Early Mass Selection Response of Two Quinoa Landraces under Highlands Conditions in Northern Chile

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Abstract

Quinoa has been cultivated since centuries in the Andean region as a seed crop by indigenous communities. The crop has gained renewed interest because of its highly nutritious grain with high-quality protein rich in essential amino acids, and several bioactive compounds, along with its ability to grow under stress conditions. Despite the importance of the crop, limited research work on breeding aspects has been undertaken, leading to lack of information on the understanding of levels of variability of genotypes for different traits and their interactions. The aim of the present study was to assess and quantify the early response to mass selection in two quinoa landraces in highland conditions. Mass selection experiments were conducted during two successive crop seasons using eleven morphological traits. Correlation, genetic gain (gg) per selection cycle and principal component analysis was carried out. Only plant height (PH) and number of branches (NB) presented changes between selection cycles in both germplasm lines. Grain yield per plant (GYP) was positively correlated with inflorescence length (IL), stem diameter (SD) and plant weight (PW) for both quinoa lines. The results obtained would be useful to facilitate selection of the most relevant variables of quinoa considering its variation and interactions in the highland environment in Chile.

Keywords: mass selection, highland, saponin

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an ancient crop that has been cultivated for the past 7,000-8,000 years in the mountain regions of the Andes in South America (Fuentes *et al.*, 2009). The crop has gained worldwide attention and renewed popularity because of its highly nutritious grain with high-quality protein (particularly rich in essential amino acids) and several bioactive compounds, along with its ability to grow under stress conditions (Fuentes and Bhargava, 2011; Fuentes and Paredes-Gonzalez, 2015; Martínez *et al.,* 2015). Quinoa grain is considered the most important component of the food chain across a broad area of the Andes, including parts of Bolivia, Peru, Ecuador, Colombia, Argentina and Chile (Vázquez-Luna *et al.,* 2019; Angeli *et al.*, 2020). In consideration of all these factors, the year 2013 was declared "The International Year of Quinoa" by the United Nations Food and Agriculture Organization (FAO), in recognition of its role in attaining food and nutritional security, and its potential for eradicating poverty (United Nations, 2011).

In Chile, quinoa is produced on small-scale on approximately 220 ha in the highlands of Atacama Desert, representing 31.2% of the national area

under its cultivation (Fuentes *et al.*, 2017). Given the enormous international demand for quinoa, indigenous communities have transformed their crop management system towards intensive production, including grain transformation for diversification of value-added products. However, the development of these initiatives is still hampered by the heterogeneous quality of its grain, primarily due to the use of a mixture of landraces for cultivation by the farmers who presume that a broader genetic base may reduce the risk of crop to variations in the environment (Fuentes *et al.*, 2005, 2009). Due to the above mentioned production strategy, this practice is characterized by low grain yields (~500 kg ha^{-1}) and its heterogeneity that makes it non-suitable for marketable production (Bazile *et al.*, 2014).

Grain yield and grain size are frequently used as selection criteria for quinoa breeding programs because of their influence on commercial quality (Bhargava *et al.*, 2006; Madrid *et al.*, 2018; Murphy *et al.*, 2018). The main breeding methods used in quinoa include mass selection, individual selection, pedigree method, hybridization and backcrossing (Murphy *et al.*, 2018; Peterson *et al.*, 2015). The intra- and inter-population selection have been quite successful in exploiting the local adaptation of selected genotypes for increasing grain production (Bertero *et al.*, 2004).

In quinoa, different analytical approaches have been employed to analyse the germplasm variability based on different morphological traits. An investigation of genotype × environment (G × E) interaction of ten varieties of quinoa in England demonstrated that variables of grain yield, number of days to anthesis and maturity were strongly dependent on the variety that led to the conclusion that earliness and grain yield were strongly associated at the level of variety, but the pattern of G × E interaction differed among the variables measured (Risi and Galwey, 1991). Another study of stability for quantitative traits in fourteen quinoa lines suggested that selection for height, inflorescence size and developmental stage could be easily performed at an early stage of a breeding program (Jacobsen *et al.*, 1996). Similarly, an investigation on developmental stability under North European conditions has suggested the selection of early, uniformly maturing plant with more branches, low saponin content and high seed yield (Jacobsen, 1998). A comprehensive multi-environment trial, involving multiple quinoa cultivars under irrigation conditions across three continents assessed the grain yield and grain size nature of the genotypes and the G × E interaction effects (Bertero *et al.*, 2004). In this study, no single quinoa genotype group displayed consistently superior grain yield across all the environments, and the genotype and the G × E interaction effects observed for the duration of the crop cycle had major influence on the cultivar performance and on the form of G × E interaction observed for the total above-ground biomass and grain yield. It was concluded that a good average performance and broad adaptation of quinoa could come from the combination of medium–late maturity and high harvest index, and that simultaneous progress for grain yield and grain size can be expected from selection.

In spite of the great importance of quinoa cultivation in the highland areas in Northern Chile, limited research work on breeding aspects has been done, leading to lack of information on the understanding of levels of variability of genotypes for different traits and their interactions (Fuentes *et al.*, 2009). In this context, the only report about Chilean quinoa germplasm evaluated under highland conditions displayed significant differences among variables describing grain yields and morphological traits of two quinoa genotypes, providing the basal germplasm material for this study (Fuentes *et al.*, 2005). The aim of the present study was to assess and quantify the early response to mass selection in two quinoa landraces in highland conditions. The results obtained would be useful to facilitate selection of the most relevant variables of quinoa considering its variation and interactions in the highland environment in Chile.

Materials and methods

Experimental site and plant material

Field experiments were conducted during crop season 2015-16 and 2016-17 on Ancovinto farm (19°23'23.44"S, 68°32'24.37"W, 3681 m above sea level) belonging to indigenous community of Ancovinto (Tarapacá Region), Chile. The zone is characterized by highland desert climate, with temperatures ranging between -17.6 and 24.4°C, an average of 194 days with frost, and precipitation between 119.0 and 159.6 mm per year (Arenas, 2011). The soil type was classified as Aridisol with sandy loam texture and the following soil parameters at 0-40 cm depth: pH (1:2.5) = 8.8; electrical conductivity 0.82 mmhos cm^{-1}; exchangeable sodium 2%; and organic matter 0.7 % (analysed by standard methods at Agroanalisis UC laboratories).

The plant material used comprised two quinoa lines namely, *red* and *yellow*, because of the

inflorescence colour at grain filling stage. During the first cycle (C_1), red and yellow lines consisted in a bulked seed material of 38 and 39 quinoa genotypes respectively, which were selected according to high grain yield per plant from a mixed crop (C_0, traditional smallholder conditions), where no prior selection had taken place (Fuentes *et al.*, 2005). For the second cycle (C_2) was used a new bulked seeds selection, which comprised 26 red and 24 yellow genotypes, selected from 100 quinoa plants according to phenotypic similarity group obtained after multivariate analysis of morphological traits (supplementary material). Both experiments were established during November and assessed morphologically at April when grain reached physiological maturity. Crop management involved soil preparation with disc plough and rotovator. Plants were thinned at three-leaf stage and weed control was managed by hand removal when required. No chemical fertilizers were used during the experiments. Irrigation was done by furrow when soil water reached 50 % of field capacity at 30 cm depth.

The experiments were conducted in a randomized complete block design with five replications. The plot size was 50 m^2 (5m × 10m) with a distance of 1 m between rows and 0.4 m between plants. Twenty plants were randomly selected in each replication to assess 11 morphological descriptors as follows: PH = plant height (cm); IW = inflorescence width (cm); IL = inflorescence length (cm); SD = stem diameter (mm); NB = number of branches (n°); PW = plant weight (g); 100SW = 100 seed weight (g); GD = grain diameter (mm); GYP = grain yield plant^{-1} (g); HI = harvest index (%) and SC = saponin content (mg g^{-1}). The definition of variation among each descriptor was made according to (IBPGR, 1981). Saponin content was measured using Koziol's standardized afrosimetric test (Koziol, 1991).

Data analysis

An analysis of variance, combining quinoa lines and selection cycles was performed by the statistical software INFOSTAT® (Di Rienzo *et al.*, 2016). Means of each trait for two lines and two cycles were used to make mean comparison with Tukey's test at p = 0.05 and 0.01. Genetic gain (gg) per selection cycle for each morphological descriptor was estimated using the following equation (Molina, 1992):

$$gg = 100b_{y/x} / y_0$$

where $b_{y/x}$ is the coefficient of regression of the expression of trait (y) over selection cycles (x), and y_0 is the expression of the trait in cycle C_0.

Multivariate analysis was carried out for each quinoa line to establish phenotypic similarity group using Pearson's coefficient for total correlation among variables (Clifford and Stephenson, 1975), principal component analysis (PCA) (Hair *et al.*, 1998) and cluster analysis.

Results and discussion

In the general ANOVA (Tab. 1) an effect of the cycle was observed for PH, NB, GD, GYP, and

Table 1. General ANOVA of two quinoa lines in two selection cycles. Mean sum of squares (MSS) for eleven morphological traits.

Source of variation	df	PH	IW	IL	SD	NB	PW	100SW	GD	GYP	HI	SC
Model	7	1584.63**	1134.05*	143.83**	9.95	151.93**	20594.77*	0.0027	0.14**	7041.23**	0.05**	0.86*
Cycle	1	1408.51**	5.60	11.36	0.05	113.29**	3151.56	0.0016	0.10**	1888.79**	0.01*	0.19
Line	1	64.94	679.31**	0.26	1.71	9.80	3724.81	0,00000	0.0039	263.83	0.0039	0.27
Block	4	47.94	135.66	11.53	4.06	20.90	2764.39	0.001	0.01	596.74	0.01	0.12
Year x Cycle	1	63.23	313.47*	120.69**	4.13	7.94	10954.01*	0.00008	0.02*	4291.86**	0.02**	0.27
Error	12	276.20	689.33	46.41	12.12	30.93	15860.44	0.01	0.05	1860.03	0.02	0.88
Total	19											
CV		5.23	11.51	6.12	6.76	9.51	21.05	4.48	2.59	20.20	10.60	14.73

Note: * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; CV = Coefficient of variation; PH = Plant height (cm); IW = Inflorescence width (cm); IL = Inflorescence length (cm); SD = Stem diameter (mm); NB = Number of branches (n°); PW = Plant weight (g); 100SW = 100 seed weight (g); GD = Grain diameter (mm); GYP = Grain yield plant^{-1} (g); HI = Harvest index (%); SC = Saponin content (mg g^{-1}).

Table 2. Mean ± SE of eleven morphological traits of two quinoa lines in two selection cycles

Trait	Cycle	Red line	Yellow line		CV (%)
PH	C_1	86.98 ± 2.36	79.82 ± 2.70	ns	6.80
	C_2	100.21 ± 1.40	100.16 ± 1.17	ns	2.89
		**	**		
	CV (%)	4.64	5.17		
IW	C_1	64.52 ± 2.36	68.26 ± 1.93	ns	7.25
	C_2	55.54 ± 2.95	75.12 ± 4.82	*	13.69
		*	ns		
	CV (%)	9.95	11.45		
IL	C_1	35.46 ± 1.16	30.32 ± 0.72	**	6.58
	C_2	29.04 ± 0.85	33.73 ± 0.56	*	5.10
		**	**		
	CV (%)	7.05	4.50		
SD	C_1	14.98 ± 0.81	14.65 ± 0.23	ns	8.94
	C_2	14.16 ± 0.22	15.66 ± 0.24	*	3.49
		ns	*		
	CV (%)	9.06	3.50		
NB	C_1	14.44 ± 0.78	14.58 ± 0.34	ns	9.32
	C_2	17.94 ± 1.29	20.60 ± 0.46	ns	11.19
		*	**		
	CV (%)	14.70	5.14		
PW	C_1	169.90 ± 25.68	150.39 ± 8.22	ns	26.62
	C_2	148.20 ± 8.25	222.30 ± 11.67	**	12.20
		ns	**		
	CV (%)	26.81	12.11		
100SW	C_1	0.51 ± 0.01	0.51 ± 0.01	ns	5.08
	C_2	0.49 ± 0.004	0.49 ± 0.01	ns	2.98
		ns	ns		
	CV (%)	3.60	4.72		
GD	C_1	2.54 ± 0.02	2.45 ± 0.04	ns	2.66
	C_2	2.33 ± 0.01	2.37 ± 0.03	ns	2.00
		**	ns		
	CV (%)	1.34	3.09		
GYP	C_1	62.92 ± 6.87	40.89 ± 6.52	*	28.87
	C_2	53.06 ± 2.12	89.62 ± 5.34	**	12.74
		ns	**		
	CV (%)	19.61	20.43		
HI	C_1	0.38 ± 0.02	0.29 ± 0.03	*	17.67
	C_2	0.37 ± 0.01	0.41 ± 0.01	**	4.20
		ns	**		
	CV (%)	8.54	15.04		
SC	C_1	2.17 ± 0.17	1.71 ± 0.12	ns	17.16
	C_2	1.74 ± 0.07	1.74 ± 0.04	ns	7.06
		*	ns		
	CV (%)	15.01	11.53		

Note: * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; ns = Non-significant; CV = Coefficient of variation;
PH = Plant height (cm); IW = Inflorescence width (cm); IL = Inflorescence length (cm); SD = Stem diameter (mm); NB = Number of branches (n°); PW = Plant weight (g); 100SW = 100 seed weight (g); GD = Grain diameter (mm); GYP = Grain yield plant^{-1} (g); HI = Harvest index (%); SC = Saponin content (mg g^{-1}).

HI variables ($p \leq 0.01$ and 0.05). The IW variable was significantly influenced by the line effect ($p \leq 0.01$), while the other traits did not show significant differences. For IW, IL, PW, GD, GYP and HI variables, an interaction of cycle with line was observed ($p \leq 0.01$ and 0.05). These findings and the high coefficient of variation values observed in GYP (20.20%) and PW (21.05%) indicate the existence of a high degree of variability for these two morphological descriptors influenced by the cycle and the interaction cycle × line, respectively. The selection of the best progenies for next cycles should therefore be based on the average of analysis of variance of cycles.

The variance analysis of eleven morphological descriptors in both quinoa lines and selections cycles are shown in Table 2. Among the variables assessed in this study, only PH and NB presented increased values ($p \leq 0.05$) between selection cycles in both quinoa lines. The variable of 100SW did not show differences ($p \leq 0.05$) between cycles for both quinoa lines, being in addition the only variable that did not register significant changes during the selection process. This shows that mass selection can be safely practiced in quinoa and this would not lead to decrease in grain weight. The remaining variables in this study showed differences ($p \leq 0.05$) indistinctly between cycles in one or both lines, standing out in the red line the decreasing of GD and SC variables, and in the yellow line the increasing of PW, GYP and HI. The comparison between lines in both selection cycles showed significant differences ($p \leq 0.05$) only for IL, GYP and HI variables, which were not consistent with a distinctive morphological pattern between lines.

Genetic gain, a product of heritability and selection differential, has been frequently used as a guiding factor in selection programmes in a number of plants (Badigannavar and Ganapathi, 2018; Kumar and Das, 2018; Mishra *et al.*, 2015) including quinoa (Bhargava *et al.,* 2007, 2008; Madrid *et al.*, 2018). After two selection cycles, the results demonstrated that the red line presented a positive genetic gain ($p \leq 0.05$) for the PH and NB variables and a negative genetic gain for IW, IL, GD and SC (Tab. 3). Similarly, in the yellow line the genetic gain was positive for the PH, IL, NB, PW, GYP and HI variables; and negative only for GD. Furthermore, it was observed that the coefficients of variation for most of variables in C_2 decreased in comparison with C_1 for both quinoa lines, excepting for IW and NB variables in the red line and only in IW in the yellow one, which showed an increase of

Table 3. Genetic gain (gg) and coefficient of variation (CV) for eleven morphological traits of two selection cycles in two quinoa lines.

Descriptor	Red line					Yellow line				
	$b_{y/x}$	r^2	gg (%)	CV C_1	CV C_2	$b_{y/x}$	r^2	gg (%)	CV C_1	CV C_2
Plant height (cm)	13.3**	0.3	15.29**	6.07	3.13	20.46**	0.4	25.63**	7.57	2.62
Inflorescence width (cm)	-9.23**	0.11	-14.31**	8.17	11.89	7.14 ns	0.03	10.46 ns	6.31	14.36
Inflorescence length (cm)	-6.4**	0.27	-18.05**	7.33	6.51	3.57*	0.07	11.77**	5.33	3.68
Stem diameter (mm)	-0.76 ns	0.02	-5.07 ns	12.02	3.48	1.04 ns	0.03	7.10 ns	3.51	3.49
Number of branches (n°)	3.38**	0.12	23.41**	12.12	16.02	6.13**	0.33	42.04**	5.28	4.96
Plant weight (g)	-21.23 ns	0.02	-12.50 ns	33.79	12.45	73.83**	0.19	49.09**	12.23	11.74
100 seed weight (g)	-0.02 ns	0.03	-3.92 ns	4.70	1.81	-0.02 ns	0.03	-3.92 ns	5.42	3.82
Grain diameter (mm)	-0.21**	0.33	-8.27**	1.38	1.30	-0.07*	0.05	-2.86*	3.55	2.50
Grain yield plant^{-1} (g)	-9.79 ns	0.03	-15.56 ns	24.43	8.94	49**	0.39	119.83**	35.67	13.33
Harvest index (%)	-0.01 ns	0.0043	-2.63 ns	10.85	5.06	0.12**	0.21	41.38**	25.21	3.30
Saponin content (mg g^{-1})	-0.43**	0.1	-19.82**	17.89	8.45	0.03 ns	0.00042	1.75 ns	15.55	5.33

Note: $b_{y/x}$ = coefficient of regression; r^2 = coefficient of determination; gg (%) = genetic gain (Molina, 1992); * $p \leq 0.05$; ** $p \leq 0.01$; ns = non-significant

their values. The moderate to high positive genetic gain observed for the abovementioned traits indicate that improvement could be made in the aforesaid characters.

Correlation coefficients show relationships among various traits along with the degree of linear relation between these characters (Bhargava *et al.*, 2008). The correlation analysis in both cycles (Tab. 4) exhibited 14 (25.5%) significant associations ($p \leq 0.05$) in the red line, of which three were negative (PH/GD, R = -0.77; NB/100SW, R = -0.65 and NB/GD, R = -0.68). Contrary to the observed in the red line, the yellow line did not show any significant negative correlations. The yellow line had 21 (38.2%) significant correlations ($p \leq 0.05$) all of them being positive. Grain yield per plant (GYP) had a large number of significant associations, positively correlating with IL, SD and PW for both the germplasm lines. High significant positive correlation values between inflorescence length and seed yield have also been observed in plants like barnyard grass (Norris, 1992), sorghum (Kenga *et al.*, 2006) and perennial ryegrass (Abel *et al.*, 2017). Likewise, significant positive association between seed yield and stem diameter as obtained in the present study has also been observed in other economically important plants like maize (Ali *et al.*, 2017), wheat (Okuyama *et al.,* 2005) and quinoa (Bhargava *et al.*, 2008; Madrid *et al.*, 2018). Thus, IL, SD and PW should be given proper attention in selection programmes for increasing grain yield in quinoa. Another interesting feature of the study was the non-significant association of HI and SC for the red line (Tab. 4).

The principal component analysis (PCA) of the red line accounted 43.8% and 31.4% of the variability accounted for by PC1 and PC2, respectively (75.2% of total variation) (Fig. 1A). Morphological descriptors that contributed most to variability in PC1 were IL and IW (collectively termed the inflorescence components); GYP, SD and PW (collectively termed the above-ground biomass components); and SC and GD (collectively termed the grain quality components); while in PC2, PH and NB (collectively termed the plant architecture components) were the largest contributors to variability. In the yellow line PC1 and PC2 accounted for 57.0% and 19.4% of the variability (76.4% of total variation) (Fig. 1B). Traits that contributed most to variability in PC1 were GYP, PW, NB, PH and HI (collectively termed the above-ground biomass components); and IL, SD and IW (collectively termed the inflorescence components); while in PC2, GD, 100SW and SC (collectively termed the grain quality components) were the largest contributors to the component.

Table 4. Pearson coefficient of correlation among morphological variables of quinoa accessions (yellow line\red line)*

	PH	IW	IL	SD	NB	PW	100SW	GD	GYP	HI	SC
PH	1	-0.36	-0.51	0.05	0.62	0.16	-0.55	**-0.77**	-0.02	-0.39	-0.5
IW	0.39	1	**0.84**	**0.67**	-0.15	**0.66**	0.06	0.61	**0.76**	-0.1	0.37
IL	**0.9**	0.59	1	**0.65**	-0.32	**0.65**	0.01	**0.82**	**0.78**	-0.0023	0.55
SD	**0.72**	0.59	**0.72**	1	0.21	**0.87**	-0.41	0.3	**0.9**	-0.38	0.21
NB	**0.85**	0.51	**0.74**	**0.65**	1	0.22	**-0.65**	**-0.68**	0.16	-0.2	-0.28
PW	**0.77**	0.59	**0.75**	**0.83**	**0.85**	1	-0.37	0.31	**0.95**	-0.56	0.06
100SW	-0.19	0.16	0.16	-0.14	-0.47	-0.33	1	0.47	-0.41	-0.11	0.15
GD	-0.34	0.36	-0.08	0.05	-0.44	-0.37	**0.69**	1	0.42	0.01	0.56
GYP	**0.84**	0.51	**0.81**	**0.76**	**0.86**	**0.97**	-0.27	-0.45	1	-0.3	0.29
HI	**0.78**	0.36	**0.71**	0.56	**0.79**	**0.82**	-0.18	-0.48	**0.92**	1	0.48
SC	0.08	0.24	-0.01	0.22	0.1	-0.13	-0.24	0.37	-0.23	-0.44	1

Note: *Values in bold represent significant correlations ($p \leq 0.05$); PH = Plant height (cm); IW = Inflorescence width (cm); IL = Inflorescence length (cm); SD = Stem diameter (mm); NB = Number of branches (n°); PW = Plant weight (g); 100SW = 100 seed weight (g); GD = Grain diameter (mm); GYP = Grain yield plant^{-1} (g); HI = Harvest index (%); SC = Saponin content (mg g^{-1}).

These findings revealed that both quinoa lines were mainly influenced by above-ground biomass and inflorescence component and secondary by grain quality components.

Saponin content in quinoa grain has been described under qualitative and quantitative genetic control, representing a main breeding goal in the development of saponin-free varieties (Mastebroek *et al.*, 2000; Murphy *et al.*, 2018). Even though the efforts to breed low-saponin varieties quantitatively have not been successful, due to a lack of sufficient response to selection (Ward, 2001), considerable genetic variation for saponin content on-farm is still available for selection purposes (Bhargava and Ohri, 2016; El Hazzam *et al.*, 2020). In our study, the frequency

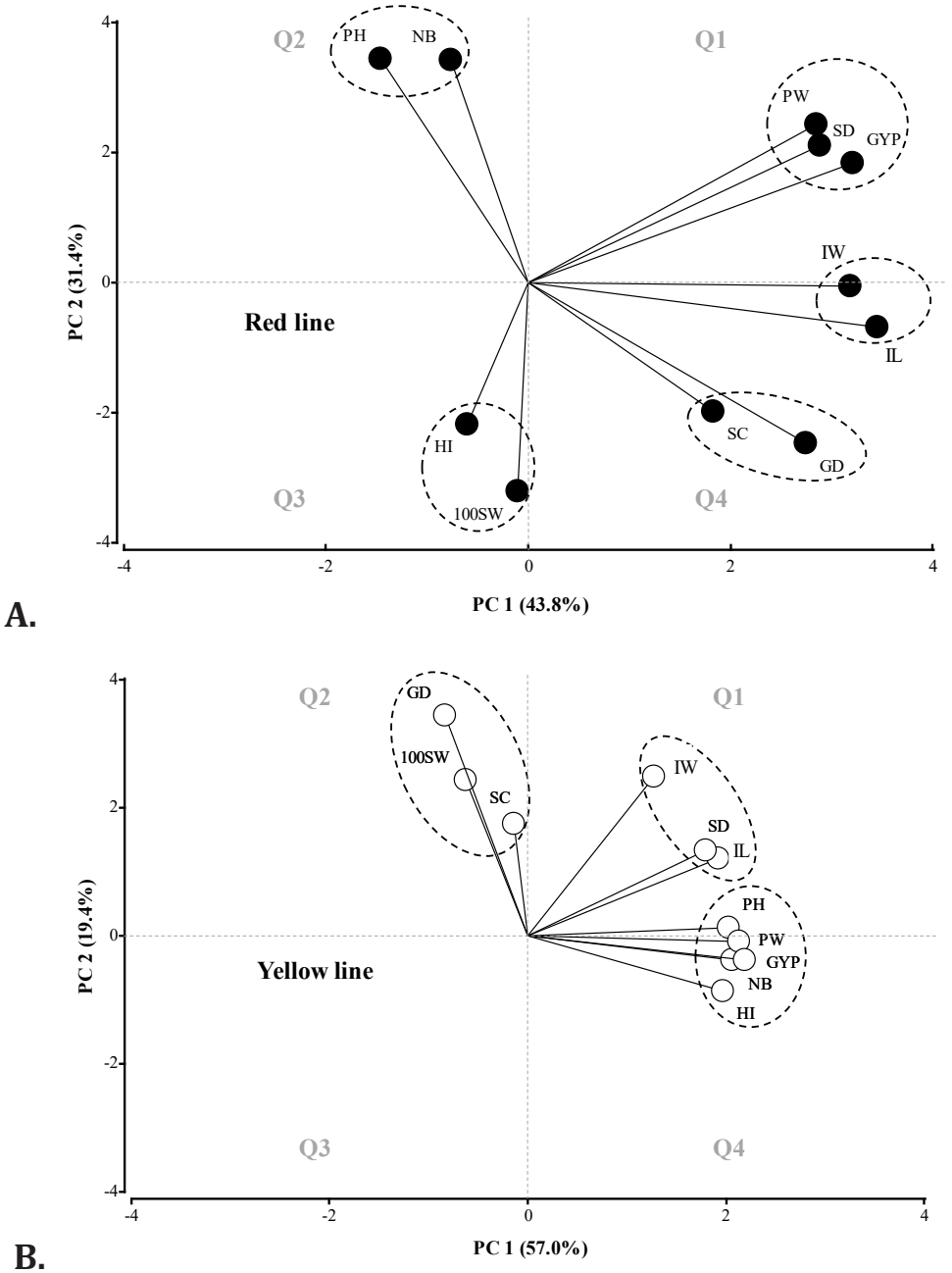

Figure 1. Principal component analysis for red (A) and yellow (B) quinoa lines under highlands conditions in northern Chile

PH = Plant height (cm); IW = Inflorescence width (cm); IL = Inflorescence length (cm); SD = Stem diameter (mm); NB = Number of branches (n°); PW = Plant weight (g); 100SW = 100 seed weight (g); GD = Grain diameter (mm); GYP = Grain yield plant^{-1} (g); HI = Harvest index (%); SC = Saponin content (mg g^{-1}).

distribution of saponin content for both genetic lines in the first cycle showed a wide dispersion (being the red line more bitter than the yellow), exhibiting several sweet genotypes with a low saponin content (Fig. 2). These observations reveal that further improvements could be made in new selection cycles.

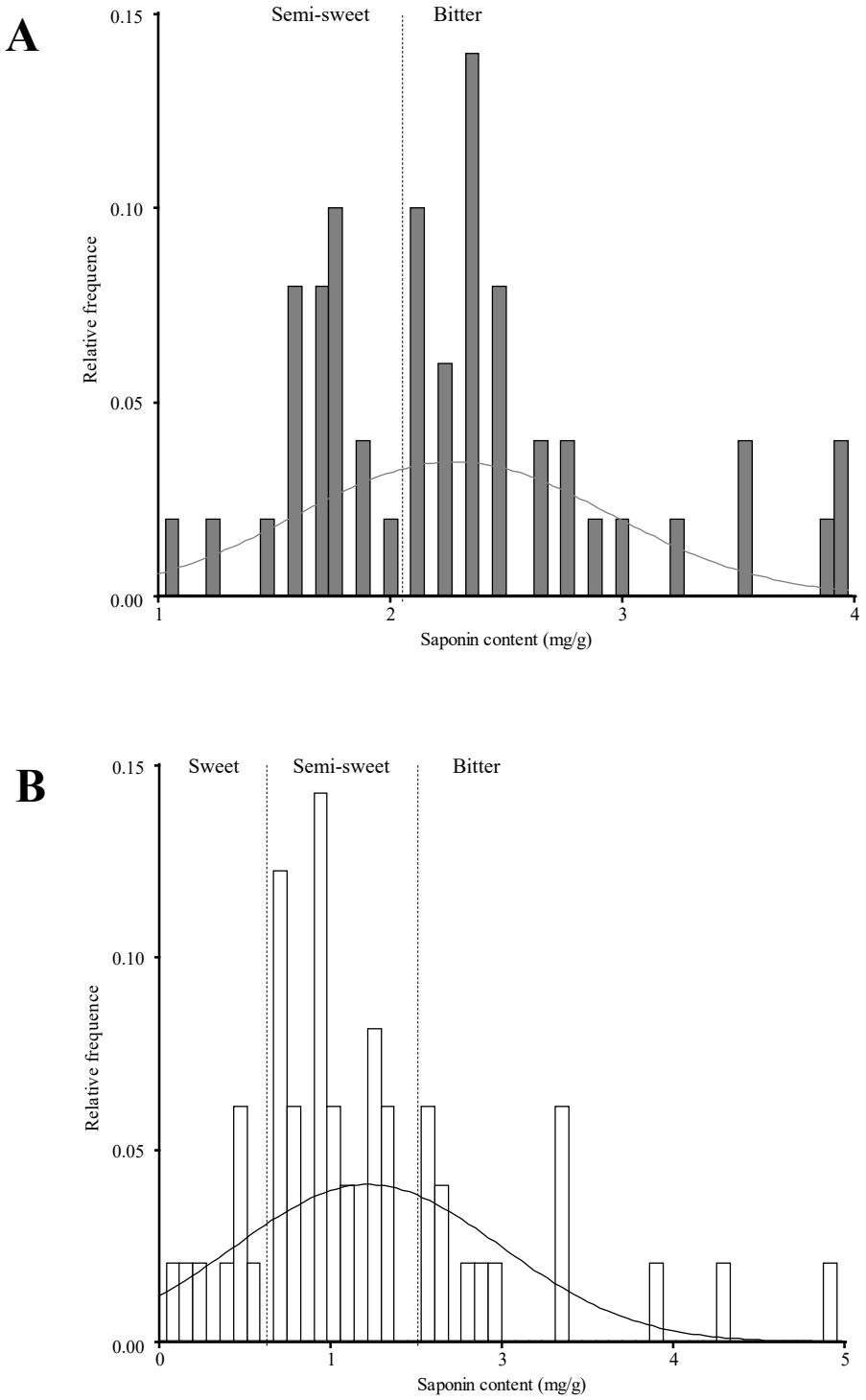

Figure 2. Frequency distribution of saponin content for red (A) and yellow (B) quinoa lines in C_1

Conclusions

The results indicate that a substantial part of the observed phenotypic variance for grain yield is additive genetic variance. Therefore, mass selection for grain yield in quinoa is likely to be effective. More effective improvement would be observed in grain quality component such as saponin content if selection on the improved populations is carried out beyond the two cycles.

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