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EFFECT OF SEED STIMULATION AND SEED AGE OF SORGHUM ON THE GERMINATION AND TRAITS OF SEEDLINGS

Mohammed Qassim Safi AL-hade and Razaq Lifta Attiya Alselawy*

Agriculture College, University of Kerbala, Iraq.

Abstract

A laboratory experiment was conducted at the seed technology lab, Department of Field Crops, College of Agriculture, University of Karbala to study the effect of stimulating seeds on the standard laboratory germination percentage and seedling vigor produced by seeds stored for a number of years and determine the best concentration of gibberellic acid to stimulate them. The Completely Randomized Design (CRD) within the order of factorial experiments included three replicates. The experiment consisted of two factors, the first factor was the treatments of seed stimulation (dry non-soaked seeds, seeds soaked in distilled water for 24 hours, and seeds soaked in gibberellic acid (GA_3) at the concentrations 20, 40, and 60 $mg.l^{-1}$) and the second factor was the age of the seeds (one year, two years, three years, and four years). Sorghum seeds, cultivar In Path, produced by Agricultural Research Department, Abu-Ghraib during the fall season were used. Results showed the significant superiority of the treatment of stimulating seeds by the concentration 60mg.l-1 of gibberellic acid giving the highest averages of the traits: standard laboratory germination percentage (73.67%), radical length (6.55 cm), seedling dry weight (11.40 mg), and seedling vigor at the second count (1103) compared to the control treatment giving the lowest of averages. The treatment of storing seeds for one year was superior in the studied traits giving the highest averages of radical length (6.38 cm), plumule length (7.77 cm), seedling dry weight (11.25 mg), and seedling vigor (1202) compared to the treatment of storing seeds for four years that gave the lowest averages. The results showed a significant effect of the interaction between the treatments of seed stimulation and seed age on the most traits in studied. We conclude from the study that stimulating seeds, in general, increased the standard laboratory germination percentage of all seed ages.

***Key words* :** sorghum, seed stimulation, Completely Randomized Design (CRD)

Introduction

One of the problems facing sorghum growing is the seed germination weakness (Hamza and Mohswen, 2017), as well as seed dormancy (Fenner and Thompson, 2005) and entering into a case of secondary dormancy immediately after harvest (Selvaraju and Krishnasamy, 2005).

phytohormones are considered the most important endogenous substances for modulating physiological and molecular responses, a critical requirement for plant survival as sessile organisms, (Al-Taey and Majid, 2018), The gibberellins (GAs) are a large group of tetracyclic diterpenoid carboxylic acids, The GAs show positive effects on seed germination, leaf expansion, stem elongation (Al-Taey, 2017), flower and trichome initiation, and flower and fruit development, They are essential for plants throughout their life cycle for growth-stimulatory functions (AlTaey *et al.,* 2018). The highest germination percentage can be obtained after harvest immediately, where the percentage declines gradually during the storage period; however, treating the sorghum seeds with gibberellic acid improves seed performance and germination as it increases the activity of enzymes (Azadi *et al.*, 2013). Moyo *et al.*, (2015) clarified that seed vigor is a parameter for the accumulated damage in seeds. He observed significant differences in the radical and plumule length when he was studying sorghum seeds kept in temperature 0-4 ú C for 10 years (2004 -2014). Stimulating seeds of three sorghum varieties by gibberellic acid increased the dry weight of seedlings significantly (NAJIM, 2016). Rasoolzadeh *et al.*, (2017) referred that stimulating seeds stored for more than 10 years with plant hormones enhanced the germination traits. This research aims to investigate the effect of stimulating seeds on germination and seedling vigor of seeds stored for several years.

***Author for correspondence :** E-mail : razzaq.ateha@uokerbala.edu.iq

Materials and Methods

A factorial experiment was conducted at the seed technology laboratory affiliating to, department of field crop, College of Agriculture, University of Karbala in 2018. The Completely Randomized Design (CRD) within a factorial experiment order including two factors and three replicates was used where the first factor was the treatments of seed stimulation (dry seeds, seeds soaked in water for 24 hours, and seeds soaked in three concentrations (20, 40, and 60 $mg.l^{-1}$) of gibberellic acid (GA3) and the second factor was seed age (one year, two years, three years, and four years). Having the solutions prepared according the required concentrations, seeds were soaked for 24 hours next 150 seeds of each treatment were taken from each variety and put in paper towels in Petridishes and replicated three times, and finally they were put in a germinator under temperature 25°C and moisture 45% (ISTA, 2103)

Studied traits

Standard laboratory examination of germination %

This examination was estimated by calculating the total number of normal seedlings 10 days after putting seeds in the germinator (ISTA, 2013). The germination percentage was calculated by dividing the number of normal seedlings by the total number of seeds.

Radical and plumule length (cm)

After the period of examining germination (10 days), five normal seedlings were chosen randomly. A ruler was used to measure the radical length after separating it from point of contact with the seed and plumule length after separating it from point of contact with epicotyl (AOSA, 1983; AOSA, 1988)

Seedling dry weight (mg)

Having the examination period (10 days) ended, ten normal seedlings resulted from the standard laboratory examination of germination were chosen. Seedlings, after seed coats had been removed away, were put in perforated paper bags in an electric oven at 80 C for 24 hours, next they were weighted by a sensitive electric balance, and then the average of the seedling dry weight was calculated (ISTA, 2013).

Seedling vigor at the percentage of standard laboratory germination

This trait was calculated according the equation of Murti *et al.,* (2004) as following.

Seedling vigor = germination percentage at the laboratory examination × (radical length + plumule length)

Statistical analysis

Data of the studied traits were statistically analyzed and means were compared based on the least significant difference (LSD) at the probability 0.05 (Steel *et al.*, 1981).

Results and Discussion

Standard laboratory germination percentage

Table 1 shows the significant superiority of stimulating seeds by 60 $mg.^{-1}$ of gibberellic acid that gave the highest standard laboratory germination percentage (73.67%) compared to other stimulation treatments, while the control treatment (dry seeds) produced the lowest average of standard laboratory germination percentage (50.67%). This superiority is attributed to the influence of gibberellic acid in breaking the seed dormancy and increasing germination percentage by stimulating creation of enzymes such as - α amylase that helps to digest starch, protease that helps to digest protein, and nuclease that helps to digest nucleic acid and transferring digested materials to the embryo (Attiya and Jaddoa, 1999). This was confirmed by the study findings of Sheykhbaglou *et al.*, (2014). They found revealed that stimulating seed by gibberellic acid significantly increased the standard germination percentage. Seed ages also affected significantly the standard germination percentage where the seeds stored for two years and for one year were superior in this trait giving the highest germination percentage (85.07 and 82.13 % respectively) compared the other treatments of seed storage, however, these two treatments did not significantly differ from each other, while they were significantly different from the seed stored for three years giving the lowest average of seed germination (39.13%) which in turn did not differ from the seeds stored for four years producing (41.60%).

The superiority of seeds stored for less than two years compared to the seeds stored for more than two years may be due to the high vitality and non-deterioration of some components, that was confirmed by findings of Azadi and Younesi (2013) and Staphen (2014) who referred to that the standard laboratory germination percentage and the seedling ability for survival significantly decreases as a result of increasing the storage period. The interaction between gibberellic acid concentration and seed age affected this trait significantly where 60mg.l-1 of gibberellic acid with seeds stored for two years produced the highest average of standard laboratory germination percentage (96.00%) and did not differ significantly from the interactions between each of gibberellic acid concentrations (60, 40, and 20) with the seeds stored for one year nor from the interaction between the treatment of gibberellic acid at 20 mgl^{-1} with seeds stored for two years. The lowest standard laboratory germination percentage (32.00%) was obtained from the interaction between the control treatment (dry seeds) with the seeds stored for three years and the seeds stored for four years. The results of the interaction indicated that the treatments of stimulation significantly affected the standard laboratory germination percentage of seeds at all ages, consequently, stimulating seeds by gibberellic acid is an important factor effective in improving the standard laboratory germination percentage of stored seeds as well as in getting seed germination comparable to the newly harvested seeds.

Radical length

Table 2 showed that the treatment of stimulating sorghum seeds by $60mg.l^{-1}$ of gibberellic acid was significantly superior in the radical length producing the highest average (6.55 cm) compared to the other concentration treatments, while the control treatment produced the lowest average of radical length (2.22 cm). The superiority of this concentration of gibberellic acid was due to the effect of gibberellic acid on increasing the processes of cell division and cell extension, as it increases the size of the meristematic area as well as increasing the number of the dividing cells (Attiya and Jaddoa, 1999). These results are consistent with those of Najim (2016) and Forghani *et al.,* (2018) who referred that stimulating seeds by gibberellic acid increased the radical lengths significantly. The table also showed that the seed age affected the radical length significantly. The treatment of one-year stored seeds was superior to the other storage treatments in the radical length giving the highest average (6.38 cm), whereas the treatment of four-years stored seeds gave the lowest average of radical length (3.50 cm).

The superiority of one - year stored seeds producing the highest radical length was due to the seed vitality and keeping the components with no deterioration. This was confirmed by Moyo *et al.*, (2015) and Yousif (2018) who indicated that the long storage period affected the radical length negatively.

Regarding the effect of interaction between stimulating seeds with gibberellic acid and seed age, the treatment of $40mg.l^{-1}$ of gibberellic acid with seeds stored for one year was superior in this trait producing the highest average of radical length (7.69 cm), however, it did not differ significantly from the treatment of gibberellic acid at $60mg.l^{-1}$ with seeds stored for one year that produced radical length averaged 7.57 cm, while the lowest average of the radical length (1.80 cm) was produced by storing the dry seeds (the control) for four years. The interaction results indicate that the treatments of stimulation increased the radical length significantly in all seed ages, so stimulating the seeds is an important factor affecting the radical length of the stored seeds and giving them length comparable to that of recently harvested seedlings.

Plumule length

(Table 3) shows the effect of stimulating seeds by gibberellic acid on the plumule length. The two concentrations 40 and 60 $mg.^{-1}$ of gibberellic acid were superior in this trait producing the highest length (8.13 and 7.87 cm respectively), however, the difference between them was not significant. The control treatment (dry seeds) produced the lowest value of plumule length averaged 3.13 cm. The superiority of stimulation by gibberellic at the concentrations 40 and 60 $mg.l^{-1}$ in giving the highest average of plumule length is attributed to the effect of gibberellic acid on increasing the processes of cell division and cell resulting in producing large seedlings and this is consistent with results of Cheyed (2008), Najim (2016), and Forghani *et al.*, (2018) who reported that stimulating seeds by gibberellic acid leads to a significant increment in the plumule length. Table 3 also reveals that the seed age has a significant effect on the plumule length.

Table 1: Effect of seed stimulation and seed age on the standard laboratory germination percentage (%).

Treatments	Seed age				Mean
	One year	Two years	Three years	Four years	
Dry seeds	66.67	72.00	32.00	32.00	50.67
Soaking in distilled water	74.67	80.00	45.33	40.67	60.17
20 $mg.l^{-1}$	86.67	92.00	34.00	34.00	61.67
40 $mg.l^{-1}$	89.33	85.33	37.33	46.67	64.67
60$mg.l^{-1}$	93.33	96.00	50.67	54.67	73.67
LSD 0.05 of interaction	10.199				LSD 0.05 of treatments 5.100
Mean	82.13	85.07	39.87	41.60	
LSD 0.05 of seed age	4.561				

The seeds stored for one year was superior in this trait giving the highest length (7.77 cm) compared to the other storage treatments especially the treatment of storing seeds for four years producing the lowest length of plumule (4.58cm). The superiority of storing seeds for one year and producing the highest plumule length compared to the other storage period was for the high vitality they have and staying their component with no deterioration and this was confirmed by Moyo (2015) who reported that the plumule length was decreased when the storage period lengthened. We observe from the same table that the interactions between the treatments of gibberellic acid (40 and 60 mg.$^{-1}$) and the seeds stored for one year, though the difference between them was insignificant, were significantly superior in giving the highest plumlule lengths (10.45 and 9.89 cm respectively) compared to the treatment of storing seeds for four years which produced the lowest plumule length averaged 2.34 cm. The interaction results indicate that stimulating seed treatments were significantly effective on increasing the plumule length for all seed ages, so this factor is important and effective in increasing the length of plumules resulted from stored seeds *i.e.* enabling them to produce long plumule comparable to plumules produced from seeds harvested recently.

Seedling dry weight (mg)

Table 4 illustrates the significant superiority of stimulating the seeds treated by 60 mg.l^{-1} of gibberellic acid producing the highest dry weight of seedlings (11.40 mg) compared to the other treatment of seed stimulation, while the lowest average of seedling dry weight (5.97mg) was obtained by the control treatment (dry seeds). The superiority of this treatment was due to the physiological effect of gibberellic acid on increasing in the division, elongation, and growth of the cells where this concentration of gibberellic acid led to an increment in the radical length table 2 and in the plumule length, consequently, it led to a significant increment in the dry weight of the seedlings. These results were consistent with those of Jaddoa and Cheyed (2013) and Sheykhbaglou *et al.*, (2014) who indicated that gibberellic acid increases seedling dry weight.

Table 4 showed that seed age also affect the seedling dry weight significantly where the treatment of storing seeds for one year was superior to other storage treatments in this trait giving the highest dry weight of the seedlings (11.25mg), whereas the treatment of storing seeds for four years produced the lowest weight of the seedlings (6.66 mg). This result was confirmed by Yousif (2018) who reported that the new seeds produced dry

Table 2: Effect of seed stimulation and seed age on the radical length.

Treatments	Seed age				Mean
	One year	Two years	Three years	Four years	
Dry seeds	3.21	2.89	2.16	1.80	2.52
Soaking in distilled water	6.73	6.59	6.89	3.88	6.02
20 mg.l^{-1}	6.66	5.31	4.48	3.71	5.04
40 mg.l^{-1}	7.69	6.74	5.33	3.25	5.75
60mg.l^{-1}	7.57	6.81	6.94	4.86	6.55
LSD 0.05 of interaction	0.943				LSD 0.05 of treatments 0.471
Mean	6.38	5.67	5.16	3.50	
LSD 0.05 of seed age	0.422				

Table 3: Effect of seed stimulation and seed age on plumule length.

Treatments	Seed age				Mean
	One year	Two years	Three years	Four years	
Dry seeds	3.27	3.51	3.42	2.34	3.13
Soaking in distilled water	6.13	4.04	4.91	3.00	4.52
20 mg.l^{-1}	9.12	8.96	5.85	5.43	7.34
40 mg.l^{-1}	10.45	9.08	6.47	6.53	8.13
60mg.l^{-1}	9.89	8.88	7.12	5.58	7.87
LSD 0.05 of interaction	1.130				LSD 0.05 of treatments 0.565
Mean	7.77	6.89	5.56	4.58	
LSD 0.05 of seed age	0.505				

weight more than seeds stored for a long time. The superiority of the seeds stored for one year in the trait of seedling dry weight was due to high vitality of the seeds and to non-deterioration of components, which led to the superiority of the radical length table 2 and plumule length table 3 resulting in an increase in the seedling dry weight. Concerning the interaction between the treatments of storing seed and age of seeds, the interactions between each of the gibberellic acid concentrations 40 and 60 $mg.l^{-1}$ where the difference between them was insignificant, with the seeds stored for one year were significantly superior producing the highest dry weight of the seedlings (13.10 and 13.37 mg respectively), whereas the lowest seedling dry weight (4.23mg) was produced by the dry seeds (the control treatment) stored for four years. The results of interaction refer that the treatments of stimulation significantly affected the seedling dry weight of all seed ages and thus stimulating seeds is an important factor and effective in increasing the dry weight of seedlings comparable to dry weight of the seedlings resulted from newly harvested seeds.

Seedling vigor in the standard laboratory germination percentage

Table 5 shows the significant superiority of the seeds treated by $60mg.l^{-1}$ of gibberellic acid giving the highest average value of the seedling vigor (1130) compared to the other treatment of seed stimulation, whereas the lowest seedling vigor (303) was obtained by the control treatment (dry seeds). The superiority of this treatment in the seedling vigor is attributed to its superiority in the traits of standard laboratory germination percentage (table 1), radical length table 2, and plumule length table 3, consequently it resulted in a significant increase in the seedling vigor. This result was confirmed by the results of Al-Selawy (2011) and (NAJIM) who indicated that stimulating seeds by gibberellic acid increased the seedling vigor significantly.

Table 5 shows that the age of seeds has a significant effect on the seedling vigor at the standard laboratory germination percentage where the seeds stored for one year were significantly superior in producing seedlings of the highest vigor (1202), whereas storing seeds for four years produced seedlings of the lowest vigor (353). The superiority of the seeds stored for only one year was due to their high vitality and to non-deterioration of components that gave them superiority in the traits of high germination percentage table 1, radical length table 2 plumule length table 3, eventually resulted in the significant superiority in comparison to the seeds stored

Table 4: Effect of seed stimulation and seed age on seedling dry weight.

Treatments	Seed age				Mean
	One year	Two years	Three years	Four years	
Dry seeds	7.57	6.30	5.77	4.23	5.97
Soaking in distilled water	9.93	8.53	9.17	7.17	8.70
20 $mg.l^{-1}$	12.27	10.20	8.53	7.13	9.53
40 $mg.l^{-1}$	13.37	10.40	9.80	7.57	10.28
60$mg.l^{-1}$	13.10	13.03	12.27	7.20	11.40
LSD 0.05 of interaction	0.912				LSD 0.05 of treatments 0.456
Mean	11.25	9.69	9.11	6.66	
LSD 0.05 of seed age	0.408				

Table 5: Effect of seed stimulation and seed age on seedling vigor at standard laboratory germination percentage.

Treatments	Seed age				Mean
	One year	Two years	Three years	Four years	
Dry seeds	436	461	179	136	303
Soaking in distilled water	957	849	536	282	656
20 $mg.l^{-1}$	1369	1311	353	313	836
40 $mg.l^{-1}$	1621	1351	444	464	970
60$mg.l^{-1}$	1628	1503	712	570	1103
LSD 0.05 of interaction	150.5				LSD 0.05 of treatments 75.2
Mean	1202	1095	445	353	
LSD 0.05 of seed age	67.3				

for more than a year. This was confirmed by the results of Ahamed and Sana (2010) who reported that the seed vitality declines when the storage period increases. Regarding the interaction between stimulating seeds and seed age, we observe in the same table the significant superiority of the treatment of stimulating seeds stored for one year by 60 $mg.l^{-1}$ of gibberellic acid producing the highest seedling vigor (1628), on the other hand the lowest average of the seedling vigor (136) was produced by the interaction between treatment of control (dry seeds) and seeds stored for four years. Interaction results indicate that stimulation treatments were significantly affected increasing the seedling vigor at the standard laboratory germination percentage for all ages of seeds and thus stimulating seeds is one of the important and influential factors in increasing the of vigor seedlings produced by stored seeds and giving them a vigor comparable to the seedlings produced by seeds newly harvested.

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