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# Effects of Different Salt Concentrations (NaCl) on Germination of Some Spinach Cultivars

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**Abstract:** Seeds of four spinach cultivars including Green Gold, Larisa, Mikado and Ohio were used to investigate the effects of different NaCl concentrations (0, 50, 100, 150 and 200 mM) on their germination percentage, germination energy, germination index, relative germination rate and germination time. The results showed that different treatments of salinity had statistically considerable effects on the germination percentage, germination energy, germination index, relative germination rate and germination time. Germination percentage and relative germination rate features of spinach cultivars were not influenced by 0–50 mM NaCl concentration, but germination energy values showed small decreases. In those features important decreases occurred by 100 mM concentration and the lowest values obtained at 200 mM. Despite the increase in germination time, germination index considerably decreased in accordance with the increasing salt concentrations. However, Green Gold cultivar demonstrated better performance than the other cultivars for most of measured parameters under different salinity levels.

**Key Words:** Spinach, salinity, germination percentage, germination energy, germination index.

## Farklı Tuz (NaCl) Konsantrasyonlarının Bazı Ispanak Çeşitlerinde Çimlenme Üzerine Etkisi

**Özet:** Bu çalışma, farklı tuz (NaCl) konsantrasyonlarının (0, 50, 100, 150 ve 200 mM), Green Gold, Larisa, Mikado ve Ohio ıspanak çeşitlerinde, çimlenme yüzdesi, çimlenme enerjisi, çimlenme indeksi, çimlenme oranı ve çimlenme zamanı özellikleri üzerine etkilerini araştırmak amacıyla yürütülmüştür. Araştırma sonuçlarına göre, farklı tuz konsantrasyonları, söz konusu çimlenme özellikleri üzerinde istatistiksel olarak önemli ölçüde etkiye sahip olmuştur. Ispanak çeşitlerinin çimlenme yüzdesi ve çimlenme oranı özellikleri 0–50 mM NaCl konsantrasyonlarından etkilenmemiş, çimlenme enerjisi değerleri ise düşük oranda azalmalar göstermiştir. Bu özelliklerde önemli miktarda azalmalar 100 mM konsantrasyonu ile ortaya çıkmış ve 200 mM konsantrasyonunda en düşük değerler elde edilmiştir. Çimlenme indeksi artan tuz konsantrasyonlarına bağlı olarak önemli miktarda azalmış, buna karşın çimlenme süresi de uzamıştır. Bununla birlikte; Green Gold çeşidi, farklı tuzluluk düzeylerinde ölçülen parametrelerin çoğunda diğer çeşitlere göre daha iyi performans göstermiştir.

**Anahtar Kelimeler:** Ispanak, tuzluluk, çimlenme yüzdesi, çimlenme enerjisi, çimlenme indeksi.

## Introduction

Seed germination takes the most important part in life cycle of plants (Khan et al., 2000). Like many other cultivars, the seed's germination ability in different environmental conditions and fast and uniform germination are intended charecteristics of spinach (Foolad et al., 2007). In many plant type, germination and seedling growing phase is very sensitive to salt stress. In general, the highest germination percentage occurs in non-salty conditions and it decreases depending on the ascending salt concentrations. (Khan et al., 2000). Germination is described as the rootlet's coming out of the testa (Coopland and McDonald, 1995). Seeds' germination begins with water intake but it is decreased by the salt (Othman, 2005). The decrease in water intake of the seed in salty conditions, osmotically and by the ion toxity with accumulation of Na and Cl ions highly around the seed, prevents the seed germination (Murillo-Amodor et al., 2002; Shokohhifard et al., 1989). Ascending salt concentrations not only prevent the germination of the seeds but also extend the germination time by delaying the starting of germination (Rahman et al., 2008). Generally, low salt concentration decreases the germination rate and high salt concentration decreases germinaton percentage (Shannon and Grieve, 1999).

Richards (1954) has classified the vegetables according to their salt tolerance. In this classification it is stated that asparagus, table beet and spinach can be grown in high salt concentrations (130 mM). Landale et al. (1971) defines spinach as half salt tolerant leafed plant. Therefore in the spinach cultivar that was irrigated with 4 dS/m salty water and grown in sandy soil, was not seen any decrease in the crop and acquired harvestable quality spinach. (Pasternak and De Malach, 1994). Speer and Kaiser (1991) applied 100 mM salt concentration to the spinach plants of which the authors grew in sandy soil and reported that there were small decreases in growth. In that study which authors did with some spinach, lettuce, pepper, broccoli and tomato kinds. Zapata et al. (2004) informed that the seeds which have the highest germination ability are spinach. Many studies have been done to determine the effects of salinity on germination of many cultivars. In the study he did about the effects of different salt concentrations (0, 4.7, 9.4, 14.1 dS $m^{-1}$) on germination of cabbage (*Brassica oleracea capitata*) and cauliflower (*Brassica oleracea bortrytis*) cultivars, Jamil et al. (2005) stated that increasing salt concentration decreases the germination percentage and rate at an important degree and increases germination time. In another study, the effect of sea water salinity (1500, 2500 and 3500 ppm) on the growth of tomato (*Lycopersicon esculentum*) cultivars (Trust, Grace and Plitz) was studied (Hajer et al., 2006). The researcher reported that, the sea water salinity delayed seed germination and reduced germination percentage especially with increasing salinity level. Whilst, besides melon and eggplant (Chartzoulakis and Loupassiki, 1997) cultivars, in rice (Khan et al., 1997) and wheat (Almansouri et al., 2001) cultivars, high salt concentrations increase germination time, in germination rate and in the end germination percentage there occured considerable decreases. At last, salt tolerance studies which have done during germination time are important for determining the plants' salt tolerance in the early and late growing phases (Zapata et al., 2003).

Spinach cultivation is done by direct sowing the seeds in the soil. The presence of environmental stress conditions limits the direct sowing. Depending on salinity and time, soil's water potential and the seed's genetical structure, the soil salinity delays and prevents the germination in direct sowings. And that, besides decreasing the seedling coming out and thereby the plant number in the field, endangers the economical harvesting (Cuartero and Fernandez–Munoz, 1999; Foolad et al., 2007). Crop plants are usually seeded within the top 10 cm layer of the soil where it usually contains highest amount of salt (Esechie, 1995). In lower salt concentrations, the inconveniences which may occur from decreases in germination can be removed by sowing more seeds. But in the areas where there is high concentration, the increase in the amount of ungerminated seeds and in the germination time is unfavorable. Lengthening of the germination period can be very dangerous for a direct–sown crop because the probability of crust formation on the soil surface, which would make difficult or even prevent emergence, increases with time and also because germinated seeds and young seedlings are especially susceptible to the attack of several fungi and pests (Cuartero and Fernandez–Munoz, 1999). Because of these reasons, to increase the crop in cultivars which are cultivated in salty soils, the salt tolerance of the plants must be enhanced. In this respect it is important to know the seeds' germination characteristics in different salt concentrations. In this study, the seeds', which belong to four different spinach cultivar that different salt concentrations are applied, germination ability in salt stress conditions and their salt tolerance during germination are examined. For this purpose, 0 (control), 50, 100, 150 and 200 mM salt concentrations are applied to the seeds of different spinach cultivars and at the end of the study germination percentage, germination energy, germination time, germination index and germination rate are evaluated.

## Material and Methods

This study was designed to evaluate the germination characteristics of four different spinach cultivars (*Spinacia olerecea*) under the laboratory conditions in 2009 at Mustafakemalpaşa Vocational School, Uludağ University in Turkey.

### Seed material and treatments

Experiments were made with four spinach cultivars (*Spinacia olerecea*): Green Gold, Larisa, Mikado and Ohio. The spinach seeds were provided by Agromar Seed Company, Inc. (Karacabey, Bursa, Turkey). Before sowing, seeds were soaked in 1% sodium hypochlorite (NaOCl) for ten minutes (disease prevention) and washed thoroughly with distilled water.

### Seed germination

The seeds were germinated in petri dishes (11 cm diameter) containing two sheets of Watman No. 1 filter paper moistened initially with 5 ml of distilled water (control), or with different treatment solutions 50, 100, 150 and 200 mM NaCl (saline conditions). These treatments were prepared with separately calculated amounts of NaCl (Merck) in dissolving distilled water. Distilled water was added when necessary. Germination chamber was at 20°C and with dark conditions. All petri dishes were placed in incubator. Each petri dish contained 100 seeds and each treatment was replicated 4 times.

To determine the germinated seeds, the seed counting process was begun with the day following the day on which the seeds moistened for the first time. That process was repeated everyday at the same hour. After sowing every day, the germinated seeds were removed from the Petri dishes. Germination was counted when a 5 mm radicle had emerged from the seed coat (Kabar, 1990; Akman, 2009). At the end of the twenty first day, the last counting was done and the study was concluded. In germination studies ISTA (1996) and Şehirali (2002) is benefited.

Germination percentage, germination energy, germination index, relative germination rate were determined by the following formula (Li, 2008);

Germination percentage = a/b

Germination energy = c/b

Germination index = Σ Gt/Dt

Relative germination rate = d/e

where, a for germinated seeds total in NaCl concentration every day, b for total number of seeds to germinate, c for germinated seed total in NaCl concentration in seven days. Gt for germinated seed in t days, Dt for the number of germination days corresponding. d for germination percentage in NaCl concentration, e for germination percentage in NaCl of control.

Mean germination time (MGT) was calculated using the method of Younsheng and Sziklai (1985) as follows:

MGT = Σ nidi/n

where, n = total number of seeds germinated during 21-day experimental period; ni = number of seed germinated on day di; di = day during germination period (between 0 and 21).

### Statistical analysis of data

The germination percentage, germination energy, germination index, relative germination rate and germination time data were analyzed using MSTAT–C (version 2.1, Michigan State University, 1991) and SPSS (version 10.0, SPSS Inc., 1999) software. Analysis of variance (ANOVA) was conducted and significance of differences among treatment was tested using the least significant difference (LSD). Differences were declared very significant at $P < 0.01$ and significant $P < 0.05$ probability levels by the *F* test. The *F*-protected LSD calculated at the 0.05 probability level according to Steel and Torrie (1980). Also, relationships between NaCl concentrations and germination percentage, germination energy, germination index, relative germination rate and germination time data of the cultivars were fitted to linear or quadratic regression equation. The SPSS software was used to carry out regression analysis.

## Results and Discussion

Analysis of variance revealed significant differences among spinach cultivars and salinity levels for germination percentage, germination energy, germination index, relative germination rate and germination time (Table 1).

### Germination percentage

The results revealed that the germination percentage of spinach cultivars (Green Gold, Larisa, Mikado and Ohio) was strongly affected by all salt treatments. Increased salt concentration caused a decrease in germination percentage. Strong reduction was observed mainly at the higher level of salt concentration compared to control. Regression equations for all the spinach cultivars were obtained by plotting observed germination percentage on the Y–axis and NaCl concentrations on the X–axis (Fig. 1a). Negative linear relationships were seen between NaCl concentration and germination percentage for Green Gold and Larisa cultivars ($P<0.01$). On the other hand, negative inclined quadratic regression equations were found for Mikado and Ohio cultivars at 99% level of confidence. The germination percentage was highest at the control treatment (0 mM). However, there were not significant differences in germination percentage between cultivars given the two treatments (control and 50 mM) (Table 1). These results were in agreement with Kaymak et al. (2009) who found that lowest concentration of NaCl was not significantly affected radish seed germination. In general, germination percentage decreased significantly with increasing after 100 mM NaCl concentration treatment. For 100 and 150 mM of NaCl concentration, the highest germination percentage was obtained as 83.50% and 77.00% from the Green Gold cultivar, respectively. On the other hand for same doses, the lowest germination percentage was found as 64.50% and 55.50% from the Mikado cultivar, respectively (Table 1). These results are similar in line with Jamil et al. (2005). They reported that germination of *Brassica* species (cabbage, cauliflower, canola) decreased as the salinity concentration increased. In studies on tomato genotypes (Doğan et al., 2008) and varieties (Kemer, Pala and Aydın Siyahı) of eggplant (Akıncı et al., 2004), it was found that the highest germination was in control and 50 mM salt applications and with the 100–150 mM salt applications the germination percentage has considerably decreased. In another study, 100 mM salt concentration caused 75% (Jones, 1986) germination losses in some *Lycopersicon esculentum* cultivar seeds and in cucumber seeds 150 mM salt concentration caused 90% losses (Passam and Kakouriotis, 1994). According to literature, sensitivity decreased in the following order; lettuce, pepper, tomato, melon, broccoli, spinach and beetroot. On the other hand, Zapata et al. (2004) found that in 150 mM salt concentration the highest germination percentage was obtained from the spinach seeds with respect to the control application and it was followed by lettuce, melon, pepper, broccoli and beetroot respectively. In comparison with the control treatment, the lowest germination percentage values were determined at 200 mM NaCl salinity. However, analysis of variance revealed significant differences among spinach cultivars at 200 mM salt treatment. Germination percentage of Green Gold (64.50%) is higher than Ohio (60.00%), Larisa (55.00%) and Mikado (51.00%) at same NaCl concentration, it suggest that seeds of Green Gold could germinate well at the highest concentration of NaCl (200 mM).

### Germination energy

Similar results were obtained in germination energy as germination percentage. The highest germination energy was obtained from control treatment. In control application the germination energy of species were found 99.50% in Green Gold, 98.00% in Ohio, 97.00% in Larisa and Mikado. However, there was a small decrease in germination energy as a result of salt treatment (0–50 mM). First considerable decreases in germination energy with respect to the control application occurred in 100 mM concentration and germination energy values decreased with the increasing salt concentration. The lowest germination energy values were determined at 200 mM NaCl salinity. It was determined that, in the highest concentrations (200 mM) applied to the spinach seeds, Green Gold has the highest germination energy (49.50%) and Mikado has the lowest (28.00%). In 200 mM NaCl concentration, the germination energy values obtained from Ohio and Larisa were, between the cultivars, 36.00% and 34.50%, respectively (Table 1). The relationships between NaCl concentration and germination energy have been evaluated for all the cultivars (Fig. 1b). Negative linear relationships were determined for Green Gold, Larisa and Ohio cultivars ($P<0.01$). The relationships between the germination energy and NaCl applied were linear implying that germination energy were decreasing with increase in NaCl dose. Also, a quadratic regression equation was obtained for Mikado Cultivar ($P<0.01$).

### Germination index

With the spinach cultivars, as Karagüzel (2003) stated, considerable changes occurred in germination index that calculated as a function of germination time and rate with the increase of salt concentrations (Table 1). Previous reports from other workers corroborate these results, for instance, Karagüzel (2003) Lipunus various, Khan et al. (2009) hot pepper. In this study, germination index of Green Gold, Larisa, Mikado and Ohio decreased significantly with increasing NaCl concentration (Table 1). The reduction gets stronger particularly at the highest level of salt at 200 mM NaCl concentration when compared to control. Relationships relating germination index to NaCl concentration are shown in Fig. 1c. The relationships between germination index and NaCl concentration were negative linear for Green Gold and Ohio and polynomial for Larisa and Mikado ($P<0.01$). Among the 4 spinach cultivars monitored, the highest germination index values were determined in Green Gold cultivar in all NaCl concentration. This cultivar was less affected from salt treatments. Germination index values for Green Gold cultivar were determined 38.01, 33.62, 23.63, 17.46 and 10.60 for control, 50, 100, 150 and 200 mM NaCl concentration doses, respectively. On the other hand, the most sensitive cultivar was Mikado at all salinity levels. In this cultivar, increased salt concentrations caused higher decreases in germination index values compared to the other 3 genotypes. Mikado's germination index values in control, 50, 100, 150 and 200 mM applications were measured as 32.63, 26.49, 10.63, 9.16 and 7.32, respectively. The remaining cultivars' values were between Green Gold and Mikado's.

### Relative germination rate

Relative germination rate was highest at control and 50 mM salt concentrations. Convenient salt concentrations were beneficial to germination of spinach seeds. In this experiment, increasing concentrations of NaCl reduced the germination rate of spinach cultivars, but the germination rate was not significantly different between control and 50 mM salt concentrations (Table 1). These results are similar to Karagüzel (2003) and Li (2008). Shannon and Grieve (1999) reported that salinity slowed the germination rate, at low concentrations the only effect was on germination rate, not on total percentage of seeds. The considerable amount of decreases beginning with 100 mM salt concentration continued with the 150 mM concentration. The lowest germination rate in spinach cultivars was obtained from 200 mM salt concentration. In the studies Jamil et al. (2005) did on Brassica species (canola, cabbage and cauliflower) and Al–Harbi et al. (2008) did on some Tomato cultivars (Pascal, Red Stone, Shohba, Super Marmand, and Tanshet Star) determined that germination rates show considerable decreases in high salt concentration in proportion to the control application. The highest germination rate was obtained from Green Gold in 200 mM salt concentration and it was followed respectively by Ohio, Larisa and with the lowest germination rate, Mikado. Germination rate of the other species were 0.64, 0.61, 0.56 and 0.52, respectively (Table 1). Linear relationships were observed between the relative germination rate and NaCl concentration in Green Gold and Larisa cultivars in the regression analysis. Also, quadratic regression equations were found in Mikado and Ohio cultivars (Fig. 1d).

### Germination time

It was found in this investigation that salinity had a considerable affect on germination time of spinach seeds. As Karagüzel (2003) stated, the short germination time in many plant type considerably increased with the increase of salt concentration. According to Ozcoban and Demir (2006) salinity influences germination time more dramatically than germination percentage. It means that the higher the salt concentration the longer the germination time. However, salinity caused different germination time delay amongst cultivars. Pujol et al. (2000) obtained similar results. The researchers reported that an increase in salinity induces both a reduction in the percentage of germinating seeds and a delay in the initiation of the germination process. Delayed germination has also been reported by Zapata et al. (2003) on *Lactica sativa* cultivars and Chartzoulakis and Klapaki (2000) on peper under the salt stress condition. Foolad and Lin (1999) reported that germination under salt stress and rapid germination under salt stress could be used as a criterion for salt tolerance. Furthermore, the rapid germination may contribute to salt tolerance to some extent.

**Table 1.** Effects of different NaCl concentrations on germination percentage, germination energy, germination index, relative germination rate and germination time of spinach cultivars

| Cultivars | NaCl concentration (mM) | Germination (%) | Germination energy | Germination index | Relative germination rate | Germination time (day) |
|---|---|---|---|---|---|---|
| Green Gold | 0 | 99.50 a | 99.50 a | 38.01 a | 1.00 a | 2.79 g |
| | 50 | 96.00 a | 96.00 ab | 33.62 bc | 0.97 a | 3.10 g |
| | 100 | 83.50 b | 80.50 c | 23.63 f | 0.84 b | 4.44 cd |
| | 150 | 77.00 bc | 62.00 d | 17.46 g | 0.77 b | 5.74 b |
| | 200 | 64.50 e | 49.50 e | 10.60 hi | 0.64 d | 6.87 a |
| Larisa | 0 | 97.00 a | 97.00 ab | 32.91 c | 1.00 a | 3.14 fg |
| | 50 | 95.00 a | 93.00 ab | 30.42 cd | 0.98 a | 3.72 ef |
| | 100 | 73.50 cd | 63.50 d | 15.45 g | 0.76 c | 4.94 c |
| | 150 | 66.00 de | 41.00 fg | 11.67 h | 0.68 d | 5.90 b |
| | 200 | 55.00 fg | 34.50 g-i | 8.78 ij | 0.56 ef | 7.11 a |
| Mikado | 0 | 97.00 a | 97.00 ab | 32.63 cd | 1.00 a | 3.20 fg |
| | 50 | 92.00 a | 89.50 b | 26.49 e | 0.94 a | 4.03 de |
| | 100 | 64.50 e | 43.50 ef | 10.63 hi | 0.66 d | 6.54 a |
| | 150 | 55.50 fg | 31.00 hi | 9.15 ij | 0.57 ef | 6.73 a |
| | 200 | 51.00 g | 28.00 i | 7.32 j | 0.52 f | 7.05 a |
| Ohio | 0 | 98.00 a | 98.00 a | 35.44 b | 1.00 a | 2.96 g |
| | 50 | 95.50 a | 94.50 ab | 32.50 cd | 0.97 a | 3.20 fg |
| | 100 | 80.50 bc | 76.50 c | 22.44 f | 0.82 bc | 4.49 cd |
| | 150 | 82.00 b | 62.00 d | 17.31 f | 0.83 b | 5.64 b |
| | 200 | 60.00 ef | 36.00 f-h | 9.64 h-j | 0.61 de | 6.62 a |
| LSD (5%) | | 7.51 | 7.95 | 2.40 | 0.08 | 0.59 |

Positive linear relationships were seen between germination time and NaCl concentration for Green Gold and Larisa cultivars. Polynomial regression equations were found for cultivars of Mikado and Ohio (Fig. 1e). The relationships were statistically significant at the level of $P<0.01$.

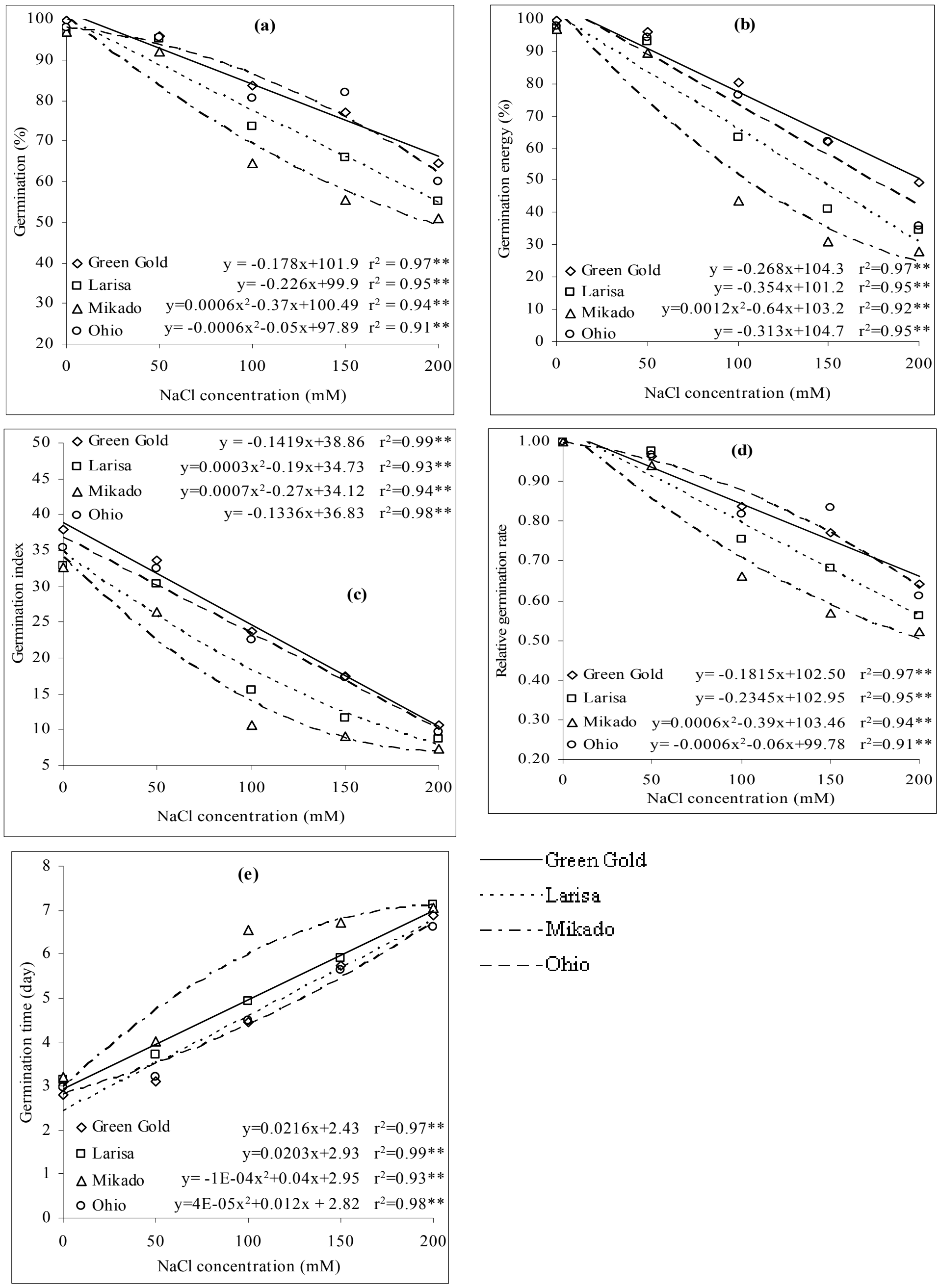


**Fig. 1a-e.** Relationships between germination percentage, germination energy, germination index, relative germination rate and germination time with NaCl concentration for spinach, ** P<0.01.

In this study, the shortest germination time for spinach seeds obtained from the control applications. In control application Green Gold and Ohio were the fastest germinating seeds with 2.79 and 2.96 days. These species were followed by Larisa and Mikado with 3.14 and 3.20 days. However, there was a small decrease in germination time as a result of salt treatment (0–50 mM). Moreover, significant differences were found between control and 50 mM applications for Green Gold. Germination time dramatically increased particularly with increasing the salt dosage to 100 and 150 mM. However, considerable germination time differences between Larisa, Green Gold and Ohio could not be found in both salt concentrations. Different from the other species, Mikado was found to have the longest germination time in 100 and 150 mM NaCl concentrations with 6.54 and 6.74 days. In the study of which determined that the spinach seeds' germination time increases depending on the salt concentration increase, also determined that the longest germination time was in 200 mM NaCl concentration. After all, there weren't significant differences in germination time between the species in 200 mM NaCl concentration (Table 1). In other words, for each cultivar the effects of the 200 mM NaCl concentration solutes were very similar. Similar results were reported by Jeannette et al. (2002). They found that the mean time to germination of almost all *phaseolus* species increased with the addition of NaCl and this increase in median germination time was greater in higher concentration as compared to low concentration.

### Conclusion

In this study, four different spinach cultivars' germination features in different salt concentrations were examined. Germination percentage, germination energy, germination index and relative germination rate values were not considerably decreased between non salty and low salt concentrations. Germination time increased with the increasing salt concentration. Increasing salt concentrations after 50 mM caused significant losses in the germination percentage, germination energy, germination index, and relative germination rate values and increased the germination time. According to the germination percentage, germination energy, germination index and relative germination rate values especially obtained from high concentration applied seeds, Green Gold has higher performance than the other spinach cultivars.

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