

Morphological Traits Defining Breeding Criteria for Coastal Quinoa in Chile

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Abstract

Coastal/lowland quinoa ecotype is an important source of germplasm due to its cultivation in cold-temperate and high latitude areas. However, the interaction of its morphological traits and yields to define breeding criteria is unknown. The present study was designed to characterize the phenotypic diversity of twelve coastal/lowland quinoas using sixteen standardized morphological descriptors under rainfed conditions in central Chile. Complementary analysis of uni- and multi-variate tools allowed a fuller understanding of interrelationships within quinoa germplasm. Through the analysis of frequency distribution, it was possible to determine that genotypes were characterized by plants having low height and medium grain yield. Cluster analysis revealed that plant morphological variables were independently grouped from grain yield components. Additionally, principal component analysis (PCA, 74.8% of total variation data), revealed the existence of three outstanding genotypes (QC01, QC02 and QC05) that were distantly located from the average dispersion of entire germplasm collection. These genotypes were associated with grain yield components, allowing the identification of two groups of high yield (VI and VII), which yielded 3337.7 and 3052.0 kg ha⁻¹, respectively. The data set presented in this study is the first report of coastal/lowland quinoas assessed in central Chile and could assist the development of breeding programmes in cold-temperate areas having similar agro-climatic conditions.

Keywords: *Chenopodium quinoa*, grain yield, landrace, multivariate analysis, rainfed

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a grain crop that is cultivated in the Andes region ranging from central valleys of Colombia to the southern zone of Chile. Its expansion through different geographical areas of Latin America has been possible due to ancestral peasant practices of domestication, use and seed exchange among Andean farmers (Fuentes, 2008). These practices have conserved a high phenotypic variability which allows its adaptation to different ecological environments, such as relative humidities from 40% to 80%, altitude ranges from sea level to 4,000 m.a.s.l. and a wide range of temperatures ranging from 8 to 38 °C (Bazile *et al.*, 2013).

According to the adaptability of quinoa to the different geographical contexts of the Andean region, five ecotypes have been described, which differ in phenology, morphology, adaptation to biotic and abiotic factors and its use. These ecotypes correspond to (i) Inter Andean valleys quinoa (in Colombia, Ecuador, and Peru); (ii) Highlands quinoa (in Peru and Bolivia); (iii) Yungas quinoa (in Bolivian subtropical forest); (iv) Salares quinoa in salt flats (in Bolivia, northern Chile, and Argentina); and (v) Coastal quinoa, from lowlands or sea level (in central and southern Chile) (Risi and Galway 1984; Fuentes *et al.*, 2012). The expansion routes from the Titicaca Lake have been supported with genetic data as revealed with the use of molecular markers and genomic resources of the quinoa genome (Fuentes *et al.*, 2009, 2012; Jarvis *et al.*, 2017).

The coastal ecotype is found along the south-central zone of Chile between the rainfed area of O'Higgins region and the rainy area of Araucanía and Los Lagos regions (Fuentes *et al.*, 2009; Sosa-Zuniga *et al.*, 2017). Quinoas from rainfed area respond to specific characteristics of their environment, including adaptation to saline and sandy soils, as well as to average annual precipitation of 500 to 650 mm distributed during autumn and winter months, while in the southern zone quinoa grows in a pre-mountain area with more than 2,000 mm of annual precipitation distributed evenly throughout the year (Bazile *et al.*, 2014). The plants are typically medium in size and minimally branched, panicles are glomerular, intermediate and amarantiform, grains varying from 1.7 to 2.2 mm in diameter and maturity period ranging from 150 to 190 days (Miranda *et al.*, 2012; 2013; Zurita-Silva *et al.*, 2014).

In the rainfed area, quinoa seed conservation is carried out by farmers who have maintained the seeds for generations, developing their own individual seed selection criteria according to their preferences (Fuentes *et al.*, 2012). In the past, quinoa was cultivated in small scale farming systems and consumed by the farm dwellers themselves with traditional practices characterized by low-external-input farm management. In this scenario, there was no priority to improve the seeds, since it was only a marginal crop within the family farms. Even though the traditional system of production persist, the scale of production has changed, thus not only the family gardens are still cultivated but larger scale of production is utilized in rotation with other crops in order to increase the income per hectare (Olguín, 2011). Nevertheless, there is no documentation regarding agro morphological traits variation of coastal quinoas for preliminary identification of local varieties with stable yields to improve quinoa productivity on-farm. The goals of the present study were to characterize the morphological diversity of twelve quinoa genotypes and to identify outstanding genotypes for quinoa production under rainfed conditions in central area of Chile.

Materials and Methods

Description of the study site

The study was carried out in a field located in the Marchigüe locality, belonging to the rainfed area of O'Higgins region in Chile, during the 2014-2015 season (34°17'9''S and 71°46'5''S, altitude: 205 m). The area is characterized by a warm temperate climate with a prolonged dry season, with temperatures reaching up to 29.5 °C (Table 1). The soil was classified as having a sandy loam texture, with a pH of 5.44; an electrical conductivity of 0.09 mS cm⁻¹ and organic matter content of 3.25% (Table 2). The plot size of the experiment was 252 m², in which each accession was established as a plot of three 3.5 m rows with 0.5 m between rows and 0.5 m between plants.

Biological material

Twelve local genotypes were used for morphological characterization (Table 3). Four accessions were collected at Paredones, two at Pumanque and six at Pichilemu cities, which have been kept in the active seed bank at Pontifical Catholic University of Chile.

Protocol for collecting data

Sixteen morphological descriptors were studied that were grouped as follows: (i) discrete variables: GH, growth habit; MSS, main stem shape; MSC, main stem colour; NPB, number of primary braches; PCPS, panicle colour at physiological stage; PS, panicle shape; PD, panicle density; PC, pericarp colour; EC, episperm colour; (ii) continuous variables: PH, plant height; StD, stem diameter; PL, panicle length; PW, panicle width; SeD, seed diameter; 1000SW, 1000 seed weight; and GY, grain yield, as defined by Biodiversity (2013).

Statistical procedures

The analysis of the data considered the analysis of variance based on a completely random block design with 12 genotypes and 3 replicates. Treatment averages at Marchigüe locality were separated using the Tukey test ($\alpha = 0.05$). Parametric and non-parametric analysis of the data was performed using the INFOSTAT (InfoStat, 2014) statistical software. Multivariate analysis was performed using the following methods: (i) the total correlation between variables using the Pearson coefficient complement (Clifford and Stephenson 1975); (ii) principal component analysis (PCA) (Hair *et al.*, 1992) and (iii) cluster analysis. A dendrogram was constructed using the complement of the Pearson coefficient and the algorithm for UPGMA (unweighted pair-group method using an arithmetic average) as described by Sokal and Michener (1958).

Results

Frequency distribution of variables

According to the morphological descriptors used in the study, nine of these corresponded to the description of discrete variables. These were: GH, growth habit; MSS, main stem shape; MSC, main stem colour; NPB, number of primary braches; PCPS, panicle colour at physiological stage; PS, panicle shape; PD, panicle density; PC, pericarp colour; EC, episperm colour. Table 4 shows the average distribution of each descriptor in the 12 genotypes evaluated in Marchigüe locality. The GH descriptor genotypes were classified into the "simple" category with 92% of accessions, meanwhile the "branched to 2/3" of plant category was represented by only one. All the genotypes evaluated for the MSS descriptor corresponded to the "angular" category. The main colour for the MSC descriptor was "yellow" (92%) and secondary, pink (8%). For the NPB descriptor the genotypes were concentrated in the category "without branches" with 92% of accessions and with "3 branches" only 8%. The PCPS descriptor considered 50% of genotypes into the "yellow" category and 50% into the "brown" one. For the PS descriptor, most of genotypes were classified into the "intermediate" category (92%), and for PD descriptor most had "intermediate" (92%) panicles. The PC descriptor presented a distribution into three categories, 42% was light coffee, 25% dark coffee and 8% yellow. Finally, for the EC descriptor 75% of the genotypes were classified as cream colour and 25% as white.

The frequency distribution of seven continuous descriptors is shown in Table 4. The PH descriptor was

Table 1. Mean and ranges of monthly maximum and minimum temperatures and relative humidity recorded at Marchigüe locality*

Month	Maximum (°C) (range)	Minimum (°C) (range)	RH (%) (range)
November	23.8 (15.8-29.6)	6.9 (1.4-10.7)	58.5 (32-89)
December	24.9 (16.3-30.8)	7.3 (1.2-11.9)	61 (29-97)
January	28.4 (19.7-34)	8.2 (3.7-13.6)	59.2 (29-95)
February	29.5 (24.9-35.3)	6.5 (1.9-9.6)	70.5 (51-92)
March	28.9 (23.4-36.8)	8.9 (2.7-15.2)	57.5 (43-92)

*Data set recorded during 2014-2015

Table 2. General soil characterization of experimental plots at Marchigüe locality (0-20 cm)*

Soil parameters	Unit	Normal ranges	Marchigüe
Chemical properties			
pH suspension	-	-	5.44
CE suspension	mS cm ⁻¹	< 0.5	0.09
M.O	%	-	3.25
Available			
N	mg kg ⁻¹	*	44
P Olsen	mg kg ⁻¹	20 - 40	6
K	mg kg ⁻¹	150 - 300	116
Cu	mg kg ⁻¹	0.6 - 11	1.3
Fe	mg kg ⁻¹	> 4.5	170
Mn	mg kg ⁻¹	> 1.0	17.7
Zn	mg kg ⁻¹	> 1.0	1.3
B	mg kg ⁻¹	1.0 - 1.5	0.1
S	mg kg ⁻¹	> 9	2
Interchangeable			
Ca	meq 100gr ⁻¹	> 4.1	3.2
Mg	meq 100gr ⁻¹	> 0.5	1.2
K	meq 100gr ⁻¹	> 0.38	0.3
Na	meq 100gr ⁻¹	< 0.5	0.1
CEC*	meq 100gr ⁻¹	-	7
Physical			
Clay	%	-	13.3
Silt	%	-	24
Sand	%	-	62.7
Texture class			Sandy loam

*CEC: cation exchange capacity

Table 3. Detail of quinoa genotypes used in the study*

Genotype	Accession ID	Place name	Latitude	Longitude	Altitude (m)
QC01	QPU001	Centro Pumanque	34°36'11.66"S	71°39'11.88"W	120
QC02	QPU002	Centro Pumanque	34°44'7.45"S	71°39'55.54"W	122
QC03	QPA001	La Queseria	34°38'58.14"S	71°52'8.26"W	66
QC04	QPA002	Paredones Centro	34°38'47.35"S	71°53'58.50"W	56
QC05	QPA003	El Peral	34°29'49.42"S	71°59'49.58"W	10
QC06	QPA004	La Vega	34°35'33.52"S	71°50'48.86"W	48
QC07	QPI001	La Palmilla	34°32'9.6"S	71°57'49.69"W	24
QC08	QPI002	Rodelillo	34°31'1.42"S	71°56'37.63"W	95
QC09	QPI003	La Plaza	34°32'9.66"S	71°59'15.16"W	15
QC10	QPI004	La Plaza	34°33'2.76"S	71°59'22.78"W	17
QC11	QPI005	La Palmilla	34°34'8.66"S	71°55'50.97"W	27
QC12	QPI006	Cáhuil	34°18'8.65"S	71°48'53.37"W	12

*Genotypes collected are registered in the active seed bank of Pontifical Catholic University of Chile

classified in a range that describes in general medium and short plants, with 58% of genotypes into the short category (<113 cm) and 33% into the medium (113 to 139 cm). However, only one genotype was classified into the tall plants category (>139 cm). The StD descriptor classified most of collection as small (83%), meanwhile medium and large diameter were found only for one genotype, respectively. For the descriptor PL, the genotypes were classified as short (50%) and medium length (42%). The genotype collection was characterized for the PW descriptor as small, with 92% into the range of 11.9 to 22.7 cm. The

SeD descriptor was considered medium for 50% of genotype collection in a range of 1.7 to 2.0 mm, meanwhile 17% and 33% of genotypes were small (1.4 to 1.7) and big (2.0 to 2.3), respectively. The 1000SW descriptor was classified as medium (3.4 to 3.8 mg) for 50% of collection and lower (<3.4 mg) and higher (>3.8 mg) for 33% and 17% of genotypes, respectively. The GY descriptor was concentrated in the range of low yields with 58% of collection between 630 to 1,613 kg ha⁻¹, meanwhile 17% and 25% of genotypes were into the medium (1,613 to 2,596 kg ha⁻¹) and high yields (2,596 to 3,579 kg ha⁻¹) ranges, respectively.

Table 4. Frequency distribution of discrete and continuous variables of morphological traits of quinoa genotypes evaluated under rainfed conditions at Marchigüe locality*

Variables	Plant traits	Category	Frequency	
			Absolute	Relative (%)
Discrete variables	GH	Simple	11	92
		Branched to 2/3	1	8
	MSS	Angular	12	100
		Cylindrical	0	0
	MSC	Yellow	11	92
		Pink	1	8
	NPB	0	11	92
		3	1	8
	PCPS	Yellow	6	50
		Brown	6	50
	PS	Glomerulate	1	8
		Intermediate	11	92
	PD	Compact	1	8
		Intermediate	11	92
	PC	Dark coffee	6	25
		Light coffee	5	42
		Yellow	1	8
	EC	Cream	9	75
White		3	25	
Continuous variables	PH (cm)	86.98 - 113.33	7	58
		113.33 - 139.68	4	33
		139.68 - 166.04	1	8
	StD (cm)	1.26 - 1.54	10	83
		1.54 - 1.83	1	8
		1.83 - 2.11	1	8
	PL (cm)	16.09 - 32.47	6	50
		32.47 - 48.86	5	42
		48.86 - 65.24	1	8
	PW (cm)	11.90 - 22.77	11	92
		22.77 - 33.63	0	0
		33.63 - 44.50	1	8
	SeD (mm)	1.43 - 1.73	2	17
		1.73 - 2.03	6	50
		2.03 - 2.33	4	33
	1000SW (mg)	3.03 - 3.41	4	33
		3.41 - 3.79	6	50
		3.79 - 4.17	2	17
GY (kg ha ⁻¹)	630.80 - 1613.60	7	58	
	1613.61 - 2596.40	2	17	
	2596.41 - 3579.20	3	25	

*GH, growth habit; MSS, main stem shape; MSC, main stem colour; NPB, number of primary braches; PCPS, panicle colour at physiological stage; PS, panicle shape; PD, panicle density; PC, pericarp colour; EC, episperm colour; PH, plant height; StD, stem diameter; PL, panicle length; PW, panicle width; SeD, seed diameter; 1000SW, 1000 seed weight; GY, grain yield

Correlation among variables

Pearson coefficient correlation among continuous variables of quinoa genotypes is shown in Table 5. Highest correlation was found between PW - StD (R = 0.82), PW - PL (R = 0.79), PW - PH (R = 0.73). Other significant correlations were StD - PH (R = 0.68), PL - PH (R = 0.68) and PL - StD (R = 0.58).

Principal component analysis

The principal component analysis shown in Fig. 1 revealed that the first two major components of the analysis accounted for 74.8% of the total qualitative variation of data, which were selected for further analysis following the criteria described by Kaiser (1960). In the figure, the genotypes QC01, QC02 and QC05 were distantly located from those associated at central point of biplot. The genotype QC05 was located at the right zone of quadrant (positive values in the PC1) and presented a positive correlation (acute angle) with seed traits (GY, PL and PW), meanwhile genotypes QC01 and QC02 were located in the right zone of quadrant, having a positive correlation with 1000SW and PW descriptors, respectively. The rest of genotypes were located at the left zone of quadrant, revealing less correlation with seed traits.

Cluster analysis

The dendrogram shown in Fig. 2, revealed the presence of two different groups among the descriptors. The first group was represented for the seed descriptors (SeD, 1000SW and GY) and the second group for variables that determined plant morphology (PL, PW, StD and PH).

A correlation matrix involving the complement of the Pearson coefficient was used to construct a dendrogram using the UPGMA algorithm. The genotypes were grouped into seven groups (Fig. 3). Group I had one genotype and was represented by a tall plant with medium yield. Group II

consisted of two genotypes characterized by having short plants with medium yields. Group III was represented by three genotypes having medium height and yields. Group IV was composed of one genotype, which was characterized by a short plant with low yield, despite the fact that it had large panicles. Group V was represented by two genotypes that had short plants with low yields. Group VI consisted of two genotypes that had tall plants and high yields. Finally Group VII was represented by one genotype with medium height and high yield.

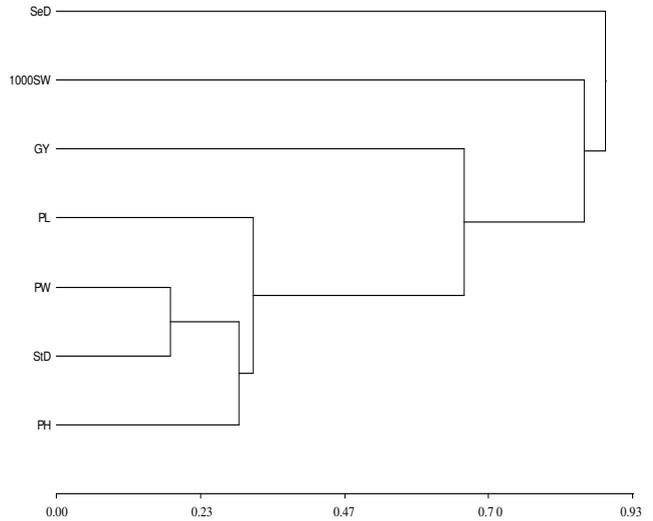


Fig. 2. Dendrogram showing distances between 7 quantitative variables of 12 quinoa genotypes evaluated under rainfed conditions at Marchigüe locality. PH, plant height; StD, stem diameter; PL, panicle length; PW, panicle width; SeD, seed diameter; 1000SW, 1000 seed weight; GY, grain yield

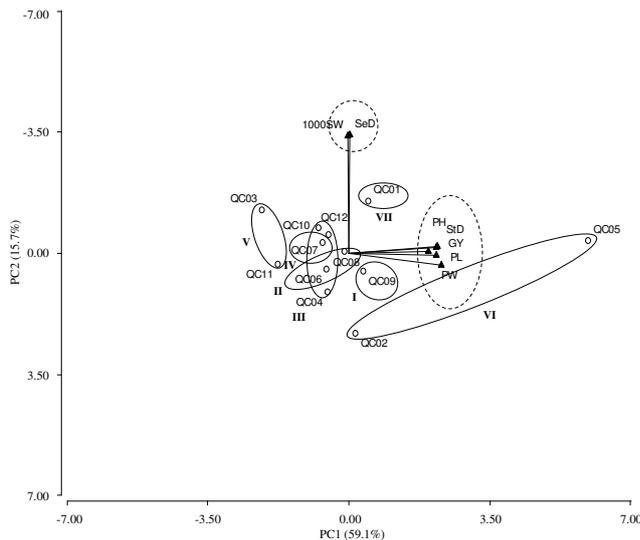


Fig. 1. Principal component analysis (PCA) biplot of twelve quinoa genotypes (white circle) and seven morphological traits (black triangle) evaluated under rainfed conditions at Marchigüe locality. PH, plant height; StD, stem diameter; PL, panicle length; PW, panicle width; SeD, seed diameter; 1000SW, 1000 seed weight; GY, grain yield

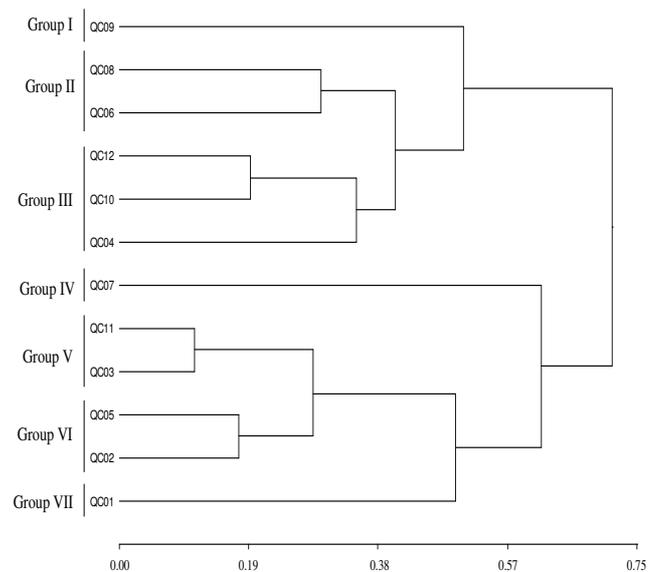


Fig. 3. Cluster analysis for characterization of 12 quinoa genotypes grown under rainfed conditions at Marchigüe locality

Discussion

The data set provided a general definition of coastal quinoa genotypes growing under Mediterranean-type climate, which express medium to small growth pattern. This definition is supported by positive correlations found among PW – PH, PW – PL and PW – PH descriptors (Table 5). Likewise, the cluster analysis (Fig. 2) also correlated with this definition, showing that PW descriptor was associated with a StD, PH and PL. These positive associations confirm the pattern observed in the field, consisting of tall plants with large panicles and thick stems.

Risi and Galwey (1989) assessing Peruvian germplasm described a large amount of variation for StD (range = 0.7–5.5 cm; mean = 2.27 cm) and PH (range = 0.36–2.56 m; mean = 1.58). In contrast, Rojas (2003) reported that morphological descriptors for Bolivian germplasm had a narrow variation for StD, ranging from 1.02 to 2.63 cm (mean = 1.71 cm) and for PH from 0.54 to 1.74 m (mean = 1.11 m). Similarly, Chilean germplasm (Salar ecotype) assessed by Fuentes & Bhargava (2011) showed a narrow variation for the StD variable, ranging from 0.88 to 1.94 cm and for PH from 1.01 to 1.91 m. A comparison of data presented in this study with the abovementioned reports confirms in general that phenotypic behaviour of coastal quinoa in central area of Chile does not show particularly extreme values for any of the variables measured.

The principal component analysis showed that three accessions were distantly located from those associated at central point of biplot (QC01, QC02 and QC05). In addition, the cluster analysis among descriptors revealed two main associations, the first related to plant morphology and the second to characteristics of grain (Fig. 2). The combined multivariate analysis of data also revealed outstanding genotypes, such as QC01 which was mainly associated with 1000SW and SeD descriptors, QC02 with PW and QC05 is associated with GY. Furthermore, these genotypes were

related and classified in group VI (QC05 and QC02 genotypes) and group VII (QC01) (Fig. 1 and 3). The association of major groups of variable-traits has been similarly described in Chilean quinoa germplasm (Salar ecotype) through leaf, grain yield and plant/inflorescence characteristics (Fuentes & Bhargava, 2011). Likewise, Franco and Hidalgo (2003) have reported in Bolivian quinoa germplasm that plant structure variables were significantly correlated with variables such as precocity (dates of mid bloom and physiological ripeness), confirming that variation in precocity influences plant shape directly, and that it is possible to combine the range variables of grain traits (e.g. harvest index) with plant phenology and plant architecture independently in one genotype.

The general classification of germplasm mediated by cluster analysis revealed that groups VI and VII had the highest grain yield, with 3,338 and 3,052 kg ha⁻¹ respectively. In contrast, groups IV and V had the lowest yields with 919.7 and 945.6 kg ha⁻¹, respectively. The wide variation observed in yields suggests a great opportunity to develop plant breeding strategies to support quinoa cultivation in rainfed areas in Chile and similar Mediterranean-type climate conditions. Similarly, the clusters analysis revealed that group VI was characterized having tall plants, large panicles and thick stems.

Interestingly, the accession QC09, belonging to group I presented similar plant height to accessions in group VI, however the grain yield of group I represented 50% of yield obtained in group VI, confirming that there is no correlation between plant size and grain yield, as previously reported by Fuentes & Bhargava (2011), using “Salar” genotypes grown under lowland desert conditions. Likewise, group VII (accession QC01) presented the highest values for SeD and 1000SW in the entire collection, however these grain characteristics did not correlate with higher yields due to presenting smaller panicles (PL and PW descriptor) than accessions in group VI.

Table 5. Pearson coefficient of correlation among morphological variables of quinoa accessions evaluated under rainfed conditions at Marchigüe locality*

Trait	PH	StD	PL	PW	SeD	1000SW	GY
PH	1						
StD	0.68	1					
PL	0.68	0.58	1				
PW	0.73	0.82	0.79	1			
SeD	0.13	0.22	0.17	-0.0015	1		
1000SW	-0.13	-0.21	-0.19	-0.1	-0.04	1	
GY	0.35	0.26	0.43	0.32	-0.11	0.1	1

*Values in bold represent significant correlations (P ≤ 0.05). PH, plant height; StD, stem diameter; PL, panicle length; PW, panicle width; SeD, seed diameter; 1000SW, 1000 seed weight; GY, grain yield

Table 6. Descriptive variables for seven groups classified by cluster analysis in quinoa genotypes evaluated under rainfed conditions at Marchigüe locality*

Descriptor	Group													
	I	II	III	IV	V	VI	VII							
PH	134.38	ab	109.89	bc	115.71	ab	112.34	bc	90.02	c	141.29	a	115.47	abc
StD	1.54	ab	1.35	b	1.31	b	1.35	b	1.35	b	1.86	a	1.43	b
PL	28.36	b	33.06	ab	30.93	b	34.24	ab	18.05	b	48.83	a	32.79	ab
PW	17.18	b	16.02	b	15.59	b	16.55	b	12.1	b	34.2	a	15.77	b
SeD	2.00	ab	1.95	ab	1.84	b	2.14	ab	2.07	ab	1.76	b	2.33	a
1000SW	3.20	a	3.45	a	3.72	a	3.44	a	3.62	a	3.52	a	3.73	a
GY	1584.27	b	1904.27	b	1406.44	b	919.7	b	945.6	b	3337.7	a	3052.0	a

*Different letters indicate significant differences (P ≤ 0.05). PH, plant height; StD, stem diameter; PL, panicle length; PW, panicle width; SeD, seed diameter; 1000SW, 1000 seed weight; GY, grain yield

Conclusions

The accessions that presented the highest yields in the entire collection were QC02 and QC05. The frequency distribution of variables allowed a general classification of quinoa accessions, which was represented by plants having low height and medium grain yield. Cluster analysis was an efficient method for germplasm classification based on yield and morphological characteristics. This approach allowed us to classify the entire collection into seven groups, identifying group VI and VII as the most outstanding in relation to productive performance. Additionally, the classification contributed to identify significant correlations between different quinoa descriptors, emphasizing the relationships between PW and StD, and between PW and PL. The use of multivariate approaches also confirmed the relationship among variables into two main groups, containing plant architecture descriptors and grain characteristics, respectively.

The data set presented in this study is the first report of quinoa assessed under rainfed conditions of central Chile, allowing the identification of promising genetic lines to generate future varieties. This research will be a great help in the development of new plant breeding programmes for quinoa in marginal areas having similar Mediterranean-type climate.

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