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Standardization of Radicle Emergence Test to Predict Seed Vigour and Field Emergence in Blackgram [*Vigna mungo* (L.) Hepper]

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ABSTRACT

Background: Blackgram is an important pulse crop grown in India. In which, prediction of planting value of seeds before sowing is important for assured yield. Standard germination test is time consuming and doesn't always show the seed lots potential performance, especially if field conditions are not optimal. Radicle emergence test is considered as one of the important physiological vigour test. This is a quick vigour test and also determines the planting value of various seed lots under wide range of climatic conditions.

Methods: The laboratory experiment was carried out at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2019-2020 to standardize the radicle emergence test to predict seed vigour and field emergence in ten different seed lots [L_1 to L_4: high vigour lots (>90% germination), L_5 to L_7: medium vigour lots (80-90% germination) and L_8 to L_{10}: low vigour lots (<80% germination)] of blackgram var. VBN 6. The radicle emergence test was conducted through top of paper method adopting ISTA rules.

Result: The results showed that the significant differences were observed between seed lots in terms of physiological parameters *viz.,* speed of germination, germination percentage, root length, shoot length, dry matter production, vigour index, mean just germination time (MJGT), mean germination time (MGT), radicle emergence and field emergence and biochemical parameters *viz.,* electrical conductivity of seed leachate and dehydrogenase enzyme activity. The results also revealed that the seed vigour can be grouped in three categories *viz.,* high, medium and low vigour based on the relationship between MGT and field emergence. When the MGT was <25 hours, the seed lots which exceeds 85 per cent field emergence could be considered as high vigour; when the MGT was 25-26 hours, the seed lots with 70-85 per cent field emergence could be considered as medium vigour and when the MGT was>26 hours, the seed lots with below 70 per cent field emergence which could be considered as low vigour.

Key words: Blackgram, Field emergence, Germination, Radicle emergence, Vigour.

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] belongs to the family Fabaceae, is one of the important pulse crop grown in India. India is the largest producer, consumer and importer of blackgram. The total production was 21.00 lakh tonnes in an area of 35.15 lakh hectares. Among the states, Madhya Pradesh stands first in respect of area (24.11%) followed by Uttar Pradesh (16.71%) and Andhra Pradesh (11.05%), whereas in terms of production, Madhya Pradesh stands first (22.32%) followed by Andhra Pradesh (15.65%) and Uttar Pradesh (14.49%) (Indiastat, 2020-21).

The standard germination test is a universal test for seed quality to evaluate the maximum potential of a particular seed lot only under a controlled favourable climatic condition. Further, the standard germination test is time consuming and doesn't always show seed lot potential performance, especially if field conditions are not optimal (Mavi *et al*., 2016).

Seed lots that do not differ in germination may differ in deterioration level and may differ substantially in field performance; thereby, a vigour test is considered as a powerful when it classify the seed lots into more groups or levels (Kolasinska *et al*., 2000). Radicle emergence test is considered as a quick test to predict varying vigour levels and field performance of seed lots than the standard germination test. Farmers easily practice for radicle emergence test and do not require sophisticated equipment or highly skilled personnel and it could be used to shorten the decision period in the seed industry management. According to ISTA, blackgram seedlings are evaluated on 7th day for its germination percentage. These long days of evaluation period was time consuming. Hence, we need an alternate technology which would give a precise result in short period. In that case, the radicle emergence test is one

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of the options for quick prediction of seed vigour and field emergence.

With this background, the present study was carried out for standardization of radicle emergence test to predict seed vigour and field emergence in blackgram seed lots.

MATERIALS AND METHODS

The laboratory experiment was carried out at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2019-2020. Genetically pure ten seed lots [L_1 to L_4: high vigour lots (>90% germination), L_5 to L_7: medium vigour lots (80-90% germination) and L_8 to L_{10}: low vigour lots (<80% germination)] of blackgram var. VBN 6 obtained from the National Pulses Research Centre (NPRC), Vamban were used as the base seed materials for this study.

Radicle emergence test

Radicle emergence test was conducted through Top of Paper (TP) method. Eight replicates of 50 seeds each in ten seed lots were placed equidistance on germination paper moistened with distilled water in Petri-dish. The Petri-dishes were kept in germination room maintained at 25±2°C and relative humidity of 95±2%. The number of seeds that had produced the radicle of 1 mm and 2 mm long were recorded manually. The image analyser measurement from the initiation of radicle emergence at two hours interval was taken until the radicle reached 2 mm length (ISTA, 2019). From the periodical observations recorded the mean just germination time (MJGT), mean germination time (MGT) and radicle emergence per cent (with 1 mm and 2 mm radicle length) were calculated by following the method of (Ellis and Roberts, 1980).

Physiological and biochemical seed quality parameters

The blackgram seed lots were tested for different physiological seed quality parameters such as speed of germination, germination percentage, shoot and root length, dry matter production, hundred seed weight, field emergence percentage and vigour index as per ISTA seed testing protocols (ISTA, 2019).

Statistical analysis

Data obtained from the experiments were analysed using an analysis of variance (ANOVA) as a factorial combination of treatments. Mean values were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at 0.05 probability level. Values in per cent data were arcsine transformed before analysis. If the F test is non-significant it was indicated by the letters NS (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Mean just germination time, Mean germination time and radicle emergence percentage

The results of the present study revealed that the significant differences in the percentage emergence of radicle with 1 mm and 2 mm length were observed among the seed lots and also due to the duration of incubation by both manual and image analyser measurements. Irrespective of seed lots, the percentage of radicle emergence was significantly increased from 16 h (16 %) to 26 h (91 %) with 1 mm length and 18 h (19%) to 28 h (91 %) with 2 mm length. However, the maximum percentage of radicle emergence with 1 mm length was recorded at 26 h (91%); whereas the percentage of radicle emergence with 2 mm length was maximum at 28 h (91%) (Table 1). Similarly, in the image analyser measurement, the percentage of radicle emergence significantly increased from 16 h (18%) to 26 h (91%) with 1 mm length and 18 h (20%) to 28 h (91%) with 2 mm length (Table 2). However, the highest percentage of radicle emergence with 1 mm length was recorded at 26 h (91%); whereas the highest percentage of radicle emergence with 2 mm length was recorded at 28 h (91%).

Highly significant variations were observed in percentage radicle emergence with 1 mm length among the seed lots. The maximum radicle emergence per cent with 1 mm length was observed at 26 h in L_2 (97%) which was on par with L_1 and L_3 (96%) while it was minimum in L_{10} (81%). Similarly, the significant differences were also observed in percentage radicle emergence with 2 mm length among the seed lots. The highest radicle emergence per cent with 2 mm length was observed at 28 h in L_1 and L_2 (100%) followed by L_3 (98%) and L_4 (97%), while it was minimum in L_{10} (79%). The MJGT and MGT was minimum in lot 1 (18.96 and 23.52 h) and maximum in lot 8 and lot 10 (23.52 h and 27.84 h), respectively (Table 3). Among the different durations of manual and image analyser measurements, 26 h counting of radicle emergence with 1 mm length and 28 h counting of radicle emergence with 2 mm length were closely correlated with other seed vigour parameters (Plate 1 and 2).

These results showed that the low vigour seed lots required more time to reach 1 mm and 2 mm length of radicle emergence when compared to high vigour seed lots. Reasons for this delay might be attributed to variations in the metabolic activity between the seeds with different vigour levels. The seed lots with high metabolic activity response quickly for imbibition and proceed further for DNA repair and enzyme synthesis, while low vigour seeds respond slowly (Matthews and Powell, 2011). These results are in agreement with the findings of Mavi *et al.* (2010) in cucurbits and sweet corn seeds. Radicle emergence test as a good indicator for predicting field emergence potential, determining seed quality and classifying seed lots into different vigour status was also confirmed in pepper, cabbage, soybean and radish seeds by Demir *et al*. (2008) and Matthews *et al.* (2011).

Attainment of MJGT, MGT and radicle emergence per cent with 1 mm and 2 mm length was faster in high vigour seed lots compared to medium and low vigour seed lots. Mean germination time relates not only the timing of the

Table 1: Standardization of duration of incubation for radicle emergence to 1 mm and 2 mm length through manual measurement in blackgram seed lots.

Seed lots	Radicle emergence with 1 mm length (%)						Radicle emergence with 2 mm length (%)					
	Duration of incubation (D)						Duration of incubation (D)					
	16 h	18 h	20 h	22 h	24 h	26 h	18 h	20 h	22 h	24 h	26 h	28 h
L_1	28 (31.94)	42 (40.39)	60 (50.76)	84 (66.42)	96 (78.46)	96 (78.46)	34 (35.66)	52 (46.14)	62 (51.94)	84 (66.42)	100 (89.71)	100 (89.71)
L_2	28 (31.94)	48 (43.85)	80 (63.43)	93 (74.66)	97 (80.02)	97 (80.02)	30 (33.21)	52 (46.14)	84 (66.42)	96 (78.46)	100 (89.71)	100 (89.71)
L_3	20 (26.56)	39 (38.64)	67 (54.94)	91 (72.54)	91 (72.54)	95 (77.08)	20 (26.56)	40 (39.23)	68 (55.55)	88 (69.73)	98 (81.87)	98 (81.87)
L_4	16 (23.57)	38 (38.05)	67 (54.94)	91 (72.54)	95 (77.08)	95 (77.08)	18 (25.10)	36 (36.87)	70 (56.79)	92 (73.57)	92 (73.57)	96 (78.46)
L_5	16 (23.57)	34 (35.66)	68 (55.55)	93 (74.66)	95 (77.08)	95 (77.08)	18 (25.10)	34 (35.66)	68 (55.55)	91 (72.54)	91 (72.54)	95 (77.08)
L_6	12 (20.26)	32 (34.45)	64 (53.13)	86 (68.02)	86 (68.02)	89 (70.63)	16 (23.57)	32 (34.45)	60 (50.76)	82 (64.89)	89 (70.63)	89 (70.63)
L_7	14 (21.94)	36 (36.87)	64 (53.13)	80 (63.43)	80 (63.43)	90 (71.56)	24 (29.33)	36 (36.87)	41 (39.81)	53 (46.72)	65 (53.73)	85 (67.21)
L_8	10 (18.43)	20 (26.56)	40 (39.23)	52 (46.14)	84 (66.42)	88 (69.73)	6 (14.14)	11 (19.37)	19 (25.84)	31 (33.83)	59 (50.18)	83 (65.65)
L_9	12 (20.26)	17 (24.35)	29 (32.58)	53 (46.72)	73 (58.69)	85 (67.21)	12 (20.26)	16 (23.57)	28 (31.94)	52 (46.14)	64 (53.13)	84 (66.42)
L_{10}	5 (12.92)	9 (17.45)	37 (37.46)	45 (42.13)	57 (49.02)	81 (64.15)	8 (16.43)	14 (21.97)	36 (36.87)	52 (46.14)	56 (48.44)	78 (62.02)
Mean	16 (23.57)	32 (34.45)	58 (49.60)	77 (61.34)	85 (67.21)	91 (72.54)	19 (28.45)	32 (34.45)	54 (47.29)	72 (58.05)	81 (64.15)	91 (72.54)
	L	D	L × D				L	D	L × D			
SEd	0.40	0.31	1.00				0.42	0.32	1.03			
CD (P=0.05)	0.80	0.62	1.97				0.83	0.64	2.04			

(Figure in parenthesis indicate arcsine values).

Table 2: Prediction of time taken for radicle emergence to 1 mm and 2 mm length through image analyser in blackgram seed lots.

Seed lots	Radicle emergence with 1 mm length (%)						Radicle emergence with 2 mm length (%)					
	Duration of incubation (D)						Duration of incubation (D)					
	16 h	18 h	20 h	22 h	24 h	26 h	18 h	20 h	22 h	24 h	26 h	28 h
L_1	28 (31.94)	45 (42.13)	60 (50.76)	84 (66.42)	94 (75.82)	96 (78.46)	35 (36.27)	55 (47.87)	62 (51.94)	82 (64.89)	98 (81.87)	100 (89.71)
L_2	30 (33.21)	48 (43.85)	80 (63.43)	93 (74.66)	97 (80.02)	97 (80.02)	30 (33.21)	52 (46.14)	84 (66.42)	96 (78.46)	99 (84.26)	100 (89.71)
L_3	23 (28.65)	39 (38.64)	67 (54.94)	91 (72.54)	91 (72.54)	96 (78.46)	20 (26.56)	40 (39.23)	68 (55.55)	88 (69.73)	98 (81.87)	98 (81.87)
L_4	16 (23.57)	38 (38.05)	67 (54.94)	92 (73.57)	95 (77.08)	95 (77.08)	18 (25.10)	39 (38.64)	70 (56.79)	92 (73.57)	90 (71.56)	97 (80.02)
L_5	18 (25.10)	34 (35.66)	69 (56.16)	93 (74.66)	95 (77.08)	94 (75.82)	20 (26.56)	34 (35.66)	65 (53.73)	91 (72.54)	91 (72.54)	95 (77.08)
L_6	15 (22.78)	32 (34.45)	66 (54.33)	86 (68.02)	86 (68.02)	90 (71.56)	19 (25.84)	30 (33.21)	60 (50.76)	80 (63.43)	88 (69.73)	89 (70.63)
L_7	14 (21.97)	36 (36.87)	64 (53.13)	80 (63.43)	80 (63.43)	90 (71.56)	27 (31.30)	34 (35.66)	39 (38.64)	53 (46.72)	70 (56.79)	86 (68.02)
L_8	10 (18.43)	20 (26.56)	40 (39.23)	52 (46.14)	84 (66.42)	87 (68.86)	8 (16.43)	14 (21.97)	22 (27.97)	30 (33.21)	59 (50.18)	83 (65.65)
L_9	15 (22.78)	17 (24.35)	29 (32.58)	53 (46.72)	73 (58.69)	85 (67.21)	14 (21.97)	17 (24.35)	30 (33.21)	52 (46.14)	66 (54.33)	83 (65.65)
L_{10}	8 (16.43)	11 (19.37)	39 (38.64)	45 (42.13)	59 (50.18)	81 (64.15)	10 (18.43)	16 (23.57)	35 (36.27)	55 (47.87)	58 (49.60)	79 (62.72)
Mean	18 (25.10)	32 (34.45)	58 (49.60)	77 (61.34)	85 (67.21)	91 (72.54)	20 (26.56)	33 (35.06)	54 (47.29)	72 (58.05)	82 (64.89)	91 (72.54)
	L	D	L × D				L	D	L × D			
SEd	0.41	0.32	1.02				0.42	0.32	1.04			
CD (P=0.05)	0.82	0.63	2.01				0.83	0.64	2.05			

(Figure in parenthesis indicate arcsine values).

Table 3: Comparison of manual and image analyzer measurement of radicle emergence to 1 mm and 2 mm length in blackgram seed lots.

Seed lots	MJGT (h)	MGT (h)	Manual measurement		Image analyser measurement	
			Radicle emergence with 1 mm length @ 26 h (%)	Radicle emergence with 2 mm length @ 28 h (%)	Radicle emergence with 1 mm length @ 26 h (%)	Radicle emergence with 2 mm length @ 28 h (%)
L_1	18.96	23.52	96 (78.46)	100 (89.71)	96 (78.46)	100 (89.71)
L_2	19.44	23.28	97 (80.02)	100 (89.71)	97 (80.02)	100 (89.71)
L_3	20.40	24.72	95 (77.08)	98 (81.87)	96 (78.46)	98 (81.87)
L_4	19.68	24.00	95 (77.08)	96 (78.46)	95 (77.08)	97 (80.02)
L_5	20.64	25.20	95 (77.08)	95 (77.08)	94 (75.82)	95 (77.08)
L_6	21.12	25.92	89 (70.63)	89 (70.63)	90 (71.56)	89 (70.63)
L_7	21.84	25.68	90 (71.56)	85 (67.21)	90 (71.56)	86 (68.02)
L_8	23.52	27.84	88 (69.73)	83 (65.65)	87 (68.86)	83 (65.65)
L_9	22.32	25.92	85 (67.21)	84 (66.42)	85 (67.21)	83 (65.65)
L_{10}	23.52	27.84	81 (64.15)	78 (62.02)	81 (64.15)	79 (62.72)
Mean	21.14	25.39	91 (72.54)	91 (72.54)	91 (72.54)	91 (72.54)
SEd	0.335	0.382	1.72	1.49	1.49	1.48
CD (P=0.05)	0.685	0.780	3.52	3.05	3.05	3.03

(Figure in parenthesis indicate arcsine values); MJGT- Mean just germination time; MGT- Mean germination time.

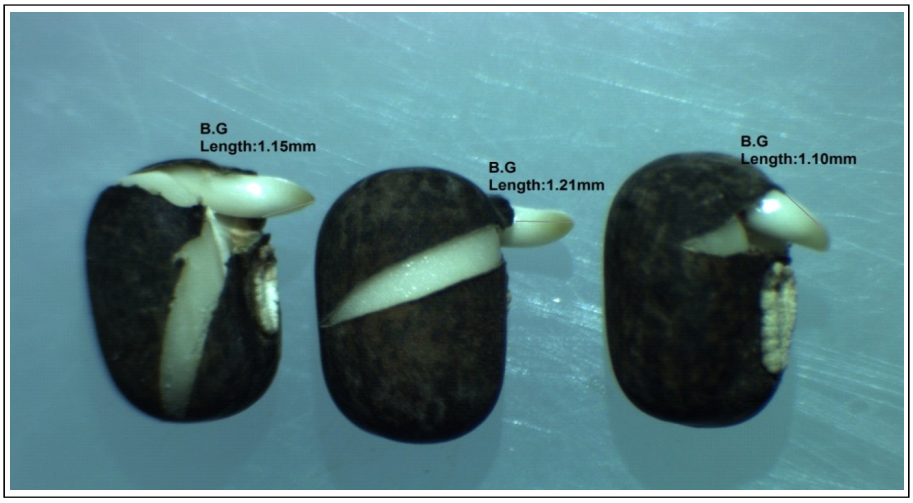

Plate 1: Radicle emergence at 26th hour in blackgram seeds.



Plate 2: Radicle emergence at 28th hour in blackgram seeds.

initiation of germination, but it also describes the spread over time for radicle emergence and seedling emergence, which related to seed size and varied among seed lots in vegetables (Demir *et al.,* 2008) and corn (Khajeh-Hosseini *et al.,* 2009). This is also a confirmation of the general proposition by Ellis and Roberts (1980) who stated that seedling size is determined by time taken to attain a required 2 mm radicle length from radicle emergence.

Physiological seed quality parameters

In high vigour seed lot (L_1), all the physiological parameters are superior to the low vigour seed lot (L_{10}). The per cent increase for all the observed parameters *viz.,* speed of germination, germination, dry matter production, vigour index and field emergence were 33, 40, 8, 76 and 46 %, respectively over the low vigour seed lot (Table 4 and Fig 1). Similar results were also reported in corn seeds by Navratil and Burris (1980), who reported that the field emergence of the seed lots over five sowings seemed to be largely determined by the time taken to emerge, which was greatly influenced by temperature, but was also significantly different among the seed lots. Similarly, the study on four seed lots of hybrid corn by TeKrony *et al*. (1989) showed that low vigour seed lots emerged slowly and resulted in low germination, dry matter production, vigour index and field emergence.

Classification of seed lots based on MGT and field emergence

The results of the study revealed that the seed vigour can be grouped in three class *viz.,* high, medium and low based on the relationship between MGT and field emergence. When the MGT was < 25 h, the field emergence exceeded 85 per cent which could be considered as high vigour; when the MGT was 25-26 h, the field emergence was 70-85 per cent which could be considered as medium vigour and when the MGT was >26 h, the field emergence was

Table 4: Evaluation of seed quality parameters in blackgram seed lots.

Seed lots	Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedling^{-1})	Vigour 100 index	seed weight (g)	Field emergence (%)
L_1	7.2	98 (81.87)	10.4	15.2	0.287	2509	4.98	95 (77.08)
L_2	7.0	97 (80.02)	10.1	15.0	0.289	2435	4.96	95 (77.08)
L_3	6.8	94 (75.82)	9.5	14.5	0.280	2256	4.91	90 (71.56)
L_4	6.4	93 (74.66)	9.0	14.4	0.275	2176	4.93	88 (69.73)
L_5	6.1	89 (70.63)	9.1	14.3	0.261	2083	4.86	84 (66.42)
L_6	6.3	88 (69.73)	9.3	14.5	0.255	2094	4.89	82 (64.89)
L_7	6.4	84 (66.42)	8.1	13.7	0.256	1831	4.90	80 (63.43)
L_8	6.0	74 (56.34)	8.0	13.3	0.231	1576	4.85	67 (54.94)
L_9	5.6	71 (57.41)	7.5	13.1	0.225	1463	4.86	65 (53.73)
L_{10}	5.4	70 (56.79)	7.3	13.0	0.210	1421	4.83	65 (53.73)
Mean	6.3	86 (68.02)	8.8	14.1	0.257	1984	4.90	81 (64.15)
SEd	0.07	1.5	0.10	0.15	0.0031	29.2	0.080	1.17
CD (P=0.05)	0.14	2.9	0.21	0.32	0.0063	59.7	NS	2.39

(Figure in parenthesis indicate arcsine values).

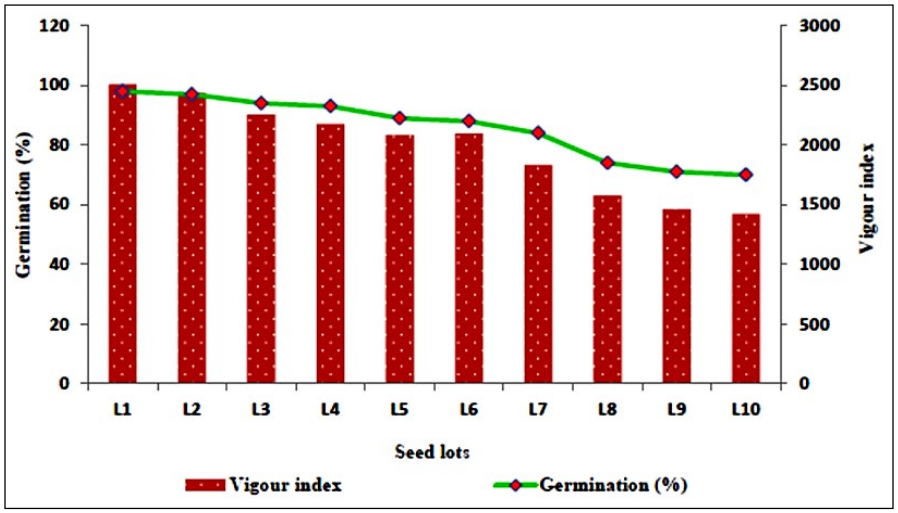

Fig 1: Evaluation of germination and vigour index in blackgram seed lots.

Table 5: Classification of seed lot vigour based on MGT and field emergence in blackgram.

Seed quality classification	Mean germination time (h)	Field emergence (%)
High vigour	<25	>85
Medium vigour	25-26	70-85
Low vigour	>26	<70

below 70 per cent which could be considered as low vigour (Table 5). Similar findings were also reported by Suganthi and Selvaraju (2017) in groundnut seeds.

CONCLUSION

Radicle emergence test is a quick test to predict seed vigour, field emergence potential and ranking seed lots. The time taken to produce 2 mm length of radicle emergence was closely associated with seed vigour and field emergence when compared to time taken for 1 mm length of radicle emergence. Finally, the study concluded that counting 2 mm radicle emergence at 28th hour could be used as quick method to assess the quality of blackgram seed lots by the seed analysts and seed industry.

Conflict of interest

All authors declared that there is no conflict of interest.

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