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EFFECT OF PRE-SOAKING AGENTS ON SALINITY STRESSED CUCUMBER SEEDLINGS

Fazali Wahid[1ǂ], Mehboob Alam[1,ǂ,*], Imran Ahmad[1,ǂ,*], Ali Raza Gurmani[2], Muhammad Sajid[1], Noor Ul Amin[1] and Amjad Ali[1]

[1]Department of Horticulture, Faculty of crop production sciences, The University of Agriculture, Peshawar, Pakistan; [2]Department of Agriculture, University of Haripur, Pakistan
[ǂ]These three authors contributed equally and are the main authors of this article
[*]Corresponding author's e-mail: mehboobalam@aup.edu.pk, imran73pk@gmail.com

An experiment to study the effect of seed pre-soaking agents on cucumber (*Cucumis sativus* L.) growth under various salinity levels was performed. Cucumber cv. Summer Green seeds were soaked in solutions of distilled water (control), salicylic acid (SA) (10^{-5} M), benzyl aminopurine (BA) (10^{-5} M) and potassium nitrate (KNO_3) (3%) for 6 hours. 14-days old seedlings were then exposed to salinity levels of 0, 50 and 100 mM NaCl. Seeds pre-soaked in SA showed significantly higher shoot fresh weight (5.0 g) and shoot (0.8 g) and root dry weight (0.1 g) under saline conditions. Similarly, plants produced from seeds pre-soaked in SA had more shoot (52.2 cm) and root length (15.8 cm) in saline and non-saline conditions compared with other pre-soaking agents. Plants procured from seeds pre-soaked in SA also attained more chlorophyll "a" (6.60 mg g^{-1} dry wt.), chlorophyll "b" (136.52 mg g^{-1} dry wt.) and total chlorophyll content (143.12 mg g^{-1} dry wt.) in both saline and non-saline conditions, followed by BA and KNO_3 pre-soaked seeds. All the three pre-soaking agents were also found helpful in decreasing Na^+ concentrations and increasing K^+ concentrations in cucumber seedlings. On the basis of overall results, it can be concluded that seed pre-soaked with SA and BA has a potential for enhancing salt tolerance in cucumber.
Keywords: Cucumber, benzyl aminopurine, potassium nitrate, salicylic acid, salinity, seed pre-soaking.

INTRODUCTION

Salinity tolerance in certain plants is a significantly cost-effective characteristic for the crops cultivated in the irrigated and marginal lands of the world. Soil salinity changed different biochemical systems within the plant by the buildup of higher absorption of Na^+ and Cl^-, which in turn limit the uptake of K^+, Ca^{2+} and P by decreasing the osmotic potential Munns (2002). Salinity also changes many various physiological and biochemical processes in the plants. Similarly, salinity affects the seed germination due to decrease in endogenous level of hormones (Debez *et al*., 2004). Therefore, plants need to adopt itself to tolerate salinity. Plants manage salinity tolerance not only by the reduction in Na^+ transportation, but also the accumulation of higher K^+ concentrations in leaf tissues (Zhang *et al*., 2010). Studies have shown that plants which have the ability to tolerate salt contain maximum K^+:Na^+ ratio as compare to salt sensitive plants (Rejili *et al*., 2007; Faried *et al*., 2016).

In the current study, the focus was on vegetable crop cucumber (*Cucumis sativus* L.), which is one of the important vegetable crops widely grown all over the world. One of the main reasons for lower yield in cucumber is, its sensitivity to salinity. Salt stress in cucumber involve both osmotic stress, by restricting water absorption from soil and ionic stress, resulting in high concentrations of toxic salt ions in plant cells (Savvas *et al*., 2005; Manan *et al*., 2016).

Crop production in salt affected soils can be improved by enhancing the crop tolerance against salt (Munns, 2002). One of the easy and cheap ways to combat against salinity is seed pre-treatment that is widely used to ameliorate the toxic effect of salinity stress on plant growth and productivity. Various seed pre-soaking agents including abscisic acid (ABA), potassium nitrate (KNO_3), benzyl aminopurine (BA), salicylic acid (SA) and gibberellic acid (GA_3) have been widely used to improve the crop performance against the decadent effect of salinity and drought in different plant species (Gurmani *et al*., 2011; Khan *et al*., 2009; Khan *et al*., 2004; Iqbal *et al*., 2015; Butt *et al*., 2016).

The seed priming and soaking techniques play key role in the yield of different vegetables. The effect of priming is quicker and lead to best crop stand and improve the production and yield, particularly under stress conditions (Halmer, 2004). SA like other plant hormones plays a vital role in many physiological processes and act as a shield against many biotic and abiotic stresses as well as regulates reactive oxygen species and antioxidant enzymes (Khan *et al*., 2003; Shi *et al*., 2008). SA also may work as signal transduction and enhance resistance against diseases in plants (Gaffney *et al*., 1993). Similarly, research has indicated that seeds pre-treatment with BA help in minimizing the adverse effect of salt stress on shoot biomass and germination percentage (Cavusoglu *et al.*, 2008).

Keeping in view the deleterious effect due to salinity on crop productivity and quality the current study was conducted with an objective to enhance crop performance against salinity. The starting hypothesis of this experiment was that exogenously applied BA, SA and KNO_3 might improve the growth of salinity stressed cucumber seedlings. The aim of this study was to find out which pre-soaking agents may help plants perform better under salinity stress.

MATERIALS AND METHODS

The experiment to determine the effect of "Exogenously applied soaking agents on growth of salinity stressed cucumber seedlings" was conducted in a glass house condition with relative humidity 60±2% and temperature of 32±2°C at National Agriculture Research Centre (NARC), Islamabad. This experiment was laid out in randomized complete block design comprising of two factors (Factor A: three soaking agents along with a control and Factor B: two salinity levels with a control). The treatment combinations were replicated four times.

***Plant material*:** Seeds of cucumber cv. Summer Green were sown in plastic pots (30 cm high and 30 cm diameter). Before sowing, pots were filled with 7 kg soil collected from the field at NARC. The physicochemical properties of the potting media were: texture of soil was sandy clay loam, with a pH of 7.8, electrical conductivity 0.80 dS m^{-1}, organic matter 1.07%, Nitrogen 0.09%, Phosphorus 4.1 mg Kg^{-1} and Potash 112 mg Kg^{-1}. Seeds were soaked for 6 h in three different soaking agents and then six seeds were sown in each pot. 15 days old seedlings were then subjected to three different salinity levels. After 30 days plants were harvested and their qualitative and quantitative attributes were analyzed.

***Seed soaking and salinity treatment*:** Before sowing in the plastic pots, seeds were soaked in aerated solutions of distilled water (control), salicylic acid (SA) (10^{-5} M), potassium nitrate (KNO_3) (3%) and benzyl aminopurine (BA) (10^{-5} M) for six hours. Seeds were then washed with tap water and distilled water.

When seedlings were 15 days old, sodium chloride (NaCl) solution was added in three split doses to the pots to develop salinity levels of 0, 50 and 100 mM. To determine the salinity levels in the soil, the electrical conductivity of soil was measured using TD Scan 4 conductivity meter (EUTECH instruments).

***Growth attributes analysis*:** After 30 days of germination plants were harvested. The shoot and root portion were separated. Then, shoot and root fresh weight of four randomly selected plants from each treatment was measured with the help of electric balance. Similarly, shoot and root length of the freshly harvested plants was determined with the help of measuring tape.

Once the fresh plant data was collected, plant samples were wrapped in aluminum foils separately and oven dried at 65°C for 72 h. Then samples were analyzed with the help of electric balance to determine the dry weight of shoot and root according to the procedure explained by Cakmak (1994).

***Determination of chlorophyll, sodium and potassium ions concentrations*:** Before harvesting, fresh leaves were analyzed for chlorophyll a and b contents. Samples were also analyzed for Na^+ and K^+ concentrations. Sixteen days after salinity treatment, fourth young leaf of four randomly selected plants in each treatment was taken and immediately immersed in 10 ml ethanol (0.80%). Samples were then heated in a water bath at 80°C for 10 min. After cooling, samples were analyzed through Pye Unichem spectrophotometer (model U08610) at 663 and 645 nm for the determination of chlorophyll a and b with the help of eq.1(a) and eq.1(b), respectively, as explained by Arnon (1949). Similarly, using eq.1(c) the total chlorophyll content was determined by Arnon (1949).

$$Chlorophyll\ "a" = \frac{(12.7(\text{Abs } 663) - 2.69(\text{Abs } 645)) \text{ x Volume}}{(1000 \text{ x dry weight})} \ldots\ldots (eq.1(a))$$

$$Chlorophyll\ "b" = \frac{(22.9(\text{Abs } 645) - 4.69(\text{Abs } 663)) \text{ x Volume}}{(1000 \text{ x dry weight})} \ldots\ldots (eq.1(b))$$

$$Total\ Chlorophyll = \frac{(20.8(\text{Abs } 645) - 8.02(\text{Abs } 663) \text{ x Volume}}{(1000 \text{ x dry weight})} \ldots\ldots (eq.1(c))$$

Once the reading for chlorophyll content was procured, acetic acid was subsequently added to make a final concentration of 100 mol m^{-3}. Samples were kept in refrigerator for 24 hours. After extraction with acetic acid, samples were analyzed for Na^+ and K^+ with the help of flame photometer (model 52-A, Perkin-Elmer corporation) using eq.2 and eq.3 respectively, as explained by Arnon (1949).

$$Na^+ concentration = \frac{\text{Abs. Na}^+ \text{ x Volume made}}{\text{Dry weight of leaf}} \ldots\ldots (eq.2)$$

$$K^+ \ concentration = \frac{\text{Abs K}^+ \text{ x Volume made}}{\text{Dry weight of leaf}} \ldots\ldots (eq.3)$$

***Statistical analysis*:** Data for growth attributes and quality parameters were collected from four randomly selected plants in each treatment that was replicated four times. Means were calculated from the four replications in each treatment. Statistical program Statistix 8.1 was used for statistical analysis and computing the analysis of variance (McGraw-Hill, 2008). When data was found significant it was further subjected to least significant test at $p<0.05$ using Tukey's test.

RESULTS

***Shoot fresh and dry weight*:** Data recorded from the experiment showed that both soaking agents and salinity levels had significantly affected the shoot fresh and dry weight (Table 1 and 2). It is evident that highest shoot fresh weight (5.0 g) was recorded in plants treated with salicylic acid (SA), followed by plants treated with potassium nitrate (KNO_3) (3.9 g). Whereas, lowest shoot fresh weight was

Table 1. Effect of salinity on growth of cucumber seedlings, obtained from seeds treated with pre-soaking agents. Each number is an average of four plants replicated four times in each treatment combination.

Salinity levels (NaCl (mM))	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot Length (cm)	Root Length (cm)
0	5.5 a	0. 8 a	0.1 a	57.4 a	13.7 a
50	4.2 b	0.6 b	0.08 ab	44.0 b	13.1 a
100	2.8 c	0.4 c	0.07 b	31.3 c	12.2 b

Numbers followed by different letter is significantly different from each other in the same parameter at $p<0.05$.

Table 2. Influence of pre-soaking agents on the growth of cucumber seedlings grown at different salinity levels. Each number is an average of four plants replicated four times in each treatment combination.

Soaking Agents	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot Length (cm)	Root Length (cm)
Distilled water (Control)	3.2 c	0.4 d	0.06 c	35.8 d	10.0 d
Salicylic Acid (10^{-5} M)	5.0 a	0.8 a	0.11 a	52.2 a	15.8 a
Potassium Nitrate (3%)	3.9 b	0.5 c	0.07 c	40.4 c	12.0 c
Benzyl Aminopurine (10^{-5} M)	4.5 a	0.7 b	0.09 ab	48.4 b	14.2 b

Numbers followed by different letter is significantly different from each other in the same parameter at $p<0.05$.

recorded in control plants (3.2 g) (Fig. 1a). Similarly, while comparing the fresh shoot weight plants grown under different salinity levels, the heavier shoot fresh weight was recorded in plants grown in non-saline media (5.5 g) followed by shoot fresh weight of plant grown at salinity level of 50 mM NaCl (4.2 g), while the lighter shoot fresh weight (2.8 g) were recorded in plants grown in media containing 100 mM NaCl (Fig. 1a).

The interactive effect of soaking agents and salinity levels showed that maximum shoot fresh weight (6.7 g) was obtained from plants grown in non-saline media, when seeds were soaked in SA (Fig. 1a). The minimum shoot fresh weight (1.9 g) was recorded in plants grown in media with 100 mM NaCl and when seeds were not treated with any pre-soaking agents (Fig. 1a).

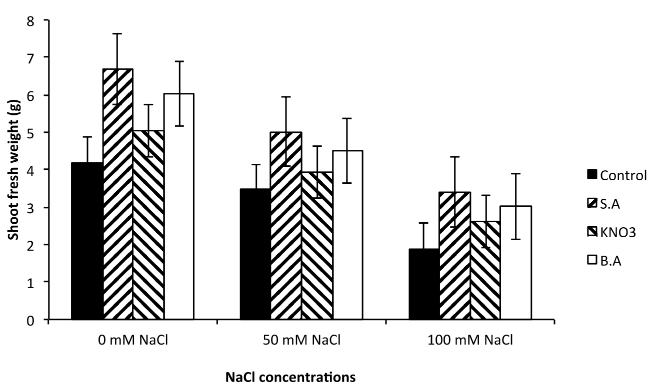

Figure 1a. Effect of salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) on shoot fresh weight (g) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment.

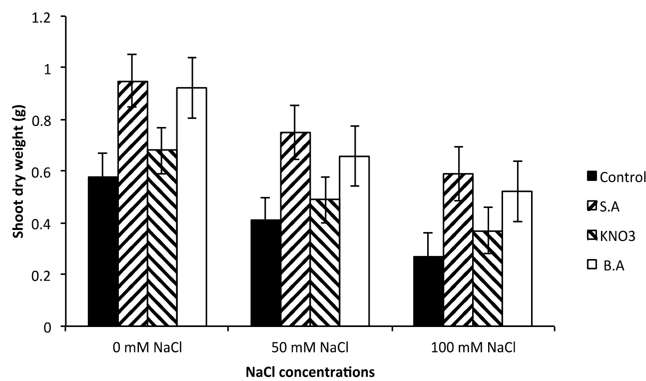

Figure 1b. Shoot dry weight (g) as affected by salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment.

Shoot dry weight was also significantly affected by soaking agents and salinity levels (Table 1 and 2). Comparing the means obtained for different soaking agents highest shoot dry weight (0.8 g) was observed in plants treated with SA with a decrease of 4.3 g in fresh weight, followed by (0.5 g) plants treated with benzyl aminopurine (BA) with a decrease of 3.8 g in fresh weight. The lowest shoot dry weight (0.4 g) was noted in plants with no treatment at all with a decrease of 2.8 g in fresh weight (Fig. 1b). Shoot dry weight was negatively affected with increase in salinity levels. The maximum shoot dry weight (0.8 g) was recorded in plants grown in media with no salinity, followed by plants grown in salinity levels of 50 mM NaCl (0.6 g), whereas the lowest shoot dry weight (0.4 g) was recorded in plants treated with 100 mM NaCl (Fig. 1b).

***Root fresh and dry weight*:** Analysis of variance (ANOVA) showed that root fresh weight was not significantly affected by soaking agents and different salinity levels. Whereas, both soaking agents and salinity levels significantly affected root dry weight (Table 1 and 2). Results indicated that, when seeds were soaked in SA the resulting plants had highest root dry weight (0.1 g), which is statistically at par (0.1 g) with plants dry weight produced from seeds soaked in BA, followed by root dry weight (0.07 g) obtained from KNO_3 treated seeds. The lowest root dry weight (0.06 g) was obtained from plants produced from seeds without any pre-soaking (Fig. 2).

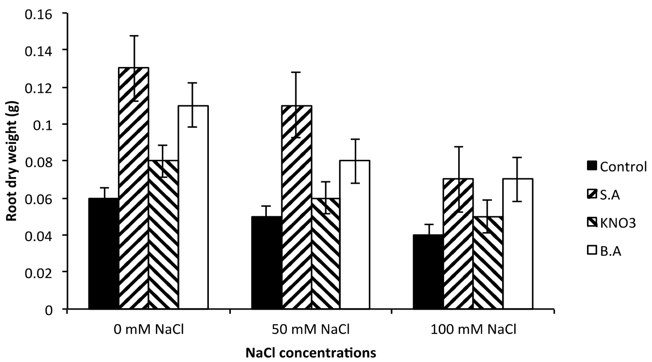

Figure 2. Effect of different pre-soaking agents (salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control)) on root dry weight (g) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100 mM NaCl. Each bar represents mean of four plants replicated four times in each treatment**.**

Plants produced at different salinity levels showed that root dry weight was significantly decreased (0.07 g) at 100 mM NaCl. The maximum root dry weight (0.1 g) was recorded in plants, which were not subjected to any salinity treatment (Fig. 2). The highest root dry weight (0.13 g) was obtained when seeds were soaked in SA and then seedlings were not subjected to any salinity levels (Fig. 2).

***Shoot and root length*:** Pre-soaking treatments of the seeds and different salinity levels significantly affected the shoot and root lengths of cucumber plants (Table 1 and 2). Mean values of shoot length as influenced by seed per-treatment shows that the highest shoot length (52.2 cm) was observed in plants produced from seeds treated with SA, followed by shoot length (48.4 cm) of the plants produced after seed treated with BA. By contrast the lowest shoot length (35.9 cm) was recorded in plants produced from seeds treated with only distilled water. Similarly, mean data recorded for salinity levels revealed that longest shoots (57.4 cm) was gained by plants produced with no salinity, followed by salinity level of 50 mM NaCl (44.0 cm) and shortest shoots were obtained at salinity level of 100 mM NaCl (31.3 cm) (Fig. 3a). The longest shoots (67.2 cm) were obtained when seeds were soaked in SA and produced in soil with no salinity, whereas, shortest shoots (23.4 cm) were obtained from plants produced from seeds treated with only distilled water and produced at 100 mM NaCl (Fig. 3a).

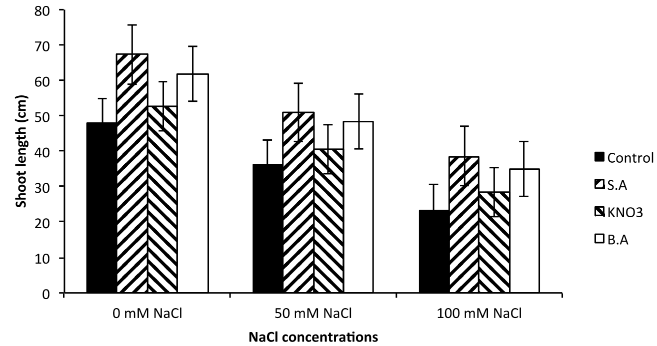

Figure 3a. Effect of salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) on shoot length (cm) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment**.**

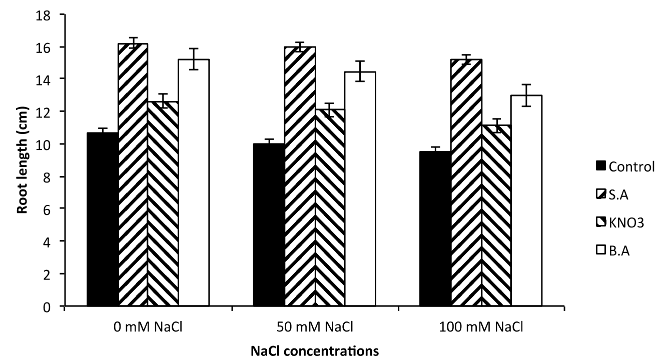

Figure 3b. Root length (cm) as affected by salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment.

Mean data of treatment showed that the longest roots (15.8 cm) was observed in cucumber plants produced from seeds treated with SA, followed by plants having root length of (14.2 cm) grown from seeds that received BA treatment. The shortest roots (10.0 cm) was procured when seeds were pre-soaked in only distilled water (Fig. 3b). Similarly, means data for different salinity levels showed that the longest roots (13.7 cm) was recorded in plants grown in media with no salinity treatments, followed by plants with root length (13.1 cm) grown in media with 50 mM NaCl, which are at par with control treatments and shortest roots (12.2 cm) were obtained

Table 3. Quality attributes as affected by different salinity levels for cucumber seedlings obtained from seeds treated with pre-soaking agents. Each number is an average of four plants replicated four times in each treatment combination.

Salinity levels (NaCl (mM))	Chlorophyll "a" (mg g^{-1} dry wt.)	Chlorophyll "b" (mg g^{-1} dry wt.)	Total Chlorophyll (mg g^{-1} dry wt.)	Na^+ conc (%)	K^+ conc (%)	K^+/Na^+
0	6.32 a	150.33 a	156.64 a	0.7 c	2.2 a	2.5 a
50	5.35 b	117.96 b	123.30 b	1.6 b	2.0 b	1.4 b
100	3.97 c	99.16 c	103.13 c	2.6 a	1.8 c	0.8 c

Numbers followed by different letter is significantly different from each other in the same parameter at $p<0.05$.

Table 4. Effect of different soaking agents on the quality attributes of cucumber seedlings obtained from plants grown at different salinity levels. Each number is an average of four plants replicated four times in each treatment combination.

Soaking Agents	Chlorophyll "a" (mg g^{-1} dry wt.)	Chlorophyll "b" (mg g^{-1} dry wt.)	Total Chlorophyll (mg g^{-1} dry wt.)	Na^+conc (%)	K^+ conc (%)	K^+/Na^+ ratio
Distilled water (Control)	4.04 d	107.09 c	111.14 c	2.5 a	1.7 d	1.1 d
Salicylic Acid (10^{-5} M)	6.60 a	136.52 a	143.12 a	1.3 bc	2.1 b	2.3 a
Potassium Nitrate (3%)	4.55 c	120.44 bc	124.98 bc	1.6 b	1.9 c	1.6 c
Benzyl Aminopurine (10^{-5} M)	5.65 b	125.87 ab	131.52 ab	1.1 c	2.3 a	1.3 b

Numbers followed by different letter is significantly different from each other in the same parameter at $p<0.05$.

when plants were grown on media treated with 100 mM NaCl (Fig. 3b). Longest roots (16.2 cm) were observed in plants produced from seeds pre-soaked in SA and grown in media with no salinity. Whereas, shortest roots (9.5 cm) were observed in plants, which seeds were soaked only in distilled water and grown in media with 100 mM NaCl (Fig. 3b).

***Plant chlorophyll content*:** The chlorophyll "a" content was significantly influenced by seed pre-soaking and different salinity levels (Table 3 and 4). The maximum chlorophyll "a" content (6.60 mg g^{-1} dry weight) was recorded in leaves of plants obtained from seeds pre-soaked with SA, followed by 5.65 mg g^{-1} dry weight, when seeds were pre-soaked in BA. However, the control treatment plants showed minimum chlorophyll "a" content (4.04 mg g^{-1} dry weight) (Fig. 4a). Mean data for salinity levels showed that minimum chlorophyll "a" content (3.97 mg g^{-1} dry weight) was recorded in plants grown on media containing 100 mM NaCl concentration, while the maximum chlorophyll "a" content (6.32 mg g^{-1} dry weight) was observed in plants grown on media with no salinity (Fig. 4a).

Pre-soaking treatments of the cucumber seeds and salinity levels significantly affected the chlorophyll "b" content (Table 3 and 4). The mean values obtained for chlorophyll "b" content revealed that higher chlorophyll "b" content (136.52 mg g^{-1} dry weight) was recorded in plants produced from seeds treated with SA, which is statistically at par with chlorophyll "b" content (125.87 mg g^{-1} dry weight) of the plants produced when seeds were pre-soaked in BA, While the least chlorophyll "b" content (107.09 mg g^{-1} dry weight) was observed in plants when seeds were pre-soaked only in distilled water, which is statistically at par with chlorophyll "b" content (120.44 mg g^{-1} dry weight) observed in plants produced from seeds soaked in KNO_3 (Fig. 4b).

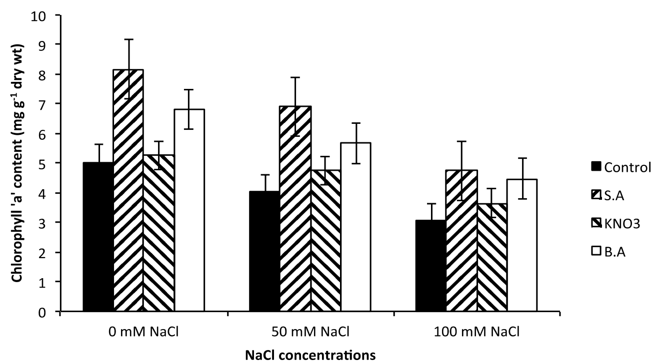

Figure 4a. Effect of different pre-soaking agents (salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control)) on chlorophyll 'a' content (mg g^{-1} dry weight) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment.

Similarly, comparing the data obtained for chlorophyll "b" content as affected by salinity levels showed that, higher chlorophyll "b" content (150.33 mg g^{-1} dry weight) was recorded in plants grown in media with no salinity and lower chlorophyll "b" content (99.16 mg g^{-1} dry weight) was observed in plants grown in media containing 100 mM NaCl (Fig. 4b).

The total chlorophyll content was also significantly affected by pre-soaking agents and salinity levels (Table 3 and 4). Statistical analysis of the data shows that seed pre-treatments with SA, KNO_3 and BA significantly influenced the total chlorophyll content (Table 4). Maximum total chlorophyll content (143.12 mg g^{-1}dry weight) was recorded for plants grown from seeds pre-treated with SA, which is statistically at par with the total chlorophyll content (131.52 mg g^{-1} dry weight) observed in plants when seeds were pre-treated with BA. The minimum total chlorophyll content (111.14 mg g^{-1} dry weight) was recorded for plants produced from seeds pre-soaked in only distilled water, which is statistically at par with the total chlorophyll content (124.98 mg g^{-1} dry weight) of the plants produced from seeds pre-soaked in KNO_3 (Fig. 4c). Mean data regarding salinity levels showed that plants grown in media treated with 100 mM NaCl salinity level showed the minimum total chlorophyll content (103.13 mg g^{-1} dry weight). While the maximum total chlorophyll content (156.64 mg g^{-1} dry weight) was observed for plants grown in media with no salinity treatment (Fig. 4c).

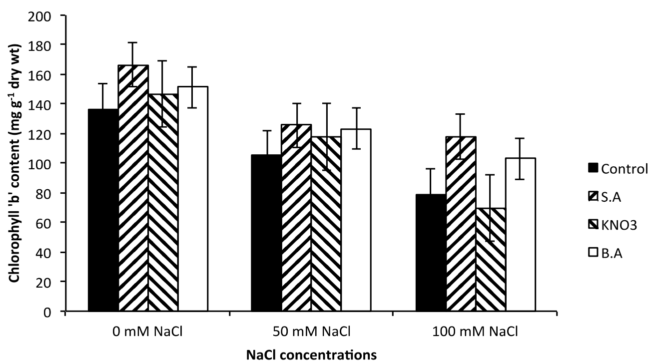

Figure 4b. Effect of salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control)on chlorophyll 'b' content (mg g^{-1} dry weight) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment**.**

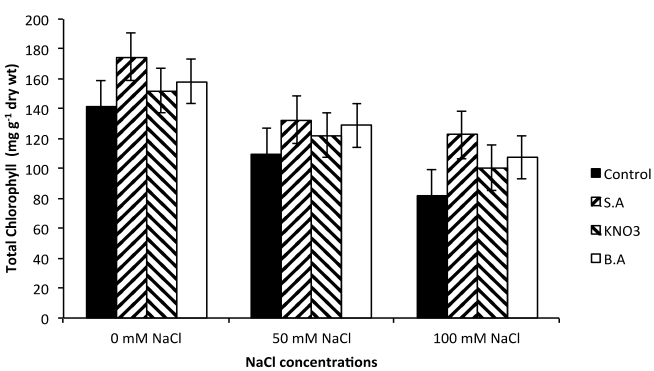

Figure 4c. Total chlorophyll content (mg g^{-1} dry weight) as affected by salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment.

***Sodium and potassium ions concentrations*:** Statistical analysis of the data showed that both pre-soaking agents and salinity levels influenced the sodium and potassium ions concentrations (Table 3 and 4). The mean values obtained for sodium ions (Na^+) concentrations revealed that lowest Na^+ concentration (1.1%) was observed in plants produced from seeds pre-soaked in BA, which is statistically at par with Na^+ concentrations (1.3%) of the plants produced from seeds pre-soaked in SA. The highest Na^+ concentrations (2.5%) was found in plants produced from seeds pre-soaked only in distilled water (control) (Fig. 5a). Similarly, the Na^+ concentration increases with increasing the salt level. The lowest Na^+ concentration (0.7%) was observed in plants grown in media with no salinity treatment. The highest Na^+ concentrations (2.6%) were observed in plants grown in media containing 100 mM NaCl (Fig. 5a).

The data pertaining potassium ion (K^+) concentration as affected by pre-soaking agents and different salinity levels showed that both have significantly affected the K^+ concentrations (Table 3 and 4). According to the statistical analysis the highest K^+ concentration (2.3%) was observed in plants, when seeds were pre-soaked in BA, followed by plants having K^+ (2.1%) while seeds were pre-soaked in SA. The lowest K^+ concentration (1.7%) was recorded in control plants (seeds treated with only distilled water) (Fig. 5b). Mean values for K^+ concentrations as affected by different salinity levels showed that highest K^+ (2.2%) was recorded in plants grown in media without salinity treatment (0 mM NaCl). Whereas, the lowest K^+ concentration (1.8%) was noted in plants grown in media containing 100 mM NaCl (Fig. 5b).

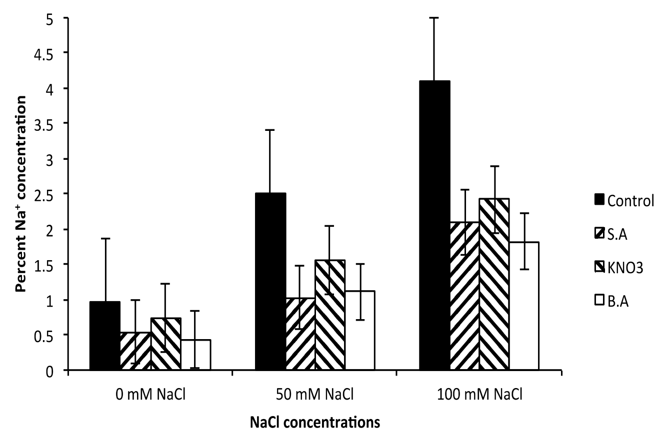

Figure 5a. Effect of different pre-soaking agents (salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) on percent Na^+ concentration under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment**.**

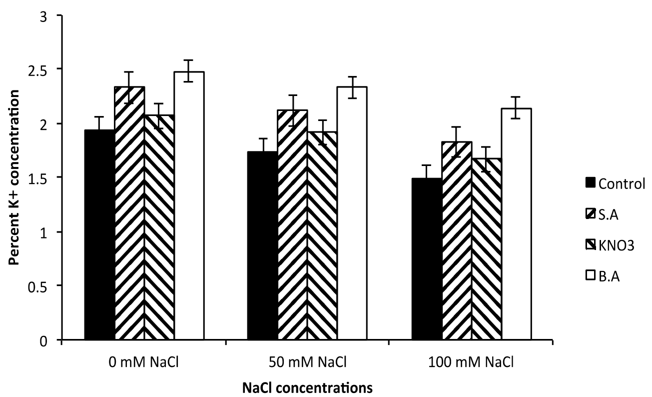

Figure 5b. Effect of salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) on percent K^+ concentration under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment**.**

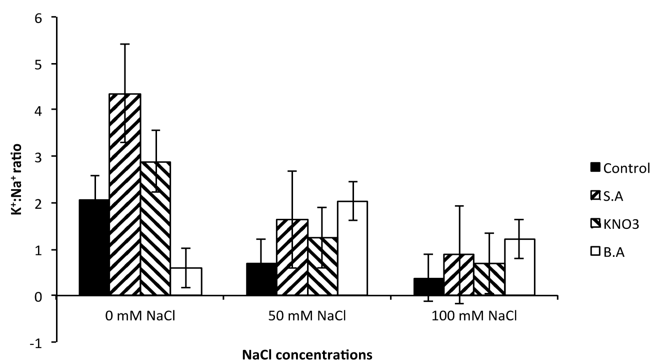

Figure 5c. K^+:Na^+ ratio as affected by salicylic acid (SA), potassium nitrate (KNO_3), benzyl aminopurine (BA) and distilled water (control) under salinity levels 0 mM NaCl (control), 50 mM NaCl and 100mM NaCl. Each bar represents mean of four plants replicated four times in each treatment.

According to the ANOVA, both factors (presoaking agents and salinity levels) significantly affected potassium/sodium ions (K^+/Na^+) ratio (Table 3 and 4). Mean data regarding different pre-soaking agents indicated that maximum K^+/Na^+ ratio (2.3) was recorded in plants that were grown from seeds pre-soaked in SA, followed by K^+/Na^+ ratio (1.3) in plants grown from seeds pre-soaked in BA. The minimum K^+/Na^+ ratio (1.1) was recorded in control treatment (seeds treated with only distilled water) (Fig. 5c). Mean data pertaining salinity levels showed that maximum K^+/Na^+ ratio (2.5) was observed in plants grown in media with no salinity (0 mM NaCl). In contrast, the minimum K^+/Na^+ ratio (0.8) was recorded in plants grown in media containing 100 mM NaCl (Fig. 5c).

DISCUSSION

The results from the current experiment indicated that salicylic acid (SA) was found most effective to obtain the highest fresh weight at all salinity levels compared to potassium nitrate (KNO_3), benzylaminopurine (BA) and control. Previous experiments have also shown that SA enhances plant growth performance under stress conditions. In an experiment, the results found by Németh *et al*. (2002) showed that addition of 0.5 mM SA in the hydroponics solution for maize, developed greater tolerance against drought.

Studies have shown that salinity in the growing media is unfavorable for growth and development of most of the plants, thereby restricting their physiological processes. Due to which stunting of plants growth occurred and thus results in lowering the weight and length of the plants. During this experiment, it was observed that both weight and length decreased with the increase in salinity of the growing media. Pirlak *et al*. (2004) also found comparable results while performing an experiment on strawberries, they stated that salinity reduce shoot dry weight, number of crowns and yield in strawberry crops.

During this experiment, it was observed that root dry weight obtained from 0 mM (0.1 g) and 50 mM NaCl (0.08 g) were statistically at par with each other, which may be due to the supporting role of SA for root development and spread for getting maximum nutrients and water. This indicates that SA induces salt tolerance ability in seedlings of cucumber. These results are similar to the studies of El-Tayeb (2005) who reported that barley presoaked seedling with SA produced higher shoot biomass under salinity. It was also supported by earlier experiments, that SA pretreated maize seeds produced maximum fresh and dry weight of shoot and roots under salt stress (Moussa *et al.*,2003).

Both shoot and root dry weights of cucumber in this experiment were higher when seeds were treated with SA, thus it shows that SA plays a key role in keeping the seedlings healthy enough during the salt stress conditions. It was also observed that when cucumber seeds were pre-soaked in SA, the resulting plants produce high shoot and root dry weights that also resulted in high total dry weight. These results are partially in agreement with the research study of Agarwal *et al*. (2005) who reported that SA increase antioxidant enzyme activities and decrease the stress, which causes increase in chlorophyll 'b' and carotenoid contents. It also increases the relative water that increased the total biomass of plants.

The maximum shoot and root length gained by plants produced from seeds treated with SA might be due to the growth regulating properties of SA, which enhances plants growth. SA promote both root and shoot growth, it helps indirectly in the process of photosynthesis. These results are similar with the finding of Gutiérrez-Coronado *et al*. (1998), who reported that SA have significant effect on shoot and root growth as well as on plant height of soybean. It was also reported that SA positively affects the plant height and yield of wheat genotypes (Agarwal *et al*., 2005). As SA promotes the growth in plants, it may encourage radicals for initiation of roots to balance the system. The positive influence of SA may be credited in increasing carbon dioxide assimilation and mineral uptake (Khan *et al*., 2003). For maximum nutrients uptake, large root system is necessary. Since strong and large root system consists of long roots. SA also helps in root formation and elongation. It is clear from the results of this experiment that seeds treated with SA have good root system even in saline condition, which helped in the survival of plants during stress condition. The finding of this experiment is in partial agreement with what was observed by Cano *et al*. (1991), that despite high level of salinity, addition of SA in the media might enhance root formation. It is further supported by Martinez *et al*. (1996), who stated that salt highly affect rooting and root growth but also positively correlate with salt tolerance at the whole plant level.

It was found that seeds pre-soaking treatments with SA provide favorable environment for different physiological and biochemical processes in plants (Turkyılmaz *et al*., 2005). Zhou *et al*. (1999) revealed that photosynthetic pigments in corn increased with the application of SA. Good vegetative growth is the result of maximum chlorophyll content in plants. The presence of high content of chlorophyll, guarantee higher amount of photosynthetic materials, which keep plants healthy. Results from earlier experiments and the current experiment showed that salinity decreased the chlorophyll content in the plants. On the other side, it was seen in the current experiment that SA positively affect cucumber seedling and encouraged it to gain more chlorophyll. These results are in line with Noreen *et al*. (2008) who stated that SA induced considerable enhancement in net photosynthetic rate even if the salinity level is at 200 mM NaCl. It is further justified by the work done by Arfan *et al*. (2007) and they reported that while NaCl was applied at the rate of 0.25 and 1 mM, SA increased the chlorophyll "a" content of leaves under salinity condition. Similarly, Pancheva *et al*. (1996) worked on long and short-term application of SA for 7 days and 2 hours respectively, on barley seedlings. The results showed that long-term treatment significantly decreased the chlorophyll "a" content whereas, the short-term treatment did not show any influence on chlorophyll "a" content as compared to untreated plants. Furthermore, De La Rosa *et al*. (1995) revealed from their experiment that photosynthetic pigments are greatly decreased under the influence of salinity as well as, chlorophyll and biomass decreased under salt stressed sorghum plants. They found that SA significantly affects photosynthetic pigments (chlorophyll "a", Chlorophyll "b" and carotenoids). In another study conducted on bean plants by Türkyılmaz *et al*. (2005), revealed that foliar application of SA increased Chlorophyll "a" content under normal field conditions. Seed pretreated with SA, BA and KNO_3 reduced Na^+ transport; however, the magnitude of reduction was higher in SA then BA and KNO_3 respectively. This reduction in Na^+ concentration might be due to the higher K^+ uptake with pre-soaking agents because of the fact those both are cations (Na+ and K+) and are therefore antagonistic to each other. Similar, results were reported by Moussa *et al*. (2003), who stated that exogenously applied SA decreased Na^+ concentration and increase chlorophyll 'a' and 'b' content in salt stressed maize plants.

Present data revealed that all the pre-soaking treatment enhanced K^+ concentration at all the salinity levels. It is well documented that salinity decreases K^+ and increases Na^+ as well as Cl^- concentration in most of the plants (Al-Harbi, 1995; Martinez *et al.*, 1989). However, increase in K^+ concentration due to BA and SA might be due to less Na^+ concentration in these treatments, as well as better shoot and root growth. An increase in K^+ concentration with the BA treated plants was already reported in rice (IR-6) under saline conditions (Gurmani *et al*., 2011).

One of the mechanisms of salt tolerant plants is the selective uptake and accumulation of ions, especially Na^+, Cl^- and K^+ Alian *et al*. (2000). Iqbal *et al*. (2006) reported that Na^+ affect the uptake of K^+ due to chemical similarities between the two cations. K^+ transport system involving good selectivity of K^+ over Na^+ can also be considered as an important salt tolerant determinant. The results from the present experiment showed higher K^+/Na^+ ratio by all the seed soaking agents; however, better K^+/Na^+ ratio was achieved with SA treated seeds, followed by KNO_3 and BA treated seeds respectively, at all the salinity levels.

Based on the results from the current experiment, it can be concluded that different soaking treatments 1) salicylic acid (SA), 2) potassium nitrate (KNO_3) and 3) benzylaminopurine (BA) improves plant performance against the adverse effect of salinity. However, seed pre-treatment with SA was more effective to escalate the growth and qualitative attributes of cucumber under salt stress conditions. In future, research work on other pre-soaking agents should be compared with SA. Similarly, different SA levels should be applied at various crop stages, to optimize the concentration of SA at specific crop stage to improve plant performance under salinity.

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