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Genetic Diversity and Protein Analysis in Greengram

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ABSTRACT: Greengram is the 3rd most important pulse crop in India next to chickpea and redgram. Though it is well-known for its nutritional value and adaptability to a variety of cropping systems, little systematic work has been done to develop better high-yielding genotypes and high seed protein content genotypes. Genetic improvement requires concerted research efforts to explore genetic diversity and to comprehend the variability in different genotypes in order to analyze and create diverse lines for future breeding operations. Therefore a set of sixty-six greengram genotypes were evaluated to study the genetic variability and diversity in order to make a judicious selection of parents for getting significant recombinations. Seed yield/plant exhibited the highest phenotypic and genotypic coefficient of variation (24.42 and 21.85%). High heritability coupled with high genetic advance was found in plant height, number of clusters/plant, number of pods/plant, hundred seed weight and seed yield/plant indicating the role of additive gene action providing thereby ample scope for effective selection. Diversity analysis led to the constellation of 6 clusters signifying that eco-geographic diversity is not a reliable index for genetic diversity. The genotypes belonging to cluster IV (AB-2557) and cluster VI (HUM-10) showing the highest inter-cluster distance (6154.40) can be selected for improvement of yield related traits in greengram. Results from protein analysis revealed the genotype HUM-10 has the highest protein content (32.68%). Therefore, it may be used as a source of germplasm for high seed protein content.

Keywords: Genetic variability, diversity analysis, greengram, protein analysis.

INTRODUCTION

Greengram (*Vigna radiata* (L.) Wilczek) is the 3rd most important pulse crop in India next to chickpea and redgram. This self-pollinating legume belongs to family fabaceae. It is a diploid crop having chromosome number 2n=22 (Karpechenko, 1925) and originated from the Indo-Burma region of the Hindustan center (Vavilov, 1926). Nutritionally, greengram is a protein-rich food containing around 23% protein. The protein is rich in lysine, minerals, and vitamins hence meeting the dietary needs of the vegetarian population of the country. The genetics of the protein or its constituents may give some valuable signs for further enhancement in protein level and quality of protein. Greengram is cultivated widely because of its adaption to short growth duration, low water requirement, restoring of soil fertility through biological nitrogen fixation, tendency to reduce greenhouse gases, capacity to increase carbon sequestration, and thus, plays an important role in sustainable agriculture. It is grown all over the country in several seasons as a sole crop as well as an intercrop or mixed crop with cereals forming an important constituent of crop rotation.

Yield is a complex trait and highly influenced by the environment due to polygenic effects (Allard, 1960). To accumulate optimum yield, it is necessary to know the presence of genetic variability among the yield contributing characters for effective selection. Genetic diversity is the basic requirement for any breeding programme which aims at genetic amelioration of yield. Effective hybridization programmes between genetically diverse parents will cause a substantial amount of heterotic response in F_1 hybrid and therefore the broad spectrum of variability in segregating generations. Therefore, an attempt was made to study the extent of the genetic divergence among 66 genotypes of greengram through D^2 statistics (Mahalanobis, 1936) to choose genetically diverse parents. Further protein analysis was done on the seed protein content of germplasms.

MATERIALS AND METHODS

The present experiment was conducted at EB-II section, Department Plant Breeding and Genetics, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, during rabi 2019-20.A set of 66 greengram germplasm lines including standard ruling varieties, important pre-released varieties and popularly adopted local landraces were laid out in randomized block design with 3 replications. The crop was raised following recommended agronomic packages & practices and observations were recorded on five randomly selected plants per replication for ten quantitative characters *viz*., days to 50 % flowering (DF), days to maturity (DM), plant height (PH) in cm, number of branches/plant (B/P), number of clusters/plant (C/P), number of pods/plant (P/P), pod length (PL) in cm, number of seeds/pod (S/P), hundred seed weight (HSW) in gram and seed yield/plant (SY/P) in gram.

The mean for all the characters of five plants in each plot was subjected to analysis of variance (ANOVA) as per the method suggested by Panse and Sukhathme (1967). Estimation of genetic parameters like phenotypic and genotypic coefficients of variation, heritability (h^2, broad sense in %) and genetic advance (GA as % of mean) were worked out according to the formulas given by Singh and Chaudhary (1977). On the basis of heritability values characters were classified to high (>60), moderate (31-60) and low (0-30) heritable (Johnson *et al.*, 1955). Likewise, characters were classified as having high (>20), moderate (10-20) and low (0-10) genetic advance as per the method of Johnson *et al.* (1955).Genetic diversity analysis was conducted using Mahalanobis D^2 statistic (1936) and the genotypes were grouped into clusters by Tocher's method (Rao, 1952). For protein analysis, matured seeds from each genotype from each replication were harvested, dried up to a safe moisture level (12-13%) and powered to estimate protein content. Nitrogen content present in seed was estimated in CHNS (O) analyzer. Then it was multiplied with a factor 6.25 to arrive at the percentage protein content of seeds (Jones, 1941).

RESULTS AND DISCUSSION

Genetic variability: Result of ANOVA revealed that all the genotypes were significantly different for all the characters studied suggesting the presence of substantial variability among the germplasm under investigation (Table 1), which will be much beneficial for the selection of breeding material. This wide range of variation may be due to diverse origins and geographical adaptation of genotypes. Estimates of phenotypic coefficient of variations (PCV) were higher than genotypic coefficient of variations (GCV) for all the characters under study (Table 2), which indicates that there was some environmental influence on these characters (Rahim *et al.*, 2010). Seed yield per plant exhibited the highest value of PCV and GCV indicating a relatively higher contribution of this character towards genotypic variability. Hence direct selection based on this trait would be effective. This result was in conformity with the findings of Srivastava and Singh (2012); Patel *et al.* (2014). It was noticed that number of branches/plant had maximum difference between GCV and PCV which indicate the environmental effect is more in this character, hence much care should be taken up while selecting this character (Varma *et al.*, 2018).

Table 1: ANOVA for 10 characters in 66 genotypes of greengram.

Sources of variation	DF	DM	PH	B/P	C/P	P/P	PL	S/P	HSW	SY/P
Genotype	21.21**	30.17**	56.88**	0.12**	0.78**	6.32**	0.64**	3.07**	0.77**	0.74**
Error	1.69	1.94	3.92	0.03	0.05	0.34	0.15	0.22	0.09	0.06
C.D (P=0.05)	2.10	2.25	2.93	0.26	0.37	0.95	0.62	0.76	0.49	0.38
SEm (±)	0.75	0.80	1.05	0.09	0.13	0.34	0.22	0.23	0.17	0.14
C.V. (%)	3.56	2.15	5.85	13.68	7.76	6.68	6.35	4.71	9.66	10.93

** indicates significance at $P_{0.01}$

Table 2: Estimates of genetic parameters for 10 morphological characters in 66 greengram germplasm.

Characters	Range	Mean	PCV (%)	GCV (%)	h^2 (bs in %)	G.A.(% of mean)
DF	31.00-42.00	36.48	7.84	6.99	79.34	12.82
DM	60.00-71.00	64.97	5.18	4.72	82.87	8.85
PH	21.16-44.98	30.98	14.84	13.64	84.86	25.83
B/P	1.00-2.13	1.18	20.30	15.00	54.60	22.84
C/P	2.13-4.33	2.98	18.25	16.51	81.88	30.78
P/P	5.86-12.06	8.58	17.82	16.44	85.10	31.24
PL	4.77-7.21	6.07	9.19	6.64	52.17	9.88
S/P	8.26-12.20	10.01	10.80	9.72	80.96	18.02
HSW	2.38-5.12	3.15	17.95	15.13	70.99	26.25
SY/P	1.33-3.73	2.18	24.42	21.85	79.98	40.25

Characters like plant height, number of clusters/plant, number of pods/plant, hundred seed weight and seed yield exhibited high heritability (broad sense in %) accompanied with high genetic advance (G.A. in % of mean) resembling the action of additive gene in controlling these characters and selection based on these characters would be rewarding for enhancement of yield. Evidently, high heritability coupled with high genetic advance suggesting the role of additive gene action for seed yield per plant was reported earlier by Patel *et al.* (2014), Raturi *et al.* (2015); Mariyammal *et al.* (2019).

Diversity analysis:

Composition of clusters: Sixty-six genotypes were grouped into 6 clusters using Tocher's method (Table 3). Cluster I containing 51 genotypes was the largest one followed by cluster III (7), cluster II (5), while the rest three clusters *viz.*, cluster IV, V and VI represented one genotype each suggesting presence of heterogeneity among the genotypes. It was observed from the results that genotypes having different sources of origin grouped into same cluster. Also, materials of the same source have been grouped in different clusters indicating eco-geographic diversity is not a reliable index for genetic diversity (Vijaya and Shekhawat 2012). Therefore, it is advised that the selection of parents during crossing programme should be based on genetic divergence rather than their geographic diversity.

Table 3: Grouping of 66 greengram genotypes into 6 clusters by Torcher's method.

I	51	AKM-9901, AKM-8802, AKM-9601, AM-2057, ADT-14, B-10-33-1, CO-6, CNO-3, CNO-35, CNO-36, Dhauli, HUM-12, KM-851, LGG-460, M-9-2, MG-12, M-9-13, MGG-349, ML-131, ML-267, ML-555, ML-682, ML-729, ML-818, ML-881, ML-1108, ML-1165, ML-1231, OUM-99-3, OUM-99-6, OUM-99-7, OUM-11-5, OUM-14-0, OUM-14-2, OUM-18, OUM-18-4, OUM-20, OUM-20-1, OUM-21,Pusa Bold, KPS-2, SML-668, TARM-1, PDM-154, Sujata, ML-1628, NM-94, IPM-02-14, ML-1299, Nayagarh-B, Nayagarh-C
II	5	CNO-59, COGG-902, COGG-912, KM-9309, PDM-139
III	7	HUM-1, HUM-6, ML-613, EC-693369, NM-92, Raipur Local, Khadabhanga-B
IV	1	AB-2557
V	1	Nayagarh-A
VI	1	HUM-10

Intra- and inter-cluster distance:

Average intra- and inter-cluster distances (D^2) values among the six clusters were calculated using divergence analysis (Table 4). The intra-cluster distance varied from 0 (cluster IV, V and VI) to 1023.36 (cluster III) and the inter-cluster distance varied from 654.34 (cluster V & VI) to 6154.40 (cluster IV & VI). Maximum intra-cluster distance indicated the presence of greater diversity among the genotypes allocated in those respective clusters. Minimum inter-cluster distance was revealed between cluster V & VI (654.34) showing that the genotypes present in them were closely related. The highest inter-cluster distance was observed between cluster IV & VI (6154.40) indicating the genotypes included in them were diverse. Greater the distance between two clusters, wider the genetic diversity among genotypes. So, hybridization between the genotypes of the cluster IV & VI having maximum inter-cluster distance would result in high heterotic combination. Similar type of findings were earlier observed by Singh *et al.* (2014); Rekha *et al.* (2015).

Table 4: Average intra- and inter-cluster (D^2) values along with the group distance ($\sqrt{D^2}$) for 6 clusters in 66 greengram genotypes.

Clusters	I	II	III	IV	V	VI
I	**260.18 (16.13)**	1300.32 (36.06)	864.95 (29.41)	808.83 (28.44)	1958.95 (44.26)	2093.06 (45.75)
II		**412.09 (20.30)**	3291.32 (57.37)	1009.97 (31.78)	1642.68 (40.53)	2461.15 (49.61)
III			**1023.36 (31.99)**	1819.88 (42.66)	3490.45 (59.08)	5811.01 (76.23)
IV				**0 (0)**	3566.48 (59.72)	6154.40 (78.45)
V					**0 (0)**	654.34 (25.58)
VI						**0 (0)**

Diagonal bold figures shows intra-cluster distance

Cluster means: Considerable variations in mean performances of various traits among the clusters suggest that quantitative traits can reveal the existing diversity (Walle *et al.*, 2019). Characterization of clusters was done as per the D^2 distances (Table 5). Cluster III recording the highest intra-cluster distance was characterized by highest pod length (6.76cm), more number of seeds per pod (11.32) and maximum 100

seed weight (3.59gm) with comparatively larger seeds and was found to be superior among all the 6 clusters studied. The traits like plant height (41.00 cm) and seed yield per plant (3.38gm) had the highest mean value in cluster IV. Cluster V had genotypes with more number of branches/plant (2.13) and more number of pods/plant (11.73). Cluster VI was characterized by less number of days to attain 50% flowering (34.33), less number of days to attain maturity (60.67) and maximum number of pods/plant (11.73). These type of findings have been earlier confirmed by Abna *et al.* (2012); Razzaque *et al.* (2016). The results indicate that for creation of variability cross between genotypes with high cluster mean values followed by selection is required.

Table 5: Cluster wise mean value of 10 quantitative characters in 66 genotypes of greengram.

Characters / Clusters	DF	DM	PH	B/P	C/P	P/P	PL	S/P	HSW	SY/P
Cluster-I	36.20	64.80	30.49	1.13	2.89	8.35	6.00	9.84	3.08	2.11
Cluster-II	38.07	66.67	39.26	1.28	3.97	10.92	6.11	10.41	3.14	2.67
Cluster-III	37.52	66.19	28.02	1.35	2.72	7.64	6.76	11.32	3.59	2.18
Cluster-IV	36.00	64.67	41.00	1.07	2.73	9.20	6.43	11.00	3.54	3.38
Cluster-V	38.33	61.67	31.06	2.13	3.80	11.73	6.15	9.53	2.89	2.46
Cluster-VI	34.33	60.67	25.14	1.33	4.07	11.73	4.77	7.27	3.12	2.26

Relative contribution of component characters to genetic divergence: According to Gupta *et al.* (2019) characters giving the highest contribution towards the diversity could be used further in the identification of the parents for the hybridization programme. Relative contribution of individual characters to overall genetic divergence among the genotypes was assessed by rank average method (Table 6). Among all the characters studied plant height (18.70%), number of seeds/pod (16.22%), days to 50% flowering (15.71%), number of pods/plant (15.48%) and seed yield/plant (10.02%) contributed maximum (60.65%) towards genetic divergence. Rest of the traits exhibiting divergence in order were number of clusters/plant (8.86%), hundred seed weight (8.11%), days to maturity (3.73%), pod length (1.68%) and number of branches/plant (1.49%). So characters like days to 50% flowering, plant height, number of seeds per pod, number of pods per plant and seed yield per plant should form criteria for selection of parents from distantly placed clusters for hybridization programme. These results have been in conformity with the findings of Garje *et al*. (2013); Jeeva and Saravanan (2017).

Protein analysis: Analysis of variance for this character showed highly significant difference among all the genotypes suggesting presence of substantial variability in the material under investigation (Table 7) which will be much beneficial for the selection of breeding material (Reddy *et al*., 2011). The seed protein content was ranged from 16.56% in PDM-154 to 35.68% in HUM-10 (Table 8). Wide range of variation in seed protein content was previously observed by Raturi *et al*. (2014). Coefficient of variation was found to be low for this character (1.74). Estimates of PCV (11.53) and GCV (11.40) were moderate. High heritability (97.73%) and high expected genetic advance as a percentage of the population mean (12.63%) indicated the role of additive gene action, hence providing the scope for enhancement of this trait through selection (Jayalakshmi *et al*., 2019 in chickpea). Further, it showed negative correlation with seed yield (-0.018), which was earlier reported by Kumar *et al.* (2013).

Table 6: Relative contribution of different characters to genetic divergence among 66 greengram genotypes.

Sr. No.	Characters	Contribution %	Times Ranked first
1.	**DF**	15.71	337
2.	**DM**	3.73	80
3.	**PH**	18.69	401
4.	**B/P**	1.49	32
5.	**C/P**	8.86	190
6.	**P/P**	15.48	332
7.	**PL**	1.68	36
8.	**S/P**	16.22	348
9.	**HSW**	8.11	174
10.	**SY/P**	10.02	215
	TOTAL	100	2145

Table 7: Analysis of variance for seed protein content in 66 genotypes of greengram.

Sources	d.f	SS	MS	F Value
Replication	2	0.06	0.03	0.17
Genotype	65	1351.97	20.80	130.39**
Error	130	8.731	0.067	

**Significant at $P_{0.01}$

Table 8: Seed protein content of 66 greengram genotypes.

Genotypes	Protein (%)	Yield (g)	Genotypes	Protein (%)	Yield (g)
AKM-9901	24.50	1.56	ML-881	21.75	2.36
AKM-8802	27.37	1.53	ML-1108	18.31	2.25
AKM-9601	23.56	2.83	ML-1165	25.06	2.44
AM-2057	24.62	1.92	ML-1231	22.88	2.28
AB-2557	23.81	3.38	OUM-99-3	19.82	2.19
ADT-14	24.31	2.04	OUM-99-6	21.68	1.54
B-10-33-1	27.93	1.46	OUM-99-7	21.50	2.04
CO-6	28.31	2.45	OUM-11-5	20.87	1.55
CNO-3	22.06	2.58	OUM-14-0	21.62	2.56
CNO-35	23.06	2.44	OUM-14-2	20.56	2.24
CNO-36	19.75	2.25	OUM-18	22.43	1.84
CNO-59	23.31	**3.73**	OUM-18-4	21.75	1.97
COGG-902	18.81	2.43	OUM-20	21.87	1.85
COGG-912	26.75	2.22	OUM-20-1	21.25	**1.33**
Dhauli	23.31	1.81	OUM-21	23.12	1.54
HUM-1	19.68	3.16	Pusa Bold	21.50	2.74
HUM-6	22.00	2.24	KPS-2	26.81	3.18
HUM-10	**32.68**	2.26	SML-668	21.13	2.98
HUM-12	24.31	2.26	EC-693369	25.06	2.83
KM-851	24.00	2.36	TARM-1	27.56	2.43
KM-9309	21.87	2.49	PDM-154	**16.56**	1.84
LGG-460	23.68	2.36	Sujata	23.13	2.74
M-9-2	19.30	2.03	NM-92	22.19	1.68
MG-12	21.56	2.37	ML-1628	21.75	2.05
M-9-13	23.18	2.20	NM-94	25.75	1.97
MGG-349	22.31	1.88	IPM-02-14	23.38	1.90
ML-131	22.50	1.83	ML-1299	22.68	2.40
ML-267	25.31	1.62	PDM-139	21.69	2.49
ML-555	24.06	2.75	Nayagarh-A	22.94	2.46
ML-613	21.93	1.59	Nayagarh-B	19.06	2.11
ML-682	25.00	1.51	Nayagarh-C	25.50	1.37
ML-729	24.00	1.79	Khadabhanga-B	25.00	1.97
ML-818	22.00	1.90	Raipur Local	21.25	1.80

Bold figure shows minimum and maximum values

CONCLUSION

In the present research, all the genotypes were significantly different for all the characters studied indicating the presence of sufficient variability in these genotypes to have an effective selection. Seed yield exhibited high magnitude in both PCV and GCV apart from high heritability and genetic advance, hence direct mass selection may be rewarded for improvement in this trait. Based on diversity analysis it can be concluded that genotypes AB-2557 and HUM-10 can be used as parents from monogenotypic clusters IV and VI in hybridization, which may produce new recombinants with desired traits. Results from protein analysis confirmed that among all the genotypes studied, HUM-10 exhibited the highest protein content (32.68%). Therefore, it may be used as a source of germplasm for high seed protein and could be crossed with high-yielding varieties for further releasing of superior variety with high yield and high seed protein content.

FUTURE SCOPE

Based on genetic parameters and D^2 statistics, the current findings have opened the way for the identification of elite germplasm lines with prospective genotypic significance for yield and yield contributing variables in greengram. The selected test genotypes would make better parent materials for recombination breeding.

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Conflict of Interest. None.

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