Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi 25 (3):434-442, 2020
(*Mustafa Kemal University Journal of Agricultural Sciences* 25 (3):434-442, 2020)
e-ISSN: 2667-7733
http://dergipark.org.tr/mkutbd

ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Radicle emergence test predicts normal germination percentages of onion seed lots with different cultivars and genotypes

İbrahim DEMIR[1], EREN ÖZDEN[2], Zeynep GÖKDAŞ[1], Ebrima S. NJIE[1], Mine AYDIN[3]

[1]*Ankara University, Agriculture Faculty, Horticulture Department 06110 Ankara, TURKEY.*
[2]*Igdır University, Agriculture Faculty, Horticulture Department, Igdır, TURKEY.*
[3]*Agriculture and Forestry Ministry, General Directorate of Agricultural Research, Yenimahalle, Ankara*

MAKALE BİLGİSİ / ARTICLE INFO

Makale tarihçesi / Article history:
DOI: 10.37908/mkutbd.697450
Geliş tarihi /Received:25.04.2020
Kabul tarihi/Accepted:18.08.2020

Keywords:
Germination test, commercial onion seed lots, seed ageing, predicting germination.

Corresponding author: İbrahim DEMİR
:mail to:demir@agri.ankara.edu.tr

Ö Z E T / A B S T R A C T

Aims: To test radicle emergence count in order to predict normal germination percentages of onion lots.
Methods and Results: This work was conducted to test the potential for 2 mm radicle emergence (RE) to predict normal seed germination percentages of 20 onion seed lots. In the first stages of the study RE counts at 72, 76 and 80 h were highly predictive (p<0.001, R2=0.94-0.95) of normal germination after 12 days. In the second stage, the regression formula (y=45.9+0.52x) developed for the 80th hour radicle emergence to predict normal germination of 20 seed lots at various aging levels collected from the market. RE counts (%) of the 20 lots at 80 h were used to predicted values by using the formula. Actual normal percentages of 20 lots were determined after 12 days of germination testing. Then regression analysis was performed on both values. Results showed that predicted and actual normal germination percentages were highly related to actual normal germination percentages (R^2=0.95, p<0.001). Means of the predicted (79.6%) and actual (80.4%) values were very similar. Results indicated that RE testing has a potential to estimate normal germination percentages of onion seed lots.
Conclusions: RE count at 80h was highly related to normal germination percentages after 12 days (p<0.001, R^2=0.95) in commercial onion seed lots.
Significance and Impact of the Study: RE testing can be used in the prediction of normal germination percentages of any commercial onion seed lots within a short period, rather than waiting until the final count (12 days) in onion germination tests.

Atıf / Citation: Demir İ, Özden E, Gökdaş Z, Njie SE, Aydın M (2020) Radicle Emergence Test Predicts Normal Germination Percentages of Onion Seed Lots with Different Cultivars and Genotypes. *MKU. Tar. Bil. Derg.* 25(3) : 434-442. DOI: 10.37908/mkutbd.697450

INTRODUCTION

The standard germination test of crop seeds for seed germination testing is performed from a count of normally developed seedlings after a certain time for any specific species (ISTA, 2016). The period in germination testing can vary, depending on the species. Vegetable seeds species like parsley may need as many as 28 days, but lettuce seeds may germinate within seven days. Quicker estimation methods for normal seedling percentages would be valuable for saving not only time but also effort, in particular when the number of seed lots to be tested is large. Moreover, faster methods may also give valuable information for ranking the seed lots regarding seedling emergence potential in the field or modules, since seed lots with higher normal germination percentages in standard laboratory tests are likely to emerge better in the field, especially under stressful conditions.

Normal germination percentages in laboratory conditions are described as those seeds that are likely to develop strong seedlings in field conditions. Differences in radicle emergence (RE) in the early stages of the germination test have been attributed to the period of the delay (lag time) from the start of imbibition to RE (Matthews and Khajeh-Hosseini, 2007). A greater delay before RE occurs in aged seed, which suggests that these require more time for the repair of deterioration. This is described as the basis of the ageing/repair hypothesis of humidification (Matthews et al., 2012). The radicle emergence (RE) test in early stages of germination test was recently set out in the ISTA Rules as a vigour test for maize (*Zea mays* L.), oilseed rape (*Brasica napus* L.) and radish (ISTA 2016; Powell and Mavi, 2016), and also was considered for other species (Lv et al., 2016). The RE test was highly related to seedling emergence potential in the field and to transplant modules in diverse seed species (Matthews and Khajeh-Hosseini, 2006; Matthews and Powell, 2011; Mavi et al., 2014; Demir et al., 2019)

More recently, there has also been some research interest in the use of the RE test for the estimation of normal seedling counts in a germination test. The basic physiological basis of the test is that seed lots with low levels of normal germination take longer (lag period) to reach the RE stage in different crop seeds (Matthews and Khajeh-Hosseini, 2006; Demir et al., 2008; Mavi et al., 2014; Ozden et al. 2018). Therefore, any seed lot having a lower radicle emerge indicates that it has less normal germination percentage. There are several species in which RE was successfully related to normal germination percentages. For example, RE was related to a wide range of percentages of normal seedlings in the standard test in oil seed rape (Khajeh-Hosseini et al., 2010), radishes (Mavi et al., 2016), and aubergines (Ozden et al., 2018). These conclusions indicated that RE would be worth investigating for the prediction of normal seedlings in commercially available lots RE can be influenced by seed ageing and pre-testing seed quality. Onion seeds are produced in various ecological regions in Turkey, so the seeds are influenced by various environmental conditions. Moreover, post-harvest conditioning such as seed moisture at harvest, drying and cleaning may vary between companies and influence the overall quality of any single onion seed lot. It is known that onion seeds are sensitive to loss of quality during storage, and so germination percentages of left-over seeds need to be tested before use (Thirusendura and Saraswathy, 2017). Therefore, if the RE test is to be used universally for a large number of seed lots, it should be tested with seed lots /genotypes / cultivars produced by different companies, and in various environmental regions, in retail markets. We aimed in this work a) to test whether the early radicle emergence count predicts normal seedlings in a germination test of commercially available onion seed lots, and to develop a regression model (y=a+bx), and b) to test to what extent this developed regression model predicts normal germination percentages of onion cultivars collected from those which are commercially available in the retail market.

MATERIALS and METHODS

A total of 20 onion (*Allium cepa* L.) seed lots, were obtained from commercial seed companies in Turkey. 12 of them are standard varieties (Bereket, Burgaz 10, Calista, Elit, Hazar, Karbeyazı, Kral, Metan 88, Naz, Oscar, Seyhan, Şampiyon) 8 of them are purified genotypes (101-36, 101-59, 101-71, 101-92, 101-99, 102-01, 102-04, 102-06). All of them had been treated with fungicide (thiram). Details of the lots are given in Table 1. The seed lots were hermetically sealed in aluminium foil packets and stored at 5°C until the experiment started.

In the first stage of the germination testing experiment, three replicates containing 50 seeds per lot were placed in Petri dishes on two germination papers (Whatman, 90 mm-diameter), and moistened with 4 ml distilled water. The Petri dishes were placed in plastic bags to prevent water loss during the test and held at 20°C in the dark. RE (radicle emergence, 2 mm) was calculated in frequent counts of radicle emergence after 72, 76 and 80 h. Then, twelve days after the commencement of the test, the seedlings were classified as normal (well-developed shoot and root structure) (ISTA, 2016). A linear regression analysis using SPSS was used to compare radicle emergence at each count time of 72, 76 and 80 h and final normal germination percentages (12 days after).

In the second part of the experiment, another 20 seed lots which were completely different than the seed lots in the first stage (Table 1) were bought from the seed market. These cultivars were from different companies and were produced in various regions of the country and in different years. A germination test was set up as described above. Then, radicle emergence percentages were counted at 80 h, and normal germination percentages after 12 days were determined in these lots as described. Subsequently, predicted values were calculated by using the regression formula that was developed in the first stage at 80 h (x axes), and actually normal germination (y axes) values were regressed by using SPSS to test whether the developed formula could predict normal germination percentages. Significance of regression was based on % levels. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) by using analyses of variance. Mean separation was made at the 5% level by the Duncan multiple range test.

RESULTS and DISCUSSION

The 20 seed lots produced a range of percentages of normal seedlings (%) in the germination tests, varying between 70 and 93 percent at 20ºC (Table 1). Total (radicle emergence) percentages were between 82 and 98%. In that sense, all seed lots were above the required minimum germination percentages for sale. The relationship between RE (radicle emergence) counts at 72, 76 and 80 h during germination and the final normal seedling percentages of the lots was examined.

Table 1. Variation in total and normal germination percentages of onion seed lots used in the study. Seed lots were obtained from different sources. Germination tests were conducted for 12 days at 20°C on top of the paper.

Cultivar /lot	Total (%)	Normal (%)
101-36	98	93
101-59	97	92
101-71	97	87
101-92	97	87
101-99	97	86
102-01	95	86
102-04	93	85
102-06	93	85
Bereket	92	84
Burgaz 10	92	82
Calista	91	81
Elit	91	81
Hazar	90	78
Karbeyazı	87	77
Kral	87	76
Metan 88	86	75
Naz	85	75
Oscar	85	75
Seyhan	84	73
Şampiyon	82	70
Mean	90.9	81.4

RE values varied between 29 and 83% after 72 hours, between 41 and 86% after 76 hours, and between 51 and 89% after 80 hours. Some cultivar and genotypes have higher and statistically different values than others at the same counting hour. This difference was found in prediction of normal germination, in regression analysis in some genotypes. High quality varieties yielded higher normal percentage and this is predictable (Table 2).

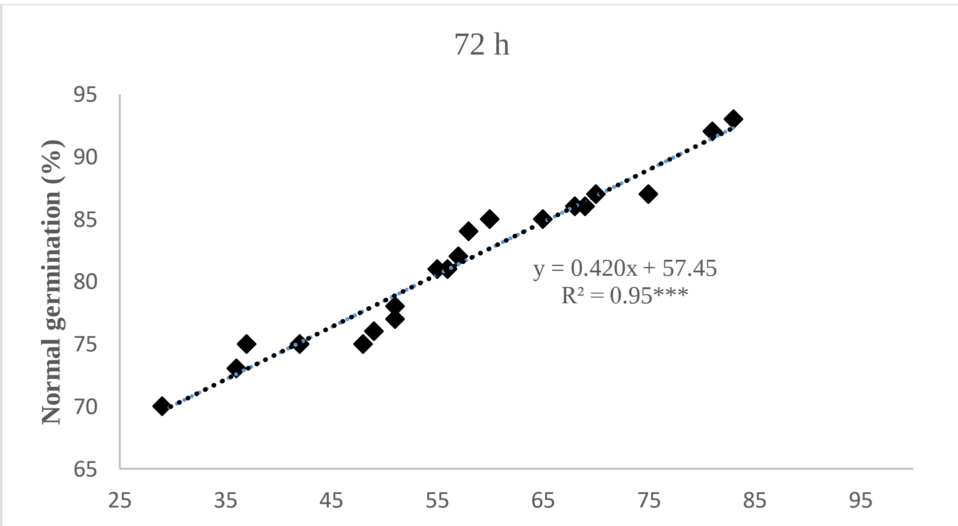
Table 2. Radicle emergence counts (%) at 72, 76 and 80 h of onion seed lots, collected from various sources as shown in Table 1 .RE test was conducted on top of the paper at 20 °C in the dark.

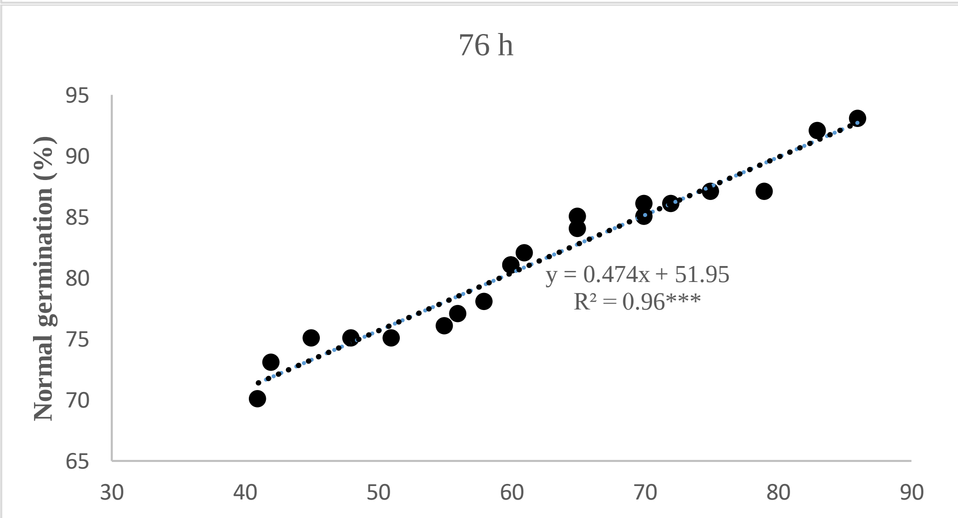
	RE (%) (hour)		
Cultivar/lot	72 h	76 h	80 h
101-36	83 a	86 a	89 a
101-59	81 a	83 a	85 b
101-71	75 b	79 b	84 b
101-92	70 c	75 c	80 c
101-99	69 cd	72 cd	77 cd
102-01	68 cd	70 d	76 cde

102-04	65 d	70 d	74 def
102-06	60 e	65 e	72 efg
Bereket	58 e	65 e	71 fgh
Burgaz 10	57 e	61 ef	68 ghı
Calista	57 e	60 fg	67 hı
Elit	56 e	60 fg	66 ıj
Hazar	51 f	58 fgh	64 ıj
Karbeyazı	51 f	56 gh	62 jk
Kral	49 f	55 h	59 kl
Metan 88	48 f	51 ı	57 lm
Naz	42 g	48 ıj	56 lm
Oscar	36 h	45 jk	51 mn
Seyhan	36 h	42 k	51 n
Şampiyon	29 ı	41 k	53 n
Mean	57	62	68

Means with different letters in the same column denote significant difference at 5% level

The mean values of the 20 lots in the counting hours were 57, 62 and 68% respectively. As the counting time extended from 72 to 80 h, mean RE percentages increased. Early counts of RE at between 72 and 80 hours were highly related to the percentage of normal seedlings (R^2>0.95, p<0.001), as seen in Figure 1.





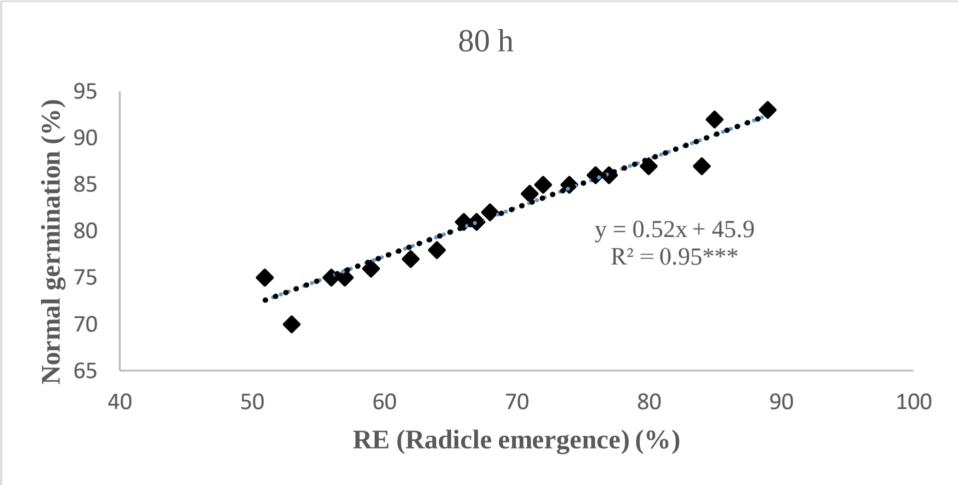


Figure 1. The relationship between two cm RE counts at 72, 76 and 80 h and normal germination percentages in 20 onion seed lots (***: p<0.001)

As well as all of them are very important, no change was observed in their significance level according to the RE + 0.52X. This formula represents 95% of variation (R^2=0.95, p<0.001) between RE at 80 h and normal germination percentages at the end of the test in 20 seed lots (Figure 1). In the second part of the work, we used counts. The regression formula that was developed in the first part of the research with RE at 80 h was y = 45.9 this formula, replacing X values with 80 h RE counts in completely different onion seed lots, and we present the results in Table 3.

Table 3. Normal seedling percentages predicted by the formula developed by 80 h RE in Figure 1 and the actual values of onion seed lots obtained from the retail market. Recounts and determination of actual values were performed at 20 °C in the dark on top of the papers.

Cultivar	Normal germination		Difference in germination (%)
	Predicted	Actual	
101-13	98	95	3
101-59	98	91	7
101-67	96	93	3
101-36	94	94	0
101-33	91	84	7
101-7	78	83	-5
101-1	74	73	1
101-8	90	90	0
101-54	90	95	5
101-20	89	92	3
101-14	89	89	0
Banka	86	87	1
Valencia 1	78	75	3
101-5	76	72	-4
Valencia 2	70	72	2
Storm	61	57	-4
101-54-4	60	61	1
Valencia 3	60	72	12
101-11	59	67	8
101-54-5	55	66	11
Mean	79.6	80.4	

In this table, predicted values calculated by the regression formula developed in the first stage are compared with actual values of normal germination percentages of lots after 12 days. As can be seen, differences between predicted and actual percentage values varied between 0 and 12%. In three cultivars (101-7, 101-5, Storm), the formula overestimated the actual values. Overall, the means of the predicted and actual values of the 20 lots were very similar: 79.6% and 80.4%

Table 4. Regression analysis was performed with the values predicted by the regression formula (second column in Table 2) and actual values (third column in Table 2) and shown in Figure 2.

Cultivar	Normal germination		Difference in germination (%)
	Predicted	Actual	
101-13	98	95	3
101-59	98	91	7
101-67	96	93	3
101-36	94	94	0
101-33	91	84	7
101-7	78	83	-5
101-1	74	73	1
101-8	90	90	0
101-54	90	95	5
101-20	89	92	3
101-14	89	89	0
Banka	86	87	1
Valencia 1	78	75	3
101-5	76	72	-4
Valencia 2	70	72	2
Storm	61	57	-4
101-54-4	60	61	1
Valencia 3	60	72	12
101-11	59	67	8
101-54-5	55	66	11
Mean	79.6	80.4	

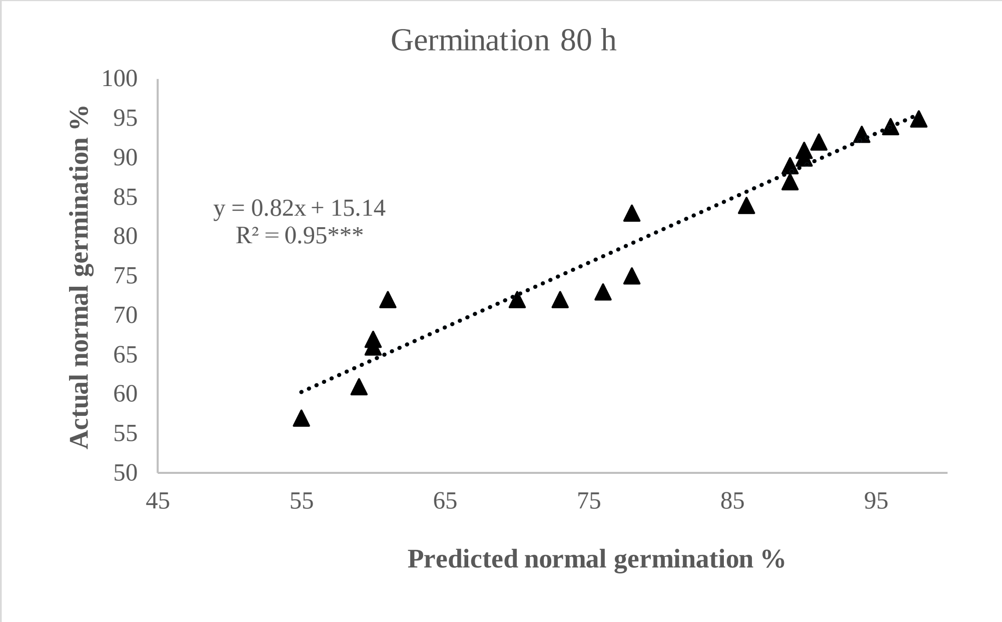


Figure 2. The relationship between predicted and actual normal germination percentages in onion lots.

The figure indicates that values predicted by 80 h RE counts were highly related (R^2=0.95, p>0.001) to actual normal percentages at the end of the germination test of onion seed lots. This indicates that by using the regression formula that was developed, normal germination percentages of onion seed lots can be predicted by 80 h of RE counting. This clearly shows that those seeds that have higher RE values after 80 h also have higher normal germination percentages.

Discussion

The results of this study revealed that a RE count at 80 h during a germination test can be used as a predictor of the percentage of normal seedlings in commercial onion seed lots. The successful use of RE count in commercially available seed lots collected from the retail market to estimate normal seedlings provided a practical approach for seed lots with a range of germination levels, which are available on the market. Seed lots in the market in most cases are not stored in appropriate storage conditions, so that high temperature and seed moisture may initiate seed ageing while seeds are in the market. During marketing, seed lots can be checked quickly as to whether their normal germination percentages fall below the minimum level of marketability. Our results confirmed that the RE test can be used for such purposes as ensuring seed quality during marketing. In that sense, the RE test is proved as the fastest germination testing method that can be used.

The potential for the use of a RE test for the assessment of seed vigour has been published in studies of many crop seeds. RE relates to field emergence in maize (Khajeh-Hosseini and Matthews, 2010), radishes (Powell and Mavi, 2016), and in the forage species (Lv et al., 2016). A single RE count was highly predictive not only of vigour but also of normal germination (%), indicating its potential in germination testing. A study was recently performed by our group on aubergine (Ozden et al., 2018) seeds. RE after 104 h gave very highly predictive results in aubergines. Similarly, RE was also found to be successful in radishes (Mavi et al., 2016) and oil seed rape (Matthews et al., 2012) in predicting normal germination. In both species, RE at 48 h gave very high prediction of normal seed germination. Naturally, the best single count time in RE testing as seen in these studies varies among the species. This may be based on the germination period, which varies between species. Moreover, slower radicle emergence was associated with seed ageing, and delay from the start of imbibition to RE draws attention to the longer delay in aged seeds (Matthews et al., 2012). According to ISTA (2016), the germination period varies from seven days in lettuce to 28 days in parsley. Clearly, the RE count will also be evaluated according to each species. In the present work, any RE count between 72 and 80 h gave the highest regression values (R^2= 0.94-95, p<0.001) with normal germination percentages. In the second part of the work, the formula developed at an 80h RE count also successfully predicted normal germination in 20 different seed cultivars collected from the market, which were not related to the first stage (Figure 3). These findings obviously indicate that the formula that was developed in this work can be used for any onion seed cultivars to estimate normal seedling percentage potential. The use of RE can be a quick and inexpensive test that can be used for fast evaluation of germination. The very similar means (80.4, 79.6%) of normal germination percentages between those predicted by the formula and actual values (Table 2) in 20 commercial seed lots show the success of the formula that was developed by the use of RE. It appeared to be that the prediction was more successful in higher germination value seed lots, since the difference between predicted and actual values was minimal (Table 2). This can be considered as an advantage for the storage of good quality seed lots regarding seed gene bank storage. Seed viability in seed banks must remain above 85% during storage in order to avoid mutatic changes (Walters et al., 2005). Moreover, a large number of seed accessions need to be tested in gene banks. This is a heavy work burden. Therefore, a quick decision about germination is very valuable in order to save time and effort. In that sense, RE can be a useful test for seed testing laboratories and seed companies in predicting final normal germination percentages.

For seed producers and farmers, seed viability based on normal seedling percentages is the most important concern, with significant economic implications. Seed viability is affected by various pre-harvest, harvest and post-harvest conditions (Basu, 1995). Basically, germination rate is a part of seed quality which is influenced by a combination of genetics and the growing environment. (Ellis and Roberts, 1981). In this work, we used various onion cultivars which have a different genetic structure and were obtained from different growing areas of the country. Some cultivars may be genetically susceptible to faster germination than others. Our results indicate that the RE counts developed by using various genetically based lots were also effectively and successfully used with other seed cultivars with regard to the estimation of normal germination percentages (Figure 3).

The mean germination time (MGT) based on serial radicle emergence during germination test was predictive of normal germination (Mavi et al., 2016). MGT determination involves several counts of radicle emergence as germination progresses. Frequent counts throughout the germination test may be hard to run for a large number of lots due to a heavy workload; however, different automated systems are available for assessing germination trends (Demilly et al., 2014). Practical automated methods used as part of a routine have been developed (Demilly et al., 2014). These include image analysis systems that assess seed size and shape changes during imbibition and subsequent germination. There is a strong effort to run such a fast and efficient method of measuring RE by automated systems (Matthews and Powell, 2011; Matthews et al., 2012; Wagner et al., 2012). An automated system has already been successfully used for almost 10 years (Wagner et al., 2012) to determine the seed germination curves, MGT and radicle emergence of seeds of over 45 crop species (Demilly et al., 2014). The effort to make it more efficient may be an outstanding prospect for seed quality evaluation systems.

Future work on these and other species as forthcoming research goals may show that normal seedling counts can also be predicted through early counts of RE, or that normal germination levels of seed lots can be estimated within a short period of a few days. Our results support the evidence that possible use and standardization of RE testing in terms of replication of the RE test and timing of RE counts will need more data from more seed lots and species, and laboratories and seed companies. We are working to develop similar data in seed lots of various species in forthcoming projects.

Mavi et al. (2016), suggest that the RE test may be applicable to many species and could be investigated further in routine testing. This may be more applicable to low quality seed lots that are left over from earlier seed production seasons, such as when seeds are stored in subtropical conditions, i.e. in high humidity and temperatures. Seed packets in retail markets are stored in ambient conditions. Even when they have high initial quality and seed moisture is reduced to a low level, packets are subjected to temperature variations at the sale points. Onion seeds usually lose viability and vigour faster than other crops, and they are short-lived under ambient conditions (Ellis et al., 1996; Yanping et al., 2000). This may accelerate in hot and humid environments, and so seed companies may need to check the quality of seed batches that are supplied to the market. Ultimately, delivery of high-quality seed lots to growers is the responsibility of the producing company. Any company that sales seed lot with germination percentage below 75%, could face a large amount of fines according to the current seed law. Therefore, frequent control of seed germination may be necessary for retailer seed companies.

The RE method has been shown to be repeatable and highly predictive of the normal germination of onion seed lots and can be used for a quick evaluation of quality rather than waiting for 12 days in a germination test. This may be tested in other crop seeds in forthcoming studies.

CONFLICT OF INTEREST DECLARATION

The author(s) declare no conflict of interest for this study.

REFERENCES

Basu RN (1995) Seed viability, In: Seed Quality: Basic mechanisms and agricultural implications (Eds. Basra AS), Food Products Press, New York. pp. 1–44.

Demilly D, Ducournau S, Wagner MH, Dürr D (2014) Digital imaging of seed germination. In: Plant Image Analysis: Fundamentals and Applications (Eds. Gupta SD, Ibaraki Y), CRC Press, Boca Raton, USA. pp.147-162

Demir I, Ermis S, Mavi K, Matthews S (2008a) Mean germination time of pepper seed lots (*Capsicum annuum*) predicts size and uniformity of seedlings in germination tests and transplant modules. Seed Sci. Technol., 36: 21-30.

Demir I, Kenanoglu BB, Ozden E (2019) Seed Vigour Tests to Estimate Seedling Emergence in Cress (*Lepidium sativum* L.) Seed Lots, Not. Bot. Horti Agrobot. Cluj-Napoca 47: 881-886.

Ellis RH, Roberts EH (1981) The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol., 9: 373–409

ISTA (2016) International Rules for Seed Testing, International Seed Testing Association, Bassersdorf, Switzerland.

Khajeh-Hosseini M, Nasehzadeh M, Matthews S (2010) Rate of physiological germination relates to the percentage normal seedlings in standard germination tests of naturally aged seed lots of oilseed rape. Seed Sci. Technol., 38: 602-611.

Lv, YY, Wang YR, Powell AA (2016) Frequent individual counts of radicle emergence and mean just germination time predict seed vigour of *Avena sativa* and *Elymus nutans*. Seed Sci. Technol., 44: 189-198.

Matthews S, Khajeh-Hosseini M (2006) Mean germination time as an indicator of emergence performance in soil of seed lots of maize (*Zea mays*). Seed Sci. Technol., 34: 339-347.

Matthews S, Khajeh-Hosseini M (2007) Length of the lag period of germination and metabolic repair explain vigour differences in seed lots of maize (*Zea mays* L.). Seed Sci. Technol., 35: 200-212.

Matthews S, Powell AA (2011) Towards automated single counts of radicle emergence to predict seed and seedling vigour. Seed Testing International, 142: 44-48.

Matthews S, Noli E, Demir I, Khajeh-Hosseini M, Wagner MH (2012) Evaluation of seed quality: from physiology to international standardisation. Seed Sci. Res., 22:69-73.

Matthews S, Wagner MH, Kerr L, McLaren G, Powell AA (2012) Automated determination of germination time courses by image capture and early counts of radicle emergence lead to a new vigour test for winter oilseed rape (*Brassica napus*). Seed Sci. Technol., 40: 413-424.

Mavi K, Mavi F, Demir I, Matthews S (2014) Electrical conductivity of seed soak water predicts seedling emergence and seed storage potential in commercial seed lots of radish. Seed Sci. Technol., 42: 76-86.

Mavi K, Powell AA, Matthews S (2016) Rate of radicle emergence and leakage of electrolytes provide quick predictions of percentage normal seedlings in standard germination tests of radish (*Raphanus sativus*). Seed Sci. Technol., 44: 393-409.

Ozden E, Ozdamar C. Demir I (2018) Radicle Emergence Test Estimates Predictions of Percentage Normal Seedlings in Standard Germination Tests of Aubergine (*Solanum melongena* L.) Seed Lots, Not. Bot. Horti Agrobot. Cluj-Napoca 46:177-182.

Powell AA, Mavi K (2016) Application of the radicle emergence test to radish (*Raphanus sativus*) seed. 31. ISTA Congress, June 15-21, Tallinn, Estnonia. pp. 43

Thirusendura SD, Saraswathy S (2017) Seed viability, seed deterioration and seed quality improvements in stored onion seeds: a review. J. Hortic. Sci. Biotechnol., 93:1–7.

Yanping Y, Ronggi G, Qingguan S, Shengfu L (2000) Vigour of welsh onion seeds in relation to storage temperature and seed moisture content. Seed Science and Technology, 28: 817–823.

Wagner MH, Ducournau S, Luciani A, Léchappé J (2012) From knowledge-based research towards accurate and rapid testing of seed quality in winter rape. Seed Sci. Res., 22: 80-85.

Walters C, Wheeler LM, Grotenhuis JM (2005) Longevity of seeds stored in a genebank: species characteristics. Seed Sci. Res., 15: 1-20.