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Effect of NaCl and PEG-Induced Osmotic Stress on Germination and Seedling Growth Properties in Wild Mustard (Sinapis arvensis L.)

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ABSTRACT: *This study was conducted to evaluate the effect of NaCl and peg-induced osmotic stress on the germination and early seedling growth of wild mustard (**Sinapis arvensis** L.) in 2016. The seeds of mustard used in this study were obtained from plants growing under wild conditions in the Konya, Turkey. Trials were carried out to study the effects of osmotic potential of polyethylene glycol (PEG-6000) solutions at seven levels (0, -2, -4, -6, -8, -10 and -12 bars) and salt concentrations at eight levels (0, 2, 5, 10, 15, 20, 25, 30 and 35 dSm^{-1}) on seedling growth of wild mustard. Under PEG and salt stress the radicula length (cm), plumula length (cm), seedling fresh weight (mg), seedling dry weight (mg), final germination percentage (%), germination index (%) and seedling vigor index (%) were measured in the study. Final germination percentage and seedling growth parameters decreased by concentration of -4 to -10 bar for PEG stress; final germination percentage decreased by 10 dSm^{-1} (-4.4 bar) and seedling growth parameters decreased by 5 dSm^{-1} (-2.2 bar) for NaCl stress. Both NaCl and PEG treatments decreased final germination percentage and seedling growth properties in wild mustard, but the effects of NaCl compared to PEG were less on germination. Seed germination was completely inhibited at -12 bar for PEG stress, 35 dSm^{-1} (-15.6 bar) for NaCl stress.*

***Keywords**: **Sinapis arvensis** L., germination, mustard, salt and drought stress.*

NaCl ve PEG'e Bağlı Osmotik Stresin Yabani Hardal (Sinapis arvensis L.)'ın Çimlenme ve Fide Gelişimi Özellikleri Üzerine Etkisi

ÖZ: *Bu çalışma farklı NaCl ve PEG uygulamalarının yabani hardalın (**Sinapis arvensis** L.) çimlenme ve erken vejetatif gelişme üzerine etkisini belirlemek amacıyla 2016 yılında yürütüldü. Çalışmada kullanılan yabani hardal tohumları Konya'da yabani koşullarda yetişen bitkilerden alınmıştır. Denemede yedi farklı polietilen glikol (PEG-6000) solüsyonu (0, -2, -4, -6, -8, -10 ve -12 bar) ve dokuz farklı NaCI solüsyonu (0, 2, 5, 10, 15, 20, 25, 30 ve 35 dSm^{-1}) kullanılmıştır. Farklı PEG ve NaCl uygulamalarında kök uzunluğu (cm), sürgün uzunluğu (cm), fide yaş ağırlığı (mg), fide kuru ağırlığı (mg), final çimlenme oranı (%), çimlenme indeksi (%) ve fide canlılık indeksi ölçümleri yapılmıştır. Çalışma sonucunda, final çimlenme oranının ve fide büyüme parametrelerinin artan PEG konsantrasyonlarına (-4 / -10 bar) bağlı olarak azaldığı; final çimlenme oranının 10 dSm^{-1} (-4,4 bar)'den itibaren azalmaya başladığı ve fide büyüme parametrelerinin 5 dSm^{-1} (-2,2 bar)'den itibaren azalmaya başladığı belirlenmiştir. Ayrıca, NaCl ve PEG uygulamalarının çimlenme ve fide gelişimini düşürdüğü; fakat PEG'e kıyasla NaCl'ün etkisinin çimlenmede daha az olduğu; çimlenmenin -12 bar PEG ve 35 dSm^{-1} (-15,6 bar) NaCl dozlarında tamamen durduğu tespit edilmiştir.*

***Anahtar kelimeler**: **Sinapis arvensis** L., çimlenme, hardal, tuz ve kuraklık stresi.*

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threat to agriculture and result in the deterioration of the environment (Odabas *et al.,* 2014; Temizel *et al.,* 2014; Toosi *et al.,* 2014).

Brassica occupy third place among the various oilseed species due to its considerable economic and nutritional value. However, their growth, yield, and oil production are markedly reduced due to salinity. In particular, seed germination and early seedling growth have been reported to be relatively more sensitive towards salinity (Ashraf and McNeilly, 2004).

Other than the economically important Brassica species, various wild relatives belonging to the genera serve as a repository of vast gene pool (Prakash *et al.,* 1999). Wild relatives, such as *Sinapis arvensis* L., also possess a number of useful agronomic traits which could be incorporated into breeding programs, including: cytoplasmic and nuclear malesterility; resistance to disease and insect and nematode pests; intermediate C3-C4 photosynthetic activity; and tolerance for cold, salt and drought conditions (Rollins, 1981; Warwick and Black 1991; Bing *et al.,* 1996).

Seed germination and seedling establishment are the most sensitive stages of plant growth in pastures and crops under environmental stresses (drought, salinity, heat and cold conditions) (Taherkhani, 2013). Salinity and drought may delay the onset, reduce the rate, and increase the dispersion of germination events, leading to reductions in plant growth and final crop yield.

Salinity and drought affect the plants in a similar way. Reduced water potential is a common consequence of both salinity and drought. Water stress acts by decreasing the percentage and rate of germination and seedling growth (Macar, 2008). The deleterious effects of salinity on plant growth are attributed to a decrease in osmotic potential of the growing medium, specific ion toxicity and nutrition deficiency (Greenway and Munns, 1980). Germination rates decrease with an increase in NaCl concentration (Murillo Amador *et al*., 2002).

Drought plays an important role not only in determining germination rate, but also influences seedling development (Macar, 2008). This stress type is one of the most important environmental stresses affecting agricultural productivity worldwide and can result in considerable yield reductions (Mohammadkhani and Heidari, 2008). It is one of the main causes for crop yield reduction in the majority of agricultural and natural regions of the world.

Mustard is sensitive to soil salinity at early stage of growth but fairly tolerant at later developmental stages. Germination and seedling growth of mustard is critical one and thus becomes a major limiting factor for full potential production of crop (Singh, 2011).

Salinity and drought stress are important environmental factors that affect different development stages of crops, especially germination stage. The objective of the present investigation was to evaluate the effect of NaCl and PEG-induced osmotic stress on the germination and early seedling growth of wild mustard (*Sinapis arvensis* L.), a commercially important weed for providing raw material for biodiesel energy (Blackshaw *et al*., 2011; Eryilmaz and Ogut, 2011; Kayacetin *et al*., 2016).

MATERIALS AND METHODS

This study was carried out at the Central Research Institute for Field Crops; oil seed crop unit, Turkey. The seeds of mustard used in this study were obtained from plants growing under wild conditions in the Konya, Turkey. They were multiplied and selected for use in the study.

Each experiment was a two-factor factorial in a Completely Randomized Design with four replication. The first factor was solutions (PEG and NaCl) and the second factor was solution levels (0, -2, -4, -6, -8, -10 and -12 bars; 0, 2, 5, 10, 15, 20, 25, 30 and 35 dSm^{-1}) (electrical conductivities of the solutions). Drought stresses were induced by polyethylene glycol (PEG-6000) treatment. Salt stresses were induced by NaCl using a conductivity meter (Model WTW Cond. 314i, Germany). Distilled water served as a control. For all investigated parameters, analysis of variance was performed using the MSTAT-C software package. Significant differences among the mean values were compared by LSD test ($P < 0.01$).

Four replications of 25 seeds of each treatment were germinated in two rolled whatman filter papers with 5 ml of respective test solutions in glass petri dishes. Then petri-dishes containing two layer filter paper were moistened by respective prepared solutions. Seeds were allowed to germinate at 22 ±1 °C (Fallah-Toosi and Baki, 2013) in the dark for 10 days. Daily germination rate was measured and filter papers were replaced, when needed. Similarly, respective NaCl and PEG solutions were added when required. Germination percentage was measured by ISTA (Anonymous, 1996) standard method. Germinated percentage was recorded on daily until a constants count was achieved (every 24 h for 10 days). Seed was considered to be germinated when radicle length exceeded 2 mm (Huang and Redmann, 1995). Tentht day, 10 seedlings were randomly selected from each replicate at the end of tenth day of standard germination test and measured parameters including radicula length (RL), plumula length (PL), seedling fresh weight (SFW), final germination percentage (FGP), germination index (GI) and seedling vigor index (SVI) were evaluated (Kandil *et al*., 2012). Seedling dry weights (SDW) were measured after drying samples at 70 °C for 48 h in an oven (Böhm, 1979).

Parameters were calculated as follows;

SFW: Radicula and plumula fresh weight of averages 10 normal seedlings at random/replicate, were determined, expressed as mg.

SDW: Radicula and plumula fresh weight of averages 10 normal seedlings at random/replicate, dry weight recorded, expressed as mg.

FGP: (number of germinated seeds/number of total seeds) x 100.

GI: (germination percentage in each treatment/ germination percentage in the control) x 100.

SVI: (average radicula length+average plumula length) x final germination percentage.

RESULTS

Effect of PEG on seed germinability and seedling growth in mustard are presented in Table 1; effect of NaCl on seed germinability and seedling growth in mustard are presented in Table 2.

Growth Parameters for Drought Stress Treatments

Statistically significant differences were found between the seven PEG treatments in mustard. PEG treatments caused significant decrease in terms of radicula length, plumula length, seedling fresh weight, seedling dry weight and final germination percentage for all treatments PEG treatment compared with control (Table 1). Radicula length was changed between 3.62-12.34 cm. The greatest decrease in radicula length was observed in -10 bar PEG treatment by 69.93%. In -8, -6 and -4 bar PEG treatments showed a decrease by 50.66%, 41.20% and 19.60% respectively. Plumula length was changed between 0.66-6.27 cm. The greatest decrease in plumula length was observed in -10 bar PEG treatment by 89.47%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 74.80%, 52.79%, 39.08% and 13.24% respectively. Seedling fresh weight was changed between 13.83-46.10 mg. The greatest decrease in seedling fresh weight was observed in -10 bar PEG treatment by 70.00%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 64.21%, 47.55%, 36.12% and 4.06% respectively. Seedling dry weight was changed between 0.42-3.03 mg. The greatest decrease in seedling dry weight was observed in -10 bar PEG treatment by 86.14%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 75.58 %, 64.69%, 42.57% and 22.44% respectively. Final germination percentage was changed between 45.83-83.33%. The greatest decrease in final germination percentage was observed in -10 bar PEG treatment by 45.00%. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 34.00%, 28.99%, 13.00% and 7.00% respectively. Germination index was changed between 55-100. The greatest decrease in germination index was observed in -10 bar PEG treatment by 45. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 34, 24, 13 and 7 respectively. Seedling vigor index was changed between 196.43-1526.14. The greatest decrease in seedling vigor index was observed in -10 bar PEG treatment by 87.13. In -8, -6, -4 and -2 bar PEG treatments showed a decrease by 72.67, 61.33, 35.80 and 9.78 respectively. -12 bar PEG treatments were excluded from this study since seed germination completely inhibited.

Table 1. Effect of PEG on germination and seedling growth in wild mustard.
Çizelge 1. Yabani hardalda çimlenme ve fide gelişimi üzerine PEG'in etkisi.

Treatment		Radicula length (cm)	Plumula length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Final germination percentage (%)	Germination index (%)	Seedling vigor index (%)
Uygulama		Kök uzunluğu (cm)	Sürgün uzunluğu (cm)	Fide yaş ağırlığı (mg)	Fide kuru ağırlığı (mg)	Final çimlenme oranı (%)	Çimlenme indeksi (%)	Fide canlılık indeksi (%)
PEG (bar)	0	12.04 a	6.27 a	46.10 a	3.03 a	83.33 a	100 a	1526.14 a
	2	12.34 a	5.44 a	44.23 a	2.35 b	77.50 a	93 a	1376.84 a
	4	9.68 b	3.82 b	29.45 b	1.74 c	72.50 ab	87 ab	979.73 b
	6	7.08 c	2.96 b	24.18 bc	1.07 d	59.17 bc	76 bc	590.09 c
	8	5.94 c	1.58 c	16.50 cd	0.74 de	55.00 c	66 cd	417.05 cd
	10	3.62 d	0.66 d	13.83 d	0.42 e	45.83 c	55 d	196.43 d
	12	0	0	0	0	0	0	0
F value		58.15	97.65	43.13	66.36	16.17	29.10	99.33
LSD value		1.38	0.66	6.27	0.37	10.85	9.53	161.95
CV (%)		10.84	12.73	14.33	15.82	10.98	9.95	12.68

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0,05$) yoktur.
Same letters in a column are not significantly difference at the 0,05 probability levels.

Growth Parameters for Salt Stress Treatments

Statistically significant differences were found among the seven NaCl treatments in mustard. NaCl treatments caused significant decrease in terms of radicula and plumula length, seedling fresh weight, seedling dry weight and final germination percentage for almost all treatments NaCl treatment compared with control (Table 2). Radicula length was changed between 0.79-12.46 cm. The greatest decrease in radicula length was observed in 30 dSm^{-1} NaCl treatment by 93.66%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 89.25%, 69.66%, 45.91%, 38.52%, 35.39% and 8.91% respectively. Plumula length was changed between 0.34-6.88 cm. The greatest decrease in plumula length was observed in 30 dSm^{-1} NaCl treatment by 95.06%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 82.56%, 66.13%, 34.30%, 30.96%, 31.25% and 5.96% respectively. Seedling fresh weight was changed between 4.85-67.63 mg. The greatest decrease in seedling fresh weight was observed in 30 dSm^{-1} NaCl treatment by 92.83%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 72.28%, 58.04%, 24.07%, 8.58%, 8.21% and 2.85% respectively. Seedling dry weight was changed between 0.12-4.60 mg. The greatest decrease in seedling dry weight was observed in 30 dSm^{-1} NaCl treatment by 97.39%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 88.26%, 73.48%, 48.26%, 29.78%, 21.96% and 8.48% respectively. Final germination percentage was changed between 29-77%. The greatest decrease in final germination percentage was observed in 30 dSm^{-1} NaCl treatment by 62.34%. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 27.27%, 19.48%, 11.69%, 10.39%, 5.19% and 5.52% respectively. Germination index was changed between 37.66-100. The greatest decrease in germination index was observed in 30 dSm^{-1} NaCl treatment by 62.34. In 25, 20, 15, 10, 5 and 2 dSm^{-1} NaCl treatments showed a decrease by 27.27, 19.48, 11.69, 11.90, 5.19 and 5.52 respectively. Seedling vigor index was changer between 34.68-1451.70. The greatest decrease in seedling vigor index was observed in 30 dSm^{-1} NaCl treatment by 97.61. In 25, 20, 15, 5, 10 and 2 dSm^{-1} NaCl treatments showed a decrease by 88.76%, 80.35%, 43.54%, 32.23%, 35.10% and 12.44% respectively. 35 dSm^{-1} NaCl treatments were excluded from this study since seed germination completely inhibited.

Table 2. Effect of NaCl on germination and seedling growth in wild mustard.
Çizelge 2. Yabani hardalda çimlenme ve fide gelişimi üzerine NaCl'nin etkisi.

Treatment		Radicula length (cm)	Plumula length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Final germination percentage (%)	Germination index (%)	Seedling vigor index (%)
Uygulama		Kök uzunluğu (cm)	Sürgün uzunluğu (cm)	Fide yaş ağırlığı (mg)	Fide kuru ağırlığı (mg)	Final çimlenme oranı (%)	Çimlenme indeksi (%)	Fide canlılık indeksi (%)
NaCl (dSm^{-1})	0	12.46 a	6.88 a	67.63 a	4.60 a	77.00 a	100.00 a	1451.70 a
	2	11.35 a	6.47 a	65.70 a	4.21 a	72.75 a	94.48 ab	1271.12 a
	5	8.05 b	4.73 b	62.08 a	3.59 ab	73.00 a	94.81 ab	942.11 b
	10	7.66 b	4.75 b	61.83 a	3.23 abc	69.00 ab	88.10 bc	983.80 b
	15	6.74 b	4.52 b	51.35 b	2.38 bc	68.00 ab	88.31 bc	819.67 b
	20	3.78 c	2.33 c	28.38 c	1.22 c	62.00 bc	80.52 cd	285.27 c
	25	1.34 d	1.20 d	18.75 d	0.54 c	56.00 c	72.73 d	163.12 cd
	30	0.79 d	0.34 e	4.85 e	0.12 d	29.00 d	37.66 e	34.68 d
	35	0	0	0	0	0	0	0
F value		97.90	284.58	179.40	180.06	41.65	51.85	72.90
LSD value		1.28	0.42	5.34	0.37	7.01	8.13	180.97
CV (%)		13.33	7.24	8.05	10.19	7.53	6.74	16.54

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0,05$) yoktur.
Same letters in a column are not significantly difference at the 0,05 probability levels.

DISCUSSION

PEG and NaCl treatments had a significant effect on all investigated characters ($P<0.01$). Germination parameters were decreased by increasing osmotic potential of PEG 6000. In distilled water, percentage of seed germination was the highest. The higher amount of PEG 6000 concentration in this research (-12 bar) completely inhibited seed germination. The first physiological disorder, which takes place during germination, is the reduction in imbibitions of water by seeds which leads to a series of metabolic changes, including general reduction in hydrolysis and utilization of the seed reserve. Upon imbibition, the quiescent dry seeds rapidly resume oxygen uptake and oxidative phosphorylation, processes required for supporting the high energy cost of germination (Baranova *et al*., 2006; Toosi *et al*., 2014).

Seed germination decreased with the increase in NaCl concentration. According to Ayaz *et al.* (2000), decrease of seed germination under conditions of salt stress is due to occur of some metabolically disorders. It seems that, decrease of germination percentage is related to reduction in water absorption into the seeds at imbibitions and seed turgoscence stages. It is reported that, radicula and shoot length are important traits in salt stress sensitivity evaluation (Jamil *et al*., 2006). Decrease of growth in root and stem can be related to NaCl toxicity and disproportion in nutrient absorption by seedlings. According to results of Werner and Finkelstein (1995) salinity decreases water absorption and growth of root and shoot. It's reported that, salinity decreases significantly nutrient absorption and root growth speed (Khan and Gulzar, 2003). Srivastava *et al*. (2004) have been reported that, proteins especially PR10 protein increases salt resistance in canola varieties at germination stages. In other research, increase of salinity from 6 to 11 dSm^{-1} decreased canola seed germination by 50% (Francois, 1996). Our result showed that seed germination was completely inhibited at 35 dSm^{-1} (-15.6 bar) for NaCl stress. Gulzar and Khan (2001) reported that, NaCl salinity prevents water absorption by seeds and decreases significantly seed germination percentage and germination pace (Bybordi and Tabatabaei, 2009). The greatest decrease in germination percentage in this study was observed in 30 dSm^{-1} treatment by 62.3% which might implicate that wild mustard seed is tolerant to salt stress.

Both NaCl and PEG treatments inhibited seed germination and seedling growth properties in wild mustard. However, seed germination parameters were better in NaCl than in PEG with the earlier observation made for alfaalfa by Tilaki *et al*. (2009); soybean by Khajeh-Hosseini *et al*. (2003); sunflower by Luan *et al*. (2014). This may be due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination (Heshmat *et al*., 2011). Our findings showed that NaCl had greater inhibitory effects on seedling growth than on germination with respect to PEG. This might be explained by more rapid water uptake in NaCl solutions and achivement of a moisture content that allowed germination. This result confirms the findings of Khajeh-Hosseini *et al*., 2003 in soybean; Okcu *et al*. (2005) in pea; Saeedipour (2009) in canola; Heasmat (2011) in canola; Asmare (2013) in harricot bean.

While seed germination and seedling growth parameters decreased by concentration of -4 to -10 bar for PEG stress; seed germination decreased by 10 dSm^{-1} (-4.4 bar) and seedling growth parameters decreased by 5 dSm^{-1} (-2.2 bar) for NaCl stress. Seed germination was completely inhibited at -12 bar for PEG stress, 35 dSm^{-1} (-15.6 bar) for NaCl stress. The marked differences in germination percentages observed with NaCl and PEG at the same osmotic potentials indicate specific ionic effects and point that germination is solely controlled by the osmotic potential.

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