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ORIGINAL RESEARCH PAPER

Can mean germination time predict seed vigor of canola (*Brassica napus* L.) seed lots?

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Abstract
The aim of this research was to test if mean germination time (MGT) can predict seed vigor in 10 seed lots of canola. There was a significant difference between the final germination percentages of the 10 seed lots tested ('Hyola 330', 'Hyola 401', 'Okapi', 'Elite', 'SLMO 46', 'Zarfam', 'RGS 003', 'Option 500', 'Echo', 'Rainbow') and a highly significant correlation between MGT and this value. Of the 10 seed lots, 'Hyola 330' had the lowest final percentage germination (62%) and the highest MGT, 3.64 days. The correlation between MGT and the proportion of "normal" seedlings was also significant. 'Hyola 330' had the lowest percentage of normal seedlings (60%). A tetrazolium test showed that this lot had some dead seeds that could not germinate; some seeds were viable, but they would produce abnormal seedlings. One of the main reasons of low final germination percentage and high MGT in 'Hyola 330' was low viability of this seed lot. Mean germination time is suggested as a good indicator of seed vigor in canola.

Keywords
oilseed; vigor; seed viability

Introduction

Canola (*Brassica napus* L.) is one of the most important oil crops in the world. The essential role of seeds in crop establishment and importance has long been recognized. Germination and seedling emergence are two processes which determine uniformity, crop stand density, degree of weed infestation, the efficient use of the nutrients and water resources available to the crop, and ultimately affect the yield and quality of the crop [1]. The major explanation for vigor differences in many species is, however, the incidence of seed ageing, which is sometimes referred to as deterioration [2,3]. Natural ageing occurs during the normal processes of harvest, transport, and seed storage [3,4]. Older maize seed had lower vigor as seen in soil emergence tests and was slower to germinate as indicated by higher mean germination time (MGT) [4]. Seed deterioration resulting from harvest conditions and prolonged storage is often cited as a physiological cause of differences between lots [5,6]. The fact that the germination of lots of maize after accelerated ageing relates to field emergence in maize [7] and sweet corn [8] suggests that deterioration is a cause of vigor differences. Ellis and Roberts [9] provided evidence for a range of crops (barley, wheat, onions, and cabbage) that when seeds deteriorated under laboratory ageing regimes, the rate of germination was slower as germination declined, indicated by significant negative relationships between mean germination time (the reciprocal of which is the rate of germination) and the final germination percentage. Similar findings were reported for naturally aged carrot seed produced from several sources [10]. Some of the reports on the maize cold test contain information suggesting that slow germination and emergence [7–11] are characteristic of lots with low field emergence. Reduced seed vigor, seen as increased MGT, has been consistently related to ageing in artificial [9] and naturally aged seeds [10]. Slow germinating lots produced shorter more variable shoots in the laboratory and emerged more slowly in the field, producing smaller and more variable plants 25 days after sowing [12]. Prediction of vigor and normal seedling development by MGT in some crops such as corn [12] and pepper [13] has been investigated but no research has been carried out in canola. The main goal of this research was therefore to determine if MGT can be used as a good indicator of seed vigor in canola seed lots.

Tab. 1 Area of production and seed weight of 10 seed lots of canola used in the treatment. All seed lots were produced in 2006.

No.	Seed lot	Production area	1000-seed weight (g)
1	'Hyola 401'	Iran (Khuzestan)	4.87
2	'Okapi'	Iran (Mashhad)	4.45
3	'Elite'	Iran (France)	4.68
4	'Slmo 46'	Iran (Karaj)	3.98
5	'Hyola 330'	Iran (Khuzestan)	5.40
6	'Zarfam'	Iran (Mashhad)	3.87
7	'RGS 003'	Iran (Karaj)	3.99
8	'Option 500'	Iran (Karaj)	3.37
9	'Echo'	France	2.44
10	'Rainbow '	France	2.83

Material and methods

Experimental procedure

This research was carried out at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. All seed lots of canola (*Brassica napus* L., Brassicaceae) were kindly provided by the Agricultural Research Center of Khorasan Razavi Province in Mashhad. Details of the 10 seed lots used in this experiment are given in Tab. 1. All were stored at room temperature (22°C) for around 60 days. Seeds were sterilized using NaOCl (5%) for 3 min and then carefully washed three times with distilled water. Four replicate batches of 25 seeds from each lot were used. This experiment was set up using the top paper method in Petri dishes (9 cm diameter) and lasted 8 days. At the beginning of experiment, 2 mL of distilled water was added to each Petri dish, and all dishes were placed in a closed box in an environmental growth chamber with a constant temperature of 22°C. The number of germinated seeds was recorded daily on the basis of a radicle length of 2 mm. The number of "normal" seedlings (those with healthy cotyledons, shoot, and root [14]) and final seed germination percentage were recorded on Day 8.

Mean germination time (days) was calculated for each lot using the daily counts according to the formula of Ellis and Roberts [9]:

$$MGT = \frac{\sum f_x}{\sum f}$$

where *f* is a number of seeds newly germinated, *x* is a number of experimental day.

Tetrazolium solution (1% TTC) was used to evaluate the viability of seeds. In this test, 50 seeds were counted and soaked in water for 17 h. After removing the seed coat, seeds were then soaked in 1% TTC for 3 h at 30°C. The viability of seeds was evaluated on the basis of seed color.

Statistical analysis

Excel software and a regression method were used to determine the relationships between MGT, final germination percentages, and the numbers of normal seedlings. MSTAT-C software, developed by the Department of Plant and Soil Sciences, Michigan State University, USA, was used for analysis of variance of the traits of seed lots, and Duncan's multiple range test (DMRT) was used for comparison of the means for all traits. The probability level was assessed at $p \leq 0.05$.

Results

The time course for the germination of the 10 seed lots of canola is shown in Fig. 1. 'Okapi' had the highest germination percentage (89%) after 1 day and 'Hyola 330' had

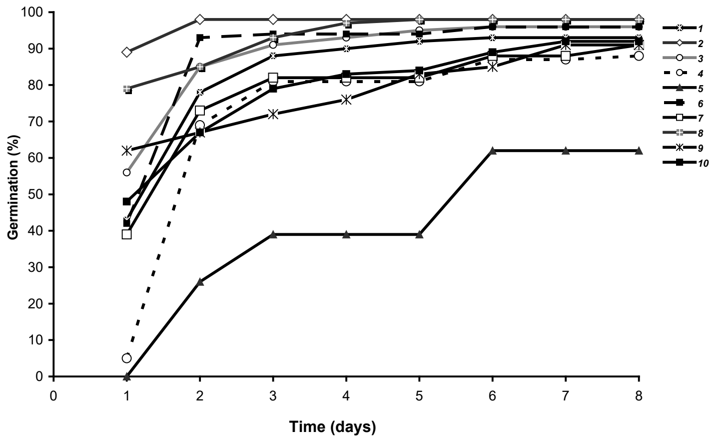

Fig. 1 Germination rate of the 10 seed lots of canola. 1 – 'Hyola 401';; 2 – 'Okapi'; 3 – 'Elite'; 4 – 'Slmo 46'; 5 – 'Hyola 330'; 6 – 'Zarfam'; 7 – 'RGS 003'; 8 – 'Option 500'; 9 – 'Echo'; 10 – 'Rainbow'.

0%. Along with 'Zarfam' (99%), the highest final germination percentage was for 'RGS 003', 'Elite', 'Option 500', 'Slmo 46', and 'Hyola 401' (95–97%), as shown in Tab. 2.

The relationship between MGT and final germination is shown in Fig. 2. When MGT increased, final germination decreased. There was a highly significant correlation ($p \leq 0.01$) between MGT and final germination percentage.

With increasing MGT, the number of normal seedling decreased. This relationship is shown in Fig. 3 for the 10 seed lots. There was a highly significant correlation between MGT and the percentage of normal seedlings ($p \leq 0.01$).

The one-way analyses of variance for the 10 seed lots showed that final germination percentage, percentage of normal seedlings, and MGT were all significant ($p \leq 0.05$). 'Hyola 330' had the highest MGT, 3.64 d, and 'Okapi' the lowest, 1.09 d (Tab. 2). 'Okapi' also had the highest percentage of normal seedlings, 90.25%, but this was not significantly different from five other seed lots. Remarkably, the lowest percentage of normal seedlings was recorded for 'Hyola 330' (60%). No significant relationship was detected between MGT and 1000-seed weight in the canola seed lots tested.

Since the seeds of 'Hyola 330' were characterized by the highest MGT, the lowest final germination percentage, and the lowest percentage of normal seedlings, the viability test using 1% TTC was performed for this seed lot. Tetrazolium test differentiates live from dead tissues of seeds staining living cells with a red color, while dead cells remain unstained. The test showed seeds of three groups of color: (1) seeds with white cotyledons and a white embryo (embryo axis with well-developed radicle and hypocotyl and very small epicotyl); (2) seeds with red cotyledons and a white embryo (embryo axis with well-developed radicle and hypocotyl and small epicotyl); (3) seeds with red cotyledons and a red embryo (embryo axis with well-developed radicle and hypocotyl and small epicotyl) (Fig. 4–Fig. 6). Therefore, Group 1 represents dead seeds, Group 3 – live seeds. The seeds of Group 2 might produce some seedlings, but their growth will be arrested and so these seeds will produce abnormal seedlings.

Tab. 2 Comparison of means of 10 seed lots for final germination percentage, the percentage of normal seedlings, and mean germination time (MGT).

No.	Seed lot	Final germination (%)	Normal seedlings (%)	MGT (days)
1	'Hyola 401'	95 ^{abc}	89.25 ^{ab}	2.01 ^{c}
2	'Okapi '	90 ^{bc}	90.25 ^{a}	1.09 ^{e}
3	'Elite'	96 ^{abc}	88.25 ^{ab}	1.62 ^{d}
4	'Slmo 46'	96 ^{abc}	83.50 ^{d}	2.42 ^{b}
5	'Hyola 330'	62 ^{d}	60 ^{e}	3.64 ^{a}
6	'Zarfam'	99 ^{a}	87.5 ^{abc}	1.65 ^{d}
7	'RGS 003'	97 ^{ab}	84.25 ^{cd}	2.12 ^{bc}
8	'Option 500'	96 ^{abc}	90 ^{a}	1.39 ^{de}
9	'Echo'	91 ^{bc}	87 ^{abc}	2.02 ^{c}
10	'Rainbow '	89 ^{c}	86 ^{bcd}	2.10 ^{bc}
CV%		5.13	2.56	1.85

Means with different letters associated are significantly different at the $p \leq 0.05$ level, as determined from Duncan's multiple range tests.

Discussion

Different seed varieties of canola have different germination characteristics. This is likely due to genetic factors, but physiological effects such as ageing can reduce germination capacity. There were differences between the seed lots tested in their germinability, but only one, 'Hyola 330', had a very low final germination percentage (62%). This is possibly due to ageing and a low vigor in this seed lot. 'Hyola 330' also germinated slower than others nine lots. A tendency for older canola seeds to have lower germination has been reported in the literature [15]. Reduced seed vigor, seen as

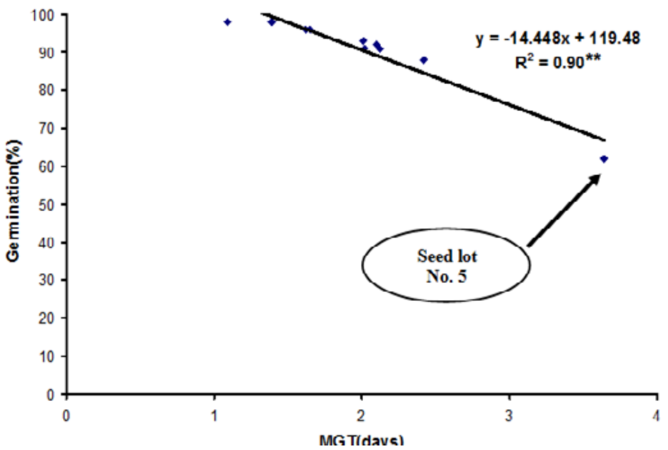

Fig. 2 Relationship between mean germination time (MGT) and final germination of the 10 seed lots.

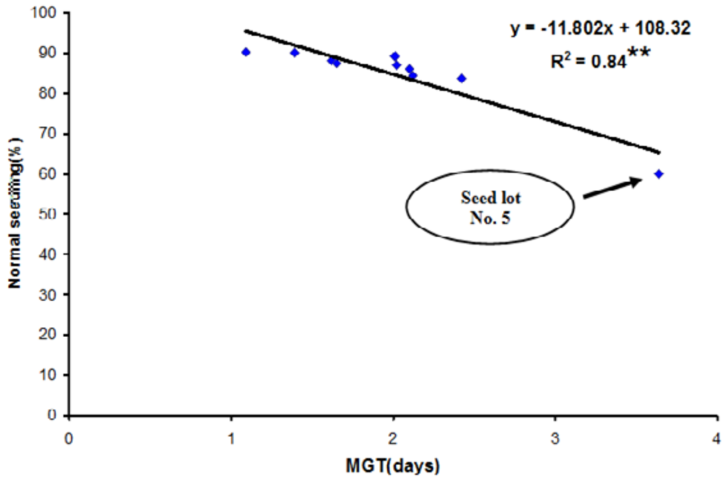

Fig. 3 Relationship between mean germination time (MGT) and the percentage of normal seedlings for the 10 seed lots.

increased MGT, has consistently been related to ageing in artificially [9] and naturally aged seeds [10]. Older maize seed had lower vigor as seen in soil emergence tests and was slower to germinate as indicated by higher MGT [16]. As MGT increases, final germination decreases; Fig. 2 shows the highest MGT (3.64 d) was recorded for 'Hyola 330'. This seed lot also had the lowest germination percentage both on Day 1 (0%) and on the last day of recording (62%). The main explanation for seed vigor differences in many species is, however, the incidence of ageing, which is sometimes referred to as deterioration [2,3]. Seed ageing is reflected as both physiological and biochemical changes [3]. A highly significant correlation between MGT and final germination was shown in Fig. 2 ($p \leq 0.01$). When seed lots deteriorate, their viability decreases and they will have a delay in germination, which is reflected in the MGT. A link between deterioration and MGT could be explained in terms of the repair hypothesis in deteriorated seeds as suggested by Matthews and Khajeh-Hosseini [4]. There is a considerable body of evidence that one of the first effects of ageing is an increase in MGT [17,18]. Osborne [17] provided evidence of DNA repair as an early event during the lag period, which leads up to germination. 'Hyola 330' had low seed vigor and so it had produced the lowest percentage of normal seedlings (60%) on the last day of the tests due to ageing. It was shown that when MGT increases, the proportion of normal seedlings decreases (Fig. 3). A significant negative correlation ($r = -0.987$, $p \leq 0.01$) was found between MGT and the percentage of normal seedlings. The slower the germination of a seed lot, the lower was the percentage of normal seedlings [15]. The fact that after accelerated ageing, the germination of maize seed related to field emergence [7,8] suggests that deterioration is a cause of differences in vigor. To test this hypothesis, a viability test of seeds of 'Hyola 330' was performed using TTC. The results of this test showed some seeds of this lot had low vigor due to deterioration. In this test, all the colored seeds of 'Hyola 330' were classified into three groups (Fig. 4). The unstained white seeds (Group 1) were dead and would never germinate. The seeds stained red (Group 3) were all viable, able to germinate and produce normal seedlings.

Fig. 4 Seeds with white cotyledons and a white embryo.

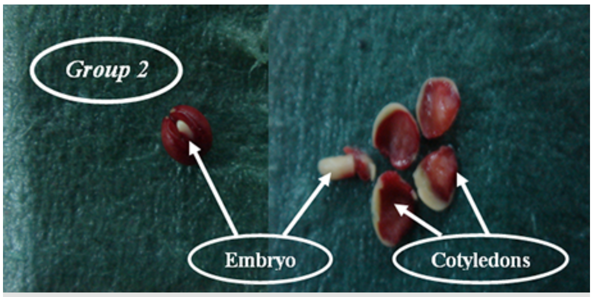

Fig. 5 Seeds with red cotyledons and a white embryo.

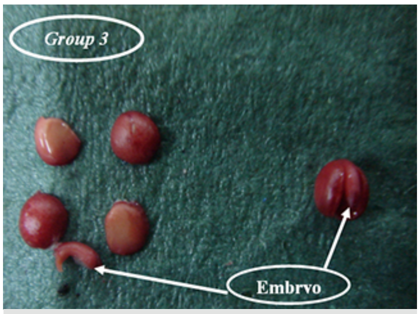

Fig. 6 Seeds with red cotyledons and a red embryo.

The seeds with red colored cotyledons but a white embryo (Group 2) might produce abnormal seedlings. Consistently, 'Hyola 330' had the lowest percentage of normal seedlings (60%). Similarly, it has been found that in mung bean cultivars [18], numbers of normal seedlings decreased with increases in the period of seed ageing. Similar results have been reported by Rodriquez and McDonald [19] for red field bean. There is much evidence that low vigor and slow germination is the result of physiological ageing in *Brassica* seed [20]. 'Hyola 330' seeds gradually decay, thus ageing in these seeds causes an increase in the germination time as shown in Fig. 3. When seeds are ageing, seed death may occur rapidly. This is why the 'Hyola 330' seed lot had such a low germination percentage and a high MGT.

Conclusion

The main aim of our study was to evaluate the mean germination time (MGT) for evaluating the 10 seed lots of canola. Slower germinating seed in our study produced a greater number of abnormal seedlings, which is a well-recognized signal of deterioration as a result of seed ageing. Our results have shown that when MGT increases, the numbers of normal seedlings decrease and there was a highly significant correlation between MGT and the percentage of normal seedlings. Therefore, seeds with a higher MGT can have been aged physiologically. One of the most important reasons for a decrease in canola yield is poor seedling emergence or establishment. An earlier count of germinated seed can be suggested for rapid evaluation of seed viability. Furthermore, seedling emergence and establishment in these seed lots also needs assessment. MGT can be used as an indicator of seed vigor in canola.

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Czy średni czas kiełkowania może być wskaźnikiem wigoru nasion rzepaku (*Brassica napus* L.)?

Streszczenie

Celem badań było sprawdzenie, czy na podstawie średniego czasu kiełkowania dziesięciu zestawów nasion rzepaku (*Brassica napus* L.) można przewidzieć wigor nasion. Stwierdzono istotne różnice w proporcji wykiełkowanych nasion pomiędzy badanymi odmianami rzepaku ('Hyola 330', 'Hyola 401', 'Okapi', 'Elite', 'Slmo 46', 'Zarfam', 'RGS 003', 'Option 500', 'Echo', 'Rainbow') oraz silną korelację między średnim czasem kiełkowania i procentem skiełkowanych nasion. Wśród przebadanych zestawów nasion, odmiana 'Hyola 330' wykazała najniższy udział skiełkowanych nasion (62%) i jednocześnie najdłuższy średni czas kiełkowania nasion – 3.64 dnia. Stwierdzono również istotną korelację pomiędzy średnim czasem kiełkowania a liczbą prawidłowo rozwijających się siewek. W przypadku odmiany 'Hyola 330' stwierdzono najniższy procent poprawnie rozwijających się siewek (60%). Test na wigor nasion (z użyciem tetrazolu) rzepaku 'Hyola 330' wykazał duży udział nasion martwych, niezdolnych do kiełkowania oraz nasion żywotnych, ale dających nieprawidłowo rozwijające się siewki. Jedną z głównych przyczyn niskiego udziału skiełkowanych nasion i długiego czasu kiełkowania nasion odmiany 'Hyola 330' była niska żywotność nasion. Wyniki badań wskazują, że średni czas kiełkowania jest dobrym wskaźnikiem wigoru nasion rzepaku.