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PRIMING EMERGENCE OF AGED SORGHUM BY ALPHA-AMYLASE

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Abstract

In a trial to improve emergence at aged day leans stored for 1, 2 and 3 years α–amylase enzyme was used in four levels. The seeds were soaked with 0, 0.25, 0.5, and 2 mg L^{-1} of enzyme solutions, along with untreated seeds. A factorial experiment of levels of enzyme and storage time was conducted on the farm of the Coll. of Agric. Eng./Univ. of Baghdad of four replicates. Seeds stored for one year were the best in first and last day of emergence, time elapsed for emergence, seedling height and seedling day weight. Seeds stored for 1and 2 years were better in emergence%, speed at emergence and chlorophyll index. The enzyme level of 1mg L^{-1} gave better values in most of traits tested without a significant difference than 0.5 and 2mg L^{-1}. Meanwhile, it gave higher value of pen cent and speed of emergence, chlorophyll index and seedling dry weight. Statistical analysis revealed that the two variables of the experiment were significantly different in last day of emergence, percent, speed–and time elapsed for emergence. It was concluded that increased time of storage had significant negative effects on traits of emergence and seedling growth and that soaking aged soybean seeds 1mg L^{-1} of α-amylase gave better results in field emergence characteristics.
Keywords : *Glycine max* L. Merrill, digestive enzymes, emergence time and speed.

Introduction

Soybean (*Glycine max* L. Merrill) is one of the important oil crops in the world, for its value in protein and oil in industry. Soybeans contain 36-40% protein and 14-26% oil. Soybean protein is a unique source amines acids, especially of methionine and cysteine (Ashraf and Foolad, 2005; Hamed, 2011). Besides being soybean as a good source of serous industries, i.e, poultry peed, it is also as a leguminous crop adds nitrogen to soil (Sousa *et al.*, 2014; Awoda, 2015). However, stored soybeans have a problem in emergence and establishment and later a non-enzyme oxidation (Elsahookie and Jannu, 2010; Cheyed and Elsahokie, 2011). When soybeans stored, some physiological changes in seeds take place plus non-enzymic oxidation (Ghassemi- Golezani *et al.*, 2014). These changes lead to damage of cellular membranes and enzymic activity (Tekrony and Hampton, 1995). Accordingly the emergence characteristics would be poor (Kausar *et al.*, 2009; Kapoor, 2010; Biabani *et al.*, 2011; Siadat, 2012; Cheyed, 2019). To solve some of this problem, many researchers tried several tests of those, soaking in water (Ashraf and Foolad, 2005; Goudarz *et al.*, 2012; Sousa *et al.*, 2014), but it did not work to stimulate mRNA and tRNA of the aleurone, but amylases can break down glycoside bonds and poly saccharinely (Jyoti *et al.*, 2011). This means that starch will be inverted into sugar and ATP which help emergence, since Weselake *et al.* (1983) showed that α-amylase in the seeds will increase one thousand times in germination. Hence, low levels of α-amylase in germinating seed is the problem of low emergence and poor seedling growth, this experiment was undertaken to evaluate α-amylase levels in improving emergence characteristics of aged soybeans.

Materials and Methods

This experiment was carried out on the Farm of Field Crop Science, College of Agricultural Engineering, University of Baghdad in 2019. That was to determine the effect of soaking aged soybeans in solutions of α-amylase enzyme in emergence characteristics. Tow variables used, levels of the enzyme: 0, 0.5, 1 and 2 mg L^{-1} of water and aged seeds of 1, 2 and 3 years. A fifth treatment was also used along which is untreated seeds. A RCBD design of 4 replicates was assigned. Plots of 1×1 m were prepared, and 400 seeds were planted for each experimental unit. Seeds planted in rows of 50 seeds and one seed per hill was planted 2-3 cm deep. Traits studied were as shown in tables. Traits were recorded as reported by Kadar (2005). Seed emergence % was calculated as:

S.E.% = (No. of seedling emerged in 12 days/No. of seeds)× 100.

Chlorophyll index was determined by using spud by taken the mean of 5 reading of each experimental unit. Germinant speed (sidling/day by counting daily emerged seeds according to Yuan-Yuan et.al. (2010):

$$GS=\sum Ni/Di$$

when Ni = daily emerged seeds and Di=No. of days.

Dry weights of seedlings were determined by taking 20 normal seedlings often 12 days from planting, oven dried at 80ºC for 24h (AOSA, 1988; Hampton and Tekrony, 1995). Results were tabulated and statistically analyzed and means compared by L.S.D at the probability level 0.05 (Steel *et al.*, 1997).

Results Discussion

First and last day of emergence

There were significant difference among years of storage in number of day to reach 1st. and last day of emergence (Table1). Seeds stored for one year were the best in there two traits. Meanwhile, the poorest values were in seeds stored 3 years, since they gave longer periods. Deterioration of stored seeds could be attributed to the negative effect of storage temperature and humidity (Biabani *et al.*, 2011). Soybeans are so sensitive to poor storage, at causes senescence (Kapilan, 2015). Data of same Table show that levels of α-amylase were significant in these traits. The treatment of soaking in 1mg L^{-1} of enzyme was the best by taking the shortest time to reach final day of emergence (7.3 day), while the two controls (soaking in water and untreated seeds) gave the longest time without a significant difference between them. At the same times the treatment of 1mg L^{-1} for seeds stored and 2 years gave significantly shortest time.

Time elapsed for emergence

Table 2 shows significant difference among α-amylase levels, time of storage and their interaction in elapsed time for emergence. Soybeans stored for one year gave shortest time for emergence (3.96 days). Soybeans stored for 3years gave longest time for emergence (5.08days). The treatment of 1mg L^{-1} of α-amylase gave shorter time for emergence (3.92), and 0.5 and 2 mg L^{-1} came after. However, soybeans soaked with water and those untreated were similar in time of emergence (4.92 and 5.0 days), respectively. Table 2 also shows that soybeans soaked with water and stored for 2 years elapsed longer time to emerge.

Emergence %

Data of Table 2 show that soybeans stored for 1 and 2 years did not differ significantly in emergence%, while those stored for 3 year gave the lowest percent. This could be attributed to speed of emergence (Table3). Long stored seeds suffer deterioration of amylase, proteolase, phospholipases and phytase (Tekrony *et al.*, 1989). Poor storage condition negatively affect DNA and rRNA, and permeability of membranes (McDonald, 1999). The level of 1mg L^{-1} of α-amylase gave higher percent of emergence, meanwhile, the soaked seeds in water and untreated seeds gave similar value of low emergence and did not differ with those of 0.25 and 0.5mg L^{-1} α-amylase treatments. Alpha–amylase encourage conversion of starch into sugar and ATP (Jyoti *et al.*, 2011). However, the best value of emergence percent was obtained from treatment of 1mg L^{-1} α-amylase on seeds stored for one and two years.

Speed of emergence

Results of speed of emergence are shown in Table 3, soybeans stored for 1 and 2 years gave better value as compared to those stored 3 years. There difference are related to value of other traits shown in Tables 1 and 2. Soybeans soaked in 1mg L^{-1} α-amylase gave higher value in speed of emergence (21.57 seedling day^{-1}), while both control treatments gave the lowest. Cheyed (2019) found that seed give lower number of days to emergence will give speedy emergence. According to interactions, the combination treatment of 1mg L^{-1} α-amylase and storage for 1 and 2 years gave the speediest emergence.

Chlorophyll index

Table 3 shows that chlorophyll index of seedling was different among several treatments. Soybeans stored for 1 and 2 years gave higher chlorophyll indices without significant difference between the two treatments (41.2 and 39.9 Spad), while those stored for three years gave 32.9 Spad. Meanwhile, the treatment of 1mg L^{-1} α-amylase gave the best value of chlorophyll index (40.38 Spad), but it was not different than of 0.25 mg L^{-1} treatment. The higher value of chlorophyll index could be related to the speed of emergence which gave seedlings longer time to capture more sunlight.

Seedling height

Table 4 shows results of seedling heights and there were significant differences among enzyme level treatments and time of storage. Stored seeds for one and two years gave higher seedling heights, while those stored for three years gave the lowest. The low value of seeds stored for three years in thought to be related to speed of emergence and the first and last day of emergence shown in Tables 1 and 3. Long storage is negatively associated with high deterioration of enzyme activities and hormones in the seeds. This also could affect the speed of seed imbibition when soaked and when planted and irrigated. Cheyed (2019) found that wheat kernels stored for different periods gave lower value of seed viability and week seedling.

Table 4 shows also that treatment of 0.5 mg L^{-1} of α-amylase gave higher seedling height but it did not differ from 0.25 and 1.0 mg L^{-1} enzyme treatments. Meanwhile, treatment of 3 mg L^{-1} enzyme and the two controls did not differ in this trait, and gave lower seedling heights. Alpha-amylase treatments as compared to both controls were different. This positive effect of this enzyme shows that it encouraged seedling growth, and this was with agreement with what was found by Alrawi and Cheyed (2018).

Seedling dry weight

Table4 shows that soybeans stored for one year gave the highs value of seedling weight (2.5 mg/seedling), while those stored three years gave lowest values (2.1 mg). This could be attributed to seedling height and accumulation of carbohydrate due to high chlorophyll in the seedling. The treatment of 1 mg L^{-1} enzyme gave heavier seedling dry weight as compared to all other value. It gave 2.60 mg $seedling^{-1}$, while of both control treatments gave the lower value, low amylase in the seed cause poor emergence and less seedling dry weight, which was due to low biochemical activities in the seedling through growing stage (Wesetlake *et al.*, 1983), at that would be negatively reflected on low growth and less dry weight.

It was concluded from this data that α–amylase has positive effect on deteriorated seeds to emerge and grow, especially the level of 1 mg L^{-1}. Time of storage deteriorate seed biochemical reactions, and causes poor emergence and low emergence characteristics.

Table 1 : Effect of soaking by α-amylase and period storage on the first day of emergence (day) and last emergence (day)

First day of emergence (day)					Last day of emergence (day)			
α-amylase (mg L^{-1})	**Period storage (year)**			**Mean**	**Period storage (year)**			**Mean**
	3	**2**	**1**		**3**	**2**	**1**	
Dry seed	5.00	3.50	3.25	3.92	10.00	8.75	7.75	8.83
0	4.50	3.25	3.00	3.58	9.50	8.75	7.50	8.58
0.25	4.00	3.25	3.25	3.50	9.50	7.50	7.00	8.00
0.50	4.00	3.75	3.50	3.75	8.75	8.00	7.25	8.00
1.00	4.25	3.00	3.00	3.41	9.50	6.25	6.25	7.33
2.00	2.00	4.25	3.25	3.58	9.25	7.25	7.75	7.92
LSD 5%	**N.S**			**N.S**	**0.78**			**0.45**
Mean	4.33	3.33	2.21		9.42	7.75	7.17	
LSD 5%	**0.26**				**0.31**			

Table 2 : Effect of soaking by α-amylase and period storage on the time of emergence (day) and field emergence (day)

Time of emergence (day)					Field emergence (%)			
α-amylase (mg L^{-1})	**Period storage (year)**			**Mean**	**Period storage (year)**			**Mean**
	3	**2**	**1**		**3**	**2**	**1**	
Dry seed	5.00	5.25	4.50	4.92	13.2	60.3	72.1	48.5
0	5.00	5.50	4.50	5.00	14.7	60.3	66.2	47.1
0.25	5.50	4.25	3.75	4.50	17.6	76.5	67.6	53.9
0.50	4.75	4.25	3.75	4.25	17.6	67.6	75.0	53.4
1.00	5.25	3.25	3.25	3.92	19.1	97.1	98.5	71.6
2.00	5.00	4.00	3.00	4.33	26.5	88.2	73.5	62.7
LSD 5%	0.94			0.54	12.5			7.2
Mean	5.08	4.41	3.96		18.1	75.0	75.5	
LSD 5%	0.38				5.1			

Table 3 : Effect of soaking by α-amylase and period storage on the on speed of emergence and chlorophyll index.

Speed of emergence (seedling day^{-1})					Chlorophyll index (Spad)			
α-amylase (mg L^{-1})	**Period storage (year)**			**Mean**	**Period storage (year)**			**Mean**
	3	**2**	**1**		**3**	**2**	**1**	
Dry seed	2.65	11.57	16.32	10.18	30.28	33.88	39.20	34.45
0	2.99	11.41	14.93	9.78	31.75	41.18	41.58	38.17
0.25	3.24	17.87	18.38	13.16	34.90	40.73	40.75	38.79
0.50	3.82	16.10	21.18	13.70	32.75	40.55	41.62	38.31
1.00	3.68	30.27	30.76	21.57	33.80	43.35	43.98	40.38
2.00	5.39	22.72	18.38	15.50	34.03	40.05	40.40	38.16
LSD 5%	**4.61**			**2.66**	**N.S**			**1.87**
Mean	3.63	18.32	19.99		32.92	39.95	41.25	
LSD 5%	**1.88**				**1.32**			

Table 4 : Effect of soaking by α-amylase and period storage on the on height seedling (cm) and dray weight of seedling (mg)

Height seedling (cm)					Dray weight of seedling (mg)			
α-amylase (mg L^{-1})	**Period storage (year)**			**Mean**	**Period storage (year)**			**Mean**
	3	**2**	**1**		**3**	**2**	**1**	
Dry seed	5.08	6.25	6.58	5.96	1.92	2.50	2.30	2.24
0	5.35	5.50	6.53	5.79	1.97	2.23	2.50	2.32
0.25	5.18	6.68	7.13	6.32	2.20	2.26	2.46	2.31
0.50	5.38	6.95	7.13	6.48	2.31	2.35	2.46	2.38
1.00	5.30	6.18	7.25	6.24	2.25	2.59	2.96	2.60
2.00	5.08	6.13	6.05	5.75	2.22	2.36	2.40	2.33
LSD 5%	**N.S**			**0.43**	**N.S**			**0.19**
Mean	5.23	6.28	6.78		2.15	2.38	2.52	
LSD 5%	**0.31**				**0.13**			

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