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Influence of Different Priming Materials on Germination and Alpha-Amylase Enzyme of Hybrids Sorghum (*Sorghum bicolor* L. Moench. x *Sorghum sudanense* Staph.) Seeds

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Abstract

Germination is considered a critical step in the development cycle of the plant. But no information is available regarding seed priming with Putrescine, Jasmonic acid, Kinetin, Potassium Nitrate (KNO_3), Salicylic acid at a time in the aged and non-aged seeds of sorghum cultivars. However, to know the effect of seed priming with the aforesaid chemicals in the aged and non-aged seeds of sorghum cultivars on the germination rate, germination vigour and alpha-amylase activity, research was conducted under laboratory condition, at Field Crops Central Research Institute, Ankara, Turkey. Aged and unaged seeds of Sugar Grazer II and Digestivo hybrid silage sorghum cultivars were used as the seed material of the experiment. Putrescine, Jasmonic acid, Kinetin, KNO_3 and Salicylic acid were used as priming chemicals. The results revealed that aged and un-aged seeds of the cultivars showed different responses to similar chemical and KNO_3 application to un-aged seeds had a positive effect on germination rate and alpha-amylase, Whereas Jasmonic acid and Putrescine applications had a positive effect on the aged seeds. Priming with putrescine, KNO_3 and jasmonic acid showed the best results in the experiment.

Keywords: Alpha-Amylase, Germination, Priming, Seed

Introduction

Seed germination forms the basis of plant formation as the period of strongest life activity in all life cycles of a plant. During this period, the metabolism of fats, proteins and carbohydrates provides energy for seedling growth (Shang and Zhao, 1993). These three major nutrient reserves are specifically hydrolysed by amylases, proteases and lipases, respectively (Barros et al., 2010; Di Girolamo and Barbanti, 2012). The carbon resources required for the growth of the embryo are obtained by the breakdown of starch. Starch is catalysed by the amylase enzyme family and broken down as a result of reactions (Sun and Henson, 1991). α-amylases are endo enzymes that can produce shorter chains called border dextrins, glucose and maltose by breaking random α- (1, 4) bonds within their polysaccharide chains. β-amylases are exohydrolases that hydrolyze α- (1, 4) bonds in polysaccharides (Catusse et al., 2012). The activities of soluble sugars and amylase enzymes are positively associated with seed germination and seedling viability (McDonald, 2000). The seed, whose metabolic activity is minimized by drying, gradually loses its germination rate and strength by being exposed to physiological and biochemical changes during improper storage conditions and prolonged storage period. As a result of aging in the seed, changes in the macromolecular structures of the enzymes that need to operate for seed germination occur in thin structure, partial curl or non-curl, condensation of polymers, lipid peroxidation through free

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radicals, enzyme inactivation, reduction of protein amount, disintegration of cell integrity and genetic damage. Problems in the activities of enzymes cause a decrease in the respiratory potential and ATP formation, resulting in a decrease in the amount of nutrients that must be provided to the seed during the germination process, and result in a decrease in the germination rate (Narayana Murthy and Sun, 2000; Goel et al., 2002; Lehner et al., 2008). Preliminary imposition of priming on seeds allows the seed to partially hydrate, causing metabolic activities to begin, increasing the activity of biochemical mechanisms required in the repair of cell damages and activation of antioxidants in the defence system. However, it does not allow root formation (Shrivastava et al. 2014). In addition, priming increases the activity of enzymes such as α-amylase, which have an important role in the germination of the seed, causing the compounds in the endosperm to break down in a shorter time and accelerates the induction of enzymes that catalyze the transfer of these degraded compounds (Di Girolamo and Barbanti, 2012). Positive effects of jasmonic acid and salicylic acid application on germination power were observed by Ebrahim Pour Mokhtari and Emeklier (2017) in hybrid sorghum, Buyukcingil (2007) in grain sorghum, and Canakci (2010) in barley. Sorghum seeds generally lose their biological properties in a short time. This situation affects plant genetic characteristics in storage conditions. Since sorghum seeds need very well prepared seedbeds for production. If planting is done in poorly prepared soils, germination and emergence rates will be very low. On the other hand, sorghum seeds should be preserved in very good conditions, as the seed vitality rapidly disappears. In this study, the effects of priming with different chemicals on the aged and unaged seeds of two different hybrid sorghum varieties (Sugar Grazer II and Digestivo) were investigated to evaluate the germination and α-amylase activity.

Materials and Method

Location and Materials

This research was conducted under laboratory conditions at Field Crops Central Research Institute, Ankara-Turkey. Aged and unaged seeds of Sugar Grazer II and Digestivo hybrid silage sorghum (*Sorghum bicolor* L. Moench. x *Sorghum sudanense* Staph.) cultivars were used as the seed material of the experiment. Five chemicals Putrescine (4 mg/L), Jasmonic acid (1 mg/L), Kinetin (10 mg/L), KNO_3 (25 mg/L) and Salicylic acid (4mg/L) were utilized for this study.

Treatments and Design

The experiment is consisted of three factors viz. a) Two cultivars (i. Sugar Grazer II, and ii. Digestivo), b) Two seed ages (aged and unaged), and c) Six priming (i. No priming-control, ii. Priming with Jasmonic acid, iii. Priming with Kinetin, iv. Priming with Potassium Nitrate (KNO_3), v. Priming with Putrescine, and vi. Priming with Salicylic acid). The experiment was laid out with RCDB and three replications.

Experimentation

For laboratory experiments, seeds were utilised for each treatment at the end of the priming session. This study was used five chemicals (Putresin (4 mg/L), Salicylic acid (4 mg/L), Kinetin (10 mg/L), KNO_3 (25 mg/L) Jasmonic acid (1 mg/L)). Chemicals used in the study were provided by Merck and Sigma Aldrich Chemical Co. (Anonymous, 2014). Depending on the variety, Sugar Grazer II variety was kept for 8 hours, Digestivo variety was kept for 6 hours. After the priming period was over, the seeds were washed with distilled water, dried in Petri dishes for 24 hours at room temperature, and then made ready for planting. The seed aging process was determined according to Matthews (1980) and Zamani et al. (2010). The seeds (100 x 3 = 300) were placed in germination cabinets with a temperature of ± 1^{o}C in special germination containers, adjusted to 25^{o}C as the standard germination temperature and left to germinate.

Data collection

Germination Rate and Germination Percentage

According to ISTA rules, the seeds germinated on the fourth day were counted, and the average of these values calculated as percentages was determined as the germination rate. Germination percentage (%): Seedling vigour is determined as seeds that germinated on the 10th day according to ISTA rules (Anonymous, 2011).

Alfa-amylase

Starch was extracted by using the method of Singh *et al*. (2009). Sorghum grain (100 g) was immersed in 200 ml of NaOH (0.25%, w/v) at 5°C for 24 h. The soaked grains were washed and ground with an equal volume of water using a blender for 3 min. The mixture was filtered through a 200-mesh screen. The remainder on the sieve was rinsed with distilled water. Grinding and filtering were repeated on this material. After rinsing, the remainder on the sieve was discarded. The filtered liquid was allowed to stand for 1 h. The filtered liquid was centrifuged at 760 × g for 10 min. The grey colored froth, top protein-rich layer was removed using a spatula. Water was added again to re-suspend the sample, and centrifugation was done for 5 min. washing and centrifugation process was repeated several times until the top starch layer was white. The starch was dried for 24 h at 40°C. After each sample of starch is extracted, for measuring alpha-amylase activity, the resultant mixture for raw starch digesting activity contains 0.3 g of raw starch, 30 ml deionized water, 5.5 ml enzyme solution (2.5 units ml^{-1}) and 4 ml of 0.1 M acetate buffer (pH 5.0). The resultant mixture was incubated for 1 h at 30°C. The absorbance of reaction mixture filtrates was analyzed in the spectrophotometer. The absorbance of the supernatant was 620 nm. The absorbance was converted to α-amylase units using a commercial α-amylase preparation with known activity.

Statistical Analysis

Statistical analyses were subjected to variance analysis using the JMP 10 program according to the Random Plots Factorial Trial Design. The significance of the differences was evaluated with the F test, and the grouping of the means was made according to the LSD 5% test.

Results and Discussion

In the study, five different chemicals of putrescine, jasmonic acid, kinetin, KNO_3, salicylic acid were applied to the seeds of two hybrid sorghum varieties (Sugar Grazer II and Digestivo) as aged and unaged conditions and compared with the control (not primed) seeds.

Table 1. Analysis of variance on the germination rate, germination vigor and alpha-amylase effect of two sorghum cultivars as seed aged and unaged conditions

Source	df	Germination rate	Seedling vigour	Alpha-amylase
Cultivar	1	1.0538	0.7149	69.6343***
Seed age	1	0.8785	0.097	11.9432**
Priming	5	22.3048***	13.518***	36.4122***
Cultivar × Seed age	1	6.9343*	0.2396	469.2659***
Cultivare×Priming	5	4.6155**	1.941	64.2289***
Seed age×Priming	5	2.4036	2.5588*	65.9455***
Cultivare×Seed age×Priming	5	6.8339***	0.4107	26.7269***
CV (%)		21	28	28

*, **, *** 5%, 1%, 0.1% significant, respectively

Germination Rate

The results of the variance analysis performed with data on the germination rate of the priming in the seeds of two sorghum hybrid varieties are given in Table 1. Cultivar x seed age interactions were found significant at 5% level, cultivar x priming at 1% level, and cultivar x seed age x priming interactions at the 0.1% level. The results of the variance analysis on the alpha-amylase of the primed seeds are given in Table 1. Seed age was found significant at 1% level and other features were found significant at the 0.1% level.

The germination rate of the un-aged seeds of the Sugar Grazer II variety varied between 86.33 and 95.00%. The highest germination rate was observed in seeds treated with Jasmonic acid, and the lowest in control seeds. In addition, seeds treated with Kinetin and control seeds were in the same group in terms of the lowest germination rate. The germination rate of aged seeds of the Sugar Grazer II variety varied between 84.00 and 98.33%. The highest germination rate was determined in seeds treated with Jasmonic acid followed by Putrescine, and the lowest germination rate was determined in control and Kinetin seeds. The germination rate in the unaged seeds of Digestivo variety varied between 83.00 and 98.00%. The highest germination rate was obtained from seeds treated with KNO_3, and the lowest germination rate from control seeds. The germination rate of aged seeds of Digestivo varied between 89.00 and 93.67%. The highest germination rate was obtained from seeds treated with Putrescine, and the lowest germination rate from control seeds (Table 2).

When we compare the responses of the aged and unaged seeds of both varieties to different chemicals in terms of germination speed characteristics, the aged and un-aged seeds of the Sugar Grazer II variety showed the highest germination rate as a result of the pre-treatment with Jasmonic acid (Figure 1). Jasmonates priming has a supportive role in morphological and physiological changes and provided the highest germination rate. The highest germination rate showed in the pre-application with KNO_3 in the unaged seeds and with Putrescine in the aged seeds of the Digestivo variety. Due to the changes in seed compounds as a result of the aging process, seeds showed different reactions to different chemicals and showed different results. The different reactions between the two cultivars are related to the physiological characteristics of the seeds of the cultivars, and this result is expected. Our findings regarding the germination rate are showed similarities with the results of Tekin and Bozcuk (1998) in sunflower, Çavuşoğlu (2007) in barley for the application Putrescine; Madakadze et al. (2000) in millet, and Buyukcingil (2007) in grain sorghum for application of KNO_3.

Table 2. The average germination rate values of aged and normal seeds of sorghum varieties of different chemical applications

	Unaged		Mean	Aged		Mean
	Digestivo	Sugar Grazer II		Digestivo	Sugar Grazer II	
Control	83.00	86.33	84.67	89.00	84.00	86.50
Jasmonic acid	93.33	95.00	94.17	93.67	98.33	96.00
Kinetin	97.00	86.33	91.67	90.00	96.00	93.00
KNO_3	98.00	93.67	95.84	92.67	92.33	92.50
Putrescine	92.00	94.00	93.00	93.67	98.33	96.00
Salicylic acid	93.67	88.00	90.84	91.67	87.67	89.67
Mean	92.83	90.56		91.78	92.78	
Unaged /aged means	91.69			92.28		

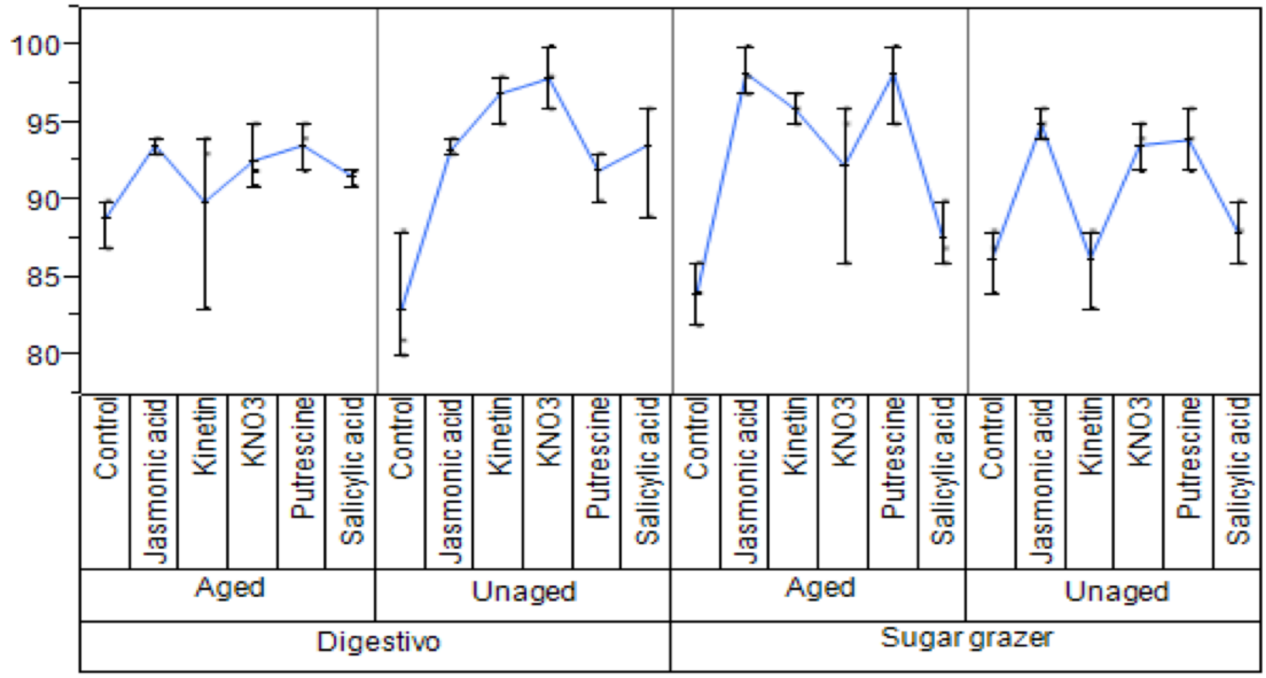

Figure 1. Germination rate values of aged and normal seeds of two sorghum varieties of different chemical applications

Germination Percentage (Vigor)

The results of variance analysis regarding the effects of priming on the germination vigor of the seeds of two different sorghum hybrid varieties are shown in Table 1.Statistically, only seed age x priming interactions were found significant at the level of 5%, the differences among the primings were significant at the 0.1% level, and differences between the varieties were found insignificant.

In terms of germination vigor, the germination power of the un-aged seeds of the Sugar Grazer II variety varied between 87.67 and 95.67%. While the highest germination vigor was determined in seeds treated with Jasmonic acid followed by Salicylic acid, and the lowest germination power was seen in control seeds. The germination power of the aged seeds of the Sugar Grazer variety varied between 87.67 and 97.67%. However, the highest germination power was determined in seeds treated with Putrescine, and the lowest germination vigor was determined in control seeds. The germination vigor of the non-aged seeds of the Digestivo variety varied between 86.67% and 96.67%. The highest germination vigor was determined in seeds treated with Salicylic acid and KNO_3, and the lowest germination vigor in control seeds. The germination strength of the aged seeds of Digestivo varied between 89.00 and 95.00%. The highest germination power was determined in seeds treated with Jasmonic acid, and the lowest germination vigor in control seeds (Table 3). When we compare the responses of aged and non-aged seeds of both varieties to different chemicals, the aged and unaged seeds of the Sugar Grazer II variety showed the highest germination vigor as a result of pre-treatment with Jasmonic acid (Figure 2). Jasmonic acid changed the morpho-physiological properties and showed the highest germination power. The non-aged and aged seeds of the Digestivo variety showed the highest germination vigor as a result of the pre-treatment with Salicylic acid and KNO_3. Ebrahim Pour Mokhtari and Emeklierin (2017) obtained the highest germination rate with the application of Jasmonic acid in both aged and un-aged seeds in hybrid sorghum varieties. Buyukcingil (2007) stated that the application of Jasmonic acid and Salicylic acid in grain sorghum provides positive effects on germination vigor. Our study showed similarities with the results obtained by Canakci (2010) in the application of Salicylic acid in barley, Xu et al. (2011) with the application of Putrescine in tobacco seeds.

Alpha-amylase in the aged seeds of the Sugar Grazer II variety varied between 0.006 and 0.057. Nonetheless, the highest alpha-amylase value was obtained in the seeds treated with Putrescine, and the lowest was found in the seeds treated with control and KNO_3. Alpha-amylase in the un-aged seeds of the Sugar Grazer II variety varied between 0.006 and 0.011. The highest alpha-amylase was determined in seeds treated with Putrescine, while the lowest alpha-amylase was determined in control seeds. The germination strength of the non-aged seeds of the Digestivo variety ranged from 0.003 to 0.005. Alpha-amylase in the aged seeds of the Digestivo variety varied between 0.005 and 0.021. The highest alpha-amylase was determined in the seeds treated with Salicylic acid, and the lowest in the seeds treated with Kinetin (Table 4 and Figure 3). The most activity of the α-amylase enzyme was obtained by Asadi Danalo et al. (2018) as a result of the priming process with 3 mM Spermidine in *Borago officinalis* plants. According to the results obtained by Lee and Kim (2000), the priming in aged seeds of rice plant increased the total sugar content in the seed and the activity of the α-amylase enzyme, resulting in an increase in the germination rate. It has been determined by many researchers that the increase in the activity of the alpha-amylase enzyme increases germination speed and strength, allowing seeds to germinate under a wider range of environmental conditions (Mukasa *et al*., 2003; Basra *et al*., 2005; Sathish and Sundareswaran, 2010; Dehghanpour Farashah *et al*., 2011).

Table 3. The average germination percentage (vigor) values of aged and normal seeds of sorghum varieties of different chemical applications

	Unaged			Aged		
	Digestivo	Sugar Grazer II	Mean	Digestivo	Sugar Grazer II	Mean
Control	86.67	87.67	87.83	89.00	87.67	87.67
Jasmonic acid	93.00	95.67	94.00	95.00	97.33	96.50
Kinetin	92.33	90.33	91.83	91.33	92.33	91.33
KNO_3	96.67	94.33	94.83	93.00	92.67	93.50
Putrescine	92.00	95.00	92.50	93.00	97.67	96.33
Salicylic acid	96.67	95.67	94.83	93.00	91.67	93.67
Mean	92.89	93.11		92.39	93.22	
Unaged /aged means	93.00			92.81		

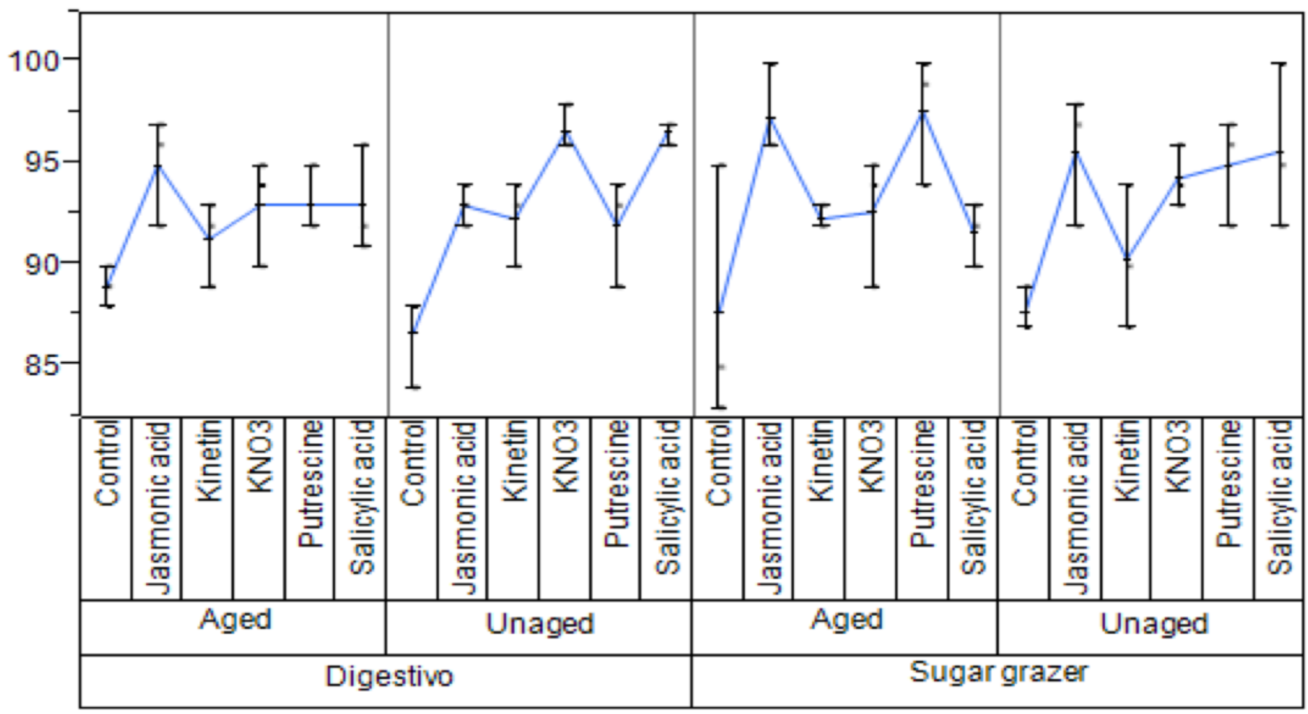

Figure 2. Germination percentage (vigor) of aged and normal seeds of two sorghum varieties of different chemical applications

Table 4. The average alpha-amylase values of aged and unaged seeds of sorghum varieties of different chemical applications

	Unaged			Aged		
	Digestivo	Sugar Grazer II	Mean	Digestivo	Sugar Grazer II	Mean
Control	0.003	0.01	0.007	0.006	0.006	0.006
Jasmonic acid	0.027	0.007	0.017	0.014	0.042	0.028
Kinetin	0.057	0.002	0.030	0.005	0.010	0.008
KNO_3	0.058	0.006	0.032	0.011	0.006	0.009
Putrescine	0.012	0.001	0.007	0.009	0.057	0.033
Salicylic acid	0.058	0.011	0.035	0.021	0.024	0.023
Mean	0.036	0.006		0.011	0.024	
Unaged /aged means	0.021			0.018		

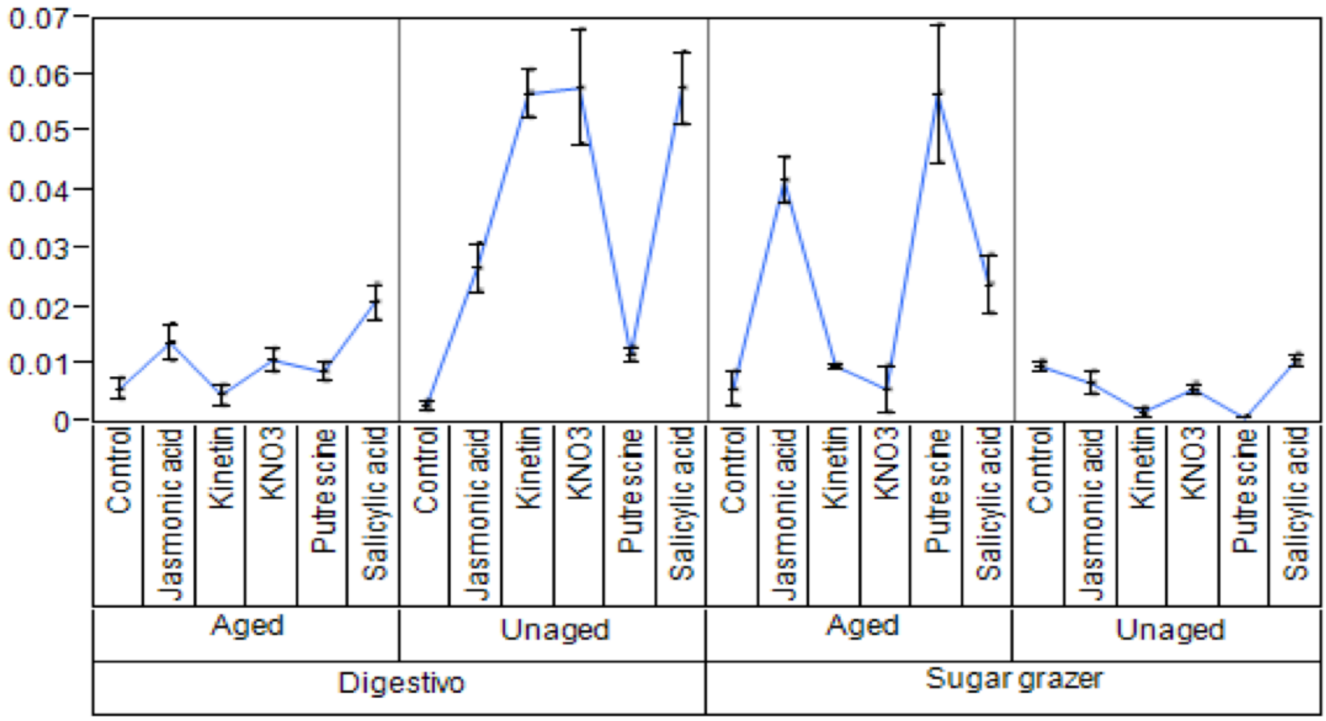

Figure 3. The Alpha-amylase of aged and unaged seeds of two sorghum varieties of different chemical applications

Conclusion

Priming with Putrescine, Jasmonic acid, Kinetin, KNO3 and Salicylic acid remarkably changed the germination rate, germination vigour and enzyme activities in the seeds of the aged and non-aged seeds of sorghum varieties. Therefore, aged and un-aged seeds of the cultivars showed differential responses to similar chemicals. Application of KNO_3 to the un-aged seeds had a positive effect on germination rate and alpha-amylase, whereas Jasmonic acid and Putrescine applications had a positive effect on the aged seeds. Priming with Purescine, KNO_3 and Jasmonic acid showed the best results in the experiment.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest

Author contribution

Negar Ebrahim Pour Mokhtari designed the study and collected the data. Ferhat Kizilgeci made the statistical analysis and wrote the original draft of the article. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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