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Assessment of genetic variation and divergence in black gram's genotypes on climatic condition of Madhya Pradesh

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Abstract

Total forty six genotypes of black gram were studied at College of Agriculture, Indore (M.P.). Wide differences found between PCV and GCV in respect of yield and yield attributing traits. Based on the estimates of genetic divergence through Mahalanobis' D^2 - statistic, total forty six genotypes of black gram were grown at College of Agriculture, Indore (M.P.) and grouped into 12 different clusters. Highest intra-cluster distance of 2.148 was observed for cluster I which comprises of five genotypes. Cluster II which contained one genotype, had lowest intra-cluster distance. The maximum inter-cluster distance of 9.900 was recorded between cluster V and cluster II. A cross between these two inter-cluster's genotypes is expected to give a heterotic hybrid and wide spectrum of variability. The lowest inter-cluster distance of 2.333 was recorded between cluster IX and VIII. Highest intra-cluster distance & lowest inter-cluster distance indicates that the genotypes of these clusters were genetically less diverse and were almost with same genetic makeup or follows more or less same evolutionary phases during development.

The cluster I (KU7-522, KU7-619, KU7-626, KU7-608 and IVU-486) had lowest mean value for earliness flower initiation, 50% flowering and maturity could be used to develop short duration black gram varieties; cluster III (KU7-632, KU8-611, KU8-612 and KU8-532) for dwarf plant type; cluster X (KU3-62, JU-840 and TPU-4) for tall plant type; cluster II (KU8-632) and cluster IV (KU7-320 and KU8-278) for more number of primary branches per plant, number of seeds per plant, number of pods per plant, biological yield per plant and seed yield per plant; cluster VI (RBU-466-D, KU8-476, JU-86, TYPE-9 and IVU-466-D) for seed weight that may be used as bold seeds. To improve any particular trait, donor may be selected from these clusters for hybridization program to evolve high yielding strains.

Keywords: black gram, variability, diversity, inter-cluster distance, intra-cluster distance

Introduction

Urdbean belongs to Phylum- Angiosperms, sub-phylum- Dicotyledones, division- Lignosae, Order- Leguminales, family- Leguminaceae, genus- Vigna and species- mungo, thus finally called Vigna mungo L. Hepper. It is vernacularly called in different names like Biri (odia), Urd, Urad, Urid, Mash and Mungo in different parts of India and abroad (Panda, 2016). The somatic chromosome number of this crop is $2n=22$. It is grown mainly in rainy and/or summer seasons. The total availability of black gram in India per person is low as compared to other pulse crops.

Blackgram is extensively used as a nutritious pulse. Its seeds may be eaten raw, roasted, parched or boiled in split form. It is parched and ground to flour for making cakes, biscuits and confectionaries. It is used for making dosa and idli amalgamated with rice, popular breakfast dishes and also used in preparing papad and bharwan. Sprouting seeds are also eaten as such. It acts as a direct source of protein in vegetarian diet and indirect source of protein in non-vegetarian diet (Panda, 2016). The productivity of urd bean in the state as well as in India is very low due to various constraints like; non-availability of quality seed of high yielding variety, seeds germinate in mature pod itself if there is rains at maturity time of crop and the crop is highly sensitive to high intensity rains etc. Thus, the crop requires due attention to increase its production and productivity.

Yield is a complex quantitative trait which cannot be improved by selecting individuals on *per se* performance basis. The knowledge of nature and magnitude of genetic variability for characters of economic importance and cause and effects of relationship of yield and yield components for the available genotypes are utmost essential which helps in planning the future breeding programme for genetic improvement for yield potential of any crop species (Gour *et al.* 2018) [5].

Hybridization programme gives an opportunity to create wide spectrum of genetic variability. Black gram is a self-pollinated crop and lacked in genetic variability.

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Therefore choice of diverse parents for hybridization was one of the important considerations for creating new genetic variability. Several biometrical approaches have been shown to be useful in selecting parents for successful hybridization programme. D² analysis has been found most effective and therefore, widely used for the classification of parental lines. The D² statistics evaluates large number of germplasm lines for genetic diversity and helps in the identification of genetically divergent genotype for their exploitation in hybridization programmes.

Further, the development of high yielding urd bean genotypes to enhances genetic diversity/variability so this investigation was carried out for fulfilment of above mentioned constraints.

Material and Methods

1. Genotypes used in the study: The experiment was carried out at Research Farm, College of Agriculture, Indore (M.P.). The experimental material used in the present study comprised of forty six genotypes including standard checks (table 1). The experiment was laid down in a Randomized Block Design with three replications with the plot size of 4 rows 4m row length and the plant geometry was maintained at 30 cm × 10 cm.

Table 1: List of black gram genotypes used in the study

S. No.	Name of Genotypes	S. No.	Name of Genotypes	S. No.	Name of Genotypes
1	KU7-522	17	KU8-278	33	IU-10
2	KU7-618	18	KU8-611	34	IU-466-9
3	KU7-619	19	KU8-638	35	JU-2
4	KU7-626	20	KU8-632	36	JU-840
5	KU7-635	21	RBU-466-D	37	JU-3
6	KU7-608	22	KU8-613	38	JU-86
7	IVU-466-9	23	KU8-621	39	TYPE-9
8	IVU-486	24	KU8-602	40	TPU-4
9	KU09-252	25	KU8-605	41	IU-466-9
10	KU7-629	26	KU8-612	42	IVU-486
11	KU7-354	27	KU8-636	43	IVU-466-D
12	KU7-632	28	KU8-532	46	LOCAL-D
13	KU7-369	29	KU8-534	45	IU-466-9
14	KU7-320	30	IU-421	46	IU-31-7
15	KU3-62	31	KU8-532		
16	KU8-606	32	KU8-476		

2. Statistical procedures

The data collected on the quantitative characters were subjected for statistical analysis and following different statistical parameters were worked out.

2.1 Estimation of genotypic and phenotypic coefficient of variation

Genotypic and phenotypic coefficients of variation were estimated according to Burton and Devane (1953) [2] based on estimate of genotypic and phenotypic variance.

Genotypic co-efficient of variation (GCV):

$$GCV(\%) = \frac{\sigma g}{\bar{X}} \times 100$$

Phenotypic co-efficient of variation (PCV):

$$PCV(\%) = \frac{\sigma p}{\bar{X}} \times 100$$

Where,

\bar{X} = General mean

σg = Genotypic standard deviation

σp = Phenotypic standard deviation

The GCV and PCV are categorized as low (10%>), moderate (10-20%) and high (20%<) as suggested by Burton and Devane (1953) [2]. The estimated GCV and PCV helped in getting a clear understanding of the variability present among various genotypes.

2.2 Estimation of Genetic divergence (D² statistic)

Mahalanobis (1928) D² statistic was used for assessing the genetic divergence between different populations. The D² analysis was carried out using the data recorded on germplasms. Mahalanobis generalized distance (D²) between any two populations is given by the formula:

$$D^2 = \sum \lambda^{ij} \sigma^i \sigma^j$$

Where,

D² = Square of generalized distance

λ^{ij} = Reciprocal of the common dispersal index

σ^i = $\mu i1 - \mu i2$ (Difference between mean value of the two lines for the ith character)

σ^j = $\mu j1 - \mu j2$ ((Difference between mean value of the two lines for the jth charecter)

μ = General mean

Since the formula for computation requires inversion of higher order determinants, transformation of the original correlated unstandardized character mean (Xs) to standard uncorrelated variable (Ys) was done to simplify the computational procedure. The D² values were obtained as the corresponding uncorrelated (Ys) values of any two uncorrelated genotypes (Rao, 1952) [25].

Test of significance (Wilk's criterion): Variances were calculated for all the characters investigated and test of significance was done. Analysis of covariance for the character pairs was estimated on the basis of mean values (Panse and Sukhatme, 1957) [19]. After testing the difference between genotypes for each of the characters, a simultaneous test of significance for differences in the mean values of a number of correlated variables with regard to the pooled effect of characters was carried out using 'V' statistic, which in turn utilizes Wilk's criterion. The sum of squares and sum of products of error and error + variety, variance – covariance matrix were used for this purpose.

Determination of population constellations

Population constellations were determined by Tocher's method described by Rao (1952) [25]. A cluster or constellation may be explained as a group of populations or genotypes such that any two populations belonging to the same cluster showed, on the average, a smaller D² value than those belonging to different clusters.

Rao (1952) [25] suggested that two closely related populations of low D² value be pooled together and then a third population of similar D² value be added to this group such that it did not increase the average D² value appreciably. This process is continued. Any population, which sharply increases the average D² value, should not be included in that group.

After formation of first cluster, the process is repeated to form second, third etc., clusters using remaining populations until all populations are included in one or the other cluster. After cluster formation average intra and inter-cluster distances were calculated as formula described by Singh and Choudhary (1977) [29]. The square root of corresponding

average D^2 values represents the distance within and between groups.

Results and Discussion

1. Phenotypic and genotypic coefficient of variation

A broad-spectrum of genetic variability is fundamental requisite for success of a plant breeding programme since it provides opportunity to breeders to make selection for desirable superior individuals from genetically diverse base population. Since, many characters of economic importance are highly influenced by environmental conditions, the improvement of a crop mainly depends upon the amount, nature and magnitude of genotypic variability present in the population. Wide range of variability existing among the genotypes to be tested for all the characters is also necessary to isolate significantly superior genotypes.

The estimates of phenotypic coefficients of variation were higher than genotypic coefficients of variation which is indicating that the environmental factors influencing those characteristics (Table 2). These results are in accordance with the findings of Panigrahi *et al.* (2014) [18] and Kumar *et al.* (2015) [10].

The high GCV of (27.33%) was recorded for seed yield per plant, followed by, number of seeds per plant (25.91%), biological yield per plant (24.57%) and number of pods per plant (20.26%), indicating the presence of variation for these characters in the materials and improvement could be possible through selection of these characters. These results are in agreement with the findings of Panigrahi *et al.* (2014) [18] except for number of primary branches per plant in blackgram.

The high PCV value was observed for seed yield per plant (36.04%), followed by, number of seeds per plant (34.07%), biological yield per plant (31.17%), number of pods per plant (29.24%) and harvest index (23.97%). The moderate PCV for plant height (18.87%), number of primary branches per plant (18.09%) and 100-seed weight (14.96%). This is suggesting that sufficient phenotypic variability was present for these traits in the materials and the favourable effect of environment. Therefore, simple direct selection could be rewarding for improving such traits. The results are in agreement with Natarajan and Rathinasamy (1999), Rajendrakumar *et al.* (2000), Kumar and Mishra (2004),

Parameswarappa and Kumar (2005), Vinay kumar *et al.* (2010) and Kumar *et al.* (2015) [16, 23, 9, 20, 32, 10].

The moderate GCV was observed for plant height (16.86%), harvest index (16.06%), 100-seed weight (11.73%) and number of primary branches per plant (11.01%). The low GCV was showed for number of seeds per pod (5.94%), days to 50% flowering (5.16%), days to maturity (3.15%) and days to flower initiation (2.21%). These results were in consonance with the findings of Veeramani *et al.* (2005); Deepshikha *et al.* (2014); Patel *et al.* (2014); Ramya *et al.* (2014); Kumar *et al.* (2015) and Gowsalya *et al.* (2016) [31, 21, 4, 24, 10, 6]. The low PCV for number of seeds per pod (9.31%), days to 50% flowering (8.19%) and days to maturity (5.51%) and days to flower initiation (4.94%). It can be concluded that such traits are highly influenced by the environment so selection for such traits may not be useful.

A comparison of difference between phenotypic coefficient of variation and genotypic coefficient of variation estimates showed wide differences in respect of number of pods per plant (8.98%), followed by, seed yield per plant (8.71%), number of seeds per plant (8.16%), harvest index (7.91%), number of primary branches per plant (7.08%) and biological yield per plant (6.60%), while for number of seeds per pod (3.37%), 100 seed weight (3.23%), days to 50% flowering (3.03%). This might have been due to larger influence of environment on the expression of these traits. This result is accordance to Konda *et al.* 2009; Kumar *et al.* 2015; and Gowsalya *et al.* 2016 [8, 10, 6]. Days to flower initiation (2.73%), days to maturity (2.36%) and plant height (2.01%) showed least differences between PCV and GCV, indicating the greater role of genetic factors influencing the expression of these characters. This indicated the utility of these characters in the selection programme.

A perusal of the genetic parameters revealed that seed yield per plant followed by biological yield per plant, number of seeds per plant and number of pods per plant exhibited high phenotypic and genotypic coefficient of variation suggesting that the existence of sufficient genetic variability for these traits in the population. Exhibiting high magnitudes as well as less influence of environment on the expression of concerned traits, so selection of such traits per heterotic group development will be very useful. These results were in consonance with the findings of Veeramani *et al.* (2005); Senapati and Mishra (2010); Meshram *et al.* (2013) [31, 27, 15].

Table 2: Genetic variation for various traits in Black gram

Characters	Days to flower initiation	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches/plant	No. of pods/plant
PCV (%)	4.94	8.19	5.51	18.87	18.09	29.24
GCV (%)	2.21	5.16	3.15	16.86	11.01	20.26
Characters	No. of seeds/plant	No. of seeds/pod	100-seed weight (g)	Biological yield/plant (g)	Harvest index (%)	Seed yield/plant (g)
PCV (%)	34.07	9.31	14.96	31.17	23.97	36.04
GCV (%)	25.91	5.94	11.73	24.57	16.06	27.33

2. Study of genetic divergence

The multivariate D^2 analysis using Mahalanobis' D^2 statistic provides a useful statistical tool for measuring the genetic diversity in germplasm collections with respect to the characters considered together. It also provides a quantitative measure of association between geographic and genetic diversity based on generalized distances (Mahalanobis, 1936) [13]. Further, the problem of selecting diverse parents for hybridization programme can be narrowed, if one can identify the characters responsible for the discrimination between the populations.

So, present study was aimed at analysis of genetic divergence among the 46 genotypes and to identify the superior and divergent lines for formulating the crossing programme.

Wilk's 'V' criterion test

Significant differences among the genotypes for the mean values of the twelve characters and the pooled effect of all the characters were carried out using the Wilk's criterion '^'. The Wilk's criterion thus obtained was used in calculations of 'V' statistic. The statistic was found significant (more than the tabulated D^2 value) indicating that genotypes differed

significantly when all the characters were considered simultaneously. This result is accordance to Kumar, 2014 and Samimy, 2014 [4, 26].

Grouping of genotypes into various clusters

Based on the estimates of genetic divergence, all the forty six genotypes of black gram were grouped into 12 different clusters using Tocher's method (Rao, 1952) [25]. Generalized distance was estimated through Mahalanobis' D^2 - statistic. Among the twelve clusters, cluster VIII was the largest including 9 genotypes followed by cluster IX, VI and I had 5 genotypes each. Clusters VII and III had 4 genotypes each. Clusters XI, X and V had 3 genotypes each. Cluster XII and IV had 2 genotypes each and cluster II including 1 genotype (table 3).

The pattern of distribution of genotypes from different eco-geographical regions into various clusters was at random indicating that there is no parallelism between geographical diversity and genetic diversity (Samimy, 2014) [26]. These suggests forces such as exchange of breeding material, natural and artificial selection, genetic drift, migration, gene flow and variation in environment may be responsible for this diversity (Kumar, 2014 and Samimy, 2014) [4, 26]. Therefore, the choice of suitable diverse parents selected on the basis of genetic diversity analysis would be more rewarding than the choice made on the basis of geographic diversity.

The intra and inter-Cluster average distance

The average distance within and between clusters and average inter and intra cluster D^2 values have been presented in Table 4. The inter-cluster distances were greater than intra-cluster distances, revealing that considerable amount of genetic diversity existed among the genotypes of different clusters. Average intra-cluster distance revealed that cluster II, which contained one genotype, had lowest intra-cluster distance (0.000). It indicated that these genotypes were closely related in their evolutionary process and passed through similar evolutionary factors. Highest intra-cluster distance of 2.148 was observed for cluster I which comprises of five genotypes. This suggests that these five genotypes possess all most same genetic makeup with minute difference due to evolutionary channel. It can be used in the yield improvement through recombination breeding.

Inter-cluster distance is the main criterion for selection of genotypes for hybridization programme using D^2 analysis. Genotypes belonging to the clusters with maximum inter-cluster distance are genetically more divergent and hybridization between genotypes of divergent clusters is likely to produce wide spectrum of genetic variability with desirable segregates. The maximum inter-cluster distance of 9.900 was recorded between cluster V and cluster II followed by cluster II and I (9.781), cluster XI and II (9.701), cluster VII and II (9.375), cluster IX and II (9.267), cluster III and II (7.459) and cluster VI and II (7.323). It is suggested that the crosses involving varieties from these clusters would give desirable recombination. The lowest inter-cluster distance of 2.333 was recorded between cluster IX and VIII which indicates that the genotypes of these clusters were genetically

less diverse and were almost with same genetic makeup or follows more or less same evolutionary phases during development. It is indicating that genotypes of these clusters had maximum number of gene complexes.

However intra and inter cluster D^2 values were worked out from the above divergence analysis indicate that the inter-cluster distances were greater than intracluster distances, revealing that considerable amount of genetic diversity existed among the accessions. Similar results were also reported by Ali *et al.* (2008), Srimathy *et al.* (2012), Singh *et al.* (2012) and Jayamani *et al.* (2013) [1, 30, 28, 7].

Contribution of characters cluster mean

Sometimes a breeder is asked to improve a particular trait of a variety which was otherwise suitable. For this a donor parent is required. Information about a range of suitable donors thus becomes inevitable. Estimates of cluster mean make information readily available. Cluster means for the twelve traits of all the twelve clusters were worked out (table 5). It was found that Cluster I had lowest mean value for earliness in flower initiation (33.73 days), days to 50% flowering (41.63 days) and days to maturity (64.40 days) which indicates the selection of the genotypes from cluster I for the development of short duration/early maturing genotypes. Cluster VI had the highest mean value for 100-seed weight (4.49 g). This suggested that genotypes from cluster VI may be selected for development bold seeded genotypes. Cluster II had the highest mean value for number of primary branches per plat (4.43), number of pods per plant (30.63), number of seeds per plant (230.03), seed yield per plant (g) (8.37) and high biological yield per plant (17.80 g). Cluster III had lowest mean value for plant height (11.75 cm) and the genotypes of this cluster will be useful for development of short stature plant in black gram. To improve any particular trait donor may be selected from these clusters for hybridization program to evolve high yielding strains.

The cluster IV was characterized for less number of pods per plant (11.99), number of seeds per plant (69.61), low biological yield per plant (6.12 g) and high harvest index (51.40 %). The cluster VII was characterized for late maturity (72.50 days), less number of primary branches per plant (2.67) and more number of seeds per pod (7.18). The cluster VIII was characterized for delayed flower initiation (36.00 days). The cluster X was characterized for tall plant type that is plant height (17.28 cm). The cluster XI was characterized for less number of seeds per pod (5.40), low harvest index (24.92 %) and low seed yield per plant (2.69 g). The cluster XII was characterized for delayed 50% flowering (51.25 days).

These results are similar with the findings of Raje and Rao *et al.* (2001) [22], Dasgupta *et al.* (2005), Majumder *et al.* (2011) [14] and Jayamani *et al.* (2013) [7]. It shows that the selection of parents for hybridization based on geographical origin would be arbitrary. The grouping of genotypes from same source into different clusters as observed in present study may be because of free exchange of breeding material among different regions (Singh *et al.* 2012) [28].

Table 3: Clustering pattern of forty six genotypes of black gram on the basis of genetic divergence

Cluster Number	Constituent genotypes	Number of genotypes
I	KU7-522, KU7-619, KU7-626, KU7-608, IVU-486	5
II	KU8-632	1
III	KU7-632, KU8-611, KU8-612, KU8-532	4
IV	KU7-320, KU8-278	2

V	KU7-618, KU7-635, IU-466-9	3
VI	RBU-466-D, KU8-476, JU-86, TYPE-9, IVU-466-D	5
VII	KU8-613, KU8-605, KU8-532, IU-421	4
VIII	IVU-486, KU7-354, KU7-369, KU8-602, IU-466-9, JU-2, IU-466-9, IVU-486, IU-31-7	9
IX	KU7-629, KU8-606, KU8-638, IU-10, JU-3	5
X	KU3-62, JU-840, TPU-4	3
XI	KU09-252, KU8-532, LOCAL-D	3
XII	KU8-621, KU8-636	2

Table 4: Average intra and inter cluster D² values between the clusters in black gram

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	2.148											
II	9.781	0.000										
III	3.797	7.459	1.290									
IV	6.512	4.176	3.746	0.724								
V	4.025	9.900	4.026	6.418	1.568							
VI	4.286	7.323	3.591	3.986	4.859	1.620						
VII	4.941	9.375	3.182	5.693	4.416	4.704	1.801					
VIII	4.834	7.445	2.558	4.053	3.634	3.013	3.303	1.578				
IX	3.853	9.267	3.518	5.756	3.147	2.883	3.757	2.333	1.768			
X	5.278	6.944	3.454	4.565	5.932	3.768	4.966	3.028	3.917	1.758		
XI	4.085	9.701	3.565	6.585	4.887	5.125	5.323	4.298	3.794	3.792	1.900	
XII	6.025	5.997	2.577	2.815	5.466	4.237	4.276	2.719	4.620	3.161	4.902	1.427

Table 5: Clusters means for 12 characters under study in black gram

Characters	Cluster mean											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Days to flower initiation	33.73	35.67	34.50	34.50	35.78	34.13	35.38	36.00	35.43	35.67	34.17	35.75
Days to 50% flowering	41.63	47.50	49.12	49.00	45.67	45.40	50.88	48.94	46.63	47.17	47.39	51.25
Days to maturity	64.40	69.50	69.88	70.50	70.22	66.70	72.50	71.31	68.70	69.22	68.56	72.25
Plant height	12.01	13.63	11.75	12.58	12.18	16.88	12.42	15.92	17.11	17.28	14.52	12.12
No of primary branches/plant	2.99	4.43	3.15	3.47	3.22	2.93	2.67	3.29	2.87	3.59	3.34	3.47
No of pods/plant	14.06	30.63	18.67	24.27	11.99	17.01	13.38	16.27	12.95	17.72	14.08	19.22
No of seeds/plant	88.56	230.03	115.30	166.38	69.61	116.99	96.31	106.64	82.16	124.91	79.57	137.30
No of seeds/pod	6.24	6.80	6.24	6.63	5.88	6.76	7.18	6.44	6.29	6.46	5.40	6.40
100-seed weight	3.53	4.03	3.63	4.20	4.41	4.49	3.54	4.15	4.42	3.59	3.58	4.00
Biological yield/plant	7.74	17.80	10.47	13.95	6.12	12.25	8.29	10.80	9.39	15.38	12.09	14.53
Harvest index	39.34	48.33	38.42	46.90	51.40	43.16	40.37	42.11	38.70	28.24	24.92	36.62
Seed yield/plant (g)	2.89	8.37	4.09	6.58	3.03	5.25	3.28	4.41	3.54	4.28	2.69	5.27

Conclusion

A perusal of the genetic parameters revealed that seed yield per plant followed by biological yield per plant, number of seeds per plant and number of pods per plant exhibited high phenotypic and genotypic coefficient of variation suggesting that the existence of sufficient genetic variability for these traits in the population. Exhibiting high magnitudes as well as less influence of environment on the expression of concerned traits, so selection of such traits per heterotic group development will be very useful.

Based on the study of genetic divergence the following entries were identified which can be used in breeding programme. To improve seed yield, genotypes from cluster II (KU8-632) and cluster IV (KU7-320 and KU8-278) would be right choice; cluster I (KU7-522, KU7-619, KU7626, KU7-608 and IVU-486) for earliness in flower initiation, 50% flowering and maturity could be used to develop short duration black gram varieties. To develop dwarf variety, genotypes from Cluster III and for tall variety genotypes from Cluster X (KU3-62, JU-840 and TPU-4) would be an ideal choice.

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