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Original Research Paper

Genetic divergence assessment in Kale (*Brassica oleracea* L var. acephala (DC.) Alef.) by using the multivariate analysis

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ABSTRACT

A total of 87 genotypes collected from different geographical areas of Kashmir valley evaluated at one site to determinate genetic variability. Considerable diversity was found in different traits of horticultural importance. High coefficient of variation and wide range and mean differences of studied traits indicated the existence of wide genetic variability. Three principal component having eigen value more than one and cumulatively accounted for 84.85 percent of total variability of evaluated horticultural traits. The leaf weight, leaf length, leaf width, leaf yield / plant and yield (q/ha) were major contributing traits towards the first principal component. Similarly number of number of leaves/plant was impotent contributed traits toward principal component -II, whereas plant height was main contributing traits to principal component -III. The maximum inter cluster D^2 value (731.04) was observed between cluster IV and cluster -I and followed by between cluster -V and cluster- I (677.29) and between cluster II and I (430.13).It indicated that genotypes belongings with these groups were genetically most divergent and may be use for hybridization to get better segregants.

Key words: Kale, genetic diversity, principal component analysis, single linkage cluster analysis.

INTRODUCTION

Kale (*Brassica oleracea* L) is one of important leafy vegetable grouped into cooked greens belongs to cole group. This is a preferred and widely grown vegetable of Kashmir valley due to cold hardiness, higher yield and better nutritive value. It is only available as a fresh vegetable in valley during extreme cold temperature and snowing period when the area is cut off with rest of the country due to heavy snowfall. The crop is grown in valley since long and have been improved by growers through a selection. Some genotypes have become popular in the valley either by the name of grower or by the name of growing locality such as G M Dari, Khaniyari and Cowdari. A wide range of genetic diversity exists due to cross pollinating nature of crop and long growing history. Large succulent and curly leaves are characteristics of consumer preference. However, no such commercial variety is available in the region. Thus development of high yielding variety with preferable quality is the need of the region.Improvement in yield and quality is normally achieved by selecting the genotype with desirable character combinations existing in the nature or by creating the diversity with hybridization. Selection of genetically diverse parents in any purposeful breeding programme on basis of divergence would be more promising to get the heterotic F1,s and broad spectrum of variability in segregating generation (Meena and Bahadur, 2015). In a varietal breeding for a particular growing conditions, it is essential to know about the local genetic population since their the relationship among the yield component are balanced and are in harmony with climatic and edaphic factors. Multivariate analysis is an effective tool for characterization and classification of plant genetic resources when a large number of accessions are assessed for several traits. Principal component analysis (PCA), one of multivariate analysis methods, depicts the traits which were decisive in genotype differentiation(Kovacic,1994). It enables an easier

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understanding of impact and relationship among the different traits. However PCA alone would not give an adequate character representation in term of relative importance when multiple characters are considered simultaneously (Shalini *et al,*2003). To complement the results of such multivariate analysis, Single Linkage Cluster Analysis(SLCA) is employed to classify the variation. SLCA is an agglomerative technique which shows the patterns of exact genotype position in population (Ariyo and Odulaja,1991) by sorting them in distinct groups. Thus, present investigation was undertaken to assess the nature and magnitude of genetic diversity in kale accessions of Kashmir valley for different morphological traits which could be utilized in further improvement programme.

MATERIAL AND METHODS

Eighty seven kale accessions (*Brassica oleracea* L var. acephala) collected from growing spots of Kashmir valley and some heterotic selection from kale lines bred at ICAR-CITH were evaluated (Table-1) . Seeds were sown in nursery in mid of August in each year and 30 days old seedling was transplanted at 45x 60 cm^2 apart in 3.0x2.70m^2 bed. The experiment was carried out during 2012 and 2013 at experimental farm of ICAR- Central Institute of Temperate Horticulture, Srinagar Jammu and Kashmir in randomized block design with three replications. Geographical position of the experimental site lies between latitude of $34^0$05 N and longitude of $74^0$50 E at an altitude of 1640 m amsl. The average maximum 19.63°C and minimum 6.52°C temperature, annual precipitation 160.72 mm and relative humidity 58.35%, evaporation 2.45mm and soil characteristics viz. pH= 6.81 and EC = 0.36 dSm^{-1} recorded during the cropping season. Recommended uniform agronomic and cultural practices were adopted to obtain better phenotypic expression of the characters. A total of seven quantitative traits representing to vegetative characteristics of plants related to yield and yield

Table 1. Accession used in study along with code number

Code	Accessions	Code	Accessions	Code	Accessions	Code	Accessions
1.	CITH-SAG-10	2.	KC-SEL-5	3.	CITH-SAG-3	4.	NW-SAG-8(s)
5.	CITH-SAG-1	6.	KC-SEL-3	7.	CITH-KC-SEL-1	8.	CITH-KC-11(s)
9.	NW-SAG-39	10.	CITH-SAG-36	11.	CITH-KC-44	12.	CITH-KC-8
13.	NW-SAG-49	14.	CITH-KC-23	15.	CITH-SAG-34	16.	CITH-KC-17
17.	NW-SAG-42	18.	CITH-KC-4	19.	CITH-KC-21	20.	2011/KLVar/3
21.	CITH-KC-6	22.	CITH-KC-40	23.	CITH-KC-24	24.	2011/KLVar/6
25.	NW-SAG-40	26.	CITH-KC-16	27.	CITH-KC-7	28.	2011/KLVar/2
29.	NW-SAG-23	30.	CITH-KC-20	31.	CITH-KC-43	32.	2011/KLVar/1
33.	NW-SAG-33	34.	CITH-KC-19	35.	CITH-SAG-4	36.	2011/KLVar/4
37.	NW-SAG-21	38.	CITH-KC-22	39.	CITH-KC-14	40.	Khanyari
41.	NW-SAG-44	42.	CITH-KC-18	43.	OP-2	44.	Kashmiri local
45.	NW-SAG-11	46.	HW-5	47.	CITH-KC-21	48.	2011/KLVar/5
49.	HW-1	50.	HW-4	51.	CITH-SAG-38	52.	CITH-KC-41
53.	CITH-KC-23	54.	CITH-KC-10	55.	CITH-SAG-50-1	56.	CITH-KC-27
57.	CITH-KC-43	58.	CITH-SAG-22	59.	CITH-KC-4	60.	CITH-KC-34
61.	CITH-KC-45	62.	CITH-SAG-8	63.	CITH-KC-13	64.	KC-SEL-5
65.	CITH-KC-3	66.	CITH-KC-5	67.	CITH-SAG-29	68.	SB-1
69.	NW-SAG-31	70.	CITH-SAG-50	71.	CITH-SAG-18	72.	OP-1
73.	CITH-SAG-41	74.	HW-4-1	75.	CITH-Khanyari	76.	CITH-KC-SEL-2
77.	CITH-SAG-24	78.	CITH-KC-38	79.	CITH-KC-47	80.	KC-12
81.	CITH-KC-48	82.	PUSA-SAG-1	83.	JP-Green Sel-1	84.	JP-Green
85.	CITH-KC-28(s)	86.	NW-SAG-18(s)	87.	NW-SAG-27(s)		

attributes were measured. Data collected on the quantitative characters were analysed using the SAS microsoft windows 9.2 (SAS Institute, 2011). Genetic diversity was studied following the Mahalanobis (1936) generalised distance (D^2) extended by Rao (1952). Average intra cluster distance was calculated by following formula as suggested by Singh and Choudhry (1985). PCA and SLCA were used for the determination of genetic variation and percentage similarity within the genotypes. The PCA produce Eigen – Vectors and principal component score were used to assess the relative discriminatory power of its axis and their associated characters. The cluster procedure was used to produce a distinct group of 87 genotypes on the basis of genetic relationship while using the character variation. SLCA summarized the position of accessions analysed the position of accessions into a dendogramme at an interval of 5% level of dissimilarity starting from 100 % of level of dissimilarity.

RESULTS AND DISCUSSION

The eighty seven genotypes evaluated varied significantly for all horticultural traits except to average leaf weight (Table 2). The phenotypic variability revealed by coefficient variation (%) was highest for average leaf weight followed by leaf yield /plant and q/ha, number of leaves per plant which was also substantiated by wider range and mean difference values. The coefficient of variation varied from 15.12 for leaf length to 33.45 for average leaf weight. High coefficient of variation and wide range and mean differences of studied traits indicated a wide range of genetic variability, which reflects the potential of improvement in kale. Similar type of variability in germplasm of kale has been reported by Maria *et al*., 2002.

To extract principal factors which do not require the assumption of normal distribution of proportion, principal component analysis was used and 87 kale genotypes based on degree of divergence of seven morphological traits were grouped into three principal components having Eigen value more than one and cumulatively accounted for 84.85 percent of total variability (Table 3). The first principal component contributed 44.59 % of total variation and was positively loaded with impotent horticultural traits *viz*., average leaf weight, leaf length, leaf width, leaf yield /plant and yield (q/ha), where as negatively loaded with number of leaves /plants. The second principal component responsible for 26.93 percent of total multivariate variation was positively loaded with number of leaves/plant, leaf yield per plant and yield (q/ha) where as negatively loaded with plant height, number of leaves and leaf width. The principal component III accounted for 13.32 % of total variation and was positively loaded with plant height ,number of leaves /plant and yield per plant where as negatively loaded with leaf weight, leaf length and leaf width. The positive and negative loading of quantitative characters reflect the positive and negative correlation trend between the components and variables and suggesting that theses principal component may be used to summarize the variables. The characters with largest absolute value closer to unit within the first component influencing the clustering than those to lower absolute value closer

Table 2.Variability for different metric traits in kale genotypes

Variable	Range		Mean	Std. deviation	CV(%)
	Minimum	Maximum			
Plant height	27.000	63.670	47.70	7.66	16.05
No. of leaves/plant	12.500	75.500	21.65	6.50	30.03
Av. leaf wt (g)	6.050	54.490	22.42	7.50	33.45
Leaf length (cm)	25.330	48.000	37.38	5.65	15.12
Leaf width (cm)	11.000	28.000	17.58	3.06	17.39
Leaf yield/plant (g)	175.000	890.000	427.32	142.17	33.27
Yield q/ha	194.440	988.890	474.80	157.97	33.27

Table 3. Latent vectors for seven traits of 87 genotypes of kale

Variable	PC-I	PC-II	PC-III
Plant height	0.254	-0.132	0.819
No. of leaves/plant	-0.007	0.697	0.135
Av. leaf wt (g)	0.452	-0.361	-0.019
Leaf length (cm)	0.350	0.058	-0.553
Leaf width (cm)	0.339	-0.401	-0.069
Leaf yield/plant (g)	0.497	0.319	0.018
Yield q/ha	0.497	0.319	0.018
Eigen value	3.10	1.90	1.01
Variability (%)	44.592	26.926	13.332
Cumulative %	44.592	71.518	84.850

to zero. Thus in present study the differentiation of genotypes in different principal component was because of high contribution of few characters rather than small contribution of each characters. The characters positively in first three principal component could be in consideration while selecting the genotype with appropriate traits and yield potential. The principal component analysis has also been used for studying the genetic variability in germplasm of many species (Ahmed *et al*.,2015, Singh *et al*., 2013). The Plot of PC- I versus PC - II indicated the that some groups of isolated genotypes clearly define the diversity among the evaluated germplasm. The genotypes CITH-KC-23, CITH-KC-25, CITH-SAG-24, 2011 KLVr-12, New SAG- 27(5), Kashmiri Local, KC-12, CITH - KC-6, CITH-KC-14, CITH-44, and 2011/KLVr-5 were most divergent (Fig-1). Usually it is customary to use one importent variable from theses identified groups for improvement programme. Hence PC-I for leaf length , leaf width and leaf yield per plant should be first choice which has a largest positive loadings for these traits. Number of leaves per plant for second principal component and plant height for third principal component. The results of study are useful as it furnish the information about the groups where certain traits are more important, allowing to breeder to execute the breeding programme for specific target. The biological implication of principal component analysis can be quantified by contribution of different variables in each PC as revealed by eigen vector. The clustering score among the component axes suggest that some relationship exist among the individuals within the cluster but do not provides the clear position of genotypes .

Based on Single Linkage Cluster Analysis, the genotypes were grouped into five clusters quantifying the share genotypes and cluster mean of all traits (Table 4). The maximum number of genotypes (81 nos) were accommodated in cluster I followed by cluster - II (3Nos) and cluster III, IV and V (1 no. in each) contributing 93.24, 3.4, 1.41, 1.14 and 1.14 % respectively. On basis of cluster mean the cluster -IV was important for maximum number of leaves per plant, leaf yield per plant and yield (q/ha) cluster- II was important for average leaf weight ,cluster- III, cluster three for plant height and cluster - V for leaf length and leaf width. The cluster having high mean values of traits would contribute more positively in their offsprings if used as a parent. Rehman and Mansur (2009) and Ahmad *et al*, 2015 also suggested that the cluster having highest mean values may be used for hybridization programme to get better segregants.

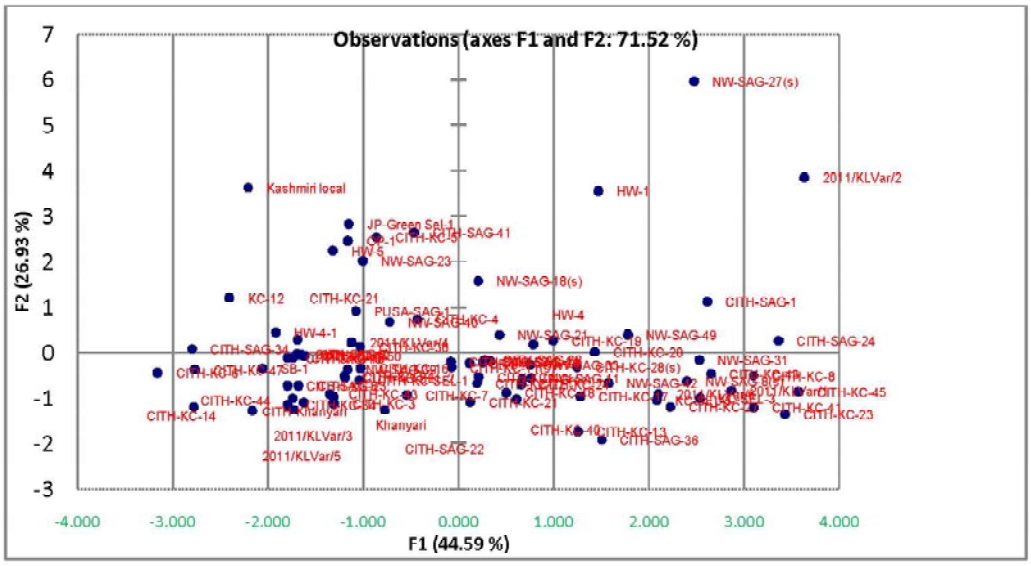

Fig. 1 Bi-plot for 1st and 2nd PC for genotypes of kale in relation horticultural traits

Table 4. Cluster means values for 7 important horticultural traits along with number and Proportion genotypes falling in each cluster

Code	Accessions	Code	Accessions	Code	Accessions	Code	Accessions
1.	CITH-SAG-10	2.	KC-SEL-5	3.	CITH-SAG-3	4.	NW-SAG-8(s)
5.	CITH-SAG-1	6.	KC-SEL-3	7.	CITH-KC-SEL-1	8.	CITH-KC-11(s)
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25.	NW-SAG-40	26.	CITH-KC-16	27.	CITH-KC-7	28.	2011/KLVar/2
29.	NW-SAG-23	30.	CITH-KC-20	31.	CITH-KC-43	32.	2011/KLVar/1
33.	NW-SAG-33	34.	CITH-KC-19	35.	CITH-SAG-4	36.	2011/KLVar/4
37.	NW-SAG-21	38.	CITH-KC-22	39.	CITH-KC-14	40.	Khanyari
41.	NW-SAG-44	42.	CITH-KC-18	43.	OP-2	44.	Kashmiri local
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53.	CITH-KC-23	54.	CITH-KC-10	55.	CITH-SAG-50-1	56.	CITH-KC-27
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61.	CITH-KC-45	62.	CITH-SAG-8	63.	CITH-KC-13	64.	KC-SEL-5
65.	CITH-KC-3	66.	CITH-KC-5	67.	CITH-SAG-29	68.	SB-1
69.	NW-SAG-31	70.	CITH-SAG-50	71.	CITH-SAG-18	72.	OP-1
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81.	CITH-KC-48	82.	PUSA-SAG-1	83.	JP-Green Sel-1	84.	JP-Green
85.	CITH-KC-28(s)	86.	NW-SAG-18(s)	87.	NW-SAG-27(s)		

D^2 value estimate of genetic divergence suggested the resolution for 87 kale genotypes in distinct five clusters with wide range of diversity in experimental material for a majority of traits (Table-5). The maximum inter cluster D^2 value (731.04) was observed between cluster IV and cluster -I and followed by between cluster -V and cluster- I (677.29) and between cluster II and I (430.13). It indicates that genotypes belongings with these groups were genetically most divergent. The selection of divergent parents based on theses cluster distance may be used in selecting the parents for the hybridization and formulating a comprehensive strategy to get a superior hybrid or superior segregants in kale. The findings are

Table 5. Average intra and inter cluster distances (D^2) of kale genotypes

Clusters	1	2	3	4	5
1	0	**430.13**	331.39	**731.04**	**677.29**
2		0	110.47	305.49	249.60
3			0	400.59	347.78
4				0	59.41
5					0

in conformity with finding of Maria, *et al*., 2002, Singh, *et al.*, 2013 and Ahmed *et al.*,2015 who had also indicated the accessions among the cluster separated by high D^2 cluster values used in hybridization programme to obtain a wide spectrum of variation among the segregants. Dendogram drawn from the Single Linkage Cluster Analysis by using the Euclidian distance depicted the relationship and exact position of genotype in the cluster (Fig.2) All the genotypes were distinct from each other at 100 % of dissimilarity and farmed 17 cluster at 75% of dissimilarity and farmed 3 cluster at 50% of dissimilarity . The dissimilarity range from 50 to 100 % among the delineated genotypes large enough to suggest the variability for crop improvement (Denton and Nwangburuka,2011) CITH-KC-23,CITH-KC-45, CITH-SAG-24, 2011 KLVr-12,New SAG-27(5),Kashmiri Local,KC-12, CITH -KC-6, CITH-KC-14, CITH- SAG-3 and 2011/KLVr-5 were most divergent in cluster position and may be use for hybridization to get better segregants. Ahmed *et al.*,2015 also reported such variability by using the single linkage cluster analysis. This genetic diversity analysis would be useful to avoid the selecting parents from genetically homogenous cluster and maintain a broad genetic base for future breeding programme.

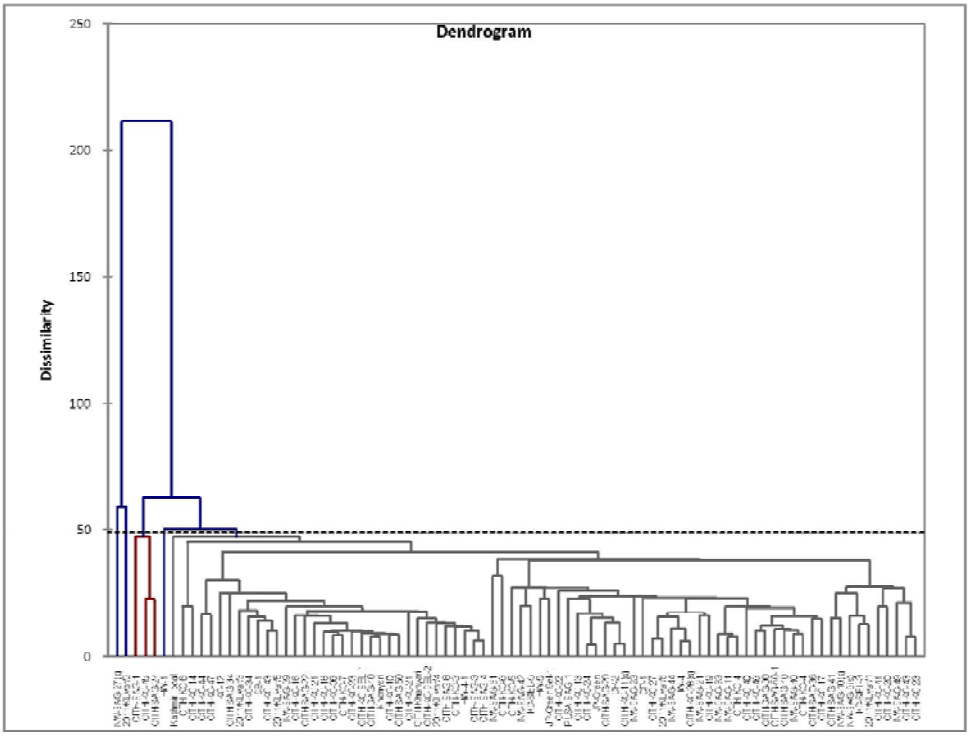

Fig 2. Dendogram depicting the genetic relationship among the kale genotypes based horticultural traits produced by ASH analysis (scale-Euclidian distance)

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