A Comparison of Germination Responses on Italian Ryegrass (diploid vs tetraploid) Seeds to Interactive Effects of Salinity and Temperature

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Received: 28 January 2022
Accepted: 24 February 2022

Abstract

The aim of this research was to determine the responses of diploid and tetraploid Italian ryegrass cultivars to interactive effects of salinity and temperature during the germination period. The seeds of diploid (cv Efe) (2×) and tetraploid (cv Baquena) (4×) Italian ryegrass were used as materials. All data obtained in the study were subjected to analysis of variance in accordance with the completely randomized design as triplicate using a three-way factorial ANOVA. Therefore, using multivariate analysis at different temperatures, provided information about the relative importance of each trait. The seeds were germinated at three different temperatures (15ºC, 25ºC, 35ºC) with 12-h photoperiod to screen and evaluate the effects of three different sodium chloride concentrations (NaCl) (0, 75, 150 mM) on the seed germination process of the Italian ryegrass cultivars. Germination energy (%) and germination percentage (%), root length (cm), shoot length (cm), fresh weight (g), dry weight (g), promptness index, and simple vigour index were measured to define germination responses. At different temperatures and salinity conditions; tetraploid Italian ryegrass had a better performance as compared to diploid Italian ryegrass. 25ºC (T_{opt}) and 75 mM (S_{2}) are the optimal temperature and salt level for both cultivars during the germination process.

Keywords: Italian ryegrass, temperature, salinity, multivariate analysis, plant response

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germination phase is highly sensitive to salinity [9-11]. Therefore, salinity may interact with temperature, and this interaction can alter the seed sensitivity to stress conditions [12]. Though increasing salinity levels decrease germination, the lethal effect of salinity is less severe at optimum germination temperature [13]. Plant responses to temperature are critical because they affect the duration of the growth cycle, expansion of the roots, and the overall fit between crop development and resource availability [14]. Also, discerning the effects of temperature on the seed germination stage can be very useful to evaluate germination responses in plants [15].

Italian ryegrass (Lolium multiflorum Lam.) is considered a very useful grass species for producing forages and lawn design. It mainly grows in temperate regions and is a cold-season forage species. It is used for grazing or production of roughage, such as hay, haylage [16, 17]. Due to its excellent quality, high yield, good palatability, and rich nutritional value, it is widely cultivated for the production of hay and silage worldwide [18] including in Turkey. The cultivation area of Italian ryegrass increased 52 times between 2014 (4832da) to 2020 (253297da) in Turkey [19]. As natural, Italian ryegrass is a diploid plant (2n = 2× = 14) [20]. Tetraploid cultivars began to use in the 1970s [21], owing to the development of agricultural breeding methods. Currently, there are numerous cultivars with different ploidy levels; diploid (2×) and tetraploid (4×). There are some differences between diploid and tetraploid cultivars of Italian ryegrass. These are seed size, leaf size, and length, plant height, seed head length when compared with its diploid species [22]. Tetraploid cultivars of Lolium species also have better tolerance to abiotic stress conditions [23].

Despite the research currently available evaluating diploid and tetraploid cultivars, limited studies are evaluating the comparison of germination responses of diploid and tetraploid Italian ryegrass cultivars under interactive effects of salinity and temperatures. The purpose of this study was to investigate the interactive effect of salinity and temperature on diploid and tetraploid Italian ryegrass cultivars by measuring germination parameters. Therefore, using multivariate analysis at different temperatures, provided information about the relative importance of each trait.

**Material and Method**

**Material**

The seeds of diploid (cv Efe) (2×) and tetraploid (cv Baquena) (4×) Italian ryegrass were used as materials. The seeds were obtained from the local market of Ankara, Turkey. The seeds were dry-stored in cloth bags at room temperature for further use. Italian ryegrass cultivars were labeled as G₃ (diploid), G₄ (tetraploid).

**Methods**

**Salt Stress and Temperature Treatments**

The seeds were germinated at three different temperatures with 12-h photoperiod (cool white fluorescent lamps, 200 µ mol m⁻² s⁻¹, 400-700 nm) to screen and evaluate the effects of sodium chloride (NaCl) (Merck, Germany) and temperature on the seed germination process of the Italian ryegrass cultivars. 0, 75, 150 mM NaCl are concentrations, which are applied to the seed for evaluation. These salt levels are divided into three different temperatures. These temperatures are 15°C, 25°C, 35°C. Salt and temperature-treated seed groups were labeled as S₀, S₁, S₂, and Tₐₐ₉, Tₐ₉, Tₐ₉ in the same order.

**Germination Experiments**

The seeds were first sterilized with 1% sodium hypochlorite solution for five minutes, then rinsed with distilled water before treating with respective salt concentrations. Seeds were germinated between filter papers (Anchor Corp, 5 × 10 cm) placed in Petri dishes as described by International Seed Testing Association [24]. These filter papers were double-layered in Petri dishes (up and down of the seeds) to avoid moisture loss. The seeds were considered germinated with the emergence of the radicle (≥2 mm). The germination experiments were applied as triplicate with 25 seeds per treatment.

Germination energy (GE) and germination percentage (GP) were recorded on 3rd and 8th day as described by International Seed Testing Association [24]. Root length (cm), shoot length (cm), fresh weight (g), and dry weight (g) were measured on the 8th day by randomly selecting 15 seeds per replication. To calculate the dry weight, the fresh shoots were dried for 72 hours at 60°C. Promptness index and simple vigour index were calculated by adopting the following formula; PI = number of day 1 (1.00) + number of day 3 (0.75) + number of day 5 (0.50) + number of day 7 (0.25) and SVI = GP (%) × Dry weight of seedling.

**Statistical Analysis**

All data obtained in the study were subjected to analysis of variance in accordance with the completely randomized design as triplicate using JMP v 13.0 [25], using a three-way factorial ANOVA (p≤0.05). The Italian ryegrass cultivars (G) were considered as the main factor and the salt levels (S), temperature treatments (T) were considered as sub-factors during the analysis of variance. Germination data were arcsine transformed before the analysis of variance. Hence, germination responses were analyzed as multivariate by using procedures of principal component analysis (PCA) and cluster analysis (CA) via computer software “JMP” v 13.0 [25]. PCA was accomplished using
a correlation matrix in order to reveal the relationship among quantitative traits that are correlated with each other by converting them into uncorrelated traits called PCs [26]. PCA provided information about the relative importance of each trait for screening the diploid and tetraploid Italian ryegrass cultivars in this study.

Results and Discussion

Germinaton Percentage

In accordance with three-way ANOVA; individually G (74.48**, T (71.45**), S (96.49**), and interaction of G × T (6.67**), G × S (63.27**), T × S (8.41**) were found to be statistically different (p≤0.01), except for T (2.54*) interaction (p≤0.05). If compared to genotypes and temperatures; G had more mean germination percentage (81.04%) than G (47.70%). For diploid and tetraploid cultivars, 35ºC (T max) affects germination percentage negatively. Maximum germination percentage of G (91.56%) and G (64.44%) was obtained at 15°C (T min), 25°C (T opt), in the same order. It can be demonstrated that T opt provided the most available germination conditions for both Italian ryegrass cultivars (Table 1). Similar to genotypes × temperature, on genotype × salinity interaction, G (81.04%) showed a better mean germination percentage than G (47.70%) cultivar. Increasing salinity level decreased germination percentage for both cultivars. Although S had a good germination percentage for both cultivars that G is 85.33% and GT are 84.00%, had the maximum germination percentage (89.33%) for GT cultivar (Table 2).

Germination Energy

In accordance with three-way ANOVA; individually G (37.45**), T (324.60**), S (36.20**), and interaction of G × T (41.87**), G × S (24.08**), T × S (20.49**) were found to be statistically different (p≤0.01). If compared to genotypes and temperatures; G had more mean germination energy (30.52%) than G (13.63%). There was no germination at Tmax on the 3rd day. For diploid and tetraploid cultivars, Tmax affects germination energy at a lethal level. Maximum germination energy of G (64.44%) and G (30.22%) was obtained at T opt. It can be demonstrated that T opt provided the most available germination conditions for both Italian ryegrass cultivars (Table 1). Similar to genotypes × temperature, on genotype × salinity interaction, G (81.04%) showed a better mean germination energy than G (13.63%) cultivar. Increasing salinity level decreased germination energy for G cultivar. But G cultivar’s best germination energy was obtained at 75mM salt level (S 2) (24.00%). It was noted that maximum germination energy was obtained in G (42.22%) at the control group salinity level. The minimum germination energy for both cultivars was 150 mM salinity level. (Table 2).

Table 1. Mean germination responses of genotype × temperature interaction.

|          | T_min | T_opt | T_max | AV  |    |          | T_min | T_opt | T_max | AV  |
|----------|-------|-------|-------|-----|----|----------|-------|-------|-------|-----|
| Germination percentage (%) |       |       |       |     |    |          |       |       |       |     |
| G       | 47.11B| 64.89AB| 31.11B| 47.70|    | 10.67C   | 30.22B| 0.00D  | 13.63 |
| GT      | 91.56A| 90.22A| 61.33B| 81.04|    | 27.11B   | 64.44A| 0.00D  | 30.52 |
| AV      | 69.33 | 77.56 | 46.22 | 64.37|    | 18.89    | 47.33 | 0.00D  | 22.07 |
| Root length (cm) |       |       |       |     |    |          |       |       |       |     |
| G       | 4.99  | 6.61  | 2.86  | 4.82 |    | 3.71     | 6.71  | 2.54   | 4.32A |
| GT      | 5.15  | 6.42  | 3.52  | 5.03 |    | 3.69     | 7.53  | 3.42   | 4.88A |
| AV      | 5.07B | 6.52A | 3.19C | 4.93 |    | 3.70B    | 7.12A | 2.98B  | 4.60  |
| Fresh weight (g) |       |       |       |     |    |          |       |       |       |     |
| G       | 0.266B| 0.155C| 0.100D| 0.174|    | 0.021A   | 0.013B| 0.013B | 0.016 |
| GT      | 0.349A| 0.147C| 0.101D| 0.199|    | 0.013B   | 0.021A| 0.014B | 0.016 |
| AV      | 0.307 | 0.151 | 0.100 | 0.186|    | 0.017    | 0.017 | 0.013  | 0.016 |
| Simple vigour index |       |       |       |     |    |          |       |       |       |     |
| G       | 1.44  | 1.07  | 0.24  | 0.92 |    | 15.33D   | 21.44C| 4.81F  | 13.86 |
| GT      | 1.57  | 3.07  | 0.39  | 1.68 |    | 29.58B   | 34.00A| 8.28E  | 23.96 |
| AV      | 1.50AB| 2.07A | 0.32B | 1.23 |    | 22.46    | 27.72 | 6.54   | 18.91 |

G: Diploid cultivar, GT: Tetraploid cultivar, T_min: 15°C, T_opt: 25°C, T_max: 35°C.
In accordance with three-way ANOVA; individually G (10.36**) and T (99.57**) were found to be statistically different (p≤0.05), while other variables were not. If compared to genotypes and temperatures; G_1 had more mean root length (5.03 cm) than G_0 (4.82 cm). For diploid and tetraploid cultivars, increasing temperature affects root length negatively. Maximum root length of G_0 (6.61 cm) and G_1 (6.42 cm) was noted at T_min. It can be determined that T_opt is the most available temperature for developing root activity (Table 1). Similar to genotypes × temperature, on genotype × salinity interaction, G_1 (5.03 cm) showed a better mean root length than G_0 (4.32 cm) cultivar. Increasing salinity level decreased root length for both cultivars. Maximum root length of G_0 (5.39 cm) and G_1 (6.39 cm) was determined at the control group, while the minimum root length of G_0 (3.30 cm) and G_1 (3.90 cm) was noted at 150 mM salinity level (Table 2). The negative effect of salinity on root length is higher as compared to the negative effect of temperature.

Shoot Length

In accordance with three-way ANOVA; individually G (20.22**) and T (372.46**), S (207.67**), and interaction of G × T (19.54**), G × S (29.44**), T × S (361.69**), G × T × S (29.55**) were found to be statistically different (p≤0.01). If compared to genotypes and temperatures; G_1 had more mean root length (4.88 cm) than G_0 (4.32 cm). Maximum root length of G_0 (6.71 cm) and G_1 (7.53 cm) obtained at T_opt. It can be noted that T_opt is the most available temperature for developing shoot activity. Minimum shoot length was obtained in T_max. The minimum shoot length of G_0 and G_1 was 2.54 cm and 3.42 cm, in the same vein (Table 1). Similar to genotypes × temperature, on genotype × salinity interaction, G_1 (4.88 cm) showed a better mean root length than G_0 (4.32 cm) cultivar. Increasing salinity level decreased shoot length for both cultivars. Maximum shoot length of G_0 (0.266 g) and G_1 (0.349 g) was obtained at T_min. Minimum shoot length was obtained in T_max. Hence, the minimum shoot length of G_0 and G_1 was 0.100 g and 0.101 g, in the same order (Table 1). Increasing

### Table 2. Mean germination responses of genotype × salinity interaction

|       | S_1     | S_2     | S_3     | AV     |       | S_1     | S_2     | S_3     | AV     |
|-------|---------|---------|---------|--------|-------|---------|---------|---------|--------|
| Germination percentage (%) |         |         |         |        |        |         |         |         |        |
| G_0   | 32.89C  | 85.33A  | 24.89C  | 47.70  | 11.56D| 24.00BC| 5.33D   | 13.63   |
| G_1   | 89.33A  | 84.00A  | 69.78B  | 81.04  | 42.22A| 28.89B  | 20.44C  | 30.52   |
| AV    | 61.11   | 84.67   | 47.33   | 64.37  | 26.89 | 26.44   | 12.89   | 22.07   |
| Shoot length (cm) |         |         |         |        |       |         |         |         |        |
| G_0   | 5.61    | 5.57    | 3.30    | 4.82   | 5.39  | 5.14    | 2.44    | 4.32A   |
| G_1   | 5.97    | 5.23    | 3.90    | 5.03   | 6.39  | 5.22    | 3.04    | 4.88A   |
| AV    | 5.79A   | 5.40A   | 3.60B   | 4.93   | 5.89A | 5.18A   | 2.74B   | 4.60    |
| Fresh weight (g) |         |         |         |        |       |         |         |         |        |
| G_0   | 0.231AB | 0.166B  | 0.124B  | 0.174  | 0.015B| 0.016AB | 0.016AB | 0.016   |
| G_1   | 0.325A  | 0.143B  | 0.129B  | 0.199  | 0.022A| 0.014B  | 0.013B  | 0.016   |
| AV    | 0.278   | 0.154   | 0.126   | 0.186  | 0.017 | 0.017   | 0.013   | 0.016   |
| Dry weight (g) |         |         |         |        |       |         |         |         |        |
| G_0   | 0.68    | 1.56    | 0.49    | 0.92   | 10.58C| 23.25AB | 7.75C   | 13.86   |
| G_1   | 2.74    | 1.24    | 1.06    | 1.68   | 26.39A| 24.01AB | 21.47B  | 23.96   |
| AV    | 1.71    | 1.41    | 0.78    | 1.30   | 18.49 | 23.63   | 14.61   | 18.91   |

G_0: Diploid cultivar, G_1: Tetraploid cultivar, S_0: distilled water, S_1: 75 mM, S_2: 150 mM.
temperature decreased fresh weight for both cultivars. Similar to genotypes × temperature, on genotype × salinity interaction, G_D (0.199 g) showed a better mean root length than G_D (0.174 g) cultivar. The heaviest fresh weight was obtained in G_D cultivar (0.325 g) in distilled water, yet the weakest fresh weight was obtained in G_D cultivar (0.124 g) at 150 mM salinity level. The negative effect of salinity on fresh weight is higher as compared to the negative effect of temperature (Table 2).

Dry Weight

In accordance with three-way ANOVA; individually G (0.01**) and T (0.65*), S (0.57*), interaction of G × T (1.89*), G × S (0.82*), T × S (1.39*) were found to be statistically different (p≤0.01) and (p≤0.05), in the same vein. If compared to genotypes × temperatures and genotypes × salinity; G_D and G_T had equal dry weight (0.016 g). The maximum dry weight of G_D and G_T was almost equal (0.021 g) at T_min and T_opt, respectively. Increasing temperature decreased dry weight for both cultivars. (Table 1). The heaviest dry weight was obtained in G_T cultivar (0.022 g) in the control group, while the weakest dry weight was obtained in G_D cultivar (0.013 g) 150 mM salinity level.

Promptness Index

In accordance with three-way ANOVA; individually G (639.17**), T (335.18**), S (56.35**), and interaction of G × T (23.14**), G × S (45.85**), T × S (8.35**), G × T × S (9.84**) were found to be statistically different (p≤0.01). If compared to genotypes and temperatures; G_T had a more mean promptness index (23.96) than G_D (13.86). For diploid and tetraploid cultivars, T_max affect germination percentage negatively. Maximum promptness index of G_D (34.00) and G_T (21.44) was obtained at T_max, while minimum promptness index of G_T (8.28) and GD (4.81) was obtained at T_min (Table 1). Similar to genotypes × temperature, on genotype × salinity interaction, G_T (23.96) showed a better mean promptness index than G_D (13.86%) cultivar. Increasing salinity level decreased promptness index of G_T cultivar. The highest promptness index of G_T (23.25) was observed at 75 mM salt level, while lowest promptness index of G_D (7.75) was obtained at 150 mM salt level. The negative effect of temperature on promptness index is higher as compared to the negative effect of salinity (Table 2).

Simple Vigour Index

In accordance with three-way ANOVA; just T (4.52*) was found to be statistically different (p≤0.05), while other variables were not. If compared to genotypes and temperatures; G_T had a more simple vigour index (1.68) than G_D (0.92). Maximum simple vigour index of G_T (3.07) and G_D (1.44) obtained at T_opt and T_min in the same vein. For both cultivars, a minimum simple vigour index was determined at T_min (Table 1). On genotype × salinity interaction, G_T (1.68) showed a better simple vigour index than G_D (0.92) cultivar. The highest simple vigour index was obtained in G_T cultivar (2.74) in the control group, while most lowest simple vigour index was obtained in G_D cultivar (0.49) at the 150 mM salinity level. Especially, increasing salinity level decreased simple vigour index for G_T cultivar (Table 2).

Multivariate Analysis of Italian Ryegrass Cultivars

The distinction examined with PCA showed that the first two principal components contributed 72.53% of the total variance among the eight germination parameters under 15°C (T_opt) conditions. At p≤0.05, GE (0.392), RL (0.378), and SL (0.361) were respectively the main contributors to the first principle component, which made up 49.75% of the total variation. Also, the main contributors of the second principal component, which is responsible for 22.78% of the total variation, were GP (0.462), PI (0.448), and FW (0.401) respectively (Fig. 1a).

The difference of diploid and tetraploid grass with PCA showed that the first two principal components contributed 83.06% of the total variance among the eight germination parameters under 25°C (T_opt) conditions. At p≤0.05, SL (0.399), PI (0.389), and GE (0.374) were respectively the main contributors to the first principle component, which made up 63.03% of the total variation. Besides, the main contributors of the second principal component, which is responsible for 20.03% of the total variation, were FW (0.604) and RL (0.582) (Fig. 1b). The variation studied with PCA showed that the first two principal components contributed 83.79% of the total variance among the eight germination parameters under T_min conditions. At p≤0.05, RL (0.470), GP (0.466), and SL (0.443) were respectively the main contributors to the first principle component, which made up 50.55% of the total variation. Furthermore, the main contributors of the second principal component, which is responsible for 33.24% of the total variation, were DW (0.548), SVI (0.393) and PI (0.377) respectively (Fig. 1c).

Discussion

The results showed that tetraploid Italian ryegrass (G_T) was greater germination percentage and germination energy under different salinity levels and temperatures as compared to diploid Italian ryegrass (G_D). It was found that 25°C (T_opt) was the optimal temperature for better seed germination, especially in diploid Italian ryegrass (G_D). The optimal germination percentage and germination energy at 25°C showed that grass species prefer relatively higher temperatures for seed germination. [13, 27, 28] studies about P. turgidum, Leymus chinesis and Lolium multiflorum
noted similar trends with this study. Previous studies have shown there is usually a negative correlation between salt tolerance and germination percentage in *Lolium* species [29, 30, 31]. Germination energy, which is called the first few days of the germination stage, in higher temperature affects more negatively than salt levels in this study. In the light of the results, higher temperature and salt levels delay the germination period. Excess salt ion with the help of temperature does not allow to absorption of water by germinating seeds [32]. Moreover, the inhibitory effects of salt ions with increasing temperature on seed germination might be due to its direct effect on the growth of the embryo [13].

Cultivating the plants substantially depends on successful root and shoot development activity under stressful conditions. After successful seed germination, plants tend to reach a powerful seedling stage. It is widely acclaimed that osmotic and ionic effects are the domination factors that inhibit these seed germination responses under salinity stress [33]. The detrimental effects of salinity generally reduced at optimal temperature [34]. Hence, interactive effects of salinity
and temperature linearly decreased root and shoot length in *Lolium* species, depending on the intensity [35-37]. Shoot length was more severely affected than the root length in this study. Therefore, mean root length and shoot length were better in tetraploid Italian ryegrass under different temperatures and salinity levels. The capability of earlier germinating of tetraploid cultivars than diploid cultivars can allow them to form the higher root and shoot lengths. In the progress of 25°C (T<sub>opt</sub>) to 30°C (T<sub>max</sub>) and 75 mM (S<sub>2</sub>) to 150 mM (S<sub>3</sub>) root length and shoot length was reduced. It is revealed that 25°C (T<sub>opt</sub>) and 75 mM (S<sub>2</sub>) was the optimal temperature and salinity level, in the same order.

Increasing temperatures and salinity levels declined fresh weight in both cultivars. Therefore, tetraploid cultivar had more fresh weight than diploid cultivar. [36], [38] and [39] mentioned that increasing salinity level affects fresh and dry weight negatively. Also, interactive effects of temperature and salinity accelerates this process. More than 50% fresh weight was loss in the progress of 15°C (T<sub>min</sub>) to 25°C (T<sub>opt</sub>) and distilled water to 75 mM (S<sub>2</sub>), respectively. Dry weight of both cultivars were very close to each other.

Promptness index (PI) was drastically declined in the progress of 25°C (T<sub>opt</sub>) to 35°C (T<sub>max</sub>) and 75 mM (S<sub>2</sub>) to 150 mM (S<sub>3</sub>). Concordantly, 25°C (T<sub>opt</sub>) and 75 mM (S<sub>2</sub>) were the optimal temperature and salinity level for promptness index, especially in diploid Italian ryegrass. On the other hand, tetraploid Italian ryegrass had a more mean PI than the diploid one. Salinity levels affected diploid and tetraploid Italian ryegrass more negatively than different temperatures. With similar trends in this study; higher temperature [40] and higher salinity [41] were sharply declined by PI. Tetraploid Italian ryegrass had more simple vigour index (SVI) than the diploid. In the progress of 25°C (T<sub>opt</sub>) to 35°C (T<sub>max</sub>) more than 50% SVI reduced for both ryegrass cultivars. [42] stated that saline stress conditions caused by reduced osmotic potential affected ryegrass seed vigor.

A different strategy such as a principal component analysis is required to classify better genotypes for both non-stressed and stressed conditions [43]. Principal component analysis (PCA) supplies significant data by converting the obtained data into its basic components and producing a new data series. PCA can be perfectly used to analyze big data from experiments to assess genotypes [44, 45]. The tolerance levels depending on the germination responses of diploid and tetraploid cultivars under interactive effect of salt stresses and different temperature were clearly determined by PCA graphs. It is seen in the PCA graph that the first salt level (control group) under T<sub>min</sub> improves the germination performance of diploid grass (Fig. 1). In PCA graphs, it can be seen from the placement of the cultivars in the graph that the initial salt level under T<sub>max</sub> had a positive effect on the germination performance of diploid and tetraploid grasses compared to the control group (distilled water) (Fig. 1c).

Several studies have shown that germination responses are significantly affected by salinity and temperature [28, 46-49]. Polyploid plants can exhibit higher adaptability, increased vigour and resistance to unfavourable environmental factors compared to their diploid ones [50, 51]. Tetraploid Italian ryegrass (G<sub>t</sub>) has been found more tolerant to salinity and temperature compared to diploid Italian ryegrass (G<sub>d</sub>) in this study. Tetraploid seeds of Italian ryegrass were bigger and heavier than their diploid seeds. Bigger seeds at a high ploidy level have more advantages than smaller seeds at a lower ploidy level [48]. For that matter; tetraploid seeds have more carbon reserves and can generate a lower osmotic potential. Due to these features, in most cases, tetraploid Italian ryegrass had higher germination responses in this study. Tetraploid Italian ryegrass appears to have a better response during the germination stage in interactive effects of salinity and temperature.

**Conclusions**

At different temperatures and salinity conditions; tetraploid Italian ryegrass had a better performance as compared to diploid Italian ryegrass. In the progress of 25°C (T<sub>opt</sub>) to 35°C (T<sub>max</sub>) and 75 mM (S<sub>2</sub>) to 150 mM (S<sub>3</sub>), germination responses of Italian ryegrass cultivars were drastically reduced. Due to this maximum temperature’s negative effects, 25°C (T<sub>opt</sub>) and 75 mM (S<sub>2</sub>) is the optimal temperature and salt level for both cultivars in the germination process.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**

1. SADDHE A.A., MALVANKAR M.R., KARLE S.B., KUMAR K. Reactive nitrogen species: Paradigms of cellular signaling and regulation of salt stress in plants. Environmental and Experimental Botany 161, 86, 2019.

2. ZHU J.K. Salt and Drought Stress Signal Transduction in Plants. Annual Review of Plant Biology 53, 247, 2002.

3. PITMAN M.G., LAUCHLI A. Global Impact of Salinity and Agricultural Ecosystems. Salinity: Environment-Plants-Molecules Published by Kluwer Academic Publishers, Dordrecht. The Netherlands. 522, 2002.

4. TUTEJA N. Mechanisms of High Salinity Tolerance in Plants. Methods in Enzymology 428, 419, 2007.

5. CRAMER G.R., URANO K., DELROT S., PEZZOTTI M., SHINOZAKI K. Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biology 11 (1), 1, 2011.

6. FENG Q., SONG S., YANG Y., AMEE M., CHEN L., XIE Y. Comparative physiological and metabolic analyzes of two Italian ryegrass (*Lolium multiflorum*) cultivars with
contrasting salinity tolerance. Physiologia Plantarum 172, 1688, 2021.
7. AZZA M., FATMA EL-QUENSI E.M., FARAHAT M.M. Responses of ornamental plants and woody trees to salinity. World J. Agriculture Science 3, 386, 2007.
8. FEIZI M., AGHAKHANI A., MOSTAFAZADEH-FRAD B., HEIDARPOUR M. Salt tolerance of wheat according to soil and drainage water salinity. Pakistan Journal of Biological Science 10, 2824, 2007.
9. BENLIOGLU B., OZKAN U. Determination of responses of some barley cultivars (Hordeum vulgare L.) to salt stresses in different doses at the germination period. J Field Crops Cent Res Inst 24 (2), 109, 2015.
10. CAMKACI S., DALLAR A. Effects of Different Temperatures and Salt Concentrations on the Germination Of Some Corn Silage Varieties. Journal of Tekirdag Agriculture Faculty 16 (2), 121, 2019.
11. BENLIOGLU B., OZKAN U. Germination and Early Growth Performances of Mung Bean (Vigna radiata (L.) Wilczek) Genotypes Under Salinity Stress. Journal of Tekirdag Agriculture Faculty 17 (3), 318, 2020.
12. BHATT A., SANTO A. Germination and recovery of heteromorphic seeds of Atriplex canescens under increasing salinity. Plant Ecology 217, 1, 2016.
13. AL-KHATEEB S.A. Effect of salinity and temperature ongermination, growth and ion relations of Panicum turgidum Forsk. Bioresource Technology 97, 292, 2006.
14. ZAKA S., AHMED L.Q., ESCOBAR-GUTIÉRREZ A.J., GASTAL F., JULIER B., LOUARN G. How variable are non-linear developmental responses to temperature in two perennial forage species?. Agricultural and Forest Meteorology 232, 433, 2017.
15. TLIG T., GORAI M., NEFFATI M. Germination responses of Diploptaxis harra to temperature and salinity. Flora 203, 421, 2008.
16. JOCHIMS F., PIRES C.C., GIBB M.J., AZEVEDO E.B., DAVID D.B., SOARES E.M. Productivity and grazing behavior of ewes with single or twin lambs raised in mixed Italian Ryegrass/White Clover pasture in Southern Brazil. American Journal of Plant Science 5, 963, 2014.
17. RODRIGUES A.M., VELOSO A., VAZ E., ALMEIDA J.F. Production and nutritional composition of two annual ryegrass cultivars (diploid and tetraploid). Current Investigations in Agriculture and Current Research 7 (2), 914, 2019.
18. GAYER T.O., KASPER N.F., TADIELO L.E., KROLOW R.H., AZEVEDO E.B., OAIGEN R.P., CASTAGNARA D.D. Different dry matters content used for the stratification in a range of temperatures, light and low water potentials under salt and drought stresses. Crop Pasture Science 68, 188, 2017.
19. LIN J., HUA X., PENG X., DONG B., YAN X. Germination responses of ryegrass (annual vs. perennial) seed to the interactive effects of temperature and salt-alkalai stress. Frontiers in Plant Science 9, 1458, 2018.
20. ALSHALLASH K.S. Seed germination of rigid ryegrass (Lolium rigidum) and sterile oat (Avena sterilis) under water salinity conditions at constant or alternating temperatures. Egyptian Journal of Botany 58 (3), 539, 2018.
21. RAHMAN A., ASADUZZAMAN M. Statistical modelling of seed germination and seedlings root response of annual ryegrass (Lolium rigidum) to different stress. Agricultural Research 8 (2), 262, 2019.
22. THOMPSON M., MAHAJAN G., CHAUHAN B.S. Seed germination ecology of southeastern Australian rigid ryegrass (Lolium rigidum) populations. Weed Science 1-7, 2021.
23. MANGWANE M., MADAKADZE I.C., NHERERA-CHOKUDA F.V., DUBE S., MANDELA, M. Germination performance of different forage grass species at different salinity (NaCl) concentrations. African Journal of Range & Forage Science 38, 98, 2021.
24. DEBEZ A., HAMED K.B., GRIGNON C., ABDELLY C. Salinity effects on germination, growth, and seed production of the halophyte cakile maritima. Plant Soil 262, 179, 2004.
25. KHAN M.A., GUL B., WEBER D.J. Seed germination in relation to salinity and temperature inSarcobatus vermiculatus. Biologia Plantarum 45, 133, 2002.
26. MARCUM KB., PESSARAKLI M. Relative Salinity Tolerance of 35 Lolium spp. Cultivars for Urban Landscape and Forage Use. In Developments in Soil Salinity Assessment and Reclamation (pp. 397-403). Springer, Dordrecht, 2013.
27. BORAWSKA-JARMULOWICZ B., MASTALERCZUK G., GOZDOWSKI D., MALUSZYŃSKA E., SZYDŁOWSKA A. The Sensitivity Of Lolium Perenne And Poa Pratensis To Salinity And Drought During The Seed Germination And Under Different Photoperiod Conditions. Zemdirbyste-Agriculture 104 (1), 2017.
28. GUO T., TIAN C., CHEN C., DUAN Z., ZHU Q., SUN L.Z. Growth and carbohydrate dynamic of perennial ryegrass seedlings during PEG-simulated drought and subsequent recovery. Plant Physiology and Biochemistry 154, 85, 2020.
29. JOSHI A.J., MALS B.S., HINGLAJIA H. Salt tolerance at germination and early growth of two forage grasses growing in marshy habitats. Environmental and Experimental Botany 54 (3), 267, 2005.
30. KIM S., RAYBURN A.L., VOIGT T., PARRISH A., LEE D.K. Salinity effects on germination and plant growth
of prairie cordgrass and switchgrass. BioEnergy Research 5 (1), 225, 2012.

40. KAZIM A.M., ABID A., SADDIA G. Response of rice (Oryza sativa L.) under elevated temperature at early growth stage: physiological markers. Russian Journal of Agricultural and Socio-Economic Sciences 20 (8), 11, 2013.

41. ALI A., IBRAHIM M., ZHOU G., NIMIR A., JIAO X., ZHU G., LU H. Ameliorative effects of jasmonic acid and humic acid on antioxidant enzymes and salt tolerance of forage sorghum under salinity conditions. Agronomy Journal 111 (6), 3099, 2019.

42. RADKE A.K., EBERHARDT P.E.R., MARTINS A.B.N., DA MOTTA XAVIER F., GONCALVES V.P., MENEGUZZO M.R.R., VILLELA F.A. Ryegrass (Lolium multiflorum Lam.) seed germination and vigor under saline stress. Australian Journal of Crop Science 12 (6), 985, 2018.

43. KACEM N.S., DELPORTE F., MUHOVSKI Y., DJEKOUN A., WATILLON B. In vitro screening of durum wheat against water-stress mediated through polyethylene glycol. Journal of Genetic Engineering and Biotechnology 15 (1), 239, 2017.

44. CHIKHA M., HESSINI K., RIM NEFISSI O., GHORBEL A., ZOGHLAMI N. Identification of barley landrace genotypes with contrasting salinity tolerance at vegetative growth stage. Plant Biotechnology 33, 287, 2016.

45. SIVAKUMAR J., PRASHANTH J.E.P., RAJESH N., REDDY S.M., PINJARI O.B. Principal component analysis approach for comprehensive screening of salt stress-tolerant tomato germplasm at the seedling stage. J Bioscience 45 (1), 1, 2020.

46. MATTHEWS S., KHAJEH-HOSSEINI M. Length of the lag period of germination and metabolic repair explain vigour differences in seed lots of maize (Zea mays). Seed Science and Technology 35, 200, 2007.

47. AHMED R., HOWLADER M., SHILA A., HAQUE M. Effect of salinity on germination and early seedling growth of maize. Progressive Agriculture 28 (1), 18, 2017.

48. AKINROLUYO O.K., URBANAVIČIŪTĖ I., JAŠKŪNĖ K., KEMEŠYTĖ V., STATKEVIČIŪTĖ G. Differences in salt tolerance between diploid and autotetraploid lines of Lolium multiflorum at the germination and vegetative stages. Zemdirbyste-Agriculture 106 (4), 329, 2019.

49. LIU Y., ZHANG S., DE BOECK H.J., HOU, F. Effects of Temperature and Salinity on Seed Germination of Three Common Grass Species. Frontiers in Plant Science 12, 731433, 2021.

50. SATTLER M., CARVALHO C., CLARINDO W. The polyploidy and its key role in plant breeding. Planta 243, 281, 2016.

51. RAUF S., ORTIZ R., MALINOWSKI D.P., CLARINDO W.R., KAINAT W., SHEHZAD M., HASSAN, S.W. Induced polyploidy: A tool for forage species improvement. Agriculture 11 (3), 210, 2021.