

Relationship of Seed Quality Tests to Field Emergence of Artificially Aged Barley Seeds in the Semiarid Mediterranean Region

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ABSTRACT

Standard germination and vigor tests can be used to predict field emergence of aged barley seeds. The objective of this experiment was to study the effect of aging on seed quality of barley and to identify the best seed quality test to predict field performance of aged barley seeds in the semiarid Mediterranean region in Jordan. Seeds of two barley cultivars, "Rum" and "ACSAD176", were subjected to artificial aging by incubating the seeds at 42°C and 100% RH for 0, 2, 4, 6, 8 and 10 days. Seeds subjected to 0 day of aging were considered as "control". Seed germination test, cold test, Accelerated Aging test (AA) and electrical conductivity test of seed leachates were conducted to evaluate the quality of aged seeds. Seedling emergence percentage, Emergence Rate Index (ERI) and Seedling Dry Weight (SDW) were measured at two locations in Northern Jordan. Aging treatments reduced seed germination and vigor. Aging treatments reduced field emergence percentage, ERI and SDW at both locations. All seed quality tests were well correlated with field emergence percentage. However, cold test predicted field emergence of aged barley better than other tests, suggesting that this test was a useful test to evaluate barley stand in the semiarid Mediterranean region.

Keywords: Barley, Seed germination, Accelerated aging test, Cold test, Electrical conductivity of seed leachate, Semiarid conditions.

INTRODUCTION

Seed aging was the major cause of lowering seed vigor in barley (Matthews, 1980). Barley is an important crop grown for forage and grain yield in the semiarid Mediterranean region (Turk, 1998; Voltas et al., 1999; Jaradat and Haddad, 1994), where high and rapid field

emergence and early establishment are essential to obtain an adequate stand and to gain an advantage of the growing season before the onset of the severe drought stress late in the season (Soltani et al., 2001; TeKrony and Egli, 1991; Khah et al., 1986). Natural and artificial aging have been reported to reduce seed quality of many species (Vieira et al., 1999; Moreno-Martinez et al., 1998; Ganguli and Sen-Mandi, 1990; Ram and Wiesner, 1988) and were associated with field emergence, growth and yield of barley (Kim et al., 1989; Matthews and Collins, 1975; Abdalla and Roberts, 1969). However, little information is available about the effect of aging on seed quality and seedling establishment of barley in the semi-arid Mediterranean region and the relationship of

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seed vigor tests (accelerated aging test, cold test, electrical conductivity test and seedling dry weight) to field performance of aged barley.

Several reports have shown that natural or artificial aging reduced seed quality and seed performance in the field. Seeds can be aged artificially by subjecting them to elevated temperature and high relative humidity (Copeland and McDonald, 1995). The physiological changes during seed aging can influence seed quality, resulting in poor stand establishment in the field (Maiti et al., 1989). Artificial seed deterioration of two maize genotypes caused drastic losses in seed germination and seedling vigor (Cruz-Garcia et al., 1995). Seeds subjected to aging had a gradual decline in vigor, leading to a slower and less uniform germination and finally the ability of seeds to germinate was lost (Alsadon et al., 1995).

Several biochemical and physiological changes have been observed in seeds during aging, resulting in a progressive decline in seed quality and an ultimate loss of viability (Marcos-Filho and McDonald, 1998). Degradation of cell membranes in the aged seeds might be the reason for the greater water uptake in the deteriorated seeds during the initial hours of imbibition (Koostra and Harrington, 1969). The release of exudates such as sugars, inorganic ions and amino acids directly affects respiration and enzymatic activities and reduces macromolecular synthesis (Bewley and Black, 1994). Poor membrane structure and leaky cells were associated with deterioration and low seed vigor (Ram and Weisiner, 1988; Wilson and McDonald, 1986). It has been suggested that membranes of aged seed were less capable of regaining their functional properties during imbibition (Simon, 1984). Lipid peroxidation within the membranes mediated by lipoxygenase was another chemical mechanism for seed aging (Wilson and McDonald, 1986). These results suggest that aging

reduced seed quality and vigor by altering many biochemical and physiological processes.

Many researchers have reported the importance of seed vigor tests in predicting field performance of seeds (Vieira et al., 1999; Hampton and Coolbear, 1990). Germination test was not a reliable test to predict seed performance when seeds were sown under unfavorable field conditions (Vieira et al., 1999). Seed vigor tests were more reliable and a sensitive indicator of seed quality and gave a better prediction of field performance than standard germination test (Hampton and Coolbear, 1990). Good correlation between field emergence and laboratory seed vigor tests, such as cold test, accelerated aging test, electrical conductivity test and seedling dry weight has been found in many crops (Vieira, 1999; Burris and Navratil, 1979; Happ et al., 1993; Steiner et al., 1989).

Identifying the best quality test to predict the actual field emergence for aged barley seeds in the semiarid region of Jordan is not well investigated and needs to be studied. Therefore, the objectives of this study were to evaluate the effect of artificial aging on seed quality of barley and to identify the best quality test to predict the actual field emergence of aged barley in the semi-arid rainfed Mediterranean region in Jordan. Such information is helpful to understand seed vigor testing in barley and to evaluate the quality of aged seeds. Identifying the best seed quality to predict field emergence in barley can be very useful for growers to adjust the seeding rate to obtain an adequate stand.

MATERIALS AND METHODS

Laboratory Experiment

Freshly harvested seeds of two most common barley (*Hordeum vulgare* L.) cultivars in Jordan, "Rum" and "ACSAD176", were obtained from the Jordanian Cooperative Corporation. Both cultivars are

recommended in the semiarid region of Jordan. The cultivar "RUM" is more tolerant to mild-drought stress than "ACSAD176" in the semiarid condition (Al-Quda, 2007). Seeds were subjected to artificial aging treatments by placing a single layer of seeds (25 g) over a wire mesh screen suspended over 40 ml of water in rounded plastic containers (11.5 cm diameter x 6 cm height) placed in an incubator at $42 \pm 0.5^\circ\text{C}$ for 0, 2, 4, 6, 8 and 10 days (at approximately 100% relative humidity). After each aging treatment, seed moisture content (on a wet weight basis) was measured as described in ISTA (1985). The initial moisture content was 8% on a wet weight basis. The seeds were left air-drying at ambient conditions ($24 \pm 2^\circ\text{C}$) until seed moisture dropped to less than 10%, then, were stored at 5°C until used for quality analysis and field studies.

Standard Germination Test

Standard germination test was conducted according to ISTA rules (1985). Four replicates of 50 seeds were placed between folded germination papers, moistened with distilled water in plastic containers (17 x 11 x 7 cm) and incubated at 20°C for 14 days. Percentage of normal seedlings was recorded at the end of incubation according to ISTA rules (1985).

Accelerated Aging Test

Four replicates of 100 seeds were exposed to 45°C and 100% relative humidity for 48 hours as described in Seed Vigor Testing Handbook (AOSA, 1983). Germination after accelerated aging test was conducted as described in the standard germination test described by ISTA rules (1985). Percentage of normal seedlings was recorded 14 days after seeding.

Cold Test

Four replicates of 25 seeds were planted in a soil mixture moistened up to 70% of the field capacity according to Seed Vigor Testing Handbook (AOSA, 1983). A soil mixture of sand, clay and peat in a volume

ratio of 8:2:1 was chosen to obtain a soil moisture content of 42% (on a dry weight basis) at field capacity. Soil was placed in plastic boxes (17 x 11 x 10 cm) to a depth of 2.5 cm. The soil mixture was moistened to 70% of field capacity and covered with sealed lids to reduce evaporation, then placed in an incubator at 5°C for 7 days. The boxes were then incubated at 20°C for 7 days. At the end of the incubation (14 days after planting), the percentage of normal seedlings was determined.

Electrical Conductivity Test

Four replicates of 50 seeds were soaked in a 200-ml Erlenmeyer flask containing 75 ml of distilled water. Flasks were covered with parafilm to reduce evaporation and placed in an incubator at 25°C for 24 hours. The electrical conductivity of seed leachates was measured using an electrical conductivity meter according to Seed Vigor Testing Handbook (AOSA, 1983) and reported as $\mu\text{S}^{-1} \text{cm}^{-1} \text{g}^{-1}$.

Field Experiments

Locations

Field experiments were conducted during the growing season of 2002/2003 in two locations in Northern Jordan. The first experiment was conducted at the Experimental Station of Jordan University of Science and Technology, Irbid ($32^\circ34'\text{N}$ latitude, $36^\circ01'\text{E}$ longitude and 520 m altitude). The soil of this experimental site is loamy, mixed, thermic calcic paleargid (Khresat et al., 1998). The second experiment was conducted in Jomha ($32^\circ55'\text{N}$ latitude, $35^\circ78'\text{E}$ longitude and 526 m altitude). The soil at Jomha site is very fine, smectitic, thermic, typic pelloxererts (Khresat, 1986).

Four replicates of 100 seeds subjected to aging treatments were sown on 1 November 2002 in both locations. Seeds were sown by hand at a depth of 8 cm. The experiment was arranged in a split plot in

randomized complete block design. The spacing was 30 cm between rows and 10 cm between seeds. Seedling emergence was counted daily after sowing until no further seedlings emerged. Seedlings were recorded as having emerged when the coleoptile appeared free from the soil surface. Seedling dry weight was determined 30 days after emergence by weighing the seedlings after oven drying at 70°C for 48 hours. Speed of emergence (as measured by emergence rate index) was calculated according to Maguire (1962) as follows:

$$\text{ERI} = \frac{\sum \text{Number of emerging seedlings at day X}}{\text{Day X}}$$

Day X: number of days after receiving a heavy rainfall. Soil moisture content was measured during the emergence period at both locations. Four samples of soil were randomly taken at a depth of 30 cm, sealed in plastic bags and brought to the lab within half an hour. Samples were weighed and oven-dried at 105°C for 3 days. Soil moisture content was calculated as the difference between the soil-wet weight and oven-dry weight, divided by the soil-dry weight. Results were expressed as a percentage of soil moisture on a dry-weight basis.

Statistical Analysis

The lab experiment was arranged as a split plot in a completely randomized design with four replications. The field experiment was arranged as a split plot in a randomized complete block design with four replications. In both experiments, the main factor was the cultivars and the split factor was the aging treatments. Data were subjected to analysis of variance (ANOVA) using MSTATC program (Michigan State University, East Lansing, MI). Probability of significance was used to indicate significant treatments and interaction effects. Means were compared using the Least Significant

Difference (LSD) at 0.05 level of probability. Simple correlation coefficients were used to determine the relationship between seed quality tests and field emergence.

RESULTS

Seed Moisture Content

As aging increased, seed moisture content increased from 8% to 29% by 6 d of aging for both cultivars (Figure 1). After 6 d of aging, no significant increase in seed moisture content was observed, suggesting that the seeds reached the equilibrium moisture content (the moisture content of the seeds at which an equilibrium with the relative humidity of the air surrounding it occurs).

Seed Quality Tests

Aging treatments decreased seed quality by decreasing the germination in the standard germination test, the germination after accelerated aging and cold tests and by increasing the electrical conductivity of seed leachate (Table 1). The standard germination percentage declined from 94 and 99% to 0% by 10 d of aging for ACSAD176 and Rum, respectively. No significant decrease in germination was observed by 2 d of aging for both cultivars. In cold test, the germination declined from 84 and 89% to 0% by 10 d of aging for ACSAD176 and Rum, respectively. In AA test, un-aged seeds (0 d) of ACSAD176 and Rum had 13 and 83% germination, respectively. The AA-germination declined to less than 5% by 2 d of aging for both cultivars. In the EC test, there was a significant increase in EC by 6 and 10 d of aging for ACSAD176 and Rum, respectively.

Field Emergence

Jomha had higher annual rainfall, higher soil moisture content and lower maximum air temperature than JUST (Figure 2). The annual rainfall for Jomha and JUST for the 2002/2003 growing season was 843 and 283 mm, which was highly above the long term average for both

locations (464 and 225 mm, respectively).

Aging reduced field emergence percentage, emergence rate index and seedling dry weight of both cultivars at both locations (Table 2). However, 2 d of aging had no effect on the field emergence of Rum at both locations and emergence rate index of both cultivars at Jomha.

In general, ACSAD176 seeds were not significantly different in field emergence between locations, while Rum seeds had lower field emergence at JUST than those at Jomha (Table 2). Both cultivars had higher emergence rate index at JUST than those at Jomha. In most cases, both cultivars had either similar or higher seedling dry weight at JUST than those at Jomha.

Although Rum had higher field emergence and emergence rate index for 2- and 4-d aged seeds than ACSAD176 at Jomha, Rum seeds had either similar or lower field emergence and emergence rate index than ACSAD176 at JUST (Table 2). The aged seeds of Rum had generally lower seedling dry weight than ACSAD176 at Jomha.

Correlation between Seed Quality Tests and Field Emergence

Field emergence strongly correlated with Standard Germination (SG), Accelerated Aging test (AA), cold test and Electrical Conductivity test (EC) (Table 3). The SG and cold test positively correlated to field emergence at $P \leq 0.001$ level. Accelerated Aging (AA) test results positively correlated with field emergence at $P \leq 0.01$ level in both locations. There was a significant negative correlation (at $P \leq 0.001$ level) between Electrical Conductivity (EC) of seed leachates and field emergence.

DISCUSSION

Seed standard germination and vigor tests have been used by many researchers to evaluate the quality of the naturally and artificially aged seeds (Hampton and

Coolbear, 1990; Moreno-Martinez et al., 1998; Ram and Wiesner, 1988). In this study, the AA-germination was the best test to evaluate the quality of the aged and un-aged seed lots (Table 1). Although the standard germination and cold germination of the aged seeds ranged from 0-97% and 0-85%, respectively, the AA-germination was less than 5%. For the un-aged seeds, the AA-germination of Rum was higher than that of ACSAD176, suggesting that the AA-germination was a useful test to evaluate the initial quality of the original seed lots. The AA-germination has been used to identify seed vigor of other crops (Ram and Wiesner, 1988; Vieira, 1999). In barley, the AA-germination was a useful test to estimate the vigor of naked barley (Kim et al., 1989), which was consistent with our results.

The artificially aged seeds were sown at two locations, varying in seedbed condition as characterized by the differences in soil type, rainfall and temperature, where barley is commonly grown in Jordan (Figure 2). The soil at Jomha was characterized as clayey, while the soil at JUST was characterized as sandy. In general, Jomha had higher rainfall, higher soil moisture content and lower maximum temperature than JUST. JUST is characterized as a drier location than Jomha.

Field emergence of both cultivars ranged from 0-84% and was generally lower than standard germination (Table 2). Field emergence has been reported to be lower than standard germination in other crops, where seedbed conditions in the field were less than ideal for germination (Makkawi et al., 1999; Happ et al., 1993). In this experiment, field emergence was generally lower at the drier location of JUST than at Jomha, with a variation between cultivars. The seeds sown at JUST had a higher emergence rate index than at Jomha, which might be due to the lighter soil of JUST. Crusting in clayey soil may provide mechanical impedance to the emerging seedlings, but loamy soil is more favorable for

seeds to emerge (Makkawi et al., 1999). In our experiment, the higher germination rate index for the seeds sown at JUST did not give the seedlings a consistent advantage in dry weight seedling⁻¹ over that observed at Jomha.

The difference between cultivars in field emergence and emergence rate index depended on the planting location (Table 2). At the wetter location (Jomha), the aged seeds of Rum had generally higher emergence and emergence rate index than ACSAD176. At the drier location (JUST), the emergence and emergence rate index of the un-aged and aged seeds of Rum were either similar or lower than those of ACSAD176. These results indicated that Rum seeds performed better at the wetter location than at the drier one, while ACSAD176 had similar performance at both locations. This response could be due to the complex interaction effect of genotype, seed quality and environmental conditions on the emergence percentage.

All seed quality tests highly correlated with field emergence percentage. Significant positive correlations of field emergence with seed quality tests (SG, cold and AA) and negative correlation with EC have been reported for barley (Kim et al., 1989) as well as for other crops (Vieira et al., 1999; Burris and Navratil, 1979; Perry, 1984). The higher EC value was an indication of the lower vigor, due to an increase in membrane permeability of the lower vigor seeds (Vieira, 1999), which may explain the negative correlation of EC with field emergence in the present study. However, the simple correlation depends on the numerical range within the paired values (Perry, 1984), so we have to investigate the best single vigor test at least, since others may correlate to field performance but are still so far from field means in terms of emergence percentage.

Means of cold germination were the closest means for the actual field emergence at both locations (Figure 3), which agreed with the finding of Ram and Wiesner (1988), who reported that cold test can be used to detect the differences in the quality of wheat seeds. In our experiment, the standard germination overestimated the field emergence percentage, while the AA-germination underestimated the field emergence percentage. These results suggested that cold test was the best test to predict field emergence of aged barley and can be used to evaluate the barley stand in the semiarid rainfed region in Jordan.

CONCLUSIONS

Artificially aging reduced barley seed quality as estimated by the standard germination, the germination after accelerated aging, cold test and the electrical conductivity test. The AA-germination was the best test to evaluate the reduction in the germination of the 2-d aged seeds in comparison with the un-aged seeds. The un-aged seeds were significantly different in AA-germination between cultivars.

All seed quality tests highly correlated with field emergence. However, the best test to predict the actual field emergence means for the aged seeds was the modified cold test described for barley in this experiment (5°C for 7 d at 70% field capacity, then 20°C for 7 d). This test can be useful to evaluate the barley stand in the semiarid Mediterranean region and needs further evaluation.

ACKNOWLEDGEMENTS

Thanks are extended for the Deanship of Research at the Jordan University of Science and Technology for financial support.

Table (1): Seed standard germination test (SG), cold test (Cold), germination after accelerated aging test (AA) and electrical conductivity of seed leachates (EC) for two barley cultivars (Rum and ACSAD176) subjected to 6 aging treatments.

Cultivars	Aging treatments ^a days	SG	Cold	AA	EC
		-----%-----			μS/cm/g
ACSAD176	0	94	84	13	121
	2	96	79	5	121
	4	78	73	2	122
	6	75	51	0	137
	8	37	29	0	137
	10	0	0	0	146
Rum	0	99	89	83	110
	2	97	85	4	117
	4	86	79	5	122
	6	79	67	2	116
	8	23	13	0	119
	10	0	0	0	128
LSD(0.05)		9	21	4.4	14

Table (2): Emergence percent, emergence rate index and seedling dry weight at two locations (Jomha and JUST) in Northern Jordan for two barley cultivars (Rum and ACSAD176) subjected to 6 aging treatments.

Locations	Cultivars	Aging treatments ^a	Emergence	ERI	SDW
		Days	%		mg/seedling
Jomha	ACSAD176	0	78	14	93
		2	72	13	85
		4	63	11	76
		6	58	9	65
		8	13	1	29
		10	0	0	0
	Rum	0	82	16	105
		2	84	15	70
		4	75	14	60
		6	56	7	45
JUST	ACSAD176	8	12	1	41
		10	0	0	0
		0	82	23	104
		2	72	21	84
		4	62	16	74
		6	52	12	68
	Rum	8	24	5	39
		10	0	0	0
		0	71	19	109
		2	71	17	85
		4	63	16	70

Locations	Cultivars	Aging treatments ^a	Emergence	ERI	SDW
		6	45	7	52
		8	15	3	38
		10	0	0	0
LSD(0.05)			5.6	1.5	7.5

Table (3): Correlation of laboratory seed quality tests to field emergence of barley sown at two locations (Jomha and JUST) in Northern Jordan.

Seed quality test	Field emergence	
	Jomha	JUST
SG	0.97***	0.96***
AA	0.40**	0.37**
Cold	0.96***	0.96***
EC	-0.51***	-0.51***

*, ** and *** indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively (n= 48).

SG: Standard Germination.

Cold: Cold test.

AA: Accelerated Aging test.

EC: Electrical Conductivity test.

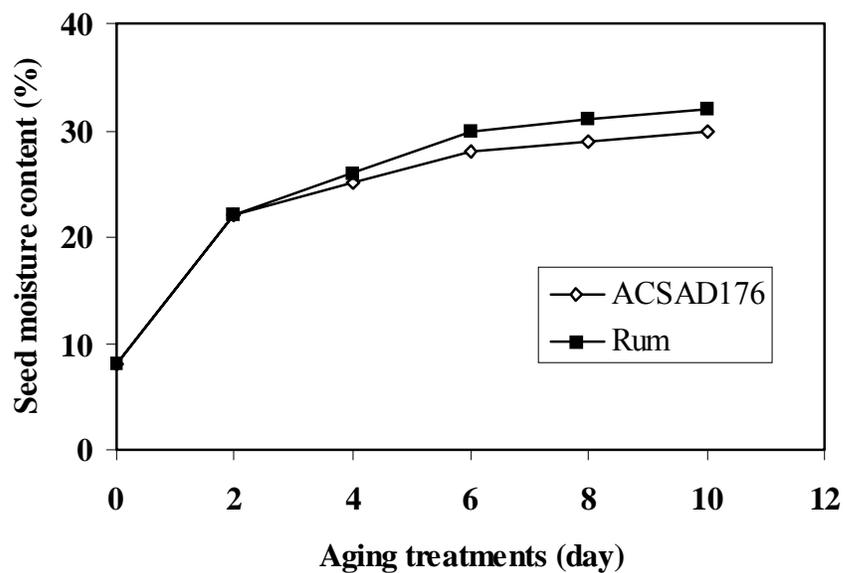


Figure (1): Seed moisture content on a wet-weight basis after 6 aging treatments.

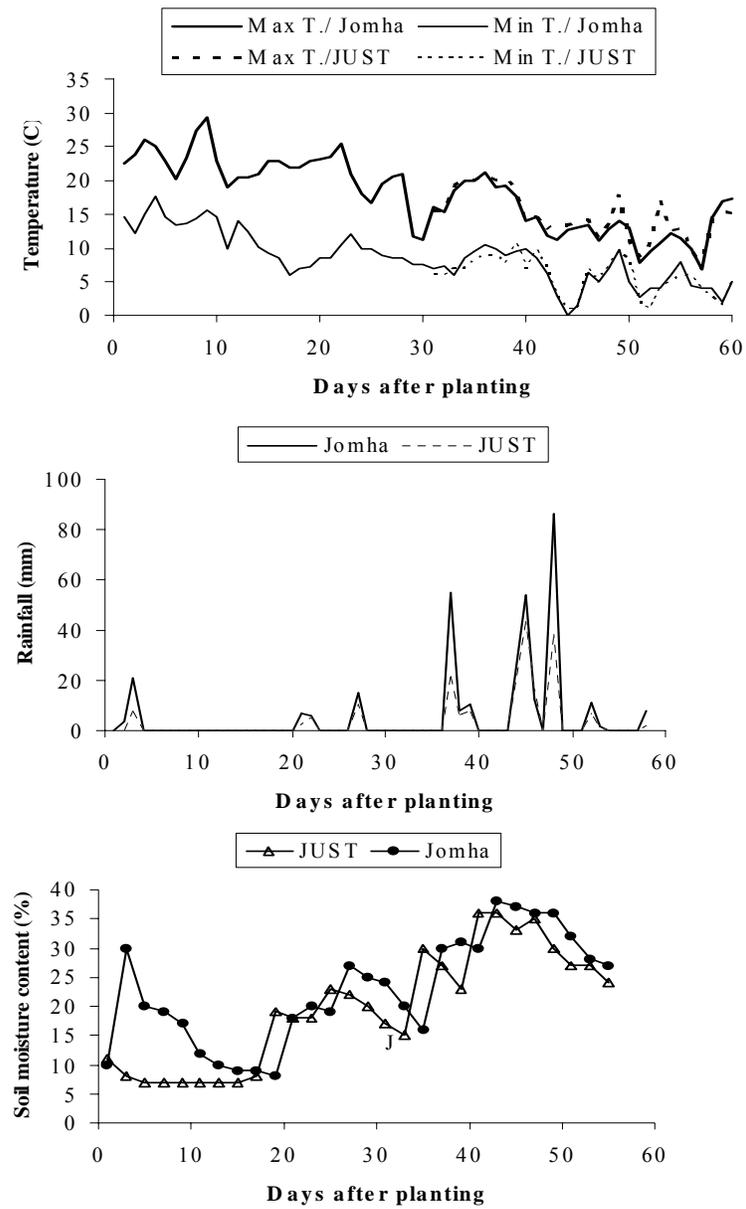


Figure (2): Daily minimum and maximum temperature, rainfall and soil moisture percentage at two locations (Jomah and JUST) in Northern Jordan.

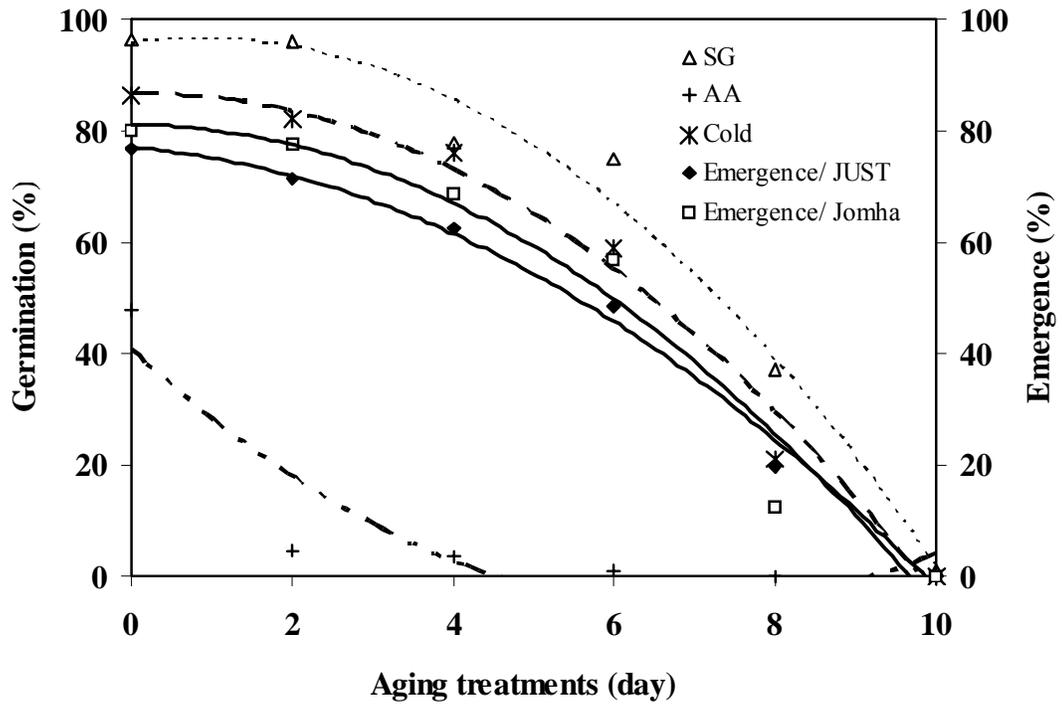


Figure (3): Prediction of field emergence percentage averaged over cultivars at two locations (Jomha and JUST) using different lab quality tests (SG, standard germination test; AA, accelerated aging test; Cold, cold test) for barely seeds subjected to 6 aging treatments (seeds aged at 42°C and 100% RH for 0, 2, 4, 6, 8 and 10 days).

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