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Seed vigour tests to predict seed quality after storage in spinach seed lots

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Abstract

A study was carried out to determine whether seed vigour tests including radicle emergence (RE), mean germination time (MGT) and accelerated ageing (AA) correlate with post-storage germination percentages (PSGP) and mean germination times (PSMGT) of spinach seed lots after one of year hermetic storage at 18°C with 11.9 and 13.9% seed moisture. The RE test counted radicle emergence percentage (2 mm) after 48, 72, 96, 120 and 144 hours of germination, and mean germination was calculated by daily counts over 21 days at 20°C. Accelerated ageing was conducted at 41°C for 72 hours at 100% RH. Correlation analysis showed significantrelationships between both PSGP and PSMGT values after storage and initial MGT ($r = 0.807$-0.840, $p < 0.001$), RE ($r = 0.681$-0.932, $p < 0.01$-0.001) and AA ($r = 0.690$-0.890, $p < 0.001$). This indicates that these three seed vigour tests can predict the quality after storage of spinach seeds.

Keywords: germination, longevity, seed storage, seed vigour tests, spinach

Introduction

Vigour is an important physiological component of seed quality which relates to the field emergence potential and storage longevity of any lot that shows high germination under laboratory conditions (Powell and Matthews, 2012; Powell, 2022). Although commercial seed lots have high normal germination, they may show large differences in emergence or longevity in storage due to differences in vigour. These originate from seed ageing, which can be due to the pre- and post-seed harvest practices such as harvest time, drying and extraction (Demir and Samit, 2001; Groot, 2022). Vigorous seed lots have higher seedling emergence when stressful environments are encountered in the field and showbetter longevity during storage (Matthews *et al*., 2012).

Various vigour tests such as accelerated ageing, cold test, controlled deterioration, electrical conductivity and saturated salt accelerated ageing have been developed and used in the seed industry (Powell, 2006, 2022). One of the oldest and most rapid vigour tests is

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accelerated ageing (AA) in which seeds are kept at high relative humidity (i.e. 100% RH) at 41°C for 72 hours to discriminate vigorous and less vigorous seed lots depending on germination tests afterwards. (TeKrony, 2003). More recently, the radicle emergence (RE) test, the proportion of seeds with a radicle ≥ 2 mm long after a certain length of time during the early stage of a laboratory germination test, was introduced as a fast, easy, repeatable and practical vigour test, and validated by ISTA (2024). The philosophy behind the RE test is based on the lag period between the start of imbibition and radicle emergence (Matthews and Khajeh Hosseini, 2007). In aged seed lots, this period is extended due to the need for more time for metabolic repair of ageing, and the radicle emerges later. There are a large number of studies that relate the AA and RE tests to the seedling emergence of diverse species in the field or in transplant plugs (Matthews and Powell, 2011; Guloksuz and Demir, 2012; Ermis *et al*., 2015; Lv *et al*., 2016; Demir *et al*., 2019; Ilbi *et al*., 2020), and to germination after storage (Ermis *et al*., 2022; Eren *et al*., 2023). Maintaining high seed viability from harvest to sowing is a critical challenge for seed companies, particularly species that exhibit rapid loss of viability during storage. International seed companies produce seeds in specific regions and distribute them globally and store them for various periods to maintain quality throughout the supply chain.

Spinach is a leafy vegetable crop. Late germination due to low vigour and quality may reduce the size and the number of plants per acre, resulting in low yield. TeKrony and Egli (1991) reported that seed vigour has influence on vegetative growth and is frequently related to yield in crops that are harvested vegetatively (i.e. leaves). The seed of spinach is in fact a fruit, consisting of an embryo surrounding the perisperm, micropylar endosperm, testa and pericarp (Hermann *et al*., 2007). A prerequisite for a vigorous seed is that the perisperm and embryo must be fully matured and developed. In any given lot, it is not possible to get all seeds to the same maturity and the same perisperm development stage. Seeds at different maturity levels are harvested during a single harvest operation (once-over harvesting). This may affect both seed-to-seed quality variation and germination after storage (Demir and Samit, 2001; Whitehouse *et al*., 2018; Hay *et al*., 2022). Furthermore, some seed coat structures such as the thicker pericarp in spinach seeds may reduce speed of germination (Nonogaki, 2006). There are several studies in which seed vigour test results were significantly related to seed longevity in different crop seeds (Ozden *et al*., 2017; Ermis *et al*., 2022; Eren *et al*., 2023). However, research on the relationship between vigour tests and the prediction of seed germination after storage in spinach is rare. In this study, we examined the AA and RE vigour tests to identify differences in seed storage potential of spinach seeds, as indicated by the percentage and rate of germination of different seed lots after storage.

Materials and methods

Fourteen seed lots of a single spinach cultivar (*Spinacia oleracea* L. cv. Matador) were obtained from different seed companies. They had all been produced over 2020-2022. After determining, the seed moisture content (mc) on % fresh weight basis (ISTA, 2024),

the seeds were kept at 5°C in hermetically sealed aluminium foil packets until use (about two months). Eight replicates of 100 seeds were weighed and means were taken and multiplied by 10 to get 1000-seed weight.

For germination tests, three replicates of 50 seeds per lot were placed between paper towels (Filtrak, Germany) (200 × 200 mm) and moistened with 10 ml distilled water. The paper towels were then rolled and placed in plastic bags in order to prevent water loss during the test. The bags were held in the dark at 20°C for 21 days. Radicle emergence (RE) test counts (number of seeds with a radicle ≥ 2 mm-long) were made after 48, 72, 96, 120 and 144 hours and daily thereafter until 21 days. At the final count, seedlings were evaluated as total germination and normal seedlings (ISTA, 2024). The number of normal seedlings was used to calculate the germination percentage (GP, %). Mean germination time (MGT, days) was calculated as

$$\sum n.t / \sum n,$$

where n is the number of seeds newly showing radicle emergence at time t (days from sowing). $\sum n$ is therefore the total number of seeds showing radicle emergence by the end of the test (21 days).

For the accelerated ageing test (AA), 40 ml of distilled water was added to each ageing box (110 cm × 110 cm × 50 cm) and 150 seeds were weighed and placed in one layer on a wire mesh tray (100 mm × 100 mm × 40 mm) inside the box (TeKrony, 2003). The boxes were kept in the dark at 41°C for 72 hours. One box was used for each seed lot. The germination test was then conducted as described above and results were expressed as a percentage normal seedling (AA, %) (ISTA, 2024). During the post AA germination test, radicle emergence (> 2 mm) counts were made daily and mean germination time after accelerated ageing (AAMGT, days) was calculated by the formula as in MGT.

For storage experiments, about 150 seeds of each cultivar were weighed (0.001 g) and equilibrated to 75% RH in plastic cups (360 mm long × 180 mm wide × 120 mm deep) by placing them on top of wire mesh over saturated NaCl solution in the dark for three days at room temperature. Saturated NaCl was prepared by adding 400 g NaCl to 1000 ml distilled water. At the end of equilibration, no more seed weight changes were recorded. Seed moisture content was determined by weight and found to range between 11.9 and 13.9%. Seeds of each lot were then hermetically sealed in aluminium foil packets and stored at 18 ± 2°C. Germination tests as described above were carried out as three replicates of 50 seeds after 12 months of storage. Post storage germination percentages (PSGP, %) were calculated based on normal germination percentages after 21 days (ISTA, 2024). During the germination period, 2 mm-radicle emergence was determined daily to calculate post storage mean germination time (PSMGT, days).

Correlation analyses between GP (%), MGT (days), RE (48, 72, 96, 120 and 144 hours) and AA (%), AAMGT (days) and post storage germination percentage PSGP (%) and post storage mean germination time PSMGT (days) were made using SPSS. Significance levels are indicated at the 5, 1 and 0.1% levels. The relationships (R^2) between the highly correlated criteria were also calculated.

Results

The seed moisture content of the lots upon receipt was between 8.1 and 11.5% (table 1). Three lots (3, 5 and 6) had seed moisture contents of 10% and above. The normal seedling percentage (GP) of the lots ranged between 71 and 98%. The first six lots had 90% and higher, four lots had 81 and 88% of germination, and the final three had 71 and 76%. All seed lots were above the commercially acceptable level of 70% of germination for spinach. The thousand seed weight ranged between 6.8 and 13.5 g. Seeds of nine out of 14 lots had a thousand seed weight exceeding 10 g while lots 7 and 12 were identified as the smallest. MGT values ranged from 2.9 and 6.2 days, with lot 7 showing the fastest germination and lot 9 the slowest.

Table 1. Production year, germination percentage (GP; normal germination, %), mean germination time (MGT, days), seed moisture content (mc, %) and thousand-seed weight (g) of the 14 commercially available spinach seed lots. Statistical analysis was performed by using SPSS to carry out ANOVA. Percentages were arcsine-transformed prior to analysis. Means with different letters in the same column denote significant difference at 5% level.

Lot	Production year	GP (%)	MGT (days)	Seed moisture (%)	Thousand seed weight (g)
1	2022	98^{a}	4.1^{df}	8.1	13.5
2	2022	96^{ab}	3.8^{ef}	9.2	8.1
3	2021	95^{ac}	3.8^{ef}	10.2	12.1
4	2021	94^{ac}	4.0^{ef}	9.3	8.9
5	2021	93^{ac}	3.1^{g}	10.5	7.2
6	2020	92^{ac}	3.5^{fg}	11.5	11.9
7	2022	88^{ad}	2.9^{g}	9.6	7.0
8	2021	85^{be}	4.1^{df}	9.4	11.7
9	2021	84^{ce}	6.2^{a}	8.1	11.8
10	2021	82^{be}	5.1^{bc}	8.6	12.6
11	2021	81^{de}	5.7^{ab}	9.6	12.1
12	2021	76^{ef}	4.8^{cd}	8.7	6.8
13	2021	75^{ef}	4.3^{de}	9.3	10.1
14	2021	71^{f}	5.5^{ac}	8.6	11.2
Range	2020-2022	71-98	2.9-6.2	8.1-11.5	6.8-13.5

The spinach seeds started to germinate after 48 hours (table 2). RE test counts showed that lots 5, 6, 7 and 10 had the earliest emergers with RE of 31 and 36% after just 48 hours, while lots 4, 8, 9, 11, 12, 13 and 14 were found to be the slowest ones, with lower than 6% RE after 48 hours. By 144 hours, lots 1 to 7 had above 83% RE, and the remaining seven lots had RE values between 48 and 75%. The statistical significance among the lots was variable at the 5% level.

Table 2. Changes in radicle emergence (RE %) percentages of 14 spinach seed lots between 48 and 144 hours, and germination percentages (normal germination, %, AA) and mean germination time (AAMGT, days) after accelerated ageing at 41°C, at 100% over 72 hours. Means with different letters in the same column denote significant difference at 5% level.

Lot	Radicle emergence (RE hours)					AA (%)	AAMGT (days)
	48	72	96	120	144		
1	13^{c}	40^{b}	67^{ab}	82^{ab}	88^{a}	84^{ac}	5.4^{c}
2	21^{bc}	49^{a}	70^{ab}	82^{ab}	87^{ab}	84^{ac}	4.6^{bc}
3	19^{bc}	56^{a}	70^{ab}	82^{ab}	83^{ab}	96^{a}	2.1^{a}
4	3^{e}	40^{bc}	66^{ab}	80^{ab}	86^{ab}	90^{ab}	3.4^{ab}
5	31^{ab}	55^{a}	73^{ab}	90^{a}	92^{a}	87^{ab}	4.4^{bc}
6	31^{ab}	63^{a}	77^{a}	82^{ab}	85^{ab}	95^{a}	2.7^{a}
7	35^{a}	58^{a}	74^{a}	86^{a}	89^{a}	84^{bc}	3.8^{ab}
8	5^{de}	39^{bc}	59^{bc}	71^{b}	75^{bc}	87^{ab}	3.4^{ab}
9	1^{ef}	5^{f}	17^{f}	39^{e}	59^{d}	57^{e}	6.0^{cd}
10	36^{a}	25^{cd}	41^{de}	57^{cd}	68^{cd}	81^{bc}	4.1^{b}
11	0^{g}	8^{ef}	37^{de}	47^{de}	56^{de}	65^{de}	5.2^{cd}
12	5^{de}	29^{bd}	41^{de}	51^{cd}	57^{de}	74^{cd}	3.3^{a}
13	6^{de}	30^{bd}	47^{cd}	59^{c}	62^{d}	57^{e}	4.9^{bc}
14	2^{e}	15^{de}	31^{e}	46^{de}	48^{e}	53^{e}	5.1^{c}
Range	0-35	5-63	17-77	39-90	48-92	53-96	2.1-6.0

Germination after the AA test ranged between 53 and 96% (table 2). The lowest germination percentages were 53 and 57% in lots 9, 13 and 14, and the highest germination was recorded in lots 6 and 3, reaching 95 and 96%, respectively. The germination percentages of lots 3, 6, 8 and 10 were not reduced by ageing at all. The fastest germination after AA was noted in lot 3 with AAMGT of 2.1 days, and the slowest (the longest time to germination) in lot 9 with 6.0 days.

Post-storage germination ranged between 3 and 95% (table 3). A substantial decline in germination was observed in seed lots 9 to 14. Lot 14 had only 3% germination after 12 months of storage. Seed lots 1 to 7 retained higher levels of germination, although PSGP ranged from 57% (lot 1) to 95% (lot 4) despite initial GP differing by only 10%.

Many of the vigour assessments were highly correlated ($r = 0.681$-0.932; $p < 0.01$) with the post storage germination percentage (PSGP) and post storage mean germination time (PSMGT) (table 4). MGT and RE counts at 72, 96, 120 and 144 hours were highly significantly correlated with both PSGP and PSMGT ($p < 0.001$). The R^2 values of 0.85 and 0.82 indicate that 85% of the variation in germination after storage and 82% of the variation in mean germination time after storage were explained by the regression on RE72 hours (figure 1). Thus, the seed lots (9, 11 and 14) with low germination after storage could be identified by the low RE counts after 72 hours of germination (table 2). MGT values were also highly related to PSGP and PSMGT with the regression of these parameters against MGT accounting for 80-84% of the variation (figure 2).

Table 3. Changes in germination percentages (normal germination, %) of spinach seed lots before (GP), after storage (PSGP) for 12 months at 18°C with seed moisture contents (%) that shown. Post storage mean germination time (PSMGT) was also presented. Means with different letters in the same column denote significant difference at 5% level.

Lot	Seed storage moisture (%)	GP (%)	PSGP (%)	PSMGT (days)
1	12.5	98^{a}	57^{d}	7.3^{ac}
2	12.4	96^{ab}	69^{bd}	5.0^{de}
3	13.4	95^{ac}	71^{bc}	4.5^{e}
4	12.8	94^{ac}	95^{a}	5.1^{de}
5	13.9	93^{ac}	77^{bc}	3.2^{de}
6	13.9	92^{ac}	76^{bc}	4.9^{de}
7	12.1	88^{ad}	83^{b}	4.9^{de}
8	12.3	85^{be}	66^{cd}	5.0^{de}
9	12.9	84^{ce}	23^{e}	8.2^{ab}
10	12.0	82^{be}	33^{e}	7.9^{bc}
11	12.7	81^{de}	23^{e}	8.8^{a}
12	11.9	76^{ef}	30^{e}	5.7^{cd}
13	12.6	75^{ef}	22^{e}	7.6^{ac}
14	11.9	71^{f}	3^{f}	6.3^{e}
Range	11.9-13.9	71-98	3-95	3.2-8.8

Table 4. Correlation coefficients (*r*) between germination percentage (normal germination GP), mean germination time (MGT, days), RE values of 48, 72, 96, 120 and 144 hours, germination (AA, %) and mean germination time (AAMGT, days) after accelerated ageing (AA, 41°C, 100%, 72 hours) and post storage germination (PSGP, %) and mean germination time (PSMGT, day) in spinach seed lots.

Vigour measure		PSGP (%)	PSMGT (days)
	GP	0.831***	-0.470^{ns}
	MGT	-0.840***	-0.807***
Radicle emergence (RE, hours)	RE48	0.681**	-0.694**
	RE72	0.850***	-0.852***
	RE96	0.889***	-0.826***
	RE120	0.908***	-0.753***
	RE144	0.932***	-0.760***
Accelerated ageing (AA)	AA	0.890***	-0.690**
	AAMGT	0.686***	-0.699**

**$p < 0.01$, ***$p < 0.001$; ns, not significant.

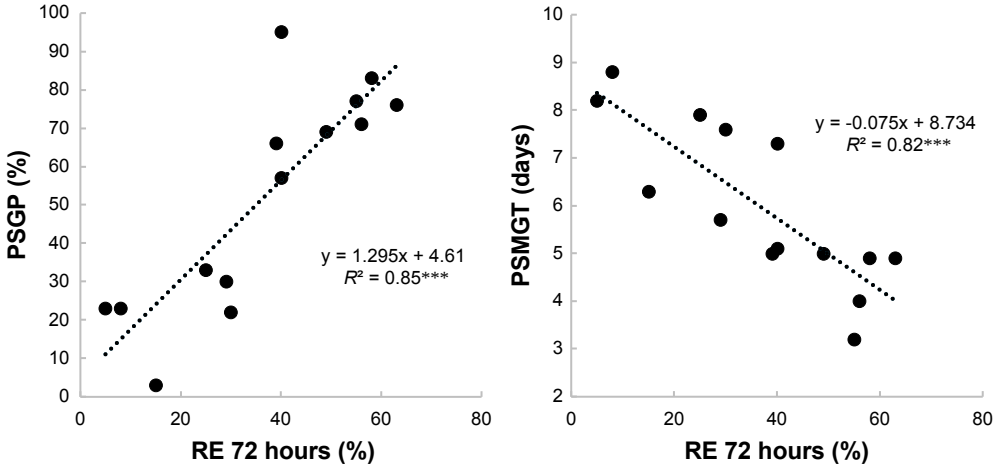

Figure 1. The relationship between radicle emergence test (RE 72 hours, %) and germination percentages (PSGP, %) and mean germination time (PSMGT, days) after 12 months of storage (18 ± 2°C, 11.9-13.9% seed mc.) for 14 spinach seed lots. ***$p < 0.001$.

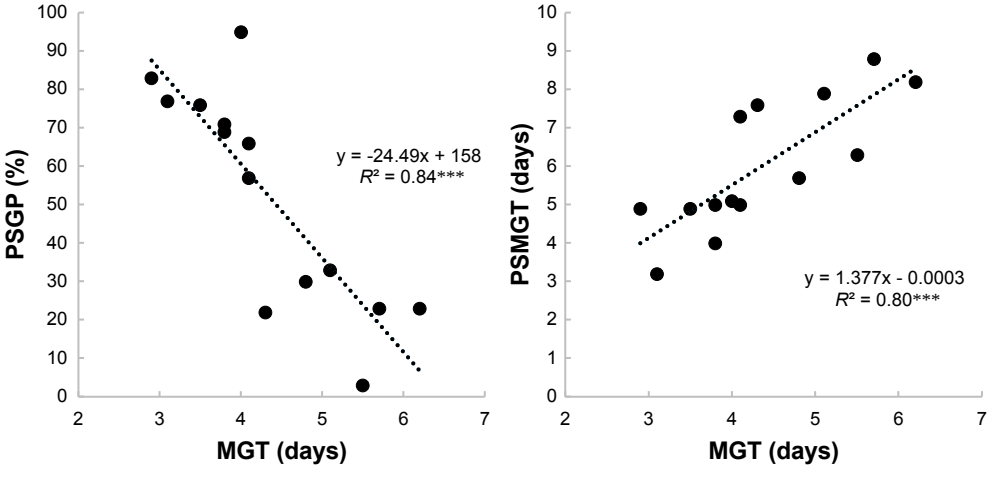

Figure 2. The relationship between mean germination time (MGT days) and germination percentages (PSGP, %) and mean germination time (PSMGT, days) after 12 months of storage (18 ± 2°C, 11.9-13.9% seed mc) for 14 spinach seed lots. ***$p < 0.001$.

The germination after AA was highly significantly related to PSGP with R^2 of 0.89 ($p < 0.001$) although less significantly related to PSMGT ($p < 0.69$, $p < 0.01$) (figure 3). AAMGT correlated with both PSGP and PSMGT, but less well than the AA germination percentage.

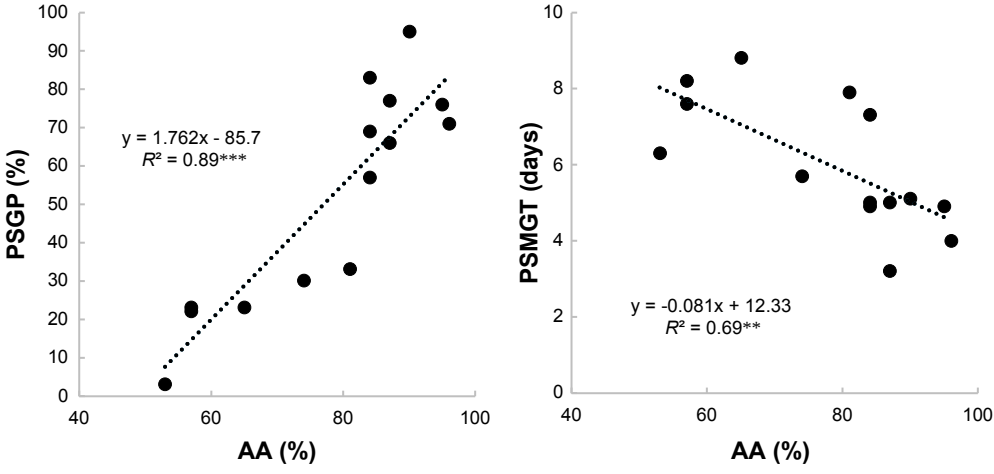

Figure 3. The relationship between accelerated ageing (AA, %) and germination percentages (PSGP, %) and mean germination time (PSMGT, days) after 12 months of storage (18 ± 2°C, 11.9-13.9% seed mc) for 14 spinach seed lots. ** $p < 0.01$, *** $p < 0.001$.

Discussion

This study aimed to identify differences in the storage potential of 14 spinach seed lots from the same cultivar, Matador, through vigour tests. The main physiological reason for low seed vigour is ageing; aged, low vigour seed lots need a longer period between the start of imbibition and radicle emergence (protrusion of the radicle) during germination. Slower RE and a lower proportion of normal seedlings have been associated with seed ageing in several crop species (Matthews and Powell, 2011; Powell, 2022). Therefore, earlier radicle emergence percentages (RE test) of any lot have been proposed as an indicator of the extent to which a seed lot has aged (Matthews and Khajeh Hosseini, 2007). A longer time between imbibition and radicle emergence is seen in low vigour lots and is necessary to allow for metabolic repair of age-induced deterioration (Osborne, 1983; Matthews and Powell, 2011). In high vigour lots – seeds with high physiological quality – the radicle emerges earlier, since there is much less ageing-induced DNA damage that needs repair (Lv *et al.*, 2016). Evidence in support of this ageing/repair hypothesis has been summarised by Matthews *et al.* (2012) and Powell and Matthews (2012). The RE test has been highly correlated with seedling emergence potential in the seeds of various contrasting species (Guloksuz and Demir, 2012; Ermis *et al.*, 2015; Lv *et al.*, 2016; Demir *et al.*, 2019; Shinohara *et al.*, 2021). However, its relationship with seed longevity has not been sufficiently explored.

Seed lots that have already aged due to pre-and post-harvest practices inevitably exhibited poorer storage potential. The present study indicated that the length of the lag period (lower RE in the early stage of germination) did reflect the extent of ageing and, in turn, seed lot germination after storage. For example, germination percentages before

storage in the first six lots were above 92%, but after storage the range were observed to be between 57 and 95% (tables 1 and 3). The RE values of these six lots ranged between 40 and 63%. Supporting our findings, Ozden *et al*. (2017) found that a RE count after 120 hours was related to p_{50} values in leek seed lots. Ermis *et al*. (2022) found that RE counts of between 30 and 34 hours gave a significant relationship ($p < 0.01$) with K_i (initial seed quality) and p_{50} (half-viability period) values for artificially aged seed lots of eight different cucurbit rootstock cultivars. Similarly, the relationship between RE 42-hour counts and K_i ($p < 0.01$, $r = 0.7538$) indicated that RE counts can be used for determining the seed quality of any seed lot in watermelon (Eren *et al*., 2023).

In this study, we used seed lots belonging to the same cultivar, so that there was no genetic variation among the lots. While it is based on the links between seed ageing and a single RE count, it is important to evaluate the RE test across diverse cultivars, including inbreds, hybrids, etc., and those seed lots that are grown in different ecosystems of the country and are produced by various production technologies (Whitehouse *et al*., 2018; Lee *et al*., 2019) to confirm applicability for evaluating which seed lots will lose viability faster during storage. In our work, seed lots were produced in consecutive years: 2020 (one lot), 2021 (10 lots) and 2022 (two lots). Moreover, the lots were collected from different regions and produced by different companies. This clearly indicates that RE can be used with diverse seed lots of spinach produced in different regions. Since all RE counts are significantly related ($p < 0.01$-0.001) to PSGP and PSMGT, we suggest RE72 hours could be a more appropriate vigour indicator due to the time convenience (earlier) than those of others. RE72 was not well related to PSGP ($r = 0.07$), PSMGT ($r = 0.58$) in those seed lots that they have 88% and above of pre-storage germination (1-7 lots). While it should be noted that commercially acceptable germination is 75% in spinach, which all seed lots except one passed in our work (table 1).

The initial MGT ($r = 0.807$-0.840, $p < 0.001$) and post-ageing MGT, (AAMGT) were significantly ($r = 0.686$-0.699, $p < 0.01$-0.001) related to the PSMGT (table 4). We determined MGT and RE counts daily and manually; MGTs counted manually have previously been shown to correlate strongly with both seedling emergence and RE after storage in several crops (Matthews and Powell, 2011; Demir *et al.*, 2019; Ermis *et al*., 2022; Eren *et al.*, 2023). However, counts in much shorter periods, in the initial stage of germination, say every four hours, particularly at high temperatures can only be possible by using automated computer aided vision capture of RE (Wagner *et al.*, 2011). Such counts could also be done manually, but this would be inconvenient, when overnight counts would be necessary. Machine vision computer aided systems are raising new horizons in RE and MGT counts (Shinohara *et al.*, 2024). This approach enables rapid evaluation of the large numbers of lots that seed companies manage.

In this study, we tested longevity at a relatively low seed moisture (11.9-13.9%) and cool environment (18°C) over 12 months. Seeds in commercial storage are typically maintained at 16-20°C and 45-60% RH. The temperature of our storage conditions is common for medium term storage, but seed moisture was slightly higher. However, some lots had about 10-11.5% seed moisture before storage. That shows that our storage environment is not so far from typical medium term storage conditions. The good range of germination after storage (3-95%) indicates that the conditions that were used were good enough to be able to discriminate based on the seed lot vigour (table 3).

In conclusion, the RE test is a fast, easy and practical vigour test that can be used to evaluate the storage potential of spinach seeds; such information could contribute to better management of commercial seed stocks. It is also useful for evaluating the effect of ecological and production factors on seed quality before and after storage.

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