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Original Research

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

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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

Introduction

Salinity is one of the most serious environmental stress factors that can affect plant growth negatively and reduce productivity [1]. Today, about 800 million hectares of farmland, 20% of the world's crop production areas, and about 50% of irrigated agricultural areas are affected by salinity [2-5]. Salinity leads to osmotic stress, ion imbalance, and nutrition deficiency. If the salinity in the soil is too high, the capability of plants to obtain water from the soil is weakened because of excessive external osmotic pressure, which slows down the growth rate and causes the leaf to start wilting [6].

Survival is one of the agronomic selection criteria to identify plant tolerance to salinity stress. Survival capability to stress factors begins with the seed germination phase. It is the first growth stage of the plants cultivating and is negatively affected by salinity [7, 8]. Several previous studies have stated that the seed

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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 G_D (diploid), G_T (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 15ºC, 25ºC, 35ºC. Salt and temperature-treated seed groups were labeled as S_0, S_1, S_2, and T_{min}, T_{opt}, T_{max} 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 8^{th} 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 ($p \leq 0.05$). 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 (744.48**), T (71.45**), S (96.49**), and interaction of G × T (6.67**), G × S (63.27**), T × S × G (8.41**) were found to be statistically different ($p \leq 0.01$), except for T × S (2.54*) interaction ($p \leq 0.05$). If compared to genotypes and temperatures; G_T had more mean germination percentage (81.04%) than G_D (47.70%). For diploid and tetraploid cultivars, 35ºC (T_{max}) affects germination percentage negatively. Maximum germination percentage of G_T (91.56%) and G_D (64.89%) 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_T (81.04%) showed a better mean germination percentage than G_D (47.70%) cultivar. Increasing salinity level decreased germination percentage for both cultivars. Although S_2 had a good germination percentage for both cultivars that G_D is 85.33% and GT are 84.00%, S_1 had the maximum germination percentage (89.33%) for G_T 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**), G × T × S (7.55**) were found to be statistically different ($p \leq 0.01$). If compared to genotypes and temperatures; G_T had more mean germination energy (30.52%) than G_D (13.63%). There was no germination at T_{max} on the 3rd day. For diploid and tetraploid cultivars, T_{max} affects germination energy at a lethal level. Maximum germination energy of G_T (64.44%) and G_D (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_T (30.52%) showed a better mean germination energy than G_D (13.63%) cultivar. Increasing salinity level decreased germination energy for G_T cultivar. But G_D 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_T (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 (%)				Germination energy (%)			
G_D	47.11**B**	64.89**AB**	31.11**B**	47.70	10.67**C**	30.22**B**	0.00**D**	13.63
G_T	91.56**A**	90.22**A**	61.33**AB**	81.04	27.11**B**	64.44**A**	0.00**D**	30.52
AV	69.33	77.56	46.22	64.37	18.89	47.33	0.00	22.07
	Root length (cm)				Shoot length (cm)			
G_D	4.99	6.61	2.86	4.82	3.71	6.71	2.54	4.32**A**
G_T	5.15	6.42	3.52	5.03	3.69	7.53	3.42	4.88**A**
AV	5.07**B**	6.52**A**	3.19**C**	4.93	3.70**B**	7.12**A**	2.98**B**	4.60
	Fresh weight (g)				Dry weight (g)			
G_D	0.266**B**	0.155**C**	0.100**D**	0.174	0.021**A**	0.013**B**	0.013**B**	0.016
G_T	0.349**A**	0.147**C**	0.101**D**	0.199	0.013**B**	0.021**A**	0.014**B**	0.016
AV	0.307	0.151	0.100	0.186	0.017	0.017	0.013	0.016
	Simple vigour index				Promptness index			
G_D	1.44	1.07	0.24	0.92	15.33**D**	21.44**C**	4.81**F**	13.86
G_T	1.57	3.07	0.39	1.68	29.58**B**	34.00**A**	8.28**E**	23.96
AV	1.50**AB**	2.07**A**	0.32**B**	1.23	22.46	27.72	6.54	18.91

G_D: Diploid cultivar, G_T: Tetraploid cultivar, T_{min}: 15ºC, T_{opt}: 25ºC, T_{max}: 35ºC.

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

G_D: Diploid cultivar, G_T: Tetraploid cultivar, S_0: distilled water, S_1: 75 mM, S_2: 150 mM.

Root Length

In accordance with three-way ANOVA; individually T (73.21**), S (35.85**) were found to be statistically different (p≤0.01), while other variables were not. If compared to genotypes and temperatures; G_T had more mean root length (5.03 cm) than G_D (4.82 cm). For diploid and tetraploid cultivars, increasing temperature affects root length negatively. Maximum root length of G_D (6.61 cm) and G_T (6.42 cm) obtained at T_{opt}. 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_T (5.03 cm) showed a better mean root length than G_D (4.82 cm) cultivar. Increasing salinity level decreased root length for both cultivars. Maximum root length of G_D (5.61 cm) and G_T (5.97 cm) was noted at the control group, while minimum root length of G_D (3.30 cm) and G_T (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 (10.36*) and T (99.57**), S (55.63**) were found to be statistically different (p≤0.05) and (p≤0.01), while other variables were not. If compared to genotypes and temperatures; G_T had more mean root length (4.88cm) than G_D (4.32cm). Maximum root length of G_D (6.71cm) and G_T (7.53cm) 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_D and G_T was 2.54 cm and 3.42 cm, in the same vein (Table 1). Similar to genotypes × temperature, on genotype × salinity interaction, G_T (4.88 cm) showed a better mean root length than G_D (4.32 cm) cultivar. Increasing salinity level decreased shoot length for both cultivars. Maximum root length of G_D (5.39 cm) and G_T (6.39 cm) was determined at the control group, while the minimum root length of G_D (2.44 cm) and G_T (3.04 cm) was noted at 150 mM salinity level (Table 2). The negative effect of salinity on root length is lower as compared to the negative effect of temperature.

Fresh Weight

In accordance with three-way ANOVA; individually G (20.22**), 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_T had more mean root length (0.199 g) than G_D (0.174 g). The maximum fresh weight of G_D (0.266 g) and G_T (0.349g) was obtained at T_{min}. Minimum shoot length was obtained in T_{max}. Hence, the minimum shoot length of G_D and G_T was 0.100 g and 0.101 g, in the same order (Table 1). Increasing temperature decreased fresh weight for both cultivars. Similar to genotypes × temperature, on genotype × salinity interaction, G_T (0.199 g) showed a better mean root length than G_D (0.174 g) cultivar. The heaviest fresh weight was obtained in G_T 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 ($p \leq 0.01$) and ($p \leq 0.05$), 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_T 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 ($p \leq 0.01$). 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_T (34.00) and G_D (21.44) was obtained at T_{opt}, while minimum promptness index of G_T (8.28) and GD (4.81) was obtained at T_{max} (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_D (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 ($p \leq 0.05$), 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_{max} (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_{min}) conditions. At $p \leq 0.05$, 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 25ºC (T_{opt}) conditions. At $p \leq 0.05$, 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_{max} conditions. At $p \leq 0.05$, 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 25ºC (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 25ºC showed that grass species prefer relatively higher temperatures for seed germination. [13, 27, 28] studies about *P. turgidum, Leymus chinensis* and *Lolium multiflorum*

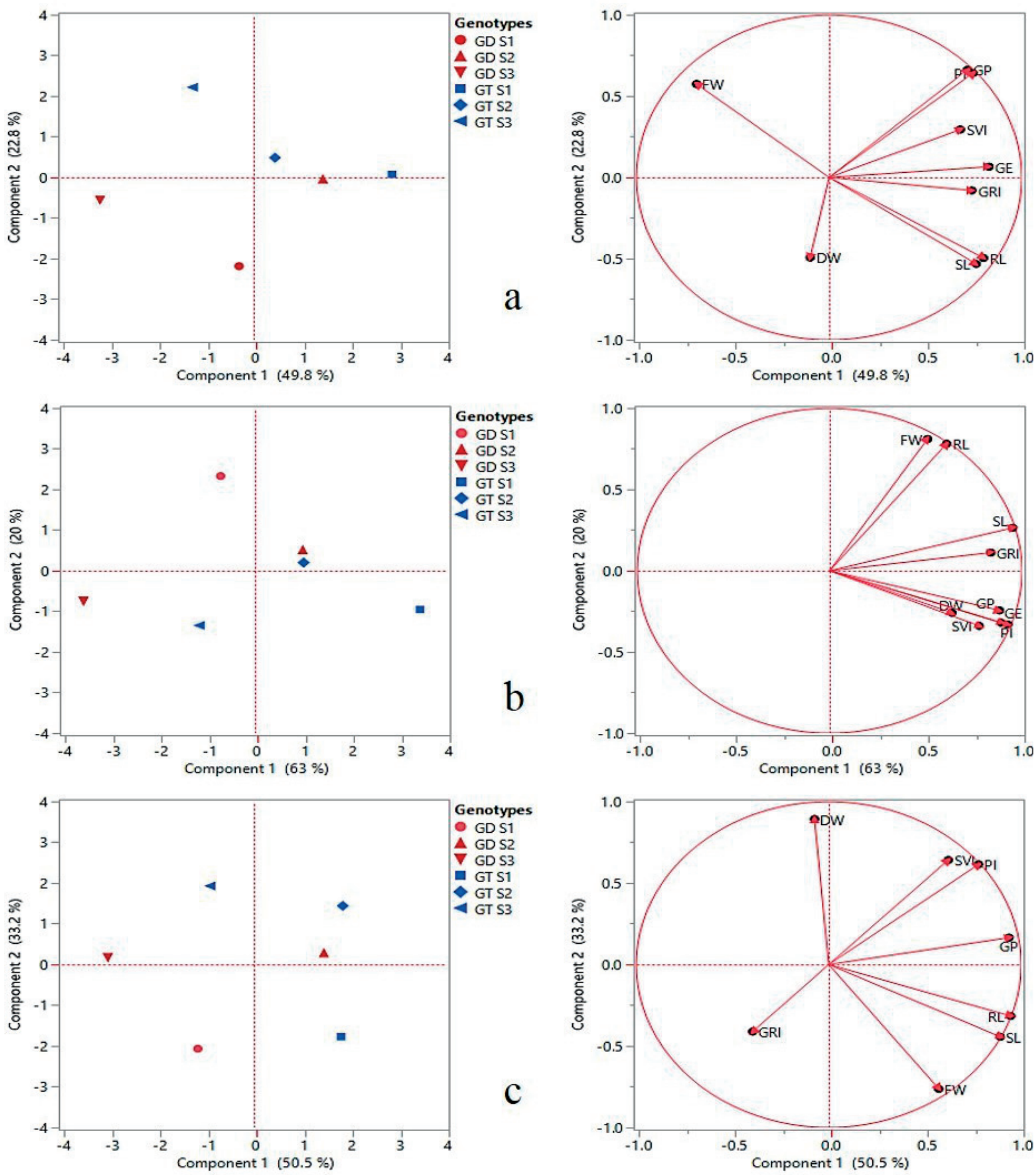

Fig. 1. Classification of Italian ryegrass cultivars along the first and second principal components based on characterization of germination traits at T_{min} (15ºC) (a), T_{opt} (25ºC) (b), T_{max} (30ºC) (c).

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 25ºC (T_{opt}) to 30ºC (T_{max}) and 75 mM (S_2) to 150 mM (S_3), root length and shoot length was reduced. It is revealed that 25ºC (T_{opt}) and 75 mM (S_2) 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 15ºC (T_{min}) to 25ºC (T_{opt}) and distilled water to 75 mM (S_2), respectively. Dry weight of both cultivars were very close to each other.

Promptness index (PI) was drastically declined in the progress of 25ºC (T_{opt}) to 35ºC (T_{max}) and 75 mM (S_2) to 150 mM (S_3). Concordantly, 25ºC (T_{opt}) and 75 mM (S_2) 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 25ºC (T_{opt}) to 35ºC (T_{max}) 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_{min} 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_{max} 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_T) has been found more tolerant to salinity and temperature compared to diploid Italian ryegrass (G_D) 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 25ºC (T_{opt}) to 35ºC (T_{max}) and 75 mM (S_2) to 150 mM (S_3), germination responses of Italian ryegrass cultivars were drastically reduced. Due to this maximum temperature's negative effects, 25ºC (T_{opt}) and 75 mM (S_2) is the optimal temperature and salt level for both cultivars in the germination process.

Conflict of Interest

The authors declare no conflict of interest.

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