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Seed Longevity Potential Predicted by Radicle Emergence (RE) Vigor Test in Watermelon Seed Cultivars

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Abstract: The study was conducted to test whether radicle emergence (RE) would correlate with the storage potential of ten seed lots of watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) hybrid cultivars (>98% normal germination). The RE test was performed by frequent counting radicle emergence (2 mm) percentage between 34 h and 60 h after germination was set up at 25 °C in the dark. Seed longevity was hermetically determined by artificial storage of seeds (air and waterproof) at 17 ± 0.3% of seed moisture and at 35 ± 2 °C over 63 days. Twelve seed samples were taken out during aging, and seed survival curves were constructed based on normal germination percentages conducted at 25 °C for seven days in the dark. The seed longevity criterion was P_{50} (time for the germination to fall to 50%), which was determined through probit analysis by using survival curves. Correlation analysis showed that RE counts at 42 h during germination were highly correlated ($p < 0.01$) with initial seed quality, *Ki* (r = 0.7538), and the half-viability period, P_{50} (r = 0.7936). Pre-storage normal germination percentages of seed lots were not related to longevity. Results showed that the RE vigor test has the potential to predict longevity in highly germinating hybrid watermelon seed lots.

Keywords: artificial seed aging; germination; hybrid seed storage; cucurbit

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1. Introduction

Watermelons belong to the *Cucurbitacea* family and are a warm-climate vegetable crop. They are grown in both open-field and glasshouse conditions. Hybrid cultivars are widely used, particularly when grown under plastic tunnels, to obtain early maturation and faster plant growth. The use of grafted transplants is also a common practice. Hybrid cultivars are produced by heterosis and are used in almost 95% of watermelon production in Turkey. Hybrid seeds are high-value commodities. They must be produced every year, i.e., crossing mother and father lines have particular advantages compared with open pollination. Successful stand establishment in the field with direct sowings and nursery production is of the utmost importance for watermelons because fast and uniform emergence provides optimum land use, uniform plant size, and early maturation. Late germination or non-uniform seed emergence causes variation in plant size and maturation time. Various factors affect successful emergence percentages and emergence times. One basic reason is the level of seed aging [1,2]. As seeds age, the time to germination (appearance of radicle) extends. Aged seeds take a longer time to radicle emergence than less aged ones. Moreover, emergence time varies among the individual seeds in an aged lot, and this causes the occurrence of various-sized seedlings in a population, reducing the overall seedling quality of the lot. Therefore, the prediction of seed longevity potential in watermelon seed lots may help seed production and nursery companies to achieve uniform high percentages of transplant production. This can be more important in hybrid seed cultivars since they are very valuable, and high transplant quality is more demanding in hybrids.

Germination tests are conducted under optimum germination conditions, i.e., temperature, light, and duration, and so do not necessarily show to what extent seed lots are aged [3]. However, seed vigor is a crucial component of seed quality, which gives further information on germination, particularly for seedling emergence and the storage longevity potential of any seed lot [4,5]. The definition of vigor by the ISTA [3] is "Seed vigor is a sum of those properties that determine the activity and level of performance of seed lots of acceptable germination in a wide range of environments". The characteristics of a seed lot associated with vigor are the uniformity of seed germination and seedling growth, the emergence ability of seeds under unfavorable environmental conditions, and seed lot performance after storage. Various seed vigor tests have been validated, namely accelerated aging and the tetrazolium test for soya beans, the controlled deterioration test for the Brassica species, the conductivity test for grain legumes, and the radicle emergence (RE) test for maize, wheat, radishes, and oil seed rape [3]. Among all these, the RE vigor test has been the most extensively studied in recent years. The RE test is based on differences in the mean germination time, i.e., the time of each seed to the protrusion of the radicle (lag period) [6]. RE is based on frequent counts of a radicle emergence of 2 mm in general, and these counts relate to seedling emergence and seed longevity potential. Results in the RE test have been successfully used as a vigor test to predict seedling emergence and seed storage longevity in agronomical [7–10], vegetable [11–14], and ornamental [15–18] plant species. More recently, Mis et al. [19] predicted normal standard laboratory germination percentages by RE test, specifically in open-pollinated watermelon seeds, and a 74 h count gave the best prediction. In that study, normal germination percentages of the lots were large, ranging between 49% and 95%. RE counts were of 30–34 h during germination in hybrid cucurbit rootstock seed lots with normal germination percentages of 79%, and 100% had the highest prediction of seed storage longevity ($p < 0.001$–0.01) [20]. Demir et al. [14], in a study on cucumbers, a species in the Cucurbitacea family, found that a RE count of 24 h discriminated hybrid seed lots with over 95% normal germination under abiotic stress conditions. These results show that the optimum RE count varies among the species in the same family or according to the initial quality. The RE test has some advantages compared with established vigor tests, such as being rapid and easily evaluated, not requiring technical skills, and being adaptable to automation. In this study, we aimed to relate the RE test to seed longevity in hybrid watermelon seed lots. These have high germination percentages (>98%) and have different backgrounds as different cultivars.

2. Materials and Methods

Hybrid watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) seed cultivars of Farfan, Elite, Joker, Üstün, Starburst, Peykan, Crimson Tide, Depar, Jet 4046, and Başkan were obtained from different commercial companies and stored at 5 °C prior to the investigation (just over a month). The study was conducted in the Seed Science Laboratory, Horticulture Department, Ankara University. Initial seed moisture content was determined for 2 g of seeds from three replicates weighed to 0.001 g by using the low-temperature oven method (103 °C, 17 h) [3]. For germination tests, four replicates of 25 seeds per cultivar were placed between wet paper towels (Filtrak, Germany) (200 mm × 200 mm) well moistened with 10 mL distilled water. The rolled paper towels were then placed in sealed plastic bags to prevent water loss during the test. The bags were held at 25 °C for fourteen days in the dark. Radicle emergence test counts (RE) (number of seeds with a radicle $\geq$ 2 mm long) were made at 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, and 60 h by the naked eye. Germination papers were opened for 10 s at maximum during counting, and they were put back into the incubator as soon as RE counts were completed. Thereafter, daily counts were carried out and cumulatively expressed (Figure 1).

Figure 1. Watermelon seed moisture equilibration among the seeds within the bag 17 ± 0.3% of moisture content (**a**), germination tests at 25 °C for fourteen days in the dark (**b**), and normal and abnormal seedling (**c**).

The mean germination time (MGT) was calculated according to the following formula:

$$\mathrm{MGT} = \sum(\mathrm{n} \times \mathrm{t})/\sum \mathrm{n},$$

where

n = number of seeds newly germinated at time t;
t = days from setting to germination.

At the final count, seedlings were evaluated as normal or abnormal [3]. The number of normal seedlings was used to calculate the normal germination percentage (GP).

For seed longevity experiments, about 950 seeds of each cultivar were weighed (0.0001 g), placed on moist (10 mL distilled water/200 mm × 200 mm) filter paper at 20 °C and allowed to imbibe to the weight calculated to achieve a 17 ± 0.3% of seed moisture content. The achievement of this weight was determined by periodic weighing of the batches. The targeted weight was achieved in about 4–5 h. The final seed moisture of the lots was calculated from the initial seed weight and moisture using the following formula:

$$\text{Seed weight (g) at desired mc (\%)} = (100 - \text{initial mc \%})/(100 - \text{desired mc \%}) \times \text{weight of seed (g)}.$$

The seeds were then kept at 5 °C in hermetic laminated foil bags for 48 h to allow uniform seed moisture equilibration among the seeds within the bag. Twelve sub-samples of 75 seeds in each lot were produced (12 sub-sample × 10 lots = 120 samples in total), and hermetically sealed laminated foil bags (air and waterproof) were incubated at 35 ± 2 °C and removed after 0, 7, 10, 14, 17, 21, 28, 35, 42, 50, 56, and 63 days. Germination was then tested (three replicates of 25 seeds) in each cultivar as previously described. The production of normally developed seedlings (developed roots and shoots) [3] for germination were determined after seven days, which made it possible to evaluate the normally developed seedlings (Figure 1).

Individual seed deaths over time show a normal distribution during storage. Thus, the determination of the time for germination to fall to 50%, P_{50}, could be then proposed as a good indicator of seed longevity of any seed population or lot [21]. Survival curves were constructed based on normal germination. The time for normal germination to decline to 50% (P_{50}), the initial theoretical germination (*Ki* indicates the pre-storage environmental and genetic factors), and the inverse of the standard deviation (σ) of the distribution of seed deaths over time were determined for each cultivar using probit analysis [22]. Correlation analyses of the RE count at each observation time as an hour and *Ki* and P_{50} were determined by using the SPSS statistical package program, and the significance was expressed at the 0.05 and 0.01 levels.

3. Results

Nine seed lots out of ten watermelon seed cultivars produced in 2021 and 2022 had normal seedling percentages of 100% except one (Üstün, 98%) before storage. These values were much higher than commercially required percentages. Seed moisture contents of the lots ranged between 5.54% and 7.39%. Not only normal germination but also germination time were very similar among the lots (Table 1). The mean time to germination (MGT) based on radicle emergence percentages had very similar values among the cultivars and ranged between 40.16 h and 47.97 h (Table 1).

Table 1. Changes in germination percentage of normal germination (GP), mean germination time (MGT, h), seed moisture content (m.c., %), 1000 seed weight (g), and production year of ten hybrid watermelon cultivars.

Cultivars	GP (%)	MGT (h)	Seed m.c. (%)	1000 Seed Weight (g)	Production Year
Farfan	100	40.16	7.25	66.07	2021
Elite	100	42.13	7.39	80.73	2021
Joker	100	45.92	6.42	65.18	2022
Üstün	98.7	47.97	5.54	63.48	2022
Starburst	100	46.83	6.06	58.78	2021
Peykan	100	40.69	6.17	64.26	2022
Crimson tide	100	42.27	6.51	72.46	2021
Depar	100	42.88	7.04	64.83	2022
Jet 4046	100	45.86	5.67	62.24	2021
Başkan	100	44.11	6.07	65.33	2022

However, there was only about just seven hours difference between the fastest and the lowest germinating lots (Farfan and Üstün). This fast germination behavior was observed in progressive, cumulative RE counts of the lots. All seed lots had very high percentages of radicle emergence (>89%) by 60 h after seeds were set to germinate (Figure 2). Joker was the slowest germinating one, while Farfan, Elite, and Peykan were the fastest. There was a very clear difference among the seed lots in different RE count times. The difference was the largest at 42 h and was 9% (Joker) and 90% (Farfan). Neither earlier nor later counts had such large RE differences among the lots.

The seed survival curves of ten watermelon cultivars are presented in Figure 3. The survival curves were produced by plotting normal germination percentages against the storage period (days). Some cultivars, such as Farfan, Peykan, and Elite, had higher values during aging. All seed lots were dead by 63 days of storage. *Ki* probit values of the initial theoretical seed quality of each cultivar are shown in Table 2. Joker had the lowest *Ki* value, 1.22 ± 0.008, while Peykan had the highest, 2.53 ± 0.035. The rest were between these two. The longest seed storage longevity, as determined by P_{50}, was seen in Farfan at 28.79 days, and the shortest one was seen in Joker at 17.17 days (Table 2). Even though they had very high normal germination percentages before storage (>98%), the difference in their longevity during storage was great. Farfan survived about 11 days more than Joker (Table 2), even though they had very similar germination percentages.

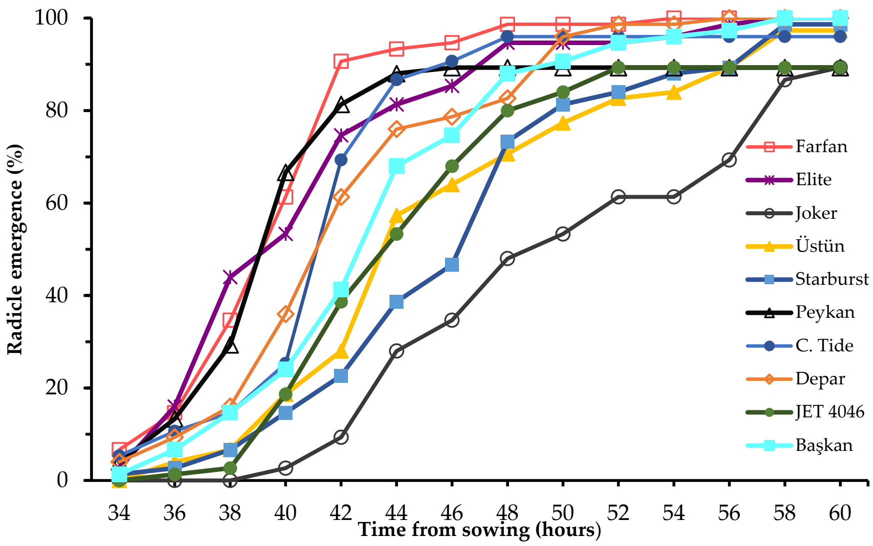


Figure 2. Ten hybrid watermelon cultivars are making progress with the appearance of 2 mm radicles.

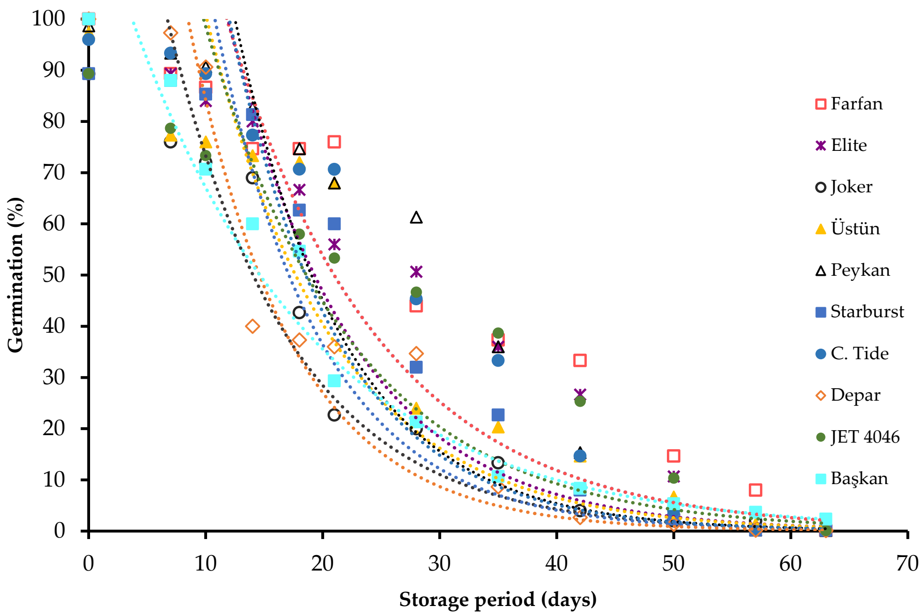


Figure 3. Survival curves of the seeds of ten watermelon hybrid cultivars stored at 35 ± 2 °C and 17% of seed moisture over 63 days.

Correlation coefficient values between RE counts and *Ki* and P_{50} showed that RE counts of 42 h had a highly significant relationship (r = 0.7383–0.7936, $p < 0.01$) in both criteria. There are some other significant relationships as well, but these are at a lower level ($p < 0.05$) than that of 42 h (Table 3). RE at 42 h was not related to pre-storage normal germination percentages (Figure 4). RE counts at 42 h varied between 9% and 91%, while normal germination before storage was very high. Therefore, RE 42 h greatly varied, but normal germination percentages did not. However, RE 42 h was related to changes in *Ki* and P_{50} values (Figure 4), which shows that the higher RE values gave, the higher *Ki* and P_{50}.

Table 2. Probit model parameters for seed cultivars of ten hybrid watermelon seed cultivars aged with 17% seed moisture content over 63 days.

Cultivars	*Ki* (s.e.) (Probits)	Slope, $-\sigma^{-1}$ (s.e.) (Probits Day^{-1})	P_{50} (s.e.) (Days)
Farfan	2.27 (0.007)	−0.0788 (0.0064)	28.79 (0.12)
Elite	2.33 (0.010)	−0.0895 (0.0078)	26.05 (0.15)
Joker	1.22 (0.008)	−0.0710 (0.0062)	17.17 (0.15)
Üstün	1.74 (0.021)	−0.0768 (0.0089)	22.71 (0.26)
Starburst	1.88 (0.031)	−0.0888 (0.0059)	21.38 (0.15)
Peykan	2.53 (0.035)	−0.0917 (0.0075)	25.67 (0.14)
Crimson tide	2.15 (0.007)	−0.0879 (0.0077)	24.42 (0.14)
Depar	2.27 (0.013)	−0.106 (0.0092)	21.45 (0.17)
Jet 4046	1.42 (0.029)	−0.0644 (0.0059)	22.05 (0.25)
Başkan	1.64 (0.031)	−0.0725 (0.0050)	22.61 (0.18)

Table 3. Correlation coefficients of RE count between 34 h and 60 h and probit values of *Ki* and P_{50} (days) for seed lots of ten hybrid watermelon cultivars. Significance: * is 0.05, and ** is 0.01.

Time of RE Count (h)	*Ki*	P_{50}
34	0.6342 *	0.5367
36	0.8063 **	0.7389 **
38	0.6527 *	0.6963 *
40	0.7513 **	0.7383 **
42	0.7538 **	0.7936 **
44	0.7008 *	0.7616 **
46	0.6356 *	0.7669 **
48	0.6100 *	0.8030 **
50	0.6668 *	0.6665 *
52	0.6072 *	0.6055 *
54	0.6050 *	0.6183 *
56	0.5612	0.6378 *
58	0.6263 *	0.6775 *
60	0.7160 *	0.7339 *

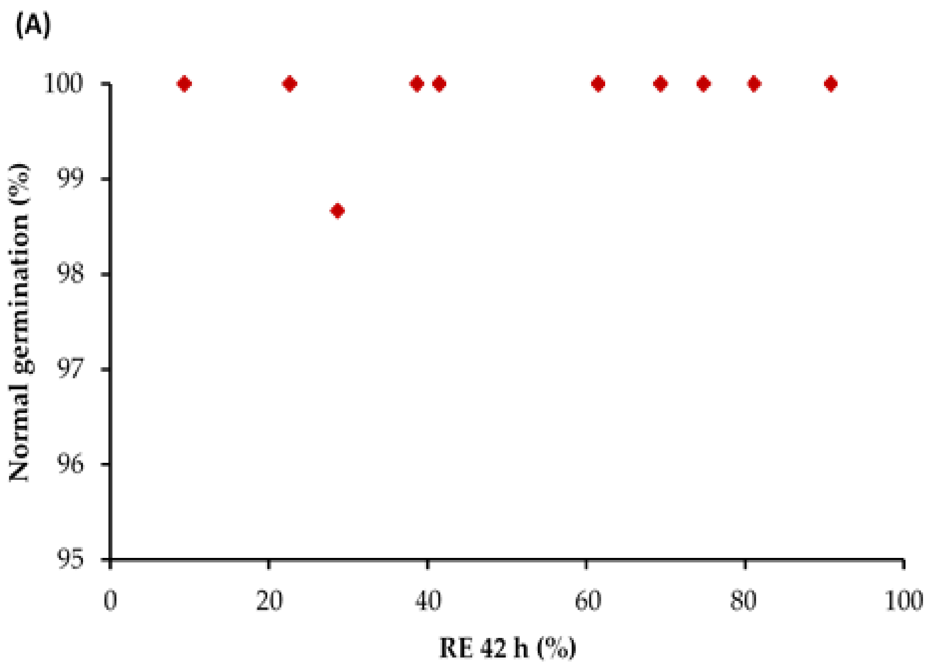
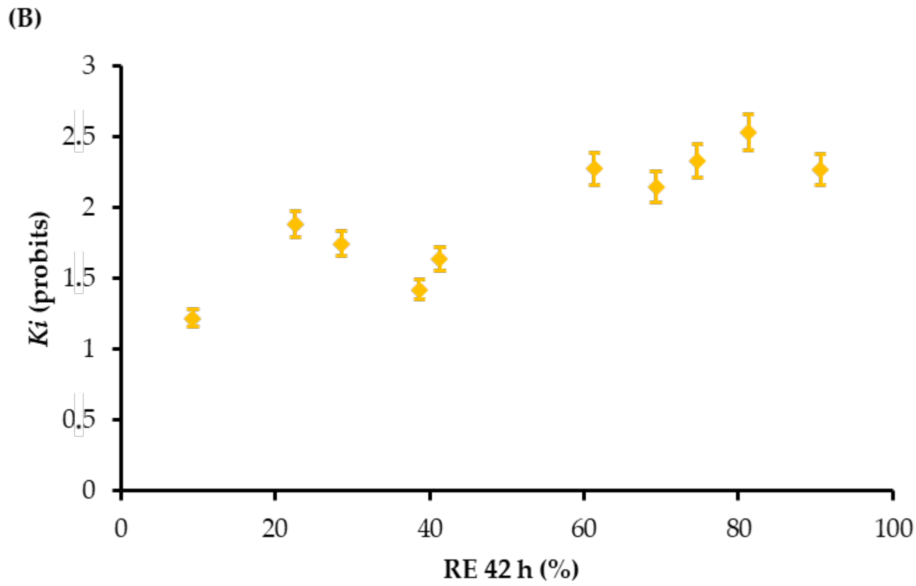


Figure 4. *Cont.*



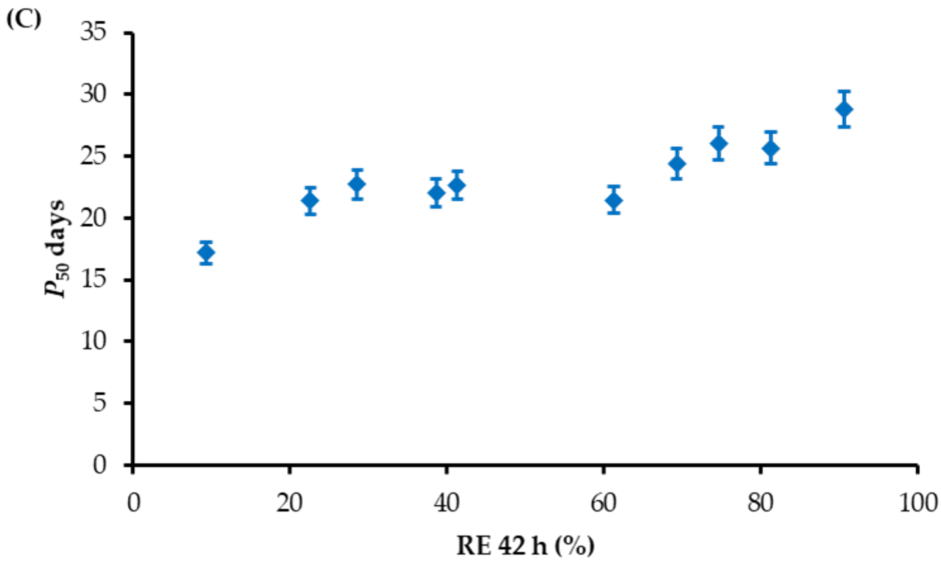


Figure 4. Correlation between radicle emergence (RE; % of seeds sown) at 42 h after germination was set up and normal germination percentage (**A**), *Ki* (**B**) and P_{50} (**C**). SEM values are shown in each symbol.

4. Discussion

This study showed that the RE test of 42 h predicted seed longevity of watermelon cultivars in artificial aging conditions ($p < 0.01$, r = 0.7936) as measured by the half viability period (P_{50}). Watermelons are produced through transplants, and seed longevity and aging are important physiological phenomena because aging, i.e., un-optimum storage conditions, reduces the transplant quality and size, i.e., shoot and root size [1,14]. Moreover, aged seeds were found to be less tolerant to abiotic stresses, such as salt, cold, and mechanical stresses, which are possible stress conditions that watermelon seeds may be subject to during seedling production in the greenhouse [14]. Such stress factors have impacts on the duration between seed germination and becoming healthy seedling stage. One other important aspect of watermelons is that grafted seedling production is a common practice to obtain tolerance to soil-originated diseases, such as Fusarium, or other merits, such as strong plant growth and higher yield. Successfully grafted watermelon seedling production requires the same size, i.e., shoot thickness of rootstock and scion [23]. Aged seeds germinate late and produce weaker and non-uniform seedlings, which reduces the overall quality of grafted seedling production. Therefore, the prediction of seed potential longevity in any lot is valuable for high-quality transplant production. Our results indicate that RE count at 42 h may distinguish which seed lots are less aged than the other and, in turn, have better transplant production potential.

Seed lots may have high normal germination percentages in laboratory conditions. However, this does not ensure high performance under field or glasshouse sowing conditions. Vigor tests provide additional information to the standard germination test to assist in the differentiation of seed lots of acceptable, high-standard germination for a particular species [4,24]. In our study, the RE test discriminated between the seed vigor levels of seed lots with normal seed germination percentages above 98%, and highly vigorous seed lots have longer longevity than less vigorous ones (Figure 2). The results of the vigor test reflect the properties of the seed that determine the germination performance of any lot after storage [12,16,18,25] as well as performance in a wide range of environments [11,14,26]. In that sense, our results are in agreement with the conclusion that even though seed lots have very high normal germination percentages, they have different longevity (vigor potential). Vigor tests validated by ISTA rules so far are conductivity, accelerated aging (AA), tetrazolium chloride, controlled deterioration, and radicle emergence tests. However, there are various advantages and disadvantages of each vigor test. Matthews [27] emphasized that a vigor test should have a theoretical background, be rapid, enable prompt reporting of results, and be repeatable both within and between laboratories. Not every vigor test has a universal use in all sorts of species. Reasonably new vigor test of RE test offers a few distinct advantages over current vigor tests as being easy and rapid, prompt reporting, being repeatable and reproducible among laboratories. Most of the vigor tests require specific conditions, such as EC or accelerated aging cabinets. Evaluation of the results in some tests, i.e., tetrazolium, may need expertise and scientific background.

However, RE based on germination tests can be regularly and routinely conducted in any laboratory. The conditions of the optimum germination environment were determined by internationally accepted organizations, such as ISTA. Prediction of seed longevity by RE test during standard laboratory germination was achieved by a 120 h count in leeks [25], an 80 h count in onions [28], and a 32–34 h count in cucurbit rootstock [20]. RE as a vigor test was also found to be successful in estimating seedling emergence in the field (rather un-optimum) and climatic room (near optimum) conditions in various species [1,6,9,10,16,17,28,29], and seed storage longevity potential, particularly under artificial aging, i.e., high humidity and temperature conditions [12,18,20,25,28]. Our results in watermelons were in agreement with these previous reports. Even though there are some reports, the relationship between RE and longevity has not been fully investigated in a large number of plant species [30]. In our earlier work [19], a RE count of 74 h was suggested to relate to normal germination percentages in laboratory testing in open-pollinated watermelon seed lots. In the present work, 42 h of RE count appears to be much earlier than that in the same species. Two reasons this may be important; One, the seed lot quality of the present work was very high (98% < normal germination), and the other hybrid cultivars in this work appeared to germinate very fast. A RE of 42 h was not related to normal germination percentages at all (Figure 4). The seed lots of Farfan and Joker had 100% normal germination (Table 1) but had very different P_{50} values of 28.76 and 17.17 days, respectively (Table 2). This obviously showed that normal seed germination percentages might not necessarily indicate how long any seed lot survives in storage. However, RE counts of 42 h correlated not only with Ki but also with P_{50} values at a very significant level ($p < 0.01$, r = 0.7383–0.7936) (Table 3).

There is a debate about whether the mechanism of aging is similar under accelerated (high temperature and humidity) or natural aging (low temperature and seed moisture) conditions [31]. McDonald [32] suggested that lipid peroxidation, which leads to reduced membrane integrity, may differ under artificial and natural aging; however, the seed vigor tests conducted under accelerated aging conditions related well to the germination percentages of naturally aged seed lots [25]. Therefore, it appears to be that aging, whether occurring under natural or artificial conditions, may have a similar mechanism [31], while artificial aging studies have to be done in a short aging period to obtain vigor differences among the lots [20,25,28]. Otherwise, this could be achieved for a very long time in natural conditions due to the low temperature and seed moisture.

Radicle emergence tests also have the potential for automation. Automated methods of counting radicle emergence using computer-aided image analysis have been successfully used to produce germination progress curves [33,34] and to give data for the calculation of germination rate [30]. Automation will give a chance for faster operation than naked eye counts when a large number of seed lots are used. That may be important for seed companies that deal with a large number of seed lots at the same time [34]. Differences in RE, as seen here, have been related to the degree of seed aging and to the metabolic repair of the deterioration caused by aging before RE can occur [4,6]. It appears to be that even though seed lots in hybrids were produced by optimum production practices (harvesting, drying, etc.), some aging may occur before RE, which influences the lag period between water imbibition and radicle appearance. Moreover, hybrid seeds were more likely to be stored in optimum storage conditions (cold storage) than low-value open-pollinated ones due to their high value. Therefore, aging is less likely to occur in hybrids than those in open-pollinated ones.

The significant relationship between with (RE vigor test) and seed vigor, i.e., longevity, in seed lots can be explained in terms of the aging/repair hypothesis [5,35]. It is argued that DNA metabolic repair of deterioration resulting from aging causes the lag period from the beginning of imbibition to radicle emergence. Khajeh-Hosseini et al. [36] described the lag period for DNA repair in high-vigor (high RE) seeds of maize as shorter than those with low vigor (Lower RE). Some other studies emphasized the repair of genome damage during the early hours of imbibition before subsequent events could lead to germination in different crop seeds [37,38]. Moreover, Weitbrecht et al. [39] stated that understanding the physiology of early seed germination not only helped to discriminate seed lot vigor but could also help in selecting more adaptive robust crop species, thus increasing crop yield and quality.

Regarding an optimum measurement of longevity during storage, P_{50} was proposed as the more acceptable method [40]. P_{50} was described as the 'mean viability period', defined as 'the point on the time scale at which the survival curve intersects the 50% level of germination', as the measure of seed longevity [41]. Probert et al. [42] published estimates of P_{50} for seeds of 195 diverse species when stored at 60% RH and 60 °C and were able to explore relationships between P_{50} and climate variables and seed traits. Similarly, Merrit et al. [43] also used a high-temperature storage environment to compare seed longevity potential in a number of species of Australian flora. *Ki* is the estimated initial viability in probits and indicates the quality of pre-storage environmental and genetic factors in seed quality [21]. This is also named the seed quality constant because it is seed lot specific. A strong relationship between RE 42 h counts and *Ki* (Figure 4) indicates that RE counts, such as vigor tests, can also be used for determining the seed quality of any seed lot before storage, such as detection of the effect of various seed production factors (environment, harvest, or drying), which will allow seed companies to influence pre-storage factors on seed quality with a very fast vigor method.

5. Conclusions

The RE test is a rapid, easy, and practical vigor test that can be used to evaluate the storage potential of watermelon seeds. It is also useful for evaluating the effect of production factors (i.e., harvest time and drying) on pre-storage seed quality (*Ki*). In this work, we tested the seed longevity in high-moisture (17%) and storage-temperature (35 °C) conditions, where seeds in commercial storage are kept at about 12–17 °C and 45–60% relative humidity. Even though aging will be slower in such conditions, it will still impact the quality of seed lots in the long term, and the RE test will have the potential to discriminate vigor.

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