Growth regulators enhance starch depletion in aged triticale (*Triticosecale Wittmack*) seeds

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Abstract

Seeds deteriorate rapidly and lose viability under poor storage conditions. This study investigated the potential of priming with various growth regulators to invigorate artificially aged triticale seeds. A factorial experiment based on a completely randomized design with three replications was conducted in 2023. Treatments included three levels of seed aging (95%, 85%, and 75%) and six priming methods (control, distilled water, cytokinin, spermidine, salicylic acid, and auxin). Aging significantly reduced germination percentage, seedling dry weight, vigor index, starch reserve mobility, and amylase activity. However, priming mitigated these effects and improved germination by 36.9%. Notably, auxin priming at the lowest seed vigor level (75%) increased starch reserve mobility and enhanced amylase activity, seedling dry weight, and seedling vigor index by 9.2, 1.5, and 1.8 times, respectively, compared to the unprimed control. While previous studies have focused primarily on the role of gibberellic acid, this study highlights the effectiveness of auxin in restoring vigor and promoting seedling development in aged triticale seeds.

Keywords: amylase, auxin, cytokinin, gibberellic acid, priming, reserve mobility, seed vigor

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Introduction

Among agricultural plants, cereals have been one of the main components of the human diet and have played an important role in the formation of human civilizations (Yang et al., 2018). Triticale (*Triticosecale Wittmack*) is a cereal grain that was created by humans through the crossbreeding of wheat as the maternal parent and rye as the paternal parent to combine the performance and quality characteristics of wheat with the disease resistance and adaptability to environmental stresses of rye, all in one plant (Bezabih et al., 2019), and can be a suitable substitute for wheat and barley in low-input systems (Mikić et al., 2015).

Seed deterioration is an important issue in seed storage, resulting in increased production costs for agricultural products. In some countries and geographic areas where seeds are exposed to high temperature and relative humidity during the maturation and storage periods, deterioration is significantly greater (Kim, 2018). Govindaraj et al. (2017) stated that during deterioration, respiration rates, the activity of enzymes involved in seed reserve degradation, and the activity of antioxidant enzymes decrease. Various research findings have shown that deterioration leads to a

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reduction in the length of the shoot and root, the dry weight of seedlings, the allometric coefficient, seedling vigor indices, the activity of seed antioxidant enzymes, and an increased sensitivity to stresses in different plants (Baharvand et al., 2017; Darabi et al., 2017; Govindaraj et al., 2017; Kapilan, 2015). This can have an impact on the primary heterotrophic growth component. It can be noted that the decrease in the weight vigor index of seedlings is due to the reduction in its components, namely germination percentage and seedling dry weight, both of which decrease under seed deterioration conditions.

Starch is an important source of energy and has a close relationship with seed vigor and the germination process. A low rate of starch metabolism during the germination stage can lead to unsuccessful seed germination (Zaynab et al., 2018). Seed deterioration leads to a decrease in the activity of lipase, amylase, and malate synthase enzymes (Khatami et al., 2019).

Seed priming is an easy, cost-effective, and safe technique to enhance germination, seedling growth, yield, and drought tolerance in agricultural crops (Hussain et al., 2015). Seed priming induces a series of biochemical changes, such as enzyme activation, hydrolysis (Iqbal et al., 2020), metabolic repair (Farooq et al., 2006), and the generation of metabolites that promote germination (Hussain et al., 2016), leading to accelerated seed germination and seedling growth, improved seed quality, and ultimately increased seedling vigor indices.

Research has shown that primed seeds with hydropriming, salicylic acid, and gibberellin have relatively higher seedling vigor length and weight indices (Saadat and Sedghi, 2021; Saadat et al., 2020). This is a controlled hydration technique that stimulates metabolic processes before germination, such as increased water uptake and the activation of amylase, cellulase, and xylanase enzymes, without actual germination occurring in the seeds, leading to the establishment and growth of seedlings (Marthandan et al., 2020). Priming increases, the activity of proteases, lipases, and amylases, which degrade food reserves to enhance the growth and development of the embryo (Acharya et al., 2020)). Additionally, priming positively affects germination, early seedling establishment, precocity, and both quantitative and qualitative yield (Souri et al., 2017). Seed priming enhances the levels of storage materials, proteins, and nucleic acids in seeds, thus improving the vigor, establishment, and final performance of the plants (Paravar and Farahani, 2017). Huang et al. (2017)found that priming seeds with spermidine increases seed germination and seed vigor indices. Priming increases the activity of proteases, lipases, and amylases, which break down food reserves to enhance embryo growth and development (Acharya et al., 2020)stated in their research that the use of seed priming technique results in an increase in seedling vigor index. Therefore, it appears that seed hydropriming compensates for some of the damaging effects caused by deterioration (Mc Donald, 2000). Primed seeds exhibit faster germination compared to non-primed seeds; hence, they can produce a more vigorous and dry matter plant within a specific time frame compared to non-primed seeds (Pawar and Laware, 2018).

According to the study by Pangestuti et al. (2021), seed priming with plant growth regulators (PGR) significantly increased starch concentration in radicle tissue. In the present study, we investigated the stimulatory effects of some PGRs on starch mobility and physiochemical traits of artificially deteriorated triticale seeds.

Materials and Methods

This experiment was conducted at the Laboratory of Seed Science and Technology, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Iran in a factorial arrangement based on a completely randomized design with three replications in 2023. Treatments were aged seeds (95%, 85%, 75%) and PGR priming (control, distilled water, cytokinin, spermidine, salicylic acid, and auxin). Triticale (*Triticosecale Wittmack*) seeds were held at 40 °C and approximately 100% relative humidity for 48 and 120 h, respectively to reach 85% and 75% aging levels in rapid aging procedure (Milivojević et al., 2018). Control seeds germination percentage (GP) was 95%. These durations were obtained from the preliminary test, which lasted for one month, at the end of which the seed GP reached zero (Delouche and Baskin, 2021).

For priming application, seeds were soaked in distilled water and PGR solutions for 24 hours at 20 °C in the dark. Concentration of PGRs was 1 mM according to the results of a preliminary test in which the concentrations of 0.25, 0.5, 1, 4.5 and 2 mM of each PGR were tested and 1 mM was selected as the best concentration based on GP results (data are not shown).

After completion of treatments, seeds were rinsed three times with tap water, surface-dried, and allowed to dry at room temperature for 2 days to reach the primary moisture content. After the priming and aging treatments, the seeds were held in room conditions to allow their moisture content to equilibrate. Unprimed and unaged seeds were used as a control.

Germination test and seedling weight vigor index (SWVI)

The germination test was performed at 25 °C for 10 days with 400 seeds (4 replicates with 100 seeds) according to ISTA (2012) rules in Petri dishes and transferred to a germinator (IKH.RI, Iran) with 8 h light period. Germinated seeds were counted every 24 h (2 mm radicle emergence was considered as germinated seed) until the number of germinated seeds was constant in three intervals days. GP was calculated by a Germin program (Soltani et al., 2013).

On the last day of counting, seedling fresh and dry weight (determined at 80 °C for 24 h) were measured on ten randomly selected normal seedlings. The seedling weight vigor index (SWVI) was calculated according to the following equation (Ebrahimi et al., 2013):

SWVI = GP (%) * seedling dry weight (g)

Biochemical measurements

Assessment of alpha-amylase activity

The enzyme activity was determined according to the method of Doman et al. (1982) four days after germination. The seeds were homogenized in 60 mM phosphate buffer (pH 6.8) and then centrifuged at 12000 g for 15 minutes. The enzyme activity was determined in the reaction medium containing 60 mM phosphate buffer (pH 6.8), 400 µg ml^{-1} calcium chloride, and 500 µg ml^{-1} starch. The enzyme extract (1 ml) was added to the assay medium after 20 minutes of incubation in a water bath. The alpha-amylase enzyme activity was determined using starch at a wavelength of 620 nm as µg of starch degraded per minute per gram of fresh material.

Starch remobilization

To analyze starch content and its depletion during imbibition, samples of dry and imbibed seeds (from day 1 to day 5 of germination test) were ground and extracted twice with 80% ethanol and then twice with 52% perchloric acid. Starch content was determined using anthrone-sulfuric acid method (Fernandes et al., 2012)at 640 nm with a spectrometer.

Statistical Analysis

Data were subjected to normality test using SPSS 19 under 1-sample K-S tab and then, regression analysis was performed on starch depletion data by Enter method in SPSS 19 software. Analyses of variance and comparison of means were performed using SAS 9.2 software. Means were compared based on the Duncan test at p<0.5.

Results

GP and growth parameters

Germination and seedling growth were affected by rapid aging and PGR treatment (Table 1). GP ranged from 93 to 71% by aging and 71 to 99.33% by priming (Table 2). GP decreased by aging, but priming significantly promoted it and the highest GP was observed in auxin pretreatment which was approximately 6, 14, and 20% higher than control in all aging levels (95, 85, and 75%, respectively).

The highest seedling dry weight (SDW) and SWVI was also related to auxin treatment in non-aged seeds (Table 2) and the effect of PGRs in low vigor seeds was greater than non-aged seeds. Interaction effect of aging and PGRs was not significant on seedling fresh weight (SFW), but simple effects were significant (Table 1). Auxin

Table 1
Variance analysis of the effect of Seed vigor and priming on some traits of triticale

Sources of variation	df	Mean Squares				
		Germination percent	Seedling fresh weight	Seedling dry weight	Seedling weight vigor index	amylase activity
Seed vigor	2	1393.5**	1.368**	0.1519**	2320.1**	0.0228**
Seed priming	5	149.12**	0.063**	0.0183**	254.6**	0.0092**
Seed vigor × seed priming	10	9.5296**	0.545ns	0.0001**	2.499**	0.0002*
Error	34	0.998	0.0153	0.00001	0.224	0.00008
C.V. (%)		1.12	11.6	0.87	1.39	9.04

* and ** represent significant differences at 5 and 1 percent probability level, respectively.

Table 2. Mean comparison for the effect of seed vigor and priming on germination percentage, seedling dry and fresh weight and amylase activity in triticale.

Treatment combination	Germination (%)	Seedling dry weight (g)	Seedling weight vigor index	Amylase activity (unit/mg protein)
V1×P1	93^{f}	0.404^{e}	37.63^{g}	0.1^{f}
V1×P2	95.66^{de}	0.442^{d}	42.28^{e}	0.116^{ed}
V1×P3	97^{cd}	0.486^{b}	47.14^{c}	0.12^{cd}
V1×P4	98.66^{ab}	0.488^{b}	48.18^{b}	0.14^{c}
V1×P5	97.66^{bc}	0.465^{c}	45.44^{d}	0.116^{de}
V1×P6	99.33^{a}	0.532^{a}	52.87^{a}	0.18^{a}
V2×P1	83^{kl}	0.311^{j}	25.81^{l}	0.07^{g}
V2×P2	85^{ij}	0.335^{h}	28.531^{k}	0.08^{g}
V2×P3	88.66^{h}	0.388^{f}	34.46^{i}	0.1^{f}
V2×P4	91^{g}	0.393^{f}	35.82^{h}	0.12^{de}
V2×P5	92.66^{f}	0.389^{f}	36.07^{h}	0.11^{ef}
V2×P6	94.66^{e}	0.438^{d}	41.466^{f}	0.16^{b}
V3×P1	71^{o}	0.223^{m}	15.87^{p}	0.013^{j}
V3×P2	75.33^{n}	0.242^{l}	18.23^{o}	0.03^{j}
V3×P3	78.33^{m}	0.289^{k}	22.69^{n}	0.05^{h}
V3×P4	82^{l}	0.322^{i}	26.4^{l}	0.07^{g}
V3×P5	83.66^{jk}	0.288^{k}	24.09^{m}	0.07^{g}
V3×P6	85.66^{i}	0.351^{g}	30.06^{j}	0.12^{de}
LSD	1.658	0.0055	0.786	0.0148

V_1, V_2, and V_3 are 95%, 85%, and 75% of viability, respectively. P1, P2, P3, P4, P5, and P6 are without priming, priming with distilled water, priming with cytokinin, priming with spermidine, priming with salicylic acid, and auxin, respectively. Means with similar letters in each column are not statistically different based on Duncan's test.

Table 3
Enter method for regression analysis of starch depletion prediction during triticale seed imbibition influenced by seed vigor and priming

Model	B	SE	Beta	t
Constant	765.58	18.02	-	42.49
Seed vigor	-1.194	5.623	-0.006 ns	-0.212
Priming	- 19.25	2.69	-0.216 **	-7.16
Imbibition time	-82.38	2.69	-0.926**	-30.64
	R= 0.95	R^2= 0.905	Adj R^2= 0.902	

ns and ** are non-significant and significant at 1% probability level, respectively.

priming increased SFW about 24% in comparison to control (Fig. I. A) and Aging decreased it about 70% (Fig. I. B). SWVI decreased by aging while priming specially with auxin had magnificent effect. Maximum SWVI was observed in non-aged seeds under auxin priming (52.87), but increase in aged seeds was greater than in control and reached 89.4, 60.7, and 40.5, respectively at 75, 85 and 95% aging levels (Table 2).

Amylase activity

Aging sharply decreased amylase activity and its reduction was 13 folds from 0.1 at 95% germinability to 0.013-unit mg^{-1} protein at 75% (Table 2). Surprisingly, auxin priming increased amylase activity by approximately 2, 2.5, and 9 folds at different aging levels, and at 75% aging the enzyme activity was more influenced by auxin (Table 2).

Starch reserves mobilization

The results of the regression analysis indicated that approximately 90% of the variance in starch reserve mobilization can be predicted by three variables including vigor, priming, and seed imbibition time (Table 3). Among these three variables, priming and seed imbibition time have a significant relationship with starch reserve mobilization. In other words, based on the beta

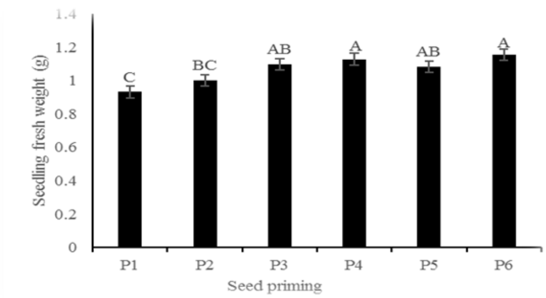

Fig. I. A. Comparison of the effect of priming on fresh weight of triticale seedlings.

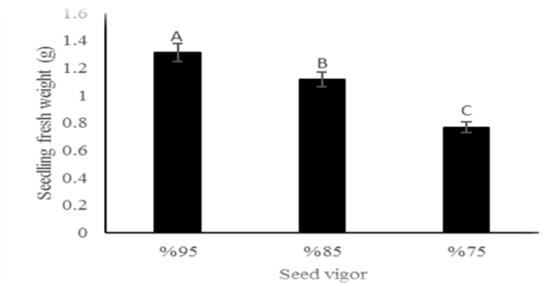

Fig. I. B. Comparison of the effect of seed vigor on the fresh weight of triticale seedlings.

coefficients, the seed imbibition time variable has the greatest impact on starch reserve mobilization in triticale seeds, followed by the type of priming. On the other hand, the non-significant effect of vigor variable may indicate an improvement in seed vigor through priming treatments and suggests that priming has been able to strengthen aged seeds and increase the utilization of starch reserves in weak seeds (Table 3).

Depletion of starch reserves through time of imbibition is presented in (Fig I). The lowest curve in each aging level (Fig. I. A-C) was related to auxin priming, indicating the influence of auxin in consuming starch, which is inconsistence with the data of amylase activity (Table 2). The Highest amylase activity, the lowest starch residual in the seeds.

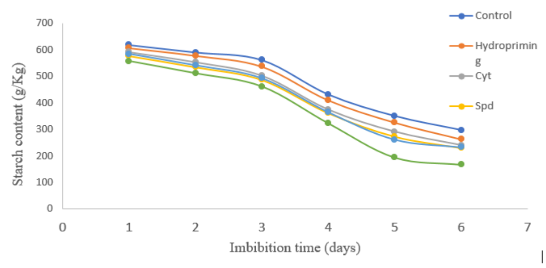

Fig. I. C. Dependence of starch content on germination time and priming treatments at high vigor (95%) of triticale seeds

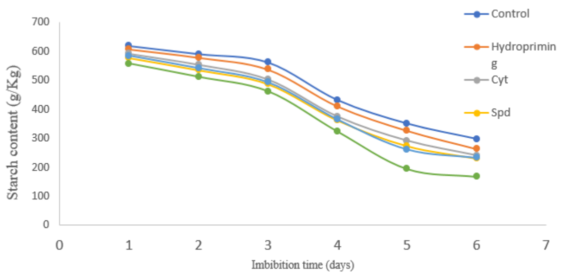

Fig. I. D. Dependence of starch content on germination time and priming treatments at medium vigor (85%) of triticale seeds

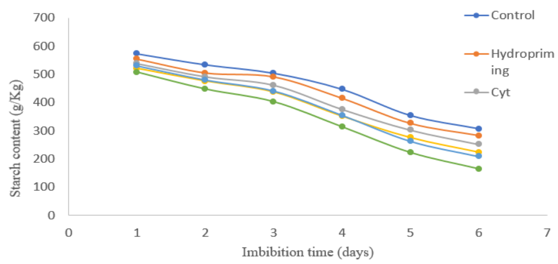

Fig. I. E. Dependence of starch content on germination time and priming treatments at low vigor (75%) of triticale seeds

Discussion

The increase in seedling weight during priming may be due to increased mitotic activity and a higher number of cells in roots and shoots, leading to greater accumulation of dry cell matter (Ahmadvand et al., 2023). The results from the comparison of means in the research by Ghasemi et al. (2014)indicated that hydropriming increased the dry weight of the produced seedlings by 35.7%. Furthermore, Jalali-Honarmand et al. (2018)reported that hydropriming increased the fresh weight and height of pea seedlings by 75.4% and 30.1%, respectively.

With an increase in salicylic acid concentration, the levels of photosynthetic pigments, particularly chlorophyll, and cell division in bean plants increased, resulting in greater height and dry weight of the plants (Shoghian and Roozbahani, 2017), which aligns with the findings of Edalateyan Kharazi and Asgharzadeh (2023). AGHLMAND et al. (2017)stated that salicylic acid increases growth and dry weight in wheat seedlings by enhancing cell division in the meristem. In basil plants, an increase in various growth indices, including fresh and dry weight of aerial parts, has been reported with salicylic acid application (Mohammadi Babazidi et al., 2013).

The superiority of pre-treated seeds in producing larger seedlings can be attributed to faster germination rates. The pre-treated seeds had a higher germination percentage compared to the control seeds, and this led to a greater production of dry matter at a given time (Mahmoudi et al., 2019). These results are consistent with those of other researchers, including Najjarzadeh et al. (2017), regarding salicylic acid and putrescine's effects on some physiological indices of peppermint (*Mentha piperita* L.).

One of the key indicators of seed quality is the seed vigor index, which is influenced by the final germination percentage and seedling length. The results of the research by Saadat and Sedghi (2022) showed that deterioration negatively affects growth indices, including root length, shoot length, fresh weight of the root and shoot, and both the length and weight vigor indices of the seeds. In this study, the length and weight vigor indices also decreased during deterioration. Factors affecting seed vigor include the environmental conditions of the parent plant, maturity stage, seed size and weight, mechanical damage, and seed deterioration (Čanak et al., 2016).

According to the results of Pour Rahim Aliabadi et al. (not published), among different priming methods, hydropriming, priming with polyethylene glycol, and priming with potassium nitrate resulted in increased dry weight of marigold seedlings. Seeds primed with a concentration of 500 µM salicylic acid produced seedlings with thick leaves and high weight. Primed seeds with a concentration of 500 µM salicylic acid had the highest seedling wet weight (0.652 g), which were significantly different from seedlings from control seeds and those primed with distilled water and a concentration of 200 µM salicylic acid. It has been reported that auxin can interact with sugars under stress by modulating the expression and activity of alpha-amylase, a key enzyme involved in carbohydrate metabolism (Khan et al., 2024). Therefore, by regulating the activity of α-amylase, auxin can affect starch degradation, leading to changes in metabolism and sugar allocation. Zhao et al. (2020)hypothesized that IAA priming could enhance seed germination by modulating the levels of endogenous hormones, including GA, ABA, and IAA itself. The role of IAA in improving seed vigor and germination is further highlighted by its regulation of sugar metabolism and starch synthesis.

Queiroz et al. (2023)report that IAA seed priming affects the activity of starch metabolism enzymes in primed soybean seeds. However, our findings are consistent with those of Abdel Latef et al. (2021), who found that IAA is an effective regulator of carbohydrate metabolism in bean seeds. At temperatures of 15 and 25 °C, the highest values for length and weight indices compared to the control were obtained from concentrations of 0.5 and 1.0 mM salicylic acid (Bahadori et al., 2017). Decreased germination temperature resulted in a significant decrease in length and weight vigor indices.

In their study, Fazeli Kakhki and Beikzadeh (2022)found that the Specific Seedling Vigor Index (SVWI) was influenced by the interaction of IBA (natural auxin) and IBA×GA3, with GA3 not showing a significant effect on this index. The maximum SVWI index (5.80) was recorded at 0 ppm IBA, which decreased with increasing concentrations of IBA to 100 and 200 ppm, but statistically, this value did not differ significantly from the treatments of GA1, GA2, and GA3. The interactive effect (IBA×GA3) showed that the SVWI index had different trends, with the maximum SVWI index (77.6) in the IBA1×GA3 treatment and the minimum in the IBA3×GA32 treatment (Fazeli Kakhki and Beikzadeh, 2022). The interactive effects of auxin and cytokinin on the weight and length vigor index of the seed were significant (Sarami et al., 2017).

Saadat and Sedghi (2022) reported that the length and weight indices decreased during aging, but priming increased these indices, and the effect of priming with gibberellin was more than hydropriming and salicylic acid.

Energy supply during the germination stage of seeds is derived from the breakdown of stored materials in the seed, which is primarily starch in the case of sorghum grains. An experiment conducted by Min Zhang et al. (2022)showed that the starch content in aged sorghum grains is lower than in normal grains before germination, indicating that starch is consumed during the aging process. The starch content in normal grains rapidly decreased after germination, while the starch content in aged grains decreased at a significantly slower rate than in normal grains. Consequently, the soluble sugar content in aged grains decreased in comparison to normal grains at later stages, indicating that seed aging may affect energy supply in germinating seeds. Spermidine exogenous treatment improved the conversion of starch to soluble sugars during germination (Zhang et al., 2022).

Use of spermidine has led to a significant increase in the activity of α and β-amylases, fructose and glucose content, and the transcription level of the β-amylase gene in seeds (Liu et al., 2016). Soaking seeds with spermidine significantly improved seed germination performance under drought and osmotic stress, regulated the activities of alpha and beta-amylases, reduced starch content, and decreased sugar and glucose content (Liu et al., 2021).

Moreover, according to Habibi et al. (2014), salicylic acid treatment significantly increased starch concentration in the root organ of Bright Yellow-2 tobacco (BY-2) cells. In Nicotiana tabacum BY-2 cultured cells, the reduction of auxin (2,4-dichlorophenoxyacetic acid) led to starch accumulation, which was accelerated by adding cytokinin (benzyladenine).

In a series of experiments, auxin and cytokinin have been shown to have different effects on the growth and development of amyloplasts. Auxin reduces the rate of amyloplast growth while cytokinin increases starch accumulation and related biosynthesis transcription. This indicates that the regulation of starch biosynthesis-related transcription by these hormones may lead to their contrasting effects (Abdulsalami et al., 2024).

Saadat and Sedghi (2023a) reported that deterioration reduces the activity of alpha-amylase, and as the intensity of deterioration increases, the enzyme's activity decreases. One of the significant effects of priming is the increased activity of α-amylase, an enzyme responsible for the degradation of stored carbohydrates. This enhanced enzymatic activity increases the availability of energy-rich compounds needed for germination (Li et al., 2017). Saadat et al. (2020) stated that priming with distilled water, salicylic acid, and gibberellin increased amylase activity in rice.

Cytokinin facilitates growth and cell division in seed embryo by stimulating DNA and RNA synthesis, aiding in germination, either by increasing alpha-amylase activity and starch hydrolysis or by enhancing the permeability of the plasma membrane, leading to faster translocation of materials during germination (Nabaei et al., 2011).

According to the study by Ellouzi et al. (2024), seedlings from IAA-primed seeds showed the highest sugar accumulation levels along with the highest α-amylase activity in both roots and coleoptile. Therefore, it can be reasonably suggested that the presence of IAA in seeds (during priming) leads to increased metabolic activation for the degradation of starch reserves.

Conclusion

Based on the results of this study, although deterioration decreased seedling dry and fresh weight, seedling weight vigor index, and amylase enzyme activity of triticale seedlings, pre-treatment of seeds with growth regulators such as salicylic acid, cytokinin, spermidine, and auxin had a significant impact on improving and enhancing seed vigor and growth parameters of triticale seeds. Moreover, based on the conducted experiments, the effect of auxin on improving the vigor of aged triticale seeds was greater than the other tested regulators. Therefore, in order to improve the growth parameters of aged triticale seeds, pre-treatment with auxin can be used to enhance seed vigor.

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