

**RADICLE EMERGENCE TEST STANDARDIZATION TO
FORECAST FIELD EMERGENCE AND SEED VIGOUR IN
HORDEUM VULGARE L.**

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Abstract

A study was conducted to discover the use of RE single counts in testing vigour and predicting seedling establishment in seed lots of barley (*Hordeum vulgare* L.). We compared the RE counts at 20 °C at intervals of 16, 24 and 40 h and also at 15 °C for 24 h, with electrical conductivity (EC), germination percentage (GP), field emergence (FE), and mean germination time (MGT), using 8 seed lots of barley. Radicle emergence counts at 15 °C for 24 h and at 20 °C for 16, 24 and 40 h showed a high correlation with two parameters of seed vigour (MGT and FE), and their correlation coefficients were greater than 0.70 ($p < 0.05$). Usable correlation coefficients were realized after 24 h both at 15 °C and 20 °C. The results of the test showed that RE single counts are useful in testing vigour in barley seeds. The results also proved that single counts of radical emergence (RE), were useful in indicating the potential of emergence in barley seedlings.

Key words: barley, radicle emergence, single counts, seed germination, seed vigour

Introduction. The germination capacity of seeds is determined using standard germination test under optimal conditions based on ISTA (International Seed Testing Association) rules. However, it may differ based on germination in field conditions because the field conditions are not always favourable and the standard germination (SG) test is a marker of physiological seed quality that can estimate

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seedling field emergence only when environmental conditions are almost ideal. The difference in the emergence of seed lots with high germination in trials under different external conditions is usually associated with seed vigour [1]. Therefore, if there is a problem that cannot be detected with the standard germination test, the vigour test determines it [2]. Among the vigour tests, electrical conductivity test is frequently used to detect vigour loss in seeds. EC and standard germination test were precise and reasonable to evaluate vigour differences in many species like *Vicia sativa* L. [1], and sorghum [3]. In contrast, EC and field emergence tests can't estimate the vigour differences of *Sorghum bicolor* and *Elymus sibiricus* L. [4]. During early stages of germination, RE test can be calculated by single radical emergence counts while MGT by regular counts of the germinated seed. The physiological basis of this method is that seed lots which have a higher mean germination time for example as a result of ageing, tend to take longer to achieve RE owing to the need for repair due to their deterioration. EBRAHIM POUR MOKHTARI [2] revealed a high correlation ($r = 0.89$; $p < 0.001$) of radicle emergence (RE) counts of eleven lentil (*Lens culinaris* L.) seed lots with field emergence. RE test has been validated for maize (*Zea mays* L.), canola (*Brassica napus*), wheat (*Triticum aestivum* L.), radish (*Raphanus sativus* L.) [5].

The research purposes were firstly, to see if there is a relationship between radicle emergence (RE) with field emergence (FE), and secondly, to determine if there are relationships between RE with FE and mean germination time (MGT); and finally, whether the single count of RE can be used as an alternative method to evaluate the vigour of barley seed lots.

Materials and methods. Seed source. The eight seed lots of *Hordeum vulgare* L. cv. Altikat were collected from the Agricultural Ministry, Turkey during 2022. The seed lots were produced in different years from 2011 to 2018. Experiments were carried out during 2022 in laboratory and research fields of the University of Gaziantep, Turkey. Seed material having minimum 88.66% germination at 15 °C and 76% at 20 °C and moisture content of the lots varied from 4.4% to 6.9% (Table 1).

Test for standard germination (SG). The SG test was carried out in accordance with the 'International Seed Testing Association Rules'. Four replication of 50 seeds were placed between moistened Whatman (No: 5, 90 mm diameter) germination papers in petri dishes, and then kept in incubator with two different temperatures (15 °C and 20 °C) in dark conditions up to 7 days [5]. Seed germination percentage was calculated by the following equation:

$$SG = NS/TS \times 100,$$

where SG refers to standard germination percentage, NS refers to number of normal seedlings, and TS refers to number of total seeds [5].

Radicle emergence (RE) test. RE test counts (number of seeds with a radicle ≥ 2 mm-long) were done at 16, 24, 40, 48, 64, 72, 88 and 96 h. The number

of seeds that showed radicle emergence [6] was converted into a percentage. The mean germination time (MGT) was computed using the equation below.

$$\text{MGT} = \left(\sum (n * t) \right) / \left(\sum n \right).$$

Here n is the number of the recently germinated seeds having 2 mm radicle, and t is the number of days the seeds were set to germinate [7].

Electrical conductivity (EC) test. Four replicates of 50 seeds from each seed lot were weighed and transferred to 50 ml deionized water. Flasks containing seeds, were shielded with cling film to avoid impurities, they were kept at 20 ± 1 °C for 24 h. After 24 h, the EC ($\mu\text{S cm}^{-1}\text{g}^{-1}$) of each replicate was calculated by using equation (3) as described by [5].

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

Seedling emergence test. In the field emergence test, 4×50 seeds from each lot were sown in randomized block design in April 2022. The percentage of seedling emergence was calculated after 40 days.

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

Results. Standard germination test. The normal seedling percentage of the eight barley seed lots varied between 76 to 89% that was above the standard commercially required level of 75% germination. Normal seedling percentages in which six lots out of eight ranged above 81% and two lots ranged between 76 and 78%. There was a statistically significant difference in SG and FE between seed lots. The field emergence (FE) of seed lots varied between 70 and 94%. There were large distinctions in field emergence of lots with normal seedling which were alike. For instance, lots H2 and H8 field emergence of 89% and 70%, respectively (Table 1). Standard germination test at 20 °C showed no significant correlation with field emergence ($r = 0.09$). The field emergences of the lots were significantly correlated with their MGT values ($r = -0.83$, $p \leq 0.01$). Seed lots with low field emergence (H4 and H6) had the highest MGT, while seed lots with high field emergence (H5, H7 and H8), had the lowest MGT (Table 1).

Electrical conductivity test. The electrical conductivity of seed lot ranged between $12.74 \mu\text{S cm}^{-1} \text{g}^{-1}$ and $27.46 \mu\text{S cm}^{-1} \text{g}^{-1}$. The highest EC was obtained for seed lot 4 ($27.46 \mu\text{S cm}^{-1} \text{g}^{-1}$) and the slowest for seed lot 8 ($12.74 \mu\text{S cm}^{-1} \text{g}^{-1}$). The EC showed a significant difference among seed lots, however, it showed poor correlation with field emergence ($r = -0.74$, $p < 0.05$). Higher EC indicated higher permeability, high respiration rate and metabolic activity (Table 1).

Radicle emergence counts at different time from the sowing period. Progress curves of radicle emergence at 15 °C and 20 °C showed differences among the eight barley seed lots (Fig. 1). Radicle emergence at 15 °C including 6–31%

T a b l e 1
Seed quality evaluation of eight barley seed lots at 15 °C and 20 °C

Seed lot	Production year	Seed mc (%)	FE (%)	EC (µS cm ⁻¹ g ⁻¹)	15 °C				20 °C				
					Normal seedlings (%)	MGT (day)	RE (%) after 24 h	RE (%) after 40 h	Normal seedlings (%)	MGT (day)	RE (%) after 16 h	RE (%) after 24 h	RE (%) after 40 h
H1	2011	6.7	78bc	27.22a	94ab	1.95ab	6d	93a	82 ab	1.67ab	4 bc	32bc	93ab
H2	2012	6.18	70c	27.07a	94ab	2.00a	8 cd	84b	84 ab	1.60b	2 cd	34abc	94ab
H3	2013	6.97	76 bc	24.70c	89b	1.95ab	8cd	84b	84 ab	1.63b	0 d	35abc	96a
H4	2014	6.71	72 c	27.46a	90ab	1.87bc	12bcd	92ab	81 ab	1.75ab	0d	22c	92ab
H5	2015	5.92	84 abc	27.04a	95a	1.84bc	17bc	90ab	76 c	1.63b	2 cd	34abc	95ab
H6	2016	6.37	73 c	25.75b	91ab	1.87bc	14bcd	86ab	78 b	1.81a	0 d	26c	90b
H7	2017	6.67	89 ab	24.32c	88b	1.80c	21b	89ab	84 ab	1.59b	6 b	42 ab	96a
H8	2018	4.43	94a	12.74d	94ab	1.68d	31a	94a	89 a	1.37c	11 a	46 a	96a
Range		4.4–6.9	70–94	12.74–27.22	88–95	1.68–2	6–31	84–94	76–89	1.37–1.81	0–11	22–46	90–96

Means with different letters in the similar column are greatly distinct at a 5% confidence level

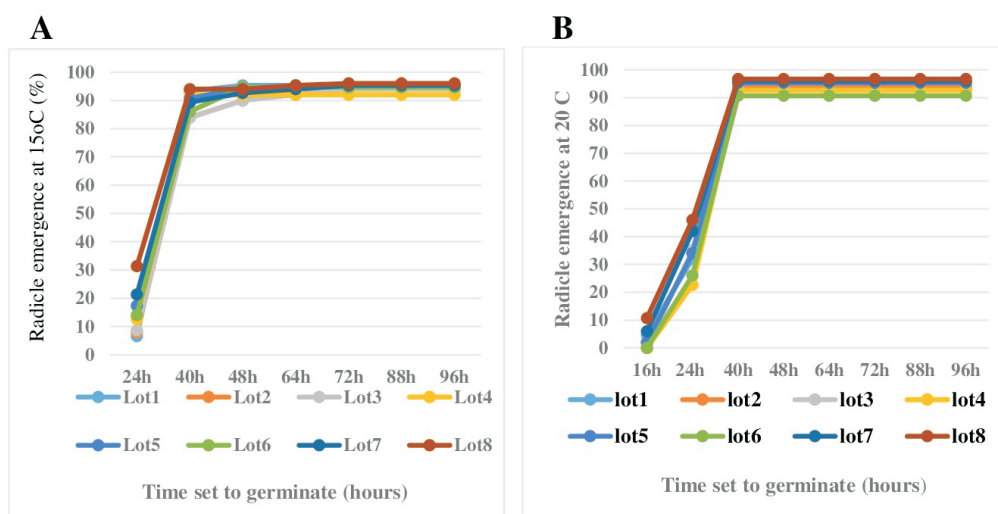


Fig. 1. Germination progress curves of eight seed lots of barley at 15 °C (A) and 20 °C (B)

at RE_{24 h}, 84–94% at RE_{40 h}, 90–95%, at RE_{48 h}, 92–95% at RE_{64 h}, and 92–96% at RE_{72 h}. The difference between germination curves of the eight seed lots were due to the duration of the appearance of the first radicle. The RE occurred 24 h after sowing, but all seed lots had high RE percentages at 72 h after sowing. The observations from RE counts at 24 h showed high germination percentage for seed lots 7 (31%) and 8 (21%), and slowest for seed lot 1 (6%), lot 2 (8%) and lot 3 (8%), which were consistent with the MGT seed lots with small MGT emerged quickly with great final emergence (Table 1, Fig. 2A, B). The field emergences of the seed lots were significantly high correlated with MGT values ($r = -0.83$, $p \leq 0.001$) (Fig. 2D). At 20 °C radicle emergence including 2–10% at RE_{16 h}, 22–46% at RE_{24 h} and 90–96% at RE_{48 h}. The RE occurred 16 h after sowing, but all seed lots had high RE percentages at 40 h after sowing. The RE percentage reached the highest levels in lot 3, lot 7 and lot 8 (96%, 96% and 96%, respectively), but was low in lot 4 and 6 (92% and 90%, respectively). Lots 7 and lot 8 with a low MGT emerged quickly with a great final emergence, while lots 4 and 6 with a larger MGT emerged more gradually to a cutailed final emergence (Table 1). The field emergence of eight seed lots varied from 70% to 94%. This result indicated that there were significant differences for seed vigour among the seed lots with similar normal seedling percentage. The field emergences of the lots were significantly correlated with their MGT values ($r = -0.83$, $p \leq 0.01$) (Fig. 2D). At 15 °C, lot 2 and lot 4 having high germination capacity of 94% and 90%, respectively, but had the lowest vigour with field emergence of 70% and 72%, respectively. The field emergences of the seed lots were significantly high correlated with MGT values ($r = -0.84$, $p \leq 0.001$) (Table 1, Fig. 2C). Lots 7 and

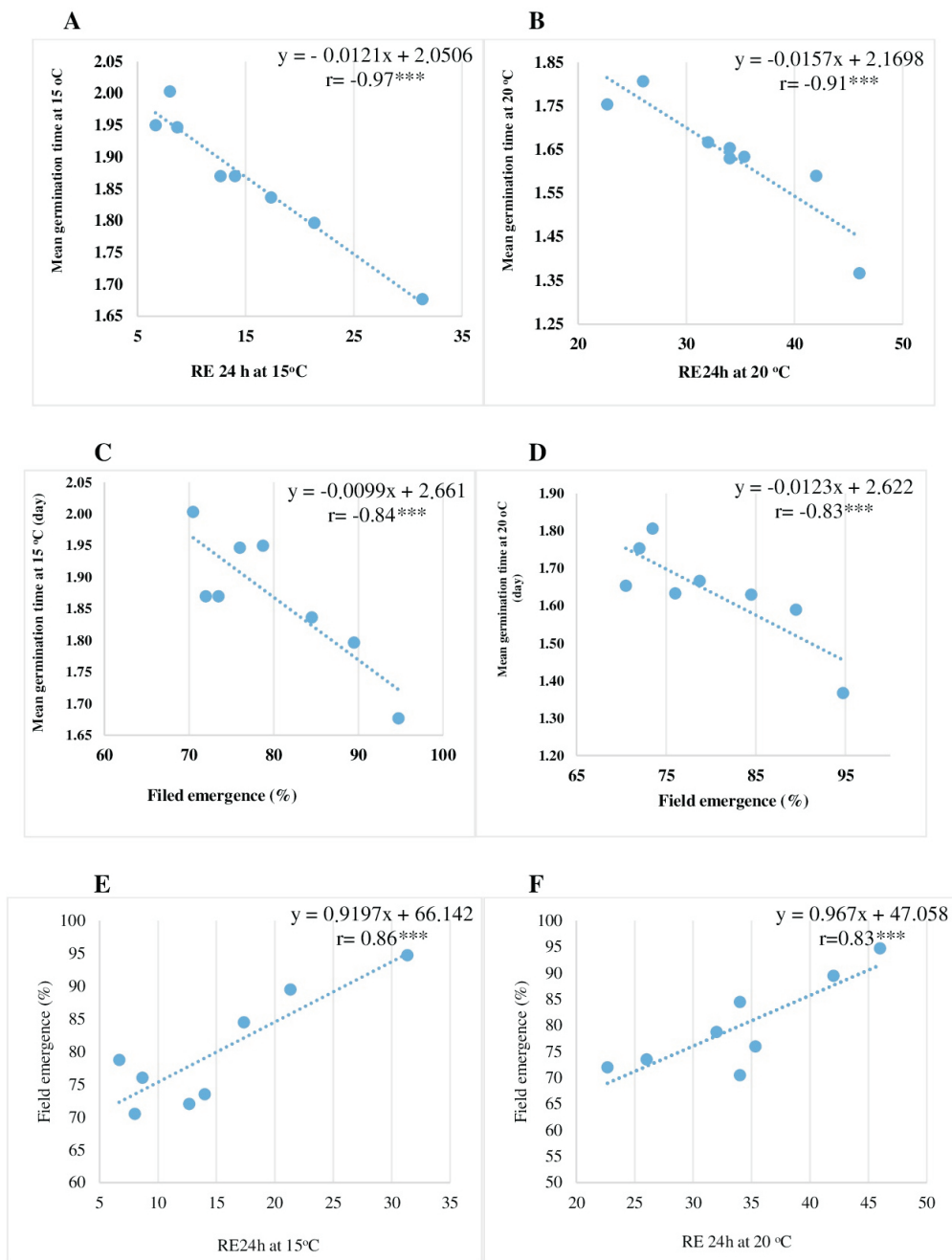


Fig. 2. The relationship between RE after 24 h with MGT (A, B), field emergence and mean germination time MGT (C, D) and RE after 24 h with field emergence (E, F) in eight barley seed lots, at 15 °C and 20 °C, *** $p < 0.001$

8 germinated fastest and lots 1, 2 and 3 the slowest, which were consistent with the MGT (Table 1). Lots 7 and 8, with low MGT emerged quickly with a great final emergence. The most significant values were for 24 h at 20 °C and at 15 °C. We selected 24 h (20 °C RE and 15 °C RE) as the optimum single counts during germination. Radicle emergence single counts at 20 °C for 24 h and counts at 15 °C for 24 h were significantly correlated with MGT and FE ($p < 0.01$), and the correlation coefficients were all higher than 0.83 (Table 1). All the correlations between radicle emergence counts and other vigour parameters were significant ($p < 0.05$; Table 1).

Radicle emergence for predicting field emergence of barley seed lots. At 15 °C the highest and significant ($p < 0.001$) count closely related to field emergence was observed at 24 h from the imbibition period, with a great correlation ($r = 0.86$, $p < 0.01$) with field emergence and RE count in other count period (16, 40, 48, 64, and 72 h) showed no significant correlation with field emergence (Table 1, Fig. 1E). At 20 °C all RE count in (16, 24, 40, 48, 64, and 72 h) showed significant correlation with field emergence but the highest and more significant ($r = 0.83$, $p < 0.001$, Table 1, Fig. 1F) count closely related to field emergence was observed at 24 h from the imbibition period, with a high correlation with field emergence.

Discussion. In this study, we measured single counts both at 20 °C for 24 h and at 15 °C for 24 h based on germination progress curves with the results from standard germination test, including MGT, electrical conductivity test, and Field emergence test. This study showed that standard germination test and EC test did not predict differences in vigour as reflected in seedling emergence in the field in each of two temperatures (15 °C and 20 °C). However, the RE test at 15 °C for 24 h and at 20 °C for 24 h could find very high relationship (0.86, 0.83, $p < 0.001$, respectively), with field emergence and can predict field emergence and vigour difference between barley seed lots. MUNYANEZA et al. [8] reported that SG test failed to estimate seeds for their physiological quality and vigour difference in *Festuca sinensis*. *Vicia sativa* L. [1], *Lens culinaris* [2], and alfalfa [9] showed poor results of the SG test to predict field emergence. Consistent with this, our study also showed that standard germination test failed to assess seeds for their physiological quality and showed a higher germination percentage ($\geq 76\%$) than seedling emergence in the field (Table 1). Principles of conductivity test are based on crippling the cell membranes of seeds with poor vigour result to discharge of water soluble compounds such as electrolytes, amino acids, and sugars when immersed in water. Sensitivity of EC to predict field emergence may depend on the species rather than the family. The EC tests separated seed physiological quality and identifying genotype induced vigour among common vetch [1], sorghum [3] and *Avena sativa* [10]. In contrast, the EC test on *Elymus nutans* Griseb. [10], *Festuca sinensis* [8], and *Lens culinaris* [2] could not rank seed lots and showed a poor results correlation with FE and could not predict field emergence. Similarly

in our study, the EC test on *Hordeum vulgare* L. could not rank vigour of seed lots and showed a poor correlation with field emergence (Table 1). Among the eight barley seed lots, the vigour levels measured by RE counts at both (15 °C and 20 °C) conditions showed that lot 8 had the highest vigour while lot 4 had the lowest vigour, consistent with the results of GP, MGT, EC, and FE (Table 1). This confirmed that the RE test possibly could be used to assess the vigour of barley seeds. The ideal time and temperature for a radicle emergence count test should be determined separately for each species. For example, RE counts were shown as a good indicator to predict sweet corn seeds, especially at 84 h at 20 °C, while the best results at 13 °C were observed at 150 h count [6]. Furthermore, based on the results, the reason why the RE test differs from other vigour tests is that when the germination percentage is similar, but 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 L4 and L8 seed lots are statistically similar (81% and 89%, respectively), the mean germination times are clearly different (1.75 days, 1.37 days, respectively). During ageing, seed lots such as L4, there is a long lag period (high MGT) to metabolic repair, DNA repair and enzymatic synthesis for repair in aged seeds before radicle emergence. Therefore, the period in which the seeds were placed to germinate up to the first radicle emergence was extended. Our results are in agreement with those obtained for *Lens culinaris* [2] and *Festuca sinensis* [8]. The high correlation between RE 24 h at 15 °C and 20 °C, respectively, with FE tests ($r = 0.86$, $r = 0.83$, $p < 0.001$) proved that RE test helps to predict field emergence and performance of barley. With the work reported, RE is validated as a vigorous test to predict field emergence in barley along with canola, maize [5] and forage species [10].

Conclusion. Vigour tests by ISTA can determine the distinctions in ageing of seed lots or deterioration levels. When weather and seed bed conditions are almost ideal, seed vigour will become less vital factor because field emergence will correspond to germination test. However, it is very rare to achieve optimum conditions in the field due to environmental factors which lead to different field performance that relies on the vigour of seed lots. Test hybrids may show varied performance due to the particular factors of seed vigour; mother plant, genetic structure, conditions of seed filling, and mechanical damage. RE test is a simple, less costly and quick test to predict vigour differences level and field performance in several species.

The extremely important values were for RE after 24 h at 20 °C and at 15 °C. We selected 24 h at both temperatures (20 °C RE and 15 °C RE) as the appropriate timing for RE test. RE single counts at 20 °C for 24 h and counts at 15 °C for 24 h were significantly correlated with MGT and FE ($p < 0.01$), and the correlation coefficients were all above 0.83 (Table 1). All the correlations between radicle emergence counts and other vigour parameters were significant ($p < 0.05$; Table 1).

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