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*Full Length Research Paper*

# Salt tolerance in red clover (*Trifolium pratense* L.) seedlings

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**This study was conducted to investigate the effect of salt stress on germination of 28 red clover (*Trifolium pratense* L.) populations collected from Black Sea Region of Turkey. Seeds were germinated in 0, 60, 120, 180 and 240 mM NaCl concentration. Germination percentage (%), mean germination time (MGT), promptness index (PI), root and shoot length (mm) were measured to determine the salinity tolerance on red clover populations. The results showed that as the salt concentration increased, germination percentage decreased in all populations, moreover, seeds could not germinate in 240 mM NaCl. Mean germination time increased with increasing NaCl level. Populations 17 and 19 were best suited for germination under the range of salinity stress in this study. The population 17 gave the highest PI, while population 19 produced the longest root at 180 mM NaCl concentration.**

**Key words:** Forage plant, germination, salinity, promptness index.

## INTRODUCTION

Although, forage plants are of great importance in sustainable agricultural systems, their proportion in total cultivated area in Turkey is approximately 7% (TurkStat, 2009). A few forage species are cultivated in Turkey; one of them is red clover. Red clover has a light compensation point at 6% of daylight (Taylor and Smith, 1995). This makes red clover an excellent small seeded legume in the northern part of Turkey which has low light compensation and short daylight period.

Soil salinity is one of the world's most serious environmental problems. About 7% of the world's total land area is affected by salt, which is similar to the percentage of its arable land (Ghassemi et al., 1995; Munns et al., 2002). It is determined that 1.5 milion ha area in Turkey has salinity problem (GDRS, 2011).

Salinity gradually increases in the northern part of Turkey. Soil salinity could be a result of natural processes, or caused by crop irrigation with saline irrigation water under poor drainage conditions (Orak and Ates, 2005). Over 50% of all irrigated lands are affected by salinization (El Swaify et al., 1983). This has led to research of salt tolerance with the aim of improving crop plants (Zhu, 2001) or soil reclamation. However, soil reclamation is a very expensive process, and hence the cultivation of tolerant species and varieties is the most practical solution when the salinity is low. It is well known that there are significant genotypic differences with respect to salt tolerance between and within plant species (Rana, 1986). It is known that legumes are generally more sensitive to salinity (Ghassemi-Golezani et al., 2009), like wise, red clover (*Trifolium pratense* L.) which has a low salt tolerance (Maas, 1990).

Germination is an important stage in the life cycle of crop plants, particularly in saline soils as it determines the degree of crop establishment. Salinity has toxic effect on germinating seeds, and excessive salt hinders seed from water uptake during germination (Kara and Kara, 2010).

Selection for salinity resistance appears as a laborious and hazardous task and plant breeders are, therefore, seeking for quick, cheap and reliable ways to assess the salt-resistance of selected material. Determination of germination potential of seeds in saline conditions could appear as a simple and useful parameter for several reasons. Firstly, salinity resistance at this stage was shown to be a heritable trait which could be used as an efficient criterion for the selection of salt-resistant-populations (Ashraf et al., 1987), although, it is a polygenic

**Abbreviations: PI,** Promptness index; **GSTI,** germination stress tolerance index; **MGT,** mean germination time.

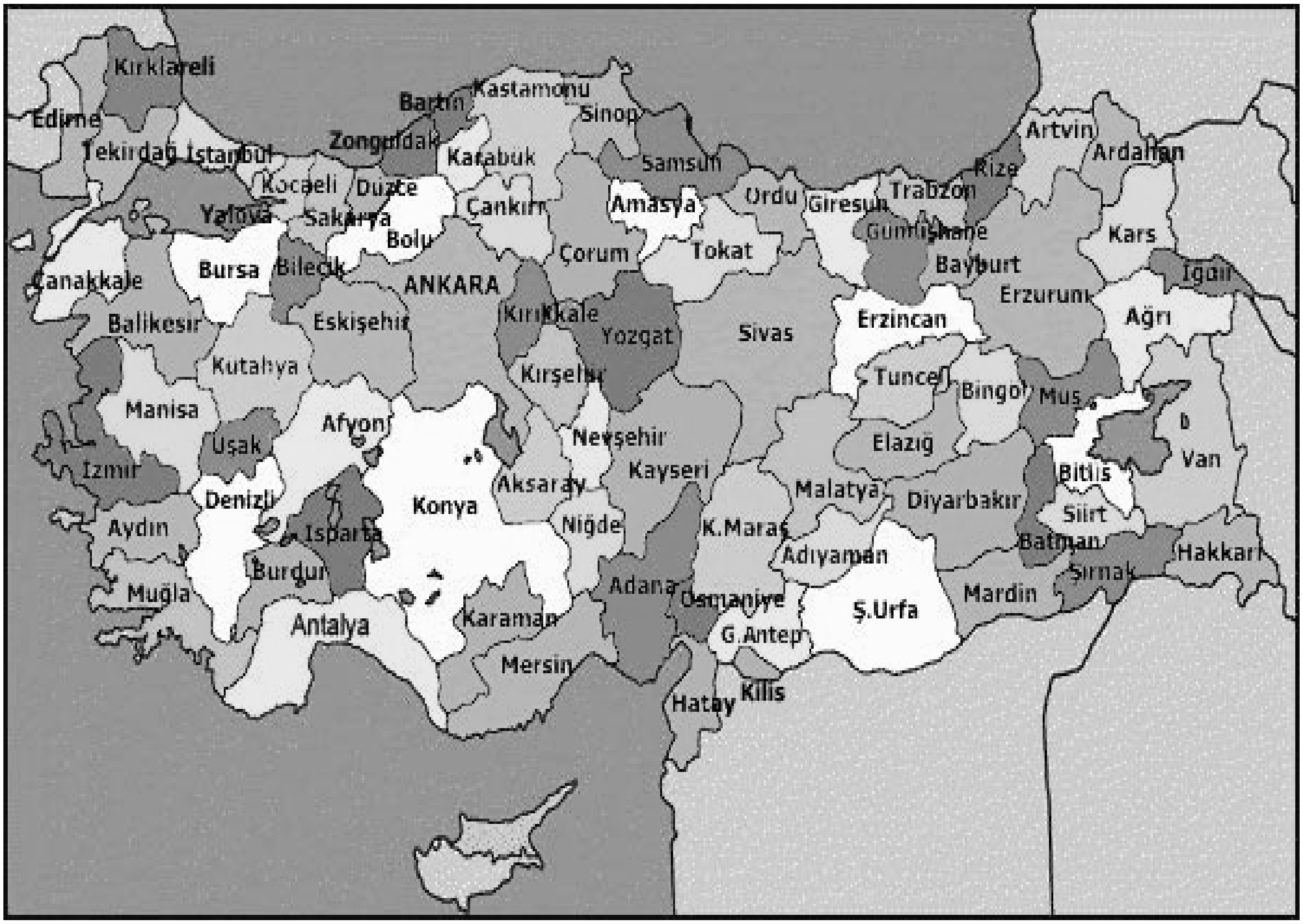


**Figure 1.** Geographic distribution of naturalized populations of red clover (*T. pratense* L.) in Central-Black Sea Region, Turkey.

character linked to a complex genetic basis (Mano and Takeda, 1997). Secondly, seeds and young seedlings are frequently confronted by much higher salinities than vigorously growing plants because germination usually occurs in surface soils which accumulate soluble salts as a result of evaporation and capillary rise of water. Selection of salt tolerant red clover genotypes would allow one to cultivate this crop on saline soils or with saline waters (Sidari et al., 2008).

Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Germination percentage, germination speed and seedling growth are the most useable criteria for cultivar selection (Bybordi and Tabatabel, 2009).

The main aim of this study was to investigate the effects of different NaCl concentrations on seed germination and seedling growth of 28 red clover populations.

## MATERIALS AND METHODS

Twenty eight (28) red clover (*Trifolium pratense* L.) populations obtained from native pastures were used in this study. Red clover seeds were collected in the period of June to September 2008 from Samsun, Ordu, Sinop, Trabzon provinces located at Black Sea Region of Turkey (Figure 1). Latitude of the locations changes between 38 and 1100 m a.s.l (Table 1). While soil contains slight salt (EC ≤ 1.37 $dSm^{-1}$) at locations where populations 17 and 19 were collected, soil at the other locations is categorized as saltless (EC ≤ 0.98 $dSm^{-1}$). The collected seeds were sown in seed trays and then seedlings were transplanted into field (70 cm row spacing and 50 cm plant spacing) at the end of March 2009 in Samsun (41°21´ N, 36°15´ E, altitude 195 m a.s.l). The experimental area had the typical Mediterranean climatic conditions during the study period (Figure 2). Seed was harvested when inflorescence colours turned to Brown (Manga et al., 2003). Harvest for seed was performed picking ripe inflorescences by hand July 2009.The harvested red clover seeds were stored in polyethylene bags for 9 months at the temperature of 20 ± 2°C.

Scarification, removing the effects of seed coat impermeability, was conducted by placing seeds between two pieces of sand paper and rubbing with gentle hand pressure. Each treatment was replicated three times. For each treatment, twenty scarified seeds were placed in 90 mm Petri dishes with filter paper and wet with 10 ml of the following concentration of NaCl: 0, 60, 120, 180, 240 mM NaCl (Nichols et al., 2009). Petri dishes were sealed with parafilm to prevent evaporation and the seeds were incubated in the dark at 20°C. The germinated seedlings were counted every 2 days for 14 days (Ries and Hofmann, 1983).

**Table 1.** Geographic distribution of naturalized populations of red clover in Black Sea Region, Turkey.

| Location | | Population | Altitude (m) |
|---|---|---|---|
| **Sinop** | Erfelek | 1 | 118 |
| | | 2 | 128 |
| | | 3 | 219 |
| | Boyabat | 4 | 1017 |
| **Samsun** | Bafra | 5 | 773 |
| | | 6 | 752 |
| | | 7 | 1085 |
| | Merkez | 8 | 744 |
| | Tekkekoy | 9 | 92 |
| | Kavak | 10 | 398 |
| | | 11 | 675 |
| | Ladik | 12 | 904 |
| | Vezirkopru | 13 | 1100 |
| | | 14 | 1105 |
| **Ordu** | Unye | 15 | 40 |
| | Merkez | 16 | 38 |
| | | 17 | 239 |
| | Ikizce | 18 | 372 |
| | | 19 | 477 |
| | | 20 | 441 |
| | | 21 | 552 |
| | | 22 | 487 |
| | Akkus | 23 | 481 |
| | Kumru | 24 | 623 |
| | Korgan | 25 | 340 |
| | Aybasti | 26 | 979 |
| | Gurgentepe | 27 | 1052 |
| **Trabzon** | Caykara | 28 | 770 |

Seeds were considered to have germinated when radicles were at 1 mm long (Voigt et al., 1998). The percentage of germinated seeds was calculated as the proportion of the initial population that imbibed after 14 days (excluding hard seeds) (Nichols et al., 2009). Mean germination time (MGT) was calculated to assess the rate of germination following Ellis and Roberts (1980). The mean germination time was calculated by the formula: MGT = $\Sigma fx/\Sigma f$, where, x is the number of the seeds newly germinated on day f, and f is the number of days from the beginning. The germination results were expresses in terms of a promptness index (PI) and then a germination stress tolerance index (GSTI) was calculated (Noreen et al., 2007).

The promptness index was calculated by the formula:

$$PI = nd2(1.00) + nd4(0.75) + nd6(0.50) + nd8(0.25)$$

Where, nd2, nd4, nd6 and nd8 are the number of seeds germinated on the 2$^{nd}$, 4$^{th}$, 6$^{th}$ and 8$^{th}$ day, respectively. While germination stres tolerance index was calculated by the formula:

$$GSTI (\%) = (PI \text{ of stressed seeds}/PI \text{ of control seeds}) \times 100.$$

Moreover, the root and shoot length along with cotyledon (Ates and

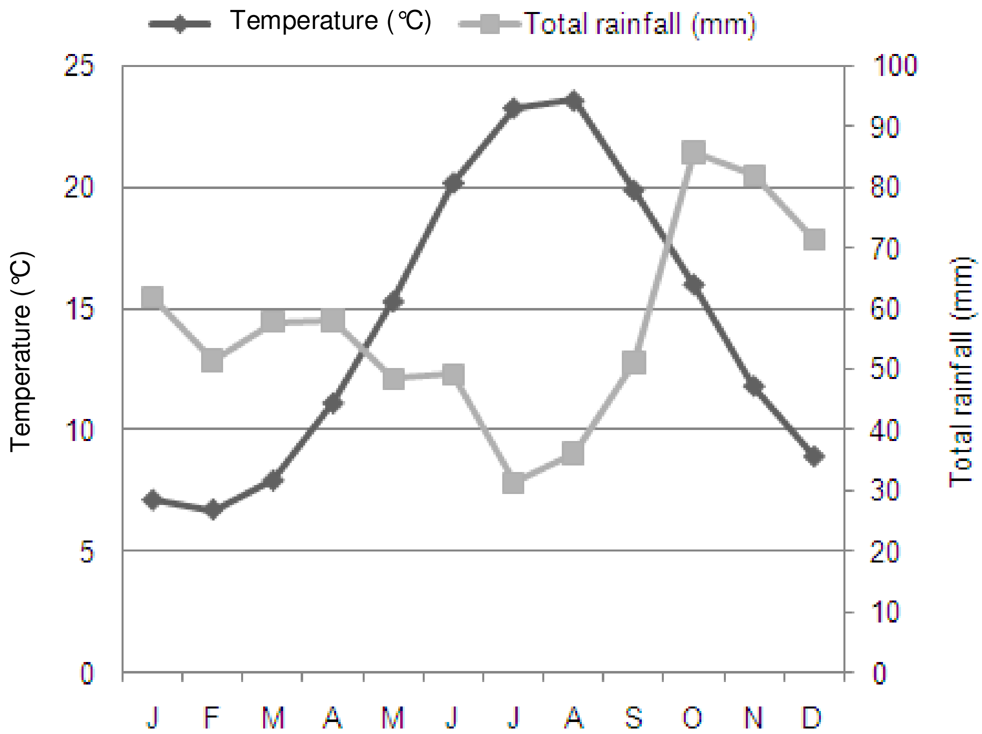


**Figure 2.** Climatic diagram of Samsun.

Tekeli, 2007) were measured on the 14th day. The layout of the experiment was a factorial based on randomized complete plot design with three replicates. Germination did not occur at 240 mM NaCl, thus, four concentrations of NaCl (0, 60, 120 and 180 mM NaCl) were compared.

Except GSTI, a general ANOVA was applied to the data from the experiment, following checking for homogeneity. A ($\sqrt{x+1}$) transformation was required for root length and mean germination time. The means were ranked according to LSD test.

## RESULTS AND DISCUSSION

The results indicated that red clover populations were significantly ($P \leq 0.01$) different from each other in terms of germination percentage, MGT and root length (Table 2). As predicted, population 19 presented the maximum germination percentage as 67.9%. Population 22 presented the minimum germination percentage as 25.5%. Plant growth is ultimately reduced by salinity stress but plant species and cultivars differ in their sensitivity or tolerance to salt stress (Winter and Lauchli, 1982; Rogers et al., 1997, 2008; Carpici et al., 2009; Nichols et al., 2009). Germination percentage differed significantly ($P \leq 0.01$) among salt concentrations. It is evident that as NaCl concentration increased, the germination percentage decreased. Germination percentage was 40.3% lower at 180 mM NaCl than control (Figure 3a). Additionally, the seeds did not germinate at 240 mM NaCl concentration. Salt concentrations did not affect the proportion of seeds imbibing for any populations (data not shown) which is in agreement with Nichols et al. (2009) report. In addition, high absorption of Na and Cl ions during seed germination could be due to cell toxicity that finally inhibits or slows the rate of germination and thus decreases germination percentage (Taiz and Zeiger, 2002). $Na^+$ inhibits many enzymes (Zhu, 2001). Winter and Lauchli (1982) also reported that *T. pratense* showed a low survival potential at salt treatment of 100 mM NaCl or higher (150 and 200 mM NaCl).

MGT was greatly affected by population and salt concentrations ($P \leq 0.01$) and in investigated red clover populations, it varied considerably between 4.1 to 7.3 days (Table 2). MGT was delayed by increasing NaCl concentration (Figure 3b) when seeds were germinated in 180 mM NaCl concentration, MGT approximately extended to twice as compared to 0 mM NaCl. The result is in line with the findings of Okcu et al. (2005) in pea, Ates and Tekeli (2007) in Persian clover and Kaya (2009) in sunflower.

The interaction of salinity and population in terms of PI was significant ($P \leq 0.01$). The maximum PI was found in population 17 at 180 mM NaCl concentration, while the minimum PI was found in populations 4, 25 and 26 (Figure 4). This result reflected on the GSTI. GSTI of populations at 180 mM NaCl ranged from 33.9 to 1.9% (Figure 5).

Although population has not significant effect on shoot length, root length of red clover, populations showed

**Table 2.** Mean germination time (MGT), germination percentage, shoot and root length of red clover populations.

| Population | MGT | Germination percentage (%) | Shoot length (mm) | Root length (mm) |
|---|---|---|---|---|
| 1 | 5.9$^{ab}$ | 37.8$^{b-f}$ | 28.9 | 7.7$^{a-e}$ |
| 2 | 4.6$^{ab}$ | 42.8$^{a-f}$ | 29.1 | 8.0$^{a-e}$ |
| 3 | 7.1$^{ab}$ | 31.4$^{def}$ | 25.7 | 6.1$^{b-f}$ |
| 4 | 6.0$^{ab}$ | 41.0$^{a-f}$ | 27.9 | 5.8$^{def}$ |
| 5 | 6.3$^{ab}$ | 40.2$^{a-f}$ | 29.1 | 6.1$^{b-f}$ |
| 6 | 6.2$^{ab}$ | 55.5$^{a-e}$ | 33.2 | 8.4$^{a-e}$ |
| 7 | 4.8$^{ab}$ | 66.2$^{ab}$ | 30.5 | 10.6$^{ab}$ |
| 8 | 6.5$^{ab}$ | 53.8$^{a-f}$ | 32.5 | 6.9$^{a-f}$ |
| 9 | 5.4$^{ab}$ | 50.6$^{a-f}$ | 30.8 | 9.4$^{a-d}$ |
| 10 | 4.1$^{b}$ | 40.2$^{a-f}$ | 27.1 | 7.4$^{a-f}$ |
| 11 | 6.2$^{ab}$ | 45.8$^{a-f}$ | 29.2 | 9.4$^{abc}$ |
| 12 | 5.1$^{ab}$ | 41.9$^{a-f}$ | 26.2 | 7.5$^{a-f}$ |
| 13 | 5.7$^{ab}$ | 47.9$^{a-f}$ | 29.1 | 6.4$^{b-f}$ |
| 14 | 6.2$^{ab}$ | 57.8$^{a-d}$ | 31.8 | 9.2$^{a-d}$ |
| 15 | 6.7$^{ab}$ | 44.8$^{a-f}$ | 29.8 | 7.6$^{a-f}$ |
| 16 | 5.4$^{ab}$ | 32.5$^{c-f}$ | 26.9 | 7.2$^{a-f}$ |
| 17 | 5.1$^{ab}$ | 61.7$^{abc}$ | 28.5 | 7.7$^{a-e}$ |
| 18 | 5.2$^{ab}$ | 51.8$^{a-f}$ | 29.1 | 7.0$^{a-f}$ |
| 19 | 5.1$^{ab}$ | 67.9$^{a}$ | 32.4 | 11.0$^{a}$ |
| 20 | 6.7$^{ab}$ | 28.6$^{def}$ | 27.1 | 5.9$^{c-f}$ |
| 21 | 5.8$^{ab}$ | 54.5$^{a-f}$ | 30.3 | 8.2$^{a-f}$ |
| 22 | 5.0$^{ab}$ | 25.5$^{f}$ | 24.0 | 4.0$^{f}$ |
| 23 | 5.7$^{ab}$ | 42.6$^{a-f}$ | 28.9 | 6.4$^{b-f}$ |
| 24 | 6.8$^{ab}$ | 38.3$^{a-f}$ | 27.2 | 7.8$^{a-f}$ |
| 25 | 7.3$^{a}$ | 26.7$^{ef}$ | 28.0 | 4.3$^{f}$ |
| 26 | 6.2$^{ab}$ | 52.4$^{a-f}$ | 30.9 | 4.9$^{ef}$ |
| 27 | 5.6$^{ab}$ | 33.5$^{c-f}$ | 26.2 | 7.0$^{a-f}$ |
| 28 | 6.1$^{ab}$ | 28.4$^{def}$ | 26.0 | 6.0$^{b-f}$ |

Means within a column followed by the same lowercase letter are not significantly different ($P \leq 0.01$) by LSD.

Significant differences ($P \leq 0.01$) in saline conditions. Populations 19 and 25 had the highest and lowest root length, respectively (Table 2). Therefore, population 19 could absorb water and nutrients from soil better than the others under salinity stress. Shoot and root production decreased with increasing levels of salinity and the lowest value for both traits was obtained at 180 mM NaCl (Figure 3c and d). Roots elongated deep to 11.8 mm under control condition, while their growth was averaged to 2.6 mm under high salt stress conditions which means a 78% reduction in root growth. The interaction of salinity and population on root length was significant ($P \leq 0.01$). The concentration of 60 mM NaCl had a stimulating effect on the root growth of populations 14 and 23 because low NaCl concentration had nutrient effect rather than deleterious effect (Kaya, 2009). The longest root was determined in populations 17 and 19 at 180 mM NaCl, while the shortest root was measured in population 25 at 180 mM NaCl (Figure 6). Shoot and root lengths are the most important traits for selection under salt stress conditions, because roots are in direct contact with soil and absorb water (Jamil and Rha, 2004) and nutrient from soil, and shoot grows until the root can fully support it. Toxic effects of NaCl as well as unbalanced nutrient uptake by seedlings may be responsible for reduction in root and shoot growth under salinty stress. High salinity may inhibit root and shoot elongation due to retardation in water and essential mineral nutrients absorption from soil by plant (Neumann, 1995). Moreover, the initial reduction in shoot growth is probably due to hormonal signals generated by the roots (Munns, 2002). Similar observations have been reported in Persian clover (Ates and Tekeli, 2007), several clover species (Rogers et al., 2008) and wheat (Moud and Maghsoudi, 2008). Winter and Lauchli (1982) indicated that *T. pratense* translocates $Na^+$ and $Cl^-$ linearly by increasing salt treatment into stem and leaves. They also reported salt-induced changes of the $K^+$ and $Ca^+$ content.

These results suggest that salinity tolerance at germination is important for successful establishment of red clover on saline soils. Germination was severely influenced by salinity stress. All populations did not

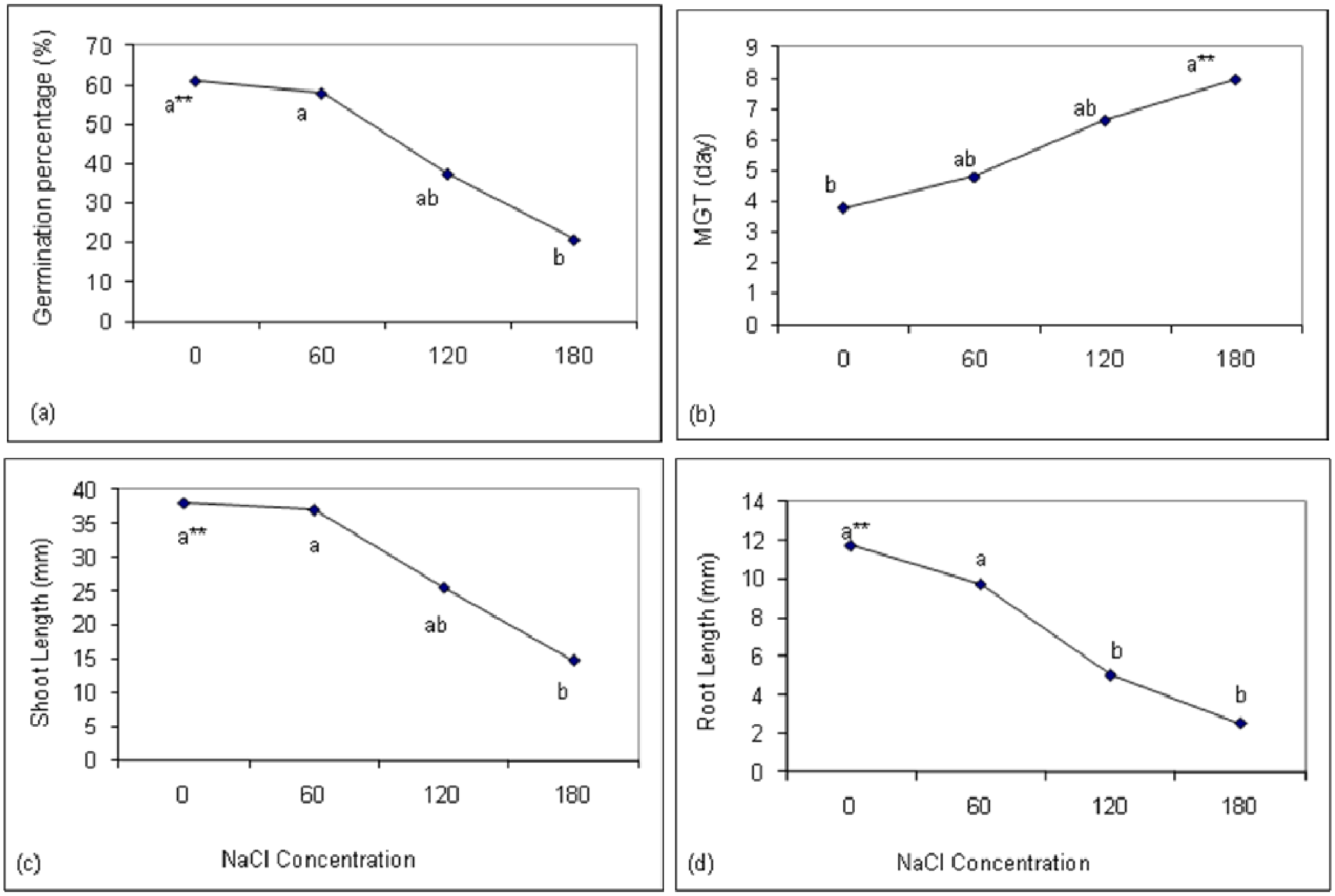


**Figure 3.** Effects of different salt concentration on red clover. Germination percentage (a), mean germination time (MGT) (b), shoot length (c) and root length (d). **: The same lowercase letter are not significantly different (P≤0.01) by LSD.

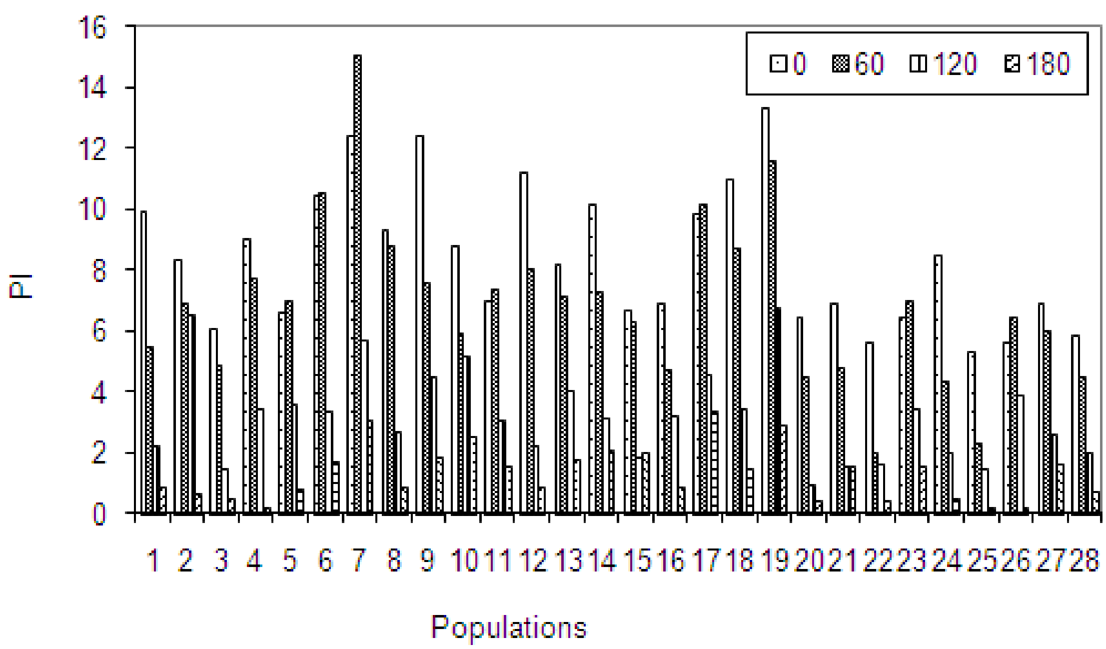


**Figure 4.** Promptness index (PI) of red clover populations grown under different NaCl concentrations. LSD $_{0.01(224\ df)}$ = 4.64.

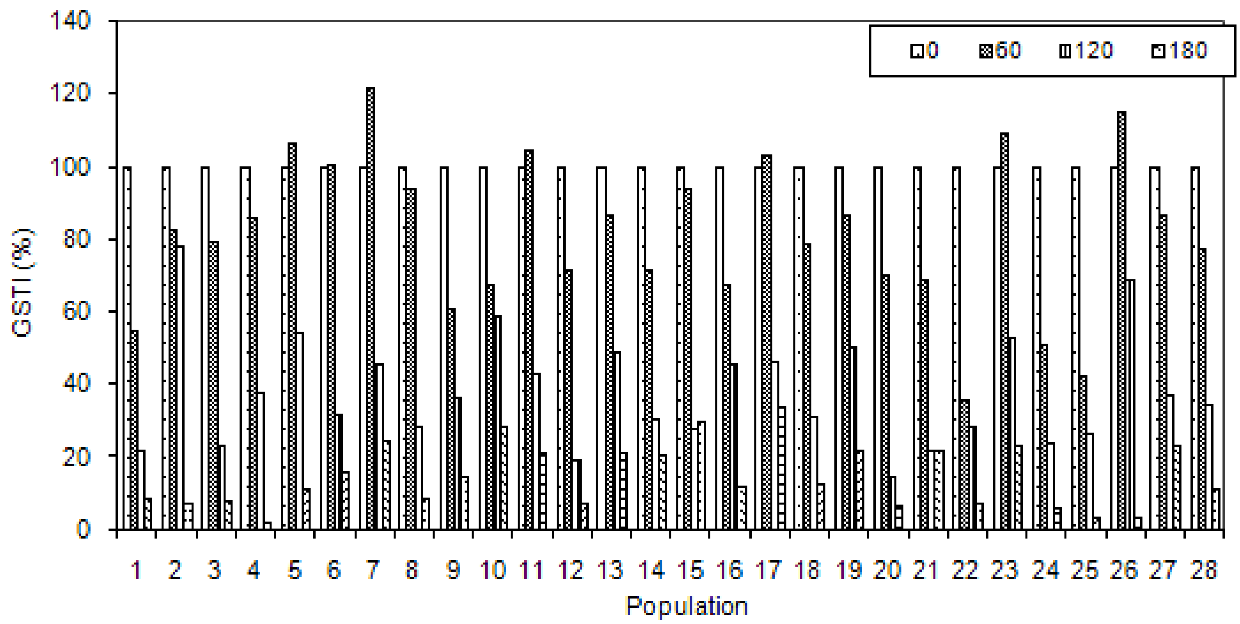


**Figure 5.** Germination stress tolerance index (GSTI) of red clover populations grown under different NaCl concentrations.

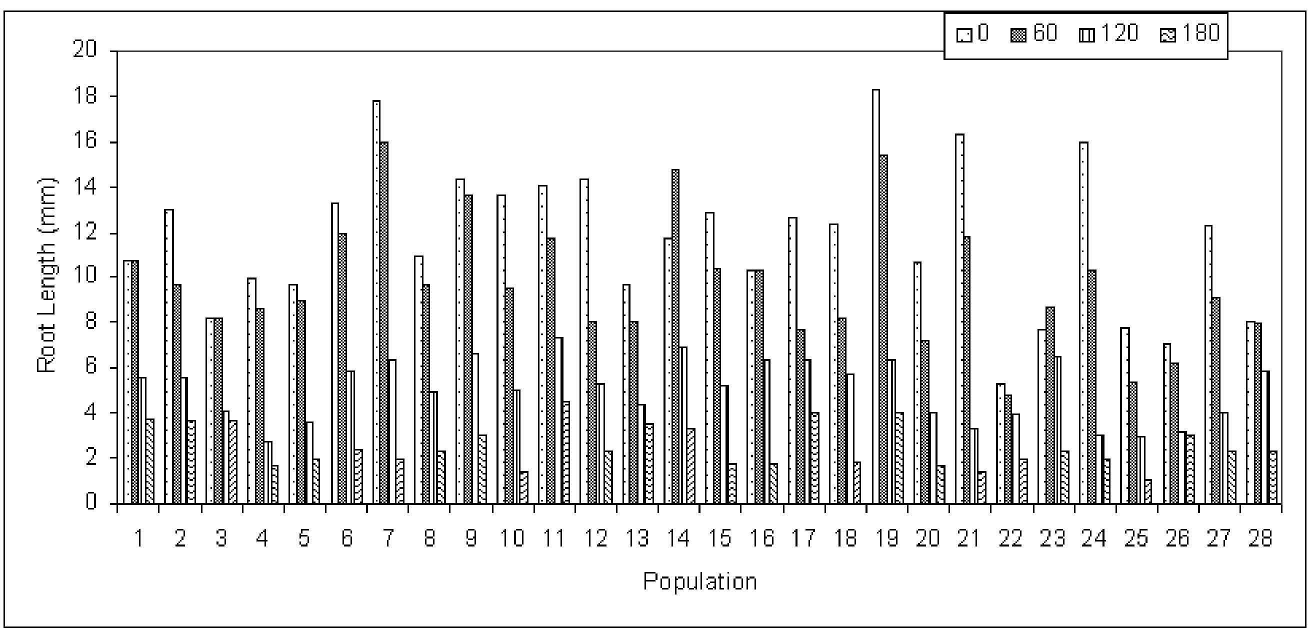


**Figure 6.** Mean root length of red clover populations grown under different NaCl concentrations. LSD $_{0.01(224\ df)}$ = 0.70

germinate at 240 mM NaCl concentration and all populations needed a considerable longer time to germinate under saline conditions. As predicted, populations 17 and 19 were best suited for germination under the range of salinity stress in this study. While population 19 produced the longest root at 180 mM NaCl concentration, population 17 had the highest GSTI at 180 mM NaCl concentration. At the end of the study, it seems that the plants, which naturally grow in the soils with high salt content, can be important genetic sources in the researches to be conducted for the purpose of releasing new cultivars tolerant to salinity.

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