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CONTENTS

Marker assisted selection for aromatic and semi-dwarf segregants in cross of aromatic Katarni rice SUNDARAM BHARTI, P.K. SINGH, KUMARI SUVIDHA, SATYENDRA, S. P. SINGH, ANAND KUMAR and MANKESH KUMAR	188
D² and principal component analysis for variability studies in <i>Vigna</i> and <i>Phaseolus</i> species PRIYANKA BHARETI, R. K. PANWAR, ANJU ARORA and S. K. VERMA	193
Assessment of genetic parameters in F₅ recombinants derived from <i>Indica</i> rice (<i>Oryza sativa</i> L.) line Pusa 6A PRACHI PRIYA, MANKESH KUMAR, TIRTARTHA CHATTOPADHYAY, BISHUN DEO PRASAD, SWETA SINHA, ANAND KUMAR and SATYENDRA	198
Genetic diversity analysis by D² clustering of fodder yield and its related traits in forage sorghum HARSH DEEP, INDRANI CHAKRABORTY, SATYAWAN ARYA, PUMMY LAMBA, S. K. PAHUJA and JAYANTI TOKAS	203
Genetic diversity for morpho-physiological and seed vigour traits in wheat (<i>Triticum aestivum</i> L.) PUNEET KUMAR, Y.P.S. SOLANKI, VIKRAM SINGH and ASHISH	209
<i>In vitro</i> plant regeneration from mature embryo using different plant growth regulators in wheat genotype HD 3059 SWATI SHARMA, ASHWANI KUMAR, ANIL SIROHI, R. S. SENGAR, KAMAL KHILARI, MUKESH KUMAR and MANOJ K. YADAV	215
Weed management and crop geometry effect on nutrient uptake and yield in aerobic rice VASUNDHRA KAUSHIK, S. P. SINGH, V. P. SINGH, TEJ PRATAP and B. S. MAHAPATRA	222
Studies on sucker control in natu tobacco (<i>Nicotiana tabacum</i> L.) under rainfed vertisols S. JAFFAR BASHA, P. PULLI BAI, S. KASTURI KRISHNA and C. CHANDRASEKHARA RAO	228
Seed and oil yield of bidi tobacco (<i>Nicotiana tabacum</i> L.) varieties as influenced by planting geometry and fertilizer levels under rainfed vertisols S. JAFFAR BASHA, P. PULLI BAI, S. KASTURI KRISHNA and C. CHANDRASEKHARA RAO	232
Comparison of non-linear models on area, production and productivity of sugarcane crop in Uttar Pradesh JHADE SUNIL and ABHISHEK SINGH	237
Performance of improved varieties of true Cinnamon (<i>Cinnamomum verum</i> J. Presl.) in Andaman Islands, India AJIT ARUN WAMAN, POOJA BOHRA and R. KARTHIKA DEVI	243
Changing climate and its effect on rice yield in Meghalaya DEOTREPHY K. DKHAR, SHEIKH MOHAMMAD FEROZE, RAM SINGH and LALA I.P. RAY	249
Age related changes in morphometrical studies on ductus deferens of guinea fowl (<i>Numida meleagris</i>) TAMILSELVAN S, B. S. DHOTE and MEENA MRIGESH	257

Occurrence of gastrointestinal nematodes in goats slaughtered at Rewa, India D. MARAVI, A. K. DIXIT and POOJA DIXIT	261
Autoimmune haemolytic anaemia in a dog-A case report NEERAJ KUMAR, MUNISH BATRA and R.S. CHAUHAN	265
Erythrocytic anaplasmosis with <i>Fasciolosis</i> in a cross-bred cattle: A case report NEERAJ KUMAR and MUNISH BATRA	269
Modification and evaluation of Pant-ICAR controlled traffic seed-cum-deep fertilizer applicator for multi-crop seeder-cum-deep placement of fertilizers applicator MANISH KUMAR, T.C THAKUR, MANOJ KUMAR and SATYA PRAKASH KUMAR	272
Drying characteristics of shrimp (<i>Metapenaeus dobsoni</i>) in electrical dryer D.S. ANIESRANI DELFIYA, S. MURALI, P.V. ALFIYA and MANOJ P. SAMUEL	281
Baur dam breach analysis using various Manning's roughness values MEENAKSHI RAMOLA, JYOTHI PRASAD and H. J. SHIVA PRASAD	286
Study of constipation and related factors among female students of Pantnagar RITA SINGH RAGHUVANSHI, NIDHI JOSHI, DIKSHA SINGH, SHIKHA SINGH, MEENAL and DASHRATH BHATI	290
Work -related musculoskeletal disorders among chikankari workers in Lucknow (U.P.) POONAM SINGH and KATYAYNI	297
Technology adoption and productivity enhancement in groundnut cultivation: An impact assessment of farm women groups K.UMA, T. NIVETHA and S. PRAVEENA	302
Health hazard and constraints of chikankari worker in Lucknow (U.P.) POONAM SINGH and KATYAYNI	310
Studies on Indigenous Agricultural Technical Knowledge prevalent among the farmers of Assam for the management of common pests and diseases in major crops DEVAMITRA TARAFDAR and NIRMAL MAZUMDER	315
Television viewing pattern among students of CCS Haryana Agricultural University, Hisar ANIL KUMAR MALIK, KRISHAN YADAV and SUNIL KUMAR	325
Media content development and it's standardization for farmers REETA DEVI YADAV, GEETAMATI DEVI and RITA GOAL	331
Analysis of learning behavior and pattern of online learners on a MOOC platform G.R.K. MURTHY, SEEMA KUJUR, S. SENTHIL VINAYAGAM, YASHAVANTH B.S., CH. SRINIVASA RAO, P. S. PANDEY, VANITA JAIN and INDRADEVI T.	338

D² and principal component analysis for variability studies in *Vigna* and *Phaseolus* species

PRIYANKA BHARETI¹, R. K. PANWAR¹, ANJU ARORA¹ and S. K. VERMA¹

Department of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar-263145 (U.S. Nagar, Uttarakhand)

ABSTRACT: Eight *Vigna* and one *Phaseolus* species were used in the present study to estimate the contribution of component traits to the total variation. The genotypes included nine each from black gram and mungbean, three wild relatives of black gram and one mungbean, three genotypes of rice bean, five genotypes of cowpea and one genotype of french bean. The contribution of different morphological traits has been evaluated by using D² and principal component analysis, which has led to the recognition of significant phenotypic variability. The relative contribution of root dry weight (9.952), shoot to root dry weight ratio (6.817), P content in seed (6.320), 100 seed weight (5.695), total P uptake at maturity (5.382) and seed yield/plant (5.248) was maximum towards the genetic divergence by D² method. The seven principal components PC1, PC2, PC3, PC4, PC5, PC6 and PC7 with eigen roots of 8.721, 5.048, 3.268, 1.941, 1.155, 1.005 and 0.812, respectively have accounted for 91.46% of total variation of which first three principal components accounted for 70.98 per cent variation. PCA analysis revealed the maximum contribution of root dry weight (0.269) followed by total biological yield/plant (0.253) in PC1, harvest index (0.369) followed by 100 seed weight (0.294) in PC2 and seed yield/plant (0.384) followed by plant height (0.268) in PC3. The eigen root of first principal component accounted for 36.338 per cent of total variation followed by second to seventh principal components, which accounted for 21.035, 13.615, 8.089, 4.813, 4.189 and 3.382 per cent of total variations present in the genotypes, respectively. These results confirmed the presence of considerable genetic diversity for use in *Vigna* and *Phaseolus* genotypes improvement program. The study revealed that principal component analysis was more effective in partitioning variation than D² analysis.

Key words: D², eigenroot, *Phaseolus*, principal component analysis (PCA), variation, *Vigna*

Pulses or grain legumes used for human consumption are particularly important in human nutrition as sources of proteins (21-26%), vitamins and minerals that complement a predominantly cereal-based diet (Tharanathan and Mahadevamma, 2003). Pulses are the most affordable vegetable protein. By virtue of this, they occupy a unique position in the World Agriculture. They are broadly defined by their unusual flower structure, podded fruit, and the ability to form nodules with rhizobia (de Faria *et al.*, 1989). Thus their well-known ability to fix atmospheric nitrogen helps reducing energy consumption while making them particularly suitable for low-input systems. The world produced an estimated 92.4 million tonnes of pulses in 2017-18. Substantial increases have been achieved in the production of common beans, chickpeas, lentils, pigeon peas, cowpeas and dry peas.

The major kharif season pulses are mungbean (*Vigna radiata* L. Wilczek), urdbean (*Vigna mungo* L. Hepper), rice bean (*Vigna umbellata* (Thunb.), french bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* (L.) Walp.) and pigeonpea (*Cajanus cajan* (L.) Millsp.) while rabi season pulses are mostly chickpea, lentil and field pea. India is the largest producer (25% of global

production), consumer (27% of world consumption) and importer (14%) of pulses in the world. The country had the world's largest area under pulses with 27-29 million hectares in two seasons and was the world's largest producer of 22-24 million tonnes in 2018-19. However, at about 800 kg per hectare, yields are low by world standards. Scaling in pulses productivity was targeted by at least an average of about 80-100 kg/ha during every 5-year interval to accomplish the achievable targeted yield of 1 tonne per ha by 2025 and 1.4 t/ha by 2050. Major pulse producing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh and Karnataka. The rice-wheat-rice cycle needs to be broken for the ecological disaster it has brought about such as deterioration in soil health and an alarming fall in the water table. Systematic inclusion of pulses in the cropping system will deliver multiple benefits, including improvement in soil health through nitrogen fixation.

Indeed, leguminous plants constitute the third largest family among flowering plants and contain no less than 18,000 species. But the specific diversity of pulses is poorly represented in most cropping systems despite the growing demand from food chains (Edwards, 2007). The

diversity implies potentially high variable responses to abiotic and biotic stresses. While more than 80 pulse species contribute to the human diet, the FAO database includes only 11 of these (Tiwari *et al.*, 2011). They are, most of the time, locally important crops maintained by cultural preferences and traditional practices because they are nutritionally rich (Padulosi *et al.*, 1999; Ghane *et al.*, 2010) and adapted to harsh environments unfit for other crops where they can still provide sustainable productions (Mal, 2007). However, as recent articles (Massawe *et al.*, 2016) demonstrate, diversity is a key component of a sustainable diet i.e., Healthy Diet for Healthy Planet (Drewnowski, 2014). The assessment and exploitation of diversity in pulses can have a significant impact on productivity. In light of these considerations, it is essential to help maintain and use pulse genetic resources, to ensure sustainable development and use by present and future generations, in order to take full advantage of the services they offer (Guiguitant *et al.*, 2020).

Among the various methods identified/developed to study the genetic divergence in a population, the Mahalanobis D^2 (Mahalanobis, 1936) is reliable and most frequently used. D^2 analysis is a useful tool in quantifying the degree of divergence between biological population at genotypic level and to assess relative contribution of different components to the total divergence, both at the inter- and intra-cluster levels. However, Principal Component Analysis (PCA) is a standard tool in modern data analysis due to its simple, non-parametric nature for extracting relevant information from various data sets. Main benefit of PCA arises from quantifying the importance of each dimension for describing the variability of a data set. It involves a mathematical procedure that transforms a number of correlated variables into a (smaller) number of uncorrelated variables called principal components (Muniraja *et al.*, 2011). It was performed by using correlation matrix in order to examine the relationships among the 24 characters that are correlated among each other's by converting into uncorrelated characters called principal components.

With this view the present investigation aims at evaluating genetic diversity for morphological traits of few cultivated and wild relatives of *Vigna* and *Phaseolus* at the interspecies level and contribution of few major traits towards total variation be identified based on D^2 and principal component analysis.

MATERIALS AND METHODS

The seeds of different *Vigna* and *Phaseolus* species were

obtained from G. B. Pant University of Agriculture and Technology, Pantnagar and BHU, Varanasi. *Vigna* and *Phaseolus* species comprised of black gram, mungbean, common bean, rice bean and cowpea. The experiment was planted in *Kharif* season 2012-13 in randomized block design with 2 replications. The data were recorded on five representative plants from each plot at 45 DAS and at the time of harvesting, which were selected randomly. Data were recorded on twenty four yield, yield contributing and root morphological traits viz., plant height (cm), root length (cm), lengthiest lateral root length (LLRL) (cm), root volume (mm^3), root collar diameter (mm), number of lateral roots, root dry weight (mg), shoot dry weight (mg), leaf area (cm^2), root surface area (mm^2), shoot to root dry weight ratio and total P uptake (mg/plant) and at the time of harvesting viz., number of pods/plant, pod length (cm), number of seeds/pod, root dry weight (mg), shoot dry weight (mg), seed yield/plant (g), 100 seed weight (g), total biological yield/plant (g), harvest index, shoot P uptake (mg/plant), P content in seeds (mg/plant) and total P uptake (mg/plant). Phosphorus concentration in plant samples was determined by following vanadomolybdate yellow colour method as outlined by Jackson (1973).

The genetic divergence was estimated by using D^2 statistics suggested by Mahalanobis (1936), which is based on multivariate analysis of quantitative traits. The genetic distances were estimated by Mahalanobis D^2 statistic. The relative contribution of different characters to the total D^2 values between each pair of genotypes was given a score of 1 to 24 (there are twenty four characters) based on the magnitude of D^2 value due to each character. A rank of 1 represented the highest contribution and 24 the lowest. Percentage contribution of each character was calculated as following:

$$\text{Percentage contribution of X character} = \frac{N(X) \times 100}{n(n-1)/2}$$

Where, $N(X)$ = number of genotypic combinations which were ranked first for characters X out of total genotypic combinations of $n(n-1)/2$ (possible combinations between total genotypes).

In principal component analysis, the mean scores on twenty four yield & yield contributing traits and root morphological traits were subjected to ordination procedure by principal component analysis. The concept of principal component which is a multivariate technique was developed by Hotelling (1933) after its original concept given by Pearson (1901). The eigenvector for all the principal components have been scaled in such a way that the largest element in each vector is unity. These were interpreted as relative weight of the variables in each component. The important variables are those which have high positive/negative relative weight values.

RESULTS AND DISCUSSION

The relative contribution of different characters towards the expression of genetic divergence as estimated through D² analysis is given in Table 1. Root dry weight at 45 DAS contributed maximum towards the genetic divergence (9.952%) followed by root dry weight at maturity (9.925%), shoot to root dry weight ratio (6.817%), P content in seed (6.320%), 100 seed weight (5.695%), total P uptake at maturity (5.382%), seed yield/plant (5.248%), total P uptake at 45 DAS (4.893%), shoot P uptake (4.459%), total biological yield/plant (3.488%), shoot dry weight at 45 DAS (3.431%), shoot dry weight at maturity (2.889%), pod length (2.769%), root volume (2.579%), leaf area (2.566%), lengthiest lateral root length (2.448%), root collar diameter (2.280%), number of seeds/pod (1.990%), plant height (1.963%), harvest index (1.809%) and root surface area (1.196%). It was observed that contribution of three characters, viz., number of pods/plant (0.978%), number of lateral roots (0.969%) and root length (0.759%) was below 1%.

Principal component analysis reflects the importance of the largest contributor to the total variation at each

Table 1: Contribution of different characters towards genetic divergence in 31 genotypes of *Vigna* and *Phaseolus* species

S. No.	Character	Per cent contribution to genetic divergence
1	Plant height (cm)	1.963
2	Root length (cm)	0.759
3	Lengthiest lateral root length (cm)	2.448
4	Root volume (mm ³)	2.579
5	Root collar diameter (mm)	2.280
6	Number of lateral roots	0.969
7	Root dry weight (mg) at 45 DAS	9.952
8	Shoot dry weight (mg) at 45 DAS	3.431
9	Leaf area (cm ²)	2.566
10	Root surface area (mm ²)	1.196
11	Shoot to root dry weight ratio	6.817
12	Total P uptake (mg/plant) at 45 DAS	4.893
13	Number of pods/plant	0.978
14	Pod length (cm)	2.769
15	Number of seeds/pod	1.990
16	Root dry weight (mg) at maturity	9.925
17	Shoot dry weight (mg) at maturity	2.889
18	Seed yield/plant	5.248
19	100 seed weight (g)	5.695
20	Total biological yield/ plant (g)	3.488
21	Harvest index	1.809
22	Shoot P uptake (mg/plant)	4.459
23	P content in seed (mg/plant)	6.320
24	Total P uptake (mg/plant) at maturity	5.382

axis for differentiation (Sharma, 1998). The principal component analysis of 31 genotypes of *Vigna* and *Phaseolus* species for 24 traits based on correlation matrix of yield, yield contributing and root morphological traits yielded the eigen roots (eigen values) and eigenvectors. These values and associated percentage of variation explained by eigen root are presented in Table 2. The study revealed that seven principal components PC1, PC2, PC3, PC4, PC5, PC6 and PC7 with eigen roots of 8.721, 5.048, 3.268, 1.941, 1.155, 1.005 and 0.812, respectively have accounted for 91.46% of the total variation. The first principal component accounted for 36.338 per cent of total variation followed by second to seventh principal components which accounted for 21.035, 13.615, 8.089, 4.813, 4.189 and 3.382 per cent of total variations present in the genotypes, respectively. First three principal components accounted for 70.987 per cent variation though the per cent variation explained by 5th, 6th and 7th components were relatively small. Mohanlal *et al.*, 2018 observed 35.44% contribution of the first PC to the total variability, whereas 20.06%, 12.39% and 11.23% were accounted by second, third and fourth principal components in blackgram. Similar studies were also conducted in field pea by Kumar *et al.*, 2018. Sharifi *et al.* (2018) indicated that in chickpea first three factors in total accounted for 69.69% of the total variability with individual contribution of 33.69%, 20.82% and 15.19% of total variability, respectively.

Traits having higher values in the first principal component such as root dry weight at maturity (0.269) followed by total biological yield/plant (0.253), and root surface area (0.247) had more contribution to the total variation. Similarly, harvest index (0.369) followed by 100 seed weight (0.294) and root collar diameter (0.288) in the second principal component; seed yield/plant (0.384) followed by plant height (0.268) and number of seeds/pod (0.256) in the third principal component; root length (0.428) followed by number of seeds/pod (0.380) and seed yield/plant (0.376) in the fourth principal component; number of seeds/pod (0.317) followed by number of pods/plant (0.292) and lengthiest lateral root length (0.288) in the fifth principal component; P content in seed (0.381) followed by total P uptake at maturity (0.281) and number of lateral roots (0.215) in the sixth principal component and P content in seed (0.554) followed by root length (0.341) and plant height (0.243) in the seventh principal component were the major contributors for variability to each principal components. Mohanlal *et al.* 2018 also reported that first principal component had higher contribution from the following traits viz., number of pods per plant, pod length, number of seeds per pod, seed yield per plant, whereas second, third and fourth principal

Table 2: Eigenvector, eigen root and associated variation for principal components in 31 genotypes of different *Vigna* and *Phaseolus* species

S. No.	Character	Eigen vector						
		1	2	3	4	5	6	7
1	Plant height (cm)	0.166	0.239	0.268	0.171	0.032	0.087	0.243
2	Root length (cm)	0.106	0.172	-0.155	0.428	-0.115	-0.125	0.341
3	Lengthiest lateral root length (cm)	0.085	-0.072	-0.410	0.133	0.288	0.129	-0.224
4	Root volume (mm ³)	0.097	-0.013	-0.476	0.249	0.120	-0.012	-0.115
5	Root collar diameter (mm)	0.163	0.288	0.088	0.219	0.053	0.171	-0.277
6	Number of lateral roots	0.004	0.107	-0.393	0.364	-0.220	0.215	0.126
7	Root dry weight (mg) at 45 DAS	0.245	0.208	-0.067	-0.160	-0.267	0.156	-0.172
8	Shoot dry weight (mg) at 45 DAS	-0.207	-0.203	0.138	0.120	-0.418	-0.252	-0.051
9	Leaf area (cm ²)	0.242	0.087	-0.070	0.093	-0.093	-0.545	0.154
10	Root surface area (mm ²)	0.247	-0.262	0.123	0.085	0.043	0.097	-0.073
11	Shoot to root dry weight ratio	0.202	-0.226	0.060	0.036	-0.356	0.168	-0.213
12	Total P uptake (mg/plant) at 45 DAS	-0.251	0.257	-0.037	0.024	-0.189	-0.138	-0.022
13	Number of pods/plant	0.225	-0.288	0.024	-0.033	0.292	0.091	0.058
14	Pod length (cm)	-0.273	0.169	0.109	0.223	0.131	-0.008	0.016
15	Number of seeds/pod	0.105	0.020	0.256	0.380	0.317	-0.294	-0.267
16	Root dry weight (mg) at maturity	0.269	0.189	-0.025	-0.108	-0.261	0.174	-0.110
17	Shoot dry weight (mg) at maturity	-0.333	-0.047	-0.026	0.057	0.008	0.019	-0.067
18	Seed yield/plant	-0.030	0.081	0.384	0.376	-0.113	0.176	-0.274
19	100 seed weight (g)	0.204	0.294	0.085	-0.147	0.146	-0.037	-0.225
20	Total biological yield/ plant (g)	0.253	-0.221	-0.007	0.094	-0.320	-0.125	-0.050
21	Harvest index	0.094	0.369	-0.053	-0.229	0.007	0.031	0.153
22	Shoot P uptake (mg/plant)	-0.268	0.248	0.021	-0.018	-0.027	0.210	-0.067
23	P content in seed (mg/plant)	0.127	-0.108	0.232	0.137	0.066	0.381	0.554
24	Total P uptake (mg/plant) at maturity	-0.263	-0.185	-0.087	0.130	-0.071	0.281	-0.087
Eigen root		8.721	5.048	3.268	1.941	1.155	1.005	0.812
Per cent variation exp.		36.338	21.035	13.615	8.089	4.813	4.189	3.382
Cum. Variation exp.		36.338	57.372	70.987	79.076	83.889	88.078	91.460

components had contributions from various other traits viz., number of branches, pod weight and 100 seed weight. Katiyar *et al.* (2009) working on mungbean also used principal component and non-hierarchical Euclidean cluster analysis to compare the genotypes and observed wide diversity among the cultivars for different attributes. Thus the present study confirmed that *Vigna* and *Phaseolus* genotypes have wide range of variations for the characters studied. It is also suggested that ample opportunities exist for genetic improvement of pulses genotypes for future utilization.

CONCLUSION

The analysis of variance showed significant differences among all twenty four characters studied. Principal component analysis showed that seven principal components PC1, PC2, PC3, PC4, PC5, PC6 and PC7 accounted for 91.46% of the total variation. The first three principal components accounted for 70.987 per cent to the total variation indicating that hybridization breeding program can be initiated by selecting

genotypes from the PC1, PC2 and PC3.

Out of all the traits evaluated, root dry weight at maturity, total biological yield/plant, root surface area in PC1 and harvest index, 100 seed weight, root collar diameter in PC2 contributed more to the total variations. On comparing D² and PC analysis, it was observed that the traits such as root dry weight, 100 seed weight and seed yield/plant contributed more to the total variation. These results further confirmed the presence of considerable genetic diversity for use in *gna* and *Phaseolus* genetic improvement program.

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