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**Response of germination and early seedling growth of phog (*Calligonum polygonoides*)
and khara lana (*Haloxylon recurvum*) to temperature and salinity**

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Woody perennial plants are better suited than annuals in the areas with a long dry season. Shrubs cover > 70 % landscape in Indian hot arid region and considered as most successful plant type in desert environment (Kumar *et al.* 2005). Shrubs are important source of fodder, food, fuel wood, fiber, medicine thatching materials, and materials for making household articles. Shrubs also play an important role in arresting soil erosion, stabilization of sand dunes, improving soil fertility, phyto-remediation of degraded soils, and promoting biodiversity (Singh *et al.* 2008). During recent years the focus has been shifted towards shrubs of arid regions for emerging need of agricultural diversification, demand for fodder, medicinal and other economic products, along with their role in environment restoration. A critical first step in utilizing the potential of these species is to establish how their germination and early seedling growth will be affected by probable abiotic factors.

Phog (*Calligonum polygonoides* L.) is commonest glycophyte shrub of psammophytes scrub desert, and occurs in the area receiving rainfall $< 400 \text{ mm yr}^{-1}$. It has been considered as valuable fodder and fuel wood species of the arid region. Its flower bud contains 16.9 % protein and used to prepare variety of food items. It is also an important species for sand dune stabilization and arresting wind erosion (Singh *et al.* 2008).

Khara lana (*Haloxylon recurvum* (Moq.) Bunge ex Boiss.) is a dwarf perennial halophytic distributed in the saline depressions of northwestern India. It has been used for making *Choa* and *Saji* (a product of sodium carbonate), which is used in local papad (a salty confectionary item) industry. It is source of medicine and alkaloids. It has been also reported as an alternative fodder for goats in arid regions (Rathore *et al.* 2008). It accumulates high quantity of salt that makes it suitable for phytoremediation of highly saline lands (Singh *et al.* 2008). Multiple uses coupled with excellent adaptations to moisture and salt stress condition makes it as an important species for cultivation in saline wastelands of hot arid region.

Seed germination is initial and the most crucial stage in the life cycle of plants. Temperature and salinity are the two most important factors that have overriding influences on the germination of seeds in hot arid regions (Khan and Ungar 1998). Temperature has significant effect on onset, potential and rate of germination and consequently a major factor limiting the establishment of plant species.

In hot arid regions, the salt concentration in surface soil is high due to higher evaporation than precipitation. Consequently the seeds are exposed to salt stress. Therefore, tolerance of salinity during germination is critical for the establishment of plants growing in these regions. Response of germination and seedling growth to salinity depends on temperature, and the response of germination to interaction of temperature and salinity is species specific (Aiazzi *et al.* 2002). Therefore, the interaction effects of temperature and salinity play an important role in regulating the germination and early seedling growth of

plants in hot arid and semi-arid regions characterized by high fluctuation in salinity and temperature near the soil surface.

Germination and early seedling growth responses of *C. polygonoides* and *H. recurvum* to temperature and salinity are poorly understood. We hypothesized that response of germination and early seedling growth to salinity and temperature would be species specific. We also tested the expectations that response to salinity would differ with changes in temperature. The aim of present study is to describe the germination and early seedling growth response of *C. polygonoides* and *H. recurvum* under various temperature and salinity regimes. Better understanding of the responses facilitates the *ex-situ* conservation and effective utilization of these species.

Seeds of *C. polygonoides* (seed weight: 1.61 ± 0.017 g, 100 seeds; $n = 5$) and *H. recurvum* (seed weight: 0.29 ± 0.005 g, 100 seeds; $n = 5$) were collected from experimental farm of CAZRI, Regional Research Station, Bikaner, India (28.4 °N latitude and 74.3 °E longitude, 228.4 m asl). Seeds were separated from inflorescences, cleaned, and dry stored at room temperature after surface sterilization with 0.85 % sodium hypochlorite for 1 minute.

Experiments were carried out during 2009 in factorial randomized block design with three replications for each treatment. Treatments consisted five constant temperatures (15, 20, 25, 30 and 35 °C) for both the species. Salinity treatments comprised six NaCl concentrations (0,100,200, 300, 400 and 500 mM NaCl) for *H. recurvum* and seven NaCl concentrations (0, 25, 50,100,200,300 and 400 mM NaCl) for *C. polygonoides*. Seeds were exposed to different salinity concentrations at each constant temperature for 20 and 30 days in *H. recurvum*, and *C. polygonoides*, respectively. Germination was carried out in plastic Petri dish (90 mm diameter). In the study, 25 seeds were placed on two layers of filter paper moistened with 10 mL of test solutions as per treatment and distilled water (0 mM NaCl) served as control. All Petri dishes were placed in an incubator at constant temperature as per treatment. Every 2day,

the solution in each Petri dish was removed again as completely as possible, before 5 mL of the test solution was added.

Germination was recorded on alternate day for 20 days in case of *H. recurvum* and for 30 days in case of *C. polygonoides*. Seed was considered to have germinated when the emerging radicle was at least 2 mm long. Germination percentage was calculated as the proportion of germinating seeds within a replicate. The proportion of seed germinated at 20 days and 30 days after incubation in *H. recurvum* and *C. polygonoides*, respectively was expressed as final percentage germination (G_F). Rate of germination was estimated with modified Timson index of germination velocity:

$$= \Sigma G/t,$$

Where, “G” is percentage of seed germinated after 2 day intervals, and “t” is total time of germination (Khan and Ungar 1998).

Five seedlings from each replicate were selected randomly at the end of incubation period (i.e. 20 days in case of *H. recurvum* and for 30 days in case of *C. polygonoides*). Length of seedling, plumule and radicle was measured and expressed in cm. Vigor index was calculated by using the formula:

$$\text{Vigor index} = (\text{mean root length} + \text{mean shoot length}) \times \text{germination (\%)}$$

The treatments were set in factorial randomized block design with three replications. All data were expressed as mean \pm S.E. Germination percentage were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were subjected to analysis of variance for factorial randomized block design (Gomez and Gomez 1984). Two-way ANOVA was used to test the significance of main effects (temperature and salinity) and their interaction on germination and seedling growth attributes. Treatment means for main effect of salinity, main effect of temperature and temperature x salinity interaction were compared using the least significant difference (LSD) mean separation procedure. For analysis of

seedling growth and vigor index of *C. polygonoides* samples from four temperature (15, 20, 25, and 30 °C) and salinity (0, 25, 50 and 100 mM NaCl) regimes were used.

Seeds germination and seedling growth of *C. polygonoides* and *H. recurvum* were significantly affected by temperature, salinity, and the interaction of the two factors (Table 1). Highest G_F of *C. polygonoides* was recorded at 20 °C, and decreased with deviation of temperature from 20 °C (Table 2). The highest G_F of *H. recurvum* was recorded at 15 °C, and it decreased progressively with an increase in temperature (table 2). The G_F of *H. recurvum* at 20, 25, 30 and 35 °C was 19.1, 28.3, 71.5 and 77.9 % lower respectively, compared to 15 °C temperature. The 15-20 °C temperature seems to be optimum for the germination of both species. Reduction in germination with deviation of temperature from optimum temperatures might be attributed to denaturation of protein, membrane dysfunction and finally the termination of metabolic activities required for germination. Reduction in germination was more at higher temperatures compared to lower temperature. Decreased germination at higher temperatures could be attributed to exposure of seeds during germination to heat, which resulted in malfunctioning of the enzymes systems. This would lead to limitation of many physiological processes essential for germination (Roberts 1989).

Salinity inhibited germination of both species (Table 2). Germination of *C. polygonoides* was significantly inhibited at > 25 mM NaCl concentrations. Average across all the temperatures, the G_F of *C. polygonoides* was 7.1, 19.5, and 43.2 % less at 25, 50 and 100 mM NaCl concentrations compared to non-saline control. Averaged across all temperatures, G_F of *H. recurvum* with 100, 200, 300 400 and 500 mM NaCl concentrations was 24.0, 58.0, 80.2, 84.9, and 95.8 % lower respectively, compared to the non-saline control. Results support that germination of both glycophytes (*C. polygonoides*) and halophytes (*H. recurvum*) is inhibited by salinity stress, but germination response to salinity is species specific. Germination of *C. polygonoides* was completely inhibited at 200 mM NaCl

concentration, in contrast *H. recurvum* showed 7.7 to 59.8 % germination at the same level of salinity. Salinity induced inhibition of germination could be attributed to reduced water absorption by seeds due to osmotic effect, decreased permeability of plasma membrane and increase influx of external ions and efflux of cytosolic solutes (Azaizeh *et al.* 1992) and specific ion toxicity.

Inhibitory effect of salinity varied with shift in temperature and salinity induced inhibition was more pronounced at higher temperatures. The finding is in agreements as those reported by Gulzar and Khan (2001) but contradicted to the finding of Aiazzi *et al.* (2002). Highest final germination percentage (90.0 %) of *H. recurvum* was recorded at 15 and 20 °C incubation temperatures with non-saline control. Germination was completely inhibited with 300 mM NaCl at ≥ 30 °C and with 500 mM NaCl at ≥ 20 °C. In *C. polygonoides* the germination was completely inhibited with 200 mM NaCl at 15-30°C, but at 35 °C no germination was recorded even with 100 mM NaCl. More detrimental effects of salinity at higher temperature might be attributed to toxicity of Na⁺ that cause irreversible damage. Higher incubation temperature could increase the ion influxes via cell membrane, particularly Na⁺ and Cl⁻, which increase the risk of toxicity inhibition to the physiological process of seed germination . Inhibitory effect of salinity at higher temperature has ecological significance, as it prevents seeds from germinating in salt affected habitats and consequently avoiding seedling mortality during the period when surface soil salinity is extremely high (Khan and Ungar 1998).

Germination rate of both the species showed significant response to temperature, salinity, and the interaction of the two factors (Table 3). Highest germination rate of *C. polygonoides* was observed at 20 °C. Rate of germination sharply decreased at temperatures > 20 °C. Rate of germination was 24.6, 50.7, 81.1 and 91.3 percent slower at 15, 25, 30 and 35 °C respectively, compared to 20 °C (Table 3). Highest rate of germination of *H. recurvum*

was observed at 15 °C temperatures. Averaged across all salinity levels, the shift of temperature from 15 °C recorded 21.5 to 74.8 percent reduction in rate of germination. Rate of germination in both the species decreased with an increase in salinity. Averaged over all incubation temperatures, rate of germination of *C. polygonoides* decreased ~ 125 percent at 100 mM NaCl compared to non-saline control. Rate of germination of *H. recurvum* under 100, 200, 300, 400 and 500 mM NaCl concentration was 34.0, 65.9, 86.5, 89.7, and 97.5 % lower respectively, compared to the non-saline control. Salinity-induced inhibitory effect on rate of germination in both species varied with change in temperature. Inhibition of rate of germination caused by salinity was more pronounced at higher incubation temperatures. Rate of germination of *H. recurvum* became zero with 300 mM NaCl at higher temperature (30 and 35°C) as compared to 500 mM NaCl at moderate temperatures (20 and 25 °C).

Temperature, salinity and the interaction of both factors had significant effect on the seedling growth of both species (Table 1). Highest seedling growth of *C. polygonoides* was recorded at 20 °C and decreased significantly both at lower and higher temperatures. Reduction in seedling growth was more at higher temperature (25 and 30 °C) than at lower temperature (15 °C) (Fig. 1). Averaged across the salinity levels (0, 25, 50 and 100 mM NaCl), seedling growth at 25 and 30 °C was 35.3 and 61.2 percent less respectively, compared to 20 °C. Unlike final germination percentage and rate of germination the highest seedling growth of *H. recurvum* was observed at 20-25 °C (Fig. 2). Averaged across all salinity levels, the seedling growth at 30 and 35 °C was 47.9 and 71.2 percent less respectively, as compared to 20 °C. Thus, the favorable temperature for the seedling growth of both the species was slightly higher than for germination. Faster seedling growth at slightly higher temperature than germination might enhance the seedling survival after germination episode in hot arid regions. This could secure seedling survival through the rapid growth of roots enabling them to reach moist layers of soils.

Seedling growth of both the species decreased with an increase in salinity (Fig 1 and 2). In *C. polygonoides*, > 25 mM NaCl concentration caused significant inhibition of seedling growth. Averaged across four incubation temperatures (15,20,25 and 30 °C), the seedling growth attained with 50 and 100 mM NaCl concentrations were 23.1 and 42.8 percent less respectively, compared to the non-saline control (Fig. 1). Seedling growth of *H. recurvum* with 100, 200, 300, 400 and 500 mM NaCl was 27.3, 54.4, 75.0, 85.5 and 94.7 percent less respectively, as compared to non-saline control (Fig. 2). Salinity-induced inhibition of seedling growth could be attributed to inability of plant to adjust osmotically, specific ion toxicity, restriction of synthesis of plant growth promoters such as cytokine and increased production of growth inhibitors such as ABA, inhibition of the enzymatic activity and photosynthesis (Levitt 1980). The inhibition of seedling growth by salinity stress was more in *C. polygonoides* than *H. recurvum*. In the present study the seedling growth of *C. polygonoides* and *H. recurvum* with 100 mM NaCl concentration was 42.8 and 27.3 percent lower respectively, compared non-saline control. Halophytes typically accumulate high concentrations of inorganic ions to lower the osmotic potential in response to increasing salinity, which enables them to absorb water from saline medium and ensure continued growth (Zhao *et al.* 2003). Response of seedling growth to salinity changed with shift in temperature. Salinity-induced inhibition of seedling growth was more pronounced at higher temperature (25 and 30 °C) than lower temperature (15 °C). In case of *H. recurvum* at the lowest temperature (15°C) the 100 mM NaCl did not caused significant inhibition of seedling growth, but at other tested temperatures the reduction in seedling growth with 100 mM NaCl was significant as compared to non-saline control.

Highest vigor index (V.I.) of *C. polygonoides* was recorded at 20 °C and decreased at low and high temperatures (Fig. 3). Averaged across the salinity levels, it's V.I. at 15, 25, and 30 °C incubation temperatures were 28.0, 59.3 and 83.8 % lower respectively, than 20 °C

temperature. Highest V.I. of *H. recurvum* was recorded with non-saline control at 25 and 20 °C incubation temperature and it decreased significantly > 25 °C. V.I. at 30 and 35 °C was 70.3 and 92.5 percent lower respectively, as compared to 25 °C (Fig. 4). V.I. of *C. polygonoides* decreased with increasing salinity particularly > 25 mM NaCl. Averaged across the incubation temperatures, the V.I. with 50 and 100 mM NaCl were 38.2 and 64.8 % lower respectively, compared to the non-saline control. V.I. of *H. recurvum* decreased with an increase in salinity, and V.I. recorded with 100, 200, 300, 400 and 500 mM NaCl were 53.8, 98.2, 117.4, 122.0 and 125.7 % less respectively, compared to the non-saline control. Inhibition of vigor in both the species was more at higher temperatures at any given level of NaCl concentration.

We conclude that the optimum temperature for seed germination and seedling growth of the species ranges from 15 to 20 °C, and *C. polygonoides* could germinate over a wide range of temperature under non-saline condition and up-to 100 mM NaCl at optimum temperature. *H. recurvum* could germinate up-to 400 mM NaCl under optimum temperature. Temperature modulates the germination and early seedling growth response of the species to salinity. Germination of seeds occurs only when both the temperature and salinity are favourable; this helps to persistence of seed bank and spreading the germination over time. Delay and postpone of germination under high salinity and temperature is part of the adaptive strategy of species to survive in the harsh environment of desert region like northwest India. Moreover, we suggest that one should take into account the interaction of temperature and salinity for deciding suitable time and site for planting of these shrub species. The management strategies to *ex-situ* conservation and utilize the potential of these species should consider the interaction of these two important environmental variables.

SUMMARY

Temperature and salinity are the two most important factors regulating germination and early seedling growth in hot arid regions. Laboratory experiments were conducted to assess the effect of temperature and salinity on germination and seedling growth of *Calligonum polygonoides* and *Haloxylon recurvum*. Seeds were incubated at five temperatures (15, 20, 25, 30 and 35 °C) with six NaCl concentrations (0, 100, 200, 300, 400 and 500 mM) for *H. recurvum* and with seven NaCl concentrations (0, 25, 50, 100, 200, 300, and 400 mM) for *C. polygonoides*. The 20-25 °C temperature seems to optimum for germination and seedling growth of *C. polygonoides* and *H. recurvum*. Germination and seedling growth were inhibited by either an increase or a decrease in temperature from the optimal temperature. The *C. polygonoides* germinated up to 100 mM NaCl concentration, whereas *H. recurvum* germinated up-to 400 mM NaCl concentration under optimal temperatures. The 50 % reduction in seedling growth of *C. polygonoides* and *H. recurvum* was recorded with > 50 mM and 300 mM NaCl, respectively. The interaction effect of temperature and salinity on all measured variables was significant. Salinity-induced inhibition of germination and seedling growth was more at higher temperatures in both species. These results provide information pertaining to germination behaviour of species, and shows that in arid region, selection of suitable planting time and site must take into account both salinity and temperature.

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Table1. Two-way ANOVA of the effect of temperature (T), salinity (S) and their interaction (T x S) on germination and seedling growth of *Calligonum polygonoides* and *Haloxylon recurvum*.

| Dependent variable | <i>Calligonum polygonoides</i> | | | <i>Haloxylon recurvum</i> | | |
|-----------------------|--------------------------------|-------------|---------|---------------------------|-------------|---------|
| | Temperature(T) | Salinity(S) | T x S | Temperature(T) | Salinity(S) | T x S |
| Final germination (%) | 396.9*** | 1024.8*** | 52.8*** | 288.3*** | 599.4*** | 18.2*** |
| Rate of germination | 572.0*** | 718.5*** | 81.9*** | 312.7*** | 1004.5*** | 29.6*** |
| Seedling growth | 138.8*** | 59.8*** | 3.1*** | 234.8*** | 728.6*** | 17.8*** |
| Vigor index | 164.6*** | 66.6*** | 7.3*** | 249.6*** | 790.6*** | 51.9*** |

Number indicates *F* value, *** $P < 0.001$

Table 3. Effect of temperature and salinity on rate of germination of *C. polygonoides* and *H. recurvum*.

| NaCl (mM) | Rate of germination | | | | | |
|-----------------|--------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|------|
| | <i>Calligonum polygonoides</i> | | | | | |
| | Temperature (°C) | | | | | |
| | 15 | 20 | 25 | 30 | 35 | Mean |
| 0 | 18.3 ± 1.4 ^a | 18.0 ± 0.4 ^a | 8.8 ± 0.3 ^{de} | 4.3 ± 0.2 ^{gh} | 2.5 ± 0.2 ^{hi} | 10.4 |
| 25 | 13.1 ± 0.7 ^b | 17.1 ± 0.6 ^a | 7.7 ± 0.1 ^{ef} | 3.5 ± 0.2 ^h | 1.4 ± 0.1 ^j | 8.6 |
| 50 | 11.9 ± 0.9 ^c | 13.3 ± 0.1 ^b | 6.9 ± 0.1 ^f | 1.8 ± 0.1 ^{ij} | 1.2 ± 0.1 ^{jk} | 7.0 |
| 100 | 7.4 ± 0.3 ^{ef} | 9.4 ± 0.2 ^d | 5.0 ± 0.3 ^g | 1.3 ± 0.1 ^{jk} | 0.0 ^{jk} | 4.6 |
| 200 | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 |
| 300 | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 |
| 400 | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 ^{jk} | 0.0 |
| Mean | 7.2 | 9.6 | 4.7 | 1.8 | 0.8 | |
| | Salinity | Temperature | Salinity x Temperature | | | |
| C.D. (P = 0.01) | 0.6 | 0.5 | 1.4 | | | |
| | <i>Haloxylon recurvum</i> | | | | | |
| 0 | 42.2 ± 0.1 ^a | 42.7 ± 0.3 ^a | 40.3 ± 1.7 ^a | 21.9 ± 0.5 ^c | 15.7 ± 0.8 ^f | 32.5 |
| 100 | 29.5 ± 1.0 ^b | 26.5 ± 0.6 ^{cd} | 28.5 ± 1.7 ^{bc} | 12.6 ± 1.1 ^{fg} | 10.3 ± 1.4 ^{gh} | 21.5 |
| 200 | 23.5 ± 0.2 ^{de} | 10.5 ± 0.2 ^{gh} | 14.0 ± 0.7 ^f | 3.7 ± 1.9 ^{jh} | 3.7 ± 1.8 ^{jh} | 11.1 |
| 300 | 10.5 ± 0.8 | 6.7 ± 0.4 ^{ij} | 4.8 ± 0.1 ^{jh} | 0.0 ^h | 0.0 ^h | 4.4 |
| 400 | 8.0 ± 0.2 ^{hi} | 6.1 ± 1.0 ^{ij} | 2.6 ± 1.3 ^h | 0.0 ^h | 0.0 ^h | 3.3 |
| 500 | 4.0 ± 0.5 ^{jh} | 0.0 ^h | 0.0 ^h | 0.0 ^h | 0.0 ^h | 0.8 |
| Mean | 19.6 | 15.4 | 15.0 | 13.7 | 10.7 | |
| | Salinity | Temperature | Salinity x Temperature | | | |
| C.D. (P = 0.01) | 1.5 | 1.3 | 3.3 | | | |

Values are mean ± S.E. and the values followed by different letters are significantly different at P < 0.01

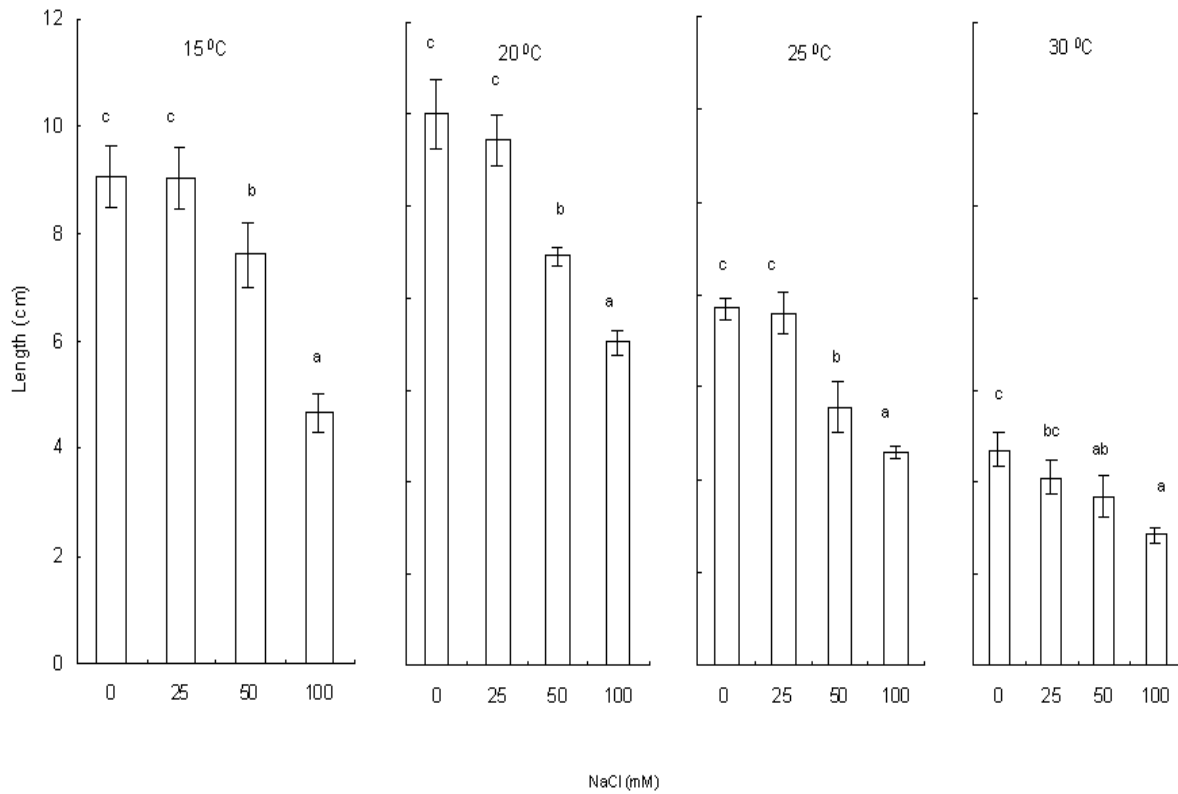


Fig. 1 Effect of temperature (15, 20, 25, and 30 °C) and NaCl concentration (0, 25, 50, and 100 mM) on the seedling growth of *C. polygonoides*. Bar represents mean \pm SE. Different letters indicate significant difference between treatments ($P < 0.001$).

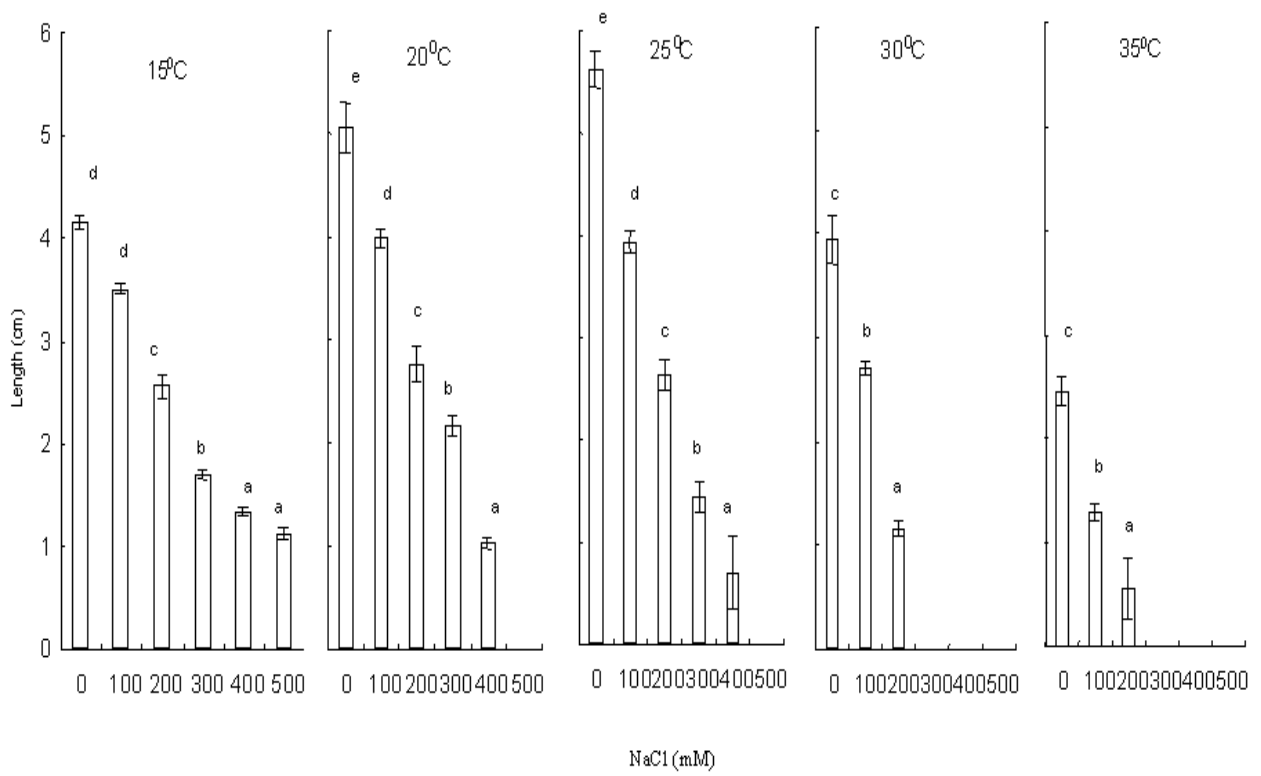


Fig. 2. Effect of temperature (15, 20, 25, 30 and 35°C) and NaCl concentration (0, 100, 200, 300 400 and 500 mM) on seedling growth of *H. recurvum*. Bar represents mean \pm SE. Different letters indicate significant difference between treatments ($P < 0.001$).

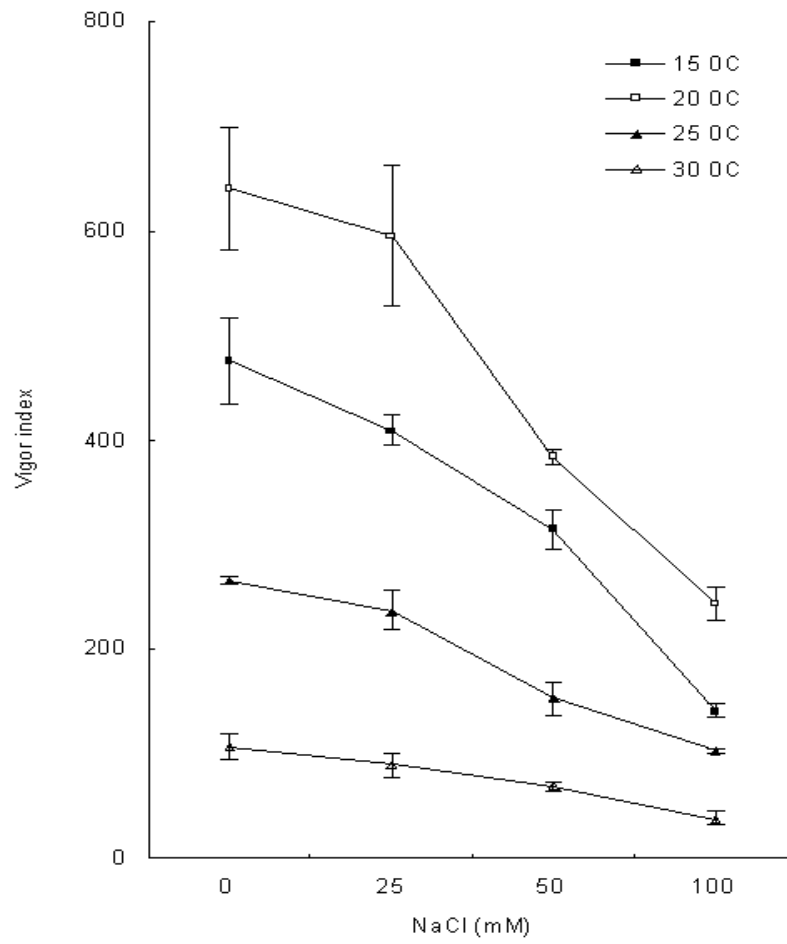


Fig. 3 Effect of temperature (15, 20, 25, and 30 °C) and NaCl concentration (0, 25, 50, and 100 mM) on the vigor index of *C. polygonoides*. Bar represents mean \pm SE.

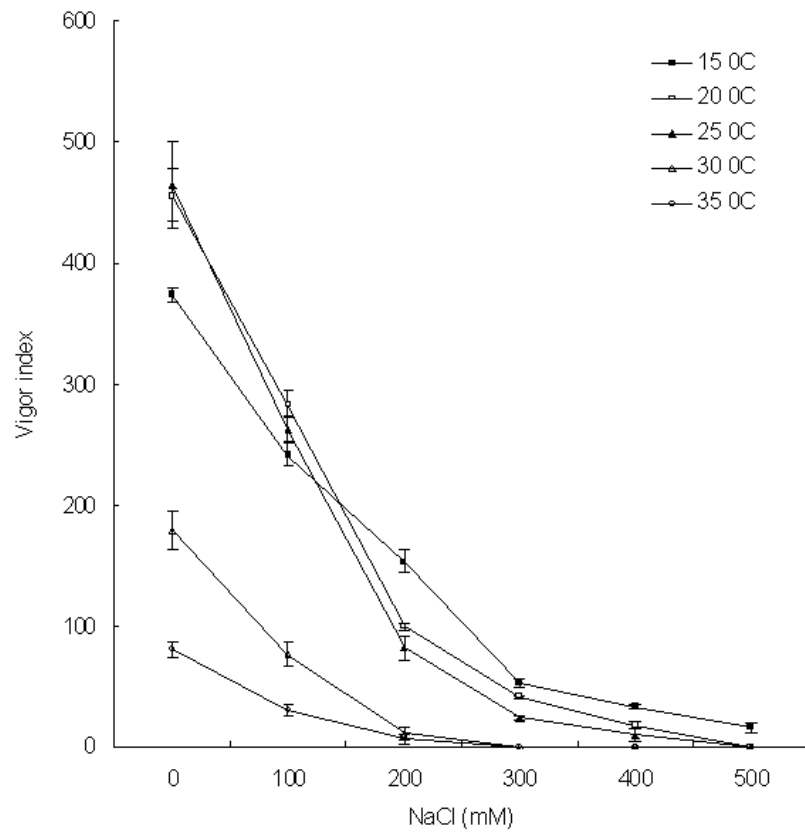


Fig. 4. Effect of temperature (15, 20, 25, 30 and 35°C) and NaCl concentration (0, 100, 200, 300 400 and 500 mM) on the vigor index of *H. recurvum*. Bar represents mean \pm SE.