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Determination of germination characteristics and salinity and drought tolerances of Mountain Swan (*Atriplex nitens* Schkuhr)

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ABSTRACT

In this study, it was aimed to determine the appropriate seed germination temperature, salt tolerance, salinity and drought tolerance of seedlings in Mountain swan (*Atriplex nitens*). For this purpose, an experiment was established in 2021 in laboratory conditions according to the factorial experiment design in random plots and in greenhouse conditions according to the random plots experiment design with three replications. Firstly, 4 constant (10, 15, 20, 25, 30°C) and 2 variable (20/15, 25/15°C) temperatures were used in the refrigerated incubator. Total germination rates and average germination times of seeds with and without pericarp were determined. Secondly, 6 different salt concentrations (0, 100, 200, 300, 400 and 500 mM NaCl) were studied considering the optimum germination temperatures (25°C and 20/15°C). At the end of the study, germination rates (%), average germination times (days) and sensitivity indices (SI) were determined. In the last two stages, seedlings were subjected to 4 different salinity (0, 100, 200, 300, 400 and 500 mM NaCl) and 5 different drought (control, low, moderate, high and severe) tests under greenhouse conditions. At this stage, plant and root length, stem thickness, leaf area index (LAI), plant and root dry weight, root/plant ratio and tolerance percentage values were measured. As a result of the laboratory study, it was determined that seeds without pericarp had a better germination percentage than seeds with pericarp. The highest total and normal germination rates were obtained from 200 mM NaCl treatment at 20/15°C. Germination rate was determined from 0, 100 and 200 mM salt treatments at 25°C. These results showed that *Atriplex nitens* seeds could germinate in high salt concentrations and that their seedlings had high tolerance to drought and salinity.

1. Introduction

Approximately 2 billion ha of the world's agricultural areas are affected by drought and 954 million ha are affected by salinity. In Turkey, 66.9 million ha of agricultural land is affected by drought and 4.2 million ha of land is affected by salinity (SSCA 2014). Therefore, many cultivated and fodder plant species cannot be cultivated economically. As a consequence, these areas are out of production due to salinity and drought. It is necessary to search and find species and varieties that are not affected by these stress factors or that are resistant to them. One of these species is *Atriplex nitens* (Mountain swan), which belongs to the *Amaranthaceae* family. This species is an annual, fringed-rooted C3 plant that can grow in extremely saline and arid areas (Dursun and Acar 2015; Doudova et al. 2017). The plant has pericarp seeds and reproduces by seed. The plant can produce high amounts of seed and stem yields under unsoiled and rainfall conditions(Temel and Keskin 2022a; Keskin et al. 2023; Temel et al. 2024). Leaves are lanceolate and shiny, ashy green. Under wet and dry conditions without any fertiliser, the plant grows 2.0-3.0 m tall at the end of the vegetative period and 2.5-3.3 m tall at full flowering (Keskin and Temel 2022; Temel and Keskin 2022b). The flowers are sparsely racemose and monoecious, located at the very tip of the plant and at the ends of the lateral branches. The leaves of the plant are preferred as human food and the above-ground biomass produced in high quantity and medium quality is preferred as animal feed (Acar et al. 2017; Keskin and Temel 2022; Temel and Keskin 2022b; Temel et al. 2022a; Temel et al. 2022b). In crop production, low germination rates are one of the main reasons that restrict cultivation and cause great economic losses. The environmental factors affecting seed germination are temperature, water and oxygen (Emiralioğlu and Acar 2022). Temperature is the most important among these factors (Erkovan et al. 2017). Because temperature affects germination by changing water absorption, diffusion of mobile substances and enzyme activity. (Kacar et al. 2022; Başaran and Aytaş Akçin 2022).

Low temperatures reduce the swelling rate, which leads to a reduced germination rate and biological damage to the seed. High temperatures can increase the swelling rate of seeds and lead to faster radicle emergence (Kenanoğlu et al. 2019). It may also reduce the germination rate of many seeds or cause seed death (Kenanoğlu et al. 2019). Variable temperatures favourably affect germination (Kenanoğlu et al. 2019; Temel et al. 2023). Seeds of some species need variable temperatures for germination (Dimen 2016). On the other hand, even if conditions such as temperature, water and oxygen are suitable for germination, the presence of some factors at extreme levels in the germination environment is not suitable. One of these factors is salinity, which causes many negative effects by inhibiting seed germination at high levels or by promoting the onset of dormancy at low levels (Gürsoy 2023). These effects may include reduced imbibition due to low osmotic potential, altered enzymatic activity due to toxicity, disruption of the balance of plant growth regulators (Yıldız et al. 2017) inhibition of protein metabolism (Dölarslan and Gül 2012), decreased utilisation of nutrients in seeds or inhibition of mitosis of cells (Julkowska and Testerink 2015; Khan et al. 2019).

Germination rates of seeds are generally high at optimum temperature values. At these temperatures, seed germination may not be affected much by salinity (Liu et al. 2021; Temel et al. 2023). Seedling period is another important plant development stage that should be emphasised in cultivation for the formation of healthy individuals. Because in this early developmental stage, seedlings are exposed to many stress factors such as weed competition, salinity and drought that limit crop diversity and productivity (Partheeban et al. 2017; Abbasdokhta et al. 2014; Yohannes and Abraha 2013). For these reasons, it is necessary to know the minimum, optimum and maximum germination temperatures of the species.

It is of great importance to bring saline and arid soils, which are very abundant in the world, into the economy. There is no detailed study on how Mountain swan, which can grow in such areas and has high economic value, responds to temperature, salinity and drought conditions in seed germination and seedling development. With this study, it was aimed to determine the appropriate temperatures and salinity tolerances of Mountain swan in seed germination and to prove that it can be grown in saline and arid soils by testing the degree of salinity and drought tolerance in seedling development.

2. Materials and Methods

The present research was conducted both in the laboratory (seed germination temperatures and germination period salinity tests) and controlled greenhouse conditions (seedling period salinity and drought tests) in 4 stages between 2020-2021. Pericarp and non-pericarp seeds of Mountain swan were used in the study (Figure 1).

The studies planned under laboratory conditions were established according to the factorial experiment design in random plots, and the studies under greenhouse conditions were established according to the random plots experiment design with three replicates. In order to determine the appropriate germination temperatures, seeds with and without pericarp were held in a refrigerated incubator at 4 constant (10±1°C, 15±1°C, 20±1°C, 25±1°C, 30±1°C) and 2 variables (20/15±1°C, 25/15±1°C) (12/12) temperatures. The seeds were sterilised with 2% sodium hypochlorite for 10 minutes and then washed with distilled water. After this process, the seeds (25 seeds in each treatment) were placed in petri dishes (12 cm) and covered with blotting paper. After that, 10 ml of 2% pomarsol solution was poured into the germination cups against fungal pathogens. Humidity controls were made during the experiment, and 5 ml of pure water was added when necessary. The experiment was maintained for 28 days. In daily controls, the seeds whose rootlets reached 2 mm in length were considered germinated and taken out of the germination cups (Shiade and Boelt 2020). In the second process, seeds were subjected to salinity tolerance tests at the two most appropriate temperatures (25°C and 20/15°C) and salt concentrations of 0, 100, 200, 300, 400 and 500 mM NaCl during germination. The procedures for determining the appropriate temperatures were observed, and 10 ml of pre-prepared appropriate salt solutions were poured over the seeds during moisture controls (Tan and Akçay 2019). In order to prevent salt accumulation in the germination dishes to which salt solution was added, the blotting papers were changed at 2-day intervals. At the end of the experiments, germination rates, average germination times and the sensitivity indices of the seeds were determined using the following formulae.

Germination Rate (%)= (Total number of seeds germinated at the end of 28 days / total number of seeds placed in germination cups) x 100.

Mean Germination Time/Rate (days)= Σ(ix) / Σ i (Bilgili et al. 2018)

i: Number of germinated seeds on the counting day, x: Number of counting days

Sensitivity Index (SI)= Mean germination time in the salt treatment / Mean germination time in the control treatment (Altuner et al. 2019).

For seedling period salinity (0, 100, 200, 300, 400 and 500 mM NaCl) and drought tests (control, low drought, moderate drought, high drought, and severe drought), the soils (garden soil + 10% burnt farmyard manure) were filled into potsnumber 8 (diameter 47x39 cm= 35 L) at the same level. The studies were carried out in 3 repetitions. For this purpose, 18 pots were used for salinity tests and 15 pots were used for drought tests. Sowing was carried out at a sowing depth of 3-4 cm. Seedlings were watered with distilled water until the 10th day of emergence. Then, in the seedling salinity tests, salt solutions prepared previously were gradually applied with adaily increase of 50 mM

Figure 1. Seed types.

until the targeted concentrations were reached (Hariadi et al. 2011; Shavrukov 2013). When the targeted concentrations (levels) were reached, the solution was applied according to the amount of salt in the concentration determined for each treatment. These treatments were made during the period when the plants needed water (when 50% of the available water was consumed).

In the low, moderate, high and severe drought tests during the seedling period, water was added until the field capacity was reached when 50%, 75%, 90%, and 95% of the available water was consumed, respectively. For this purpose, field capacity and sustained wilting points of the soils placed in pots were determined before the experiment. Then, the moisture content of the soils was measured with a soil moisture measuring device (by burying the probe of the device at a depth of 20-30 cm in 2-3 places of the potted area). The amount of moisture decreasing from the field capacity was determined using the available water-holding capacity calibration curve determined by USDA. The pots in the control group were kept constant at field capacity throughout the experiment. These treatments continued until flowering began (Keskin and Temel 2022; Temel and Keskin 2022b), which is the most suitable harvesting period for Mountain swans. In the present study, when the plants in the control treatment began flowering, the plants in the other treatments, including the control, were harvested in this period. Plant height, root length, stem thickness, leaf area index (LAI), plant dry weight, root dry weight, root/plant ratio, salt tolerance percentage (STP) and drought tolerance percentage (DTP) were determined in each treatment before harvest (Hariadi et al. 2011; Adolf et al. 2012; Raney et al. 2014) using the formulas given below:

Root/plant ratio (%)= (root dry weight / plant dry weight) * 100

Percent salt tolerance (%)= (Plant dry weight in salt treatment/plant dry weight in control treatment) * 100

Drought tolerance percentage (%)= (Plant dry weight in drought treatment/plant dry weight in control treatment) * 100

The data obtained in seed germination temperatures and germination period salinity tests were subjected to analysis of variance in SPSS statistical package program according to the factorial arrangement in random plots experimental design, and in seedling period salinity and drought tests according to random plots experimental design, and the grouping of significant means was made according to the Duncan multiple comparison test.

3. Results and Discussion

Germination rates (%) and average germination times (days) of Mountain swan seeds obtained as a result of different temperature treatments are given in Table 1. When Table 1 is examined, it is seen that seeds without pericarp germinated in a shorter time and had higher germinations rates than seeds with pericarp in terms of seed types. This may be due to the presence of germination inhibitors in the fruit shell or the slowing down of water uptake by the fruit shell (pericarp). Previous studies have shown that the seed coat and pericarp inhibit gas diffusion. In addition, it has been revealed that it restricts water uptake or physically prevents the germination of seeds due to chemical inhibitors (Baskin and Baskin 2014; Tan and Akçay 2019). In terms of temperature, it was observed that the germination rate and speed of seeds were low at low temperatures. The best germination rate (98.0%) and speed (3.8 days) were obtained at 20/15°C and 25°C, respectively. This may be due to the slowing down of water absorption into the seed at lower temperatures. As a matter of fact, decreases in germination rate at temperatures below the optimum temperature may be due to decreases in swelling rate with decreasing temperature (Kacar etal. 2022).

Regarding seed type x temperature interaction, the fastest germination was determined in seeds without a pericarp at 30°C and the slowest germination was determined in seeds with a pericarp kept at 10°C (Figure 2). This may be since seed types do not respond to different temperatures at the same rate.

As a result of the germination test, it was determined that seeds without pericarp had a higher germination percentage and speed than seeds with pericarp. However, since it is difficult and uneconomical to remove the fruit shells (pericarps) of the plant in practice, the study was continued by using seeds with pericarps in germination-seedling period salinity and seedling period drought stages.

Total germination rates of Mountain swan seeds germinated at different temperatures and salt concentrations varied between 85.3% and 98.0%, and the highest total germination percentages were obtained from 0, 100, 200 and 300 mM NaCl concentrations. However, it was observed that normal germination rates decreased with increasing salt concentrations. The highest normal germination rates were obtained from the control (0 NaCl) treatments (Table 2). This may be due to the fact

Table 1. Germination rates (%) and germination times (days) of Mountain swan seeds as a result of different seed types and temperature treatments

Treatments	Germination rates (%)	Germination times (days)
Seed types		
Perikarplı	90.3 b	5.90 a
Perikarpsız	95.6 a	4.20 b
Temperature		
10°C	88.0 c	6.4 a
15°C	90.0 bc	6.1 a
20°C	91.3 bc	4.3 d
25°C	94.7 ab	3.8 e
30°C	95.3 ab	5.3 b
20/15°C	98.0 a	4.6 cd
25/15°C	93.3 a-c	4.9 bc
P value and significance	ST: 16.69**. T: 3.92**. ST× T: n.s.	ST: 186.79**. T: 30.310**. ST × T: 39.251**

** It is significant within the 1% probability limits. ns: non-significant. a, b, c: values shown with different letters in the same column are statistically different from each other. ST: seed type, T: temperature, ST x T: seed type x temperature interaction.

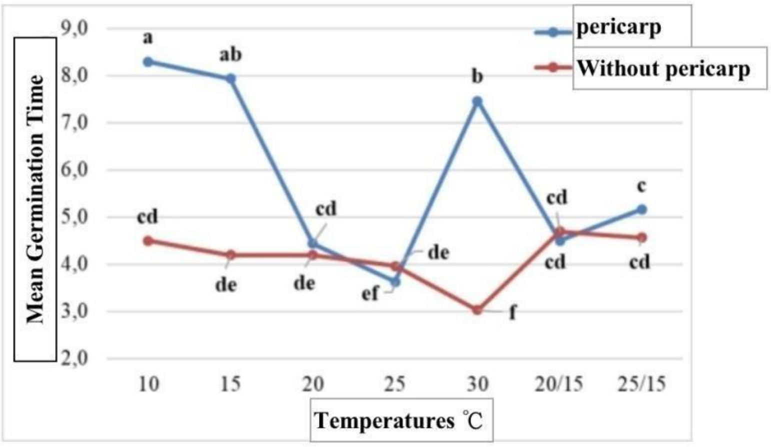

Figure 2. Effect of seed type x temperature interaction on mean germination time.

Table 2. Total and normal germination rates (%), mean germination time (day) and sensitivity index of Mountain swan seeds exposed to different temperatures and salt concentrations

Treatments	Total germination rate (%)	Normal germination rate (%)	Mean germination time (day)	Sensitivity index
Temperatures				
25°C	94.0	30.0 b	5.10 b	1.47 a
20/15°C	94.0	52.7 a	5.40 a	1.29 b
Salt concentrations				
0 mM	96.0 a	74.7 a	3.8 c	1.00 c
100 mM	98.0 a	66.0 b	3.9 c	1.01 c
200 mM	98.0 a	61.3 b	4.1 c	1.07 c
300 mM	96.0 a	34.7 c	5.5 b	1.47 b
400 mM	90.7 b	6.7 d	6.9 a	1.84 a
500 mM	85.3 c	4.7 d	7.1 a	1.89 a
P value and significance	SC: 24.3**.T: n.s. SC × T: 4.3**	SC:244.6**. T: 200.1**, SC × T: 50.1**	SC: 98.3**. T:5.4*, SC × T: 7.4**	SC: 88.8**. T:24.3*, SC × T: 9.7**

** and * are significant at the 1% and 5% probability limits, respectively. ns: non-significant. a, b, c: values shown with different letters in the same olumn are statistically different from each other. SC: salt concentration, T: temperature, SC x T: salt concentration x temperature interaction.

that increasing salt levels decreases the osmotic potential in the germination medium or damages the metabolic and physical structure of the seed. At high salt levels, osmotic potential decreases, and seed imbibition decreases (İnan et al. 2018). This results in metabolic and physical damage to the seed (Köseoğlu and Doğru 2021). It was also observed that increasing salt concentration, after a certain dose, increased the average germination time and salinity sensitivity of the seeds (Table 2).

According to these results, the average germination times and sensitivity indices obtained from 100 and 200 mM NaCl treatments were in the same statistical group as the control treatment. It is known that salinity slows down the water uptake potential of seeds in almost all plant species and thus delays germination. Because salt decreases the water potential in the environment during germination and slows down the water uptake of the seed, this, in turn, can affect the rate of biochemical events during germination and cause prolonged germination time (Tabassum et al. 2017; Öner and Kırlı 2018).

Table 2 shows that seeds at variable temperatures (20/15°C) had a higher normal germination rate and salinity tolerance level. This may be because *Atriplex nitens* may require variable temperatures for normal germination since it uses the C3 photosynthetic pathway. As a matter of fact, Kenanoğlu et al. (2019) and Temel et al. (2023) stated that seeds of some species require variable temperatures for germination. However, the average germination time of the seeds was shorter at constant temperature (25°C). This may be due to the fact that at constant temperatures, the seeds met their total temperature requirements in a shorter period and, therefore, showed rapid germination.

Accordingly, prolonged exposure to high temperatures may also increase salt damage. Indeed, Terzi et al. (2017) reported that high temperatures increased salt damage in *Salsola crassa*. The effect of temperature x salt concentration on the parameters examined was found to be statistically significant (Figure 3). Accordingly, the highest total and regular germination rates were obtained from seeds exposed to variable temperature conditions and 200 mM NaCl treatment, while the average germination time was found to be fastest in control and 100 mM NaCl treatments kept at constant temperature. It was also determined that seeds exposed to constant temperature and 100 mM NaCl treatment had higher tolerance to salinity (Figure 3).

When Table 3 was analyzed, it was observed that there were significant decreases in plant height, root length, plant stem thickness, leaf area index, plant dry weight, root dry weight and salt tolerance percentage values with increasing salt concentrations. This may be due to the decrease in salt concentrations in the growing medium, which decreases the water uptake potential of the seedlings. Accordingly, plant growth may slow down because physiological drought occurs in plants due to increased salt concentration in the growing medium, which decreases the water uptake potential of the plant (Doğan and Çarpıcı 2016). As a result, the seedling cannot compensate for the reduced water due to transpiration. The resulting decrease in turgor pressure limits growth and development (Tan and Akçay 2019). The present study showed no linear decrease in root/plant ratio with increasing salt concentration as in other parameters. On the contrary, an increasing trend was observed. Although the root/plant ratio was generally higher in the control treatment,the highest ratios were statistically obtained from 300 and 400 mM NaCl treatments (Table 3).

Statistically significant differences were observed between the parameters examined (except root length and root/plant ratio) depending on the drought levels (Table 4). Table 4 shows that plant height, stem thickness and leaf area index decreased linearly with increasing drought. Similarly, plant dry weight and drought tolerance percentage also decreased, but these two parameters occurred after moderate drought. According to these results, the highest plant dry weight and drought tolerance percentage were obtained from control and low drought treatments. These differences may be due to the inability of plants to absorb the water they need for growth and development due to increasing drought severity. In order to realize growth (elongation in length, expansion in volume and increase in weight) in plants, a sufficient amount of water must enter the cell (Gençtan 2016). In addition, as water becomes scarce, the plant usually closes its stomata to avoid losing more water. This limits the uptake of CO_2 required for fixation by photosynthesis and thus the net photosynthetic (organic matter) production (Örs and Ekinci 2015). As a result, the lack of sufficient water/humidity due to drought severity causes decreases in growth parameters. On the other hand, the highest root dry weight was determined at low and medium drought levels, which were in the same statistical group as the control group. Indeed, plants primarily react to drought stress conditions by slowing the formation of above-ground parts (Raney et al. 2014).

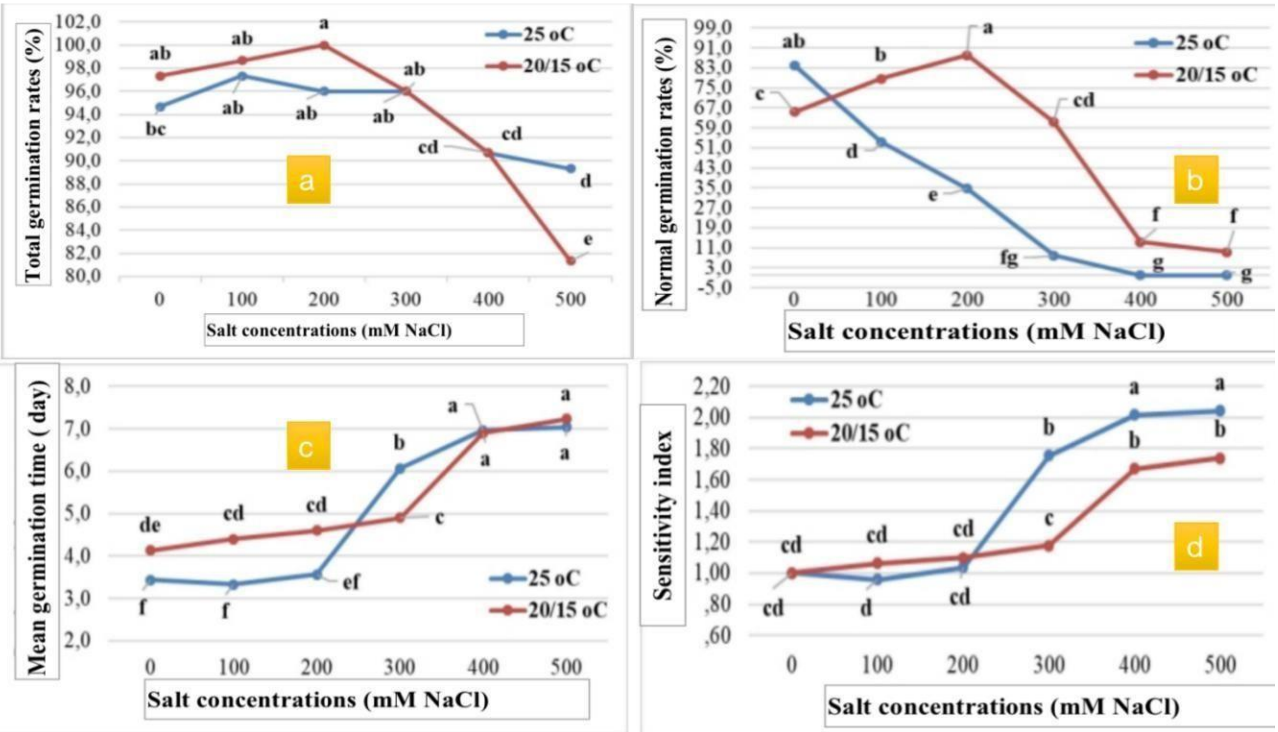

Figure 3. Effect of temperature x salt concentration interaction on total germination rate (a), normal germination rate (b), mean germination time (c) and sensitivity index (d).

Table 3. Some growth and development characteristics of Mountain swan seedlings at different salt concentrations

Doses	PH (cm)	RL (mm)	ST (mm)	LAI (cm^2)	PDW (g)	RDW (g)	RPR (%)	STP(%)
0 mM	198.7±20.1 a	1206.7±78.8 a	10.1±0.6 a	5.44±0.35 a	38.90±2.80 a	8.03±0.55 a	20.68±0.82 ab	100.00±0.00 a
100 mM	135.3±2.9 b	759.3±26.4 bc	7.8±0.3 b	1.88±0.09 b	20.10±1.29 b	3.40±0.12 b	16.98±0.52 c	51.66±3.30 b
200 mM	114.3±5.9 bc	855.3±19.9 b	6.6±0.2 c	1.37±0.07 bc	16.48±0.58 bc	3.27±0.12 b	19.82±0.04 ab	42.37±1.50 c
300 mM	104.7±2.3 c	817.3±24.0 b	6.0±0.3 cd	0.94±0.09 cd	14.84±0.48 c	3.13±0.07 bc	21.13±0.31 a	38.15±1.23 cd
400 mM	97.7±4.3 c	535.7±62.5 c	5.9±0.1 cd	0.79±0.09 d	14.14±0.18 c	2.97±0.15 bc	20.97±0.80 a	36.34±0.45 d
500 mM	88.0±5.1 c	189.3±21.9 d	5.5±0.3 d	0.53±0.12 d	12.78±0.49 c	2.40±0.21 c	18.71±0.96 bc	32.85±1.25 d
Avg.	123.1±9.5	727.3±38.9	7.0±0.4	1.82±0.41	19.54±2.22	3.87±0.47	19.72±0.42	50.23±5.61
P value and significance	19.72**	20.07**	27.85**	121.99**	56.01**	63.61**	6.00**	232.45**

**$P < 0.01$ is significant within the probability limits. a, b, c, values indicated with different letters in the same column are statistically different from each other. PH: Plant height, RL: Root length, ST: Stem thickness, LAI: Leaf area index, PDW: Plant dry weight, RDW: Root dry weight, RPR: Root/plant ratio, STP: Salinity tolerance percentage.

Table 4. Some growth and development characteristics of Mountain swan seedlings at different drought levels

DL	PH (cm)	RL (mm)	ST (mm)	LAI (cm^2)	PDW (g)	RDW (g)	RPR(%)	DTP (%)
0	218.7±10.1 a	1318.67±137.7	11.8±0.2 a	5.20±0.48 a	40.11±1.79 a	8.23±0.39 a	20.70±1.96	100.0±0.00 a
%50	198.3±13.1 ab	1350.33±101.9	11.0±0.3 ab	4.84±0.29 ab	35.37±2.14 a	7.17±0.22 a	20.44±1.57	88.16±3.19 a
%75	178.3±8.6 bc	1765.67±170.7	10.3±0.5 b	4.81±0.14 ab	29.62±1.73 b	7.30±0.72 a	24.53±1.11	74.41±7.06 b
%90	159.7±9.2 c	1281.0±161.1	8.8±0.2 c	4.03±0.33 bc	21.10±0.58 c	4.83±0.77 b	22.74±2.97	53.56±1.48 c
%95	151.0±0.6 c	1004.33±210.7	8.3±0.5 c	3.68±0.04 c	18.46±1.51 c	3.93±0.48 b	21.44±2.46	46.85±3.83 c
Ort	**181.2**±7.5	**1344.0**±89.1	**10.0**±0.4	**4.51**±0.19	**28.93**±2.28 **c**	**6.29**±0.48	**21.97**±0.90	**72.60**±5.57
P value and significance	8.87**	2.89$^{n.s}$	16.33**	4.53*	31.48**	10.69**	0.63$^{n.s.}$	32.89**

** and * are significant, n.s., insignificant at the 1% and 5% probability limits, respectively. a, b, c, values indicated with different letters in the same column are statistically different from each other. DL: drought level, PH: Plant height, RL: Root length, ST: Stem thickness, LAI: Leaf area index, PDW: Plant dry weight, RDW: Root dry weight, RPR: Root/plant ratio, DTP: Drought tolerance percentage.

4. Conclusion

The results of this study showed that Mountain swan seeds germinated faster and had a higher germination percentage at high temperatures (25, 30, 20/15 and 25/15℃) compared to low temperatures (10, 15 and 20℃) and seeds without pericarp have high germination rate compared to seeds with pericarp.

Although the germination percentage decreased with increasing salt concentration, it was revealed that seeds could germinate easily at high salt concentrations, and seedlings could grow at high salt and drought levels. These results suggest that *Atriplex nitens* can play an essential role in planting marginal areas abandoned due to salinity and drought, especially in bringing these areas into production as an alternative feed/food source.

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