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Original Research Article

Tz test: A quick and reliable predictor for seed quality assessment in guar (Cyamopsis tetragonoloba (L.) Taub)

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ABSTRACT
A total of fifteen lots of five distinct types were put through a series of viability and vigour tests to determine which ones were good indicators of various quality characteristics. Standard germination, seedling length, the Vigor index-1, the electrical conductivity test, the tetrazolium test, the dehydrogenase activity test, and field emergence were all observed. For all of the criteria examined, there was a significant amount of variation between these seed batches and cultivars. For the conventional germination, electrical conductivity, tetrazolium, dehydrogenase, and field emergence tests, variety HG-884 performed best. Lot-2 (HG-563) performed better than the other lots for standard germination and the tetrazolium test, while lot-3 (GH563) performed best for seedling length and dry weight, lot-7 (GH-870) performed better than the other lots for electrical conductivity, and lot-11 (HG-884) performed best for the dehydrogenase test and field emergence. Except for electrical conductivity, most of the variables showed a substantial positive correlation with typical germination and field emergence. While dehydrogenase activity testing had the most reliability for predicting standard germination, tetrazolium testing had the highest reliability for predicting field emergence. The criteria seedling length, seedling dry weight, electrical conductivity, tetrazolium test, and dehydrogenase activity test can be utilised as predictors of standard germination and field emergence, according to correlation coefficient analysis and regression analysis.

1. Introduction

Cyamopsis tetragonoloba (L.) Taub, sometimes known as clusterbean, is a significant pulse crop grown in both rainfed and irrigated regions of the world. Clustrbean seeds have a high commercial value and the possibility of losses in germination and vigour over time emphasizes the importance of the development of satisfactory seed vigour tests for this species. The key factor limiting their productivity and output is the poor quality of their seed, which prevents proper crop stand establishment. Quality seeds provide the best possible use of all other inputs, including fertilisers, irrigation, pesticides, etc., in addition to providing the maximum economic and social returns of any input. Because high-quality seed functions as a catalyst for maximising the potential of other inputs, its availability at planting time is crucial for reaching agricultural production goals. There are several criteria that have been devised to assess seed quality. Some of these evaluations have been called "viabilty" and "vigour" tests. These assays could be used to accurately forecast seedling emergence in the field. Since the Tz test has been successfully used for many species and produces results within a relatively short time, it is important to develop or improve existing methodology. Therefore, it was intended for the current study to: (1) assess the various guar seed lots for various characteristics relating to seed quality; and (2) compare the effectiveness of various seed viability and vigour tests as a predictor of typical germination and seedling establishment.

2. Materials and methods

The research material of present study comprised of fifteen seed lots of five varieties of guar viz. HG-563, HG-365, HG-870, HG-884 & HG-2-20. Three seed lots of each variety was collected from different sources i.e. RDS Farm-CCS HAU Hisar, KVK-Sirsa, CRS- Sirsa, Deptt. of Seed Science & Technology and different private sources. The experiment was carried out at the Seed Science & Technology farm and lab at CCS HAU Hisar. The following criteria were applied to all samples:

Standard Germination (%): Standard seed testing was done in accordance with international guidelines [1]. One hundred seed from each seed lot were placed between germination papers replicated three times at 25°C. standard germination was recorded after 14 days.

Seedling Length (cm): At the time of the final count of the standard germination, three replicates of 10 seedlings were chosen at random from the normal seedlings, and the average length was measured in cm.

Vigour index-1: From the observations under various tests, the vigour index was calculated by:

Vigour index-I = Standard Germination (%) X Average Seedling Length (cm)

Electrical Conductivity Test (ms/cm/seed) : A 100 ml beaker containing 75 ml of distilled water was used to soak three replicates of 50 normal seeds while keeping the temperature at 25°C [2]. After 24 hours, the electrical conductivity was assessed using a conductivity metre and expressed as ms/cm/seed.

Tetrazolium Test (%): Tetrazolium testing was done in accordance with Moore [3]. For 16 hours at 25°C, three duplicates of 50 seeds were ingested between papers to activate the dehydrogenase enzymes. After the seed coat was removed, the seeds were dyed for 4 hours at 38 degrees Celsius in Petri plates with a 0.5 percent tetrazolium solution (2, 3, 5-triphenyl tetrazolium chloride). The percentage of seeds that were fully dyed red were regarded as viable seeds.

Dehydrogenase activity test (O.D.): DHA testing was done in accordance with Kittock and Law [4]. Each seed lot's three copies of 25 seeds each were pulverised to fit through a 20 mesh screen. 2000 mg of flour were soaked for two hours in 5 ml of a 0.5% tetrazolium solution that had a pH of 7 and was 35°C. The supernatant was then drained off after 3 minutes of centrifuging at 1000 rpm. Formazan was extracted using 10 ml of acetone for 16 hours, then the solution's absorbance was measured at 520 nm using a Systronics spectrophotometer-169. The measurements were made using an optical density scale (O.D.).

Field emergence (%):Up until seedling establishment, the number of seedlings that emerged each day was counted (21days).

The standard procedure recommended by Panse and Sukhatme [5] was used to examine the data obtained from studies performed in CRD and RBD.

3. Results and discussion

The mean squares due to lots were highly significant for all the seven traits revealing the presence of sufficient amount of variability among all the lots for all the characters studied.

Table-1. ANOVA for different quality parameters:

Sr.No.	Characters	Mean squares		
		Mean squares	Error	CV%
	Degree of freedom	14	30	
1	Standard Germination	1052.260**	3.089	2.290
2	Seedling Length	10.809**	0.475	3.510
3	Vigour index-1	1390.927**	9.737	6.314
4	Electrical Conductivity	0.054**	0.001	3.728
5	Tetrazolium Test	895.517**	4.644	2.750
6	Dehydrogenase Test	0.075**	0.000	3.486
7	Field Emergence	561679.281**	2854.071	3.480

**Significant at 1%

Standard Germination (%): The standard germination test had overall mean 77 and range varied from 23 to 93. Maximum germination percentage was observed in lot-2 whereas minimum was observed in lot-4. Varietal mean was found maximum for genotype HG-884 (83) and minimum for HG-365 (64).

Seedling Length (cm): Seedling length showed variation from 13.93 to 22.37 with a overall mean of 19.64.The maximum seedling length was observed in lot-3 and lowest in lot-4. The varietal mean was found maximum for HG-563 and HG-2-20 (20.41) while minimum for HG-365 (18.13).

Vigour index-1: The vigour index-I showed a variation ranging from 314.80 to 2065.77. The highest and lowest value of vigour index-I were recorded for lot-3 and lot-4 respectively, whereas maximum and minimum varietal mean was recorded for genotype HG-884(1635.49) and HG-365 (1254.05) respectively.

Electrical Conductivity Test (ms/cm/seed): The range of electrical conductivity of seed exudates varied from 0.593 to 1.010 with an overall mean of 0.708. Lot-7 was considered best as it had lowest amount of leachates whereas lot-4 considered as poor. Among varieties, HG-884 was considered superior whereas HG-563 was considered as inferior (table-2).

Tetrazolium Test (%): The range of viable seeds through tetrazolium test varied from 29 to 94 with an overall mean 78. Lot-2 showed highest viability percentage (94) whereas lot-4 showed lowest (29).The genotype HG-884 showed highest viability percentage (84) whereas HG-365 showed lowest value (67).

Dehydrogenase activity test (O.D.): The character DHA showed a variation ranging from 0.267 to 0.800. The highest and lowest value of dehydrogenase were recorded for lot-11(0.800) and lot-4(0.267) respectively, whereas maximum and minimum varietal mean was recorded for genotype HG-884(0.727) and HG-365 (0.540) respectively.

Field emergence (%): The field emergence showed variation ranging from 14 to 80 with an overall mean of 50. Lot 11 showed highest field emergence (80) whereas, lot-4 showed lowest field emergence (14). Among the varieties, HG-884 showed highest field emergence (63) while HG-870 showed lowest field emergence (30).

Correlation Coefficient (r) between field and laboratory parameters: Table 3 shows the correlation coefficient between laboratory and field characteristics for five guar types. With the exception of electrical conductivity, all of the seed quality indicators and field emergence were strongly and positively associated. The standard germination demonstrated a significant positive correlation with seedling length (0.844), field emergence (0.755), Vigour index-1 (0.985), tetrazolium test (0.998), and dehydrogenase activity test (0.916), while it demonstrated a significant negative correlation with electrical conductivity (-0.829). The dependability of germination test as a predictor of field emergence was demonstrated by the positive, substantial relationship between the standard germination test and field emergence. Verma et al. [6] reported

similar outcomes in barley. Standard germination also demonstrated a substantial favourable correlation with the TZ test. Dahiya et al. [7] in oat also came to similar conclusions.

The seedling length was significantly associated with standard germination (0.844**), vigour index-1 (0.908**), tetrazolium test (0.829**), dehydrogenase activity test (0.671**) and field emergence (0.513*) while it was negatively correlated with electrical conductivity (-0.596*). Vigour index-1 was significantly associated with standard germination (0.985**), seedling length (0.908**), tetrazolium test (0.979**) dehydrogenase activity test (0.888**), field emergence (0.735**).

All of the parameters under study showed a negative correlation with the electrical conductivity test results. The amount of electrolytes that seep out of the seeds as they decay was determined by the electrical conductivity test. Genotypes with declining and low vigour are linked to poor membrane structure and leaky cells. Verma et al. [8] in barley similarly came to the same conclusions.

Table 2. Mean values of different quality parameters for seeds of guar.

Varieties	Seed Lot	SG	SL	Vigour index-I	FE	EC	TZ	DHA
HG-563	1	47	18.07	854.50	26	0.994	52	0.350
	2	93	20.80	1,941.03	73	0.784	94	0.790
	3	92	22.37	2,065.77	73	0.711	92	0.767
Varietal mean		78	20.41	1620.43	57	0.829	79	0.636
HG-365	4	23	13.93	314.80	14	1.010	29	0.267
	5	88	20.37	1,792.47	67	0.593	89	0.717
	6	82	20.10	1,654.87	63	0.601	83	0.637
Varietal mean		64	18.13	1254.05	48	0.735	67	0.540
HG-870	7	88	19.80	1,735.77	40	0.579	91	0.630
	8	73	20.03	1,461.10	24	0.726	74	0.523
	9	75	18.87	1,408.77	25	0.671	76	0.543
Varietal mean		78	19.57	1535.21	30	0.658	80	0.565
HG-884	10	76	20.07	1,525.27	56	0.668	77	0.710
	11	90	19.20	1,734.30	80	0.633	91	0.800
	12	83	19.77	1,646.90	54	0.596	85	0.670
Varietal mean		83	19.68	1635.49	63	0.632	84	0.727
HG-2-20	13	84	19.30	1,627.50	68	0.675	86	0.763
	14	81	20.23	1,639.07	51	0.630	82	0.650
	15	75	21.70	1,627.50	28	0.760	76	0.500
Varietal mean		80	20.41	1631.36	49	0.688	81	0.638
Overall mean		77	19.64	1535.31	50	0.708	78	0.621
Range		23-93	13.93-22.37	314.80-2065.77	14-80	0.593-1.010	29-94	0.267-0.800
CD		2.945	1.155	89.515	5.386	0.044	3.611	0.036

SG= Standard Germination, SL= Seedling Length, FEI=Field Emergence, EC= Electrical Conductivity, TZ= Tetrazolium Test, DHA= Dehydrogenase activity.

Table 3. Correlation Coefficient (r) for quality parameters of all seed lots of guar.

Tests	SG	SL	FE	EC	TZ	DHA	V-I
SG							
SL	0.844**						
FE	0.755**	0.513*					
EC	-0.829**	-0.596*	-0.545*				
TZ	0.998**	0.829**	0.752**	-0.831**			
DHA	0.916**	0.671**	0.916**	-0.739**	0.913**		
V-I	0.985**	0.908**	0.735**	-0.775**	0.979**	0.888**	

*Significant at 5% , **Significant at 1%, NS= non significant.

SG= Standard Germination, SL=Seedling Length, FEI=Field Emergence, EC= Electrical Conductivity, TZ= Tetrazolium Test, DHA= Dehydrogenase activity.

Tetrazolium test have been observed significantly associated with standard germination (0.998**), Vigour index-1 (0.979**), dehydrogenase activity test (0.913**), field emergence (0.752**) and seedling length (0.829**) while it was negatively significanct with electrical conductivity (-0.831**). Dehydroganase activity test have been observed significantly associated with standard germination (0.916**), seedling length (0.671**), Vigour index-1 (0.888**), tetrazolium test (0.913**), field emergence (0.916**) and negatively significant with electrical conductivity (-0.739**).

Prediction of standard germination (%) by different viability and vigour parameters: Regression analysis was used to examine the simultaneous fluctuation of all seven characteristics that had a strong correlation with typical germination. The estimated mean germination percentage determined by a number of factors, including seedling length (76.75567), Vigour index-1 (76.7556), electrical conductivity (76.7554), tetrazolium test (76.75553), dehydrogenase activity (76.75567), and field emergence (76.7556, were comparable to the value determined by standard germination methods (76.7556). Tetrazolium test (R2=0.9970), DHA (R2=0.8399), seedling length (R2=0.7126), electrical conductivity (R2=0.6870), field emergence index (R2=0.5703), and Vigour index-1 (R2=0.9701) all had the highest R2 values since they were shown to be substantially connected with conventional germination. The greatest value (R2=0.9988) was observed when the data were pooled. Tetrazolium test and DHA test can be utilised as trustworthy predictors of standard germination, according to the regression analysis between standard germination and other viability and vigour indices. The tetrazolium test showed good correlation with germination indicating its suitability for estimation of viability. The highest value of R2 in multiple regression indicated that all the parameters may be used individually or together to get more accurate information.

Table 4. Relationship between standard germination and other parameters

Kind of test	Mean and Range		Correlation	Regression	R-square
	Actual	Estimated			
SL	19.64(13.93-22.37)	76.75567 (29.224-99.476)	0.844**	-86.829+(8.325)x	0.7126
V-I	1535.31(314.80-2065.77)	76.7556 (24.723-99.370)	0.985**	11.303+(0.043)x	0.9701
FE	50 (14-80)	76.7556 (53.708-96.621)	0.755**	44.294+(0.657)x	0.5703
EC	0.708(0.593-1.010)	76.7554 (41.859-91.746)	-0.829**	158.799+(-115.748)x	0.6870
TZ	78(29-94)	76.75553 (22.950-90.417)	0.998**	-8.077+(1.082)x	0.9970
DHA	0.621(0.267-0.800)	76.75567 (38.387-96.122)	0.916**	9.514+(108.259)x	0.8399
Pooled		76.7556			0.9988
SG	76.7556(22.950-92.991)	29.224-99.467			

** Significant at1%

Table 5. Relation between Field Emergence and other parameters

Kind of test	Mean and Range		Correlation	Regression	R-square
	Actual	Estimated			
SG	77(23-93)	49.4222 (2.461-63.815)	0.755**	-17.219+(868)x	0.5703
SL	19.64 (13.93-22.37)	49.42233 (16.199-65.297)	0.513**	-64.919+(5.822)x	0.2634
V-I	1535.31(314.80-2065.77)	49.42227 (4.761-68.833)	0.735**	-6.758+(0.037)x	0.5407
EC	0.708(0.593-1.010)	49.42227 (23.038-60.757)	-0.545*	11.454+(-87.515)x	0.2971
TZ	78 (29-94)	49.42213 (2.854-63.7440	0.752**	-24.000+(0.937)x	0.5650
DHA	0.621(0.267-0.800)	49.4222 (5.344-71.670)	0.916**	-27.825+(124.369)x	0.8386
Pooled		49.42227			0.8941
FE	49.42227(15.796-76.25)				

Prediction of field emergence (%) by different viability and vigour parameters:

The estimated mean of field emergence, obtained by various viability and vigour viz.standard germination (49.4222), seedling length (49.42233), Vigour index-1

(49.42227), electrical conductivity (49.42227), tetrazolium test (49.42213), dehydrogenase activity (49.4222), were at par with the value obtained by field emergence value (49.42227) as shown in table-5. Maximum value of R2 was obtained for DHA (R2=0.8386) followed by standard germination (R2=0.5703) as these tests are found highly correlated with field emergence. The regression analysis between field emergence and various viability and vigour parameters shows maximum reliability with DHA test and standard germination. When data were pooled the value is observed to be maximum indicating thereby that all these multiple tests may be used to get more accurate information as compared to single test for estimating the field emergence. Dahiya *et al*. [9] in oat and Dahiya *et al*. [10] in sunflower reported similar findings.

Regression and viability tests such seedling length, seedling dry weight, electrical conductivity, tetrazolium test, and dehydrogenase test can all be employed as accurate predictors of typical germination and field emergence in guar based on correlation. Tetrazolium and dehydrogenase tests had excellent correlations in terms of forecasting typical germination and field emergence.

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