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## Standardization of vigour test to predict field emergence and difference of seed vigour among lentils (*Lens culinaris* L.) seed lots

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**Abstract:** Selecting seeds with high vigour eminently affects initial plant growth and determines plant stand. Various tests including electrical conductivity (EC), radicle emergence counts (RE), and standard germination (SG) tests were used to assess the vigour of eleven lentils (*Lens culinaris* L.) seed lots. Lentil seed lots with over 75% normal germination, were germinated with the use of an ISTA standard germination test. The radicle emergence (2 mm) was tallied at constant intervals from 24–120 h. The seed lots were sown in the fields and 40 days after sowing, the final seedling emergence was evaluated. The standard germination test demonstrated that there was no substantial correlation between standard germination and field emergence. However, after 24-h of germination, there was a highly significant correlation between seed emergence and radicle emergence count ( $r = 0.89$ ;  $p < 0.001$ ). Hence, at 24 h, radicle emergence counts attributed to variations in field emergence by 80%. Results revealed that at 24 h, radicle emergence count during germination could be used as a vigour test to evaluate the full potential of field emergence of lentil seed lots.

**Key words:** Field emergence test, lentil seed, mean germination time, radicle emergence

### 1. Introduction

Food plants including horticulture and field crops were historically the first to be harvested and cultivated. They are grown for human consumption for centuries in various parts of the world and show great diversity. Food plants, particularly field crops, are strategically important and food plant cultivation has many benefits for both farmers and consumers (Ercisli et al., 2005; Nadeem et al., 2018; Fawad et al., 2021; Azam, MD Gulam et al., 2022; Novikova et al., 2022; Taskesenlioglu et al., 2022).

Healthy and strong plants are produced from high vigour seeds that ensure uniform crop establishment in fields. The inability of a seed to germinate is called seed aging and it causes variances in seed vigour (Powell, 2006; Sun et al., 2007; Mokhtari and Emeklier, 2018). The main cause of variations in vigour that result in poor emergence of seedlings both in the field and laboratories is seed deterioration that culminates from factors affecting production and seed storage. Seed vigour is defined by ISTA as the sum of those properties of the seed which determine the level of activity and performance of the seed of a seed lot during germination and seedling emergence. It is a significant aspect of plant growth and development that requires evaluation to acquire knowledge on seed lot performance during storage or in

the field by tests of viability (Finch-Savage et al., 2010; Mokhtari and Kizilsimsek, 2019; ISTA, 2020). Seed vigour is the measure of damage built up as vitality reduces. Seeds with high vigour can increase the emergence ability under unfavorable environmental conditions, like drought, high temperature, and irregular rainfalls, and alleviate the negative effects of these factors as well as increase crop yield. Low-vigour seeds are those seed lots with high germination but poor emergence. Under optimum conditions, low-vigour seeds may germinate well even with their higher levels of deterioration caused by aging, however, they have a low storage potential and poor performance in the field. Seed lots that are similar in germination may be dissimilar in the level of deterioration and significantly differ in field emergence. Therefore, a vigour test is deliberately a powerful process that is used during the classification of seed lots into the number of levels or groups (Kolasinska et al., 2000). Acquiring accurate knowledge regarding seed lots is the first step, for most technical and commercial decisions regarding seed lots and their performances. Vigor tests can identify seed lot aging and variances in deterioration levels. Hence, testing seed vigour has increasingly become vital in the classification of seed lots in accordance with physiological potential (Fatonah et al., 2017). The sensitivity of different vigour testing methods

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has shown high dependency on crop plants and species. Predicting vigor requires reliability and effectiveness and any test carried out must be reproducible, recapitulative, and meet the conditions of vigor tests for plant species mentioned in the ISTA Rules (ISTA, 2020). However, each test must be proved to be fit for the desired purpose, which essentially means that tests conducted must show evident distinctions between seed lots during field emergence with reference to storage potential, seedling uniformity and size, final emergence, and rate of emergence (Powell, 2022). Several studies showed the development of vigor tests in seeds, and aging or deterioration levels in seeds i.e. standard germination tests, electrical conductivity (EC), controlled deterioration test (CD) and accelerated aging (AA) just alike methods of vigor testing (Powell and Matthews, 2005; Mokhtari and Kizilsimsek, 2019). However, these tests are laborious, require a long time, and involve a number of steps. Standard germination test which is less sensitive to assessing physiological seed quality, only provides predictive information in seedling emergence under ideal conditions which cannot represent field conditions for different plant species (Soares et al., 2018, Alahakoon et al., 2021). For example, in *Festuca sinensis* L., standard germination test could not assess the seeds for their physiological quality and showed a higher germination percentage ( $\geq 86\%$ ) than the field emergence (Munyaneza et al., 2022). An electrical conductivity test is based on the principle that the deterioration process involves the leaching of cells of seeds immersed in water because of the destruction of the integrity of their cellular systems. During the development of seeds, alteration of cell membranes occurs preceding physiological maturity, and seed desiccation prior to harvest. During the rehydration of seeds in early imbibition, the capability of the seed's cellular membranes to repair damages or reorganize affects the degree of electrolyte leakage from the seeds and determines their vigor. The existence of dead seeds in a sample test may influence the results of conductivity resulting in error. Hence, it is required that damaged seeds should not be included when preparing a sample. The higher the speed of seeds to form membrane integrity, the lesser the electrolyte leakage. Seeds with higher vigor are capable of organizing their membranes faster and repairing damages effectively as compared to seeds with low vigor. Therefore, low conductivity is a result of high-quality seed and low electrolyte leakage whereas, high conductivity results in higher output seed leachate and low seed quality. Exudates leakage after sowing, showing destruction in cell membrane organization and selective permeability, under field conditions can boost the development of pathogenic microorganisms and undermines seedling emergence substantially at lower temperatures (Vieira and Krzyzanowski, 1999). The electrical conductivity

test has achieved satisfactory results in the separation of seed lots with different levels of vigor for some crops, such as chickpea (Khajeh-Hosseini and Rezazadeh, 2011) and common vetch (Mokhtari and Kizilsimsek, 2019). In contrast, EC test could not predict field emergence performance and difference in vigor for *Sorghum bicolor* (Fatonah et al., 2017), *Elymus sibiricus* L. (Wang et al., 2004) seed lots.

An ISTA vigor test, which is a 'single count' of radicle emergence (RE) is a new method that has been developed and established to assess seed vigor in radish, wheat, maize, oilseed canola (ISTA, 2020), *Festuca sinensis* (Munyaneza et al., 2022), marigold (Ilbi et al., 2020) and cucumber and watermelon (Mavi et al., 2010). It is considered as a quick method for predicting varying levels of seed vigor and field performance of seed lots as compared to the standard germination tests. RE single counts as a method of testing seed vigor provides a quick, cheap, and simple way of establishing seed lots. Farmers can easily understand the radicle emergence test which does not need highly skilled personnel or complex equipment. It can also be used to minimize the period of decision-making in the seed management industry. The RE tests are specific tests to quantify the moment of root protrusion which can provide valuable insights into the total germination capacity, vigor, and uniformity of the seed lots. In addition, they have the highest capability for their use in various species when involved with image analysis (Shinohara et al., 2021). This has undoubtedly shown the potential for the application of updated technology in the vigor test. The basis for radicle emergence testing is explained through repair hypotheses and differences in the rate of germination due to aging (Matthews and Khajeh Hosseini, 2006; Khajeh Hosseini et al., 2009; Matthews et al., 2012). The variances in RE test in the preliminary stages of testing germination have been ascribed by the time of delay (lag time) from the beginning of imbibition to the radicle emergence (Matthews and Khajeh-Hosseini, 2007). A huge delay in aged seeds before radicle emergence occurs which suggested that there should be additional time for repairing deterioration (Matthews et al., 2012). The fundamental physiological foundation of this test is that the seed lots of which normal germination is low, have a longer lag period in various crop seeds as they take a long time to achieve the radicle emergence stage (Matthews and Khajeh-Hosseini, 2006; Demir et al., 2008; Mavi et al., 2014; Ozden et al., 2018). These establishments indicate that it is worth looking into radicle emergence for the forecasting of normal seedlings in the lots that are commercially available. MGT can be used during the preliminary stages of germination to forecast the seed vigor of various plant species by a single radicle emergence count (Khajeh Hosseini et al., 2009; Mavi et al., 2010).

Keeping in view the importance, this research aimed to identify the suitable vigour test to be recommended as a vigour assessment method for estimating differences in field emergence, and finally to develop the identified procedure as a routine vigor test and protocol for lentil seed lots.

## 2. Materials and methods

### 2.1. Seed collection

Eleven seed lots of lentils were collected from the General Directorate of Agricultural Research and Policies, Turkey during 2020. The collected seeds were protected in zip bags and were stored at +5 °C until their use in experiments. Experiments were conducted in the seed testing laboratory and research field of Islahiye Vocational School, University of Gaziantep, Turkey, in 2021. Eleven seed lots of lentil having minimum 80% germination were used in this study. The seed lots were produced in different years from 2012 to 2016. The seed moisture content of the lots varied from 6.81%–7.57%.

### 2.2. Standard germination test

The standard germination test was conducted according to ISTA Rules (ISTA, 2020). Four replicates of 50 seeds were placed between moistened Whatman (No: 5, 90 mm diameter) filter papers in the petri dishes. Petri dishes were kept in an incubator at 20 °C in the seed germinator up to 10 days. Normal and abnormal seedlings were counted in the final count based on ISTA rules (ISTA, 2020). Seed germination percentage was calculated by following equation (1).

$$SG = NS/SS \times 100 \quad (1)$$

where, SG refers to standard germination percentage, NS refers to number of normal seedlings and SS refers to number of total seeds (Hu et al., 2015).

### 2.3. Radicle emergence test

During the germination test, radicle emergence (radicle emergence; 2 mm) was recorded every 24 h (at 24, 48, 72, 96, and 120 h) until the end of the experiment. The mean germination time (MGT) was calculated by Ellis and Roberts (1980).

$$MGT = (\sum (nd))/(\sum n) \quad (2)$$

where, “n” represents the number of newly germinated seeds having a 2 mm radicle; and “d” is the number of days, when the seeds were taken to germinate.

### 2.4. Conductivity test

During the conductivity test, all seed lots showed different germination percentage. Four replicates of 50 seeds per seed lot were weighted, transferred to 50 mL of deionized water. The flasks containing seeds, were covered with cling film to avoid contamination, and were kept at  $20 \pm 1$  °C for 24 h. Two Erlenmeyer flasks containing only deionized water were used as control. After 24 h, the leachate solution

was mixed by shaking the flask containing seeds for 10 s. The electrical conductivity readings of each sample ( $\mu\text{S cm}^{-1}$ ) were determined without filtration of the solution. The EC ( $\mu\text{S cm}^{-1}\text{g}^{-1}$ ) of each replicate was calculated by using equation (3) as described by (TeKrony, 1995).

$$EC = \frac{(\text{EC reading after 24 h} - \text{EC reading of deionised water only})}{\text{weight of seed sample (g)}} \quad (3)$$

### 2.5. Seedling emergence test

In the seedling emergence test,  $4 \times 50$  seeds for each lot were sown by hand in a randomized block design during April 2021. Since the seedling emergence percentage was calculated after 40 days, emergence was defined as the appearance of cotyledons above the soil surface which was counted on a daily basis.

### 2.6. Statistical analysis

Version 25 of the IBM SPSS statistical program was used to carry out regression analysis and Pearson correlation analysis.

## 3. Results

### 3.1. Standard germination test

According to Table 1, the germination capacity (Normal Seedling %) of the eleven seed lots of lentil ranged from 80% to 99.5%, i.e. normal seedling percentages in which nine lots out of eleven ranged above 90% and two lots ranged between 80% and 87%. All seed lots were above the standard commercially required level of 75% germination (Ilbi et al., 2020) (Figure 1). Standard germination test showed a low significant correlation with field emergence ( $r = 0.634$ ,  $p < 0.05$ ) (Table 2).

### 3.2. Conductivity test

The highest EC was obtained from seed lot 9 ( $34.59 \mu\text{S cm}^{-1}\text{g}^{-1}$ ) and the lowest EC was obtained from seed lot 7 with a value of  $21.47 \mu\text{S cm}^{-1}\text{g}^{-1}$  and seed lot 8 with a value of  $24.61 \mu\text{S cm}^{-1}\text{g}^{-1}$ , respectively (Table 1). The correlation observed between EC and field emergence was low ( $r = -0.617$ ,  $p < 0.05$ ) but significant (Table 2).

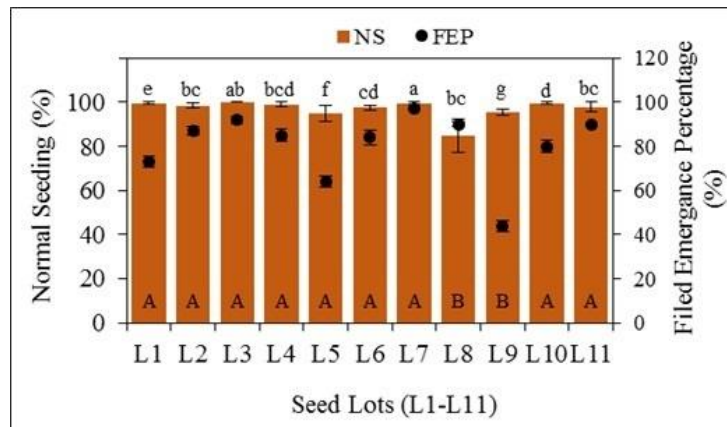
### 3.3. Radicle emergence test

Radicle emergence counts showed significant differences among the seed lots, including 4%–65% at RE24h, 17%–96% at RE48h, 53.5%–99%, at RE72h, 81.5%–99% at RE96h, and 91.5%–100% at RE120h. The field emergence of eleven seed lots varied from 44% to 97%. This result indicated that there were significant differences in seed vigour among the seed lots with similar normal seedling percentage. For example, lots L5 and L9 had high germination capacities of 95% and 80%, respectively, but had the lowest vigour with field emergence of 64% and 44%, respectively. Also, the field emergences of the seed lots were significantly highly correlated with MGT values

**Table 1.** Normal and abnormal seedlings, mean germination time (MGT), and radicle emergence (RE) assessed in the standard germination test and field emergence percentages of eleven seed lots of lentil.

Seed Lots	Variety	Year of production	Normal seedling (%)	Abnormal seedlings (%)	RE 24 h	EC ( $\mu\text{S cm}^{-1} \text{g}^{-1}$ )	MGT (d)	FE
L1	ANKARA YESILI	2013	99.5 a	0.5	32 f	30.49 ab	1.75 c	73 e
L2	ANKARA YESILI	2015	97.5 a	1	51 cd	29.00 b	1.31 d	87 bc
L3	CEREN	2016	97.5 a	0	61 ab	31.59 ab	1.2 d	92 ab
L4	CIFTCI	2013	99 a	0.5	42.75 e	31.19 ab	2.15 b	85 bcd
L5	YUSUFHAN	2013	95 a	4	23 g	32.38 ab	1.99 bc	64 f
L6	YUSUFHAN	2015	97.5 a	0	28 fg	31.93 ab	1.82 bc	84 cd
L7	AKM C:10	2012	99a	0.5	65.33 a	21.47 c	1.04 d	97 a
L8	AKM C:11	2012	87 b	13.5	55.33 bc	24.61 c	1.14 d	90 bc
L9	KAFKAS	2016	80 b	4	4 h	34.59 a	3.54 a	44 g
L10	FIRAT -87	2014	98.5 a	0	24 fg	33.10 ab	2.15 b	80 d
L11	CAGIL	2014	97 a	2	46.66 de	31.69 ab	1.74 c	90 bc
Range	-	-	80–99.5	-	4–65	21.47–34.59	1.14–3.54	44–97

Means with different letters in the same column are significantly different at a 5% confidence level.



**Figure 1.** Normal seeding (NS) and field emergence percentage (FEP) of eleven different seed lots of lentil seeds with  $\pm$  standard errors of four replications. Different capital letters inside the vertical bars indicate significances among seed lots for NS whereas, small letters above the vertical bars indicate significances among seed lots for FEP at 0.05 probability level.

( $r = -0.881$ ,  $p \leq 0.001$ ) (Figure 2). While the seed lot 7 (L7) with the highest field emergence level had the lowest MGT (1.04 day), the seed lot 9 (L9) with the lowest field emergence level had the highest MGT value (3.54 day) (Table 1).

The germination progress curves of the seed lots based on MGT indicated that differences between lots were particularly clear in the first 24 to 48 h of germination (Figure 3, Table 3) and the difference between germination

curves of the eleven seed lots were due to the duration of the appearance of the first radicle.

The correlation between the RE at 24 h and MGT was found to be the highest significance of any time ( $r = -0.894$ ,  $p < 0.001$ ) (Table 3 and Figure 4). According to Matthews and Khajeh-Hosseini (2006), seed lots with low levels of normal germination take a longer time (high lag period) to reach the RE stage. Results confirmed that as the seed vigour decreases seed germination is delayed.

**Table 2.** Correlation coefficient (r) values for the relationship between the field emergence (FE) of the eleven seed lots of *Lens culinaris* L. and their standard germination percentage (SG), mean germination time (MGT), and electrical conductivity test (EC). \*p < 0.05, \*\*\* p < 0.001.

Correlation coefficient	FE
SG	0.634*
MGT	-0.881***
EC	-0.617*

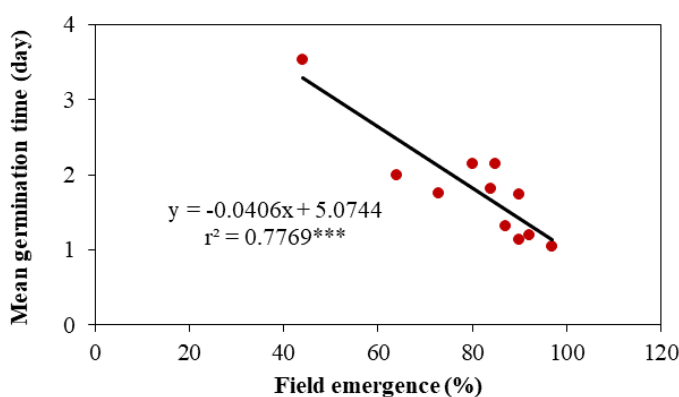
### 3.4. Radicle emergence for predicting seedling emergence of lentil lots

Counts of radicle emergence at 24 h were significantly related to field emergence data according to regression analysis. The coefficients of determination were 0.898 (p < 0.001) (Table 3) and were able to predict field emergence performance. R<sup>2</sup> value of 0.80 indicated that 80% of the variation in field emergence was accounted for by the regression on RE (Figure 5). Thus, the seed lots with low field emergence (low vigour), such as lots L5 and L9 could be identified by the low RE counts after 24 h of germination (Table 1) in comparison with the lots with high field emergence (high vigour: i.e. lots L3 and L7) which had high RE counts after 24 h.

## 4. Discussion

The ideal seed vigour tests as repeatable, fast, and uniformly applicable amongst the laboratories (Mc Donald, 1999). Differences in the level of deterioration or aging of seed lots can be determined by the ISTA-validated vigour tests based on the whole process of aging i.e. the CD test and AA test. However, these tests usually take a long time (Powell

and Matthews, 2005). Results showed that the standard germination test and electrical conductivity (EC) test could not be sufficient to predict differences in vigour as reflected in seedling emergence in the field. However, the radicle emergence test at 24 h could find a very high relationship (0.898, p < 0.001) with field emergence and can predict field emergence and vigour difference between seed lots. In the laboratory, the temperature is close to the optimum, so it decreases the influence of the other factors (light, soil condition and etc.) and could not represent field conditions (Soares et al., 2018). Therefore, standard germination test has been proven to be less sensitive to assess physiological seed quality and predict field emergence in different species (Alahakoon et al., 2021). In consistent with this, our study showed that seed germination failed to assess seeds for their physiological quality and showed a higher germination percentage (≥80%) than field emergence (≥44%). Agro-ecological conditions of the parent seed can cause differences in vigour. Environmental factors cause 80% productivity variation for seeds with different agro-ecological conditions. While 10% of agro-ecological conditions are caused by the genotype itself, and its interaction with the environment (Yan, 2001; Nowosad et al., 2016). Loss of integrity of cell membranes and leakage of electrolytes are the first symptoms of seed aging. For this reason, the EC test is an important and effective vigor test, which determines the deterioration of seeds with high and low vigor (Fatonah et al., 2017). For example, the EC tests separated seed physiological quality among chickpea (Khajeh-Hosseini and Rezazadeh, 2011) and common vetch (Mokhtari and Kizilsimsek, 2019) seed lots. However, in Siberian wild ryegrass and Sudan grass (Wang et al., 2004), *Festuca sinensis* (Munyaneza et al., 2022) showed poor results of the EC test to predict field emergence. Similarly, in our study, the EC test on *Lens culinaris* could not specify vigour difference among seed lots and showed a poor correlation with field emergence



**Figure 2.** The relationship between field emergence and mean germination time (MGT) in eleven lentil seed lots. \*\*\* p < 0.001.

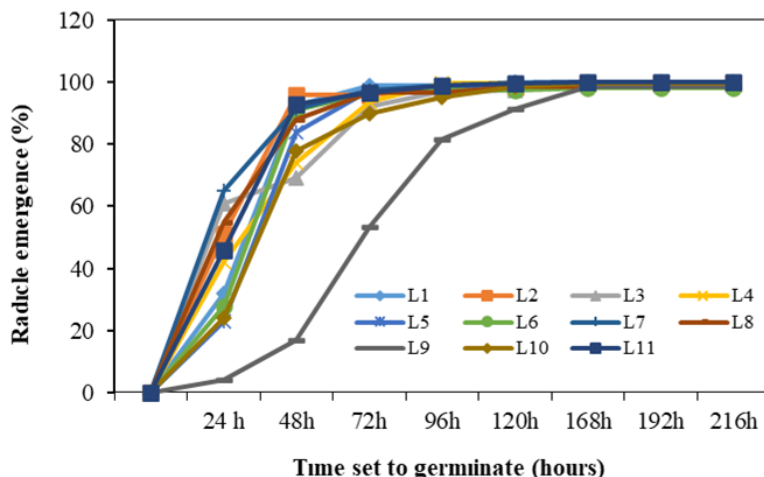


Figure 3. Germination (radicle emergence) progress curves of eleven seed lots of lentil. Each data point is the mean of four replicates of 100 seeds.

Table 3. Correlation coefficient (r) values for the relationship between the radicle emergences of eleven seed lots of lentil. Their mean germination time (MGT) and field emergence (FE) assessment after different periods during germination. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

Correlation coefficient	RE at 24 h	RE at 48 h	RE at 72 h	RE at 96 h	RE at 120 h
MGT	-0.894***	-0.812**	-0.834**	-0.778**	-0.730 *
FE	0.898***	0.750**	0.754**	0.745**	0.704*

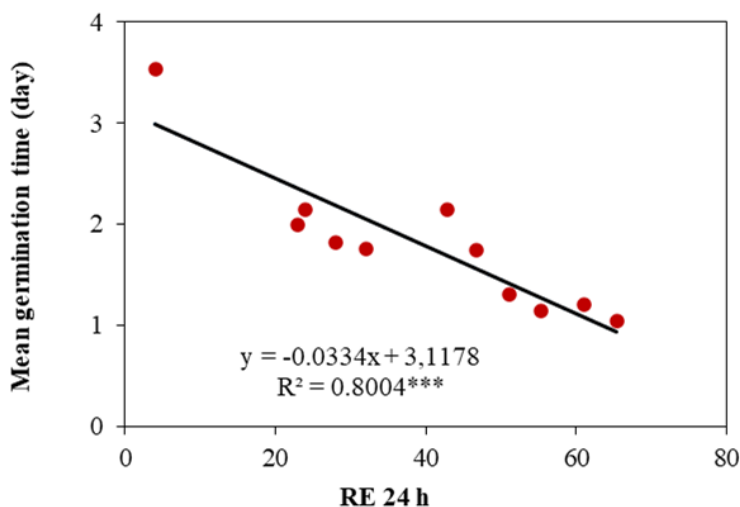
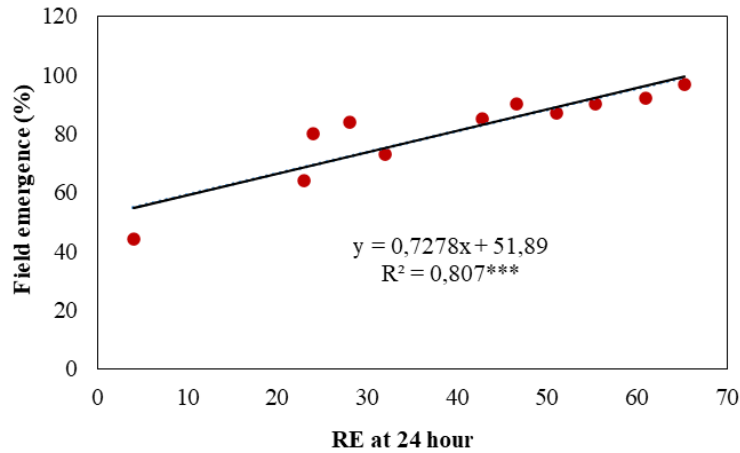


Figure 4. The relationship between RE counts at 24 h and mean germination time (MGT) of eleven different lentil seed lots. \*\*\* p < 0.001.

percentage (Table 2). These results suggested that the relationship between field emergence and conductivity test may be influenced by environmental conditions, genotype, seed size, integrity, and moisture contents. Piercing the seed coat to enhance the permeability of the

semipermeable membrane may allow EC measurement for quality ranking of seed lot physiology (Lv et al., 2017). Also, EC sensitivity in predicting field emergence may rely on species rather than family, for example in soybean, the brown seed coat highly affected EC results (Santos et al.,



**Figure 5.** The relationship between RE count at 24 h and field emergence (FE) of 11 different *Lens culinaris* L. seed lots. \*\*\*  $p < 0.001$ .

2007), due to higher protein and lignin contents, which resulted in lower absorption rate and difference in vigour among genotypes. The ideal time and temperature for the RE count test should be identified individually for each species (Guan et al., 2018). The ideal time for radicle emergence counts may vary according to the temperature used. For example, RE counts were shown as a good indicator to predict sweet corn seeds, especially at 84 h and 20 °C, while the best results at 13 °C were observed at 150 h count (Luo et al., 2015). Furthermore, based on the results, the reason why the RE test differs from other vigour tests is that when the germination percentage is similar, it helps in detecting seeds (due to low vigour) that differ in mean germination times (MGT). In our study, while the normal germination percentages in seed lots L8 and L9 are statistically similar (87% and 80%, respectively), the mean germination times are clearly different (1.14 versus 3.54 days, respectively). During aging, such as L9, there is a long lag period (high MGT) and accumulation of damages, including damage to membranes (Powell and Matthews, 1977) organelles (Cheah and Osborne, 1978), and DNA (Berjak and Villiers, 1977). Therefore, the time when seeds were set to germinate until the first radicle emergence was extended (Matthews and Khajeh Hosseini, 2007). According to the highly significant correlation between RE and MGT ( $r = -0.898$ ,  $p < 0.001$ ) a higher MGT reveals a longer delay or lag period before RE which has been interpreted as being due to the need for more time for physical, biochemical, cytological, and physiological changes, and metabolic repair to deteriorated seeds before the germination processes can begin. As the seed lot has a

low RE, the mean germination time of this lot is high, and it shows low field emergence. Our results are in agreement with those obtained for *Festuca sinensis* (Munyaneza et al., 2022), marigold (Ilbi et al., 2020), and corn (Matthews and Khajeh Hosseini, 2007). Generally, the seed lots that have low RE, have low field emergence because of low vigour and performance. The high correlation between RE and FE tests ( $r = 0.898$ ,  $p < 0.001$ ) proved that the RE test helps to predict field emergence and performance of lentils. The high RE value indicated high field emergence and low MGT. With the work reported, RE is validated as a vigorous test to predict field emergence in lentils along with canola and maize (ISTA, 2017), forage species (Lv et al., 2016), radish (Powell and Mavi, 2016), bean (Ozden et al., 2019), and cress (Demir et al., 2019).

## 5. Conclusion

This study was performed to predict the differences between seed lots of lentil plant whose seeds have been stored for different time periods before transferring them to field. Since a strong correlation could not be established between SG test and EC test results and field emergence results, it is thought that these tests could not detect vigour differences between lentil seeds. However, it should be considered that the soil temperature and moisture contents in the soil affected these results during the field emergency test and should be considered as a factor in future studies. According to the results obtained, the lightness difference in the seed lots appeared with MGT value (lag period) and it was determined that the RE result at 24th h and 20 °C may predict the vigour of the seed lots and field emergence.



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**Table S1.** List of abbreviations.

<b>Abbreviation</b>	<b>Definition</b>
AA	Accelerated aging
CD	Controlled deterioration test
EC	Electrical conductivity test
FE	Field emergency
ISTA	International seed testing association
L	Lot
MGT	Mean germination time
NS	Normal seedling
RE	Radicle emergence counts
SG	Standard germination