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RESEARCH ARTICLE

The halopriming of seeds improves the germination, growth, physiological and phytochemical attributes of tomato under saline conditions

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ABSTRACT

The low seed germination response and seedling growth of crops to salinity is common problem in Pakistan. To overcome this problem, seed priming is used as an economic and effective approach. Present study was conducted to evaluate the effects of halopriming of seeds on germination, growth and yield of tomato under saline conditions. The seeds of tomato variety 'Raja' were primed with 0, 50 mM and 100mM of NH_4NO_3, KNO_3, and $(NH_4)_2SO_4$ solutions for 12 and 24 hours. The primed seeds were irrigated separately with tap water, saline water, and Hoagland solution with saline water. The results indicated that halopriming of tomato seeds with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ significantly increased the germination percentage (GP) from 58% (unprimed) to 95% (primed) while decreased the mean germination time (MGT) from 7.44 days (unprimed) to 4.6 days (primed). A significant increase was observed in the height of plant, leaf area, number of leaves, shoot and root biomass, number of fruits per plant, etc. in primed plants irrigated with Hoagland solution under saline conditions. Similarly, the halopriming with NH_4NO_3 and KNO_3 also enhanced the chlorophyll pigments, lycopene contents, total soluble sugars, total proteins, total phenolic contents and total flavonoids in primed plants irrigated with Hoagland solutions under salt stress. Furthermore, proline contents were increased in primed plants irrigated with NaCl solution as compared to other treatments and control. In conclusion, KNO_3 and NH_4NO_3 are more effective priming agents than $(NH_4)_2SO_4$ were positively affected the germination, plant height, physiological and biochemical attributes in tomato plants under saline and non-saline conditions. The supplement of Hoagland solution under salt stress further improves the morphological, physiological and biochemical attributes of tomato.

Keywords: Tomato, seed priming, salinity, Hoagland solution, growth attributes

INTRODUCTION

Among many environmental stresses, soil salinity is a critical problem worldwide. The soil salinity may delay the germination events leading to effects on plant physiology and final crop yield (Ashraf and Foolad, 2005). The soil salinity is characterized into three types; saline, sodic and saline-sodic soils. Over 800 million hectares, lands in the world are severely affected by soil salinity. Salt stress affected 34 million hectares of irrigation soil worldwide with a loss of 50% by the mid-twentieth century of grown soil (Wang et al., 2003). Pakistan is the 8th country worldwide which is severely affected by salinity in terms of area (6174.5 thousand ha.). There are two main reasons of the inhibition or delayed seed germination; the ionic and osmotic stress which affect the metabolic processes in plants including carbohydrate metabolism, lipid metabolism and protein synthesis (Parida and Das, 2005). The high salinity level generates an osmotic pressure which in turn significantly reduces the yield of most of the crops. The accumulation of high levels of Na^+ results a reduction in the water potential, membrane dysfunction and inhibition of cellular metabolism (Munns and Tester, 2008).

The use of seeds priming technique activates the metabolic processes prior to seed germination. (Parera and Cantliffe, 1994) found that seed priming reduced the time of seed germination and emergence of seedlings. The seeds primed with water showed early germination, rapidly developed shoot and root, more vigorously grow and significantly greater seedling length than non-primed

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seeds. Seed priming not only increases the seed efficiency but also coordinates with positive changes in biochemical, physiological, cellular and molecular level (Manonmani et al., 2014).

The seed germination process is categorized into three stages; the first is imbibition phase where rapid water uptake occurs by the seeds, the second is lag phase where reactivation of metabolism and eventually starts the germination, while in the third phase, root and hypocotyl emerge from seed (Ibrahim, 2016). Several seed priming techniques are commonly employed to enhance the germination including hydro-priming, halo-priming (seed soaking in inorganic salt solution), osmo-priming (seed soaking in osmotic solution with low water potential), solid matrix priming (seed treatment with chemical and physical characteristics of solid material), thermo-priming (exposing seeds to high or low temperatures), and bio-priming (hydration of seeds using microorganisms or their biological compounds) (Ashraf and Foolad, 2005). Srivastava et al. (2017) found that rice seeds priming with KNO_3 and $Mg(NO_3)_2$ showed improved germination and production by enhancing the Nitrate reductase activity (NRA) and nitrogen absorption as compared with control (unprimed).The tomato seed priming with KNO_3 and $CaCl_2$ lead to considerable reductions in germination time and increase seedling growth and development under high salinity ((Ebrahimi et al., 2014).

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family 'Solanaceae' and the genus Lycopersicon, is ranked as the 2nd most important vegetable crop in the world particularly in China, USA, Japan, India, Pakistan, and Bangladesh. Tomato is very important component of food consumed in Pakistan on daily basis. Tomato is mostly used as fresh vegetable and in processed products such as canning, ketchup and a variety of sauces. Tomato contains a wide array of beneficial nutrients and antioxidants including vitamin A, vitamin C, calcium, folic acid, alpha-lipoic acid, lycopene, choline, beta-carotene and lutein (Nawaz et al., 2011). Lycopene is a dominant antioxidant that gives rich red colour to tomatoes and prevent the growth of various types of cancers (Adenuga et al., 2013).

The world production of tomato was 186 million tons in 2020 while in Pakistan, tomato was cultivated on an area of about 150 thousand hectares with 594 thousand tons production (FAO, 2020), whereas it was grown on more than 67.46 thousand hectares in Sindh province with the highest production. Tomato is mainly cultivated by small farms with relatively lower yield compared to the other countries (Mari et al., 2007). The demand of tomato will be increasing with population growth and urbanization (Mari, 2009) but salinization of soil has become one of the most destructive stress that badly reduces the growth and yield of plants (Ma et al., 2008). Because of the increasing demand and economic importance of tomato, the current study was carried out to examine the influence of halopriming of seeds with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ solutions on some morphological and physiological attributes of tomato.

MATERIAL AND METHODS

The study was carried out to assess the germination, growth and physiological attributes of halo-primed seeds of tomato under saline conditions at the Institute of Biotechnology and Genetic Engineering, University of Sindh, Pakistan.

Sterilization and halopriming of seeds

The germplasm of tomato cultivar 'Raja' was collected from Plant Protection Department, Karachi, Sindh, Pakistan. The healthy tomato seeds "cultivar Raja" were surface sterilized with sodium hypochlorite (1.5%) solution for 5 min, after then washed with distilled water for 3 times (5 min/wash) to removed disinfectant and placed on filter paper to dry. The surface sterilized seeds were then saturated separately in aerated solutions of 00 mM (control), 50 mM and 100 mM of NH_4NO_3, KNO_3, and $(NH_4)_2SO_4$ at 25°C for 12 and 24 hours. The details of treatments are given in Table 1. After respective priming treatment for specific period, seeds were washed with distilled water and then dried on filter paper at room temperature.

Laboratory scale experiment

The experiment was performed to investigate the effect of halopriming with nitrogenous compounds on germination of tomato seeds. The experiment was conducted as a completely randomized design (CRD), all the treatments consisted of five replications with 20 seeds each sown in Petri dishes lined with four layers of Whatman 1 filter paper moistened with 25 ml distilled water and incubated at 25°C. The germination was assumed to occur in moistened seeds as 2 mm or more sized radical was emerged. The germinated seeds were counted regularly after every 24 hours for 15 days (Association, 1985).

The germination percentage was calculated at the 15th day of sowing through the formula;

$$GP\left(\%\right) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} X 100$$

The mean germination time was determined according to the equation reported by (Ellis and Roberts, 1981).

Table 1: Priming treatments of tomato cv. Raja seeds

Treatment	Priming solution (mM)		Priming duration
RC		00	00
T1	KNO_3	50	12 h
T2		50	24 h
T3		100	12 h
T4		100	24 h
T5	NH_4NO_3	50	12 h
T6		50	24 h
T7		100	12 h
T8		100	24 h
T9	$(NH_4)_2SO_4$	50	12 h
T10		50	24 h
T11		100	12 h
T12		100	24 h

RC: Raja control, mM: Milimole, h: hour

$$\text{Mean germination time (days)} = \frac{\Sigma Dn}{\Sigma n}$$

n = number of germinated seeds on days

D = days counted from the sowing to germination.

The average seeds germinated per day was calculated as;

Mean germination rate (MGR)= $\sum Ni\,Ti$

Whereas N - Number of germinated seeds in T time (day).

Green house experiment

The experiment was conducted in the green house as randomized complete block design (RCBD) with 5 replications. The tomato seeds primed with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ were sown in the pots containing sandy loam soil and irrigated with tap water. Physiochemical properties of soil were analyzed before sowing and after completion of experiment as shown in supplementary data. After 25 days of sowing, the tomato seedlings (8-10 cm height) were transplanted in garden according to RCBD and initially irrigated with tap water. After 10 days of transplantation, the seedlings were divided into three groups for irrigation; i) the first group was irrigated with normal tap water, ii) the second group was irrigated with increasing concentrations of saline water, and iii) the third group was irrigated with a combination of increasing concentrations of saline water and Hoagland solution (Hoagland and Arnon, 1950). The concentration of saline water (50,100,150 and 200 mM NaCl) was gradually increased after 10 days of interval. The seedlings of unprimed seeds transplanted in soil and irrigated with tap water were used as control. The plants were harvested after fifty days of transplanting and various morphological attributes were assessed including shoot and root lengths of plants, number of leaves and fruits per plant, leaf area, average fruit weight, shoot and root fresh and dry weights. For biochemical attributes, 70% ethanol extracts of fresh leaves of treated and control plants were prepared.

Determination of chlorophyll contents

For this purpose, 0.1 g of fresh leaf was crushed into small pieces in test tube containing 10.0 ml of 95% acetone. The test tube was then sealed with paraffin and incubated in the refrigerator at 4ºC for 48h. Finally, the absorbance of extract was read on UV-visible spectrophotometer at 662, 644 and 470 nm for chlorophyll a, b and carotenes respectively against 95% acetone as blank, was calculated according to the method of (Shabala et al., 1998) and Chlorophyll pigments (µg/g fresh weight of leaf tissues) were calculated through the equations (Lichtenthaler, 1987);

$$\text{Chl a} = 9.784D662 - 0.99D644$$

$$\text{Chl b} = 21.42D644 - 4.65D662$$

$$\text{T. Chl} = \text{Chla} + \text{Chlb}$$

$$CX + C = \frac{1000\,D470 - 1.90\,Chla - 63.14\,Chlb}{214}$$

Determination of leaf proline

About 500 mg of a frozen leaf sample (-40°C) was homogenized in 5.0 ml of sulfosalicylic acid (3.0% w/v) and filtered using Whatman 2 filter paper. About 2.0 ml of extract was taken in a test tube along with acid ninhydrin (1.0 ml) and glacial acetic acid (1.0 ml). The mixture was incubated in a water bath at 100 °C for 60 minutes and then the reaction was terminated by keeping the reaction mixture at 4°C followed by the addition of toluene (2.0 ml) with vigorous stirring for 15-20 min until separation of two phases. The upper phase was collected and read the absorbance at 520 nm on UV-visible spectrophotometer using toluene as blank (Bates et al., 1973) using D-proline for standard graph. Finally, the amount of free proline was determined through using the formula;

$$\text{Proline}(\mu M / g\ \text{tissue}) = \frac{[(\mu g\ \text{proline} / ml) x\ ml\ \text{toluene} / 115.5\ \mu g / \mu moles}{[(g\ \text{sample}) / 5]}$$

Determination of sugar contents

Free total free sugars were determined by Hedge and Hofreiter, (1962) method. For this purpose, 1.0 ml of test sample was mixed thoroughly with 5.0 ml of anthrone reagent a test tube, then incubated in boiling water bath for 10 minutes and then cooled at room temperature to complete the reaction. Finally, absorbance was determined at 620 nm against blank. The absorbance was interpreted into total sugars by standard graph which was prepared using glucose as standard sugar. The reducing sugars were determined by DNSA method (Miller, 1959). In a test tube, 2.0ml of test solution was mixed well with 2.0ml of 3,5-Dinitrosalicyclic acid (DNS), then incubated for 5 minutes in a boiling water bath and then cooled at room temperature. Finally, the absorbance was measured at 540 nm on spectrophotometer against blank. The amount of reducing sugars was calculated from standard curve which was plotted using various concentrations of glucose.

Determination of total proteins

Total proteins in the extracts of tomato leaves were measured by Braford method (Bradford, 1976). For this purpose, 100μl sample extract was mixed with 5 ml Bradford solution and incubated for 10 min at room temperature. Finally, absorbance was read at 595 nm on spectrophotometer against blank. The absorbance was interpreted into total proteins from standard calibration curve using bovine serum albumin as a standard protein.

Determination of lycopene contents

For this purpose, 0.1 g ripened fruit tissue (frozen at -40°C) was ground in pestle and motor, the powder was collected in amber vial and then added 0.05% w/v BHT (butylated hydroxytolulene) in hexane: acetone: ethanol (6.0ml:3.0ml:3.0ml) solvent mixture and the reaction vial was stirred for 15 min at 4°C. Later, 3.0 ml of deionized water was added, mixed vigorously for 5 minutes, and then phase separation was allowed to occur at room temperature. Finally, the supernatant was collected and the absorbance was read at 503 nm on UV-visible spectrophotometer (Fish et al., 2002). The concentration of lycopene in the hexane extracts was calculated using the formula (Anthon and Barrett, 2006).

$$\text{Lycopene}\left(mg/kg\right)=\frac{A503\ x\ \left(537\ x\ 8\ x\ 0.55\right)}{\left(0.10\ x\ 172\right)}$$

whereas 537 g/mole is lycopene molecular weight, 8.0 ml is the volume of solvent mixed, 0.1 g is tomato sample, 0.55 is the volume ratio of the upper layer of mixed solvents, while 172 mM^{-1} is extinction coefficient used for lycopene in hexane.

Determination of total phenolic contents (TPC)

The phenolic contents in ethanol extractions of leaves from treated and control plants of tomato were determined by Folin-Ciocalteu method (Folin and Ciocalteu, 1927) modified by (Singleton et al., 1999). Briefly, 0.3 ml plant extract was added in a test tube containing 1.5 ml of 10-fold diluted Folin-Ciocalteu, and then added 1.2 ml of Na_2CO_3, mixed thoroughly and stayed at room temperature for 30 min to complete the reaction. Finally, the absorbance was measured against the blank at 765 nm on UV-visible spectrophotometer using Gallic acid as a standard phenolic compound.

Determination of total flavonoid contents (TFC)

Total flavonoid contents (TFC) in sample extracts were determined through following (Kim et al., 2003) method. Briefly, 0.1 ml of sample extract was mixed with 0.3 ml of 5% $NaNO_2$ in a test tube, wait for 5 min at room temperature, and then 0.3 ml of $AlCl_3$ was added in the reaction mixture. After 5 min, 2.0 ml of 1M NaOH was added and the volume of reaction mixture was raised up to 10.0 ml with distilled water. The absorbance was measured against the blank at 510 nm on UV-visible spectrophotometer using rutin as a standard flavonoid.

Statistical analysis

The findings recorded in Tables and Figs. were the mean of triplicates with standard deviation (Mean ± S.D.) calculated through Microsoft excel 2010. The replicated data was analyzed using analysis of variance (ANOVA) through IBM SPSS statistics 26 software. The treatment means were compared according to LSD ($P \leq 0.05$) test.

RESULTS

Effect of halo-priming on germination of tomato seeds

The analysis of variance revealed a significant difference in germination of tomato seeds of cultivar Raja among the treatments of halo-primed seeds and control (Table 2). It was observed in the laboratory experiment that the germination percentage was significantly increased while the mean germination time (MGT) and time duration (in days) to 50% germinations (T50) were decreased in the seeds treated with various concentrations of KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ as compared to unprimed seeds (Table 2).

Effect of halo-priming on morphological attributes of tomato plants

In the green house experiment, significant interactive effects were observed in all the growth and yield parameters under study in tomato grown in varying conditions (Tables 3,4 and 5). In all primed seed treatments, the mean values of all morphological attributes such as plants height, leaf area, number of leaves, shoot and root biomass were significantly improved compared to unprimed tomato plants (Table 3 and 4). Among all, the treatments irrigated with a mixture of Hoagland's solution and NaCl showed significantly improved morphological attributes as compared to primed seeds plants irrigated with tap water and tap water with NaCl and control plants (unprimed) as presented in Tables 3 and 4. It was observed that the maximum plant height (109 cm) was found in T4 treatment followed by 105.8 cm in T2 treatment plants (seeds primed with KNO_3 for 24h) supplemented with Hoagland supplement and increasing concentration of NaCl as compared with control and other treatments. Moreover, positive increase in the number of leaves per plant and leaf area was also noted in primed seeds plants supplemented with Hoagland supplement and increasing concentration of NaCl (Table 3).

Table 2: Effect of halopriming of tomato seeds of variety Raja on germination

Treatment	GP (%)	MGT (days)	MGR (day^{-1})	T_{50} (days)
T1	95±5	4.83±0.06	0.2±0.003	4.45±0.04
T2	93±4.3	4.6±0.09	0.21±0.004	4.2±0.13
T3	89±6.7	4.87±0.06	0.2±0.002	4.5±0.07
T4	82±6	4.74±0.09	0.21±0.004	4.3±0.13
T5	95±5	4.64±0.08	0.21±0.003	4.2±0.07
T6	88±9.5	4.69±0.1	0.21±0.004	4.17±0.19
T7	88±12	4.57±0.11	0.22±0.005	4±0.13
T8	88±6.4	4.63±0.12	0.21±0.005	4.2±0.2
T9	92±6	4.77±0.05	0.2±0.002	4.4±0.048
T10	76±6.9	4.76±0.12	0.21±0.005	4.3±0.11
T11	67±3	4.8±0.03	0.21±0.001	4.45±0.05
T12	68±3	4.65±0.01	0.21±0.0007	4.2±0.11
Unprimed	58±3.3	7.44±0.05	0.13±0.0008	6.81±0.05

*mean±SD
**GP- % germination, MGR-mean germination rate (day^{-1}),
MGT-mean germination time (days), T_{50}-time to 50% germination of seeds

The results presented in Tables 4 and 5 also showed a significant increase in shoot and root biomass, number of fruits per plant and average fruit weight as compared to control. Among all treatments, T6 primed seeds with NH_4NO_3 for 24 h showed 77% increase in shoot fresh weight when irrigated with Hoagland solution under increasing concentrations of NaCl. The fresh weight of root was also improved almost in all treatment and conditions as compared to control (Table 4).The results indicated that the maximum root fresh weight (12.5g) was recorded in T6 treatment irrigated with tap water and with Hoagland supplement under saline conditions. However, most of the treatments showed a decrease in dry root weight as compared to control (Table 4).

The number of fruits $plant^{-1}$ of tomato variety 'Raja' was also positively affected by seed priming and salinity. A significant ($P<0.05$) variation in number of fruits per plant was observed due to seed priming and salinity (Table 5). The result indicated that the highest number of fruits was noted in T7 and T8 treatments while an increase in average fruit weight was noted in treatment T1 (45.5g) irrigated with Hoagland solution and increasing concentrations of NaCl as compared to other treatments and control.

Physiological and Biochemical analysis

The results documented in Fig. 1 showed that the chlorophyll contents and carotenoids were significantly enhanced in the treated plants by halopriming of tomato seeds with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$. The results indicated that T6 treatment showed significant higher amount of

Table 3: The effect of halopriming of seeds on plant height, leaves per plant and leaf area of tomato cv. Raja

Treatment	Height of Plant (cm)			Leaf area (cm^2)			Leaves per plant		
	Primed seeds	Primed* Saline	Primed* Saline* Hoagland	Primed seeds	Primed* Saline	Primed* Saline* Hoagland	Primed seeds	Primed* Saline	Primed* Saline* Hoagland
T1	78.6±2.29	61.1±11	76.2±6.83	15±0.5	19.7±0.4	17.9±0.9	126±6.5	92.4±8.3	69.6±1.5
T2	97.9±2.07	53.5±10.8	105.8±6.0	15.±0.5	12.9±0.6	19±0.5	127±5.8	98±5.5	106.6±11
T3	67.4±1.33	75.1±2.6	99±1.92	16.5±1.1	13.3±0.4	18.05±0.4	95±10	87.6±7.9	104±5.9
T4	77.9±3.14	63±4.6	109±6.7	13.3±0.6	13.9±0.5	17.6±0.5	90±4.9	85.8±4.	115±5.1
T5	71.2±9.5	56.3±5.45	94±14.8	20.4±0.5	16.9±0.3	19.6±0.4	88.2±6	110±3.7	98.4±9.6
T6	79±2.16	75±4.2	65.7±12.8	16.9±0.7	19±0.8	17.9±0.4	82.2±5.8	92±7.5	80±6.8
T7	82.5±4.9	75.7±2.9	84.3±4.5	15.1±0.7	18.5±0.7	17.1±0.6	89±7.8	96.8±3.1	108.4±6.6
T8	91±2.2	75.9±2.7	84.2±0.9	15.3±0.4	16.7±1	19.7±0.3	82±5.7	102±6.7	102.8±6.9
T9	75.2±3.1	66.3±10.9	68.9±7.56	13.4±0.6	12.2±0.3	18.4±1.3	78.4±4.3	89.2±10	74.8±4.7
T10	76.2±1.08	74.4±4.1	74.1±11.3	13±0.4	13.4±0.2	18.2±0.3	78.4±3.6	81±2.9	87.8±1.9
T11	76.8±3.7	66.8±2.9	71.8±6.53	13.1±0.5	13.4±0.8	19.7±0.3	88±4.6	93.6±3.	91±2
T12	76.8±2.04	55.8±3.2	70±3.8	12.9±0.4	14.5±0.9	17.9±0.5	72.2±7.3	92.6±1.8	87.2±3
Unprimed	47.18±0.2	47.18±0.2	47.18±0.2	11.9±0.9	11.9±0.9	11.9±0.9	40±3.7	40±3.7	40±3.7

*mean±SD

Table 4: The effect of halopriming of seeds on shoot and root biomass in tomato cv. Raja

Treatment	Fresh shoot weight (g plant^{-1})			Dry shoot weight (g plant^{-1})			Fresh root weight (g plant^{-1})			Dry root weight (g plant^{-1})		
	Primed seeds	Primed* Saline	Primed* Saline* Hoagland	Primed seeds	Primed* Saline	Primed* Saline* Hoagland	Primed seeds	Primed* Saline	Primed* Saline* Hoagland	Primed seeds	Primed* Saline	Primed* Saline* Hoagland
T1	71.7±0.7	33.5±2.4	68.3±7.6	6.64±0.5	13.2±0.3	1.05±0.1	0.78±0.14	1.76±0.2	7.81±2.2	10.4±0.55	7.5±1.1	15.6±0.92
T2	29±3.8	33±2.73	53.8±15.5	6.85±0.8	16±2.3	1.24±0.3	1.05±0.45	2.09±0.3	8.35±0.2	9.7±1.4	10.2±1.7	16.9±2.2
T3	42.9±3.7	43.2±12	53.7±6.5	7.93±0.2	10.8±1.1	1.26±0.02	1.03±0.05	1.28±0.2	10.1±0.02	12±1.2	9.16±0.54	13.2±0.82
T4	40.3±4	37.4±4.1	50.1±5.1	9.4±0.44	16.7±1.5	1.11±0.06	1.17±0.05	2.13±0.07	8.94±0.5	11.3±2.6	10.6±0.23	18.5±1.6
T5	51.9±16.	41.8±3.9	36.51±1.5	4.74±0.5	6.62±0.6	1.03±0.05	0.59±0.06	0.95±0.09	8.24±0.4	8.2±2.2	6.8±2.7	7.8±0.725
T6	60.8±2.1	44.32±	110±17.1	8.76±0.3	9.35±0.6	1.5±0.05	1.09±0.04	1.41±1	12.5±0.4	10.4±1	14.4±2.8	11.1±1.3
T7	38.2±2.6	24.8±3.9	80.9±5.1	8.04±1.6	13±0.19	1.3±0.13	1±0.2	1.87±0.2	10.8±1.1	12.6±0.6	7.7±2.2	15.1±0.64
T8	60.7±3.5	43±1.9	59±2.1	5.3±1.1	9.27±1.8	1.1±0.06	0.66±0.14	1.06±0.4	8.99±0.5	10.7±0.72	8.34±0.65	9.9±1.6
T9	21.7±2	41.6±2.4	26±1.7	9.66±0.5	8.48±0.6	0.78±0.07	1.2±0.05	1.16±0.05	6.27±0.6	7.2±0.76	10.2±1.2	9.5±0.53
T10	23.5±2.4	58.5±2.5	49.6±1.4	7±0.62	9.87±1.3	0.72±0.03	0.87±0.07	1.26±0.3	5.83±0.2	7.02±1.3	9.52±0.82	10.7±1.77
T11	17.5±2	44.4±2.8	47.90±0.6	5.66±0.5	10±0.8	0.53±0.09	0.70±0.07	1.24±0.1	4.25±0.8	4.7±0.75	6.9±0.44	10.7±0.25
T12	44.8±1.6	56±9.7	48.4±0.45	11±0.2	10.2±3.3	0.96±0.06	1.38±0.03	1.48±0.2	7.7±0.5	8.5±1.2	8.43±0.32	11.3±3.3
Unprimed	24.4±2.	24.4±2.	24.4±2	6.26±0.6	6.26±0.6	1.6±0.38	1.6±0.38	1.6±0.38	6.26±0.6	6.14±0.58	6.14±0.58	6.14±0.58

*mean±SD

chlorophyll a (28.08 μg/g of FW) and total chlorophyll pigments (39.64 μg/g FW) when supplemented with Hoagland solutions under saline condition (Fig. 1A and 1C) while an increased amount of chlorophyll b was noted in T4 (17.5μg/g. FW) irrigated with increasing concentrations of NaCl (Fig. 1B). Furthermore, the increased amounts of carotenes were also observed in all treated plants, then unprimed plants (Fig.-1D).

According to the data presented in Fig. 2 about the lycopene assay from tomato fruit, a significant result was shown ($p < 0.05$) almost in all primed seed treatments as compared to control. Among all, the increased amount of lycopene contents was noted in treatments irrigated with tap water as well as with Hoagland solution under saline conditions (Fig.-2). T4 treatment showed the highest amount of lycopene (28.95mg/kg. FW) when irrigated with tap water comparing with other treatments and control.

The results described in Fig. 3 showed changes in sugar contents in the leaves of treated tomato plants. Among three mode of irrigations, Hoagland solutions under saline conditions showed significantly positive increase in total soluble sugars and reducing sugars in all treatments as compared to other types of irrigations. The results indicated that the highest quantity of total soluble sugars and reducing sugars (30.23 mg/ml and 14.39 mg/ml respectively) were found in T11 treatment irrigated with Hoagland solutions under saline conditions (Fig. 3A and B). Similarly, total soluble proteins were also enhanced in the treated plants irrigated with Hoagland solutions and increasing concentrations of NaCl as compared to other treatments irrigated with tap water or saline water and control (Fig. 4A).

Proline is an amino acid which accumulate in plants under different abiotic stresses including salinity. Besides acting as osmolyte for osmotic adjustment, proline also improves the salt tolerance to plants, act as an antioxidative defense molecule and protect against reactive oxygen species (ROS) effects. In present study, the accumulation of proline in fresh leaves of tomato variety 'Raja' was significant ($p<0.05$) as described in Fig. 4B. The results indicated that the halopriming of seeds followed by irrigation with or without NaCl stimulated the accumulation of free proline in leaves. Among all treatments, the highest accumulation of proline (15.51μmoles/g. FW) was noted in T2 treatment under saline conditions (Fig. 4B).

Effect of halo-priming on flavonoid contents and phenolic acids

The effect of halopriming on phytochemicals was positive as presented in Fig. 5. The treatments irrigated with Hoagland solutions and increasing salt concentrations

Table 5: The effect of halopriming of seeds in number of fruits per plant and fruit weight in tomato cv. Raja

Treatment	Number of fruits ($Plant^{-1}$)			Fruit weight (g $fruit^{-1}$)		
	Primed seeds	Primed* Saline	Primed* Saline* Hoagland	Primed seeds	Primed* Saline	Primed* Saline* Hoagland
T1	14±0.6	11±1.0	16±1.2	28.8±0.7	14.7±1.3	45.6±6.6
T2	15±1	13±0.8	14±1	41.7±1.3	23.1±0.6	34.4±3.5
T3	14±0.7	5.8±0.8	14±1.3	40.2±2.0	24.9±1.1	34.1±3.4
T4	18±0.6	11±0.8	18±1.2	24.3±1.4	18.1±0.8	28.1±4.2
T5	13±1.2	6±0.8	9±1	32.5±3.9	22.1±1.1	32.8±1.6
T6	9±0.8	7±0.6	15±0.9	28.8±3.6	19.4±1.6	33.2±1.4
T7	9±0.6	4±0.7	21±0.7	43±3.5	25.04±1.3	31. ±2.2
T8	7±0.6	2±0.6	21±0.8	21.6±1.9	15.5±0.8	38.1±5.6
T9	13±0.6	8±0.0	13±0.6	34.9±4.1	19.0±1.2	18.4±1.3
T10	16±0.6	10±1	15±0.7	36.4±1.6	24.8±1.3	23.8±1.9
T11	10±0.6	4±0.6	15±0.6	29.9±1.4	16.8±1.7	21.3±1.7
T12	12±0.7	6±0.6	10±0.6	30.8±1.5	23.4±1.2	18.9±2.1
Unprimed	3.4±0.89	3.4±0.89	3.4±0.89	12.2±1.2	12.2±1.2	12.2±1.2

*mean±SD

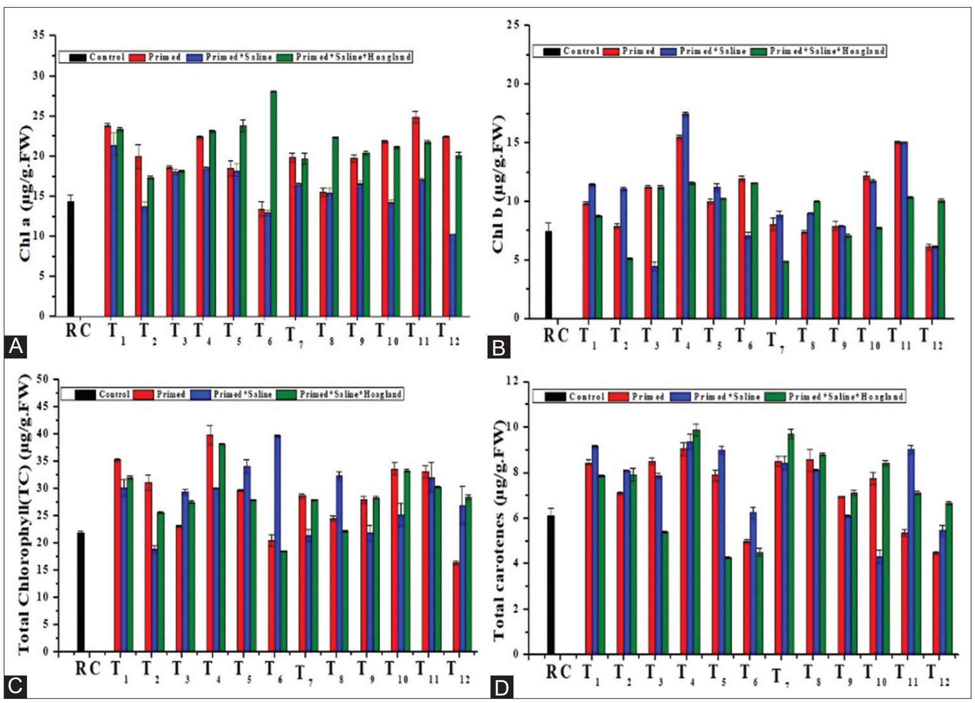

Fig 1. Effect of halopriming on photosynthetic pigments in leaves of tomato variety Raja irrigated with different water conditions; A, B, C and D are showing chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, and total carotenes respectively.

showed the enhanced amounts of total flavonoid contents as compared to other mode of types of watering. The results revealed that total flavonoid contents were higher in T7 treatment (13.08 mg/ml) irrigated with Hoagland solution under saline condition (Fig. 5A). Similarly, a statistical evaluation of data demonstrated that total phenolic acids were also significantly influenced by seed priming. The T10 treatment showed the maximum amount of total phenolic acids (0.90 mg/ml) when irrigated with Hoagland solutions under NaCl stress (Fig. 5B).

DISCUSSION

Priming is a technique of controlled seeds hydration and drying which triggers pre-germinated metabolic processes for fast germination (Hussain et al., 2016). In present study, the seed germination capacity and seedling growth were positively improved in tomato because of seeds priming with KNO_3. There are problems facing the seed industry and growers including poor quality and uncertified tomato seeds leading to lower crop yield. Pre-sowing treatments with exogenous substances stimulate the growth of plants.

The priming of tomato seeds with 0.75% of potassium nitrate (KNO_3) showed more successful results than other units and non-primed control in the greenhouse and growth chamber studies (Ali et al., 2020). Our results are also in agreement with another report, in which tomato seeds of two varieties (Mersa and Tekeze-1) were primed with 0.5% and 1.5% KNO_3 performed better in terms of germination percent (G%), germination index (GI), and mean germination rate (MGR) than the control and 1% KNO_3 (Mebratu, 2022). According to Mahmoud (2015), The germination and seedling growth of tomato cv. Ace 55VF were improved by halo-primed treatments with KNO_3 under normal and saline conditions. The treatments also had the most significant values for the number of leaves, stem length and diameter, fresh and dry weights of shoot and root, and leaf chlorophyll content. In our study, the germination percentage (GP) and mean germination rate (MGR) were improved while MGT and T_{50} were reduced by the influence of seed priming with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ in laboratory experiment (Table 2). Similarly, various growth attributes such as plant height, leaf area, number of leaves, biomass, fruit number and average fruit weight were significantly increased as contrasted with unprimed seeds in green house experiment (Tables 3 and 4). The priming technique is

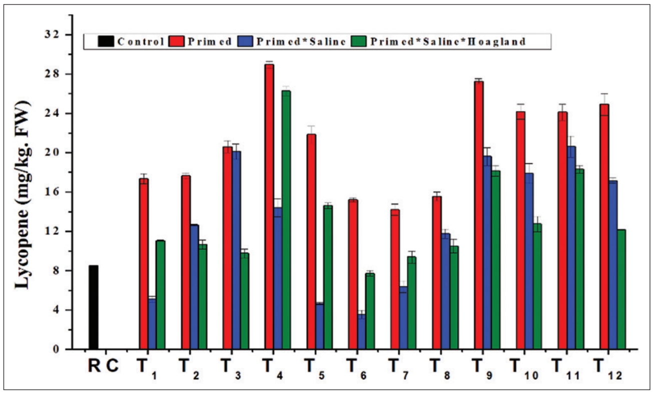

Fig 2. Effect of halopriming on lycopene contents in ripened fruits of tomato variety 'Raja' irrigated with different water conditions.

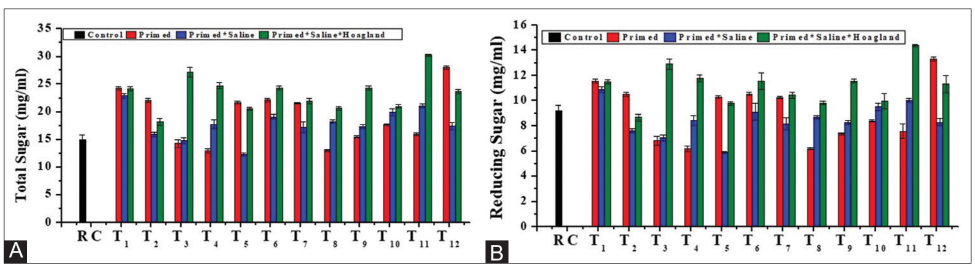

Fig 3. Effect of halopriming on sugar contents of tomato leaves variety 'Raja' irrigated with different water conditions; A – total soluble sugars, B – reducing sugars.

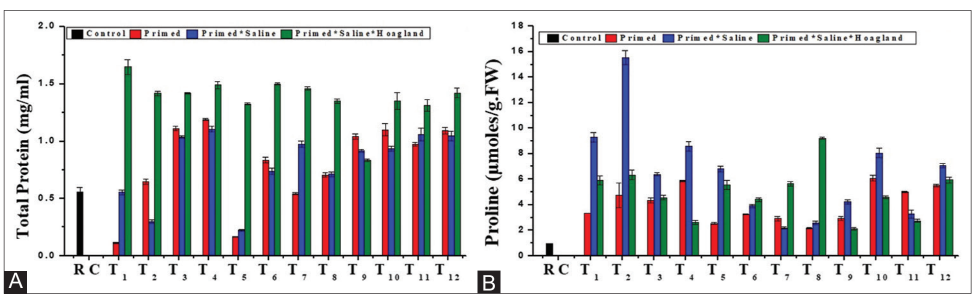

Fig 4. Effect of halopriming total proteins and proline contents in the leaves of tomato variety 'Raja' irrigated with different water conditions; A - total Protein, and B - proline contents

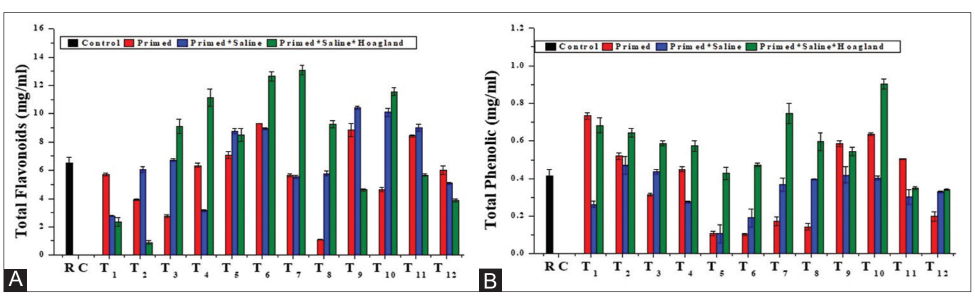

Fig 5. Effect of halopriming on phytochemicals in the leaves of tomato variety 'Raja' irrigated with different water conditions; A - total flavonoids, and B - total phenolic acids.

used for improving seed germination capacity and seedling growth. These findings are in agreement with the previous reports. According to (Santos and Buckeridge, 2004), the germination of seeds, growth and development of seedlings relies on the use of soluble sugar from stem and radical tissue used to maintain osmotic homeostasis and the growth of plants. More recently, Anwar et al. (2020) reported that seeds primed with 200 ppm of GA_3 and 5% of KNO_3 significantly improved plant height, seedling index, biomass, leaf macro and micro-nutrients in cucumber. Similarly, Vaktabhai and Kumar (2017) also reported that the growth, nutrients, biochemical and enzyme activities were significantly increased in tomato due to different combinations of halo-priming, priming duration and salinity. The tomato seeds can be revitalized by halo-priming with 1 or 2 % of KNO_3 for 24 and 48 h against salt stress.

In present study, the halopriming of tomato seeds with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ significantly enhanced the chlorophyll pigments and carotenoids when irrigated with tap water, saline water or Hoagland solutions under saline conditions (Fig. 1). These results are in agreement with previous studies such as in a finding reported by (Anwar et al., 2020), the seeds primed with GA_3 and KNO_3 significantly increased the chlorophyll pigments, net photosynthetic (Pn), stomatal conductance (Gs), leaf macro and micro nutrient content in the cucumber seedlings and plants. Similarly, Parida et al. (2002) found that salinity affected photosynthesis significantly as chlorophyll levels were reduced and membrane stability was also adversely affected in the leaves of *Bruguiera parviflora*. The increased salinity levels reduced plant content of chlorophyll (Scalet et al., 1995). Photosynthetic pigment contents decreased with salinity and the electric transportation activity in plants in photosystems II (Potiuri and Prasad, 1993). In present study, the chlorophyll pigments were increased in all treatments, this increment in chlorophyll contents may be due to halopriming of seeds with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ and then irrigation with saline water or Hoagland solutions under saline conditions (Fig. 1).

In present study, seed priming increased the lycopene contents, total soluble sugars, reducing sugars and total proteins in the leaves of treated tomato plants as compared to control (Figs. 2-4). Other researchers also reported the changes in sugar contents due to environmental stress. For example, researchers reported a decrease or consistency of sugar contents due to stress (Hanson and Hitz, 1982; Morgan, 1992) while others noted a rises of sugar content under water stress (Parida et al., 2007). The increase of chlorophyll pigments and photosynthetic activity in the treatments enhanced the lycopene, sugar and protein contents in the plants due to halopriming of seeds. In present study, increasing concentrations of salts during watering increased the accumulation of proline in the leaves (Fig. 4B). Karimi et al. (2017) reported that plants accumulate proline due to environmental stresses which protects the plants against salinity and osmotic stress. A higher proteolysis or a decreased protein synthesis may cause proline accumulation. The high proline accumulation in plants due to salt stress is beneficial because proline has osmotic leaf potential and therefore result in osmotic change. All primed seeds treatments under saline conditions indicated an increase of proline.

The plants generate mainly secondary metabolites like total polyphenols, phenylpropanoid due to abiotic stresses (Dixon and Paiva, 1995; Oueslati et al., 2010), increase in total polyphenols were different throughout the ripening of fruit in pepper (Navarro et al., 2006). The salinity has significant effect on phenolic contents in different organs of *Nigella sativa* such as the phenolic contents in shoots were increased while in roots were decreased (Bourgou et al., 2012). Our study showed significant increase in total phenolic contents and total flavonoids of tomato variety 'Raja' primed seeds under increasing concentrations of NaCl as compared to control (Fig. 5).

In this study, both macro- and micro-nutrients are used in balanced combination in Hoagland solution, while saline solutions produce one form of salt that causes particular ion toxicity. Hoagland solution has been used to mitigate ion toxicity and classify the effects of osmotic pressures, regardless of salt form. Punjab 2011 and FSD-08 wheat varieties were grown in sand-containing pots in half-strength Hoagland's nutrient solution, with distilled water containing sodium chloride (150 mM) chilling (5°C), heating (60°C), and feeding at 60°C. Both varieties of wheat responded better in both saline and non-saline media compared to other priming treatments. In salt stress of the wheat varieties Punjab 2011 and FSD-08, levels of sodium and total free amino acids had substantially increased (Abida et al., 2019). In primed seeds of tomato under saline condition with Hoagland supplement, all growth and biochemical parameters were improved as compared with unprimed and other watering conditions.

CONCLUSION

Tomato production has been reduced by the low seed quality and other abiotic factors. The seed vigor was enhanced through halopriming of the seeds of tomato variety 'Raja' with KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$. The outcomes concluded that KNO_3 and NH_4NO_3 are more effective priming agents than $(NH_4)_2SO_4$ in improving the seed vigor in tomato. The supplement of Hoagland solution further improved the growth and yield under saline condition. The study will provide a reference line to the researchers to enhance seed vigor, growth and yield of crops under saline environment.

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CONFLICT OF INTEREST

No competing interest

FUNDING/SPONSORSHIP

None

Author's contribution

Najam ul Sahar and Naseem Khatoon contributed in the experimentation, Abdul Majeed Mangrio, Nadir Ali Rind and Muhammad Rafiq contributed in design the experiments, data analysis and writing the manuscript. Muhammad Umar Dahot critically reviewed and revised the manuscript. All authors finally approved the version to be submitted for publication.

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