

Effect of Salinity Stress on Seed Germination *Catharanthus roseus* Don. Cvs. Rosea and Alba

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Abstract: This experiment was carried out aiming to determine the *Catharanthus roseus* Don resistance against salinity, due to the increasing salinity of soil, and the importance of this plant as an ornamental flower, as well as the little information available on its tolerance against salinity during the germination period. In order to an experiment was conducted in randomized completely design factorial. Sodium chloride was used for induce salinity stress. These factors include cultivar and salinity levels with Electrical Conductivity (EC) of 1, 2, 4, 6, 8, and 10 dS/m. The results of variance analysis showed that salinity effect at level 1% on the germination percentage, germination rate, radicle and plumule length, seedling wet and dry weight and ratio of plumule length to radicle length were significant and they were reduced. While the cultivar and interaction of cultivar and salinity treatments were non significant. According to the results, response of two cultivars to the salinity was the same and there was no difference between them.

Key words: *Catharanthus roseus* Don, germination, salinity stress, sodium chloride

INTRODUCTION

Madagascar periwinkle (*Catharanthus roseus* Don.) is an ornamental, medical plant belonging to the Apocynaceae family. This family contains 114 genera and 4650 species that most of them are both ornamental with medical value (Simpson, 2006). Madagascar periwinkle is a warm season, perennial, cold-sensitive plant which could be preserved given its proper conditions. It could be planted as a marginal flower in gardens during spring and in autumn it would perish due to the cold weather. It has extended, ellipsoid, glossy, dark-green colored leaves and its veins are light colored which is helpful in its identification. The flowers have five petals with pink, white, red, and purple colors (Ghasemi Ghahsare and Kafi, 2009). In horticulture it is considered as an annual plant (Khalighi, 2008). Three different cultivars of this flower are discriminated by their colors including "Rosea" with pink flowers, "Alba" with white flowers and the third one has white flowers with pink or yellow rings (Kamboj, 2000). Madagascar periwinkle is known as a medical plant which contains a number of terpenoid indole alkaloids with more than 130 separated and identified compounds (Van der hejden *et al.*, 2004). It is categorized as a glycophyte plant (Jaleel *et al.*, 2007). Germination is one the most sensitive stages of its growth cycle, since this stage plays a major role in determining the plant's final density. During salinity and moisture stresses conditions, plant's germination effect on determining the plant's final density is of utmost important (Hampson

et al., 1990). The high salt concentration in soil may impede the germination; seeds germinate whenever the proper temperature and soil conditions are established (Ungar, 1996). Halophyte and non-halophyte plants seeds in dicote similar responses to salinity stress; including delays in primary germination process by this stress (Almansouri, 2001). The effect of sodium chloride on seed germination is due to osmotic effects and/or ions toxicity (Tobe, 2004). In halophytes, the decrease in germination usually is due to osmotic effects while this happens due to ion toxicity in non-halophytes (Bajji, 2002). Researches show that there is a decline in germination percentage of Alba and Rosea cultivars of Madagascar periwinkle as the salinity increases, in such a way that there is a large decrease in germination at 60 mM of salt concentration which is more significant in Rosea cultivar. Also by increasing salinity intensity, the length of radicle and plumule decreases (Grieve and Munnes, 1980). In an investigation on Bodegold variety of *Matricaria chamomilla* L. germination it was observed that increasing the amount of salinity, the germination percentage significantly decreases, as in 200 and 250 mM concentrations, the germination was decreased to 29 and 17%, respectively (Zehtab Salmasi, 2008). Increasing the salinity level decreases the germination percentage in valerian and cumin plants. As for valerian, the germination percentage decreased by 67.91% in 150 mM salinity treatment in comparison with the control treatment. The germination for cumin genotypes (Mashhad, Sarayan and Torbat - eJam) in dicoted

Table 1. Variance analysis of properties under salinity stress in *Catharanthus roseus* Don

Source	d.f	Mean square					
		Germination percentage	Germination rate	Plumule length	Wet weight of seedling	Dry weight of seedling	Length of plumule to radicle
Cultivar	1	1.7781 ^{ns}	0.001 ^{ns}	0.039 ^{ns}	0.001 ^{ns}	0.000 ^{ns}	0.033 ^{ns}
Salinity	5	6034.311**	413.971**	35.370**	0.370**	0.001**	11.091**
Cultivar×Salinity	5	7.911 ^{ns}	0.119 ^{ns}	0.008 ^{ns}	0.001 ^{ns}	0.000 ^{ns}	0.008 ^{ns}
Error	24	16.667	1.222	0.024	0.002	0.000125	0.050 ^{ns}
(%)C.V	5.88	8.21	5.28	6.01	13.51	16.15	8.52

** Significant in level % 1; ns: non significant respectively

decreases by 100, 95.24, and 93.94% in 250 mM of sodium chloride treatment (Salami *et al.*, 2005). The effect of different salinity levels caused by sodium chloride on germination percentage, radicle and plumule lengths, and germination rate was investigated and it was found that sodium chloride decreases the examined characteristics (Tajbakhsh, 2000). This experiment was carried out aiming to determine the Madagascar periwinkle's resistance against salinity, considering the saline soil of the Khuzestan region and the importance of this plant as an ornamental flower, as well as the little information available on its tolerance against salinity during the germination period.

MATERIALS AND METHODS

This experiment was carried out as a factorial in form of a randomized completely design with three replications, in order to investigate the effect of salinity stress caused by sodium chloride on germination components of two cultivars of Madagascar periwinkle flower. These factors include cultivar and salinity levels with Electrical Conductivity (EC) of 1, 2, 4, 6, 8, and 10 dS/m. First, the seeds were disinfected using sodium hypochlorite for 10 min and then were washed several times with distilled water. After sterilizing the petri dishes and specifying the treatments, 50 seeds placed between two sheets of filter papers (sterilized using autoclave) were put inside the petri dishes, and 5 mL of the solutions with the desired EC was added to each of them. Then the petri dishes were transferred to the germinator at 34±1°C (Jaleel *et al.*, 2007). The seeds were examined daily and seeds with noticeable radicles were counted as the germinated seeds. At the experiment's last day, the lengths of radicles and plumules were measured. Also the germination percentage (Camberato and Mc carty, 1999) and germination rate (Maguirw, 1962) were calculated using the following equations:

$$%GP = \frac{\sum G}{N} \times 100$$

G: The number of germinated seeds; N: The total number of seeds

$$GR = \sum_i^n = 1 \frac{S_i}{D_i}$$

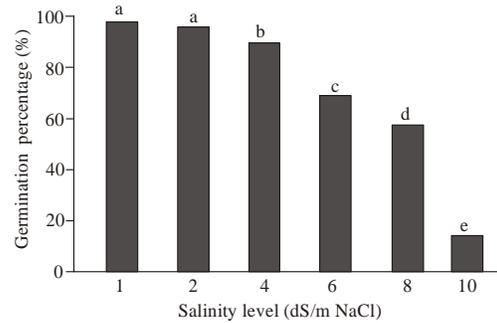


Fig. 1: Effect of salinity stress on the germination percentage of Madagascar periwinkle

S_i: The number of germinated seeds in each counted; D_i: Counting the number of days n; n: Frequency counts.

Statistical analysis: The statistical analysis was performed using the MSTATC software and the diagrams were drawn using the EXCEL software. Also, the Duncan's multiple range test was applied for the averages comparison at level of 1%.

RESULTS AND DISCUSSION

The results from the variance analysis Table1 show that the effect of cultivar and also the interaction of cultivar and salinity level on the germination percentage and germination rate, radicle and plumule length, seedling wet and dry weight and ratio of plumule length to radicle length were not significant, while the salinity stress levels had a significant effect on the characteristics and were significant at the level of 1%.

Effect of salinity stress on the germination percentage and germination rate: Figure 1 indicates that the different levels of salinity stress caused by sodium chloride salt decrease the germination percentage. The maximum seed germinations occurred at the levels of 1 (96%) and 2 (94.6%) dS/m and these two levels had no significant difference, the minimum germination is related to the 10 dS/m level which caused a significant decrease, since the germination dropped from 96 to 13%. The salinity during germination period imposes damages to

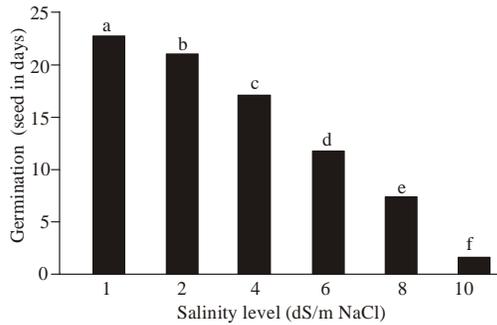


Fig. 2: Effect of salinity stress on the germination rate of Madagascar periwinkle

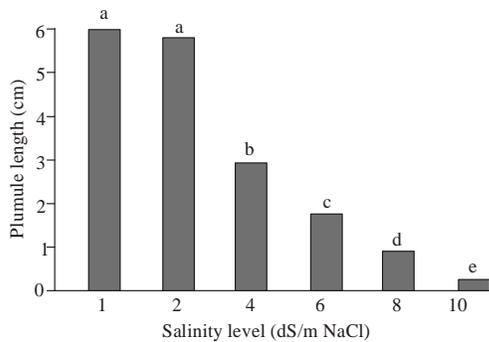


Fig. 3: Effect of salinity stress on the plumule length of Madagascar periwinkle

cell membranes, especially to the cytoplasm membrane, and consequently increases the cells permeability due to replacing Ca^{+} with Na^{+} which ultimately increases the K^{+} losses (Takel, 2000). The decreased germination percentage of plants in addition to the osmosis effect which decreases absorption due to the ions specific toxicity also could be caused by the nutrients absorption disorder (Penuelas *et al.*, 1997).

The germination rate has also been influenced by salinity stress, as by increasing the amount of salinity, it decreases. Figure 2 indicates that the maximum germination rate is related to the 1 dS/m level (22.8) and the minimum has occurred in the 10 dS/m level (1.23). Here, all the salinity stress levels showed significant differences with each other at the level of 1%. The reason for decreased germination rate and germination percentage could be due to the extensive presence of anions and cations. This presence, in addition to the increase in toxicity, though soluble in water, decreases the water's potential, so that despite water presence in the environment, plants are unable to absorb water and are faced with a sort of water deficit, since the water's reaction capacity is occupied by ions (Singh *et al.*, 1988).

Effect of salinity stress on the plumule and radicle length: Salinity caused a decrease in the plumule length. From Fig. 3 it could be observed that the salinity stress

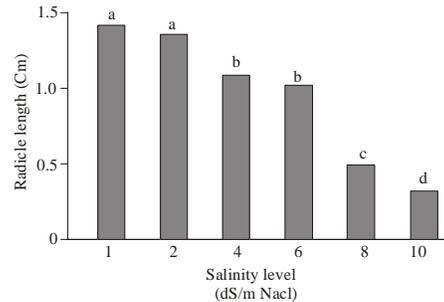


Fig. 4: Effect of salinity stress on the radical length of Madagascar periwinkle

decreases plumule length. The maximum plumule length occurred in 1 and 2 dS/m level and the minimum is related to the highest salinity level (10 dS/m) which decreased from 5.9 to 0.3 cm. All the salinity levels showed significant differences at the level of 1%.

The salinity stress decreased the radicle length, but the significant differences are lower than the plumule length, that is, the length of radicles is less influenced by the salinity stress rather than the length of plumules and has lower sensitivity to the salinity (Fig. 4). The maximum radicle lengths are related to the 1 dS/m (1.4 cm) and 2 dS/m (1.36 cm) salinity levels and the minimum is related to the 10 dS/m salinity level, also there is no significant difference between the 4 and 6 levels. Plants need osmotic adjustment and one way to achieve osmotic adjustment is to form organic substances, such as sorbitol, proline, and glycine, within the tissues. Formation of such substances consumes energy in plants, and therefore the consumed energy for osmotic adjustment decreases the growth of plant's aerial organs (Penuelas *et al.*, 1997). Generally, the decreases in the germination and seedling growth, due to the increase in the environment's salt concentration, are caused by physicochemical effects or by osmotic-toxic salts which exist in saline solutions. In fact, increasing the osmotic pressure (more negative osmotic pressure) resulted from the increased environment's salinity, on one hand disrupts the seed hydration, and on the other hand, the high concentrations of anions and cations (especially Na^{+} and Cl^{-}) in the environment impede the seed germination by imposing toxicity in seeds (Rajabi and Postini, 2005).

Effect of salinity stress on the seedling wet and dry weight: As it could be observed from Fig. 5, the seedling's wet weight decreases as the salinity level increases. The maximum seedling's wet weights belong to the 1 and 2 dS/m levels with 0.67 and 0.64 g, respectively, while the minimum occurred in the 10 dS/m level with 0.007 g. Other levels also had significant differences with each other.

The salinity could influence the seedling's dry weight and decrease it. As it is shown in Fig. 6, the maximum dry weights are observed in 1 dS/m (0.11 g) level and the

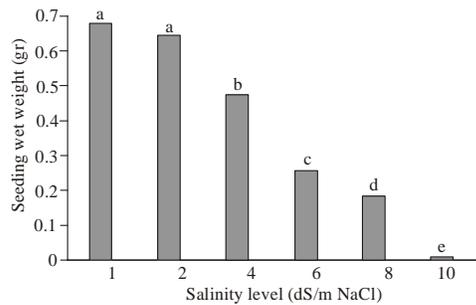


Fig. 5: Effect of salinity stress on the wet weight of seedling of Madagascar periwinkle

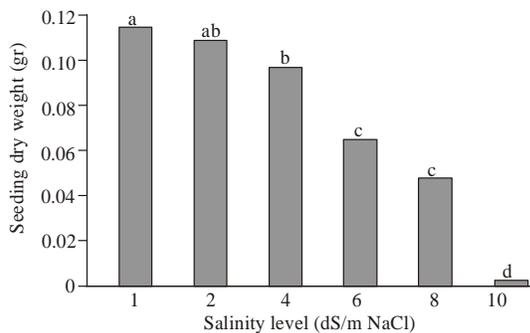


Fig. 6: Effect of salinity stress on the dry weight of seedling of Madagascar periwinkle

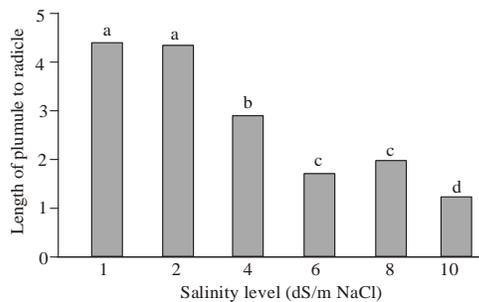


Fig. 7: Effect of salinity stress on the ratio of plumule length to radicle length of Madagascar periwinkle

minimum is related to the 10 dS/m (0.0013 g) level. Other levels showed significant differences with each other. It seems that the negative effects of salinity on plants are the result of creating osmotic potential using salt in the plant's growth environment. Therefore, the root's cells are unable to absorb water needed for growth from the environment, thus the absorption of some water soluble mineral substances is limited which limits the plant's growth and development due to the difficulties in the metabolism. Some authors believe that metabolism present negative effects because of ion accumulation resulted from membrane permeability (Grieve and Fujiyama, 1987).

Effect of salinity stress on ratio of plumule length to radicle length: The ratio of plumule length to radicle length is affected by salinity and is decreased as the salinity increases. Figure 7 shows that the maximum plumule length to radicle length occurs in the 1 dS/m (4.26) and 2 dS/m (4.21) levels and the minimum is related to the 10 dS/m (1.04) level. It is expected that during salinity conditions, the ratio of aerial organs to root would decrease and it is reported through many sources (Flowers *et al.*, 1977).

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