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Controlled Deterioration and Accelerated Aging Tests to Estimate the Relative Storage Potential of Cucurbit Seed Lots

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Abstract. **The laboratory germination (radicle emergence) percentages of 9 watermelon, 12 melon, and 7 cucumber seed lots were tested after storing in relatively adverse storage conditions of 25 °C and 12% mc for 6, 12, and 18 months in sealed aluminum foil packets. The laboratory germination (radicle emergence) of lots was determined after controlled deterioration (CD) at 45 °C with 20% or 24% moisture content (mc) for 48, 72, 96, 120, and 144 h. The accelerated aging test (AA) was conducted at 45 °C for the same aging periods. A number of seed lots was dead by 12 and 18 months in watermelon and cucumber, respectively. Various combinations of test regimens were found to be correlated with laboratory germination after 6 months storage, but the most consistent regimens for AA tests was 96 h at 45 °C in all species (r = 0.71 to 0.98). In the CD tests, 72 h with 20% mc at 45 °C gave the best correlation (r = 0.86 to 0.96). These conditions of highest correlation were observed after laboratory germination after 6 months storage and are suggested as good predictors of storage life in cucurbit seed lots. The initial standard germination before storage was also significantly correlated with seed longevity, but the correlation coefficient was generally lower (r = 0.60 to 0.83) than the AA and CD test results and separation of lots less clear.**

Differences in the field emergence of seed lots with high laboratory germination or in germination after storage are referred to as seed vigor (Dornbos, 1995; TeKrony, 2003), a concept that comprises various aspects of quality and indicates the stage of seed deterioration.

Low-vigor lots (i.e., highly deteriorated) having poor field emergence may not necessarily be detected by standard germination. Vigor tests thus provide additional information on the relative emergence potential and longevity of seed lots (Powell and Matthews, 2005; TeKrony, 2003). Several such tests have been used to indicate emergence potential and longevity in crop seeds among which controlled deterioration (CD) and accelerated aging (AA) are widely used (Hampton and TeKrony, 1995). Both these tests are based on differentiating seed lots by testing their germination after a period of controlled aging. Standard aging conditions proposed for a number of crop seeds in CD were 45 °C and 20% mc for 24 h (Matthews, 1993) and in AA at 41 to 43 °C for 48 to 72 h (Hampton and TeKrony, 1995).

Although various studies have been conducted in cucurbit seeds (Pesis and Ng, 1983; Torres and Marcos-Filho, 2005), no consistent aging environment has been proposed for predicting cucurbit seed longevity. These studies compared the vigor of seed lots but did not correlate the results with emergence and longevity. Recently, we identified vigor test regimens that were well correlated with field emergence potential (Mavi and Demir, 2007a, 2007b), but these were not evaluated as predictors of longevity.

The use of transplanted modules in cucurbit production places more emphasis on high germination to maximize the efficiency of module production. If carryover seeds are used for transplant production, the vigor may be lower, although the standard germination remains high. Rapid emergence and uniform seedling growth are important attributes in modular plant production and they have also been shown to depend on seed vigor in peppers (Basak et al., 2006) and Brassica spp (Matthews, 1980; Powell et al., 1991). Therefore, information about the vigor of the seed lots before storage is valuable for producers. Several studies as mentioned have ranked the vigor of cucurbit seed lots by means of CD and AA tests, but there is little work to correlate these tests with seed storage potential. The work described here was conducted to fill this information gap and thus improve the management of seed stocks for the benefit of seed companies and plant producers. The objective of the study was then to find the optimum CD and AA duration to rank the seed lots and to correlate laboratory germination (radicle emergence) after AA and CD test with relative storage longevity.

Material and Methods

Nine seed lots of watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai], cv. Crimson Sweet, 12 seed lots of melon (*Cucumis melo* L.) cv. Kirkagac, and seven seed lots of cucumber (*Cucumis sativus* L.) cv. Beith Alpha were obtained from commercial companies in Turkey in Oct. 2004. All seeds were kept in temporary storage at 5 °C during the initial testing.

Standard germinations [International Seed Testing Association (ISTA), 2007] were conducted using four replicates of 50 seeds per seed lot. Seeds of each replicate were placed between three 20 × 20-cm filter papers (Filtrak, Germany), two below and one above, each wetted with 6 mL of distilled water.

These papers were then rolled and placed in plastic bags in a dark germination cabinet at 25 °C. Standard germinations were evaluated after 14 d in watermelon and after 8 d in melon and cucumber. Seed moisture content was determined using the high oven method (130 °C, 1 h) using duplicate samples of 4 g per seed lot (ISTA, 2007).

The CD test was conducted on samples of the seed lots held at moisture contents of 20% and 24%. Details of the procedure for seed moisture equilibration were described in Mavi and Demir (2007a). Five subsamples of 150 seeds per lot and moisture content were then sealed in laminated foil bags and were incubated at 45 °C. A total of 120 samples in melon, 70 samples in cucumber, and 90 samples in watermelon were prepared for two seed moistures, five aging durations and 12, seven, and nine seed lots in melon, cucumber, and watermelon, respectively (Table 1). Subsamples (24 in melon, 14 in cucumber, and 18 in watermelon in each aging period) were removed after 48, 72, 96, 120, or 144 h and germination were then tested using the emergence of a 2-mm-long radicle as the criterion for germination (laboratory germination).

For the accelerated ageing test, 40 mL of distilled water was added to each plastic box (11 × 11 × 4 cm) and 150 seeds were placed on a wire mesh tray (10 × 10 × 3 cm) inside the box. Seeds were aged at 45 °C for 48, 72, 96, 120, and 144 h using one box is used for each aging/time combination. The germination test was then conducted, again using radicle emergence (laboratory germination) for assessment.

The retention of seed viability in storage was determined for all seed lots between Feb. 2005 and Aug. 2006. Seed moisture of all seed lots was adjusted initially to 12 ± 0.2% equilibrated at 5 °C for over 3 d and three subsamples of each lot for 6, 12, and 18 months of storage were stored at 25 °C in a laminated and hermetically sealed aluminum

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Table 1. Changes in standard germination (SG) and seed moisture (Smc) of watermelon, melon, and cucumber seed lots used in the experiment[z].

	Watermelon		Melon		Cucumber	
Lot	SG (%)	Smc (%)	SG (%)	Smc (%)	SG (%)	Smc (%)
1	90 b	6.4	100 a	7.3	94 b	5.8
2	98 a	9.1	91 b	5.8	95 b	6.2
3	90 b	7.5	99 a	5.6	97 a	6.9
4	100 a	7.9	99 a	5.9	98 a	4.9
5	98 a	5.8	91 b	8.1	100 a	7.2
6	99 a	6.7	100 a	4.9	100 a	7.0
7	92 b	9.0	99 a	4.9	97 a	6.7
8	100 a	6.7	99 a	4.1	NA	NA
9	97 a	8.4	96 a	9.1	NA	NA
10	NA	NA	95 a	3.6	NA	NA
11	NA	NA	90 b	8.0	NA	NA
12	NA	NA	95 a	7.4	NA	NA

[z]Laboratory germination was based on 2-mm radicle emergence and seed moisture was determined by a high oven method (130 °C, 1 h) according to ISTA (2007) rules.
NA = no seed available.

foil packet. Each subsample consisted of 200 seeds. The reason 25 °C was chosen is that seed deterioration at this temperature would be high and the difference in germination among the lots would be seen clearly in a short storage time. Such a high temperature would not be used in commercial storage but would give a measure of relative longevity of the lots. One sample of 200 seeds was taken from each lot in each species after 6, 12, and 18 months storage and germination tests were carried out using radicle emergence as the criterion. Germination refers to laboratory germination (radicle emergence) throughout the text except when the term standard germination is used.

Statistical analysis was conducted using the Statistical Package for Social Sciences (Chicago, IL). Means were compared using the Duncan multiple range test at the 5% level. Correlation coefficients (r) of vigor tests with storage longevity were calculated.

Results

The standard germination test in watermelon and melon varied between 90% and 100% and 94% and 100% in cucumber seed lots. Moisture content varied between 4.1% and 9.1%; details for each species and lot are shown in Table 1. Standard germination percentages of all lots were much higher than that necessary for commercial sale in the market, which is 75% in cucumber and 80% in watermelon and melon seeds.

In all three species, seed lots showed large differences in their retention of germinability during storage. Some watermelon seed lots showed a large decrease in germination during storage, whereas other lots retained high germination; for example, lot 2 showed a fall in germination of only 1%, 28%, and 43% after 6, 12, and 18 months. Thus, despite the high initial germination in all lots, there were considerable differences in the rate of deterioration in storage. Very low germinations (less than 10%) were seen in three watermelon lots after 12 months and in eight lots after 18 months (Table 2).

A more gradual decline in germination of melon seed was observed during storage, but again with variation between the different lots. In general, the higher germinating ones after 6 months also had higher germinations after 12 and 18 months. After 18 months storage, germination of melon lots ranged from 0% to 66%; lot 1 had the highest germination and was significantly ($P < 0.05$) higher than the others (Table 3).

In cucumber, the fastest reduction in germination was observed in lots 1, 4, and 7, in which germination fell from 94%, 98%, and 97% to 1% or 0% by 12 months, whereas lots 5 and 6 maintained their germination reasonably well within that period (Table 4). Several significant differences were observed among lots in all three species ($P < 0.05$). Lot 1 in watermelon, lots 1, 3, and 9 in melon, and lots 5 and 6 in cucumber all retained high germinations compared with the others.

The standard germinations after the standard ISTA test (ISTA, 2007) and the laboratory germination (radicle emergence) after AA and CD were correlated with laboratory germination (radicle emergence) after 6 months in watermelon and cucumber and after 6 and 12 months in melon (Table 5). Longer storage periods were not included in Table 5 whenever zero germination resulted for seed lots after storage because separation of the lots with zero germination was not possible. All treatment regimens in both tests were significantly (mostly $P < 0.001$) correlated with laboratory germination (radicle emergence) after storage in 6 months storage (Table 5) in watermelon (r = 0.87 to 0.97,

Table 2. Laboratory germination percentages (radicle emergence) after 6, 12, and 18 months of storage and accelerated aging (AA) test of 45 °C for 96 h and controlled deterioration (CD) conditions of 45 °C, 20%, 72 h in watermelon seed lots[z].

	Germination after storage			AA	CD
Lot	6 months (%)	12 months (%)	18 months (%)	45 °C, 96 h	45 °C, 72 h, 20%
1	6 e	0 e	0 c	67 b	45 d
2	97 a	70 a	55 a	98 a	99 a
3	65 d	0 e	0 c	73 b	71 c
4	91 b	19 c	0 c	93 a	98 a
5	86 b	61 b	10 b	97 a	98 a
6	94 b	63 b	0 c	98 a	99 a
7	67 c	4 e	4 c	75 b	80 b
8	85 b	65 ab	0 c	96 a	97 a
9	78 c	44 d	0 c	92 a	94 a

[z]Means with different letters are significantly different at 5% level in the same column.

Table 3. Changes in laboratory germination percentages (radicle emergence) after 6, 12, and 18 months of storage and accelerated aging (AA) test of 45 °C for 96 h and controlled deterioration (CD) conditions of 45 °C, 20%, 72 h in melon seed lots[z].

	Germination after storage			AA	CD
Lot	6 months (%)	12 months (%)	18 months (%)	45 °C, 96 h	45 °C, 72 h, 20%
1	98 a	88 a	66 a	97 a	100 a
2	68 ef	46 d	1 g	34 f	53 e
3	81 cd	70 b	52 c	98 a	98 ab
4	91 b	83 a	30 e	94 a	91 bc
5	42 h	38 e	15 f	47 e	54 e
6	77 de	82 a	48 c	91 ab	88 c
7	47 h	58 c	0 g	67 d	49 e
8	98 a	83 a	29 e	99 a	98 ab
9	89 bc	84 a	60 b	90 ab	96 abc
10	85 bcd	74 b	4 g	92 ab	69 d
11	53 gh	44 de	0 g	75 cd	54 e
12	59 fg	62 c	38 d	83 bc	77 d

[z]Means with different letters are significantly different at 5% level in the same column.

Table 4. Changes in laboratory germination percentages (radicle emergence) after 6, 12, and 18 months of storage and accelerated aging (AA) test of 45 °C for 96 h and controlled deterioration (CD) conditions of 45 °C, 20%, 72 h in cucumber seed lots[z].

	Germination after storage			AA	CD
Lot	6 months (%)	12 months (%)	18 months (%)	45 °C, 96 h	45 °C, 72 h, 20%
1	37 e	0 f	0 d	35 d	4 e
2	81 c	23 d	15 c	74 b	65 b
3	67 d	41 c	0 d	69 b	62 b
4	63 d	1 e	0 d	58 c	39 c
5	92 b	66 b	26 b	93 a	60 b
6	97 a	73 a	43 a	88 a	86 a
7	71 d	0 f	0 d	68 b	25 d

[z]Means with different letters are significantly different at 5% level in the same column.

Table 5. Correlation coefficients calculated for laboratory germination (radicle emergence) and controlled deterioration (CD) and accelerated aging (AA) tests and initial laboratory germination (LG) as predictors of watermelon and cucumber seed storage longevity for 6 months and 12 months in melon seeds which were stored at 25 °C with 12% mc in hermetic aluminum foil packets.

Test	Temp. RH	Hour	Watermelon 6 months	Melon 6 months	Melon 12 months	Cucumber 6 months
		48	0.95***	0.73**	0.88***	0.85*
		72	***0.96*****	***0.86*****	***0.88*****	***0.87*****
	45 °C	96	0.96***	0.85***	0.89***	0.85*
	20%	120	0.96***	0.79**	0.83***	0.75*
CD		144	0.96***	0.84***	0.86***	0.66
		48	0.95***	0.79**	0.80**	0.39
		72	0.97***	0.76**	0.73**	0.81*
	45 °C	96	0.93***	0.92***	0.94***	0.76*
	24%	120	0.96***	0.94***	0.84***	0.91**
		144	0.94***	0.87***	0.93***	0.62
		48	0.87**	0.66*	0.83***	0.56
		72	0.87**	0.73**	0.84***	0.90**
AA	45 °C	96	***0.93*****	***0.71*****	***0.85*****	***0.98*****
		120	0.94***	0.67*	0.85***	0.83*
		144	0.94***	0.48	0.69*	0.75*
SG			0.83**	0.60*	0.83***	0.77*

*, **, ***Indicates significance at $P \leq 0.05$, 0.01, and 0.001, respectively.
SG, standard germination; RH, relative humidity.
Bold italic indicates recommended aging conditions for CD and AA tests.

$P < 0.01$ to 0.001) and exceptionally one of 30 in melon (r = 0.66 to 0.94, $P < 0.05$ to 0.001) after 6 and 12 months storage. Large number of correlations were correlated in cucumber (r = 0.75 to 0.98, $P < 0.05$), but four combinations were found not to correlate significantly when seeds were stored for a relatively short period, i.e., 6 months. Standard germination was also significantly correlated (r = 0.60 to 0.83, $P < 0.05$) with germination after 6 months of storage in watermelon and cucumber and 6 and 12 months in melon (Table 5). However, correlation values were much lower than laboratory germination after AA and CD tests in all storage periods. Thus, seed lots with high germination after AA and CD tests retained their germinability in storage better than those with low germinations after the aging tests.

The highest and most consistent correlation values in the CD test in all three species were found at 45 °C and 20% for 72 h (r = 0.86 to 0.96, $P < 0.01$). In the AA test, 96 h aging of all three species at 45 °C gave the highest and the most consistent correlation values (r = 0.71 to 0.98, $P < 0.05$) with laboratory germination (radicle emergence) after storage (Table 5). These relationships are illustrated for all three cucurbits after 6 months storage (Fig. 1) and often 12 months for melon (Fig. 2) alongside the relationship for initial standard germination. The lower the CD and AA germination, the more rapid the loss of germination in storage. Initial standard germination tests showed lower R^2 values (R^2 = 0.36 to 0.69, $P < 0.05$) than either of the vigor tests in all storage durations and a less clear separation of lots.

Discussion

Differences in the ability of watermelon, melon, and cucumber seed lots to retain germination during adverse and controlled environment (high temperature and seed moisture, 25 °C, 12% mc) storage were detected by CD and AA vigor tests. The storage conditions used in this experiment are more adverse than usual commercial storage conditions. Cucurbit seeds stored in the usual conditions of 10 to 15 °C with 6% to 8% mc remain viable for a long time (Bates and Robinson, 1995) presumably longer than 18 months. Supporting this assumption, in our previous study, at 20 °C with 5% mc, melon seeds had 90% and higher even after 5 years of storage (Demir and Ozcoban, 2007). Similarly, watermelon seeds had reasonably high germination percentages (80% and less) when they were stored at 20 °C with 8% to 10% mc over 18 months (Ozcoban and Demir, 2002). However, we aimed to detect the relative differences among the lots within 18 months and the ranking of the lots under adverse storage conditions would be indicative of the relative differences in more usual storage conditions. In support of this view, Powell and Matthews (1984a, 1984b) used three different storage environments and many samplings in seeds of onion and Brussels sprouts and relative germination between lots were similar at each sampling and for each store.

Seed lots, which had low initial vigor test results, showed a more rapid loss of germination during storage. This conclusion is in agreement with previous reports in various crops (Basak et al., 2006; Delouche and Baskin, 1973; Hampton and Bell, 1989; Ibrahim et al., 1993; Powell and Matthews, 1984a, 1984b). This implies that low seed vigor lots have undergone some physiological deterioration before storage (Matthews, 1980), which may not be clearly indicated by the standard germination test and such lots were first to show loss of viability. This is consistent with the conclusion of Ellis (1992) that the loss of vigor precedes loss of germination, which would occur both before and after storage.

Normal seedling production also decreases before the ability to produce a radicle. Although one precedes the other, the two measurements are consistently related. Thus, differences in radicle emergence among the lots (ranking) after storage would have been relatively the same as the differences in normals.

Tables 2, 3, and 4 reveal that lots with lower levels of initial standard germination, close to 90%, store less well. This would be expected from the fall in germination from aging before the receipt of the seed. The germination would be following the typical initial slow decline seen in the seed survival curve (Matthews, 1993). The correlations with initial standard germination are lower than those with germination (radicle emergence) after AA and CD (Table 5) and the separation of the lots is much less than that seen after aging tests.

Various reports have indicated that higher seed vigor results in a higher seedling emergence percentage, size, and uniformity in modules or field conditions (Demir et al., 2008; Marshall and Naylor, 1985; Powell et al., 1991). The use of high-value hybrid seeds in cucurbit transplant production demands a plant of uniform size in each module. Recently, vigor testing in cucurbits gained another dimension through advances in commercial production technology. The grafting of seedlings, particularly watermelon, to protect against diseases and increase yield requires rapid and uniform emergence for use as a scion (Yetisir and Sari, 2003). Rapid emergence provides seedlings with uniform hypocotyl thickness, which makes grafting both quicker and more efficient. If carryover seeds are used for transplant production, rapid emergence and uniform seedling growth depend on seed vigor as was shown in peppers (Basak et al., 2006) and Brassica spp (Matthews, 1980; Powell et al., 1991). Therefore, information about the vigor of the seed lots before storage is valuable for producers to reduce the risk of using even lower vigor seed after storage.

Controlled deterioration and accelerated aging tests have been used for predicting storability of various crop seeds. Previously, 45 °C with 20% mc for 24 h in CD (Matthews, 1993) and 41 °C for 72 h in AA (Hampton and TeKrony, 1995) were considered as generally acceptable regimens. Our earlier findings (Mavi and Demir, 2007a, 2007b) indicated that cucurbit seeds appeared to be reasonably resistant to aging compared with various small, seeded vegetable crops such as brassicas and lettuce. Consequently, aging temperatures ≈40 °C did not reduce the germination sufficiently to discriminate vigor differences in watermelon and melon seed lots. For that reason, we used 45 °C in our aging regimens in these experiments. Jianhua and McDonald (1996) affirmed that this may be the result of differences in species (e.g., oil content) as well as seed size; smaller seeds may deteriorate faster than larger ones as a result of the surface/volume ratio, particularly in the AA test. Pesis and Ng (1983) also suggested a much longer aging period (144 h, 45 °C) for melon seed lots than suggested for

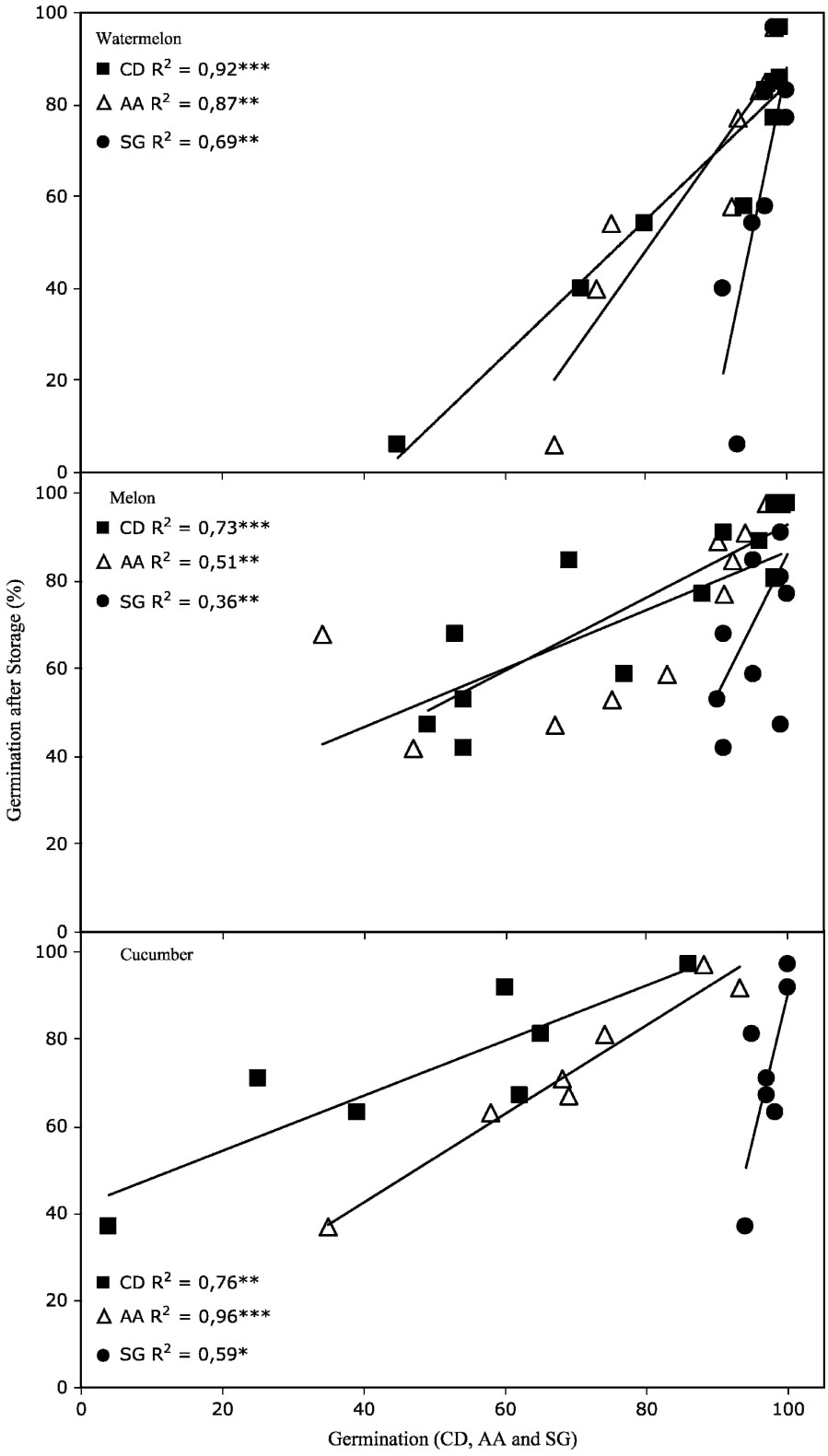

Fig. 1. Relationship between controlled deterioration, CD (■), at 20% mc for 72 h and accelerated aging, AA (△), at 96 h in watermelon, melon, and cucumber seed lots at 45 °C and laboratory germination (radicle emergence) at 12% mc for 6 months of hermetic storage at 25 °C and initial laboratory germination (●). Seed lots with zero laboratory germination after any storage period were not included in correlations. *, **, ***Indicates significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

the AA test (41 °C, 72 h) for a large number of crops.

Our previous findings showed that germination after CD conditions of 48 h and 24% mc at 45 °C, and AA conditions of 120 h at 45 °C in watermelon is related to seedling emergence potential of the lots in stressed sowing environments (Mavi and Demir, 2007a). Likewise, in melon seeds, CD at 48 h and 20% mc at 45 °C and AA conditions of 120 h at 45 to 47 °C (Mavi and Demir, 2007b) also correlated with emergence potential of the lots in stressed environments. In the work reported here, we used the same seed lots. These aging environments were also related to seed storability, but they were not as consistent and highly correlated as 96 h in AA at 45 °C or 20% with 72 h in CD at 45 °C in all species (Table 5). Previously, in the AA test, we proposed 72 h and 120 h at 45 °C based on work with two cucumber and three melon seed lots (Demir et al., 2004). However, the present conclusions here are based on more seed lots and can be considered more robust.

The controlled deterioration test has been recommended for small-seeded crops. Although cucurbit seeds are larger, CD works well in vigor discrimination. Seed moisture is raised to 24% in ≈25 to 30 min at room temperature on top of wet paper. The large surface area of the flat seeds may help fast water absorption. Moreover, using an extended seed equilibration period of 72 h before placing seeds in aluminum foil packets can be an additional precaution. AA requires a longer aging period of 96 h to discriminate the vigor. This may be the result of the longer time required for seeds to reach a defined moisture content because they must absorb the water from a high relative humidity (90% to 100% relative humidity).

Despite the assumption that differences in the initial seed moisture percentage may affect the aging rate in AA (TeKrony, 2003), our results showed that this is not the case. There are a number of examples in which seed lots with the same initial germination, but very different moisture contents showed no significant difference in germination percentage after accelerated aging. Low initial moisture did not provide higher germination compared with lots with high moisture content. For instance, lots 3 and 9 of melon had 99% initial viability but 5.6% and 9.1% moisture content, whereas after AA, they had 98% and 90% germination and were both highly vigorous lots (Tables 1 and 3). A similar trend was observed in various seed lots among the other species, i.e., lots 2, 5, 7, and 9 in watermelon and lots 4, 8, and 10 in melon.

When the seed storability potential was compared, it appears that watermelon is the most sensitive among the three species. Almost all lots were dead within 18 months, whereas in melon and cucumber, some lots still had comparatively high germination (Tables 2, 3, and 4). Not much is known about watermelon seed longevity, but previous studies indicated that watermelon seeds may have a relatively short life under suboptimum conditions (Delouche and Baskin, 1973; Ozcoban and Demir, 2002). One reason may be the high oil content of seeds. Watermelon seeds had ≈20% to 45% of oil content (Pursglove, 1984) and can be considered oily seeds like peanut, soybean, and

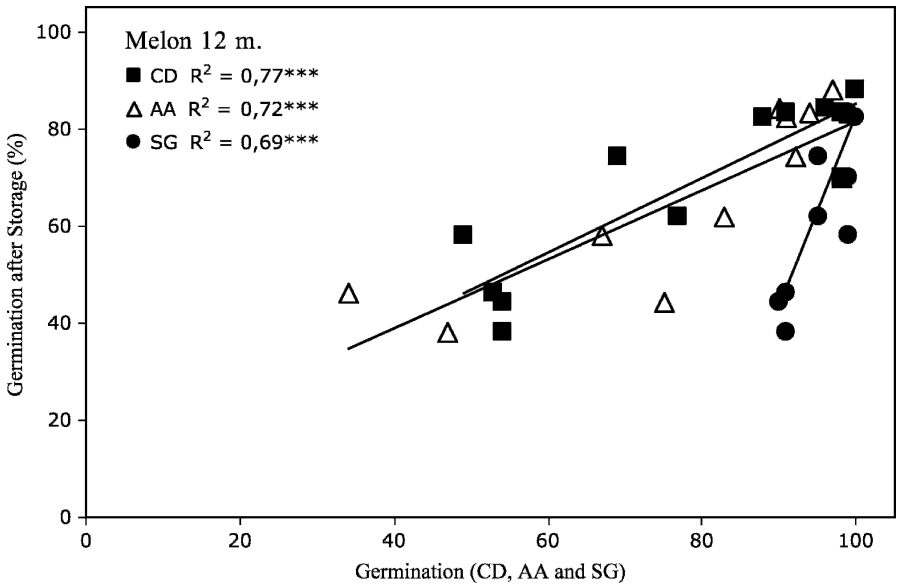

Fig. 2. Relationship between controlled deterioration, CD (■), at 20% mc for 72 h and accelerated aging, AA (△), at 45 °C for 96 h and laboratory germination (radicle emergence) at 12% mc for 12 months of hermetic storage at 25 °C and initial laboratory germination (●) in melon seed lots. Seed lots with zero laboratory germination after any storage period were not included in correlations. *, **, ***Indicates significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

rape. Priestley (1986) indicated that there is a negative relationship between high oil content and seed longevity.

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