



Research Note

Multivariate approach to analyse genetic diversity in some basmati rice genotypes (*Oryza sativa* L.)

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Abstract

Twenty three genotypes of basmati representing the broad spectrum of variation were assessed for ten yield attributing characters and ten quality characters using principal component analysis and cluster analysis. Principal component analysis identified five principal components with eigen values more than one for four components which contributed 90.40 per cent of the cumulative variance. The genotypes were grouped into six clusters by using cluster analysis. Cluster II was the largest, consisting of six genotypes followed by five genotypes each in clusters III, IV and V, two genotypes each in clusters I and VI. The results indicated that there was some degree of similarity of genotypes clustered together on the basis of their origin. However, the pattern of distribution of some genotypes from different eco-geographical regions was found random, indicating that geographical diversity and genetic diversity were not related. The maximum intra cluster distance was observed for the cluster III. The highest genetic divergence was observed between the clusters IV and III exhibiting wide diversity. Among different traits, plant height, days to 50% flowering, spikelets per panicle, KLAC and amylose content had maximum contribution towards total divergence may be used as selection parameters in segregating generations. Judicious selection of genotypes from the clusters may be used as potential donors for future hybridization programmes to develop varieties and hybrids of high yield without much compromise on quality.

Key words

Basmati rice, genetic diversity, principle component analysis, cluster analysis, eigen value

Basmati Rice, indigenous to the Indian sub-continent and endowed with unique quality traits are palatal delights of the rice connoisseurs' world over. These virtues of Basmati Rice command them premium price in domestic and international markets (Siddiq *et al.*, 2012). Improving productivity and quality traits of basmati rice always remain crucial. Traditional basmati rice varieties are very low yielding due to their poor harvest index, tendency to lodging and increasing susceptibility to foliar diseases, hence there is a need to develop new varieties combining the grain quality attributes of basmati with high yield potential (Amarawathi *et al.*, 2008).

Genetic variability is the pre requisite for any crop improvement programme. Improvement in any trait is solely depends on the amount of variability present in the base material of that trait. Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including, analysis of genetic variability in cultivars (Smith, 1984; Cox *et al.*, 1986), identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barett and Kidwell, 1998), and introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al.*, 1998). An understanding of genetic relationships among inbred lines or pure lines can be particularly useful in planning crosses, in assigning lines to specific heterotic groups, and

for precise identification with respect to plant varietal protection (Hallauer and Miranda, 1988). Significant emphasis is being paid to comprehensive analysis of genetic diversity in numerous field crops for long-term success of breeding program and maximum exploitation of the genetic resources (Belaj *et al.*, 2002). If the structure of the genetic diversity is known within a large collection of germplasm which may be of great help to make decisions on management procedures and breeding strategies to be used in breeding programs.

With the development of advanced biometrical techniques such as multivariate analysis, quantification of degree of divergence among the biological populations and assessing the relative contribution of different components to the total divergence at intra- and inter cluster levels have now become possible. Such a study also permits to select the genetically diverse parents to obtain the desirable recombinant in the segregating populations upon crossing. Multivariate statistical techniques which simultaneously analyze multiple measurements on each individual under investigation, are widely used in analysis of genetic diversity irrespective of whether it is morphological, biochemical or molecular marker-based and subsequently, classification of germplasm collections. Among the multivariate techniques, cluster analysis, principle component analysis (PCA), principal co-ordinate analysis

(PCoA) and Multi-Dimensional Scaling (MDS) are at present, most commonly employed and appear particularly useful (Mohammadi and Prasanna, 2003). The present study was, therefore, undertaken to assess the extent of genetic diversity in 25 rice genotypes using multivariate analysis approaches like principle component analysis (PCA) and cluster analysis.

The experimental material used in the study comprised of twenty three basmati rice genotypes grown in different agro-ecological zones of India. Two non-basmati genotypes were also included in the study making a total of twenty five genotypes. Genotype name, parentage and origin details are presented in Table 1. All genotypes were evaluated for grain yield and its attributing characters following randomized complete block design (RBD) with three replications during *kharif* season of 2011 and 2012 at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India, which is situated at 25° 18' North latitude and 83° 03' East longitude, at an altitude of 123.3m from mean sea level. The soil of the experimental plot was fertile, alluvial loam and was characterized as the type soil of Indo-Gangetic Plains. Transplanting was done 25 days after sowing of seeds in nursery bed in a 4m² plot. Plant to plant distance was 15cm, row to row distance was 20cm and the crop was raised as per recommended package of practices to ensure normal crop.

Observations were recorded on yield attributes viz., days to maturity, plant height (cm), panicle length (cm), effective panicles per plant (no.), spikelets per panicle (no.), spikelet fertility (%), test weight (gm) and yield per plant (gm) of ten randomly selected plants in each entry in a replication. Observations were also recorded to study grain quality characters viz., kernel length (mm), kernel breadth (mm), kernel L/B ratio, kernel length after cooking (mm), elongation ratio, alkali spread value (Little *et al.*, 1958) and amylose content (Juliano 1971). For statistical analysis, INDOSTAT software version 8.6 was used. The mean of the twenty five genotypes were analyzed statistically by the method outlined by Ostle (1966). The analysis of variance for different characters was carried out in order to assess the genetic variability among genotypes as given by Cochran and Cox (1950). The level of significance was tested at 5% and 1% using F table values given by Fisher and Yates (1963). The data were statistically analyzed to study the genetic diversity by principal component analysis as described by Jackson (1991). The genetic diversity between the genotypes was worked out using Mahalanobis D² analysis (1936) and grouping of genotypes into clusters was carried out following Tocher's method (Rao, 1948 and 1952).

The analysis of variance revealed a significant difference among twenty five genotypes for all ten yield characters indicating the existence of high variability among the genotypes (Table 2). However, there is little variation exist for quality traits since basmati quality traits are unique and should not vary (Table 3).

Principle component analysis identified five principle components with Eigen values which contributed 93.08 percent of cumulative variance (Table 4). The first principal component (PC1) contributed maximum towards variability (47.015) with high significant positive loading of number brown rice breadth (0.320) followed by kernel breadth (0.308), kernel length (0.288) and filled grains (0.287). The second principal component (PC 2) accounted 25.145 per cent of total variance and it reflected significant positive loading of days to 50% flowering (0.398) followed by spikelets per panicle (0.299), KLAC (0.222) and kernel L/B ratio (0.202). The third principle component (PC 3) accounted for 13.246 percent of total variation and was characterized conspicuously by high loading of test weight (0.543) followed by brown rice L/B ratio (0.299) and brown rice length (0.294). The fourth principle component (PC 4) accommodates only one character, days to maturity (1.000). The fifth principle component (PC 5) reflected highest contribution from alkali spread value (0.728). PC4 and PC5 accounted for 5 and 2.67 per cent total variation respectively. Based on the five principle components, mean genotypic scores or PCA score were computed (Table 5). The plot of PC 1, PC 2, PC 3 and PC 4 showed clear differentiation of genotypes according to their cluster membership of each cluster. Genotypes belonging to a common cluster have fallen nearer to each other and *vice versa*. Fig. 1. shows the two dimensional PCA plot with twenty five genotypes of rice clustered among themselves based on PCA scores. Thus the principal component scores of genotypes were used as input for clustering procedures in order to group the genotypes into various clusters.

Based on the relative magnitude of D² estimates, twenty five genotypes were grouped in to six clusters (Table 6). Cluster II was the largest, consisting of six genotypes followed by five genotypes each in clusters III, IV and V, two genotypes each in clusters I and VI. The clustering pattern indicated that there was some degree of similarity of genotypes clubbed together in a cluster on the basis of their origin. Similar findings were reported by Singh *et al.* (2008). However, some genotypes were placed in clusters independent of geographical origin. Similar kind of results were reported in other studies (Sharma *et al.*, 2002; Datt and Mani, 2003; Pradhan and Mani, 2005; Sharma *et al.*, 2008 and Sharma *et al.*, 2011).

Average intra and inter cluster distance (D^2 and D) values among six clusters were presented in Table 7. The average intra cluster D values ranged from 0 to 51.294. The highest intra cluster distance ($D=51.294$) was observed in the cluster IV, indicating wide genetic variation among the genotypes included in the cluster. The inter cluster D values ranged from 19.44 to 110.013. The maximum genetic distance was between cluster IV and III ($D=110.013$) followed by cluster III and V ($D=102.48$), cluster III and VI ($D=94.592$) and cluster VI and I ($D=85.810$), revealing that genotypes included in these clusters are genetically diverse and may give rise to superior recombinants and high heterotic response. However, it was noted that cluster I and VI included two genotypes each which are traditional basmati genotypes. Emphasis should be given to this cluster while selections of parents for hybridization programme since traditional basmati cultivars are excellent in basmati cooking qualities.

Percentage contribution of the characters towards total divergence (Table 8) revealed that maximum percentage of contribution came from the trait amylose content (39.33%), followed by KLAC (10.67%), days to 50% flowering (10.67%), plant height (10 %), spikelets per panicle (10 %) and elongation ratio (5.33%). Relative importance of some of these characters in inter varietal divergence on basmati rice was reported in other study (Datt and Mani, 2003; Pradhan and Mani, 2005; and Singh et al., 2008). The other traits had very low contribution to genetic divergence. Contribution of each character towards genetic divergence has been estimated from the number of times that each character appeared in the first rank (Table 8). Hence, amylose content, KLAC, days to 50% flowering, plant height, spikelets per panicle and elongation ratio may be used as selection parameters in the segregating generations.

The cluster mean values showed a wide range of variation for all the yield traits under study (Table 9) however, variation among quality traits was less (Table 10). Cluster means are helpful in selecting the desirable traits among clusters and it serves as a quick glance of clusters carrying desirable traits.

Considering the importance of principle components, Euclidean genetic distance, relative contributions of characters towards total divergence, the present investigation suggests that parental lines selected from cluster IV for plant height, panicle length and effective panicles; cluster I for spikelets/panicle, filled grains and alkali spread value; cluster II for cooking quality traits viz., brown rice L/B ratio, kernel length and kernel L/B ratio could be used in breeding programme to isolate superior recombinant genotypes with higher yield and best basmati

quality. However, it was noted that, unlike the development of non-basmati hybrids, the task of developing basmati quality hybrids was challenging as the development of parental lines was required to be incorporated with the basmati quality traits in order to improve the yield potential without sacrificing the special quality features of basmati.

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Table 1. Details of rice genotypes under study under study

S. No.	Variety	Parentage	Origin
1	TBD-1	Mutant of Taroari Basmati	BHU
2	TBD-2	Mutant of Taroari Basmati	BHU
3	TAROARI BASMATI	Pureline selection from local Basmati	Haryana
4	BASMATI 370	Pureline selection from local agro commercial group	Punjab
5	KASTURI BASMATI	Basmati 370/CR 88-17-1-5	DRR, Hyderabad
6	SONASAL BASMATI	-	Jammu & Kashmir
7	RANBIR BASMATI	Selection from Bas 370-90-95	Jammu & Kashmir
8	PUSA 2517-2-51-1	-	IARI, New Delhi
9	PUSA BASMATI-1	Pusa 167/Karnal local	IARI, New Delhi
10	PUSA BASMATI-1S-97	Selection from Pusa Basmati-1	BHU
11	PUSA 44	IARI 5901-2/IR-8	IARI, New Delhi
12	PUSA SUGANDHA-3	-	IARI, New Delhi
13	PUSA SUGANDHA-5	Pusa 3A/Haryana Basmati	IARI, New Delhi
14	HUBR-2-1	HBR 92/Pusa Basmati-1/Kasturi	BHU
15	BASMATI-24-1	Local land race	Maharaj ganj, U.P
16	BASMATI-24-5	Local land race	Partawal, U.P
17	BASMATI-24-7	Local land race	Siddardh nagar, U.P
18	VASUMATI	-	
19	PUSA SUGANDHA-2	-	IARI, New Delhi
20	CSR-30(YAMINI)	Selection from Taroari Basmati	
21	JP-2	Collection from Basti	Uttar Pradesh
22	PUSA 1460	Improved Pusa Basmati-1	IARI, New Delhi
23	PUSA 1121(Pusa Sugandha-4)	Pusa 614-1-2/Pusa 614-2-4-3	IARI, New Delhi
24	MAHI SUGANDHA	BK 79/Basmati 370	Rajasthan
25	TYPE-3	Selection from Dehradun Basmati	Uttar Pradesh

Table 2. Analysis of variance for yield attributing characters in Basmati rice

Source	d.f	Days to 50% Flowering	Days to maturity	Plant Height (cm)	Panicle Length (cm)	Effective Panicles (no.)	Spikelets/ Panicle (no.)	Filled Grains (no.)	Spikelet Fertility(%)	Test Weight (gms)	Yield per plant(gms)
Replication	2	0.413	0.093	0.982	1.279	0.213	0.373	25.120	0.492	0.010	0.538
Treatment	24	204.120**	202.396**	1117.06**	24.205**	11.264**	2425.97**	1734.05**	23.470**	0.315	18.988**
Error	48	0.705	0.675	0.573	0.353	0.074	5.901	11.38	2.969	0.013	0.447

*, ** significant at 5 and 1 per cent level, respectively

Table 3. Analysis of variance for quality characters in Basmati rice

Source	d.f	Brown Rice Length(mm)	Brown Rice Breadth(mm)	Brown Rice L/B Ratio	Kernel Length(mm)	Kernel Breadth(mm)	Kernel L/B Ratio	KLAC(mm)	Elongation Ratio	Alkali Spread Value	Amylose Content(%)
Replication	2	0.005	0.000	0.000	0.000	0.003	0.042	0.036	0.000	0.053	0.108
Treatment	24	2.330**	0.005	0.596	2.187*	0.034	0.606	6.048**	0.075	7.692**	4.296**
Error	48	0.004	0.002	0.000	0.001	0.002	0.016	0.025	0.000	0.025	0.083

*, ** significant at 5 and 1 per cent level, respectively

Table 4. The eigen values, proportion of total variance, cumulative per cent variance and component loading of different characters for five principal components

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen Value (Root)	9.403	5.029	2.649	1.000	0.535
% Var. Exp.	47.015	25.145	13.246	5.000	2.675
Cum. Var. Exp.	47.015	72.160	85.407	90.407	93.082
Days to 50 % flowering	0.058	0.398	0.232	0.000	0.106
Days to Maturity	0.000	0.000	0.000	1.000	0.000
Plant Height (cm)	-0.312	-0.101	0.013	0.000	-0.078
Panicle Length (cm)	-0.279	-0.052	0.087	0.000	0.171
Effective Panicles	-0.295	0.142	0.048	0.000	0.164
Spikelets/ Panicle	-0.079	0.299	-0.308	0.000	-0.112
Filled Grains	0.287	0.173	-0.006	0.000	-0.007
Spikelet Fertility %	-0.241	-0.219	-0.089	0.000	-0.302
Test Weight (100 Grain Wt)	0.134	0.037	0.543	0.000	0.119
Yield/ Plant (gms)	0.109	0.334	-0.243	0.000	0.075
Brown Rice Length (mm)	-0.161	-0.319	0.294	0.000	-0.037
Brown Rice Breadth (mm)	0.320	-0.065	0.007	0.000	0.044
Brown Rice L/B Ratio	0.155	-0.297	0.299	0.000	-0.077
Kernel Length (mm)	0.288	0.185	0.050	0.000	0.050
Kernel Breadth (mm)	0.308	0.022	0.178	0.000	0.011
Kernel L/B Ratio	-0.206	0.202	0.344	0.000	0.260
KLAC (mm)	-0.227	0.222	0.281	0.000	0.040
Elongation ratio	0.239	-0.275	0.053	0.000	-0.249
Alkali Spread Value	-0.053	-0.313	-0.249	0.000	0.728
Amylose Content (%)	-0.269	0.191	0.088	0.000	-0.360

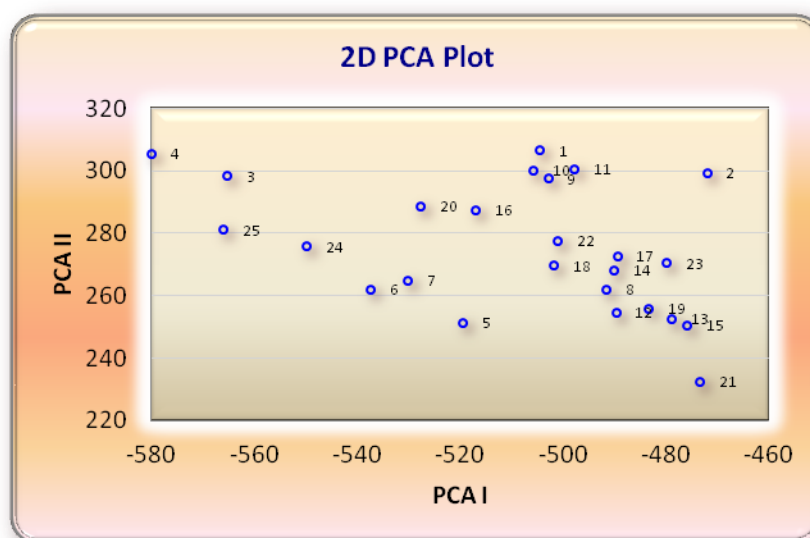


Fig. 1. Two dimensional graph showing relative position of 25 genotypes of basmati rice based on PCA scores (numbers 1 to 25 represent genotype serial number in table 1)

Table 5. The mean genotypic scores or PCA scores for 25 genotypes

S. No.	Genotypes	PC 1	PC 2	PC 3	PC 4	PC 5
1	TBD-1	-504.491	306.493	275.938	30.000	-254.302
2	TBD-2	-472.038	299.318	278.876	30.000	-243.664
3	TAROARI BASMATI	-565.160	298.339	308.140	30.000	-264.165
4	BASMATI-370	-579.919	305.398	274.454	30.000	-263.387
5	KASTURI BASMATI	-519.428	251.318	274.685	30.000	-253.576
6	SONASAL BASMATI	-537.372	261.984	261.822	30.000	-256.246
7	RANBIR BASMATI	-530.121	264.733	278.470	30.000	-259.070
8	PUSA 2517-2-51-1	-491.466	261.903	279.493	30.000	-251.670
9	PUSA BASMATI-1	-502.865	297.430	286.575	30.000	-241.820
10	PUSA BASMATI-1S-97	-505.831	300.189	283.441	30.000	-243.822
11	PUSA 44	-497.798	300.651	280.324	30.000	-256.101
12	PUSA SUGANDH-3	-489.697	254.544	278.112	30.000	-246.098
13	PUSA SUGANDH-5	-478.902	252.547	276.249	30.000	-246.630
14	HUBR-2-1	-490.089	268.136	279.444	30.000	-236.767
15	BASMATI 24-1	-475.971	250.424	253.655	30.000	-244.260
16	BASMATI 24-5	-516.905	287.454	287.130	30.000	-268.020
17	BASMATI 24-7	-489.453	272.724	277.017	30.000	-251.708
18	VASUMATI	-501.773	269.593	285.762	30.000	-257.142
19	PUSA SUGANDH-2	-483.380	255.929	287.714	30.000	-251.318
20	CSR-30	-527.586	288.751	302.172	30.000	-255.670
21	JP-2	-473.352	232.515	279.038	30.000	-240.247
22	PUSA 1460	-501.050	277.524	303.626	30.000	-251.795
23	PUSA 1121	-479.981	270.450	301.648	30.000	-249.227
24	MAHI SUGANDHA	-549.878	275.699	288.952	30.000	-255.507
25	TYPE-3	-566.055	281.030	294.936	30.000	-250.313

Table 6. Distribution of 25 genotypes of Basmati rice in different clusters

Cluster	Genotypes included	No. of genotypes
I	Taroari Basmati, Basmati 370.	2
II	Pusa 2571-1-51-1, HUBR-2-1, Basmati 24-7, Vasumati, Pusa 1460, Pusa 1121.	6
III	Pusa Sugandha-3, Pusa Sugandha-5, Pusa Sugandha-2, Basmati 24-1, J.P-2.	5
IV	TBD-1, TBD-2, Pusa Basmati-1, Pusa Basmati-1S-97, Pusa 44.	5
V	Kasturi Basmati, Ranbir Basmati, Sonasal Basmati, Basmati 24-5, CSR-30	5
VI	Mahi Sugandha, Type-3.	2

Table 7. Average intra and Inter Cluster distance (D^2 and D) values among six clusters

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	378.103 (19.444)	2523.309 (50.232)	4299.450 (65.570)	7363.450 (85.810)	5343.192 (73.074)	1570.968 (39.635)
Cluster II		1163.680 (34.112)	5970.428 (77.268)	2560.090 (50.597)	2311.110 (48.074)	2656.271 (51.539)
Cluster III			2631.606 (51.299)	12102.923 (110.013)	10502.781 (102.483)	8947.732 (94.592)
Cluster IV				0.000 (0)	1155.605 (33.994)	5399.063 (73.478)
Cluster V					0.000 (0)	3682.947 (60.687)
Cluster VI						0.000 (0)

D values are in parenthesis.

Table 8. Times ranked first and percentage contribution to total D^2

S. No.	Characters	Times Ranked 1 ST	Percentage contribution to total D^2
1	Days to 50%flowering	44	10.67
2	Days to Maturity	0	0.00
3	Plant Height (cm)	41	10.00
4	Panicle Length (cm)	0	0.00
5	Effective Panicles	0	0.00
6	Spikelets/ Panicle	57	12.33
7	Filled Grains	0	0.00
8	Spikelet Fertility %	0	0.00
9	Test Weight (100 Grain Wt)	0	0.00
10	Yield/ Plant (gms)	2	0.67
11	Brown Rice Length (mm)	7	2.33
12	Brown Rice Breadth (mm)	10	3.33
13	Brown Rice L/B Ratio	0	0.00
14	Kernel Length (mm)	7	2.33
15	Kernel Breadth (mm)	9	3.00
16	Kernel L/B Ratio	0	0.00
17	KLAC (mm)	50	10.67
18	Elongation ratio	16	5.33
19	Alkali Spread Value	0	0.00
20	Amylose Content(%)	108	39.33

Table 9. Mean values of different clusters with respect to ten yield traits

	Days to 50% flowering	Days to Maturity	Plant Height (cm)	Panicle Length (cm)	Effective Panicles	Spikelets/ Panicle	Filled Grains	Spikelet Fertility %	Test Weight (100 Grain Wt)	Yield/ Plant (gms)
I Cluster	110.100**	140.100**	98.450*	26.600	10.600	202.200**	171.200**	84.710*	1.986	11.903
II Cluster	97.111	127.111	105.606	27.222	8.556	152.778*	133.000*	87.089	2.361	9.482
III Cluster	87.750	117.750	104.825	25.500*	6.750*	156.750	138.250	87.675	2.158	6.415*
IV Cluster	98.625	128.625	149.000**	30.875**	10.875**	175.500	152.750	86.813	2.310	12.586**
V Cluster	85.667*	115.667*	131.017	30.667	9.333	163.167	145.500	89.017**	1.875*	8.563
VI Cluster	103.500	133.500	119.600	26.500	10.000	169.750	145.750	85.900	2.467**	11.630

*lowest values; **highest values

Table 10. Mean values of different clusters with respect to ten quality traits

	Brown Rice Length (mm)	Brown Rice Breadth (mm)	Brown Rice L/B Ratio	Kernel Length (mm)	Kernel Breadth (mm)	Kernel L/B Ratio	KLAC (mm)	Elongation ratio	Alkali Spread Value	Amylose Content (%)
I Cluster	7.502	1.960*	3.851	7.199	1.741	4.077	13.877	1.872	6.200**	23.675
II Cluster	8.268**	2.000	4.132**	8.136**	1.786	4.499**	14.201	1.722	5.444	23.697
III Cluster	7.050*	2.030**	3.457*	6.950*	1.775	3.915*	11.540*	1.692*	6.000	21.620*
IV Cluster	7.609	1.995	3.825	7.400	1.760	4.229	14.667**	1.920	3.625	24.170
V Cluster	7.582	1.988	3.708	7.160	1.693*	4.148	14.053	1.955**	2.833	24.327
VI Cluster	8.142	2.000	3.818	7.545	1.835**	4.128	13.520	1.793	2.750*	25.432**

*lowest values; **highest values