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Effects of NaCl on the Germination, Seedling Growth and Water Uptake of Triticale

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Abstract: The lack of information about the effects of salinity on vegetative growth of triticale prompted us to study the salt tolerance levels of 3 newly registered cultivars, Karma-2000, Presto, and Tatlıcak-97. Moreover, the relative importance of the osmotic or toxic effects of NaCl on seed germination are not clear in triticale. In this study, germination percentage, seedling fresh and dry weight and water uptake of the seeds of 3 triticale cultivars were determined under various salt (NaCl) stresses with electrical conductivities of 2.4, 4.2, 5.9, 7.7, 10.6 and 13.2 dS m^{-1}. Roots and shoots were analyzed for uptake of Na^+ and K^+, and seeds for Na^+, K^+ and Cl^-. The results showed that increased NaCl significantly affected mean germination time without affecting final germination percentage. In each salt concentration, water uptake was not significantly changed. Salinity increased the accumulation of Na^+ and decreased the K^+ content in the roots and shoots. Moreover, the Na^+ and Cl^- content of germinating seeds gradually increased, while K^+ diminished. Karma-2000 appeared to be more tolerant to salt stress than the others. It was concluded that the delay in germination was mainly due to higher Na^+ accumulation in the seeds rather than osmotic stress in triticale cultivars, while final germination percentage was not changed by NaCl. However, it appeared that NaCl adversely influenced triticale seedling characters.

Key Words: Triticale (x *Triticosecale* Wittmack), salinity, water uptake, germination

Tritikale'de Çimlenme, Fide Gelişimi ve Su Alımı Üzerine NaCl'nin Etkileri

Özet: Tritikale'nin vejetatif gelişimi üzerine tuzluluğun etkileri yönündeki bilgilerin yetersizliği nedeniyle, yeni tescil edilen üç tritikale çeşidinde (Karma-2000, Presto ve Tatlıcak-97) tuza tolerans seviyelerini tespit amacıyla bu çalışma yürütülmüştür. Bununla birlikte, NaCl'nin çimlenme üzerine osmotik ya da toksik etkisi olup olmadığı da belli değildir. Bu araştırmada, tritikale çeşitlerine ait tohumların 2.4, 4.2, 5.9, 7.7, 10.6 ve 13.2 dS m^{-1} elektriksel iletkenliğe sahip tuz streslerindeki çimlenme yüzdeleri, fide yaş ve kuru ağırlıkları ile su alımları belirlenmiştir. Kök ve sürgünlerde Na^+ ve Cl^-, tohumlarda Na^+, Cl^- ve K^+ ion alımları analiz edilmiştir. Sonuçlar artan NaCl dozları toplam çimlenme yüzdesinde önemli değişikliğe neden olmadan, ortalama çimlenme zamanını önemli şekilde etkilediğini göstermiştir. Farklı tuz konsantrasyonunda tohumların su alımı oranı değişmemiştir. Artan tuz konsantrasyonlarıyla kök ve sürgündeki Na^+ ionlarının konsantrasyonun arttığını, K^+ konsantrasyonun azaldığı belirlenmiştir. Ayrıca, çimlenmekte olan tohumlarda Na^+ ve Cl^- oranları artarken, K^+ oranı azalmıştır. Karma-2000 çeşidinin diğer çeşitlere göre tuza daha toleranslı olduğu görülmüştür. Çimlenmedeki gecikme düşük su alımından çok tohumda biriken aşırı Na^+ iyonunun etkisinden olduğu fakat toplam çimlenme yüzdesinin değişmediği belirlenmiştir. Bununla birlikte, NaCl'nin çimlenmeden sonraki gelişme dönemlerinde daha etkili olduğu söylenebilir.

Anahtar Sözcükler: Triticale (x *Triticosecale* Wittmack), tuzluluk, su alımı, çimlenme

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Introduction

Triticale (x *Triticosecale* Wittmack) is a cereal crop, high yielding and well adapted to extreme cold, drought and acidic soils, and grown in almost all geographic regions where the parental species are grown (Briggle, 1969). In many semi-arid and arid parts of the world, including Turkey, salt accumulation in the soil profile due to high evapotranspiration is common (Meiri, 1984; Pessarakli, 1999). Salt accumulation in soils affects plant growth to different degrees (Bernstein, 1975). However, in the same saline environment, different plant species may exhibit different growth response (Mass and Hoffman, 1977).

A prerequisite for successful production is stand establishment. One of the major environmental stress factors adversely affecting uniform germination is salinity in arid and semi-arid regions (Demir et al., 2003). Many researchers have reported that several plants are sensitive to high salinity during germination and the seedling stage (Jones, 1986; van Hoorn, 1991; Ghoulam and Fares, 2001). The source of the sensitivity to salinity is not fully understood. Some researchers have indicated that the main reason for germination failure was the inhibition of seed water uptake due to a high salt concentration (Coons et al., 1990; Mansour, 1994), whereas others have suggested that germination was affected by salt toxicity (Leopold and Willing, 1986; Khajeh-Hosseini et al., 2003).

Although preliminary studies on the salt tolerance of triticale have been conducted (Francois et al., 1988; Karim et al., 1993), the responses of newly released triticale to salinity are not well known. Francois et al. (1988) found that 7.3 dS m^{-1} reduced triticale yield by 2.8%. In addition, Karim et al. (1993) indicated that triticale cultivars gave different responses to varying NaCl (0-200 mM). Furthermore, the relative importance of the osmotic or toxic effects of NaCl on seed germination is not clear in triticale. Therefore, the present study was conducted to determine the effect of NaCl on the seedling growth of triticale cultivars on germination, and the reasons for seed germination failure in saline conditions and to find out mineral uptake by seeds and seedlings during germination at various concentrations of NaCl.

Materials and Methods

Seeds of the triticale cultivars Karma-2000, Presto and Tatlıcak-97 were used. Seeds were obtained from the Anatolian Agricultural Research Institute, Eskişehir, Turkey. The experiment was conducted in the laboratory of the Department of Field Crops, Faculty of Agriculture, University of Ankara. Salinity levels with electrical conductivity of the solution at 20 °C were adjusted to 2.4, 4.2, 5.9, 7.7, 10.6 and 13.2 dS m^{-1} (deciSiemens m^{-1}) using different NaCl concentrations (Rhoades et al., 1992). Distilled water served as a control.

Three replicates of 20 seeds were germinated on 2 sheets of Whatman No.1 filter paper in petri dishes with 10 ml each of the respective test solution and the paper was altered once after every 2 days to prevent accumulation of salt (Rehman et al., 1996). Twenty pre-weighed seeds of each variety with known moisture content were placed in petri dishes (9 cm diameter) as described for the germination test to measure the rate of imbibitions from NaCl treatments. In order to prevent evaporation, the edges of the petri dishes were tightly sealed with Parafilm. The seeds were allowed to germinate at 20 ± 2 °C in the dark for 8 days (ISTA, 1996). A seed was considered to have germinated when the emerging radicle elongated to 1 mm. Germination percentages were recorded every 24 h for 4 days. The seeds were weighed after surface water was removed and compared to determine the water uptake at each concentration, after 6, 12 and 24 h of treatment. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980). Water uptake was expressed as the percentage increase in moisture content on a fresh weight basis (Khajeh-Hosseini et al., 2003). The seedlings were thinned to 10 plantlets per petri dish after the fourth day (Ghorashy et al., 1972). Root number, root length and shoot length, shoot dry weight, root dry weight and root/shoot dry weight were measured on the eighth day (Bray, 1963). Dry matter were measured after drying samples at 70 °C for 48 h in an oven (Böhm, 1979).

A randomized complete block design was used with a factorial arrangement of treatments (cultivar and NaCl level) with 3 replications and 20 seeds in each replicate. Data were analyzed by 2 way analysis of variance using the statistical package MSTAT-C, and the differences between the means were compared using Duncan's multiple range test ($P < 0.05$).

Two grams of seeds of each cultivar were placed on petri dishes containing the same NaCl levels as used for Experiment 1 in order to determine mineral uptake (K^+,

Na^+ and Cl^-) of seeds after 14 h. The seeds were dried and mineral analysis was performed as follows: the shoot and root parts from Experiment 1 and seeds from Experiment 2 were weighed and separately dried at 70 °C for 48 h for mineral analysis (Na^+ and K^+ in Experiment 1; Na^+, K^+ and Cl^- analysis in Experiment 2). Chloride contents were determined with a titrimetric method and sodium and potassium analysis was performed using a flamephotometric method (Johnson and Ulrich, 1959).

Results

Germination and water uptake

In each level of NaCl, 100% final germination was observed for all cultivars (data not shown). The effect of increasing NaCl levels on final germination percentage was essentially the same for all cultivars. All cultivars germinated at all levels of NaCl, but MGT differed relative to cultivars and NaCl (Figure 1). Increasing NaCl level delayed germination time rather than affecting the final germination percentage. When the NaCl level increased, water uptake of the cultivars did not differ considerably (Figure 2). Seeds absorbed water much faster during the first 6 h. Water uptake of cultivars did not vary much with NaCl levels at each measurement time.

Seedling characters

NaCl levels and variety x NaCl level interaction were significant ($P < 0.05$) for shoot length (Table 1). Generally, shoot length declined as NaCl concentration increased. The longest shoot length was detected in the control for Presto and Tatlıcak-97, whereas Karma-2000 gave it at 2.4 dS m^{-1}. With increasing NaCl concentrations, shoot length of Karma-2000, Presto and Tatlıcak-97 decreased 45.2%, 52.0% and 53.0%, respectively. This showed that the shoot growth of Tatlıcak-97 was the most affected by NaCl levels.

Mean root length varied between 102.3 and 70.5 mm for various NaCl concentrations (Table 1). As expected, the control had the longest root length, while the shortest value was at 13.2 dS m^{-1}. Generally, root length decreased as NaCl concentration increased. However, Karma-2000 gave the longest root length on 4.2 dS m^{-1}.

Increasing NaCl concentrations adversely affected shoot dry weight (Table 1) although 2.4 dS m^{-1} gave the highest value of 8.27 mg $plant^{-1}$. Considering each cultivar, shoot dry weight fluctuated with varying NaCl concentrations. The lowest values were determined at 10.6 dS m^{-1} or higher.

In terms of root dry weight, differences among the cultivars were not significant; however, it was adversely affected by different levels of NaCl. No significant change in the root dry weight was observed up to 7.7 dS m^{-1}; however, higher NaCl concentrations resulted in a significant reduction in root dry weight (Table 1).

There were significant differences for the cultivars and NaCl levels with respect to the root to shoot dry weight ratio. Increased NaCl levels caused a remarkable increase in the root to shoot dry weights, and the highest

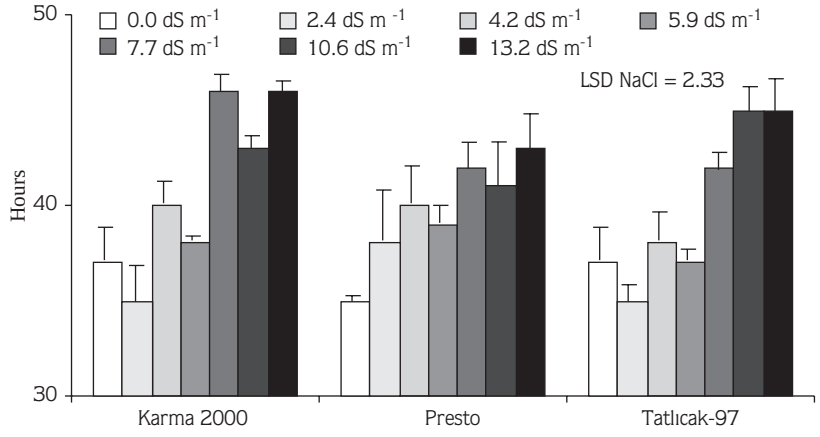

Figure 1. Mean germination time (MGT) of cultivars. The bars on each column show standard error.

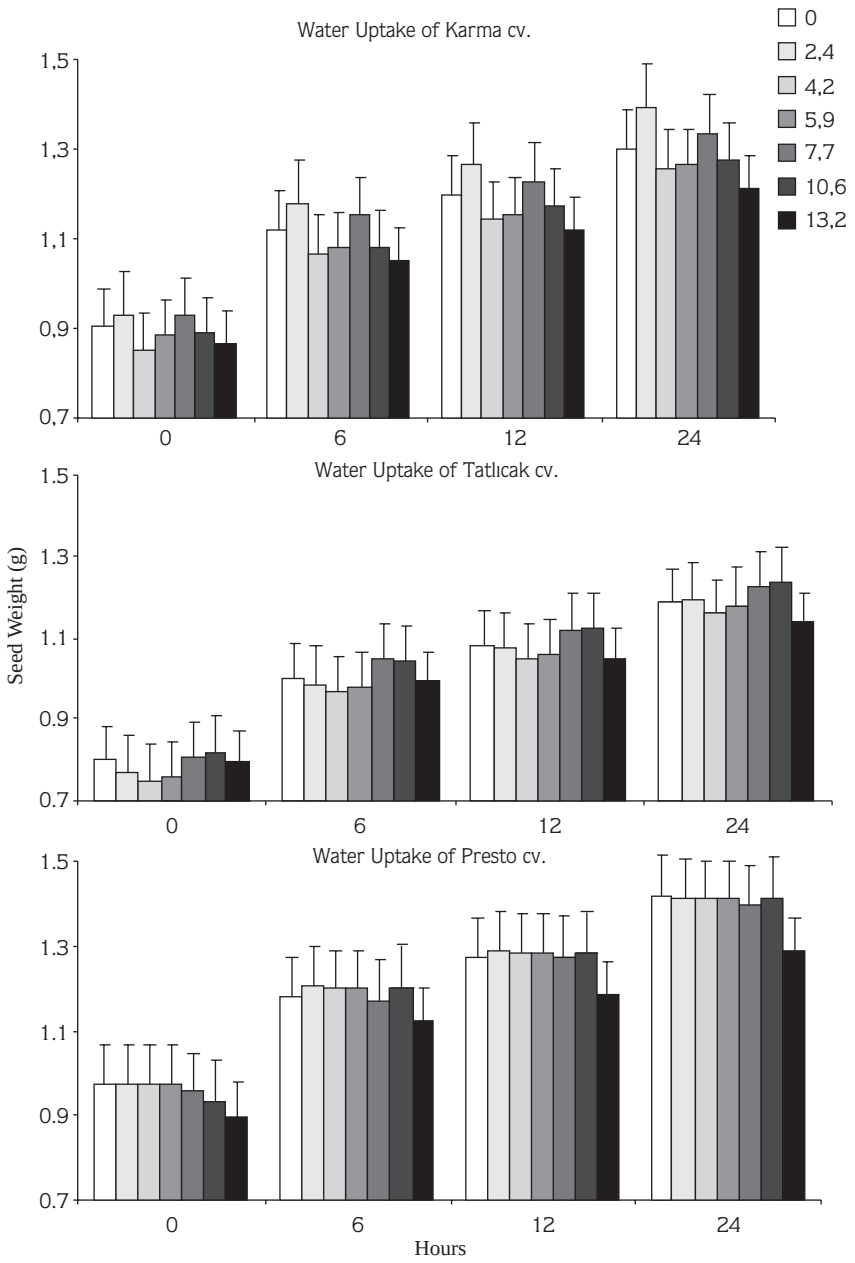

Figure 2. Increase in seed weight of triticale cultivars in relation to time and NaCl stress. The bars on each column show standard error.

ratio (1.24), was obtained at the 13.2 dS m^{-1}. Tatlıcak-97 gave the highest ratio (1.12) (Table 1).

There was a significant cultivar x NaCl level interaction on shoot dry matter. Mostly, increasing NaCl levels caused an increase in shoot dry matter. Concerning the cultivars, the shoot dry weight of Karma-2000, Presto and Tatlıcak-97 increased by 83.0%, 37.0% and 12.5%, respectively. This means that accumulated dry matter in the shoots was higher at higher NaCl concentrations.

Root dry matter was affected by NaCl concentration without a significant linear decrease or increase. The highest root dry matter (12.36%) was obtained from 13.2 dS m^{-1}, which was not significantly different from 10.6 dS m^{-1} and the control (Table 1).

Table 1. Effect of different NaCl levels on seedling characteristics of triticale cultivars.

Varieties	Shoot Length (mm)							
	Control	2.4 dS m^{-1}	4.2 dS m^{-1}	5.9 dS m^{-1}	7.7 dS m^{-1}	10.6 dS m^{-1}	13.2 dS m^{-1}	Mean
Karma 2000	75.0	88.6	76.5	67.0	65.0	48.9	41.1	66.0
Presto	80.5	73.9	71.7	61.2	64.2	46.4	38.6	62.4
Tatlıcak-97	79.3	76.5	67.5	79.1	57.1	43.8	37.0	62.9
Mean	78.3	79.7	71.9	69.1	62.1	46.4	38.9	
LSD	NaCl= 6.24		Int.= 10.81					
	Root Length (mm)							
Karma 2000	96.9	99.6	112.4	94.5	97.4	79.7	77.0	93.9
Presto	111.3	106.6	104.1	84.5	97.3	79.5	67.1	92.9
Tatlıcak-97	98.6	93.2	89.3	85.9	80.7	70.5	67.3	83.6
Mean	102.3	99.8	101.9	88.3	91.8	76.6	70.5	
LSD	NaCl=7.12		Cult=NS	Int.= NS				
	Shoot Dry Weight (mg $plant^{-1}$)							
Karma 2000	9.00	9.63	8.30	7.93	8.80	7.50	5.90	8.15
Presto	8.10	7.90	7.46	7.53	7.60	6.20	5.60	7.20
Tatlıcak-97	6.13	7.30	6.10	7.36	6.30	5.23	4.60	6.15
Mean	7.74	8.27	7.28	7.61	7.56	6.31	5.36	
LSD	NaCl=0.60		Cult=0.48	Int.= NS				
	Root Dry Weight (mg $plant^{-1}$)							
Karma 2000	7.80	8.63	7.13	7.50	7.90	7.27	6.50	7.53
Presto	8.10	8.03	7.67	7.10	7.80	6.80	7.30	7.54
Tatlıcak-97	6.30	6.60	6.83	7.23	7.77	6.33	6.10	6.74
Mean	7.40	7.76	7.21	7.28	8.82	6.80	6.63	
LSD	NaCl = 0.66		Cult= NS	Int.= NS				
	Root / Shoot Dry Weight Ratio							
Karma 2000	0.93	0.89	0.84	0.94	0.89	0.97	1.10	0.94
Presto	1.00	1.01	1.02	0.95	1.01	1.10	1.30	1.06
Tatlıcak-97	1.02	0.91	1.14	0.98	1.23	1.21	1.33	1.12
Mean	0.98	0.94	1.00	0.96	1.05	1.09	1.24	
LSD	NaCl= 0.09		Cult= 0.12	Int.= NS				
	Shoot Dry Matter (%)							
Karma 2000	7.31	10.68	10.12	10.83	11.09	12.08	13.40	10.78
Presto	10.19	10.38	10.17	10.92	11.45	11.98	13.95	11.29
Tatlıcak-97	11.09	12.22	11.30	11.31	12.29	12.40	12.48	11.87
Mean	9.53	11.09	10.53	11.02	11.61	12.17	13.28	
LSD	NaCl= 0.93		Cult= 0.30	Int.=1.61				
	Root Dry Matter (%)							
Karma 2000	13.09	9.88	10.65	10.03	10.30	10.79	11.47	10.89
Presto	10.19	9.23	9.95	10.34	9.26	10.92	13.02	10.42
Tatlıcak-97	12.48	11.56	11.77	10.52	12.59	12.97	12.58	12.07
Mean	11.92	10.22	10.79	10.30	10.72	11.56	12.36	
LSD	NaCl= 1.17		Cult= 1.23	Int.= NS				

Mineral analysis

Mineral analysis of root samples from the 3 cultivars showed that increased levels of NaCl affected the Na^+ and K^+ composition of triticale (Table 2). As expected, accumulation of Na^+ increased with increasing NaCl levels in the roots. K^+ was also higher in increased NaCl levels. Similar trends were noted in all cultivars.

Shoots accumulated less Na^+ than did the roots (Table 3) because the roots come into contact with NaCl and they absorb it directly. Higher NaCl levels resulted in a higher Na^+ accumulation in the shoots. K^+ concentration was also affected by increased NaCl levels, but levels higher than 4.2 dSm^{-1} resulted in increased K^+.

Na^+ accumulation in the seeds increased linearly with increasing NaCl levels (Table 4). Cultivars had different responses to Na^+ uptake, and the highest value was observed from Karma-2000. Furthermore, the control showed that Na^+ content differed between cultivars. Differences between the control and the highest NaCl were maximum in Tatlıcak-97. K^+ concentration in the seeds decreased as NaCl increased, but Presto did not show the same trend. Chlorine analysis showed that Cl^- accumulation in the seeds changed moderately with the increase in NaCl. All cultivars showed roughly the same response to NaCl levels, but accumulation of Cl^- in Tatlıcak-97 was higher than that in the others. Differences between the control and the highest NaCl were maximum in Tatlıcak-97.

Table 2. Sodium (Na^+) and potassium (K^+) in roots of triticale cultivars germinated at different NaCl concentrations.

	Karma-2000		Presto		Tatlıcak-97	
	Na (g kg^{-1})	K (g kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})
Control	2.39	7.37	2.73	7.93	2.75	7.98
2.4 dS m^{-1}	14.10	7.79	11.89	7.67	11.04	6.15
4.2 dS m^{-1}	15.46	7.62	16.41	7.52	13.11	7.42
5.9 dS m^{-1}	18.29	7.79	19.03	8.92	16.69	7.87
7.7 dS m^{-1}	23.01	8.77	19.97	8.73	15.38	8.41
10.6 dS m^{-1}	22.32	9.57	21.65	9.89	18.77	9.12
13.2 dS m^{-1}	25.00	10.81	18.98	10.83	22.62	10.02
$LSD_{int.}$=	1.79	0.128				

Table 3. Sodium (Na^+) and potassium (K^+) in shoots of triticale cultivars germinated at different NaCl concentrations.

	Karma-2000		Presto		Tatlıcak-97	
	Na (g kg^{-1})	K (g kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})	Na (g kg^{-1})	K (g kg^{-1})
Control	0.23	7.97	0.13	8.56	0.20	7.90
2.4 dS m^{-1}	3.19	7.29	1.80	8.84	1.19	8.22
4.2 dS m^{-1}	3.49	8.17	2.55	9.16	2.77	9.87
5.9 dS m^{-1}	4.77	9.63	3.11	9.13	3.10	6.51
7.7 dS m^{-1}	4.54	8.91	3.34	8.30	4.47	7.59
10.6 dS m^{-1}	4.59	9.18	4.17	7.78	6.40	6.95
13.2 dS m^{-1}	5.31	9.12	3.97	8.02	5.91	7.01
$LSD_{int.}$=	0.182	0.016				

Table 4. Sodium (Na^+), potassium (K^+) and chloride (Cl^-) in germinating seeds of triticale cultivars at different of NaCl concentrations.

	Karma-2000			Presto			Tatlıcak-97		
	Na (ppm)	K (g kg^{-1})	Cl (g kg^{-1})	Na (ppm)	K (g kg^{-1})	Cl (g kg^{-1})	Na (ppm)	K (g kg^{-1})	Cl l (g kg^{-1})
Control	44	3.79	0.7	68	3.34	0.7	20	3.26	0.5
2.4 dS m^{-1}	162	3.67	0.8	123	3.18	0.8	158	3.18	0.6
4.2 dS m^{-1}	207	3.51	0.8	171	3.23	0.8	276	3.06	0.6
5.9 dS m^{-1}	295	3.59	0.9	253	3.18	0.9	309	3.02	0.7
7.7 dS m^{-1}	216	3.58	1.0	324	3.29	0.9	403	2.96	0.9
10.6 dS m^{-1}	408	3.29	1.1	348	3.40	0.9	319	3.25	1.0
13.2 dS m^{-1}	495	3.24	1.2	383	3.31	1.0	474	2.81	1.1
$LSD_{int.}$=	6.57	0.416							

Discussion

Final germination percentage was not noticeably changed by NaCl concentration, but mean germination time was increased as NaCl increased. Similar results were noted in several crops (Bewley and Black, 1982; Demir et al., 2003; Khajeh-Hosseini et al., 2003). Water uptake of the investigated cultivars did not vary although a slight decrease in water uptake was observed with increasing NaCl levels. This showed that the osmotic barrier due to NaCl level affected water uptake and mean germination time but not final germination. Our findings agree with those of Leopold and Willing (1986), Hampson and Simpson (1990) and Perez-Alfocea et al. (1993), who determined that germination and seedling growth were reduced in saline soils with varying responses for cultivars while NaCl affected the germination of seeds by creating an external osmotic potential preventing water uptake. However, a number of studies demonstrated that water uptake in bean (Frota and Tucker, 1978), cotton (Pesserakli and Tucker, 1985), tomato (Pesserakli and Tucker, 1988), barley and wheat (Pesserakli et al., 1991) plants is significantly reduced under salt or water stress conditions.

Increasing NaCl level delays germination time rather than affecting final germination percentage, in agreement with van Hoorn (1991), who determined that an increase in salt concentration delayed germination time in several crops. These results also revealed that the levels of NaCl used in this study did not have a toxic effect on germination although they had a detrimental effect on the rate of germination. In particular, 5.9 dS m^{-1} or higher levels caused considerable delays in germination without changing final germination percentage in the investigated cultivars (Figure 1).

Root and shoot length decreased with increasing NaCl; at 10.6 dS m^{-1} they decreased drastically. Furthermore, the shoots were more sensitive than the roots as the NaCl increased. These results are similar to those reported by Gupta and Srivastava (1989), who found that the root parts were less affected than the shoots in wheat. It was reported that root growth in triticale was much better than that in rye and wheat plants in varying (0, 75 and 150 mM NaCl) salt treatments (Salim, 1991), and the relative grain yield of triticale cultivars was unaffected by soil NaCl up to 7.3 dS m^{-1} (Francois et al., 1986).

Increasing NaCl concentration antagonistically affected shoot and root dry weight. Reductions in dry weights depended relatively on the decrease in the lengths while the lengths declined severely compared to dry weights. These results are similar to those reported by Gupta and Srivastava (1989) and Salim (1991).

Increased NaCl levels caused a remarkable increase in the root/shoot dry weight ratio. This means that triticale shoots were more severely affected by NaCl than the roots, as reported by Salim (1991). Triticale cultivar shoots accumulated a high sodium content, primarily due to higher rates of net ion transport from root to shoot.

The significant cultivar x NaCl level interaction on shoot and root dry matter indicated that the cultivars did not respond similarly as NaCl increased.

Shoot parts accumulated less Na^+ than did the root parts (Table 3), but the increase in Na^+ content in the shoots was higher than that in the roots. This shows that Na^+ transport from root to shoot accelerated when NaCl increased as reported by Begum et al. (1992). Moreover, Na^+ content in the seeds resulted in a delay of mean germination time because no change in water uptake was determined. This means that Na^+ accumulation has a toxic effect on germination time, but does not inhibit germination (van Hoorn, 1991; Begum et al., 1992).

K^+ concentration in the roots increased surprisingly while K^+ in the shoots and seeds decreased relatively as NaCl increased. It seems that Na^+ and K^+ were exchanged by water uptake during germination. K^+ in the seeds was released into the germination medium while Na^+ in the medium was absorbed by the seeds. Rehman et al. (1996) and Craig et al. (1990) reported that increasing NaCl concentrations resulted in increasing K^+ leakage from seeds.

Cl^- analysis showed that Cl^- accumulation in seeds changed as the NaCl increased (Begum et al., 1992). All cultivars showed nearly the same response to NaCl levels. However, accumulation of Cl^- in Tatlıcak-97 was higher than in the others.

In conclusion, our findings revealed that the accumulation of Na^+ in seeds may have an adverse effect on germination with delaying mean germination time in triticale. It seems that the main reason for the delayed germination was higher Na^+ accumulation in seed rather than lower water uptake in triticale cultivars. However, final germination percentage was not significantly changed by increasing NaCl concentrations. In terms of seedling characters, shoot growth is more sensitive than root growth. Karma-2000 should be considered more tolerant to NaCl stress.

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