

## Use of the radicle emergence test (RE) to estimate germination and emergence potential in sponge gourd (*Luffa aegyptiaca* Mill.) seed genotypes

Kazım Mavi<sup>1</sup>  Ahmet Hakan Eker<sup>2</sup>  Ibrahim Demir<sup>2</sup> 

<sup>1</sup>Department of Horticulture, Faculty of Agriculture, University of Mustafa Kemal, Antakya, Hatay Türkiye

<sup>2</sup>Department of Horticulture, Faculty of Agriculture, University of Ankara, 06110 Ankara, Türkiye

### Article History

Received: August 7, 2024

Revised: October 28, 2024

Accepted: November 1, 2024

Published Online: November 24, 2024

### Article Info

Article Type: Research Article

Article Subject: Vegetable Growing and Treatment

### Corresponding Author

Kazım Mavi

✉ [kazimmavi@hotmail.com](mailto:kazimmavi@hotmail.com)

### Available at

<https://dergipark.org.tr/jaefs/issue/87864/1528541>

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### Abstract

The study was carried out to test whether the radicle emergence (RE) test could be used to rank 12 *Luffa* (*Luffa aegyptiaca* Mill.) seed genotypes according to total (TG, %) and normal (NG, %) germination percentages in laboratory conditions (25°C, 14 days, dark), and mean germination time (MGT, day), seedling emergence percentage (SE, %), seedling shoot weight (SSW, g/plant) and hypocotyl thickness (HT, mm/plant) in climatic room conditions (23±2°C, 70% relative humidity), in peat moss medium after 15 days. RE (2 mm radicle) counts were made at 24, 48, 72, 96, 120 and 144 hours at 25°C in the dark after germination was set up. RE values were correlated to seed germination and seedling quality parameters. The highest correlation values occurred after 72h and onwards. RE 72h and seed quality parameters were regressed and were found to be significantly related to TG ( $R^2=0.732$ ,  $P<0.001$ ), NG ( $R^2=0.751$ ,  $P<0.001$ ), MGT ( $R^2=0.842$ ,  $P<0.001$ ), SE ( $R^2=0.754$ ,  $P<0.001$ ), SSW ( $R^2=0.349$ ,  $P<0.05$ ), and HT ( $R^2=0.757$ ,  $P<0.001$ ). The potential of the RE test to discriminate between *Luffa* genotypes according to their germination and emergence potential is discussed.

**Keywords:** *Luffa* genotypes, RE test, Germination, Seedling emergence, Seed vigour

**Cite this article as:** Mavi, K., Eker, A.H., Demir, I. (2024). Use of the radicle emergence test (RE) to estimate germination and emergence potential in sponge gourd (*Luffa aegyptiaca* Mill.) seed genotypes. International Journal of Agriculture, Environment and Food Sciences, 8(4): 779-785. <https://doi.org/10.31015/jaefs.2024.4.6>

## INTRODUCTION

The sponge gourd (*Luffa*) belongs to the Cucurbitaceae family and is cultivated widely as a vegetable in India, China and Asian countries. Its immature fruits are used as vegetables and the mature fruits as fibres. The genus *Luffa* consists of ten species but specifically *Luffa aegyptiaca* Mill. and *Luffa acutangula* Roxb. are economically cultivated in different parts of the world (Marr et al., 2005).

Vegetable transplant grafting has been widely practiced in Cucurbitaceae for various purposes, including the enhancement of tolerance to abiotic and biotic stresses (Savvas et al., 2010; Lee et al., 2010; Jang et al., 2022). Grafting is also considered to be an environment-friendly operation (Rivard and Louws, 2008) for sustainable vegetable growing systems. One of the main purposes of grafting is to improve tolerance to soil pathogens such as Fusarium wilt as well as to low temperature and high salinity (Yetisir et al., 2007; Mavi et al., 2006). In this way, rootstocks that are tolerant to such soil borne diseases can be valuable. *Luffa* species have the merit of nematode tolerance and have the potential to be used as a rootstock for watermelons and melons (Schwarz et al., 2010). For successful grafting, high and uniform seedling emergence and longer hypocotyl length are important since the occurrence of more similar sized seedlings gives a chance for the production of a higher number of grafts at the same time (Mavi et al., 2006). Thus, estimation of seed germination and emergence potential of seed lots/genotypes is valuable. This study highlights the importance of uniform seedling emergence and hypocotyl length for successful grafting. This is directly relevant to focus on optimizing seedling growth conditions. Estimating the seed germination and emergence potential of different genotypes is a critical aspect of my research, as it allows for better planning and execution of grafting procedures, ultimately leading to higher grafting success rates.

Ability to emerge successfully in a wide range of environments is reflected in the seed vigour characteristics of any lot (Powell, 2022). Vigorous seed lots produce higher seedling emergence percentages and stronger seedlings than those of less vigorous ones. In most cases standard laboratory germination tests at optimum conditions may not necessarily indicate the seedling emergence performance of any lots in the field or glasshouse. Therefore, vigour tests were developed to test seed lot performance under a wide range of environmental conditions. Seed vigour tests of accelerated ageing, controlled deterioration and mean germination time were successfully used to estimate laboratory germination and seedling emergence in various crop seeds (Mavi and Demir, 2007; Mis et al., 2022). RE, the radicle emergence test, was introduced and validated by ISTA and used to predict not only laboratory germination (Mavi et al., 2016; Shinohara et al., 2021) but also seedling emergence in both modules and the field environment (Demir et al., 2008; Khajeh-Hosseini et al., 2009; Mavi et al., 2010; Matthews et al., 2011; Ermis et al., 2022), as well as seed longevity (Ermis et al., 2022; Eren et al., 2023). RE has various advantages compared to ageing tests, as it is easy to evaluate, faster and simpler, and is suitable for machine evaluation (Matthews et al., 2011). However, the RE test has rarely been used for landraces or genotypes that have a different genetic background (Demir et al., 2020). Faster and easier evaluation of seed germination and vigour potential of Luffa seed genotypes may help to decide faster and to select the right material for further breeding purposes as using for the next year's breeding programme. In this study, we tested whether RE can be used to estimate laboratory germination and seedling size in Luffa seed genotypes.

## MATERIALS AND METHODS

### Plant materials

Twelve seed lots of Luffa (*Luffa aegyptiaca* Mill.) genotypes were collected from various parts of Hatay, Turkiye (Mavi et al., 2018; Mavi et al., 2020). Seeds of genotypes from the S3 stage were used. They were harvested from mature yellow fruits. Seeds were dried at room temperature and kept at 5°C. Seeds were distinctively different in appearance and size. Six lots were black (1, 3, 5, 7, 10, 12), five (4, 6, 8, 9, 11) were brown, and one (2) was a white coated genotype. The weight of one hundred seeds varied between 10.4 g, lot 9, and 15.4 g, lot 2. Seed moisture content was between 6.7 and 7.3%.

### Germination and emergence procedure

The germination of three replicates of 50 seeds from each seed lot was assessed using the between-paper method (ISTA, 2022) at 25°C in the dark. Radicle emergence test counts (the number of seeds with a radicle  $\geq 2$  mm long) were made at 48, 72, 96, 120, 144 hours. At the final count, 14 days after the beginning of the test, seedlings were evaluated as total (TG) and normal germination (NG) percentages (ISTA, 2022). Mean germination time (MGT) was calculated on the daily radicle emergence counts by using the formula below:

$$\Sigma (n.t) / \Sigma n$$

where n is the number of seeds newly showing radicle emergence ( $>2$  mm) at time t (hours).  $\Sigma n$  is the total number of seeds showing radicle emergence by the end of the test.

The seedling emergence tests were conducted with three replicates of 25 seeds in each seed lot. The seeds were sown 4 cm deep in compost (Plantaflor, Humus Verkaufs, GmBH, Vechta, Germany) in sandwich boxes (360 x 220 x 60 mm), and all boxes were transferred to a growing cabinet for 16 days at  $22 \pm 2$  °C. Light was provided by cool fluorescent lamps (Philips, Hamburg, Germany) at a rate of  $78 \mu\text{mol m}^2 \text{s}^{-1}$  for  $12 \text{ h d}^{-1}$  at the seedling level. The relative humidity in the cabinet was maintained above 65-70% throughout the experiment to minimize water loss from the boxes. The appearance of a hypocotyl hook on the compost surface was used as an emergence criterion, and emerged seedlings were recorded daily at the same time of the day. Seedling emergence (SE) percentages were determined 16 days after sowing. To determine seedling fresh weight, hypocotyl length and seedling length, destructive harvests were taken 16 days after sowing on 15 randomly seedlings (5 seedlings x 3 replicates) selected among normally developed seedlings of each genotype. Seedlings were taken out of the peat moss, cut just above the root and cleaned, and seedling fresh weight (SSW, 0.001 g/plant), and hypocotyl thickness (HT, 2 cm above the soil surface, mm/plant) were determined in each plant. The measurements were made within 10 seconds of destructive harvest.

### Statistical analysis

Correlation and regression coefficients ( $R_2$ ) were performed between radicle emergence test (RE) and total, normal germination percentages, mean germination time and seedling parameters by using SPSS (IBM version 25). Level of significance was determined as various significant levels.

## RESULTS AND DISCUSSION

Final total germination (TG) of the seed lots ranged between 55 and 97%, and normal germination was between 53 and 93%. Statistically significant differences were determined between the genotypes in terms of the examined traits (Table 1). Cumulative RE percentages are shown in Figure 1. There are great range among the twelve seed lots starting by 24 hours towards 144 hours (Figure 1). The fastest germinating lots were lots 1 and 4. Both reached

97% over 144 hours. However, lots 10, 11 and 12 had the lowest values. They had lower than 65% germination over 144 hours. Lot 12 had just 47% germination over 144 hours. Seed lots had different MGT values of between 2.5 and 4.3 days, the lowest being in lot 10 and the highest in lot 1.

Table 1. Variation in total (TG, %), normal (NG, %), germination, mean germination time (MGT, d), seedling emergence (SE, %), Seedling shoot weight (SSW, g/plant), hypocotyl thickness (HT, mm/plant) of *Luffa* genotypes. Means with different letters in the same column are significantly different at the 5% level.

Seed Lot	TG	NG	MGT	SE	SSW	HT
1	97 <sup>a</sup>	93 <sup>a</sup>	2.5 <sup>a</sup>	100 <sup>a</sup>	1.21 <sup>a</sup>	2.61 <sup>a</sup>
2	97 <sup>a</sup>	92 <sup>a</sup>	3.0 <sup>ab</sup>	97 <sup>a</sup>	1.15 <sup>ab</sup>	2.44 <sup>ab</sup>
3	92 <sup>ab</sup>	90 <sup>ab</sup>	3.1 <sup>ac</sup>	73 <sup>bc</sup>	0.60 <sup>d</sup>	2.38 <sup>ac</sup>
4	93 <sup>ab</sup>	88 <sup>ac</sup>	3.3 <sup>ad</sup>	92 <sup>a</sup>	0.61 <sup>d</sup>	2.43 <sup>ab</sup>
5	92 <sup>ab</sup>	88 <sup>ac</sup>	3.8 <sup>bd</sup>	85 <sup>ab</sup>	0.57 <sup>d</sup>	2.42 <sup>ab</sup>
6	89 <sup>ac</sup>	87 <sup>ac</sup>	3.2 <sup>ac</sup>	82 <sup>ab</sup>	0.83 <sup>cd</sup>	2.30 <sup>bc</sup>
7	95 <sup>ab</sup>	86 <sup>ac</sup>	3.4 <sup>ad</sup>	88 <sup>ab</sup>	0.80 <sup>cd</sup>	2.43 <sup>ab</sup>
8	88 <sup>ac</sup>	80 <sup>bd</sup>	3.9 <sup>bd</sup>	50 <sup>d</sup>	0.74 <sup>cd</sup>	2.34 <sup>ac</sup>
9	84 <sup>bd</sup>	77 <sup>cd</sup>	3.3 <sup>ad</sup>	72 <sup>bc</sup>	0.56 <sup>d</sup>	2.40 <sup>ac</sup>
10	79 <sup>cd</sup>	69 <sup>de</sup>	4.3 <sup>d</sup>	48 <sup>d</sup>	0.90 <sup>bc</sup>	2.36 <sup>ac</sup>
11	76 <sup>d</sup>	64 <sup>e</sup>	3.6 <sup>bd</sup>	64 <sup>cd</sup>	0.93 <sup>bc</sup>	2.37 <sup>ac</sup>
12	55 <sup>e</sup>	53 <sup>f</sup>	4.1 <sup>cd</sup>	53 <sup>d</sup>	0.65 <sup>cd</sup>	2.13 <sup>c</sup>
Range	55-97	53-93	2.5-4.3	53-100	0.60-1.21	2.13-2.61

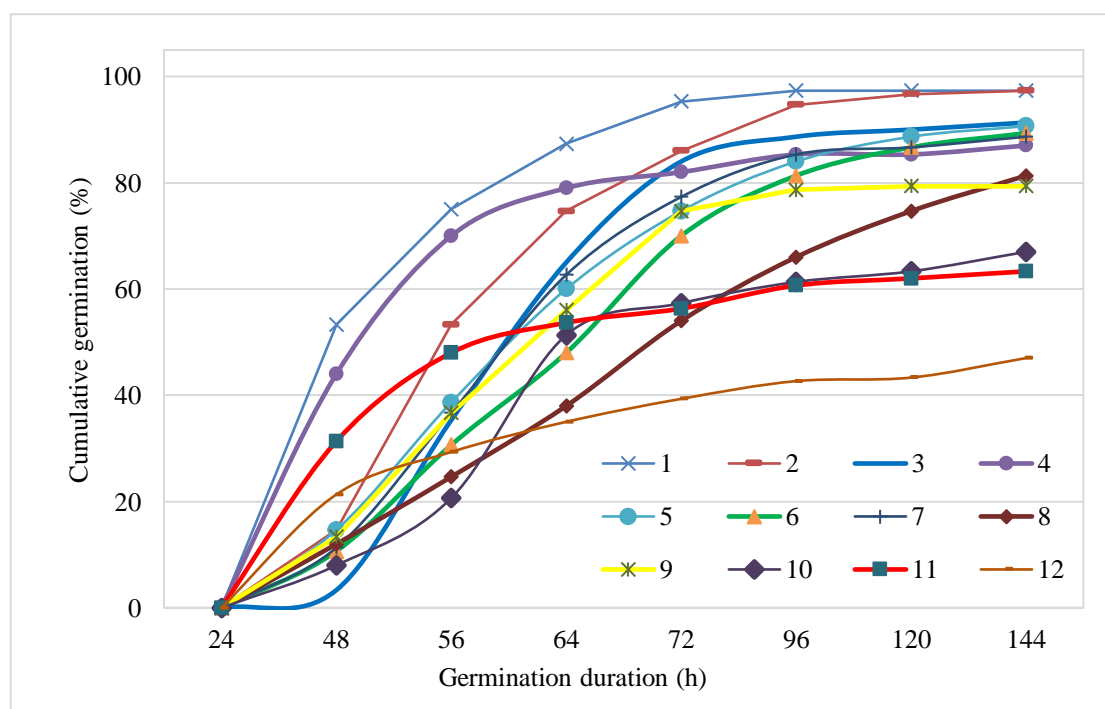


Figure 1. RE counts in the 144 hours after the germination test was set up in twelve *Luffa* genotypes.

Seed lot 1 had the highest seedling emergence percentages, the lowest mean emergence time, seedling shoot weight and hypocotyl thickness among all lots (Table 1). Lot 12 had 53% of seedling emergence and the thinnest of HT at 2.13 mm/plant.

The highest coefficient values between RE values and seed germination and seedling quality factors were found at 72, 96, 120 and 144 h (Table 2). We chose 72 hours for the sake of shorter evaluation time. RE72h was highly related to total ( $P < 0.001$ ,  $R^2 = 0.732$ ) and normal ( $P < 0.001$ ,  $R^2 = 0.751$ ) germination in laboratory conditions and MGT ( $P < 0.001$ ,  $R^2 = 0.842$ ) values (Figure 2).

Table 2. Correlation coefficient values (r) for the relationship between RE and Total Germination (TG, %), Normal Germination, (NG, %), Mean Germination Time (MGT, day) Seedling emergence (SE %), Seedling Shoot weight (SSW, g/plant) and Hypocotyl Thickness (HT, mm/plant) in twelve *Luffa* genotypes. Significance: \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

RE hours	TG	NG	MGT	SE	SSW	HT
RE48	0.081	0.097	-0.371	0.424	0.422	0.454
RE56	0.446	0.467	-0.679*	0.762**	0.490	0.652**
RE64	0.736**	0.746**	-0.808**	0.793**	0.627*	0.836***
RE72	0.855***	0.866***	-0.917***	0.868***	0.591*	0.870***
RE96	0.937***	0.960***	-0.900***	0.872***	0.507	0.829***
RE120	0.959***	0.993***	-0.873**	0.827***	0.455	0.800**
RE144	0.971***	0.982***	-0.832***	0.768**	0.447	0.777**

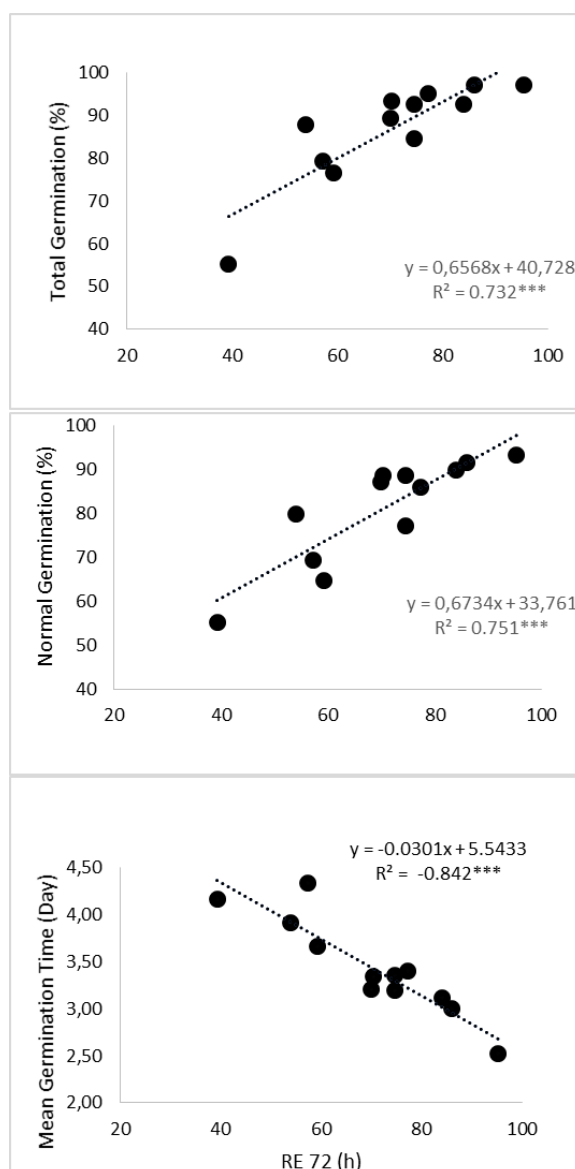


Figure 2. The relationship between RE72h and total (TG), normal (NG) germination percentages and mean germination time (MGT) in twelve *Luffa* genotypes. Significance \*\*\*:  $P < 0.001$ .

The period in which the RE test showed the highest correlation with six different characteristics (72h) was determined as the most ideal period for the power test (Table 2). In previous studies, the highest correlation was determined in 48-hour RE tests in radish (Mavi et al., 2016) and 120-hour RE tests in carrot (Mis et al., 2022).

The results obtained in this study show that RE tests are successful at discriminating both the laboratory germination percentages (total /normal) in ideal germination conditions and seedling growth in modules, i.e. vigour of sponge gourd seed genotypes. Our work supports previous reports in which a single count of RE, identified from a germination progress curve, correlates with laboratory germination in many diverse crop seeds (Matthews and Khajeh-Hosseini, 2006; Khajeh-Hosseini et al., 2010; Matthews et al., 2018; Mavi et al., 2016; Powell and Mavi, 2016; Ozden et al., 2018; Demir et al., 2020; Mis et al., 2022). A single RE count after 48 hours at 13°C or 24 and 30 hours at 20°C in oilseed rape (Matthews et al., 2012), after 48 hours at 20°C in radish (Mavi et al., 2016) and cauliflower (Shinohara et al., 2021), and after 104 hours at both 25°C and alternating 20/30°C in aubergine (Ozden et al., 2018) is significantly related with NG. Work on onion seed and the application of an 80 hour RE count successfully predicted the NG of commercial lots (Demir et al., 2020): 40 h in lettuces, 74 h in watermelons and 120 h in carrots were found to be successful (Mis et al., 2022). The RE test has been also found to be successful in discriminating lots according to seed vigour, i.e. emergence in modules and the field in several species (Demir et al., 2008; Khajeh-Hosseini et al., 2009; Mavi et al., 2010; Matthews et al., 2011, 2012; Ermis et al., 2022). The lower RE values were associated with longer time to germination as a consequence of seed deterioration (Matthews and Khajeh-Hosseini, 2006). This was proposed as the basis of the ageing-repair hypothesis and the basis of all vigour tests (Matthews et al., 2011).

The seed lots with lower RE count are the result of a longer lag period before radicle emergence begins (Matthews et al., 2012; Shinohara et al., 2021), which is due to the increased need to repair the deterioration caused by seed ageing. Various vigour tests can be used to discriminate differences in the level of ageing of seed lots, such as accelerated ageing (ISTA, 2022). The RE test is a valuable test for predicting vigour differences due to its simplicity and rapidity.

RE values showed a significant relationship with seedling quality parameters. The relations varied. RE72 was found to have a very highly predictive potential for seedling emergence ( $P < 0.001$ ,  $R^2 = 0.754$ ), hypocotyl thickness ( $P < 0.001$ ,  $R^2 = 0.757$ ) and seedling shoot weight in the seed lots (Figure 3).

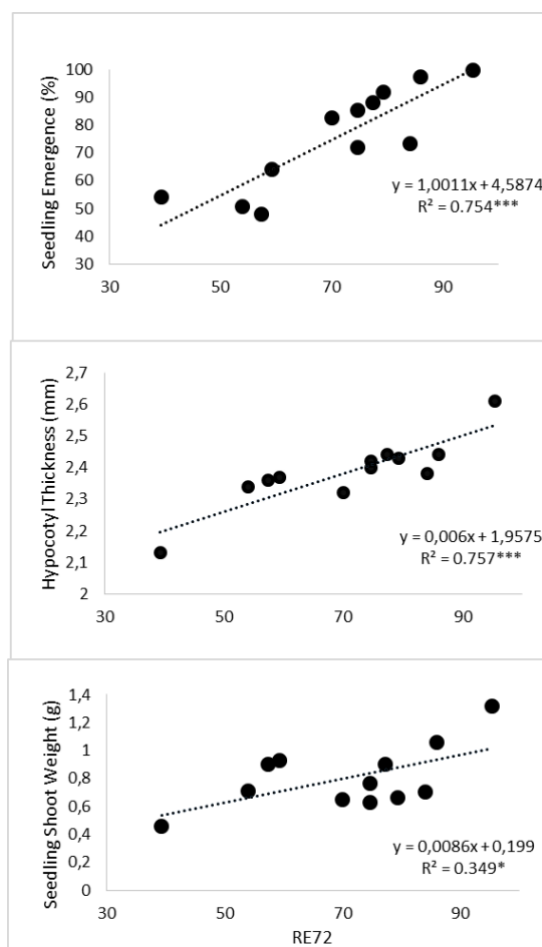


Figure 3. The relationship between RE72h and seedling emergence (SE), hypocotyl thickness (HT) and seedling shoot weight (SSW) in twelve *Luffa* genotypes. Significance \*:  $P < 0.05$ , \*\*\*:  $P < 0.001$ .

Seed lots with higher RE values had higher total and normal germination and germination time but also had higher seedling quality parameters as reflected in seedling emergence, higher seedling shoot weight and hypocotyl thickness (Figure 3). RE results after 72 hours at 25°C show correlation with the results of seedling emergence percentages, seedling shoot weight and hypocotyl length. This suggests that the RE could be used to rank lots according to seedling emergence potential (Figure 3). Both criteria are important for grafted transplant production in cucurbits. Thicker hypocotyls provide a higher number of seedlings for graftable levels, which increase the efficiency of grafting. In this study, the seed lots were genotypes collected from various parts of the Hatay region. They were not genetically stable (homogeneous). For breeders, selecting higher quality seed lots is valuable. In our study, RE72 h indicates the higher total and normal germination of any seed lots very significantly (Figure 2). This can be a very helpful selecting method for breeders when a large number of seed lots are used in breeding programmes.

Sponge gourd is a heat-resistant cucurbit species, mainly used for grafting in cucumbers. Therefore, it is recommended to use sponge gourd as a rootstock for cucumber cultivation in high-temperature conditions. It has been reported that cucumbers grafted on to luffa rootstocks significantly alleviate growth reduction at high temperatures (Li et al., 2014). This study is valuable for determining the rootstock potential of the sponge gourd genotypes used and selecting a suitable rootstock genotype for this country. Particularly, genotypes with higher emergence percentages, seedling dry weight and hypocotyl thickness are considered to have potential rootstock characteristics (Figure 3). Sponge gourd genetic materials are very important both in the breeding of commercial varieties and in the breeding of rootstocks. For this reason, genotypes selected from Hatay should be evaluated both as vegetables and as rootstocks (Mavi et al., 2020). It has been determined that some of the genotypes can be evaluated as vegetables. Genotypes with high emergence and high hypocotyl thickness (Figure 3) can be used in experiments as rootstocks. When vegetable rootstock reach the graftable level in a shorter time, that is, they have higher values in the RE test, the efficiency of grafting can be higher. Fast germination results in thicker hypocotyls, and this makes it easier to do more grafting at one time, which helps to produce equally-sized transplants (Ermis et al., 2022).

## CONCLUSION

In conclusion, the RE test was able to assess the seed germination percentages and seed vigour of seed genotypes in Luffa. The RE test has the advantages of being fast, easy and practical, and the analyst does not require the special training necessary for other vigour tests. Also, there is no use of chemicals. A single radicle emergence count after 72 hours at 25°C is therefore a successful alternative vigour test for Luffa seeds. This conclusion can be useful for ranking seed lots in relation to their germination and seedling emergence potential.

## Compliance with Ethical Standards

### Peer-review

Externally peer-reviewed.

### Declaration of Interests

All authors declare that they have no conflicts of interest.

### Author contribution

Ahmet Hakan Eker (AHE), and Ibrahim Demir (ID) carried out experimental part of the study. Kazım Mavi (KM), and ID reviewed the manuscript, AHE done statistical analysis, KM and ID designed the experiments and ID conceived the principal idea and wrote the paper.

### Funding

This study did not obtain any external funding.

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