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Genetic diversity for morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.) germplasm

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Abstract

Twenty nine germplasm lines of *Chenopodium quinoa* and two of *Chenopodium berlandieri* subsp. *nuttalliae* were evaluated for 12 morphological and 7 quality traits for two test seasons. The 19 traits were analyzed for cluster and principal component analysis. The first four PCs contributed 78.70 % of the variability among the germplasm lines. The first PC accounted for 39.5% of the variation and had inflorescence/plant, plant height and stem diameter as the traits with largest coefficients, all with positive sign. The characters with greatest positive weight on PC_2 were days to maturity (0.309), inflorescence length (0.260) and branches/plant. All the germplasm lines were grouped into six clusters based on average linkage method. Cluster III had high values for seed yield and most of the quality traits but showed a small seed size. The dendrogram separated the two lines of *C. berlandieri* subsp. *nuttalliae* from the quinoa lines.

Introduction

Huauzontle (*Chenopodium berlandieri* Moq. subsp. *nuttalliae* (Safford) Wilson et Heiser) and quinoa (*C. quinoa* Willd.) have been cultivated since long as food plants in America (Risi and Galwey 1984). Quinoa, an Andean grain crop, has gained worldwide attention because of its ability to grow in various stress conditions like soil salinity, acidity, drought, frost, etc., (Jacobsen et al. 2003). Its grain is a rich source of a wide range of minerals, vitamins, oil and high quality protein containing ample amounts of sulphur rich amino acids (Koziol 1992; Ruales and Nair 1992). These benefits necessitated the introduction of quinoa to newer areas outside its native region, especially in the subtropical regions of the world. However, despite the immense possibilities of quinoa as a food crop, significant efforts have not been made for its genetic improvement. The emphasis has been mainly on its introduction to newer agro-ecological zones. Initial reports on quinoa trials from Europe and Africa are encouraging (Mujica et al. 2001), but most of these are centered around three traits, namely, seed yield, biomass production and maturity period. Other yield contributing traits seem to have been overlooked which has resulted in absence of breeding plans to enhance yield and quality traits like seed protein and carotenoids.

Multivariate statistical methods have been successfully used to classify quantitative and qualitative variations in many crop species like pea (Amurrio et al. 1995), mustard (Rabbani et al. 1998), Russian wildrye (Berdahl et al. 1999) and

Arachis (Chandran and Padya 2000). Although reports on morphological diversity in quinoa are available (Risi and Galwey 1989a; Ortiz et al. 1999), but detailed agronomic recommendations for yield and quality enhancement are rare (Bhargava et al. 2003). Therefore, the present study was conducted with the following objectives: (a) to analyze the degree of similarity/dissimilarity among germplasm lines of *C. quinoa* and its distant relative *C. berlandieri* subsp. *nuttalliae* through various morphological and quality traits and (b) to determine the extent of genetic diversity for effective germplasm management and proper utilization in breeding programs.

Materials and methods

The experimental material comprised 27 germplasm lines of *C. quinoa* and two lines of *C. berlandieri* subsp. *nuttalliae*. All the lines were tetraploid ($2n = 36$) and were procured from USDA and IPK, Gatersleben Germany. The sources and origin of various lines are provided in Table 1. These lines were evaluated in a randomized block design with three replications during crop years 2002–2003 and 2003–2004 at the experimental field of National Botanical Research Institute, Lucknow. The plot size for each line representing a single replication was 4 m^2 with row-to-row and plant-to-plant distance was 30 and 20 cm, respectively. The data on 10 plants in each replication were recorded for 12 morphological and 7 quality traits. The quality traits were estimated according to standard methods.

The mean data of two seasons were standardized and then subjected to combine analysis of variance. Multivariate analysis was done by numerical taxonomic techniques using the procedure of principal component analysis (Sneath and Sokal 1973). To bring out the patterns of similarity and dissimilarity, data was subjected to cluster analysis using the average linkage method to group the 29 germplasm lines.

Results

Analysis of variance exhibited highly significant differences for all the 19 traits among the 29 germplasm lines (data not shown) indicating the presence of high degree of morphological and qualitative variations among the lines studied. Mean, range and coefficient of variability for various traits are presented in Table 2. Days to maturity ranged from 109.33 to 163.33 days, while plant height ranged from 11.27 to 144.03 cm. Seed protein among the lines ranged from 12.55 to 21.02% with an average of $16.22 \pm 0.47\%$, while seed carotenoid was in the range of 1.69–5.52 mg/kg with a mean of 2.83 ± 0.16 mg/kg. Leaf moisture, days to flowering and days to maturity had lower CV values in comparison to other traits.

Principal component analysis

In order to assess the patterns of variation, principal component analysis (PCA) was done by simultaneously considering all the 19 variables. The first four principal components (PCs) accounted for 78.70% of the variability amongst the 29 lines under study (Table 2). PC_1 accounted for 39.5% of the total morphological and qualitative variation for the traits included in the two test seasons. The first PC has inflorescence/plant, plant height and stem diameter as the traits with the largest coefficients, all with positive sign. Therefore, first component distinguished tall lines with thick stems and more inflorescences. The first PC seems to be more related to yield and yield contributing traits as PC_1 had high positive values for these traits. Traits related to seed morphology had low values for PC_1, while leaf pigments exhibited moderate to high positive weight on PC_1.

The second component accounted for 18.94% of the variance and was more related to seed morphological traits, both of which contributed with high coefficients but having negative signs. The characters with greatest positive weight on PC_2 were days to maturity (0.309), inflorescence length (0.260) and branches/plant. All traits related to leaf quality had negative values for PC_2 while all yield-contributing traits did not occur strongly in the second component. Seed protein had negative values for the first three components but contributes to the IVth component with highest positive value.

Cluster analysis

The germplasm lines were grouped into six clusters based on average linkage method (Figure 1).

Table 1. Germplasm lines, their source, origin and seed color.

Germplasm line	Source	Status*	Origin*	Altitude* (m)	Seed color
C. quinoa Willd. CHEN 58/77	IPK, Germany	–	–	4000	Light
C. quinoa Willd. CHEN 67/78	IPK, Germany	–	Puno, Peru	–	Dark
C. quinoa Willd. CHEN 71/78	IPK, Germany	–	Bolivia	–	Light
C. quinoa Willd. CHEN 33/84	IPK, Germany	–	–	–	Light
C. quinoa Willd. CHEN 84/79	IPK, Germany	–	Cuzco, Peru	3200	Light
C. quinoa Willd. CHEN 92/91	IPK, Germany	–	Columbia	–	Light
C. quinoa Willd. CHEN 7/81	IPK, Germany	–	–	–	Light
C. quinoa Willd. PI 614938	USDA	–	Oruro, Bolivia	–	Light
C. quinoa Willd. PI 478408	USDA	Cultivar	La Paz, Bolivia	3800	Light
C. quinoa Willd. PI 478414	USDA	Cultivar	La Paz, Bolivia	3800	Dark
C. quinoa Willd. PI 596498	USDA	Landrace	Cuzco, Peru	3030	Light
C. quinoa Willd. Ames 13219	USDA	–	La Paz, Bolivia	3700	Light
C. quinoa Willd. Ames 13719	USDA	–	New Mexico, USA	–	Light
C. quinoa Willd. PI 587173	USDA	Cultivated	Jujuy,Argentina	–	Light
C. quinoa Willd. PI 510532	USDA	Cultivated	Peru	3000	Light
C. quinoa Willd. PI 614883	USDA	–	Jujuy, Argentina	–	Light
C. quinoa Willd. PI 584524	USDA	Cultivated	Chile	–	Light
C. quinoa Willd. Ames 22156	USDA	Cultivar	Chile	–	Light
C. quinoa Willd. Ames 13762	USDA	–	New Mexico, USA	–	Light
C. quinoa Willd. PI 614881	USDA	–	Jujuy, Argentina	–	Light
C. quinoa Willd. PI 510537	USDA	Cultivated	Peru	–	Dark
C. quinoa Willd. PI 510547	USDA	Cultivated	Peru	–	Dark
C. quinoa Willd. Ames 22158	USDA	Landrace	Chile	–	Light
C. quinoa Willd. PI 510536	USDA	Cultivated	Peru	–	Dark
C. quinoa Willd. PI 478410	USDA	Cultivar	La Paz, Bolivia	3800	Light
C. quinoa Willd. PI 433232	USDA	–	Chile	–	Light
C. quinoa Willd. Ames 21909	USDA	Landrace	Oruro, Bolivia	3870	Light
C. berlandieri subsp. *nuttalliae* (Saff.) Wilson et Heiser PI 568155	USDA	Landrace	Mexico	1680	Dark
C. berlandieri subsp. *nuttalliae* (Saff.) Wilson et Heiser PI 568156	USDA	Landrace	Mexico	2700	Dark

*From germplasm database.

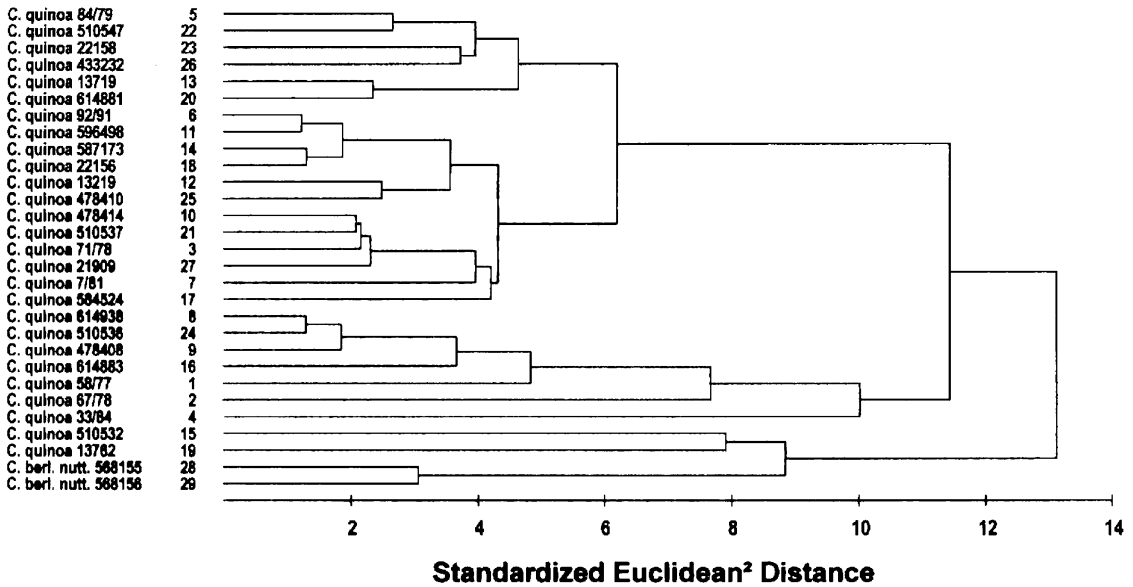

Figure 1. Dendrogram of 29 germplasm lines derived from average linkage cluster analysis.

Clusters I, II and III consisted of 6 germplasm lines each; cluster IV of 7 and clusters V and VI of 2 lines each. Lines from Peru, Chile and Bolivia were distributed in various clusters due to which it was difficult to establish any relationship between origin and clustering pattern. However, the two

Table 2. Basic statistics and Principal components for 12 morphological and 7 quality traits in 29 germplasm lines of *Chenopodium* spp. pooled over two seasons.

Traits	Mean ± SE	Range	CV	Coefficients of variates			
				PC_1	PC_2	PC_3	PC_4
Days to flowering	81.76 ± 1.18	70.78–101.55	7.82	0.177	0.142	0.185	0.036
Days to maturity	129.51 ± 2.51	109.33–163.33	10.44	0.282	0.309	0.069	0.001
Plant height (cm)	83.76 ± 6.79	11.27–144.03	43.67	0.477	0.174	0.058	0.026
Leaf size (cm^2)	18.15 ± 1.44	4.42–30.91	42.75	0.408	−0.165	0.084	−0.211
Stem diameter (cm)	0.86 ± 0.05	0.32–1.32	31.39	0.468	0.114	0.127	0.062
Branches/plant	20.62 ± 1.08	8.55–35.74	28.32	0.306	0.217	0.023	0.087
Dry weight/plant (g)	16.37 ± 2.24	1.11–52.89	73.85	0.363	0.102	0.126	0.046
Inflorescence length (cm)	2.64 ± 0.24	0.84–6.47	49.62	0.187	0.260	−0.007	−0.055
Inflorescence/plant	88.59 ± 7.81	11.67–141.55	47.48	0.559	−0.001	−0.062	0.177
Seed yield (g/plant)	16.27 ± 2.06	1.29–39.39	68.40	0.404	−0.192	0.071	0.253
Seed size (mm)	1.84 ± 0.03	1.34–2.21	11.41	0.020	−0.336	0.180	0.017
1000 seed weight (g)	2.69 ± 0.15	0.78–4.09	31.97	0.143	−0.416	0.200	−0.007
Leaf moisture (%)	86.17 ± 0.32	81.84–89.11	2.01	−0.012	−0.263	0.330	0.068
Chlorophyll a (mg/g)	1.26 ± 0.05	0.48–1.82	23.01	0.290	−0.227	−0.207	−0.059
Chlorophyll b (mg/g)	0.17 ± 0.007	0.07–0.25	23.52	0.271	−0.285	−0.270	−0.083
Total chlorophyll (mg/g)	1.43 ± 0.06	0.55–2.04	23.07	0.294	−0.239	−0.219	−0.063
Leaf carotenoid (mg/kg)	484.09 ± 18.37	230.23–669.56	20.42	0.380	−0.063	−0.190	−0.076
Seed carotenoid (mg/kg)	2.83 ± 0.16	1.69–5.52	31.80	0.143	0.158	−0.278	−0.060
Seed protein (%)	16.22 ± 0.47	12.55–21.02	15.90	−0.230	−0.093	−0.274	0.429
Components							
Root				1.95	0.93	0.63	0.37
% variance explained				39.50	18.94	12.72	7.55
Cumulative variance				39.50	58.44	71.15	78.70

SE, standard error of the means; CV, coefficient of variability.

Mexican lines formed a separate cluster that indicates that these lines are distinct from the rest of the material.

The lines in cluster I were early maturing and high yielding but had low carotenoid content. Cluster II comprised lines having low seed quality but were higher in leaf quality components. Cluster III had highest seed yield and high values for protein and carotenoids. The lines in cluster IV matured earliest and had high seed protein, while cluster V had high seed yield, dry weight/plant, stem diameter and maximum number of inflorescences. Cluster VI had low values for traits related to seed morphology and quality except for carotenoid content. It was noticed that clusters IV, V and VI showed more clear separation than the rest of the clusters. There was considerable overlapping between clusters I, II and III suggesting that principal components do not effectively separate the lines.

The intra and inter cluster distances are depicted in Figure 2. Clusters IV and V had high intra-cluster distances, while minimum intra-cluster distance was observed for cluster II. Maximum intercluster distance was found between cluster IV and VI (19.96), followed by clusters IV and V (17.89) and clusters III and IV (16.07).

Discussion

The results revealed that an enormous amount of genetic variability existed in the quinoa germplasm lines. Cluster analysis grouped together those lines that had greater genetic similarity but the clusters did not include lines from the same origin indicating heterogeneity of the lines within a given geographical region. This is most effectively exemplified by *C. quinoa* PI 510536 and *C. quinoa* PI 510537; *C. quinoa* PI 478408 and *C. quinoa* PI 478410; and *C. quinoa* PI 614881 and *C. quinoa* PI 614883. Each of the above mentioned pairs were collected from the same place but fall in separate clusters. Such diversity of population within geographical region might be due to factors like heterogeneity, genetic architecture of population, history of selection and/or developmental traits (Singh 1991) and has been reported in different crop species (Ghafoor et al. 2001; Alemayehu and Becker 2002; Singh et al. 2004).

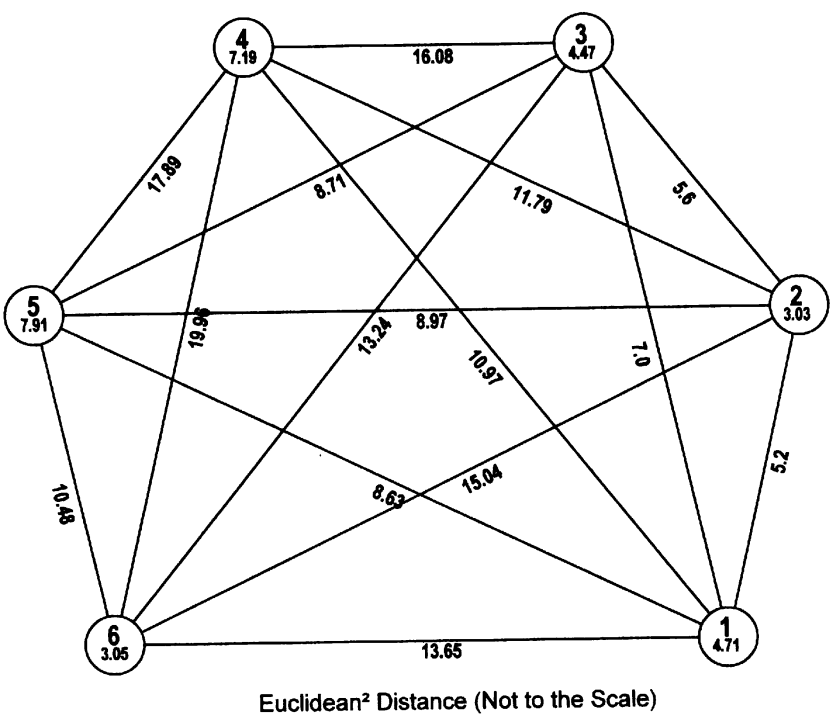

Figure 2. Diagram depicting the intra and inter-cluster distances among six clusters.

Multivariate analysis for 19 traits showed that most of the variations were accounted by the first four PCs. The main traits that accounted for more variability in both PC_1 and PC_2 include days to maturity, primary branches/plant, chlorophyll content and seed yield/plant. Thus, these traits are important in distinguishing the material under study. Good vegetative growth and high seed yield/plant characterized the lines with high PC_1 values, whereas lines with high PC_2 values were characterized by late maturity and larger seed size.

Quinoa seeds are a good source of seed protein and appreciable amounts of seed carotenoids that is in agreement with earlier reports on this plant (Cardozo and Tapia 1979; Wright et al. 2002). *C. berlandieri* subsp. *nuttalliae* is cultivated in Mexico primarily as a vegetable crop (Risi and Galwey 1989b). The present study shows that this species had low seed yield and small, light weighted seeds with low protein content. However, the plants are tall, late maturing, heavily branched and rich in leaf carotenoids due to which it is used as a vegetable crop. Although quinoa is generally utilized as a grain crop, our study shows that its leaves are a rich source of carotenoids and have higher leaf carotenoid in comparison to other foliage crops like *Amaranthus* (318 mg/kg) (Shukla et al. 2003), *Sonchus* (158 mg/kg) (Guil-Guerrero et al. 1999) and *C. album* (0.119 mg/kg) (Prakash et al. 1993; Bhargava et al. unpublished). A fodder yielding plant should be tall, leafy, thin stemmed, with a longer vegetative phase and high biomass. Lines of clusters II and III have most of the abovementioned characteristics along with high leaf carotenoid, which makes them suitable as green fodder crop. The present investigation therefore emphasizes the potential of quinoa as a fodder crop.

C. berlandieri subsp. *nuttalliae* was earlier considered to be conspecific with *C. quinoa* (Aellen 1929), which was further corroborated on the basis of grain characters (Simmonds 1976). However, various other evidences like genetic complementation of light fruited condition (Heiser and Nelson 1974), morphological and electrophoretic differences and crossability data indicate independent origin of both the cultivated species (Wilson and Heiser 1979). Similar results have also been obtained on the basis of RAPD profiles (Ruas et al. 1999), SDS-PAGE of seed proteins (Bhargava et al. 2005a) and karyotypic studies (Bhargava et al. 2005b). The present investigation based on morphological and quality characters also clusters two lines of *C. berlandieri* subsp. *nuttalliae* separately from the quinoa lines. The difference between the two species is also evident by the large intercluster distances shown by Cluster VI with all the clusters.

The lines of a particular cluster having desirable genes for a specific trait can be hybridized with the other promising lines of different clusters, which may facilitate to accumulate favorable genes in hybrids. The hybrids thus obtained may be fixed by selecting transgressive segregants, followed by recurrent selections in advanced generations, which may lead to development of high yielding varieties with desirable components. However, it is important to keep inter-cluster distances in mind while performing hybridization as magnitude of heterosis largely depends on the degree of diversity in parental lines and higher statistical distance between two clusters.

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