# ARTICLE



# Hybridization between progenies and agronomic characterization of the F2 generation in quinoa

# Wilson Anchico-Jojoa<sup>1,2\*</sup>, José Ricardo Peixoto<sup>2</sup> and Carlos Roberto Spehar<sup>2</sup>

**Abstract:** Quinoa is a self-pollinating species with very small flowers, which hinders efficiency in crossbreeding. Therefore, developing an efficient crossbreeding method should be considered an important step for breeding programs. In this regard, the objective of this study was to obtain hybrids, estimate the rate of natural hybridization, and estimate genetic parameters and agronomic characteristics of the  $F_2$  generation. Crossbreeding assays were set up in three environments with four parents, using two methodologies, pot planting and field planting. Morphological markers were used to identify the hybrids, which were self-pollinated to obtain the  $F_2$  generation. The results indicated that cross-pollination ranged from 3.81% to 19.88% depending on the planting methodology. The presence of pigmentation was dominant compared to its absence. The most prominent hybrid was obtained from the cross P88 x BX4, showing grain yield of 3000 kg ha<sup>-1</sup>. Broad-sense heritability was identified with values exceeding 81.27% for all variables.

**Keywords:** Chenopodium quinoa, genetic parameters, quinoa genetic improvement

# INTRODUCTION

Quinoa (*Chenopodium quinoa*) is an allotetraploid plant (2n = 4x = 36) with diploid segregation. Native to South America, it has great potential for innovation and development of food products due to its protein content and nutritional benefits (Ayub et al. 2021). It has a protein content between 14 and 18.7%, with a wide range of amino acids, rich in arginine, lysine, and unsaturated fatty acids (Rodríguez Gómez et al. 2021, Anchico-Jojoa et al. 2023). Quinoa can adapt to different agroecological conditions thanks to its high phenotypic variability, which can be recognized by plant pigmentation, inflorescences, seeds, early, intermediate and late cycles, grain size, panicle type, and resistance to abiotic factors such as drought, frost, high humidity, and salinity (Anchico et al. 2020, Manjarres-Hernández et al. 2021). Additionally, quinoa is a low-demand product and a great alternative for marginal environments (Fuentes and Bhargava 2011). It can develop at different altitudes ranging from 0 to 4000 m (González et al. 2011).

The genetic variability of quinoa has allowed producers to sow it in different agroecological regions. Desirable characteristics in genetic improvement include shorter plant cycles, larger seed size, and higher yields (Fuentes and Crop Breeding and Applied Biotechnology 23(4): e46292345, 2023 Brazilian Society of Plant Breeding. Printed in Brazil http://dx.doi.org/10.1590/1984-70332023v23n4a40



\*Corresponding author: E-mail: anchico25@gmail.com DRCID: 0000-0002-1993-1644

> Received: 20 May 2023 Accepted: 18 August 2023 Published: 10 October 2023

 <sup>1</sup> Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de Investigación Turipaná, Vía Montería - Cereté km 13, 230558, Cereté, Córdoba, Colombia.
<sup>2</sup> Universidade de Brasília, Campus Universitário Darcy Ribeiro, s/n, Asa Norte, 70910-900, Brasília, DF, Brazil

Paredes-González et al. 2013). Breeding programs have relied on mass selection, progeny selection, obtaining strains by hybridization, and backcrossing (Murphy et al. 2018). Crossing techniques and population studies have been used to determine mechanisms of plant color inheritance, a predominant morphological characteristic for use in genetic improvement programs, as well as axillary stem pigmentation, type of inflorescence, saponin content, grain color, earliness, plant height, and male sterility (Murphy et al. 2018).

Quinoa is a gynomonoecious species, containing hermaphrodite and female flowers on the same plant (Risi 1984). Hermaphrodite flowers contain a single pistil usually surrounded by five anthers, although the number of anthers may vary considerably. Female flowers are smaller and do not have anthers (Peterson et al. 2015). Quinoa crossing processes can be done in the field and in the greenhouse, although it is very important to have favorable environmental conditions for plant growth and development (Peterson et al. 2015). Due to the small size of the flowers, manual emasculation has been shown to be very difficult for artificial hybridizations (Jacobsen and Stølen 1993). However, natural crossing can be induced even though quinoa is predominantly autogamous, with variable rates of natural hybridization (Murphy et al. 2016). This hybridization depends on the coincidence in flowering, distance between plantations, wind, and presence of pollinators (Silvestri and Gil 2000).

Different strategies have been proposed for hybridizations and identification of  $F_1$  hybrids using molecular and qualitative morphological markers that are easily detectable and dominant in inheritance, such as stem color, inflorescence color, saponin content, seed color, hypocotyl color, and axil pigmentation (Peterson et al. 2015, Emrani et al. 2020). In quinoa, hybridization has been used in genetic improvement with the aim of gathering desired traits from different recombinant hybrid accessions (Gomez-Pando 2015). This work aimed to estimate the rate of natural hybridization, inheritance of morphological traits, genetic parameters, and agronomic characteristics of the  $F_2$  generation, for future genetic improvement programs of quinoa.

# **MATERIAL AND METHODS**

The experiments were conducted in two locations in Brazil and one in Colombia, according to the descriptions below.

# **Experimental site in Brazil**

The experiment was conducted at the Experimental Biological Station of the University of Brasília and at the Água Limpa Farm of the Faculty of Agronomy and Veterinary Medicine, located at lat 15° 56' S, long 47° 55' W, alt 1100 m asl. The region has an AW-type climate, according to Köppen's classification, with a rainy summer from October to April and a dry winter from May to September (Kottek et al. 2006). During the experiments, the average temperature was 22.0 °C, and the precipitation was 451.7 mm.

# **Experimental site in Colombia**

The experiment was carried out at the Prosperidad Experimental Farm, Municipality of Popayán, located at lat 2° 27' N, long 76° 37' 18" W, alt 1850 m asl (Popayán 2023). During the experiments, the average temperature was 19.0 °C, and the precipitation was 600 mm.

# Selection of progenitors

The selection of progenitors (progenies) was carried out taking into account agronomic characteristics of interest such as earliness, grain weight, plant size, grain yield, grain color, and harvest index, using a morphological characteristic of monogenic inheritance defined for hybrid identification and differentiation (Peterson et al. 2015). Thus, progenitors (progenies) with a dominant characteristic of pigment presence in leaves, inflorescences, and axils and progenies with a recessive characteristic of pigment absence (Emrani et al. 2020) were used. Additionally, the progenitors were selected from pure lines evaluated in experiments in Colombia and in the Brazilian Cerrado (Anchico et al. 2020).

# **Characteristics of progenitors (progenies)**

BRS Syetetuba - a cultivar developed in Brazil. It has a cycle of 120 days, an average plant height of 180 cm, grains with a white pericarp covered by the perigonium, which opens during maturation. The seeds have high quality and

tolerance to high-stress environments (Alencar et al. 2021), an average weight of 2.5 g and 3.3 g (weight of 1000 grains) in summer and winter crops, respectively, containing up to 18 g 100<sup>-1</sup> g of protein, grain yield of 2.3 t ha<sup>-1</sup>, and dry matter of 7.5 t ha<sup>-1</sup>. It has no pigmentation (Spehar et al. 2011). The progenies used from this cultivar were: BX4 and BX8.

Aurora - a cultivar obtained by the Faculty of Agricultural Sciences of the Universidad de Nariño - Colombia, adapted to altitudes between 2300 and 3000 m. It has earliness with an approximate cycle of 85 to 140 days. It has low height, from 90 cm to 130 cm, and yield from 1.8 to 2.4 t ha<sup>-1</sup>. The grains are uncovered at maturity, showing less tolerance to threshing. The seeds are white and small, with a diameter smaller than 2 mm (Sañudo 2005), and the plants are pigmented (purple color). The A88 progeny was selected from Aurora.

Piartal - a cultivar that originated in the province of Carchi, northern Ecuador. The plant is equally pigmented (purple), can reach up to 220 cm in height, and is susceptible to mildew. The grain is opaque white, with approximately 2 mm in diameter. It has an average yield of 2.3 t ha<sup>-1</sup> (Anchico et al. 2020). The progeny selected from Piartal was P88.

# **Cultivation of parents**

Progenies with morphological characteristics of the cultivars BRS Syetetuba from Brazil, Aurora from Colombia, and Piartal from Ecuador were grown in the experimental field of the University of Brasília (UnB). Progenies with presence of pigmentation in the inflorescence and axils (dominant) were originated from Aurora (A88) and Piartal (P88), male parents. Progenies with absence of pigmentation (recessive) (Emrani et al. 2020), were originated from BRS Syetetuba (BX4 and BX8) and were used as female parents.

As mentioned before, quinoa is a self-pollinating species with very small flowers, which hinders the effectiveness of crosses. Therefore, developing an efficient crossing method should serve as the first step in quinoa breeding programs (Emrani et al. 2020). In that regard, the following two crossing methods are described, which were developed in the present study:

# Sowing in pots (Methodology 1)

In February 2019, 5 pots of 2 L were arranged, and half of them were seeded weekly to increase the chances of coinciding in flowering. Progenies of Aurora (A88) and Piartal (P88) pigmented plants surrounded BRS Syetetuba progenies (BX4 and BX8). The pots of male parents (pigmented) were placed on the ends, and the female parent was placed in the center as the receptor (non-pigmented). The plants were kept under protection during their vegetative period until the beginning of panicle formation and flowering. Later, they were transferred to an open, ventilated environment, allowing the visit of pollinating insects. The procedure was repeated 3 times to ensure successful crosses.

# Sowing in the field (Methodology 2)

Four experiments were set up in the field to determine the percentage of natural crossing and obtain the hybrids. In July 2019, two experiments were set up at Água Limpa Farm, University of Brasília (UnB), Brazil, and On August 1, 2020, two experiments were set up at Prosperidad Experimental Farm, Popayán – Colombia. Pigmented and nonpigmented parents were identified for these crosses as a contrasting characteristic. The green color was shown by the BX4 progeny as the receptor and recessive. The A88 and P88 progenies of the Aurora and Piartal cultivars, with dominant pigmentation, acted as donors. In both locations, 10 rows of genotypes were alternately sown. Each row was 5 m long and had a spacing of 0.5 m, with 25 plants per square meter.

# Harvest of F<sub>1</sub> seeds

In both methodologies, seeds of the receptor progenies (recessive) were harvested. For the  $F_1$  seed harvest in methodology 1, all panicles of the receptor plants (non-pigmented); BX4 and BX8, which originate from the BRS Syetetuba cultivar, were selected. For the  $F_1$  seed harvest in methodology 2, from the 5 sown rows of the BX4 genotype (receptor), 10 plants were randomly selected from each row, and 100 seeds from each plant were subsequently threshed and counted for sowing.

Sowing of F<sub>1</sub> generation seeds

In methodology 1, in January 2020, all seeds obtained from the receptor plants were planted in the field at Água Limpa Farm, UnB. Thirty days before sowing, the soil was prepared by adding limestone and organic fertilizer, applied in the planting furrow in the amount of 2 tons per ha<sup>-1</sup>, calculated according to the chemical analysis of the soil and cultivation recommendations. They were sown in 5 m long furrows with a spacing of 0.5 m.

Sowing methodology 2: in January 2020, 50 furrows were sown for each receptor plant, using the same soil management as in the previous methodology. The furrows were 5 m long, spaced 0.5 m apart, with a density of 20 seeds m<sup>-1</sup>.

#### Plant selection for methodologies 1 and 2

The percentage of open pollination was calculated using the methodology described by Silvestri and Gil (2000). All plants showing pigmentation in the inflorescences, leaves, axils, and stems were harvested, and the proportion was determined using equation 1:

cnd = <u>Pigmented plants (Rr)</u> × 100 (Pigmented plants (Rr)+ Non-pigmented plants (rr)

At the beginning of anthesis, the panicles were covered with a paper bag to avoid natural cross-pollination. Subsequently, the seeds were collected and stored in a cold chamber at 5 °C.

#### Sowing of the F, generation

Sowing was carried out in July 2020 at the Prosperidad Experimental Farm, in the municipality of Popayán, Colombia. The experiments were conducted in a randomized block design, with four replications and eight treatments (genotypes): four progenies (A88, P88, BX8, BX4) and the hybrids obtained from the crosses A88 x BX8, A88 x BX4, P88 x BX8, and P88 x BX4. Each plot consisted of 3 rows, 2.5 m long, spaced 0.5 m apart. Each row was sown with 30 plants m<sup>-1</sup>, and an area of 1.5 m<sup>2</sup> was used for evaluation. The soil was prepared 30 days before sowing, with the application of organic fertilizer and lime. Planting and top-dressing fertilizations were carried out according to the soil chemical analysis and following the recommendations of Spehar (2007), using 60 kg ha<sup>-1</sup> of N, 60 kg ha<sup>-1</sup> of P, and 60 kg ha<sup>-1</sup> of K.

#### Agronomic evaluations of F<sub>2</sub> generation

Characteristics such as plant height, panicle length, dry matter yield, harvest index, weight of 1000 grains, and yield were evaluated.

#### **Statistical analyses**

Genes software (Cruz 2013) was used for statistical analysis. Analysis of variance was performed for all traits, with an F-test ( $p \le 0.05$ ), and means were grouped by Scott-Knott test. Genetic parameters were calculated. Additionally, frequencies (phenotypic and genotypic) were calculated for plants with and without pigmentation, in a phenotypic ratio of 3:1. The Chi-square test ( $X^2$ ) was used to check if the observed frequencies fitted the expected frequencies (Ramalho et al. 2012).

# **RESULTS AND DISCUSSION**

Considering that the parent plants used in the experiment develop well under short-day conditions (Anchico et al. 2020) and that they were alternately sown in each of the methodologies, synchrony in the flowering of the parents with and without pigmentation was achieved. The average daily temperature was between 19 °C and 22 °C in all environments, which does not affect the flowering stages of quinoa (Lesjak and Calderini 2017). This condition favored natural hybridization. The progenies of Aurora, Piartal, and Syetetuba produced seeds with good quality, as measured by the high percentage of germination, as reported by Jojoa et al. (2021). Additionally, they showed a similar cycle (number of days to flowering and maturity), allowing synchrony in plant stages, including flowering, as reported in studies on calculation of thermal accumulation in these genotypes (Anchico-Jojoa et al. 2021). Samples of  $F_1$  generation seeds obtained from methodology 1 were sown to evaluate the percentages of natural crossing in a total of 2000  $F_1$  seeds per hybrid. From these samples, 1705 were evaluated, representing 85.27% of germination. Therefore, the average natural crossing (NC) rate was 12.33%, with the hybrid P88 x BX8 standing out with 19.88%. However, in the A88 x BX8 cross,

Hybridization between progenies and agronomic characterization of the F2 generation in quinoa

Methodology 1 (Sowing in pots). Brasília, DF-Brazil									
Date of sowing	parents	Seeds sown	Evaluated seedlings	Non-pigmented seedlings	Pigmented seedlings	% of NC <sup>1</sup>			
08/15/2019	P88 x BX8	2000	1670	1338	332	19.88			
08/27/2019	P88 x BX4	2000	1601	1401	200	12.49			
08/15/2019	A88 x BX8	2000	1801	1743	58	3.22			
08/27/2019	A88 x BX4	2000	1750	1510	240	13.71			
Overall Mean		2000	1706	1498	208	12.33			
	Met	hodology 2 (Sow	ing in the field). Brasília	a, DF-Brazil/Popayán-Colomb	bia				
UNB (Brazil)									
08/23/2019	P88 x BX4	5000	4055	3705	350	8.63			
05/20/2019	A88 x BX4	5000	3990	3838	152	3.81			
		Prosp	eridad Experimental Fa	arm (Colombia)					
07/17/2020	P88 x BX4	5000	4253	4059	194	4.56			
03/01/2020	A88 x BX4	5000	4150	3861	289	6.96			
Overall Mean		5000	4112	3866	246	5.99			

Table 1. Natural crossing values. Methodology 1 and methodology 2

<sup>1</sup> NC: Natural crossing

the rate was only 3.22% (Table 1). Studies have shown that natural crossing in quinoa depends on many factors, such as wind, sowing distance, and the presence of insects such as thrips, green aphids, *Diptera* spp., and bees (*Apis* sp.) (Murphy et al. 2018).

Of the  $F_1$  seeds obtained from methodology 2, 5000 were used from selected receptor plants (without pigmentation). Out of these seeds, 4112 plants were evaluated, representing 82.24% of germination (Table 1). It was found that 246.25 plants (5.99% of the total) showed pigmentation in the axils, hypocotyl, and leaves, derived from dominant monogenic inheritance, with an efficiency rate of 70.86% for real identification of  $F_1$  in the axil color characteristic and 88.89% in hypocotyl color (Emrani et al. 2020). A higher percentage of natural crossing (NC) was observed in the experimental trial in Brazil, in the P88 x BX4 hybrid, with 8.63% NC. On the other hand, in the experiment carried out in 2020 at the Prosperidad Experimental Farm, Colombia, the average NC percentage on both sowing dates was 5.76% (Table 1). The average percentage of natural crossing that occurred with both sowing methodologies was 9.16%, located between 0.5 and 17% (Silvestri and Gil 2000, Murphy et al. 2016, Emrani et al. 2020). The hybrid P88 x BX8 showed a hybridization percentage higher than 17%, demonstrating that the efficiency of hybridization can vary according to crossing arrangements and synchronization in flowering (Emrani et al. 2020). The methods used to evaluate natural crossing in this experiment proved to be efficient in natural hybridization. Artificial hybridization methods can also be employed, such as manual emasculation or immersion in hot water (Peterson et al. 2015).

Selection was continued in the  $F_2$  generation by identifying segregation that represents possible recombinants (Silvestri 2000, Peterson et al. 2015). In addition to segregation by color, variations in saponin content, grain size, inflorescence type, and seed quality were identified (Murphy et al. 2018). Considering that plant color is controlled by a pair of alleles with dominance for pigment presence, the expected segregation in  $F_2$  is 3:1 (Ramalho et al. 2012). In this context, it was checked whether the observed values fitted the expected proportions based on the probability of occurrence of the event through the Chi-square ( $X^2$ ) test (Table 2).

The Chi-square values found ( $X^2 = 3.5851$ ,  $X^2 = 3.675$ ,  $X^2 = 3.6170$ ,  $X^2 = 2.9793$ ) (Table 2) for all generations were lower than the tabulated value ( $X^2 = 3.84$ ), at a 5% probability level (Ramalho et al. 2012). Thus, the observed frequencies fit the expected proportion, with non-significant deviations. Therefore, pigmentation in quinoa is determined by dominant monogenic inheritance for the presence of pigmentation over its absence (Silvestri and Gil 2000, Peterson et al. 2015, Emrani et al. 2020).

In the experimental field of the Prosperidad Experimental Farm, both parents and  $F_2$  progenies were sown, and all genotypes showed statistical differences according to the F test ( $p \le 0.01$  and  $p \le 0.05$ ) in the evaluation of plant height (PH), panicle length (PL), dry matter yield (DM), grain yield (GY), weight of 1000 grains (WTG), and harvest index (HI) (Table 3). The Scott-Knott test of means ( $p \le 0.05$ ) showed the existence of significant differences. In the PH characteristic, the

*Table 2.* Estimate of  $\chi^2$  (Chi-square) for the presence of pigmentation in the stem, leaves, axils, and inflorescence color of the F<sub>2</sub> generation of the crosses P88 x BX8, P88 x BX4, A88 x BX8 and A88 x BX4

(P88 x BX8)					
Phenotype of F <sub>2</sub>	PR	Frequ	iency	Deviation	(OF-EF) <sup>2</sup>
Generation	Expected	Observed	Expected	(OF–EF)	EF
Presence of pigmentation	3	562	540	22	0.8962
Absence of pigmentation	1	158	180	-22	2.6888
Total	4	720	720		χ <sup>2</sup> =3.5851
(P88 x BX4)					
Phenotype of F,	PR	Frequency		Deviation	(OF-EF) <sup>2</sup>
Generation	Expected	Observed	Expected	(OF–EF)	EF
Presence of pigmentation	3	501	480	21	0.91875
Absence of pigmentation	1	139	160	-21	2.7562
Total	4	640	640		χ <sup>2</sup> =3.675
(A88 x BX8)					
Phenotype of F,	PR	Frequency		Deviation	(OF-EF) <sup>2</sup>
Generation	Expected	Observed	Expected	(OF–EF)	EF
Presence of pigmentation	3	608	585	23	0.9042
Absence of pigmentation	1	172	195	-23	2.7128
Total	4	780	780		X <sup>2</sup> =3.6170
(A88 x BX4)					
Phenotype of F <sub>2</sub>	PR	Frequency		Deviation	(OF-EF) <sup>2</sup>
Generation	Expected	Observed	Expected	(OF–EF)	EF
Presence of pigmentation	3	417	435	-18	0.7448
Absence of pigmentation	1	163	145	18	2.2344
Total	4	580	580		χ <sup>2</sup> =2.9793

Degrees of freedom (df) = 1; Probability = 5%; PR: Phenotypic Relationship; OF: Observed Frequency; EF: Expected Frequency; Tabulated Chi-square (X<sup>2</sup>): 3.84.

mean value was 139.87 cm, with P88 x BX4 and P88 x BX8 being the hybrids with the highest PH values, 155.75 cm and 155 cm, respectively. However, the A88 x BX8 hybrid showed the lowest PH value, 117.25 cm (Table 3). In the evaluation of panicle length (PL), the mean was 35.18 cm, with the P88 x BX8 hybrid standing out, with 45.50 cm, and the lowest value shown by the A88 progeny, 29.25 cm. In quinoa breeding, methodologies are sought to establish selection for larger panicle size to increase grain yield and quality (Zurita-Silva et al. 2014). The dry matter yield (DM) was 8411.96 kg ha<sup>-1</sup>, with the P88 x BX8 and P88 (parent) hybrids predominating, with 11065 and 10853.25 kg ha<sup>-1</sup>, respectively. The A88 and A88 x BX8 genotypes showed the lowest values, 5912.50 and 5950 kg ha<sup>-1</sup>. In quinoa, grain yield and size are decisive selection criteria and should be addressed in genetic improvement (Bertero et al. 2004). The average grain yield (GY) was 2269.3 kg ha<sup>-1</sup>, with the hybrid P88 x BX8 standing out, showing values above the average with 3100.25 kg ha<sup>-1</sup>. However, the hybrid A88 x BX8 showed lower grain yields, with 1305 kg ha<sup>-1</sup>. The most outstanding parent was P88, with 2814 kg ha<sup>-1</sup>. Similar data were found for this same genotype under conditions of 1100 and 1800 m of latitude in Colombia, making it promising for genetic improvement in other regions (Anchico et al. 2020). On the other hand, the parent with the lowest results was A88, with 1305 kg ha<sup>-1</sup> (Table 3). Superior quinoa progenies have also been obtained through pedigree (genealogical) or modified genealogical selection (Emrani et al. 2020).

The weight of 1000 grains (WTG) had an average of 2.17 g, with the parents BX4 with 2.87 g and P88 with 2.82 g showing the best results. In  $F_2$  with higher WTG are the crosses P88 x BX8 and P88 x BX4, with 2.78 and 2.74 g, respectively. Progenies originating from the cultivar BRS Syetetuba (BX4 and BX8) had values similar to those reported under Brazilian Savanna conditions (Spehar et al. 2011). The harvest index (HI) showed an average of 26.83%, with the parent BX4 standing out with 30.07%. There were statistical differences in all crosses, possibly due to the segregation result and the genetic characteristics of each cross. Thus, the progenies obtained from the cross P88 x BX8 are promising for future genetic improvement programs due to their high production potential (Table 3).

Genetic parameter estimation was performed for plant height (PH), panicle length (PL), dry matter yield (DM),

Hybridization between progenies and agronomic characterization of the F2 generation in quinoa

Treatment (G)	<b>PH</b> (cm)	<b>PL</b> (cm)	<b>DM</b> (kg ha <sup>-1</sup> )	<b>GY</b> (kg ha <sup>-1</sup> )	WTG (g)	HI (%)
P88 x BX4	155.75 a	39.75 b	9775.00 b	2595.00 c	2.74 b	26.55 b
P88 x BX8	155.00 a	45.50 a	11065.00 a	3100.25 a	2.78 b	28.01 a
BX4	145.75 b	34.00 d	8325.00 c	2485.00 c	2.87 a	30.07 a
BX8	145.50 b	34.00 d	8167.50 c	2285.25 d	2.52 c	27.98 a
P88	144.25 b	35.75 c	10853.20 a	2814.50 b	2.82 a	25.97 b
A88 x BX4	132.00 c	29.75 e	7247.50 d	2027.50 e	2.71 b	27.97 a
A88	123.50 d	29.25 e	5912.50 e	1542.50 f	2.47 c	26.12 b
A88 x BX8	117.25 e	33.50 d	5950.00 e	1305.00 g	2.40 d	21.98 c
Mean	139.87	35.18	8411.96	2269.37	2.66	26.83
CV (%)	1.28	3.01	5.11	3.69	2.00	5.58
F test	18.25**	7.55**	5.34**	7.92**	12.69**	10.03**

*Table 3.* Means grouping test of four quinoa parents and four F, progenies in the evaluation of agronomic characteristics: plant height (PH), panicle length (PL), dry matter yield (DM), grain yield (GY), weight of 1000 grains (WTG) and harvest index (HI)

Means followed by the same letters in each column do not differ from each other by the Scott-Knott Test at 5% probability.

\*\* Significant by F test ( $p \le 0.01$ ). CV: Coefficient of variation.

*Table 4.* Genetic parameters for agronomic characteristics: plant height (PH), panicle length (PL), dry matter yield (DM), grain yield (GY), weight of 1000 grains (WTG), and harvest index (HI) of four parents and four F, progenies of quinoa

Genetic parameters	РН	PL	DM	GY	WTG	н
σ2f	198.978	28.780	4085819.450	379192.749	0.031	5.627
σ2e	10.901	3.809	764982.466	47845.805	0.002	0.561
σ2g	188.077	24.971	3320836.980	331346.944	0.028	5.066
ha²(%)	94.522	86.764	81.277	87.382	92.120	90.030
CVg (%)	9.800	14.202	21.664	25.356	6.306	8.388
CVe (%)	4.719	11.095	20.795	19.271	3.688	5.583
CVg/CVe (%)	2.077	1.280	1.042	1.316	1.710	1.503

Phenotypic variance ( $\sigma 2f$ ), genotypic variance ( $\sigma 2g$ ), environmental variance ( $\sigma 2e$ ), broad-sense heritability ( $ha^2$ ), genetic coefficient of variation (*CVg*), environmental coefficient of variation (*CVg*), genetic to environmental coefficient of variation ratio (CVg/CVe).

grain yield (GY), weight of 1000 grains (WTG), and harvest index (HI) (Table 4). The calculation of genetic parameters in quinoa has helped genetic improvement programs to be more successful in selecting superior genotypes, considering quantitative characteristics (Yadav 2021). In addition, the relationships between the genotypic coefficient of variation (*CVg*), environmental coefficient of variation (*CVe*), and heritability (ha<sup>2</sup>) are parameters that measure data reliability and the success in selecting superior genotypes (Oliveira et al. 2021, Santos et al. 2023). The genotypic coefficient of variation is the proportionality of gain relative to the average, and therefore is expected to be higher than the environmental coefficient of variation (*CVe*). All variables had higher (*CVg*) data compared to (*CVe*) (Table 4), showing that genetic contribution was greater than the environmental effect, making the selection effective.

The broad-sense heritability coefficients (ha<sup>2</sup>) showed high values for the analyzed variables, ranging from 81.27% to 94.52% (Table 4). High heritability coefficients are related to greater genetic variability and higher selective precision (Cargnelutti Filho et al. 2009), increasing the probability of success in quinoa selection (Vasconcelos et al. 2016). Estimates of the *CVg/CVe* ratio in the evaluated characteristics expressed values of 2.07 (PH), 1.28 (PL), 1.04 (DM), 1.31 (GY), 1.71 (WTG), and 1.5 (HI). All variables showed values higher than 1.0. These progenies have a favorable selection index, measured by the *CVg/CVe* ratio, with values close to or higher than 1.0 (Vasconcelos et al. 2016).

# CONCLUSIONS

Quinoa showed open pollination percentages ranging from 3.81% to 19.88%, depending on the crossing methodology.

The trait presence of pigmentation in the plant exhibited complete dominance over absence of pigmentation.

Genetic parameters such as high heritability and high coefficients of genetic variation found in this study suggest

the potential for more efficient selection processes starting from the F, generation.

The genotype resulting from the cross P88 x BX4 showed the best agronomic characteristics, being considered promising to continue in the quinoa breeding program.

#### ACKNOWLEDGEMENTS

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), to the company Green Line Productos de Origen and to COLFUTURO for funding the research.

#### REFERENCES

- Alencar ER, Jojoa WA, Silva KN and Silva NO (2021) Ozonation of quinoa seeds (*Chenopodium quinoa* Willd.): Saturation and decomposition kinetics of ozone and physiological quality of seeds. Semina: Ciencias Agrarias 42: 1019-1032.
- Amabile R, Faleiro G and Ricardo J (2015) Estimation of genetic parameters, phenotypic, genotypic and environmental correlations on barley (*Hordeum vulgare* L.) grown under irrigation conditions in the Brazilian savannah. **Interciencia 40**: 255-262.
- Anchico W, Spehar CR and Vilela MS (2020) Adaptability of quinoa genotypes to altitudes and population densities in Colombia. Bioscience Journal 36: 14-21.
- Anchico-Jojoa W, Peixoto JR and Júnior AAO (2023) Physicochemical characterization and antioxidant capacity of quinoa progenies from Colombia, Brazil and Ecuador produced in the Brazilian Savannah. Revista Colombiana de Ciencias Hortícolas 17: e15696.
- Anchico-Jojoa W, Peixoto JR, Spehar CR and Vilela MS (2021) Calculation of the thermal units for 13 codes of the BBCH scale of 12 progenies of quinoa in the growing conditions of the Brazilian savanna. **Revista Colombiana de Ciencias Hortícolas 15**: e13109-e13109.
- Ayub M, Castro-Alba V and Lazarte CE (2021) Development of an instantmix probiotic beverage based on fermented quinoa with reduced phytate content. **Journal of Functional Foods 87**: 104831.
- Bertero HD, De La Vega AJ, Correa G, Jacobsen SE and Mujica A (2004) Genotype and genotype-by-environment interaction effects for grain yield and grain size of quinoa (*Chenopodium quinoa* Willd.) as revealed by pattern analysis of international multi-environment trials. **Field Crops Research 89**: 299-318.
- Cruz CD (2013) Genes a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum. Agronomy 35: 271-276.
- Emrani N, Hasler M, Patiranage DSR, Nathaly MT, Rey E and Jung C (2020) An efficient method to produce segregating populations in quinoa (*Chenopodium quinoa*). **Plant Breeding 139**: 1190-1200.
- Cargnelutti Filho A, Storck L and Ribeiro ND (2009) Medidas da precisão experimental em ensaios com genótipos de feijão e de soja. **Pesquisa** Agropecuária Brasileira 44: 1225-1231.
- Fuentes F and Bhargava A (2011) Morphological analysis of quinoa germplasm grown under lowland desert conditions. Journal of Agronomy and Crop Science 197: 124-134.

- Fuentes F and Paredes-González X (2013) Perspectivas nutracéuticas de la quinua: propiedades biológicas y aplicaciones funcionales. Estado del arte de la quinua en el mundo. In Bazile D, Bertero D and Nieto C (eds) Estado del arte de la quinua en el mundo. FAO/Santiago de Chile and CIRAD/Montpellier, p. 341-357.
- Gomez-Pando L (2015) Quinoa breeding. In Murphy KS and Matanguihan J (eds) Quinoa: Improvement and sustainable production. John Wiley & Sons, New York, p. 87-108.
- González JA, Bruno M, Valoy M and Prado FE (2011) Genotypic variation of gas exchange parameters and leaf stable carbon and nitrogen isotopes in ten quinoa cultivars grown under drought. Journal of Agronomy and Crop Science 197: 81-93.
- Jacobsen SE and Stølen O (1993) Quinoa Morphology, phenology and prospects for its production as a new crop in Europe. European Journal of Agronomy 2: 19-29.
- Jojoa WA, Peixoto JR, Spehar CR, Vilela MS, Fagioli M, Nobrega D, Cruz J and Oliveira A (2021) Evaluation of the physiological quality of quinoa seeds. African Journal of Agricultural Research 17: 802-808.
- Kottek M, Grieser J, Beck C, Rudolf B and Rubel F (2006) World map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift 15: 259-263.
- Lesjak J and Calderini DF (2017) Increased night temperature negatively affects grain yield, biomass and grain number in Chilean quinoa. Frontiers in Plant Science 8: 352.
- Manjarres-Hernández EH, Arias-Moreno DM, Morillo-Coronado AC, Zarely Ojeda-Pérez Z, Cárdenas-Chaparro A and Arias-Moreno EH (2021) Phenotypic characterization of quinoa (*Chenopodium quinoa* Willd.) for the selection of promising materials for breeding programs.
  Plants 10: 1339.
- Murphy KM, Bazile D, Kellogg J and Rahmanian M (2016) Development of a worldwide consortium on evolutionary participatory breeding in quinoa. Frontiers in Plant Science 7: 608.
- Murphy KM, Matanguihan JB, Fuentes FF, Gómez-Pando LR, Jellen EN, Maughan PJ and Jarvis DE (2018) Quinoa breeding and genomics. Plant Breeding Reviews: 257-320.
- Oliveira CRSD, Borel JC, Pereira DA, Carvalho BPD, Medrado EDS, Ishikawa FH and Oliveira EJD (2021) Genetic parameters and path analysis for root yield of cassava under drought and early harvest. **Crop Breeding and Applied Biotechnology 21**: e36162137

Peterson A, Jacobsen SE, Bonifacio A and Murphy K (2015) A crossing

#### Hybridization between progenies and agronomic characterization of the F2 generation in quinoa

method for quinoa. Sustainability 7: 3230-3243.

- Popayán Alcaldia Municipal de Popayán (2023) Historico clima. Available at <http://popayan.gov.co/ciudadanos/popayan/nuestra-geografia>. Accessed on February 18, 2023.
- Ramalho MAP, Santos JD, Pinto CABP, Souza ED, Gonçalves FMA and Souza JD (2012) Genética na agropecuária. UFLA, Lavras, 566p.
- Risi JC (1984) The chenopodium grains of the Andes: Inca crops for modern agriculture. Advances in Applied Biology 10: 145-216.
- Rodríguez GMJ, Prieto MJ, Cruz SV and Calvo MP (2021) Nutritional characterization of six quinoa (*Chenopodium quinoa* Willd) varieties cultivated in Southern Europe. Journal of Food Composition and Analysis 99: 103876.
- Santos VDS, Fukuda WMG, Oliveira LAD, Pereira MEC, Nuti MR, Carvalho JLVD and Pereira HD (2023) Genetic parameters considering traits of importance for cassava biofortification. Crop Breeding and Applied Biotechnology 23: e447923211.
- Sañudo B, Arteaga G, Betancourth C, Zambrano J and Burbano J (2005) Perspectivas de la quinua dulce para la región andina de

Nariño. Unigraf, Pasto, 30p.

- Silvestri V and Gil F (2000) Allogamy in quinua. Revista de La Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo 1: 71-76.
- Spehar C (2007) Quinoa: alternativa para a diversificação agrícola e alimentar. Embrapa, Planaltina, 101p.
- Spehar CR, Rocha JEDS and Santos RLDB (2011) Desempenho agronômico e recomendações para cultivo de quinoa (BRS Syetetuba) no cerrado. Pesquisa Agropecuária Tropical 41: 145-147.
- Vasconcelos ES, Hoepers LML, Amaral RG, Egewarth VA and Strenske A (2016) Genetic parameters and productivity of quinoa in western Paraná State, Brazil. Acta Scientiarum. Agronomy 38: 185-191.
- Yadav RK (2021) Genetic parameters and their association with grain yield in quinoa (*Chenopodium quinoa* Willd) under late sown condition. International Journal of Farm Sciences 11: 37-39.
- Zurita-Silva A, Fuentes F, Zamora P, Jacobsen SE and Schwember AR (2014) Breeding quinoa (*Chenopodium quinoa* Willd.): Potential and perspectives. **Molecular Breeding 34**: 13-30.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.