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RESEARCH ARTICLE

Evaluation of Radicle Emergence Test to Predict Seed Vigor and Field Emergence in Different Seed Lots of Cluster bean (*Cyamopsis tetragonoloba L.)*

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ABSTRACT

The study was conducted to evaluate and correlate the radicle emergence test with other vigour parameters to predict the seed vigour and field emergence potential in ten seed lots of cluster bean with varying vigour. The results showed that the significant differences were observed among the seed lots in terms of physiological parameters *viz.,* speed of germination, germination percentage (GER), root length, shoot length, dry matter production, vigour index, mean just germination time (MJGT), mean germination time (MGT), radicle emergence (RE) and field emergence (FE) and biochemical parameters *viz.,* electrical conductivity of seed leachate (EC) and dehydrogenase activity (DA). Relationship between seed vigour parameters and radicle emergence per cent with 1mm and 2mm radicle length was also evaluated. The highest R^2 values were observed in 2 mm length of radicle emergence per cent (R^2 values *viz.*, MJGT = 0.8832, MGT = 0.9379, EC = 0.8320, DA = 0.7810, GER = 0.8323 and FE = 0.8796) compared with 1mm length of radicle emergence per cent (R^2 values *viz.,* MJGT = 0.8777, MGT = 0.8826, EC = 0.8107, DA = 0.8113, GER = 0.8292 and FE = 0.8699) for most of the seed vigour parameters in ten different seed lots. The study concluded that counting 2 mm radicle emergence at 32 hours could be used for a quick evaluation to assess the seed vigour in terms of field emergence in cluster bean seed lots.

Keywords: *Radicle emergence, cluster bean, germination, vigour and field emergence*

INTRODUCTION

Cluster bean (*Cyamopsis tetragonoloba L.)* a bushy annual herb, have a deep- rooted system. and It is an important leguminous crop grown on sandy soils of arid and semi-arid regions. It is belongs to the family Fabaceae. It commonly known as *Guar, Kothavarangai,* etc*.,* Guar is a photosensitive crop and grows well in specific climate conditions, which ensure a soil temperature around 21–25 °C for proper germination. The guar seed consists of a seed coat (14-17 %), the endosperm (35-42 %), and the germ (43-47 %).

The cultivation of cluster bean is dominated by India as a leading producer of the crop contributing to around 75–82 % of the total world production. In North Indian states like Rajasthan, Haryana, Gujarat and Punjab, it is mainly cultivated for guar gum production and for forage, whereas in South India it is being cultivated for the vegetable purpose. India exports cluster bean mainly to USA, Germany, Netherlands, UK, Japan, and France to a tune of Rs. 200 million annually (Singh *et al*., 2009).

The seed is considered as the prime input in agriculture. Quality of the seed is most important to produce vigorous and healthy plants in the field. A key component of the performance of crop seeds in field largely dependent on the seed vigour. Seed vigour, an important factor governing the seed quality, reflects the potential seed germination, seedling growth, seed longevity and tolerance to adversity (Sun *et al.,* 2007).

International Seed Testing Association defined that "Seed vigour is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments" (ISTA, 2012). Seed vigour is qualitative in concept than a quantifiable seed quality character, but it is often evaluated through various vigour tests where the vigour status is often indicated through comparative values. Vanderlip *et al.* (1973) observed that seed lots differed widely between each variable measured on standard germination and other vigour tests. A vigour test should provide a reproducible result which accurately

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describes the potential for rapid, uniform emergence under field conditions and describes the ranking of a seed lots. A practical seed vigour test should give a good indication of the field performance potential of the seed lot and the test results should be reproducible (Hampton and TeKrony, 1995).

The seed lots with the same germination per cent varying vigour in their performance either in storage and field (Carvalho and Nakagawa, 2000). Deterioration of seed lots is inevitable, which results in loss of seed vigour and viability (McDonald, 1999). Reduced vigor and viability of seeds may affect field performance and productivity of the crop (TeKrony and Egli, 1989). Therefore, use of high-quality seed is mandatory, which necessitates the evaluation of its quality before introducing into market. Seed vigour tests should be inexpensive, rapid, simple, objective, reproducible, and should have high correlation with field performance (Copeland and McDonald, 2001).

The standard germination test is considered as a universal test of seed quality to evaluate the maximum potential of a particular seed lot under an ideal set of environmental conditions (ISTA, 1987). A standard germination test is time-consuming and doesn't always show the potential performance of a seed lot, especially if field conditions are not optimal (Hampton and TeKrony, 1995).

Radicle emergence test (RE) is considered as a quick test to predict varying vigour level and field performance of seed lots than the standard germination test in several crops. Radicle emergence is defined as the appearance of a 2mm radicle after breaking through the seed coat. The radicle emergence test has been accepted as a valid seed quality test by the International Seed Testing Association (ISTA) in the Annual Meeting held at Zurich in June 2011 for *Zea mays* (Matthews and Powell, 2011).

According to the Indian Minimum Seed Certification Standards (IMSCS),cluster bean seedlings are evaluated on 14thday for its germination percentage. Once the seeds meet IMSCS it can be sold in the market. Since seed is a living entity and kept for this evaluation period not only reduced the germination percentage but also the vigour. We need to advance technology which would give a precise result in short period.

Against this back drop, the present study was undertaken with the aim to evaluate the radicle emergence test to predict seed vigour and field emergence in cluster bean seed lots.

MATERIAL AND METHODS

The present experiment was conducted to evaluate and correlate the radicle emergence test with other vigour parameters in ten different seed lots (L1 to L10) of cluster bean.Genetically pure ten seed lots of cluster bean var. Pusa Naubahar obtained from Dharani seeds, Udumalaipet formed the base material for this study. The laboratory and green house experiments studies were carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2018-2019.

The germination test was conducted with 100 seeds in four replications for each lot in roll towel paper medium. The test conditions of 25 ± 2° C temperature and 95 ± 2 % RH were maintained in the germination room. At 14th day end of the germination test period, the number of normal seedlings were counted and the mean was expressed as germination percent (ISTA, 2016). The seeds showing plumule emergence in each lot, replication wise were counted daily from the third day after sowing till end of a germination test. From the number of seeds germinated on each day, the speed of germination was calculated as per the method suggested by Maguire, (1962) and the results were expressed in a number. At the time of germination count, ten normal seedlings were selected at random from each lot replication wise and the root length was measured from the collar region to the tip of primary root. The mean values were calculated and expressed in centimeter. The seedlings used to measure root length were also used for measuring shoot length. The shoot length was measured from the collar region to the tip of the primary leaves and the mean values were expressed in centimetre. For dry matter production, the seedlings selected for root and shoot length measurements were put inside a paper cover, first shade dried for 24 h and then dried in a hot air oven maintained at 80 ± 2°C for 24 h. After cooled in silica gel desiccator for 30 min, the dry weight of seedlings was weighed using an electronic balance and the mean values were expressed in g 10 seedling^{-1}.Vigour index values were computed using the formula suggested by Abdul-Baki and Anderson (1973). The mean values were expressed as whole numbers.

Radicle emergence test was conducted through Top of the paper method. Eight replicates of 25 seeds in each lot were placed on germination paper moistened with distilled water in petri-dish. The petri-dishes were kept in the germination room maintained at 25±2°C and relative humidity of 95 ± 2 %. The number of seeds that had produced the radicle of 1mm and 2 mm long was recorded from the initiation of radicle emergence at two hours interval up to 24 hours for each replication (ISTA, 2012).

From the daily count data, the percentage radicle emergence (1mm and 2mm), Mean Just Germination Time (MJGT) and Mean Germination

Time (MGT) were calculated using the following formula.

$$\text{Radicle emergence with 1mm length(\%)} = \frac{\text{No. of seeds with 1mm radicle length}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{Radicle emergence with 2 mm length(\%)} = \frac{\text{No. of seeds with 2mm radicle length}}{\text{Total no. of seeds sown}} \times 100$$

The first appearance of the radicle, which is termed as Mean Just Germination Time and the Mean Germination Time, which is the mean lag period, to radicle emergence was calculated using the following formula proposed by Ellis and Roberts (1980) and expressed in hours.

$$MJGT = \Sigma nD / \Sigma n$$

{Where, n= number of seeds germinated (first appearance of the radicle) at time D, D= hours from the beginning of the germination test, Σn= final number of radicle emergence}

$$MGT = \Sigma nD / \Sigma n$$

{Where, n= number of seeds germinated (with 2 mm radicle emergence) at time D, D= hours from the beginning of the germination test, Σn= final number of radicle emergence}

In order to estimate the electrical conductivity of the seed leachate,four replicates of twenty-five seeds in each seed lot were prewashed with distilled water to remove the adhering chemicals and then soaked in 25 ml of distilled water for 8 hours at room temperature. After soaking, the seed steep water was decanted to obtain the seed leachate. The electrical conductivity of the seed leachate was measured in a digital conductivity meter with a cell constant of one and expressed as dSm^{-1} (Presley, 1958).

The dehydrogenase activity test was conducted with four replicates of twenty-five seeds in each seed lot were pre-conditioned by soaking in water for 8 hours at room temperature. Out of this, 10 seeds were taken at random and prepared by removing the seed coat. Then the seeds were soaked in 0.5% of 2, 3, 5 – Triphenyl tetrazolium chloride solution and kept in the dark at 40^0C for 4 hours for staining. After staining, the seeds were soaked in 10 ml of 2-methaxy ethanol (Methyl cellosolve) solution for 4 hours with occasional stirring till the extraction of red colour formation was completed. The extract was decanted and the intensity of colour was read in a spectrophotometer (ELICO SL 159) at 470 nm. The OD values were reported as dehydrogenase activity (Kittock and law, 1968).

In order to conductfield emergence test, four replicates of hundred seeds in each seed lot were sown in raised nursery beds and the seedlings emerged with normal root and shoot were counted after 15 days replication wise and the mean values were expressed in percentage.

$$\text{Field emergence (\%)} = \frac{\text{No. of normal seedlings}}{\text{Total no. of seeds sown}} \times 100$$

Statistical Analysis

Data obtained from the experiments were analyzed using an analysis of variance (ANOVA) as a factorial combination of treatments. Means were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at the 0.05 probability level. Values in percent data were arcsine transformed before analysis. Significance of correlation coefficients was tested by Pearson correlation method using SPSS software. The R^2 values were computed in the scatter diagram.

RESULTS AND DISCUSSION

Physiological seed quality parameters

In the present study, the radicle emergence test and other tests of seed vigour parameters were evaluated for ten different seed lots. Statistically significant differences were observed among seed lots for all the physiological parameters. Among the seed lots, L_1 recorded the highest values for all the observed parameters, *viz.,* speed of germination, germination per cent, root length, shoot length, dry matter production and vigour index (8.6, 96 %, 6.8 cm, 12.2 cm, 0.229 g 10 $seedling^{-1}$ and 1824) respectively followed by L_2 (8.5, 93%, 6.5cm, 11.6cm, 0.223 g 10 $seedling^{-1}$ and 1683) respectively. While L_{10} recorded lowest values (7.0, 71%, 4.8cm, 10.1 cm, 0.182g10 $seedling^{-1}$ and 1058) and L_9 (7.0, 72%, 5.1cm, 10.6cm, 0.190 g 10 $seedling^{-1}$ and 1130), respectively (Table 1).

The Mean Just Germination Time (MJGT) and Mean Germination Time (MGT) significantly increased with medium and low vigour seed lots compared with high vigour seed lots. The MJGT was minimum in L_1 (21.60 h) and L_2 (28.32 h) with short MJGT and the maximum in L_{10} (24.96 h) followed by L_8 (24.24 h). However, the minimum MGT was registered in L_1 and L_2 (28.32) and the maximum MGT was L_{10} (31.20 h) compared with other seed lots (Table 2). The current findings are in accordance with the results of radicle emergence studies in brinjal seed lots (Ozden *et al.,*2018).

Significant variations were observed in

percentage radicle emergence (1mm and 2mm radicle length) among seed lots. The maximum radicle emergence per cent with 1mm length was observed within the prescribed time of 28 h in L_1 (98 %), while it was minimum in L_{10} (82 %). The maximum radicle emergence per cent of 2mm length was observed within the prescribed time of 32 h in L_1 (98 %), while it was minimum in L_{10} (78 %) (Table 2).

Table 1. Evaluation of physiological seed quality parameters in different lots of cluster bean seeds cv. Pusa Naubahar

Seed lots	Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedling^{-1})	Vigour index
L_1	8.6	96 (78.46)	6.8	12.2	0.229	1824
L_2	8.5	93 (74.66)	6.5	11.6	0.223	1683
L_3	8.2	91 (72.54)	6.6	11.2	0.225	1620
L_4	8.2	90 (71.56)	6.5	11.0	0.220	1575
L_5	8.1	89 (70.63)	5.8	11.5	0.212	1540
L_6	7.7	88 (69.73)	5.8	11.3	0.204	1505
L_7	7.5	83 (65.65)	5.5	10.7	0.200	1345
L_8	7.1	74 (59.34)	5.0	10.3	0.195	1132
L_9	7.0	72 (58.05)	5.1	10.6	0.190	1130
L_{10}	7.0	71 (57.41)	4.8	10.1	0.182	1058
Mean	7.8	85 (67.21)	5.8	11.1	0.208	1431
SEd	0.08	1.2	0.08	0.13	0.0032	23.7
CD (P=0.05)	0.18	2.6	0.17	0.27	0.0065	48.5

(Figure in parenthesis indicate arcsine values)

Low vigour seed lots require more time to reach1mm and 2mm length of radicle emergence compared to high vigour seed lots.Reasons behind this delay have been interpreted as the time required for metabolic repair, DNA repair and enzymatic synthesis for before radicle emergence (Matthews and Powell, 2012). The long the lag period, higher the mean germination time, in the seed lots of cucurbits and cabbage this can be explained in terms of requirement of longer time for repair in aged seeds. Measurement of radicle emergence to 2 mm after 84 hours at 20°C or 150 hours at 13°C could be used as a vigour test method for sweet corn seed. This method needs less time to complete compared with other vigour test methods (Mavi *et al.,* 2010; Demir *et al.,* 2008).

Table 2. Comparison of physiological parameters in different lots of cluster bean seeds cv. Pusa Naubahar

Seed lots	MJGT (h)	MGT (h)	1mm Length of radicle emergence (%)	2mm Length of radicle emergence (%)	Field emergence (%)
L1	21.60	28.32	98 (81.87)	98 (81.87)	92 (73.57)
L2	22.08	28.32	94 (75.82)	98 (81.87)	90 (71.56)
L3	22.08	28.80	92 (73.57)	95 (77.08)	87 (68.86)
L4	22.80	29.28	95 (77.08)	95 (77.08)	85 (67.21)
L5	22.80	29.28	90 (71.56)	87 (68.86)	82 (64.89)
L6	23.04	30.00	88 (69.73)	85 (67.21)	82 (64.89)
L7	23.76	30.48	87 (68.86)	83 (65.65)	80 (63.43)
L8	24.24	30.72	83 (65.65)	80 (63.43)	71 (57.41)
L9	24.00	30.72	85 (67.21)	80 (63.43)	68 (55.55)
L10	24.96	31.20	82 (64.89)	78 (62.02)	68 (55.55)
Mean	23.14	29.71	89 (70.63)	88 (69.73)	81 (64.15)
SEd	0.383	0.390	1.5	1.1	0.9
CD (P=0.05)	0.784	0.797	3.1	2.4	1.8

(Figure in parenthesis indicate arcsine values)

MJGT- Mean just germination time MGT- Mean germination time

Among the ten different seed lots significant differences were observed in field emergence percent. Irrespective of the seed lots L_1 (92) registered highest field emergence, while L_9, L_{10} (68) registered the lowest field emergence compared with other seed lots (Table 2). The current findings are in accordance with the results of Ilbi and Eser (2006) in onion seed lots.

Table 3. Correlation between seed vigour parameters and radicle emergence test in different seed lots of cluster bean var. Pusa Naubahar

	GER (%)	MJGT (days)	MGT (days)	EC (dsm^{-1})	DA (OD value)	FE (%)	RE 1MM (%)	RE 2MM (%)
GER (%)	1							
MJGT (days)	-.953**	1						
MGT (days)	-.935**	.977**	1					
EC (dsm^{-1})	-.985**	.929**	.923**	1				
DA (OD value)	.986**	-.937**	-.920**	-.977**	1			
FE (%)	.989**	-.951**	-.943**	-.976**	.967**	1		
RE 1MM (%)	.927**	-.937**	-.939**	-.899**	.901**	.933**	1	
RE 2MM (%)	.912**	-.940**	-.968**	-.911**	.884**	.938**	.964**	1

**Correlation is significant at the 0.01 level

GER - Germination test
MJGT- Mean just germination time
MGT- Mean germination time
EC - Electrical conductivity test
DA- Dehydrogenase activity test
FE - Field emergence test
RE 1MM - Radicle emergence test (% of 1MM radicle length)
RE 2MM - Radicle emergence test (% of 2MM radicle length)

Biochemical seed quality parameters

Statistically significant differences were observed for electrical conductivity (EC) of seed leachate (dsm^{-1}) in among seed lots. The seed lots L_1 and L_3 (0.190 dsm^{-1}) registered the lowest EC value. While L_9 registered the highest value (0.248 dsm^{-1}) of electrical conductivity of seed leachate, which was on par with L_{10} (0.245 dsm^{-1}) (Fig. 1).

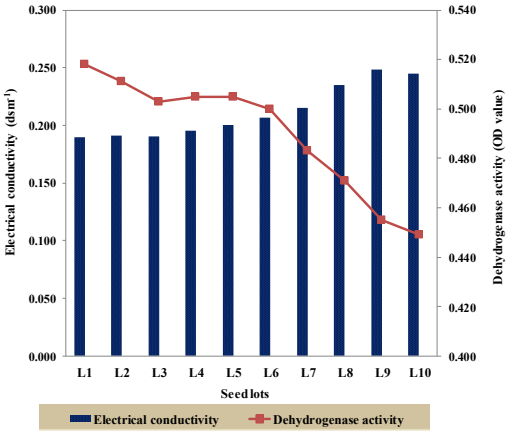

Figure 1. Evaluation of biochemical seed quality parameters in different lots of cluster bean seeds cv. Pusa Naubahar

Similar results were also reported in groundnut seeds by Suganthi and Selvaraju (2017). The electrical conductivity of seed leachate was mainly governed by cell wall permeability. Higher EC indicated higher permeability, high respiration rate and metabolic activity (Doijode, 1985). The increase in electrical conductivity might be due to the loss of selective permeability of cell membrane by auto oxidation of polyunsaturated fatty acids, free radical peroxidation via auto-oxidation, lipo-oxygenase and hydrolytic damage (Francis and Coolbear, 1984)

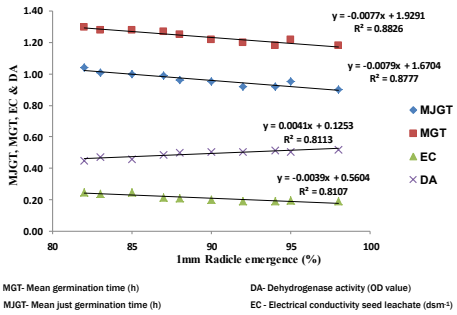

Figure 2a. Relationship between seed vigour parameters and radicle emergence % (1mm) in different lots of cluster bean seeds cv. Pusa Naubahar

Significant variations were also observed in dehydrogenase activity (OD value) among seed lots. The highest dehydrogenase activity was registered in L_1 (0.518), while L_{10} (0.449) registered the lowest value, which was on par with L_9 (0.455) compared with other seed lots (Fig. 1). Similar results were also reported in groundnut seeds. The activity of the dehydrogenase enzyme which is responsible for the respiration of the seed reduced with the ageing of the seeds, which is also used as a vigour indicator (Suganthi and Selvaraju, 2017).

Correlation between seed vigour parameters and radicle emergence test

Correlation analysis was carried out to assess the relationship between seed vigour parameters

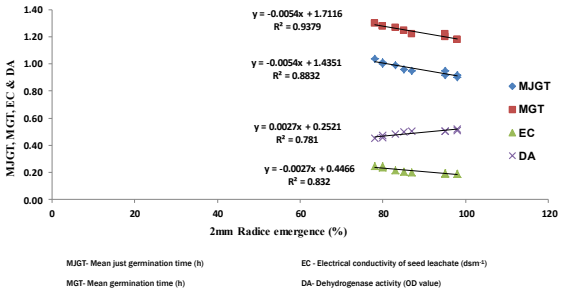

Figure 2b. Relationship between seed vigour parameters and radicle emergence % (2mm) in different lots cluster bean seeds cv. Pusa Naubahar

viz., germination (%), mean just germination time (h), mean germination time (h), electrical conductivity of seed leachate (dsm^{-1}), dehydrogenase activity (OD Value), field emergence (%) with radicle emergence per cent (1mm and 2mm radicle length) in ten different seed lots. However, field emergence (0.938**) followed by germination (0.912**) and dehydrogenase activity (0.884*) had a significant positive correlation with radicle emergence per cent (2mm radicle length).

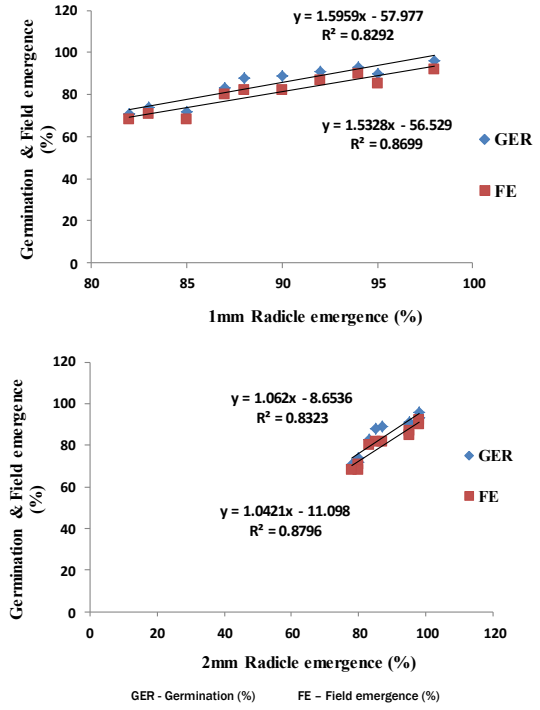

Figure 3. Relationship between germination (%), field emergence (%) and radicle emergence % (1mm & 2mm) in different lots of cluster bean seeds cv. Pusa Naubahar

Mean germination time (-0.968**) followed by mean just germination time (-0.940**) and Electrical conductivity of seed leachate (-0.911**) had a highly significant negative correlation with radicle emergence per cent (2mm radicle length). Highest correlation coefficient values are observed in 2mm length of radicle emergence per cent compared with the 1mm length of radicle emergence per cent for most of the seed vigour parameters in ten different seed lots (Table 3).

Significant correlations were also seen between the mean germination time of 31 seed lots of maize and both the cold test and field emergence (Lovato *et al.,* 2005) The field emergence showed positive and significant correlation with radicle emergence test, accelerating ageing test and dehydrogenase activity test where it was negatively and significantly correlated with electrical conductivity of seed leachate in coriander (Kumar *et al.*, 2015)

Relationship between seed vigour parameters and radicle emergence test

Relationship between seed vigour parameters and radicle emergence per cent (1mm and 2mm radicle length) were analyzed. Highest R^2 Valuesare observed in 2mm length of radicle emergence per cent (R^2 Values: MJGT = 0.8832, MGT = 0.9379, EC = 0.832, DA = 0.781, GER = 0.8323 and FE = 0.8796) comparedwith 1mm length of radicle emergence per cent (R^2 Values: MJGT = 0.8777, MGT = 0.8826, EC = 0.8107, DA = 0.8113, GER = 0.8292 and FE = 0.8699) for most of the seed vigour parameters in ten different vigour lots (Fig. 2a, 2b& Fig. 3). The current findings are in accordance with the results of Matthews and Powell (2011) in maize and Mavi *et al.* (2010, 2016) in cucumber, radish and cotton.

Classification of seed lots based on field emergence, 2mm length of radicle emergence and mean germination time (MGT)

In the present investigation, the seed vigour was classified into three groups *viz.,* high, medium and low vigour based on field emergence per cent, 2mm length of radicle emergence per cent and Mean germination time (MGT) relationships. When field emergence was exceeding 85 per cent, the radicle emergence was > 95 per cent (MGT: < 29 h) and it would be considered as “high vigour”; when field emergence was between 75 - 85 per cent, the radicle emergence was between 80 - 90 per cent (MGT: 29 - 30 h) and it would be considered as “medium vigour”; and when field emergence fell below 75 per cent the seeds are considered as “low vigour” with radicle emergence was < 80 per cent (MGT: > 30 h).

CONCLUSION

Radicle emergence test is a quick test to predict seed vigour and field emergence. It provides accuracy in the ranking of the seed lots and showed a better correlation to predicted field emergence potential of seed lots.The R^2 values between seed vigour parameters and radicle emergence testwere higher in 2mm length of radicle emergence

compared to 1mm length of radicle emergence. The result revealed that counting seedlings with 2 mm radicle emergence at 32 hours could be used for quick assessment of the seed vigour in terms of field emergence.

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