Tikrit Journal for Agricultural Sciences (2023) 23 (3): 87-93
DOI: https://doi.org/10.25130/tjas.23.3.10

IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية

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

Using α -amylase enzyme with the seed pre-soaking to develop emergence and yield of triticale

Balsam Salih Muhammed and Labed Sharief Mohammed
Department of Field Crops, College of Agriculture, Tikrit University, Iraq

* *Corresponding author: E-mail*: **Balsam.s.mohammed@st.tu.edu.iq**

KEY WORDS:
Emergence, Grain yield, α – amylase, Triticale, leaf area

Received: 26/07/2022
Accepted: 25/09/2022
Available online: 30/09/2023

ABSTRACT

In order to investigate the efficiency of stimulating stored seeds of Triticale (X *Triticosecale wittmack*) by α -amylase enzyme, a field experiment was carried out using split plot arrangements according to a randomized complete block design in three replications. The storage period of seeds 6 months, 1.5 years, and 2.5 years represented the main plots. The stimulation treatments was secondary plots included dry seeds (control), stimulated seeds with distilled water, 1 and 2 mg L^{-1} of α -amylase enzyme. The results showed a significant decrease in the percentage of emergence, plant leaf area, plant dry weight, number of tillers m^{-2}, number of spikes m^{-2}, weight of one thousand grains, and grain yield with the increasing of storage period. The enzyme concentration of 1 mg L^{-1} was the superior in % emergence (85.02), Plant leaf area (244.88) cm^2, plant dry weight (2.087) g, number of tillers (448.8) tiller m^{-2}, number of spikes (372.9) spike m^{-2}, number of grains $spike^{-1}$ (44.13) grain $spike^{-1}$ and grain yield (5.842) ton h^{-1}. However, there was on significant differences between the two concentration 1 and 2 mg L^{-1} of enzyme in leaf area, plant dry weight, 1000 grain weight and grain yield.

استخدام نقع البذور بأنزيم الفا أميليز لتحسين البزوغ وحاصل القمح الشيلمي

بلسم صالح محمد ولبيد شريف محمد
جامعة تكريت – كلية الزراعة – قسم المحاصيل الحقلية

الخلاصة

بهدف استكشاف كفاءة تحفيز البذور المخزونة للقمح الشيلمي بأنزيم الفا أميليز، اجريت تجربة حقلية باستخدام نظام الالواح المنشقة بحسب تصميم القطاعات العشوائية الكاملة بثلاثة مكررات. مثلت مدد خزن البذور 6 أشهر و 1.5 سنة و 2.5 سنة الالواح الرئيسية ، ومثلت معاملات التحفيز الالواح الثانوية والتي شملت البذور الجافة (المقارنة) والتحفيز بالماء المقطر والتركيزين 1 و 2 ملغم لتر$^{-1}$ من أنزيم الفا أميليز. بينت النتائج انخفاض النسبة المئوية للبزوغ والمساحة الورقية و وزن النبات الجاف و عدد الاشطاء والسنابل م$^{-2}$ وعدد الحبوب سنبلة$^{-1}$ وحاصل الحبوب بزيادة مدة الخزن. تفوق التركيز 1 ملغم لتر$^{-1}$ من الانزيم في النسبة المئوية للبزوغ (84.73)% والمساحة الورقية (244.88) سم^2 و وزن النبات الجاف (2.078) غم وعدد الاشطاء (448.8) شطأ م$^{-2}$ و عدد السنابل (372.9) سنبلة م$^{-2}$ وعدد الحبوب (44.13) حبة سنبلة$^{-1}$ وحاصل الحبوب (5.842) طن هـ$^{-1}$. لم يكن هناك فرق معنوي بين تأثير التركيزين 1 و 2 ملغم لتر-1 في صفات المساحة الورقية و ووزن النبات الجاف ووزن 1000 حبة وحاصل الحبوب.

الكلمات المفتاحية: كلمة 1 ، كلمة 2 ، كلمة 3 البزوغ، حاصل الحبوب، الفا أميليز، القمح الشيلمي، المساحة الور.

INTRODUCTION

Triticale is one of cereal crops, which was produced by cross-breeding between genus *Triticum* and genus *Secale* by humans and then doubling the cells chromosomes of the plants resulting from the cultivation of hybrid seeds (Khalil et al. 2015; Al-Dulaimi 2020). Using the stored seeds of this crop for planting can save a lot of costs economically, but the viability of these seeds may be affected by many factors that weaken their viability and limit their ability to field emerge, which negatively affects the field establishment on the one hand, the plants activity and their subsequent growth and yield. One of these factors is the length of the storage period, which may extend for months or many years (Moyo, 2015). It was found that the longer the storage period, the more affected the viability of the seeds and consequently the strength of the seedlings and the growth characteristics of plants and their yield (Gebeyehu, 2020). Many chemical, physiological and biological changes occur during the storage period, which increases with the lengthening of this period. These changes leads to the aging of the seeds, increase in respiration processes, an increase in the level of vital processes in the embryo, and negative impact on the hormonal and enzymatic system that is important for the decomposition of stored compounds in endosperm which necessary for emergence, strength of seedlings, growth and yield of plants (Portey, 2016).

In order to overcome the decrease in the viability of the stored seeds, the degraded seeds are stimulated before planting with some stimulant agents such as the salts of some elements, growth regulators, vitamins as well as enzymes , especially the α -amylase enzyme because of its role in the decomposition of compounds in the endosperm of stored cereal crops such as triticale to make the seeds close to the start of germination process in addition to reducing the time required for seeds germination and emergence of seedlings. The raising the rates of germination and emergence and enhancing the strength of seedlings and growth characteristics of plants with a homogeneity in the plant density in the field, which is reflected in the homogeneity in the stages of plant growth In order to provide the appropriate time and environment for the healthy growth of plants and raise their productivity. The effectiveness of the α-amylase enzyme is affected by the concentration used for this purpose, because the seeds need very few concentrations to achieve the desired effect without having an adverse effect due to the high concentration of the enzyme resulting from the effect on its activating role and turning it into an inhibitor in addition to its effect on the effectiveness of other hormones or enzymes (Al-Tareihi, 2020).

Determining the most effective concentration is important in stimulating and raising the viability of stored seeds. Therefore, the research aims to improve the emergence of seedlings of stored seeds of triticale for different periods by using several concentrations of a-amylase enzyme and determining the best concentration to effect improvement in growth and yield.

MATERIAL AND METHODS

A field experiment was carried out at the Research Station of the Field Crops Department - College of Agriculture - Tikrit University to determine the effect of the storage period of triticale seeds and the stimulation with different concentrations of a-amylase enzyme on seedling emergence, growth and yield. The experiment was carried out using the split plot arrangement according to the randomized complete blocks design in three replications. The storage periods represented main plots and included storage for 6 months, 1.5 years and 2.5 years. These seeds were produced in the research station in successive seasons 2020-2021, 2019-2020 and 2018-2019 respectively. The seeds were storage under normal environment of laboratory and not in standard condition of seeds storage. The secondary treatments included the stimulation treatments dry seeds, stimulated seeds with distilled water, stimulated seeds with concentrations 1 and 2 mg L^{-1} of α – amylase enzyme. The seeds were presoaking with water and concentrations of enzyme for 12 hours, and then sown at 11/17/2021. The dimensions of plots was 1 x 2 m, with 5 lines each plot of 2 meters in length, and 20 cm between them. Calcium superphosphate fertilizer was added at the rate of 100 kg P_2O_5 h^{-1} before planting and urea fertilizer (46%). N at 200 kg N h^{-1} in two batches, the first at emergence and the second at the stage of tillers formation.

Studied characters:

Field emergence (%):

Field emergence estimated according to the number of normal seedlings produced after 14 days of planting, as a percentage of the number of planted seeds, which averaged 81 seeds in each line, according to the following equation

$$\% \ \boldsymbol{emergence} = \frac{\textbf{Number of seedlings produced after 14 days}}{\textbf{Total number of planted seeds}}$$

Plant leaf area (cm^2)

The average of five marked plants leaf area from each treatment was estimated after 90 days of planting according to the equation: (Thomas, 1975)

$$\textbf{Plant leaf area} = \boldsymbol{Leaf\ length} * \boldsymbol{Leaf\ wedght} * \mathbf{0.95}$$

Plant dry weight (g):

The five plants measured by their leaf area were dried in an electric oven at a temperature of 70 ° C until the weight was stable.

Number of tillers and spikes m^{-2}:

It was calculated from the number of tillers and spikes in the three middle lines and converted to square meter for each treatment at ripening stage .

Number of grains $spike^{-1}$:

To estimate these traits the number of grains of plants located at a distance of 50 cm from one of the median lines for each treatment was calculated and divided by the number of spikes.

Weight of 1000 grains (g):

A sample of 1000 grains was taken from the yield of each experimental unit and weighed with a sensitive scale based on a moisture rate of 14%.

Grain yield:

The three median lines of each experimental unit were harvested, their grains were weighted with grains of the plants in 50 cm harvested to calculate the number of grains $spike^{-1}$.

The results were analyzed according to split plot arrangement in RCBD using the SAS program, and Duncan's polynomial test was used to test the significant differences between treatments at the 5% level.

Results and discussion

The storage period and stimulation treatments were significantly affected in all traits except for the weight 0f 1000 grains. The interaction between the two factors was significant in the number of tillers and spikes m^{-2} (Table 1).

The storage period treatment of 6 months had the highest field emergence rate of 88.77%, significantly superior to the storage treatments of 1.5 and 2.5 years which recorded 81.52 and

71.85%, respectively (Table 2). The percentage of emergence is related to the seeds physiology, seeds viability and the extent of the seeds ability to maintain the viability and an acceptable physiological level during the storage period to be able to germinate and emerge at high rates in addition to continue with high-level of growth characteristics. The long storage period leads to a weakness of the enzymatic system, an increase in the storage compounds consumption and decrease of seedling vigor and emergence (Azavedo et al., 2003 and Badawi et al., 2017).

Table 1: Analysis of variance of studied characters in triticale

Characters	Storage period	Error A	Enzyme Con.	S*E	Error B
DF	2	4	3	6	18
Emergenc %	865.2**	2.104	132.4**	1.98ns	3.154
Leaf area (cm2)	2549.6**	478.04	997.2**	2.144ns	210.93
Plant dry weight(g)	0.4831**	0.0206	0.0642**	0.0066ns	0.0228
Tillers No.m^{-2}	21441**	95.45	12617**	190.49*	55.11
Spikes No.m^{-2}	28340**	63.88	13275**	291.4**	21.97
No. grains $spike^{-1}$	101.0**	0.0195	17.456**	0.179ns	0.692
1000 grains weight (g)	0.0823ns	0.232	0.066ns	0.219ns	0.577
Grain yield(ton h^{-1})	17.209**	0.0681	5.899**	0.0333ns	0.0752

These negative effects of prolonging the storage period were found by Gebeyehu, 2020. Prolonging the storage period led to a decrease in the plant leaf area. The storage period of 2.5 years recorded the lowest value of 218.42 cm^2, while the plants obtained from the seeds stored for 6 months had the highest leaf area (247.46) cm^2. The superiority of plants produced from seeds stored for 6 months may be because its ability to cell division, elongate, expand the leaf surface during the measurement period, capable of effectively intercepting solar radiation, active photosynthesis and active accumulation of dry matter, represented by the superiority of this treatment by the plants dry weight, as it increased to 2.156 g, which did not differ significantly from the value of 2.072 g for treatment 1.5 years (Table 2). The longer storage period, in which the dry weight decreased significantly due to the weak vitality of the seeds that produced plants with low leaf area, which negatively affected the photosynthesis and dry matter accumulation (Al- Hadi, 2019).

The decrease in the number of plants per unit area due to the decrease in the percentage of emergence as the storage period increases. The effect of a decrease in the characteristics of leaf area and plant dry weight, indicated that seeds stored for a longer period, produced weakest plants in providing the necessary feeding and week hormonal balance necessary to break the apical dominance to produce higher numbers of tillers. The stored seeds of 2.5 years recorded 371.5 tiller m^{-2}, while in stored seeds of 1.5 years and 6 months reached 417.1 and 455.9 tillers m^{-2} respectively. This superiority of treatment stored for 6 months may due to of their high values of emergence, leaf area, active photosynthesis and accumulation of dry matter. Jyoti and Malik (2013) mentioned that the storage period is an important factor in determining the vitality of seeds and its reflection on growth parameters. Ghassemi-Golezani (2014) confirmed the effect of seed viability and storage period on field emergence and its relationship to growth parameters (leaf area and plant dry weight).

The number of tillers is an effective factor in determining the number of spikes per unit area, so this trait affected by storage period in the same pattern of number of tillers. Treatment of 6 months excelled with the number of spikes, which amounted to 390.9 spikes m^{-2}, and it decreased to 330.7 and 294.7 spikes m^{-2} in treatments of 1.5 and 2.5 years respectively. By observing the number of tillers and spikes for each treatment, 14.2%, 20.7% and 20.6% of the tillers for the three treatments were not produced spikes. This reflects the extent of the decline in the ability of plants to produce spikes from tillers by increasing the storage period, which is due to the decline in growth characteristics and the ability of the plant to cover growth requirements. The strong competition between vegetative and reproductive parts for the growth requirements was one of factors

eliminated the formation of spikes in weaker plants. The decrease in the number of tillers in plants which their seeds stored for a longer period, was mentioned by Biabani et al. (2011).

The superiority of treatment 6 months storage seeds in growth characters lead to superiority in number of grains spikes^{-1} with a value of 45.6, while the number of grains spikes^{-1} decreased to 42.22 and 39.82 in the treatments 1.5 and 2.5 years respectively. The superiority of the storage treatment in the shorter storage period in the crop components resulted in its superiority in the grain yield with a value of 6.399 tons ha^{-1} (Table 2).

Table 2 : Effect of storage period on studied characters in triticale

Storage period	Emergence %	Leaf area(cm^2)	Plant dry weight (g)	No. tillers m^{-2}	No. spike m^{-2}	No.grains Spike^{-1}	1000 grains weight(g)	Yield ton h^{-1}
6 months	88.77 a	247.4 a	2.156 a	455.9 a	390.9a	45.60 a	39.50a	6.399 a
1.5 years	81.52 b	235.1 ab	2.072 a	417.1 b	330.7 b	42.22b	39.67a	4.933 b
2.5 years	71.85 b	218.4 b	1.774 b	371.5 c	294.7 c	39.82c	39.56a	4.025 c

Stimulation with α–amylase improved the percentage of emergence with a significant superiority of treatment 1 mg L^{-1} with a value of 84.73% (Table 3). The improvement in this characteristic reflects the role of the enzyme in reducing the negative effects of seed storage, increasing the ability of enzyme to accelerating the processes of endosperm compounds decomposition, transfer to the developing embryo and raising the effectiveness of vegetative part for elongation and emergence outside the soil (Moyo et al., 2015).

This improvement in the ability and speed of the seedlings emerging activated leaves cell division and elongation to form a wider leaf area and providing more active absorption of water and elements, which also encouraged raising the cell turgor pressure for cell elongation. This in turn encouraged raising the levels of light interception, increasing the activities of photosynthesis and dry matter accumulation, providing the necessary energy and better hormonal activity. Therefor treatment of 1 mg L^{-1} also excelled in leaf area and plant dry weight, which amounted to 244.88 cm and 2.087 g respectively. These effects of the enzyme were found by Al-Tareihi (2020).

Increasing the vitality of the seeds by using the enzyme α-amylase stimulation especially in concentration 1 mg L^{-1} had an important role in raising the efficiency, speed and percentage of emergence, which led to an increase in the number of plants per unit area, as well as its role in improving the leaf area and dry weight was reflected in providing requirements for producing a higher number of tillers because of lack of competition for growth requirements with a better hormonal balance to break the apical dominance and produce a higher number of tillers. Therefor the superiority was for the concentration treatment of 1mg L^{-1} which produced 448.8 tiller m^{-2}. Number of tillers is one of the characteristics that determine the number of spikes in the unit area, so treatment 1mg L^{-1} produced 372.9 spike m-2. Number of spikes gradually decreased significantly in stimulation treatments 2 mg L-1, distilled water and dry seeds to 366.2, 325.5 and 290.5 spikes m^{-2} respectively. Due to the superiority of the stimulation treatment of 1 mg L^{-1} in vegetative growth characteristics and their ability to form fertile spikelets and florets during the stage of reproductive parts. This treatment gave the highest value in number of grains spike^{-1} of 44.13 grain spike^{-1}. The superiority in yield components of treatment 1mg L^{-1} led to the production of the highest grain yield amounted to 5.842 tons h^{-1}, with a superiority of 40% compared to the treatment of dry seeds.

Table (4) shows that the best interaction between storage period and stimulation treatment was in storage period for 6 months × stimulation with 1 mg L^{-1} for all characters studied.

Table 3 : Effect of enzyme concentration on studied characters.

Stimulation treatments	**Emergence%**	**Leaf area(cm^2)**	**Plant dry weight(g)**	**No. tillers m^{-2}**	**No. spikes m^{-2}**	**No.grains Spike^{-2}**	**1000 grains weight(g)**	**Yield ton h^{-1}**
Dry seeds	76.20 d	220.21 b	1.896 b	364.8 d	290.5 d	41.26 c	39.67 a	4.170 c
Distilled water	79.03 c	231.43 ab	1.971 ab	408.3 c	325.5 c	41.50 c	39.50 a	4.724 b
1 mg L^{-1}	84.73 a	244.88 a	2.087 a	448.8 a	372.9 a	44.13 a	39.63 a	5.842 a
2 mg L^{-1}	82.90 b	238.18 a	2.047 a	437.5 b	366.2 b	43.30 b	39.51 a	5.741 a

Table 4:Effect of interaction between storage period and stimulation treatment on characters studied

Storage period	Stimulation treatments			
	Dry seeds	Distilled water	1 mg L^{-1}	2 mg L^{-1}
Emergence%				
6 months	83.467 bcde	84.767 bc	89.167 a	86.333 ab
1.5 years	78.900 fg	81.633 cdef	84.400 bc	83.767 bcd
2.5 years	75.867 g	79.567 efg	81.500 cdef	80.033 def
Plant leaf area(cm^2)				
6 months	234.43 bcd	244.00 abc	259.57 a	251.87 ab
1.5 years	221.30 cde	233.60 bcd	245.60 abc	240.10 abcd
2.5 years	204.90 e	216.70 de	229.50 bcde	222.60 cde
Plant dry weight(g)				
6 months	2.0883 abc	2.1520 ab	2.1987 a	2.1850 a
1.5 years	1.9860 abc	2.0510 abc	2.1610 ab	2.0910 abc
2.5 years	1.6160 e	1.7110 de	1.9030 bcd	1.8670 cde
Number of Tillers m^{-2}				
6 months	393.300 d	450.400 b	495.100 a	485.100 a
1.5 years	370.000 e	410.300 c	450.100 b	438.200 b
2.5 years	331.300 f	364.400 e	401.200 cd	389.200 d
Number of Spike m^{-2}				
6 months	349.433 e	390.000 b	415.100 a	409.200 a
1.5 years	280.100 h	311.300 g	370.600 c	360.900 d
2.5 years	242.000 l	275.300 h	333.000 f	328.700 f
No. of grains Spike-2				
6 months	44.30 bc	44.60 b	47.40 a	46.10 a
1.5 years	41.10 d	41.30 d	43.60 bc	42.90 c
2.5 years	38.40 e	38.60 c	41.40 d	40.90 d
1000 weight grains (g)				
6 months	39.87 a	39.20 a	39.45 a	39.51 a
1.5 years	39.55 a	39.77 a	40.01 a	39.35 a
2.5 years	39.61 a	39.55 a	39 43 a	39.67 a
Grain yield ton h^{-1}				
6 months	5.440 c	6.140 b	7.010 a	7.007 a
1.5 years	3.923 e	4.463 d	5.786 bc	5.560 c
2.5 years	3.146 f	3.570 ef	4.730 d	4.656 d

.

CONCLUSION

It is concluded that prolonging the storage period negatively affects the vitality of the seeds and their ability to emergence and field establishment. Therefor early plants growth, previous growth stages, growth characteristics, yield and its components were negatively affected with increasing of storage period. Stimulation by α–amylase enzyme improved growth characteristics and yield in comparison with dry seeds based on the improvement in seedling vigorous in the early stages of growth and its reflection on successive previous growth stages and growth characteristics to reach the best yield with a preference for concentration 1 mg L^{-1}.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

REFERENCES

Al-Dulaimi, O.Z.A. (2020). Influence of seed variety, activation by salicylic acid and addition of humic acid on growth characters, yield and quality of triticale varieties. Ph.D Thesis, Tikrit University, College of Agriculture.

Al-Hadi, M.Q.S. (2019). Effect of seed storage priming with different storage periods on seed vigor, growth and yield of *Sorghum bicolor* L. Ms.c Thesis, College of Agriculture, University of Karbala.

Al-Tareihi, M.S.M. (2020). Stimulation of deteriorated seeds during storage by alpha-amylase to improve growth and yield in soybean. Ph.D. Thesis, University of Baghdad, College of Agriculture Engineering Sciences.

Azavedo, M.R.Q.A., Josivanada, P.G.G., Dilma, M.M.T. and Vicentw, P.Q. (2003). Influencia das embalagrnse condicoes de amazenamento on vigor sementes de gergelim. Rev. Bras. Eng. Agric. Ambient. 7(3): 519-524.

Badawi, M.S., Kandle, A.A. and Sharief, A.E.(2013). Effect of seed storage treatments on wheat storage . Int. Jour. Adv. Res. Bio. Sci. 4(1): 78-91.

Biabani, A., Mollashahi, K. M., Bahlake, M.G., Hali, A. and Khani, A.G. (2011). Correlation and relationships between seed yield and other characteristics in chickpea (*Cicer arietinum* L.) cultivars under deterioration . African Jour. Agric.Res. 6: 1359-1362.

Gebeyehu, B. (2020). Review on: Effect of seed storage period and storage environment on seed quality. Int. Jour. App. Agri. Sci. 6(6): 185-190.

Ghassemi-Golezani, K., Khomari, S., Dalili, B., Hosseinzadeh-Mahootchy, B. and Chadordooz-Jedi, A. (2010). Effect of seed aging on field Performance of winter oil seed rap. Jour.Food Agric.Envir. 8(1):175-178.

Jyoti, J., Lal, N. and Malik, A. (2013). Partial purification characterization of an amylase from *Bacillus licheniformis* JAR-26. IJABR2(3):315-320.

Khalil, N.A., Al-Mitwaly, A.A., Shafik, M.M. and Al- Morshidy, W.A. (2015). Cereals and Legumes Crops. College of Agriculture, Cair, University, 1st Press, 83-84.

Moyo, R., Ndlovu, E., Moyo, N., Kudita, S. and Maphose, M. (2015). Physiological parameters of seed vigor in exsitu stored sorghum Germplasm. Jour. Of cereal and oil seeds.6(6):31-38 .

Portey, H.M., Sadia, A.O. and Asibuo, J.Y. (2016). Effect of seed storage period and temperature upon seed and seedling traits penial rye grass (*Lolium perinn* L.) .polopojerida . 21(2): 3-9 .