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Morphological Characterization and Genetic Diversity Studies in Blackgram

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ABSTRACT

Morphological characterization of 40 blackgram genotypes using 11 morphological descriptors namely, hypocotyl anthocyanin coloration, plant growth habit, stem color, stem pubescence, leaflet terminal shape, foliage color, leaf pubescence, petiole color, pod pubescence, seed color and seed lusture was undertaken in the present investigation using standard descriptors of blackgram. Principal component analysis revealed four principal components with eigen value >1. These components contributed for a total variability of 64.882%. Component 1 (PC 1) had contributed maximum of 22.479% towards the total variability. Shannon-Weaver diversity index results revealed high diversity index value (H' ≥ 0.75) for the morphological traits, namely, hypocotyl anthocyanin coloration, plant growth habit, stem color, petiole color, seed color and seed lusture, while stem pubescence, leaflet terminal shape, foliage color and seed color had intermediate (H'= 0.5-0.75) phenotypic diversity.

Keywords Characterization, Blackgram, Principal component analysis, Shannon-Weaver diversity index, Gentic diversity.

INTRODUCTION

Blackgram is an important source of protein and is widely cultivated in the Indian subcontinent, mostly as fallow crop after rice cultivation. A sound knowledge of the morphological marker traits with respect to the germplasm and breeding material helps in their classification, identification and documentation and hastens their utilization in the crop improvement programs. Characterization consists of recording those characters which are highly heritable and can easily be seen by the naked eye and are expressed in all environments (IBPGR 1985). In this context, qualitative morphological characters are important for plant description, since they are less influenced by environment. Further, many of the qualitative traits are related to biotic/abiotic stress resistance/ tolerance. The blackgram plants with lanceolate

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leaf shape (narrow leaves) in most cases have been observed to be drought tolerant, similarly, pubescent are noticed to be more tolerant against some insects, whereas glabrous cultivars are easy for harvesting and threshing. Morphological characterization of urdbean varieties is also essential for their protection under Plant Variety Protection (PVP) legislation, because varietal testing for Distinctness, Uniformity and Stability (DUS) is the basis for granting protection of new variety under Protection of Plant Variety and Farmers Right (PPV and FR) Act. Testing of DUS assures that the new variety is distinct from other varieties and ensures that the users will grow a variety with uniform properties. In this context, the present investigation was undertaken with objective of morphological characterization and estimation of genetic diversity in few blackgram genotypes for their use in crop improvement programs.

Table 1. Details of blackgram genotypes studied in the present investigation.

Sl. No.	Germplasm No.	Genotype	Source
1.	GP9	TU-94-2	ARS, Ghantasala, Andhra Pradesh
2.	GP11	TGBG-26	ARS, Ghantasala, Andhra Pradesh
3.	GP12	TGBG-344	ARS, Ghantasala, Andhra Pradesh
4.	GP13	TGBG-74	ARS, Ghantasala, Andhra Pradesh
5.	GP14	TGBG-143	ARS, Ghantasala, Andhra Pradesh
6.	GP15	TGBG-281	ARS, Ghantasala, Andhra Pradesh
7.	GP16	KUG-216 × BG 018-2	ARS, Ghantasala, Andhra Pradesh
8.	GP17	DPU-8831 × VBG 4-088	ARS, Ghantasala, Andhra Pradesh
9.	GP18	LBG-645	RARS, Lam Farm, Guntur, Andhra Pradesh
10.	GP19	TBG-104	RARS, Tirupati, Andhra Pradesh
11.	GP20	WBG-108	ARS, Ghantasala, Andhra Pradesh
12.	GP21	KUG-216 × PU 40	ARS, Ghantasala, Andhra Pradesh
13.	GP23	PU-40	GB Pant University of Agricultural Sciences and Technology, Pantnagar, Uttarakhand
14.	GP24	TU-18	ARS, Ghantasala, Andhra Pradesh
15.	GP26	PU-31	GB Pant University of Agricultural Sciences and Technology, Pantnagar, Uttarakhand
16.	GP27	TGBG-258	ARS, Ghantasala, Andhra Pradesh
17.	GP28	IPU-2-43	ARS, Ghantasala, Andhra Pradesh
18.	GP29	LBG-788	RARS, Lam Farm, Guntur, Andhra Pradesh
19.	GP30	KUG-216 × SPS 5	ARS, Ghantasala, Andhra Pradesh
20.	GP31	TGBG-136	ARS, Ghantasala, Andhra Pradesh
21.	GP32	T9	ARS, Ghantasala, Andhra Pradesh
22.	GP33	TGBG-401	ARS, Ghantasala, Andhra Pradesh
23.	GP34	LBG-752	RARS, Lam Farm, Guntur, Andhra Pradesh
24.	GP36	LBG-20	RARS, Lam Farm, Guntur, Andhra Pradesh
25.	GP37	Tutiminumu	ARS, Ghantasala, Andhra Pradesh
26.	GP39	TBG-106	RARS, Tirupati, Andhra Pradesh
27.	GP40	SRI	ARS, Ghantasala, Andhra Pradesh
28.	GP41	Maruthi	ARS, Ghantasala, Andhra Pradesh
29.	GP42	Nandhi	ARS, Ghantasala, Andhra Pradesh
30.	GP43	GBG-47	ARS, Ghantasala, Andhra Pradesh
31.	GP44	GBG-45	ARS, Ghantasala, Andhra Pradesh
32.	GP46	GBG-1	ARS, Ghantasala, Andhra Pradesh
33.	GP50	GKB-4	ARS, Ghantasala, Andhra Pradesh
34.	GP51	VBG-12-110	NRPC, Vamban, Tamil Nadu
35.	GP52	VBG-13-3	NRPC, Vamban, Tamil Nadu
36.	GP53	VBG-14-16	NRPC, Vamban, Tamil Nadu
37.	GP54	VBG-12-034	NRPC, Vamban, Tamil Nadu
38.	GP56	IPU-11-2	ARS, Ghantasala, Andhra Pradesh
39.	GP58	VBG-17-026	NRPC, Vamban, Tamil Nadu
40.	GP59	ADT-6	ARS, Ghantasala, Andhra Pradesh

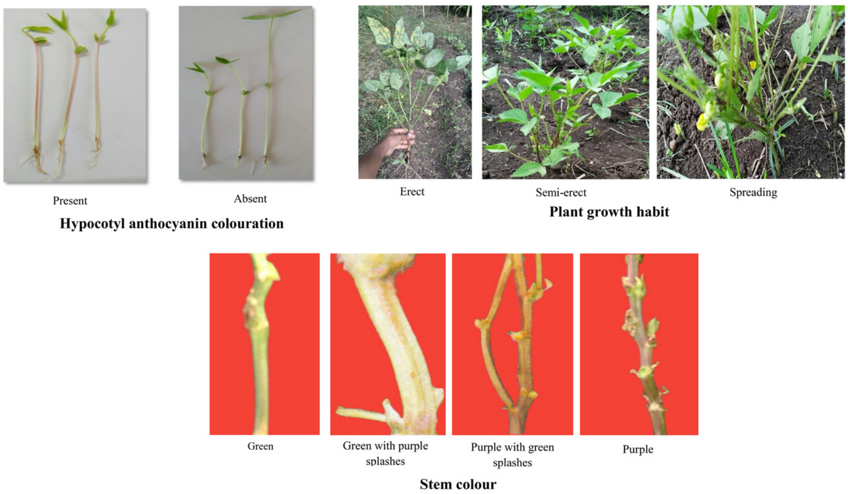

Plate 1. Descriptors for hypocotyl anthocyanin coloration, plant growth habit and stem color.

MATERIALS AND METHODS

Experimental material for the present investigation comprised of 40 blackgram genotypes obtained from Agricultural Research Station, Ghantasala (27 Nos.), Regional Agricultural Research Station, Lam Farm, Guntur (4 Nos.) and Regional Agricultural Research Station, Tirupati (2 Nos.), Andhra Pradesh State in addition to collections from G.B Pant University of Agricultural Sciences and Technology, Pantnagar (2 Nos.) and National Pulses Research Center, Vamban (5 Nos.) (Table 1). These genotypes were sown during *rabi* 2020-21, Agricultural College, Bapatla. The experiment was laid out in a Randomized Complete Block Design with three replications. Each genotype was grown in 6 rows of 4 m length with a spacing 30 cm between rows and 10 cm between plants within the row. The crop was raised under irrigated conditions and all recommended practices were followed to raise a healthy crop. Data was collected on 40 genotypes with respect to the DUS descriptors of 11 morphological characters and was used for characterization of the genotypes. The descriptors like plant growth habit, Stem color, stem pubescence, leaflet terminal shape, foliage color and leaf pubescence were recorded at 50 % flowering stage. Petiole color and pod pubescence were recorded when the green pods were fully developed. Seed color was recorded from mature seeds under sunlight while, seed lusture data was recorded from mature and dried seeds also under light conditions. Hypocotyl anthocyanin coloration was recorded when the cotyledons unfolded as per Rao *et al*. (2007). Phenotypic diversity for the above morphological descriptors was determined using Shannon-Weaver Diversity Index (H') as per the procedure described by Perry and McIntosh (1991). The Shannon-Weaver diversity index was calculated as follows.

$$H' = 1 - \sum_{i=1}^{n} pi \; loge \; pi$$

Where pi is the proportion of accessions in the i^{th} class of an n-class character and n is the number of phenotypic classes for a character. Shannon-Weaver Diversity indices were, standardized by dividing each value of H' by loge n to keep the value in a range of 0 to 1 in order to estimate the importance of phenotypic diversity. The analysis was carried out using MSEXCEL. Further, an arbitrary scale of diversity indices adopted from Jamago (2000) was used to categorize the computed indices into low (H'<0.50), intermediate (H'=0.5-0.75) and high (H' ≥ 0.75). Principal component analysis was also carried out using XLSTAT version 2021.2.2 software.

RESULTS AND DISCUSSION

The frequency distribution of each phenotypic class,

Table 2. Frequency distribution of blackgram genotypes for different morphological traits.

Sl. No.	Character	Descriptor	Frequency	%
1.	Hypocotyl anthocyanin coloration	Present	11	27.5
		Absent	29	72.5
		Total	40	100
2.	Plant growth habit	Erect	7	17.5
		Semi-erect	12	30
		Spreading	21	52.5
		Total	40	100
3.	Stem color	Green	1	2.5
		Purple	18	45
		Purple with green splashes	12	30
		Green with purple splashes	9	22.5
		Total	40	100
4.	Stem pubescence	Presence	33	82.5
		Absence	7	17.5
		Total	40	100
5.	Leaflet terminal shape	Ovate	25	62.5
		Deltoid	13	32.5
		Cuneate	1	2.5
		Lanceolate	1	2.5
		Total	40	100
6.	Foliage color	Green	34	85
		Dark green	6	15
		Total	40	40
7.	Leaf pubescence	Presence	0	0
		Absence	40	100
		Total	40	100
8.	Petiole color	Green	4	10
		Purple	13	32.5
		Green with purple splashes	23	57.5
		Total	40	100
9.	Pod pubescence	Presence	36	90
		Absence	4	10
		Total	40	100
10.	Seed color	Green	8	20
		Greenish brown	6	15
		Brown	11	27.5
		Black	14	35
		Mottled	1	2.5
		Total	40	100
11.	Seed lusture	Shiny	19	47.5
		Dull	21	52.5
		Total	40	100

estimated for each character for the genotypes studied, are presented in Table 2 and Plates 1-4 for the 11 morphological descriptors studied in the present investigation.

Hypocotyl anthocyanin coloration

Hypocotyl anthocyanin pigmentation is a useful morphological marker in genetic studies and cultivar identification. The genotypes are grouped based on the presence or absence of the hypocotyl anthocyanin pigmentation. In the present study, as many as 29 (72.5%) genotypes showed absence of hypocotyl anthocyanin coloration. Only 11 (27.5%) genotypes, showed the presence of hypocotyl anthocyanin coloration.

Plant growth habit

Growth habit is also considered to be an important DUS characteristic in blackgram. The genotypes were classified into those possessing erect, semi-erect or spreading growth habit. In the present study, majority of the genotypes (52.5%) exhibited spreading growth habit, followed semi-erect (30%) and erect (17.5%) growth habit.

Stem color

In the present investigation four classes of stem color namely, green, green with purple splashes, purple with green splashes and purple (Plate 3) were observed among the 40 genotypes in the studied. Most of the genotypes recorded purple stem color (45%), followed by purple with green splashes (30%) and green with purple splashes (22.5%). Only one genotype (2.5%) studied had recorded green stem color in the present investigation.

Stem pubescence

Pubescence on stem is a useful trait which makes the plant more tolerant against some insect pests. In the present investigation, two classes of stem pubescence namely, presence and absence of pubescence on stem (Plate 2) were observed among all genotypes. Most of the genotypes exhibited presence of pubescence (82.5%) on stem, while 17.5% genotypes exhibited absence of pubescence on stem.

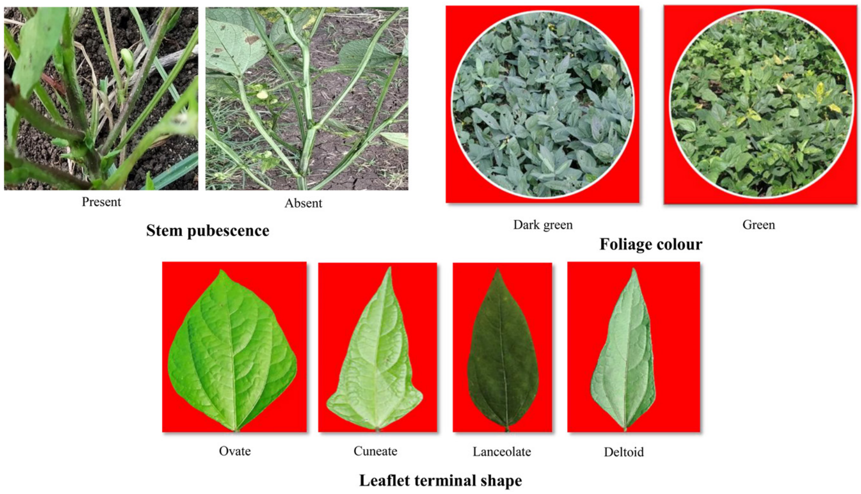

Plate 2. Descriptors for stem pubescence, foliage color and leaflet terminal shape.

Leaflet terminal shape

Four classes of terminal leaflet shape namely, ovate, cuneate, lanceolate and deltoid were observed among the genotypes studied and the results are depicted in Plate 2. As many as 25 genotypes (62.5%) showed ovate, leaflet terminal shape, followed by 13 genotypes (32.5%) with deltoid, one genotype (2.5%) with cuneate and one genotype (2.5%) with lanceolate leaflet terminal shape. Plants with lanceolate leaf shape (narrow leaves) have been reported to be associated with drought tolerance and hence, may be utilized for breeding blackgram varieties suitable for rainfed and moisture stress conditions (Ghafoor *et al*. 2001).

Foliage color

The foliage color ranged from green to dark green (Plate 2) in the genotypes studied in the present investigation. As many as 34 genotypes (85%) showed green stem color whereas six genotypes (15%)

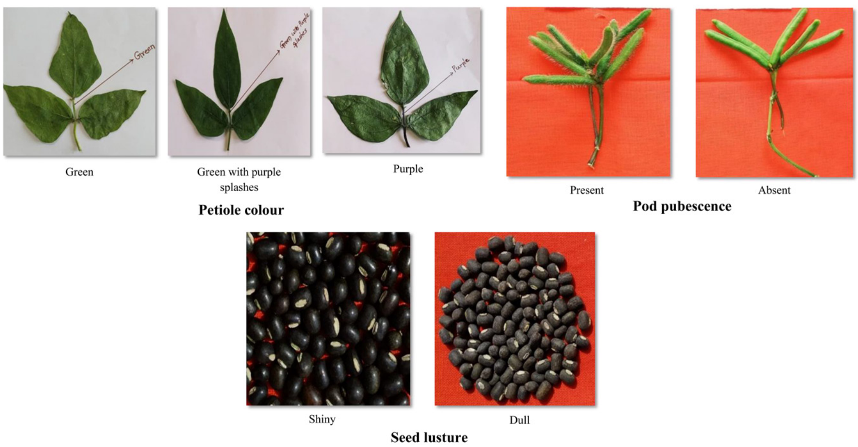

Plate 3. Descriptors for petiole color, pod pubescence and seed lusture.

Plate 4. Descriptors for seed color.

showed dark green stem color.

Leaf pubescence

Pubescence on plant is an important trait which makes the plant more tolerant against biotic stresses. In the present study, absence of leaf pubescence was observed for all the 40 genotypes studied.

Petiole color

Three classes of petiole color, namely, green, green with purple splashes and purple (Plate 3) were observed among the genotypes studied in the present investigation. As many as 23 genotypes (57.5%) showed green with purple splashes, followed by 13 genotypes (32.5%) with purple and four genotypes (10%) with green pigmentation. Devi *et al*. (2019) reported that genotypes with purple petiole pigmentation and purple splashes showed resistant to moderately resistant reaction to Mung Bean Yellow Mosaic Virus (MYMV) infection, while the genotypes with green colour petiole were susceptible to highly susceptible for MYMV infection. In the present study, 36 genotypes had exhibited purple and purple splashes petiole color. Hence, these genotypes may be utilized in the development of varieties resistant to MYMV infection.

Pod pubescence

Two classes of pod pubescence namely, presence and absence of pubescence on pod (Plate 3) was recorded in the genotypes studied in the present investigation. Majority of the genotypes studied (90%) recorded presence of pubescence on pod. Only four genotypes (10%) recorded absence of pubescence on pod.

Seed color

Seed color is the primary morphological characteristic used to discriminate between greengram and blackgram. In the present investigation, five classes of seed color namely, black, brown, greenish brown and mottled (Plate 4) were observed among the genotypes under study. 14 genotypes (35%) showed black seed color, while 11 genotypes (27.5%) recorded brown seed color. However, eight genotypes (20%) exhibited green seed color, while six genotypes (15%) recorded greenish brown seed color. Only one genotype (2.5%) had recorded mottled seed color.

Seed lusture

In the present investigation two classes of seed lusture

Table 3. Eigen values, proportion of total variance represented by first four principal components, cumulative per cent variance and component loading of different characters in blackgram genotypes.

Components	PC1	PC2	PC3	PC4
Eigen value	2.248	1.730	1.443	1.068
Variability (%)	22.479	17.297	14.429	10.677
Cumulative %	22.479	39.779	54.205	64.882
Hypocotyl anthocyanin coloration	0.460	-0.172	-0.026	-0.362
Plant growth habit	0.406	0.256	-0.111	-0.307
Stem color	-0.139	-0.125	0.373	0.255
Stem pubescence	0.158	-0.077	0.567	0.029
Terminal leaflet shape	0.353	-0.188	0.193	0.614
Foliage color	-0.450	-0.178	0.079	-0.174
Petiole color	-0.436	-0.239	-0.251	0.080
Pod pubescence	-0.079	-0.292	0.551	-0.390
Seed color	-0.082	0.612	0.168	0.286
Seed lusture	-0.220	0.547	0.298	-0.246

namely, dull and shiny were observed among the 40 genotypes studied (Plate 3). As many as 21 genotypes (52.5%) showed dull seed lusture, while19 genotypes (47.5%) recorded shiny seed lusture.

Similar kind of morphological differences among the genotypes were revealed by Islam *et al*. (2019) for hypocotyl anthocyanin coloration, growth habit, leaflet terminal shape, pod pubescence and seed color.

Principal component analysis

Principal component analysis for the above morphological characters, excluding leaf pubescence that did not show any variation was performed to investigate the importance of different traits in explaining multivariate polymorphism. The percentage of variance explained by principal components (PCs) and eigen values are given in Table 3. A perusal of results revealed first four principal components with eigen value more than one. These principal components were observed to contribute to 64.88 % towards the total variability. Similar results were reported earlier in blackgram by Prathyusha *et al*. (2017). The first principal component (PC 1) contributed maximum towards divergence (22.479%). The characters, hypocotyl anthocyanin coloration (0.460), plant growth habit (0.406), stem pubescence (0.158) and terminal leaflet shape (0.353) had contributed positively for the variation in the principal component, while the other characters studied had contributed negatively. The second principal component (PC 2) contributed to 17.297 % of total variance. Plant growth habit (0.256), seed color (0.612) and seed lusture (0.547) were noticed to be positively loaded for the principal component, while other traits were noticed to be negatively loaded. The third principal component (PC 3) was characterized by 14.429 % contribution towards the total variability. The characters, stem color (0.373), stem pubescence (0.567), terminal leaflet shape (0.193), foliage color (0.079), pod pubescence (0.551), seed color (0.168) and seed lusture (0.298) were noticed to contribute positively for the variability explained by the principal component, while other traits studied recorded negative loadings for the principal component. The fourth principal component (PC 4) was characterized by 10.677 % contribution towards the total variability. Stem color (0.255), stem pubescence (0.029), terminal leaflet shape (0.614), petiole color (0.080) and seed color (0.286) were noticed to contribute positively for the variability explained by the principal component, while other traits studied recorded negative loadings for the principal component. The PCA analysis thus identified hypocotyl anthocyanin coloration and plant growth as the maximum contributing traits towards the total variability.

Shannon-Weaver diversity index

The Shannon-Weaver diversity index (H') was calculated to measure phenotypic diversity for 10 morphological traits as given in the Table 4. The indices for 10 qualitative characters were grouped into three

Table 4. Shannon-Weaver diversity indexes for qualitative characters in blackgram genotypes.

Sl. No.	Character	Shannon-Weaver diversity index (H')
1.	Hypocotyl anthocyanin coloration	0.85
2.	Plant growth habit	0.91
3.	Stem color	0.83
4.	Stem pubescence	0.66
5.	Leaflet terminal shape	0.61
6.	Foliage color	0.61
7.	Petiole color	0.83
8.	Pod pubescence	0.48
9.	Seed color	0.88
10.	Seed lusture	1.00

categories namely low, intermediate and high diversity as per the classification given by Jamago (2000). A perusal of these results revealed intermediate to high phenotypic diversity for most of the characters studied. The lowest diversity index value of zero was recorded for leaf pubescence, while maximum diversity index value of 1.00 was recorded for seed lusture. Hypocotyl anthocyanin coloration (0.85), plant growth habit (0.91), stem color (0.83), petiole color (0.83) and seed color (0.88) showed high diversity index (H') values, while stem pubescence (0.66), leaflet terminal shape (0.61) and foliage color (0.61) showed moderate diversity index (H') values. Similar kind of Shannon-Weaver diversity indices were revealed by Islam *et al*. (2019) for terminal leaflet shape, petiole color, stem color and pod pubescence. The above results for morphological traits studied reveal considerable amount of morphological diversity, that could be exploited for developing blackgram cultivars with unique features.

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REFERENCES

Devi HC, Kumari VP, Devi PS (2019) Morphological and phenotypic variability in blackgram genotypes with varying reaction to mungbean yellow Mosaic virus infection. *J Pharmacog Phytochemi* 8(4): 1606-1610.

Ghafoor A, Sharif A, Ahmad Z, Zahid MA, Rabbani MA (2001) Genetic diversity in blackgram (*Vigna mungo* L. Hepper). *Field Crops Res* 69(2): 183-190.

IBPGR. Descriptors for *Vigna mungo* and *Vigna radiata* (Revised) (1985) International Board for Plant Genetic Resources, Rome.

Islam MT, Rahaman S, Malek MA, Ahmed I, Jahans T (2019) Characterization and diversity of blackgram germplasm. *Bangladesh J Agric Res* 44(2): 239-251.

Jamago JM (2000) Morpho-agronomic and molecular diversity of the Philippine mungbean (*Vigna radiata* L.) germplasm. University of the Philippines Los Banos. The Philippines, pp174.

Perry MC, McIntosh MS (1991) Geographical patterns of variation in the USDA soybean germplasm collection: I. Morphological traits. *Crop Sci* 31(5): 1350-1355.

Prathyusha BV, Koteswara Rao Y, Prasada Rao GMV, Prasanthi L, Hari Y, Mallikarjuna G, Bentur JS (2017) SSR polymorphism and distinctiveness, uniformity and stability (DUS) features in selected genotypes of blackgram (*Vigna mungo* (L.) Hepper). *J Food Leg* 30(2): 1-8.

Rao MV, BalaRavi S, Seetharam, Makhija OP, Sharma SP, Dhillon BS, Singh RV, Tikko JL, Malathi Laxmi Kumaran, Roshini Nair, Chakrabarty SK (2007) Guidelines for the conduct of test for distinctiveness, uniformity and stability on blackgram (*Vigna mungo* (L.) Hepper). *Pl Var J Ind* 1(1): 1-9.