



Evaluation of Tolerance of Trans Chromosomal Primary Tritipyrum Lines to NaCl Salinity for Germination and Seedling Emergence

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Abstract

Cell death, oxidative stress and nutrient deficiency are the major stress factors induced by soil salinity and are a source of crop and forest plant losses. By mid of 21st century, 50% of arable lands are expected to be affected, by salinity induced losses costing a loss of > \$12 billion annually. The development of salt-tolerant plants such as Tritipyrum (a hybrid between *Triticum durum* and *Thinopyrum besarabicum*) could be used to alleviate the problem. It has a high grain yield potential and high salt tolerance. This plant species may become an important source of natural transchromosomal gene transformation and recommended for cultivation on saline soils. The germination and seedling emergence percentage of the primary Tritipyrum line were checked under 24 dS m⁻² induced salt stress to determine their salt tolerance. The results showed that all segregating lines could germinate with 85% at EC=24 dS m⁻². The data was retrieved on seedling length and seed morphology in of TCPT F1 segregating lines. The results support the idea of creating resistant varieties against salinity stress in future wheat breeding programs for inducing salt tolerance.

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1. Introduction

Climate conditions are gradually increasing stress on present food production systems. Soil salinity is an important environmental abiotic stress factor that has negative effects on crop and forest plants' production leading to imbalances in cellular homeostasis, cell death, leading to oxidative stress, nutrient deficiency, retarding growth and cell death.

Currently, about 10% of the arable lands are salt-affected and about 50% of arable lands are liable to salinity by the mid of the 21st century. This will cause a loss of about US\$12 billion per annual in the agricultural sector. A careful estimate has predicted to increase in the world population by 9.2 billions in 2050, which clearly indicate the need to develop new strategies to stop these developments (Arsenovic, 2024). Therefore new innovations should be integrated into plant breeding programs to decrease yield loss per unit area. Interspecific hybrids can contribute to improving crop performance (Llewellyn, 2018). Triticale, and tritordeum are important interspecific crosses in cereals. Tritipyrum is new synthetic amphidiploid cross that could be used as alternative new wheat and has been developed in last 30 years.

This interspecific hybrid, is highly salt and drought tolerant developed after crossing *Triticum durum* ($2n=4x=28$, AABB) as mother parent and salty coastal grass *Thinopyrum bessarabicum* ($2n=2x=14$, EbEb) as father parent. The superiority of interspecific hybrid is attributed to the induction of hybrid vigor and is expressed as higher salt and drought resistance (Koemel et al., 2004; Hassani et al., 2006; Farokhzadeh et al., 2022). *Thinopyrum bessarabicum* which is considered tolerant to 250-350 mM NaCl. The Tritipyrum ($2n=6x=42$, AABBEbEb) can be used to introduce salt and drought-tolerant traits in high-yielding wheat varieties.

The genome of *Thinopyrum* is closely related to the ABD genome of wheat *Triticum aestivum* L. and harbors genes that confer beneficial traits such as high yield, disease resistance and unique end-use quality to wheat. This can reduce the use of excessive inputs and

mechanization that can negatively affect sustainable agricultural developments (Kamyab et al., 2018; Shanazari et al., 2018). This interspecific hybrid will form a gorgeous area of study for researchers.

The experiments show a relative success of the crop plant in Turkey. The global area of interspecific wheat hybrid Tritipyrum is expected to increase, with the passage of time in a competitive way. This study builds a new knowledge base and covers seed germination technologies using trans chromosomal Tritipyrum. Germination percentage is crucial for understanding plant adaptation to saline conditions, especially in determining the correct amount of seeds used in field trials. It helps prevent waste and ensures optimum results. Environmental factors such as temperature, humidity and salinity level also affect seed germination percentage in saline areas. A study examining the effects of different levels of salinity stress on the germination of non-Iranian Tritipyrum lines and wheat varieties revealed that with increasing salinity levels, germination percentages, root length, shoot length, dry and fresh root weight and shoot dry weight decreased. The Az/b line showed the highest germination percentage under stressed and non-stressed conditions. The simplest and hybrid lines of the plant showed salinity tolerance at the germination stage, with selected lines being better suited for further studies (Pirsalami et al., 2021).

The study had a goal to germinate new primary trans chromosomal hybrid cereal 8 Tritipyrum and 6 *Thinopyrum bessarabicum* lines. This study aimed to evaluate the germination and seedling emergence percentages regulated by NaCl (24 dS m^{-2}) to ascertain the resistance level of salt resistance of some primary Tritipyrum lines.

2. Materials and Methods

Six *Thinopyrum bessarabicum* lines namely (i) Az/b = Azizih, (ii) Cr/b = Creso/, (iii) Ka/b = Karim/, (iv) La/b =Langdon/, (v) Ma/b = Mancoun/, (vi) St/b =Stewart (6 lines) and eight Trans Chromosomal Tritipyrum lines

(PTCT namely (a) $Ka/b \times Cr/b$ -2, (b)3, (c)5, (d) 6, (4 lines) (e) $La\ 4B/4D \times/b$, (f) $Ma/b \times Cr/b$ -3 (g) 4 (2 lines, and (h) $St/b \times Cr/b$ -4 [8 lines] were used in the study. These were obtained from Associate Professor Dr. Hossein Shahsavand Hassani of the Department of Agronomy at Shiraz University Iran.

The Primary Trans Chromosomal Tritipyrum (PTCT) genotypes were obtained by crossing with *Triticum durum* ($2n=4x=28$, AABB) varieties. The study was carried out at the Department of Field Crops Ankara University and the Department of Biotechnology of Central Field Crops Research Institute (FCCRI). The seed samples used in the study; were tested against salinity level of $EC=0-24\ dS\ m^{-2}$ (Figure 1). The evaluation included measurement of the number of germinated seeds over a period of 3 weeks. To assess the level of salinity tolerance, 100 seeds per genotype (Table 1) were used. These were equally subdivided into 5 subsets containing 20 seeds each in a replication. The seeds belonging to each genotype were disinfected with 5% sodium hypochlorite (NaOCl) for 15 min using a magnetic stirrer. Following this step, each seed sample was individually rinsed $3\ min \times 5$ times in tap water. Each set of 100 disinfected seeds (replication) was placed on filter papers in $20 \times 10 \times 10$ cm germination boxes and set up in five replicates. They were screened against salt tolerance levels of $EC=24\ dS\ m^{-2}$ (NaCl) for each genotype, over 21 days at $25 \pm 1^\circ C$. The amounts of NaCl salts were determined according to Munns and James (2003).

2.1. Preparation of Samples

The seeds of each genotype were sandwiched in two layers of filter papers and kept in a zip-lock polythene bag for incubation at $20^\circ C$ for 21 days.

The EC values of each germination container were checked every 3 to 4 days using an EC meter (Mettler Toledo) and germinated seeds were counted over a 3 weeks period (Figure 1).

2.2. Statistical analysis

A randomized complete block design (RCBD) was used to evaluate 6 *Thinopyrum bessarabicum* and 8 primary Tritipyrum (AABBEbEb, $2n=6x=42$) lines. The germination test values were analyzed using IBM SPSS 27 for Windows 27 using a one-factor randomly complete design. Germination percentage means were subjected to arcsine transformation before statistical analysis. The means were compared using Duncans Multiple Range test.

3. Results and Discussion

The study screened 8 Tritipyrum genotypes using salt stress of $EC=24\ dS\ m^{-2}$ the first time. The results were compared with the germination percentage of *Thinopyrum bessarabicum* used as father. The results are shown in Table 1 and 2. The results show improvement in the salt tolerance behavior of hybrid lines. Analysis of variance (ANOVA) showed significant differences among the lines. The factor analysis results revealed five factors explaining almost 89% of the total variance. The results indicated that the number of grains per ear and harvest index can be used during selection (Farokhzadeh et al., 2023).

Tritipyrum, obtained from large hybrids of *Triticum* and *Thinopyrum*, exhibited high salt tolerance in agreement with Yuan and Tomita, (2015). The results are very encouraging and show successful improvement in the salt tolerance behavior of Tritipyrum genotypes. Mean comparisons of germination percentages (%) and number of germinated seeds of genotypes are shown in (Table 2). The results showed a germination range of 69.63 ± 1.01 to $92.26 \pm 1.14\%$ for *Thinopyrum besarabicum* seeds (Table 1). Whereas an improvement in maximum and minimum seed germination percentage was noted on seeds of primary Tritipyrum genotypes. The minimum germination ($85.16 \pm 1.37\%$) was indicated on $La\ (4B/4D \times/b)$. A maximum germination of 91.22 ± 1.09 was noted on $Ka/b \times Cr/b-5$ for primary Tritipyrum seeds (Table 2).



Figure 1. Effects of different EC percentages on germination and seedling development in PTCT genotype

Table 1. Germination percentage of 8 *Thinopyrum bessarabicum* lines used in the study which were subjected to germination studies

Serial Number of Genotypes	<i>Thinopyrum</i> <i>bessarabicum</i> lines	Gene Bank Code in Iran		No of seeds per replication in a treatment of 100×5 seeds (control treatment)	Germination percentage (%)
		QR codes	Temporary codes		
1	Az/b = Azizih	61526	01-13364	100.00±0.00	69.63±1.01E
2	Cr/b = Creso/	61527	01-13365	100.00±0.00	85.54±1.09B
3	Ka/b = Karim/	61528	01-13366	100.00±0.00	81.34±1.02D
4	La/b =Langdon/	61533	01-13371	100.00±0.00	83.12±1.15C
5	Ma/b = Mancoun/	61535	01-13373	100.00±0.00	92.26±1.14 A
6	St/b =Stewart /	61538	01-13376	100.00±0.00	81.38±1.13D

±standard error

*All values shown in a single column shown by different capital letters are statistically different using Duncan's Multiple Range Test (using $\alpha=5\%$).

Table 2. Germination percentage of 8 *Tritipyrum* lines used in the study which were subjected to germination studies

Serial No.	<i>Tritipyrum</i> lines	Gene Bank Code in Iran		No of seeds per replication in a treatment of 100×5 seeds (control treatment)	Germination percentage
		QR code	Temporary code		
1	(Ka/b ×(Cr/b -2	61529	01-13367	100.00±0.00	85.28±1.37D
2	(Ka/b ×(Cr/b -3	61530	01-13368	100.00±0.00	85.32±1.28D
3	(Ka/b ×(Cr/b -5	61531	01-13369	100.00±0.00	91.22±1.09A
4	(Ka/b ×(Cr/b -6	61532	01-13370	100.00±0.00	85.19±1.16C
5	La(4B/4D ×/b	61534	01-13372	100.00±0.00	85.16±1.37C
6	(Ma/b ×(Cr/b -3	61536	01-13374	100.00±0.00	91.14±1.48A
7	(Ma/b ×(Cr/b -4	61537	01-13375	100.00±0.00	88.15±1.59B
8	(St/b ×(Cr/b -4	61539	01-13377	100.00±0.00	89.55±1.25B

±standard error

*All values shown in a single column shown by different capital letters are statistically different using Duncan's Multiple Range Test (using $\alpha=5\%$).

4. Conclusion

Tritipyrum is an amphidiploid cross between *Triticum durum* and *Thinopyrum bessarabicum*, which is a novel salinity

tolerant cereal. Tritipyrum is a future crop that is comparable to currently used wheat varieties in terms of yield and increased tolerance against salinity. There is a need to study this

plant on molecular, cellular and whole plant level. Detailed studies are needed to understand the functions of this plant through genomic, transcriptomic, proteomic and metabolomic techniques. The results of the study confirm that the reported Tritipyrum lines have a reasonable biomass and grain yield potential, for growing in areas with soil and water salinity issues. The experimental results indicate further experiments are needed to evaluate the potential of Tritipyrum amphidiploids in industry and its use as human food and animal feed.

Declaration of Author Contributions

The authors declare that they contributed equally to the article, GP, SS and KMK have seen/read and approved the final version ready for publication. Author GP, SS and KMK MF conducted the statistical analysis, GP and MF have checked the final pre-publication version of the article.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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