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ACCELERATED AGEING TEST FOR ASSESSING VIABILITY OF FABA BEAN SEED UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT

Accelerated ageing test is considered a reliable viability routine test of official seed inspection in most countries of the world, despite its importance; it hasn't been used in Egypt yet. The international Seed Testing Association (ISTA) standardized this test for many species excepting for faba bean. Therefore, the main objective of this study was to determine the ideal test conditions including the relative humidity (75% and 100%), temperature degrees (39, 42 and 45°C) and time of exposure (3, 4 and 5 days), to which the seeds are undergo in the incubator, then assessing the physiological quality of faba bean seed under laboratory and field conditions. The results indicated that increasing the relative humidity up to 100% and temperature to 45°C and exposure period for 5 days increased seed moisture content and reduce the capability of seed to germinate and produce normal seedling under laboratory and field conditions, reduce seedling length and increase the mean germination time. The results indicated also that the correlation coefficient for the relationship between laboratory germination and field emergence was positive and highly significant (R=0.948) when the seeds were aged at RH 75%, while it was negative and significant (R=–0.921) at RH 100% regardless of other aging conditions. Obviously, the accelerated aging test could be a practical and an accurate determinant of faba bean seed quality under laboratory or field conditions provided that the relative humidity is 75% and the temperature is between 39-42°C for 3-5days.

INTRODUCTION

It is very important to have reliable methods and tests to be used for evaluating seed viability (McDonald, 1999). The accelerating ageing test, in which seed is incubated for a short period (a few days) under high relative humidity and high temperature conditions, was first developed by Delouche and Baskin (1973) for predicting the storability of seed. Although, this test is often used as a seed vigor test by seed testing stations in most countries (Ferguson, 1990), however, in Egypt it hasn't used yet. In 2007 the International Seed Testing Association (ISTA) standardized this method for many species excepting faba bean.

Likhlatchev et al. (1984) reported that the physiological changes in seeds subjected to accelerated ageing test were largely similar to those occurred during natural ageing with the main difference being the rate at which they taken place. Such rate depends on the ability of seed to resist degradation changes and protection mechanisms, which are specific for each plant species (Sisman and Delibas, 2004; Mersal, 2011; Mohammadi *et al.,* 2011). During the test, the seeds absorb moisture from the humid environment along with the high temperature, causing rapid seed aging.

High vigor seed lots withstand these stress conditions and deteriorate more slowly than low vigor seed lots. The possible sequence of events, occurring during the seed ageing has been described by (McDonald, 1999). Generally it is believed that the damage to cellular membranes is one of the key changes, followed by impairment of cellular repair and biosynthetic processes, reduction in the speed of germination and the rate of seedling growth, increase the susceptibility to abiotic and biotic stresses, poor field emergence and finally the loss of viability. The stress caused by the interaction among relative humidity, temperature degrees and exposure period is widely studied. However, deteriorative reactions proceed more readily in seeds at higher moisture content and subsequently the high moisture conditions constitute threat to the longevity of seed survival (Vertucci 1990). TeKrony (1995) recommended 41 ºC for 72 hr can be used for the accelerated Hampton and aging (AA) test for soybean [Glycine max L. (Merrill)] and canola (Brassica napus L.). For peanut (Arachis hypogaea L.), Duangpatra and Homdork (1986) proposed 42 ºC and 100% relative humidity (RH) for 192 hr, while 42 ºC and 100% RH for 264 hours was suggested by Promchote and Duangpatra,and Siri et al. (2002) and Phyo et al. (2004). Differences in aging periods for peanut seed from the previous studies may have been attributable to genotypic differences (Phyo et al., 2004). It was proposed that environment of 45 ºC and 20% seed moisture content for 24 h in control deterioration and 41 to 43 ºC for 24 to 72 h in AA is applicable for a large number of species (Hampton and TeKrony, 1995; Matthews, 1993). Therefore, the main objective of this study was to determine the optimal aging conditions, relative humidity, temperature degrees and exposure periods, on viability of faba bean seed as expressed by germination traits and field emergence.

MATERIALS AND METHODS

A laboratory experiment was carried out under the laboratory conditions of Seed Technology Research Unit in Mansoura, Dakahlia Governorate, Seed Technology Research Department, Field Crops Research Institute, Agricultural Research Center during 2014 year. Seed sample of faba bean (Vicia faba L.) variety (Nobaria 1) was obtained from Seed Testing Station in Mansoura, The mean of 100 grain weight was 125g. The seeds were subjected to the following tests:

Determination of seed moisture content: The standard moisture test is done before and after conducting accelerated aging test by the oven method according to the procedures outlined by ISTA (1996).

Standard germination: Four replications of 50 seed from each treatment were planted in sand soil which placed into plastic box (25×13×10 cm) at the depth of 3 cm. Normal seedlings were counted after 7 days and continued daily until constant. Germination percentage was expressed by the percentage of seed capable of producing normal seedlings after 14 days according to ISTA (1996).

Mean germination time (MGT): It was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds germinated on day, and D is the number of days counted from the beginning of germination.

Seedling length: 10 normal seedlings were taken at random from each replicate at the end of standard germination test to evaluate seedling length (cm).

Accelerated aging test: Seeds of faba bean cultivar were weighed, placed on a screen tray and speared in a single layer and placed in plastic boxes (inner chamber) containing 40ml of distilled water (100% relative humidity). The inner chambers and screen trays were washed thoroughly in 20% sodium Hypo-chlorite and then dried. This was done after each use to prevent fungal contamination. The inner chambers were placed in covered water bath room (outer chamber) and the seeds were aged at 39, 42 and 45 ºC for 3, 4 and 5 days.

Accelerated aging test (Modified method): This test is a modification method to the traditional accelerated aging method according to Tekrony (1995) and Jianhua and McDonald (1996), while seeds of faba bean were weighed, placed on a screen tray and speared in a single layer and placed in plastic boxes (inner chamber) containing 40ml of the saturated salt solutions of sodium chloride (Nacl) which give (75% relative humidity). The inner chambers and screen trays were washed thoroughly in 20% sodium Hypo-chlorite and then dried. This was done after each use to prevent fungal contamination. The inner chambers were placed in covered water bath room (outer chamber) and the seeds were aged at 39, 42 and 45 ºC for 3, 4 and 5 days. After these aging periods, seeds were removed from the inner chamber, the moisture content of the seed was determined and the standard germination test was conducted as described above. At the end of the aging periods the samples were removed and seeds were set out for seed moisture content, standard germination, mean germination time, seedling length as mentioned previous.

Field emergence: Field experiment was carried out at Tag AL-Ezz, Agricultural Research Station, Dakahlia Governorate, Agricultural Research Center. Four replications of 100 seeds were counted from each treatment and manually planted in 1.5 m long and 0.25 m apart rows at about 3 cm depth in field conditions. Emerged seedlings were counted (during 14 days) when the seedlings showed well characterized apparent plumule over the soil surface and it was continued until the number of emerged seedling remained constant.

Data were subjected to the statistical analysis according to the technique of analysis of variance (ANOVA) of Completely Randomized Design, as described by Gomez and Gomez (1984). Correlation coefficient was computed according to Svap (1937).

RESULTS AND DISCUSSION

The interaction among RH, temperature degree and exposure period on seed moisture content was significant (Table 1). Seed moisture content of aged seeds was increased as compared with non-aged seeds (control), such increase was higher as the Relative Humidity (RH) of 100% and at the temperature of 42C° for 5 days. This revealed that aging test conditions were positively interacting to increase seed moisture content or the positive effect of one and two factors such as the higher RH and exposure period was higher than temperature degree. The seed moisture content of (18.88%) was obtained when the seeds were aged at RH 75% at 45 C° for 4 days as compared with the control (14.98%). The highest seed moisture content (30.65%) was produced from incubated the seeds at RH 100% and at 45 C° for 5 days. Similar findings were observed by Mersal, (2011). The effect of seed moisture content on its viability is well established where higher seed moisture content above the optimal degree leading to more rapid seed deterioration, particularly at the moisture content above 12%. The elevated moisture intake by seed under ageing conditions was an indicator of losing viability, while the slight increase was an indicator of less effect during storage that was revealed on germination and seedling vigor. Since seed of faba bean was more deteriorated under higher RH and temperature for long incubation period. Such conclusion is in conformity with the findings of Vertucci (1990) who observed that deteriorative reactions proceed more readily in seeds at higher moisture content and subsequently, the high moisture conditions constitute threat to the longevity of seed survival.

Table (1): Moisture content (%) of faba bean seeds as affected by relative humidity, temperature degrees and exposure periods of accelerated aging test.

Treatments		Moisture content%	
Temperature degrees (C°)	Exposure periods (days)	Relative humidity%	
		75%	100%
Control		14.98	14.98
39C°	3	18.35	24.33
	4	18.68	27.35
	5	18.70	28.08
42C°	3	18.45	26.70
	4	18.52	28.28
	5	18.55	29.43
45C°	3	18.45	26.90
	4	18.88	30.20
	5	18.68	30.65
LSD at 5%		0.37	

Table 2 shows that seed germination percentages after aging at different temperatures and relative humilities for various testing periods. Seed germination percentages were ranged between 90 to 93% when the seed were subjected to RH 75% and 39 C° and for 3 to 5 days. But, as the RH was 100% seed germination percentages was ranged between 90 to 92% under

the same test conditions. This may revealed the crucial influence of the RH% on seed germination. The effect of test temperature was obvious at 45 C°, where seed germination were ranged between 74 to 80% and from 47 to 64% at RH 75% and 100%, respectively. Generally, the higher RH% and the higher temperature the more adverse effect on seed germination. The findings of this attempt were in agreement with Akhter et al., (1992) and Mersal (2011) who suggested that under adverse conditions in AA test such as the high temperature, relative humidity and exposure period, the variation in seed germination and seedling vigor can be high. While, Hampton and TeKrony (1995) suggested that for canola, small oil seed rape (Brassica napus L), a temperature of 41°C and an aging period of 72 h should be used for the accelerated aging vigor test. The reduction in germination might be due to degradation of mitochondrial membrane leading to reduction in energy supply necessary for germination (Gidrol et al., 1998). Seed ageing is generally marked by reduction in vigor (Gupta and Aneja, 2004), viability, rate and capacity of germination (Arefi and Abdi, 2003).

The mean germination time increased as the RH was increased regardless of the other test conditions (temperature degrees and exposure period (Table2). However, such increase was high to some extant as temperature degree and exposure period were increased which indicated a high interaction effect of the three factors included in aging test and consequently they should be considered for aging faba bean seed. These results are supported by Hampton and TeKrony (1995) and Matthews (1993) they stated that AA is applicable for a large number of species however, the most appropriate aging environment needs to be determined RH for each species.

Seedling vigor as expressed by seedling length was also affected by aging test condition (Table 2) high relative humidity (100%) combined with high temperature (45C°) reduced considerably seedling length i.e. seedling length was about 20.0 cm (an average) when the seed were aged at (39C°), 100% RH whereas it was about 7.0 cm at 45C° and 100% RH However the adverse effect of RH% on seedling length was greatly higher than that of temperature degree i.e., as an average of different exposure period (3,4 and 5 days), seedling length at RH 75% was (about 24cm) compared with seedling length at RH 100% (about 7.0 cm). This indicated that the RH % of test conditions should be considered so that intermediate degrees between 75 to 100 % are included in aging test since the RH 75 % had a slight adverse effect on seedling length in contrast with a significant effect of the RH 100%. Similar results of decrease in seedling length were also reported by Roy et al. (1994) in chickpea, Perez and Arguello (1995), in peanuts.

Table (2): Standard germination, mean germination time and seedling length of faba bean seed as affected by accelerated aging test conditions.

Treatments		Germination (%)		Mean germination time (day)		Seedling length (cm)	
Temperature degrees (C)	Exposure periods (days)	Relative humidity		Relative humidity		Relative humidity	
		75%	100%	75%	100%	75%	100%
Control		92	92	6.2	6.2	24.2	24.2
39C°	3	90	92	6.2	6.7	24.1	23.3
	4	92	92	6.0	6.8	23.1	20.6
	5	93	90	6.2	6.8	23.1	16.7
42C°	3	92	74	6.2	6.9	26.0	19.2
	4	93	72	6.4	7.1	26.2	11.9
	5	91	66	6.8	7.4	25.6	7.2
45C°	3	80	64	6.7	7.2	23.3	8.9
	4	76	66	6.8	7.5	23.2	6.7
	5	74	47	6.9	7.9	26.2	5.3
LSD at 5%		2		0.2		1.1	

The results of field emergence of faba bean seeds resulted from different aging conditions showed similar trend as those of laboratory germination. Field emergence was ranged between 68-82 % when the seeds were aged at RH75% whereas it was ranged between 29-80% when the seeds were aged at RH 100% .This indicated that there was greater adverse effect on germination traits and field emergence at RH 100% than at RH 75% regardless the other aging conditions. The value of relative field emergence of great importance because it indicated the percentage of viable seeds capable of producing seedlings under field conditions.

Table (3): Field emergence and relative field emergence of faba bean seed as affected by aging test conditions.

Treatments		Field emergence %		Relative field emergence %	
Temperature degrees (C)	Exposure periods (days)	Relative humidity		Relative humidity	
		75%	100%	75%	100%
Control		80	80	87	87
39C°	3	81	80	90	87
	4	80	79	87	86
	5	82	76	88	85
42C°	3	79	59	86	80
	4	81	49	87	67
	5	76	39	84	60
45C°	3	74	56	93	88
	4	70	40	92	70
	5	68	29	93	61
LSD at 5%		3.o		4.0	

Table (4) showed the correlation coefficient for the relationship between laboratory germination traits and field emergence. The correlation coefficient for the relationship between laboratory germination and field emergence at RH 75% was positive and highly significant at 1% level (R=0.٩٤٨**), but at RH 100% it was negative and highly significant at 1% (R=-0.953**). This mean that the RH 75% of the aging test was more effective for explaining seed performance under field conditions. The correlation coefficient for the relationship between laboratory germination and mean germination time was also highly significant (R= ٠.870**) when the seeds were aging at RH 75%, in contrast with negative value at 100%, (R=-o.921**). Generally Accelerated aging test is a practical and an accurate determinant of faba bean seed quality under laboratory and field conditions provided that the RH does not exceed 75% and temperature degrees between 39-42 C° for 3-5 days. These results are in agreement with those stated by Adebisi (2008) and Khan et al. (2010).

Table (4): The Correlation coefficients between germination traits and field emergence of Faba bean aged seed.

Characters	R.H %	Seedling length (cm)	Seed moisture %	Mean germination time(day)	S. Germination %	Relative field emergence
Seed moisture	75	0.089 NS				
	100	-0.790**				
Mean germination time	75	0.256 NS	0.395 NS			
	100	-0.921**	0.787**			
S. germination	75	0.028 NS	-0.370 NS	-0.751**		
	100	0.923**	-0.683*	-0.903**		
Relative field emergence	75	0.٢٨٦ NS	0.225 NS	0.٤٦٩*	-0.٨٥٩**	
	100	0.٧٥٦**	-0.624*	-0.7٧٩**	0.٧٤٣**	
Field emergence	75	-0.12٦ NS	-0.335 NS	٠.٨٧٠**	0.٩٤٨**	-0.٦٥٣*
	100	0.٩٢٤**	-0.714**	-0.9٢١**	-0.9٥٣**	-0.8٨٩**

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إختبار الإسراع بالتدهور فى تقييم جودة تقاوى الفول البلدي تحت ظروف المعمل والحقل

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يعتبر إختبار الإسراع بالتدهور (Accelerated Aging AA) من الإختبارات الهامة التى يتم تطبيقها فى كثير من الدول إلا أنه لا يطبق فى محطات فحص التقاوى فى مصر، وعلى الرغم من أنه تم تحديد شروط إجراء الإختبار من حيث المدة ودرجات الرطوبة النسبية والحرارة التى تتعرض لها البذور أثناء الإختبار لمعظم بذور المحاصيل بواسطة الإتحاد الدولى لفحص البذور ISTA إلا أنه ليس من بينها الفول البلدى. ولذلك أجريت هذه الدراسة عام ٢٠١٤ بوحدة بحوث تكنولوجيا البذور بالمنصورة بهدف تحديد شروط الإختبار المناسبة لهذا الإختبار من خلال تعريض تقاوى الفول البلدى صنف (نوبارية1) فى المعمل (حضانة) إلى درجتى رطوبة نسبية (٧٥ ، ١٠٠%) ، وثلاث درجات حرارة (٣٩ ،٤٢ و٤٥ م°) و مدة التعرض لهذه الظروف (٣ ،٤ و٥ أيام) بالإضافة إلى تحديد نسبة الإنبات تحت ظروف الحقل. أوضحت النتائج أن ظروف الإختبار من حيث زيادة الرطوبة النسبية إلى ١٠٠% ، ودرجة الحرارة الى ٤٥ م° و مدة التعريض الى ٥ ايام) قد أدى إلى زيادة المحتوى الرطوبى للبذور وإنخفاض كبير فى قدرة البذور على الانبات ونقص طول البادرة وزيادة متوسط زمن الإنبات، مقارنة بالبذور الغير المعاملة وتلك التى تعرضت لرطوبة نسبية ٧٥% ودرجتى الحرارة ٣٩،٤٢ م° ولمدة ٣-٥ أيام. وقد أوضحت النتائج أيضا وجود علاقة قوية وموجبة بين إنبات البذور فى المعمل وإنباتها فى الحقل حيث بلغ معامل الارتباط (R= ٠.٩٥٠)، بينما كان الإرتباط سالبا مع المحتوى الرطوبى للبذرة بعد تعرضها لظروف الإختبار (0.777-). ويقترح إجراء إختبار الإسراع بالتدهور تحت شروط ٧٥ % رطوبة نسبية ودرجات حرارة بين ٣٩-٤٢ م° لمدة ٣-٥ أيام.