SEED POLYMORPHISM OF *RHYNCOSIA CAPITATA* (ROTH) DC. ENHANCE ITS TOLERANCE TO VARYING TYPES AND INTENSITY OF SALT STRESSES

Khalid Ali[1], Asif Tanveer[1], Naila Farooq[2], Tasawer Abbas[3*], Ghulam Sarwar[2], Muhammad Ather Nadeem[4], Ishtiaq Hassan[3], Muhammad Mansoor Javaid[4], Anees-ul-Hussnain Shah[5], Bilal Ahmad Khan[4] , Ali Raza[6]

DOI: https://doi.org/10.28941/pjwsr.v26i4.852

ABSTRACT

Increased soil salinity under changing climate has complicated weed management. *Rhynchosia capitata* (Clustered-flower Snoutbean) has become problematic weed in summer crops, such as cotton, soybean, pearl millet and mungbean worldwide. Current study was conducted to evaluate the impact of four types of salts stresses (NaCl, Na_2SO_4, $CaCl_2$ and $NaHCO_3$) at six different levels (0, 50, 100, 150, 200 and 250 mM) on *R. capitata* seeds of different sizes including small, medium and large. Results revealed that *R. capitata* can germinate over a wide range of salt stress but as the salinity level was increased to 250 mM the germination percentage and seedling growth decreased significantly. Larger seeds have more potential to germinate and grow vigorously at an increased salt concentration as compared to medium and small seeds. Salt stress caused 40-73%, 59-96% and 40-100% inhibition in seed germination, seedling length and dry weight, respectively. Among various salt stresses $CaCl_2$ showed less inhibition of *R. capitata*. The higher tolerance of this weed to wide range of salt stresses is alarming factor under current and anticipated increase in salinity, as it will disturb management plans by changing critical completion period and threshold level due to more adaptability of weed under stress than crop plants.

Keywords: Climate change, *Rhynchosia capitata,* salt tolerance in weed, soil salinity, lsummer weeds

Citation: Ali, K., A. Tanveer, N. Farooq, T. Abbas, G. Sarwar, M. A. Nadeem, I. Hassan, M. M. Javaid, A.H. Shah, B. A. Khan, A. Raza. 2020. Seed polymorphism of *rhyncosia capitata* (roth) dc. Enhance its tolerance to varying types and intensity of salt stresses. Pak. J. Weed Sci. Res. 26(4): 393- 401.

[1] Department of Agronomy, University of Agriculture Faisalabad, 38040, Pakistan.
[2] Department of Soil & Environmental Sciences, College of Agriculture, University of Sargodha, Pakistan
[3] In-service Agriculture Training Institute, Sargodha, Pakistan
[4] Department of Agronomy, College of Agriculture, University of Sargodha, Sargodha, Pakistan
[5] Fodder Research Institute Sargodha.
[6] Adaptive Research Farm Sargodha.
*Corresponding Author, Email: tagondaluaf@gmail.com, ORCID of corresponding author: 0000-0002-9533-0795

INTRODUCTION

Changing climate conditions and land use activities are causing various hazards to agricultural sustainability. The major anticipated issue to sustainable agriculture is the increase in soil salinity (Dasgupta *et al.,* 2015). In addition to various harmful effects of salinity on crop growth and yield it will also alter the weed management strategies. For example, the more tolerance of weed species to salt stresses as compared to crop plants will give advantage to weed plants and alter the critical weed competition periods and economic threshold level of weed species. Generally, weeds show more tolerance to stress conditions than crop plants, however tolerance varies depending on type of salinity and weed species (Tanveer and Shah*,* 2017).

Weeds from fabaceae family especially genus Rhyncosia are distributed to various tropical areas. *Rhyncosia capitata* is fast spreading weed of summer season and found in locations in hilly areas of Pakistan (Jahan *et al.,* 1994) and other tropical regions in Asia (Dogra *et al.,* 2009; IIdis*,* 2010). Furthermore, this weed has become one of the tops troublesome weeds in southern Punjab, Pakistan and is emerging threat to the sustainability of cropping system (Ali *et al.,* 2011). The way of prorogation of this weed is seeds, that normally germinate after first irrigation to crop. It is spreading weed with flexible horizontal stem and each node produce branches and his own roots. Early flowering potential just at age of one month and high numbers of seeds make this weed common and fast spreading (Sharma *et al.,* 1978). Additionally, seeds of this weed have high dormancy that keeps these seeds viable for long periods of time and survive after exposure to herbicides application in soil (Ali *et al.,* 2011).

Soil salinity is major factor to influence the weed seed germination and invasion in new areas. Salt stress is widespread problem in Asia, especially irrigated areas due to deteriorated quality of underground water (Azhar, Tariq*,* 2003). Osmotic effect due to high salt contents in soil and toxicity of iron negatively influence the germination of seeds (Hajlaoui *et al.,* 2007). Water stress reduced the water using ability of plants which caused metabolic changes and inhibit plant development (Munns*,* 2002). Plant species showed different response to salt stresses, for example legumes are more sensitive to salt stresses which is major yield reducing factor in salt effected areas (Lluch *et al.,* 2007). Salt stress exert more negative impact at germination and early seedling growth as compared to mature crop plants (Dodd, Donovan, 1999). Salt stress hinders the intake of water in germinating seeds and inhibit germination (Keiffer*,* Ungar*,* 1995). High concentration of salts in germination media negatively influence the weight of radical (Sedghi and Nemati*,* 2010). Furthermore, high salt concentration reduces photosynthetic process and cause decrease in production. This reduction may be either because of closer of stomata and decline in observation of CO_2, hence low fixation of Carbon, it ultimately reduces photosynthetic activity (Rivelli *et al.,* 2002).

To best of our knowledge the effect of salt stresses on *R. capitata* is not determined yet. Therefore, we used four types of salts at six different concentrations to determine their effect on germination and seedling growth of *R. capitata* seeds of different sizes. The outcomes of this study will help to optimize the weed management strategies to tackle crop yield losses due to *R. capitata* in saline soils and to determine the role of salt tolerance in weed invasion. As increase in soil salinity is anticipated due to climate change.

MATERIAL AND METHODS

The study was conducted in Laboratory, to determine the influence of various types of salt stresses and their doses on seed germination and growth *R*. *capitata* seeds having different size*.* Experiment was laid out in completely randomized design with factorial arrangement having three replications. Seed was graded into three categories, small (360 × 180 μm), medium (384 × 262 μm) and large (450 × 283.5 μm) based on visual observation and the seed size was determined using micrometer. Seed weight was determined by unitary method. Each and every seed was scarified with sand paper to break seed coat dormancy. Four types of salts including NaCl, Na_2SO_4, $CaCl_2$ and $NaHCO_3$ were used. Stock solution (500 ml) of each salt was prepared. For that purpose, 29.25 g of NaCl, 71 g of Na_2SO_4, 55.5 g of $CaCl_2$ and 42 g of $NaHCO_3$ were dissolved separately in small amount of distilled water and made the volume up to 500 ml.

Experiment was performed using blotting papers. Blotting papers were laid down and small amount of water was sprinkled with shower and 10 seeds for each treatment were placed. Blotting paper was folded four times, stapled and kept erect in a zipper bag and then 30 ml of various concentrations NaCl was poured in each bag separately. After that zipper bag was sealed. Three replications of each treatment were prepared in the same way. This method is called rag-doll method. Experiments for other three salts (Na_2SO_4, $CaCl_2$ and $NaHCO_3$) were performed in the same way. The temperature range during the experiment was 30 ± 2°C and 33 ± 2°C, in laboratory conditions.

Data on parameters including germination percentage, seedling length and dry weight of weed were recorded during study by using standard procedures and analyzed statistically by Fisher's analysis of various techniques. Tukey's Honestly Significant Difference (HSD) test at 5% probability was used to compare treatment's means (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Germination of *R. Capitata*

NaCl stress. The data given in Table 1 indicate that germination percentage of *R. capitata* was significantly influenced by NaCl concentration and seed size. Germination percentage of *R. capitata* decreased with an increase in salt concentrations but increased with an increased seed size. Maximum germination percentage (58.88%) was obtained at 50 mM NaCl concentration which was statistically similar with germination percentage at 100, 150 and 200 mM NaCl concentration and control as well. The significantly minimum germination percentage (24.44%) was recorded at 250 mM NaCl concentration. Large seeds gave maximum germination percentage (57.78%) which was statistically not different from that of medium seeds, while lowest germination percentage (42.22%) was obtained in small seeds.

Na_2SO_4 stress- Na_2SO_4 concentration and seed size also influenced the germination percentage of *R. capitata* significantly. Germination percentage enhanced with enhancement in seed size but as salt concentration raised, germination percentage declined. Maximum germination percentage (56.78 %) was obtained at 50 mM Na_2SO_4 concentration. Lowest germination percentage (12.67 %) was obtained at 250 mM Na_2SO_4 which was statistically alike to that at 200 mM Na_2SO_4 concentration.

$CaCl_2$ stress- Various concentrations of $CaCl_2$ affected the germination percentage of *R. capiata* significantly but effect of seed size was non-significant. As the salt concentration enhanced, germination percentage of *R. capiata* decreased. Highest germination percentage (56.66 %) was recorded at 50 mM $CaCl_2$ concentration which was statistically not different from germination percentage at 100, 150 and 200 mM $CaCl_2$ concentration and control as well. Minimum germination percentage (36.66%) was observed at 250 mM $CaCl_2$ concentration.

$NaHCO_3$ stress- Various concentrations of $NaHCO_3$ affected the germination percentage of *R. capiata* significantly. Germination percentage of *R. capiata* decreased as the concentration of $NaHCO_3$ enhanced. Highest germination percentage (64.44%) was recorded at 50 mM $NaHCO_3$ concentration and it was statistically at par with germination percentage at 100 and 150 mM $NaHCO_3$ concentration and control as well. Minimum germination percentage (20%) was observed at 250 mM $NaHCO_3$ concentration.

Seedling length

NaCl stress- The data given in Table 2 illustrate that NaCl concentrations and seed size influenced the seedling length of *R. capitata* significantly. Seedling length increased with an increase in seed size but when salt concentration went higher, seedling length declined. Longest seedling (18.31 cm) was obtained at 50 mM NaCl concentration. Lowest seedling length (3.86 cm) was recorded at 250 mM NaCl concentration. Highest seedling length (12.63 cm) was recorded in large seeds which was statistically similar to seedling length in medium seeds while shortest seedling length (11.58 cm) was recorded in small seeds.

Na_2SO_4 stress- Various Na_2SO_4 concentrations significantly affected the seedling length of *R. capiata*. As salt concentration increased, seedling length decreased. Highest seedling length (13.52 cm) was recorded at 50 mM Na_2SO_4 concentration which was statistically similar to seedling length at 100 mM Na_2SO_4 concentration and control as well. Minimum seedling length (5.32 cm) was observed at 250 mM which was statistically not different from seedling length at 100, 150 and 200 mM Na_2SO_4 concentration. Effect of seed size on seedling length was non-significant in case of Na_2SO_4.

$CaCl_2$ stress- Different $CaCl_2$ concentrations and seed size influenced the seedling length of *R. capitata* significantly. Seedling length increased with an increase in seed size but when salt concentration raised, seedling length declined. Maximum seedling length (15.24 cm) was obtained at 50 mM $CaCl_2$ concentration. Lowest seedling length (7.62 cm) was recorded at 250 mM $CaCl_2$ which was statistically not different from seedling length at 200 mM $CaCl_2$ concentration. Highest seedling length (13.78 cm) was recorded in large seeds which was statistically similar to seedling length in medium seeds while shortest seedling length (11.94 cm) was recorded in small seeds.

$NaHCO_3$ stress- Seedling length of *R. capitata* was significantly influenced by $NaHCO_3$ concentrations and seed size. Seedling length decreased with an increase in salt concentration but increased with an increased seed size. Maximum seedling length **(**16.85 cm) was observed at 50 mM $NaHCO_3$ concentration which was statistically at par with seedling length in check. The significantly minimum seedling length (0.72 cm) was recorded at 250 mM $NaHCO_3$ concentration. Effect of seed size on seedling length was non-significant.

Reduction in germination of *R. capitata* due to salinity might be because of its effect on imbibition process of germinating seeds by changing external osmotic potential or due to toxicity that occurred because of increase in Na^+ and Cl^- ions (Khajeh-Hosseini *et al.,* 2003). Understanding regarding effect of stress on weed germination and determination optimum conditions for growth of any specific weed is important to determine of competitive potential of any weed (Chauhan and Johnson, 2010). In relation to ecological situations, estimate of weed seed germination and its emergence is necessary to practice suitable weed control method (Ghorbani *et al.,* 1999). According to Rao *et al.,* (2008) as the concentration of NaCl was enhanced from 0 to 320 mM the germination of American sloughgrass (*Bechmannia syzigachne*) seeds reduced accordingly. Germination was about 80% at 40 mM NaCl level but reduction in germination occurred to 36% at 160 mM NaCl and no germination was observed at 320 mM NaCl concentration. Alatar*,* (2011) conducted a research to assess the impact of salinity on seed germination of *Achillea fragrantissima* and *Moringa peregrine* and stated that as the concentration of salt was enhanced the germination percentage declined. The minimum germination percentage was recorded at 5000 ppm (15.3 and 60.7% for *A. fragrantissma* and *M. peregrine*, respectively).

Dry weight of *R. capitata*

NaCl stress- The data given in Table 3 illustrate that seedling dry weight of *R. capitata* was significantly influenced by NaCl concentration and seed size. Maximum seedling dry weight (0.05 mg) was recorded at 50, 100 and 150 mM NaCl concentration and control. These treatments did not differ statistically. Minimum seedling dry weight (0.01mg) was recorded at 250 mM NaCl concentration which was statistically not different from seedling dry weight at 200 mM NaCl concentration. Large seeds gave more seedling dry weight (0.05 mg) which was statistically not different from seedling dry weight in medium seeds. Minimum seedling dry weight (0.03 mg) was obtained in small seeds.

Na_2SO_4 stress- Seedling dry weight of *R. capitata* was significantly influenced by Na_2SO_4 concentration and seed size. Maximum seedling dry weight **(**0.05 mg) of *R. capitata* was obtained at 50 mM Na_2SO_4 concentration which was statistically at par with seedling dry weight at 100 mM Na_2SO_4 concentration and control also. Minimum seedling dry weight (0.01mg) was recorded at 250 mM Na_2SO_4 concentration which was statistically alike to seedling dry weight at 100 and 200 mM Na_2SO_4 concentration. Large seeds gave significantly maximum seedling dry weight (0.04 mg). Minimum seedling dry weight (0.03 mg) was obtained in small seeds which was statistically similar to seedling dry weight in medium seed.

$CaCl_2$ stress- Various salt concentrations of $CaCl_2$ affected the seedling dry weight of *R. capiata* significantly. Maximum seedling dry weight (0.05 mg) was recorded at 50 mM $CaCl_2$ concentration which was statistically similar to seedling dry weight at 100 and 150 mM $CaCl_2$ concentration and control as well. Minimum seedling dry weight (0.03 mg) was observed at 250 mM $CaCl_2$ concentration. Effect of seed size on seedling dry weight was non-significant.

$NaHCO_3$ stress- Seedling dry weight of *R. capitata* was significantly influenced by $NaHCO_3$ concentration and seed size. Seedling dry weight **(**0.04 mg) was maximum at 50 mM $NaHCO_3$ concentration and it was statistically similar with seedling dry weight at 100, and 150 mM $NaHCO_3$ concentration and control as well. Minimum seedling dry weight (0.00 mg) was recorded at 250 mM $NaHCO_3$ concentration. Large seeds gave maximum seedling dry weight (0.04 mg) which was statistically at par with seedling dry weight in medium seeds. Minimum seedling dry weight (0.02 mg) was obtained in small seeds.

Differential response of germination and growth due to different size of weed seeds is important factor for successful weed spread and adaptability under different conditions. More tolerance of large size seeds of *R. capitata* to salt stresses will increase the adaptability of this weed under saline conditions. According to Tanveer *et al.,* (2013), bigger seeds of field bindweed (*Convolvulus arvensis* L.) gave more seedling establishment and rapid germination compared to smaller seeds despite of water stress or deeper seeding depth. Our results support the findings of Bentley (1980) who reported that large seeds contain more available resources against the small seeds, so the seedlings emerging from these larger seeds have length higher than those of seedlings emerging from small seeds. Sedghi and Nemati (2010) reported that germination and seedling dry weight of milk thistle (*Silybum marianum* L.) was badly influenced by enhanced levels (10.0 dS m^{-1} EC) of NaCl salinity. As area under salt effected soil is increasing due to changing climate and land use activities, therefore understanding on impact of salt stress on weeds is important to estimate weed crop competition and to optimize potential weed management tactics.

The tolerance of *R. capitata* to salt stress as compared to crop plants will make this weed more competitive and troublesome. It will also change the critical competition period and economic threshold level under saline conditions. The present study considering *R. capitata* as test weed plant will give new direction to weed researchers to study the response of other weeds under saline conditions and to develop effective weed management strategies to tackle crop yield losses in future (Dasgupta *et al.,* 2015).

Present findings conclude that *R. capitata* has strong tolerance against different levels of NaCl, Na_2SO_4, $CaCl_2$ and $NaHCO_3$ stress. Larger seeds have more potential to germinate and grow vigorously at an increased salt concentration as compared to medium and small seeds. Salt stress caused 40-73%, 59-96% and 40-100% inhibition in seed germination, seedling length and dry weight, respectively.

REFERENCES CITED

Ali, H.H., A. Tanveer, M.A. Nadeem and H.N. Asghar. 2011. Methods to break seed dormancy of *R. capitata,* a summer annual weed. Chil. J. Agr. Res., (71): 483-487.

Azhar, M.J and M.Y. Tariq. 2003. Water crises and crop production gaps in Punjab: identification, risk analysis and mitigation measures (Wheat perspectives: Improving resource management to sustain food security). Pak. J. Bot., (41): 2209-2216.

Bentley, S., J.B. Whittacker and A.J.C. Malloch. 1980. Field experiments on the effects of grazing by a chrysomelid beetle (*Gastrophysa Viridula*) on seed production and quality in *Rumex obtusifolius* and *Rumex crispus.* J. Ecol., (68): 671-674.

Chauhan, B.S., and D.E. Johnson. The role of seed ecology in improving weed management strategies in the tropics. Adv. Agron., 2010(105): 221-262.

Dasgupta, S., M.M. Hossain, M. Huq and D.Wheeler. 2015. Climate change and soil salinity: The case of coastal Bangladesh. Ambio., 44(1): 815-826.

Dodd, G.L. and L.Donovan. 1999. Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. Am. J. Bot., 86: 1146-1153.

Dogra, K.S., S.K. Sood, P.K. Dobhal and S. Kumar. 2009. Comparison of understory vegetation in exotic and indigenous tree plantations in Shivalik Hills of N.W. Indian Himalayas (Himachal Pradesh). J. Ecol. Nat. Environ., 1:130-136.

Ghorbani, R., W. Seel and C. Leiferr. 1999. Effects of environmental factors on germination and emergence of *Amaranthus retroflexus*. Weed Sci., 47: 505-510.

Hajlaoui, H., M. Denden and M. Bouslama. 2007. Etude de la variabilité intraspécifique de tolérance au stress salin du pois chiche (*Cicer arietinum* L.) au stade germination. Tropicultura., 25:168-173.

ILDIS (International Legume Database and Information Service). 2010. Available from: http://www.ildis.org/LegumeWeb, 2010.

Jahan, B., A.A Vahidy and S.I. Ali. 1994. Chromosome numbers in some taxa of Fabaceae mostly native to Pakistan. Ann. Missouri. Bot. Gard., 81: 792-799.

Keiffer, C.H and I.A. Ungar. Germination responses of halophytes exposed to prolonged hyper saline conditions. In biology of salt tolerant plants. Khan MA, Ungar IA (eds.). Department of Botany, University of Karachi, Karachi, Pakistan. 1995;43-50.

Lluch, C, N. Tejera, J.A Herrera-cervera, M. Lopez, J.R. Barranco-gresa, F.J. Palma, M. Gozalvez, C. Iribarne, E. Moreno, and A. Ocana. 2007. Saline stress tolerance in legumes. Lotus Newslett., 37: 76-77.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell and Environment., 25: 239-250.

Rao, N., L. Dong, J. Li and H. Zhang. 2008.Influence of environmental factors seed germination and seedling emergence of American sloughgrass (*Bechmannia syzigachne*). Weed Sci., 56*:* 529-533.

Rivelli, A.R., S. Lovelli and M. Perniola. 2002. Effects of salinity on gas exchange, water relations and growth of sunflower (*Helianthus annuus*). Funct. Plant Biol., 29: 1405–1415.

Sedghi, M., and A. Nemati 2010. Influence of different priming materials on germination and seedling establishment of Milk thistle (*Silybum marianum*) under salinity stress. World Appl. Sci. J., 11: 604-609.

Sharma, N.K., M.M. Sharma and D.N Sen. 1978. Seed perpetuation in *Rhynchosia capitata* DC. Biologia. Plantarum., 20: 225-228.

Steel RCD, Torrie JH, Dickey DA. Principles and Procedures of Statistics: A Biometrical Approach. McGraw Hill Book Co., Inc. New York. 1997;3: 400-428.

Tanveer, A., M. Tasneem, A. Khaliq, M.M. Javaid and M. N. 2013. Chaudhry. Influence of seed size and ecological factors on the germination and emergence of field bindweed (*convolvulus arvensis*) planta daninha, viçosa-MG., 1: 39-51.

Tanveer, M and A.N. Shah. 2017. An insight into salt stress tolerance mechanisms of Chenopodium album. Environ Sci Poll Res., 24: 16531-16535.

Table.1 Impact of salt stress on germination percentage (%) of *Rhyncosia capitata* (DC) seed of different sizes.

Seed size	**NaCl**	**Na_2SO_4**	**$CaCl_2$**	**$NaHCO_3$**
Small	42.22 B	35.00 B	45.55	44.44
Medium	45.56 AB	40.00 AB	51.11	46.11
Large	57.78 A	48.33 A	57.22	52.22
LSD	13.21	9.98	NS	NS
Salt concentration				
0 mM (control)	61.11 A	61.33 A	60.00 A	65.56 A
50 mM	58.88 A (3.64%)	56.78 A (7.41%)	56.66 A (5.56%)	64.44 A (1.70%)
100 mM	53.33 A (12.73%)	51.11 AB (16.66%)	54.44 AB (9.26%)	61.11 A (6.78%)
150 mM	50.00 A (18.18%)	36.67 BC (36.26%)	51.11 AB (14.81%)	55.55 AB (15.26%)
200 mM	43.33 AB (29.09%)	21.11 CD (65.56%)	46.88 AB (21.86%)	28.78 B (56.10%)
250 mM	24.44 B (60.00%)	12.67 D (72.80)	36.66 B (40.00%)	20.00 C (69.49%)
HSD value 5%	22.99	17.37	19.86	19.77

Values in parentheses indicate the decrease over check. Means not sharing a letter in a common differ significantly at 5% level of significance.

Table 2. Impact of salt stress on seedling length (cm) of *Rhyncosia capitata* (DC) seed of different sizes.

Seed size	**NaCl**	**Na_2SO_4**	**$CaCl_2$**	**$NaHCO_3$**
Small	11.58 B	8.55	11.94 B	11.31
Medium	12.58 AB	8.92	13.26 AB	12.75
Large	12.63 A	11.89	13.78 A	12.80
LSD	0.09	NS	1.60	NS
Salt concentration				
0 mM (control)	18.91 A	15.68 A	18.61 A	17.79 A
50 mM	18.31 A (3.17%)	13.52 A (13.77%)	15.24 B (18.10%)	16.85 AB (5.28%)
100 mM	10.52 B (44.36%)	12.14 AB (22.57%)	14.26 B (23.37%)	14.26 BC (19.83%)
150 mM	10.38 B (45.10%)	6.24 B (60.20%)	13.13 B (29.44%)	13.06CD (26.58%)
200 mM	10.25 B (45.79%)	5.83 B (62.81%)	9.10 C (51.10%)	11.20 D (37.04%)
250 mM	3.86 C (81.96%)	5.32 B (66.07%)	7.62 C (59.05%)	0.72 E (95.95%)
HSD value 5%	2.07	6.86	2.78	2.88

Values in parentheses indicate the decrease over check. Means not sharing a letter in a common differ significantly at 5% level of significance.

Table 3. Impact of salt stress on seedling dry weight (mg) of *Rhyncosia capitata* (DC) seed of different sizes.

Seed size	NaCl	Na_2SO_4	$CaCl_2$	$NaHCO_3$
Small	0.03 B	0.03 B	0.04	0.02 B
Medium	0.04 AB	0.03 B	0.05	0.03 AB
Large	0.05 A	0.04 A	0.05	0.04 A
HSD value 5%	0.01	0.01	NS	0.01
Salt concentration				
0 mM (control)	0.05 A	0.06 A	0.05 A	0.04 A
50 mM	0.05 A (00.00%)	0.05 A (16.66%)	0.05 (00.00%)	0.04 A (00.00%)
100 mM	0.05 A (00.00%)	0.04 AB (33.33%)	0.05 A (00.00%)	0.03 AB (25.00%)
150 mM	0.05 A (00.00%)	0.02 BC (66.66%)	0.05 A (00.00%)	0.03 AB (25.00%)
200 mM	0.02 B (60.00%)	0.01 C (83.33%)	0.03 AB (40.00%)	0.02 B (50.00%)
250 mM	0.01 B (80.00%)	0.01 C n(83.33%)	0.03 AB (40.00%)	0.00 C (100.00%)
HSD value 5%	0.02	0.02	0.02	0.01

Figures in parentheses indicate the decrease over check. Means not sharing a letter in a common differ significantly at 5% level of significance**.**