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Improvement the Germination Characteristics in Aged Seeds of *Hordeum vulgare* Plants by Some Invigoration Solutions

Rabha B. Hamad

Botany Department, Science Faculty, Omar Al Mukhtar University, Al bayda, Libya.

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A B S T R A C T

Seeds viability and seed vigour decline during storage, **The aim** of this research was the improvement of seed quality by treatment with KNO3and ZnSO4 of *Hordeum vulgare* L. plants aged seeds.**Experiment** was on barley seeds storage for different periods of (1year and 4 years) in open storage type and treatment these by using seeds invigoration solutions , 3% zinc sulfate ($ZnSO_4$),and 3% potassium nitrate (KNO_3) in addition to non primed seed (control) to know their effect on viability and vigour of Seeds and determine the best one on them. **The results** showed that growth parametres, total available carbohydrate (TAC) content and starch (St) contents decrease under long storage,while total soluble sugars (TSS) increased. While at treatment by ZnSO4 and KNO3 there wasan increase in total germination , mean germination time, radical and plumule lengths, seedling fresh and dry weight, and vigour index , also increasing in TSS, St, and TAC contents. However, the treatment with KNO3 was the best compare with treatment by ZnSO4. **Conclusions**: It can be concluded from this study that using invigoration solutions led to improve germination and vigour of barley seeds for all the seed storage.

1 Introduction

The most important factor affecting crop productions the quality of the seed, Seeds deteriorating during the period of prolonged storage. It is one of the most intriguing and challenging scientific problems of universal concern**. (Raj *et al.*, 2013)**. **(Abnavi and Ghobadi , 2012)** studied the effects of seed storing on germination of two wheat cultivars, which is a loss in quality in terms of seed viability and vigour during seed storage**.(Alam et *al.*,2021**)reported that the biochemical properties like amino acid, lipid, oil, soluble sugar and enzymatic activities are changed in aged seeds. The Quality of the seeds has to be protected for a prescribed period of storage without quality deterioration until sowing. Seed deterioration is triggered by many biotic and abiotic factors such as temperature, relative humidity, seed moisture content and storage pathogen & insects **(Pallavi *et al.*, 2003).** All these factors are directly or indirectly associated with lipid oxidation, a process that happens both through enzymatic and non-enzymatic pathways, that causes cell membrane disintegration and eventually causes seed death **(Oenel *et al.*, 2017).**

Different methods can be used to improve agricultural production while seed priming is the most suitable and simple technique to increase germination, emergence and yield **(Dalil . 2014)**. Seed priming theory was proposed by **(Heydecker *et al.*, 1973)** it is a technique which is used before seed sowing, it includes seeds hydration to permit metabolic events prior to germination while preventing the emergence of radicals. **(Adnan *et al.*,2020)** Improvement in metabolic events improves the speed of seed germination in vegetables, ornamental species and some small-seeded grasses. There are many

Corresponding author: E-mail: *rabha.buhwish@omu.edu.ly*

methods of seed priming such as halo priming, hydro priming, Osmo priming, and matrix priming. Seed priming is a simply easy, low-cost, highly effective and low-risk method. Halo priming is the soaking of seeds in salts (KNO3, NaCl, CaSO4 and CaCl2) These techniques improves seed germination. **(Khan *et al* ., 2009).**

(Abnavi and Ghobadi , 2012) reported that the seed priming with potassium nitrate (KNO3) gibberellins (GA3), led to improve seeds germination and seedling growth s in Wheat plants.

Zinc is one of the most important nutrients that play a key role in all living organisms for their growth and development. It is an abundant trace element and acts as a co-factor for more than 300 enzymes, also plays a vital role in cell division, protein formation, and nucleic acid metabolism, and increases the chlorophyll contents **(Cakmak, 2008).**

Potassium is the third most important primary macronutrient, after Nitrogen (N) and phosphorus (P). It has a key role in many plant physiological processes such as the translocation of photo-assimilates stomata regulation, and enzymatic activation **(Ahmed *et al.,* 2021).**

Barley is number four in terms of the area cultivated in cereal grains in the world at 49.24 million hectares. The major uses of the barley grown are for malting and as a feed source own at a wide variety of locations**. (Wolf *et al*., 2019)**. Barley (*Hordeum vulgare* L.) has been a central and staple commodity crop in Libya. Barley's usages include its ground flour for making Bazine, a famous traditional Libyan cuisine and bread. Furthermore, barley grains and hay are used extensively for feeding livestock, and malting. Libya's production of barley was significantly low compared with other neighbouring countries' yields, 260 thousand tons in 2005, therefore, the country relies completely on importing barley seeds from the foreign markets, and Barley plays a major role in Libya's agricultural sector. It is considered a principal food grain in the daily life of the Libyan people (**Elbeydi *et al.,* 2007**). Storage is essential for food security or as a product bank for exchange into cash when required **(Yusuf and He, 2011).**

Seed quality reduces during the period of prolonged storage for this reason was the aim of this research improvement of seed quality by treatment with KNO3 and ZnSO4 aged seed of seedling *Hordeum vulgare* plants.

2 Materials and Methods

Seed Material

Barley (*Hordeum vulgare* verity Acsad 176) seeds obtained from the crops department, agriculture faculty, Omar Al-Mukhtar university, were employed in the current study for different periods of storage non-aged seed (1 year) , and aged seed, (4 years). The seeds were selected for uniformity of size, shape and color. Before germination, seeds were surface sterilized by soaking for two minutes in 4% (v/v) sodium hypochlorite, then washed several times with distilled water.

Priming Treatment

Before germination, each group of seeds (non-aged seed, and aged seed) was soaked in 8 ml of 3% KNO3 3 and 3% ZnSO4n of solution (3 grams/100 ml) and kept at 20°C for 24 hours. In addition, to controlling (unprimed non-aged seed and aged seed), to know their effect on the viability and vigour of Seeds and determine the best one for them, after priming, seeds were washed with distilled water and then left to dry for 24 hours at room temperature between two filter papers. Then the seeds (control and treatment) were placed in 12 cm Petri dishes on a layer of filter paper. Ten seeds were placed in each Petri dish with 50ml distilled water. At 25°C, three replications in each treatment were measured parameter germination was calculated by recording some of germinated seeds in all treatments starting from the second day, on which the first germination occurred, germination criterion is the appearance of radical outside seed cover **(Ganatsas, *et al*., 2008):** at end of the experiment took final results of following qualities'

Measured in this experiment were:

• Germination percentage (GP) is measured on the seventh day using the formula GP (%) = (total number of germinated seeds/ total seed) x 100. **(Ashraf and Abu-Shakra, 1978).**

• Mean germination time (MGT) calculated according to the formula of **(Ellis and Roberts, 1981):**

MGT= Σ (ni/di). With ni: number of germinated seeds and di: day of counting.

• Radical length (RL) in (mm), length (plumule PL) in (mm), radical and plumule lengths: The radical and plumule lengths were taken using a graduated ruler; the

averages were calculated by taking five seedlings from each plate.

•Seedling fresh weight (SFW) in mg, Seedling dry weight (SDW) in mg. Seedling's dry weights were measured after oven drying at 70°C for 72h of seedlings

• Vigour Index (VI) using the formula of **(Abdul- Baki and Anderson, 1973).**

VI= [TG (%) x seedlings length (mm)] /100]

: Seedlings length= radical length + plumule length.

Extraction and estimation of carbohydrate constituents: Total available carbohydrate (TAC), total soluble sugars (TSS), and polysaccharides (starch).

Extraction method

This was done by the alcoholic extraction method as described by **Younis (1963)** in which an aliquot of oven-dry biomass (100 mg) was extracted twice (2x2h) with 80% ethanol in a reflux apparatus on a boiling water bath. The two alcoholic extracts and washings were added together, evaporated to few mls in an air-dry oven at 50°C and the residue taken in water and made to known volume.

Clarification of the extract

The common lead acetate method was done in which to an aliquot of the extract, basic lead acetate solution was added drop wise with continuous stirring until another drop of lead acetate gave no further turbidity. The deleading agent, disodium hydrogen phosphate (Na2HPO4) was added drop wise to the supernatant solution with continuous stirring until the precipitation of lead phosphate was completed. The solution was then centrifuged. The clear supernatant was neutralized to phenol red end point then made to known volume and used for the determination of soluble sugars.

Estimation of total available carbohydrate content (TAC)

This is usually referred to as the total available carbohydrate (TAC) since it does not include cellulose. This fraction was estimated by the procedure described by **(**Murata ***et al.*, 1968),** in which an aliquot (100 mg) of the finely powdered oven-dry plant material was introduced into a boiling tube. Ten ml of 0.7 N HCl were then added and the tube placed in a boiling water bath for 30 min. The hydrolyzed was neutralized to phenol red end point and made to known volume. An aliquot of hydrolyzes was assayed as glucose according the method described by **(Dubois *et al.*, 1956).** To two ml of sugar extract one ml of 5% phenol was added then 5 ml of concentrated sulphuric acid were added rapidly. The tubes were allowed to stand for 10 min shaken gently and placed for 10-20 min in water bath at 30°C. Absorbance was read at 490 nm. A calibration curve using pure glucose was made from which the amount of sugar was calculated as mg g-1 d.m.

Estimation of total soluble sugars (TSS)

An aliquot (5ml) of the purified sugar extract was mixed with 5ml of 1 N HCl in a boiling tube and placed in boiling water bath for 60 min and then the hydrolyzate was neutralized to phenol red end point and made to known volume. An aliquot (2 ml) was taken and assayed as glucose by the method described before.

Estimation of polysaccharides (St.)

This is referred mainly to starch content (St) and it was deduced from the differences between the total available carbohydrate and the total soluble sugars (TAC- TSS).

Statistical analysis:

Statistical analysis of the results was done using Excel 2007 in this study; ANOVA was used for comparison between independent samples. LSD was estimated $p \leq 0.05$.

3 Results

Changes of germination percentage (GP), and mean germination time (MGT):

The results showed that decrease in total germination (GP), under long storage conditions(control) where to reduce this value in (GP)from77.4% in non-aged seed to 58.3% in aged seed , while at treatment by ZnSO4 and KN03 for aged seed (4years old) there was increased in(GP)these values were 61.23%,84.45%

compared with control 58.3%. Figer. (1) Showed that decrease in germination (GP), in aged seed and compared to non-aged seed, and increase in GP after treatment.

Mean germination time (MGT) in seed *Hordeum vulgare* L. was reduce in aged seeds compared with non-aged seeds (control), where reduced these values from 1.34 to 0.77 seedlings. day^{-1}, while there was increasing in the mean germination time (MGT) in aged seed (4years old) at treatment with ZnSO4 and KN03compared with control. The values were 1.65 seedlings. day^{-1} and 2.82 seedlings.day^{-1}compared with control 0.77 seedlings.day^{-1} compared to control Figer.2showed that decrease in

(MGT), in aged seed and compered to non-aged seed, and increase in MGT after treatment (figer.2).

Figure. (1). Changes in germination percentage:

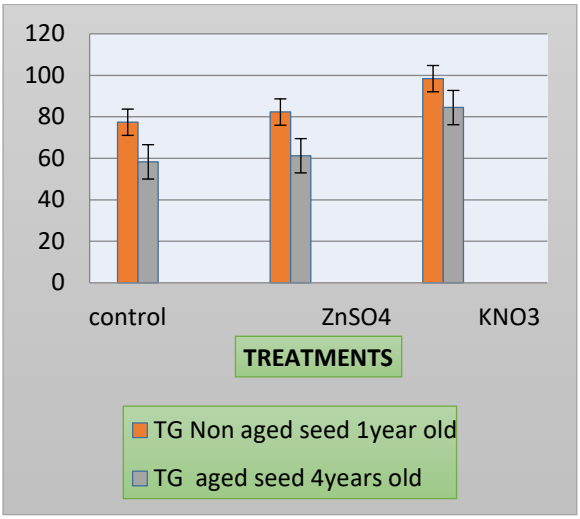
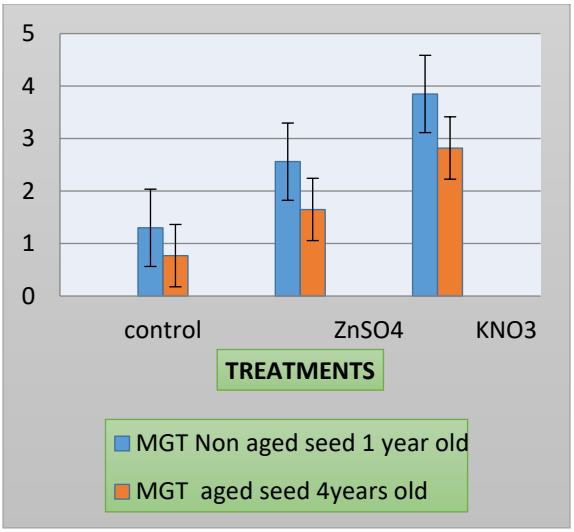


Figure. (2). Changes in Mean germination time:



Each datum indicates the mean value and vertical lines on top of the bars indicate the standard error of means.

Changes Radical length (RL) and a plumule length (PL):

(Table 2) showed that the radical length and plumule length of seedling barley plants significantly decreased in response to storage conditions. Where report these values of (RL) 49.3 mm and 26.4mm in aged seed and non-aged seed (control.) respectively. While the values of (PL) were 36.23mm, and 13.83 mm in aged seed and non-aged seed (control.) respectively (Table 1). But after treatment by ZnSO4 and KN03 the results showed to increasing in radical length and plumule length in seedling of *Hordeum vulgare* L plants where the values of (RL) were 41.6 mm, 64.3mm compared to control 26.4mm.While (PL) report these values 33.52 mm, 46.56mm compared to control 13.83mm. The values in (Table1) showed to improve growth parameter of seedlings aged seed by invigoration solutions.

Table. (1). Changes of radical length (RL) in (mm) and plumule length (PL) in (mm) of seedlings barely plants.

Parameter	**RL(mm)**		**PL(mm)**	
treatment	Non aged seed	Aged seed	Non aged seed	Aged seed
Control	49.3^{d}	26.4^{f}	36.23^{c}	13.83^{f}
ZnS04	52.5^{c}	41.6^{d}	45.45^{b}	33.52^{c}
KNO3	79.7^{a}	64.3^{b}	62.47^{a}	46.56^{b}
P-Value	0.042		0.031	
LSD	18.45		16.08	

P-value: was considered significant at P≤0.05.

LSD: Mean indexed by different superscripts is significantly different at P≤0.05

Changes in seedling fresh weight (SFW), and Seedling dry weight (SDW) of seedling barley plants.

The results showed that decrease in seedling fresh weight (SFW) and Seedling dry weight (SDW) under long storage conditions, where reports these values were 2.13mg, 1.13 mg of (control) none aged seed and aged seed, respectively. While at treatment by ZnSO4 and KN03 for aged, seed (4years old) there was an increase in (SFW) these values were 1.45mg, and 2.68 mg compared with control 1.13mg.Seedling dry weight (SDW) of seedling barley plant was reduced in aged seeds compared with non-aged seeds (control), where reports these values were 0.34mg, 0.21mg of (SDW) in non-aged seed and aged seed, respectively .While there was increasing in Seedling dry weight (SDW) of aged seed(4 years old) the treatment with ZnSO4 and K N 03 compared with control these values were 0.23 mg, 0.47

mg compared with control 0.21mg. The data in (Table, 2) report a decrease in seedling fresh weight (SFW), and Seedling dry weight (SDW) in aged seed compared to non-aged seed and an increase in these values after treatment.

Table. 2. Changes of seedling fresh weight (SFW) and Seedling dry weight (SDW) of seedling barely plants.

Parameter	SFW(mg)		SDW(mg)	
Treatment	Non aged seed	Aged seed	Non aged seed	Aged seed
Control	2.13^{c}	1.13^{f}	0.34^{c}	0.21^{d}
ZnSO4	2.56^{b}	1.45^{d}	0.46^{b}	0.23^{d}
KNO3	3.83^{a}	2.68^{b}	0.66^{a}	0.47^{b}
P-Value	0.001		0.024	
LSD	0.27		0.15	

P-value: Was considered significant at P≤0.05

.LSD: Mean indexed by different superscripts is significantly different at P≤0.05

Changes of Seedling Vigour Index (SVI) of seedling barley plants.

The results in (table 3) found a significant decrease in Seedling vigour index, in seed storage for 4 years compared to seed storage for 1years where this value reduce from 66.2 to 23.5. While vigour index of seedling was a significant increase at stored seeds for 4 years and treatment with ZNSO4 (46) compared with seedlings from control seeds (23.5), and stored seeds for 4 years and treatment with KNO3 was increasing significantly (93.6) compared with seedlings from control seeds (23.5). At (Table 3) showed that: the seedling vigour index (SVI) of barley plants significantly increased in response to treatment by KNO3.

*Significant levels, in aged seeds (93.6)

**Very significant levels represented in non-aged seed (127).

Table.3. Changes of Seedling Vigor Index (SVI) of seedling barely plants.

Treatment	Aged seed	Non aged seed
Control	66.2^{c}	23.5^{e}
ZnSo4	80.6^{b}	46^{d}
KNO3	127^{a**}	93.6^{a*}
P-Value	0.006	
LSD	15.4	

P-value: Was considered significant at P≤0.05

LSD: Mean indexed by different superscripts is significantly different at P≤0.05

Changes of carbohydrate constituents

Data in Table 4 showed decreasing of total available carbohydrate (TAC) content and starch (St) contents, in radical of untreated plants of seed storage 4years compared to seed storage 1years the decrease of (St) and TAC was 15% and 1% respectively. **Table. (4a)**

Similarly, the results reported decreasing of starch (St) and (TAC) content in pulumela of seed storage 4years compared to seed storage 1years this reduce was 16% and 10%. **Table. (4b)**

Conversely, increase the TSS content in radical and pulumela of seed storage 4years compared to seed storage 1years was 1.28-,1.3- fold . **Table. (4a &b)**

While during treatment by ZnSO4 the increase of the TSS, St, and TAC content in radical and pulumela of barley plants.

Increased in TSS, St, and TAC content in radical (seed storage 4years) were2.4-, 1.2 -, 1.5 - fold respectively compared to control. The corresponding values for pulumela were 1.9-and 1.3-, 1.4-fold, respectively (Table 4a &b).

Similarly. At treatment by KNO3 the results report increasing of TSS, St, and TAC content in radical and pulumela, of seed storage 4years compared to control. The values in radical were3.2-, 1.2-, 1.7-fold respectively compared to control. The corresponding values for pulumela were 2.3-, 1.4- , 1.5-fold, respectively (Table 4a &b). The content of total soluble sugar (TSS), total available carbohydrate (TAC) and starch (St) at treatment by KNO3 was higher than treatment by ZnSO4.

Table. (4a) Changes of carbohydrate constituents of seedling barley plants.

Seeds old & Treatments		Radical		
		TSS	ST	TAC
1y	Control	10.54[d]	39.48[b]	50.02[d]
	ZnSO4	29.06[c]	41.51[a]	70.57[b]
	KNO3	41.53[a]	47.90[a]	89.43[a]
4y	Control	13.44[d]	33.71[b]	47.15[d]
	ZnSO4	32.86[b]	38.97[b]	71.83[b]
	KNO3	43.14[a]	39.26[b]	82.40[a]
P-Value		0.031*	0.001*	0.001*
LSD		4.16	6.1	7.2

P-value: was considered significant at P≤0.05.

LSD: Mean indexed by different superscripts is significantly different at P≤0.05.

Table. (4b) Changes of carbohydrate constituents of seedling barley plants.

Seeds old & Treatments		Plumule		
		TSS	ST	TAC
1y	Control	10.26[d]	74.95[c]	85.21[b]
	ZnSO4	22.66[b]	83.43[a]	106.09[a]
	KNO3	29.23[b]	94.27[a]	123.50[a]
4y	Control	13.56[d]	63.04[c]	76.60[c]
	ZnSO4	25.28[b]	79.19[b]	104.47[a]
	KNO3	31.19[a]	85.26[a]	116.45[a]
P-Value		0.008*	0.0001*	0.001*
LSD		4.50	6.5	7.1

P-value: was considered significant at P≤0.05.

LSD: Mean indexed by different superscript is significantly different at P≤0.05

Discussion

Seed characteristics decrease under long storage conditions due to ageing. It is the reason of declining in germination, emergence and seedling growth **(Soltani *et al.*, 2016).** The results in this study showed that Seed characteristics decrease under long storage, including germination percentage (GP); mean germination time (MGT), Plumule length (PL), Radical length (RL), fresh and dry weight of seedling, and seedling vigour index of barely plants. **Table 1, 2 and 3)**

Following these results, other studies reported a decline of growth parameters during the storage of seed the other plants such as **(Pallavi *et al.*, 2003)** on sunflower plants and **(Yousif, 2010)** on sorghum.

Exposure of seeds to high temperatures and relative humidity during storage cause the reactive oxygen species to accumulate in the bilayer phospholipid membrane. Reactive oxygen species have been widely recognized as the main factor of seed ageing causing seed deterioration **(Laloi *et al.*, 2004).** The species include free radicals like superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (•OH); non-radical molecules like hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), **(Sharma *et al.*, 2012)**. **(Kibinza *et al.*, 2006)** reported the loss of viability in sunflower seeds during ageing was associated with the decline of antioxidant enzyme activity caused by the accumulation of free radicals hydrogen peroxide and lipid peroxidation. Once the seeds are imbibed in the water, enzymatic mechanisms in the seeds initiate the production of reactive oxygen species, especially in the mitochondrial respiratory chain of the metabolically active seeds **(Bailly, 2004).** Excess of ROS oxidised and denatured protein structures in the cells, caused cellular membrane starts to disorganize, and gradually lose its integrity and selectivity, leading to the rapid increment of water imbibition in the cells and affecting embryo viability **(Kapoor *et al.*, 2011; Peng *et al.*, 2011).**

Seed invigoration techniques with, chemicals, are used to reduce a seed deterioration **(Duraimurugan *et al.*,**

2011) in the present study, using invigoration solutions KNO3and ZnSO4 led to increase of the growth parameters including total germination (TG), mean germination time (MGT), Seedling length(SL), Radicle length (RL), fresh and dry weight of seedling, and vigour index, for all the seed storage. At (Table 3) showed that: the seedling vigour index (SVI) of barley plants significantly increased in response to treatment by KNO3. Potassium plays vital roles in most the biochemical and physiological processes as enzyme activation, protein synthesis, photosynthesis is, movement, energy transfer, phloem transport, and stress resistance **(Meharg, 2011)**.

The results of this study contribute to the understanding of germination seeds through the carbohydrate metabolism in seeds during storage, total available carbohydrate content in radical and pulumela were declined.(**Table 4 a & b)** Of barley seeds storage for different periods of (1year and 4 years) this was accompanied with increasing of total soluble sugars reflecting their roles as regulators. **(Alam *et al.*, 2021).**during storage, biochemical properties like, carbohydrate, and soluble sugar are changed. The process of seed deterioration is most pronounced. In carbohydrate rich seeds the processes are described by the degree of concentration of soluble sugar. Generally, it is known that total carbohydrate declines with seed ageing **Bernal-Lugo and Leopold, (1992)**.reported that the soluble sugars present in embryo may serve as important components of protection or may contnbute to the deteriorative changes occurring during seeds storage. Examination of the changes in sugars during accelerated aging of seeds with a marked decline in mono saccharides and in raffinose Sucrose content remains relatively stable.

Using invigoration solutions KNO3and ZnSO4 led to an increasing of all carbohydrate constituents in barely plants compared to those untreated plants. Increase in germination of KNO3 primed seeds recorded over control. This increase in germination may be due to the activity of α-amylase due to osmopriming. Amylases are key enzymes that play a vital role in hydrolyzing the seed starch reserve, thereby supplying sugars to the developing embryo **Abnavi, and Ghobadi, (2012).**

4 Conclusions

Conclusions: It can be concluded from this study that using invigoration solutions led to improve germination and vigour of barley seeds for all the seed storage duration. In addition, stored barley seed treatment by KNO3 was the best compared treatment by ZnSO4The priming may be an effective method to meet the demands of farmers during the culture in the field for this reason, further studies are needed to use invigoration solutions to assess the efficacy during the later stages of plant growth.

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Conflict of Interest: The author declares that there are no conflicts of interest.

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