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Seed Germination in Lucerne (*Medicago sativa* L. cv Ek Sali) under NaCl Stress at Winter and Summer Temperatures

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A B S T R A C T

Seed germination experiments were conducted with 10, 20, 50, 100, 200 and 500 mM NaCl stress at 34.3 ± 5.2°C of summer and 17.6 ± 3.4°C of winter in laboratory conditions. Highest germination (92.33±4.50%) was recorded with 50mM NaCl at 34.3 ± 5.2°C. Seed germination (70.00 ± 3.00%) significantly (P=0.05) decreased in 500mM at 34.3 ± 5.2°C whereas seeds failed to germinate with this NaCl concentration at 17.6 ± 3.4°C. Radicle length (6.73 ± 2.23cm), dry weight (0.69 ± 0.06g) of 100 seeds and germination index (773.00 ± 9.6) were also significantly (P=0.05) increased under 50mM NaCl at 34.3 ± 5.2°C when compared with the control. Seed germination and seedling growth parameters were significantly (P=0.01) decreased under 500Mm salinity at 34.3 ± 5.2°C. Comparatively, the seed germination decreased in control and all the salinity treatments at lower temperature (17.6 ± 3.4°C). Seed dry weight and germination index also significantly (P=0.01) decreased whereas total spread of germination increased at 17.6 ± 3.4°C.

***Key words*:** Salinity stress, Tolerance, Radicle length, Germination index, Coefficient of velocity of germination

Salinity is a major environmental problem that has affected approximately 7% of world land which is nearly 20% of 230 million hectare of irrigated land. Several workers [1-4] have observed maximum seed germination in distilled water whereas low germination was recorded under elevated salinity. Salinity stress has caused the loss of agricultural production in arid and semi-arid regions by reducing the seed germination and delaying the seedling establishment [5]. Physiologists have described four essential stages in the germination. It begins with imbibition of water during which water penetrates into the embryo and hydrates the proteins and other colloids. The second stage is the reactivation of enzymes leading to increased metabolic activity. The third stage is the elongation of radicle cells followed by emergence of radicle from the seed coat, termed as "germination proper" finally, the subsequent growth of seedling. But it is a complex process. During the germination the role of growth promoting hormone GA has been found significant. Germination occurs under strict hormonal regulation, especially GA and ABA. ABA promotes seed dormancy whereas GA is a dormancy releasing hormone and stimulates the seed germination, thus controlling inhibitory effects of ABA. Ethylene (ET) and Brassinosteroids (BRs) also have a positive effect on seed germination, controlling the inhibitory effect of ABA [6-9]. Salt stress affects the seed germination and seedling establishment by creating osmotic stress, ion toxicity and oxidative stress [10]. Alfalfa also called lucerne is worldwide forage originated in Iran, Turkey, Turkmenistan and Caucasus [11] cultivated in whole Asia and parts of India as a green fodder for the horses, cows, buffalos and other cattle. Ek Sali is a popular cultivar of *Medicago sativa* L. grown in most of the north-west states of India as a winter crop. This paper deals with the seed germination of Ek Sali cultivar of lucerne under varying levels of NaCl stress at 34.3 ± 5.2°C (summer) and 17.6 ± 3.4°C (winter) temperatures.

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MATERIALS AND METHODS

Seeds of Ek Sali cultivar of lucerne (*Medicago sativa* L.) marketed by Gujarat based company (Narayani seeds of Shah Bhupendra Kumar Ramniklal, Ahmedabad) in India, were procured from the company outlet of old Delhi seed market. The experiments were conducted in plant physiology and ecology lab at Government Science College, Gwalior, M.P. India. The seed germination studies were carried out under 0, 10, 20, 50, 100, 200 and 500mM stress of NaCl at two temperatures, between 1st-9th January 2021 at 17.6 ± 3.4°C and 20th-29th May 2021 at 34.3 ± 5.2°C. The seeds in the lots of 100 were pre-soaked overnight in respective NaCl concentrations and subsequently planted on the Whatman's filter paper no. 1 duly moistened with respective salt concentrations in glass petri-plates of 15 cm size. Three replicates of treatments were used in the experiment. Standard seed germinator was used for germination. 1ml of NaCl solution of different concentrations was added over respective filter papers at 3 days interval. Radicle emergence was considered as the criterion for seed germination. Seed germination was studied for a period of 9

days. The numbers of germinated seeds were counted on daily basis till further no radicle emergence was observed. The following parameters were studied:

Final Germination Percentage (FGP) and Dry Weights (DW) of germinated seeds were recorded. Radicle and hypocotyl lengths were also measured by a millimetre scale after 9 days of germination.

Mean Germination Time (MGT)

It is an index of seed germination speed and velocity. It was calculated by the formula of Ellis and Roberts [12].

$$MGT = \sum(n \times d) / N$$

Where, n= number of seeds germinated corresponding to day d
N= Total number of seeds germinated

*Germination index (*GI)

This was calculated as per the equation given by Kader [13]

$$GI= (9 \times n_1) + (8 \times n_2) + (7 \times n_3) + \text{-------} (1 \times n_{100})$$

Where n1, n2…. N9 are number of germinated seeds on 1st, 2nd and subsequent days until 9th day.

Coefficient of Velocity of Germination (CVG)

This is the index of seed germination speed & velocity and was calculated by the formula of Scott *et al*. [14]

$$CVG = \sum N_i/N_iT_i \times 100$$

Where, N = number of seeds germinated on day i
T= Number of days from sowing corresponding to N

Total Spread of Germination (TSG)

This is the total span of seed germination from first day to last day occurring in a seed lot.

Statistical test of analysis of variance (ANOVA) was performed to compare the mean values of control and treatments for different parameters of germination by using SPSS version 23 software of IBM.

RESULTS AND DISCUSSION

At the lower temperature (17.6 ± 3.4°C) of winter, highest germination (82.33 ± 3.51%) was recorded in the control whereas germination percentage sharply decreased with all the NaCl treatments (Table 1, Fig 1). In a study carried out by Fu *et al*. [15], low temperature (5°C) negatively affects the seed germination percentage, germination energy and first germination time in *Elymus nutans* whereas it increased the mortality percentage when compared with control plants at 25°C. In our study, seeds failed to germinate at 500mM salinity. Peak (5.90 ± 0.75cm) of radicle length was recorded with 20mM NaCl treatment at lower temperature (17.6 ± 3.4°C) after which it successively (5.50 ± 0.75, 4.66 ± 0.70, 3.00 ± 0.70cm) decreased with 50, 100, 200mM salinity respectively (Table 1, Fig 2). Hypocotyl length (10.25 ± 1.1cm) was found to be significantly (P=0.05) increased with 20mM concentration but significantly (P=0.01) decreased with 200mM NaCl and recorded as 5.26 ± 0.40cm at 17.6 ± 3.4°C (Table 1, Fig 3). Seed dry weights (0.43 ± 0.01, 0.54 ± 0.01, 0.55 ± 0.02g) were found significantly (P=0.01) increased with salinity treatments respectively at 10, 20, 50mM of NaCl when compared with 0.39g of the control. Mean germination time (1.65 ± 0.03, 1.69 ± 0.07, 1.74 ± 0.10, 1.79 ± 0.10, 1.91 ± 0.08) successively increased with increased NaCl concentrations of 10, 20, 50, 100 and 200mM respectively. Thus, showing that the higher salt concentration adversely affects the germination and seeds take more time to germinate. At the lower temperature of 17.6 ± 3.4°C, peak (696.00 ± 12.2) of germination index was recorded in control and significantly (P=0.01) decreased with all the NaCl concentrations at 17.6 ± 3.4°C (Table 1). Highest coefficient of velocity (66.39 ± 4.71) was also observed in the control germination and significantly (P=0.05) decreased with 50 and 100mM salinity. Thus, highest seed germination speed was observed in the control germination. Total span of germination significantly (P=0.01) increased with salinity treatments and recorded as 6.00 days under 20, 50,100 and 200mM of NaCl salinity when compared with 5.00 days of the control at 17.6 ± 3.4°C.

Table 1 Germination and seedling growth parameters of lucerne (*Medicago sativa* L. cv. Ek sali) at 17.6 ± 3.4°C

NaCl (Mm)	Germination percentage	Radicle length (cm)	Hypocotyl length (cm)	Dry Weight of germinated seeds (g)	Mean germination time	Germination index	Coefficient of velocity of germination	Total spread of germination
0	82.33±3.51	3.80±0.34	8.76±0.61	0.39±0.00	1.50±0.08	696.00±12.2	66.39±4.71	5.00±00
10	69.00**±5.56	4.80^{NS}±0.70	9.91^{NS}±0.54	0.43**±0.01	1.65*±0.03	566.00**±6.08	60.52^{NS}±5.78	5.66**±0.57
20	70.00**±3.0	5.90**±0.75	10.25*±1.1	0.54**±0.01	1.69**±0.07	583.00**±8.18	58.97^{NS}±4.83	6.00**±00
50	75.33*±4.04	5.50**±0.75	9.76^{NS}±0.65	0.55**±0.02	1.74**±0.10	618.00**±11.78	57.37*±4.31	6.00**±00
100	74.66*±3.21	4.66^{NS}±0.70	8.60^{NS}±1.08	0.41^{NS}±0.01	1.79**±0.10	606.00**±8.18	55.71*±3.74	6.00**±00
200	69.00**±3.00	3.00^{NS}±0.70	5.26**±0.40	0.30**±0.01	1.91**±0.08	568.00**±14.73	52.27**±5.44	6.00**±00
500	NG	NG	NG	NG	NG	NG	NG	NG

Not Germinated (NG), Non-Significant (NS), *Significant at 5% level; **Significant at 1% level; ±SD

Seed germination (92.33 ± 4.50%) was found to be significantly (P= 0.01) increased under 50mM salinity when compared with 83.33 ± 3.05% of control at 34.3 ± 5.2°C (Table 1, Fig 1). Germination (77.00 ± 3.00%) was found to be significantly (P=0.05) decreased at 500mM salinity. In a study carried out by Mamo *et al*. [16] in chickpea, low levels (<100mM) of salt were found stimulant in germination, however, a considerable decrease was recorded at higher salinity levels of 200 and 250mM, similar results were obtained by Croser *et al*. [17] in *Picea* and *Pinus* species in their studies. Peak of radicle length (6.73 ± 2.23cm) was recorded under 50mM NaCl stress with significant (P=0.05) increase over the 4.00 ± 1.00cm of control (Table 2, Fig 2). Hypocotyl length (6.00 ± 1.00cm) under 200mM salinity was found to be significantly (P=0.05) decreased and further significantly (P=0.01) decreased (4.33 ± 0.57cm) with 500mM salinity stress

when compared with 9.33 ± 2.08cm of the control (Fig 3). Highest dry weight (0.69 ± 0.006g) of 100 seeds was recorded at 50mM salinity. Seed dry weights (0.53 ± 0.008, 0.61 ± 0.008, 0.56 ± 0.007g) were found to be significantly (P=0.01) increased at 10, 20 and 100mM salinity respectively when compared with control (0.40 ± 0.006 g), however, seed dry weights (0.39 ± 0.007 and 0.28 ± 0.01g) were significantly decreased with 200 and 500mM NaCl concentrations at 34.3 ± 5.2°C as shown in (Table 2). Lowest mean germination time (1.12 ± 0.05) was recorded for the control whereas it was significantly increased either at 5% or 1% salinity with different NaCl concentrations. This shows that under the salinity stress germination speed and velocity gradually decreased against the control (Table 2). Thus, the speed and velocity of germination was adversely affected with all salinity levels either at 5% or 1% level of significance. Peak (773.00 ± 9.16) of germination index was recorded at 50mM NaCl concentration at 34.3 ± 5.2°C (Table 2). Highest coefficient of velocity of germination (89.00 ± 4.58) was observed in control germination against all the salinity concentrations. Total span of germination (5 days each) significantly (P=0.05) increased with 200 and 500mM salinity when compared with 4 days of control at 34.3 ± 5.2°C (Table 2). This shows delayed germination under the salinity stress.

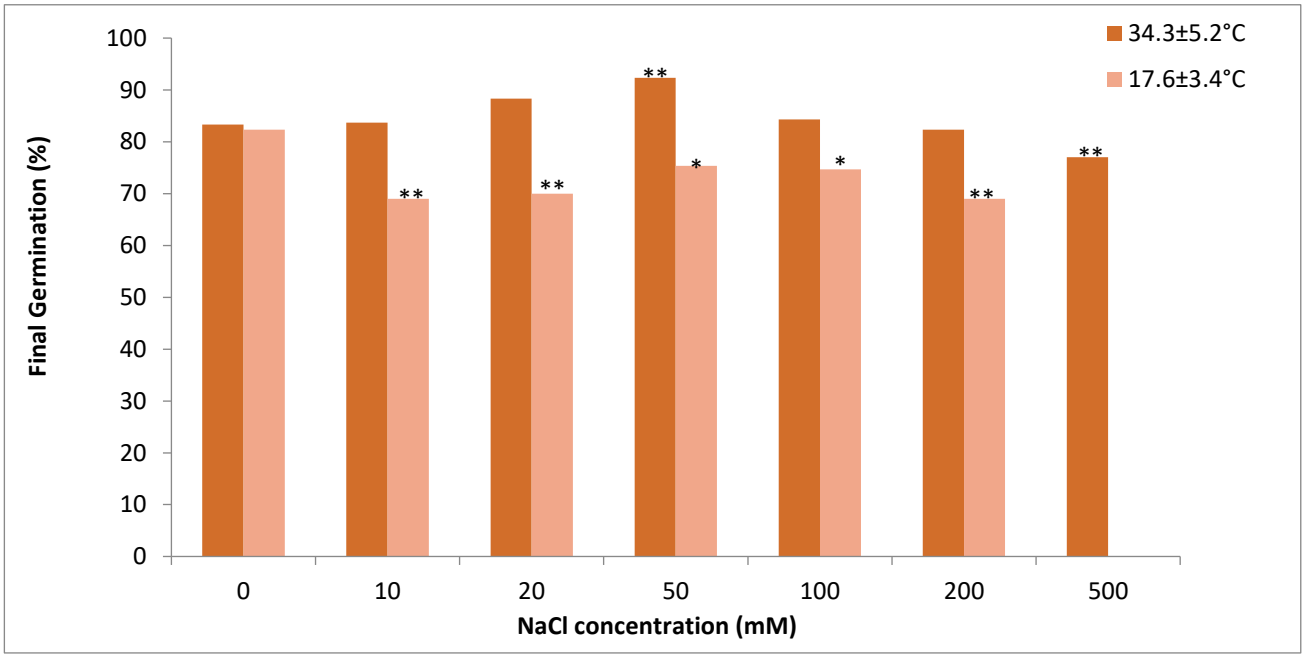

Fig 1 Final germination percentage (FGP) of *Medicago sativa* L. under NaCl salinity at 34.3±5.2°C and 17.6±3.4°C
*Significant at 5% level; **Significant at 1% level

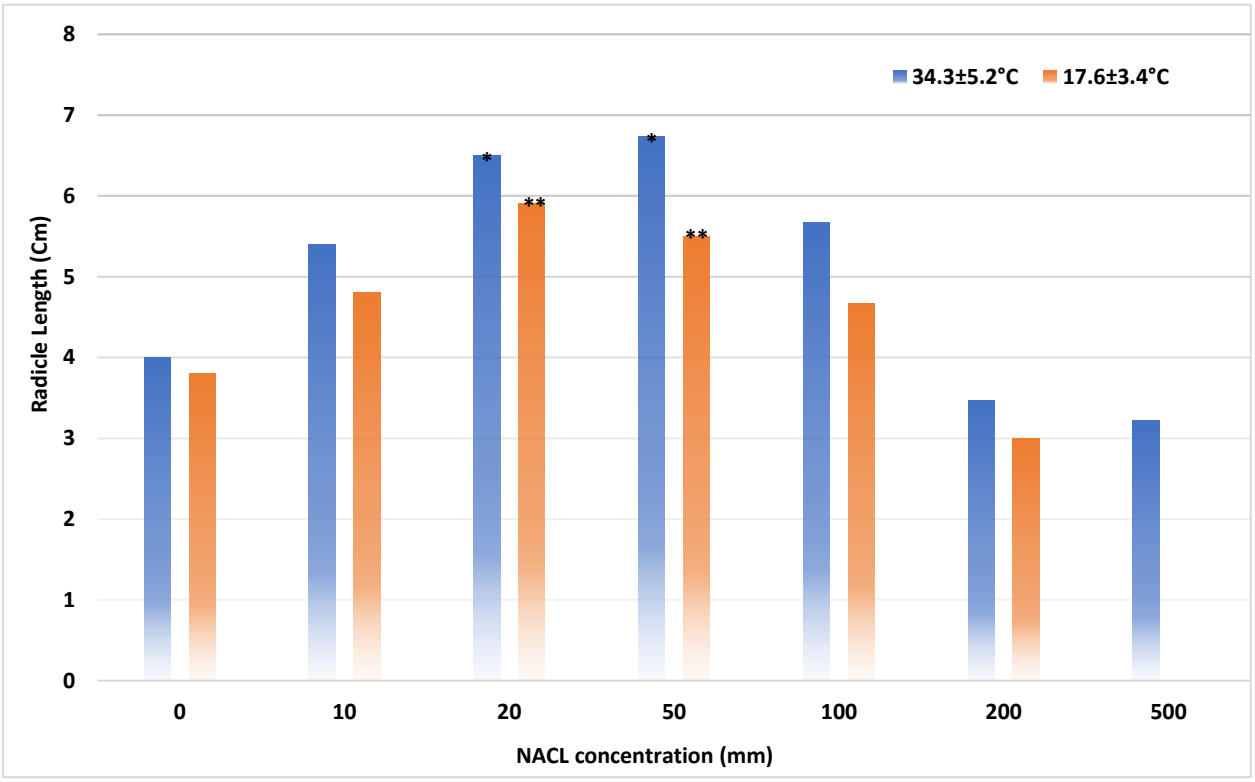

Fig 2 Radicle length (RL) of *Medicago sativa* L. under NaCl salinity at 34.3±5.2°C and 17.6±3.4°C
*Significant at 5% level; **Significant at 1% level

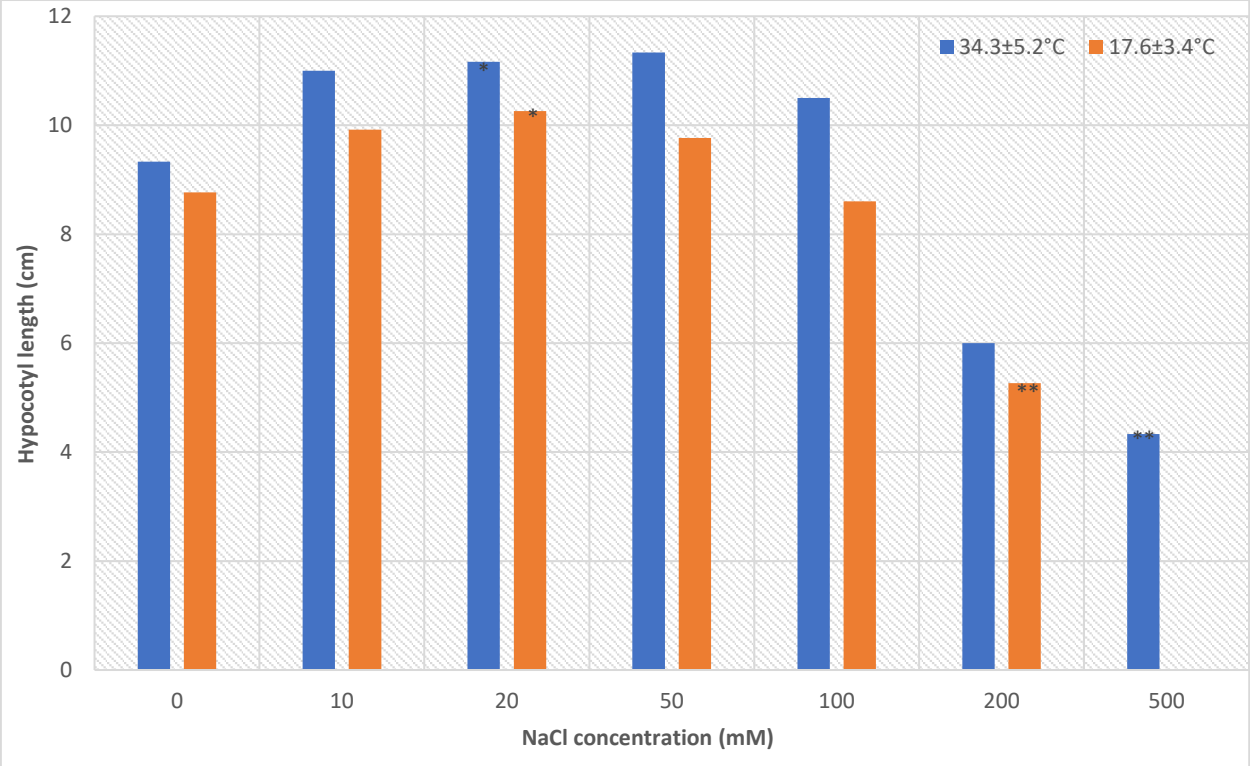

Fig 3 Hypocotyl length (HL) of *Medicago sativa* L. under NaCl salinity at 34.3±5.2°C and 17.6±3.4°C
*Significant at 5% level; **Significant at 1% level

Table 2 Germination and seedling growth parameters of lucerne (*Medicago sativa* L. cv. Ek sali) at 34.3 ± 5.2°C

NaCl (mM)	Germination percentage	Radicle length (cm)	Hypocotyl length (cm)	Dry weight of germinated seeds (g)	Mean germination time	Germination index	Coefficient of velocity of germination	Total spread of germination
0	83.33±3.05	4.00±1.00	9.33±2.08	0.40±0.006	1.12±0.05	746.00±14.10	89.00±4.58	4.00±00
10	83.66^{NS}±2.08	5.40^{NS}±0.65	11.00^{NS}±1.00	0.53**±0.008	1.29*±0.07	732.00*±8.18	77.77*±6.51	4.00^{NS}±00
20	88.33^{NS}±2.51	6.50*±1.22	11.16^{NS}±1.44	0.61**±0.008	1.41**±0.06	723.00*±9.84	56.77**±7.70	4.00^{NS}±00
50	92.33**±4.50	6.73*±2.23	11.33^{NS}±2.51	0.69**±0.006	1.59**±0.08	773.00**±9.16	61.58**±7.41	4.00^{NS}±00
100	84.33^{NS}±4.72	5.66^{NS}±1.04	10.50^{NS}±1.80	0.56**±0.007	1.70**±0.05	706.00**±9.64	59.02**±5.34	4.33^{NS}±0.57
200	82.33^{NS}±4.72	3.46^{NS}±0.55	6.00*±1.00	0.39*±0.007	1.73**±0.08	687.00**±11.53	57.04**±4.35	5.00**±00
500	77.00*±3.00	3.21^{NS}±0.19	4.33**±0.577	0.28**±0.01	1.88**±0.09	625.00**±9.53	53.10**±4.30	5.00**±00

Not Germinated (NG), Non-Significant (NS), *Significant at 5% level; **Significant at 1% level; ±SD

The seed germination was found to be significantly increased with lower and medium doses of 10, 20, 50mM NaCl concentrations at higher temperature of 34.3 ± 5.2°C of summer. However, this was not true at the lower temperature (17.6 ± 3.4°C) of winter because temperature negatively affects the germination. There is an optimum range of temperature (15-30°C) of germination for most of the seeds [18-20]. Low temperature is a limiting factor for germination and plant growth. Cold stress commonly causes delayed seed germination and growth [21]. Chlorine is absorbed from soils as the chloride ion (Cl^-) and remains in this form in plant tissue without becoming structural part of organic molecules. Most of the species absorbed 10 to 100 times chloride than they need [22]. Chloride stimulates the split of H_20 during photosynthesis, also essential for roots. In the deficiency of chloride roots become stunted in length [23]. There may be the possible role of Cl^- ions in the elongation of radicle during seed germination and it may help in germination to a limited extent as observed in our experiments under NaCl stress of 50Mm at the temperature of 34.3 ± 5.2°C.

CONCLUSION

Plant physiologists have classified the plants into two groups on the basis of their susceptibility to salt damage, namely the halophytes and glycophytes. The former can tolerate relatively high salt concentrations whereas the later can relatively tolerate low concentrations. *Medicago sativa* L. cv Ek Sali appears to be moderately salt lover as observed in present study, the tested cultivar is salt tolerant for moderate salinity stress (10, 20 and 50mM). The 50Mm NaCl treatment was found to be germination stimulant as the final germination percentage (92.33 ± 4.50), radicle length (6.73 ± 2.23cm), hypocotyl length (11.33 ± 2.51cm) and dry weight (0.69 ± 0.006g) were found significantly increased at this level of salinity at higher temperature of 34.3 ± 5.2°C. The seed germination was decreased in control and further decreased with increasing salinity at winter temperature (17.6 ± 3.4°C). There was a decreasing trend observed for different germination parameters at low temperature both in control and in treatments. In India plant is cultivated as a winter crop and the seed sowing

is completed in the last week of October or the first week of November by the time the temperature does not tend to be too much low thus the seed germination is not affected by lower doses (10 and 20mM) rather a moderate dose of 50mM significantly increases the germination but it decreases afterwards.

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