Tikrit Journal for Agricultural Sciences (2022) 22 (4): 116-122
https://doi.org/10.25130/tjas.22.4.14

IRAQI Academic Scientific Journals
المجلات الأكاديمية العلمية العراقية

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

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Germination improving of Stored Triticale Seeds and and seedling growth by α – Amylase Enzym

KEY WORDS:

Triticale, amylase, germination

ARTICLE HISTORY:

Received: 26/06/2022

Accepted: 25/07/2022

Available online: 31/12/2022

ABSTRACT

An experiment was carried out at the Department of Field Crops laboratory, College of Agriculture, University of Tikrit, in order to know the effect of stimulating the stored seeds of triticale Farah cultivar by α -amylase enzyme using a complete random design (CRD) in a factorial experiment. The first factor was the storage periods of 6 months, 1.5 years and 2.5 years (S1,S2 and S3) and the second factor was α -amylase enzyme concentrations of 0 (dry seeds), seeds soaked in distilled water, 1 and 2 mg L-1 of the enzyme (C1,C2,C3 and C4). Results showed that increasing the storage period reduces the viability of seeds and their ability to germinate and seedling characters. The percentage of germination, plumule length, radicale, seedling vigor, plumule dry weight and radical dry weight reduced from 93.5%, 10.68 cm,11.05 cm, 2033.42, 0.1149 g and 0.1231 g in S1 treatment to 83.88%, 7.53cm, 7.5 cm, 1267.1, 0.0977 g and 0.1022 g in S3 treatment. The concentration 1 mg L-1 was superior in germination, plumule length, seedling vigor and plumule dry weight and the values were 92.44, 9.46cm, 1805.78 and 0.1111 g. However the concentration of 2 mg L-1 was superior in radical length and its dry weight. The best interaction was between storage period S1 and enzyme concentration C3 with values of 96.6%, 11.50 cm, 2212.33 and 0.1288 g for germination percentage, plumule length, seedling vigor and plumule dry weight and with the concentration C4 that recorded 11.67 cm and 0.1353 g for radical length and dry weight respectively.

INTRODUCTION

Triticale (X *Trititcosecal wittmack*) is a cereal crop that includes characters of two genus, wheat (*Triticum*) and rye (*Secale*) and induced by a human through multiplication the chromosomal number of the hybrid between the two genus. However, it is distinguished from wheat by the high percentage of protein and lysine, which reaches 4% of the total protein, as well as the high content of carbohydrates (Khalil et al. 2015; Al-Dulaimi 2020). Therefore, it is a source of nutrition for humans as well as feeding animals through using it as green forage in addition the use of its seeds for poultry feeding. Seeds of this crop, like any other, are affected by the duration of storage which, in turn, affects the viability and then germination of these seeds and their use as planting seeds. Seeds are normally stored for different periods in order to be used for the next agricultural season, and that can save a lot of costs economically, taking into account the activation of these seeds to

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obtain high viability for germination and field establishment and the production of vigorous and healthy seedlings and plants. One of the essential and important factors, that determine the percentages of seed germination, is the viability of the seeds used in agriculture for planting which is strongly related to the ability of seeds to germinate, emerge and produce vigorous seedlings that establish the growth of plants with high quality characters in respect of vegetative growth and physiological characteristics. The length of storage duration, that deteriorate the viability of seeds which varies from several months to several years is regarded one of the most important factors accompanied with chemical, physiological and biological changes that affect the germination process. The deterioration in the viability of the seeds increases with the increase in the storage duration because it causes a significant decrease in the effectiveness of the enzymes that stimulate germination and seed aging, which is negatively reflected on the vigorous of the seeds, germination, establishment and then the final outcome (Azadi and Younesi, 2013; Sibande, 2015).The technique of seed stimulation before planting is one of the procedures implemented by soaking the crop seeds in water or different concentrations of hormones, enzymes or salts of some elements in order to activate the seeds to start the primary metabolic processes of the germination p to be close to the germination point and the aim of this process is to raise the percentages of germination and field emergence reducing the time required for germination and emergence, and then enhancing the strength of the resulting seedling growth and the homogeneity of the field establishment, which is reflected in a close synchronization of the stages of growth of all plants and promoting high yield and good quality. The hormonal and enzymatic system inside the seeds, especially the effectiveness of the α -amylase enzyme in the decomposition of endosperm stored compounds to make them ready for use by the embryo. Therefore, processing the seeds with diluted solutions of enzymes related to germination is an important and effective technology that is also used to overcome the decrease in the effectiveness of enzymes by adding an activating dose of this enzyme to speed up and activate the germination process and in less time while giving vigorous to seedling growth (Ciornea et al., 2008). As a result, resorting to revitalizing crop seeds using the α -amylase enzyme may be effective in raising the viability of the seeds, the percentage of their germination and the seedling vigorous. It is more feasible and effective than non-stored seeds, because the changes that occur in stored seeds may cause these seeds to deteriorate or lose their vitality even under good conditions of storage. Therefore, the research aims to know the effect of using seed stimulation with α -amylase enzyme in improving the viability of triticale seeds stored for different periods through germination and seedling growth characteristics and determining the best enzyme concentration for that.

MATERIALS AND METHODS

An experiment was carried out in the laboratory of the Department of Field Crops, College of Agriculture, University of Tikrit, in order to stimulate stored seeds of triticale Farah variety (from Ministry of Sciences and Technology) using different concentrations of α-amylase enzyme in addition to soaking with distilled water and dry seeds. The factorial experiment was designed using a complete random design (CRD) and every treatment replicated 4 times. The first factor was the storage periods of 6 months, 1.5 years and 2.5 years, which were indicated by the symbols S1, S2 and S3. The previously stored seeds were obtained from the variety yield of seasons 2017- 2018, 2018-2019 and 2019-20 and stored at room temperature. The second factor was α -amylase enzyme concentrations 0 (dry seeds), seeds soaked in distilled water, 1 and 2 mg L^{-1} of the enzyme (C1, C2, C3 and C4). The seeds were soaked in distilled water and enzyme concentrations for 12 hours, then 25 seeds from each treatment were planted directly between two moistened filter papers in a petri dishes 13 cm diameter. The experiment was carried out at a temperature of 20 ± 2 °C and 14 hours light (ISTA, 2013).

Studied characteristics

Germination percentage

According to the number of normal seedlings after 8 days, as a percentage according to the following equation (ISTA, 2013)

$$\%\ germination = \frac{\text{Number of seedlings produced after 8 days}}{\text{Total number of planted seeds}}$$

Plumule and Radical length (cm)

Ten normal seedlings were taken after the end of the experiment (14 days), then each of the plumule and radical were separated from their point of contact with the seed and estimate the length (ISTA, 2013)

Seedling vigor

The seedling vigor was calculated using the equation

Seed vigor= Percentage of germination * {Plumule length (cm) + Radical length (cm)} (Murti et al., 2004)

Plumule and Radical dry weight (g)

Ten natural seedlings were taken after the end of the experiment (14 days), then both the plumule and the radical were separated from their point of contact with the seed. Then the two parts of seedlings were placed in perforated paper bags and the samples were dried in an electric oven at a temperature of 70 °C until the weight was stable, then the ten dry plumules and radicals were weighted by sensitive balance. The dry weight average of two parts of seedlings were estimated. .

RESULTS AND DISCUSSION

The storage period and the concentration of a-amylase enzyme affected the percentage of germination and growth characteristics of seedlings significantly, but the interaction between the two factors was not significant (Table 1)

Effect of storage period

The percentage of germination of triticale seeds significantly decreased from 93.5% (S1) to the lowest value of 83.8% when seeds were stored for 2.5 years (S3) (Table 2). This probably is due to the negative impact of storage on the viability of the seeds, as increasing the storage period reduces the viability of the seeds and their ability to germination, and the reason is due to the physical, chemical and physiological changes to which the contents of the seed are exposed, which leads to the aging of the seeds and the decrease in the effectiveness of hormones and enzymes that encourage germination and the consequent slow decomposition of the stored food into the simple forms needed by the axes of the embryo for germination (Tiwari and Das, 2014).

Table (1): Analysis of variance of studied characteristics in triticale

S.O.V	DF	% germination	Plumule length	Radical length	Seedling vigor	Plumule dry weight	Radical dry weight
Storage Period (A)	2	282.1**	30.0**	37.8**	1765070.3**	0.000945 **	0.0014 **
Enzyme concentration(B)	3	169.4*	3.25**	4.31**	309788.23**	0.000396 **	0.00481 **
A*B	6	17.3n.s	0.18n.s	0.141 n.s	8392.805 n.s	0.000112 n.s	0.000153 n.s
Error	24	6.77	0.588	0.575	12057.3	0.000161	0.000107

*significant at 0.05 (P≤0.05) **significant at 0.01 (P≤0.01) (n.s) non significant

The storage period (S1) recorded the highest mean of seedling plumule length of 10.68 cm. However it decreased significantly with increasing storage period to 8.85 cm in treatment S2 and 7.53 cm in treatment S3 (Table 2). The reason may be related to the fact that increasing the storage period causes an acceleration in the aging of seeds that negatively affect the activity of the hormones that are formed during the germination process, especially auxins and cytokinins, which activate the processes of cell division and elongation. That results in a significant slowdown of these two processes and then the reduction in the length of the plumule as well as a decrease in energy levels caused by the lack of storage compounds in endosperm needed for the respiration process due to the damage caused to it as a result of storage and as a result of imbalance the enzymatic system needed to break down endosperm compounds, which in turn reduces the energy levels available for the process of division and elongation (Tilebei and Golpayegani, 2011).the same results was found by Ghassemi-Golezani (2011) in corn and Al-Tareihi (2020) in soybean.

The same reasons that led to the decrease in the length of the plumule caused the decrease in the length of the radicale with an increase in the storage period, as it decreased from 11.05 cm in treatment S1 to the lowest value in storage period (S3) 7.5 cm (Table 3), with a significant difference from the value of 9.27 cm for storage treatment S2. These results was agree with the Moyo et al.(2015) results in sorghum.

The vigor of the seedling is a reflection of the plumule length, radicale length and the percentage of germination, and the storage period treatment S1 was significantly superior in the above three traits. Therefore, this treatment excelled in the trait of the seedling vigor (2033.42) and the decrease was gradual in the two treatments S2 and S3 in proportion to the decrease in the length of the seedling. The vegetative and root total and the percentage of germination with an increase in the storage period in treatments S2 and S3, as the seedling vigor in these two treatments decreased to 1621.33 and 1267.10 (Table 2) for the two treatments, respectively. The storage period reflects the behavior of the seedlings by the effect of the storage period. Therefore, the measurement of these traits reflects the extent of the resulting seedling vigor and the extent of the deterioration that can occur in the growth of the seedling and then the plants growing in the field in the successive stages of vegetative and fruitful growth. These effects were referred to by Said (2019) in wheat.

It appears from table (2) that the storage period had a negative effect on the dry weight of plumule and radical. The dry weight of the two parts was the highest the lowest period of storage (S1) and recorded 0.1149 and 0.1231 g, respectively, and gradually decreased when storage period increased in treatments S2 and S3 and reached the lowest values of 0.0977 and 0.1022 g, respectively. The reason for this decrease in both traits with an increase in the storage period is the significant decrease in the length of the two parts of seedlings, which resulted from a decrease in the activity of the embryo axial activity upon germination and a decrease in the accumulation of dry matter in them. The same results were obtained by Cheyed (2020)

Table (2): Effect of storage period on studied characteristics in Triticale

Storage period	% Germination	Plumule length (cm)	Radical length (cm)	Seedling vigor	Plumule dry weight (g)	Radical dry weight (g)
S1	93.5a	10.68a	11.05a	2033.42a	0.1149a	0.1231a
S2	89.3b	8.85b	9.27b	1621.33b	0.1023b	0.1070b
S3	83.8c	7.53c	7.50c	1267.10c	0.0977c	0.1022c

Effect of seed stimulation

The use of the seed stimulation process led to an increase in the percentage of germination from 83.11% in dry seeds (C1) to 88.0% when stimulated with distilled water (C2) with a significant increase of 5.8%, while the superiority of the stimulation treatment was at a concentration of 1 mg L^{-1} (C3) with a value of 92.44%, which did not differ significantly from the stimulation treatment with a concentration of 2 mg L^{-1} (C4) (92.00%), and a higher percentage of 11.22 and 10.69%, respectively, compared to dry seeds. The reason for this is due to the role of the enzyme stimulation of α -amylase in reducing the viscosity of starchy materials stored in the endosperm of seeds and analyzing the bonds between glucose-type α- 1,4-glucose (Pandey et al., 2000), which causes the stored endosperm compounds to be converted in simple forms that move to the plumule and radicale to initiate the processes of division and elongation for germination to occur with the emergence of the plumule and the radicale outside the seed coat, so the deteriorated seeds as a result of the increase in the storage period and the weak activity of the hormones, especially gibberellin and the enzyme α -amylase, improve their germination rate (Table 3) by preparing the enzyme externally with diluted solutions, which compensates for a decrease in the enzyme in stored seeds

Table (3) indicates that the stimulation with distilled water (C2) did not affect the length of the plumule significantly compared to the treatment of dry seeds (C1) and the means where 8.80 and 8.29 cm, respectively. The two treatments of stimulation with α -amylase enzyme did not differ significantly at a concentration of 1 and 2 mg L^{-1} (C3 and C4) as well and record 9.64 and 9.36 cm and over the two treatments C1 and C2. However there was some decrease when increasing the concentration to 2 mg L^{-1} compared to the concentration 1 mg L^{-1}, and that the positive effect of using the enzyme prepares the seeds In the germination stage, the enzymes needed to break down endosperm compounds into single units of sugars, especially glucose and maltose, to compensate for the deficiency in the activity of the enzyme produced internally as a result of storage with its role in the production of energy compounds ATP, and all this as a result speeds up the processes of division and elongation and the effectiveness of the hormones responsible for it and increase the length of the plumule (Jyoti et al., 2011)

The radical length characteristic behaved similarly to the plumule length characteristic, as it is noted in Table (3) that soaking treatment with concentration 2 mg L^{-1} (C4) was superior to the root length (9.91) cm, which did not differ significantly with C3 treatment (concentration 1 mg L^{-1}), and this is consistent with what Declerck et al. (2000) mentioned about the positive effects of the activity of the enzyme α -amylase in accelerating the exploitation of the decomposing food in the endosperm in the growth of the radicale outside the germinated seed coat by accelerating the processes of division and elongation

Table (3) showed the extent of improvement in seedling vigor resulting from seeds stimulated with the α -amylase enzyme, as the value of this trait increased from 1411.02 in the treatment of dry seeds (C1) to 1805.78 and 1775.78 in the two stimulation treatments C3 and C4 at concentrations of 1 and 2 mg L^{-1} respectively, with an improvement rate of 28% and 25.8% for the two treatments, respectively, compared with C1, and that reflects the role of this enzyme in reducing the results of the deterioration of stored seeds, improving their performance at germination and the growth of the embryo axial in terms of lengths and dry weight of seedlings, as well as high rates of germination. These results and effects were reported by Al-Tareihi (2020)

Table (3): Effect of α -amylase enzyme concentration on studied characteristics in triticale

Enzyme con.	Germination %	Plumule length (cm)	Radical length (cm)	Seedling vigor	Plumule dry weight (g)	Radical dry weight (g)
C_1	83.11 c	8.29 c	8.48 b	1411.02 c	0.0972 b	0.1004 b
C2	88.00 b	8.80 bc	8.91 b	1569.89 b	0.1017 ab	0.1108 a
C_3	92.44 a	9.64 a	9.79 a	1805.78 a	0.1111 a	0.1158 a
C_4	92.00 a	9.36 ab	9.91 a	1775.78 a	0.1098 a	0.1159 a

Treatment (C3) at concentration 1 mg L^{-1} was superior to the dry weight characteristic of the plumule with the highest value of 0.1111 g, which did not differ significantly from C4 treatment at concentration 2 mg L^{-1} (0.1098) g (Table 3), while the superiority in the dry weight characteristic of the radical at concentration 2 mg L^{-1} was 0.1159 g, which did not differ significantly from the value 0.1158 g for concentration 1 mg L^{-1}.The reason for this is due to the superiority of these two treatments in the lengths of the plumule and the radicale resulting from high level of food decomposition, embryo axial growth and high level of vital activities, including raising the level of dry matter aggregation. These effects were found by Al-Tareihi (2020)

Effect of interaction between storage period and stimulation treatments

Table (4) showed that the highest significant interaction was between storage period S1 and enzyme concentration C3 with values 96.9%, 11.5 cm, 2212.33 and 0.1288 g in germination percentage, plumule length, seedling vigor and plumule dry weight or C4 (11.67cm and 0.1353 g) in radical length and dry weight respectively.

Table (4): Effect of Interaction between storage period and α -amylase enzyme concentration on studied characteristics in triticale

Storage period	Enzyme concentration mg L^{-1}			
	C_1	C_2	C_3	C_4
Germination %				
S_1	90.6 abc	92.6 abc	96.6 a	94.0 ab
S_2	83.3 e	89.3 cd	92.6 abc	92.0 bcd
S_3	75.3 f	82.000 e	88.0 d	90.0 a
Plumule length (cm)				
S_1	10.07 b	10.53 ab	11.50 a	10.63 ab
S_2	8.07 d	8.47 cd	9.50b c	9.37 bc
S_3	6.73 e	7.40 de	7.93d e	8.07 d
Radical length(cm)				
S_1	10.40 ab	10.77 ab	11.37 a	11.67 a
S_2	8.63 dc	8.87 dc	9.83 bc	9.73 bc
S_3	6.40 f	7.10 ef	8.17 de	8.33 de
Seedling vigor				
S_1	1854.33 cd	1973.33 bc	2212.33 a	2093.67 ab
S_2	1389.00 e	1548.33 e	1791.33 cd	1756.67 d
S_3	989.73 g	1188.00 f	1413.67 e	1477.00 e
Plumule dry weight (g)				
S_1	0.1040 bc	0.1045 bc	0.1288 a	0.1222 ab
S_2	0.097 c	0.104 bc	0.1044b c	0.1026b c
S_3	0.0900 c	0.0963 c	0.1002 c	0.1046b c
Radical dry weight (g)				
S_1	0.1078 bc	0.1150 b	0.1340 a	0.1353 a
S_2	0.1028 bc	0.1084 b	0.1087 b	0.10 80 b
S_3	0.0904 c	0.1091 b	0.1049 bc	0.1044 bc

CONCLUSION

Storage period had a significant negative effects on triticale seeds germination and seedling growth characteristics. The highest storage period the lowest characteristics values. The stimulation by α -amylase enzyme reduced the negative effects og storage period and improved germination and seedling growth. Stimulating treatment at concentration 1 mg L^{-1} was superior in all studied characteristics.

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تحسين انبات بذور القمح الشيلمي المخزونة ونمو البادرات بأنزيم الفا – اميليز

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الخلاصة

الكلمات المفتاحية: إنبات ، تريتيكال ، اميليز، القمح الشيلمي

اجريت تجربة مختبرية في مختبرات قسم المحاصيل الحقلية – كلية الزراعة – جامعة تكريت ، لمعرفة تأثير تنشيط بذور القمح الشيلمي صنف فرح بواسطة أنزيم الفا – أميليز ، باستخدام التصميم العشوائي الكامل في تجربة عاملية . مثل العامل الاول مدد خزن بذور القمح الشيلمي 6 أشهر و 1.5 سنة و2.5 سنة رمز لها S1 و S2 و S3 ، والعامل الثاني تراكيز أنزيم الفا أمليز التي تضمنت البذور الجافة (بدون تنشيط) و تحفيز بالماء المقطر و 1 و 2 ملغم لتر-1 من الانزيم (C1 و C2 و C3 و C4). اظهرت النتائج ان زيادة مدة الخزن خفضت حيوية البذور ومقدرتها على الانبات وصفات نمو البادرة. انخفضت النسبة المئوية للانبات وطول الرويشة والجذير ووزنهما الجاف وقوة البادرة من 93.5% و 10.68 سم و 11.05 سم و 0.1149 غم و 0.1231 غم و 2033.42 في المعاملة S1 الى 83.88% و 7.53 سم و 7.5 سم و0.0977 غم و 0.1022 غم و 1267.1 في المعاملة S3 على التوالي. كانت الافضلية والتفوق في معاملات التحفيز لمعاملة التركيز 1 ملغم لتر-1 من الانزيم في صفات النسبة المئوية للانبات (92.44)% و طول الرويشة (9.64) سم و والوزن الجاف للرويشة (0.1111) غم و و قوة البادرة (1805.78) في حين كان التفوق للمعاملة 2 ملغم لتر-1 من الانزيم في صفتي طول الجذير ووزنه الجاف. افضل تداخل كان بين معاملة الخزن S1 ومعاملة التحفيز C3 بقيم 96.6% و 11.50سم و 0.1288 غم و 2212.33 في صفات النسبة المئوية للانبات وطول الرويشة ووزنها الجاف وقوة البادرة ومع معاملة التحفيز C4 في صفتي طول الجذير ووزنه الجاف (11.67سم و 0.1353 غم) على التوالي.