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Using Chromosomal Abnormalities and Germination Traits for the Assessment of Tritipyrum Amphiploid Lines under Seed-Aging and Germination Priming Treatments

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Abstract: Primary Trans Chromosomal Tritipyrum (PTCT) amphiploid is a new cereal grown in saline soil and brackish water for grain and forage production. We evaluated the tolerance to seed deterioration in 13 promising PTCT lines, assessing accelerated aging (AA) tests by using AA boxes with 100% relative humidity at 40 °C for 72 h. The (Ma/b)(Cr/b)F4 and (St/b)(Cr/b)F4) PTCT lines, more sensitive to seed aging, were primed with NaCl, Salicylic Acid (SA), and Polyethylene Glycol (PEG) to increase the seed vigor of artificially aged seeds. Germination and emergence traits, biochemical parameters, and chromosomal abnormalities induced by artificial aging were measured in deteriorated and not-deteriorated seeds. The highest reduction percentages related to seed vigor were observed in (Ka/b)(Cr/b)F2 (34.52) and La(4B,4D)/b (28.15) lines, while the lowest was found in (Ma/b)(Cr/b)F4 (7.65) and (St/b)(Cr/b)F4 (7.46) lines. Seed aging also increases electrolytes, potassium, and protein leakages. Chromosomal abnormalities are caused by seed aging that interferes with chromosome behaviors during cell division. Seed priming on aged seeds revealed an increase in the germination percentage (GP) with PEG treatment, while the priming by SA showed an increase in seedling traits, such as the seedling length (SL2). In conclusion, we highlighted the potential use of different PTCT lines and the effective use of seed priming on deteriorated seed to enhance seed viability and seedling vigor as a useful tool for sustainable agriculture.

Keywords: PTCT lines; seed deterioration; leakage; pre-sowing; chromosomal aberration

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1. Introduction

The development and selection of new amphiploid lines of cereals are crucial to identify the most performing ones in terms of crop production and survival in harsh environmental conditions [1,2]. Nowadays, one of the main factors reducing plant productivity is the poor seedling establishment in dry and semi-arid areas due to different abiotic stresses, such as rare annual precipitation, high evaporation, water scarcity, and soil salinity [3,4]. Primary Trans Chromosomal Tritipyrum (PTCT) lines, derived from the natural hybridization of *Triticum aestivum* L. and *Thinopyrum bessarabicum*, are recombinant chromosomal lines of hexaploid Tritipyrum, a new salt-tolerant species of cereal [5,6]. It was demonstrated that Tritipyrum lines could tolerate a salt concentration of about 250 mmol NaCl [7,8]. Thus, these novel amphiploid lines provide a new opportunity for producing grain and forage in saline soils and brackish waters in arid and semi-arid areas.

The first step toward reaching high production yields is the effective establishment of a crop. Seed germination dynamics play a crucial role in this process [9]. The uniformity of development, yield, and quality of the harvested products are all greatly influenced by the quality of the seeds and how they are stored [10]. The seed's nature and its physical, morphological, and nutrient storage conditions are crucial to enable the proper seedling establishment and the least amount of mechanical and biological losses [11,12]. Understanding the intricate factors affecting seed longevity is extremely important from an ecological, agronomical, and economic perspective [13]. It is recognized that the aging rate of the seeds is strongly influenced by environmental and genetic factors, such as storage temperature, seed moisture content, and seed quality [14]. These factors could affect all the seed properties that determine the successful plant establishment. Seed vigor is the sum of the seed properties which determine the potential for high germination abilities, rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions, as defined by the Association of Official Seed Analysts (AOSA) [15,16].

The uniformity of emergence and seedling establishment, with consequently farmers' income, could be reduced by seed deterioration. Almost every year, 25% of the harvested seeds lose their quality due to deterioration [17]. Seed deterioration may occur as a natural phenomenon, starting with a chain of biochemical events, such as membrane damage and disruption of biochemical processes [18]. Thereby, most of the vital properties of seeds are diminished, starting with a decline in germination and emergence [19], leading to poor seedling establishment [20]. During seed deterioration, chromosomal aberration and permanent chemical and structural changes occur at the cellular level that leads to a decreased viability of seeds [21].

In natural conditions, seed deterioration induces decreased germination percentages, poor seedling production, lowered vigor, declined viability, and seed death [22]. Furthermore, deteriorated seeds have a more non-uniform establishment than healthy seeds [23]. These issues lead to low seedling emergence, and few plants per hectare occur in the form of spots in the field [23]. The viability of damaged seeds can be influenced by a decrease in total carbohydrates and an increase in lipid peroxidation during storage [24]. Seed quality and viability decrease are further triggered by adverse environmental conditions, leading to a decay rate variation among varieties of the same species [10,25,26]. Under high salt concentrations, for example, seed decay induces chromosome abnormalities during cell division (e.g., chromosome stickiness, laggard chromosome, disturbed and irregular anaphase, and anaphase bridge formation) [27,28]. Katabale and colleagues [29] observed high rates of chromosomal abnormalities after exposing onion (*Allium cepa* L.) seedlings to aqueous extracts of neem (*Azadirachta indica*) leaf. Another study by Pavlova [30] evaluated nickel's toxic effects on root-meristem cell division in the seedlings of *Plantago lanceolata* L., observing anaphase bridges, chromosome adhesion, retardation, and extrusion of nuclear material into the cytoplasm of these cells. The percentage of chromosome alterations usually increases in a concentration and time-dependent manner.

Seed storability is one of the key factors that assure plant propagation and crop production [31]. This important characteristic has not been described for PTCT lines developed in our previous work [11]. Currently, conventional methods to evaluate seed storability include seed vigor accelerated aging (AA), followed by germination and seedling growth tests [31,32]. Additionally, after AA, deterioration tests and electrical conductivity (EC), pH level, and cellular leakage of solutes during seeds' water uptake can be measured [33]. Biochemical processes augment cell membrane permeability in seeds during storage. As a result, some cellular solutes (sugars, amino acids, fatty acids, proteins, enzymes, and inorganic ions like K^+, Ca^{+2}, Mg^{+2}, Na^+, and Mn^{+2}) are released to the external environment during water uptake for germination [31]. The amount of solute leakage can be used as an indicator for screening the storability of cultivars [34]. The frequencies of chromosomal abnormalities arising from chromosomal fusions formed through errors in re-joining DNA double-strand breaks (DSBs) by the cell's recombination pathways can occur in AA tests [35].

One of the strategies used to improve plant growth in semi-arid and salinity-affected environments is the priming of seeds. Many works highlighted the use of seed priming to increase germination rate and crop development uniformity for overcoming poor germination and erratic crop stand [36]. This treatment is provided before planting, in the early germination stage, when water is absorbed but prevents roots from coming out. In this process, seeds are soaked in water or various osmotic solutions and then dried until the initial moisture is achieved [37]. Some biochemical processes are required during priming to initiate germination (e.g., water absorption, hydrolysis or metabolism of inhibitory materials, and enzymatic activities) [38]. The induced effects remain even after redrying the seeds. Materials of high molecular weights are usually used for priming. Polyethylene glycol (PEG6000-8000) is the most used for seed priming. PEG is a non-toxic substance with a high molecular weight, osmotic pressure, and water solubility, which does not penetrate plant tissues while serving as a suitable compound for inducing drought stress [39,40]. Salicylic Acid (SA) is a phenolic growth-regulating compound that is present in low quantities (mg of fresh weight or less) in growing plants and applied for seed priming [41]. It was demonstrated that rice seed priming by SA increases seed vitality [42]. Salts are frequently utilized for seed priming [43,44]. Pretreatment with mineral salts (halo-priming) is an easy, low-cost, and low-risk technique effective in seed priming for plantations under salinity stress conditions [45]. Priming is a simple, low-cost, low-risk approach to increase the vigor of deteriorated seeds.

The storage-tolerant lines development is one of the key characteristics of the ongoing plant breeding program. However, this method of introducing current lines has been little investigated. In this work, we hypothesized that 13 germplasms of *Tritipyrum* could be valid tolerant storage lines.

To test this hypothesis, we analyzed 13 germplasms of *Tritipyrum* to determine seed vigor by assessing germination and emergence traits, cell leakage, and cytogenetic abnormalities. Three different seed priming treatments were performed on the artificially aged seeds, and germination and emergence traits were measured to evaluate the potential increase in germination vitality of aged seeds.

2. Materials and Methods

2.1. Plant Materials

In this study, 13 PTCT (Primary Trans Chromosomal Tritipyrum) lines (*Tritipyrum*, 2n = 6x = 42, *AABBEbEb*) were provided by the Department of Crop Production and Plant Genetics, School of Agriculture, Shiraz, Iran and transferred to the laboratory to evaluate their viabilities. The Tritipyrum lines used were La/b (1), (Ka/b)(Cr/b)F5 (2), (Ka/b)(Cr/b)F2 (3), La(4B,4D)/b (4), Ka/b (5), St/b (6), (Ma/b)(Cr/b)F3 (7), (Ma/b)(Cr/b)F4 (8), (St/b)(Cr/b)F4 (9), Cr/b (10), Az/b (11), (Ka/b)(Cr/b)F3 (12), (Ka/b)(Cr/b)F6 (13), and kavir (14) as a control. Then, the homogeneous and uniform seeds were selected and stored in plastic bags with minimum permeability at 5 °C.

2.2. Accelerated Aging (AA) Analysis

The experiments were carried out in a completely randomized factorial design with three replications at the laboratory and greenhouse of the College of Agriculture, Shiraz University, Shiraz, Iran.

Seeds were placed in mesh bags on a sieve suspended above water inside a plastic Accelerated Aging (AA) box [28]. The box was kept in the incubator with 100% relative humidity at 40 °C for 72 h [46]. After this aging period, four replicates of 25 seeds for each treatment were analyzed for seed germination and emergence as described. The mean average seedling percentage for each batch was calculated ten days after sowing based on an evaluation of seedling development. Seed moisture content (oven-drying method at 105 °C/24 h) was also measured before and after the aging period to assess the accuracy of the AA results. For cytogenetic studies, the seeds were subjected to AA stress adjusted in a desiccator with 100% humidity in Petri dishes above wire meshes, sealed, and maintained

at 5 °C (T1), 40 °C (T2), 50 °C (T3), 60 °C (T4), and 70 °C (T5) for 24 h. In the control treatments (T1), the seeds were kept in a cold chamber at 5 °C. After applying each heat treatment, a cytogenetic analysis of artificially aged seeds was performed.

2.2.1. Germination Test

Three replicates of 25 seeds were analyzed in 90-mm glass Petri dishes on 1-layered filter paper (No. 1) at 25 °C. Each plate received 10 mL of deionized water, enough to cover each seed by roughly half its content [47].

2.2.2. Emergence Test

The emergence test was conducted in a greenhouse with four replicates of 25 seeds. Each germplasm was hand-sowed on trays of sand at a depth of 20 mm in 1-m-long furrows in 60-mm-spaced rows. The emerged seedlings were counted daily. Evaluation of seedling development was performed ten days after starting the tests. Emergence tests were carried out at the deterioration levels of deteriorated seeds and not-aged seeds of the different Tritipyrum lines for ten days. Tritipyrum lines, a new salt-tolerant cereal, are derived from the natural hybridization of *Triticum aestivum* L. and *Thinopyrum bessarabicum* [5,6].

2.3. Plant Traits

2.3.1. Germination Percentage (GP)

Germination Percentage (GP) was calculated through the following formula:

$$GP = \frac{N}{n} * 100$$

where N is the number of germinated seeds, and n is the number of experimental seeds.

2.3.2. Germination Rate (GR)

Germination count was performed daily at the same time, and Germination Rate (GR) was calculated by using the following equations [48]:

$$GR = 1/MGT$$

$$MGT = \sum(ni * di)/N$$

where ni shows the number of seeds germinated per day, di represents the incubation period, and N denotes the total number of seeds germinated in each treatment.

2.3.3. Shoot Length (SL) and Root Length (RL)

Shoot Length (SL) and Root Length (RL) was measured with a ruler with 1-mm accuracy, and then, their average lengths were recorded for each treatment in each repetition [46].

2.3.4. Seedling Length Vigor Index (SLVI)

Seedling Length Vigor Index (SLVI) was calculated via the following equation [49]:

$$SLVI = \frac{(RL + SL) * GP}{100}$$

where RL, SL, and GP stand for root length (cm), shoot length (cm), and germination percentage, respectively. Length measurements were performed by considering the total seedling in each treatment and replication.

2.3.5. Shoot Dry Weight (SDW) and Root Dry Weight (RDW)

Shoot and root were separated to measure their dry weights. They were heated in an air-heated oven at 105 °C for 30 min, dried in the oven at 70 °C for 24 h, and then determined their dry weights with an accuracy of 0.0001 g.

2.3.6. Tolerance Index (TI)

We also calculated the Tolerance Index (TI) through the following equation [50]:

$$TI = \frac{Mean\ traits\ after\ AA}{Mean\ traits\ before\ AA} * 100$$

2.3.7. Reduction Percentage (RP)

Reduction Percentage (RP) was calculated for GP, GR, GE, SDW, RDW, SVI, EP, and ER by applying the following formula:

$$RP = \left(1 - \frac{Nx}{Mc}\right) * 100 \quad (1)$$

where *Nx* and *Mc* are the amounts of germination and emergence traits after and before seed aging (aged and not-aged seeds) since the measurement units of germination traits were different, the declining changes in each trait were first calculated and converted into percentages after applying artificial seed aging. Then, their means were determined for each cultivar.

2.4. Biochemical Parameters

2.4.1. Electrical Conductivity (EC)

Electrolyte leakage was measured based on the leakage of solutes from the cell membranes of all the seeds in deionized distilled water. Fifty undamaged seed samples per lot were weighed on an analytical balance (0.01 g) and soaked in 25 mL of distilled water at 25 ± 1 °C for 24 h [51]. Then, the Electrical Conductivities (ECs) of the leachates were measured with a conductivity meter (K220 Consort, DDS-11A model, Nanjing T-bota Scietech Instruments & Equipment Co., Ltd. (TBT), Nanjing, China).

2.4.2. Potassium Leakage

Fifty undamaged seed samples per lot were weighed on an analytical balance (0.01 g) in 4 replicates and then soaked in 75 mL of distilled water at 25 ± 1 °C. Then, they were placed in disposable plastic cups and kept in a germinator at 25 °C. After 48 h (imbibition period), leached potassium was determined with a flame photometer adjusted to 50 g K^+ mL^{-1} and reading 50 [52]. The results were expressed in g K^+ g^{-1} seeds.

2.5. Cytogenetic Analysis

Cytogenetic analysis was carried out through the root-tip smear method [53,54]. Root cell chromosomes of plants are suitably utilized for research purposes like studying chromosomal abnormalities due to their ease of access, large size, small number in each batch, and detectable characteristics [55]. Twenty-five seeds with three replicates in each treatment were used in the germination test. Seed germination rates varied due to the different temperatures applied in each treatment. Once the radicles were 1–2 cm long, the root tips containing meristem tissues were collected after keeping them in Lewitsky's fixative solution for 16 h. Then, the samples were hydrolyzed (1N HCl was maintained at 60 °C for 10 min), washed in distilled water, and stained with 2% acetic orcein using the squashing method [56]. The slides were observed through a light microscope (Zeiss, Axiolab 5, Jena, Germany), and the best chromosomal plates were captured at 100× magnification using an Axiocam 208 color camera (Zeiss, Jena, Germany). Five hundred cells per treatment (100 cells with five replicates) were counted and analyzed. Mitotic Index (MI) was calculated by dividing the total number of cells under division by the total number of cells analyzed multiplied by 100.

2.6. Seed Priming

The seeds of PTCT lines, including (Ka/b)(Cr/b)F2 and La(4B/4D)/b that resulted in the more sensitive to seed aging, were primed at four levels of priming treatments,

including control, NaCl (350 mM), SA (1100 ppm), and osmo-priming with PEG6000 (−1.2 Mpa) for 9 h [57]. Then, the germination test was performed as described in the previous section.

2.7. Statistical Analysis

The data were tested for normality via the Kolmogorov–Smirnov normality test [58]. They were then analyzed using SAS software (version 9.3), and the graphs were plotted in Excel. To show the effect of aging on the slope of germination percentage and emergence percentage, the regression test was performed by Excel 2010 software. The means were compared by Duncan's multiple range test (DMRT) in the case where the F-test of the two-way ANOVA was at least at the $p < 0.05$ and $p < 0.01$ level of confidence.

3. Results

3.1. Accelerated Aging (AA) Effects on Germination and Emergence Traits

Seed vigor was affected by the accelerated aging test (Table 1). The seed accelerated aging reduced the germination percentage (GP), seedling traits emergence percentage, and emergence rate. The percentage of reduction of seedling traits and the percentage and speed of emergence to seed aging did not follow the same trend. So that the highest percentage reduction (PR) of germination percentage (36.9), germination rate (31.75 seed/day), and the lowest percentage reduction (PR) of germination percentage (2), germination rate (5.40 seed/day) were observed in line 3 and line 9, respectively. The highest percentage reduction (PR) of the shoot length (33.06) and the lowest percentage reduction (PR) of it (0.18) were observed in line 1 and line 6, respectively. The highest percentage reduction (PR) of root length (65.22) and the lowest percentage reduction (PR) of it (6.46) were observed in line 3 and line 8, respectively. The highest percentage reduction (PR) of the seedling vigor index (41.69) and the lowest percentage reduction (PR) of it (1.48) were observed in line 3 and line 10, respectively. The highest percentage reduction (PR) of the root shoot ratio (8.36) and the lowest percentage reduction (PR) of it (0.42) was observed in line 2 and line 5, respectively. The highest percentage reduction (PR) of the root dry weight (55.42) and the lowest percentage reduction (PR) of it (2.83) were observed in line 3 and line 10, respectively. The highest percentage reduction (PR) of the shoot dry weight (44.66) and the lowest percentage reduction (PR) of it (2) were observed in line 3 and line 11, respectively. The highest percentage reduction (PR) of the emergence percentage (19.46) and the lowest percentage reduction (PR) of it (3.73) were observed in line 3 and line 12, respectively. The highest percentage reduction (PR) of the emergence rate (27.27 seed/day) and the lowest percentage reduction (PR) of it (20 seed/day) were observed in line 2 and line 12, respectively (Table 1).

We can use the average reduction percentage for the final evaluation of cultivars regarding the effect of aging on germination percentage, germination rate, seedling traits, emergence percentage, and emergence rate. The highest and lowest mean reductions percentage of germination percentage (GP), the germination rate (GR), the shoot length (SL), the root length (RL), the seedling vigor index (SVI), the root shoot ratio (R/S), the root dry weight (RDW), the shoot dry weight (SDW), the emergence percentage (EP) and the emergence rate (ER) occurred in (Ka/b)(Cr/b)F2, La (4B,4D)/b lines and (Ma/b)(Cr/b)F4, (St/b)(Cr/b)F4 lines, respectively (Figure 1). So (Ma/b)(Cr/b)F4, (St/b)(Cr/b)F4, and (Ka/b)(Cr/b)F2, La(4B,4D)/b lines were introduced as the tolerant and sensitive lines to seed deterioration, respectively.

Table 1. Effect of seed accelerated aging on germination and emergence traits of PTCT (Primary Trans Chromosomal Tritipyrum) lines.

	Line	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
GP	NA	100 a	98 a	97.33 a	89.33 a	94.67 a	100 a	98.67 a	100 a	100 a	100 a	100 a	97.33 a	97.33 a	97 a
	A	80 b	93 b	61.33 b	70.67 b	86.67 b	81.33 b	92 b	95.33 b	98 b	98.67 b	92 b	84 b	93.33 b	96 b
PR (%)		20	5.10	36.9	20.88	8.45	18.67	6.76	4.67	2	1.33	8	13.69	4.10	1.03
GR	NA	0.24 a	0.39 a	0.38 a	0.35 a	0.35 a	0.41 a	0.43 a	0.38 a	0.37 a	0.36 a	0.43 a	0.39 a	0.33 a	0.42 a
	A	0.2 b	0.3 b	0.26 b	0.3 b	0.311 b	0.27 b	0.3 b	0.33 ab	0.35 ab	0.25 b	0.24 b	0.29 b	0.3 b	0.38 b
PR (%)		16.66	23.07	31.57	14.28	11.14	34.14	30.23	13.15	5.40	30.55	44.18	25.64	9.09	9.52
SL	NA	9.98 a	10.80 a	7.12 a	9.29 a	9.41 a	9.96 a	11.08 a	11.5 a	11.8 a	10.18 a	10.23 a	9.77 a	10.37 a	11.98 a
	A	6.68 b	9.86 b	6.62 b	7.47 b	9.3 ab	9.95 ab	8.1 b	11.4 a	10.35 ab	9.36 b	10.03 a	9.55 a	6.72 b	10.1 b
PR (%)		33.06	99.08	7.02	19.59	1.16	0.18	26.89	0.86	12.28	8.05	1.95	2.25	35.19	15.69
RL	NA	10.81 a	13.17 a	9.72 a	7.77 a	9.06 a	9.92 a	8.49 a	14.07 a	15.13 a	12 a	9.07 a	11.32 a	9.84 a	14.45 a
	A	9.86 b	8.45 b	3.38 b	5.67 b	8.41 b	8.22 b	7.77 b	13.16 ab	13.87 b	7.91 b	8.4 b	9.1 b	7.41 b	13.25 b
PR (%)		8.788	35.83	65.22	27.02	7.17	17.13	8.48	6.46	8.32	34.08	7.38	19.61	24.69	8.30
SVI	NA	797 a	1045 a	694.6 a	719.5 a	889.4 a	940.6 a	1091.7 a	1017 a	1186.6 a	936.66 a	959 a	923.81 a	971.21 a	1394 a
	A	766 a	834 b	405 b	589 b	816.8 a	822.45 b	898.9 b	956 ab	1035 ab	922.80 b	846 b	836.82 b	658.26 b	1234 b
PR (%)		3.76 b	20.19	41.69	18.13	8.16	12.56	17.65	15.83	12.78	1.48	11.78	9.41	32.22	11.47
R/S	NA	1.22 a	1.22 a	1.05 a	0.70 a	0.95 a	1.02 a	0.98 a	1.24 a	1.34 a	1.29 a	0.98 a	1.35 a	1.87 a	1.21 a
	A	0.89 b	0.2 b	0.45 b	0.62 a	0.91 a	0.79 b	0.74 ab	1.16 a	1.28 a	0.76 ab	0.88 ab	0.92 b	0.95 b	1.10 ab
PR (%)		2.70	8.36	5.71	1.14	0.42	2.25	2.44	0.64	0.44	4.10	1.02	3.18	4.91	0.90
RDW	NA	0.08 a	0.14 a	0.08 a	0.08 a	0.09 a	0.1 a	0.1 a	0.06 a	0.11 a	0.14 a	0.12 a	0.09 a	0.11 a	0.14 a
	A	0.06 b	0.10 ab	0.04 b	0.06 bc	0.06 ab	0.08 ab	0.07 ab	0.06 a	0.10 a	0.14 a	0.11 a	0.08 a	0.10 ab	0.13 a
PR (%)		25	27.97	55.42	25.97	31.03	14.43	27.83	3.38	5.50	2.83	6.77	8.04	8.84	7.14
SDW	NA	0.11 a	0.17 a	0.103 a	0.93 a	0.1 a	0.13 a	0.15 a	0.08 a	0.16 a	0.18 a	0.15 a	0.123 a	0.15 a	0.13 a
	A	0.1 ab	0.15 ab	0.06 b	0.08 b	0.08 b	0.12 a	0.10 ab	0.08 a	0.15 b	0.17 ab	0.14 ab	0.11 b	0.14 b	0.13 a
PR (%)		11.81	10	44.66	91.39	20.61	5.51	31.33	8.045	4.37	5.55	2	13.00	4.66	3.70
EP	NA	91.33 a	83.66 a	87.33 a	79.33 a	85 a	90.66 a	89.66	92.33 a	93 a	91 a	90.33 a	80.33 a	87.33 a	89 a
	A	80 b	73.66 b	70.33 c	66 b	74.66 b	80 b	78.66 b	89 a	89.6 a	80 b	80 b	77.33 b	77.3 b	79.6 b
PR(%)		12.40	11.95	19.46	16.80	12.16	11.76	12.27	6.85	6.88	12.08	11.43	3.73	11.48	10.49
ER	NA	0.18 a	0.22 a	0.16 a	0.18 a	0.22 a	0.19 a	0.19 a	0.24 a	0.24 a	0.2 a	0.2 a	0.20 a	0.22 a	0.24 a
	A	0.14 b	0.16 b	0.1 b	0.12 b	0.16 b	0.14 bb	0.14 bb	0.2°	0.2 a	0.15 b	0.15 b	0.16 b	0.17 b	0.18 b
PR (%)		22.22	27.27	37.5	33.33	27.27	26.31	26.31	16.66	16.66	25	25	20	22.72	25
Mean of PR (%)		15.64	26.88	34.52	28.15	12.76	14.29	19.02	7.65	7.46	12.50	11.95	11.85	15.79	9.32

Means that sharing the same letter does not differ significantly at $p \leq 0.05$ between the lines. The traits represent the germination percentage (GP), the germination rate (GR), the shoot length (SL), the root length (RL), the seedling vigor index (SVI), the root shoot ratio (R/S), the root dry weight (RDW), the shoot dry weight (SDW), the emergence percentage (EP), the emergence rate (ER), the percentage reduction (PR), aged seeds (A) and not-aged seeds (NA).

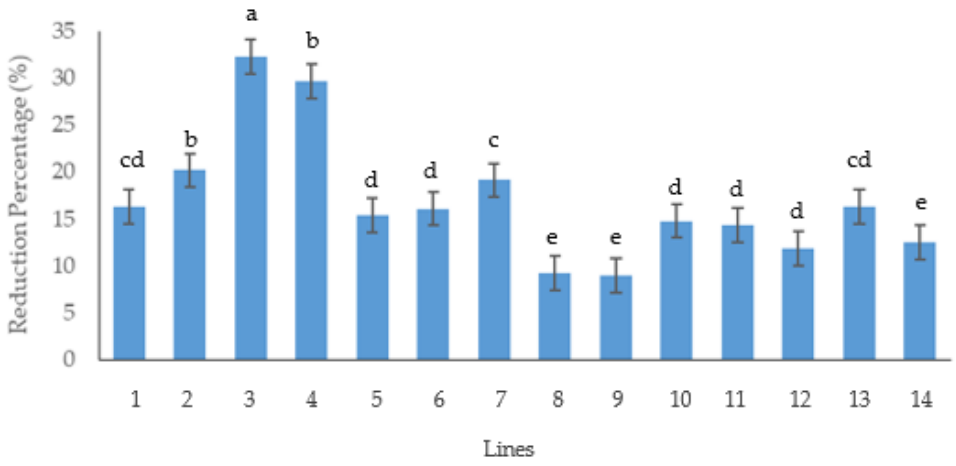

Figure 1. Reduction Percentage (RP) of the germination and emergence traits after seed aging of PTCT (Primary Trans Chromosomal Tritipyrum) lines. Results followed by the same case letter are not significantly different ($p > 0.05$).

The trends of average germination and emergence percentage were affected by seed aging of Tritipyrum lines.

Seed aging reduced germination and emergence rate (slope of germination curve over time) (Figure 2). The germination process in control seeds (not aged) and aged seeds follows the logarithmic model with 93% accuracy, and seed aging has not reduced the prediction accuracy. However, the prediction accuracy of emergence, a situation similar to a farm, has decreased by 90%.

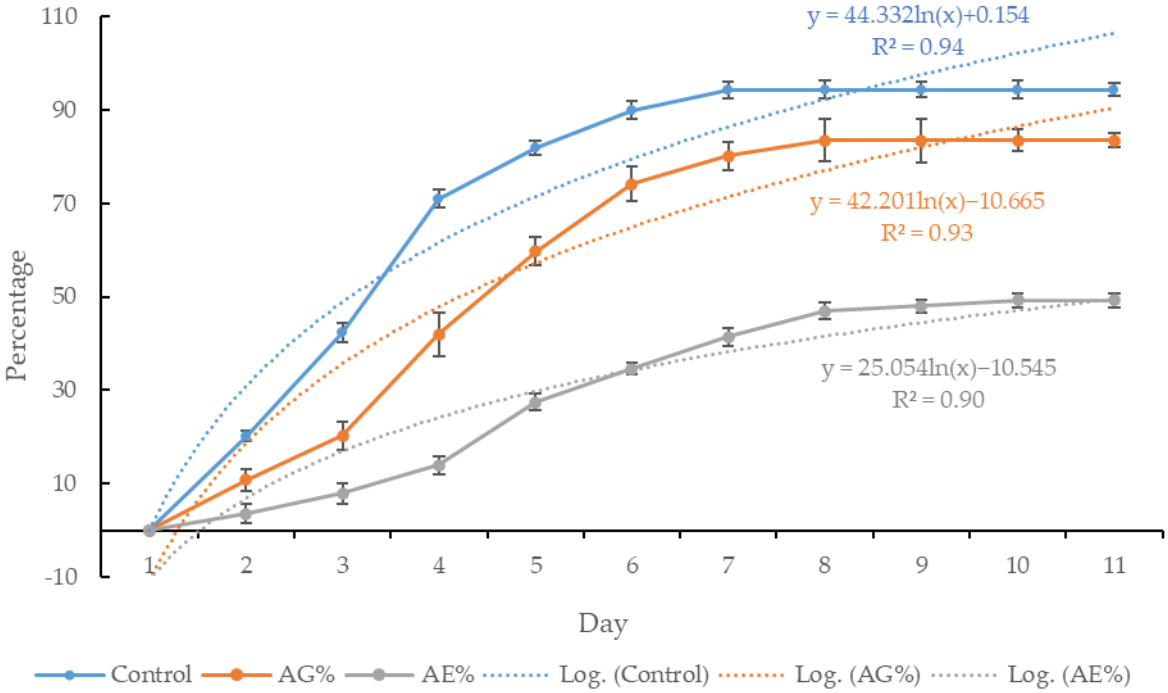

Figure 2. The effect of seed aging on the average trend germination and emergence percentage of PTCT (Primary Trans Chromosomal Tritipyrum) lines. Data represented germination and emergence from three and four replication, respectively. Control: germination percentage of non-aged seeds; AG%: germination percentage of aged seed in the laboratory; AE%: emergence percentage of aged seed in the greenhouse.

Seed aging reduced the percentage of final germination (83.5) and the percentage of final emergence (49.28) compared to the control (94.4). Additionally, seed aging decreased

the germination slope from 44.33 (control) to 42.2 and reduced the emergence percentage to 25 in the aged seeds.

Seed aging delayed reaching maximum germination and reaching maximum emergence by one and two days, respectively. Thus, the maximum germination in the control seed occurred on the sixth day, but in the aged seeds, it occurred on the seventh day, and the maximum emergence occurred on the ninth day (Table 2).

Table 2. Comparison trend of germination and germination percentage of aged seeds with control of PTCT (Primary Trans Chromosomal Tritipyrum) lines.

Day	1	2	3	4	5	6	7	8	9	DM	Slope
Control	20.2	42.4	71	82	90	94.4	94.4	94.4	94.4	6	44.33
AG%	10.8	20.3	42	59.8	74.2	80.2	83.5	83.5	83.5	7	42.2
AE%	3.57	7.85	13.92	27.5	34.64	41.42	47	48	49.28	9	25

Tolerance Index (TI) to aging was evaluated to introduce aging-tolerant cultivars. The PTCT (Primary Trans Chromosomal Tritipyrum) lines showed different responses to aging tolerance. The lowest and highest TI manifested in the germination percentage (GP), the germination rate (GR), the shoot length (SL), the root length (RL), the seedling vigor index (R/S), the root shoot ratio (RSW), the root dry weight (RDW), the shoot dry weight (SDW), the emergence percentage (EP) and the emergence rate (ER) occurred in (Ka/b)(Cr/b)F2 (56.3), La(4B,4D)/b (67) lines and (Ma/b)(Cr/b)F4 (99), (St/b)(Cr/b)F4 (98) lines, respectively (Figure 3). Finally, (Ma/b)(Cr/b)F4, (St/b)(Cr/b)F4, and (Ka/b)(Cr/b)F2, La(4B,4D)/b was introduced as the tolerant and sensitive lines to seed deterioration, respectively.

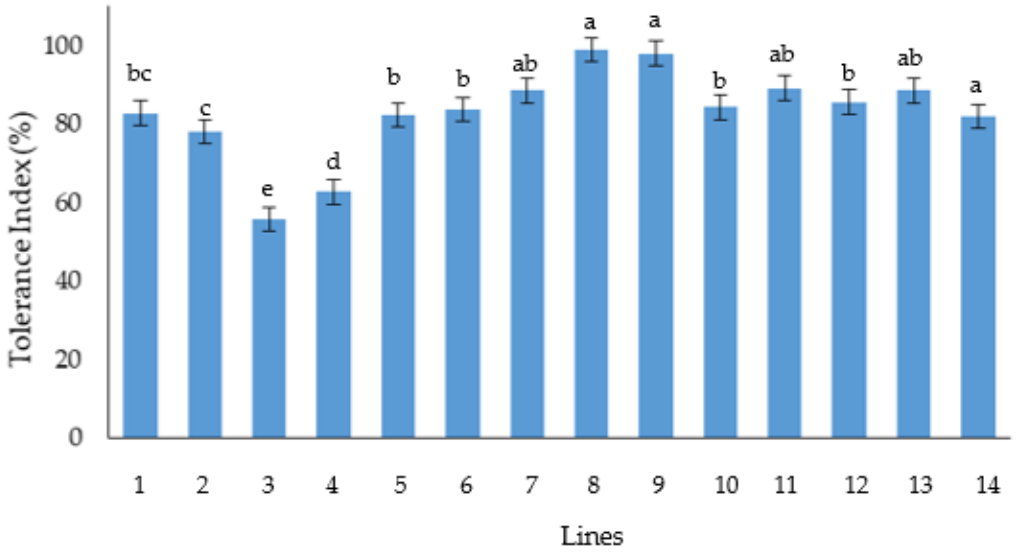

Figure 3. Tolerance index (TI) of germination and emergence traits after seed accelerated aging of PTCT (Primary Trans Chromosomal Tritipyrum) lines. Results followed by the same case letter are not significantly different ($p > 0.05$).

3.2. Biochemical Parameters and Membrane Permeability

The effect of aging on membrane permeability was estimated based on the electrolyte, protein, and potassium leakages. The EC values recorded for the seeds of PTCT lines were different. The ECs were enhanced by seed aging, so their measurements of the leachates from the aged seed were significantly more than the non-aged seed. The highest and lowest ECs were recorded in (Ka/b)(Cr/b)F2 (132.33 μS cm^{-1} g^{-1}) and La(4B,4D)/b (145.44 μS cm^{-1} g^{-1}) lines and (Ma/b)(Cr/b)F4 (58.66) and (St/b)(Cr/b)F4 (59.33) lines, respectively. In this study, protein and potassium leakage values differed after

the seed lines' aging treatments. The seed protein and potassium leakages were affected by its aging. The values of protein and potassium leakages from the aged seeds were significantly higher than those of the control treatments (not aged) (Table 3).

Table 3. Effect of seed accelerated aging effect on electrical conductivity and the protein and potassium leakage values of PTCT (Primary Trans Chromosomal Tritipyrum) lines.

	Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14
EC	**NA**	19.85	27	30.69	43.11	11.74	27.51	30.66	19.33	19.60	36.71	27	18.03	10	17
	A	109.70	97	132.33	145.44	101.19	118.69	69.23	58.66	59.33	125.60	96.04	105.38	105	74
KL	**NA**	1398	1354	1539	1553	1482	1536	1298	1003	1038	1529	1366	1422	1297	1005
(%)	**A**	2598	2553	2900	2942	2782	2700	2300	1603	1703	2728	2562	2622	2554	1953
PL	**NA**	60	64	64	59	63	61	67	561	49	19	65	62	56	67
(%)	**A**	80	86	96	95	87	76	82	68	61	63	86	82	80	79

3.3. Cytogenetic Analysis

The mitotic results followed by observing the root-tip cells demonstrated mitotic depression, various types of chromosomal abnormalities, and changes in the Mitotic Index (MI) using different artificial aging treatments to the seeds. Since the cytological qualities of the seeds were negatively influenced by Accelerated-Aging (AA) stress through increasing temperatures, using artificial aging stress was an efficient method to study the alterations caused by the seed deterioration process. Different temperatures induced various mitotic abnormalities, including chromosome bridge (a, b, c, d), chromosome fragment (e, f), chromosome stickiness (g, h), disorganized cell (i), laggard chromosome (j), chromosome break (k, l, m), unequal anaphase (n, o), and disturbed metaphase (*p*) in the root-tip cells (Figure 4, Tables 4 and 5). The highest and lowest percentages of chromosomal abnormalities were evidenced in the seeds treated at 70 °C and the control treatments. In this study, MI was an efficient index for detecting the seed deterioration process, which ranged from 2.40 to 12.00%, with a sharp decrease in rate throughout the treatments due to applying higher stress temperatures (Table 4). The highest depression in cell division was witnessed in the seed's root tips of the seeds, which were stored at 70 °C with an MI value of not more than 2.4% compared with 12% in the control treatments. In general, the rate of mitotic division is precisely related to ATP levels. Therefore, cell division can be an energy-dependent process. Chromosome movement mainly depends on the energy-generating system. It can be assumed that any toxic materials in the aged seeds may disturb their respiratory pathways, producing low-energy and other essential ATP compounds like sugars and protein molecules. PTCT lines also showed a susceptible attitude toward long-term storage in the gene bank and maintained germination and vigor for a longer period. This aspect is in line with the chromosomal abnormalities induced by AA.

Table 4. Different mitotic phases of root cells under five increasing aging treatments.

Treatment *	TA	CD		CI	CP		CM		CA		CT		MI (%)
		RE	IR		RE	IR	RE	IR	RE	IR	RE	IR	
T1	500	59	1	440	14	-	17	-	16	1	12	-	12.00
T2	500	38	3	459	15	-	16	-	1	3	6	0	8.20
T3	500	30	9	461	12	1	12	5	1	3	5	-	7.80
T4	500	16	9	475	15	-	-	3	1	3	-	3	5.00
T5	500	6	6	488	5	1	-	1	-	2	1	2	2.40

* T1: control; T2: 24 h at 40 °C; T3: 24 h at 50 °C; T4: 24 h at 60 °C; T5: 24 h at 70 °C. Regular Cells (RE); Irregular Cells (IR); Total number of cells analyzed (TA); the number of cells under different phases of mitotic division (MD) at cell interphase (CI), cell prophase (CP), cell metaphase (CM), cell anaphase (CA), cell telophase (CT) and the mitotic index (MI) in seeds submitted to five different aging treatments.

Table 5. Types of cell division abnormalities mitotic phases of root cells under five increasing aging treatments.

Treatment *	CD	CB	B	DC	TIC	Irregular Cells (%)
T1	60	1	-	-	1	1.66
T2	41	2	-	1	3	7.31
T3	39	1	-	8	9	23.07
T4	25	1	7	1	9	36.00
T5	12	1	5	-	6	50.00

* T1: control; T2: 24 h at 40 °C; T3: 24 h at 50 °C; T4: 24 h at 60 °C; T5: 24 h at 70 °C; Total Number of cells under mitotic division (CD); number and types of cell division abnormalities: Chromosome Breaks (CB); Bridge (B); Disorganized Cells (DC) and Total number of Irregular Cells (TIC) in seeds submitted to five different aging treatments.

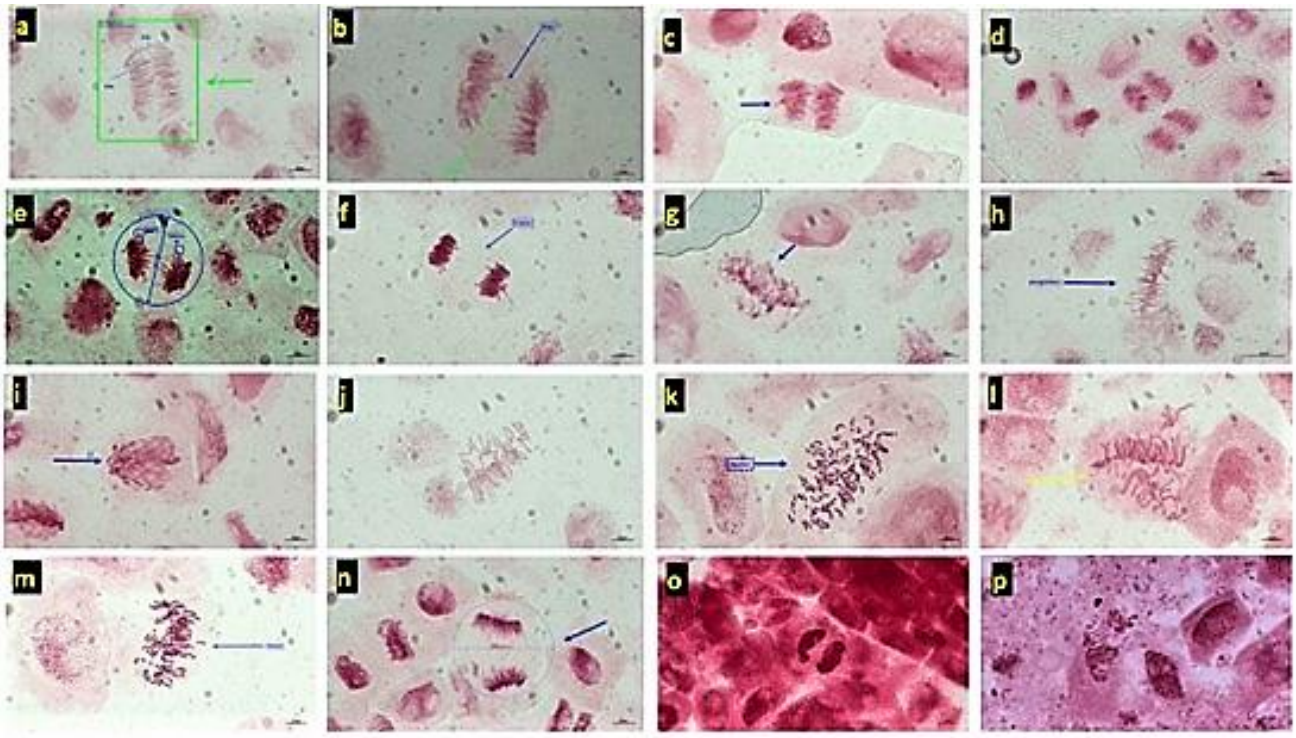

Figure 4. Different abnormalities in mitosis and chromosomes on aged seeds; Chromosome Bridge (**a**–**d**); Chromosome Fragment (**e**,**f**); Chromosome Stickiness (**g**,**h**); Disorganized cell (**i**); Laggard Chromosomes (**j**); Chromosome Break (**k**–**m**); Unequal anaphase (**n**,**o**); Disturbed Metaphase (**p**).

3.4. Seed Priming

GP, GR, and seedling traits of the aged seeds of PTCT (Primary Trans Chromosomal Tritipyrum) lines were improved by pretreatments. GP and GR were significantly augmented by priming. Their highest values were observed in the seeds primed with PEG6000 and NaCl, respectively. Treatment with polyethylene glycol ameliorated GP up to 69/58 compared to the control treatments (unprimed seeds). Seed priming of the deteriorated seeds significantly impacted the SVI and GE values of the seedlings. The highest SVI and GE values were observed in the deteriorated seeds primed with PEG6000. Therefore, treatment with PEG6000 enhanced SVI and GE in the seedlings up to 13/15 and 2/23, respectively, compared to the control treatments. Root Dry Weight (RDW) and Shoot Dry Weight (SDW) were also significantly augmented by priming (Table 6).

Table 6. Effect of different seed priming treatments on PTCT lines for germination percentage, germination rate, and seedling traits of aged seeds.

Germination Traits	Control	NaCl	Salicylic Acid	Polyethylene Glycol
GP	40 b	57.92 ab	65.00 ab	69.58 a
GR	0.30 b	0.39 a	0.37 a	0.37 a
SL1	7.69 b	11.11 a	12.25 a	11.84 a
GE	1.19 b	1.65 ab	2.00 a	2.23 a
RL	4.04 b	6.47 a	6.89 a	6.97 a
SL2	11.73 b	17.49 a	19.14 a	18.81 a
SVI	4.72 b	10.50 ab	12.36 ab	13.15 a
RDW	0.03 b	0.07 a	0.08 a	0.07 a
SDW1	0.04 b	0.08 a	0.10 a	0.10 a
SDW2	0.07 b	0.15 a	0.17 a	0.16 a

Means with the same letter are not significant at $p \leq 0.01$. The traits represent the germination percentage (GP), the germination rate (GR), the shoot length (SL1), the germination energy (GE), the root length (RL), the seedling length (SL2), the seedling vigor index (SVI), the root dry weight (RDW), the shoot dry weight (SDW1), the dry seedling weight (SDW2).

4. Discussion

The reduction percentage and the Tolerance Index of germination and emergence traits of the aged seed showed that (Ma/b)(Cr/b)F4, (St/b)(Cr/b)F4 PTCT lines are the most tolerant lines to seed deterioration. Instead, the more sensitive lines were (Ka/b)(Cr/b)F2 and La(4B,4D)/b. The results obtained from seed aging about (St/b)(Cr/b)F3 line is promising, also considering its potential in salinity tolerance, as demonstrated by Pirsalami et al. (2021) [59]. Furthermore, seed aging decreased cell membrane stability and increased the electrolyte, protein, and potassium leakages. This physiological parameter was inversely related to seed quality [60]. Differences in the EC values indicated that the membrane's nature and extent of protection were not the same in the studied seed lines. Seed deterioration is associated with increased permeability of the seed membrane, leading to higher electrolyte leakage during imbibition [61]. During storage, the seeds gradually get old and lose their strength through coagulation and the breakdown of proteins [62]. The more sensitive the storage condition, the faster the process develops as the temperature of the seed mass increases [63]. We noticed that artificial aging causes cytological damage in seeds and chromosomal abnormalities. It is known that chromosomal aberrations increase during seed aging [64]. We observed that, at mitosis, the enhancing seed age led to the percentage reduction of the dividing cells. With increasing temperature, the number of cells under division decreases while chromosomal abnormalities and injuries increase, leading, for example, to the formation of anaphase and telophase bridges [65–67]. The chromosomal bridge may be caused by chromosomal adhesion and the subsequent inability of chromosomes to initiate a normal anaphase separation. It may be attributed to unequal transmission or the origin of chromosomal fragments [68]. Hence, cell division phases are considered helpful for detecting the seed deterioration process. This study's cell numbers at the interphase stage ranged from 440 to 488. MI indicates the frequency of mitotic division, in which temperature increase and reduces. With increasing deterioration time, a decrease in cell division was observed.

Chromosomal abnormalities in the mitotic cells of degraded seeds are detected via micronucleosis and chromosome breakage and bridge [69]. Chromosome fragment formation might be due to chromosomes' stickiness followed by separation failure. Breakage and reunion of the broken ends lead to chromosome bridges, which could be observed until early telophase. Our results of cytological changes in the root-tip cells of the aged seeds confirmed the previous studies [69,70]. In prophase, chromosomes are already visible, preparing to enter metaphase. When they exhibit a distinct pattern of organization, any irregularities occur probably due to mitotic spindle formation and chromosome condensation triggered by already duplicated chromosomes in the interphase. Chromosome disorganization at this stage would interfere with the regularity of metaphase chromosomes

due to disturbances in mitotic spindle organization. Disorganized metaphase chromosomes appear due to their diffuse arrangements in the equatorial planes of the cells. This chromosome aberration is considered a sign of spindle malformation or partial inactivation. However, little is known about the activities of the directly regulated zones [71]. Therefore, the chromosomes organized in the equatorial plates represent regular cell divisions in the metaphase [72]. Only low cell irregularities were detected in the control treatments in this research, while aberrations were observed in the stress treatments.

Applying priming in the accelerated seed of PTCT lines was promising (Table 6). It was observed that primed seeds' germination traits were greater than untreated seeds. In addition, the germination traits were enhanced by applying all three priming treatments (halo-priming, hormone-priming, and osmo-priming). Seed priming promotes efficient germination by inducing a more favorable physiological state. It regulates hydration that starts the normal metabolic process during the early stages of germination before the protrusion of the radicle [73]. In this study, osmo-priming increased germination traits further than hydropriming and hormone-priming (Table 6), as Mohajeri et al. (2016) observed. They reported that polyethylene glycol application for seed priming improved vetch seeds' root dry weight and shot dry weight values [74].

Furthermore, Chen et al. (2021) observed that PEG6000 application for seed priming improved germination percentage and germination rate [75]. Osmo-priming can benefit abiotic stresses like drought, extreme temperature, and salinity, generating different pre-germination metabolic activities, improving the antioxidant system, and preparing the seed for radicle protrusion [76]. In our study, priming significantly affected shoot and root length (Table 6). The highest and lowest mean values of these traits were observed in hormonal priming with SA and the control treatments, respectively (Table 6). These outcomes match with those attained by Tabatabaei [77]. Many field crops have reported increasing SVI and GE values in seedlings by priming seeds [78–81]. Rosinska et al. (2023) reported the benefits of osmo-priming on germination and seedling vigor in carrots seeds. They demonstrated the potentiality of this treatment also to mitigate biotic stress [82]. PTCT lines' best germination percentage, germination rate, shoot length, root length, seedling vigor index, and dry seedling weight were observed in seeds treated by osmo-priming compared to the control. Possible mechanisms for improved germination traits by halo-priming, hormone-priming, and osmo-priming are activation of water-induced metabolic processes, improved repair due to enzyme activity, and production of hormones. Osmo-priming with PEG enhances some of the antioxidant enzymes, such as the activities of catalase-peroxidase in seedlings exposed to stress, thus increasing seed germination and stand establishment [83]. In other research, Zhang and colleagues found that osmo-priming with PEG improved cell membrane stability, enhanced superoxide dismutase, peroxidase, and catalase, and decreased lipid peroxidation, compared to non-primed seeds [84].

5. Conclusions

It is a well-known fact that some seeds of Tritipyrum lines are susceptible to aging. The latter significantly decreases germination and emergence traits and enhances leakage of cellular solutes of PTCT (Primary Trans Chromosomal Tritipyrum) lines. In this research, these responses to seed deterioration were varied, indicating genetic diversities of the PTCT lines. These genetic diversities were also underlined by the different Tolerance Indexes (TI) obtained after aging. Some PTCT lines were prone to long-term storage in the gene bank and could maintain germination and vigor, as also evidenced by the chromosomal abnormalities induced by the artificial aging of seeds. Nevertheless, the vigor of the deteriorated seeds of the PTCT lines was significantly improved by priming. The treatment could ameliorate the germination and seedling establishment rate of the PTCT lines of the aged seeds. These findings are in line with our previous studies on other cereals like wheat and triticale. We observed that these PTCT lines gave a positive response to priming. In addition, we observed an enhancement of the early vigor of Tritipyrum line seeds by using priming.

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