P L A N T B R E E D I N G A N D S E E D S C I E N C E
Volume 67 2013

DOI 10.2478/v10129-011-0074-2

[1]H.Saeb, [1]M.Khayyat, [1]A.Zarezadeh, [1]F.Moradinezhad, [2]A.Samadzadeh, [2]M.Safaee

[1]Department of Horticultural Science, College of Agriculture, Birjand University, Birjand, Iran;
[2]Department of Agronomy and Plant Breeding, College of Agriculture,
Birjand University, Birjand, Iran

EFFECTS OF NACL STRESS ON SEED GERMINATION ATTRIBUTES OF PERIWINKLE (*CATHARANTHUS ROSEUS* L.) AND CORN POPPY (*PAPAVER RHOEAS L.) PLANTS*

ABSTRACT

The present research was conducted to evaluate the effects of salinized water with NaCl on seed germination of Periwinkle and Corn Poppy. Treatments were: A) H_2O- distilled water (control); B) sodium chloride solutions (5, 10, 20, 40 and 80 mM). Application of the highest NaCl concentration (80mM) significantly reduced germination percentage and mean germination time of both species, although increased the day of 50% germination. Increment of salinity concentration was led to the reduction of radicle length in Periwinkle. The seedling fresh weight and water content and radicle length of Corn Poppy were decreased in both levels of 20 and 80 mM, and seedling dry weight was unaffected by treatments. It is concluded that both species are tolerant to NaCl salinity up to 80 mM during germination stage.

Key words: corn poppy*;* germination percentage; periwinkle; radicle length; salinity;

INTRODUCTION

Periwinkle *(Catharanthus roseus)*, family *Apocynaceae*, is a perennial herbaceous plants with creeping stems that root at the nodes and with short ascending flowering shoots. The opposite, short-stalked leaves are evergreen, leathery and elliptic. The flowering stems are used medicinally, containing several alkaloids, tannins, saponins, pectin and organic pigments. These substances give the plant tonic, astringent, hypotensive, vasodilating and diuretic properties (Stodola and Volak, 1992). It is used in some pro-

Communicated by Andrzej Anioł

prietary preparations for cardiovascular disorders and in herbalism for treating bleeding from the nose and gums, for diarrhea, coughing spasms and stomatitis, and in gynaecology (Stodola and Volak, 1992). It has also been found that its two alkaloids vincristine and vinblastine inhibit the growth of certain cancer-forming cells (Stodola and Volak, 1992).

Corn poppy (*Papaver rhoeas* L.), family *Papaveraceae*, is an annual herb indigenous to numerous regions in the world. In traditional medicine until synthetic drugs are developed, extracts of this plant have been used for the treatment of a wide range of diseases including inflammation, diarrhea, sleep disorders and, moreover, for cough, analgesia and also the reduction of withdrawal signs of the opioid addiction. It is also claimed that this plant exhibits sedative, narcotic, and emollient effects (Zargari, 1994).

Salinity is one of the most important limiting factors in production of horticultural crops, which affect the germination rate, percentage and seedling growth in different ways depending on the plant species (Murrilo-Amador *et al.*, 2000; Almansouri *et al.*, 2001) and/or cultivars, which may lead to uneven stand establishment and reduced crop yields (Foolad and Lin, 1997). Naturally occurring salt stress is generally due to NaCl (Levitt, 1972). More than 900 million hectares of land world-wide, approximately 20% of the total agricultural land (FAO, 2007), are affected by salinity, accounting for more than 6% of the world's total land area. NaCl is the predominant salt causing salinization, and it is unsurprising that plants have evolved mechanisms to regulate its accumulation (Munns and Tester, 2008).

Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth. Despite the importance of seed germination under salt stress (Ungar, 1995), the mechanism (s) of salt tolerance in seeds is relatively poorly understood, especially when compared with the amount of information currently available about salt tolerance physiology and biochemistry in plants (Kanai *et al.*, 2007; Khyyat *et al.*, 2009). In plants, salt stress causes reduced cell turgor and depressed rates of root and leaf elongation (Fricke *et al.*, 2006), showing the primary impact of salinity on water uptake. Furthermore, high intracellular concentrations of both Na^+ and Cl^- can inhibit the metabolism of dividing and expanding cells (Neumann, 1997), retarding germination and even leading to seed death. Although, there are some reports on the effects of salinity on Periwinkle plants (Jaleel *et al.*, 2008; Jaleel *et al.*, 2008), however, there is no investigation about seed germination of periwinkle and corn poppy plants under NaCl stress. So, the main aims of this study were to find out the seed germination response of these plants and germination attributes under NaCl stress.

MATERIALS AND METHODS

Seed and petri dishes preparation

This laboratory experiment was carried out using Periwinkle and Corn Poppy seeds in the Department of Horticultural Science, Birjand University, Iran, during February of 2011. Before starting the experiment, 48 petri dishes were prepared and dipped in 60°C distilled-water for about 20 min. The dishes disinfected using spray of 25% ethyl alcohol. Fifty seeds from each species were placed in each petri dish (80 mm) over two filter papers. The dishes were moistened with 5 ml of distilled water (control) or with an equal quantity of the respective solution.

Treatments

The following treatments were used (Table 1):

A) H_2O- distilled water (control),

B) sodium chloride solutions (5, 10, 20, 40 and 80 mM),

Table 1

NaCl concentrations and yielded electrical conductivities

NaCl Concentration [mM]	Electrical Conductivity [μs]
0	0
5	343
10	764
20	1532
40	3210
80	5750

The dishes were placed in an incubation chamber under dark, and temperature of 25 ±1°C. Distilled water or test solutions were added to each petri dish, during the experiment according to their water requirements. The experiment lasted for 18 days.

Measurement

Germination percentage (GP), speed of germination (SG), mean daily germination (MDG), the day of 50% emergence (G50%), seedling fresh (FW) and dry weight (DW), seedling water content (WC), radicle length (RL), peak value (PV), germination value (GV) and mean germination time (MGT) were measured in this experiment. The germination percentage was recorded every day, starting from the first day after the seeds were initially

placed in the petri dishes. With appearance of cotyledons in each seedling, radicle length was recorded; then, the respective seedling was weighted for fresh and dry weights, and removed from the experiment. Speed of germination was calculated by the following formula given by Czabator (1962):

$$S_G = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \cdots + \frac{n_n}{d_n}$$

where,
S_G — speed of germination
n_n — number of germinated seeds per each calculation,
d_n — number of day until calculation,

Mean daily germination was assessed using Hartmann *et al.* (1990) method:

$$MDG = \sum \frac{(N_1 \times T_1 + N_2 \times T_2 + \cdots + N_x \times T_x)}{Total\ number\ of\ germinated\ seeds}$$

where,
MDG — mean daily germination
N_x —number of seeds germinated within consecutive intervals of time;
T_x — time between the beginning of the test and the end of a particular interval or measurement.

Day of 50% emergence was calculated based on Heydecker and Wainwright (1976):

$$D_{E50\%} = \frac{[(t_2 - t_1) \times 50\% + (p_2 \times t_1 - p_1 \times t_2)]}{p_2 - p_1}$$

where,
$D_{E50\%}$ — day of 50% emergence
t_1 — time at which the germination percentage is less than 50%;
t_2 — time at which the germination percentage is more than 50%;
p_1 — the measurements of germination percentage occurring at t_1,
p_2 — the measurements of germination percentage occurring at t_2,

For fresh weight, seedlings were weighted after appearance of cotyledons using analytical single-pan balance with 0.0001 accuracy. Then, the mentioned seedlings were dried in oven with the temperature of 70°C for 24 h and dry weight was assessed. Moisture content of seedlings was determined by the following formula given by Evan (1972):

$$M_{\%} = \frac{F_{wgt} - D_{wgt}}{F_{wgt}} \times 100$$

where,
M% — moisture percentage,
F_{wgt} — fresh weight,
D_{wgt} — dry weight,
Radicle lengths were measured using a ruler.

Peak value was calculated by the following formula given by Czabator (1962):

$$PV = \frac{MDG_{max}}{N_d}$$

where,
PV — peak value,
MDG_{max} — maximum mean daily germination (cumulative percentage of full seed germination),
N_d — number of days elapsed since sowing date reached at any time during the period of the test

Germination value was calculated by the formula given by Czabator (1962):

$$GV = PV \times MDG$$

where,
GV — germination value,
PV — peak value,
MDG — mean daily germination,

Mean germination time was calculated based on Schelin *et al.* (2003) as followed:

$$MGT = \frac{\sum f_i \times n_i}{N}$$

where,
f_i — day during germination period (between 0 and 18 day),
n_i — the number of germinated seeds per day,
N — sum of germinated seeds,

Statistics

The experiment was arranged in a completely randomized design with 6 treatments and 4 replications, each replication consisted of one petri dish and 50 seeds in each. Data were analyzed using Gen-STAT software. Means were separated with least significant difference (LSD) at *P*= 0.05.

RESULTS AND DISCUSSION

Application of the highest NaCl concentration (80mM) significantly reduced germination percentage and mean germination time of both species, although increased the day of 50% emergence (Table 2).

Table 2

Effects of NaCl concentrations on germination percentage (GP), speed of germination (SG), mean daily germination (MDG) and the day of 50% germination (G50) of periwinkle and corn poppy

Species	Periwinkle				Corn Poppy			
Treatments	GP	SG	MDG	G50	GP	SG	MDG	G50
Control	94.50ab	3.14ab	5.20ab	3.00ab	80.00a	2.88a	4.37a	2.25b
5 mM	97.00ab	3.18ab	5.35ab	3.00ab	84.50a	2.85a	4.65a	2.50b
10 mM	100.00a	3.11ab	5.50 a	2.75 b	75.00a	3.37a	4.10a	3.25b
20 mM	97.00ab	2.98 b	5.35ab	3.00ab	75.75a	3.29a	4.15a	3.25b
40 mM	96.25ab	3.23ab	5.30ab	3.00ab	73.50a	3.37a	4.05a	3.25b
80 mM	92.50 b	3.49 a	5.07 b	3.25 a	62.00b	3.36a	3.37b	6.00a

Within each column, same letter indicates no significant difference between treatments at 5% levels

These data indicated that salinity conditions to some extent and dependently on species-, increase germination. It was in agreement with Zhang *et al.* (2010) who found that under salinity conditions, seeds were able to germinate faster and to higher percentages, because of taking up rapidly both salt and water. The highest and lowest values of speed of periwinkle germination were found for treatments 80 mM and 20 mM , respectively. However, both of these treatments no significant effect on this variable was found in the case of corn poppy (Table 2).

Neither fresh nor dry weight of periwinkle seedlings were significantly affected by salinity treatments in comparison to control (Table 3). The highest and lowest water content in seedlings of periwinkle were resulted at the concentration of 20 and 80 mM NaCl solution, respectively (Table 3). Increment of salinity concentration led to the reduction of radicle length in this species, which showed the lowest value for this variable in the highest NaCl level (Table 3). The seedling fresh weight, water content and radicle length of corn poppy were decreased both for 20 and 80 mM, and seedling dry weight was unaffected by treatments (Table 3).

Table 3

Effects of NaCl concentrations on seedling fresh weight (FW), seedling dry weight (DW), seedling water content (WC) and radicle length (RL) of periwinkle and corn poppy

Species	Periwinkle				Corn Poppy			
Treatments	FW	DW	WC	RL	FW	DW	WC	RL
Control	0.010a	0.0006a	93.75ab	3.35a	0.009a	0.0012a	86.30a	4.99 a
5mM	0.011a	0.0006a	94.25ab	3.37a	0.009a	0.0003a	96.80a	4.39 ab
10mM	0.008a	0.0005a	93.25ab	2.43b	0.011a	0.0016a	88.25a	3.62abc
20mM	0.009a	0.0005a	94.50a	2.50b	0.005b	0.0010a	80.90b	2.11 bc
40mM	0.010a	0.0006a	93.50ab	2.62ab	0.011a	0.0003a	97.13a	3.66abc
80mM	0.008a	0.0006a	92.75 b	1.82b	0.004b	0.0001a	23.75b	1.70 c

Within each column, same letter indicates no significant difference between treatments at 5% levels

Table 4

Effects of NaCl concentrations on peak value (PV), germination value (GV), and mean germination time (MGT) of periwinkle and corn poppy

Species	Periwinkle			Corn Poppy		
Treatments	PV	GV	MGT	PV	GV	MGT
Control	1.37ab	7.12bc	8.10a	1.37 a	6.05a	6.44ab
5mM	1.42ab	7.60ab	8.42a	1.32ab	6.34a	6.62ab
10mM	1.37ab	7.52ab	8.52a	0.97bc	4.07a	6.82 a
20mM	1.35 b	7.17bc	8.02a	0.90 c	3.75a	6.72ab
40mM	1.60 a	8.45 a	8.62a	0.97bc	3.93a	6.70ab
80mM	1.25 b	6.35 c	8.77a	0.75 c	2.72b	5.47 b

Within each column, same letter indicates no significant difference between treatments at 5% levels

Salinity affects seed germination through osmotic effects (Bliss *et al.*, 1986), ion toxicity (Hampson and Simpson, 1990) or combination of them (Huang and Redmann, 1995). Regarding to data, it is suggested that ion toxicity may be the stronger cause of radicle growth inhibition in both species.

The highest peak and germination values of periwinkle were resulted from 40 mM. There was no significant difference among treatments on mean germination time of this species (Table 4). Both control and 5 mM treatments were led to the highest peak value of corn poppy (Table 4). All treatments had not significant effects on the germination value and mean

germination time of this species, with the exception of 80 mM, which significantly reduced the values of these variables (Table 4).

In rice, wheat and barley salinity has been shown to negatively affect the rate of starch remobilization by causing a decrease in α-amylase activity (Lin and Kao, 1995; Almansouri *et al.*, 2001; Zhang *et al.*, 2010). Plants can be classified into two main groups based on their response to saline stress, salt-tolerant halophytes and salt-intolerant glycophytes. However, this classification is somewhat artificial as the implied discreteness of response does not exist in reality, with responses occurring along a gradient (Greenway and Munns, 1980).

Salinity-induced reduction in the germination of halophytes is mainly due to osmotic effects only, whereas glycophytes are more likely to exhibit additional ion toxicity (Romo and Haferkamp, 1987; Dodd and Donovan, 1999). Furthermore, the seeds of salt-tolerant species tend to have lower osmotic potentials, allowing them to absorb water from the environment. This decrease in osmotic potential can be achieved in two ways: exclusion of salt from the cells while maintaining osmotic potential using organic solutes, or by allowing Na^+ and Cl^- to enter the cells and using them as osmolites while having mechanisms for mitigating the toxic effects of salt within the cell (Zhang *et al.*, 2010).

Regarding to results, it is concluded that both species are NaCl tolerant during germination and cotyledon development, and can be easily established using salinized water containing up to 80 mM NaCl.

REFERENCES

Abdul Jaleel, C., B. Sankar, R. Sridharan, R. Panneerselvam, 2008, Soil Salinity Alters Growth, Chlorophyll Content, and Secondary Metabolite Accumulation in Catharanthus roseus, *Turk. J. Biol.,* 32, 79-83.

Abdul Jaleel, C., R. Gopi, P. Manivannan, R. Panneerselvam, 2008, Soil salinity alters the morphology in *Catharanthus roseus* and its effects on endogenous mineral constituents, *EurAsian J.BioSci.,* 2, 18-25.

Almansouri, M., J.M. Kinet, S. Lutts, 2001, Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.), *Plant Soil,* 231, 243-254.

Bliss, R.D., K.A. Plattaloia, W.W. Thomson, 1986, Osmotic sensitivity in relation to salt sensitivity in germinating barley seeds, *Plant Cell. Environ.,* 9, 721–725.

Dodd G.L., L.A. Donovan, 1999, Water potential and ionic effects on germination and seedling growth of two cold desert shrubs, *Amer. J. Bot.,* 86, 1146–1153.

G.C. Evans, 1972, The Quantitative Analysis of Plant Growth, Blackwell Scientific Publication, oxford.

FAO, 2007, FAO Agristat, www.fao.org (accessed on 10 June 2010).

Foolad, M.R. and G.Y. Lin, 1997, Genetic potential for salt tolerance during germination in *Lycopersicon species*, *HortSci.,* 32(2), 296-300.

Fricke, W., G. Akhiyarova, W.X. Wei, E. Alexandersson, A. Miller, P.O. Kjellbom, A. Richardson, T. Wojciechowski, L. Schreiber, D. Veselov, G. Kudoyarova, V. Volkov, 2006, The short-term growth response to salt of the developing barley leaf, *J. Experiment. Bot.,* 57, 1079–1095.

Greenway, H., R. Munns, 1980, Mechanisms of salt tolerance in nonhalophytes, *Annual Rev. Plant Physiol. Plant Molecul. Bio.,* 31, 149–190.

Hampson, C.R., G.M. Simpson, 1990, Effects of temperature, salt, and osmotic potential on early growth of wheat (*Triticum aestivum*). 1. *Germination. Can. J. Botany-Revue Canadienne De Botanique,* 68, 524–528.

Hartmann, H.T., D.E. Kester, Jr, F.T. Davies, 1990, *Plant propagation, principles and practices. Prentice-Hall International,* Inc. Englewood Cliffs, New Jersey, USA.

Heydecker, W., H. Wainwright, 1976, More rapid uniform germination of *Cyclamen persicum* L. *Sci. Hortic.,* 5, 183–189.

Huang, J., R.E. Redmann, 1995, Salt tolerance of Hordeum and Brassica species during germination and early seedling growth, *Can. J. Plant Sci.,* 75, 815–819.

Kanai, M., K. Higuchi, T. Hagihara, T. Konishi, T. Ishii, N. Fujita, Y. Nakamura, Y. Maeda, M. Yoshiba, T. Tadano, 2007, Common reed produces starch granules at the shoot base in response to salt stress, *New Phytol.,* 176, 572–580.

Khayyat, M., E. Tafazoli, S. Rajaee, M. Vazifeshenas, M.R. Mahmoodabadi, A. Sajjadinia, 2009, Effects of NaCl and supplementary potassium on gas exchange, ionic content, and growth of salt-stressed strawberry plants, *J. Plant Nutr.,* 32, 907-918.

Levitt, J, 1972, *Salt and ion stresses.* In: Physiological ecology: a series of monographs texts and treatises. Academic press, London, pp: 489-530.

Lin, C.C., C.H. Kao, 1995, NaCl stress in rice seedlings – starch mobilization and the influence of gibberellic acid on seedling growth, *Bot. Bull. Acad. Sinica,* 36, 169–173.

Munns, R., M. Tester. 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.,* 59: 651–681.

Murillo-Amador, B., E. Troyo-Dieguez, H.G. Jones, F. Ayala-Chairez, C.L. Tinoco-Ojanguren, A. Lopez-Cortes, 2000, Screening and classification of cowpea genotypes for salt tolerance during germination, *Phyton-International J. Experim. Bot.,* 67, 71-84.

Neumann, P, 1997, Salinity resistance and plant growth revisited, *Plant Cell. Environ.,* 20, 1193–1198.

Romo, J.T., M.R. Haferkamp, 1987, Effects of osmotic potential, potassium chloride and sodium chloride on germination of greasewood (*Sarcobatus vermiculatus*), *Great Basin Natur.,* 47, 110–116.

Schelin, M., M. Tigabu, I. Eriksson, L. Swadago, and P.C. Oden, 2003, Effect of scarification, gibberllic acid and dry heat treatments on the germination of *Balanties Egyptian* seed from the Sudanian savanna in Burkina Faso, *Seed Sci. Tech,* 31, 605–617.

Stodola, J. And Volák, J, 1992, The Illustrated Encyclopedia of Herbs, p. 299. Chancellor Press, Michelin House, London.

Ungar I.A. , 1995, Seed germination and seed-bank ecology of halophytes. In: Kigel J, Galili G. eds. Seed development and germination, New York: Marcel Dekker, 599–627.

Zargari A. , 1994, Medical Plants. vol. 1. Tehran University, Tehran, pp, 91–102.

Zhang, H., L.J. Irving, C. Mcgill, C. Matthew, D. Zhou, P. Kemp, 2010, The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator, *Ann. Bot.,* 106, 1027-1035.