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Research Note

Pattern of agro-morphological trait relationship and genetic divergence in greengram [*Vigna radiata* (L.) Wilczek]

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Abstract

Thirty six greengram genotypes were evaluated during kharif 2012 in three environments for analysis of quantitative traits. Association analysis showed significant positive relationship between seed yield and some of the agro-morphological traits *viz*., days to 50% flowering, primary branches per plant, secondary branches per plant, clusters per plant, pods per cluster, pod length, seeds per pod, pod mass, pod wall mass, seed mass, selling percentage, seed index and harvest index. Hence these traits may be used as selection criteria for yield improvement. Based on principle component analysis, pod length, seeds per pod, pod mass, seed mass, pod wall mass, selling percentage, seed index and harvest index were found important and can be added in breeding program. Sona selection had moderate to more diversity with all the genotypes due to their solitary nature, and can be used in breeding program for greengram improvement. Besides this, representative genotypes from each cluster may be selected for future hybridization to get good segregants in segregating generations.

Key words: Greengram, Bi-plot analysis, genetic distance, principle component analysis, selection criteria.

Yield is a complex trait depends on their component traits and highly influenced by the environment. The main cause of low yield in greengram is due to its indeterminate growth, non-synchronous maturity and losses due to pests and diseases. In most of the breeding programme, major emphasis is given on the improvement of yield *via* agro-morphological traits. The knowledge of genetic variation and trait relationship should lead to more understanding of yield component traits and yield potential of greengram. It is important to examine the contribution of various agro-morphological traits towards the seed yield. Therefore, information on trait relationship is greater importance. The primary objective of any breeding programme is to identify the superior genotypes on the basis of multiple traits over wide range of environments in targeted location. In such situation, creation of environments by extending the sowing dates has greater importance to save the time and gives clear idea about nature of traits for targeted location. Several methods are available to understand the variation and association of traits. Yan et al. (2000) developed the GGE bi-plot methodology for graphical analysis of multi environmental data. Yan and Rajan (2002) used GT (genotype by trait) bi-plot to study the genotype by trait data. A bi-plot analysis displays the effect of genotypes and the environment (Gabriel 1971).

Genetic diversity analysis helps in isolating the suitable genotypes for future hybridization to generate the more genetic variability for trait of interest and isolate the desirable recombinants/ segregants. Several methods are available for diversity analysis but here, Principle Component Analysis (PCA) was used for this purpose. PCA helps to isolate the major yield components, which helps in covering the maximum variability. The two way cluster analysis helps in grouping of genotypes as well as grouping of traits in various clusters. Keeping the above facts under consideration the present experiment was conducted to identify the important yield contributing traits and their relationship with each other and to isolate the suitable genotypes for recombination breeding.

The experimental material comprised of thirty-six genotypes of greengram listed in Table 1. The entries were received from Pulse Breeding Section, Department of Plant Breeding and Genetics, Tirhut College of Agriculture, Dholi, Muzaffarpur, Bihar, India. The experiment was conducted at Crop Research Farm, TCA, Dholi (RAU, Pusa), which is situated (25.5^0N, 35.4^0E, 52.12 m MSL) in district Muzaffarpur of North Bihar, India. The experiment was conducted in randomized complete block design (RCBD) with three replications under three environments by adjusting the sowing dates at 15 days interval viz., 10 July 2012 (early sown as E_1), 25 July 2012(timely sown as E_2) and 11 August 2012 (late sown as E_3). Each genotype was sown in six rows in plot of 4 m length with 30 cm plant geometry.

Five random plants were selected from each genotype in each replication to record the data for all the yield and yield component traits (except days to 50% flowering) viz., plant height (PH), number of primary branches per plant (NPBP), number of secondary branches per plant (NSBP), number of clusters per plant (NCP), number of pods per cluster (NPC), pod length (PL), number of seeds per pod (NSP), selling percentage (SP), seed index (SI), biological yield per plant (BYP), harvest index (HI) and seed yield per plant (SYP). Days to 50% flowering (DFF) was recorded on plot basis. Pod mass (PM) and seed mass (SM) were recorded by weighing the 10 pods and seeds from these 10 pods from five randomly selected plants and averaged. Pod wall mass (PWM) obtained by subtracting the seed mass from pod mass. Pod wall proportion (PWP) is an index obtained by dividing the weight of pod wall by weight of whole pod. The data for all the traits were subjected to pooled analysis of all the three environments. Correlation coefficient among yield and their component traits (R, version 2.15), bi-plot analysis, Principle Component Analysis (PCA), and cluster analysis (SAS, version 9.3) were performed by using statistical package given in parenthesis.

In order to maintain and effective utilization of breeding materials, it is important to investigate the extent of genetic variability and diversity available in gene pool (Mohammadi et al. 2003). In present investigation the significant variability was found for almost all the agro-morphological traits studied, indicated the possibility to utilize the genotypes for trait manipulation and other breeding programs (data not shown). Quantitative traits are highly influenced by environment and selection of the traits based on single season/ year/ environment taken under consideration for varietal improvement may be misleading. So, to meet this problem, crop was grown in three environments created by adjusting the sowing dates and data was subjected to pooled analysis to minimize the environmental effect and collect the right information about traits relationship and genetic divergence.

Association analysis: Knowledge of trait relationship would helps to identify the traits to be given important during selection. Correlation coefficients were worked out between 17 yield component traits with SYP and among themselves; have been presented in Table 2. SYP showed positive significant association with DFF, NPBP, NSBP, NCP, NPC, PL, NSP, PM, SM, SP, SI and HI, whereas PH and PWP were negatively associated with SYP. Similar pattern of relationship of SYP with agro-morphological given in parenthesis have earlier been reported by Venkateswarlu (2001) for SI, NSP and PL, Rao et al. (2006) for NCP, Siddique et al. (2006) for NSP and SI, Biradar et al. (2007) for NCP, NPC, PL, NSP and SI, Rahim et al. (2010) for PL and NSP and Tabasum et al. (2010) for PL, SI and HI and Khajudparn and Tantasawat (2011) for NSP and PL. The negative association between SYP and PH have earlier been reported by Singh et al. (2009), whereas several researchers viz., Hakim (2008); Zaid et al. (2011) have earlier reported the positive association between these traits. This deviation may be noted due to breeding material and environmental conditions. Yimram et al. (2009) observed the high environmental effect on PH. Branching behaviour helps in making plant canopy and enhances the yield by accumulating the photosynthate. The positive association between number of branches and other agro-morphological traits *viz.*, indicated that genotypes with more NPBP and NSBP had more NCP, NPC, PL, high SP, SI & HI; helps to enhance their yield potential. NPC, PL and NSP exhibited negative correlation with PWM and PWP. The positive association of PWM, SM and SI were recorded with PM. Among the pod characters PWM and SM; both had positive correlation with PM indicating the importance of both traits in PM enhancement. Green pods also contributed in photosynthesis and food material stored in seeds from source to sink. Thus, these agro-morphological traits may be taken under consideration during the selection for yield improvement of greengram. HI showed strong positive association with most of the traits viz., NPBP, NSBP, NCP, NPC, PL, NSP, SM, SP, SI and SYP, whereas negative association was noticed between HI and BYP. Thus, HI with optimized biomass (BYP) may also be used as selection criteria for yield improvement.

Principle component analysis (PCA) and interaction bi-plot **:** In order to find out the relationship among genotypes and traits, principle component analysis (PCA) was also performed. The relationship between genotypes and yield component traits also plotted in same graph using genotype by trait bi-plot interaction as shown in Fig 1. Bi-plot analysis is an important tool for analysis of data and their interpretation. Bi-plot showed relationship between traits by angle form between two or more variables. According to bi-plot analysis, there was positive association between DFF, NPBP, NSBP, NCP, NPC, PL, NSP, PM, SM, SP, SI, HI with SYP (<90˚ angle); whereas PH and PWP make >90˚ angle with SYP, showed the negative association between them. The angle between two traits <90˚, showed positive association and vice versa. According to bi-plot analysis, genotypes with high PC_1 score and low PC_2 score gave high and consistent performance for SYP and vice versa. Dividing the variance into its components helps in conservation and utilization of specific traits for crop improvement (Ghafoor et al. 2001). Thus, multivariate analysis may be effective to cover all the important agro-morphological traits by transforming the PCs into a single index. A total 16 principle components (PCs) were formed, which explained 100% variation as presented in Table 3. The first five PCs with >1 eigen values contributed 80.32% of total variation. Among the agro-morphological traits, pod related (reproductive) traits i.e. PL, SM and PWM were noted as key components for PC_1, PC_2 and PC_3, respectively. But other agro-morphological traits had also high weight given in parenthesis for PC_1 (PL, NSP, PM & SI), PC_2 (PM, SM & SP) and PC_3 (PWM & PWP), suggesting the importance of these traits to reflects the yield potential of genotypes (Table 4).

Cluster analysis**:** Genetic divergence analysis was widely used to determine the genetic relationship among the genotypes and find out the suitable genotypes for future breeding programme. Genetic diversity analysis also helps in tagging and elimination of the duplicate accessions from genetic stock (Dwivedi and Gaibriyal 2009). The two way hierarchical cluster analysis was done for grouping of genotypes as well as yield component traits using Ward's method to assess the similarity or dissimilarity and clarify the relationship among them as shown in Fig 2. Distributions of genotypes into various clusters and cluster distances have been presented in Table 5 and 6 respectively. Cluster analysis indicated that all the 36 genotypes were grouped in 4 main clusters (Three major and one minor). Cluster I comprised 6 genotypes, cluster II comprised 11 genotypes, cluster III comprised 17 genotypes and cluster IV formed a mono genotypic cluster. It was also noted that the greengram germplasm of differences in origin were grouped in the same cluster, indicating absence of relationship between genetic diversity and geographic diversity. The results were in agreement with Singh et al. (2013). The maximum intra cluster distance was recorded for cluster I followed by cluster III and cluster II, whereas maximum inter cluster distance was found between cluster II-IV followed by cluster I-IV and III-IV, indicating the possibility of greengram improvement through crossing between members of these clusters. The high genetic distance of solitary cluster with rest clusters indicating the possibility of greengram improvement through crossing between members of these clusters. The minimum inter cluster distance was found between cluster II-III. The mean characteristics of various clusters have been presented in Table 7. For clear understanding about characterization of clusters has also been done by parallel plot analysis as shown in Fig 3. Cluster I is characterized by higher NPC, SP, BYP and SYP; cluster II is by early flowering, less NPBP, NSBP, NCP, NPC and BYP, whereas it had highest values for PL, NSP, PM, PWM, SM, HI and SI. Cluster III is characterized by high PWP and PH. Cluster IV had minimum values for almost all the traits studied except DFF, NPBP, NSBP, NCP, NPC and PWP.

However, developing the guidelines to select the parents is crucial but it is major problem in heterosis breeding. It is generally assume that the parents with more diversity involved in crossing programme give more heterosis than the closely ones (Singh, 1991). Several researchers *viz.*, Katiyar et al. (2009); Abna et al. (2012); Patel and Patel (2012); Jayamani and Sathya (2013) also gave emphasis on need of high genetic diversity to create the high genetic variation and genetic gain under selection. The theoretical considerations support this statement but in practice some reports stated the relationship between heterosis and parental diversity is contradictory. Behl et al. (1995) suggested that heterotic response occurs within a restricted range of diversity. Shukla and Singh (2006); Yadav et al. (2007) have also observed the no correspondence between F_1 performance and their parents performance (except some traits). Thus, the moderately diverse genotypes can also be included in breeding programme to isolate the good recombinants. Similar statement has earlier been given by Parameshwarappa et al. (2009). In this situation representative genotype(s) from each cluster may be selected and involve in crossing program for yield improvement. Based on cluster mean, the promising donors for some important agro-morphological traits may also be isolated for trait manipulation and/ or recombination and/ or transgressive breeding.

Based on trait relationship and bi-plot analysis, it was noted that traits *viz*., DFF, NPBP, NSBP, NCP, NPC, PL, NSP, PM, SM, SP, SI and HI were identified as important traits for yield improvement. The association of agro-morphological traits with PCs indicated that the traits *viz*., PL, NSP, PM, SM, PWM, SP, SI and HI were found with high weight and these traits may be taken under consideration as key components during the selection for yield improvement of greengram. Sona selection had moderate to more diversity with all the genotypes due to their solitary nature and may be used in breeding programme. Besides this, representative genotypes from clusters II-IV, I-IV and II-IV may be selected and added in hybridization programme for greengram improvement.

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Table 1. List of greengram genotypes and their origin used in present experiment

Code	Genotype Name	Origin	Code	Genotype Name	Origin
G1	HUM-12	BHU, Varanasi	G19	DMS 02-11-4	TCA, Dholi
G2	IPM-02-14	IIPR, Kanpur	G20	IPM 99-1-6	IIPR, Kanpur
G3	NDM-09-18	NDUAT, Faizabad	G21	Pusa 1232	IARI, New Delhi
G4	ML-1666	PAU, Ludhiana	G22	Pusa Vishal	IARI, New Delhi
G5	DM 05-12-1-42-3	TCA, Dholi	G23	Pusa 1231	IARI, New Delhi
G6	DMS 01-34-2	TCA, Dholi	G24	IPM2K-15-4	IIPR, Kanpur
G7	DMS 03-17-2	TCA, Dholi	G25	Pusa 9531	IARI, New Delhi
G8	DM 99-11-5	TCA, Dholi	G26	PM 08-2	GVPUAT, Pantnagar
G9	SML-668	PAU, Ludhiana	G27	NDM12-308	NDUAT, Faizabad
G10	Samrat	IIPR, Kanpur	G28	DMS 02-11-13	TCA, Dholi
G11	DMC 17	TCA, Dholi	G29	IPM 99-394	IIPR, Kanpur
G12	Meha	IIPR, Kanpur	G30	SML 1186	PAU, Ludhiana
G13	Sona selection	TCA, Dholi	G31	PM 5	GVPUAT, Pantnagar
G14	IPM 2K-14-9	IIPR, Kanpur	G32	SML 1151	PAU, Ludhiana
G15	DM 05-74-11	TCA, Dholi	G33	Pusa Baishakhi	IARI, New Delhi
G16	IPM 99-01-10	IIPR, Kanpur	G34	AKM 8803	PKV, Akola
G17	PM 2	GVPUAT, Pantnagar	G35	HUM 16	BHU, Varanasi
G18	Pusa 1131	IARI, New Delhi	G36	TMB 37	BARC, Mumbai

Table 2. Phenotypic correlation among various agro-morphological traits with seed yield and among themselves in greengram

Traits	DFF	PH	NPBP	NSBP	NCP	NPC	PL	NSP	PM	PWM	PWP	SM	SP	SI	BYP	HI
DFF	1.000															
PH	0.063	1.000														
NPBP	0.516**	-0.111*	1.000													
NSBP	0.591**	-0.05	0.681**	1.000												
NCP	0.504**	0.084	0.313**	0.397**	1.000											
NPC	0.301**	0.078	0.193**	0.237**	0.483**	1.000										
PL	0.218**	0.204**	0.181**	0.110*	0.431**	0.305**	1.000									
NSP	0.236**	0.208**	0.018	0.086	0.584**	0.328**	0.532**	1.000								
PM	0.041	0.139*	0.09	-0.017	0.150**	0.071	0.402**	0.162**	1.000							
PWM	-0.306**	-0.015	-0.176**	-0.256**	-0.380**	-0.296**	-0.146**	-0.332**	0.451**	1.000						
PWP	-0.368**	-0.099	-0.246**	-0.273**	-0.502**	-0.361**	-0.384**	-0.457**	-0.093	0.832**	1.000					
SM	0.274**	0.162**	0.230**	0.174**	0.448**	0.298**	0.544**	0.424**	0.740**	-0.266**	-0.726**	1.000				
SP	0.368**	0.111*	0.264**	0.268**	0.497**	0.365**	0.391**	0.456**	0.09	-0.824**	-0.985**	0.718**	1.000			
SI	0.400**	0.258**	0.220**	0.218**	0.536**	0.437**	0.607**	0.603**	0.336**	-0.319**	-0.550**	0.603**	0.550**	1.000		
BYP	-0.088	-0.06	0.002	-0.029	-0.189**	-0.009	-0.206**	-0.370**	0.075	0.206**	0.177**	-0.074	-0.165**	-0.213**	1.000	
HI	0.209**	0.012	0.112*	0.158**	0.472**	0.290**	0.371**	0.498**	0.088	-0.350**	-0.413**	0.358**	0.410**	0.440**	-0.580**	1.000
SYP	0.219**	-0.008	0.134*	0.175**	0.464**	0.366**	0.326**	0.343**	0.140*	0.282**	-0.372**	0.364**	0.367**	0.441**	0.004	0.735**

*= P<0.05, **= P<0.01

Days to 50% flowering (DFF), Plant height (PH), number of primary branches per plant (NPBP), number of secondary branches per plant (NSBP), number of clusters per plant (NCP), number of pods per cluster (NPC), pod length (PL), number of seeds per pod (NSP), Pod mass (PM), Pod wall mass (PWM), Pod wall proportion (PWP), shelling percentage (SP), seed index (SI), biological yield per plant (BYP), harvest index (HI) and seed yield per plant (SYP).

Table 3. Eigen values and variability explained by each principle components (PCs)

PCs	Eigen value	Percent variation	Cumulative Percent variation
1	4.32	25.44	25.44
2	3.57	20.95	46.38
3	2.24	13.16	59.55
4	1.94	11.43	70.98
5	1.59	9.34	80.32
6	0.85	5.01	85.32
7	0.77	4.50	89.83
8	0.49	2.89	92.72
9	0.36	2.11	94.83
10	0.35	2.06	96.89
11	0.23	1.33	98.22
12	0.15	0.91	99.12
13	0.10	0.56	99.68
14	0.03	0.16	99.84
15	0.02	0.14	99.98
16	0.01	0.02	100.00

Table 4. Correlation coefficient of each agro-morphological trait with respect to its principle components (PCs)

Traits	PC 1	PC 2	PC 3
DFF	-0.756	0.313	0.306
PH	0.309	-0.297	0.094
NPBP	-0.766	0.285	0.211
NSBP	-0.849	0.252	0.123
NCP	-0.265	0.260	0.179
NPC	-0.104	0.314	0.169
PL	0.767	0.182	0.215
NSP	0.708	-0.327	-0.309
PM	0.578	0.636	0.417
PWM	0.453	-0.066	0.844
PWP	-0.028	-0.765	0.594
SM	0.470	0.835	0.048
SP	0.012	0.758	-0.595
SI	0.554	0.472	0.216
BYP	-0.134	0.564	0.269
HI	0.279	-0.105	-0.383
SYP	0.114	0.326	-0.111

Days to 50% flowering (DFF), Plant height (PH), number of primary branches per plant (NPBP), number of secondary branches per plant (NSBP), number of clusters per plant (NCP), number of pods per cluster (NPC), pod length (PL), number of seeds per pod (NSP), Pod mass (PM), Pod wall mass (PWM), Pod wall proportion (PWP), shelling percentage (SP), seed index (SI), biological yield per plant (BYP), harvest index (HI) and seed yield per plant (SYP).

Table 5. Distribution of greengram genotypes in various clusters

Cluster	Number of genotypes	Genotype code	Name of genotypes
I	6	1, 2, 3, 4, 8, 14	HUM 12, IPM 02-4, NDM 09-18, ML 1666, DM 9911-5, IPM 2K-14-9
II	7	12, 22, 24, 36, 31, 23, 30	Meha, Pusa Vishal, IPM 2K-15-4, TMB 37, PM 5, Pusa 1132, SML 1186
III	20	5, 15, 29, 26, 9, 34, 11, 17, 16, 18, 6, 33, 7, 20, 21, 25, 35, 10, 28, 32, 19, 27	DM 05-12-1-42-3, DM 05-74-11, IPM 99-394, PM 08-2, SML 668, AKM 8803, DMC 17, PM 2, IPM 99-01-10, Pusa 1131, DMS 01-34-2, Pusa Baishakhi, DMS 03-17-2, IPM 99-1-6, Pusa 1232, Pusa 95.1, HUM 16, Samrat, DMS 02-11-13, SML 1151, DMS 02-11-4, NDM 12-308
IV	1	13	Sona selection

Table 6. Intra (diagonal) and Inter-cluster (below diagonal) distances for four clusters in greengram

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	**4.93**			
Cluster II	5.80	**4.11**		
Cluster III	5.96	5.36	**4.46**	
Cluster IV	11.58	12.63	11.53	**0.00**

Table 7.Cluster means for various agro-morphological traits in greengram

	Clusters			
Characters	I	II	III	IV
DFF	32.78	31.89^{+}	32.21	77.11*
PH	39.7	48.31	50.50*	33.15^{+}
NPBP	2.98	2.97^{+}	3	7.48*
NSBP	3.03	2.77^{+}	2.98	9.30*
NCP	11.16	8.93^{+}	9.93	11.20*
NPC	5.07*	3.85^{+}	4.15	4.28
PL	6.47	6.73*	6.15	5.13^{+}
NSP	9.09	10.12*	9.56	4.79^{+}
PM	0.42	0.45*	0.38	0.36^{+}
PWM	0.15	0.17*	0.16	0.14^{+}
PWP	37.20^{+}	38.28	41.98*	38.73
SM	0.26	0.28*	0.22^{+}	0.22^{+}
SP	62.67*	61.6	58.21^{+}	61.42
SI	3.18	3.36*	2.95	2.77^{+}
BYP	25.54*	18.61^{+}	18.84	21.66
HI	24.79	25.22*	22.87	17.12^{+}
SYP	5.90*	4.03	3.87	3.74^{+}

*Highest cluster mean, +Lowest cluster mean

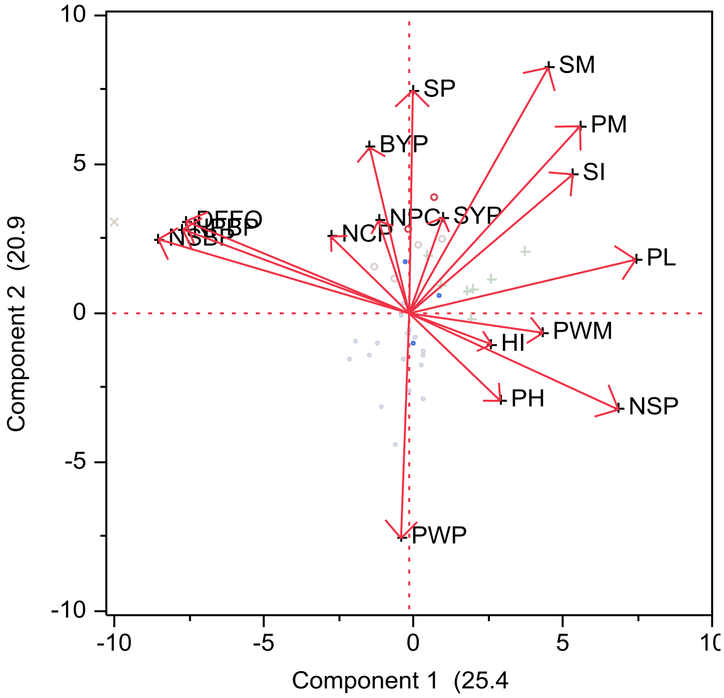

Fig 1. Genotype by trait interaction bi-plot for trait relationship among various agro-morphological traits in greengram

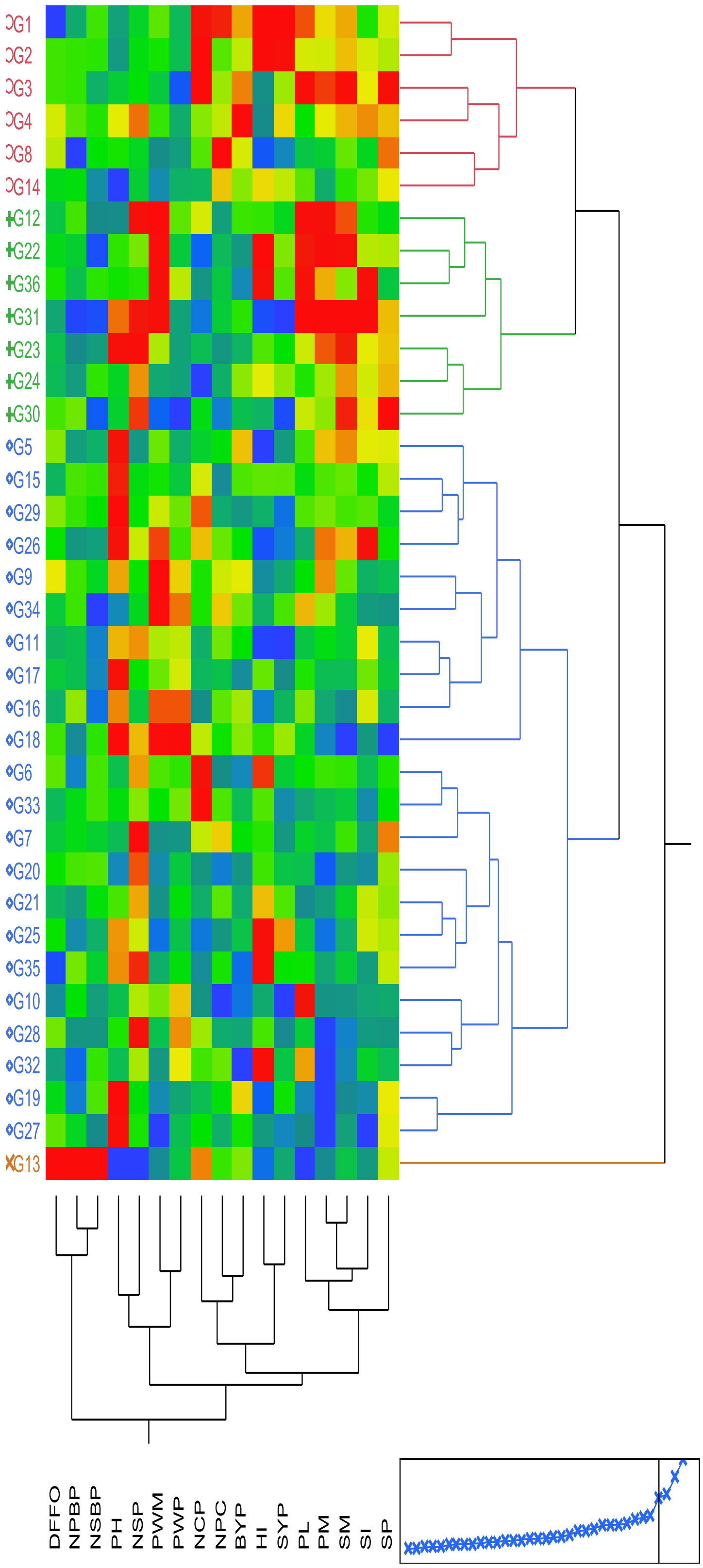

Fig 2. Two-way clustering of 36 greengram genotypes* and 17 agro-morphological traits using Ward's method (*Name of genotypes have been shown as Table 5)

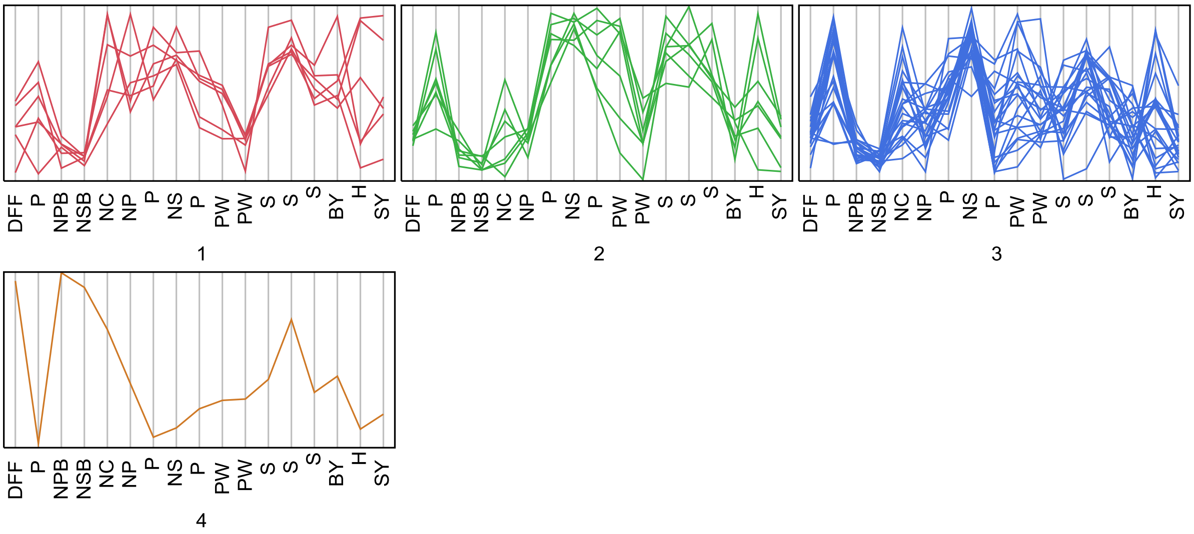

Fig 3. Parallel plot for four clusters [(1) Cluster I, (2) Cluster II, (3) Cluster III, (4) Cluster IV] based on various agro-morphological traits in greengram