Este articulo tiene una buena estructura, porque contine lo siguiente:

Enhancing quinoa cultivation in the Andean highlands of Peru: a breeding strategy for improved yield and early maturity adaptation to climate change using traditional cultivars

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Abstract Quinoa (*Chenopodium quinoa* Willd.) is an ancient food crop that originated in the Andes. It has good nutritional properties that increasingly attract interest around the world and it is considered as a future crop for food security and climate change adaptation. Currently, there is a great need to develop new quinoa varieties with higher yield, tolerance to biotic and abiotic stresses, and adaptation to new growing areas. Despite the existence of breeding programs, quantitative genetic parameters that are relevant for selection gain have hardly been investigated for quinoa. We estimated these parameters for important agronomic traits in six segregating populations of 96 lines each, derived from crosses of popular traditional Peruvian quinoa varieties. The traits were evaluated in trials at several locations in the Peruvian highlands over 3 years. Maturity was the best phenological stage for trait evaluation because at this stage we obtained high estimates of heritability and high correlation of panicle trait indices with grain yield. Based on these traits, we selected the 18 best lines and characterized them in additional field trials for the same traits. Three lines (HUA×KAN53, SAL×NCO46 and SAL×PAN171) combined advantageous traits of their parents and showed lower plant height, earlier maturity and higher yield. Our work shows that the estimation of variance and variance components in the amphidiploid crop quinoa provides useful information for the design of breeding programs and the selection of improved genotypes for cultivation in the Peruvian highlands.

Las keyword están en orden alfabético

Abbreviation
MLT   Multilocation trial

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Introduction

Quinoa (*Chenopodium quinoa* Willd., $2n = 4\times = 36$) is an ancient food crop that was domesticated in the Andean region (Hellin and Higman 2005; Jacobsen et al. 2003). It is of great importance for agriculture

and nutrition in several regions of South America including Peru, Bolivia, Ecuador, and Chile (Bazile et al. 2016). Andean farmers have been involved in the domestication and selection of quinoa varieties, which resulted in a high level of genetic diversity (Ortiz et al. 1998; Jarvis et al. 2017; Winkel et al. 2018; Salazar et al. 2019). Quinoa grains contain all essential amino acids such as lysine in a favorable composition and they are gluten-free (Repo-Carrasco et al. 2003; Alvarez-Jubete et al. 2010; Nowak et al. 2016). In addition to its good nutritional qualities, quinoa is tolerant to diverse abiotic stresses such as drought, heat, frost, and salinity. High resistance to abiotic stress likely reflects evolutionary adaptation to very different environmental conditions in the area of origin of this crop (Hinojosa et al. 2018; Mahdavi Rad et al. 2022). The number of countries outside the native Andean region, in which quinoa is cultivated, is increasing although the production volume remains very small compared to the Andes (Bazile et al. 2016; Alandia et al. 2020).

Ongoing climate warming is affecting crop production worldwide due to changes in precipitation patterns and temperatures (Hasegawa et al. 2021). For this reason, varieties with stable yields and adaptation to new environmental conditions need to be developed. This requirement also exists for quinoa in its original growing region. The Andean highlands of Peru experience adverse environmental conditions with frequent hail storms, droughts, and ongoing changes in rainfall patterns (Flubacher et al. 2017; Hasegawa et al. 2021), which is a problem for the food security and income of smallholder farmers. The selection of lines with improved characteristics such as yield, plant height, and days to maturity are therefore important breeding goals to reduce the risks caused by an adverse environment.

The Andean region hosts a great diversity of native quinoa genetic resources (Bazile et al. 2016) that offers plant breeders the opportunity to develop new and improved varieties with desirable traits for a target production region. Breeding of new varieties also needs to consider the preferences and requirements of end-users, including smallholder farmers and industry (Gamboa et al. 2018). Currently, the main target traits of most quinoa breeding programs are seed yield and quality (Bertero et al. 2004). Farmers in the Peruvian highlands have a strong preference for varieties characterized by higher yields, larger grain size,

lower saponin content, and earliness (Gamboa et al. 2018). Introducing improved varieties tailored to the needs of smallholder farmers contributes to improved food security in the Andean region.

The utilization of genetic diversity by breeding new varieties is a crucial step in the improvement of this crop. Although several quinoa breeding programs have been established, quantitative genetic parameters for traits targeted for improvement were not estimated to our best knowledge. The main objective of this work was therefore (1) To estimate quantitative genetic parameters and estimate selection gain in six populations based on crosses of six widely cultivated varieties in the Peruvian highlands to evaluate the potential for breeding new varieties that are adapted to the future climatic conditions of this region. (2) To implement a breeding program and select genotypes with improved agronomic traits related to the yield, early maturity, and lower plant height for cultivation in the Peruvian highlands that combine key traits of adapted varieties.

> En la intrucción se abarca el tema de lo general a los especifico (forma de embudo) y al final se mensionan los objetivos del estudio.

Materials and methods

Selection of parents and development of segregating populations

> Se desarrolla en base a los objetivos planteados

Seeds of six quinoa traditional varieties and modern cultivars were provided by the Germplasm Bank of the Universidad Nacional del Altiplano, Puno, Peru (Table 1). They were selected based on their main agronomic traits and their different origins and pedigrees. Salcedo INIA (SAL) shows high yield performance with resistance to frost and powdery mildew (*Peronospora variabilis;* Mujica et al. 2001). Huariponcho (HUA) has a high saponin content and a compact panicle with tolerance to hailstorms and frost and resistance to mildew (Reinoso and Paredes 1998). Pasankalla (PAS) is characterized by medium height, low saponin content, and resistance to powdery mildew (Grace 1985). Negra Collana (NCO) is frost and drought tolerant and has a high yield (Catacora and Canahua 1991). Kancolla (KAN) has a medium height, high saponin content, and is resistant to cold and hail (Tapia et al. 2000), and Pandela Rosada (PAN) has a large grain size (Tapia et al. 2000).

The six traditional varieties were used to generate six segregating populations derived from

Table 1 Characteristics and description of the six quinoa (*Chenopodium quinoa*) parents used to produce six segregating populations evaluated during the experiments 2017–2020 in the Peruvian Highlands

| Variety Characteristic	Salcedo INIA	Pasankalla	Kancolla	Negra Collana	Pandela Rosada	Huariponcho
Release name	Salcedo INIA	INIA 415—Pasankalla	Kancolla	INIA 420—Negra Collana	Pandela Rosada	Huariponcho
Place, year of release	Puno, 1995	Puno, 2006	Puno, 1975	Puno, 2008	Bolivia, 2000	Puno, 1998
Origin	Real Boliviana×Sajama	GenBank: PIQ031069	Mass selection	Compound accessions	Mass selection	Mass selection
Breeder	INIA-Puno	INIA-Puno	INIA-Puno	INIA-Puno	PROINPA	INIA-Puno
Cycle (days)	150.00	144.00	170.00	138.00	191.00	186.00
Plant height (m)	1.61	1.35	1.55	1.25	1.46	1.20
Yield (t/ha)	2.50	3.54	1.75	2.80	2.50	2.20
Panicle shape	Glomerulate	Amarantiform	Amarantiform	Glomerulate	Glomerulate	Glomerulate
Panicle length (cm)	37.00	32.50	30.20	32.50	20.60	21.00
Panicle diameter (cm)	9.65	6.00	7.00	6.00	5.90	5.20
Grain color	White	Purple	White	Shiny black	White	Yellow
Grain diameter (mm)	2.00	2.10	1.80	1.60	2.10	1.90
Protein (%)	16.23	17.83	16.11	17.62	15.10	16.70
Saponin (%)	0.02	0.00	0.35	0.00	0.29	0.33
Mildew resistance	Moderate	Resistant	Resistant	Resistant	Susceptible	Resistant
Cold tolerance	Moderate	Moderate	Tolerant	Tolerant	Moderate	Tolerant
Drought tolerance	Tolerant	Tolerant	Tolerant	Tolerant	Moderate	Tolerant
Source	Mujica et al. (2001); FAO (2013)	Grace (1985); FAO (2013)	Tapia (2000); FAO (2013)	Catacora and Canahua (1991) FAO (2013)	Reynagan (2011)	Reinoso and Paredes (1998)

the following crosses: Salcedo INIA×Huariponcho (SAL×HUA), Huariponcho×Kancolla (HUA×KAN), Pasankalla×Kancolla (PAS×KAN), Salcedo INIA×Pandela Rosada (SAL×PAN), Salcedo INIA×Negra Collana (SAL×NCO), and Negra Collana×Kancolla (NCO×KAN).

Manual crosses were performed in 2012 in a greenhouse at the National University of Altiplano, Puno, Peru. Hermaphrodite flowers were emasculated and then pollinated with the pollen grain collected from the male parent (Emrani et al. 2020). Grains obtained from each cross were grown and self-pollinated using a bulk method until the F5 generation to increase homozygosity. In the F6 generation, the lines were selected by rows according to their yield performance. During the F7 to F9 generation variety selection and registration trials were performed to identify new cultivars within each segregating population (Fig. 1).

Trait evaluation

In both series of trials, phenotypic traits were recorded during four phenological stages, namely at (i) flowering (flw, stage 69), (ii) maturity (mtr, stage 89), (iii) harvest (hrv, stage 99) and (iv) post-harvest (phrv), according to standard descriptors for *Chenopodium quinoa* (Bioversity International et al. 2013) and the BBCH scale (Sosa-Zuniga et al. 2017). Data were separately recorded for each experimental unit. The recorded phenotypic traits were: number of days to 50% flowering (dap), number of days to 50% physiological maturity (dap), plant height (cm), panicle length (cm), panicle width (cm). Yield (g/plant) was
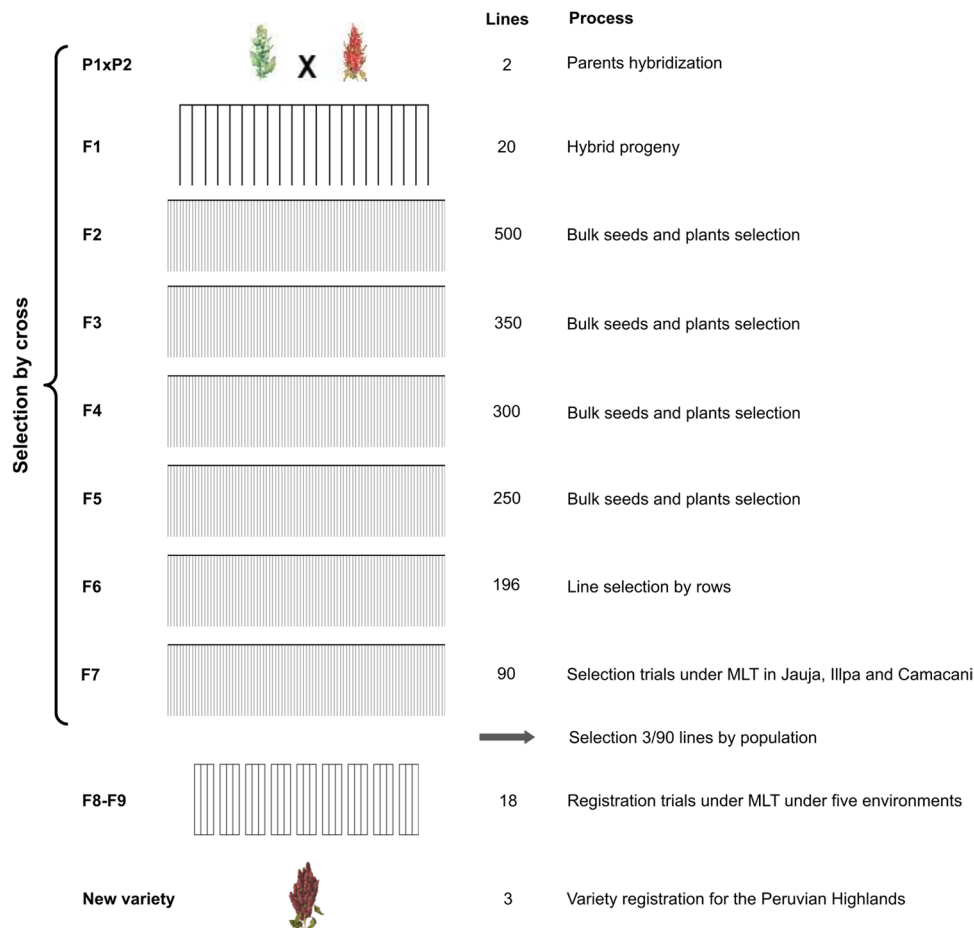
Fig. 1 Breeding scheme based on a bulk method used for quinoa. Six traditional varieties from the populations were generated based on simple crosses (F1): SAL×HUA, HUA×KAN, PAS×KAN, SAL×PAN, SAL×NCO, and NCO×KAN. Bulking and self-fertilization of lines were carried out from F2 throughout F6 generations. For selection trials in F7, the three best lines were selected by population based on high yield, pre-cocity, and medium plant height. For registration trials in F8 and F9, the best performing three lines per cross were evaluated in multi-location trials to select three lines as candidates for variety registration. Abbreviations: Salcedo INIA (SAL), Huariponcho (HUA), Kancolla (KAN), Pasankalla (PAS), Pandela Rosada (PAN), and Negra Collana (NCO)

evaluated at harvest with two sub-samples per experimental unit from an average of 10 plants selected randomly in each experimental unit. In the registration trials, additional traits were recorded as grain width (mm), biomass weight (stover weight in g/plant), and the harvest index was calculated for each subsample as the relation between the yield and biomass.

Different panicle indices were calculated based on the panicle traits at flowering and maturity for indirect selection to the yield. The indices were the ratio length and width (PanicleLength|Width), ratio width and length (PanicleWidth|Length), the product of width and length (PanicleWidth×Length), length squared by width (PanicleLength2Width), and the width squared by length (PanicleWidth2Length).

Multi-location field trials

Field trials were conducted in three successive growing seasons from 2017/18 to 2019/2020 at multiple locations in the Peruvian highlands (Table 2). Two series of multilocation trials (MLTs) were performed. The first series was used to select the best lines/experimental cultivars of each population

Table 2 Quinoa multi-location field trials conducted for selection and registration in three successive growing seasons from 2017/18 to 2019/2020 in the Andean highlands of South and Central Peru

Type of trial	Season	N° Entries	Plot size (m2)	Experimental design	Location	Latitude	Longitude	Altitude (masl)
Selection	2017–2018	(F7) 600	4.8	Lattice 10×10 Rep: 2	Jauja, Junin	11° 51′ 21.9″	75° 23′ 43.01″	3322
					Camacani, Puno	15° 56′ 54.45″	69° 51′ 31.13″	3842
					Illpa, Puno	15° 42′ 49.2″	70° 04′ 20.33″	3815
Registration	2018–2019	(F8) 25	30	Lattice 5×5 Rep: 4	Camacani, Puno	15° 56′ 54.45″	69° 51′ 31.13″	3842
					Illpa, Puno	15° 42′ 49.24″	70° 04′ 20.33″	3815
	2019–2020	(F9) 18	30	Lattice 4×4 Rep: 4	Potojani, Puno	15°56′ 23″	69° 52′ 46.8″	3850
					Illpa, Puno	15° 42′ 49.24″	70° 04′ 20.33″	3815
					Camata, Puno	15°56′ 34.8″	69° 50′ 47.4″	3838

The first series of trials was used to select three lines of each population based on yield, plant height, and precocity, and the second series of trials were performed for variety registration, with the selection being based on yield

in one year (2017/18) and three locations (Illpa, Camacani, and Jauja). These trials will be referred to as "selection trials" in the following. The second series of MLTs was implemented for a variety registration ("registration trials" in the following) over two years (2018/19 and 2019/20) in a total of five location-year combinations, involving four different locations (Illpa, Camacani, Camata, and Potojani). Jauja is located in the Junin region from Peru above the 3300 m.a.s.l. and has clay soil. The four locations Illpa, Camacani, Camata, and Potojani are located in the Puno province of the Peruvian Highlands with an altitude range between 3815 and 3850 m.a.s.l. with silty clay, sandy clay loam, and clay loam soils. All experiments were carried out using a lattice design. In both series of MLTs, the selection of lines was based on the Elston index (Elston 1963) using the function elston() in the *st4gi* package (Eyzaguirre 2022).

In the selection trials, we used separate 10×10 lattice designs with two replications for each of the six segregating populations. A total of 18 experiments were performed (six populations×three locations). Each experiment included 90 F7 lines, six parents, and four checks, and each experimental unit consisted of 4.8 m^2 (4 rows at 0.6 m spacing with 2 m

length). From each population, three lines combining high yield, small plant height, and precocity were selected for the registration trials (Online Resource 2, Table S1).

In the registration trials, in the first season (2018–19), experiments were performed with a 5×5 lattice design with four replications. Each experiment consisted of 18 experimental lines (F8), six parents, and one check. For the second season (2019–20), a lattice design 4×4 with four replications was used. The 16 entries consisted of nine experimental lines (F9), six parents, and one check. In the registration trials, each experimental unit (plot) had a size of 30 m^2 (5 rows at 0.6 m spacing with 10 m length). The line selection in this stage was performed based on yield performance.

Field management

Trials were sown between September to October with the beginning of the rainy season in each location (Online Resource 1, Fig. S1). The field experiments were conducted under rainfed conditions with a 12:12 photoperiod. At sowing, 5 g of seeds from each line were used per linear meter. For soil fertilization, 4 t/ha organic manure was

applied, complemented with 190 kg/ha urea (46% N) and 170 kg/ha ammonium di-phosphate (18% N-46% P_2O_5). The total amount of ammonium di-phosphate was applied at the sowing. The nitrogen fertilizer was divided into two equal parts, one applied at planting and the other before ridging. At 35–45 days after emergence, rows were thinned to ten plants by a linear meter to achieve a density of ca. 167,000 plants/ha. In Jauja, no density control was done. The plots were ridged at 50–60 days after planting (DAP). Harvests were made after the physiological maturity when seeds showed between 13 and 15% humidity. The soil preparation was performed by tractor and all agricultural activities were carried out manually with the support of local farmers (Online Resource 1, Fig. S2).

Model for stagewise analysis and selection strategy

Analyses of selection trials were performed with a stagewise approach according to Schmidt et al. (2019b) and Buntaran et al. (2020). In stage 1, a linear mixed model with lines as fixed effect was used for each experiment according to the lattice design (Zystro et al. 2018) to estimate the Best Linear Unbiased Estimators (BLUEs) with the following model:

$$\gamma_{ijk} = \mu + g_i + rep_j + block_{jk} + plot_{ijk}$$

where γ_{ijk} is the response variable of the ith genotype in the kth block of the jth replicate, μ is the first-stage intercept, g_i is the effect for the ith genotype in the first stage, rep_j is the effect of the jth replicate, $block_{jk}$ is the effect of the kth incomplete block of the jth replicate, and $plot_{ijk}$ is the plot error effect corresponding to γ_{ijk}.

In stage 2, a linear mixed model for line by environment interaction was used where the lines were set as random effects to calculate the Best Linear Unbiased Predictors (BLUPs) with the following model:

$$\overline{\gamma}_{im} = \mu + g_i + l_m + gl_{im} + \overline{e}_{im}$$

where $\overline{\gamma}_{ihm}$ is the adjusted mean of the ith genotype in the mth location obtained in the first stage, μ is the intercept, l_m is the main effect for the mth location, g_i is the main effect of the ith genotype, gl_{im} is the im-th

genotype × location interaction effect and $\overline{e}_{im}$ is the error of the mean g_i obtained in the first stage. For the registration trials, we performed a one-stage analysis to calculate the BLUPs (Buntaran et al. 2020).

Broad-sense heritability and repeatability

Broad-sense heritability (H^2) and variance components were calculated based on genotype-difference using linear mixed models (Schmidt et al. 2019a) in which lines were considered as random effects in the second stage of the analysis. The heritability was estimated as proposed by Cullis et al. (2006) and involves the variance of a difference between genotypes:

$$H^2_{Cullis} = 1 - \frac{\overline{V}_\Delta^{BLUP}}{2\sigma_g^2}$$

where σ_g^2 is the genotypic variance and $\overline{V}_\Delta^{BLUP}$ is the mean-variance of a difference between two genotypic BLUPs.

Plot-based repeatability (w) for each environment was calculated in the first stage from the stagewise analyses according to the formula:

$$w = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_\in^2}{R}}$$

where σ_g^2 is the genotypic variance, σ_ϵ^2 is the residual error variance, and R is the number of replicates.

Statistical analysis and software implementation

Statistical analyses and plotting of graphs were performed with the statistical package R version 4.2.1 (R Core Team 2020). Broad-sense heritabilities, repeatability, variance components, BLUEs, and BLUPs were estimated based on the function H2cal() implemented in the package *inti* (Lozano-Isla 2020). The function uses a linear mixed model for random and fixed effects for the genotypes based on the *lme4* package (Bates et al. 2014). The outlier removal for multilocation trials was based on method 4, Bonferroni-Holm using re-scaled median absolute deviation for standardizing residuals described in Bernal-Vasquez et al. (2016) that were implemented in the same function. The *FactoMineR* package (Lê et al.
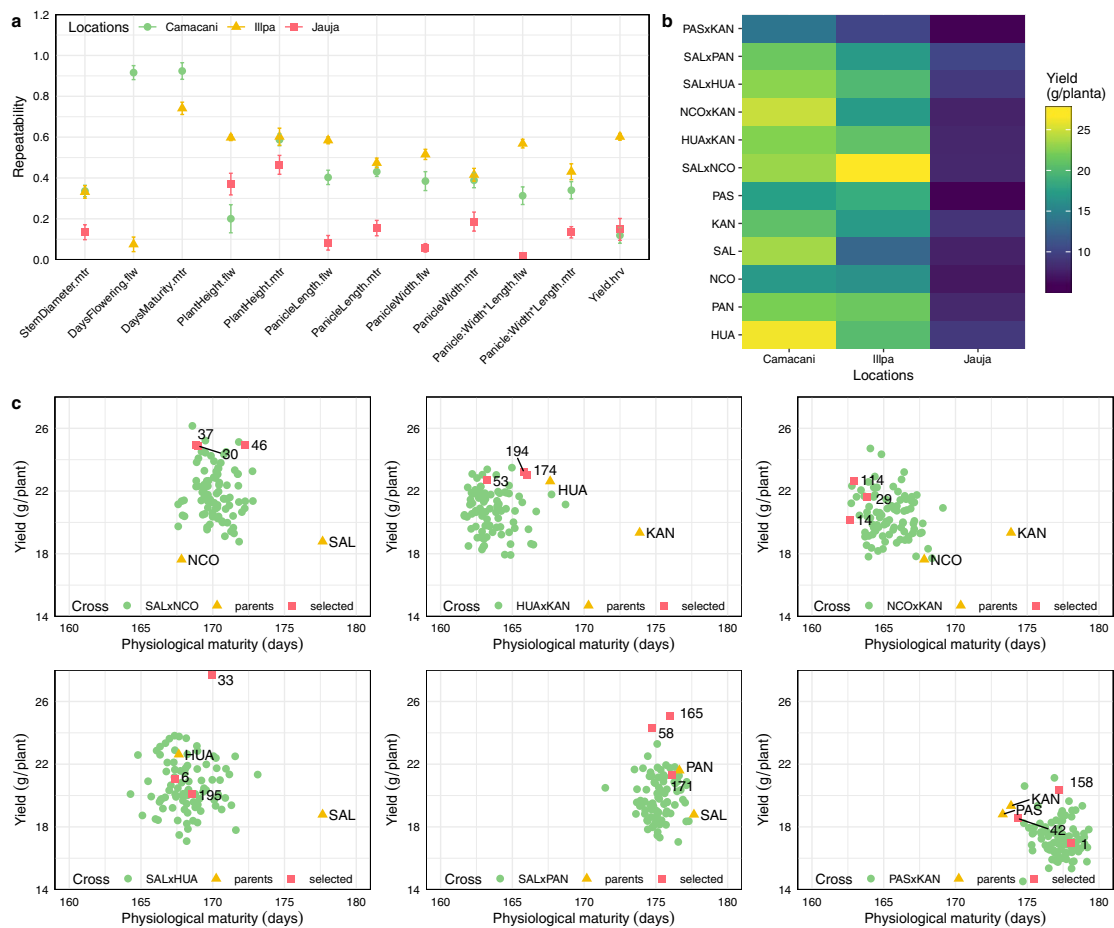
Fig. 2 Selection of candidate elite lines from segregating populations across multi-location trials using stagewise analysis in the F7 generation. **a** Estimated repeatabilities for various traits at different locations. **b** Mean yield performance of parents and crosses at the different locations. **c** Elite selected lines by population based on yield, plant height, and precocity in the selection trials at Camacani and Illpa in 2017–2018. Repeatability and yield were calculated based on BLUEs in stage I. Line selection was based on the BLUPs in stage II

2008) was used for principal components analysis (PCA) and graphics. The correlation plot was made with the *psych* package (Revelle 2021). The code and the reproducible data analysis were performed with the Quarto scientific publishing system and are available as supplementary information (Online Resource 3; Allaire et al. 2022).

Results

Elite line selection based on yield, small plant height, and precocity

To select the best genotypes for both selection and variety registration trials, experiments were performed in three different locations (Table 2). The repeatability of the experiment was estimated by location to perform line selection. The three best lines of each population were selected based on high yield, small plant height, and precocity.

In the selection trials, repeatability differed among traits and locations. Eight out of ten phenotypic
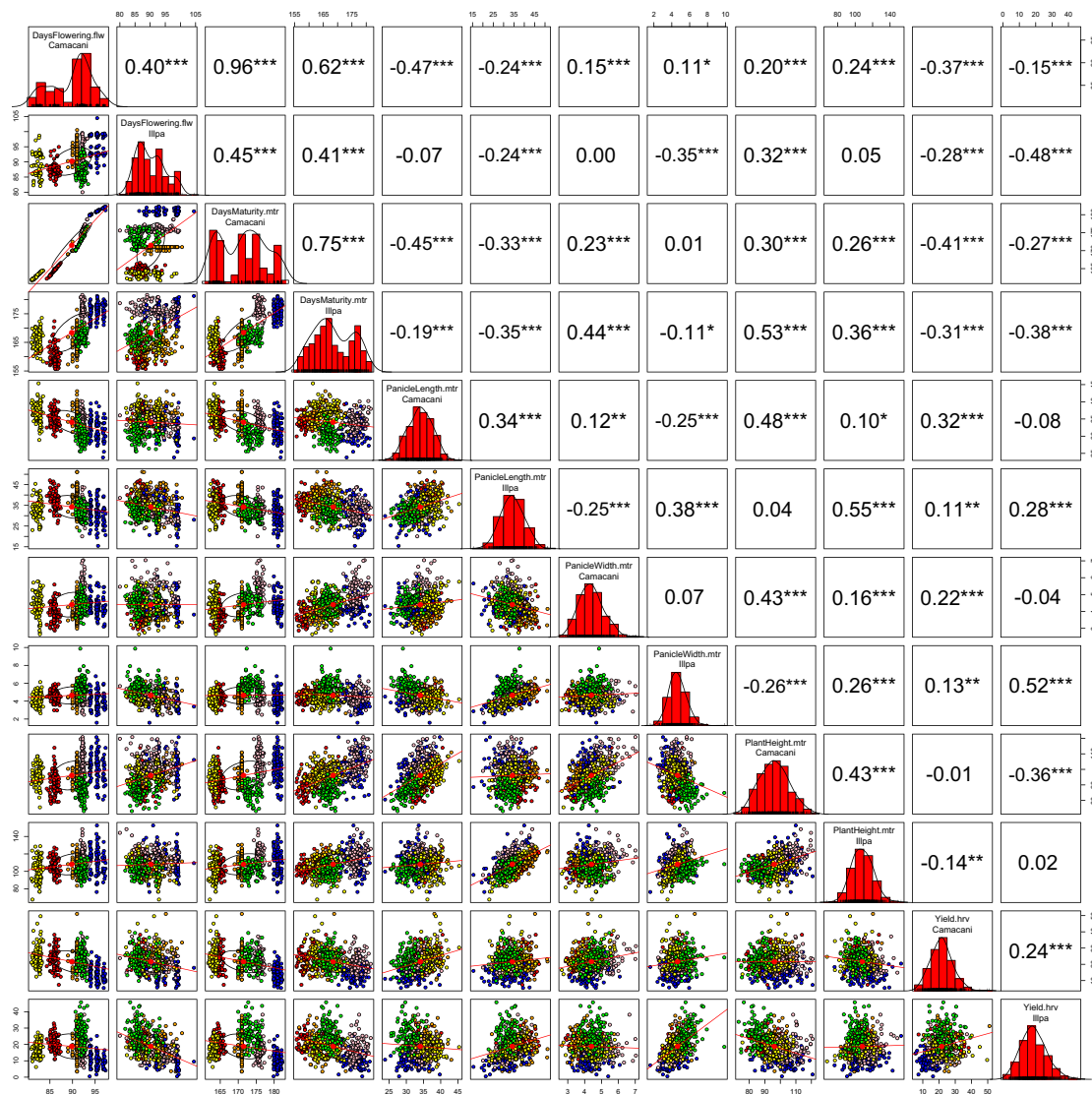
Fig. 3 Correlation coefficients (above diagonal) and linear regressions with scatter plot represented by the six populations (below diagonal in colors) among days to flowering and maturity, panicle length and width, plant height, and yield across the three locations (Camacani, Illpa, and Jauja) of the selection trials for 540 quinoa experimental lines derives from 6 segregating populations. *p* value: 0.05*, 0.01**, 0.001***

traits showed the highest repeatability in Camacani, whereas Jauja was the location with the lowest values for the same traits (Fig. 2A). The experiment at Jauja had a higher plant density, which favored the appearance of the powdery mildew pathogen. Both factors likely contribute to low heritabilities and reduced yield in this location (Fig. 2B).

The multivariate analysis for the selection of candidate elite lines was based on yield, precocity,

and reduction in plant height (Online Resource 2, Table S1). The selection and ranking of candidate lines were based on field trials from Illpa and Camacani, since the genetic differentiation among lines at Jauja was poor. The selection intensity applied for each population was 1.75 (i.e. three out of ninety lines per population). These lines were selected for the next stage of the variety of registration trials (Fig. 2C; Online Resource 2, Table S1).

To evaluate the potential to improve the studied traits a correlation between the traits and locations was performed. The traits days to flowering and maturity showed a high correlation between phenological stages in each location. There was no significant correlation between plant height and yield for Camacani (r = −0.01, p > 0.05) and Illpa (r = 0.02, p > 0.05; Fig. 3), which indicates that selection may reduce plant height without effects on yield. While days to flowering and maturity were negatively correlated with yield, panicle length and width showed a positive correlation with grain yield (Fig. 3). With the exception of days to flowering and maturity in Camacani, all traits were normally distributed (Fig. 3).

To study the performance of the crosses between populations, we plotted segregation variation segregation and calculated the selection gain for the traits to be used for selection. All populations showed segregation for steam diameter, days to flowering and maturity, plant height, panicle length and width, and yield (Fig. 4). The progeny of crosses differed strongly from each other with respect to different trait values. For example, SAL×PAN showed the highest value for stem diameter, whereas PAS×KAN showed very late flowering and maturity in combination with low yield (Fig. 4). SAL×NCO progeny showed a low plant height at maturity with high panicle width at maturity and a high yield in comparison to the other crosses (Fig. 4).

The main goal of a breeding program is to obtain the highest rates of genetic gain (R) in response to selection (Rutkoski 2019). To estimate genetic gain, response to selection was evaluated at physiological maturity for each population, at which the highest heritabilities for days to maturity, plant height, and yield were observed. The mean value for day to maturity was between 147.6 and 161.1 days and heritability ranged from 0.5 to 0.8 across populations with SAL×HUA, NCO×KAN, and HUA×KAN showing the largest response to selection. For the trait plant height, average values ranged between 100 to 116.1 cm, and the highest heritability ranged from 0.6 and 0.8. For yield, the highest response to selection was observed in the crosses with INIA Salcedo as maternal parent, i.e. SAL×HUA, SAL×NCO, and SAL×PAN (Table 3). Average values for yield performance ranged from 10.2 to 19.5 among crosses.

Elite line performance in the variety registration trials

To release and characterize new quinoa varieties adapted to the conditions of the Peruvian Highlands, 18 selected lines (i.e. 3 per population) were further evaluated under multi-location trials to assess their yield performance (Fig. 5). The three lines by population were compared with their parents for yield, days to maturity, and plant height based on the BLUPs. In these trials, the parents exhibited substantial phenotypic differences. Salcedo INIA was characterized by a high yield (53.3 g) and plant height (152.5 cm) with late maturity (192.9 days), whereas Pandela Rosada showed low values for the same traits with 30.6 g, 109.3 cm, and 146.57 days, respectively (Fig. 5). Accordingly, progeny with the highest gain for yield were SAL×PAN 171, SAL×NCO 46, and HUAxKAN 53 with 67.7, 54.3, and 52.8 g, respectively, and the progeny with the lowest yield was PASxKAN 1 (29.1 g) (Fig. 5A; Online Resource 2, Table S2). Progenies with early maturity were HUA×KAN 194 and NCO×KAN 29 with 122 and 122.5 days respectively, and SAL×HUA 33 (190.3 days) and PAS×KAN 158 (189.42) with late maturity (Fig. 5B). For plant height, at least one progeny from each cross was smaller than one of its parents (Fig. 5C). One of the outstanding progenies is line SALxPAN 171, because its yield was 27% higher than Salcedo INIA which is the crossing parent with the highest yield.

Determining the optimal phenological stage for trait evaluation is important to maximize selection gain and phenotyping efficiency. We phenotyped traits at flowering and maturity. The traits, plant height ($H^2 = 0.88$), panicle length (0.78), and width (0.86) showed higher heritabilities at maturity than at flowering with 0.45, 0.7, and 0.46 respectively (Table 4). All traits related to maturity were characterized by high heritability ($H^2 > 0.86$), whereas yield components showed higher heritabilities at the harvest stage, such as biomass ($H^2 = 0.79$), harvest index ($H^2 = 0.72$), and yield ($H^2 = 0.84$; Table 4).

Association of grain yield with panicle indices, biomass, and harvest index

Variation of inflorescence architecture influences grain yield. Since phenotyping of quinoa panicles is time-consuming for large populations frequently used
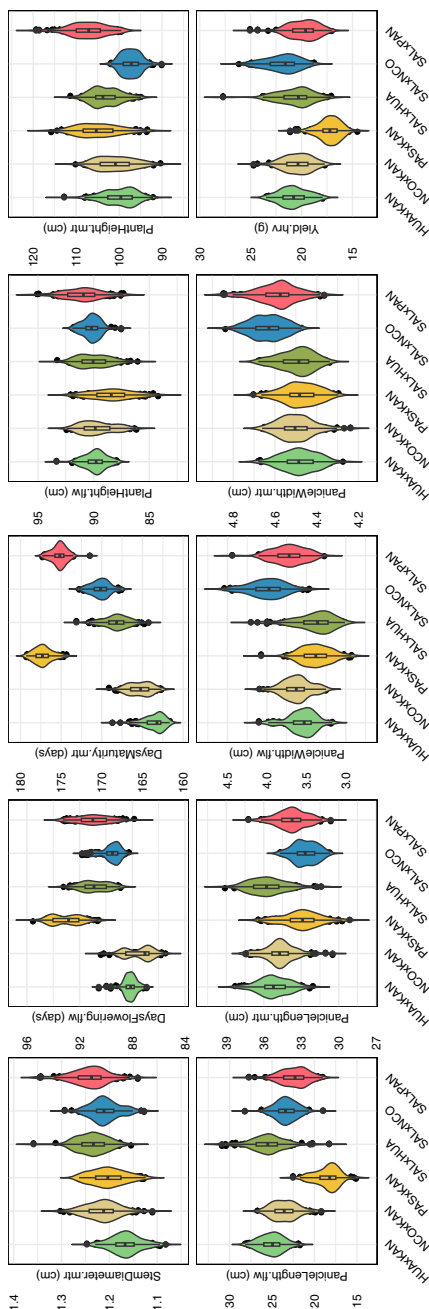
Fig. 4 Trait distribution among the six segregating populations in the F7 generation obtained in the selection trials. The distributions of each cross include 90 lines that were evaluated at two locations (Camacani and Illpa) per population

in genetic mapping studies or in breeding programs, identifying traits or indices associated with grain yield is useful for high-throughput phenotyping applications. To evaluate trait associations, we used a PCA and calculated the heritability of six panicle traits indices from the selection and registration trials and associated these with grain yield. In the registration trials, the two traits biomass and harvest index were additionally included in the analysis (Fig. 6).

In both the selection and registration trials, the indices PanicleWidth*Length, PanicleLength2Width, and PanicleWidth2Length at flowering and maturity were associated with yield but differed in their heritabilities between trials (0.06 to 0.32 vs. 0.78–0.81). In contrast, the traits panicle Width|Length, and panicle Length|Width were not associated with grain yield despite medium to high heritabilities (0.47–0.74 vs. 0.65–0.87) in both trials (Fig. 6A). Biomass was correlated with yield with a heritability of 0.79 and 0.84, respectively. Panicle length at maturity was associated with harvest index with a heritability of 0.72 and 0.78, respectively. Grain yield was more strongly associated with biomass than the harvest index. Finally, in both selection and registration trials, the index panicle Width * Length at maturity was strongly correlated with grain yield.

Discussion

> Los autores realizan un breve resumen de lo que se realizo en el estudio y lo que se ha obtenido.

Our analysis of a conventional breeding program for quinoa under the conditions of the Andean highlands of Peru reveals that considering the stage of selection, correlations between traits and the traits used for selection strongly influence selection gain. The breeding program was based on six segregating populations generated from single crosses of widely cultivated quinoa traditional varieties. The improved genotypes were selected based on multi-location field trials from 2017 to 2020. The analysis of variance components revealed that physiological maturity is the optimal phenological stage to perform trait evaluation when trait heritabilities were the highest. Among evaluated traits, the product of panicle width and length (Width * Length) at physiological maturity was strongly associated with grain yield and can be used for indirect selection.

Table 3 Estimated genetic gain (*R*) in six segregating quinoa populations (F7 generation) during the selection trials

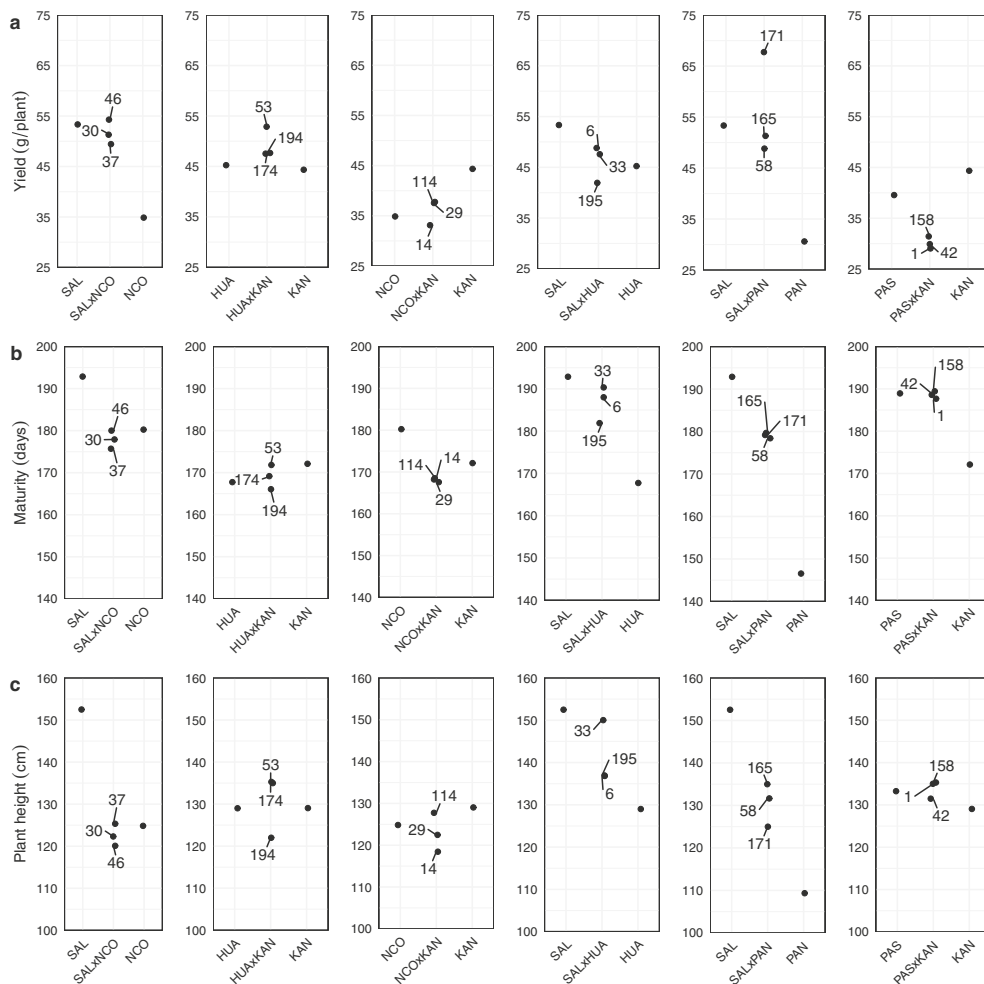| Trait	Population	mean	std	min	max	V·g	V·e	H²	R
DaysMaturity.mtr	PAS × KAN	161.1	1.5	157.0	163.9	1.1	8.6	0.5	1.7
DaysMaturity.mtr	SAL × HUA	157.3	2.3	152.3	164.0	3.5	10.1	0.7	3.6
DaysMaturity.mtr	SAL × NCO	153.8	1.3	150.2	156.9	1.0	5.7	0.6	1.7
DaysMaturity.mtr	NCO × KAN	154.3	2.0	150.4	158.9	2.9	7.5	0.8	3.3
DaysMaturity.mtr	HUA × KAN	147.6	1.8	144.5	153.4	2.4	5.6	0.8	3.0
DaysMaturity.mtr	SAL × PAN	160.8	1.3	155.3	164.0	0.0	14.0		
PlantHeight.mtr	PAS × KAN	115.7	7.6	99.3	132.8	32.2	284.0	0.6	9.7
PlantHeight.mtr	SAL × HUA	111.0	5.5	97.6	127.1	20.4	107.5	0.7	8.3
PlantHeight.mtr	SAL × NCO	100.0	3.4	90.4	108.3	4.3	88.8	0.4	2.8
PlantHeight.mtr	NCO × KAN	108.0	6.7	92.0	121.2	28.7	149.5	0.7	9.9
PlantHeight.mtr	HUA × KAN	102.6	6.4	89.8	125.0	30.7	86.1	0.8	11.1
PlantHeight.mtr	SAL × PAN	116.1	6.5	102.8	134.2	30.7	144.5	0.7	10.4
Yield.hrv	PAS × KAN	10.2	2.9	4.2	18.9	4.4	31.8	0.6	3.5
Yield.hrv	SAL × HUA	17.5	4.5	7.0	30.1	11.8	71.2	0.6	5.8
Yield.hrv	SAL × NCO	19.5	4.5	11.7	32.5	11.5	75.9	0.6	5.7
Yield.hrv	NCO × KAN	16.6	3.0	10.8	25.1	3.8	44.2	0.4	2.9
Yield.hrv	HUA × KAN	17.0	3.1	10.9	24.6	5.3	31.8	0.6	3.9
Yield.hrv	SAL × PAN	16.6	3.9	9.1	31.5	8.9	48.2	0.6	5.2

Where: Standard deviation (std), Minimum (min), Maximum (max), Genetic variance (V.g), and Error variance (V.e) and broad-sense heritability based on Cullis' approach (H2). $R = i*r*V.g$, where: i = selection intensity (1.75), r = accuracy ($\sqrt{H^2}$). Calculation based on 90 genotypes evaluated at two locations per population. Where: Days to maturity (DaysMaturity.mtr), panicle length at maturity (PanicleLength.mtr), panicle width to maturity (PanicleWidth.mtr), and yield (Yield.hrv)

A breeding program for the Andean highlands of Peru

The objective of this work was to select promising lines with better characteristics for farmers in the Andean highlands in South and central Peru. A key breeding goal was to reduce the time to maturity while maintaining a high yield to adapt cultivars to changes in rainfall patterns caused by the ongoing climate change (Hasegawa et al. 2021). In addition, a shorter time to maturity also reduces the effects of pests and diseases on crop yield. We were able to show that selection and identification of promising lines in a conventional breeding program based on interconnected single crosses of multiple parents and subsequent generations of self-fertilization is possible. The lines selected for the official registration trials, HUA × KAN 53, SAL × NCO 46, and SAL × PAN 171 showed a substantial improvement in plant height reduction, precocity, and yield. The initial selection of the lines in the breeding program was based on these three traits using the Elston index, which enables the selection and improvement of multiple traits (Elston 1963). The comparison of selection gain among the six breeding populations revealed that Salcedo INIA was the best parent with respect to the genetic gain of the selected traits. Salcedo INIA is one of the varieties with the highest yield in Peru but shows later maturity and increased susceptibility to mildew infections. Reguera et al. (2018) evaluated the yield of Salcedo INIA in Chile, Peru, and Spain and found that INIA Salcedo had the highest yield per hectare.

We observed a low correlation between plant height or days to maturity with the grain yield for quinoa. Santis et al. (2018) found a negative correlation between days to flowering and maturity with the grain yield. Other studies found a positive correlation between plant height and days to maturity with grain yield (Spehar and Santos 2005; Maliro et al. 2017; Manjarres-Hernández et al. 2021a). Based on these different results various indices have been used for the selection of genotypes in quinoa based on yield, plant height, grain diameter, number of panicles, precocity, and grain color (Manjarres-Hernández et al. 2021a, b). Using the multivariate selection index in the selection trials, we were able to increase the yield,

Realizan comparaciones de los resultados obtenidos con estudios realizados por otros autores

Fig. 5 Performance of the three selected-F8-9 lines in each of six crosses compared to their parents in the registration trials in two seasons from 2018/19 to 2019/20. **a** Yield. **b** Days to physiological maturity. **c** Plant height. Values are represented by the BLUP for each line

precocity and reduce plant height at the same time. In quinoa, plant height and stem diameter are favored by an adequate planting density. For this reason, density management could be an important factor to maintain grain yields and avoid the problem of lodging by birds during physiological maturity.

Since our goal was to develop improved varieties for the Andean highlands of Peru, we carried out field trials in this environment to ensure local adaptation. This was challenging because of limited access to suitable field trial locations with a size of more than 0.3 hectares. We used the minimum number of trials (i.e. two years in three locations) required for the registration of new varieties in the Peruvian highlands.

To account for variation in plant development and small plot sizes during some stages of the breeding program, we calculated grain yield by measuring 10 individual plants to be able to compare among experiments. Selection bias occurred in the first stages of the breeding program for the lack of application of proper statistical analysis (Schmidt et al. 2019b) reflected in negative selection gain for grain yield in the selected lines (e.g.PAS×KAN). The yield can be influenced by plant density and planting techniques (Dao et al. 2020). For this reason, future research should include this information to allow comparisons among trials.

Physiological maturity optimum time for trait evaluations

Heritability is an important quantitative genetic parameter for breeding programs to predict phenotypic values of future offspring (Schmidt et al. 2019a). A sufficiently high heritability is also the main requirement for selection gain for a trait. We observed a higher heritability at physiological maturity compared to the flowering stage.

Our analyses revealed two types of variation in heritability, the first referred to the plant physiological stage and the second related to advance in the population generation. Benlhabib et al. (2016) studied recombinant-bred lines in generations F2 to F6 and obtained similar results for the heritability of plant height, panicle length, and width at different stages of plant development, with the highest values found at physiological maturity. Low heritabilities were observed in this early generation for biomass and seed diameter ($H^2 < 0.46$). We obtained a similar result in the selection trials (i.e., F7) and the heritability increased in the next generations during the registration trials (i.e. F8 to F9). These two types of variation are explained by Visscher et al. (2008): the first source of variation for the same trait measured over the lifetime of an individual may have different genetic and environmental influences so that variances become a function of age. The second source of variation is related to the fact that heritability is not a static trait and its influence on genetic background, environmental conditions, selection, and inbreeding can change. The interaction between these factors can also change over generations. It is important to mention that many studies do not mention the phenological stage at which the traits were evaluated (i.e. plant height and panicle length and width). It is recommended to use the BBCH scale (Sosa-Zuniga et al. 2017) to compare trait evaluation during crop development in different studies. This problem is also mentioned by Stanschewski et al. (2021) in the recommendations for quinoa phenotyping methods.

Although heritability estimates are population specific, we found that estimates for quinoa varied during plant development and increased with phenological maturity. A proper estimation of heritability therefore improves the efficiency of more efficient selection and increase the selection gain for future breeding populations.

Table 4 Registration trial for quinoa across multi-location trials derived from crosses of six populations under two seasons 2018–2019 and 2019–2020

Trait	Stage	Mean	Std	Min	Max	V.g	V.gxl	V.e	Ratio	H2
PlantHeight	Flowering	111.3	6.0	102.2	124.8	20.3	64.8	139.11	1:3.2:6.8	0.45
PanicleLength	Flowering	32.6	3.2	24.9	37.2	7.7	6.7	31.37	1:0.9:4.1	0.7
PanicleWidth	Flowering	33.6	3.0	27.3	38.8	4.0	11.0	44.12	1:2.8:11	0.46
DaysFlowering	Flowering	94.9	4.8	82.4	102.1	21.2	3.8	1.96	1:0.2:0.1	0.91
PanicleWidth * Length	Flowering	1112.6	162.9	735.4	1423.5	13,354.3	37,672.4	117,853.96	1:2.8:8.8	0.47
PlantHeight	Maturity	130.8	11.3	108.0	155.5	119.5	26.0	109.17	1:0.2:0.9	0.88
PanicleLength	Maturity	48.6	3.8	38.4	55.2	12.0	8.0	26.88	1:0.7:2.2	0.78
PanicleWidth	Maturity	72.5	10.2	49.9	88.9	90.2	29.1	81.82	1:0.3:0.9	0.86
DaysMaturity	Maturity	178.3	11.1	145.9	193.2	118.3	11.5	4.48	1:0.1:0	0.95
PanicleWidth * Length	Maturity	3565.6	615.4	2193.4	4762.2	303,126.5	150,660.0	467,396.64	1:0.5:1.5	0.81
HarvestIndex	Harvest	35.9	3.2	29.8	42.3	7.4	2.4	45.53	1:0.3:6.2	0.72
Yield	Harvest	43.7	10.8	25.0	69.7	98.6	29.1	108.29	1:0.3:1.1	0.84
Biomass	Harvest	125.3	28.3	72.3	181.9	634.3	328.0	869.74	1:0.5:1.4	0.79
GrainWidth	Post-harvest	2.2	0.2	2.0	2.6	0.0	0.0	0	1:0.1:0.1	0.94

Traits by phenological stage for variance components and broad-sense heritability based on Cullis' approach (H^2) in the top of the bars. V·g = genetic variance, V·gxl = genetic variance by location, V·e = error variance and the Ratio = variance ratio base in the V·g for each variance component (V·g/V·g:V·gxl/V·g:V·e/V·g). Calculations are based on 18 genotypes under five locations in the Andean highlands of Peru
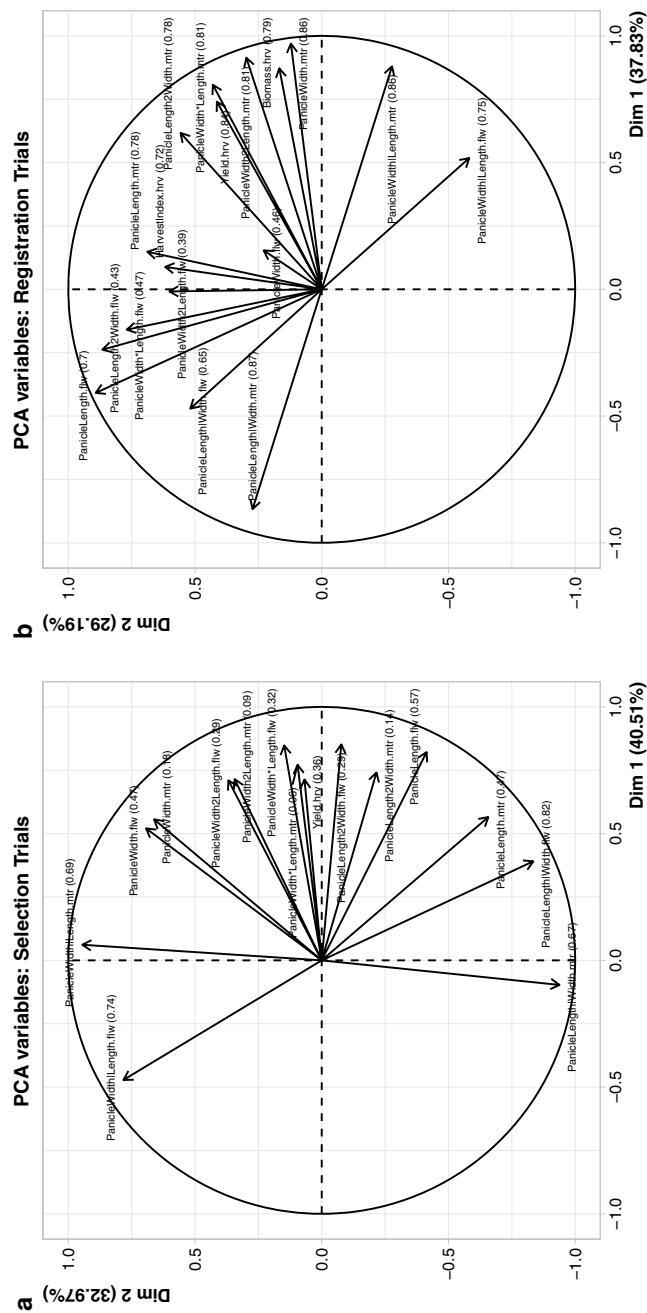
Fig. 6 Principal component analysis (PCA) from different panicle indices to correlate with grain yield in quinoa. **a** Panicle indices from the selection trials ($n = 18$ lines). Heritability values are in brackets. **b** Panicle indices from the registration trials ($n = 540$ lines). **b** Panicle indices from the selection trials ($n = 18$ lines). Heritability values are in brackets. Values represent the BLUP estimates of traits

Grain yield is associated with the product of panicle width and length

Yield is an important trait for the selection of promising lines in breeding programs. Therefore, identifying traits that aid in the selection of genotypes with a high yield potential is required. At physiological maturity, we observed a strong association of grain yield with biomass and the product of panicle width and length, respectively. This differs from Manjarres-Hernández et al. (2021a, b) who observed no relationship between phenological states and yield components, and also a low association between panicle traits and grain yield. Similar to our results, other studies found a positive correlation between grain yield with panicle length and width (Benlhabib et al. 2016; Maliro et al. 2017; Santis et al. 2018; Spehar and Santos 2005), and biomass (Benlhabib et al. 2016; Bertero et al. 2004; Bhargava et al. 2007). Other traits such as panicle density and plant density also are associated with the productivity of quinoa (Manjarres-Hernández et al. 2021b).

The evaluation of panicle traits may support quinoa breeding by selecting lines with higher yields and particular panicle characteristics such as panicle color or panicle shape idiotype. The latter trait is likely important because it may influence susceptibility to pathogens and insect pests by providing a suitable microenvironment. In modern breeding programs, high throughput phenotyping will be crucial for improving traits (Rohila et al. 2021). Automatic scoring of panicle traits via high-throughput phenotyping pipelines that use computer vision for trait extraction will allow to predict yield more efficiently.

Tambien contiene las contribuciones de cada autor y los conflictos de intereses

Conclusion

We showed that evaluation of the quantitative genetic parameters is important for maximizing efficiency and reducing the time for line selection. Physiological maturity is the optimal stage for trait evaluation because heritabilities showed the highest values. Panicle indices such as the product of panicle width and length (Width * Length) at physiological maturity support the selection of lines with higher grain yield potential. The lines HUAxKAN 53, SAL×NCO 46, and SAL×PAN 171 were identified as promising lines for cultivation in the Peruvian Highlands.

The release of these new lines as varieties will help improve incomes for farmers and help them cope with climate change and increased pest and disease pressure in the Peruvian highlands. The information generated in this work will be useful for increasing quinoa diversity and supporting future breeding programs in quinoa.

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