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Diversity Analysis of a Quinoa (*Chenopodium quinoa* Willd.) Germplasm during Two Seasons

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***Abstract*—**The present work has been carried out to evaluate the diversity of a collection of 78 quinoa accessions developed through recurrent selection from Andean germplasm introduced to Morocco in the winter of 2000. Twenty-three quantitative and qualitative characters were used for the evaluation of genetic diversity and the relationship between the accessions, and also for the establishment of a core collection in Morocco. Important variation was found among the accessions in terms of plant morphology and growth behavior. Data analysis showed positive correlation of the plant height, the plant fresh and the dry weight with the grain yield, while days to flowering was found to be negatively correlated with grain yield. The first four PCs contributed 74.76% of the variability; the first PC showed significant variation with 42.86% of the total variation, PC2 with 15.37%, PC3 with 9.05% and PC4 contributed 7.49% of the total variation. Plant size, days to grain filling and days to maturity are correlated to the PC1; and seed size, inflorescence density and mildew resistance are correlated to the PC2. Hierarchical cluster analysis rearranged the 78 quinoa accessions into four main groups and ten sub-clusters. Clustering was found in associations with days to maturity and also with plant size and seed-size traits.

***Keywords*—**Character association, *Chenopodium quinoa*, Diversity analysis, Morphotypic cluster, Multivariate analysis.

I. Introduction

QUINOA (*Chenopodium quinoa*) is an Andean grain crop which has been grown for more than 5000 years as food crop in the Americas [1]. Quinoa is an allotetraploid with 2n= 4x=36 chromosomes. The genus *Chenopodium* includes 150 species that are diploids, tetraploids or hexaploids and are native to both Old World and New World. Quinoa is a highly nutritious crop [2], [3]. It has a typical adaptability to adverse climate and soil conditions [4]. Its genetic variability is very important for developing suitable cultivars with desirable traits such as earliness, large white seeds, resistance to biotic and abiotic adverse factors and grain yield.

Quinoa diversity is due to phenotypic elasticity; intensive selection for adaptation and productivity in highly diverse environments; and genetic mechanisms like transposable elements that generate variation at the DNA level [5]. All of these factors together explain quinoa's distribution in different types of climates and soil conditions.

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The main objective of this study is to evaluate genetic diversity among developed germplasm of quinoa for proper utilization in breeding programs and select the most important traits for further breeding efforts.

II. Materials and Methods

A total of 78 quinoa accessions developed through a recurrent selection from primarily Bolivian and Peruvian germplasm introduced in 2000 to Morocco were evaluated on the basis of agro-morphological traits. The experimental work was performed under field conditions during two years (2012 and 2013) in Rabat, Morocco.

Conventional pest control and manual weeding were undertaken when it was required. The experiment plot was irrigated 3 times a week due to the loamy soil texture. Accessions were harvested when the seed reached physiological maturity.

The experimental design was three blocks where accessions were randomly planted in a 2m row of 0.50m inter-row. Twenty-three morphological descriptors were recorded during the plant growing cycle [6]; traits were grouped into quantitative and qualitative characters (Tables I and II). Five measurements were taken per accession per block. Absolute frequency and relative frequency were calculated. Quantitative trait variation was analyzed through frequency distributions and descriptive statistics.

The analysis of variance was performed based on a completely randomized design with one factor, the genotype, and three replicates. Multivariate analysis was carried out using the following methods: (i) Pearson coefficient of correlation between the variables [7]; (ii) Principal Component Analysis (PCA) for the quantitative traits; (iii) Cluster analysis using UPGMA (Unweighted Pair-Group Method Analysis) as described by reference [8] (1958). All the analyses were performed using PAST software [9].

III. Results

Quinoa collection was characterized through several morphological traits, grouped into two classes [nine qualitative traits (Table I) and 14 quantitative traits (Table II)] and three trait categories in relation to seeds, plant and inflorescence morphology. The statistical analysis showed highly significant differences between accessions for all the quantitative traits in Table II.

The descriptors of the seed traits showed that most of the accessions were of medium seed size and had diameters ranging from 1.50 to 1.80mm. Eighty-four percent of the

accessions were early emerging; they took between 6 and 7 days to emerge. Half of the accessions were brown seeded. Fifty percent were vitreous and all the selected accessions for Moroccan conditions (W11, W119, W142 and W143) belonged to this group.

Nine plant descriptors were used: leaf color (LC), ramifications (Rs), branch position (PB), plant size (PZ), length of the root (LR), plant fresh weight (FW), plant dry weight (DW), reaction to downy mildew (RMil) and indeterminate growth (IG) as a qualitative trait. Twenty-two percent of the accessions had a size (PZ) between 150 and 200cm. Most plants were in mid-range between 50 and 150cm (70%). Eighty-seven percent of the accessions presented ramification and 36% had spread branches, showing their wild-type traits. In general, about 92% had a determinate growth showing their adaptation to the coastal conditions of Rabat.

The inflorescence descriptors revealed an important diversity in the inflorescence shape, color, size, and grain weight. Sixty-two percent of the accession inflorescences were medium density (ID). Yellow inflorescences were the most frequent and represented 57% of the total, 32% were red, while orange, pink and purple inflorescences were about 4% each. There was also significant variation among accessions for harvest index and grain yield, which varied from 8 to 67% and 14.0 to 195.9 g/plant, respectively.

TABLE I
MORPHOLOGICAL TRAITS RECORDED FOR QUALITATIVE TRAITS

Trait	Scale
Seed color (SC)	Beige, Black, Brown, Dark brown, Pink, White, Yellow
Seed form (SF)	Flat, Round
Seed type (ST)	Opaque, Vitreous
Panicle color (PC)	Byzantine, Byzantium, Coral, Green, Lavender, Pink, Violin, Yellow
Leaves color (LC)	Dark green, Green, Lime green
Ramifications (Rs)	Present, Absent
Inflorescence form (IF)	Amarantiform, Glomeriform
Position of the branches (PB)	Very spread, Spread, Tight , Very tight
Indeterminate growth (IG)	Yes, No

Table II provides the Pearson correlation coefficients between grain yield and the reaction to downy mildew with the other traits. Several correlations were regenerated; the highest correlation (R=0.67***) was found between grain yield and the fresh biomass (Table II). Significant correlations were observed also between days to harvest and plant size at harvest (R=0.64***) and between grain yield and plant size (R=0.54**). Other significant correlations were between inflorescence density and days to flowering (R=0.47**); days to flowering and plant size (R=0.40**); and grain yield and days to early flowering (R=0.34**). Negative correlations were found between harvest index and fresh biomass (R=-0.52); reaction to mildew and days to flowering (R=-0.35**); and grain yield and seed size (R = -0.22**).

TABLE II
DESCRIPTIVE STATISTICS OF MORPHOLOGICAL TRAITS IN QUINOA AND THE CORRELATION COEFFICIENTS TO GRAIN YIELD AND REACTION TO DOWNY MILDEW

Traits	Mean	Correlation to grain yield	Correlation to resistance to mildew
Seed size (SZ)	1.53	-0.22**	0.06
Inflorescence density (ID)	1.65	0.38**	-0.31**
Days before emergence (DBE)	6.59	0.28**	0.15*
Days before early flowering (DBeF)	60.31	0.14*	-0.20**
Days before flowering (DBF)	70.14	0.18*	-0.35**
Days before filling stage (DBFil)	92.10	0.34*	-0.20**
Days before harvest (DBH)	126.17	0.43**	-0.22**
Grain yield (GY)	54.15	-	0.09
Plants size at harvest (PZ)	139.02	0.54***	-0.14*
Plant fresh weight (FW)	206.18	0.67***	-0.03
Plant dry weight (DW)	50.94	0.57***	-0.06
Response to mildew (RMil)	2.31	0.09	-
Length of the root at harvest (LD)	14.02	0.14*	-0.23**
Harvest index (HI)	0.32	0.19**	0.02

**: p < .05; **: p < .01; ***: p < .001.*

The first four principal components for quantitative traits represented 74.76% of the total variability among the accessions. The PC1 accounted for 42.86% of the total quantitative variation and was correlated to plant size, days to harvest, days to flowering and inflorescence density, but was negatively correlated with the response to downy mildew (Table III). The PC2 explained an additional 15.37% of the total variation, and reflected primarily the patterns of variation in grain yield and harvest index; both of which had high positive values (Fig. 1). PC3 accounted for 9.05% of the variability present among the accessions and had the highest positive values for the reaction to downy mildew and seed size, while having high negative values for the inflorescence density and days to flowering. Finally, PC4 represented 7.49% of total variance; it showed positive values for the reaction to downy mildew and days to grain filling stage, and negative coefficients for length of the root and fresh biomass (Table III).

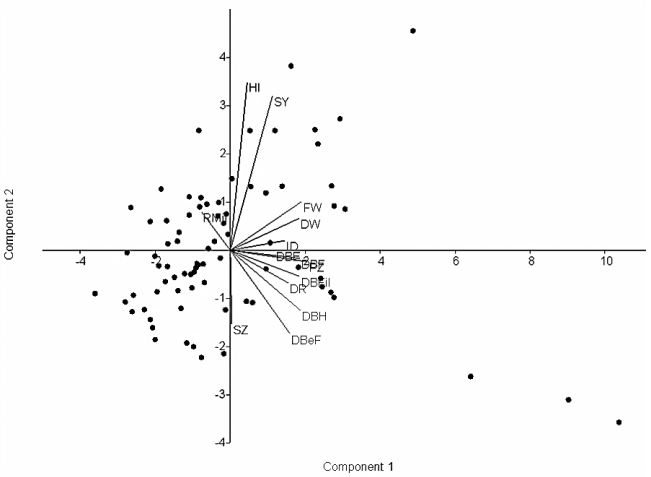

Fig. 1 Principal component analysis (PCA) of 78 quinoa's accessions using PAST software [9]

TABLE III
FIRST FOUR PRINCIPAL COMPONENTS FOR 14 TRAITS IN THE QUINOA COLLECTION

Traits	PC1	PC2	PC3	PC4
Seed size (SZ)	0.01	-0.40	0.50	0.06
Inflorescence density (ID)	0.63	0.05	-0.42	0.01
Days before emergence (DBE)	0.51	-0.00	0.39	0.47
Days before early flowering (DBeF)	0.68	-0.45	-0.13	0.19
Days before flowering (DBF)	0.80	-0.04	-0.33	0.30
Days before filling stage (DBFil)	0.80	-0.14	-0.12	0.35
Days before harvest (DBH)	0.81	-0.32	0.04	0.09
Grain yield (GY)	0.48	0.83	-0.01	0.04
Plants size at harvest (PZ)	0.89	-0.06	0.09	-0.14
Plant fresh weight (FW)	0.82	0.26	0.25	-0.29
Plant dry weight (DW)	0.79	0.17	0.26	-0.31
Response to mildew (RMil)	-0.33	0.21	0.59	0.40
Length of the root at harvest (LD)	0.67	-0.17	0.27	-0.41
Harvest index (HI)	0.19	0.90	-0.02	0.14
% Variance explained	42.86	15.37	9.05	7.49
% Cumulative variance	42.86	58.23	67.28	74.76

The trait "reaction to downy mildew" exhibited high positive weight on PC3 (0.587) and PC4 (0.405) and a moderate weight on PC1 (-0.329). Traits related to grain yield did not occur strongly in the first component while traits related to the plant phenology had the highest coefficients. Therefore, the first component distinguishes plant phenology, while the second relates mainly to grain yield.

The phylogenic tree displayed in Fig. 2 reveals the presence of four distinct clusters and ten sub-clusters. The cluster I represents 5% of the germplasm and gathered the very late accessions that exhibited the biggest size, the lowest harvest indexes and high resistance to downy mildew; for the seed traits, all accessions had opaque grains (Table IV). Cluster II gathered late-maturing accessions with high levels of resistance to downy mildew. Within this cluster, sub-cluster 3 grouped the vitreous-seeded accessions. Cluster III gathered 37 % of the accessions; the corresponding three sub-clusters shared two common traits, early maturing and short plant size. This cluster grouped most advanced selection lines and the two Danish cultivars (Table IV). The last cluster VI included early maturing, high harvest index and high yielding accessions; this group represents 28% of the quinoa germplasm (Table IV).

TABLE IV
PERCENT OF ACCESSIONS PER CLUSTER

Main Cluster	Sub Cluster	No. of accessions	% of accessions per sub-cluster	% of accessions per cluster
I	1	4	5.12 %	5.12 %
	1	6	7.69 %	
II	2	6	7.69 %	29,48 %
	3	11	14.10 %	
	1	7	8.97 %	
III	2	12	15.39 %	37.18 %
	3	10	12.82 %	
	1	12	15.38 %	
IV	2	4	5.12 %	28.20 %
	3	6	7.69 %	

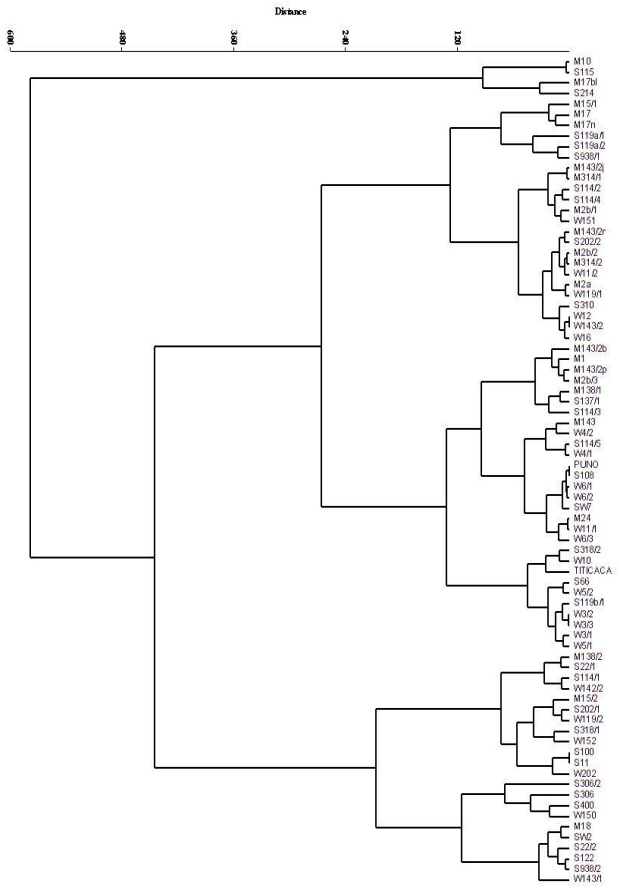

Fig. 2Dendrogram based on agronomic and morphological traits using UPGMA

IV. DISCUSSIONS

The comparisons of UPGMA clustering and PCA revealed that the highland, non-adapted accessions to Rabat's coastal climate are grouped separately and distantly from the other clusters. Highland accessions had tall stature and indeterminate growth when cultivated in coastal environment. They were, however, highly resistant to downy mildew; they developed small lesions and had a long latent period when inoculated with oospores of *Peronospora farinosa* under controlled conditions [10]. They therefore represent potential gene sources to enhance downy mildew resistance in quinoa breeding.

The results reveal that all quinoa accessions selected for the Moroccan growing environment have vitreous seeds. Therefore, it could be relevant to look for the specificity of this vitreous grain type in terms of protein content, salt tolerance and heat tolerance. Moreover, all the advanced-selection lines belong to the sea level, salt flat and Altiplano types described as dryland and salt tolerant genotypes [11]. Those accessions were distributed across the dendrogram, indicating a high degree of diversification in terms of quantitative and qualitative traits. The existence of such variability is essential for the establishment of a local quinoa core collection and also for the identification of the best adapted genotypes. The agro-morphological characterization

of crop germplasm is the first step in a crop breeding program; it is also an important tool to describe and sort out the existing diversity.

The multivariate analysis of the 14 traits showed that most variation was accounted for by the four first PCs. Days before flowering, days to maturity, grain yield and plant size at maturity are the main traits that accounted for more variability in PC1 and PC2. Therefore, these traits are central criteria to evaluate the variability and to discriminate between accessions. Both days to grain filling and inflorescence density differentiated the accessions through their high PC1 values; accessions with high PC2 values were distinguished by having a high harvest index.

Within several accessions, weedy traits were still persistent, like shoot ramification, indeterminate growth and black seed color. This indicates probable gene flow through continuous hybridization between cultivated quinoa and wild species of Chenopodium due to the absence of genetic and non-genetic hybridization barriers between weedy and cultivated quinoa [12], [13]. This would also explain quinoa's extensive variability and its adaptation to abiotic and biotic stresses, along with its high phenotypic plasticity in response to climate changes [14]-[16].

This study revealed an extensive phenotypic diversity within the quinoa collection. The evaluation of the agro-morphological diversity through univariate and multivariate analysis pointed out the most performing genotypes. Accessions belonging to the same cluster and having desirable traits (genes) could be crossed to accessions from other clusters to gather favorable genes in one elite variety.

Based on the pheno-morphological traits, every accession was associated to one of the five-quinoa groups defined by reference [17] and [11] (1990). The results showed that all the selected accessions (W11, W119, W142, W143…) belong to the sea level, salt flat or Altiplano types.

Grain yield, earliness, large diameter seeds and short plant size were the main traits common to the advanced lines; therefore, they can be taken as relevant selection criteria for the quinoa national breeding program.

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