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Determination of genetic diversity in *Chenopodium* spp.

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Abstract

Field experiments were conducted for two successive seasons to assess the genetic diversity in 44 germplasm lines of *Chenopodium*. Eleven agronomic traits were analyzed for cluster and principal component (PC) analysis. The first 4 PCs contributed 88.10 % of the variability present among the lines. Three characters contributed positively to all the 4 components. The first principal component (PC_1) had plant height, inflorescence length and stem diameter as the variables with largest coefficients. The germplasm was categorized in 4 clusters following Ward's method. A wide range of diversity for most of the traits were observed that would enable breeders to identify lines with suitable traits to be used in hybridization programmes for broadening the genetic base.

Key words: *Chenopodium*, genetic diversity, clustering, principal components

Introduction

The genus *Chenopodium* (family *Chenopodiaceae*) includes herbaceous, suffrutescent and arborescent perennials, although most species are colonizing annuals [1]. *Chenopodium* spp. have been cultivated for centuries as leafy vegetable (*C. album*) as well as an important subsidiary grain crop (*C. quinoa* and *C. album*) for human and animal foodstuff due to high protein (10-14%) [2] and fat (30-62 g/kg) [3] contents. Moreover, the seed proteins have a balanced amino acid spectrum with high lysine (5.1-6.4%) and methionine (0.4-1.0%) contents [4]. Many forms are referable to *C. album*, which has three ploidy levels $2n$ = 18 ($2x$), 36 ($4x$), 54 ($6x$) [5, 6]. The crop is gaining worldwide attention due to its rich nutrient content and the ability to grow in various stress conditions [7].

The information available on genetic diversity in germplasm lines plays an important role in deciding appropriate plant breeding methods for crop improvement. Multivariate statistical methods have been successfully used to classify variation in many crop germplasm collections like pea [8], Russian wildrye [9], blackgram [10] and Ethopian mustard [11]. However, such reports in *Chenopodium* are rare [12, 13] and all are centered on a single species, *C. quinoa*. Thus, the present investigation was undertaken to determine the distribution and extent of genetic diversity in *Chenopodium* spp. based on agronomic traits so that the generated information could be utilized in future breeding programmes.

Materials and methods

The materials consisted of 44 exotic and indigenous germplasm lines of *Chenopodium* spp. representing 10 lines of *C. quinoa*, 23 lines of *C. album*, one line each of *C. bmhianum, C. amaranticolor, C. murale, C. opulifolium, C. strictum, C. berlandieri, C. ugandae*, 2 lines of *C. giganteum* and two selections from 2 separate cross progenies (Table 1). These lines are being maintained at N.B.R.I., Lucknow and were pure and homozygous. A perusal of Table 1 shows that the germplasm lines selected were of different ploidy levels and were collected from diverse sources.

The experiment was conducted at the experimental field of National Botanical Research Institute, Lucknow (26.5°N and 80.5°E), situated at an elevation of 120 m above sea level. The seeds of each line were sown in a randomized block design with three replications in the crop year 2000-2001 and 2001-2002. The seeds for subsequent years were maintained by usual method of selfing (bagging of inflorescences by bags of muslin cloth) to avoid outcrossing, which is negligible in this genus (5.8%). Each germplasm line was sown in two rows of 3 m long. The plant-to-plant and row-to-row distance was maintained at 15 and 45 cm respectively. 5 random plants in each replication were selected and data on 11 quantitative traits namely days to flowering, days to maturity, plant height (cm), leaf size (cm^2), stem diameter (cm), primary branches/plant, inflorescence length (cm), 100 seed weight (g), dry weight/plant (g), inflorescence/plant and seed yield/plant (g) were recorded.

Data analysis: The observations recorded on 11 agronomic characters were analyzed by numerical taxonomic techniques using the procedure of cluster and principal component analysis [14]. Eigenvectors and eigenvalues from the first four principal component

Table 1. Germplasm lines, their source, chromosome number and ploidy level

S. No.	Germplasm line	Origin	Chrmo-some no.	Ploidy level
1.	*C. album* PRC 9801	H.P.,India	54	6x
2.	*C. album* PRC 9803	H.P.,India	54	6x
3.	*C. album* PRC 9804	H.P.,India	54	6x
4.	*C. album* PRC 9802	H.P.,India	54	6x
5.	*C. album* IC 107295	H.P.,India	54	6x
6.	*C. album* IC 107297	H.P.,India	54	6x
7.	*C. album* IC 107299	H.P.,India	54	6x
8.	*C. album* 1C 107296	H.P.,India	54	6x
9.	*C. quinoa* PI 587173	Jujuy,Argentina*	36	4x
10.	*C. album* 'local red'	Lucknow,India	18	2x
11.	*C. bushianum* Ames 22376	Illinois,USA*	36	4x
12.	*C. album* 'Iowa'	Iowa,USA	54	6x
13.	*C. album* 'H.P'	H.P.,India	54	6x
14.	*C. quinoa* PI 510537	Peru*	36	4x
15.	*C. quinoa* CHEN 92/91	Columbia**	36	4x
16.	Progenitor of quinoa	Mexico	36	4x
17.	*C. quinoa* PI 478414	La Paz,Bolivia*	36	4x
18.	*C. album* (local) × *C. quinoa*	Hybrid	54	6x
19.	*C. quinoa* PI 584524	Chile*	36	4x
20.	*C. giganteum* 'local'	Lucknow,India	54	6x
21.	*C. album* 'Mexico'	Mexico	36	4x
22.	*C. album* × *C. album* 'Siliguri'	Hybrid	18	2x
23.	*C. album* 'Siliguri'	Siliguri,India	18	2x
24.	*C. quinoa* PI 596498	Cuzco,Peru*	36	4x
25.	*C. quinoa* Ames 22158	Chile*	36	4x
26.	*C. album* 'chandanbathua'	U.P.,India	18	2x
27.	*C. quinoa* CHEN 67/78	Puno,Peru**	36	4x
28.	*C. album* 'amaranticolor'	H.P.,India	54	6x
29.	*C. quinoa* CHEN71/78	Bolivia**	36	4x
30.	*C. album* CHEN 60/76	Belgium**	54	6x
31.	*C. album* CHEN 85/82	Unknown**	54	6x
32.	*C. album* 'Czech'	Czech Republic	54	6x
33.	*C. album* × *C. quinoa* (colchiploid)	Hybrid	54	6x
34.	*C. murale* 'local'	Lucknow,India	18	2x
35.	*C. opulifolium* CHEN 43/96	Unknown**	36	4x
36.	*C. album* PI 605700	Michigan,USA*	54	6x
37.	*C. album* 'local 6x'	Lucknow,India	54	6x
38.	*C. giganteum* PI 596371	Oklahoma,USA*	54	6x
39.	*C. giganteum* PI 596112	California,USA*	54	6x
40.	*C. album* 'local'	Lucknow,India	18	2x
41.	*C. strictum* CHEN 47/79	Unknown**	54	6x
42.	*C. berlandieri* PI 568156	Mexico*	36	4x
43.	*C. album* CHEN 63/80	Unknown**	54	6x
44.	*C. ugandae* CHEN 77/78	Rwanda**	36	4x

*Source-USDA, **Source-Gatersleben, Germany

axes were calculated from a similarity correlation matrix. The 44 entries were clustered using Ward's method [15]. Means of each variable were standardized prior to cluster and principal component analyzes to avoid the effect due to difference in scale.

Results and discussion

Principal component analysis: In the principal components analysis (PCA), the values are first scaled to make their variances equal. A new set of axes is then chosen in the multivariate space so that the variances on the first and second axes is as large as possible, but are at right angles to each other. The coefficient of the data points on each new axis is a weighted sum of its coefficients on the originally scaled axis. Table 2 presents the latent root i.e. variance on each axis, the percentage of total variance that each represents and the coefficients used in the weighted sum (eigenvectors or loadings). The first 4 components contributed 88.10% of the total variability amongst the 44-germplasm lines for 11 agronomic characters. Only 3 traits namely days to flowering, days to maturity and leaf size contributed positively to all the 4 PCs.

Table 2. Eigenvalues, proportion of variability and agronomic traits that contributed to the first four PCs of *Chenopodium* spp.

Components	PC_1	PC_2	PC_3	PC_4
Root	2.031	0.398	0.210	0.180
% variance explained	63.477	12.439	6.555	5.637
Cumulative variance	63.477	75.916	82.471	88.108
	Coefficients of variates			
Days to flowering	0.410	0.268	0.028	0.162
Days to maturity	0.449	0.207	0.053	0.155
Plant height (cm)	0.610	0.063	–0.023	–0.090
Leaf size (cm^2)	0.256	0.161	0.014	0.056
Stem diameter (cm)	0.493	0.003	–0.112	–0.063
Primary branches/plant	0.402	–0.038	–0.135	–0.082
Inflorescence length (cm)	0.559	–0.113	–0.025	–0.204
100 seed weight (g)	–0.387	0.141	–0.405	0.046
Dry weight/plant (g)	0.380	–0.050	–0.045	0.006
Inflorescence/plant	0.183	–0.449	–0.042	0.209
Seed yield/plant (g)	0.422	–0.120	–0.078	0.143

The first principal component (PC_1) had plant height, inflorescence length and stem diameter as the variables with largest coefficients. This means that the first component distinguished those lines that were tall and had thick stem and longer inflorescences from those with converse characteristics. 100 seed weight was the only trait contributing to the first component with negative sign, reflecting the low seed weight of the tall plants. The second and third components (PC_2 and PC_3) accounted for 12.43 % and 6.55 % of the total variance. The variable that contributed to PC_2 with the largest coefficient was inflorescence/plant, but

with a negative sign. All the traits associated with reproductive development *viz.*, inflorescence/plant, inflorescence length and grain yield/plant contributed negatively to PC_2. However, both variables related to vegetative growth *viz.*, days to flowering and days to maturity contributed positively to PC_2. Therefore, this component reflects the tendency of the lines to emphasize vegetative, as opposed to reproductive growth. Ghafoor *et al.* [10] also reported positive contribution of both these traits associated with vegetative growth, while studying genetic diversity in blackgram through PCA. Leaf size had moderate positive weight on PC_2 while inflorescence length exhibited negative weight. This suggests that the lines that emphasize vegetative growth tend to have larger leaves but fewer numbers of inflorescences.

The pattern of divergence between the 44 lines for the first two principal components is given in Figure 1. Most of the indigenous lines of *C. album* occupied the center i.e. the point of the lines passing through the respective averages of the component axes. 4 lines namely *C. album* 'Iowa', *C. giganteum* 'local', *C. album* 'amaranticolor' and *C. ugandae* CHEN 77/78 occupied the upper right portion of the figure which designates high positive coefficient values of both PC_1 and PC_2. All the germplasm lines of *C. quinoa* except *C. quinoa* CHEN 92/91 occupied the lower region of the figure indicating that these lines had low values for the first component.

Cluster analysis: Figure 2 shows a dendrogram constructed following Ward's minimum variance method [15] that is based on a combined analysis of the data of two test seasons. The dendrogram supports the findings of the principal component analysis. The cluster analysis showed that the germplasm could be grouped into 4 clusters. Cluster I consisted of 10 germplasm lines, half of which were the indigenous lines of *C. album* (Table 3). Cluster II included 13 lines of which 10 were indigenous to India. 9 of the 16 (56.25%) indigenous *C. album* lines were grouped in cluster II. The IIIrd cluster constituting 13 lines included bulk of the exotic accessions (12 out of 13). Also 8 of the 9 lines of *C. quinoa* formed a part of this cluster. Cluster IV was heterogeneous with 8 lines comprising 2 hybrids, 2 accessions of *C. album* and 1 accession each of *C. strictum, C. murale, C. opulifolium* and 1 Progenitor of quinoa. The tendency of lines occurring in clusters cutting across geographical boundaries demonstrates that geographical isolation was not directly related to genetic diversity and had been reported in various crop species [16, 17].

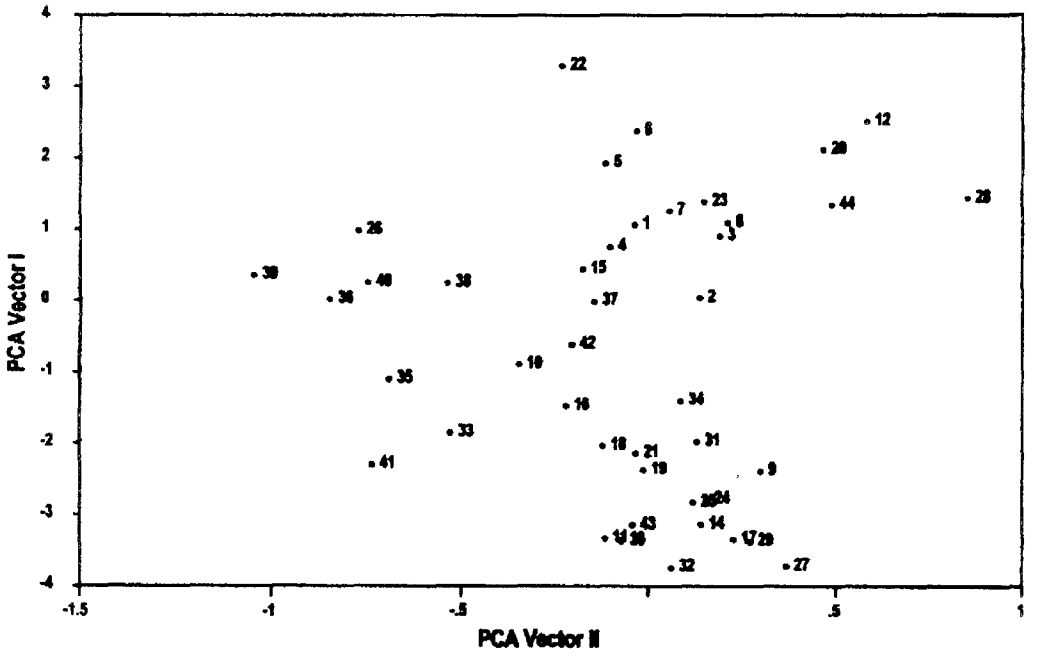

Fig. 1. Plot of the first and second component scores for 44 germplasm lines of *Chenopodium* spp.

Table 4 presents the cluster mean for various traits. Cluster II seems to be most promising in terms of yield (56.91 ± 6.63 g/plant) and had high values for all the agronomic traits except for 100 seed weight (0.05 ± 0.004 g). The lines of this cluster were late maturing and also had longest grain filling period (38.81 days). On the other hand, lines of cluster III were low

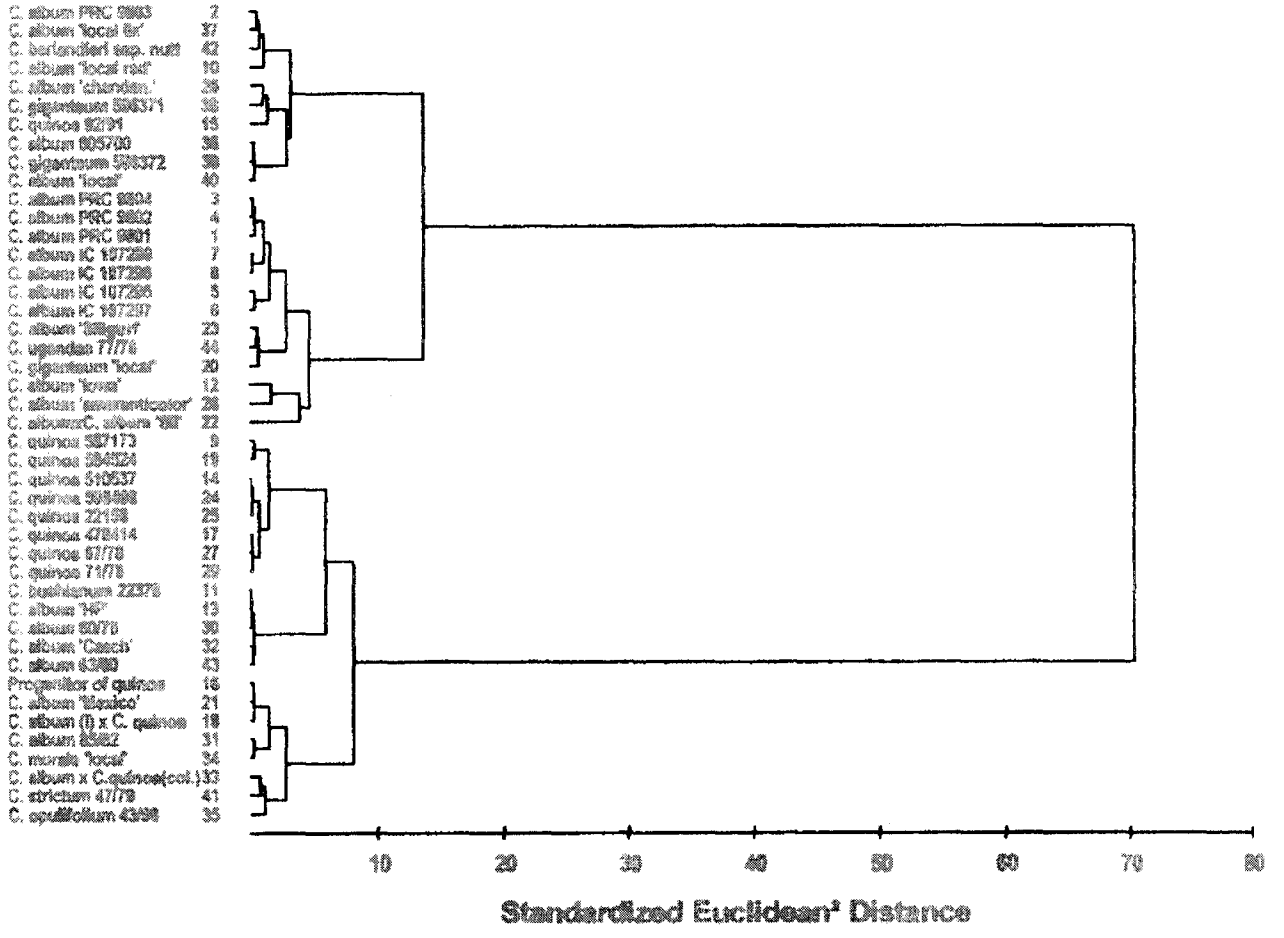

Fig. 2. Dendrogram of 44 germplasm lines of *Chenopodium* spp. following Ward's method

Table 3. Composition of different clusters of 44 germplasm lines of *Chenopodium* spp.

Cluster	No. of lines	Source	Species composition
I	10	India-5	*C. album* - 6
		USDA-4	*C. quinoa* - 1
		IPK,Germany-1	*C. berlandieri* subsp. *nuttalliae*-1 *C. giganteum*-2
II	13	India-10	*C. album* - 10
		IPK,Germany-1	*C. giganteum*-1
		USA-1	*C. ugandae*-1
		Hybrid-1	*C. ugandae*-1 Hybrid-1
III	13	USDA-7	*C. quinoa*-8
		IPK,Germany-4	*C. album*-4
		India-1	*C. bushianum*-1
		Czech Rep.-I	
IV	8	IPK,Germany-3	*C album*-2
		Mexico-2	Hybrid-2
		Hybrid-2	*C. strictum*-1
		India-1	*C. murale*-1
			C. opulifolium-1
			Progenitor of quinoa-1

yielding and early maturing. Thus, cluster III could be considered as a potential source of earliness but is unsuitable for improvement of yield components. For improvement in yield, lines of cluster I and III could be effective.

For maintaining and utilizing germplasm effectively, it is imperative for the breeder to ascertain the extent of diversity present in the material. The results have established the presence of a large amount of genetic diversity among the 44 lines for all the traits. Cluster analysis has proved to be an effective method in grouping germplasm lines for facilitating selection of a workable collection. Some of the lines possessing good genes for more than one trait could be included in hybrid programmes for varietal development. However, in *Chenopodium*, the crossability among the species must also be kept in mind for any hybridization programme. The lines of *C. album, C. guinea* and most of the species are cross compatible among themselves. Further, the diploid lines of *C. album* are cross compatible with *C. quinoa* [18] personal communication

Table 4. Mean and standard error (S.E.) for 4 clusters based on 11 agronomic characters.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV
Days to flowering	102.84±3.13	132.15±4.14	96.07±1.79	103.11±3.36
Days to maturity	139.64±3.79	170.96±4.24	121.95±2.09	136.02±5.21
Plant height (cm)	173.35±16.88	263.71±6.84	55.87±5.85	91.94±9.93
Leaf size (cm^2)	20.48±3.39	67.36±12.19	7.82±2.04	14.36±1.96
Stem diameter (cm)	1.57±0.09	1.92±0.07	0.82±0.07	1.14±0.09
Primary branches/plant	36.72±3.00	37.56±1.63	15.17±2.21	24.23±1.60
Inflorescence length (cm)	26.67±1.06	28.93±2.01	5.30±0.87	9.93±1.20
100 seed weight (g)	0.06±0.01	0.05±0.004	0.19±0.02	0.10±0.01
Dry weight/plant (g)	98.06±18.66	119.55±17.94	9.78±1.57	26.73±3.02
Inflorescence/plant	402.25±63.36	238.52±36.12	106.56±16.16	389.08±77.35
Seed yield/plant (g)	46.27±5.41	56.91±6.63	13.18±3.19	20.74±5.78

by Dr. M. Pal. The present study would help to identify, select and combine germplasm lines to obtain important traits in one line with a broad genetic base.

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