D= Chlorophyll extract's absorbance reading at a specific wavelength.

V= 80% acetone-chlorophyll extract final volume.

W= Tissue extracts fresh weight.

The terminal leaflets of the fully expanded leaves of black gram plants from each treatment were used to record fresh, dry and turgid weights of leaf segments for estimating the relative water content (RWC), water retention capacity (WRC), water saturation deficit (WSD) and water uptake capacity (WUC) (Islam *et al*., 2021b).

$$RWC = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100 \quad ...(6)$$

$$WSD = 100 - RWC \quad ...(7)$$

$$WRC = \frac{\text{Turgid weight}}{\text{Dry weight}} \quad ...(8)$$

$$WUC = \frac{\text{Turgid weight - Fresh weight}}{\text{Dry weight}} \quad ...(9)$$

The oven dried grains were subjected to mechanical grinding with the help of a Willy grinding machine for estimation of protein (P) and nitrogen (N) content.

Subsequently, 1.0 g of finely ground grains were taken into a 250 ml conical flask and 10 ml if di-acid mixture (HNO_3:$HClO_4$ = 2:1) by following wet oxidation method (Jackson 1973), whereas the N content was determined by using the Kjeldhal method.

Statistical analysis

The recorded data were statistically analyzed to determine the significance among employed treatments of salinity levels and citric acid doses using MSTAT-C statistical package. For this purpose, two-way analysis of variance (ANOVA) was performed to determine the overall significance of employed treatment factors (salinity levels and citric acid doses) and thereafter, significance among treatment means was estimated by employing the least significant difference (LSD) test at 5% probability level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Plant height and number of leaves per plant

The plant height and number of leaves plant^{-1} showed significant variation in saline environment at all sampling stages (Table 1). The results indicated that these traits of black gram decreased with increasing levels of SS. However, application of CA (100 µM) significantly increased plant height, which were statistically similar to 50 µM CA. The tallest plants of 37.83, 69.00 and 71.67 cm were observed from S_0CA_2 (0 mM NaCl + 100 µM CA) at 35, 55 DAS and FH, respectively, while the minimum plant height (13.83, 23.33 and 23.83 cm) values were observed for S_2CA_0 (100 mM NaCl + 0 µM CA) treatment at 35, 55 DAS and FH, respectively. The results also showed that the highest number of leaves per plant (6.00, 8.33 and 8.67) was exhibited by S_0CA_2 (0 mM NaCl + 100 µM CA) and the lowest number of leaves per plant (3.00, 5.00 and 5.33) was observed in S_2CA_0 (100 mM NaCl + 0 µM CA) *i.e.* without CA at 35, 55 DAS and FH, respectively. Nevertheless, addition of CA enhanced the number of leaves per plant and the treatment S_1CA_2 (50 mM NaCl + 100 µM CA) remarkably increased the number of leaves per plant (4.33, 7.67 and 8.33 at 35, 55 DAS and FH, respectively).

Roots fresh and dry weights and chlorophyll a, b and total contents

The SS significantly diminished the root fresh and weights, along with chlorophyll a, b and total contents of black gram (Table 2). The CA application enhanced root fresh weight and the highest values (0.074 and 0.427 g plant^{-1}) were recorded by CA_2 treatment which was statistically at par with CA_1 (0.063 and 0.356 g plant^{-1}) at 35 and 55 DAS, respectively. Regarding root dry weight, the highest root dry weight (0.024, 0.130 and 0.204 g plant^{-1} at 35, 55 DAS and FH, respectively) was observed in S_1CA_2 (50 mM NaCl

Table 1: Effect of citric acid doses on the plant height and number of leaves per plant of black gram (cv. BARI Mash-3) under saline conditions.

Treatments	Plant height (cm)			Leaves number plant^{-1}		
	35 DAS	55 DAS	Final harvest	35 DAS	55 DAS	Final harvest
Salinity levels						
S_0	36.67a	67.83a	68.83a	5.67a	7.33a	8.11a
S_1	20.78b	38.83b	39.11b	4.00b	6.68b	7.22ab
S_2	19.83b	34.11b	34.39c	3.33b	6.11b	6.45b
LSD	4.367	11.87	3.477	1.480	2.217	1.259
CA doses						
CA_0	21.28b	40.50b	40.78c	4.00b	5.45b	6.33b
CA_1	27.33a	49.00a	49.28b	4.33a	7.00a	7.33a
CA_2	28.67a	51.28a	52.28a	4.67a	7.67a	8.11a
LSD	2.251	4.722	2.691	0.803	1.152	0.803
Interaction effect of salinity and CA levels						
S_0CA_0	35.67a	66.50a	66.67b	5.33abc	6.07bcd	7.67ab
S_0CA_1	36.50a	68.00a	68.17ab	5.66ab	7.67ab	8.00ab
S_0CA_2	37.83a	69.00a	71.67a	6.00a	8.33a	8.67a
S_1CA_0	14.33c	31.67c	31.83e	3.67d	5.33cd	6.00cd
S_1CA_1	23.50b	41.83b	42.33c	4.00cd	7.00abc	7.33abc
S_1CA_2	24.50b	43.00b	43.17c	4.33bcd	7.67ab	8.33a
S_2CA_0	13.83c	23.33d	23.83f	3.00d	5.00d	5.33d
S_2CA_1	22.00b	38.17bc	39.33c	3.33d	6.33bcd	6.67bcd
S_2CA_2	23.67b	41.83b	42.00c	3.67d	7.00abc	7.33abc
LSD	3.898	8.178	4.662	1.391	1.996	1.391
CV (%)	8.51	9.80	5.52	18.04	16.74	10.77

The values followed by the same letter did not differ significantly at $p<0.05$, CV: Coefficient of Variation, LSD: Least significant difference, S_0= 0 mM, S_1= 50 mM and S_2= 100 mM NaCl, CA_0= 0 µM, CA_1= 50 µM and CA_2= 100 µM citric acid.

+ 100 μM CA), while the lowest dry weight of root (0.010, 0.097 and 0.098 g plant^{-1}, respectively) was found in S_2CA_0 (100 mM NaCl + 0 μM CA) treatment. Combined effect of SS and CA on the Chl a was statistically significant and SS-induced reduction of Chl a was improved due to CA. Under higher SS, the highest Chl a (1.39 mg g^{-1} FW) was recorded with the application of CA_2 (S_2CA_2) which was alike (1.35 mg g^{-1} FW) to CA_1 (S_2CA_1). Additionally, CA application increased Chl b concentrations as the highest Chl b (0.77 mg g^{-1} FW) was recorded in S_2CA_2 (100 mM NaCl + 100 μM CA) treatment which was similar to S_2CA_1 (0.73 mg g^{-1} FW). The results revealed that TChl content showed a significant increase as the level of CA was increased as due to combined effect of salt and CA, the highest TChl (2.44 mg g^{-1} FW) was noted for S_1CA_2 (50 mM NaCl + 100 μM CA) treatment (Table 2).

Yield contributing traits and water status

The interaction effect of SS and CA showed that the maximum pods number per plant (11.33) was found in S_0CA_2 (0 mM NaCl + 100 μM CA) (Table 3). It was also observed that the highest pod length (4.68 cm) was recorded from S_0CA_2 (0 mM + 100 μM) and the lowest corresponding value (3.03 cm) was found in S_2CA_0 (100 mM NaCl + 0 μM CA). Moreover, the highest grains number per pod (5.12) was recorded from without SS and CA condition *i.e.* S_0CA_0 (0 mM NaCl NaCl + 0 μM CA) and the lowest corresponding value (3.39) was found in higher salt without CA *i.e.* S_2CA_0 (100 mM NaCl + 0 μM CA). Under higher SS condition, the highest grains number per pod (4.20) was recorded in CA_2 (S_2CA_2) which significantly differed with CA_1 (S_2CA_1). The SS (100 mM) reduced the grains number per pod, while CA application increased its corresponding value (7.67 and 23.89%, respectively). Furthermore, Application of CA increased the 100-grains weight over without CA. The combined effect revealed that the treatment combination S_0CA_0 (0 mM NaCl + 0 μM CA) produced the highest grain weight (3.63 g), whereas the treatment combination S_2CA_0 (100 mM NaCl + 0 μM) showed the lowest corresponding grain weight (1.54 g). Application of CA (100 μM CA) increased the HGW by 72.72% under 100 mM NaCl-induced saline stress in (S_2CA_2).

Regarding water relations, S_2CA_2 combination exhibited the highest relative water content of 69.16% which was statistically equal to S_2CA_1 and the lowest value of 62.98% was given by S_2CA_0. Likewise, the interaction effect of salinity levels and CA doses revealed that the highest water saturation deficit (37.02) was recorded for S_2CA_0

Table 2: Effect of citric acid doses on roots fresh and dry weights along with chlorophyll content of black gram (cv. BARI Mash-3) under saline conditions.

Treatments	Fresh weight of root (g plant^{-1})		Dry weight of root (g plant^{-1})			Chlorophyll (mg g^{-1} FW)		
	35 DAS	55 DAS	35 DAS	55 DAS	Final harvest	Chl a	Chl b	Total Chl
					Levels of salt stress			
S_0	0.087a	0.463a	0.027a	0.153a	0.210a	1.99a	1.12a	3.11a
S_1	0.059ab	0.329b	0.020b	0.125ab	0.172b	1.50a	0.88b	2.38ab
S_2	0.035b	0.289b	0.017b	0.114b	0.153b	1.31b	0.72c	2.03b
LSD	0.032	0.845	0.123	0.442	0.422	1.047	0.093	0.072
					Levels of CA			
CA_0	0.042a	0.299a	0.016a	0.115a	0.136b	1.45b	0.82a	2.32b
CA_1	0.063a	0.356b	0.023a	0.136a	0.190a	1.66a	0.91a	2.24ab
CA_2	0.074a	0.427c	0.025a	0.141a	0.207a	1.69a	0.94a	2.63a
LSD	0.032	0.045	0.099	0. 312	0.234	0.276	0.098	0.056
					Interaction effect of salt stress and CA			
S_0CA_0	0.063abc	0.404b	0.023a	0.130ab	0.187a	1.74ab	1.06ab	2.8abc
S_0CA_1	0.096ab	0.426b	0.028a	0.163a	0.214a	2.09a	1.14a	3.23ab
S_0CA_2	0.102a	0.559a	0.030a	0.168a	0.229a	2.17a	1.17a	3.34a
S_1CA_0	0.040bc	0.275de	0.013b	0.117ab	0.125bc	1.43b	0.85cde	2.28bcd
S_1CA_1	0.060abc	0.351bcd	0.023a	0.127ab	0.185a	1.53bc	0.88cd	2.41abc
S_1CA_2	0.077abc	0.362bc	0.024a	0.130ab	0.204a	1.54bc	0.90bc	2.44abc
S_2CA_0	0.024c	0.219e	0.010b	0.097b	0.098c	1.18c	0.69e	1.87d
S_2CA_1	0.033c	0.292cde	0.018a	0.117ab	0.140ab	1.35bc	0.73de	2.08cd
S_2CA_2	0.043abc	0.359bc	0.021a	0.127ab	0.190a	1.39bc	0.77cde	2.16bcd
LSD	0.055	0.078	0. 556	0.725	0.732	0.481	0.169	0.098
CV (%)	22.88	11.35	21.86	20.02	10.56	16.88	10.16	21.11

The values followed by same letter did not differe significant at $p<0.05$, CV: Coefficient of Variation, LSD: Least significant difference, S_0= 0 mM, S_1= 50 mM and S_2= 100 mM NaCl, CA_0= 0 μM, CA_1= 50 μM and CA_2= 100 μM citric acid.

combination and the lowest corresponding value (24.17) was observed in S_1CA_2 combination. Additionally, the CA application increased the water retention capacity in plants as the highest value of 7.05 was observed in plants treated with 100 µM CA (CA_2), indicating a better ability to retain water. In contrast, the lowest water retention capacity of 6.34 was recorded in plants treated with 0 µM CA (CA_0). Moreover, spraying of CA relieved the plants from SS and decreased the water uptake capacity. The highest water uptake capacity (2.67) was observed without CA and the lowest (1.65) was in CA_2 which was statistically dissimilar to CA_1 (2.14).

Grain, stover and biological yields, harvest index, nitrogen and protein contents

Under varying salinity levels, the highest and lowest grain yields (0.64 and 0.35 g plant^{-1}) were observed for S_2CA_2 (100 mM NaCl NaCl + 100 µM CA) and S_2CA_0 (100 mM NaCl NaCl + 0 µM CA), respectively (Table 4). Nonetheless, the maximum stover yield of 1.90 g plant^{-1} under severe SS was found with spraying CA_2 (S_2CA_2) (100 mM NaCl + 100 µM CA) which was statistically alike to S_2CA_1. Interestingly, the results revealed that the maximum biological yield (4.69 g plant^{-1}) was recorded by S_0CA_2 (0 mM NaCl +100 µM CA). Additionally, the highest biological yield (2.69 g plant^{-1}) was found in S_1CA_2 (50 mM + 100 µM CA). Contrastingly, the lowest biological yield of 1.54 g plant^{-1} was observed for S_2CA_0 (100 mM + 0 µM CA). Furthermore, the CA application increased the harvest index from 31.66% (CA_0) to 34.11 and 35.54% at 50 and 100 µM CA, respectively. Moreover, the results showed that CA remarkably increased nitrogen content from 3.06 to 3.54 mg g^{-1} DW accounting by 15.69% (S_2CA_2) which was statistically similar to S_2CA_1. Moreover, the CA application significantly increased protein content as the treatment combination S_2CA_2 (100 mM + 100 µM) showed the highest value of protein content (22.13%) under severe SS (100 mM) (Table 4).

Correlation analysis

The results showed that stem dry weight at final harvest had positive non-significant correlation with plant height at harvest, PL, grain weight, stover yield, biological yield, harvest index and grain yield of black gram sown in saline environment. However, rest of the traits showed positive significant associated with one to another indicating p=0.05, *; P=0.01, ** and p=0.001, *** probability levels (Fig 1). On the contrary, positive significant associations were found among the photosythetic pigments and plant water relation traits with grain yield (Fig 1). In this case, water uptake capacity and water saturation deficit showed

Table 3: Effect of citric acid doses on pod number, pod length, number of grains pod^{-1}, 100 grains weight and water status of black gram (cv. BARI Mash-3) under saline conditions.

Treatments	Number of pods	Pod length (cm)	Number of grains pods^{-1}	100 grains weight (g)	Relative water content	Water saturation deficit	Water retention capacity	Water uptake capacity
Levels of salt								
S_0	9.56a	4.48a	5.23a	3.70a	82.76a	17.24c	7.19a	1.69b
S_1	7.28b	4.07b	4.28ab	2.56b	73.14b	26.86b	6.79a	2.11b
S_2	6.11c	3.22c	3.75b	1.98b	66.50c	33.50a	6.07b	2.65a
LSD	1.165	0.268	1.204	0.756	2.539	2.536	0.480	0.484
Levels of CA								
CA_0	6.83b	3.73b	4.01a	3.65a	71.61b	28.39a	6.34b	2.67a
CA_1	7.61ab	3.98ab	4.46a	3.66a	74.32a	25.68b	6.65ab	2.14b
CA_2	8.50a	4.08a	4.78a	3.68a	76.47a	23.53b	7.05a	1.65c
LSD	1.165	0.268	1.204	0.756	2.539	2.539	0.480	0.484
Interaction effect of salt and CA								
S_0CA_0	8.00bc	4.23ab	5.12a	3.63a	81.20a	18.80e	6.89a	2.33bcd
S_0CA_1	9.33ab	4.58ab	5.16a	3.69a	82.12a	17.88e	7.21a	1.56d
S_0CA_2	11.33a	4.68a	5.40a	3.73a	84.97a	15.03e	7.45a	1.31d
S_1CA_0	6.67c	3.93c	3.75c	2.17bc	70.66cd	29.34bc	6.58ab	2.56abc
S_1CA_1	7.33bc	4.13bc	4.27b	2.62ab	73.48bc	26.52cd	6.82a	2.08bcd
S_1CA_2	7.83bc	4.16bc	4.66ab	2.88ab	75.29b	24.71d	6.95a	1.67cd
S_2CA_0	5.83d	3.03e	3.39d	1.54c	62.98e	37.02a	5.33c	3.21a
S_2CA_1	6.17c	3.27d	3.95bc	1.96bc	67.39de	32.64ab	5.91bc	2.78ab
S_2CA_2	6.33c	3.37d	4.20b	2.46b	69.16cd	30.84bc	6.75ab	1.96bcd
LSD	2.019	0.465	2.085	1.309	4.397	4.397	0.831	0.837
CV (%)	15.25	6.82	27.26	27.50	3.43	9.82	7.20	22.50

The values followed by same letter did not differ at $p<0.05$, CV: Coefficient of Variation, LSD: Least significant difference, S_0= 0 mM, S_1= 50 mM and S_2= 100 mM NaCl, CA_0= 0 µM, CA_1= 50 µM and CA_2= 100 µM citric acid.

significant negative associated with relative water content, water retention capacity, Chl a, Chl b, Tchl and grain yield following different probability levels (p=0.05, *; P=0.01, ** and p=0.001, ***). Other traits recorded significant positive associated with one to another as water retention capacity with relative water content, Chl a, Chl b, Tchl, grain yield, water uptake capacity and water saturation deficit.

The obtained results indicated that the SS decreased the yield attributes of black gram, while CA application remained effective in boosting these traits of black gram plants by alleviating the adverse effects of SS. The CA foliar spraying stimulated the photosynthesis process which led to increased synthesis and accumulation of carbohydrates (Abd El-Al and Faten, 2009). Likewise, CA triggered the biosynthesis of antioxidants that stimulated cell division which led to taller plants (Chakrobortty *et al.,* (2022). In another study, CA improved the root system of wheat under saline-sodic soils by decreasing sodium absorption ratio (SAR), soil pH, exchangeable sodium percentage (ESP) and electrical conductivity (ECe) which increased plant height significantly (Aslam *et al*., 2022). These findings are in agreement with the previous results whereby plant fresh weight of tomato was increased under SS by organic acid sprays (Ghoohestani *et al*., 2012). CA could counteract the harmful effect of SS on growth by improving soil properties, photosynthetic pigments, photosynthetic rate, transpiration rate, stomatal conductance and activity of enzymes like catalase (CAT), proline dehydrogenase (ProDH), Ascorbate oxidase (AO) (Behairy *et al*., 2017) leading to increase yield attributes.

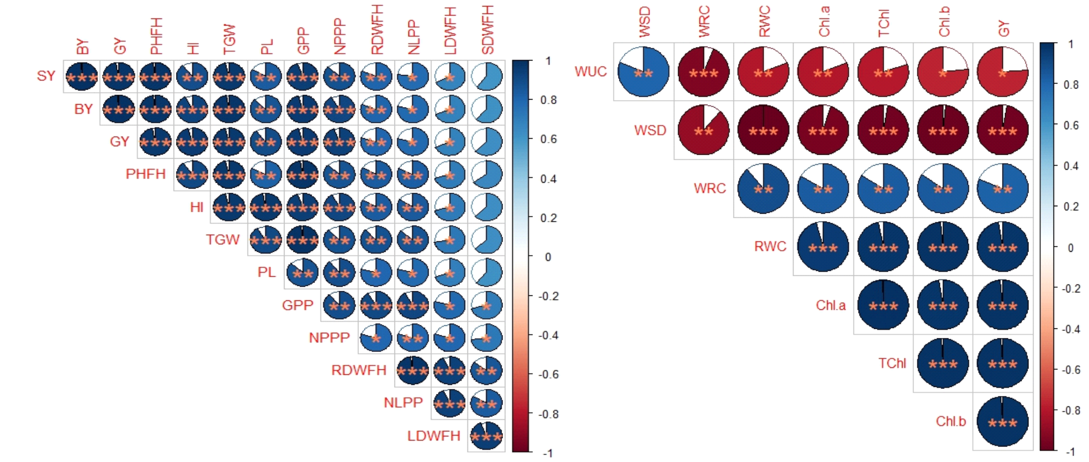
The chlorophyll content of leaves determines plant's photosynthesis capability and growth rate, while the SS declines the chlorophyll content in plants by inhibiting translocation and assimilation of photosynthetic products, contributing to early leaf senescence and photosynthesis inhibition. The SS damaged the chloroplast structure and inhibited photosynthetic activity (Fidalgo *et al*., 2004). However, it could be attributed that CA exogenous application effectively reduced the adverse effects of SS on photosynthetic machinery by improving the chlorophyll biosynthesis and the mobilization of internal tissue nitrate (Behairy *et al*., 2017).

The plant cell's capacity to retain water is termed as relative water content which demonstrates cellular water deficit caused by SS (Islam *et al*., 2021a). The highest relative water content (76.47%) was observed in plants treated with 100 µM CA, indicating efficient water uptake and retention and the value was statistically identical to 50 µM CA. It was also concluded that CA application in saline-stressed plants mitigated the adverse effects of SS

Table 4: Effect of citric acid doses on the grain yield, stover yield, biological yield, harvest index, nitrogen and protein content of black gram (cv. BARI Mash-3) under saline conditions.

Treatments	Grain yield (g plant^{-1})	Stover yield (g plant^{-1})	Biological yield (g plant^{-1})	Harvest index (%)	Nitrogen (mg g^{-1} DW)	Protein (%)
			Levels of salt			
S_0	1.72a	2.61a	4.33a	39.72a	4.29a	26.81a
S_1	0.81b	1.69b	2.50b	32.40b	3.77ab	23.56b
S_2	0.45c	1.60b	2.05c	21.95c	3.12b	19.56c
LSD	0.118	0.268	0.299	2.291	0.507	2.786
			Levels of CA			
C_0	0.82c	1.77b	2.59c	31.66b	3.89b	24.31b
C_1	1.02b	1.97ab	2.99b	34.11a	4.32a	27.00a
C_2	1.18a	2.14a	3.32a	35.54a	4.39a	27.44a
LSD	0.118	0.268	0.299	2.291	0.507	2.786
			Interaction effect of salt and CA			
S_0CA_0	1.49b	2.54b	4.03b	36.97ab	4.08b	25.50b
S_0CA_1	1.76a	2.75a	4.51a	39.02a	4.43a	27.69ab
S_0CA_2	1.90a	2.79a	4.69a	40.51a	4.67a	29.90a
S_1CA_0	0.63d	1.66c	2.29cd	27.51d	3.57cd	22.31c
S_1CA_1	0.79cd	1.66c	2.45c	32.24c	3.88bc	24.25b
S_1CA_2	0.92c	1.77c	2.69c	34.20bc	3.99bc	24.94b
S_2CA_0	0.35e	1.19d	1.54e	22.73e	3.06e	19.13d
S_2CA_1	0.47de	1.47c	1.94de	24.23d	3.47d	21.69c
S_2CA_2	0.64d	1.90c	2.54c	25.20d	3.54d	22.13c
LSD	0.209	0.465	0.519	3.968	0.878	4.826
CV (%)	12.12	13.58	10.10	5.52	10.94%	10.94%

The values followed by same letter did not differ at $p<0.05$, CV: Coefficient of Variation, LSD: Least significant difference, S_0= 0 mM, S_1= 50 mM and S_2= 100 mM NaCl, CA_0= 0 µM, CA_1= 50 µM and CA_2= 100 µM citric acid.



A B

PHFH= Plant height at final harvest; NLPP= Number of leaves per plant; LDWFH= Leaves dry weight at final harvest; SDWFH= Stem dry weight at final harvest; RDWFH= Root dry weight at final harvest; NPPP= Number of pods plant^{-1}; L= Pod length; GPP= Grains per pod; TGW= Thousand grain weight; SY= Stover yield; BY= Biological yield; HI= Harvest index and GY= Grain yield. Chl a = Chlorophyll a; Chl b = Chlorophyll b; TChl= Total chlorophyll; RWC= Relative water content; WSD= Water saturation deficit; WRC= Water retention capacity; WUC= Water uptake capacity; GY= Grain yield.

Fig 1: A: Association of different morphological and growth traits with seed yield of black gram (cv. BARI Mash-3) influenced by citric acid under salt stress conditions. B: Association of photosythetic pigments and plant water relation traits with seed yield of black gram influenced by CA under salt stress.

and increased the relative water content in plants (Sun and Hong, 2011). The CA might have neutralized the NaCl-induced toxic effects in plants which promoted water use efficiency (WUE) under SS along with reducing water saturation deficit under SS by osmotic adjustment (Chakrobortty *et al*., 2022).

The results of the present findings demonstrated that SS reduced the pods number per plant, while CA application alleviated SS and increased the pod number plant^{-1}. These results remained in agreement with Khan *et al*. (2010), who reported that plant growth hormones significantly increased the pod length of mungbean under SS. The pod length of bean (*Phaseolus vulgaris* L.) is increased with CA under drought stress as reported by El-Tohamy *et al*. (2013).

Yield is the final manifestation of the growth and photosynthetic processes and the results of the current study demonstrated that SS declined the grain yield, but application of CA increased the grain yield under non-stress and NaCl-induced SS conditions by alleviating the adverse effect of stress. The increased grain yield by the application of CA might be attributed to an increase in yield attributes especially number of pods and grains per pod and 1000 grain weight. Previously, it was reported that CA application significantly increased the grain yield by neutralizing the deleterious effects of saline environment and promoted the yield attributes in different field crops (Dadrwal *et al*., 2022).

The N content was declined due to the incidence of SS, whereas its content was enhanced significantly by the foliar applied CA in different doses. Previously, it was depicted that SS reduced the N content in wheat while application of ascobin (ascorbic acid and citric acid in 2:1 ratio) significantly increased the N content (Elhamid *et al*., 2014)). In this study, various levels of CA application in saline soil had a significant effect on the protein content of black gram grains. The SS impaired the nitrogen accumulation and reduced protein content, whereas CA exogenous application enhanced the accumulation of nitrogen, consequently increasing the value of protein in soybean (Sheteaw, 2007). This study also assessed the relative performance of yield variables under SS by employing the correlation analysis as reported by previous studies (Islam *et al*., 2021b; Islam *et al*., 2023). We noted a strong positive correlation between the grain yield and plant growth, yield-related traits as well as photosynthetic and water status owing to CA application under SS and non-stress conditions. Furthermore, these results remained in agreement with those of Islam *et al*. (2023), who recorded that SS significantly reduced vital yield attributes that had linear association with grain yield and ultimatley grain yield was reduced drastically in saline environment.

CONCLUSION

The recorded findings were in accordance with the research hypothesis as saline environment negatively affected blakgram yield contributing characteristics and grain yield, whereas citric acid application significantly alleviated the deleterious effects of varying levels of salt stress. According to recorded findings, citric acid (100 μM) remained unmatched in terms of morphology, growth, physiology, yield contributing traits, yield and grain quality of black gram, whereas it was statistically identical with 50 µM concentration for different yield attributes and grain yield. Therefore, black gram (cv. BARI Mash-3) could be sustainably grown under mild salt stress (50 mM stress) by using citric acid (50 µM) foliar application. These encouraging findings necessitate conducting further in-depth studies for evaluating higher doses of citric acid in terms of their efficacy for boosting black gram yield by alleviating the deleterious effects of a saline environment. Moreover, nutritional quality assessment of black gram grain must be studied in response to foliage applied citric acid doses and varying levels of salinity along with exploring the underlying citric acid associated mechanisms which neutralize the adverse effects of salinity.

ACKNOWLEDGEMENT AND FUNDING

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2024R298), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abbas, R.N., Arshad, M.A., Iqbal, A., Iqbal, M.A., Imran, M., Raza, A., Chen, J.T., Alyemeni, M.N., Hefft, D.I. (2021). Weeds spectrum, productivity and land-use efficiency in maize-gram intercropping systems under semi-arid environment. Agronomy. 11: 1615. https://doi.org/10.3390/agronomy 11081615.

Abd El-Al, Faton, S. (2009). Effect of urea some organic acids on plant growth, fruit yield and its quality of sweet pepper (*Capsicam annuns*). Resesearch Journal of Agriculture and Biological Sciences. 5: 372-379.

Ahmad, Z., Khaliq, A., Waraich, E.A., Artyszak, A., Zaman, Q., Abbasi, A., Iqbal, M.A. (2023). Exogenously applied silicon and zinc mitigates salt stress by improving leaf pigments and antioxidant activities in canola cultivars. Silicon. 15: 5435-5444.

Arthanari, M.P. (2024). Growth and productivity of black gram (*Vigna mungo* L.) as influenced by residual effect of triafamone herbicide applied for transplanted rice (*Oryza sativa* L.). Legume Research. 47(1): 142-146. doi: 10.18805/LR-4884.

Aslam, A., Nawaz, H., Khan, A., Ghaffar, R., Abbas, G. (2022). Effect of exogenous application of citric acid on growth of maize (*Zea mays* L.) under sodium fluoride stress. Fluoride. 56(1): 329-350.

Behairy, R.T., El-Hamamsy, S.M.A., El-khamissi, H.A.Z. (2017). Alleviation of salinity stress on Fenugreek seedling growth using salicylic acid, citric acid and proline. Middle East Journal of Agricultural Research. 6(2): 474-483.

Chakrobortty, J., Imran, S., Mahamud, M.A., Sarker, P., Paul, N.C. (2022). Effect of citric acid (CA) priming and exogenous application on germination and early seedling growth of okra (*Abelmoschus esculentus* L.) plants under salinity stress condition. Archives in Agriculture and Environmental Sciences. 7: 318-326.

Dadrwal, B.K., Bagdi, D.L., Kakralya, B.L., Sharma, M.K. (2022). Alleviation of adverse effects of salt stress on growth, yield and yield attributes in wheat by foliar treatment with ascobin. The Pharma Innovation Journal. 11: 2112-2116.

EL Sabagh, A., Islam, M.S., Skalicky, M., Raza, M.A., Singh, K., *et al.* (2021). Salinity Stress in Wheat (*Triticum aestivum* L.) in the Changing Climate: Adaptation and Management Strategies. Frontiers in Agronomy. 3: 661932.

Elhamid, E.M.A., Sadak, M.S., Tawfik, M.M. (2014). Alleviation of adverse effects of salt stress in wheat cultivars by foliar treatment with antioxidant 2-Changes in some biochemical aspects, lipid peroxidation, antioxidant enzymes and amino acid contents. Agricultural Science. 5: 1269-1280.

El-Hawary, M.M., Nashed, M.E. (2019). Effect of foliar application by some antioxidants on growth and productivity of maize under saline soil conditions. Journal of Plant Production. 10(2): 93-99.

El-Tohamy, W.A., El-Abagy, H.M., Badr, M.A., Gruda, N. (2013). Drought tolerance and water status of bean plants (*Phaseolus vulgaris* L.) as affected by citric acid application. Journal of Applied Botany and Food Quality. 86: 212-216.

Fidalgo, F., Santos, A., Santos, I., Salema, R. (2004). Effects of long term salt stress on antioxidant defence systems, leaf water relations and chloroplast ultrastructure of potato plants. Annals of Applied Biology. 145: 185-192.

Ghoohestani, A., Gheisary, H., Zahedi, S.M., Dolatkhahi, A. (2012). Effect of seed priming of tomato with salicylic acid, ascorbic acid and hydrogen peroxide on germination and plantlet growth in saline conditions. International Journal of Agronomy and Plant Production. 3: 700-704.

Gomez, K.A., Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. 2nd Edition, John Wiley and Sons, New York, USA. pp. 680.

Hema, S., Vijayalakshmi, A., Silpa, M. (2024). Impact of paddy and coffee husk as the bio compost and its effect on the growth and yield of black gram [*Vigna mungo* (L.) Hepper]. Agricultural Science Digest. 44(1): 184-188. doi: 10.18805/ag.D-5623.

Iqbal, A., Abbas, R.N., Al Zoubi, O.M., Alasasfa, M.A., Rahim, N., Tarikuzzaman, M., Aydemir, S.K., Iqbal, M.A. (2024). Harnessing the mineral fertilization regimes for bolstering biomass productivity and nutritional quality of cowpea [*Vigna unguiculata* (L.) Walp]. Journal of Ecological Engineering. 25(7): 340-351.

Iqbal, M.A., Hamid, A., Hussain, I., Siddiqui, M.H., Ahmad, T., Khaliq, A., Ahmad, Z. (2019). Competitive indices in cereal and legume mixtures in a South Asian environment. Agronomy Journal. 111(1): 242-249.

Iqbal, M.A., Iqbal, A. (2015). A study on dwindling agricultural water availability in irrigated plains of Pakistan and drip irrigation as a future lifeline. American-Eurasian Journal of Agricultural and Environmental Sciences. 15(2): 184-190.

Iqbal, M.A., Iqbal, A., Siddiqui, M.H., Maqbool, Z. (2018). Bio-agronomic evaluation of forage sorghum-legumes binary crops on Haplic Yermosol soil of Pakistan. Pakistan Journal of Botany. 50(5): 1991-1997.

Islam, M.S., Fahad, S., Hossain, A., Chowdhury, M.K., Iqbal, M.A., Dubey, A. (2021a). Legumes Under Drought Stress: Plant Responses, Adaptive Mechanisms and Management Strategies in Relation to Nitrogen Fixation. In: Engineering Tolerance in Crop Plants against Abiotic Stress. [Fahad, S., Sönmez, O., Saud, S., Wang, D., Wu, C., Adnan, M., Arif, M., Amanullah (Eds)]. CRC Press, Taylor and Francis Group, USA. pp. 179-208.

Islam, M.S., Hasan, M.K., Islam, B., Renu, N.A., Hakim, M.A., *et al.* (2021b). Responses of water and pigments status, dry matter partitioning, seed production and traits of yield and quality to foliar application of GA_3 in mungbean (*Vigna radiata* L.). Frontiers in Agronomy. 2: 596850.

Islam, M.S., Hasan, M.K., Islam, M.R., Chowdhury, M.K., Pramanik, M.H., Iqbal, M.A., Rajendran, K. (2023). Water relations and yield characteristics of mungbean as influenced by foliar application of gibberellic acid (GA_3). Frontiers in Ecological Evolution. 11: 1048768.

Jackson, M.L. (1973). Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd. New Dheli, India. pp. 10-144.

Khan, N.A., Syeed, S., Masood, A., Nazar, R., Iqbal, N. (2010). Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. International Journal of Plant Biology. 1: 1-8.

Khatun, M.R., Mukta, R.H., Islam, M.A., Huda, A.N. (2019). Insight into citric acid-induced chromium detoxification in rice (*Oryza sativa*. L). International of Journal of Phytoremediation. 21(12): 1234-1240.

Li, C., Iqbal, M.A. (2024). Leveraging the sugarcane CRISPR/Cas9 technique for genetic improvement of non-cultivated grasses. Frontiers in Plant Science. 15: 1369416.

Ramamoorthy, P., Ariraman, R. (2023). Effect of phosphorus and sulphur application on growth, yield attributes, nutrient uptake, quality and economics of blackgram (*Vigna mungo* L.): A review. Agricultural Reviews. 44(2): 252-258. doi: 10.18805/ag.R-2180.

Sadiq, M., Rahim, N., Iqbal, M.A., Alqahtani, M.D., Tahir, M.M., Majeed, A., Ahmed, R. (2023). Rhizobia inoculation supplemented with nitrogen fertilization enhances root nodulation, productivity and nitrogen dynamics in soil and black gram [*Vigna mungo* (L.) Hepper]. Land. 12: 1434.

Sagar, A., Hossain, M.A., Uddin, M.N., Tajkia, J.E., Mia, M.A., Iqbal, M.A. (2023). Genotypic divergence, photosynthetic efficiency, sodium extrusion and osmoprotectant regulation conferred salt tolerance in sorghum. Phyton-International Journal of Experimental Botany. 92(8): 2349-2368.

Sheteaw, S.A. (2007). Improving growth and yield of salt-stressed soybean by exogenous application of jasmonic acid and ascobin. International Journal of Agriculture and Biology. 9: 473-478.

Sun, Y.L., Hong, S.K. (2011). Effects of citric acid as an important component of the responses to saline and alkaline stress in the halophyte *Leymus Chinensis* (Trin.). Plant Growth Regululation. 64: 129-139.

Witham, H., Blades, D.F., Devin, R.H. (1986). Exercise in Plant Physiology. 2nd edition, PWS Publishers. Boston, USA. pp. 128-131.

Yasir, T.A., Khan, A., Skalicky, M., Wasaya, A., Rehmani, M.I.A., Sarwar, N., *et al.* (2021). Exogenous sodium nitroprusside mitigates salt stress in lentil (*Lens culinaris* Medik.) by affecting the growth, yield and biochemical properties. Molecules. 26: 2576.