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GENETIC DIVERSITY ASSESSMENT OF INDIGENOUS RICE GERMPLASM FROM NORTHERN PAKISTAN USING AGRO MORPHOMETRIC TRAITS

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Abstract

The, study aims to ascertain the extent of genetic variability among the *Oryza sativa* germplasm using agro-morphological characters. The germplasm assayed comprised of 116 accessions acquired from Northern Areas of Pakistan. All the genotypes were characterized for 19 agro-morphological traits. The data were analyzed by cluster analysis and principal component analyses. A significant level of variability was noticed for a number of agro-morphological traits. The largest variation was observed in seed yield per plant, (588.32), sterile culms per plant (341.25) and flag leaf length (291.09). The 116 accessions in this study were grouped into seven clusters based on hierarchical clustering method. PCI which is most important explained 28.41%, PCII contributed 13.38%, PCIII accounted for 11.69% of total morphological variability On the basis of greater, seed yield per plant, 1000-seed weight, early maturity, lowest no of sterile culms, highest no of panicle per plant, three promising genotypes 7821,7814,7593 can be used for general cultivation under Islamabad conditions. Genotypes 7757, 7837, could be evaluated further to be used in future breeding programs.

Key words: Genetic diversity, Principal component analysis, Cluster analysis, Rice accessions**.**

Introduction

Rice, *Oryza sativa* L. (2n = 24) is modelled species, largely developed in the zone of tropical, subtropical and monsoon regions of the old world (Ezuka & Kak, 2000). Rice is major staple food ranks second for Asian countries as well as in Pakistan (Ashfaq, 2012). Asian countries are the main rice producer, with its rice production accounting for nearly 92% of the world's total production (Ohtsubo *et al.,* 2005). It is widely cultivated in more than 112 countries, including Pakistan. It has also been used as an important source of income and employment for rural masses and as well foreign exchange earning commodity and hence named "Golden Grain of Pakistan" (Galani *et al.,* 2011). Rice accounts 0.6% of the value added in GDP. The total paddy production area is about 2311 000 hectares in 2013, 10.1 percent less than 2012 year's area. Production of the crop is 5541 000 tones, and to compare 2012's year production shows a decrease of 10.0 percent (Anon., 2013). It is grown on largest areas in Punjab and Sindh. Khyber Pakhtoonkhwa is also growing rice, Short grain Begami rice is grown in Swat having good scent and aroma retain for longer time. The quality traits are better than basmati. There is also high demand of this rice in national as well as international markets. The ten years forecasted area under rice trends of decreasing in the coming years in KPK, which is an alarming situation for poor Pakistani consumers of rice. While forecasted yield of rice for coming ten years tend to low in KPK. As there is a decrease in yield and also there is a province requirement to increase the production of rice in KPK, to fulfill poor people supply of protein, carbohydrates, fat, calcium and iron that do not have enough resources to buy expensive protein based diet (Bibi *et al.,* 2014). Like any other crop species, for rice it is a source of variation, which is a raw material for any improvement as it assists in the development of superior recombinants. Genetic diversity analysis is used for estimating and establishing of genetic relationship in germplasm collection, identifying more promising diverse parental combinations to create segregating progenies with maximum genetic variability for further selection, and introgressing desirable genes, from diverse germplasm into the available genetic base, (Smith, 1984; Thompson *et al*., 1998; Islam *et al.,* 2012). A lot of work is done in this respect for other areas of Pakistan (Akram *et al.,* 1995; Zafar *et al.,* 2000; Tehreem *et al.,* 2012). In this study we specifically explored rice germplasm from Northern Areas of Pakistan. Hence, in this study indigenous genotype from Northern Areas of Pakistan were used to assess the level of genetic diversity exhibited by germplasm collection of rice on phenotypic basis using multivariate analysis.

Materials and Methods

The research work was done at, Plant Genetic Resources Institute (PGRI), National Agriculture Research, Centre, Islamabad (NARC), Islamabad. Pakistan during the year 2011-12. One hundred and sixteen rice accessions including three check varieties (JP5, Super Basmati, and IR6) are used for morphological study. Seeds collected from the National Gene bank, Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The experiment conducted in Randomized Complete Block Design, (RCBD) with three replications. Twenty days, old seedlings were transferred from pots to field. The distance between rows and plants were kept 20cm. Standard agronomic practices and plant protection measures were adopted. The Data collected for 19 quantitative traits on the particular growth stages of the rice plant (Table 1). Following the descriptor for Rice *Oryza sativa* L. (IBRGR-IRRI Advisory Committee, 1980) all the agronomic and morphological traits are recorded. The data collected on agro-botanical traits from the rice accessions were subjected to statistical analysis using software SPSS v17.0. Cluster analysis with the SAHN subprogram using the UPGMA clustering as implemented in NTSYS-PC version 2.2e was used to construct a dendogram showing the relationship among the genotypes. Principal Components Analysis (PCA) of the traits was used to examine, the percent contribution, of each trait to the total genetic variation. For graphical representation, of the pattern of variation among all the accessions of rice scatter plots of first three principal components were formed using Statistica, version 7.0.

Table 1. Traits abbreviation list.

S. No.	Quantitative traits	Abbreviations
1.	Flag leaf length	FLL
2.	Flag leaf width	FLW
3.	Leaf length	LL
4.	Leaf width	LW
5.	Legule length	LgL
6.	Number of culms	NoC
7.	Culm length	CL
8.	Culm diameter	CD
9.	Sterile culm per plant	SC/P
10.	Panicles per plant	P/P
11.	Panical length	PL
12.	Plant height	PH
13.	Days to maturity	DM
14.	Seed yield per plant	SY/P
15.	1000-grain weight	TSW
16.	Paddy grain length	PGL
17.	Paddy grain breadth	PGB
18.	Paddy grain thickness	PGT
19.	Grain length/breadth ratio	GL/B

Results and Discussion

Morphological traits: Basic statistics for measured agronomical characters is tabulated, in (Table 2) revealed a considerable level of coefficient of variation for a number of Agro-morphological traits among different accessions of Rice in this study. Seed yield being an ultimate product of all traits is very important (Saleem *et al.,* 2010). The high coefficient of variation (CV) was observed for seed yield per plant, (588.32), sterile culms per plant (341.25). Flag leaf is very important for preparation of food. CV recorded for flag leaf length and culm length is (291.09), (248.21) respectively. In rice plant height is a complex character and is the final product of several genetically controlled factors such as no of internodes (Cheema *et al*., 1987) The semi dwarf plant type has been extensively utilized in the improvement of rice (*Oryza sativa* L.) cultivars throughout the world. Coefficient of variation recorded in this study for plant height is (234.49). Panicles per plant is also yield attributing trait and observed CV value is (231.93), but Panicle per plant alone does not determine the high grain yield as traits such as, grain shape, grain size, longer panicles and higher number of grains/panicle ultimately contribute to grain yield (Akram *et al.,* 1994). Number of culms is yield attributing trait (Abbasi *et al.,* 1995). Its variance is (221.23). Ali *et al.* (2000) also observed high coefficient of variability for number of culms per plant. CV for leaf length is (220.89). While lowest level of CVs was observed for other traits. Analysis of sample variance and standard deviation for these traits showed that variation was highly significant so it was concluded that a lot of variation existed among the cultivars.

Table 2. Descriptive statistics of agro-morphological traits in rice accessions.

Traits	Mean	Minimum	Maximum	SD	Variance	CV (%)
Flag leaf length	40.89	23.60	67.40	119.01	10.91	291.09
Flag leaf width	1.32	0.74	2.12	0.07	0.26	5.13
Leaf length	48.17	28.00	71.20	106.41	10.32	220.89
Leaf width	1.18	0.72	1.86	0.04	0.21	3.81
Legule length	1.45	0.54	2.50	0.12	0.34	8.11
Number of culms	21.36	9.20	41.00	47.25	6.87	221.23
Culm length	105.17	74.20	141.40	261.04	16.16	248.21
Culm diameter	2.61	1.66	4.04	0.18	0.43	6.93
Sterile culm per plant	4.29	0.40	26.40	14.62	3.82	341.25
Panicles per plant	17.07	7.40	38.00	39.60	6.29	231.93
Panical length	27.58	19.40	38.40	11.95	3.46	43.33
Plant height	132.75	95.00	174.20	311.28	17.64	234.49
Days to maturity	84.14	79.00	131.00	38.09	6.17	45.27
Seed yield per plant	73.76	35.57	146.00	433.93	20.83	588.32
1000-grain weight	27.73	19.04	35.06	12.63	3.55	45.55
Paddy grain length	5.89	4.74	8.02	0.32	0.57	5.47
Paddy grain breadth	2.81	1.86	3.37	0.10	0.31	3.43
Paddy grain thickness	2.01	1.54	3.01	0.04	0.20	2.00
Grain length/breadth ratio	2.14	1.61	3.36	0.15	0.39	7.10

Cluster analysis: On basis of agronomic and morphologic characters the unweighted. pair group method using, arithmetic averages (UPGMA) is most commonly used (Mohammadi & Prasanna, 2003). The 116 accession in this study were grouped into seven clusters based on hierarchical clustering (Fig. 1) Cluster I comprised of 17 genotypes, Cluster II includes 56 genotypes, Cluster Ill comprises of 3 genotypes, Cluster IV comprises of 9 genotypes. Cluster V comprises of 5 genotypes. Cluster VI comprises of 20 genotypes and Cluster VII comprises of 6 genotypes (Fig. 1). Group V accessions had longest leaf length, while leaf width, flag leaf length did not varied considerably (Fig. 2A). The number of culms per plant was highest (37) for the accessions in Clusters VII (Fig. 2B). But members of group III produced lowest no of culms per plant. Group VII had highest no of panicles per plant. Members of group IV and V had longest panicle length. No of sterile culms per plant was also lowest for members of group III. (Fig. 2B).The genotypes in Cluster III were late maturing. The mean duration to days to maturity for each group was 82-84 days for all clusters except for cluster III which is 115 days (Fig. 2C). All genotypes of Northern areas performed well in Islamabad’s hot conditions and were earliest in days to maturity. Means for plant height per group varied significantly, and was between 122 and 154 cm across the groups (Fig. 2C). Members of group V, and IV were tallest in plant height and group I members were dwarf in plant height. Seed yield per plant was highest for members of group VII. Genotypes belonging to group III and IV had longest paddy grain length, while characters like paddy grain breadth and paddy grain thickness did not give enough variation to data (Fig. 2D). In this study we did not get any single cluster which may be appropriate for all agro morphological traits recorded at NARC, Islamabad. The overall objective of this study is utility of the genotypes in rice development work. Therefore accessions on important agronomical characters are identified (Table 3). No duplicates were identified among the studied accessions for quantitative traits in the cluster analysis (Fig. 1), which means there is a high diversity among the accessions for these traits. Cluster analysis based on agro-morphological diversity assessment in rice was also reported in several studies, such as (Ghalain, 2006; Durai, 2009; Zhang *et al.,* 2010; Tehreem *et al.,* 2012).

Pca analysis: Principal, component analysis based on nineteen agro-morphological, traits during year 2012 showed that five of the 19 principal components with an eigenvalue was, higher than one accounted for 67.86 of the total variation among 116 accessions of Rice (*Oryza sativa.* L). PCI which is most important explained 28.41%, PCII contributed 13.38%, PCIII accounted for 11.69% of total morphological variability (Table 4). First three PCs accounted for 53% of total variance, traits that accounted for most of the observed variations of the first three principal components considering the coefficients with an absolute value equal or higher than 0.64, were flag leaf length, flag leaf width, leaf length, culm length, plant height and grain length/grain breadth ratio. Whereas paddy grain breadth, paddy grain thickness and 1000-grain weight had substantially negative weight. Our results also agreed with Caldo *et al.* (1996) who reported that leaf length, plant height, culm length, maturity, tillering ability and heading were the major factors contributing to the total variation of parental lines of Philipine modern cultivars of rice. Principal component analysis of the (*O. sativa)* accessions revealed diverse grouping pattern which in general supported cluster analysis (Fig. 3). The first 3 principal components were plotted to detect the associations between the accessions. The separation on the basis of PC1 and PC2 showed that the genotypes were scattered in all the quarters, which show the high level of genetic diversity in the evaluated genotypes (Fig. 3). Among the 114 accessions were found diversely scattered on the scattered plot. In the first and second principal components 7803, 7593, 7660, 7814, 7779, 7646, 7837, 7786, 7642, 7232, 7670, 7802 and 7814 showed greater genetic diversity based on accessions numbers. In the first and third principal components 7803, 7617, 7814, 7660, 7598, super basmati, 7624, 7623, 7205, 7618, 7802, 7622 and 7630 showed greater genetic diversity based on accessions numbers (Fig. 4). Though principal component analysis organized accessions with more morphological similarities but clusters also included the accessions from different or far by sites. All data presented in this study allowed the identification of cultivars with diverse morphological traits that could be used as possible parents for rice improvement program. Our results also agree with Lie *et al.* (2010).

Conclusions and Recommendations

For most of the important traits highly significant differences among genotypes were disclosed by cluster analysis and presence of diversity among *O.sativa* genotypes. Genotypes 7821,7814,7593, have high yield per plant, highest no of culms, high no of panicles per plant, Genotype 7757 have high thousand weight, lowest sterile culms per plant, 7837 have low sterile culms per plant, early maturing, maximum grain length/breadth ratio. From the above conclusions it was recommended that: Genotype 7821, 7814, and 7593 can be used for general cultivation under Islamabad conditions. Genotypes 7757, 7837, could be evaluated further to be used in future breeding programs.

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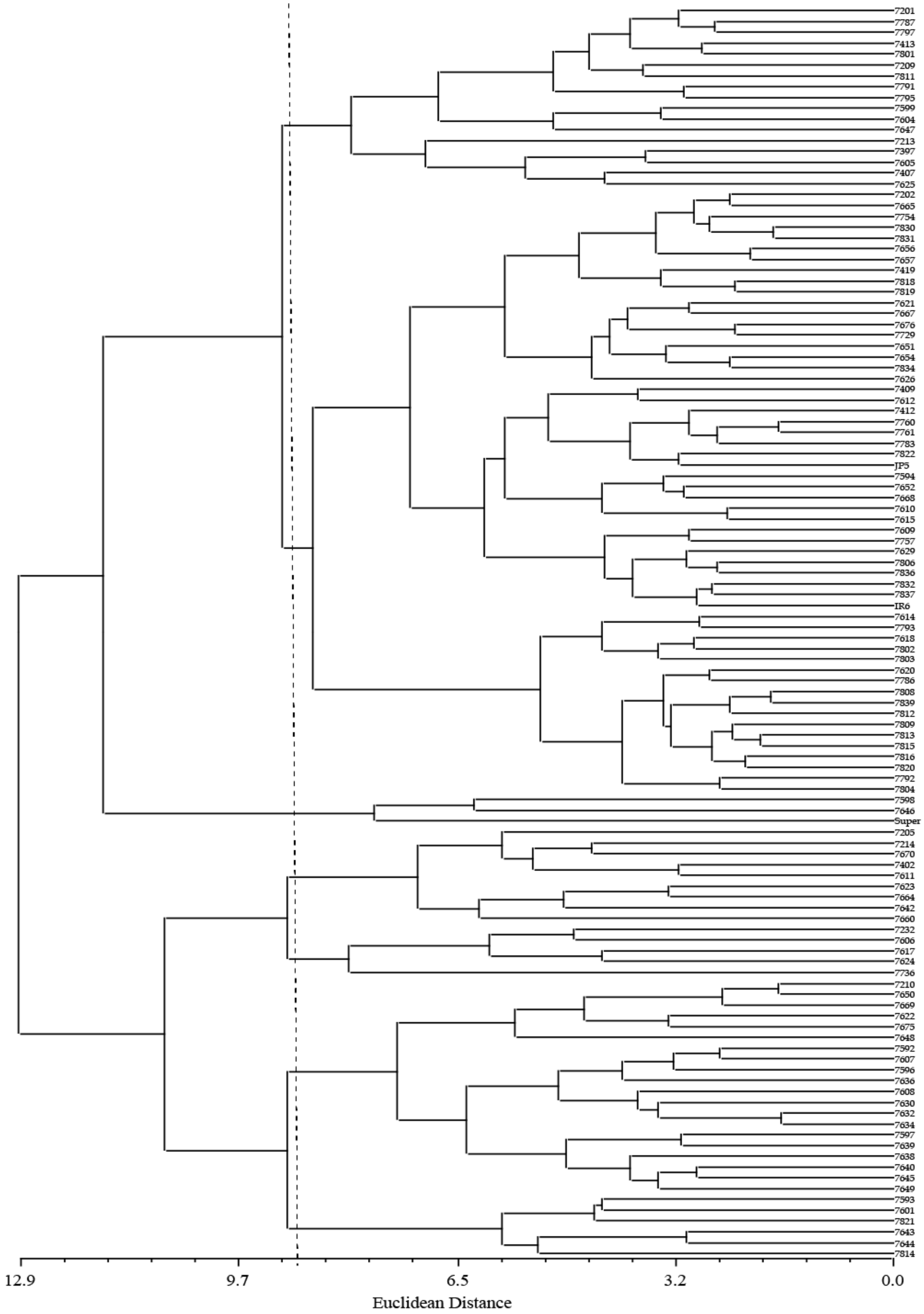

Fig. 1. Dendrogram showing the relationship among 113 accessions and 3 check varieties of *O. sativa* accessions based on 12 quantitative characters during year 2012- 2013.

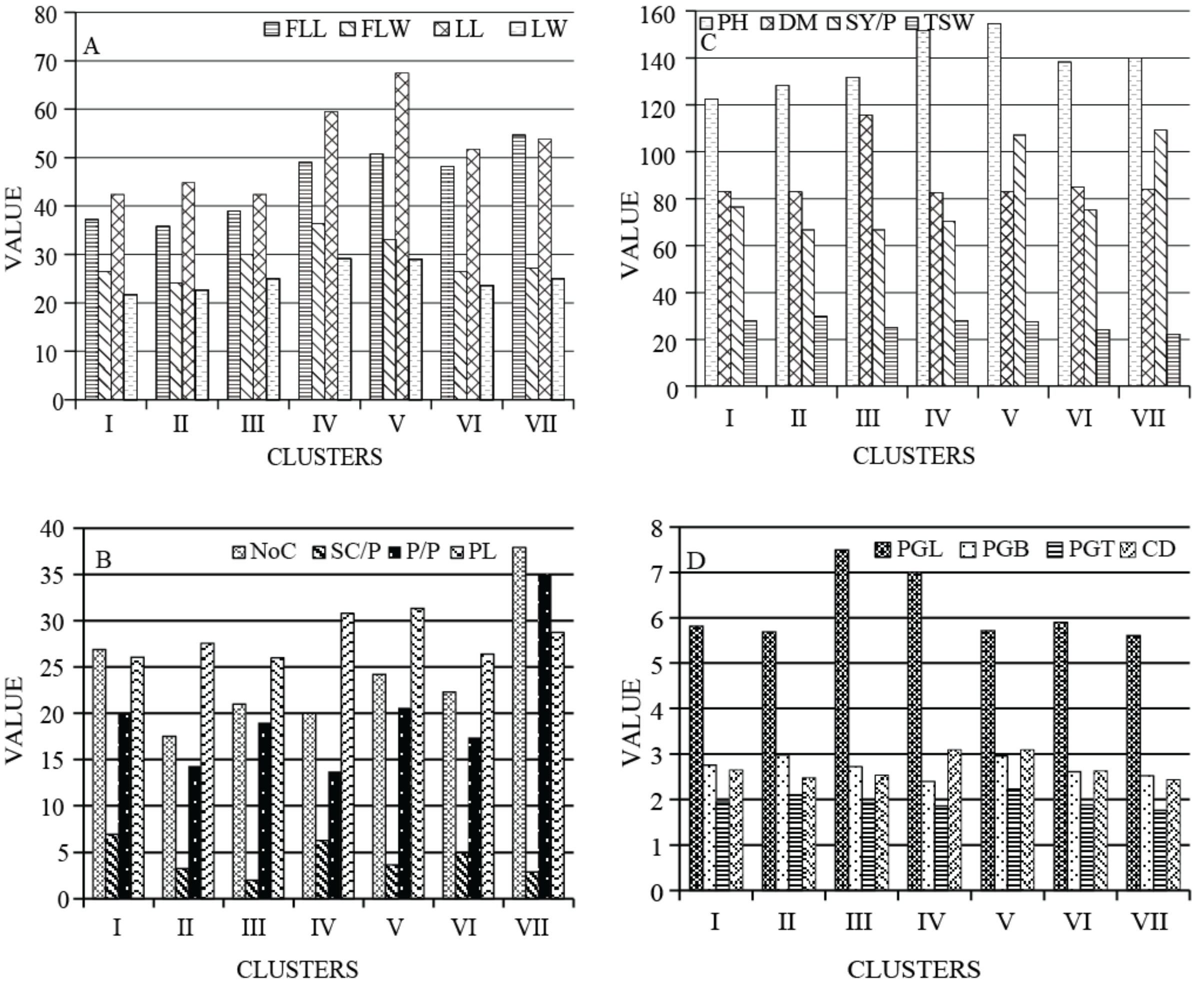

Fig. 2(A, B, C, D). Characteristic means of seven similarity clusters generated by UPGMA based on the classification of 114 genotypes of *O. sativa*, using different agro-botanical characteristics. For the purpose of scaling, data for flag leaf width and leaf width were multiplied by 20, all other data are means for cluster groups.

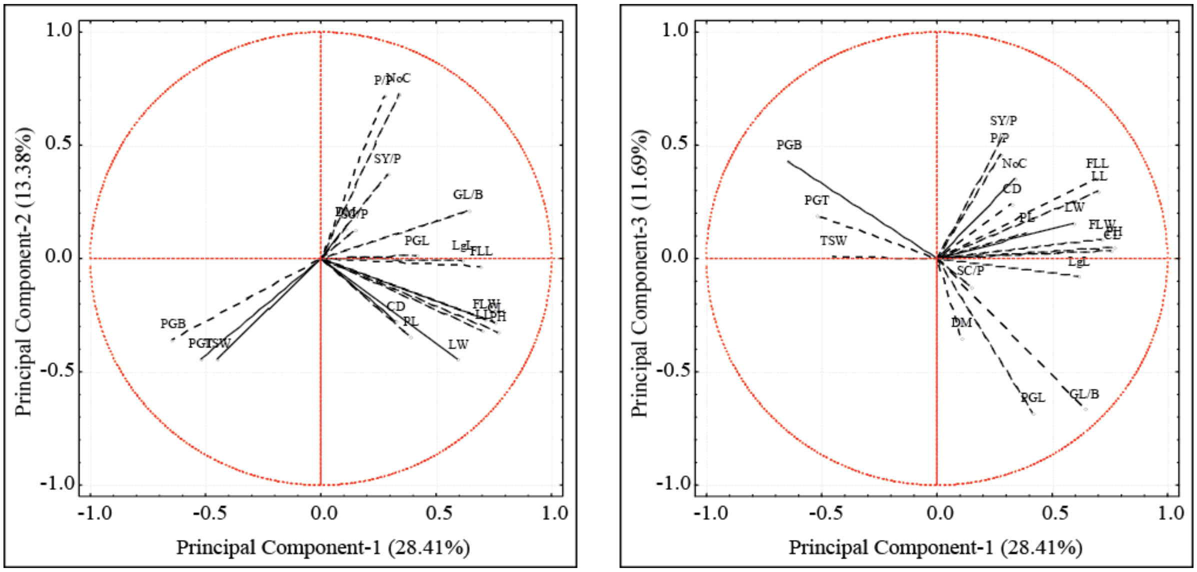

Fig. 3. Principal components analysis plot showing the contribution of 19 traits to total variation in first three PCs in a collection of *O. sativa* accessions.

Table 3. Elite accessions identified on the basis of important agro-morphological traits for future breading program.

Traits of interest	Range	Accessions identified
Number of culms	≥38	7821, 7814, 7593, 7213, 7643, 7644
Sterile culm per plant	≥0	7610, 7620,7757,7760,7205,7837
Panicles per plant	≥29	7646,7593,7643,7644,7814,7821
Panical length	≥33	7606,7624,7611,7667,7605,7397
Plant height	≤110	7804,7802,7620,7614,7629,7407
Days to maturity	≥79	7839,7837,7831,7830,7813,7812,7808
Seed yield per plant	≥121.5	7821,7814,7593,7636,7624,7617
1000-grain weight	≥34	Jp5,7836,7786,7757,7629,7609
Paddy grain length	≥7.3	7646,Super Basmati, 7205, 7402,7611,7660
Paddy grain breadth	≥3.0	7647,7623,7670,7622,7648,7675
Grain length/breadth ratio	≤4	Super Basmati, 7837,7832,7803,7760,7594

Table 4. Eigenvectors, Eigen values, total variance, and cumulative variance for 114 accessions based on 19 agro-morphological traits.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	5.40	2.54	2.22	1.47	1.26
Cumulative Eigenvalue	5.40	7.94	10.16	11.63	12.89
% Total variance	28.41	13.38	11.69	7.74	6.64
Cumulative %	28.41	41.79	53.48	61.22	67.86
Traits			**Eigenvectors**		
Flag leaf length	0.691	-0.035	0.351	-0.068	0.065
Flag leaf width	0.713	-0.269	0.083	-0.408	-0.145
Leaf length	0.698	-0.317	0.298	-0.018	0.109
Leaf width	0.592	-0.445	0.158	-0.160	-0.031
Legule length	0.612	-0.010	-0.079	-0.308	-0.005
Number of culms	0.337	0.728	0.354	-0.083	-0.391
Culm length	0.755	-0.283	0.035	0.366	0.134
Culm diameter	0.321	-0.277	0.242	-0.418	-0.090
Sterile culm per plant	0.149	0.129	-0.123	0.061	-0.903
Panicles per plant	0.277	0.717	0.461	-0.127	0.121
Panical length	0.386	-0.345	0.112	0.273	-0.289
Plant height	0.767	-0.327	0.054	0.389	0.066
Days to maturity	0.105	0.136	-0.349	-0.527	0.232
Seed yield per plant	0.290	0.371	0.541	-0.127	0.145
1000-grain weight	-0.454	-0.444	0.010	-0.167	-0.200
Paddy grain length	0.414	0.012	-0.680	-0.384	-0.047
Paddy grain breadth	-0.646	-0.358	0.433	-0.319	-0.053
Paddy grain thickness	-0.524	-0.445	0.191	-0.214	-0.083
Grain length/breadth	0.642	0.214	-0.665	-0.002	0.015

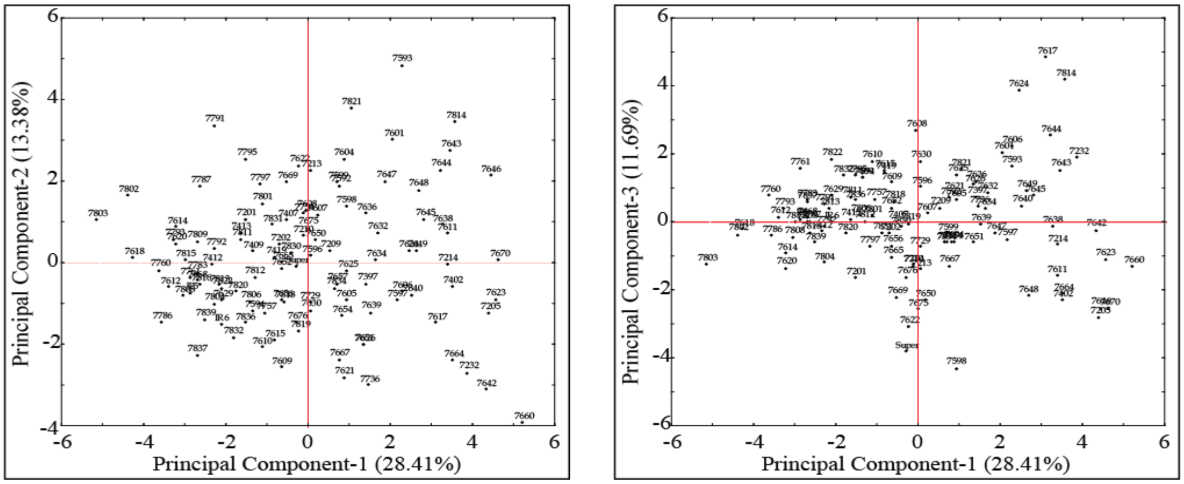

Fig. 4. Two dimensional scatter plot of the genetic relationship among 114 *O. sativa* accessions as revealed by first three principal componentsns.

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