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Effect of salinity stress on germination and heterotrophic growth of wild okra (*Corchorus olitorius* L.) seeds

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ABSTRACT:

An experiment was laid out in order to evaluate the effect of salinity stress on seed germination, seedling growth and heterotrophic growth of wild okra at the laboratory of horticulture, Faculty of Gorgan Agricultural and Natural Resources University, Iran, March 2015. In this experiment treatments were different salinity stress levels of NaCl (0, -2, -4, -6, -8 and -10 bar). 25 seeds of each lot evenly placed on Whatman filter paper No.1 in sterilized 9-cm petri dishes separately and 10 ml of Nacl solution at different concentration were added . Seed germination parameters, seedling growth and heterotrophic growth components were determined. The results showed that, the effect of salinity stress was significant on germination percentage, germination rate, hypocotyl and radicle dry weight, seedling length, seed vigor index, seed reserves utilization, conversion efficiency of mobilized seed reserve and ratio of utilized seed reserve. Mean comparison data showed that maximum germination percentage, germination rate, radicle dry weight, seedling length, seed vigor index and seed reserves utilization were recorded for -2 bar water potential and the lowest of them was recorded for the -10 bar water potential (severe salinity stress) for all traits. Maximum hypocotyl dry weight were recorded at -2 bar saline stress treatment. Moreover, maximum conversion efficiency of mobilized seed reserve and ratio of utilized seed reserve was founded at -8 bar saline stress treatment and minimum of them was recorded for -10 bar water potential. The results of the study showed that increasing of saline stress in wild okra decreased seed germination parameters, seedling growth and heterotrophic growth components.

Keywords:

Heterotrophic growth, salinity and seed germination

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INTRODUCTION

Corchorus olitorius is a popular vegetable, grown in both dry and semi-arid regions and in the humid areas of world, because of its importance in giving the nutrients (Oladiran, 1986). Wild okra belongs to the Tiliaceae family and is an erect annual herb that varies from 60 cm to approximately 150 cm in height depending on the cultivar (Husselman and Sizane 2006).Wild okra is a wild leafy vegetable with potential for development as a crop is one of the annual dicotyledonous herbs (Zare *et al.,* 2007). Studies have shown that *Corchorus olitorius* among many indigenous vegetable species have suboptimal germination capabilities that hinder cultivation efforts (Modi, 2007). The edible leaves are rich sources of vitamins and minerals including: iron, calcium, thiamine, riboflavin, niacin, foliate, dietary fiber and protein (Leong *et al.,* 1968) Denton (Denton, 1997) recommended a solution to the incidence of seed dormancy through the application of seed parboiling process. Its seeds tend to show poor germination (Vorster, 2002). Several studies have been carried out on pre-germination treatments of seeds (Farooq *et al.,* 2005). For example, Nkomo and Kambizi (Nkomo and Kambizi, 2009) reported that pre-chilling followed by exposure to temperature higher than 30°C encourages germination of *C. olitorius* seeds.

Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth. Germination is one of the most salt-sensitive plant growth stages severely inhibited with increasing salinity. This negative response of seed germination under salt stress was reported by many authors on *Ocimum basilicum, Eruca sativa, Petroselinum hortense*, *chamomile*, sweet marjoram and *Thymus maroccanus* (Miceli *et al.,* 2003 and Ramin, 2006). In spite of the reputation of seed germination in salt stress (Chapman, 1974 and Van, 1996) the mechanism of salt acceptance in seeds is comparatively poorly understood, particularly when compared with the quantity of information presently available about salt tolerance physiology and biochemistry in vegetative plants (Hu *et al.,* 2005 and Ren *et al.,* 2005). Salinity disturbs seed germination through osmotic effects and ion toxicity (Hampson and Simpson, 1990) or both(Huang and Redmann, 1995) Salinity-induced decrease in the germination of halophytes is mostly due to osmotic effects, whereas glycophytes are further likely to display additional ion toxicity (Dodd and Donovan, 1996). Sodium chloride has been used to put on osmotic stress effects in petri dish (*in vitro*) for plants to uphold uniform water potential all over the experimental period (Kulkarni and Deshpande, 2007). Production of green buds and seeds are strongly affected by drought and salinity stresses, resulting in poor plant establishment in dry and saline soils. Therefore, this study was conducted to evaluate the response of wild okra (*Corchorus olitorius* L.) to saline stress.

MATERIALS AND METHODS

This experiment was laid out in order to evaluate the response of wild okra to combat salt stress. This experiment was laid out in a completely randomized design with four replicates at the laboratory of Horticulture, Faculty of Gorgan Agricultural and Natural Resources University, Iran, March, 2015. Seeds were surface-sterilised with 3% sodium hypochlorite solution, rinsed in distilled water for three times and dried for 36h before the experiment. Treatments were different for the irrigation of drought stress levels of NaCl (0, -2, -4, -6, -8 and -10 bar). 25 seeds of every lot consistently placed on Whatman filter paper No.1 in disinfected 9-cm petri dishes distinctly and 10 ml of each solution were added to the related treatment. All petri dishes were wrapped to check the loss of moisture and to avoid contamination, and then positioned in a plant growth chamber for 12 days. The seeds were allowed to germinate at 27°C with 12/12 h light/dark periodicity. The photosynthetic photon flux density was 340-mol m^{-2} s^{-1}, delivered by metal halide lamps, with a comparative humidity of 45%. Germination was determined by calculating the number of germinated seeds at 24h intervals over a 12-d period and articulated as total percent germination. Seeds were well-thought-out to be germinated at the development of the radicle. Radicle and hypocotyl lengths were measured 12 days after sprouting. The root and stem dry weight were determined by desiccating the plant material in an oven at 80°C for 24h prior to weighing. Seed vigor index determined by using radicle length (Ungar, 1995).

Germination percentage and germination rate were calculated using the following formula (Ungar, 1995).

$G\% = (n/N)*100$

$RG = \sum(Ni / Di)$

G: Germination percentage, n: Number of seeds germinated, N: Total number of seed in each petri dishes, RG: Rate of Germination (seed /day), Ni: Germinated seeds in each numeration, Di: Day of each numeration.

After germination test, oven-dried weight of seedlings were determined. The weight of utilized (mobilized) seed reserve was calculated as the dry weight of the original seed minus the dry weight of the seed remnant. Conversion efficiency of mobilized seed reserve and ratio of utilized seed reserve to initial seed dry weight was considered as Seed Reserve Depletion Percentage (SRDP) (Ungar, 1995).

The statistical studies were done to find out the distinct and interactive effects of treatments were using JMP 5.0.1.2 SAS Institute,(1990). Statistical significance was professed at P≤0.05 and P≤0.01. Treatment effects from the two runs of tests followed a similar drift, and thus the data from the two independent runs were pooled in the investigation (Seyyed, 2007).

RESULTS AND DISCUSSION

Germination percentage: Results of analysis of variance showed that the effect of salt stress on germination percentage was significant at 1% (Table 1). Mean comparison table showed that the Germination Percentage (GP) reduced with increasing of drought level so that the highest GP was recorded for zero water potential (97.3%) and the lowest GP was recorded for the -10 bar water potential (33%) (Table 2).

Germination rate: The results showed that the effect of saline stress on Germination Rate was significant at 1% (Table 1). Mean comparison table showed that the Germination Rate (GR) reduced with increasing salinity level so that the highest GR was recorded for 0 bar water potential (1.74) and the lowest GR was recorded for the -10 bar water potential (0.6) (Table 2).

Hypocotyl dry weight: The effect of salinity stress on hypocotyl dry weight was not significant (Table 1).

Radicle dry weight: The effect of salinity stress on radicle dry weight was significant (Table 1). Radicle dry weight decreased with increase in saline levels. Maximum radicle dry weight was obtained at 0 bar water potential while the minimum of this trait resulted from the lowest level of water potential (-10 bar) (Table 2).

Seedling length: Results of analysis of variance showed that the effect of salinity stress on seedling length was significant at 1% level (Table 1). Mean comparison table

Table 1. Analysis of variance of wild okra seeds germination under salt stress

S.O.V	df	Germination percentage %	Germination rate	Hypocotyl dry weight (g)	Radicle dry weight (g)	Seedling length (mm)	Seed vigor	Seed reserves utilization ($mg.g^{-1}$)	Conversion efficiency of mobilized seed reserve ($mg.g^{-1}$)	Ratio of utilized seed reserve (mg)
Salinity stress	5	1677^{**}	0.6**	0.0003^{ns}	0.000005^{**}	918^{**}	13175644^{**}	188801992^{ns}	755^{ns}	0.0000003^{**}
Error	15	20	0.001	0.0002	0.0000004	18	126615	95988799	383	0.00000004
CV(%)		3.9	0.16	0. 3	0. 1	5.1	18.06	9.3	13.5	4

**, * and ns: Significant at 1% and 5% levels of probability showed that the seedling length reduced with increase in drought level so that the highest seedling length was recorded for 0 bar water potential (63.3mm) and the lowest seedling length was recorded for the -10 bar water potential (16.6mm) (Table 2).

Seed vigor index: The effect of salinity stress on seed vigor index was significant at 1% level (Table 1). Mean comparison showed that the highest seed vigor index was recorded for -0 bar water potential and the lowest seed vigor index was recorded for the -10 bar water potential (Table 2).

Heterotrophic growth: Results for heterotrophic growth showed that the effect of drought stress on seed reserves utilization and conversion efficiency of mobilized seed reserve was not significant but the ratio of utilized seed reserve was significant (Table 1). Mean comparison for ratio of utilized seed reserve showed that the highest ratio of utilized seed reserve was recorded for 0 bar water potential and the lowest ratio of utilized seed reserve was recorded for the -10 bar water potential (Table 2).

Results showed that there were significant differences between treatments based on rate of germination, germination percent, shoot length and root length ($p<0.01$) (Table 1). Salinity impressed seed germination through osmotic pressure and therefore reduction of water absorption by seeds and also from the toxic effects of Na^+ and Cl^- (Rehman *et al.,* 1996). With increasing in salinity stress levels, there was significant difference between treatments in all evaluated traits ($p<0.01$). The salinity in the seed germination phase harm the membrane of the cell, especially the cytoplasmic membrane that fallouts in increasing penetrability of the membrane due to the addition of Ca^{2+} by Na^+ that resulted to the collective K^+ losses (Ungar, 1995). Also, Salehi (Sardoei *et al.,* 2008) reported that with an upsurge in salinity from 50 to 150 Mm, germination of *Lycopersicon esculentum* L. var Cal-ji abridged about 18.21%. With increasing of NaCl concentration, germination percentage, germination rate, radicle and hypocotyl dry weight, seedling length and heterotrophic growth of wild okra were decreased (Table 2). Sharifi (Seyyed *et al.,* 2007) in an experiment with study on four salinity levels (0, 25, 50 and 100 mM) using NaCl on *Silybum marianum* germination showed that salinity stress has significant effect on germination percentage and germination uniformity with average germination percentage in salinity potential 25 and 50 mM NaCl which was decreased compared to control (distilled water) with 17.20 and 43.00 percent respectively.

In the present study, the maximum of all traits was obtained at 0 bar treatment (control) and minimum of them was obtained at -10 bar treatment (Table 2). Decrease in root length wass reported with increase in water potential and one of the causes of shoot length reduction in drought stress conditions has been found as reduction or non-transfer of nutrients from seed storage tissues to the embryo by Takel (Ungar, 1995). Salinity may inactivate the germination-affecting enzymes, especially by increasing the uptake of K^+ which brings about a secondary peak which as a result, inhibits the

Table 2. Means comparison of wild okra seeds germination under salt stress

Treatments	Germination percentage(%)	Germination rate	Hypocotyl dry weight (g)	Radicle dry weight (g)	Seedling length (mm)	Seed vigor index	Seed reserves utilization ($mg.g^{-1}$)	Conversion efficiency of mobilized seed reserve($mg.g^{-1}$)	Ratio of utilized seed reserve(mg)
0	97.3^a	1.74^a	0.009^a	0.004^a	63.3^a	6152^a	6173^{ab}	12.4^{ab}	0.0011^a
-2 bar	86.7^b	1.55^b	0.029^a	0.003^b	43.6^b	3779^b	16498^{ab}	33^{ab}	0.0010^a
-4 bar	73.3^c	1.31^c	0.005^a	0.002^{cb}	30.9^c	2266^c	4442^b	8.9^b	0.0008^{ab}
-6 bar	60^d	1.07^d	0.006^a	0.002^{cb}	25.2^{dc}	1511^d	9497^{ab}	19^{ab}	0.0005^{bc}
-8 bar	50.7^e	0.9^e	0.006^a	0.001^{cd}	20.1^{de}	1021^{de}	22778^a	45.6^a	0.0002^c
-10 bar	33.3^f	0.6^f	0.003^a	0.0001^d	16.6^e	555^e	2018^b	4^b	0.0009^a

Means with at least one similar letter in each column have no signification difference at %5 of probability level activation and/or synthesis of germination-affecting enzymes and the uptake of Ca^{2+} increases in a confrontation with Na^{+}. Nabizadeh (Nabizadeh, 2002). stated that the adverse effect of salinity on plants can be caused by the loss of osmotic potential of root medium, specific ion toxicity and the lack of nutritional ions.

Also, in the present study, germination rate decreased with increase in salinity level and the reduction of different germination parameters can be related to reduction of speed and rate of water absorption. In addition to the osmotic effect which reduces the uptake due to the specific toxicity effect of ions, the loss of germination percentage is caused by the interruption of nutrients uptake which has been confirmed by Safarnejad *et al*.

Decrease of hydrolytic enzyme activity laid to the decrease of heterotrophic growth components under drought stress. In the present study, heterotrophic growth components such as seed reserves utilization, conversion efficiency of mobilized seed reserve and ratio of utilized seed reserve decreased with increase in salinity levels (Table 1). The highest seed reserves utilization was recorded for -2 bar water potential and the lowest seed reserves utilization was recorded for the -10 bar water potential (Table 2). (Soltani *et al*., 2006 and Takel, 2000). showed that the increase of osmotic pressure under drought and salinity stresses lead to reduction of seed reserve mobilization, but no significant differences were found between stressed and non-stressed plants with respect to the conversion efficiency of mobilized reserves. However, decreasing in water absorption by seed under salinity and drought stress causes to decrease in hormones secretion and enzymes that result to impair seedling growth (root and shoot) (Asghari, 2002). The results showed that seed vigor index had more reaction to moisture by increase in NaCl concentration and was adversely affected when moisture stress reached -10 bars. Decreasing seed vigor index is probably due to the decreasing moisture availability, which causes enzyme activity by some problems in the transfer of endosperm reserves in the form used for the growth of embryonic axes and synthesis of compounds of seed (Velempin *et al.,* 2003). The results of this study showed that with increase in salinity levels in wild okra, seed germination parameters, seedling growth and heterotrophic growth components decreased significantly.

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