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Diversity Analysis of Bitter Gourd (*Momordica charantia* L.) Germplasm from Tribal Belts of India

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ABSTRACT

Forty six landraces collected from tribal belts of Gujarat, Tamilnadu, Kerala, Karnataka and Maharastra states of India were examined for 14 quantitative characters during vegetative and reproductive stages to assess the magnitude of genetic diversity. Data that were subjected to univariate and multivariate statistical analyses indicated ample variability for most traits. Correlation studies indicated a significant positive correlation of fruit length, fruit girth, seed weight and number of seeds per fruit with fruit yield. Accession MCC-23 collected from Gujarat was found to be the highest yielding landrace among the bitter gourd collection. Principal component analysis revealed that the first five principal components exhibited eigen values that explained 70% of the total variability. Hierarchical cluster analysis grouped the accessions into six clusters at the level of 5.10 euclidean distances. Clusters I to VI possessed 22, 12, 1, 9, 1 and 1 accessions, respectively. The major clusters, viz. I, II and IV possessed accessions of common geographical origin indicating that clinal variation still exists among the landraces of this under-exploited species and a lack of exchange of landraces between the tribal belts. Strategies for collection and conservation of this nutritionally and medicinally important vegetable are discussed.

Keywords: cluster analysis, correlation, principal component analysis

INTRODUCTION

Bitter gourd (*Momordica charantia.* L) is an important cucurbitaceous vegetable crop grown throughout the world, especially in India, Pakistan, Sri Lanka and China where it is being grown in larger area. Presently, bitter gourd is grown in an area of 0.26 million ha with production of 1.62 million tons; productivity is 6.23 tons/ha in India (Sidhu 1998). In addition to its significant use as a vegetable, each and every part of this plant has nutritive or medical significance, and has a long association with human beings (Morton 1967). Chang *et al.* (1996) reported that bitter gourd seeds contain 41-45% of essential oil and it is 10 times greater than the industrially important Tung oil in respect of the ratio of oleostearic and stearic acids. The ratio of oleostearic and stearic acids is inversely related to the rate of drying in cross-lining in the paint industry (Chang *et al.* 1996). The seed protein of bitter gourd was found to inhibit the growth of immuno deficiency virus (HIV-1) in human cell cultures (Bodeker *et al*. 2006).

Bitter gourd is considered to have a hypoglycemic effect (Miura *et al.* 2001). The lipid oxidative enzyme present in bitter gourd reduces cholesterol deposition in the body (Chan *et al.* 2005; Chen *et al*. 2005). The anti-fertility property of bitter gourd extracts also makes it useful as an organic contraceptive (Girini 2005). It is also used for its anti-mutagenesis, antiviral and antibacterial properties (Chiyampanichayakul *et al.* 2001; Grover and Yadav 2004). In spite of numerous uses, not many concentrated efforts have been made to genetically improve this crop. Bitter gourd is grown in semi-arid and arid ecosystems that face water stress during critical periods, the flowering and late fruit development stages, resulting in drastic yield loss. The tribal farmers are comparatively less adopters of modern varieties and they preserves landraces according to their preference for years together through local methods (Rhodes and Booth 1982). Hence, collection and exploitation of bitter gourd landraces from these belts for the development of new varieties with desirable horticultural traits could improve yield under semi-arid and arid ecosystems. Hence, this investigation was initiated with the objective of collecting and assessing the magnitude of genetic diversity existing among bitter gourd landraces from tribal belts of India for their capacity to perform under drought stress conditions.

MATERIALS AND METHODS

Bitter gourd landraces were collected from 27 geographically diversified sites of tribal belts in Dahod and Panchmahals (Gujarat), Salem and Coimbatore (Tamilnadu), Palghat and Thirchur (Kerala) and some parts of Karnataka and Maharastra states, in 2000 (**Table 1**). Forty-six bitter gourd accessions were evaluated at the Central Horticultural Experiment Station (CIAH), Vejalpur, Gujarat over three seasons (2001-2004). The experimental site was located at 22° 41′ 33″ and 73° 33′ 22″ and lies between 110-115 m asl. The annual rainfall mainly is confined to three months (July to September) with an average of 35 rainy days a year. The annual maximum and minimum temperature ranged from 42-43°C in May and 6-7°C in January, respectively. The annual potential evapo-transpiration ranged from 1500-1600 mm against the annual precipitation of 750 mm. The experiment was laid out in a randomized block design and each accession was replicated in triplicate. All cultural practices and plant protection measures were followed based on Singh (2001), except for irrigation, as the crop has been maintained under rainfed and no supplementary irrigation has been provided to the crop for whole cropping period. In addition to 25 tons of Farm Yard Manure, the crop has been supplied with 40:30:30 Kg NPK per ha. Fruit fly damage has been effectively controlled by spraying of 0.1% Endosulfan. Observations were recorded on main vine length (MVL), number of primary branches (NPB), days to first female flower anthesis (DFA), node to first female flowers (NFA), number of female flowers per plant (NFFP), days to first fruit harvest (DFH), number of fruits

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Table 1 Location of the accessions included in the study.

Accession number	District	Location name	Latitude	Longitude
MCC-1	Panchmahals	Vejalpur	22° 40″ N	73° 33″ E
MCC-2	Panchmahals	Vejalpur	22° 40″ N	73° 33″ E
MCC-3	Panchmahals	Vejalpur	22° 40″ N	73° 33″ E
MCC-4	Panchmahals	Vejalpur	22° 40″ N	73° 33″ E
MCC-5	Panchmahals	Vejalpur	22° 40″ N	73° 33″ E
MCC-6	Panchmahals	Kalol	23° 14″ N	72° 30″ E
MCC-7	Panchmahals	Kalol	23° 14″ N	72° 30″ E
MCC-8	Panchmahals	Kalol	23° 14″ N	72° 30″ E
MCC-9	Panchmahals	Kalol	23° 14″ N	72° 30″ E
MCC-10	Panchmahals	Halol	22° 30″ N	73° 28″ E
MCC-11	Panchmahals	Halol	22° 30″ N	73° 28″ E
MCC-12	Panchmahals	Halol	22° 30″ N	73° 28″ E
MCC-13	Dahod	Devgard baria	22° 41″ N	73° 54″ E
MCC-14	Dahod	Devgard baria	22° 41″ N	73° 54″ E
MCC-15	Dahod	Dhanpur	22° 38″ N	74° 05″ E
MCC-16	Dahod	Wagbhet	22° 37″ N	74° 16″ E
MCC-17	Dahod	Piplod	22° 40″ N	73° 53″ E
MCC-18	Dahod	Piplod	22° 40″ N	73° 53″ E
MCC-19	Dahod	Piplod	22° 40″ N	73° 53″ E
MCC-20	Dahod	Dahod	22° 49″ N	74° 15″ E
MCC-21	Dahod	Dahod	22° 49″ N	74° 15″ E
MCC-22	Dahod	Dahod	22° 49″ N	74° 15″ E
MCC-23	Dahod	Dahod	22° 49″ N	74° 15″ E
MCC-24	Dahod	Dahod	22° 49″ N	74° 15″ E
MCC-25	Ahmadnagar	Rahuri	19° 22″ N	74° 38″ E
MCC-26	Ahmadnagar	Rahuri	19° 22″ N	74° 38″ E
MCC-27	Ahmadnagar	Tarabad	19° 24″ N	74° 29″ E
MCC-28	Ahmadnagar	Tarabad	19° 24″ N	74° 29″ E
MCC-29	Ahmadnagar	Sonai	19° 23″ N	74° 49″ E
MCC-30	Ahmadnagar	Sonai	19° 23″ N	74° 49″ E
MCC-31	Ahmadnagar	Musalvadi	19° 27″ N	74° 40″ E
MCC-32	Ahmadnagar	Sirur	19° 27″ N	74° 40″ E
MCC-33	Ahmadnagar	Chas	19° 04″ N	75° 54″ E
MCC-34	Ahmadnagar	Shendi	19° 10″ N	74° 47″ E
MCC-35	Ahmadnagar	Vilad	19° 12″ N	74° 39″ E
MCC-36	Palghat	Palghat	10° 45″ N	76° 39″ E
MCC-37	Thirchur	Thirchur	10° 31″ N	76° 16″ E
MCC-38	Bangalore	Nelamangala	13° 05″ N	77° 23″ E
MCC-39	Coimbatore	Coimbatore	11° 01″ N	76° 58″ E
MCC-40	Coimbatore	Alandurai	10° 55″ N	76° 47″ E
MCC-41	Coimbatore	Madukkarai	10° 54″ N	76° 58″ E
MCC-42	Coimbatore	Walayar	10° 51″ N	76° 50″ E
MCC-43	Salem	Sendamangalam	11° 16″ N	78° 14″ E
MCC-44	Salem	Sendamangalam	11° 16″ N	78° 14″ E
MCC-45	Salem	Sekkadi	11° 26″ N	78° 21″ E
MCC-46	Salem	Tammampatti	11° 26″ N	78° 29″ E

per plot (NFP), yield per plot (YPP), Average fruit weight (AFW), Average fruit length (AFL), Average fruit girth (AFG), fruit flesh thickness (FFT), 100-seed weight (HSW) and number of seeds per fruit (NSF).

Statistical analysis

Descriptive statistics viz. mean, range standard deviation and coefficient of variability were computed for all morphometric traits. The multivariate analysis of variance was used to verify genetic variability among the accessions using Wilk's criteria (Wilks 1932). Pearson's correlation was performed to assess the most significantly contributing character towards yield and to verify the need to discard traits. Hierarchical cluster analysis adopting ward's minimum variance method (Ward 1963) was applied over all 14 quantitative traits to produce a dendrogram showing successive fusion of individuals which ultimately culminates into a single cluster. The data were also subjected to principal component analysis (PCA) procedures of SAS software Version 8.0 (SAS 1999) to identify potential traits contributing to variability. 'Statistica 6.0' software was employed for statistical analysis of the data.

RESULTS

Variability analysis

Forty-six accessions were collected from diversified locations (**Table 1**) and analyzed for plant, fruit and seed morphological characters; the descriptive statistics are presented in **Table 2**. The analysis of variance estimated for different characters showed that the highly significant mean sum of square estimates for all the characters was evidence of a great wealth of variability among the accessions. All 14 quantitative traits showed wide variation for different characters as illustrated by the high COV, SD and range values. The range observed among the accessions for MVL (2.60-6.60 m), NPB (12.811-38.708), DFA (35.586-55.488), DFH (12.69-20.65), NFP (35.68-76.45), AFW (75.57-227.73 g), FFT (3.962-7.926 mm), HSW (13.86-24.15g) and YPP (1.970-7.771Kg) indicating maximum variability present in these traits. The traits, NFFP, NBP, AFW, YPP and MVL had exhibited the higher coefficient of variation shows a greater scope for selection among the existing genotypes. However, AFG showed the lowest coefficient of variation indicating its least scope for improvement through selection.

With regards to vegetative parameters, vine length, leaf colour and size is considered pivotal characters under rainfed condition. Most of the accessions studied have a green and moderate sized leaves. Some of the accessions produced large and dark green leaves. A few of the accessions produced many large leaves showing advantage of producing high foliage than most of the accessions with in the population. Such as MCC-46 recorded the highest mean value of MVL coupled with dark green leaves. MCC-29 showed the lowest time taken for anthesis of first female flower. The accession MCC-23 exhibited the highest NBP, AFW and YPP. The highest AFG, FFT and HSW were observed in MCC-21. These characters are responsive indicators for healthiness of plants, earliness and high yield under stress conditions and hence the worthiness of these collections for developing varieties with early maturity coupled with high yield potential under drought is promising.

Correlation analysis

Pearson's correlation coefficients (**Table 3**) revealed that few traits such as NFP, AFW, AFL, DFA and DFH showed significant correlation with high YPP, indicating that these traits could contribute for high yields. The trait NFFP had a strong positive correlation with NFP ($r = 0.513$), AFW ($r = 0.479$) and FFT($r = 0.373$). The fruit parameters like AFW exhibited the strong positive correlation with NFP ($r = 0.517$), AFG ($r = 0.379$) and FFT ($r = 0.427$). AFL had strong positive correlation with YPP ($r = 0.588$) and NSF ($r = 0.549$). The trait, NFA was found to have strong negative correlation with YPP ($r = -0.487$), however, it showed strong positive relation with DFH ($r = 0.440$). This indicates that under drought stress all the early female flowers produced in the plant come for early harvest and thereby higher yields.

Cluster analysis

Cluster analysis revealed six distinct major clusters at the level of 5.10 Euclidean distances (**Fig. 1**). Among these six clusters, three major clusters namely cluster I, cluster II and cluster IV were constituted of 22, 12 and 9 accessions, respectively. Accessions collected from diverse areas formed the major clusters and exhibited a reasonable level of locational groupings. Majority of the accessions collected from Panchmahals and Dahod district were accommodated in cluster I and accessions collected from Kerala were grouped in cluster IV. This could be due to the fact that quantitative clustering obtained from each location is related to similarity in adaptation. However, one exception that was observed in the accessions collected from Dahod district of Gujarat showed the highest phenotypic dispersion consti-

Table 2 Summary of the analysis of variance for 14 descriptors evaluated in forty six landraces evaluated over three years.

Source of variation	df	MVL	NPB	DFA	NFA	NFFP	DFH	NFP	AFW	AFL	AFG	FFT	HSW	NSF	YPP
Block	2	0.416	41.411	15.678	50.452	602.430	5.817	260.460	566.952	2.688	3.437	1.659	70.416	20.085	1.264
Accession	45	3.2**	118.3**	81.2**	46.6**	4389.6**	12.5**	516.1**	5855.7**	102.5**	3.4**	2.7**	23.7**	89.7**	5.5**
Error	90	0.107	3.810	5.438	4.692	107.671	0.673	10.071	67.652	1.947	0.669	0.079	3.137	9.261	0.095
Mean		4.442	20.262	44.586	20.557	101.419	16.622	53.389	143.01	24.370	15.075	5.843	20.267	23.223	4.756
F value		30.096	31.067	14.940	9.939	40.769	18.567	51.262	86.555	52.647	5.136	34.757	7.568	9.695	58.073

main vine length (m) (MVL), number of primary branches (NPB), days to first female flower anthesis (DFA), node to first female flowers (NFA), number of female flowers per plant (NFFP), days to first fruit harvest (DFH), number of fruits per plot (NFP), yield per plot (kg)(YPP), average fruit weight (g) (AFW), average fruit length (cm) (AFL), average fruit girth (cm) (AFG), fruit flesh thickness (mm) (FFT), 100 seed weight (g) (HSW), number of seeds per fruit (NSF).
* F test value = Significant at 0.05 probability level, ** = Significant at 0.01 probability level, *** = Significant at 0.001 probability level

Table 3 Correlation coefficients among various quantitative traits of Bitter gourd accessions collected from various tribal belts of India.

Characters	MVL	NPB	DFA	NFA	NFFP	DFH	NFP	AFW	AFL	AFG	FFT	HSW	NSF	PPY
MVL	1.00	0.312*	0.068	-0.281	0.382**	-0.061	0.253	0.380**	0.280	0.192	0.179	0.125	0.284**	0.256
NPB		1.00	0.062	-0.153	0.257	-0.094	0.213	0.397*	0.362*	0.178	0.144	0.019	0.080	0.054
DFA			1.000	0.055	0.166	-0.268	-0.058	0.096	0.112	-0.018	0.094	-0.148	0.033	-0.070
NFA				1.000	0.035	0.414**	0.131	-0.143	-0.571	-0.263	-0.089	-0.051	-0.289	-0.428**
NFFP					1.000	-0.040	0.513***	0.479***	0.135	0.198	0.373*	0.246	0.086	0.057
DFH						1.000	0.122	-0.258	-0.213	0.062	-0.233	0.047	0.051	-0.047
NFP							1.000	0.517***	0.080	0.242	0.292	0.346*	0.193	0.079
AFW								1.000	0.280	0.379**	0.421**	0.299	0.174	0.049
AFL									1.000	0.309*	0.288	0.229	0.549***	0.558***
AFG										1.000	0.015	0.356*	0.232	0.467**
FFT											1.000	0.353*	0.331*	0.059
FSW												1.000	0.368*	0.340*
NSF													1.000	0.671***

main vine length (m) (MVL), number of primary branches (NPB), days to first female flower anthesis (DFA), node to first female flowers (NFA), number of female flowers per plant (NFFP), days to first fruit harvest (DFH), number of fruits per plot (NFP), yield per plot (kg)(YPP), Average fruit weight (g) (AFW), Average fruit length (cm) (AFL), Average fruit girth (cm) (AFG), fruit flesh thickness (mm) (FFT), 100 seed weight (g) (HSW) and number of seeds per fruit (NSF).
*= Significant at 0.05 probability level, **= Significant at 0.01 probability level, ***= Significant at 0.001 probability level

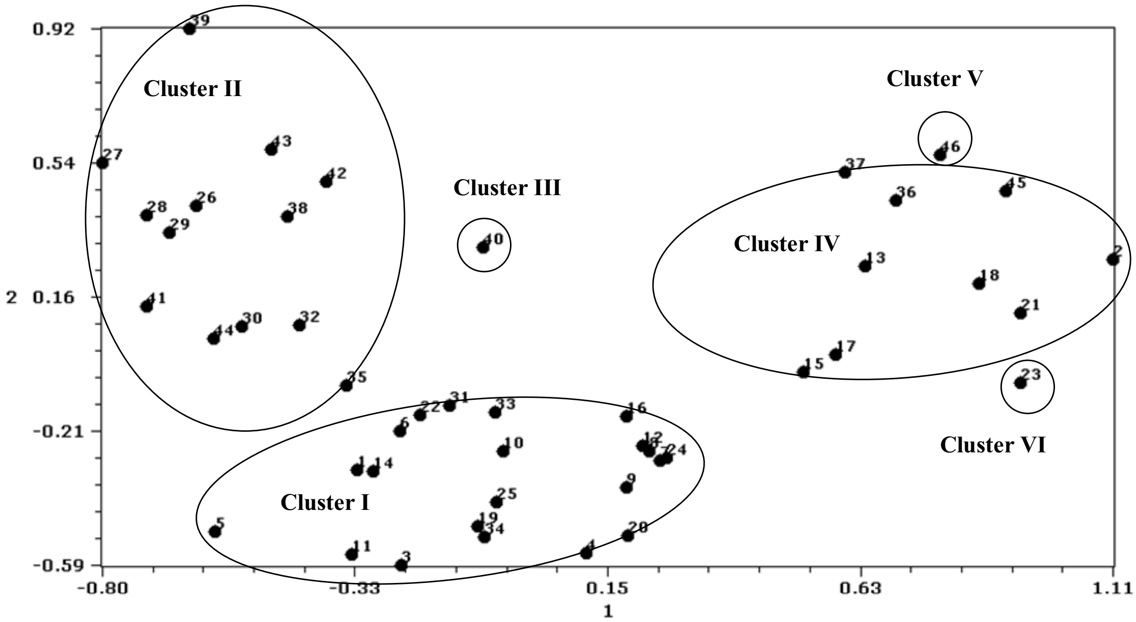

Fig. 1 Clustering of accessions used in this study. Accessions and distinct character in each cluster of bitter gourd germplasm collection using the Gower method.

tuting a part of cluster I, cluster IV and cluster VI, thus indicating a high phenotypic variation among germplasm found growing in this location. Distinct morphological characters were observed between clusters. Cluster I was characterized with low YPP, high NSF, lower NFA. Cluster II showed the lowest MVL, cluster III possessed the highest AFL and the lowest HSW, cluster IV exhibited the highest NSF, cluster V contains the highest MVL and cluster VI possessed the highest YPP, lowest NFA, high AFW and high FFT.

Principal component analysis

The identification of principal component is based on the correlation among different characters, their eigen values, and eigen vectors of principal components (**Table 4**). The characters in the principal components were identified on

Table 4 The first five principal components showing Eigen values and their contributions to the variability among accessions of bitter gourd.

Principle component	Eigen values	Proportion	Cumulative
Node to first female flower appeared	3.93	28.09	28.09
Days to fruit harvest	2.06	14.77	42.87
Average Fruit weight	1.68	12.04	54.91
100 Seed weight	1.15	8.25	63.16
No of seed per fruit	0.96	6.89	70.06

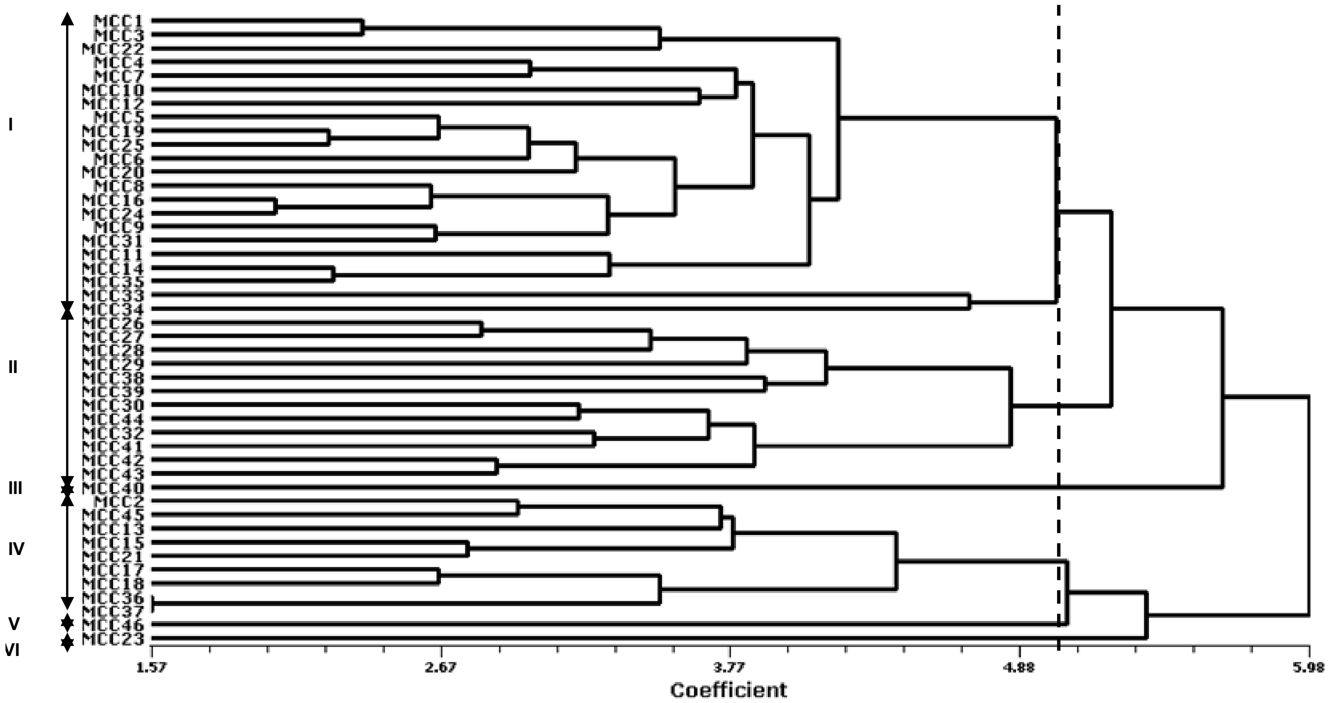

Fig. 2 Dendrogram generated by Ward's method of cluster analysis of morphological data of bitter gourd accessions.

the basis of eigen vectors. The principal component analysis revealed that the first five principal components explained 70% of the total variability. The first principal component comprised of NFA and DFH and explained 28% of the total variation, whereas the second principal component comprised of YPP, AFL and NSF and explained 14.7% of total variation. The third principal component comprised of DFH, HSW and NFA explained 12% of total variation, whereas the fourth principal component comprised of FFT, HSW, NSF that explained 8.25%. The fifth principal component comprised of HSW and AFW and explained 6.8% of total variation.

DISCUSSION

The environmental conditions in marginal areas make the cultivation of landraces competitive with that of improved cultivars (Limongelli *et al.* 1996; Negri 2003).Where socio-economic conditions of tribal belt are weak, modern agricultural methods cannot be applied and consequently agriculture retains traditional farming practices. Landraces are dynamic genetic entities that show many desirable traits, which enhance their overall quality, thus posing a need for exploitation. For efficient breeding, knowledge of the level on distribution of genetic variation within and among landraces is extremely important for both actions aimed at genetic improvement and for conservation purposes in order to avoid risks of genetic erosion.

The quantitative characters of 46 bitter gourd landraces collected and investigated in the present study, showed a great extent of variation, which are more useful in developing varietal descriptors for identification and classification. Characters like YPP, AFW, AFL, AFG and NFA is of particular interest in bitter gourd improvement. The levels of variation found in these collections have shown the potentiality for development of varieties with earliness and high yield. All the quantitative characters exhibited a greater variability as evidenced by high coefficient of variation observed in most of the traits, indicating the possibility of finding desirable traits to meet the demands of both researchers and farmers interested in the development of promising cultivars of bitter gourd. Similar studies have also been reported in other gourds concerning the morphological diversity among landraces from different centers of diversity, such as squash types in Cuba and Korea (Rios Labrada *et al.* 1997; Chung *et al.* 1998), pumpkin in Puerto Rico (Wessel-Beaver 1998), sponge gourd in India (Rana *et al.* 2000) and Nepal (Joshi *et al.* 2004).

In the present study on correlation and eigen values, the plant characters played a major role in deciding 70% variability towards yield indicates the importance of plant vigor under stress condition. The higher MVL and the early NFA found to facilitate early fruit harvest and delay or late appearance of flower would coincide with stress period and the plant might not produce marketable fruit size due to stress condition. This result is in consonance with finding of Rajanarayanan *et al.* (1996) in bottle gourd. There is a positive correlation between YPP and NFP, AFW, AFL, AFG, HSW and NSF in bitter gourd is in concordance with the finding of Sharma and Bhutani (2001).

In PCA, major characters contributing more towards diversity were NFA, DFH, AFW, HSW and NSF. Five major principal components were identified to cluster the accessions of the present collection. The cluster analysis grouped the accessions into six clusters (**Fig. 2**). A clear grouping according to geographical origin was observed. Cluster I included the accessions collected from Gujarat, Cluster II grouped Maharastra accessions. Cluster IV included all the accessions of Tamil Nadu. Similar parallelism between genetic diversity and geographic distribution was observed in garlic of Brazil investigated by Menezes-Sobrinho *et al.* (1999). The reason for existence of relationship between clusters and geographical region could be due to the restricted movement of planting material from one tribal region to another. This could also be explained by the fact that the quantitative clustering obtained from each location is related to similarity of adaptation (Van Hintum 1995). This also indicates monopoly preference of tribal belt farmers specific to the material available in their locality, rather than obtaining from other regions. However, the collection from Dahod district of Gujarat showed the highest phenotypic dispersion constituting a part of three clusters (I, IV and VI) out of the six clustering groups obtained from the quantitative phenogram, thus indicating a high phenotypic variation among germplasm found growing in this region. A possible explanation for this is the adaptation of the germplasm to natural selection been driven by high selection pressure (Pignone *et al.* 1997; Omolaja *et al.* 2000; Vilaro *et al.* 2004). The ample variability present in the collected germplasm could produce valuable breeding lines upon using appropriate recombinant breeding methods among the diversified landraces. A successful approach in the tribal belt to dynamically preserve the genetic variation present in the landraces is to use participatory breeding approaches (Rhoades and Booth 1982), which might be much appropriate to solve farmer's problems in a scientific way.

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