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CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

CLASSIFICATION OF SOME LINSEED (*Linum usitatissimum* L.) GENOTYPES FOR SALINITY TOLERANCE USING GERMINATION, SEEDLING GROWTH, AND ION CONTENT

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Salinity reduces germination, delays emergence, and inhibits seedling growth of linseed (*Linum usitatissimum* L.) while some varieties are less affected by salinity than others. In this study, we aimed to determine the effects of NaCl levels (0, 5, 10, 20 and 30 dS m^{-1}) on germination and seedling growth of 10 linseed genotypes (lines 87, 89, 104, 114, 193, 194, 209, 215, C-90 and cv. Sarı-85) and to classify the genotypes for salinity tolerance using germination and seedling characteristics. Germination percentage, mean germination time, root and shoot length, seedling fresh and dry weight, Na^+ content and Na:K ratio of seedlings were investigated. Classification of linseed genotypes for salinity tolerance was done according to (i) combination of Principal Component and Cluster Analysis, (ii) Na^+ content, and (iii) Na:K ratio of seedling. The results showed that the highest values were obtained from lines 193, 194 and 215 except for mean germination time, while germination percentage was not adversely influenced by NaCl up to 20 dS m^{-1}. Seedling growth was inhibited at 20 dS m^{-1} although genotypes exhibited varying responses. Na^+ content was enhanced by NaCl, but seedling from lines 194, 193 and 215 had the lowest Na^+ content at all NaCl levels. Cluster analysis performed by multiple parameters revealed three groups for salinity tolerance. It was concluded that lines 193, 194, and 215 were tolerant, lines 87, 209, C-90, and cv. Sarı-85 were moderately tolerant and lines 89, 104, and 114 were salt-sensitive genotypes. Classification of genotypes for Na^+ content and Na:K ratio showed similar result for tolerant genotypes while different genotypes for sensitive group were detected.

Key words: *Linum usitatissimum*, NaCl, tolerance, cluster, ion accumulation.

Linseed (*Linum usitatissimum* L.) is a cool temperate annual herb with erect stems. Although there are several utilization purposes, it is cultivated commercially for its seed, which is processed into oil and a high protein stock feed after oil extraction (Sankari, 2000; Kurt and Bozkurt, 2006; Berti *et al*., 2010) and for its fibers, which are made into linen and other cloths (El-Nagdy *et al*., 2010). In addition, linseed varieties with oils suitable for culinary use are available (Hosseinian *et al*., 2004).

Linseed has a shallow root system and needs sufficient moisture during the growing season (Hocking *et al*., 1997). Seedling establishment is generally slow and seedlings have poor competitive ability. Germination and seedling emergence may be influenced by temperature, sowing depth and seedbed conditions like available moisture and salinity (O'Connor and Gusta, 1994; Saeidi and Rowland, 1997; Couture *et al*., 2004; Kurt and Bozkurt, 2006). In arid and semi-arid regions where rainfall is insufficient to leach salts out of the root zone, the salinity is a major problem which limits plant growth (Khajeh-Hosseini *et al*., 2003), since evaporation tends to exceed rainfall (Pessarakli, 1999; Kaya *et al*., 2003). Salinity leads to delayed germination and emergence, low seedling survival, irregular crop stand and lower yield due to abnormal morphological, physiological and biochemical changes (Ashraf and Fatima, 1994; Munns, 2002; Muhammad and Hussain, 2010).

The main approach of screening the cultivars for salinity tolerance is growing or cultivating them on the salt affected soils. Several researches on the classification of crop plants for salinity have been performed using various criteria such as reduction in plant growth (Bassil and Kaffka, 2002; Akhtar *et al*., 2003; Hakim *et al*., 2010), water stress day index (Katerji *et al*., 2000), biochemical activities (Johnson *et al*., 2003; Naureen and Naqvi, 2010), ion balance (Ashraf and O'Leary, 1997; Alian *et al*., 2000; Munns *et al*., 2006), leaf water potential and stomatal conductance (Ashraf and O'Leary, 1997), and yield reduction (Natarajan *et al*., 2005). Selection of a salt tolerant genotype based on germination and seedling growth under controlled conditions is simple, quick, precise and not time consuming. Consequently, this research focused on classification of linseed genotypes

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Received: 27 April 2011.
Accepted: 20 December 2011.

for salt tolerance based on germination and seedling growth along with Na^+ content and Na:K ratio instead of searching a selection criterion.

MATERIALS AND METHODS

This study was carried out at the Faculty of Agriculture, Ankara University, Turkey. Totally nine linseed genotypes, which lines 87, 89, 104, 114, and C-90 (inbred line from cvs. Sueloef, Verum, Aoyagi, Svetoc and Wiera originated from Germany respectively), lines 193 (K-5843) and 194 (K-6970) from Russia, lines 209 (Kul5215) and 215 (inbred from cv. At125) from Sweden, and one native cultivar Sarı-85 from Turkey improved by single seed selection methods in Ankara University, Turkey, were used as material. Germination and early seedling growth of the genotypes were studied using distilled water (control) and under NaCl concentrations with the electrical conductivity (EC) values of 5, 10, 20, and 30 dS m^{-1}, respectively.

Four replicates of 50 seeds were germinated between three layered rolled filter paper with 21 mL of respective test solutions and the papers were replaced every 2 d to prevent accumulation of salts. The rolled paper with seeds was put into sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate at 20 ± 1 °C in the dark. A seed was considered to be germinated when the emerging radicle elongated to 2 mm. Germination percentage was recorded every 24 h for 10 d. Mean germination time (MGT) was calculated for the speed of germination according to ISTA (2003). Root length, shoot length, seedling fresh and dry weight of 10 seedlings randomly selected from each replicate were measured after the 10^{th} day. Dry weight was measured after drying samples at 70 °C for 48 h in an oven. One thousand seed weight of each genotype was determined as an average of 4 × 100 seeds.

In order to evaluate the toxic effect of Na^+ accumulation, all seedlings from each replicate were sampled for mineral analysis. The samples were weighed and separately dried at 70 °C for 48 h for mineral analysis (Na^+ and K^+ analysis). Sodium and K analysis were performed using a flame photometric method (Kacar and Inal, 2008).

The experimental design was two factors factorial (10 × 5) arranged in a completely randomized design with four replicates and 50 seeds per replicate. The first factor was linseed genotypes and the second was NaCl levels. Data for germination percentage were subjected to arcsin transformation before ANOVA was made using MSTAT-C program (Michigan State University). The differences between the means were compared using LSD values ($P < 0.05$). For grouping the genotypes, all measured parameters of the genotypes exposed to various NaCl levels were considered. Principle Component and Cluster Analysis was performed by classifying the genotypes for salinity tolerance.

RESULTS AND DISCUSSION

There is a significant difference for one thousand seed weight of linseed genotypes ($P < 0.05$). Heavier seed weight was measured in lines 193 and 215, followed by line 194, cv. Sarı-85 and line 209 (Figure 1). A significant two way interaction (genotype and NaCl) was found ($P < 0.05$) for all investigated characters. Germination percentage was not significantly reduced at NaCl levels between control and 20 dS m^{-1} while it declined considerably at 30 dS m^{-1}. The highest decrease in germination was determined in lines 87, 89, 114 and cv. Sarı-85 while the highest germination percentage (72.5%) at NaCl concentration of 30 dS m^{-1} was detected in line 194 (Table 1). Mean germination time (MGT) was delayed by increasing salinity stress; however, NaCl level of 20 dS m^{-1} retarded it much more compared to lower level of NaCl. The MGT was not calculated at 30 dS m^{-1} because of insufficient germination. The fastest germination at 20 dS m^{-1} was recorded in line 104 with 1.73 d. NaCl adversely influenced germination percentage, mean time to germination and seedling growth of linseed genotypes, but any inhibitory effects of NaCl lower than 30 dS m^{-1} on germination percentage were not determined. The results of this study are in agreement with the observations of Muhammad and Hussain (2010), who observed that NaCl levels between 0 and 15 dS m^{-1} did not adversely affect germination percentage and the least affected species for decreasing germination percentage was *L. usitatissimum*. El-Nakhlaway and El-Fawal (1989) reported significantly reduced germination with increasing salinity in *Linum*. This result could be explained osmotic stress constituted by salt concentrations or specific ion effects which delay or/and inhibit germination as reported by Almansouri *et al*. (2001), Kaya *et al*. (2006) and Atak *et al*. (2006). On the other hand, germination of large or heavy seeded varieties is influenced deeply by the osmotic stress created by salinity (Kaya *et al*., 2008) while large seeds produced vigorous seedlings compared to small seeds (Soltani *et al*., 2002). The lower MGT in control (0.0 dS m^{-1}) and reduced MGT in heavier seeds compared to light seeded genotypes under NaCl stress were observed in this study.

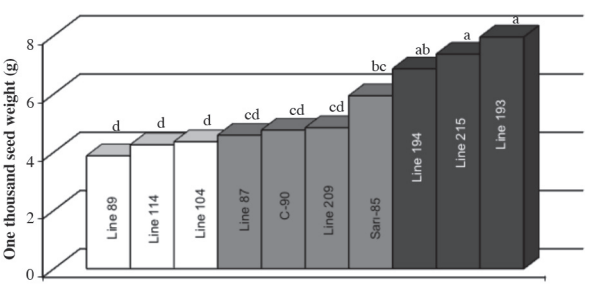

Figure 1. One thousand seed weight of 10 linseed genotypes calculated as an average of 4 × 100 seeds. Letters on each bar show significance level of means at p < 0.05 level.

Table 1. Germination and seedling characteristics of 10 linseed genotypes used for grouping for salinity tolerance under various NaCl levels.

Genotype	NaCl	Germination	MGT	Root length	Shoot length	Seedling fresh weight	Seedling dry weight
	dS m^{-1}	%	d	cm		mg plant^{-1}	
	0	99.5 ± 0.5	1.22 ± 0.06	2.33 ± 0.27	7.18 ± 0.23	48.8 ± 0.95	2.50 ± 0.28
	5	100.0 ± 0.0	1.10 ± 0.06	3.86 ± 0.14	10.93 ± 0.14	57.0 ± 1.22	2.75 ± 0.25
Line 87	10	100.0 ± 0.0	1.21 ± 0.07	5.07 ± 0.24	9.70 ± 0.16	52.8 ± 0.85	2.50 ± 0.28
	20	100.0 ± 0.0	2.12 ± 0.03	6.30 ± 0.52	6.38 ± 0.19	43.8 ± 1.25	2.50 ± 0.28
	30	3.5 ± 1.0	-	-	-	-	-
	0	100.0 ± 0.0	1.05 ± 0.01	5.37 ± 0.22	12.73 ± 0.79	83.3 ± 3.59	4.00 ± 1.15
	5	99.0 ± 0.6	1.10 ± 0.06	5.01 ± 0.37	15.35 ± 0.79	89.3 ± 5.70	3.75 ± 0.75
Line 193	10	100.0 ± 0.0	1.20 ± 0.01	6.20 ± 0.60	15.16 ± 0.27	91.5 ± 4.65	4.75 ± 0.47
	20	100.0 ± 0.0	2.07 ± 0.05	6.08 ± 0.39	6.98 ± 0.19	68.8 ± 2.43	4.75 ± 0.25
	30	14.5 ± 2.7	-	-	-	-	-
	0	97.0 ± 1.9	1.47 ± 0.04	3.16 ± 0.31	6.49 ± 0.45	55.3 ± 2.50	3.25 ± 0.25
	5	100.0 ± 0.0	1.19 ± 0.02	6.80 ± 0.41	10.33 ± 0.12	66.0 ± 2.17	3.25 ± 0.25
Sarı-85	10	100.0 ± 0.0	1.65 ± 0.08	4.73 ± 0.34	9.15 ± 0.28	67.8 ± 1.65	4.00 ± 0.41
	20	99.5 ± 0.5	2.83 ± 0.09	6.43 ± 0.20	2.38 ± 0.50	44.5 ± 1.32	5.00 ± 0.00
	30	0.0 ± 0.0	-	-	-	-	-
	0	100.0 ± 0.0	1.03 ± 0.00	4.71 ± 0.16	14.55 ± 0.53	77.0 ± 3.46	4.00 ± 0.00
	5	98.0 ± 1.1	1.01 ± 0.00	5.21 ± 0.47	16.12 ± 0.12	77.8 ± 3.54	3.75 ± 0.25
Line 194	10	100.0 ± 0.0	1.07 ± 0.03	7.75 ± 1.14	15.28 ± 0.23	76.8 ± 1.03	4.25 ± 0.25
	20	98.5 ± 1.5	2.37 ± 0.03	5.46 ± 0.24	9.74 ± 0.39	59.0 ± 2.34	4.25 ± 0.47
	30	72.5 ± 8.1	-	-	-	-	-
	0	100.0 ± 0.0	1.04 ± 0.02	4.75 ± 0.08	5.38 ± 0.15	38.5 ± 2.06	2.00 ± 0.00
	5	100.0 ± 0.0	1.04 ± 0.02	4.55 ± 0.25	10.95 ± 0.31	53.5 ± 0.65	2.50 ± 0.28
Line 114	10	100.0 ± 0.0	1.14 ± 0.05	4.35 ± 0.47	9.45 ± 0.34	45.3 ± 1.84	2.75 ± 0.25
	20	100.0 ± 0.0	2.61 ± 0.05	6.40 ± 0.15	6.28 ± 0.14	42.0 ± 1.47	3.25 ± 0.25
	30	1.0 ± 0.5	-	-	-	-	-
	0	100.0 ± 0.0	1.02 ± 0.01	4.87 ± 0.08	9.05 ± 0.15	61.5 ± 0.50	2.75 ± 0.25
	5	100.0 ± 0.0	1.06 ± 0.02	4.03 ± 0.36	10.78 ± 0.19	58.5 ± 1.19	3.25 ± 0.25
Line 209	10	100.0 ± 0.0	1.11 ± 0.04	5.15 ± 0.77	10.70 ± 0.31	60.8 ± 0.85	3.00 ± 0.00
	20	100.0 ± 0.0	2.58 ± 0.17	7.60 ± 0.25	6.35 ± 0.37	48.0 ± 1.08	3.50 ± 0.28
	30	10.0 ± 0.8	-	-	-	-	-
	0	100.0 ± 0.0	1.07 ± 0.02	2.44 ± 0.64	7.99 ± 0.22	72.0 ± 3.72	5.25 ± 0.25
	5	100.0 ± 0.0	1.06 ± 0.02	4.28 ± 0.14	11.80 ± 0.41	86.8 ± 3.54	4.25 ± 0.25
Line 215	10	100.0 ± 0.0	1.10 ± 0.04	7.25 ± 0.67	10.70 ± 0.55	77.3 ± 2.29	5.25 ± 0.25
	20	100.0 ± 0.0	2.03 ± 0.04	6.53 ± 0.17	6.48 ± 0.24	60.0 ± 1.73	4.75 ± 0.47
	30	37.0 ± 2.5	-	-	-	-	-
	0	100.0 ± 0.0	1.05 ± 0.03	7.23 ± 0.41	9.25 ± 0.23	50.0 ± 0.91	2.25 ± 0.25
	5	100.0 ± 0.0	1.05 ± 0.02	3.28 ± 0.14	11.18 ± 0.07	53.8 ± 1.31	1.25 ± 0.14
Line 89	10	100.0 ± 0.0	1.27 ± 0.07	5.50 ± 0.24	12.23 ± 0.17	58.0 ± 2.89	2.50 ± 0.28
	20	99.0 ± 1.0	5.19 ± 0.20	2.56 ± 0.18	2.44 ± 0.43	27.8 ± 2.12	2.25 ± 0.25
	30	0.0 ± 0.0	-	-	-	-	-
	0	100.0 ± 0.0	1.03 ± 0.01	5.06 ± 0.53	8.19 ± 0.05	56.8 ± 1.11	2.75 ± 0.25
	5	100.0 ± 0.0	1.05 ± 0.01	4.69 ± 0.65	12.92 ± 0.16	69.0 ± 2.48	2.75 ± 0.25
C-90	10	100.0 ± 0.0	1.11 ± 0.02	7.34 ± 0.18	12.53 ± 0.36	64.0 ± 2.00	3.25 ± 0.25
	20	99.5 ± 0.5	2.97 ± 0.44	3.93 ± 0.41	5.74 ± 0.57	46.5 ± 1.66	2.75 ± 0.47
	30	5.5 ± 1.2	-	-	-	-	-
	0	100.0 ± 0.0	1.00 ± 0.00	4.88 ± 0.47	9.69 ± 0.38	47.8 ± 1.49	2.25 ± 0.25
	5	100.0 ± 0.0	1.01 ± 0.00	4.54 ± 0.23	13.38 ± 1.22	58.5 ± 1.04	2.25 ± 0.25
Line 104	10	100.0 ± 0.0	1.07 ± 0.04	6.41 ± 0.25	11.55 ± 0.26	50.3 ± 1.11	2.75 ± 0.47
	20	100.0 ± 0.0	1.73 ± 0.09	4.29 ± 0.40	5.95 ± 0.21	43.8 ± 1.70	2.00 ± 0.40
	30	9.0 ± 3.0	-	-	-	-	-
$LSD_{int.}$ ($p < 0.05$)		2.62	0.26	1.03	1.11	6.15	0.91

Data represent mean ± standard error (SE) of four replicates.
MGT: Mean germination time.

Increasing NaCl resulted in increase in root length of almost all of the linseed genotypes up to 20 dS m^{-1} except for line 89. None of the genotypes were able to grow roots at 30 dS m^{-1}. Line 209 was superior to the others at 20 dS m^{-1} and had a root length of 7.65 cm. Greater reduction in shoot length due to NaCl was very evident at 20 dS m^{-1} ($P < 0.05$) with no recorded shoot growth at 30 dS m^{-1}. NaCl enhanced shoot growth up to 10 dS m^{-1} while, it was inhibited dramatically at 20 dS m^{-1}. Soltani *et al*. (2002) observed that root length of chickpea was diminished by increasing NaCl concentration while large seeds gave vigorous seedling growth compared to small seeds. It is assumed that variation in seed reserve of linseed genotypes we used was responsible for the differences in seedling growth because higher seed weight resulted in higher germination and vigorous seedling growth. Although Diederichsen and Jones-Flory (2005) stated that seed vigor was not correlated with 1000 seed weight of

linseed cultivars, seed weight would be responsible for seedling growth as supported by Soltani *et al*. (2002) and Kaya *et al*. (2008) in chickpea, Kaya and Day (2008) in sunflower, who found relationship between seed weight and seedling growth.

Depending on decrease in shoot and root length, seedling fresh weight gradually declined with the increasing salinity stress. Considering each genotype higher seedling fresh weights were recorded from lines 193, 194, and 215 under salinity stress. Increasing salinity levels did not cause remarkably decreases in seedling dry weight. Although our genotypes showed different responses to each salt level and produced varying levels of dry weight, the highest dry weight in all salinity levels were usually obtained from line 215. These results were similar to those observed by Chandru *et al*. (1993) in sunflower (*Helianthus annuus* L.), Ghorashy *et al*. (1972) and Kaya *et al*. (2003) in safflower (*Carthamus tinctorius* L.), Ashraf and Fatima (1994) in linseed and Saboora and Kiarostami (2006) in wheat (*Triticum aestivum* L.). In our study, the linseed genotypes we used could keep up with the soil salinity up to 20 dS m^{-1} during the germination and early growth stages, though, both the germination percentage and seedling growth fell sharply down at higher salinity level of 20 dS m^{-1}.

Seedling samples from 10 linseed genotypes showed that increased levels of NaCl affected significantly the Na^+ content of linseed seedlings (Figure 2). Considering linseed genotypes, seedling from lines 193, 215, and 194 had lower Na^+ content than the others. Linseed genotypes gave different Na:K ratio while lower Na:K ratio was recorded in lines 194, 193, 215, C-90, and 104, respectively (Figure 3). Reducing cell division and plant growth metabolism induced by accumulation of Na^+ ion caused changes in ion balances and the imbalance of mineral nutrients resulted in a reduction or an inhibition of plant growth were reported by Mer *et al*. (2000). Na^+ content increased with increasing salinity level and peaked at 20 dS m^{-1}. This means that Na^+ accumulation was harmful because the genotypes with low Na^+ content like lines 193, 194, and 215, possessed more vigorous seedling growth than the other genotypes. Na:K ratio

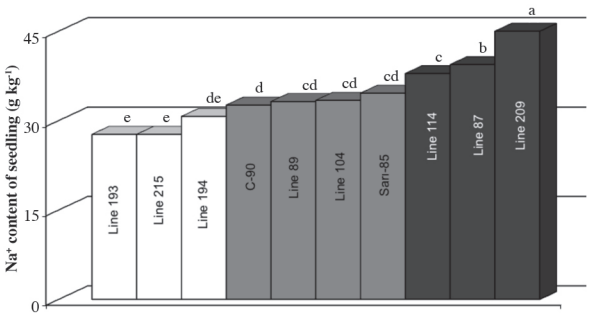

Figure 2. Classification of 10 linseed genotypes for salinity tolerance using Na^+ content measured at 10 d old seedlings. Letters on each bar show significance level of means at p < 0.05 level.

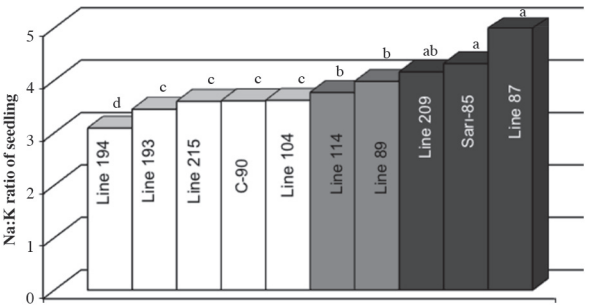

Figure 3. Classification of 10 linseed genotypes for salinity tolerance using Na:K ratio measured at 10 d old seedlings. Letters on each bar show significance level of means at p < 0.05 level.

shows ion balance of linseed seedling and an important criteria for seedling survival under NaCl stress. Increased NaCl promoted Na:K ratio while this increase varied with linseed genotypes. The minimum Na:K ratio was determined in line 194, followed by lines 193 and 215. Natarajan *et al*. (2005) reported that the genotypes with lower Na:K ratio resulted in higher grain yield in rice.

According to the classification for salinity tolerance based on Na^+ content of seedling, lines 193, 215, and 194 were tolerant and C-90, lines 89, 104, and cv. Sarı-85 were moderately tolerant, while lines 114, 87, and 209 could be rank as sensitive to salinity (Figure 2). On the basis of Na:K ratio, lines 193, 194, 215, C-90, and 104 could be regarded as tolerant and lines 114 and 89 were moderately tolerant, while line 209, cv. Sarı-85, and line 87 could be classified as salt-sensitive genotypes (Figure 3). Principal Component and Cluster Analysis based on mean values of germination and seedling characteristics showed that lines 193, 194, and 215 could be grouped as tolerant, and lines 89, 104, C-90, and cv. Sarı-85 were moderately tolerant while lines 114, 87, and 209 could be arranged as salt-sensitive (Figure 4).

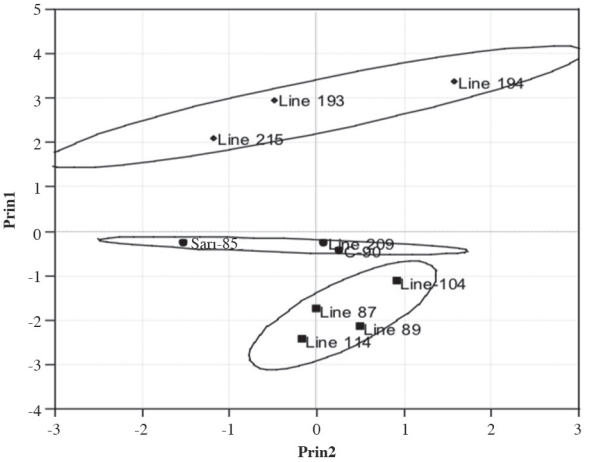

Figure 4. Classification of 10 linseed genotypes subjected to various NaCl concentrations for salinity tolerance using combination of Principal Component and Cluster Analysis. Prin represents Principal Component Analysis.

CONCLUSIONS

Laboratory screening of 10 linseed genotypes for salt tolerance at germination and early seedling growth indicated a large variation among genotypes. Also, there were differences among the linseed genotypes for salt tolerance and three classification methods confirmed that lines 194, 193, and 215 were more tolerant to high salt concentrations than the others while lines 114, 87, and 209 were the susceptible. Combination of Principal Component and Cluster Analysis could be successfully used for classification of the genotypes against salinity. However, further study should be performed to classify linseed for salt tolerance under field conditions to evaluate the possibility of linseed cultivation on salt-contaminated areas.

Clasificación de algunos genotipos de linaza (*Linum usitatissimum* L.) para tolerancia a salinidad usando germinación, crecimiento de plántulas y contenido de iones. La salinidad reduce germinación, retrasa emergencia, e inhibe el crecimiento de plántulas de lino (*Linum usitatissimum* L.) mientras algunas variedades son menos afectadas por la salinidad que otras. El objetivo de este estudio fue determinar los efectos de niveles de NaCl (0, 5, 10, 20 and 30 dS m^{-1}) en germinación y crecimiento de plántulas de 10 genotipos de lino (líneas 87, 89, 104, 114, 193, 194, 209, 215, C-90 y cv. Sarı-85) y clasificar los genotipos por tolerancia a salinidad usando características de germinación y de plántulas. Se evaluaron porcentaje de germinación, tiempo medio de germinación, longitud de raíces y brotes, peso fresco y seco de plántulas, contenido de Na^+ y relación Na:K de las plántulas. La clasificación de los genotipos de lino por tolerancia a salinidad se hizo de acuerdo a (i) combinación de Análisis de Cluster y Componentes Principales, (ii) contenido de Na^+, y (iii) relación Na:K de plántulas. Los resultados muestran que los valores mayores se obtuvieron en las líneas 193, 194, y 215 excepto para tiempo medio de germinación, mientras el porcentaje de germinación no fue adversamente influenciado por NaCl hasta 20 dS m^{-1}. El crecimiento de plántulas fue inhibido a 20 dS m^{-1} aunque los genotipos exhibieron variadas respuestas. El contenido de Na^+ fue elevado por NaCl, pero plántulas de las líneas 194, 193, y 215 tuvieron los menores contenidos de Na^+ en todos los niveles de NaCl. El análisis de cluster realizado por parámetros múltiples reveló tres grupos para tolerancia a salinidad. Se concluyó que las líneas 193, 194, y 215 eran tolerantes, las líneas 87, 209, C-90, y cv. Sarı-85 fueron moderadamente tolerantes, y las líneas 89, 104, y 114 fueron genotipos sensibles a sal. La clasificación de genotipos por contenido de Na^+ y relación Na:K mostró resultados similares para genotipos tolerantes mientras se detectaron genotipos diferentes para el grupo sensible.

Palabras clave: *Linum usitatissimum*, NaCl, tolerancia, cluster, acumulación de iones.

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