Agrociencia

GENETIC PARAMETERS AND SELECTION CRITERIA IN QUINOA

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Citation: Valverde-Ramos MG, Benítez-Riquelme I, Miranda-Colín S, Espitia-Rangel E. 2022. Genetic parameters and selection criteria in quinoa. **Agrociencia.** https://doi.org/ 10.47163/agrociencia.v56i7.2501

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: February 24, 2022.
Approved: August 23, 2022.
Published in Agrociencia:
November 16, 2022.

ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) is a crop with high nutritional and agronomic potential, adapted to environmental stress conditions. It is grown on a small scale in Mexico, and currently improved varieties are not available. In order to establish the basis to guide genetic improvement in Mexico, the objective was to determine the genetic variability, heritability and interrelationships of yield and its components in 16 quinoa genotypes, in a randomized complete block design, in six environments in the central high valleys of Mexico. The proportion of participation of the sources of variation genetic, environmental, and genotype × environment interaction was determined; as well as heritability, response to selection, and phenotypic correlations among morphological characters, yield and its components. Out of 15 variables evaluated, the genetic effects on thousand-grains weight and grain filling period were the most important; whereas for plant height at flowering, harvest index, and stem diameter, environmental effects were the most relevant. The genotype × environment interaction is not greater than 20 % for the 15 variables evaluated. The highest values for additive variance, heritability, and response to selection were for grains per square meter, yield, thousand-grains weight, grain filling period, and panicle emergence. The variables stem diameter, plant height at maturity, grains per square meter, yield per day, grain filling period, and panicle emergence were the most correlated with yield. Therefore, they can be used as indirect yield selection criteria in breeding programs to obtain materials with better agronomic characteristics. Based on the genetic parameters obtained and the association of the variables with yield, grains per square meter, yield per day and plant height at maturity are the most appropriate traits for indirect selection for yield.

Keywords: *Chenopodium quinoa*, phenotypic correlations, genotypes, heritability, response to selection.

INTRODUCTION

The increase in world population and climate change are factors influencing the search for new plant species or genotypes capable of producing under conditions of environmental stress (Howell, 2001). Quinoa (*Chenopodium quinoa* Willd.) is an annual, dicotyledonous species of the Amaranthaceae family, adapted to water-restricted environments (Garcia *et al*., 2003; Bazile *et al*., 2016).

The greatest diversity of genotypes and wild progenitors is reported around lake Titicaca in Peru and Bolivia, where it is native (Apaza *et al*., 2013; Manjarres *et al*., 2021). In its center of origin, Quinoa develops in a wide range of environments, from desert areas to regions with high rainfall; its cycle to physiological maturity of the grain is four to six months (Mujica *et al*., 2001; Rojas *et al*., 2014; Curti *et al*., 2016); the colour of plants, inflorescence and seeds, inflorescence type and protein and saponin content are variable (Zurita-Silva *et al*., 2014). This variability reflects the adaptation of the species to different agroecological conditions of soil, precipitation, nutrients, temperature, altitude, drought, salinity and environments rich in ultraviolet radiation (González *et al*., 2015); all of which implies a high genetic plasticity of the crop (Ayala *et al*., 2004, Morillo *et al*., 2022).

Quinoa grain provides humans with all essential aminoacids (FAO, 2011); it is high in protein, fat and dietary fibre (Nowak *et al*., 2016), as well as a high content of unsaturated fats, isoflavones, vitamins and minerals (Jaikishun *et al*., 2019; Sampaio *et al*., 2020); medicinal properties are also attributed to it (Valenzuela *et al*., 2015).

In crop genetic improvement, the estimation of genetic parameters associated with yield is required to define selection schemes (Fuentes *et al*., 2009). Crop yield is often analysed in terms of its yield components, and a high association between these components is an advantage to increase selection gains (Ligarreto and Ospina, 2009; Slafer *et al*., 2014).

In order to define the genetic response to selection, it is necessary to quantify the genetic variability and heritability of the characters of the genetic materials or population to be improved. This variability is recorded in values of dispersion, range, phenotypic and genotypic variances; however, the coefficient of genetic variation is the appropriate parameter for this purpose (Ehdaie and Waines, 1989).

In Mexico, quinoa is currently a small-scale crop for which no improved varieties are available (Espitia *et al*, 2019); thus, a genetic improvement program was initiated through the characterization of 100 collections introduced through the Amaranth Network (SADER) from Ecuador, Peru, Japan and Bolivia. From these, 15 materials were selected which showed the best behaviour and adaptation to Mexican conditions, and one huauzontle (*Chenopodium berlandieri* ssp *nutalliae*), also known as Mexican quinoa. The hypothesis of the study is based on the fact that the cultivars of *Chenopodium quinoa* Willd. evaluated have a wide genetic variation under the conditions studied; likewise, plant characteristics are positively correlated with yield, which allows defining selection criteria. The objective of this study was to determine the genetic variability, heritability and interrelationships of yield and its components in 16 quinoa genotypes.

MATERIALS AND METHODS

Plant material: Sixteen quinoa genotypes from different origins with varying fruit and grain color characteristics were evaluated (Table 1).

Table 1. *Chenopodium quinoa* genotypes used to determine genetic variability in six environments in Mexico.

Num.	Name	Fruit color	Grain color	Origin
1	Huauzontle	Yellow	White	Mexico
2	Tunkahuan	White	White	Ecuador
3	Tokio rosa	White	White	Japan
4	Blanca Ayacucho	White	White	Peru
5	Choclo	White	White	Peru
6	CP-forraje	White	White	Mexico
7	Pata de venado	White	White	Ecuador
8	Amarilla	Yellow	White	Peru
9	Misa amarilla	Yellow	White	Peru
10	Marangani	Yellow	White	Peru
11	Roja ku	Red	White	Peru
12	Rojita	Red	White	Peru
13	Pasankalla lila	Gray	Brown	Peru
14	Negra	Green	Black	Peru
15	Pasankalla reventona	Gray	Brown	Peru
16	Pasankalla plomo	Gray	Brown	Peru

Location of the experimental sites

Genotypes were evaluated at two locations, Santa Lucía de Prías, Texcoco, State of Mexico, located at 19° 26′ 42′′ N, 98° 53′15′′ W, and at an altitude of 2272 m in 2019, and Nanacamilpa, Tlaxcala, in 2018 and 2019, located at 19° 29′22′′ N, 98° 33′46′′ W, and at an altitude of 2825 m. These locations are similar in altitude and climate to the inter-Andean valleys where most of the genotypes in the trial originate. Two experiments were established in each environment. One in which fungicide was applied to the seed and the plant, and another without fungicide which were used to simulate an environment with and without disease (for a total of six environments) because *Peronospora variabilis* is the cause of a disease present throughout the world where is grown and represents the major health problem of the crop. In all three environments the soil texture is sandy loam. Rainfall in the crop cycle was 264 mm in Sta. Lucia with a planting date on June 28, 300 mm in Nanacamilpa 2018 with a planting date on July 25, and 364 mm in Nanacamilpa 2019 with a planting date on June 18.

Experimental design and crop management

In each experiment, the genotypes were distributed in a randomized complete block design with three replicates; the experimental plot consisted of three furrows 5 m long and 0.8 m apart. The crop was grown under rainfed conditions, without chemical fertilization or pest control. In all experiments, two manual weeding were carried out at 25 and 45 days after sowing (dds). In the environments with fungicide, Carboxin® and Captan® were applied before sowing at a dose of 1 g kg^{-1} of seed; in addition, Metalaxyl® was applied 30 d after plant emergence, and Mancozeb® was applied 20 d later at a dose of 1.1 kg ha^{-1} and 1.5 kg ha^{-1}, respectively.

Evaluted variables

The variables days to panicle emergence (EPA), days to maturity (MAD) and grain filling period (PLLG = MAD-EPA, days) were recorded, the first two when 50 % of the plants in each plot presented the inflorescence primordium or physiological maturity of the grain, respectively. At flowering, stem diameter (DIT, cm) was measured with a digital vernier (Stainless Hardened) and plant height (APF, cm) with a 3 m ruler. At grain maturity, plant height at maturity (ALM, cm) and panicle length (LPA, cm) were recorded; all measurements were taken from panicle emergence on 10 representative plants per plot.

At harvest, the number of panicles per square meter (PMC) of the plot was counted, which corresponded to the primary panicles. Biological yield (RB) was obtained by multiplying the fresh weight of the plot × dry weight, which was determined by drying a 500 g fresh weight sample in an oven at 60 °C until a constant weight was reached and extrapolated to one hectare (ha). Grain yield (economic yield, RE) (KGH, kg ha^{-1}) was determined in 3 m of the central furrow, and extrapolated to one ha. The harvest index (IC) was estimated by the ratio of economic yield to biological yield. The yield per day (RPD, g) was calculated by the ratio of grain yield to days to maturity.

Seed diameter (DIS, mm) was obtained in 10 groups of 10 seeds from each plot, measured with a digital vernier (Stainless Hardened). The hectolitre weight (PEH, kg hL^{-1}) was determined by weighing a known volume of seed, which was extrapolated to 100 L weight. The thousand-grains weight (PMG, g) was based on the weight of five groups of 100 grains from each plot and extrapolated to one thousand. The number of grains per square meter (GMC) was estimated from the yield per plot divided by the thousand-grains weight.

Genetic parameter estimation and data analysis

The variances for the estimation of genetic parameters were obtained with the application of the VARCOM procedure of the SAS package, by the REML method (SAS 9.4, 2012). The simple correlation analysis of yield and its components was performed with the CORR procedure of SAS (SAS 9.4, 2012). The coefficient of genetic variation was calculated as the quotient of the genetic standard deviation by the mean. Villaseñor *et al*. (2017) classified coefficients greater than 20 % as high genetic variability, from 12 to 20, intermediate, and less than 10, low. Since quinoa is a self-pollinating plant, the genetic variance of the material evaluated corresponds to that present among homozygous genotypes; therefore, the one calculated is the narrow sense heritability. Which was obtained by dividing the additive genetic variance by the phenotypic variance, and the response to selection by multiplying the standardized selection differential (5 %) by the heritability, and this product by the phenotypic standard deviation.

RESULTS AND DISCUSSION

Origin of the variation

Variation due to environmental factors was the main factor in 10 of the 15 variables evaluated (Figure 1), of which plant height at flowering was the one with the highest percentage (84 %). With values between 51 and 60 % were stem diameter, days to maturity, plant height at maturity, harvest index, and panicle emergence; wthin the range of 30 and 50 % there were panicle length, yield per day, grains per square meter, and grain yield. It is worth mentioning that grain yield and its proximate components were least affected by the environment, because the evaluation of genotypes was very high in these traits, contrary to the findings by Garrido *et al*. (2013), who indicated that for the same traits the most relevant variation was caused by the environment, which may be due to the fact that the environments of this study were less contrasting than the levels of drought tested by those authors.

Variation due to genotypes was of greater relevance than that attributed to the environment in thousand-grains weight and grain filling period (53 and 50 %, respectively), while for seed diameter and hectolitre weight the genotypic variation

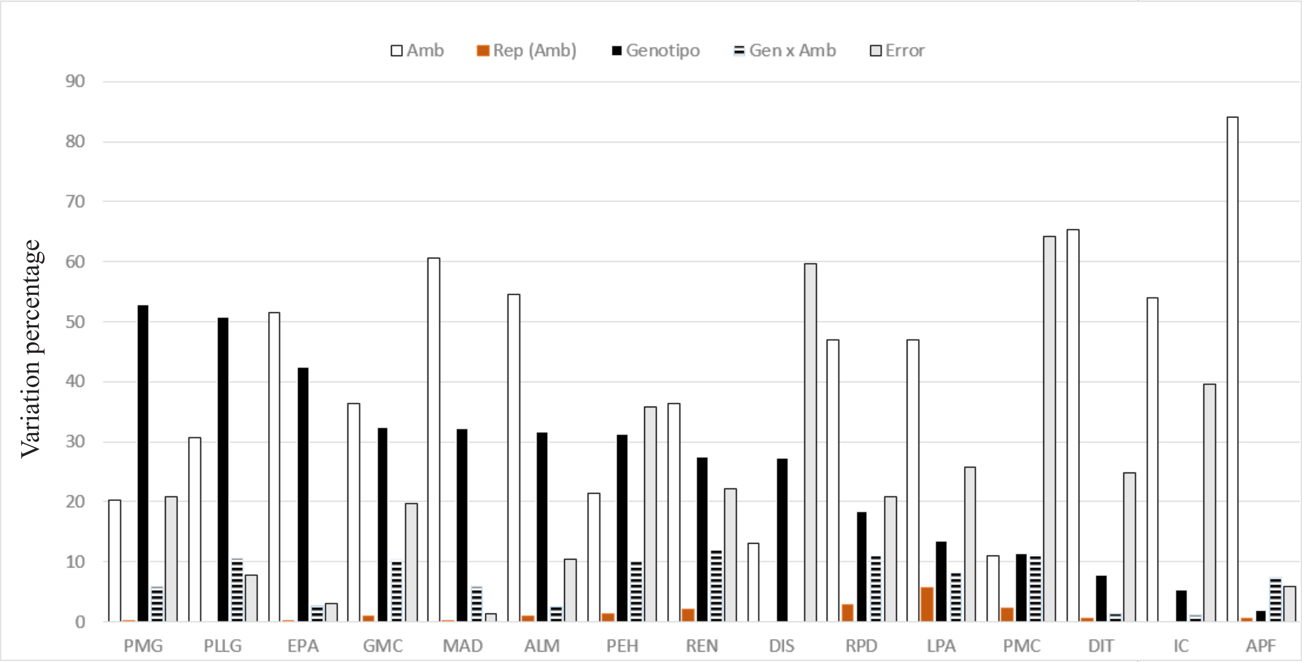

Figure 1. Proportion of variability due to sources of variation in 16 quinoa genotypes evaluated in six rainfed environments in the high valleys of central Mexico. EPA: panicle emergence, MAD: days to maturity, PLLG: grain filling period, LPA: panicle length, DIT: stem diameter, APF: plant height at flowering, ALM: plant height at maturity, DIS: seed diameter, RPD: yield per day, PHE: hectoliter weight, IC: harvest index, PMC: panicle per square meter, PMG: thousand-grains weight, GMC: grains per square meter and REN: grain yield.

was 42 and 32 % in regard to the total. The variation due to genotypes was greater in two of 15 variables in this study, contrary to what was reported by Espitia *et al*. (1992) that in 22 of 25 variables, variation attributed to genotypes was the most important, when evaluating 60 amaranth genotypes in two environments; this can be explained by the difference in the number of genotypes and environments studied in other researches.

The variation due to genotype × environment interaction was lower than that of the two main sources and less than 20 %. The literature, overall indicates the opposite, a greater variation due to genotype × environment interaction than that due to genotypes; especially for yield, harvest index, grains per square meter, and thousand-grains weight in quinoa (Garrido *et al*., 2013). The similarity in the environments, characterized by almost the same precipitation and the inclusion of only two localities of high valleys of central Mexico with little longitude, altitude and latitude variation could be the cause to understand the low variation of the genotype × environment interaction.

The importance of identifying the origin of variability is that the plant breeder knows the proportion of variability that can be capitalized on in genetic improvement. For example, in this study grain yield showed 27.4 % variability due to genotype effect. In contrast, the harvest index showed 5.2 %, thus it will be easier for the breeder to increase the grain yield than the harvest index.

The coefficients of genetic variation (CVG) were high for GMC (31.18) and REN (24.51) (Table 2). In a second group were RPD, EPA, ALM, and PLLG with their respective

Table 2. Genetic parameters of 15 variables in 16 quinoa genotypes evaluated in six rainfed environments in high valleys of central Mexico, P-V 2018-2019.

Variables	σ^2_P	σ^2_G	CVG	h^2	R
Emergence of panicle (d)	126.41	53.7	14.219	0.425	9.852
Days to maturity (d)	258	83	6.718	0.322	10.656
Grain filling period (d)	126.6	64.27	10.994	0.508	11.784
Panicle length (cm)	61.51	8.26	6.945	0.134	2.173
Stem diameter (cm)	8.72	0.68	6.937	0.079	0.479
Plant height at flowering (cm)	1265.7	24.65	5.545	0.019	1.430
Plant height at maturity (cm)	1405	443.48	14.03	0.316	24.409
Seed diameter (mm)	0.058	0.016	6.122	0.273	0.135
Yield per day (kg)	80.24	14.77	19.629	0.184	3.401
Hectolitre weight (kg hL^{-1})	8.16	2.55	2.357	0.313	1.844
Harvest index	0.015	0.001	8.469	0.053	0.013
Panicles per square meter	12.53	1.42	7.63	0.113	0.825
One thousand-grains weight (g)	0.25	0.131	12.884	0.528	0.544
Grains per square meter	2.8E+09	908971332	31.186	0.325	35439.193
Grain yield (kg ha^{-1})	1493901.5	410372.1	24.516	0.275	692.654

σ^2_P: phenotypic variance, σ^2_G: genotypic variance, CVG: coefficient of genetic variation, h^2: heritability in the strict sense, R: response to selection.

values from 10.99 to 19.62; that is, the genetic variability is medium. The other variables presented values lower than 10 %, which indicates that these are the variables with less genetic variability. In these latter variables with low variability and genetic gain, the breeding program should be focused on the introduction of new germplasm that contributes new allelic variants to favour the presence of individuals with desirable agronomic traits (Villaseñor *et al.*, 2017), or targeted crosses between germplasm with large genetic distances to generate new recombinants, and thus favor heterotic effects (Chura *et al.*, 2019).

The CVG are associated with a mean of the trait expression presented in Table 3. Within each CVG group there was variation in the means of the evaluated characters, which makes it possible to choose not only characters with high CVG, but also genotypes with better average for use as base germplasm, and to initiate with them a genetic improvement program. The averages are very similar to those of quinoa in its place of origin, since yields of 1500 to 3500 kg ha^{-1} are reported in the Highland and Inter-Andean Valleys (Mujica *et al.*, 2022a). Therefore, it is feasible to initiate a breeding program with the genotypes evaluated in this study.

The highest heritabilities were found in PMG, PLLG and EPA (greater than 0.40) (Table 2), suggesting that these traits can be easily improved, since their variation is mainly due to genetic effects. In contrast, APF, IC, DIT, PMC, LPA, and RPD had low heritabilities (less than 0.25); these traits will be difficult to improve as they have considerable environmental influence. In an intermediate group are GMC, MAD, ALM, PHE, REN, and DIS, with heritability values from 0.27 to 0.32. Overall, the values found in this study differ to what was reported by Benlhabib *et al.* (2016), who estimated high heritability values for ALM, LPA, DIS, MAD, and REN, ranging from

Table 3. Means (M), standard deviation (DS), and maximum and minimum values of 15 variables in 16 quinoa genotypes evaluated in six rainfed environments of the high valleys of central Mexico, P-V 2018-2019.

Variables	Mean	DS	Minimum	Maximum
Emergence of panicle (d)	51	10.61	34	85
Days to maturity (d)	135	15.08	101	168
Grain filling period (d)	73	11	48	97
Panicle length (cm)	41.3	7.49	26.4	57.7
Stem diameter (cm)	11.9	2.78	6.3	19.6
Plant height at flowering (cm)	89.5	33	31	159
Plant height at maturity (cm)	150	35.39	78	240
Seed diameter (mm)	2	0.24	2	4
Yield per day (kg)	19.58	8.55	2.4	53.9
Hectolitre weight (kg hL^{-1})	67	2.78	59	75
Harvest index	0.33	0.12	0.05	0.77
Panicles per square meter	15.6	3.49	7.5	30
One thousand-grains weight (g)	2.81	0.48	1.59	4.38
Grains per square meter	96676	50749	8833	301877
Grain yield (kg ha^{-1})	2613	1174	325	7979

0.42 to 0.89, probably because these authors evaluated 72 recombinant lines in a single environment; therefore, their values over represented genetic effects in relation to environmental effects. This is an evident result of the differences due to the germplasm studied and, above all, to the evaluation environments.

In contrast, De Santis *et al*. (2016) found heritabilities for plant height at maturity, thousand-grains weight, harvest index, and grain yield ranging from 0.33 to 0.86 when studying 25 genotypes in two environments. This is an evident result of the differences due to the germplasm studied and, above all, to the evaluation environments. The germplasm response is differential in the particular environmental conditions in which it is evaluated. Results in this study agree with the heritability values for MAD, ALM, REN, and LPA reported by Mujica *et al*. (2022b), but differ for ALF, DIT, IC, and PMG. All of which indicates that the estimated values will largely depend on the genotypes or base populations used.

Regarding the response to selection, based on a selection pressure of 5 %, grains per square meter and grain yield, the gains would be greater than 25 % in regard to the average (Table 2). Within average values of response to selection there were yield per day, plant height at maturity, grain filling period, thousand-grains weight, and days to panicle emergence, since their response to selection corresponded to 15-20 % compared to the mean of each variable. The rest of the variables presented a low response to selection. The responses to selection found in this study are lower than those reported by De Santis *et al*. (2016). The greater the response to selection, the easier it is to improve that trait, while for low response it is necessary to introduce germplasm with greater variability for the trait of interest.

Correlations between variables

REN presented the highest correlations (r = 0.65** to 0.91**) with DIT, ALM, GMC, and RPD, and medium correlations with ALF and LPA (r =0.44** and 0.49**, respectively) (Table 4), which is partly in agreement with those reported by Bhargava *et al.* (2006), who found correlations with yield of r = 0.60** for panicle length, r = 0.50** for plant height at maturity and r = 0.49** for thousand-grains weight. Significant, although low, correlations of REN with PLLG and IC were also found (r = 0.12** and = 0.23** respectively); some authors reported high correlations of harvest index with grain yield (r = 0.78 **) (De Santis *et al*., 2016; Murphy *et al*., 2019). In this study, no association of EPA, MAD, PMC, and PMC with REN was found, which is in agreement with the results by Madrid *et al*. (2018), who reported no association of grain yield with thousand-grains weight, and De Santis *et al*. (2016) and Benlhabib *et al*. (2016) for yield to days to maturity. As for GMC, it presented the highest correlations with REN (r = 0.91 **); in addition, it showed high association with RPD (r = 0.82**), and ALM (r = 0.70**).

Another important component of performance is PMG, which in this study presented no association with REN, showed a negative mean association with MAD, PHE, APF, EPA, and LPA with r values between -0.32** and -0.44**, and it also showed a positive

Table 4. Pearson's correlations of 15 variables in 16 quinua genotypes evaluated in six rainfed environments in high valleys of central Mexico, P-V 2018-2019.

Variables	M1AD	PLLG	LPA	DIT	ALF	ALM	DIS	RPD	PEH	IC	PMC	PMG	GMC	REN
EPA	0.69	-0.07 ns	-0.37	-0.34	>0.00 ns	-0.07 ns	-0.21	-0.28	0.43	0.17	0.07	-0.37	0.056 ns	-0.11 ns
MAD		0.65	-0.063 ns	-0.17	0.31	0.13	-0.32	-0.24	0.44	-0.13	0.20	-0.44	0.17	0.03 ns
PLLG			0.29	0.10 ns	0.43	0.21	-0.26	-0.07 ns	0.22	-0.33	0.22	-0.24	0.15	0.12
LPA				0.57	0.47	0.62	-0.23	0.47	-0.06 ns	-0.38	-0.11 ns	-0.32	0.52	0.49
DIT					0.55	0.76	-0.021 ns	0.67	-0.11 ns	-0.56	-0.27	-0.04 ns	0.59	0.65
ALF						0.63	-0.28	0.32	0.24	-0.63	-0.07 ns	-0.38	0.53	0.44
ALM							-0.13	0.66	0.01 ns	-0.57	-0.18	-0.23	0.70	0.73
DIS								0.02 ns	-0.37	0.11 ns	-0.12	0.53	-0.28	-0.08 ns
RPD									-0.182	-0.19	-0.11 ns	0.05 ns	0.82	0.95
PEH										-0.04 ns	-0.10 ns	-0.44	0.09 ns	-0.08 ns
IC											0.14	0.23	-0.30	0.23
PMC												-0.02 ns	-0.03 ns	-0.06 ns
PMG													-0.45	-0.09 ns
GMC														0.91

EPA: panicle emergence, MAD: days to maturity, PLLG: grain filling period, LPA: panicle length, DIT: stem diameter, ALF: plant height at flowering, ALM: plant height at maturity, DIS: seed diameter, RPD: yield per day, PHE: hectolitre weight, IC: harvest index, PMC: panicle per square meter, PMG: thousand-grains weight, GMC: grains per square meter, and REN: grain yield; ns: non-significant values.

mean association with DIS (r = 0.53**). This differs from the results reported by Madrid *et al.* (2018), who found no association of thousand-grains weight and seed diameter with none of the variables of Chilean quinoa. Upon this, it follows that higher values of GMC, RPD, ALM, LPA, and ALF would be expressed as higher yield in quinoa genotypes.

Selection criteria

Genetic gains and selection methods depend on genetic variability, heritability and the association of the trait of interest with other variables. In the genotypes of this study, in order to increase quinua yield, it shall be necessary to select plants with greater panicle length, stem diameter, plant height at flowering, plant height at maturity, yield per day, and grains per square meter. However, the low coefficients of genetic variation for panicle length, stem diameter, and plant height at flowering suggest the need to introduce germplasm with these traits. Plant height at maturity and grains per square meter showed variability, heritability and correlation appropriate for the indirect selection of germplasm for yield. On the other hand, it is necessary to improve seed size, since it is fundamental in the commercialization and form of consumption of quinua, since those that produce large grains are preferred for direct consumption and those with small grains for flour (Bonifacio *et al.*, 2015). If the objective is to increase seed size, it shall be necessary to select plants with higher thousand-grains weight, panicle weight per square meter, hectolitre weight, plant height at maturity, plant height at flowering, panicle length, grain filling period, days to maturity and panicle emergence.

Grain yield showed high variability in the quinua populations in this study, but had low heritability, thus a moderate response to selection would be expected. To improve the grain yield of these populations, it would be advisable to introduce more germplasm to improve not only yield but also other characteristics such as seed diameter, thousand-grains weight, maturity, and plant height.

CONCLUSIONS

It was found in this study that some quinoa variables are more affected by the environment, such as plant height at flowering and stem diameter, while other variables were more affected by the genotype, such as thousand-grains weight and grain filling period. Regarding the genetic parameters, it was found that grains per square meter, grain yield, and grain yield per day showed the greatest genetic variability.

The variables with the highest heritability were thousand-grains weight, grain filling period, and panicle emergence, while the best response to selection was for the variables grains per square meter, grain yield, thousand-grains weight, and panicle emergence. The greatest association of variables with grain yield was found with stem diameter, plant height at maturity, grains per square meter, yield per day, grain filling period, and panicle emergence.

Based on genetic parameters and association with yield, grains per square meter, yield per day, and plant height at maturity are the most appropriate traits for indirect selection for yield in *Chenopodium quinoa*.

ACKNOWLEDGEMENTS

To the Consejo Nacional de Ciencia y Tecnología (CONACYT) for funding the master’s degree on science studies for MG Valverde-Ramos. To the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) for its support to implement this research.

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