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Genotype-dependent phenotypic variation for agronomic traits, seed composition, and root architecture of Chilean Quinoa (*Chenopodium quinoa* Willd.)

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ABBREVIATIONS

%	Percent
°C	Celsius
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
AZ	Arid Zone
C. quinoa	*Chenopodium quinoa*
ca.	Circa
CHA	Convex Hull Area
cm	Centimeters
Cq	*Chenopodium quinoa*
DAD	Diode Array Detection
DTF	Days to Flowering
DTM	Days to Maturity
DW	Dry Weight
EI	Electron Impact
ESI	Electrospray Ionization
FAO	Food and Agriculture Organization
FD	Fresh Weight
FT	*Flowering Locus T*
g	Grams
GAE	Gallic Acid Equivalents
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GWAS	Genome-Wide Association Study
h	Hours
HD	Hederagenin
HPLC	High-Pressure Liquid Chromatography
HSD	Tukey's Honestly Significant Difference
i.e.,	That is to say (used to add Explanatory Information)
INIA	Instituto de Investigaciones Agropecuarias
IQBP	INIA's Quinoa Breeding Program
Kg	Kilograms

kV	Kilovolts
LA	Leaf Area
LAR	Leaf Area Ratio
LC-FTICR-MS	Liquid Chromatography-Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
LDM	Leaves Dry Mass
LMF	Mass Fractions Of The Leaves
LPLC	Low-Pressure Lipid Chromatography
LRL	Lateral Roots Length
LSD	Fischer's Least Significant Difference
mg	Milligrams
min	Minutes
mL	Milliliters
mM	Millimolar
µL	Microliter
µM	Micromolar
NMR	Nuclear Magnetic Resonance
NOR	Nucleolar Organizer Regions
OA	Oleanolic Acid
PA	Phytolaccagenic Acid
PCA	Principle Component Analysis
PRL	Primary Root Length
RA/LA	Root Area To Leaf Area
RAPD	Random Amplified Polymorphic DNA
RDM	Roots Dry Mass
REML	Restricted Maximum Likelihood
RIL	Recombinant Inbred Lines
RMF	Mass Fraction Of Roots
RSA	Root System Architecture
RSD	Root System Depth
RSW	Root System Width
SA	Serjanic Acid
SD	Standard Deviation
SDM	Stems Dry Mass

SMF	Mass Fraction Of Stems
SNPs	Single-Nucleotide Polymorphisms
SSR	Simple Sequence Repeat
TKW	Thousand Kernel Weight
TMS	Tandem Mass Spectrometry
TPM	Total Plant Dry Mass
TRL	Total Root Length
TSAR	*Triterpene Saponin Biosynthesis Activating Regulator*
UV	Ultraviolet energy
V	Volts
Vg	Genetic Variance
WHO	World Health Organization

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ABSTRACT

Today, climate change is one of the biggest challenges facing agriculture practices, and it has significant implications for the sustainable provision of nutritious food in sufficient amounts. To meet such challenges, there is a need for cultivars that have high yields and desirable agronomic characteristics as well as the ability to use resources more efficiently. In recent years, quinoa (*Chenopodium quinoa* Willd.), a pseudocereal crop has become increasingly popular due to its high nutritional value and adaptability to a range of harsh environmental conditions such as drought, frost, and high salinity making a promising alternative crop for cereals. Understanding the genotype-dependent phenotypic mechanism and existing variation of important agronomic traits is crucial for the development of modern quinoa breeding. The present research work was designed to characterize the existing genotype-dependent phenotypic variation of Chilean quinoa germplasm using standardized above- and below-ground related agro-morphological descriptors. The combined analysis of uni- and multivariate analysis showed a good wide significant variation ($p < 0.05$) among the evaluated *C. quinoa* accessions for agro-morphological and root-related descriptors, and allowed a deeper understanding of the interrelationship within genotypes for evaluated traits. Also, the Principle Component Analysis (PCA) and cluster analysis, revealed the appearance of some candidate genotypes that were distinctly grouped and located from the average dispersion of entire studied accessions. These accessions were associated with early precocity, high seed yield, and vigorous rooting pattern. Other than that, the content of antinutrient triterpenoid saponins and bioactive phenolic components were assessed using GC-MS/LC-MS for the available *C. quinoa* germplasm. The variation in the content and type of these metabolites in quinoa genetic resources were considered significant. The relative content for the saponins and phenolics were in ranged between 0.22 mg/g to 15.04 mg/g and 35.51 mg/100 g to 93.23 mg/100 g of seed dry weight, respectively. Our study found significant variability concerning agro-morphological descriptors, secondary metabolites content, and rooting pattern in *C. quinoa*, which can contribute to the value of genetic resources for the identification of ideal genotypes that might be used in current and future *C. quinoa* breeding programs.

Keywords: Quinoa, aro-morphological traits, saponins, phenolic compounds, phenotyping, GC-MS/LC-MS, root traits, rhizobox.

KURZFASSUNG

Der Klimawandel ist heute eine der größten Herausforderungen für die landwirtschaftliche Praxis und hat erhebliche Auswirkungen auf die nachhaltige Bereitstellung von nahrhaften Lebensmitteln in ausreichender Menge. Um diese Herausforderungen zu meistern, werden Sorten mit hohen Erträgen und wünschenswerten agronomischen Eigenschaften sowie der Fähigkeit, Ressourcen effizienter zu nutzen, benötigt. In den letzten Jahren hat Quinoa (*Chenopodium quinoa* Willd.), eine Pseudogetreidepflanze, aufgrund ihres hohen Nährwerts und ihrer Anpassungsfähigkeit an eine Reihe rauer Umweltbedingungen wie Trockenheit, Frost und hoher Salzgehalt zunehmend an Beliebtheit gewonnen und ist damit eine vielversprechende Alternative zu Getreide. Das Verständnis der genotypabhängigen phänotypischen Mechanismen und der bestehenden Variation wichtiger agronomischer Merkmale ist für die Entwicklung einer modernen Quinoa-Züchtung von entscheidender Bedeutung. Die vorliegende Forschungsarbeit diente der Charakterisierung der bestehenden genotypabhängigen phänotypischen Variation des chilenischen Quinoa-Keimplasmas unter Verwendung standardisierter agromorphologischer Deskriptoren, die über und unter dem Boden liegen. Die kombinierte Analyse von uni- und multivariaten Analysen zeigte eine große signifikante Variation ($p < 0.05$) zwischen den bewerteten *C. quinoa* Akzessionen für agromorphologische und wurzelbezogene Deskriptoren und ermöglichte ein tieferes Verständnis der Wechselbeziehungen innerhalb der Genotypen für die bewerteten Merkmale. Auch die Hauptkomponentenanalyse (PCA) und die Clusteranalyse ergaben das Auftreten einiger Kandidatengenotypen, die deutlich gruppiert waren und sich von der durchschnittlichen Streuung der gesamten untersuchten Akzessionen abhoben. Diese Akzessionen zeichneten sich durch eine frühe Frühreife, einen hohen Samenertrag und eine kräftige Bewurzelung aus. Darüber hinaus wurde der Gehalt an Triterpensaponinen und bioaktiven phenolischen Komponenten mittels GC-MS/LC-MS für das verfügbare *C. quinoa* Keimplasma bestimmt. Die Unterschiede im Gehalt und in der Art dieser Metaboliten in den genetischen Ressourcen von Quinoa wurden als signifikant angesehen. Der relative Gehalt an Saponinen und Phenolen lag zwischen 0.22 mg/g und 15.04 mg/g bzw. 35.51 mg/100 g und 93.23 mg/100 g Saatentrockengewicht. Unsere Studie ergab eine signifikante Variabilität in Bezug auf agromorphologische Deskriptoren, den Gehalt an sekundären Metaboliten und das Bewurzelungsmuster von *C. quinoa*, was zum Wert der genetischen Ressourcen für die Identifizierung idealer Genotypen beitragen kann, die in aktuellen und zukünftigen Zuchtprogrammen für *C. quinoa* verwendet werden könnten.

Stichworte: Quinoa, agro-morphologische Merkmale, Saponine, phenolische Verbindungen, Phänotypisierung, GC-MS/LC-MS, Wurzelmerkmale, Rhizobox.

Introductory Remarks

1 Introduction

In recent years, there has been a growing trend for the utilization of *Chenopodium quinoa* Willd. (*C. quinoa*) based on the increasing need for its nutritious seeds in the international market and its desirable tolerance to abiotic stresses. *C. quinoa*, an Andean crop, could be an excellent alternative crop in many countries to partly face global climate challenges in agriculture.

C. quinoa is an allotetraploid (*2n* = 36), which belongs to the Amaranthaceae family. *C. quinoa* has been adapted and cultivated by indigenous people in the Andean regions at different altitude levels. Since its domestication began several thousand years ago, *C. quinoa* has turned out to be an essential component of the diet of the Andean civilizations. *C. quinoa* seeds contain higher protein levels compared with all the major cereal crops, are gluten-free, and also provide an excellent balance of vitamins, carbohydrates, lipids, fiber, and minerals (Mohamed Ahmed et al. 2021; Bastidas et al. 2016; Vega-Gálvez et al. 2010). Furthermore, these seeds are also a good source of phenolic compounds that possess antioxidant properties (Pereira et al. 2020). Today, Bolivia and Peru are the main exporters of *C. quinoa* next to the USA, Ecuador, Argentina, and Chile. The cultivation of quinoa has been extended to other regions including China, Vietnam, Canada, India, and several EU countries. In 2018, *C. quinoa*'s worldwide production amounted to 158,920 metric tons and has increased in the following years[1]. However, such an increase in production is largely achieved due to the expansion in *C. quinoa* cultivation area. Even though there were improvements in production, the yield per hectare showed fluctuations in the last 20 years[1]. At present, Europe, as a major *C. quinoa* consumer[2], is also seeking alternative modes to increase *C. quinoa* production to meet demand and develop new markets. Overall, there is an increased interest in expanding the growth of *C. quinoa* worldwide. New varieties have started to be bred to adapt the growth of *C. quinoa* to new climatic conditions such as in north-western Europe and the USA. However, the genetic diversity of *C. quinoa* and the establishment of new breeding programs to develop new varieties better adapted to different environmental conditions remain largely unexplored.

1.1 Adaptation to different geographical regions and genetic variability of various morphological traits in *C. quinoa*

Crop adaptation to growth-limiting environmental conditions results from the allelic variation seen in the species that live in these conditions, which ensures stable growth in the offspring. Phenotypic plasticity and possessing a wide genetic diversity contribute to the potential adaptation of crops under

[1] FAOSTAT (https://www.fao.org/faostat/en/#home)
[2] The European market potential for quinoa | CBI

various environmental conditions. *C. quinoa* is a good example of a crop that has large diversity and phenotypic plasticity ensuing from vast adaptation. The widespread distribution of *C. quinoa* throughout the Andean area, which includes Colombia, Ecuador, Peru, Bolivia, Chile, and northern Argentina, attests to both the genetic and phenotypic diversity of the plant and to its adaptability to agro-ecological regions. *C. quinoa* is able to survive adverse climatic events and thrive in areas where only a few crops can (Murphy et al. 2018; Bonifacio 2003). The ecotypes of *C. quinoa* are adapted to different geographical regions that have been defined as; (1) Valley (the inter-Andean valleys); (2) Altiplano (the highland plateau of Bolivia and Peru); (3) Salares (the salt flats of the high Andean plateau, region of Bolivia and Chile); (4) Coastal or Sea-level (the central and south Chile); (5) Subtropical (the Bolivian Yungas region); (Hinojosa et al. 2018; Tapia 2015). The genetic and phenotypic diversity of *C. quinoa* has been shown regarding morphological and physiological traits, growth habits, and seed composition.

Over time, researchers have revealed how the genetic diversity of *C. quinoa* can be used to understand its genetic structure and to predict its biological diversity depending on its eco-geographical distribution (Patiranage et al. 2022; Schmöckel 2021; Murphy et al. 2018). Researchers have explored the genetic diversity in *C. quinoa* through morphological markers (EL-Harty et al. 2021; Emrani et al. 2020; Peterson et al. 2015; Ward 2000a; Wilson 1988a). Such information is needed in *C. quinoa* germplasm management and evaluation, and conservation approaches. For *C. quinoa* breeding programs, knowledge of genetic diversity is also crucial, especially to introduce elite genotypes through a selection of parental combinations.

During the 1980s, Hugh Wilson initiated molecular studies in *C. quinoa*. These studies highlighted the genetic variability using morphological and isozyme data among *C. quinoa* germplasm from both Andean highland and coastal-lowland zones (Wilson 1988a, b; Wilson 1981). Towards a similar goal, in another study, morphological traits and agronomic performance were evaluated to understand the genetic diversity of *C. quinoa* germplasm collected from different geographic regions ranging between highland (Bolivia) and coastal-lowland (Chile) (Rojas 2003). In this study using multivariate approaches including principal component analysis and cluster analysis, *C. quinoa* germplasm was classified into seven individual groups. Two clusters were found to be the center of the lower altitude locality of the eastern Andean mountain range whereas, five clusters were found to be the centers within the Altiplano region. However, the traits evaluated in the study did not distinguish clearly for the Chilean germplasm, which is categorized among the Altiplano clusters.

Bhargava et al. (2007a) used various morphological and qualitative traits to analyze genetic variability and the interrelationship of variables among diverse *C. quinoa* germplasm in the Indian subtropical

region. Such morphological and quality traits were reviewed to determine their selection criteria for *C. quinoa* breeding efforts. Moreover, Bhargava et al. (2007b) used various morphological and qualitative traits to investigate genetic variability in *C. quinoa* germplasm lines. In this study principal component and cluster analysis were carried out for variables that revealed great genetic variability existing in *C. quinoa* lines. However, multivariate analysis grouped those lines with great genetic similarity but did not group those lines of the same origin, implying the heterogenicity of studied lines within a geographical area. Bhargava et al. (2007b) suggested that selection processes, population genetic structure, heterogenicity and/or developmental traits might explain such population variability within a geographic area.

Fuentes and Bhargava (2011) published the first research work on morphological traits analysis of *C. quinoa* germplasm grown in a lowland desert environment. In this study, diverse morphological descriptors, i.e., inflorescence-related traits, shoot architecture, yield, and harvest index, were assessed for *C. quinoa* accessions collected from the northern highland regions of Chile, and cluster analysis grouped the studied accession into six distinct groups. Significantly, the reported data would enable breeders to expand the genetic base of elite accessions through a selection of genotypes based on important morphological characteristics.

In the northwestern Argentinian region, *C. quinoa* is seen as marginal from the perspective of its cultivation. Curti et al. (2012) studied the phenotypic variability in quantitative and qualitative traits within a set of cultivated *C. quinoa* populations from northwestern Argentina. In this study, the data set was analyzed by using descriptive and multivariate techniques. Based upon quantitative traits, principal component analysis and clustering differentiated among accessions from contrasted ecogeographic zones in particular highlands, transition zone, central dry valleys, and eastern valleys. In contrast, based upon qualitative traits multivariate analysis differentiated accessions from a transition zone and eastern valleys. The authors suggested that the accessions from highlands and dry valleys showed advanced domesticated characteristics, whereas accession from the transition zone and eastern valleys showed characteristics similar to wild-type Chenopods from the Andean regions.

More recently Madrid et al. (2018) studied the phenotypic variation of coastal-lowland *C. quinoa* genotypes using plant morphological and structural variables (i.e., shoot architecture, panicle structure, growth habit, yield-related traits, etc.) to identify potential candidate genotypes for better agronomic performance in a rainfed environment in central Chile. Principal component analysis and clustering techniques were used as an efficient method to distinguish *C. quinoa* germplasm and, as a result, the entire germplasm was grouped into seven groups based on their morphological performance.

Multivariate analysis has also shown the inter-relationship among variables that could significantly be improved and classified promising genetic lines for further breeding efforts.

Similarly, EL-Harty et al. (2021) performed the morphological and molecular characterization of *C. quinoa* genotypes using several descriptors such as days to maturity, shoot architecture, panicle structure, and growth habit in Saudi Arabia. This study has confirmed the differences among the genotypes through morphological and molecular markers. Cluster analysis based on the Euclidean distance coefficient grouped the studied *C. quinoa* genotypes into four and two distinct groups respective to their origin or genetic background via both phenotypic and molecular assessments. This work addressed the utility of undertaking molecular-assisted breeding. The output of this study confirmed the existing genetic variation due to the significant differences within the population. In another study on the selection of promising material for *C. quinoa* breeding, Manjarres-Hernández et al. (2021) used quantitative and qualitative morphological descriptors for the phenotypic assessment of *C. quinoa* accessions. Discrimination of the studied accessions based on their agronomic characteristics suggested candidate genotypes as potential parents for crosses to be introduced in *C. quinoa* breeding programs.

Overall, the characterization of *C. quinoa* genotypes using morphological and molecular descriptors allows the effective selection of candidate genotypes that exhibit desired characteristics and address the needs of farmers and breeders for the development of genetic improvement strategies.

1.2 Antinutrient and bioactive components in *C. quinoa*

Despite the balanced nutritional composition of its seeds, *C. quinoa* contains a mixture of secondary glycosylated metabolites named saponins (El Hazzam et al. 2020). Saponins are seen as antinutrient components that confer bitterness to seeds and derived products and are considered toxic when ingested in high amounts. Until now, to the best of our knowledge, there have not been published reviews that confirm the role of *C. quinoa* saponins and the exact factors that determine their biosynthesis during *C. quinoa* growth. However, their presence is mainly considered as a plant defense mechanism against pathogens. (Gee et al. 1993; Ridout et al. 1991). Saponins show various biological and physico-chemical properties such as antioxidant, antimicrobial, antiviral, immunostimulant, anti-inflammatory, and hemolytic activities (El Hazzam et al. 2020). Recently, saponins have received close attention through a wide array of pharmacological and biological properties (El Hazzam et al. 2020; Ahumada et al. 2016; Chwalek et al. 2006; Escalante et al. 2002; Southeeswaran and Kenchington 1989). As mentioned above, saponins give a bitter taste to seeds which varies between genotypes, from bitter to sweet or low saponins varieties. Since saponins confer bitterness, they must be removed before the

consumption of seeds. Several processes have been described to wash off saponins from the seed pericarp.

As early as 1991, Ridout and his group set out the water-based washing method for saponins separation and analysis from raw *C. quinoa* seed. The reported work was also the first to classify the degree of bitterness of *C. quinoa* based on its saponin content (Ridout et al. 1991). In other experiments, it has been stated that the afrosimetric method, which involves stirring *C. quinoa* seeds vigorously with distilled water on a magnetic stirrer, had made it possible to sort sweet and bitter *C. quinoa* varieties through taste testing. The conventional afrosimetric method was established to save considerable time to extract saponins from seeds (Koziol 1991; Kozioł 1992). However, this method has been proven to be more effective in the field when distinguishing the low saponins varieties or testing the performance of the abrasive seed peeling. Unlike aqueous extracts used in the lab, a commercial washing approach eliminated around 72% of the saponins from the seeds, and the saponin content varied from 0% to 2% based on sweet or bitter variety (Gee et al. 1993). Further, it has been reported that the saponin content in seeds washed only with water was reduced by about 20% of the original content, whereas saponin content was reduced to less than 0.06% in seeds treated by the industrial wet approach that involves thorough cleaning with water, combined with a dry method involving mechanical abrasion referred to as scarification (Pappier et al. 2008). The aforementioned study suggested that the commercial approach was much more severe than the laboratory approach (Gee et al. 1993). From a different perspective, Ruales and his colleagues reported the effect of the wet approach on the nutritional quality of *C. quinoa* seeds and their research work did not identify any significant change in the composition of amino acids after washing, which suggests that the wet approach of saponin removal does not affect the protein quality of *C. quinoa* seeds (Ruales and Nair 1993).

Progressively, various analytical approaches such as low-pressure lipid chromatography (LPLC), high-pressure liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS) have been introduced for the detection of saponins in *C. quinoa* seeds. For example, the effects of the heat treatments on the chemical profile of *C. quinoa* flour have been analyzed by using HPLC (Brady et al. 2007). This study showed that heating treatments can deteriorate saponins, which could lower the bitterness conferred by saponins. In another study, Medina-Meza and his team studied GC-MS profiling of saponins from several *C. quinoa* varieties and maps the content of saponins, and reported the differences in saponin content among the studied genotypes (Medina-Meza et al. 2016). In his research work, a multivariate analysis was carried out to group the *C. quinoa* genotypes based on their sapogenin profile, which classified the genotypes into “bitter varieties” and “low saponin content varieties”.

Moreover, other approaches for saponins removal have been tested, including genetic approaches. A study on the heritability of saponin content from the parental breed to its progeny has been already published (Ward 2000b). This research work showed a slow reduction in saponins content in the self-pollinated S4 generation, and the reason for this slow reduction was found to be due to the dominance of the allele that is responsible for the expression of saponins. This implies that the recessive alleles for saponins expression need to be present at the concerned loci (Ward 2000b). Later, Maughan et al. (2004) and Jarvis et al. (2008) published linkage maps contingent on several molecular tools prepared using *C. quinoa* recombinant inbred lines (RILs), including Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP), and Random Amplified Polymorphic DNA (RAPD) markers; the Nucleolar Organizer Regions (NOR) and morphological color loci (Jarvis et al. 2008; Maughan et al. 2004). A new epoch of *C. quinoa* research started in early 2017 when a genome sequence of *C. quinoa* was published, which also accelerated the identification of saponin biosynthesis genes and their regulators such as *triterpene saponin biosynthesis activating regulator 1* and *2* (*TSAR 1* and *TSAR 2*); (Jarvis et al. 2017). Such research work facilitated the uncovering of the transcriptional mechanisms that regulate saponins production, including alternative splicing, which is a cause of premature stop codon in sweet varieties. Yet, genes regulating the scarcity of saponins in sweet varieties are unexplored. Recently, researchers have been able to unravel the genetic mechanisms of agronomically important *C. quinoa* traits, including saponins (Patiranage et al. 2022). In this research work, a genome-wide association mapping uncovered SNPs stably associated with the relevant loci for agronomically important traits, including saponins. The results showed a significant marker – traits association for saponin content on the Cq5B chromosome which covered genes that being reported to control saponin content in *C. quinoa* (Patiranage et al. 2022; Jarvis et al. 2017).

Besides the antinutrient properties, *C. quinoa* seed is a reliable source of bioactive phenolic compounds. Accordingly, during the last years, phenolic substances have been the most explored health-beneficial compounds in *C. quinoa*. In *C. quinoa* seeds, phenolic compounds have been mainly characterized and quantified by spectrophotometric or chromatographic methods. Such assessment for phenolic compounds is determined as total phenolics, and both free and bound forms of phenolics. Referring to spectrophotometric assay, a simple and relatively rapid Folin–Ciocalteu assay is mainly used for the evaluation of phenolic compounds. Other than that, the Prussian Blue assay is also commonly used to assess the content of phenolics in *C. quinoa*. A study on plant secondary metabolites and genetic evaluation of *C. quinoa* germplasm have been reported before (Saad-Allah and Youssef 2018). In this study, a variation in antioxidant phenolic compounds was likely due to the differences in *C. quinoa* genotypes. In other studies, phenolic compounds had also been uncovered in *C. quinoa* collected from different Asian territories, in particular, India, China, and Korea (Lim et al. 2020; Liu et al. 2020; Han et al. 2019; Li et al. 2018; Kaur et al. 2016). Such surveys showed that the contrast in

the phenolics content among *C. quinoa* samples could be due to different genetic backgrounds, as well as different pedo-climatic conditions, and the effect of sample processing approaches. Phenolic contents have been often studied on *C. quinoa* of diverse geographical origins, in particular, the USA, Peru, Chile, Argentina, Denmark, and Poland (Sobota et al. 2020; Vega Gálvez et al. 2018; Lee and Sim 2018). These research efforts offer a valuable basis for assessing the nutritional quality, and antioxidant and antimicrobial properties of *C. quinoa* seed. Flavonoids, a specific sub-group of phenolic compounds, have also been assessed by spectrophotometric methods, and their amount was assigned in their both free and bound fractions as total flavonoid contents. Beyond the spectrophotometric approach, profiling-based methods such as high-pressure liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS) have been introduced for the identification and quantification of phenolic compounds in *C. quinoa*. Such approaches overcome certain limitations of the Folin-Ciocalteu assay, such as possible overestimation of compounds (upon the reaction of non-polyphenolic interfering substances) and decrease in sugar moiety through the Folin-Ciocalteu reagents. Further, such approaches allow the quantification of a comparatively larger amount of phenolic components as possible in one extract. Like most other grains, various studies on the antioxidant compounds of *C. quinoa* seeds using a profiling-based technique have been published. In *C. quinoa,* profiling of free and bound fractions of polyphenol has been performed by liquid chromatographic approach by Gomez-Caravaca and collaborators (Gómez-Caravaca et al. 2011). The mass spectrum data tentatively reported altogether 30 bioactive phenolic compounds present in various amounts in *C. quinoa* seeds (Gómez-Caravaca et al. 2011). Later, Carciochi et al. (2016) used a reversed-phase HPLC technique for the determination of various phenolic acids and flavonoids of *C. quinoa* seeds from Argentina. This work addressed the effect of temperature on the phenolic contents and the antioxidant properties. The analysis detected and validated the natural presence of six phenolic compounds in seed extracts. Also, a study on the flavonoid glycosides content of Chilean *C. quinoa* germplasm has been reported earlier (Graf et al. 2016). In this study, differences in the flavonoids content among distinct *C. quinoa* genotypes were determined and revealed a correlation with the genetic background. The results showed significant variations in flavonoids content across studied samples. Furthermore, highland varieties had a total flavonoids content 2.6-fold greater compared with coastal-lowland varieties grown under the same environmental conditions. The authors suggested that the content of flavonoids might be genotypically controlled in *C. quinoa*. At a later time, a study by Vega Gálvez concluded that the *C. quinoa* ecotypes hold a great potential source of bioactive compounds and dietary fibers (Vega Gálvez et al. 2018). Such research work contributes to detailed knowledge of phenolics, flavonoids, and isoflavones content in Chilean *C. quinoa* seeds. Paucar-Menacho and his colleagues carried out phenolic profiles of Peruvian *C. quinoa* grains using LC-MS and HPLC (Paucar-Menacho et al. 2018).

In this study, the complete analysis allowed the detection of 18 phenolic derivatives in *C. quinoa*. The results of the study concluded outstanding variations in the group of phenolic acids and flavonoids in both raw and heat expanded samples. Also known commercial cultivars such as Titicaca and Puno, have been shown as rich sources of bioactive compounds and as having important antioxidant activities (Stikić et al. 2020). Profiling analysis detected and quantified 13 phenolic compounds in the extracts of seeds of both cultivars that were grown in Southeastern European agri-environmental conditions. However, the profile of individual phenolic compounds and their concentration differed greatly. Recently, an article on free and conjugated phenolic compounds profiles in *C. quinoa* seeds and their interconnection with genetic background has been also reported (Antognoni et al. 2021). The results of this study showed genotype-dependent differences in polyphenols and also proposed that agroecological conditions may alter the polyphenols contents, at least to some extent.

In general, the information contained in the literature gives an overview of saponins and polyphenols in *C. quinoa* including extraction methods, variously identified chemical structures, and genotype-dependent differences in secondary metabolite content.

1.3 Root system growth in *C. quinoa*

Root systems vary significantly among species, and within species that are subject to different environmental and genetic factors. The root system is often undervalued largely due to its difficult accessibility for direct observations. Recently, researchers have become more interested in the hidden half of plants - their roots. Various attempts are being made to phenotype these systems. (Waisel et al. 2005).

Roots play an important role in plant growth by providing essential nutrients and water, and anchoring the plant to the ground. They are also the site of interaction with beneficial organisms in the rhizosphere. The resilience of root system growth in response to various agroecological conditions provides the potential for exploring natural variation to ensure root traits to improve plant productivity in agricultural systems (Paez-Garcia et al. 2015; Alvarez-Flores et al. 2014a; Smith and De Smet 2012). In both natural and agricultural systems, plant growth and survival depend directly on root traits that allow the plants to acquire available soil resources.

Comparative studies of plant phenotyping and morphology could confer certain insights referring to various plant traits evolved under human and natural selection among the Andean *Chenopodium* species. In *C. quinoa*, morphological characteristics and/or molecular markers have suggested that genetic diversity is associated with ecogeographic structures. (Maldonado-Taipe et al. 2022; Patiranage et al. 2022; Emrani et al. 2020; Costa Tártara et al. 2012; Leonardo et al. 2009; Bhargava et al. 2007a). However, detailed information on the root traits of *C. quinoa* remains mostly unknown despite few

studies on root architecture and morphology and on the crucial role of roots in seizing nutrients and soil water resources. Specifically in adverse environments, wherein early, fast, and vigorous rooting is essential to acquire scarce soil resources, root traits such as total root length, rooting depth, and root diameter are crucial for successful plant growth at later development stages (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b; Lamb et al. 2012).

In the Andes region, under climatically severe and contrasting habitats, a diversity of little-known species of *Chenopodium* species is present, as chenopods (i.e. *Chenopodium hircinum*, *Chenopodium carnosolum*, *Chenopodium petiolare*), and as quinoa (*Chenopodium quinoa*), and Cañahua[3] (*Chenopodium pallidicaule*) (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b; Bonifacio 2003). *Chenopodium* species, specifically, *C. quinoa* from low-resource habitats have faster root growth during the early development stages and a larger and more elongated root system (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b). Alvarez-Flores et al. (2014a) studied the root morphology of cultivated *Chenopodium* populations (*C. quinoa*) and the population of their wild relatives. In this study, *C. quinoa* has shown higher relative root growth and better potential to explore soil resources at depth during early developmental stages. Alvarez-Flores et al. (2014b) scrutinized the variations in root architecture and rooting patterns in *Chenopodium* species including two ecotypes of *C. quinoa*. *C. quinoa* holds a herringbone root structure, and a fast and vigorous rooting system compare to other *Chenopodium* species (Alvarez-Flores et al. 2014b). Rooting plasticity is important for crops capitalizing on erratic soil water resources. A critical perspective article by Alvarez-Flores et al. (2018), clearly unravels rooting plasticity in wild and cultivated *Chenopodium* species under both wet-dry soil profiles in a controlled environment. Variation in innate root traits and resilience responses among *C. quinoa* ecotypes affects its root foraging capability for nutrient and soil water resources (Alvarez-Flores et al. 2018). All studies on root system architecture and root morphology of Andean *Chenopodium* species showed that *C. quinoa* has comparatively more root biomass placed at superficial soil horizons surface level, but better plasticity with depth which contributed to improved plant growth and adaptation to varying soil resource availability (Alvarez-Flores et al. 2018; Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b). The inconsistent improvement between shoots and roots is also noted as the necessity for supporting targets to enhance the progressions of both biomass and yield-related shoot traits, and root traits relating to nutrient use efficiency in soybean (Li et al. 2019).

In the plant model organism *Arabidopsis thaliana*, it is well-known that root system growth could differ based on the environmental conditions that impact the three-dimensional distribution of the primary and lateral roots (Deja-Muylle et al. 2021; Del Bianco and Kepinski 2018). However, the plasticity of

[3] Also written as cañihua, cañiwa, kañihua, kaniwa, cañigua, etc.

traits is also dependent on genetic variation responsible for the formation of root architecture. Indigenous populations within a species may have adapted the growth of their root systems in response to common environmental factors, which could become an inherited trait. Plant species and genotypes among species vary in root trait expression, and in their ability to access nutrients and soil water. Genetically diverse panels are now been used to explore variability in root system growth (Deja-Muylle et al. 2020; Chen et al. 2017). However, low spatial resolution and a relatively low-throughput hamper the measurement of root system growth and open questions concerning which methodologies can be employed for the phenotyping of a large set of genotypes for mapping studies. At present, high-throughput phenotyping approaches to root systems are important for recognizing genotypes with distinct root system growth that has resulted in an increased ability to adapt plant growth to ever-changing climatic conditions (Nagel et al. 2012). Some root traits such as total root length, root length at relative depths, convex hull area, and root diameter can be used as a framework for breeding programs intended for a promising cultivar with better resource use efficiency and adaptation to harsh environmental conditions.

In conclusion, root systems play a significant role in resource acquisition and adaptation to environmental stress. Various technologies are being developed to study root traits more accurately, which will help us understand the relationship between root architecture and function and the complexity of root-soil interactions. This knowledge will lead to improved crop performance and productivity.

The main intent of the reported research work is to examine genotype-dependent phenotypic variation in agronomic traits, seed composition, and root architecture of Chilean quinoa germplasm (*C. quinoa* Willd.). The coastal-lowland quinoa genotypes are mostly day-length neutral, therefore they can be cultivated at a wider range of Northern latitudes including in Europe. This aspect makes them a good choice for future variety development, since other ecotypes that are strongly affected by photoperiod may not be suitable for certain regions. To address this research, the overall scientific work is separated independently into four substantial chapters, as follows.

Chapter 1 outlines the existing phenotypic diversity in a large collection of Chilean *C. quinoa* germplasm over agronomically important traits. In addition, an understanding of inter-correlation among agronomic traits might be useful to improve the selection criteria for the *C. quinoa* breeding program.

Chapter 2 provides an overview of the variability in saponins content among *C. quinoa* germplasm. Besides, structure elucidation of saponins and examination of their fragmentation spectrum enables the detection of aglycones as well as sugar moieties and their positions in the saponins.

Chapter 3 examines both total phenolics and individual phenolic compounds among *C. quinoa* genotypes to assess the genetic variance of *C. quinoa* seeds. Detail statistics of phenolic compounds will assist in the initial recognition of candidate accessions relative to higher phenolic contents which could help to establish a good source of bioactive compounds to combat health problems. In the context of breeding programs, the overall statistics of bioactive compounds can be used to develop molecular markers and specify genomic regions that are linked to bioactive compounds for the *C. quinoa* breeding program.

Chapter 4 investigates the variability of *C. quinoa* root system growth in controlled environment conditions in rhizoboxes using a subset of the Chilean germplasm, as well as previously characterized genotypes. The knowledge of root trait growth will allow us to unveil genotypes with a vigorous root system and with comparatively higher resource use efficiency.

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Chapter 1: Phenotypic diversity for key agronomic and morphological traits of Chilean quinoa (*Chenopodium quinoa* Willd.) germplasm

1 Introduction

One of the biggest challenges for today's agriculture is the sustainable provision of nutritious food in sufficient amounts in face of climate change. Such a goal results in increased demand for the development of cultivars that have high yield and desirable agronomic characteristics, as well as the ability to exploit more efficiently the limited resources available.

In recent years, *Chenopodium quinoa* Willd. (quinoa) has gained attention for the high nutritional content of its seeds and its tolerance to abiotic stresses making it a potential alternative crop to cereals, for example in defined crop rotations or on marginal lands. *C. quinoa* is an annual pseudocereal crop and it belongs to the genus *Chenopodium* (Amaranthaceae). *C. quinoa* is native to the Andean region of South America, where it has been grown for more than 7,000 years (Fleming and Galwey 1995). It was an important pre-Columbian crop known as the "Mother Grain" in Incan culture (Risi C and Galwey 1984). However, due to the cultural and religious significance of *C. quinoa*, Spanish conquistadores suppressed its use. Only recently *C. quinoa* production has increased, due largely to international recognition of the high nutritional value of its seeds, which have high protein levels compared to major cereal crops. In addition, *C. quinoa* seeds also provide an excellent balance of amino acids, vitamins, lipids, fibers, carbohydrates, and minerals (Vega-Gálvez et al. 2010). Because *C. quinoa* has the potential to provide a staple food source, the Food and Agriculture Organization (FAO) referred to quinoa as the "super grain of the future" and declared 2013 as the international year of quinoa.[4] Together with seed composition, it is of great interest that, quinoa is tolerant to many adverse environmental conditions, including salinity, drought, frost, heat, and high UV irradiance (Dumschott et al. 2022; Adolf et al. 2012; Jacobsen et al. 2003; Fleming and Galwey 1995). Because of these reasons, *C. quinoa* cultivation has remarkably increased from South America to other world regions including Asia, Africa, Europe, and North America.

Based on its geographical adaptation and distribution, *C. quinoa* is broadly classified into five ecotypes, as (1) Inter-Andean valley: ecotypes which are grown at 2000 to 3500 m.a.s.l.; (2) Altiplano: ecotypes grown at high altitudes around lake Titicaca (more than 3500 m.a.s.l.); (3) Salares: ecotypes grown in the salt flats of the high Andean plateau (regions of Chile and Bolivia) and have a high salinity tolerance; (4) Coastal or sea-level: ecotypes grown in the low altitude regions of central and southern

[4] Nutritional value- International Year of Quinoa 2013 (fao.org)

Chile; (5) Subtropical or Yungas: ecotypes grown in the eastern slope of the Andes region along humid and rainy valleys of Bolivia (**Figure 1.1**) (Hinojosa et al. 2018; Tapia 2015).

Despite *C. quinoa*'s potential, it is still an underutilized crop (Massawe et al. 2016), with active breeding programs having only recently emerged (Patirange et al. 2020; Jarvis et al. 2017; Zurita-Silva et al. 2014). At present, the significance has become specifically on its introduction to promote agroecological areas. Relatively few efforts have been made to improve the crop for important agronomic and morphological traits and to understand the mechanisms of its notable tolerance to abiotic stresses (Manjarres-Hernández et al. 2021a; EL-Harty et al. 2021; Mizuno et al. 2020; Fuentes and Bhargava 2011; Leonardo et al. 2009). The key intention of the *C. quinoa* breeders is to introduce *C. quinoa* cultivars with high yields adapted to adverse agro-climatic conditions. Plant breeding for targeted traits and for target cultivation environments needs to access widely diverse genotypes as it allows us to exploit the within-species variability of different traits and their interactions. Therefore, the success of these developing breeding programs will depend on assessing the genetic and phenotypic variability of populations encompassing suitable diversity. The amount of existing diversity in the germplasm defines the limits of the selection process necessary to achieve genetic improvement. *C. quinoa* holds an extensive range of both genetic and morphological variations (Murphy et al. 2018; Bioversity International 2013). Improvement of important agronomical traits such as shoot svariation

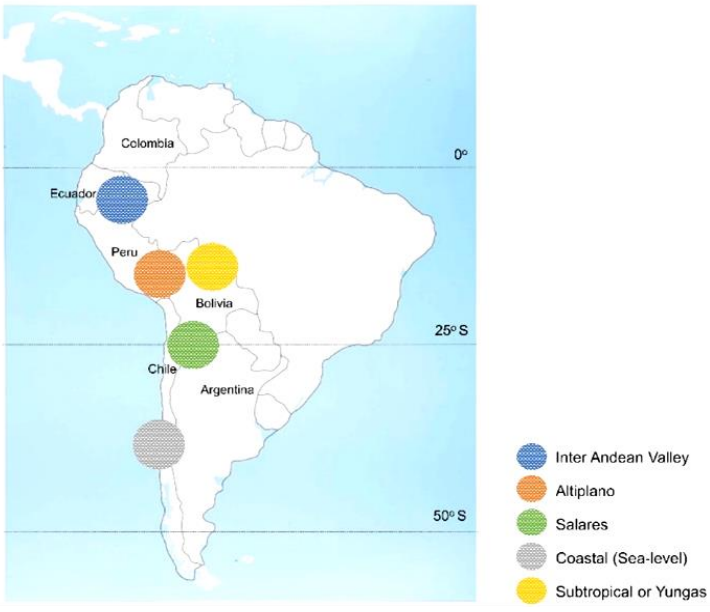

Figure 1.1. Geographic adaptation and distribution of *C. quinoa* ecotypes. Quinoa ecotypes are classified in to 1. Inter-Andean valley plants adapted to grow at 2000-3500 m in regions having annual rainfall of 800-900 mm. Plants belonging to the Andean valleys are long, thick-stemmed and branched, and have small to large seed sizes. 2. Altiplano plants grow at higher altitudes of 3850 m where annual rainfall is between 700-800 mm. Altiplano plants have a comparatively shorter height and produce small- to medium-size seeds. 3. Salares plants adapted to grow in the southern highlands at altitudes of over 3800-4200 m with low annual rainfall. Plants are large with more branches and produce bigger seeds. 4. Coastal region plants are cultivated near sea areas where annual rainfall is about 500 mm. Plants belonging to this region have medium branching and produce small size seeds. 5. Subtropical landraces grow at altitudes of 1500-2000 m and in rainy or humid areas. These are tall plants and produce small size seeds (Hinojosa et al. 2018; Tapia 2015).

existing in the available germplasm, interdependency of qualitative and quantitative morphological characters with physiological traits, as well as traits heritability and percentage of genetic variance.

In this study, the assessment of phenotypic diversity was carried out based on a large collection of the Chilean *C. quinoa* germplasm. In the northern part of Chile, *C. quinoa* is cultivated by indigenous Aymara Indians in the northern Altiplano of Chile, however, *C. quinoa* cultivation extends to central and southern regions of Chile. Chilean *C. quinoa* ecotypes are distinguished by a wide variety of morphological variations that resulted from natural or human selection and genetic drift as ecotypes were introduced to central and south Chile via the trade and movement of indigenous peoples. Studies of agronomic and morphological traits variation of Chilean highland and coastal ecotypes for initial recognition of local varieties, and for quinoa breeding to improve quinoa performance on-farm have only been recently started. In the current study, we characterized the variation of the agronomic and morphological traits among the Chilean *C. quinoa* germplasm focusing on coastal-lowland ecotypes with the following research objectives:

1. To determine the existing genetic diversity among *C. quinoa* germplasm for agronomically important quantitative and qualitative traits that can be selected directly or indirectly.
2. To interpret the interrelation among morphological traits using correlation analysis to strengthen selection criteria with a multi-trait approach for developing *C. quinoa* breeding program.

To our knowledge, coastal-lowland genotypes are mostly day-length-neutral, and because of this feature they can be cultivated at more Northern latitudes including in Europe (Patiranage et al. 2021). For these reasons, it is attractive to focus on these genotypes for future variety development, whereas other ecotypes with flowering time strongly dependent on photoperiod may be a source of genes and alleles for increased tolerance to both abiotic and biotic stresses. The output of the present experimental work provides insight into existing phenotypic variability to distinguish candidate accessions with high potential for agronomic and morphological traits. Also, predicting genetic correlation with multi-traits development might enhance the identification of parent combinations, a significant move for breeding, that will offer favorable genetic interrelationship architecture. Such information as trait heritability, population size, and phenotypic-genotypic correlation architecture could also be utilized in future marker-assisted quinoa breeding programs.

2 Materials and Methods

2.1 Chenopodium quinoa germplasm

In the reported work, a total of 114 Chilean *C. quinoa* accessions including a few candidate breeding lines were used for the assessment of morphological traits variation. The *C. quinoa* germplasm used in the current study was established by Instituto de Investigaciones Agropecuarias (INIA), Chile. The quinoa germplasms were initially collected based on their agro-morphological traits performance, that observed in the field trials and further developed in INIA's breeding program through a combination of mass selection and it was self-pollinated for at least two field seasons. All germplasms belong to the two groups of genotypes that represent variation from two different biomes of Chile: there are 105 coastal-lowland and 9 Salares ecotypes, thereby Salares ecotypes are under-represented compared to the coastal-lowland ones. **Table 1.1** shows detailed information about the *C. quinoa* germplasm used in the current study.

2.2 Experimental setup and growth conditions

The experiment was conducted in a research greenhouse (50° 54' 36.022" N 6° 24' 46.145" E) located at the IBG2 Plant Sciences Institute at Forschungszentrum Jülich, Germany, between April and September 2019. In total 114 *C. quinoa* germplasm lines were initially stratified at 4^{o}C to synchronize the germination of seeds of each genotype. Four seeds from all lines were sown into 2L pots containing peat soil 'ED 73' (Einheitserde, Balster Einheitserdewerk, Fröndenberg, Germany; N, approx. 250 mg l^{-1}, P2O5, approx. 300 mg l^{-1}, K2O, approx. 400 mg l^{-1}) and grown under long-day conditions (16h light/ 8h dark). After seedlings were established, thinning was done to obtain a single plant per pot. The experimental setup was in a randomized layout with four replicates per genotype. Each experimental table had 5 rows spaced 10 cm apart from other rows and each row had 10 pots. Each plant was placed 10 cm from each other. The average temperature during the daytime was 22 ± 2^{o}C and 18 ± 2^{o}C at night throughout the study. Irrigation was provided by a drip irrigation system to keep plants well-watered. No additional fertilizer was applied to the pots before the beginning of plant cultivation or during the experimental period.

2.3 Assessment of agronomic and morphological traits of *C. quinoa*

To evaluate agronomically important yield-related and morphological traits variation in *C. quinoa*, in total 114 *C. quinoa* lines were grown as mentioned in chapter 1, section 2.2. The plants from each line in each replication were monitored and recorded for the following agro-morphological traits, according to Sosa-Zuniga et al. (2017). Both quantitative (i.e., number of days to flowering and maturity, plant height, yield, and Thousand Kernel Weight), and qualitative (i.e., panicle shape) traits were characterized.

Table 1.1. Accession name, seed source, and collection region of the *C. quinoa* lines studied in the present work (Pandya et al. 2021)**.**

Quinoa line	Accession name	Type	Seed source	Collection region		Quinoa line	Accession name	Type	Seed source	Collection region	
				Latitude	Longitude					Latitude	Longitude
AZ-1	Javi	Selfed line	INIA	-34.49778	-72.00444	AZ-19	Villarrica	Selfed line	INIA	-39.81944	-73.24528
AZ-2	Javi	Selfed line	INIA	-34.49778	-72.00444	AZ-20	Villarrica	Selfed line	INIA	-39.81944	-73.24528
AZ-3 [b]	Cancosa	Selfed line	INIA	-20.49083	-69.32917	AZ-21	KM21	Selfed line	INIA	-39.48194	-72.13417
AZ-4	Cáhuil	Selfed line	INIA	-34.28111	-71.85722	AZ-22	KM23	Selfed line	INIA	-39.48194	-72.13417
AZ-5 [b]	Cancosa	Selfed line	INIA	-20.49083	-69.32917	AZ-23	KM23	Selfed lin e	INIA	-39.48194	-72.13417
AZ-6	U de C9	Selfed line	INIA	-35.73306	-72.53306	AZ-24	KM30	Selfed line	INIA	-39.48194	-72.13417
AZ-7	Palmilla	Selfed line	INIA	-34.57722	-71.38000	AZ-25	CHENO 042	Selfed line	INIA	-34.78667	-72.04917
AZ-8	Palmilla	Selfed line	INIA	-34.57722	-71.38000	AZ-26	CHENO 046	Breeding line	INIA	-34.70000	-72.01667
AZ-9 [a]	R49	Selfed line	INIA	-19.27639	-68.64000	AZ-27	CHENO 047	Selfed line	INIA	-34.70000	-72.01667
AZ-10	U de C9	Selfed line	INIA	-35.73306	-72.53306	AZ-29	CHENO 207	Selfed line	INIA	-34.49750	-72.02111
AZ-11 [a]	R49	Drought tolerant line	INIA	-19.27639	-68.64000	AZ-30	CHENO 207	Selfed line	INIA	-34.49750	-72.02111
AZ-12	Peñablanca-VI	Selfed line	INIA	-34.61139	-71.64083	AZ-31	FARO	Selfed line	INIA	-34.46778	-71.82583
AZ-13	--	Selfed line	INIA	-	-	AZ-32	FARO	Selfed line	INIA	-34.46778	-71.82583
AZ-14 [a]	Mix	Selfed line	INIA	-19.27639	-68.64000	AZ-33	EM10-1	Selfed line	INIA	-34.53167	-71.98722
AZ-15	Peñablanca-VI	Selfed line	INIA	-34.61139	-71.64083	AZ-34	EM10-1	Selfed line	INIA	-34.53167	-71.98722
AZ-16	Dorada P Paredones	Selfed line	INIA	-34.65750	-71.97889	AZ-35	EMPO 10-17	Selfed line	INIA	-34.65083	-71.89500
AZ-17 [a]	Mix	Selfed line	INIA	-19.27639	-68.64000	AZ-36	EMPO 10-15	Selfed line	INIA	-34.64667	-71.90806
AZ-18	Cáhuil	Breeding line	INIA	-34.28111	-71.85722	AZ-37	EMPO 10-14	Selfed line	INIA	-34.52833	-71.99278

AZ-38	EMPO 10-14	Selfed line	INIA	-34.52833	-71.99278	AZ-60	PJEV 009	Selfed line	INIA	-34.68417	-71.99667
AZ-39	EMPO 10-10	Selfed line	INIA	-34.51861	-71.98028	AZ-61	PJEV010	Selfed line	INIA	-34.68583	-72.00139
AZ-40	EMPO 10-9	Selfed line	INIA	-39.48194	-72.13416	AZ-62	EAM 1	Breeding line	INIA	-34.76833	-72.07556
AZ-41	EMPO 10-8	Selfed line	INIA	-34.51444	-71.69722	AZ-63	EAM 1	Selfed line	INIA	-34.76833	-72.07556
AZ-42	EMPO 10-7	Selfed line	INIA	-34.64139	-71.91194	AZ-64	EAM 1	Selfed line	INIA	-34.76833	-72.07556
AZ-43	EMPO 10-6	Selfed line	INIA	-34.65806	-71.92889	AZ-65	EAM 2	Selfed line	INIA	-34.98639	-71.42750
AZ-44	EMPO 10-5	Selfed line	INIA	-34.63667	-71.95944	AZ-66	PJEV 011	Selfed line	INIA	-34.76472	-72.07806
AZ-45	EMPO 10-4	Selfed line	INIA	-34.62361	-71.68778	AZ-67	PJEV012	Selfed line	INIA	-34.83667	-72.05944
AZ-46	EMPO 10-2	Selfed line	INIA	-34.69389	-71.91917	AZ-68	EAM 3	Selfed line	INIA	-34.49778	-72.00444
AZ-47	EMPO 10-1	Breeding line	INIA	-34.53639	-71.96917	AZ-69	EAM 4	Selfed line	INIA	--	--
AZ-48	PJEV 029	Selfed line	INIA	-36.05806	-72.47306	AZ-70	PJEV 013	Selfed line	INIA	-34.84194	-72.14194
AZ-49	PJEV 028	Selfed line	INIA	-35.95444	-72.42139	AZ-71	EAM 5	Selfed line	INIA	-34.53556	-71.58583
AZ-50	PJEV 027	Selfed line	INIA	-35.93667	-72.70639	AZ-72	PJEV 014	Selfed line	INIA	-34.92806	-72.17944
AZ-51	PJEV 026	Breeding line	INIA	-35.93528	-72.70694	AZ-73	PJEV 015	Selfed line	INIA	-35.00944	-71.91833
AZ-52	PJEV 025	Selfed line	INIA	-35.91444	-72.68972	AZ-74	JML01	Selfed line	INIA	-35.86611	-71.59694
AZ-53	PJEV 006	Selfed line	INIA	-34.52778	-71.94611	AZ-76	PRJ3	Selfed line	INIA	-34.49778	-72.00444
AZ-54	PJ001	Selfed line	INIA	-34.70000	-72.01667	AZ-77	PRJ3	Selfed line	INIA	-34.49778	-72.00444
AZ-55	PJ002	Selfed line	INIA	-34.53917	-71.92833	AZ-78	PJEV 016	Breeding line	INIA	-35.12694	-71.91722
AZ-56	PJEV 007	Selfed line	INIA	-34.53222	-71.98556	AZ-79	PJEV 017	Selfed line	INIA	-35.01667	-71.91833
AZ-57	PJEV 008	Selfed line	INIA	-34.49833	-72.02278	AZ-80	Palmilla	Selfed line	INIA	-34.57722	-71.38000
AZ-58	PJ003	Selfed line	INIA	-34.52028	-71.97722	AZ-81	PJEV 018	Selfed line	INIA	-35.00889	-71.92611
AZ-59	PJ005	Selfed line	INIA	-34.53556	-71.58583	AZ-82	PJEV 019	Selfed line	INIA	-35.04583	-71.91194

AZ-83	PJEV 020	Selfed line	INIA	-35.50111	-72.08278	AZ-101	Javi	Selfed line	INIA	-39.48194	-72.13417
AZ-84	Dorada y Paredones	Selfed line	INIA	-34.65750	-71.97889	AZ-102	Cancosa	Selfed line	INIA	-34.57722	-71.38000
AZ-85	PJEV 021	Selfed line	INIA	-35.50361	-72.08306	AZ-103	Cáhuil	Selfed line	INIA	-34.65750	-71.978888
AZ-86	PJEV 022	Selfed line	INIA	-35.59028	-72.60917	AZ-104	Cáhuil	Selfed line	INIA	-35.91083	-72.68555
AZ-87	Roja Paredones	Selfed line	INIA	-34.65750	-71.97889	AZ-105	Javi	Selfed line	INIA	-34.49777	-72.00444
AZ-88	PJEV 003	Selfed line	INIA	-34.61278	-71.65139	AZ-107	Palmilla	Selfed line	INIA	-34.57722	-71.38000
AZ-89	PJEV 003	Selfed line	INIA	-34.61278	-71.65139	AZ-108 [a]	R49	Selfed line	INIA	-34.57722	-71.38000
AZ-91	PJEV 023	Selfed line	INIA	-35.91083	-72.68556	AZ-110	Palmilla	Selfed line	INIA	-34.65750	-71.97888
AZ-92	PJEV 23	Selfed line	INIA	-35.91083	-72.68556	AZ-111 [a]	R49	Selfed line	INIA	-35.91083	-72.68555
AZ-93	PJEV 024	Selfed line	INIA	-35.91028	-72.68667	AZ-112	Dorada P Paredones	Selfed line	INIA	-34.49777	-72.00444
AZ-94	PJEV 024	Selfed line	INIA	-35.91028	-72.68667	AZ-113	Peñablanca-VI	Selfed line	INIA	-34.57722	-71.38000
AZ-95	PJEV 05	Selfed line	INIA	-34.53583	-71.95694	AZ-114 [a]	MIX	Selfed line	INIA	-34.57722	-71.38000
AZ-96	PJEV 05	Selfed line	INIA	-34.53583	-71.95694	AZ-115	Dorada P Paredones	Selfed line	INIA	-34.65750	-71.97888
AZ-97	Plantas Verdes	Breeding line	INIA	--	--	AZ-129	CHEN0207	Selfed line	INIA	-34.49750	-72.02111
AZ-98	Plantas Moradas	Breeding line	INIA	-39.81944	-73.24528	Cq-1	Vikinga	Variety	Uni. of Copenhagen	--	--
AZ-99	Kinia	Breeding line	INIA	-39.48194	-72.13417	Cq-2	Titicaca	Variety	Uni. of Copenhagen	--	--
AZ-100	Javi	Selfed line	INIA	-39.48194	-72.13417	Cq-3	ATLAS	Variety	INIA	--	--

[a] Selected salares quinoa genotypes from the Altiplano region of Chile (~ 19º S and ~ 68º W) and [b]locality of Cancosa (~ 20º S and ~ 69º W). AZ: Arid Zone.

Note: Table 1.1 was adapted from the published article (Agronomy 2021, 11(9), 1867; https://doi.org/10.3390/agronomy11091867) to better suit the context and the purpose of the current work.

1. Seed germination: seeds were considered germinated once cotyledons emerged from the soil surface (Principal growth stage: 0; Sosa-Zuniga et al. 2017).
2. Days to flowering: the number of days from the date of seed emergence to the date of inflorescence development. Flowering was considered as starting when the first anthers were extruded from the inflorescence on the main stem inflorescence (Principal growth stage: 6; Sosa-Zuniga et al. 2017).
3. Days to maturity (physiological maturity): the number of days from the seed germination to the date when grains had become mature, i.e., ripening of the seeds classified into milky, thick, and finally ripe grains which are difficult to crush (Principal growth stage: 8; Sosa-Zuniga et al. 2017)
4. Plant height (cm): the height from the soil level to the tip of the panicle on the main stem at the time of plant maturity.
5. Seed yield (g): the seeds of each replicated plant of each line were bulked and weighed for each accession.
6. Thousand Kernel Weight (TKW in g): an average weight of 1000 seeds from the total measured seeds of each accession.
7. Panicle shape: panicle shape was classified as glomerular, intermediate, and amarantiform, as mentioned in Bioversity International (2013).

2.4 Statistical analyses

A descriptive analysis was performed for each variable. By calculating a mean ± standard deviation (SD) of all the plant replicates for different agro-morphological traits, statistical parametric tests were performed. All data were subjected to the assumptions of normality and homoscedasticity of variances of residuals by the Shapiro-Wilks and the Levene tests, respectively. Variables that failed to meet these assumptions were transformed to the natural logarithm ($ln(x+1)$). A one-way analysis of variance ANOVA followed by a *post-hoc* test (Tukey's – Honestly Significant Difference multiple comparisons, $\alpha = 0.05$) was carried out using the R-package Agricolae (De Mendiburu 2014). A broad-sense heritability (H_b), a ratio of genotypic and phenotypic variance, was estimated using the following formula proposed by Singh (2010).

$$H_b = \frac{\sigma^2 g}{\sigma^2 p}$$

Where H_b is an estimated heritability, $\sigma^2 g$ is genotypic variance, and $\sigma^2 p$ is phenotypic variance. The multivariate analysis for the whole dataset was performed through (1) Pairwise correlation among variables using Pearson's correlation coefficient; (2) Principal Components Analysis (PCA) and clustering based on correlation distance matrix using R-packages (factoextra and FactoMineR)

(Kassambara and Mundt 2017; Lê et al. 2008); (3) Hierarchical clustering. A dendrogram was constructed using the Euclidean distance and complete grouping method by means of PCA scores (Madhulatha 2012). Further, the percentage of genetic variance (Vg) within the phenotypic variance for agro-morphological traits was estimated. The genotypic effect was defined according to a linear additive model:

$$y_{ij} = \mu + G_i + \varepsilon_{ij}$$

where *yij* is the phenotypic value measured for the trait *y* on the plant *j* of the genotype *i*; μ corresponds to the overall mean; *Gi* is the random effect of genotype *i* representing the effect of each genotype or genotypic effect on trait *y*, and εij is the random residual error per plant *j* of the genotype *i*. Vg was estimated according to the restricted maximum likelihood (REML) variance components using the lme4 library of R (Bates et al. 2014). The significance of the effects was assessed using the Akaike and Bayesian criteria and tested by the likelihood ratio (Sillanpää and Corander 2002).

3 Results and Discussion

To study the agro-morphological traits variation among the *C. quinoa* genotypes, principal agro-morphological traits were analyzed twice a week for days to flowering and maturity, while plant height and grain-related traits were recorded at maturity time and at harvesting time, respectively according to Sosa-Zuniga et al. (2017). In this study, the emergence rate was noted in the range of 0% to 92%, which gave us an overview of the viability of *C. quinoa* seeds and hence how to take care of seedling establishment during further studies. It was found that *C. quinoa* shows two distinguished phenological phases, the initial phase is referred to as the vegetative phase (from sowing to 20 - 25 days later) which covers seed emergence to the side shoot formation. The second phase is the reproductive phase which covers the appearance of the inflorescence bud, anthesis on the main inflorescence (flowering), grain filling, and ripening (physiological maturity; **Figure S1**). During the reproductive phase, significant phenotypic variations were observed in days to flowering, days to physiological maturity, plant height at maturity stage, seed yield, and thousand kernel weight.

3.1 Phenotypic assessment and frequency distribution of variables

Based on the phenological and morphological variables evaluated in this study, a broad phenotypic variation among genotypes was observed in the Chilean *C. quinoa* panel. The mean values and frequency distribution of the principal agronomic and morphological traits are presented in **Table 1.2**. Early flowering and maturity are some of the desired characteristics of *C. quinoa* grown in European long-day photoperiod conditions. In the current study, the days to flowering was observed in the range of 34 to 71 days, whereas the accession AZ-62 was the earliest to reach flowering at a mean of 34 days

after sowing, which was 36 days earlier than the accession AZ-4 that flowered at a mean of 71 days after sowing (**Figure 1.2, Table S1**). Further, the days to physiological maturity were observed in the range of 74 to 150 days, whereas AZ-62 showed early maturity at a mean of 74 days after sowing. On the other hand, the accession AZ-4 was found to be the latest-maturing genotype with a mean of 150 days to maturity after sowing (**Figure 1.2, Table S1**). A significant difference ($p < 0.05$) for both variables was detected among the *C. quinoa* lines used in the study and we were able to distinguish the genotypes into different groups. These results are comparable to those data reported by Jacobsen (1998). A study by Jacobsen and Stølen (1993) reported that the germplasm originating from Chile can be adapted to grow under long-day conditions and showed a range of 126 to 143 days after sowing to maturity. Overall, in our experimental work, the life cycle of all *C. quinoa* genotypes under long-day conditions was found having an average of 140 days (up to the senescence stage). The usual growth period of *C. quinoa* in the European field conditions is suggested to be < 150 days, especially Chilean *C. quinoa* lines (Jacobsen 2003, 1998). In *C. quinoa* breeding, early maturity is one of the most prominent traits, and therefore a short growth cycle is considered to be useful in the high-latitude regions and northern Europe where a short growing season is the main constraint (Jacobsen 2003). Interestingly, under long-day conditions, we found three forms of photoperiodic responsive genotypes, early, mid, and late precocity genotypes (days to flower and physiological ripeness). A thorough assessment showed that 84% of the genotypes responded promptly or moderately to the long-day photoperiod, suggesting they do not have any specific photoperiodic needs. These findings reflect the low photoperiodic sensitivity of the Chilean *C. quinoa* germplasm and its potential adaptability to long-day conditions (Patiranage et al. 2021; Patirange et al. 2020; Bhargava and Ohri 2016; Jacobsen and Stølen 1993). By using the obtained data, we compared the flowering and maturity traits with the geographical origin of the accessions. However, we did not observe any clear separation of the *C. quinoa* accessions relating to the origin location and measured flowering time as well as maturity time (**Figure S3).** These findings also provided a good explanation for the low sensitivity of the evaluated genotypes to photoperiod and may support a potential expansion of Chilean ecotype for cultivation to higher latitudes, as previously described (Patiranage et al. 2021; Murphy and Matanguihan 2015).

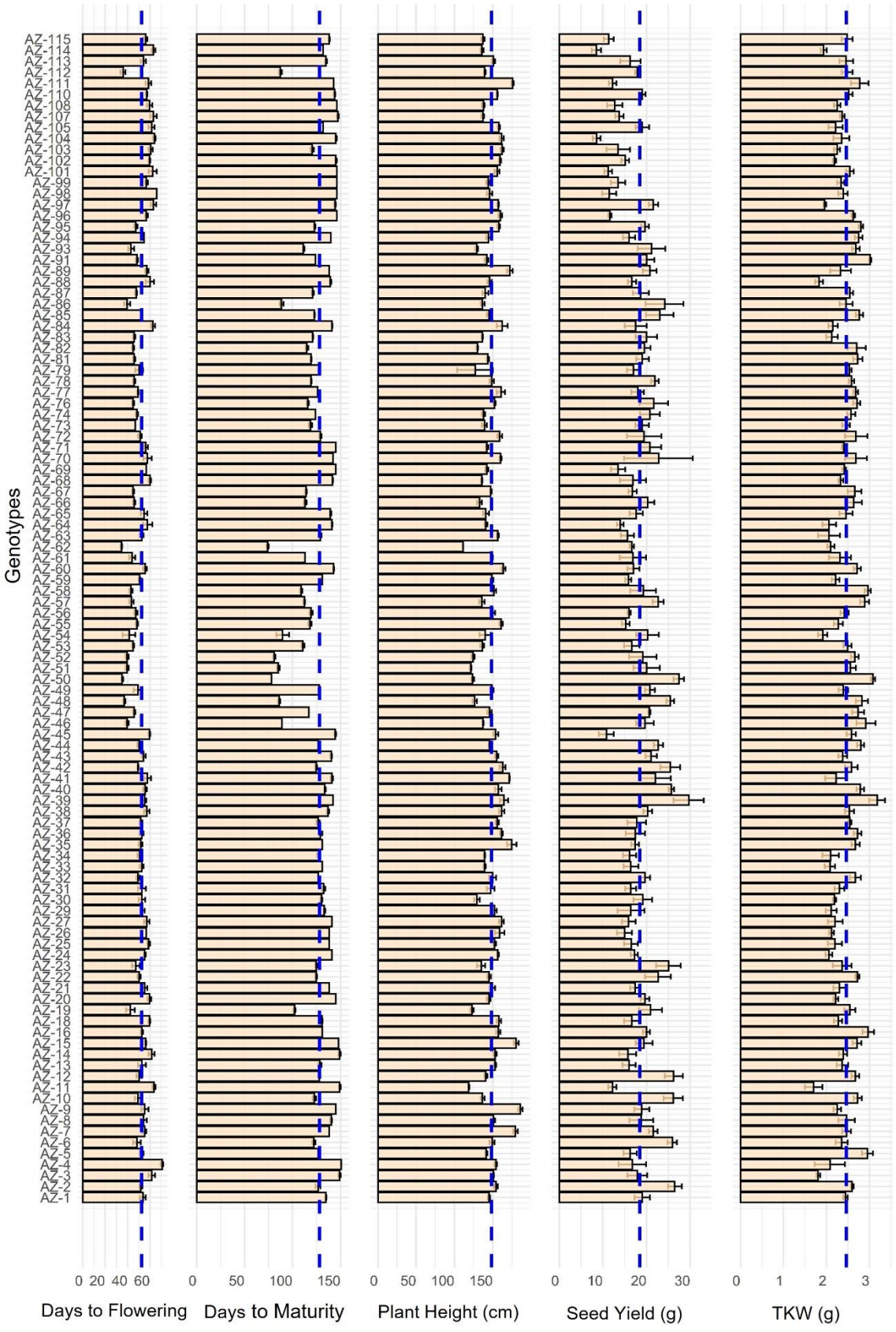

Figure 1.2. Morphological traits variation among *C. quinoa* accessions. To evaluate trait variation all 114 *C. quinoa* accessions were grown in the greenhouse under long-day conditions. The plants from each accession in each replication were monitored and recorded for days to flowering, days to maturity, plant height, and for yield-related traits such as seed yield, and thousand kernel weight (TKW). The y-axis shows the *C. quinoa* accessions and the x-axis shows the performance of the evaluated traits. Each column is the mean of four replicates, whereas error bars denote the standard error of the mean of replicates from each evaluated accession. The vertical dashed blue line shows the average of all assessed accessions for the respective trait.

Table 1.2. Descriptive statistics of mean performance and dispersion for each evaluated morphological trait.

Traits	Mean ± SD	Range	CV	Category	Frequency		H_b
					Absolute	Relative (%)	
Days to flowering	52.49 ± 3.61	34 - 71	6.8%	30 - 40	8	7.5	73.5
				40 - 50	29	27.3	
				50 - 60	56	52.8	
				60 - 70	12	11.3	
				≥ 70	1	0.9	
Days to maturity	128.06 ± 1.50	74 - 150	1.1%	≤ 80	2	1.8	98.2
				80 - 100	7	6.6	
				100 - 120	16	15.1	
				120 - 140	49	46.2	
				140 - 160	32	30.1	
Plant height (cm)	148.24 ± 5.44	111 - 186	3.6%	≤ 120	2	1.8	76.6
				120-140	28	26.4	
				140-160	57	53.7	
				160-180	5	4.7	
				180-200	2	1.8	
Seed yield (g)	18.46 ± 3.54	8.53 - 29.68	19.1%	≤ 10	2	1.8	41.9
				10 - 15	21	19.8	
				15 - 20	49	46.2	
				20 - 25	29	27.3	
				25 - 30	5	4.7	
Thousand kernel weight (g)	2.45 ± 0.20	1.70 - 3.17	9.4%	≤ 02	6	5.6	49.4
				2.0 - 2.5	52	49	
				2.5 - 3.0	45	42.4	
				≥ 3.0	3	2.8	
Panicle shape	-	-	-	Glomerulate	91	86	
				Intermediate	12	11	
				Amarantiform	3	3	

SD: standard deviation, CV: coefficient of variation, H_b: heritability

Aside from earliness, *C. quinoa* plant ideotypes should have a shorter main stem to avoid lodging and to enable or facilitate mechanical harvest (Murphy et al. 2018). To assess plant height variation, the distance from the soil level to the tip of the panicle on the main stem was measured at the maturity stage. A significant variation was observed in plant height among all *C. quinoa* lines. At maturity, plant height ranged from 111 cm to 186 cm among the lines we studied. Plant height showed a mean of 148 cm, whereas the *C. quinoa* line AZ-62 showed the lowest height (111 cm). On the other hand, the highest height was noted for *C. quinoa* line AZ-9 (186 cm). Morphological characterization of Chilean germplasm for plant height done by Madrid et al. (2018) showed a considerable variation in plant height, ranging from 86 cm to 166 cm. Another study was done by Fuentes and Bhargava (2011) where Chilean Salares germplasm showed plant height in a range of 101 cm to 191 cm. Quinoa ecotypes differ significantly in terms of plant height. Taller plants (those that are more than 2 m high) are typically found in the Valley regions, while shorter plants are typically found in the Altiplano and Salares regions (Murphy and Matanguihan 2015). Our data confirm that the phenotypic behavior for plant height under long-day conditions in the greenhouse is very similar to that of other published reports.

Today seed yield is often used as a selection parameter for *C. quinoa* breeding (Bertero et al. 2004). As a result, to measure another quantitative agronomic trait, the seeds of each replicated plant of each accession were bulked and weighed. The average weight of 1000 seeds from the total seeds of each line was evaluated. The most variable traits in the presented study were seed yield and TKW with a coefficient of variation of 19.1% and 9.4%, respectively. Likewise, Manjarres-Hernández et al. (2021b) have reported the highest coefficient of variation for seed yield among the other measured variables for the Bolivian *C. quinoa* germplasm. The seed yield among the *C. quinoa* lines ranged from 8.53 g to 29.68 g per genotype with an overall mean of 18.46 g, while TKW was in the range of 1.70 g to 3.17 g per genotype with an overall mean of 2.45 g. Such as our data, previously published literature also noted a considerable variation in the seed yield in their various experiments (Manjarres-Hernández et al. 2021a; Madrid et al. 2018; Bhargava et al. 2007b). However, these experiments were conducted under field trial conditions and therefore the direct comparison of those data with our data is not straightforward. However, considering this variation in yield might result in the opportunity to develop breeding strategies to sustain *C. quinoa* cultivation under long-day climate conditions. Concerning the other yield-related descriptor such as TKW, it ranged between 1.70 g and 3.17 g per genotype with the next largest coefficient of variation. For panicle structure, most of the *C. quinoa* lines were sorted into a glomerular form with 86%, while the intermediate and amarantiform shapes were less common appearing with 11% and 3%, respectively, among the studied accessions (**Figure S2**). A similar trend for panicle shape was also observed in another article, where the glomerular form was the main panicle form compared to intermediate- and amaranti- forms (Manjarres-Hernández et al. 2021a).

Knowledge of broad-sense heritability (H_b) is significant as it shows the potential and to what extent the improvement could be carried out using phenotypic selection. As heritability (H_b) is the ratio of genotypic and phenotypic variance, it can also be interpreted as the heritable percentage of phenotypic variance. In the present study, studied traits showed moderate to high broad-sense heritability (H_b) values which suggest that these traits have significant underlying genetic components. The heritability (H_b) values were highest for day to flowering (73.5%), days to maturity (98.2%), and plant height (76.6%), whereas moderate heritabilities (H_b) were observed for yield-related traits such as seed yield (41.9%), and TKW (49.4%; **Table 1.2**). Earlier several studies have also reported high estimated heritability (H_b) for *C. quinoa* (Benlhabib et al. 2016; Bhargava et al. 2012; Bhargava et al. 2007a). However, high heritability alone does not ensure significant gains from selection until adequate genetic advance due to additive gene action is present.

The analysis of variance on the agronomic and morphological variables has shown statistically significant differences ($p < 0.05$) among the evaluated *C. quinoa* accessions for the measured agro-morphological characters. Likewise, Tukey's – Honestly Significant Difference multiple comparisons test ($p < 0.05$) has shown the differences between the studied accessions and distinguished them into different groups. Such agro-morphological trait variations are essential to understand the growth cycle and genetic variability of these accessions and, in perspective, to introducing new elite cultivars through suitable selection processes.

3.2 Correlation among variables

Evaluating trait correlation is considered to be a significant move in breeding programs to enable directional selection and formulating selection indices (Neyhart et al. 2019; Fernandes et al. 2018). In our study, we computed correlation coefficients to understand the interrelationship between various agronomic and morphological traits in *C. quinoa* accessions (**Figure 1.3)**. Among all traits, a strong positive correlation was found between days to flowering and physiological maturity (r = 0.83). This result is comparable with Bhargava et al. (2007a) who studied the genetic variability and correlations between various morphological characters in *C. quinoa* including Chilean genotypes and reported a strong positive interrelationship among days to flowering and maturity (r = 0.70). Additionally, other recently published data also confirmed a strong correlation between days to flowering and days to maturity (Patirange et al. 2020). Further, weaker but significant positive correlations were noted between days to flowering and plant height (r = 0.41), days to maturity and plant height (r = 0.49), and between seed yield and TKW (r = 0.36). These results are similar to those of previous studies reporting

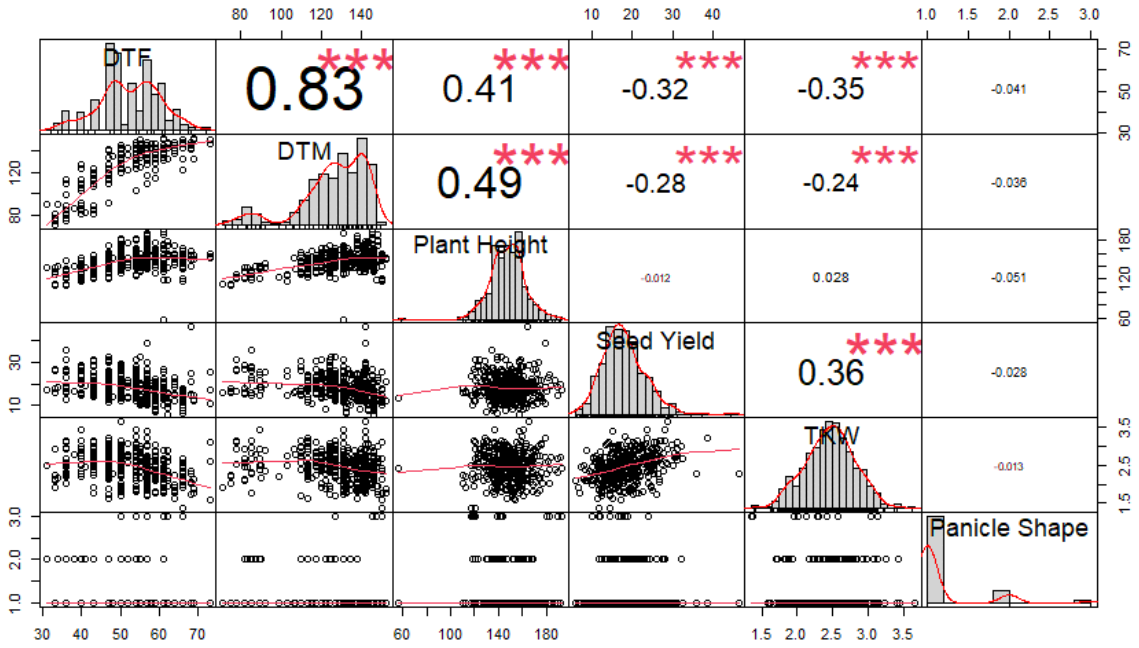

Figure 1.3. Interrelationship among morphological traits. Pearson's correlation coefficient was computed to explore the associations among all variables. A histogram shows the distribution frequency of phenotypic variance in *C. quinoa*. Below the diagonal, scatter plots are shown that present the distribution of accessions with the fitted line and the relationship between two variables. For example, two variables DTF and DTM show good correlations for the studied accessions. The stars at the top right of the panels show the correlation coefficients with highly significant levels. '***'0.001. '**'0.01, '*'0.05, '.'0.1, ' ' 1. DTF: Days to Flowering, DTM: Days to Maturity, TKW: Thousand Kernel Weight.

positive correlations between various agro-morphological traits and also between yield-related traits for direct and indirect selection (Manjarres-Hernández et al. 2021b; Patirange et al. 2020; Bhargava et al. 2012; Bhargava et al. 2007a). High heritability together with genetic advance is a significant criterion for direct selection, while the genotypic association among traits creates ground for indirect selection. The significance of direct and indirect selection for improving quality characteristics and grain yield in *C. quinoa* has been well studied earlier. Bhargava et al. (2012) proposed that grain yield could be increased with indirect selection for stem diameter, while days to flowering and maturity might be of little importance. Likewise, the levels of pigmentation have played a major role in the improvement of quality traits such as seed and leaf carotenoid, whereas indirect selection for high protein content in *C. quinoa* grain appears to be difficult to achieve. A yield-related parameter, TKW was positively correlated with grain yield. In wheat, increasing grain yield via improvement of TKW has been studied earlier (Zhang et al. 2022). Zhang et al. (2022) suggested that improving the net photosynthesis rate plays a significant role in improving TKW, and therefore developing high-yielding cultivars. For these reasons, TKW could also be an important trait for the selection of genotypes to be introduced in genetic improvement programs of *C. quinoa*. On the other hand, we found a negative and significant correlation between phenological growth stages such as days to flowering and maturity, and yield-related traits, i.e., seed yield and TKW. We also noticed that the accessions with higher than average plant height produced seed yield below average, as reported in previous literature, and found

an overall negative association between plant height and seed yield (Manjarres-Hernández et al. 2021a; De Santis et al. 2016). These findings imply a negative influence of a delay in phenological development on yield-related traits. This outcome could be explained by the source-sink interrelation in crops and its influence on seed yield (Smith et al. 2018). Because seed yield potential is the combined effect of source and sink strength and the rate of remobilization of carbon towards developing seeds, we hypothesize that tall plants may have comparatively large root systems acting as a sink for carbon that cannot be remobilized during the reproductive stage.

3.3 Principal component and hierarchical clustering analyses

To evaluate the pattern of variation that exists in our dataset, principal component analysis (PCA) was performed by considering all the agronomic and morphological variables. As shown in **Figure 1.4A**, the first two main components PC1 and PC2 of the principal component analysis explained about 45% and 20% of the total variation, respectively, with a cumulative percentage of the total value of about 65%. The linear functions with their relevant loading factors defining the principal components are the following:

$$\begin{aligned}\mathrm{PC1} = &-0.5739 \times \mathrm{DTF} - 0.5519 \times \mathrm{DTM} - 0.3409 \times \mathrm{Plant\ Height} + 0.3737 \times \mathrm{Seed\ Yield} \\ &+ 0.3305 \times \mathrm{TKW} + 0.0267 \times \mathrm{Panicle\ Shape}\end{aligned}$$

$$\begin{aligned}\mathrm{PC2} = &\ 0.0633 \times \mathrm{DTF} + 0.2013 \times \mathrm{DTM} + 0.5916 \times \mathrm{Plant\ Height} + 0.4671 \times \mathrm{Seed\ Yield} \\ &+ 0.5515 \times \mathrm{TKW} - 0.2881 \times \mathrm{Panicle\ Shape}\end{aligned}$$

Based on the loading factors, variables such as days to flowering, days to maturity, and plant height are well defined by PC1, whereas yield-related characteristics such as seed yield and TKW are well defined by PC2. According to scores of principal components, the interdependence of PC1 to variables such as days to flowering, days to maturity, and plant height has an opposite direction, which is negative. As a result, accessions that displayed earliness in days to flowering and maturity but had lower plant height are grouped on the right side of the quadrant. However, PC1 holds positive values for yield-related traits, i.e., seed yield and TKW. In other terms, accessions that show higher seed yield and TKW are also grouped on the right side of the quadrant. Next, hierarchical clustering was carried out based on the Euclidean distance and complete grouping method using the PCA score (**Figure 1.4B**). The *C. quinoa* accessions in the presented study were grouped into two main clusters corresponding to early and late phenological development. The *C. quinoa* lines grouped under the late phenological developmental phase were further divided into three sub-groups, characterized by early, mid, and late precocity. As illustrated in **Figure 1.4B**, clusters 3 and 1 show the lowest value for PC1. Considering that the first component of PCA was negatively associated with the days to flowering, days to maturity, and plant height, clusters 3 and 1 grouped those accessions that showed mid-late and late flowering

and maturing characteristics, and had the tallest plants. Because we found a negative association between measured agronomically important traits (i.e., flowering, maturity, and plant height) and yield-related traits (i.e., seed yield and TKW), both clusters include those accessions showing below than average seed production. Also, cluster 4 grouped those accessions that showed significant variation relating to precocity and yield-related traits compared to the other ones. In other terms, those accessions which have shown earliness during the developmental phases, and good performance in yield characteristics are categorized into cluster 4. Finally, cluster 2 grouped the accessions, which are early in days to flowering and maturity as well as are characterized by close-to-average seed production. The

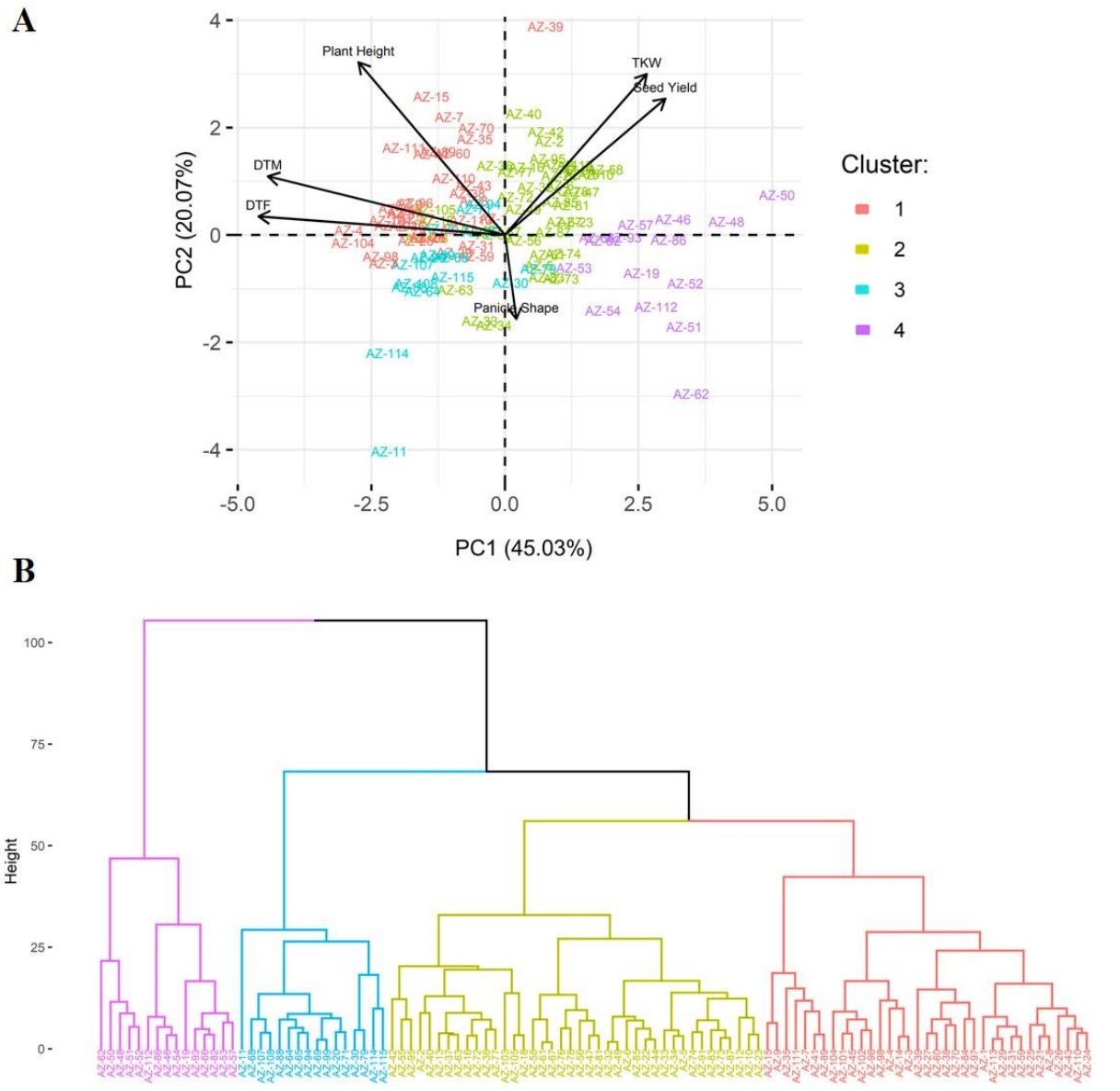

Figure 1.4. Principal component analysis (A) and hierarchical clustering (B) of various morphological traits. The *C. quinoa* accessions were grouped into distinct clusters corresponding to the performance of their morphological traits. The bi-plot shows two main components PC1 and PC2 that explained about 65% of the total variation of traits in the studied accessions. Arrows represent each variable, and the length of arrows approximates the variance of the variables, whereas the angle between arrows indicates their correlation. The scores of each genotype are outlined as genotype ID and the distance between genotype ID explains how similar the observations are. Both PCA and cluster analysis pooled together those accessions that had greater phenotypic similarity. The cluster colors in panel B correspond to the cluster number in the legend of Panel A. DTF: Days to Flowering, DTM: Days to Maturity, TKW: Thousand Kernel Weight.

late phenological developmental cluster shared some accessions within the sub-groups, without clear separation into distinct groups. Although most of the *C. quinoa* accessions were collected from the same origin location, they fall into distinct clusters based on their agronomic and morphological trait performance. Such diversity of *C. quinoa* lines within geographical locations could be due to factors such as a history of a selection of cultivars and/or developmental traits, heterogeneity of lines, and the genetic background of *C. quinoa* accessions (Fuentes et al. 2009; Bhargava et al. 2007b; Christensen et al. 2007) and has been also noted in different crop species (Singh et al. 2004; Alemayehu and Becker 2002).

3.4 Variance explained by a genetic effect

To assess the genotypic effect underlying trait variation patterns, the percentages of the total phenotypic variance of agro-morphological traits described by a genetic effect (Vg) were estimated using a linear additive model. In the present study, we noted a significant genetic effect ($p \leq 0.05$) which shows that each accession expresses the phenotype of measured agronomic and morphological traits distinctly, i.e., there is an existing genetic diversity that could explain the variation of studied agronomically important traits. Accordingly, genetic diversity up to 74.5% for days to flowering, 98.1% for days to maturity, and 68.7% for plant height were observed. For seed yield and thousand kernel weight, the variance explained by Vg was found to be 48.5% and 50.1%, respectively. The presence of a good percentage of genetic variance (Vg) for agronomic and morphological traits indicates that the present diversity within the Chilean *C. quinoa* lines could become a source for *C. quinoa* breeding programs to advance toward the development of new cultivars.

4 Conclusions

The results of this study highlight the existence of a wide phenotypic variance among the Chilean *C. quinoa* accessions, which forms the basis for the selection of new varieties for *C. quinoa* genetic improvement strategy. Furthermore, the present study has shown differences in phenotypic variables and interrelationships among such variables which could be helpful to develop selection tools oriented to characteristics of agronomic importance. Future work will help in defining their usefulness in increasing the efficiency of the selection of parental lines for specific crosses to introduce a mapping population for *C. quinoa* to be used in breeding programs. In this view, accessions of individual clusters carrying desired genes for a specific agro-morphological trait can be crossed with another promising accession of another cluster and help to introduce new genes into the mapping population. Hybrid genotypes could then potentially be introduced by selecting segregating lines, followed by a recurrent selection process for several generations. These processes would support the development of elite varieties with desired characteristics. Our results indicate that the accessions belonging to cluster 4 are

promising parental lines with good performance that could be considered further towards breeding programs. However, it is important to consider the experimental conditions of the present work, which was performed in the greenhouse under long-day conditions during a single season. Therefore, it will be necessary to conduct field trials in different environments for two or more seasons and estimate the genotype-environment interaction of the selected accessions.

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Chapter 2: Determination and metabolite profiling of mixtures of triterpenoid saponins from seeds of Chilean quinoa (*Chenopodium quinoa*) germplasm.

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1 Introduction

Saponins, glycosylated secondary metabolites, are present in a wide range of plant species (Vincken et al. 2007). They consist of a triterpenoid (C_{30}) or steroid (C_{27}) aglycon (sapogenin) attached to sugar units varying by type, number, and position (Cheok et al. 2014). Saponins have been widely studied for their potential applications in agriculture due to their antifungal activity and in the food industry for use as preservatives, food additives, and flavor modifying properties (Stuardo and Martin 2008; State of the Art Report of Quinoa in the World in 2013 2015). Moreover, due to their significant role in anticholesterol activity and other bioactive properties including antioxidant, antiviral, antimicrobial, and anti-inflammatory activities, saponins have been widely used in the cosmetic and pharmacology industry (Güclü-Üstündag and Mazza 2007).

In recent years quinoa (*Chenopodium quinoa* Willd.), a member of the Amaranthaceae family, has gained attention for its high nutritional content (Vega-Galvez et al. 2010; Rodríguez Gómez et al. 2021; Mhada et al. 2020) and its tolerance to abiotic stresses (Adolf et al. 2012; Hariadi et al. 2011; Jacobsen et al. 2003), showing potential to become an alternative crop to cereals, for example in defined crop rotations or on marginal lands. However, *C. quinoa* seeds contain a mixture of triterpenoid saponins in the seed pericarp that leads to bitterness. Saponin accumulation is not only limited to seeds but also other to plant organs including leaves, flowers, and fruits. The saponins in *C. quinoa* are a commixture of triterpenoid glycosides which are mainly derivatives of oleanolic acid (OA), hederagenin (HD), serjanic acid (SA), and phytolaccagenic acid (PA) (Burnoufradosevich et al. 1985; Cuadrado et al. 1995; Mastebroek et al. 2000; Ridout et al. 1991). A fifth sapogenin was characterized as 3,23,30-trihydroxy olean-12-en-28 oic acid (Madl et al. 2006). These sapogenins carry a hydroxy and carboxylic group at C-3 and C-28 position, respectively, which are linked to sugar units. Arabinose, glucose, and galactose are the major saccharides whereas glucuronic acid and xylose are present to a minor extent (Madl et al. 2006; Dini et al. 2001a; Dini et al. 2002; Dini et al. 2001b; Kuljanabhagavad et al. 2008; Mizui et al. 1990; Zhu et al. 2002; El Hazzam et al. 2020). Up to the present, nearly 140 different triterpene saponins have been identified and annotated in *C. quinoa* seed samples (Madl et al. 2006; Kuljanabhagavad et al. 2008; Jarvis et al. 2017a; El Hazzam et al. 2020). Saponins confer a bitter flavor and, based on their content, *C. quinoa* varieties have been classified into “sweet-varieties” or “low saponin varieties” (<0.11% or <0.11 g/100 g FW, and ca of ≤0.6% or ≤6.0 mg/g of DW) and “bitter-varieties” or “high saponin varieties” (≥0.11% or ≥0.11 g/100 g of FW, and ca of ≥1.0% or

≥10.00 mg/g of DW) (Vega-Galvez et al. 2010; Koziol 1991; Medina-Meza et al. 2016; Martinez et al. 2009). Since saponin compounds confer bitterness and other organoleptic properties to *C. quinoa* seeds, they must be removed before seeds can be used for human consumption. In general, saponins can be removed either by simple washing due to their amphiphilic nature or by a dehulling- abrasion process (Chauhan et al. 1992; Gomez-Caravaca et al. 2014; Lundberg 2019; El Hazzam et al. 2020). Because these processes consume water and require the use of dedicated machinery, the development of low saponin *C. quinoa* varieties is an important aim of current plant breeding programs. Today, several programs are in the process of developing saponin-free or low saponins varieties to be introduced in *C. quinoa* cultivation practice (Zurita-Silva et al. 2014; van Erp 2016; Murphy et al. 2018). Saponin content in *C.quinoa* has been assessed routinely by hemolytic, gravimetric, and foam-based approaches (Koziol 1991; Chauhan et al. 1992; Reichert et al. 1986). However, these assays are mostly qualitative or at best semi-quantitative and are likely to lead to substantial errors resulting from overestimation or low recovery of saponins (Ward 2000; Reichert et al. 1986). Therefore, recently, sophisticated technologies such as low-pressure lipid chromatography (LPLC), high-pressure liquid chromatography (HPLC), gas chromatography (GC), UV–vis spectroscopy, and gas chromatography-mass spectrometry (GC-MS) have emerged as appropriate methods for saponin separation and detection (Kuljanabhagavad et al. 2008; Jarvis et al. 2017a; Gómez-Caravaca et al. 2012; Medina-Meza et al. 2016; Ruales and Nair 1993; Woldemichael and Wink 2001; El Hazzam et al. 2020).

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For plant breeding purposes, it is important to consider both the total saponin content and the individual saponin compounds. In the current study, we hypothesized that the abundance of saponins and their composition can differ depending on the ecotypes and genetic background. Therefore we aim;

1. To characterize the extent of variability in saponins content among 114 Chilean *C. quinoa* accessions representing variations within the coastal-lowland and salares ecotypes.

2. Additionally, we focused on structure elucidation of saponins and on their fragmentation spectrum which enables the detection of aglycones as well as sugar moieties and their positions in the saponins for these genotypes.

These resources will be used in further studies to identify underlying genomic regions linked to saponins for future plant breeding efforts.

2 Materials and Methods

2.1 Chemicals

Oleanolic acid (OA) and Hederagenin (HD) were purchased from Sigma-Aldrich/Merck, (Darmstadt, Germany), and VWR, (Darmstadt, Germany), respectively. Silyl-991 (bis(trimethylsilyl)trifluoroacetamide (BSTFA)–trimethylchlorosilane (TMCS) (99:1)) was obtained from Chromatographie Service, (Langerwehe, Germany). Methanol (LC-MS grade), ethyl acetate, acetonitrile, water (LC-MS grade), pyridine, hydrochloric acid, sodium chloride, and sodium carbonate were supplied by VWR, (Darmstadt, Germany).

2.2 Chenopodium quinoa diversity panel

In reported study, the saponins content was evaluated in 114 different *C. quinoa* accessions including advanced breeding lines of the ongoing INIA's Quinoa Breeding Program (IQBP) in Chile. The *C. quinoa* diversity panel used for the experimental work was initially selected based on morphological characteristics (i.e., plant height, branching growth habit, panicle height, panicle shape) and yield traits (i.e., seed yield, seed diameter), and subsequently established by the Instituto de Investigaciones Agropecuarias (INIA), Chile. The *C. quinoa* panel (AZ = Arid Zones) was obtained by a combination of mass selection, self–pollination of individual lines (through at least two seasons), and panicle-furrow selection. The entire *C. quinoa* panel belongs to the two groups of genotype that represent variations from two different biomes in Chile: the coastal-lowland and the salares ecotypes. Here, salares genotypes are under-represented compared to the coastal-lowland type. Furthermore, *C. quinoa* diversity panel covered, 7 genotypes (salares ecotype) from the Chilean Altiplano (highlands region), 2 south Altiplano genotypes from Cancosa area, along 102 genotypes from the Chilean coastal-lowland regions (Chapter 1**, Table 1.1**). Most of all genotypes were cultivated and harvested from Huasco experimental station (28°3' S, 70°4' W). In the studied genotypes, we also included the known *C. quinoa* cultivars (cv.) Titicaca (moderate to high saponins content, drought, and salinity tolerant), Vikinga (low saponins content, and drought and salinity tolerant), and ATLAS (sweet cv) (Medina-Meza et al. 2016; Gómez-Caravaca et al. 2012; Ruiz et al. 2017; Jacobsen). These cultivars were

provided by Dr. Sven Erik Jacobsen from the University of Copenhagen, Denmark, and INIA, Chile, respectively.

2.3 Extraction and hydrolysis of saponins from *C.quinoa* germplasm

Saponins were indirectly quantified via their corresponding sapogenins derived from hydrolysis, according to Jarvis et al. (Jarvis et al. 2017a). Briefly, 50 mg ground *C. quinoa* seeds were suspended in 1 mL methanol then vortexed for 1 min and treated in an ultrasonic bath for 10 min. After centrifugation for 2 min at 14°C at 14,000 rpm using Eppendorf mini-spin (Hamburg, Germany), 900 µL of supernatant was withdrawn. The remaining solvent including the pellet was extracted a second time as mentioned above and the withdrawn supernatant (1000 µL) was combined with the first. The supernatant was evaporated to dryness and the residue hydrolyzed using 2 mL of 2.5 N hydrochloric acid at 90°C for 2 h. After the addition of 0.25 g of NaCl, the solution was extracted twice with 1 mL of ethyl acetate by vigorous vortexing for 1 min. The combined ethyl acetate extracts (800 and 900 µL) were treated with 0.25 g of sodium carbonate. After centrifugation 1 mL solution was withdrawn and evaporated to dryness. For derivatization, 1 mL acetonitrile, 100 µL Silyl-991, and 100 µL pyridine were added to the residue and heated to 90°C for 1 h. Afterward, derivatized samples were analyzed by gas chromatography-mass spectrometry (GC-MS).

2.4 Quantification of sapogenins by gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS system consisted of an Agilent 7890B gas chromatograph and 7693 autosampler (Santa Clara, CA, USA) as well as a Jeol JMS-T200GC AccuTOF GCx mass spectrometer (Tokyo, Japan). Analytes were separated on a Zebron ZB-5 HT Inferno column (30 m x 0.25 mm i.d., 0.25 µm film thickness) (Phenomenex, Torrance, CA, USA). Helium was used as carrier gas at a constant gas flow of 1.0 mL/min. The oven temperature program employed for the analysis of silylated sapogenins was as follows: 180°C (1 min), with 6°C/min to 350°C (3 min). The injector temperature was held at 340°C, and all injections (1 µL) were made in the split mode (1:10). The mass spectrometer was used in the electron impact (EI, 70 eV) mode and scanned over the range m/z 35 - 750 with a sampling interval of 0.25 ns and a recording interval of 0.4 s. The GC interface and ion chamber were kept at 340°C and 250°C, respectively. Data processing was performed by use of the software MSAxel (Jeol) and XCalibur 2.0.7 (ThermoFisher Scientific, USA). Analysis of each quinoa genotype/cultivar was performed in duplicate. Quantification was done by the method of external calibration with standard solutions in the concentration range 25 – 200 µM. As phytolaccagenic acid (PA) was not commercially available, its quantification was carried out based on a linear regression equation with HD. This method was justified because of almost congruent regression lines for OA and HD.

2.5 Identification of saponins by LC-FTICR-MS and LC-MS/MS

Liquid chromatography-Fourier transform ion cyclotron resonance mass spectrometry (LC-FTICR-MS) experiments were carried out using an Agilent 1200 series HPLC system consisting of a binary pump, autosampler, and column oven (Santa Clara, CA, USA). Saponins from the extraction with methanol (chapter 2, section 2.3) were separated on an Aqua 3 µm C18 column (150 x 2 mm, 3 µm particle size) equipped with a pre-column filter from Phenomenex. The mobile phase consisted of 1 mM aqueous ammonium acetate (A) and methanol + 1 mM ammonium acetate (B). Samples were separated at 40°C and a flow rate of 0.3 ml/min using gradient elution: isocratic at 90% A for 1 min, linear gradient to 1 % A over 29 min, isocratic at 1 % A for 10 min, linear gradient to 90% A over 1 min and equilibration at 90% A for 4 min (total run time: 45 min). The injection volume was 10 µL. Mass spectrometry was performed using a hybrid linear ion trap-FTICR-mass spectrometer LTQ-FT Ultra (ThermoFisher Scientific, Bremen, Germany) equipped with a 7 T supra-conducting magnet. The electrospray ionization (ESI) source was operated in the negative mode with a spray voltage of 2.80 kV. Nitrogen was employed as both sheath gas (8.0 arbitrary) and auxiliary gas (0 arbitrary). The transfer capillary temperature was set to 275 ºC. Voltages for capillary and tube lens were set to -33 V and -135 V, respectively. Mass spectra were recorded in a full scan from 150 to 1500 Da with a mass resolution of 100,000 at m/z 400 (full width at half maximum). The automatic gain control for providing a constant ion population in the ICR cell was set to 5E5 for the FTMS full scan mode. The maximum ion trap fill time was set to 10.0 ms and the maximum ICR cell fill time to 500 ms. The accurate masses of quasi-molecular ions $[M - H]^-$ were used for the calculation of chemical formulae with the Qual Browser in Xcalibur software version 2.0.7. The search algorithm contained the isotopes ^{1}H, ^{12}C, ^{13}C, and ^{16}O. Each compound had to be represented by 3 mass peaks: the base peak and the peaks of the corresponding ^{13}C- and $^{13}C_2$-isotopologues. Search results were restricted to mass errors of 3.0 ppm for the ^{12}C- and the corresponding ^{13}C- and $^{13}C_2$-isotopologues.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was done on a Waters ACQUITY® UHPLC system (binary pump, autosampler) coupled to a Waters Xevo TQ-S® triple-quadrupole mass spectrometer (Waters Technologies Corp., MA, USA). Separation of saponins from the extraction with methanol (Chapter 2, Section 2.3) was achieved on a Nucleoshell RP18 column (100 x 4.6 mm, 2.7 µm; Macherey-Nagel, Germany). The column was equipped with a pre-column (Macherey –Nagel, Germany). The mobile phases were water (A) and acetonitrile (B) each containing 0.1% formic acid, at a flow rate of 1.0 mL/min. The gradient program was as follows: 85% A, to 15 % A within 20 min, back to 85% A within 0.1 min, and holding for 2.9 min. The injection volume was 10 µL. The electrospray ionization (ESI) interface of the mass spectrometer was driven in the negative mode. The capillary voltage was set to 2.0 kV. The cone voltage was 35 V. The desolvation temperature and source

temperature were 600°C and 150°C, respectively. The desolvation gas flow was set to 1000 l/h and the cone gas flow at 150 l/h using nitrogen in both cases. MS/MS spectra were obtained in the daughter ion scan mode on precursor ions which were determined by LC-FTICR-MS before. Nitrogen was used as the collision gas at a flow of 0.15 mL/min and ionization voltage was set to 30 and 50 eV, respectively.

2.6 Statistical analysis

All analyzed results are reported as mean ± standard deviation (SD) values of the independent sample extractions (n = 4). To determine the significant difference in saponins content among *C. quinoa* accessions, the dataset was analyzed as a one-way analysis of variance ANOVA (Tukey's – Honestly Significant Difference multiple comparisons) using the R-package Agricolae (de Mendiburu 2020). A *p*-value ≤ 0.05 was considered as a statistically significant difference. Principal component analysis (PCA) and cluster analysis were done for the complete dataset to present the entire correlation distance matrix, and to group according to specific variables. Hierarchical clustering was performed based on the euclidean distance and complete grouping method using PCA scores. Also, the percentage of variance explained by the genotypic effect (Vg) in the total phenotypic variance for saponins content was estimated according to the restricted maximum likelihood (REML) variance components using the lme4 library of R (Bates et al. 2016).

3 Results and Discussion

3.1 Extraction and quantification of triterpenoid saponins in *C. quinoa* germplasm

Saponins content in *C. quinoa* seeds was evaluated in terms of their sapogenins (aglycons) derived from hydrolysis of seed samples. In total 114 different *C. quinoa* genotypes including breeding lines from the INIA breeding program and cultivars were analyzed for the saponins content. Quantification of sapogenins was carried out by the use of external calibration curves for OA and HD. For this analysis, standard solutions were prepared over a concentration range of 25 – 200 µM which were similar to those in the extracts. The obtained correlation coefficients (r^2) were in the range of 0.986 – 0.999. Next, the calibration curve of HD was used for the quantification of PA since the calibration curves of OA and HD were almost identical.

The ion mass chromatogram of silylated sapogenins from *C. quinoa* obtained by GC-MS is shown in **Figure 2.1**. The gas chromatogram shows three main and two minor peaks. Four of them could unambiguously be identified as silylated OA, HD, SA, and PA by comparison of their retention times (25.7, 26.6, 27.9, and 28.7 min, respectively) and mass spectra to the standard compounds and published data, respectively (Ridout et al. 1991; Gómez-Caravaca et al. 2012; Medina-Meza et al. 2016). The mass spectrum of the fifth sapogenin was insufficient to obtain any information about its

structure. Mass spectra of silylated sapogenins are characterized by very low abundances of their molecular peaks M^+. Fragmentation reactions mainly occurred under the elimination of CH_3, $Me_3SiO(H)$, and $Me_3SiOOCH$. Further, the mass spectrum peaks of TMS-OA were detected at m/z 600 (M^+), 585 (M^+ - Me), 482 (M^+ - $Me_3SiOOCH$), and 393 (M^+ - $Me_3SiOOCH$ - Me_3SiO). TMS-HD fragment ions were found at m/z 688 (M^+), 673 (M^+ - Me), 570 (M^+ - $Me_3SiOOCH$), and 481 (M^+ - $Me_3SiOOCH$ - Me_3SiO). The fragmentation pattern of TMS-SA was found at m/z 644 (M^+), 629 (M^+ - Me), 554 (M^+ - Me_3SiOH), 526 (M^+ - $Me_3SiOOCH$), and 467 (M^+ - $Me_3SiOOCH$ - MeOCO). TMS-PA peaks were spotted at m/z 732 (M^+), 717 (M^+ - Me), and 614 (M^+ - $Me_3SiOOCH$). These mass spectra are similar to those in the literature (Ridout et al. 1991; Gómez-Caravaca et al. 2012; Medina-Meza et al. 2016).

Because saponins content was, to our knowledge, never determined in earlier work with a few

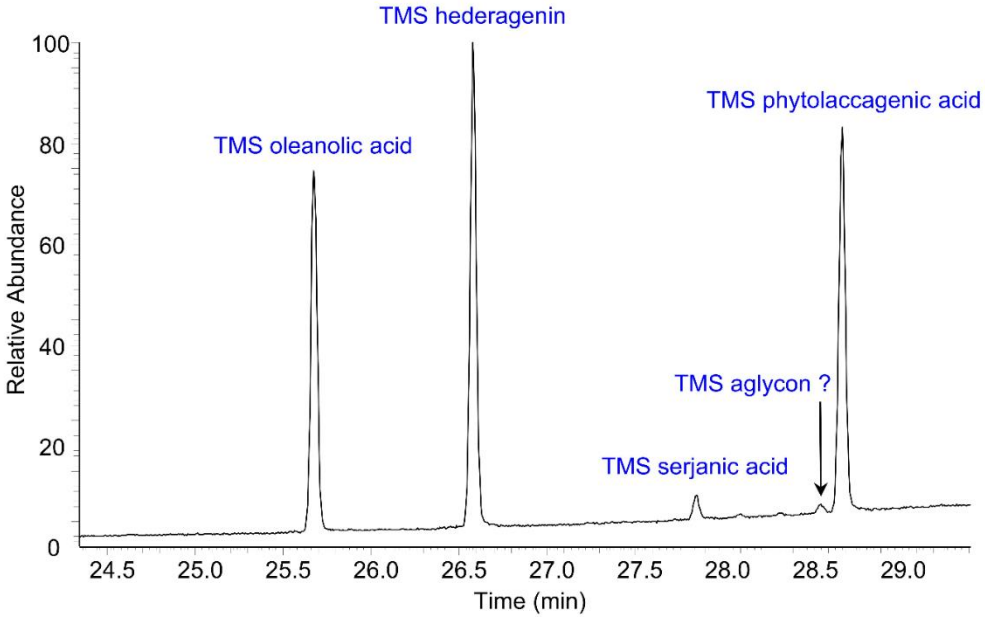

Figure 2.1. Ion mass chromatogram of trimethylsilylated sapogenins from *C. quinoa* obtained by GC-MS. GC-MS chromatogram profile shows the separated sapogenins from *C. quinoa* seed extract. Saponins were quantified by indirect quantification of their corresponding sapogenins derived from the hydrolysis of saponins from *C. quinoa* seed. Mass spectra of silylated oleanolic acid, hederagenin, serjanic acid and phytolaccagenic acid were assigned by comparison of retention times (25.7, 26.6, 27.9 and 28.7 mins, respectively) and mass spectra to the corresponding standards.

exceptions, for the studied genotypes originating from Chile, direct comparisons with previously published values is not currently possible for the whole dataset. However, we were able to compare our results with previously published data for the positive and negative control lines. Certain quinoa lines (AZ-18, AZ-26, AZ-47, AZ-51, AZ-62, AZ-78, AZ-97, AZ-98, and AZ-99) are advanced breeding lines of IQBP. In our experiment, 26.3% of the genotypes were noted as high saponin quinoa lines, whereas 73.6% of the genotypes were noted as low saponin quinoa lines. The relative saponins content among the genotypes studied ranged from 0.22 mg/g to 15.04 mg/g of seed dry weight. We found that variation in total saponins content was highly significant ($p < 0.001$) (**Figure 2.2A**, **Table**

S2, Table S3). Considering the breeding lines we studied (**Table 1.1**), AZ-51 and AZ-26 revealed a high content of saponins with 11.60 mg/g and 9.42 mg/g of seed dry weight, respectively. In contrast, the remaining breeding lines had lower saponins content in the range of 4.33 mg/g to 6.79 mg/g of seed dry weight (**Figure 2.2A**, **Table S2**). Furthermore, the Danish cultivar Titicaca (Cq-2) showed very high saponins content with a concentration of 15.04 mg/g of seed dry weight among all genotypes. We detected a higher concentration of PA with 7.76 mg/g of total saponins in comparison to other sapogenins in this cultivar (**Table S2**).

Our data is comparable with data from Medina-Meza et al. (2016) in which the Titicaca variety had an average total saponins content of 16.75 mg/g. Additionally, another study also revealed a high percentage of PA compared with the other sapogenins OA and HA in the bitter variety Titicaca, which

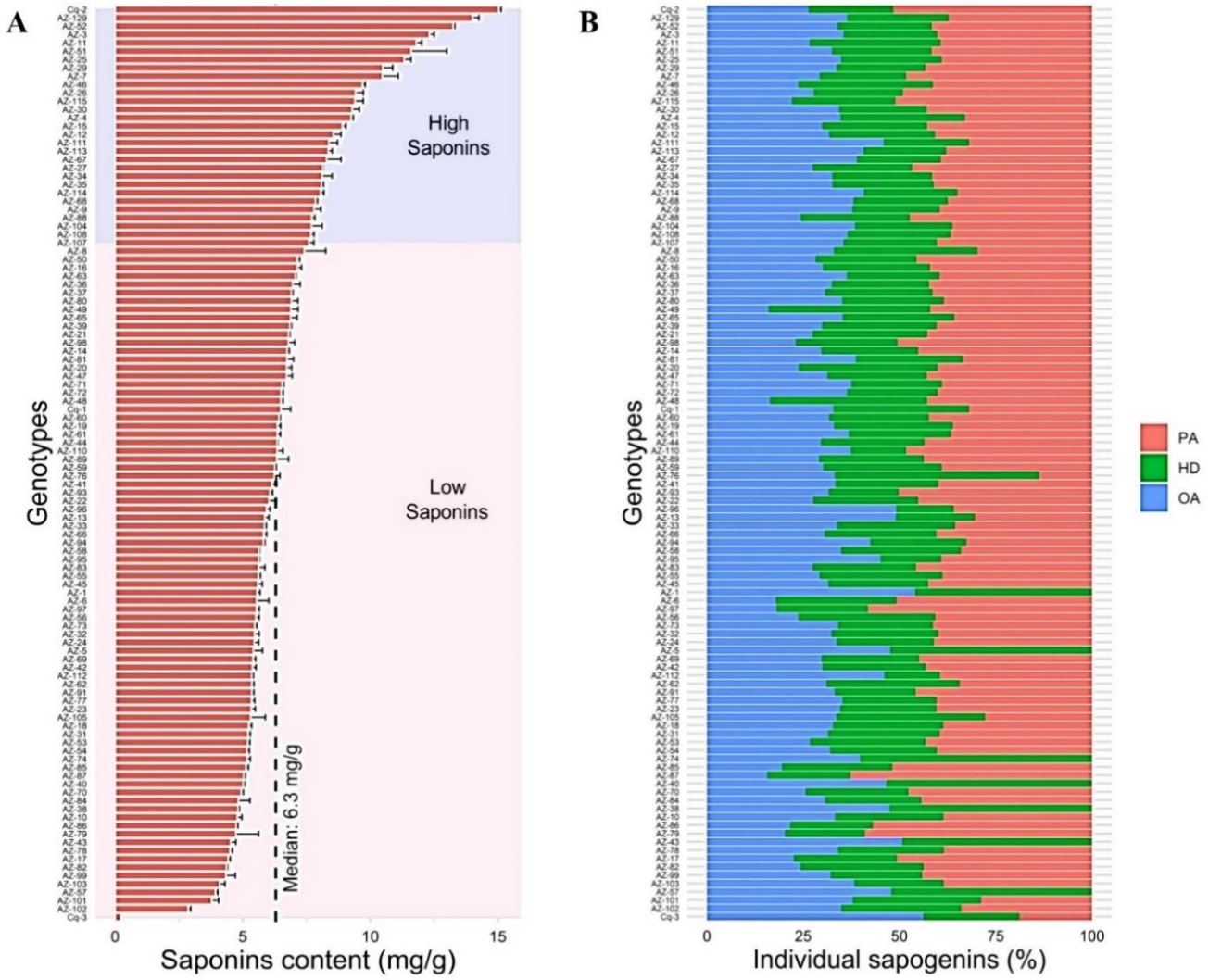

Figure 2.2. **Total saponins content (A) and percentage of individual sapogenins (B) in the *C. quinoa* panel.** (A) A bar graph showing relative saponin content in different *C. quinoa* accessions. Quantification of saponins was indirectly performed via their corresponding sapogenins. Analyzed data are reported as mean ± standard deviation of independent sample extractions. Statistical analysis was performed by ANOVA test (Tukey's – HSD, α=0.05). A significant difference ($p < 0.001$) in relative saponins content was noticed and saponins concentration ranged from 0.22 mg/g to 15.04 mg/g of seed DW. The median line shows the average of the total saponins content of all studied genotypes. (B) Stacked bar graph showing individual sapogenins and outlined in % of total saponins. Light blue: Oleanolic acid (OA), Green: Hederagenin (HD) and Light red: Phytolaccagenic acid (PA).

is also in agreement with our result (Ruiz et al. 2017). For the few previously investigated *C. quinoa* genotypes, total average saponins among Cáhuil accessions AZ-4, AZ-18, AZ-103, and AZ-104 were 9.24 mg/g, 5.23 mg/g, 4.08 mg/g, and 7.69 mg/g of seed dry weight, respectively. The reported average amount of saponins in this coastal-lowland Chilean genotype was comparable to the average values reported by Medina-Meza et al. (2016) and Ward (2000) (10.95 mg/g and 4.65 mg/g, respectively). However, the average saponins amount for the Cáhuil genotype in our case was higher compared with the one reported in another study (0.39 mg/g of saponins) (Miranda et al. 2012). Possible discrepancies in the variation in total saponins reported in various studies might occur due to numerous factors including agronomical and environmental conditions as well as the handling of the standardized procedures that have been used for the extraction and quantification of saponins. Concerning these points, the seeds of the Cáhuil genotype used in Medina-Meza's and Ward's experiments have been cultivated at different experimental locations in the United States. Furthermore, in Miranda's experiment, saponins were analyzed based on an HPLC procedure although seeds of the Cáhuil genotype were all collected in Chile. Moreover, another central Chilean genotype, FARO (AZ-31 and AZ-32) showed on average 5.33 mg/g of total saponins. This value was higher compared with the one found by Miranda et al. (2012), which was ~ 0.30 mg/g of total saponins. Similarly, Cancosa corresponding to south Altiplano ecotype (AZ-3, AZ-5) and south Chilean coastal-lowland ecotypes from Villarrica locality (AZ-19, AZ-20) contained on average 8.87 mg/g and 6.53 mg/g of saponins, values which were higher than those reported by Miranda et al. (2012) which were 0.20 mg/g and 0.89 mg/g), respectively. Such contradictory results relating to saponins amount can be concerned with the fact that in Miranda's study the characterization of saponins was carried out based on the reversed-phase HPLC approach. In the studied low saponins cultivar that we included as negative controls, Vikinga (Cq-1) showed an average saponins content of 6.49 mg/g of seed dry weight, which is lower than 1% of total saponins. The relative concentration of sapogenins OA and HD in Vikinga were detected in a 1:1 ratio, while the concentration of PA was in comparison slightly lower than OA and HD (**Table S2**). This result is nearly identical to the findings of a previous study where the authors reported a 1:1 OA:HD ratio with a relatively low amount of PA in Vikinga.(Ruiz et al. 2017) Among the entire panel of genotypes, we found that cv ATLAS (Cq-3) had a very low amount of saponins, as expected (0.22 mg/g of seed dry weight). The GC-MS chromatogram of this variety had 56% and 25% of OA and HD, respectively, while PA was 19% of total sapogenins (**Figure 2.2B**). This result implies a high ratio of OA:HD. This result can be explained by the fact that a high ratio of OA to HD is connected, to some extent, with the sweetness of seeds and a low ratio, to some extent, with the bitterness of seeds (Mastebroek et al. 2000; Ruiz et al. 2017).

Our GC-MS results show that PA was the main compound of sapogenins as such representing 38% of the total saponins content followed by OA and HD with 33% and 28%, respectively (**Figure 2.2B**).

This result is very similar to other previous experiments where PA was reported as the main class of sapogenins (42% - 43%) followed by OA (30% - 34%) and HD (24% - 27%) in coastal-lowland ecotypes (Ridout et al. 1991). Overall, we found a positive correlation between PA and total saponins ($p < 0.001$, $r = 0.854$; (**Figure S4**), as previously reported (Medina-Meza et al. 2016). It has been also noted that the bitterness of *C. quinoa* seeds can be linked with a higher content of PA, whereby sweet *C. quinoa* genotypes have low or no apparent PA content (Medina-Meza et al. 2016; Ruiz et al. 2017; Ng et al. 1994). However, in other studies, OA was identified as the main class of sapogenins in *C. quinoa* (Gómez-Caravaca et al. 2012; Mastebroek et al. 2000). The variation in the relative amount of sapogenins can be linked with cross-pollination which has been noted up to 9.9% in the *C. quinoa* and also it's accumulation in the various portion of the grain (Mastebroek et al. 2000; Ando et al. 2002).

In general, results revealed that the seven *C. quinoa* genotypes Cq-2, AZ-3, AZ-11, AZ-25, AZ-51, AZ-52, AZ-129 had a total saponins content above the threshold percentage (> 1%) among the genotypes we studied in this experimental trial. We adopted this empirical threshold based on a prior study (Medina-Meza et al. 2016). Thus, these genotypes can be categorized as bitter genotypes and must need to be passed through the post-harvesting process to remove saponins before products can be used for human consumption. Besides, twenty-two genotypes (AZ-4, AZ-7, AZ-9, AZ-12, AZ-15, AZ-26, AZ-27, AZ-29, AZ-30, AZ-34, AZ-35, AZ-46, AZ-67, AZ-68, AZ-88, AZ-104, AZ-107, AZ-108, AZ-111, AZ-113, AZ-114, AZ-115) showed total saponins level near to this threshold percentage (close to 1%), and these can also can be considered as high saponins content genotypes. The rest of the genotypes with a lower percentage of total saponins content, i.e. below the median of ca 0.6%, can be classified as low saponins genotypes. Among all genotypes and cultivars, ATLAS (Cq-3) can be classified as a sweet genotype due to the fact of its very low percentage of total saponins content. This complete assessment was similar to a previous study (Medina-Meza et al. 2016).

3.2 Evaluation of triterpenoid saponins of *C. quinoa* seeds

Saponins not only confer bitterness but also, comprise various biological activities which include cytotoxic, immunomodulatory, hepatoprotective, antidiabetic, hypolipidemic, antiosteoporosis, antiviral, antifungal as well as anthelmintic actions (Mroczek 2015). Therefore we also characterized saponins from *C. quinoa* by choosing the following analytical approach. First, saponins were separated by HPLC (**Figure 2.3**) and detected by high-resolution FTICR-MS in the full scan mode. The high accurate masses obtained allowed the calculation of chemical compositions with mass deviations lower than 3.0 ppm). Additional calculation of the corresponding ^{13}C- and $^{13}C_2$-isotopologues confirmed their correctness. In consideration of the molecular ion adduct $[M - H]^-$ formed by ESI(-) ionization

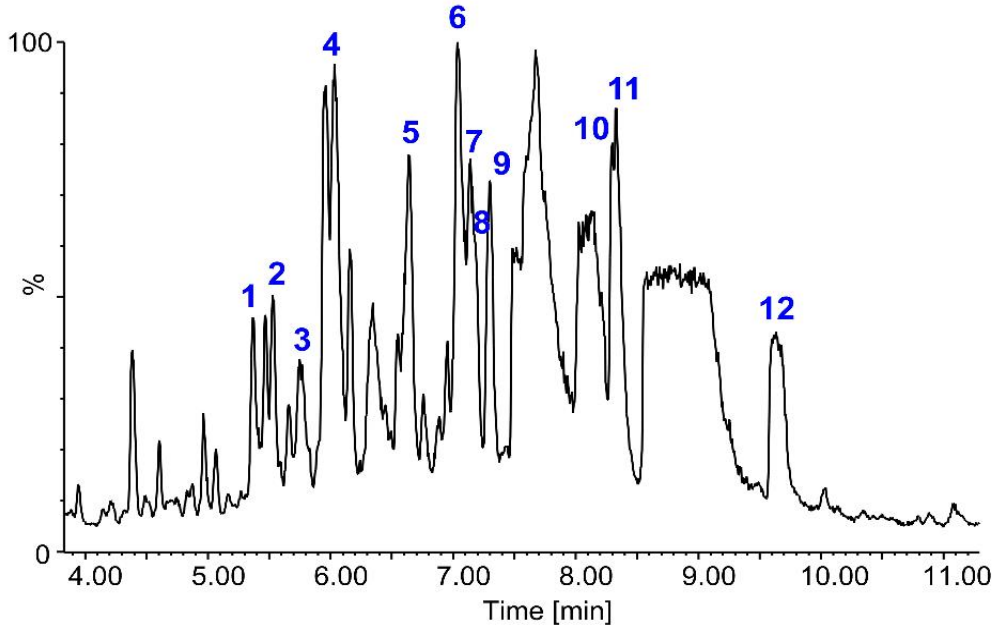

Figure 2.3. LC-ESI(-)-MS chromatogram of saponins from seeds of quinoa genotype AZ-129. Available saponins in seeds of quinoa accession AZ-129, were detected by FTICR-MS. The LC-MS chromatogram shows separated saponins obtained from quinoa seeds after extraction with methanol. The saponins 1 to 12 are assigned in Table 2.1.

molecular formulas had been derived. In the second step LC-MS/MS was used to obtain product ion spectra from the formerly determined precursor ions $[M - H]^-$ and additionally the corresponding formate adducts $[M + FA - H]^-$. The fragmentation patterns were used to identify the aglycones as well as the types of sugars and their sequence in the saponins. Out of the 12 identified saponins, 11 had been previously reported (**Table 2.1**). Compound 8 was assigned as a novel saponin. The product ion spectrum of compound 4 (**Figure 2.4**) chosen as an example for the other saponins is characterized by the gradual neutral losses of sugar units representing their sequence and the aglycone-specific product ion m/z 515, $[PA - H]^-$. The initial fragmentation of 4 (*m/z* 971) resulted in *m/z* 809 correspondings to $[M - H - 162]^-$ or the loss of a hexose residue. The loss of 28-*O*-linked hexose was also observed for nine other saponins as it is the energetically favored fragmentation pattern in comparison to 3-*O*-linked saccharides.(Madl et al. 2006) However, compounds 11 and 12 differ from that pattern displaying losses of pentose $[M - H - 132]^-$ as the initial step. This result might be due to the negative ESI mode whereas published data are based on ESI(+) conditions (Madl et al. 2006). Another possibility is a 28-*O*-linked pentose instead of hexose which has not been described in the literature so far. The majority of saponins were bearing the common aglycons OA (*m/z* 455), HD (*m/z* 471), SA (*m/z* 499), and PA (*m/z* 515). Compound 2 showed an aglycone at *m/z* 485 which suggests a structure similar to HD but with an ethyl instead of a methyl group at C-20 (Madl et al. 2006).

Table 2.1. Existing triterpenoid saponins from seeds of *C. quinoa* accession AZ-129.

		LC-FTICR-MS						LC-MS/MS
No.	**Saponin[a]**	**t_R[b] [min]**	**m/z experimental**	**Rel. Int. [%]**	**Composition $[M - H]^-$**	**Δ[c] [ppm]**	**Molecular formula**	**m/z**
1	3-*O*-Hex-Hex-***PA*** 28-*O*-Hex	5.36	1001.49539	100	C49 H77 O21	-0.89	$C_{49}H_{78}O_{21}$	1001 $[M - H]^-$, 839 $[M - H$ - Hex$]^-$, 677 $[M - H - 2$ Hex$]^-$, 515 $[M - H - 3$ Hex$]^-$ or $[PA - H]^-$
			1002.49931	59.07	C48 [13]C H77 O21	-0.33		
			1003.50333	14.02	C47 [13]C2 H77 O21	0.34		
2	3-*O*-Hex-Pent-***AG485*** 28-*O*-Hex	5.53	941.43785	100	C46 H69 O20	-0.98	$C_{46}H_{70}O_{20}$	941 $[M - H]^-$, 779 $[M - H$ - Hex$]^-$, 617 $[M - H - 2$ Hex$]^-$, 485 $[M - H -$ 2 Hex - Pent$]^-$ or $[AG485 - H]^-$
			942.44187	52.49	C45 [13]C H69 O20	-0.27		
			943.44482	15.17	C44 [13]C2 H69 O20	-0.7		
3	3-*O*-HexA-Pent-***PA*** 28-*O*-Hex	5.75	985.46400	100	C48 H73 O21	-1	$C_{48}H_{74}O_{21}$	985 $[M - H]^-$, 823 $[M - H$ - Hex$]^-$, 647 $[M - H -$ Hex - HexA$]^-$, 515 $[M - H -$ Hex – HexA - Pent$]^-$ or $[PA - H]^-$
			986.46742	60.18	C47 [13]C H73 O21	-0.93		
			987.47110	8.02	C46 [13]C2 H73 O21	-0.6		
4	3-*O*-Hex-Pent-***PA*** 28-*O*-Hex	6.03	971.48585	100	C48 H75 O20	0.14	$C_{48}H_{76}O_{20}$	(1017 $[M + FA - H]^-$)[d], 971 $[M - H]^-$,809 $[M - H$ - Hex$]^-$, 647 $[M - H - 2$ Hex$]^-$, 515 $[M - H - 2$ Hex - Pent$]^-$ or $[PA - H]^-$
			972.48963	52.7	C47 [13]C H75 O20	0.57		
			973.49181	17.3	C46 [13]C2 H75 O20	-0.63		
5	3-*O*-Hex-Hex-***SA*** 28-*O*-Hex	6.63	985.46519	100	C48 H73 O21	0.2	$C_{48}H_{74}O_{21}$	(1031 $[M + FA - H]^-$)[d], 985 $[M - H]^-$, 823 $[M - H$ - Hex$]^-$, 661 $[M - H - 2$ Hex$]^-$, 499 $[M - H - 3$ Hex$]^-$ or $[SA - H]^-$
			986.46874	54.6	C47 [13]C H73 O21	0.41		
			987.47249	11.4	C46 [13]C2 H73 O21	0.79		
6	3-*O*-Hex-Pent-***SA*** 28-*O*-Hex	7.03	955.49035	100	C48 H75 O19	-0.47	$C_{48}H_{76}O_{19}$	(1001 $[M + FA - H]^-$)[d], 955 $[M - H]^-$, 793 $[M - H$ - Hex$]^-$, 631 $[M - H - 2$
			956.49438	56.18	C47 [13]C H75 O19	0.23		

			957.49710	16.08	C46 [13]C2 H75 O19	-0.43		Hex]$^-$, 499 [M – H – 2 Hex - Pent]$^-$ or [SA – H]$^-$
7	3-*O*-HexA-***Hed*** 28-*O*-Hex	7.14	809.43306 810.43673 811.43956	100 43.43 10.93	C42 H65 O15 C41 [13]C H65 O15 C40 [13]C2 H65 O15	0.2 0.59 -0.05	$C_{42}H_{66}O_{15}$	809 [M – H]$^-$, 647 [M – H - Hex]$^-$, 471 [M – H – Hex - HexA]$^-$ or [Hed – H]$^-$
8	3-*O*-HexA-***SA*** 28-*O*-Hex	7.27	837.42806 838.43165 839.43421	100 57.59 11.22	C43 H65 O16 C42 [13]C H65 O16 C41 [13]C2 H65 O16	0.3 0.58 -0.37	$C_{43}H_{66}O_{16}$	837 [M – H]$^-$, 675 [M – H - Hex]$^-$, 499 [M – H – Hex - HexA]$^-$ or [SA – H]$^-$
9	3-*O*-Hex-Pent-***Hed*** 28-*O*-Hex	7.30	927.43863 928.50152	100 44.27	C47 H75 O18 C46 [13]C H75 O18	2.96 2.45	$C_{47}H_{76}O_{18}$	(973 [M + FA – H]$^-$)[d] 927 [M – H]$^-$, 765 [M – H - Hex]$^-$, 603 [M – H – 2 Hex]$^-$, 471 [M – H – 2 Hex - Pent]$^-$ or [Hed – H]$^-$
10	3-*O*-HexA-***OA*** 28-*O*-Hex	8.30	793.43723 794.44077 795.44454	100 48.31 9.08	C42 H65 O14 C41 [13]C H65 O14 C40 [13]C2 H65 O14	-0.94 -0.71 -0.19	$C_{42}H_{66}O_{14}$	793 [M – H]$^-$, 631 [M – H - Hex]$^-$, 455 [M – H – Hex - HexA]$^-$ or [OA – H]$^-$
11	3-*O*-Pent-HexA-***Hed*** 28-*O*-Hex	8.38	941.44005 942.44388 943.44724	100 50.0 16.2	C46 H69 O20 C45 [13]C H69 O20 C44 [13]C2 H69 O20	1.28 1.75 1.77	$C_{46}H_{70}O_{20}$	941 [M – H]$^-$, 809 [M – H - Pent]$^-$, 647 [M – H – Pent - Hex]$^-$, 471 [M – H – Pent - Hex - HexA]$^-$ or [Hed – H]$^-$
12	3-*O*-Pent-HexA-***OA*** 28-*O*-Hex	9.60	925.47997 926.48382 927.48743	100 53.69 15.5	C47 H73 O18 C46 [13]C H73 O18 C45 [13]C2 H73 O18	-0.29 0.24 0.52	$C_{47}H_{74}O_{18}$	925 [M – H]$^-$, 793 [M – H - Pent]$^-$, 631 [M – H – Pent - Hex]$^-$, 455 [M – H – Pent - Hex - HexA]$^-$ or [OA – H]$^-$

[a] PA: phytolaccagenic acid; SA: serjanic acid; HD: hederagenin; OA: oleanolic acid
Pent: pentose; Hex: hexose; HexA: corresponding sugar acid

[b] t_R: retention time

[c] Δ: deviation between experimental and theoretical mass in ppm

[d] ESI(-) molecular ion adduct formation with formate: [M + FA – H]

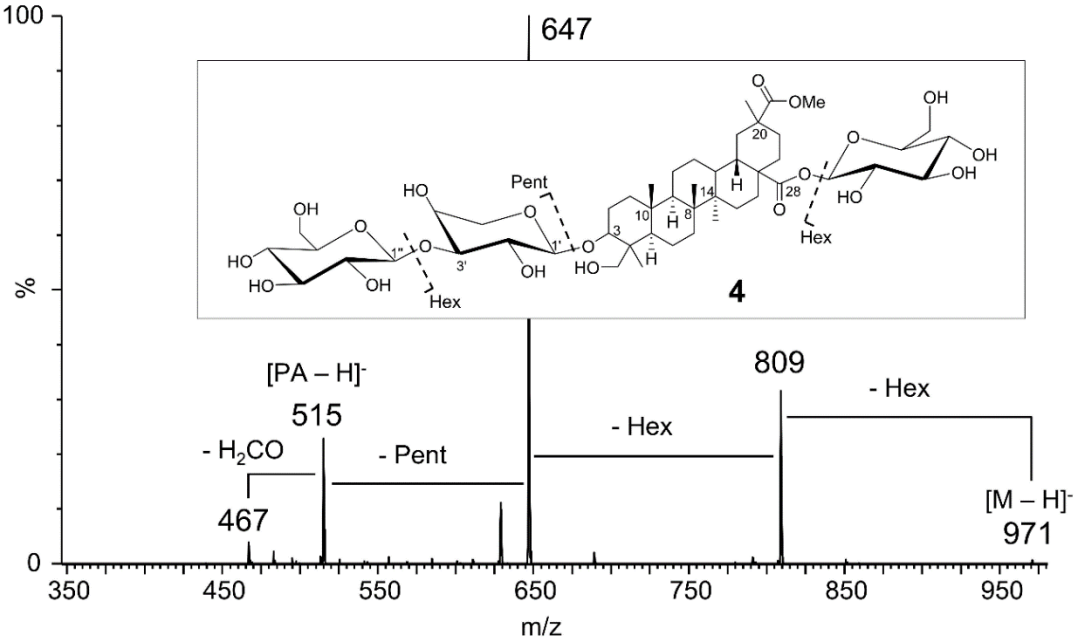

Figure 2.4. Product ion spectrum on [M - H]- of compound 4 (m/z 971) that was identified in genotype AZ-129. The fragment ion series m/z 809, 647 and 515 shows the consecutive loss of sugar units from the saponin as it is indicated in the inserted structural formula. The fragment ion m/z 515 is characteristic for phytolaccagenic acid as the aglycon.

3.3 Principal component analysis and Clustering

Principal component analysis (PCA) was performed to find a small number of linear orthogonal combinations of all variables that captured the greatest amount of variation present in our dataset as a whole. The overall dataset of total saponins and individual sapogenins was used to outline the main axes of the principal component analysis.

The PCA analysis revealed a 75% of variation for PC1 and 16% of variation for PC2 among all *C. quinoa* genotypes studied (**Figure 2.5A**). As stated in **Figure 5A**, the total saponins content is well described by PC1, while the content of OA and HD are well described by PC2. Further, cluster analysis was done based on the euclidean distance and complete grouping method using the PCA score (**Figure 2.5B**). The data output of each variable and for each of the genotypes grouped into two major clusters in proportion to the total saponins content. Based on the PCA score, cluster 4 had the lowest value for PC1. Since the main component of PC1 was negatively correlated with the PA and total saponins content, cluster 4 grouped the genotypes that have a high content of saponins. Cluster 3 pooled those genotypes that had a high amount of PA compared to the rest of the genotypes from other clusters, except cluster 4. On the contrary, cluster 5 contained a very low score PA and total saponins, which confirms the known genotypes with extremely low saponins content. Further, the remaining clusters (clusters 2 and 1) showed scattering nearby the core boundary of PC2. These clusters grouped those genotypes which characterized with low PA contents and thus low saponins content. **Figure 2.5** shows uninterpreted divergence among sub-groups of the major two groups without clear separation.

Interpretation of the high saponins cluster showed consistent variation within and among sub-groups, which represented accessions from both salares and coastal-lowland ecotypes (and regions). Moreover, the low saponin content genotypes scattered at close range distance compared with the genotypes that had high saponin content. The low saponins cluster segregated into PC1, including accessions from coastal-lowland regions and European cultivars. However, sub-groups shared some accessions from salares (AZ-14 and AZ-17) and south-altiplano (AZ-5). These data revealed an interpolation of coastal-lowland and salares genotypes in both PCA and dendrogram analysis. An interpolation of genotypes in two major clusters could likely be due to the genetic similarity between highland and coastal-lowland *C.quinoa* genotypes (Christensen et al. 2007). Another study also revealed the existing genetic diversity

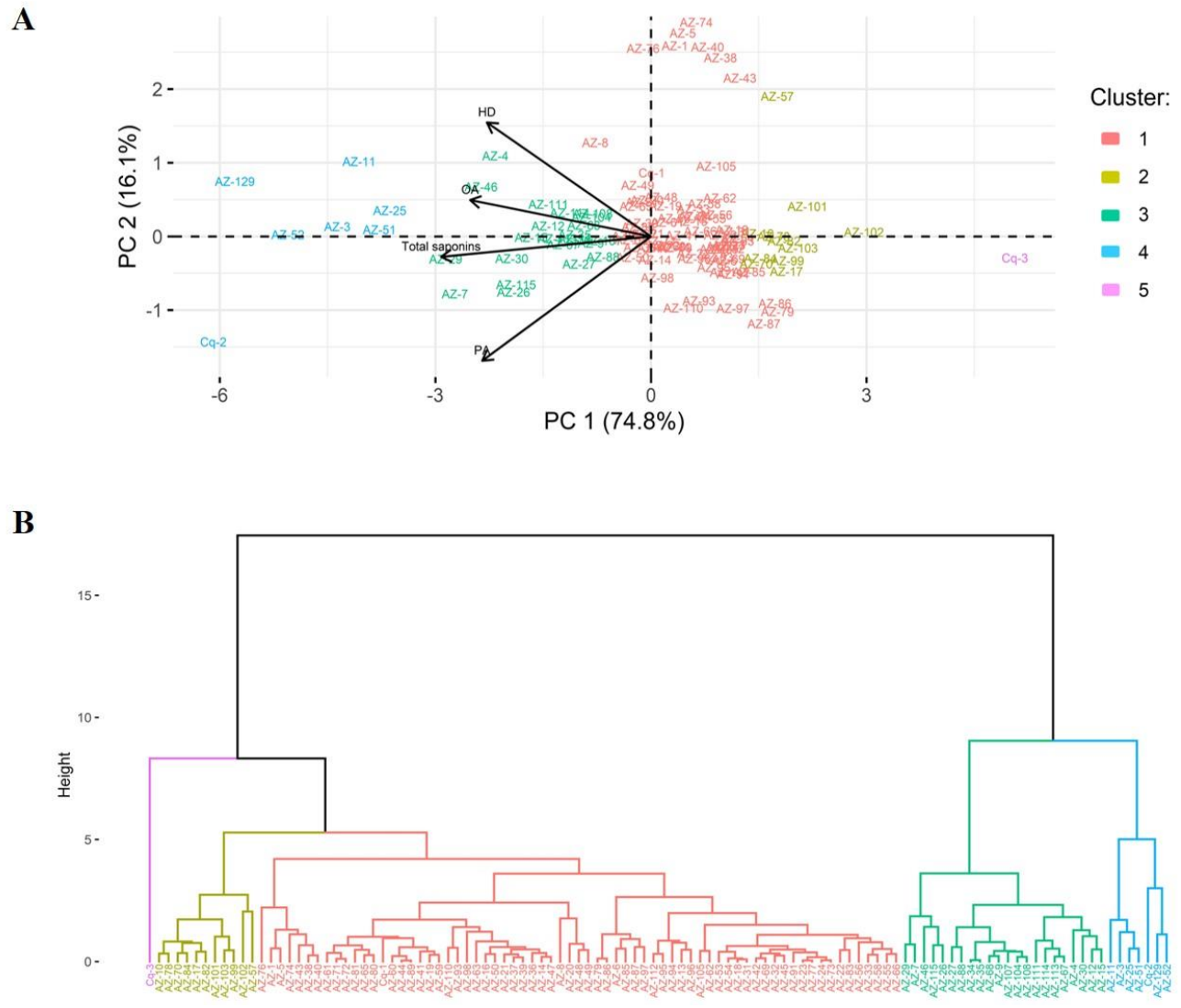

Figure 2.5. Principal component analysis (a) and hierarchical cluster (b) of triterpenoid saponins of *C. quinoa*. Data of total saponins and individual sapogenins was used to perform PCA analysis. Bi-plot showing two main components PC1 and PC2 explained 90.9% of total variation in saponins content. Arrows represent each variable and the length of arrows approximates the variance of the variables, whereas the angle between arrows indicates their correlation. The scores of each quinoa genotype are outlined as points. The distance between each point explained how similar the observations are. The content of total saponins and phytolaccagenic (PA) are well explained by PC1, while the contents of oleanolic acid (OA) and hederagenin (HD) are well explained by PC2. Cluster analysis was performed based on Euclidean distance and complete grouping method. The dendrogram from hierarchical cluster analysis shows two major clusters based on the total saponins which are further divided into five sub-groups. The cluster colors correspond to the cluster numbers in the legend.

within and among the different *C. quinoa* genotypes from different biomes of Chile, and such genetic diversity could be a primary reason for this possible variation in saponin content (Fuentes et al. 2009). Therefore, it is interesting to study the genetic background of these accessions to understand potential genomic variation resulting in relative saponins content in *C. quinoa*.

3.4 Variance by the genotypic effect

To estimate the effect of genetic diversity pattern, the percentages of variance explained by genotypic effect (Vg) in the total phenotypic variance for saponins content were estimated using a mixed linear model. This variance explained by genotypic effect (Vg) was calculated according to the restricted maximum likelihood (REML) variance components using the lme4 library of R (Bates et al. 2016). As a result, we found a significant genotypic effect ($p \leq 0.05$) which shows that each genotype express the phenotype of the measured traits differently, i.e., there is a genetic diversity that would explain the variation of saponins content. The significant effect of genotype represented by Vg validated the existence of genetic diversity up to 94.5% within the tested population for saponin content (**Table S4)**.

4 Conclusions

Conclusively, the GC-MS profiling addressed the high degree of significant variance in relative saponins content which ranges from 0.22 mg/g to 15.04 mg/g among the 114 different *C. quinoa* genotypes. In total twenty-nine genotypes were categorized as high saponins content, and thus required to remove saponins before products can be used for human consumption, while the rest of the genotypes were categorized as low-saponins or saponins free lines. In this study, PA was reported as a prominent sapogenin among others. Overall cluster data revealed uninterpreted divergence among sub-groups of major two groups without clear separation. These dissimilarities in the sapogenins can be attributed to the existing genetic diversity in *C. quinoa*. The high percentages of variance explained by the genotypic effect (Vg) in the total phenotypic variance for saponin content confirmed the existing diversity in terms of saponin content, which could become a target for future plant breeding efforts based on these accessions. Linked to this consideration, further genetic investigation such as using genome-wide association studies (GWAS) will be used in future studies to identify underlying genomic regions linked with saponins content to be employed in plant breeding of quinoa through marker-assisted selection.

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Chapter 3: Characterization of bioactive phenolic compounds in seeds of Chilean quinoa (*Chenopodium quinoa* Willd.) germplasm.

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1 Introduction

A healthy diet helps to protect against malnutrition in all its forms, as well as reducing the risk and incidence of non-communicable diseases (NCDs), including diabetes, heart disease, stroke, and cancer. Together with fruits, vegetables, and legumes whole grains are recommended by the WHO for a balanced and healthy diet [WHO]. Grain foods are a good source of valuable mineral nutrients, proteins, vitamins, and polyphenols. At present, the interest of agriculture sectors in functional foods has been on the rise for shaping healthy diets and improving human nutrition, and thereby seek for highly valuable foods has increased (Jones and Ejeta 2016; Fan and Pandya-Lorch 2012).

Chenopodium quinoa Willd. (quinoa) originated from the Andean region in South America and its seeds are not only rich in carbohydrates and proteins but also in vitamins, minerals, and polyphenols (Le et al. 2021; Repo-Carrasco-Valencia et al. 2010; Vega-Galvez et al. 2010). Bioactive phenolic compounds, including phenolic acids and flavonoids, are plant secondary metabolites that are considered to have potential health-beneficial effects, in particular, because of their reported antioxidant, anti-carcinogenic, anti-microbial, and anti-inflammatory activities (Benavente-García and Castillo 2008). Moreover, these compounds have been reported to have anti-herbivory characteristics in Amaranthus species of the Chenopodiaceae family (Niveyro et al. 2013). For example, rutin and kaempferol act as phagostimulant and feeding deterrents, respectively, and can alter the growth of aphids. Vanillic acid sugar ester glucoside is more effective on the offspring of aphids, while tannins reduce the savoriness of tissues due to their astringent characteristics (Murphy and Matanguihan 2015; Steffensen et al. 2011; Wink and Schimmer 2010; Wink 2006; Lattanzio et al. 2006).

In recent years, several studies on phenolic compounds in *C. quinoa* seed have shown that phenolic compounds exist in both free and bound forms. Usually, free phenolics are flavonoids or proanthocyanidins and their glycoside derivatives and, to a smaller extent, glucosides of ferulic and vanillic acid. In the fraction of bound phenolics phenolic acids such as e.g. benzoic acid, ferulic acid, and vanillic acid were identified which are building blocks of lignin as part of the cell walls. (Murphy and Matanguihan 2015; Gomez-Caravaca et al. 2011). Both free and bound phenolic forms can be extracted for analytical assessment by alkaline and acid hydrolysis. To date, phenolic compounds from *C. quinoa* have been evaluated mainly by spectrophotometric methods like the Folin-Ciocalteu assay (Nsimba et al. 2008; Tang et al. 2015; Pasko et al. 2009; Gorinstein et al. 2007). However, the accuracy

of this assay can be influenced by a number of interfering substances and, in addition, there is a lack of standardization. These aspects lead to an overestimation of phenolic compounds and insufficient comparability, respectively. To improve analyses, over the past few years, methodologies based on nuclear magnetic resonance (NMR), high-pressure liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS) have been used for identifying and quantifying phenolic compounds in *C. quinoa* seeds (Melini and Melini 2021; Hur et al. 2018; Christophoridou and Dais 2009; Zhu et al. 2001; del Hierro et al. 2020; Liu et al. 2020). High-performance liquid chromatography with diode array detection (DAD) and electrospray ionization- (ESI) tandem mass spectrometry (MS/MS) are currently the methods of choice for the analysis of phenolic compounds. The use of MS/MS not only allows the identification of individual phenolic compounds by their fragmentation patterns but also their quantification with high reliability (Sampaio et al. 2020; Stikic et al. 2020; Balakrishnan and Schneider 2020; Gomez-Caravaca et al. 2011).

Because phenolic compounds are reported to have significant dietary and industrial importance, it is valuable to characterize in detail genetic diversity in seed composition in quinoa in order to identify candidate genotypes with desirable phenolic content profiles. It is important to note that, to this extent, the dietary composition of *C. quinoa* seeds and their phenolic compounds, such as phenolic acids and flavonoids may differ among different cultivars.(Mhada et al. 2020; Tang et al. 2015; Gomez-Caravaca et al. 2011; Pasko et al. 2008; Repo-Carrasco-Valencia et al. 2010).

This knowledge, in turn, could lead to potential applications in agriculture and pharmaceutical practices. However, it should be taken into account that secondary metabolites are formed in the plant as an adaptive response to environmental factors and that their concentrations depend on genetic background-specific environmental factors and their interactions (Antognoni et al. 2021b; Brunetti et al. 2018; Chandrasekara and Shahidi 2011). Recent investigations revealed the effects of agroecological factors on bioactive phenolic compounds in other diverse crops such as *Brassica* species, baby leaf lettuces, and strawberry fruits (Biondi et al. 2021; Marin et al. 2015; Anttonen et al. 2006).

Previous research on quinoa highlighted the importance of considering these aspects in detail. Significant effects of agronomical or/and environmental factors including salinity, drought, and cultivation region in influencing nutritional values of *C. quinoa* seed (Reguera et al. 2018). Abiotic factors such as salinity and drought have a great influence on the content of bioactive phenolic compounds (Gómez-Caravaca et al. 2012). It has been reported that water deficit is likely to increase the content of phenolic compounds, while irrigation with saline water is responsible for a gradual drop

in the phenolics content. Also, weed interference and its effect on total polyphenol content in *C. quinoa* was studied before (Merino et al. 2019). Several studies reported the influence of contrasting environmental conditions on phenolics content. For example, Miranda et al. (2013) noted that several *C. quinoa* genotypes that were grown in different agroecological locations in Chile contained varying amounts of bioactive polyphenols, which ranged from 12.39 mg GAE/100 g to 22.87 mg GAE/100 g. In the same study, it was reported that there was a significant increase, which means 1.5 folds in phenolics content under a water-deficit environment (Miranda et al. 2013). In addition, it has been shown that agroecological conditions, particularly the light intensity/ UV-B could have altered the phytochemical content in *C. quinoa* seed (Antognoni et al. 2021b; Treutter 2005). Even though phenolic contents have not been studied comprehensively for Chilean *C. quinoa* genotypes, with a few exceptions, previous studies have shown variations in phenolic content among different *C. quinoa* landraces (Antognoni et al. 2021a; Miranda et al. 2012)

In the present work, we hypothesized that the content of these bioactive compounds is strongly influenced by inherent genetic variation, and as a result, such bioactive phytochemicals may vary in different quinoa accessions. To this purpose, we analyzed the phenolic profiles of *C. quinoa* germplasm collected from two different biomes within Chile to verify if such bioactive phytochemicals in seed extracts vary according to geographical origin. So, we aim;

1. To evaluate the composition of both free and conjugated phenolics among 111 *C. quinoa* genotypes.

Their seeds were collected during field trials under the same agroecological conditions in the southern Atacama desert region in Chile. Our results show that the existing variation in phenolic compounds in these accessions supports the selection of candidate genotypes for further research and development. In a plant breeding context, the present knowledge of both the total phenolics and individual compounds can be used short term to develop molecular markers and determine genomic regions that are linked to bioactive phenolic compounds to support future *C. quinoa* genetic improvement programs.

2 Materials and Methods

2.1 Chenopodium quinoa germplasm

In the present study, the phenolic compounds were assessed in 111 different *C. quinoa* accessions including advanced breeding lines in the framework of the ongoing Instituto de Investigaciones Agropecuarias's Quinoa Breeding Program (INIA's - IQBP) in Chile. This *C. quinoa* collection was initially established by INIA, Chile through mass selection and self–pollination of individual lines for at least two growing seasons. This diversity panel included 7 genotypes (salares ecotype) originally

collected in the Chilean Altiplano (highlands region), 2 south Altiplano genotypes from the Cancosa area, and 102 genotypes originating from the Chilean coastal-lowland regions (**Figure 3.1**). Most of the genotypes were cultivated and harvested at Huasco experimental station (28°3' S, 70°4' W) located in the southern Atacama desert region. Typically, environmental conditions in this research station during the quinoa growing season are characterized by high solar irradiance, cool nights, and virtually no precipitation requiring irrigation throughout crop growth. The soil at the experiment station consensus to La Compañia string, a sandy loamy class of soil with low organic matter, and showed alkaline pH and with the following macronutrient amounts; N (45 mg/kg), P (21 mg/kg), and K (311 mg/kg).

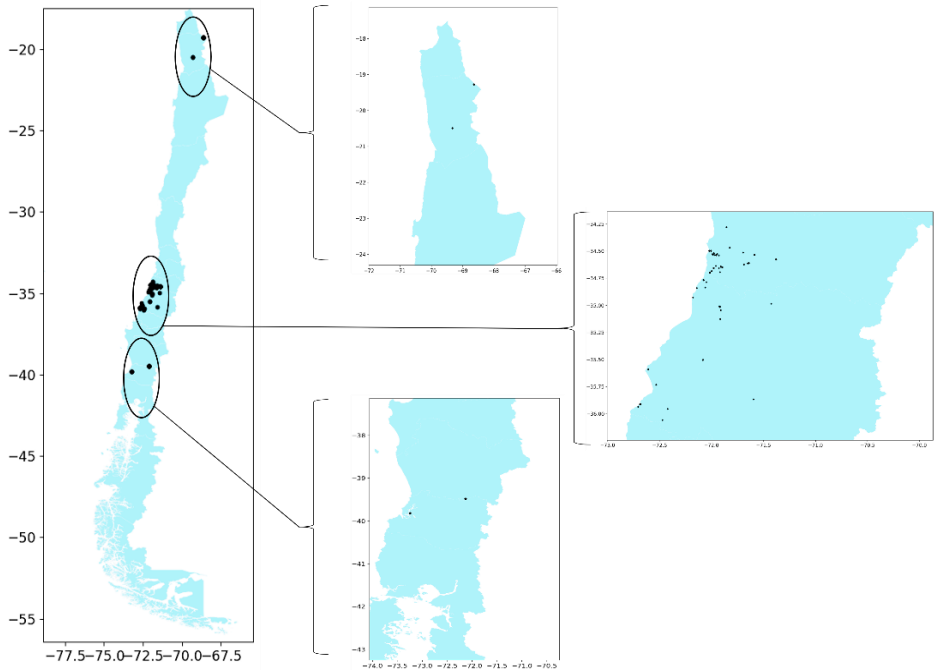

Figure 3.1. Localities of. *C. quinoa* germplasm. *C. quinoa* germplasm belongs to two groups of genotypes representing the large variation from two different regions, coastal-lowland as well as from highland, of Chile. The diversity panel included 7 genotypes (salares ecotype) originally collected in the Chilean Altiplano (highlands region), 2 south Altiplano genotypes from the Cancosa area, and 102 genotypes originating from the Chilean coastal-lowland regions.

2.2 Chemicals

Caffeic acid, cinnamic acid, coumaric acid, ferulic acid, gallic acid, 4-hydroxybenzoic acid, kaempferol, quercetin, quercetin-3-O-glucuronide, quercetin 3-O-sambubioside, rutin, syringic acid, and vanillic acid were purchased from VWR (Darmstadt, Germany). Acetonitrile, ethyl acetate, hexane, methanol, water (all LC-MS grade), hydrochloric acid, and sodium hydroxide were supplied by VWR (Darmstadt, Germany).

2.3 Extraction of free phenolic compounds from quinoa seeds

Free phenolic compounds were extracted from quinoa seeds according to Gomez-Caravaca et al. (2011) with some modifications. 100 mg ground quinoa seeds were extracted with 1.5 mL water: methanol

(3:1) containing 0.1% formic acid by use of a vortex for 1 min followed by an ultrasonic bath for 20 min. After centrifugation at 12000 rpm for 2 min, 1 mL supernatant was withdrawn and filtered through a 0.2µm PTFE filter. The pellet was dried in a stream of nitrogen gas. Samples were stored at -20°C until analysis.

2.4 Extraction of bound phenolic compounds from quinoa seeds

The remaining pellet from the extraction of the free phenolic compounds was used for the extraction of the bound phenolic compounds following the method of Gomez-Caravaca et al. (2011). Briefly, the dried pellet was resuspended in 1 mL water and transferred into a bigger sample tube. Powder adherent to the wall of the storage tube was removed three times with 1.5 mL 2 M sodium hydroxide which was combined with the suspension. After gently blowing nitrogen gas into the tubes, these were vigorously mixed on a vortex for 1 min and thereafter placed in an ultrasonic bath for 30 min. Then the suspensions were shaken at room temperature for 20 h. After cooling on ice the sample solutions were brought to pH 1 – 2 by dropwise addition of 32% hydrochloric acid. For the removal of lipids, the samples were extracted with 20 mL hexane. The phenolic compounds were finally extracted three times with 4 mL ethyl acetate by vortexing for 1 min. The combined organic fractions were evaporated to dryness and reconstituted in 2 mL water: methanol (3:1) mixture. Prior to analysis, the samples were filtered through 0.2 µM PTFE filters.

2.5 Identification and quantification of phenolic compounds by liquid chromatography-diode array detection-tandem mass spectrometry (LC-DAD-MS/MS) analysis

LC-DAD-MS/MS was done on a Waters ACQUITY® UHPLC system (binary pump, autosampler, diode array detector) coupled to a Waters Xevo TQ-S® triple-quadrupole mass spectrometer (Waters Technologies Corp., Milford, MA, USA). Separation of phenolic compounds was achieved on a Nucleoshell RP18 column (100 x 4.6 mm, 2.7 µm; Macherey-Nagel, Düren, Germany). The column was equipped with a pre-column (Macherey –Nagel, Düren, Germany) and maintained at 40°C. The mobile phases were water (A) and acetonitrile (B) each containing 0.1% formic acid, at a flow rate of 1.0 mL/min. The gradient program was as follows: 85% A, to 60% A within 7 min, to 2% A within 0.5 min and holding for 2.5 min, back to 85% A within 0.1 min, and holding for 2.9 min. The injection volume was 1 µL. UV spectra were recorded in the wavelength range 200 – 400 nm. For the identification of phenolic compounds, the electrospray ionization (ESI) interface of the mass spectrometer was driven in the positive as well as negative mode. The capillary voltage was set to 2.5 (ESI(+)) and 2.0 kV (ESI(-)), respectively. The desolvation temperature and source temperature were 600°C and 150°C, respectively. The desolvation gas flow was set to 1000 L/h and the cone gas flow at 150 L/h using nitrogen in both cases. MS detection was carried out in full scan mode (m/z 50 – 1000).

Identified molecular ion adducts were submitted to collision-induced dissociation in the daughter ion scan mode at collision energies of 8, 10, 15, 20, and 30 eV.

Quantification was performed in the ESI(-) mode by applying the same ESI setting as mentioned above. The triple-quadrupole mass spectrometer was driven in the multiple reaction monitoring (MRM) mode for the detection of selected phenolic acids and flavonoid glycosides (**Table 3.1**). Nitrogen was used as the collision gas at a flow of 0.15 mL/min.

Data acquisition and processing were performed by use of the software MassLynx 4.2 (Waters Technologies Corp., Milford, MA, USA). Analysis of each quinoa genotype or cultivar was performed in technical duplicates. Quantification was done by the method of external calibration with standard solutions in the concentration range 0.1 – 25 µM.

Table 3.1. MRM parameters of analyzed phenolic compounds.

No.	Compound	t_R [min]	Precursor ion $[M - H]^-$ m/z	Product ion (Quant./Qual.) m/z	Cone [V]	Collision energy (Quant./Qual.) [V]
1	4-Hydroxybenzoic acid	2.33	137.0	93.6/65.0	46	20/26
2	Vanillic acid	2.61	167.0	152.0/108.0	22	14/18
3	Syringic acid	2.63	197.0	182.0/123.0	54	14/22
4A,B	Coumaric acid	3.51/3.76	163.0	119.4/93.0	42	17/28
5A,B	Ferulic acid	3.87/4.10	193.0	134.0/178.0	52	16/14
6A,B	Quer-Hex-(DHex-Pent)[a]	[c]	741.0	300.1[d]/271.0	94	36/62
7A-E	Quer-Hex-DHex[a]	[c]	609.0	300.1[d]/271.0	86	34/60
8A,B	Quer-Hex-Pent[a]	[c]	595.0	300.1[d]/271.0	74	30/52
9A,B	Quer-Hex[a]	[c]	463.0	300.1[d]/271.0	54	36/58
10	Quer-HexA[a]	[c]	477.0	301.1/151.0	80	20/36
11	Quercetin	6.21	301.0	151.0/179.0	76	20/18
I[b]	Cinnamic acid	6.76	147.0	103.0/77.0	32	10/20
II[b]	Gallic acid	1.18	169.0	97.0/69.0	24	18/22
III[b]	Caffeic acid	2.49	179.0	79.0/107.0	20	24/22
IV[b]	Kaempferol	7.57	285.0	151.0/93.0	82	18/30

[a] Que: Quercetin
Pent: pentose; Hex: hexose; DHex: deoxyhexose; HexA: hexuronic acid

[b] Compounds I – IV were < LoD in all samples

[c] see table 3

[d] Radical aglycon product ion from homolytic cleavage of the glycosidic bond ($[Y_0 - H]^{-\bullet}$, m/z 300) (Hvattum and Ekeberg 2003)

2.6 Statistical analysis

All analyzed data are recorded as mean ± standard deviation (SD) of single extractions (n = 4). The complete dataset was subjected to the Shapiro-Wilk normality test. To address statistical significance in phenolic content among the studied *C. quinoa* genotypes, the dataset was analyzed with a one-way analysis of variance ANOVA (Tukey's – Honestly Significant Difference multiple comparisons, α = 0.05) using the R-package Agricolae (De Mendiburu 2020). Principal component analysis (PCA) was performed to summarize the whole dataset by means of a smaller set of concise indexes of specific variables based on a correlation distance matrix using R-packages (factoextra and FactoMineR) (Kassambara and Mundt 2020; Le et al. 2008). Cluster analysis was done based on the euclidean distance and complete grouping method using PCA scores. In addition, the percentage of variance explained by the genotypic effect (Vg) in the total phenotypic variance for phenolic compounds was calculated based on the restricted maximum likelihood (REML) variance components using the lme4 library of R (Bates et al. 2016).

3 Results and Discussion

3.1 Detection of phenolic compounds by HPLC-DAD-MS/MS

In the current work, a total of 111 *C. quinoa* genotypes were analyzed for both free and bound fractions of phenolics. To achieve this goal, a C18 core-shell column was selected to determine the existing phenolic compounds, as described previously (Gomez-Caravaca et al. 2011). All accessible free and

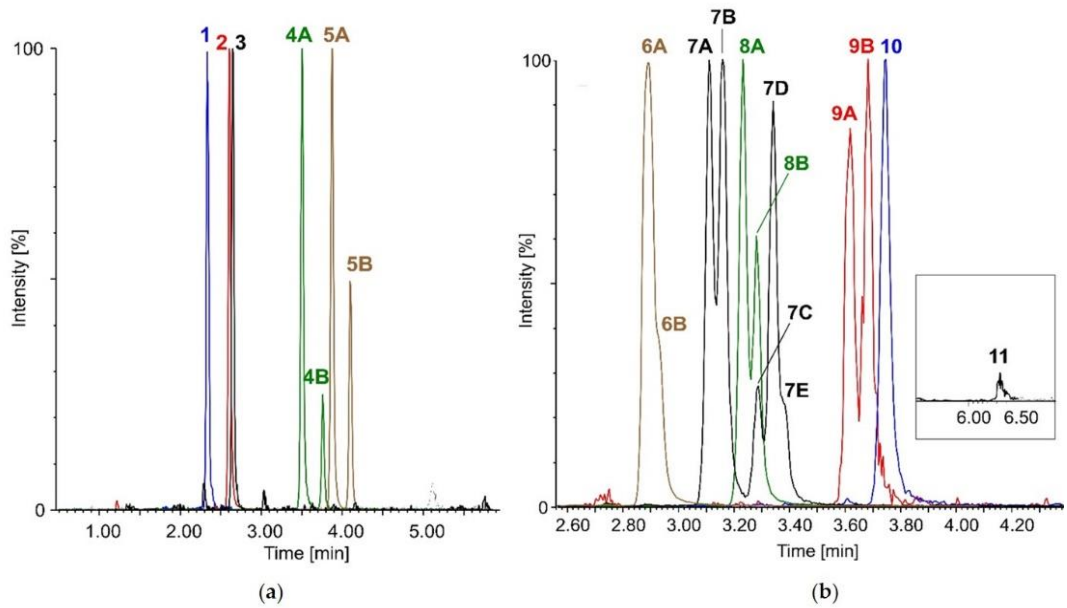

Figure 3.2. Overlaid MRM chromatograms of phenolic acids from the bound phenolic fraction (a) and flavonoid glycosides from the free phenolic fraction (b) of quinoa seeds obtained by LC-ESI(-)-MS/MS. HPLC chromatogram profile shows the separated free and bound phenolic derivatives from *C. quinoa* seed extract. Total phenolic content was quantified by means of their free and bound fractions of phenolics. Fragments of phenolic derivatives were assigned by mass spectra and comparing retention times to the corresponding standards. For peak assignment of (a) see Table 3.1 and of (b) see Table 3.2.

bound phenolic fractions were detected (**Figure 3.2**). However, an isocratic step with 100% acetonitrile was added to the gradient program to flush out potential lipophilic compounds from the column. An important factor was the choice of solvent composition for the samples. Water:methanol (3:1) turned out to have the best influence on both the sharpness and symmetry of the peaks. However, with an increasing amount of methanol peak broadening and/or splitting was observed.

3.2 Assessment of free phenolic fraction in *C. quinoa* seed

By extraction of quinoa seeds with water:methanol three main families of compounds could be obtained: phenolic acids, flavonoid glycosides, and saponins. The results for saponins are described in a previous publication (Pandya et al. 2021).

Nineteen different phenolic compounds have been found and quantified by LC-MS among studied genotypes (**Table 3.1**). **Figure 3.2a** illustrates the overlaid MRM chromatograms of seven phenolic compounds which were found in the free and in much larger quantities in the bound phenolic fraction of *C. quinoa* seeds. Therefore this phenolic fraction will be discussed in detail in section 3.3.

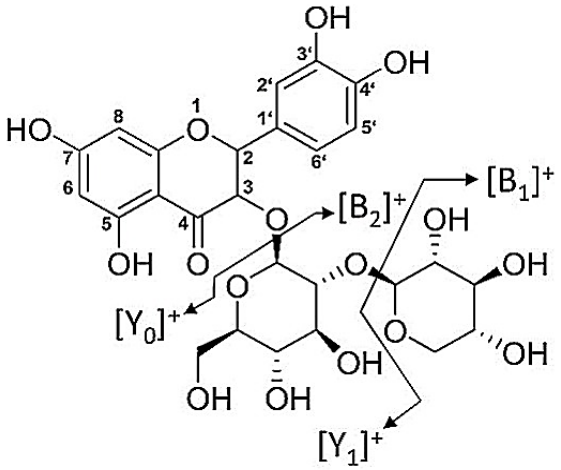

Figure 3.3. Nomenclature of formed product ions from the fragmentation of flavonoid glycosides. Y_j represents the product ions still containing the aglycon, where j is the number of the inter-glycosidic bond broken, counting from the aglycone. The glycosidic bond between the sugar unit and the aglycon is numbered 0. B_i are the cleaved sugar moieties where i represents the number of the glycosidic bond cleaved, counting from the last sugar unit in the molecule.

The other twelve phenolic compounds could be assigned to the class of flavonoid glycosides (**Figure 3.2b**). For their unambiguous characterization, LC-DAD-MS/MS analysis was performed in the positive ESI mode since this mode is more informative than ESI(-) mode for the structural evaluation (Abad-Garcia et al. 2009). UV spectra of compounds 6-10 showed two absorption bands at 250 – 255 nm and 350 – 355 nm, respectively, which is typical for quercetin glycosides (Djoukeng et al. 2008). After identification of the $[M + H]^+$ ions in the full scan mode, they were submitted to MS^2 fragmentation in order to obtain their product ion spectra (**Table 3.2**). Subsequently, the nomenclature described by Domon and Costello (1988) was employed for the assignment of the product ions of flavonoid glycosides (**Figure 3.3**).

Table 3.2. Product ions of $[M + H]^+$ for quercetin glycosides from the free phenolic fraction of quinoa seed extracts.

No.	t_R [min]	Flavonoid glycoside[a]		m/z (% base peak intensity)							CE [eV]	Lit.
				$[M + H]^+$	$[Y_0]^+$	$[Y^*]^+$	$[Y_1]^+$	$[Y_2]^+$	$[B_1]^+$	$[B_2]^+$		
6A	2.89	Quer-Hex-DHex-Pent	Quer-7-*O*-Glc-Rha-Xyl	742.76 (100)	303.17 (82.5)	-	465.06 (19.3)	610.83 (5.1)	-	-	15	
6B	2.93		Quer-3-*O*-Glc-Rha-Xyl	742.82 (100)	303.50 (10.6)	-	465.12 (7.5)	-	-	-	15	
7A	3.11	Quer-Hex-DHex or Quer-HexA-Pent	Quer-7-*O*-Glc (6→1)Rha	611.23 (18.8)	303.06 (100)	449.10 (12.1)	465.12 (30.7)	-	147.38 (5.0)	309.03 (11.1)	10	(Abad-Garcia et al. 2009)
7B	3.16		Quer-7-*O*-Glc (2→1)Rha	611.10 (40.6)	303.25 (100)	449.10 (32.6)	465.12 (65.0)	-	146.92 (4.3)	-	10	(Abad-Garcia et al. 2009)
7C	3.29		Quer-3-*O*-Glc (2→1)Rha	611.17 (40.5)	303.0 (100)	449.17 (6.1)	465.18 (30.3)	-	147.38 (2.3)	309.29 (0.7)	10	(Abad-Garcia et al. 2009)
7D	3.34		Quer-3-*O*-GlcA(2→1)Xyl	611.23 (17.2)	303.06 (45.6)	435.32 (2.7)	479.29 (100)	-	-	-	10	
7E	3.38		Quer-3-*O*-Glc (6→1)Rha[b]	611.10 (41.8)	303.25 (100)	449.0 (3.1)	465.18 (44.9)	-	-	-	10	(Abad-Garcia et al. 2009)
8A	3.23	Quer-Hex-Pent	Quer-3-*O*-Gal(6→1)Xyl	597.19 (27.1)	303.19 (100)	435.18 (4.3)	465.18 (35.8)	-	133.34 (3.3)	295.11 (3.9)	8	(Grayer et al. 2002)
8B	3.28		Quer-3-*O*-Glc(6→1)Xyl	597.25 (28.9)	303.19 (100)	435.12 (0.7)	465.25 (20.3)	-	133.34 (0.5)	295.25 (0.8)	8	(Unuofin and Lebelo 2021; Shui and Peng 2004)

9A	3.62		Quer-3-*O*-Gal	464.99 (88.2)	303.22 (100)	-	-	-	-	-	8	(Ersan et al. 2016)
9B	3.68	Quer-Hex	Quer-3-*O*-Glc	465.19 (27.3)	303.22 (100)	-	-	-	-	-	8	(Ersan et al. 2016; Gomez-Caravaca et al. 2011; Gomez-Caravaca et al. 2012)
10	3.74	Quer-HexA	Quer-3-*O*-GlcA[b]	479.15 (100)	303.17 (99.5)	-	-	-	-	-	8	(Ersan et al. 2016; Gomez-Caravaca et al. 2011)

All MS^2 mass spectra have a fragment profile at m/z 303 ($[Y_0]^+$) indicating quercetin as the only aglycon in all identified flavonoid glycosides. In summary, five groups of quercetin glycosides were noted which were differentiated in the number and type of sugar units (**Table 3.2**). Quercetin glycosides with a high degree of glycosylation elute first followed by those with decreasing glycoside units (**Figure 3.2b**). This elution pattern corresponds to the one reported previously (Gomez-Caravaca et al. 2011). MS/MS analyses of both isomers of tri-glycosylated compounds (compound 6A/B) that were detected at 2.89/2.93 min have shown unfragmented $[M + H]^+$ at *m/z* 743 as base peaks at CE = 15 eV. However, an increase in collision energy only led to not interpretable mass spectra. Fragmentation of 6A showed the product ions $[Y_2]^+$, loss of Xyl), $[Y_1]^+$ ($[M + H – 132 - 146]^+$, loss of Xyl-Rha) and $[Y_0]^+$ ($[M + H – 132 –146 - 162]^+$, loss of Xyl-Rha-Glc). In literature flavonoid, tri-glycosides are described with glycosylation positions for naringenin and quercetin at C-7 and C-4'. However, an O-glycosylated glucose at C-4' would be led to a strong $[Y^{4'}{}_0]^+$ product ion at m/z 581, which is not present in the MS^2 spectra of 6A/B (Abad-Garcia et al. 2009; Gil-Izquierdo et al. 2004). Therefore, compound 6A was tentatively considered to be **quercetin 7-*O*-xylosyl-rutinoside**. The product ion spectrum of 6B was characterized by weak peaks at m/z 465 and 303 which tentatively suggests a similar structure as for 6A but with a 3-O-glycosylation. Further, mass spectra of five diglycosylated quercetins (compound 7A-E) were eluted between 3.11 and 3.38 min. The MS^2 spectra of 7A, B, C and E looked very similar with $[Y_1]^+$ ($[M + H - 146]^+$, loss of Rha) and $[Y_0]^+$ ($[M + H – 146 - 162]^+$, loss of Rha-Glc). Furthermore $[Y^*]^+$ could be observed which is formed by an internal glucose residue loss ($[M + H – 162]^+$) as a consequence of a rearrangement (Ma et al. 2000) as well as $[B_1]^+$ and $[B_2]^+$. The structural differences between these four quercetin *O*-glucosyl rhamnosides could result from the inter-glycosidic linkage between rhamnose and glucose (Rha(1→6)Glc (rutinose) / Rha(1→2)Glc (neohesperidose)) and the O-glycosylation position of quercetin at C-3 or C-7. Based on the guideline for characterization of O-di-glycosyl flavonoid isomers with respect to their inter-glycosidic linkage isomery and glycosylation positions, 7A could be tentatively assigned as **quercetin 7-*O*-rutinoside** and 7E as **quercetin 3-*O*-rutinoside** (rutin) because of their retention order (Abad-Garcia et al. 2009). 7E was confirmed by comparison with the retention time and MS^2 spectrum of rutin as a reference substance. 7B and 7C are assigned as **quercetin 7-*O*-neohesperidoside** and **quercetin 3-*O*-neohesperidoside,** respectively. However, the intensities of the fragment ions in the mass spectra of 7A, 7B, 7C, and 7E differ from those in the literature (Abad-Garcia et al. 2009). The order of elution of 7C and 7E is also inverse to the general elution order for O-diglycosyl flavonoids (Abad-Garcia et al. 2009). These differences might be explained by the use of different mass spectrometric systems and HPLC columns. Compound 7D totally differs from the other showing the fragment ions $[Y_1]^+$ ($[M + H - 132]^+$, loss of Xyl), $[Y_0]^+$ ($[M + H – 132 - 176]^+$, loss of Xyl-GlcA) and $[Y^*]^+$ ($[M + H - 176]^+$, loss of GlcA). It was tentatively identified as **quercetin 3-*O*-[xylosyl-(1→2)-glucuronide]**. Next,

compounds 8A and 8B appeared at 3.23 and 3.28 min, respectively. The product ions $[Y_1]^+$ ([M + H - 132]$^+$), $[Y_0]^+$ ([M + H – 132 - 162]$^+$) and $[Y^*]^+$ ([M + H - 162]$^+$) indicated sequential loss of pentose and hexose. Quercetin 3-O-[xylosyl-(1→2)-glucoside] (quercetin 3-O-sambubioside) could be excluded as a possible isomer because the reference standard eluted earlier than 8A/B. Based on the former literature compound 8A was tentatively identified as **quercetin 3-O-[xylosyl-(1→6)-galactoside]** (Grayer et al. 2002) and 8B as **quercetin 3-O-[xylosyl-(1→6)-glucoside]** (Unuofin and Lebelo 2021; Shui and Peng 2004). Compounds 9A and 9B eluted at retention times of 3.62 and 3.68 min, respectively. They showed the loss of hexose ([M + H – 162]$^+$). Compound 9A was tentatively identified as **quercetin 3-*O*-galactoside** and 9B as **quercetin 3-*O*-glucoside** as it was already reported in pistachio hulls by Erşan et al. (2016) The presence of 9B in quinoa has already been described by Gomez-Caravaca et al. (2012; 2011). The last quercetin glycoside 10 which occurred at a retention time of 3.74 min was unambiguously identified as **quercetin 3-*O*-glucuronide** by comparison to the reference compound. Its presence in quinoa was also reported by Gomez-Caravaca et al. (2012; 2011).

3.3 Assessment of bound phenolic fraction in *C. quinoa* seed

The fraction of bound phenolic compounds was obtained after alkaline hydrolysis of the residue from the water/methanol extraction of ground *C. quinoa* seeds (Gomez-Caravaca et al. 2012). Five different types of phenolic acids have been identified in this fraction by comparison with retention times and mass spectra of the corresponding reference substances (**Figure 3.2a**, **Table 3.1 - No. 1 – 5**). Compounds 1 – 5 were assigned according to published literature as hydroxybenzoic acid, vanillic acid, syringic acid, coumaric acid, and ferulic acid, respectively (del Hierro et al. 2020; Han et al. 2019; Gomez-Caravaca et al. 2011). Remarkably, two fragments at the retention times of 3.51 min and 3.76 min have been noted for coumaric acid (**Figure 3.2a**, peaks 4A/B) as well as for ferulic acid (**Figure 3.2a**, peaks 5A/B) at 3.87 and 4.10 min. Such phenomenon is likely due to the E/Z-isomerism of the double bond in the vinyl carboxylic acid side chain (Renard et al. 1999; Borges et al. 1991). It is important to note that for the quantification of both phenolics, the fragment areas of both isomers were summed up. Among the five phenolic acids, ferulic acid showed the highest concentration which is in agreement with previous literature (Gomez-Caravaca et al. 2011). This result could be explained by its high amount in the cell walls of *C. quinoa* seeds (Renard et al. 1999).

3.4 Phenolic profile in *C. quinoa* seed

In our experimental work, the content of total phenolic compounds in *C. quinoa* seed was evaluated as the sum of both bound and free fractions. The class of phenolic acids including hydroxybenzoic acid, vanillic acid, syringic acid, coumaric acid, and ferulic acid was determined and evaluated in the

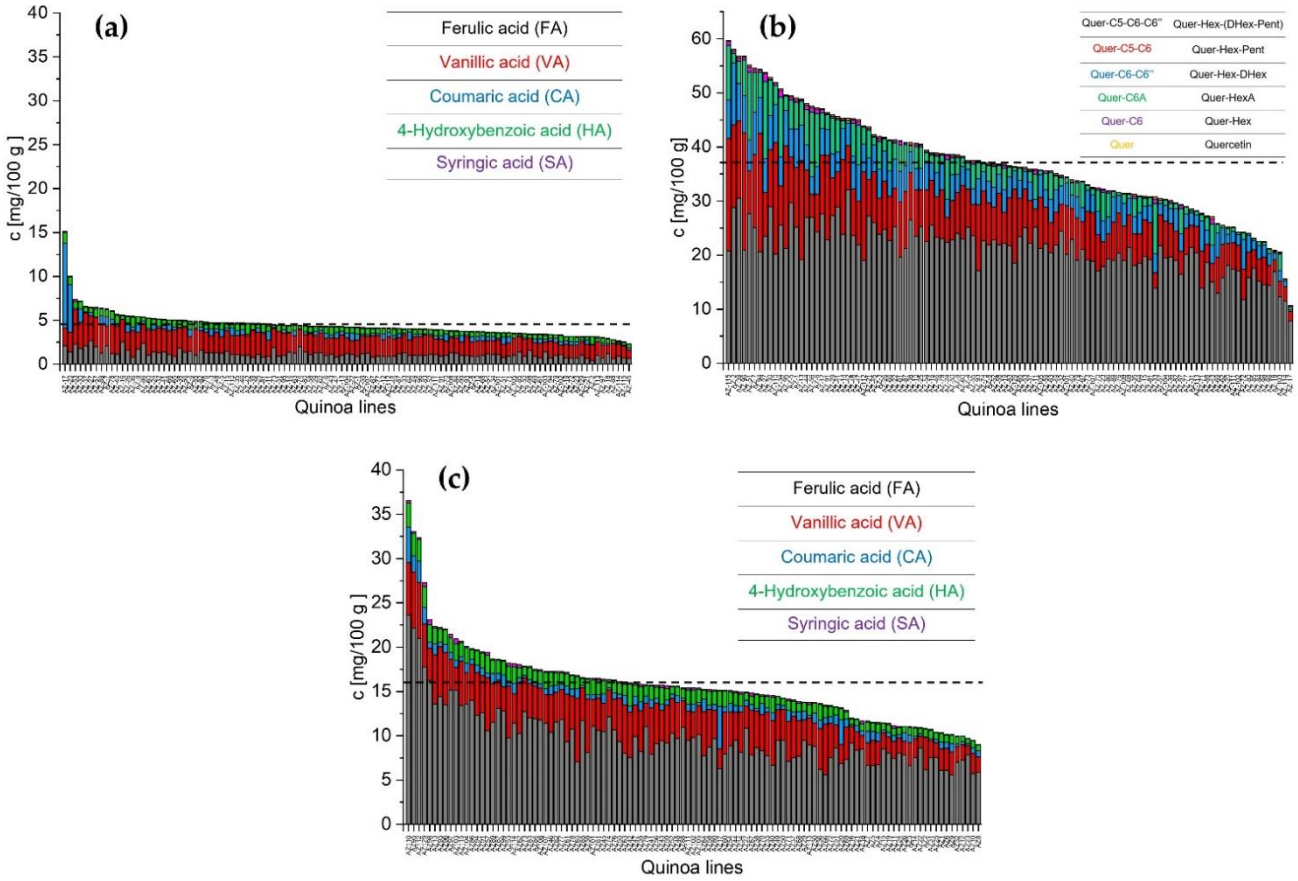

Figure 3.4. Fractions of free (a, b) and bound phenolics (c) in *C. quinoa* genotypes. The stacked columns show the contents of individual phenolic acids (a, c) as well as quercetin glycosides (b) for all investigated 111 quinoa lines. Dashed lines in the figures indicate the average values of the summed concentrations of phenolic acids and quercetin glycosides, respectively.

fractions of free and bound phenolics. The class of quercetin glycosides was only found and quantified in the fraction of free penolics. Overall, the existence of available phenolic derivatives was in agreement with the previously published literature (Han et al. 2019; Gomez-Caravaca et al. 2011; Ersan et al. 2016). To the best of our knowledge, individual, as well as total phenolic contents have not been studied systematically for this collection of Chilean *C. quinoa* genotypes. Therefore, direct comparisons with formerly published articles are not possible at present for the whole dataset. Taking this fact into account, we were nonetheless able to compare results with formerly published results with a few previously studied genotypes. The total phenolics content among the studied genotypes ranged from 35.51 mg/100 g to 93.23 mg/100 g of seed dry weight. In the present work, 41% of the *C. quinoa* genotypes were found which have an above-average content of phenolic derivatives and therefore comparatively rich in phenolics content, while 58% were found as comparatively poor in phenolics content with below-average content. Detailed statistics are presented as supporting materials (**Table S5**, **Table S6**), and **Figure 3.4** shows the overall variation in phenolic compositions among the studied genotypes. We noted that the differences in total phenolics in studied *C. quinoa* genotypes were significant ($p < 0.001$) (**Figure 3.4**, **Table S5**, **Table S6**). In our study, we detected a higher content of

total phenolic compounds with 93.23 mg/100 g of seed dry weight in AZ-110 among all genotypes, while the lowest total phenolics content was observed in AZ-18 with 35.51 mg/100 g of seed dry weight. For the few earlier reported *C. quinoa* genotypes, the total mean phenolics content among both central Chilean genotypes Cáhuil (AZ-4, AZ-18, AZ-103, and AZ-104) and FARO (AZ-31 and AZ-32) were noted up to 47.25 mg/100 g and 46.79 mg/100 g of seed dry weight, respectively, a lower amounts than those reported by Vega-Galvez et al. (2018) (194.01 mg/100 g for Cáhuil and 187.79 mg/100 g for FARO). One more experiment carried out by Sobota et al. (2020) has also shown a higher value for total phenolics for FARO in comparison with the one reported in our present analysis. These systematic differences in the total phenolics content can be explained by the fact that the determination of phenolic compounds was carried out by a classical approach involving the Folin-Ciocalteu method. Such an assay may be overestimating the content of phenolics because of interference of non-phenolic components (Melini and Melini 2021). It is important to underline that in both studies reported by Vega-Galvez et al. (2018) and Sobota et al. (2020), the total phenolics were expressed as gallic acid equivalent (GAE). Similarly, other south Chilean genotypes from the Villarrica region (AZ-19 and AZ-20) have shown a mean of 51.39 mg/100 g and 48.40 mg/100 g of total phenolics content, respectively, which was lower as well than the one reported in a previously published article (Vega-Galvez et al. 2018). As stated above, total phenolic compounds in the reported Villarrica ecotype were analyzed by the Folin-Ciocalteus assay (Vega-Galvez et al. 2018). Finally, south Altiplano ecotypes AZ-3 and AZ-5 that originate from the Cancosa region, had a mean of 57.80 mg/100 g and 65.74 mg/100 g of total phenolics content, respectively, values which were less than the ones reported for Cancosa with 112 mg GAE/100 g in Vega-Galvez et al. (2018) experiment. Such contradictory results for these previously reported *C. quinoa* genotypes can arise from several factors including agronomical conditions as well as the protocols that have been used for the assessment of total phenolic compounds.

Concerning individual compounds, free phenolics have been reported in a range of 18.28 mg/100 g to 62.27 mg/100 g of seed dry weight ($p < 0.001$) (**Figure 3.4a, b**). As reported by Gomez-Caravaca et al. (2011), the flavonoid derivatives were the most abundant free phenolics in proportion to 81.35% of the total free phenolics for the studied genotypes. Among previously reported *C. quinoa* genotypes (Cancosa, Cáhuil, FARO, and Villarrica), south Altiplano ecotypes from the Cancosa region had the highest flavonoid glycosides content (mean of 45.68 mg/100 g), while central-southern ecotypes had the lowest flavonoid glycosides content (mean of 29.98 mg/100 g) (**Figure 3.4a, b**). Relating to these genotypes, the mean flavonoids content in the south Altiplano ecotypes was 1.5-fold higher compared with the central-southern ecotypes. In general, this trend is comparable with the results from Graf et al. (2016). In Graf's experiment, flavonoid glycosides were analyzed in the Chilean *C. quinoa* genotypes that originate from different biomes of Chile. In their study, the relative flavonoids content was 2.6

times higher in northern genotypes compared with those originating from the central-southern region of Chile. Further, the bound phenolics have been reported in a range of 9.03 mg/100 g to 36.57 mg/100 g of seed dry weight ($p < 0.001$) (**Figure 3.4c**). Amongst the five phenolic acids mentioned above, ferulic acid was identified as the main compound with 63% of the total bound phenolics since the derivatives of hydroxycinnamic acid are monomeric components of lignin which forms plant cell walls together with cellulose. This result is identical to the previously published articles where ferulic acid was found to be a major compound of bound phenolics in *C. quinoa* (Han et al. 2019; Tang et al. 2015; Gomez-Caravaca et al. 2011). In addition, the pool of the free phenolic fractions was higher as such being 72% compared to the bound fractions with 28% of the total phenolics content. Our result is identical to those results reported in previous literature where the free fractions of phenolics were reported as a significant contributor to the total phenolics content compared with the bound fractions (Li et al. 2021; Han et al. 2019; Vega-Galvez et al. 2018; Gomez-Caravaca et al. 2011). These results could be explained by the fact that free phenols are available on the outer surface of the seed pericarp, whereas bound phenols are attached to cell wall components (Sumczynski et al. 2016; Abderrahim et al. 2015; Murphy and Matanguihan 2015). However, on the contrary, the higher content of bound fractions in comparison to free fractions was reported in the Peruvian Altiplano genotypes (Abderrahim et al. 2015). Such variation in different phenolic fractions of *C. quinoa* seeds that have been reported in the previous studies could occur due to several factors particularly different germplasm, and environmental as well as agronomical conditions (Abderrahim et al. 2015; Vega-Galvez et al. 2018).

3.5 Principal component analysis and hierarchical clustering

The principal component analysis (PCA) was performed using a dataset for all variables for each line to represent existing phenotypic variation patterns in the studied *C. quinoa* panel. Principal component analysis was executed with a correlation distance matrix and outlined into a two-dimensional scatter plot. The specific loading factors that outline the principal component are listed in **Table S7.** As shown in **Figure 3.5a**, the first two components PC1 and PC2 of the principal component analysis explained 26% and 22% of the total variation, respectively. Based upon the loading factors, phenotypic variables such as total phenolics, several flavonoids, and phenolic acids such as hydroxybenzoic acid, and vanillic acid in both free and bound fractions, and the bound ferulic acid showed important loading components for PC1. Considering the remaining variables, syringic acid, coumaric acid in both free and bound forms, and the free ferulic acid showed important loading components for PC2. The output

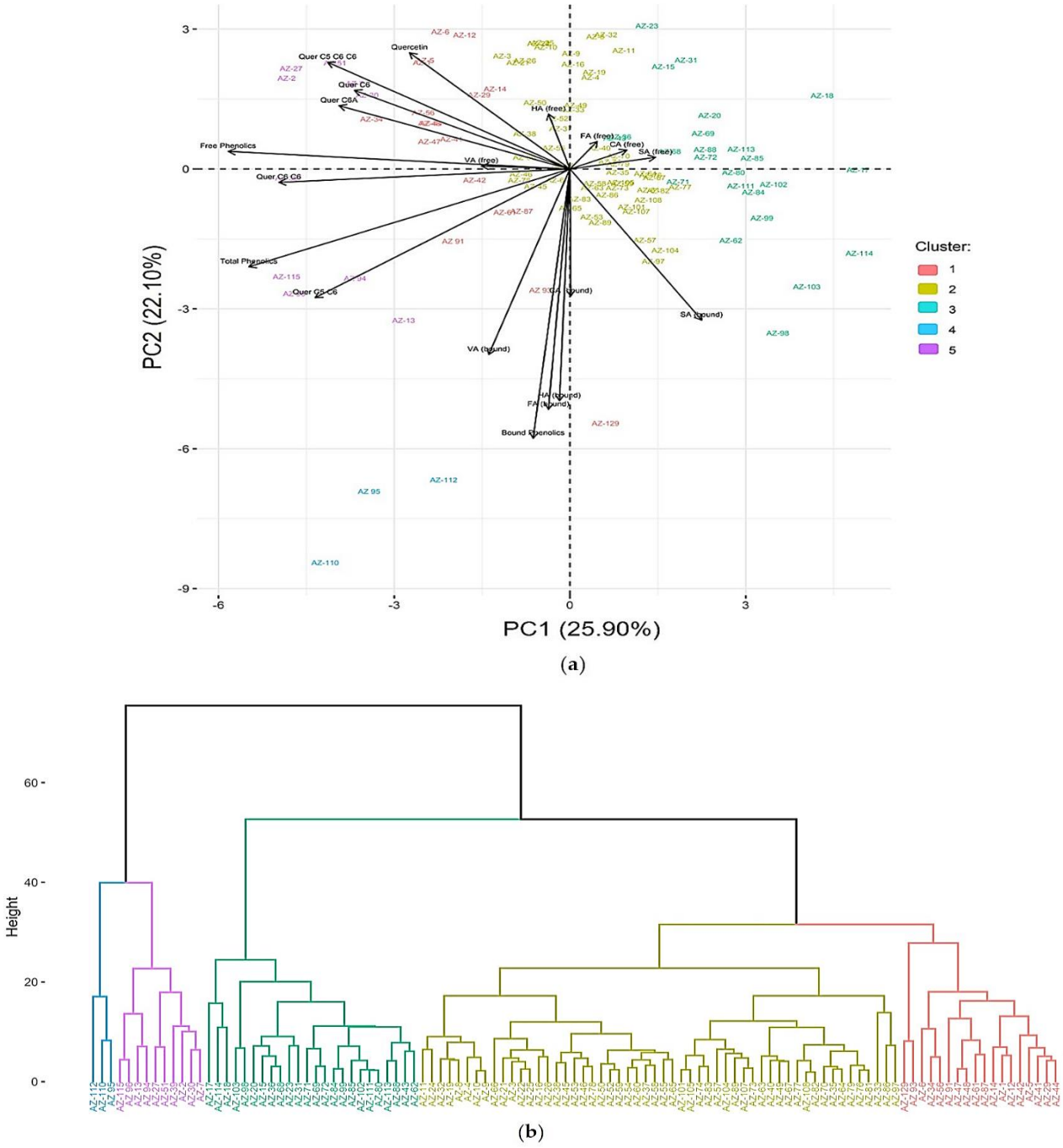

Figure 3.5. Principal component analysis (a) and hierarchical cluster (b) of phenolic compounds of *C. quinoa*. Bi-plot shows the main components PC1 and PC2 of PCA, and that explains 48% of the total phenolics content in *C. quinoa*. Arrows show the phenolic derivatives and the length of the arrow approximates the variance of the derivatives. The distance between each point explains how similar the observation is and colors correspond to the clusters.

data of each characteristic and for each accession clustered into two major clusters consistent with the content of phenolic compounds. Based on the above data, accessions that were abundant in phenolics content are present on the left side of the quadrant, while accessions that had a mean or lower than the mean amount of phenolic compounds are showing on the right side of the quadrant. Further, hierarchical clustering was carried out based on the euclidean distance and complete grouping method using the PCA score (**Figure 3.5b**). According to the PCA score, cluster 4 showed the smallest value

for PC1. Because the first principal component PC1 correlates negatively with the number of free and bound phenolic acids, flavonoids, and total phenolics content, cluster 4 pooled together those genotypes having a high phenolics content. Moreover, clusters 5 and 1 pooled genotypes that possessed a high content of free fractions of phenolics and consequently total phenolics related to the remaining genotypes from other clusters. Contrarily, cluster 3 contains those genotypes which are characterized by low phenolics in both fractions. Finally, the remaining cluster 2 showed scattering in proximity to the central border of PC2. This cluster pooled those genotypes which showed an average amount of total phenolic compounds. However, principal components analysis shows a partially interlinked scattering for sub-groups of the main clusters. The low phenolics cluster revealed variation within and among sub-groups, which presented genotypes from the south Altiplano and also from coastal-lowland regions. Interestingly, the south Altiplano genotypes are dispersed among different sub-groups within the same cluster, which suggests that the low-land group may have a comparatively higher genetic diversity. Such a result, in the context of existing genetic diversity and comparison of both quality and quantitative traits in *C. quinoa* germplasm, is in agreement with previously published work where data confirmed a comparatively higher genetic variation in lowland genotypes compared with highland ones (Christensen et al. 2007; Miranda et al. 2012). Overall our data showed interlinking of the south Altiplano and coastal-lowland genotypes in both PCA and dendrogram. However, interlinking among the genotypes from two different biomes could be due to existing shared alleles (Fuentes et al. 2009) and consequently genetic similarity (Christensen et al. 2007) between highland and coastal-lowland quinoa genotypes. Several studies have been carried out which confirmed the extant genetic diversity across the different *C. quinoa* genotypes for various traits and such genetic variance could be a key reason for possible existing variation in phenolics content (Christensen et al. 2007; Mizuno et al. 2020; Fuentes et al. 2009). Therefore, the thorough genetic study of these accessions could help to elucidate the possible genomic variance which leads to comparatively different phenolic compounds in *C. quinoa*.

In general, our data show that the thirteen *C. quinoa* genotypes (AZ-2, AZ-7, AZ-13, AZ-27, AZ-30, AZ-39, AZ-51, AZ-94, AZ-95, AZ-96, AZ-110, AZ-112, and AZ-115) hold 50% of total phenolics content. As these genotypes showed high content of phenolic derivatives, they can be categorized as high phenolics content genotypes. Such genotypes could lead to seed enrichment in health-promoting functional bioactive compounds. Further, eighteen genotypes (AZ-1, AZ-5, AZ-6, AZ-29, AZ-34, AZ-41, AZ-42, AZ-44, AZ-47, AZ-48, AZ-53, AZ-56, AZ-61, AZ-78, AZ-87, AZ-91, AZ-93, and AZ-129) showed a total phenolics content above the mean value and any such genotypes can also be accounted for a comparatively high phenolic source than the remaining ones. Among all genotypes, AZ-15, AZ-17, AZ-18, AZ-20, AZ-23, AZ-31, AZ-36, AZ-43, AZ-62, AZ-68, AZ-69, AZ-71, AZ-72, AZ-80, AZ-84, AZ-85, AZ-88, AZ-98, AZ-99, AZ-102, AZ-103, AZ-111, AZ-113, and AZ-114 can

be categorized as low phenolics content genotypes as they showed a low amount of all assessed phenolic derivatives (i.e., *ca* 14% of total phenolics). The rest of the genotypes had a total phenolics level near the average range. It is important to note that the entire categorization was based on the available clustering statistics.

3.6 Variance explained by a genetic effect

To assess the genotypic effect on trait variation pattern, the percentages of variance described by genetic effect (Vg) in the total phenotypic variance for agro morphological traits were estimated using a mixed linear model. The variance explained by genetic effect (Vg) was measured according to the restricted maximum likelihood (REML) variance components using the lme4 library of R (Bates et al. 2016). In the current study, we noted a significant effect of genetic variance ($p \leq 0.05$), which presents that each accession shows the phenotype of assessed traits distinctly, i.e., there is a genetic variation that would interpret the existing differences of phenolics content. Such notable genotypic effect by Vg highlights the genetic diversity to 97% within the studied Chilean *C. quinoa* genotypes for total phenolic compounds (**Table S8**). Our data may further contribute to *C. quinoa* breeding programs to advance toward the development of new cultivars.

4 Conclusions

Overall the results of this study underlie the existence of a wide variation among the Chilean *C. quinoa* germplasms for total phenolics content. The total phenolics content among the studied genotypes was ranging from 35.51 mg/100 g to 93.23 mg/100 g of seed dry weight. In total thirteen *C. quinoa* genotypes were categorized as a rich source of phenolic derivatives. Also, eighteen more *C. quinoa* genotypes had a comparatively high content of phenolic compounds compared with a low phenolics group of genotypes. Such genotypes can be used as a good quality source of phenolic compounds for human consumption. As phenolic derivatives play a key role in potential health benefits, *C. quinoa* genotypes which are plentiful with such individual phenolic compounds can be used to reduce the risk of health-related acute and chronic diseases. Various phenolic compounds (i.e., rutin derivatives, vanillic acid, and quercetin derivatives) were noted to have anti-herbivory characteristics, therefore quinoa genotypes that are abundant with such bioactive compounds can be used for biological control. In the current study, the content of the free phenolic fractions was higher compared to the bound fractions in the studied genotypes. Overall PCA and cluster data showed considerable differences among sub-clusters of main clusters. Such variation in the content of phenolic derivatives can be concerned with the genetic background of *C. quinoa* accessions. The relevant effect of genotypes explained by Vg for existing phenolic derivatives highlights the existing genetic diversity in terms of total phenolic content, which could become a good source for *C. quinoa* breeding programs for the

development of new cultivars based on these genotypes. Concerning future research steps, a genetic study such as genome-wide association studies (GWAS) will be performed in further analyses to determine inherent genomic regions that are associated with phenolics content to be used in quinoa breeding programs through marker selection.

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Chapter 4: Root system growth in the Chilean quinoa (*Chenopodium quinoa*) germplasm.

1 Introduction

Root systems play a significant role in minimizing yield losses and supporting the green revolution by ensuring that staple crop provisions are sufficient to meet human food needs (Maqbool et al. 2022). The plant root system growth of individual plants overall impacts the balance between resource capture and resource usage during plant growth. Both natural and human selection have created a variety of plant structures, which has led to a diversity of morphological characteristics among plants from different habitats. This, in turn, has produced distinct characteristics and homogeneity within the plant species and genotypes (Magray et al. 2022; Alvarez-Flores et al. 2014a; Ross-Ibarra et al. 2007). Further, plant growth directly relies on morphological variation of root architecture, which not only provides sturdy mechanical support to the aboveground organs, but is also crucial for capturing ephemeral or confined resources from the neighboring soil (Alvarez-Flores et al. 2014a; Yu et al. 2007). Root properties influence the productivity of both natural and agro-ecosystems, and in many cereals, the selection criteria for grain yield have been revised to take this into account by not only considering the aerial parts but also how well the root grow (Palta et al. 2011; Wolfe et al. 2008; Siddique et al. 1990). Because of the emphasis on grain production and due to the inherent difficulty of accessing the root system for direct studies and measurements, the hidden half of plants has been substantially and frequently neglected in research on crop phenotypic variation.

At present, in face of climate change, cultivated plant species are in compelling need of progress for better yield and tolerance to environmental stress factors (Ray et al. 2013). Therefore, globally, plant researchers seek a way of modulating the hidden half of plants to enhance the yield performance and overall plant growth (Wu et al. 2022; Deja-Muylle et al. 2021; Chen et al. 2019; Rogers and Benfey 2015). To achieve such a target, researchers pay attention to studying the structure and growth dynamics of the root system, a concept used to outline every aspect of root development. Root system architecture (RSA) refers to the dimensional distribution of the root system within the heterogeneous soil matrix (Chen et al. 2015; Hinsinger et al. 2011). Recently, *Chenopodium quinoa* Willd. (quinoa) turned out to be the target of dedicated research due to its prospective contribution to food security (Bazile et al. 2016) and its capability to grow under adverse growth conditions such as drought, high salinity, and heat (Aly et al. 2018; Razzaghi et al. 2012; Ruiz-Carrasco et al. 2011). *C. quinoa* shows a wide diversity from a climatic region of the highland desert down to the intermontane plateau and temperate littoral zone, resulting into several landraces and local varieties with good potential to be cultivated in adverse environmental conditions outside of their native habitat (Bazile et al. 2016;

Bertero et al. 2004; Bonifacio 2003). Comparative studies of variation in morphological and phenotypic variation in root system growth are of ecological importance, especially in low-resource availability environments where rooting is pivotal to seedling establishment, and intake of nutrients and water from the soil. Also, the development of strong root systems during early growth phases is beneficial for the prompt colonization of deep soil layers, improving the acquisition of limited soil resources (Lamb et al. 2012) and therefore resulting in early vigor in domesticated species compared to wild ancestors (Wolfe et al. 2008). Until now, to the best of our knowledge, only a few studies have revealed rooting patterns and root growth of this little-known *Chenopodium* species. *C. quinoa* forms a herringbone root pattern i.e. root branches are mainly confined to the main axis, and such topology would favor the acquisition of nutrients, especially in low-resource habitats thereby reducing inter-plant competition (Alvarez-Flores et al. 2014b; Bouma et al. 2001). In a previous study, it has been noted *C. quinoa* has rapid taproot elongation and long and thicker root segments that result in higher total root length, and deep root growth compared to other *Chenopodium* species (Alvarez-Flores et al. 2014b). Considering ephemeral soil resources, improved root growth could increase plant productivity and lower the plant uptake needs (Lynch 2022; White et al. 2013; Passioura 2006). *C. quinoa*, a cultivated chenopod species, is a good candidate crop to study plant ideotypes consistent with low agricultural resources environment (Alvarez-Flores et al. 2014b; Rojas et al. 2009). It is known that plants can alter their root growth in response to one or several exogenous abiotic challenges, showing root architectural plasticity (Schneider and Lynch 2020; Correa et al. 2019; Alvarez-Flores et al. 2018). In previous studies, rooting plasticity has been reported for *C. quinoa* under several harsh environmental conditions such as drought and saline soil conditions (Nguyen et al. 2021; Mamedov et al. 2020; Alvarez-Flores et al. 2018). Exploring variance in morphological characteristics such as rooting length, rooting depth, and root diameter of the hidden part of the plants, particularly among the ecotypes of cultivated species, would help to define the ideal root system that enhances the acquisition of soil resources. Also, several studies have reported that root traits, such as maximum rooting depth, total root length, and root diameter, are important for the successful establishment of plants in harsh and variable environments (Lamb et al. 2012; Materechera et al. 1992). Considering that soil resources and root distributions change dynamically, evaluating root growth based on a one-time assessment does not allow us to investigate the significance of plant responses to heterogeneous soil status (Chesson et al. 2004). Considering the analysis of root morphological traits, phenotypic assessment is a challenging task as it implies manual destructive methods and often growing conditions which are often distant from those found in specific field conditions. Moreover, the low resolution and low throughput of the available approaches to study roots often restrict the measurement of root system growth. It follows that the development of advanced technologies for plant phenotyping has gained more importance to measure non-invasively (via imaging) distinct plant traits and evaluate phenotypes in a high-throughput

fashion. For that reason, GROWSCREEN-Rhizo, a novel non-destructive phenotyping robot that enables synchronic assessment of root and shoots growth using high-throughput 2-dimensional imaging analysis, was shown as a promising technique that facilitates us to explore root systems and correlate root traits to the whole plant growth (Nagel et al. 2012). The GROWSCREEN-Rhizo is a prototype to 2-dimensional analyses of the visible root system at transparent faces of rhizotrons. Several other studies also reported image based high-throughput root phenotyping of cereal crops (Tayade et al. 2022; Alsalem et al. 2021; Wu et al. 2018; Slota et al. 2017).

As of today, the potential of *C. quinoa* root trait growth has not been fully explored. Exploring the extent of ontogenetic and morphological variation in the root structure of contrasting *C. quinoa* germplasm could help in identifying ideal genotypes with improved root systems and related genotypes to the further selection for better adaptability under variable soil conditions where vigorous rooting is crucial to soil resources uptake. Initial work addressing root system growth in *C. quinoa* needs to be complemented by further studies evaluating in detail the extent of genotypic and phenotypic variation of contrasting germplasm. In the present study, we address the morphological variation in root system growth in 12 Chilean *C. quinoa* germplasm of different genetic backgrounds with the hypothesis that different ecotypes would display rooting variation. Therefore, we aim to identify those traits that describe better the genetic diversity in terms of root architecture and to assess the actual correlations among above- and below-ground traits and how these phenotypic relationships change in contrasting genotypes.

2 Materials and Methods

2.1 *C. quinoa* germplasm and experimental design

The experiment was conducted in a growth chamber under controlled climatic conditions at the Froschungzentrum Jülich, IBG2 Plant Science Institute. A preliminary field trial work at the Huasco experimental station in the Southern Atacama Desert, Chile (28°34´S, 70°47´W) was conducted for root morphology. Based on the rooting values from that preliminary experiment, in total 12 *C. quinoa* germplasm were selected and compared in detail for variance in root systems growth (**Table 4.1**). The *C. quinoa* panel of this study comprises accessions that are considered for the further ongoing INIA's Quinoa Breeding Program (IQBP) in Chile and also the re-sequenced *C. quinoa* line QQ-74 (Jarvis et al. 2017). All of these genotypes originated and were selected from different habitats in Chile: highland and coastal-lowland areas. The studied genotype set also includes the known cultivars Titicaca and Puno that were bred from parental lines that originated in Peru and southern Chile and subsequently selected in Denmark (Ruiz et al. 2017; Risi C and Galwey 1984).

Table 4.1. *C. quinoa* accessions were used in this experiment to study variation in root system growth.

No.	Accession name	Acronym	Origin	Collection region	
				Latitude	Longitude
1	R49*	AZ-11	Chile	-19.27639	-68.64000
2	CHENO 046	AZ-26	Chile	-34.70000	-72.01667
3	EMPO 10-1	AZ-47	Chile	-34.53639	-71.96917
4	PJEV 026	AZ-51	Chile	-35.93528	-72.70694
5	EAM 1	AZ-62	Chile	-34.76833	-72.07556
6	PJEV 016	AZ-78	Chile	-35.12694	-71.91722
7	Plantas Verdes	AZ-97	Chile	--	--
8	Plantas Moradas	AZ-98	Chile	-39.81944	-73.24528
9	Kinia	AZ-99	Chile	-39.48194	-72.13417
10	Titicaca	Cq-1	Denmark (Bred from parental lines that originated in Peru and southern Chile)		
11	QQ-74	Cq-2	Chile	--	--
12	Puno	Cq-3	Denmark (Bred from parental lines that originated in Peru and southern Chile)		

* Selected Salares quinoa genotypes from the Altiplano region of Chile (~ 19º S and ~ 68º W)

2.2 Experimental setup and growth conditions

In the studied *C. quinoa* population, initially, seeds were selected through a sieves-based screening process for their size consistency (1-2 mm) and exposed to cold conditions overnight at 4ºC to synchronize the germination rate. Five seeds per replicate for each genotype were sown at 2-3 cm depth in soil-filled rhizotrons. Rhizotrons had a black polyethylene plate with an internal volume of 60×30×2 cm and a transparent polycarbonate plate on the front side (**Figure 4.1**). Each rhizotron was filled with 8L of a sieved black peat soil, which had 42% of dry matter with macro-nutrient such as N_2 (111 mg/L), NH_4-N (82 mg/L), NO_3-N (29 mg/L), P_2O_5 (20 mg/L), and K_2O (208 mg/L). Rhizotrons were placed into open polyethylene boxes (32×60×40 cm) with a 45º inclination to promote root growth on the front windowpane. Rhizotron-containing boxes were arranged in a random block design and each box was considered as a statistical replication block. All genotypes were allowed to grow in a growth chamber under long-day conditions (16h light/ 8h dark) at 22 ± 2ºC together with 50% relative air humidity. Over the time of the experiment, all plants were irrigated using an automated drip irrigation system.

2.3 Plant trait measurements

Three days after seed sprouting, seedlings in each rhizotron were thinned to one seedling and six replicate plants per genotype were followed two times a week for non-destructive root growth measurement from 3 to 30 days after sowing. The root system of each replicate was recorded twice a week by means of image acquisition of visible root growth on the front transparent windowpane of the

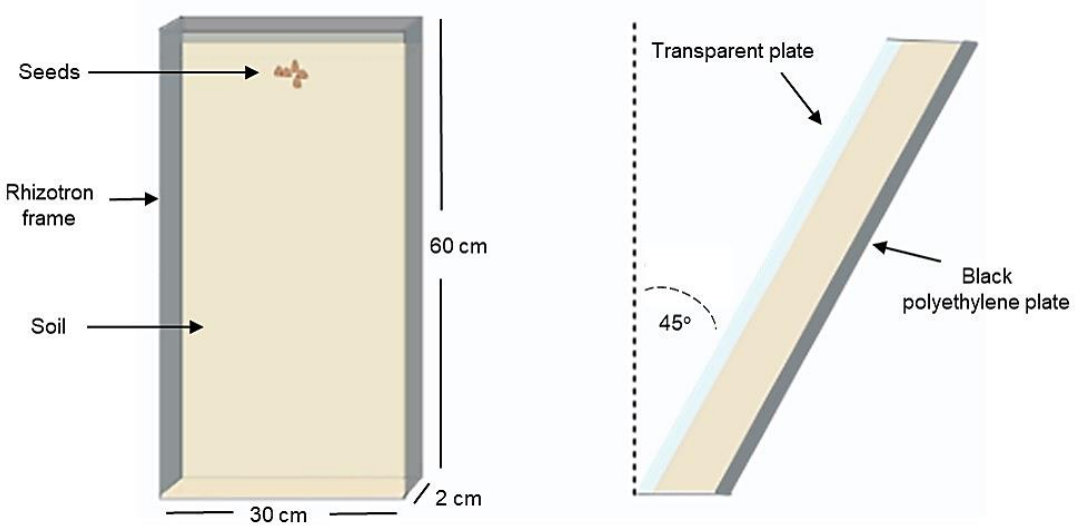

Figure 4.1. Graphic of the rhizotron used for root growth of *C. quinoa*. The rhizotron consisted of black polyethylene box transparent polycarbonate plate on the front side and filled with black peat soil. Rhizotrons were set to an inclination angle of 45°. *C. quinoa* seedlings were grown and each plant per genotype was assessed for non-destructive root growth measurements.

rhizotron. To achieve this, imaging was done in a custom-built imaging station using a digital camera (Canon EOS 70D, Canon Inc. China). For the quantification of root growth traits, root growth images were analyzed using the Paint-Rhizo software (Nagel et al. 2009). The following traits linked to root growth were analyzed: primary root length (PRL), lateral roots length (LRL), total root length (TRL), root system depth (RSD), root system width (RSW), convex hull area (CHA). At the end of the experimental period (30 days after emergence), when the deepest root had come in touch with the bottom portion of the rhizotron, plants were harvested, and individual plants were divided into stems, leaves, and roots. As a next step, dry biomass of above-ground traits was recorded and further total leaf area (LA) was measured using the Licor leaf area meter (LI-3100C, Lincoln, USA). Moreover, the plant's main stem height and radial growth of the stem were also recorded as additional biomass data. On the other side, roots were gently washed and carefully separated from the soil substrate. After studying the entire root system of each genotype, selected genotypes based on their root growth characteristics (i.e., TRL, RSD and CHA) were chosen to examine in detail root diameters. For that, roots were carefully washed to recover fine roots, scanned at a resolution of 800 dpi, and analyzed with WinRhizo Pro 2017a software (Regent Instruments Inc. Quebec, Canada) to estimate root length and diameter distribution. Root diameter distribution was used as a criterion to distinguish between main roots and lateral roots. To cover a broad range of all possible root diameters, we set up 30 different diameter classes ranging from 0.0 mm to 1.50 mm at an interval of 0.05 mm.

From the recorded various mass and length data, mass fractions of the leaves (LMF), mass fraction of stems (SMF), and mass fractions of root (RMF) relative to total plant dry mass (TPM) were estimated. In addition, the mass ratio of root to shoot (root/shoot), leaf area ratio (LAR), and root area to leaf area ratio (RA/LA) were also determined.

2.4 Statistical analysis

Descriptive statistics were performed as mean ± standard deviation (SD) values of plant replicates (n=6 replicates/genotype) to explore morphological variation in root system growth of contrasting genotypes. Differences in root and shoot growth among the studied genotypes were computed by factorial analysis of variance (ANOVA). Prior to performing ANOVA, the assumptions of normality and homoscedasticity of variances of residuals were done by the Shapiro-Wilks and the Levene tests, respectively. Variables that failed to meet these assumptions were transformed to the natural logarithm ($ln(x+1)$). Significant differences among genotypes were compared by the Tukey test ($p \leq 0.05$) using the R-package Agricolae (De Mendiburu 2014). Principal component analysis (PCA) and cluster analysis were performed for the whole dataset to compute the correlation distance matrix, and to group it according to specific variables. Further, to analyze the relationships among the selected variables, a correlation analysis was carried out using Pearson's correlation coefficient between variables.

3 Results and Discussion

The present study was focused on the growth during the initial developmental stages of Chilean *C. quinoa* germplasm, as the first few weeks after sowing are crucial for both seedling establishment and acquisition of soil resources, particularly in low-resource habitats. The results of this experiment revealed the interconnecting effects of genetic background at early stages on the development of shoot and root growth, which are important for plant growth at later developmental stages. To understand this interplay, morphological variation in the root system and shoot growth was studied among *C. quinoa* genotypes. By simultaneous measurements of the plant's shoot and root growth and by exploring trait interdependence among studied genotypes, we found significant variation in various shoot and root-associated traits related to early vigor (**Table 4.2**). Consequently, we preliminary concluded that the studied individual traits are likely to play a major role in explaining the variation in plant growth dynamics across genotypes.

Table 4.2. Analysis of variance (ANOVA) for genotypic effect on the various measured shoot and root traits in *C. quinoa* genotypes.

Shoot and root traits (Units)	Description	Significant variation (*p*-value)
Shoot height (cm)	Plant's main stem height from soil level to apical bud	$< 0.001^{***}$
Stem Diameter (mm)	Radial growth of stem	0.083
Leaf area (cm^2)	Total area of all leaves	0.041^{*}
Leaf area ratio (cm^2/g)	Total leaf area of all leaves divided by dry mass of entire plant	0.049^{*}
Shoot dry biomass (g)	Dry weight of the whole shoot including stem and leaves	0.009^{**}

Root dry biomass (g)	Dry weight of entire roots	0.014*
Total plant dry mass	Total dry weight of shoot and root	0.037*
Primary root length (cm)	Length of visible primary root	0.010*
Lateral roots length (cm)	Length of visible roots branched from the primary root	0.022*
Total root length (cm)	Total length of entire visible roots	0.019*
Root system depth (cm)	Maximal vertical depth of a root system	0.040*
Root system width (cm)	Maximal horizontal distribution of a root system	0.052
Convex hull area (cm^2)	Area of the convex hull that encompasses the whole root system	0.035*
Leaves mass fraction (%)	Leaves biomass relative to total plant dry mass	0.008**
Stem mass fraction (%)	Stem biomass relative to total plant dry mass	0.001***
Root mass fraction (%)	Root biomass relative to total plant dry mass	0.013*
Root to shoot ratio (root/shoot)	Mass ratio of root biomass to shoot biomass	0.010*
Root area to leaf area ratio (RA/LA)	Ratio of root convex hull area to leaf area	0.040*

Note: One-way analysis of variance was performed with six replicates per genotype. ANOVA was performed after the *ln* transformation of the data set when required to comply with assumptions of normality and homoscedasticity. ('***' ≤ 0.001, '**' ≤ 0.01, '*' ≤ 0.05).

3.1 Plant growth and biomass production

At the harvest date, genotypes such as Cq-1, Cq-2, Cq-3, and AZ-97 showed the most vigorous root growth and, consequently a higher plant height and longer root system during the time of the experiment. These genotypes had also a large leaf area, root area, and higher plant biomass (with averages of SH = 12 cm, LA = 293 cm^2, TRL = 1261 cm, CHA = 1191 cm^2, TPM = 1.42 g) (**Figure 4.2**). In contrast, genotypes AZ-11, AZ-26, AZ-51, and AZ-62 showed the least vigorous plant growth among the studied *C. quinoa* genotypes (with averages of SH = 8 cm, LA = 156 cm^2, TRL = 490 cm, CHA = 706 cm^2, TPM = 0.77 g) (**Figure 4.2**). The remaining genotypes showed intermediate and average values, considering the entire panel, for the shoot and root growth traits (**Figure 4.2**). As observed, differentiating in the plant's vigorous growth among the studied genotypes was related to the differences in the individual variables. This contrast between genotypes did affect the overall plant growth and such rooting patterns may be beneficial when selecting ideal genotypes which hold ecological significance, specifically in low-resource environments where rooting is significant to the seedling establishment in early growth stages. The trend of variations in shoot and root traits underlying genotypic differences in early vigor is in agreement with the findings of both studies of Alvarez-Flores et al (2014a; 2014b). In their studies on *C. quinoa,* differences in above and below-ground related traits were observed in contrasting *C. quinoa* genotypes from different habitats. Alvarez-Flores et al (2014a; 2014b) reported higher shoot-root trait values for the *C. quinoa* from arid highlands compared to the *C. quinoa* from rainy and temperate lowlands regions in Chile. An alternative basis to such variations

in shoot and root growth comes up when considering present results with those of Deja-Muylle et al. (2021). To get an understanding of the influence of genetic background on rooting patterns, Deja-Muylle et al. (2021) explored a natural variation and performed an *in vitro* study of *Arabidopsis thaliana* (*A. thaliana*). A comprehensive analysis by Deja-Muylle et al. (2021) showed high variability of the shoot traits and root system architecture over different *A. thaliana* accessions. Also, Liu et al. (2021) studied shoot and root traits that underlie genotypic variation in the early vigor of spring wheat. In their study, they explored root traits that could improve nutrient uptake and early vigor in wheat grown at high latitudes. In this study, shoot and root traits of nine different spring wheat genotypes were quantified together with nutrient accumulation in rhizoboxes grown under controlled conditions. Results showed that the genotypes differed significantly in their shoot and root traits, as well as in their ability to accumulate nutrients.

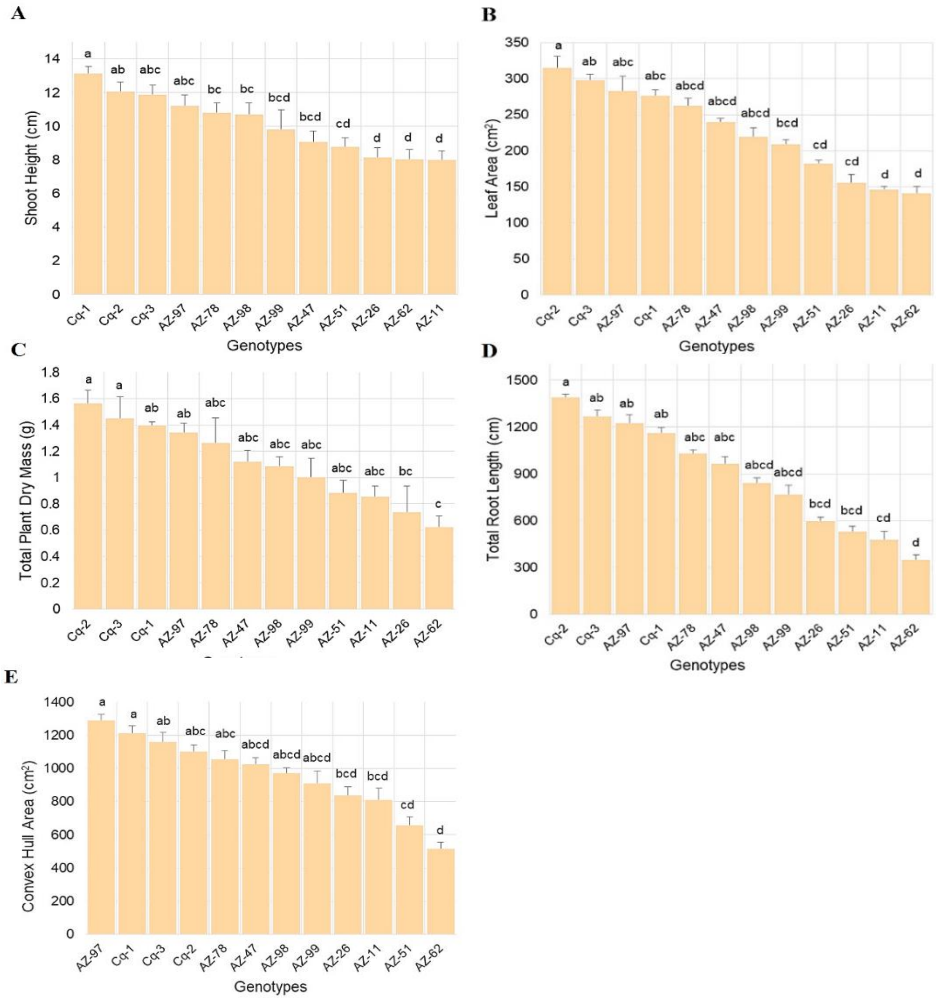

Figure 4.2. Phenotypic root growth variations among *C. quinoa* genotypes. To evaluate root trait variation all *C. quinoa* genotypes were grown in Rhizotron under non-limiting growth conditions. Each plant per genotype was assessed for shoot and root growth such as shoot height, leaf area, plant dry mass, total root length, and convex hull area. Each column is the mean of six replicates, whereas error bars denote the standard error of the mean of replicates from each evaluated genotype. Different letters indicate significant differences in evaluated traits ($p \leq 0.05$, Tukey test).

To our knowledge, this is the first study that addresses phenotypic variation in the root system growth among the various Chilean *C. quinoa* genotypes. Our results suggest that all genotypes shared a broad

range of variations of growth and morphology traits. Starting with biomass allocation, the total plant biomass among all genotypes varied from 1.56 g to 0.62 g of plant dry mass, with a major part allocated to leaves at ~ 65%, followed by stems and roots that represented ~ 20% and ~ 15% of the total plant dry mass (**Figure 4.3A**). Such biomass allocation pattern could be due to biomass allocation dynamics occurring at three phases during growth: 1) leaves: as a result of leaves' autonomous carbon fixation, 2) stems: lateral root proliferation sustains accelerated shoot growth, and 3) roots: elongation in deep soil by the main root and root segments. Likewise, Alvarez-Flores et al. (2014a) found that in *Chenopodium* species the majority of the biomass (≥50%) was allocated to leaves in their study. Another study also revealed biomass distribution patterns in leaves, stems, and roots in wild and cultivated populations, conform to the ontogenetic trend that is influenced by environmental and evolutionary history (Poorter et al. 2012). At harvest time, among all studied genotypes, the LMF was lowest (below average) in Cq-1, Cq-2, Cq-3, and AZ-97 with a mean ratio of 0.62 relative to TPM. On the opposite, the RMF was higher in these genotypes with a mean ratio of 0.17 relative to TPM (**Figure 4.3B**). On the other hand, the least vigorous genotypes (AZ-11, AZ-26, AZ-51, and AZ-62) showed higher LMF but lower RMF relatives to TPM, reaching mean ratios up to 0.68 and 0.12, respectively (**Figure 4.3B**). In the present study, under non-limiting resource conditions, the LMF was maximum in AZ-62, reaching a value of 71%. However, during the study, the RMF had never exceeded 18% of TPM among all genotypes. Overall, the biomass allocation pattern is similar to that noted in the previous literature where, in *C. quinoa* and its wild relative species, the mass fraction of leaves has been recognized as an influential attribute relating to the total biomass distribution (Alvarez-Flores et al. 2014a).

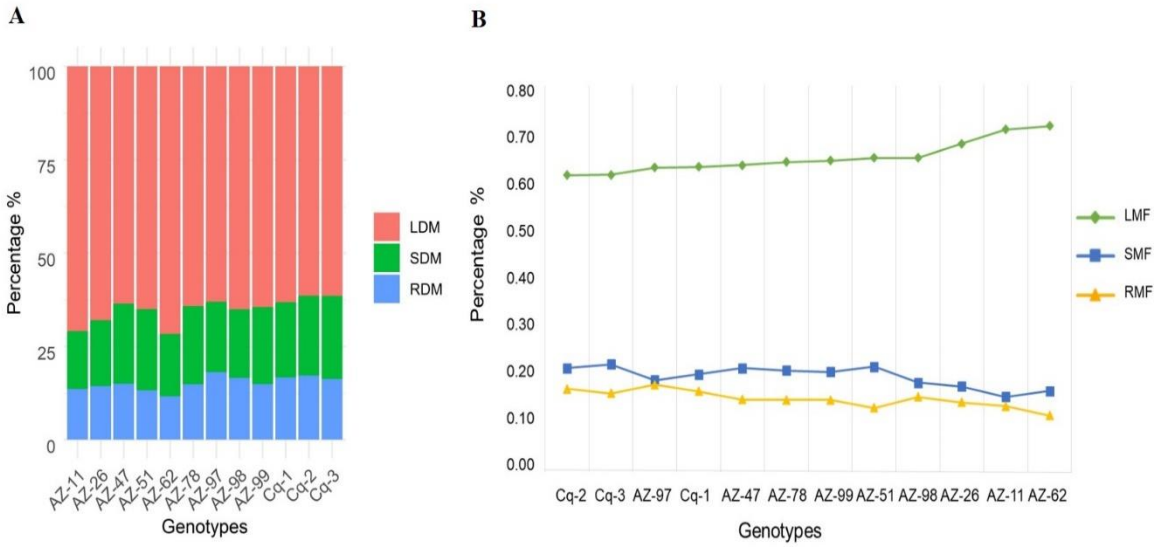

Figure 4.3. Plant biomass allocation (A) and mass fractions of the leaves, stems, and roots (B) in *C. quinoa*. At the harvest, biomass allocation was observed with a major part into leaves, followed by stems, and roots. Stacked bar graph showing biomass distribution and outlined in % of total plant dry biomass. Light blue: root dry mass (RDM), Green: stem dry mass (SDM), and Light red: leaves dry mass (LDM). (B) Line graph showing the mass fraction of leaves, stems, and roots relative to total plant dry biomass at the harvest time. Yellow: root mass fraction, Light blue: stem mass fraction, Light green: leaves mass fraction.

Leaf morphology at both levels, at the leaf level (LA) and whole plant level (LAR), was determined among all *C. quinoa* genotypes. The total LA ranged between 141 cm^2 and 315 cm^2 (**Figure 4.2B**). At the end of the experimental period, the maximum LA was observed in Cq-2, reaching a value of 315 cm^2, while the minimum LA was observed in AZ-62, reaching a value of 141 cm^2. The data showed relatively high values of leaf area in the plants that were superior in terms of biomass, plant height, and dense root growth (**Figure 4.2B**). Similarly, in the previously published literature, it has been shown that productive genotypes concerning plant height, biomass yield, and deeper root system had a higher LA among studied *C. quinoa* ecotypes and their wild relatives (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b). In *A. thaliana* and wheat plants, it has been noted that the lower LA could influence plant biomass and root growth as slow leaf development can make considerable differences in lateral root density, as the leaves have been a source of photosynthates, and auxin to foster root growth (Smith and De Smet 2012; Boyer et al. 2010). On another side, the LAR showed comparatively high values in the smaller plants which is similar to the one found by Alvarez-Flores et al. (2014a), where the authors reported LAR declined consistently as plant mass increased. Considering the studied genotypes and the entire growth period, the LAR showed a minor negative correlation with plant size and root growth. The influence of specific leaf area (SLA: leaf area/ leaf dry mass) on alterations in LAR has been observed across different species, and also retains correlation over a plant size in addressing the ontogenetic variations in LAR relevant to changes in leaf morphology and plant size during vegetative stage (Poorter et al. 2012). Over the experimental period, CHA was higher than LA during the vegetative phase, and RA/LA values varied significantly among studied *C. quinoa* genotypes ($p \leq 0.05$). Like other studied parameters, Cq-1, Cq-2, Cq-3, and AZ-97 had higher RA/LA, reaching a value of 4.97 on average. On the contrary, AZ-11, AZ-26, AZ-51, and AZ-62 had lower RA/LA, reaching a value of 3.55 on average. Such a pattern of growth reflects an equal or larger exchange rate in roots than in leaves, implying an offset between soil foraging and light capture during the early growth stages of the plant cycle. This pattern of biomass allocation in root and leaf areas is similar to the findings of Alvarez-Flores et al. (2014a), assuming the initial importance of leaf growth related to biomass does not entail a limited role of the root system. Among the *Chenopodium* populations, Alvarez-Flores et al. (2014a) noted a high values of RA/LA at early growth stages that put forward larger exchange area in roots compared to leaves in emerging seedlings.

3.2 Root system growth

To assess the natural variation in root system growth parameters, root system traits were examined by image acquisition of visible root growth and quantified using the Paint-Rhizo software. In the present study, all genotypes showed a similar herringbone topology with only a main axis and primary laterals (**Figure 4.4**). However, for the reason of the constraint of time, a detailed report concerning differences in the root system topology, particularly branching patterns and branching angles among genotypes is lacking in our study. The overall root system wherein the root branches were mainly confined to the main axis is comparable with the previously reported study (Alvarez-Flores et al. 2014b). The experiment of Alvarez-Flores and his co-authors revealed a high value of the topological index in *C. quinoa* (0.76), which simply reflects the characteristics of herringbone morphology (Alvarez-Flores et al. 2014b). The root topology index is a slope of the regression of the altitude of the root system (i.e., number of links in a prolonged path from root crown to external tips) on magnitude (i.e., the total number of root system tips). A similar form of the herringbone root system has been also observed in other *Chenopodium* species (Fitter 1987). In an ecological view, the herringbone root structure of young seedlings can be linked to being obliged to rapidly get to the deep soil layers, considering they compete with other plants (Paula and Pausas 2011). Such hypothesis has been also put forward in the growth of the sprouting of *C. quinoa* and its wild relatives where the fast root growth would ensure soil resource acquisition and young plant survival at an early growth phase (Alvarez-Flores et al. 2014b; León et al. 2011).

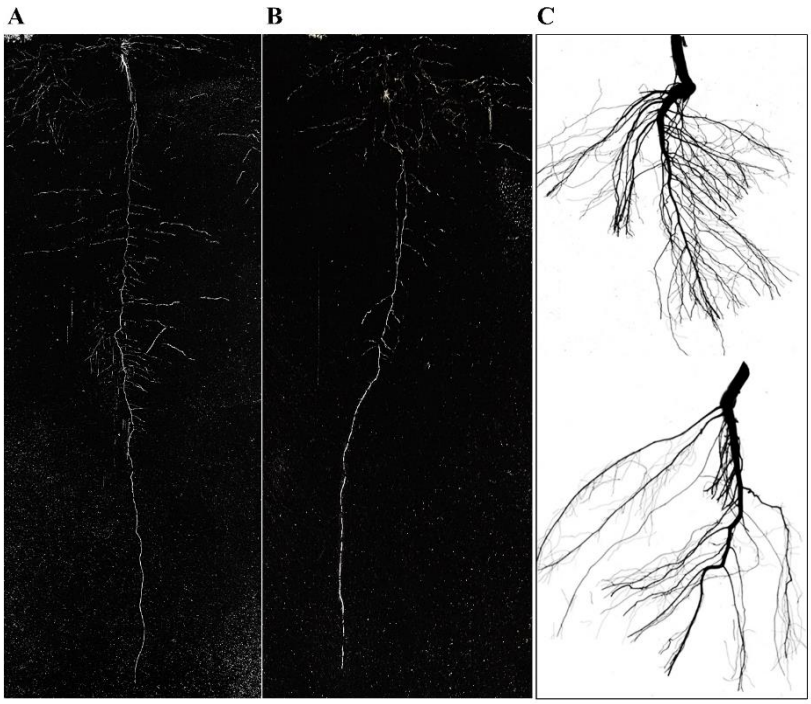

Figure 4.4. Root system growth of *C. quinoa*. The root system growth was examined by image acquisition of visible root growth on the front transparent windowpane of the Rhizotron and analyzed using the Paint-Rhizo software. *C. quinoa* genotypes have shown a herringbone root topology with a main axis and primary lateral root.

Despite the uniform herringbone root structure, studied genotypes differed significantly in their detailed root trait growth. In all genotypes, Cq-1, Cq-2, Cq-3, and AZ-97 had higher TRL values with an average of 1261 cm, which differs significantly from all other genotypes. This corresponded to the enormous development of lateral roots, which represented almost 94% of TRL and RDM. Also, non-destructive image analysis of visible root growth revealed a higher proportion of external root links compared to internal root links, resulting in higher TRL in these genotypes. Such ontogenetic effect on root growth could be interpreted as the external-internal root links consolidating the large degree of the TRL in *C. quinoa* and other *Chenopodium* species (Alvarez-Flores et al. 2014b). Alvarez-Flores et al. (2014b) found more abundant and longer external root links in *C. quinoa*, resulting in the highest TRL among the studied population. At the time of harvest, vertical root distribution of the root growth showed significant differences among soil layers and genotypes (**Figure 4.5**). The present results showed genotypes Cq-1, Cq-2, Cq-3, and AZ-97 formed a higher vertical root growth (on an average of 52 cm), and had significantly greater CHA (on an average of 1191 cm^2) compared to the least vigorous genotypes AZ-11, AZ-26, AZ-51, and AZ-62, referring to root growth, which in turn showed one-third times lower vertical root growth and nearly one-sixth times smaller CHA. Among all genotypes, AZ-97, Cq-1, Cq-3, and Cq-2 had a bigger root system where vertical growth was extended up to an average of 54 cm, 53 cm, 51 cm, and 49 cm depth, respectively, with maximum root distribution in the 11-25 cm soil layer (-2 to -4 soil layer, on an average of *ca*. 6.3 m root length in that layer, equivalent to *ca*. 4 cm/cm^2 root length density). On the other hand, AZ-62, AZ-51, AZ-11, and AZ-26 had a much smaller root system where vertical taproot growth was extended up to an average of 34 cm depth, on an average of *ca*. 2.6 m root length in the 11-25 cm soil layer (equivalent to *ca*. 1 cm/cm^2 root length density). Beyond a 45 cm depth (-9 to -10 soil layer), AZ-97, Cq-1, Cq-3, and Cq-2 showed considerably higher root colonization compared to the rest of the genotypes. In arid soil conditions where soil water is restored during the rainy season and where the deepest soil layers may store moisture, the fast vertical root growth is indeed considered as suitable for the acquisition of limited soil resources at depth rather than dense root system in the upper soil layers (Alvarez-Flores et al. 2018; Alvarez-Flores et al. 2014b). The results revealed that among all genotypes, Cq-1 and Cq-3 had statistically dense inter-root distances and deeper root growth distribution, which could confer advantages over other genotypes for both dry- and wet- habitats. Such root foraging capability can be attributed to the fact that both genotypes are bred from parental lines that originated in dry-habitat (Peru) and wet-habitat (southern Chile) (Ruiz et al. 2017; Risi C and Galwey 1984). Furthermore, coastal-lowland genotypes AZ-97 and Cq-2 also had analogous root foraging forms. Here, such root growth habits can be concerned with the fact that Peruvian - Bolivian southern highlands are considered as the center of morphological diversity and domestication (Murphy and Matanguihan 2015; Fuentes et al. 2012; Christensen et al. 2007). It also coincides with the genetic diversity of Chilean *C. quinoa*

germplasm likely evolved through seed exchange and the domestication process in Andean societies (Fuentes et al. 2012). Contrarily, the least vigorous genotypes in particular AZ-11, AZ-26, AZ-51, and AZ-62 had dense root development in the superficial soil layers which contain water- and air-containing substrates are essential for sustaining biological activity and accumulate nutrients such as P and K, which seems more advantageous to temperate rainy coastal-lowland regions as soil moisture in superficial layers being more frequent. Such similar root distribution had been reported in previous literature whereas, the *C. quinoa* genotype from high-resource habitat showed slower root growth colonization at depth than the *C. quinoa* genotype from the low-resource habitat of the arid region (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b). Under common non-limiting growth conditions, such variations during the early vegetative stages imply that these differences in root traits growth are genetically controlled in the studied genotypes and might have been influenced by human

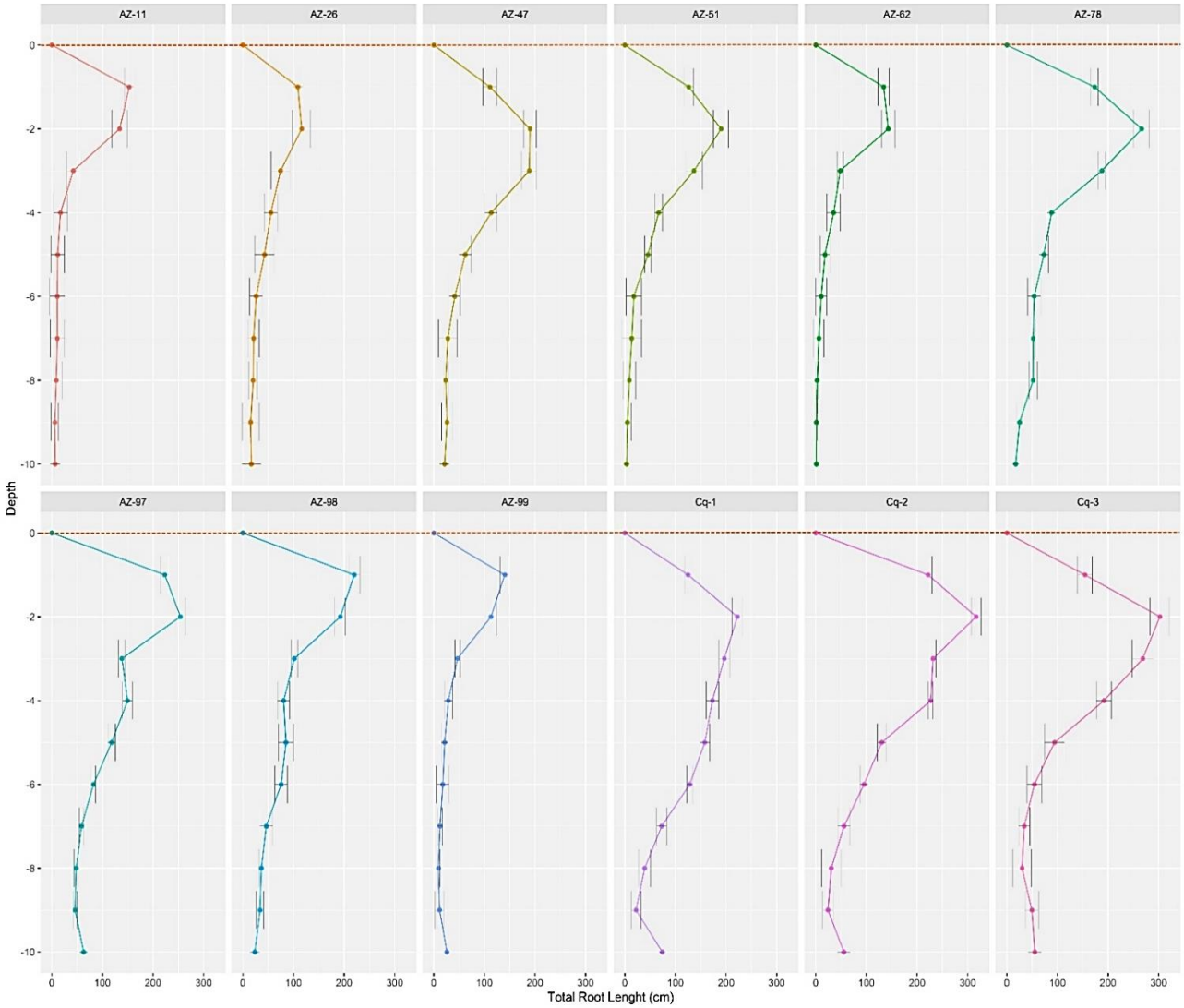

Figure 4.5. Total root length distribution in the soil profile at the harvest time. Spatial distribution of visible primary and lateral roots at the transparent surface of soil filled Rhizotron analyzed by Paint Rhizo software. Plants were grown under non-limiting growth conditions in a long-day environment. The vertical direction shows relative soil layers performance of the evaluated traits and the vertical direction shows performance of the visible root length. Statistics is the mean of six replicates, whereas error bars denote the standard error of the mean of replicates from each evaluated genotype.

selection to some extent. Identical conclusions have been put forward comparing Andean *Chenopodium* species under non-limiting environments (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b). These experiments, on Andean chenopods including *C. quinoa*, found that human selection through the domestication process and genetic variation contribute to root morphological differences. Even with contrasted various root traits growth, the root/shoot mass ratio was highly variable as well among the *C. quinoa* genotypes. In the context of convex hull area, the genotypes Cq-1, Cq-2, Cq-3, and AZ-97 showed contrast over AZ-11, AZ-26, AZ-51, and AZ-62 with the first set showing larger convex hull area (**Figure 4.6**). Yet again, Cq-1, Cq-2, Cq-3, and AZ-97 showed the greatest values of root/shoot ratio (root/shoot = *ca*. 0.20 on an average in these genotypes), while AZ-11, AZ-26, AZ-51, and AZ-62 showed less values of root/shoot ratio (root/shoot = *ca* 0.15 on an average in these genotypes. This higher stake in the root structure might interpret the overall higher productivity in Cq-1, Cq-2, Cq-3, and AZ-97 compared with AZ-11, AZ-26, AZ-51, and AZ-62. Such a similar opinion has been suggested by Alvarez-Flores et al. (2014a). Alvarez-Flores and his colleagues suggested that the improved overall plant production in cultivated and wild *Chenopodium* species could be attributed to their greater root system investment and lower root maintenance costs.

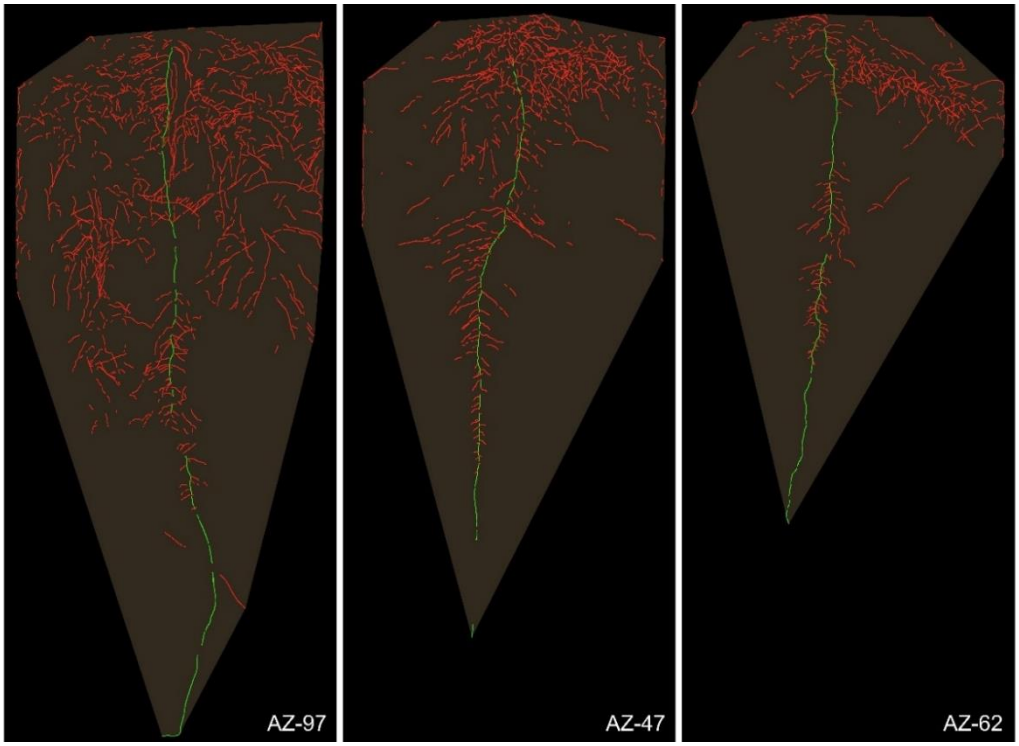

Figure 4.6. *C. quinoa* genotypes contrasted width to to-depth ratio measured in the Rhizotron study. The projected convex hull area from spatial distribution of root traits was calculated from each image for each genotype. Convex hull area represents the combination of both the depth and width of root growth. The most vigorous root system shows a high width-to-depth ratio, and a high convex hull area (AZ-97). In contrast, least vigorous root systems show a comparatively reduced width-to-depth ratio and a low convex hull area (AZ-62).

In the present study, root diameter distribution was studied for selected genotypes based on their rooting phenotypes. Existing results showed root diameter differed among selected studied genotypes which

correspond to previous studies in *C. quinoa* and in wheat (Liu et al. 2021; Alvarez-Flores et al. 2014a). Roots with diameters >0.3 mm were distinguished as the main root, whereas <0.3 mm as lateral roots, which is similar to a previously published research article (Alvarez-Flores et al. 2014a). Furthermore, our data showed clear correlations among several traits (**Figure S6**). For example, shoot biomass is strongly associated with root biomass, total root length, and convex hull area, which confirms the close linkage between above- and below-ground traits. Other root-related traits are also positively linked with shoot traits growth, to some extent. Root biomass is positively linked to total root length and convex hull area as well. Logically, the convex hull area represents both the width and depth of the root system. From the correlation data, we found that root depth has a strong influence on the convex hull area as compared to the root width whose correlation is relatively modest. This could be because root depth and primary root length are strongly linked, which supports the hypothesis that these two variables are related. This corresponds to the correlation found between the convex area and primary root length (Deja-Muylle et al. 2021). Understanding the correlations between above- and below-ground traits is desirable to contribute to plant improvement (Paez-Garcia et al. 2015).

3.3 Principal component analysis and hierarchical cluster analyses

Principal component analysis (PCA) was performed to evaluate the pattern of variation that exists in our dataset. As shown in **Figure 4.7A**, PC1 and PC2 of the principal component analysis interpreted 59% and 15% of the entire variation, respectively, with a cumulative percentage of the total value of 75%. The linear functions through their relevant loading factors defining the principal components are listed in **Table S9**. Based on the PCA score, cluster 3 had the lowest value for PC1. Because PC1 was negatively linked with nearly all the shoot and root-related traits except LAR, LMF, and RA/LA, cluster 3 grouped the genotypes that have vigorous shoot and root growth. Contrarily, cluster 1 grouped those genotypes that had a considerably low score for the shoot and root growth compared with genotypes in clusters 2 and 3. Furthermore, cluster 2 grouped those genotypes which revealed average performance for the shoot and root traits development. Each of the studied genotypes was collected from the same Chilean coastal-lowland region together with the Chilean Altiplano ecotypes and inbred lines but fell into separate clusters. Such diversity of germplasm lines within a geographic region might be owing to factors like heterogeneity of lines within geographic regions, the genetic architecture of accessions, and/or the history of selection patterns for developmental traits (Fuentes et al. 2009; Christensen et al. 2007; Bhargava et al. 2007) and has been also reported in different crop species (Singh et al. 2004; Alemayehu and Becker 2002). The genotypes of a particular cluster have desirable characteristics for specific traits which may facilitate the selection process as well as define the ideal root system that enhances seedling establishment and acquisition of soil resources during early growth stages.

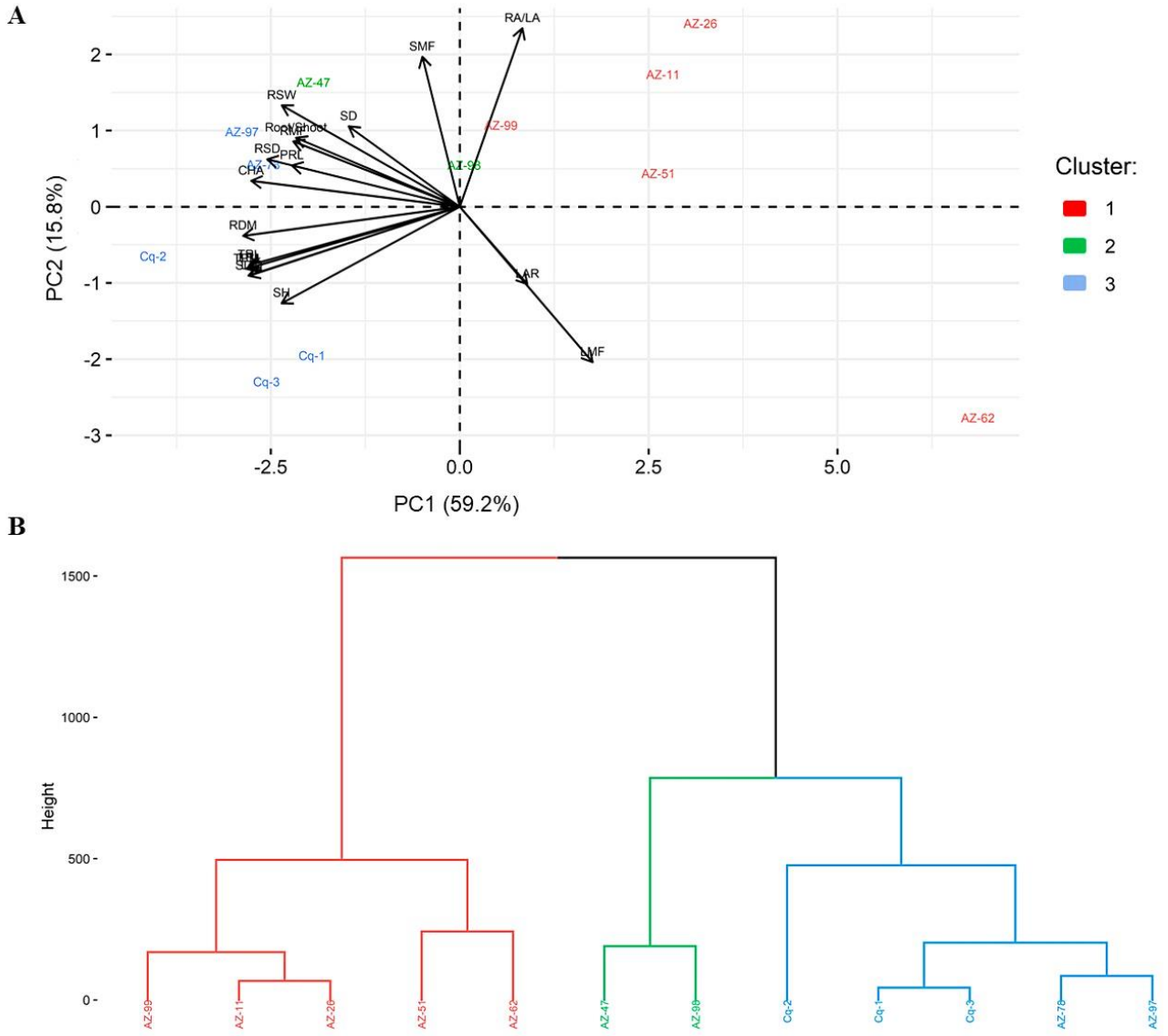

Figure 4.7. Principal component analysis (A) and hierarchical cluster (B) of root system traits of *C. quinoa*. Bi-plot shows main components PC1 and PC2 of PCA, and that explained 75% of the total root phenotypic variation in *C. quinoa*. Arrows show the root var and the length of arrows approximates the variance of the derivatives. The distance between each point explains how similar the observation is and colors correspond to the clusters.

4 Conclusions

In conclusion, the present study brings comprehensive information on root system growth in known *C. quinoa* genotypes, including two cultivated inbred lines of *C. quinoa*. In general, the results show both similarities to some extent and differences in shoot and root growth for *C. quinoa* seedlings of contrasting genotypes under non-limiting growth conditions. A vigorous root system with thicker roots allows deep soil root penetration and an efficient soil exploration that could substantially strengthen the root foraging capacity of *C. quinoa* germplasm. In non-limiting growth conditions, overall data indicated that Cq-1, Cq-2, Cq-3, and AZ-97 showed potentially positive root foraging traits, which prosper bottom level and also adapted rooting into a top-soil level within rhizobox. The specific appearance of such root growth from early developmental stages sustains seedling growth and enhances

the acquisition of soil resources in both dry- and temperate wet- habitats. Deep roots provide access to nutrients and water at depth whereas shallow rooting explores top-soil layers which are potentially beneficial for capturing seasonal rain. On the contrary, the rooting morphology of AZ-11, AZ-26, AZ-51, and AZ-62 improve their ability to explore soil at more superficial layers rather than at depth, which seems beneficial to mainly coastal-lowland habitats as soil moisture in superficial layers is more frequent. The present study identifies certain main root system attributes and ecological significance of the studied Chilean *C. quino*a genotypes in such habitats where rooting is important for seedling establishment. The present study was conducted in non-limiting environmental conditions, however, data on phenotypic variations among genotypes need to be assessed in different agronomical conditions in the field.

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Discussing Remarks

Chenopodium quinoa is a crop that is adapted to various habitats and has tremendous potential for diversification of agroecosystems in Andean highland regions and coastal-lowland environments in central and southern Chile. *C. quinoa* has gained interest among farmers, researchers, and agro-industries in its native region as well as in other regions of the world due to the valuable nutritional properties of its seeds and its tolerance to abiotic stresses. The demand for *C. quinoa* has grown significantly in recent years, but the current global supply of quinoa from its native regions is not yet adequate to meet this demand. As a consequence, *C. quinoa* possible cultivation outside of its native Andean regions is of particular interest in this respect. The two ecotypes of quinoa cultivated in Chile, the highland (salares) and coastal-lowland ecotypes, differ notably in terms of their adaptation to altitude, tolerance to abiotic stress, and day-length sensitivity. Regarding the latter aspect, genotypes which are day-neutral or have suitable photoperiodic requirements could be cultivated at more Northern latitudes including in Europe. Moreover, the genetic backgrounds of these two ecotypes grown in Chile also vary. Interestingly, the genetic backgrounds within the southern coastal-lowland quinoa are highly diverse (Fuentes et al. 2012). Therefore, it is attractive to focus on these genotypes for future quinoa variety development programs. The main purpose of this research work was to evaluate the genotype-dependent phenotypic variations for agronomically important traits, seed composition, and root architecture of Chilean quinoa germplasm.

The results of the present study allow the preliminary selection of candidate genotypes that exhibit desired agronomic and seed characteristics and address the needs of farmers and researchers for the efficient improvement of *C. quinoa*. Such results were obtained through a comprehensive phenotypic characterization of diverse Chilean quinoa germplasms. In the present study, we analyzed key agronomical and morphological traits for over 100 accessions of Chilean germplasm which belong to two groups of genotypes that represent the variations from the coastal-lowland regions and highland regions in Chile. However, in our study, the highland region accessions were underrepresented compared to the coastal-lowland ones. Also, antinutrient saponins as well as health-promoting phytochemicals phenolic and flavonoid compounds were comprehensively evaluated within this core diversity panel. Furthermore, the variability of *C. quinoa* root system growth was also studied using a contrasting subset of *C. quinoa* genotypes grown in controlled environment experiments in rhizoboxes.

1.1 Agronomical and morphological traits variability among *C. quinoa* germplasm

The present study contributed several interesting results with regard to agro- morphological traits. The results of agronomic and morphological traits showed large variation ($p < 0.05$) among *C. quinoa* germplasm (**Figure 1.2, Table S1**). This variation can be useful and form the basis for developing new

varieties with distinct agronomic and morphological traits. Our assessment of agro-morphological traits shows that most of the genotypes were promptly or intermediately responsive to the long-day photoperiod in our greenhouse environmental conditions, which reflects that these genotypes do not have any specific photoperiodic requirements. The present results further support earlier reports indicating that the Chilean ecotypes are insensitive to day-length responses (Patiranage et al. 2021; Patirange et al. 2020; Bhargava and Ohri 2016; Jacobsen and Stølen 1993). In recent years, two likely functional *FT* homologs *CqFT2B* and *CqFT1A* have been found in *C. quinoa* which regulates the flowering time under different photoperiodic conditions (Patiranage et al. 2021). In their research work, Patiranage et al. proposed that the *FT* homologs *CqFT2B* might play a role as a floral activator under short-day conditions but not under long-day conditions, whereas *CqFT1A* might play a role as a floral repressor under long-day conditions but not under short-day conditions. As a result, two different mechanisms control the flowering time in quinoa under different photoperiodic environments. Moreover, we noticed that the variability in photoperiod-dependent traits was not related to the geographical origin of these accessions **(Figure S4)**. Such results also provided a plausible possibility that the plants may become less sensitive to photoperiod and could suggest a possible extension of cultivation of Chilean ecotypes towards lower latitudes, as reported previously by other research groups (Patiranage et al. 2021; Murphy and Matanguihan 2015). The evaluated accessions had an average growth period of about 140 days in greenhouse conditions which is comparable to that reported by Jacobsen (1998), and is similar to the usually suggested < 150 days growth period for field cultivation of *C. quinoa* in the Northern European conditions (Jacobsen 2003).

Simple stems or unbranched plants with reduced plant height are ideally suitable for mechanized agriculture. Notably, our results show statistically significant differences in plant height for the evaluated *C. quinoa* accessions. Interestingly, plant height ranged from 111 cm for accession AZ-62 to 186 cm for accession AZ-9 of studied accessions (**Figure 1.2, Table S1**). Our results are also similar to those obtained by Madrid et al. (2018) and Fuentes and Bhargava (2011) for Chilean *C. quinoa* germplasm. However, both experiments were performed under filed trial conditions. Madrid et al. (2018) carried out a field experiment in Chile under temperate climate conditions, and based on the plant height descriptor plants were classified into short (< 113 cm), medium (113 - 139 cm), and tall (> 139 cm) categories. Similarly, Fuentes and Bhargava (2011) assessed various morphological traits for the Chilean quinoa germplasm under lowland desert conditions, and reported plant height variation which ranged from 100 cm to 191 cm for the studied germplasm. Previously published literature reported a considerable variation also in yield-related descriptors (i.e., seed yield. Thousand Kernel Weight - TKW) in their various performed experiments (Manjarres-Hernández et al. 2021a; Manjarres-Hernández et al. 2021b; Madrid et al. 2018; Bhargava et al. 2007b). Our results support these data as seed yield was noted up to 29.68 g per genotype, with the highest coefficient of variation of 19% among

evaluated variables for *C. quinoa* genotypes (**Figure 1.2, Table 1.2**). Also, TKW showed the second-largest coefficient of variation for studied *C. quinoa* genotypes. However, the data presented in previously published literature were collected from their greenhouse and field trial experiments under different environmental conditions therefore the direct comparison of those data with our data is not straightforward. Manjarres-Hernández et al. (2021a; 2021b) performed the phenotypic characterization of Colombian quinoa germplasm under controlled greenhouse conditions. In their study, they reported a seed yield of between 12.26 g and 87.53 g of seeds per plant. Such differences in seed yield with our data could be due to the different seed materials used for the phenotypic assessment and experimental greenhouse conditions. In another article, Bhargava et al. (2007b) studied genetic diversity in *C. quinoa* germplasm for agro-morphological traits, and reported a seed yield of up to 39.39 g per plant in their field experiment. Regarding panicle structure, 86% of the genotypes were glomerular forms, while the intermediate and amarantiform forms were less prominent in our study. This trend in panicle structure is similar to another study reported by Manjarres-Hernández et al. (2021a). Manjarres-Hernández et al. (2021a) reported the glomerular form was the main panicle form compared to intermediate- and amaranti- forms. The broad-sense heritability (H_b) values for most of the evaluated variables were moderate to high which suggests that these traits are restrained under genetic effects (**Table 1.2**). Such high broad-sense heritability (H_b) values for various traits have been reported for *C. quinoa* (Benlhabib et al. 2016; Bhargava et al. 2012; Bhargava et al. 2007a). Similar to our data, Benlhabib et al. (2016) reported that days to maturity and plant height had the highest heritability (H_b) values (89% and 73%, respectively), whereas yield-related traits had intermediate to lower heritability (H_b) values. Likewise, Bhargava et al.(2012; 2007a) reported a high heritability that exceeded 80% for all evaluated agro-morphological traits for quinoa germplasm in both experiments.

An interesting observation relates to the correlation between days to flowering and maturity, which was positive and highly significant (r = 0.83), thus indicating a linear correlation and a positive impact of the flowering period on the physiological maturity of the crop. So, the earlier a plant flowers, the sooner it reaches physiological maturity (**Figure 1.3**). In irrigated systems, a shorter time to maturity is also a significant advantage, as this reduces the time required for irrigation, and thus reduces water consumption. This result is similar to that obtained by Bhargava et al. (2007a) who reported a positive interrelationship between days to flowering and maturity. Similarly, a published article by Patirange et al. (2020) also confirmed a positive correlation between days to flowering and the physiological maturity in *C. quinoa* genotypes. Interestingly, in *C. quinoa* a number of studies have been published that reported a positive correlation between various agro-morphological traits and also between yield-related traits for direct and indirect selection (Manjarres-Hernández et al. 2021b; Patirange et al. 2020; Bhargava et al. 2012; Bhargava et al. 2007a). Our study confirms the interrelationship among the various agro-morphological traits in *C. quinoa* for developing direct or indirect selection criteria. The

direct and indirect selection for improving quality characteristics and grain yield in *C. quinoa* has been well studied earlier (Bhargava et al. 2012). Improving the net photosynthesis rate could have a significant role in improving TKW, and therefore developing high-yielding cultivars (Zhang et al. 2022). A significant positive correlation existed between grain yield and TKW in our study which would support the above-reported statement (**Figure 1.3**). For these reasons, TKW could also be an important trait for the selection of candidate materials in the genetic improvement of high-yielding *C. quinoa* varieties. A negative and significant correlation was observed between growth stages such as days to flowering and to reach maturity, and yield-related traits, i.e., seed yield and TKW (**Figure 1.3**). Furthermore, most of the accessions having above average plant height also had a low seed yield which highlights the trade-off in investment of resources between vegetative (leaves and stems) and reproductive (inflorescences and seeds) biomass. Such results could be explained by the negative influence of a delay in phenological growth on yield-related traits, which strongly support the findings of the source-sink interrelation in crops and its influence on seed yield (Smith et al. 2018).

Our principal component analysis (PCA) showed that five accessions (AZ-4, AZ-11, AZ-39. AZ-50, and AZ-62) were distantly located from those at the center of the bi-plot (**Figure 1.4A**). In addition, PCA revealed two main components, the first well explained by the plant agro-morphological traits and the second by the yield-related descriptors. The existing variation in the dataset of agronomic and morphological traits has been similarly described by Madrid et al. (2018) for coastal-lowland Chilean *C. quinoa* germplasm. In the study performed by Madrid et al. (2018), the multivariate approaches confirmed the inter-relationship among evaluated variables into two major groups, including plant morphological characteristics and seed-related characteristics. In the present work, cluster analysis grouped together those genotypes that had higher phenotypic similarities. The general classification of *C. quinoa* germplasm made by cluster analysis grouped these genotypes into two main clusters corresponding to early and late phenological development. The *C. quinoa* lines grouped under the late phenological developmental phase were further divided into three sub-groups, characterized by early, mid, and late flowering and maturity (**Figure 1.4B**). The cluster analysis revealed that cluster 4 grouped those genotypes that had high precocity (early in days to flowering and physiological maturity), and high seed yield. On the other hand, clusters 3 and 1 grouped those genotypes that had mid-late and late precocity (mid-late or late flowering and maturity), and below-average seed production. Further, cluster 2 had those genotypes that had early flowering and physiological maturity but had an average seed production. Similar analyses based on agronomic and morphological characteristics using Chilean *C. quinoa* germplasm have been published before (Madrid et al. 2018; Fuentes and Bhargava 2011). The multivariate techniques performed by Madrid et al. (2018) and Fuentes and Bhargava (2011) have allowed for elucidating the genotype-dependent phenotypic relationship among Chilean quinoa accessions based on several agro-morphological descriptors. Likewise, Patirange et al. (2020) have

seen a related clustering pattern for agronomically important variables for different *C. quinoa* germplasm. Interestingly, a significant value ($p \leq 0.05$) of the variance explained by the genetic effects (Vg) revealed the existing genetic diversity that could explain the variation of evaluated agronomically important traits. The wide genotype-dependent phenotypic variation observed for agro-morphological traits suggests that the present diversity within the Chilean *C. quinoa* lines could become a source for *C. quinoa* breeding programs to advance toward the development of new cultivars.

1.2 Antinutrient triterpenoid saponins and bioactive polyphenol components in *C. quinoa*

Chapter 2 of this research work reports the relative concentration of saponins in terms of their aglycons obtained by gas chromatography-mass spectrometry (GC-MS) analysis. In the present study, three major saponin aglycons oleanolic acid (OA), hederagenin (HD), and phytolaccagenic acid (PA) were reported to be present in all studied *C. quinoa* genotypes (**Figure 2.1**). A number of studies have reported these three sapogenins are the key triterpenoid saponins in both sweet and bitter varieties (Medina-Meza et al. 2016; Gómez-Caravaca et al. 2012; Ridout et al. 1991). Similar to our study several other authors have also identified a fourth saponin aglycon serjanic acid (SA) in seeds and other organs of *C. quinoa* as being abundant compared to other sapogenins (Medina-Meza et al. 2016; Gómez-Caravaca et al. 2012). To our knowledge, saponins content was never determined before for these Chilean *C. quinoa* genotypes and with a few exceptions, direct comparisons with the previously published data is not possible for the available dataset. In the present study, our data revealed two main groups, the first one having high saponins content (26% of the genotypes), and the second one having low saponins content (74% of the genotypes). The results of the saponins quantification showed large absolute content variation that ranged from 0.22 mg/g to 15.04 mg/g of seed dry weight among the studied *C. quinoa* germplasm (**Figure 2.2A**). Medina-Meza et al. (2016) assessed *C. quinoa* varieties and found a significant amount of variation for triterpenoid saponins. The known Danish cultivate Titicaca (Cq-2) showed high saponins content (15.04 mg/g of seed dry weight) with a higher concentration of PA of total saponins in comparison to other sapogenins. Similar to our result, Medina-Meza et al. (2016) reported an average total saponins content of 16.75 mg/g in Titicaca among the evaluated 28 quinoa varieties. In another study, the presence of a high percentage of PA compared with the remaining sapogenins in the bitter variety Titicaca supports our data (Ruiz et al. 2017). In our experiment, Cáhuil accessions had total saponins content in a range of 4.08 mg/g to 9.24 mg/g which was similar to that calculated by Medina-Meza et al. (2016) and Ward (2000) who reported 10.95 mg/g and 4.65 mg/g, respectively. However, our values were generally higher compared to the ones reported by Miranda et al. (2012) for Chilean Cáhuil genotypes. Such differences in saponins content among various studies could be explained by several factors including agroecological conditions as well as the protocols and methods that have been used for the extraction and quantification of saponins. Our data

revealed a slightly higher content of saponins in the central Chilean ecotype (FARO), the south Altiplano ecotype (Cancosa), and ecotypes from the Villarrica locality compared to the values reported by Miranda et al. (2012), who assessed the saponins in Chilean ecotypes by reversed-phase HPLC approach. A high ratio of OA to HD is concerned with the sweetness of seeds and a low ratio is concerned with the bitterness of seeds, at least to some extent (Ruiz et al. 2017; Mastebroek et al. 2000). In this study, a high OA:HD ratio with a relatively low amount of PA for the total saponins in Vikinga (Cq-1) and cvATLAS (Cq-3) supports the above-mentioned statements (**Figure 2.2B**). An interesting observation was found relating to the interrelationship between PA and total saponins, which was positive and highly significant ($r = 0.85$), thus indicating a linear relation of PA on the saponins content (**Figure S4**). This implies a higher concentration of PA results in a relatively high amount of total saponins. Supporting our data, a previous study reported PA as the main class of sapogenins followed by OA and HD in coastal-lowland ecotypes (Ridout et al. 1991).

Besides conferring bitterness, saponins play multiple roles in various biological activities, including immunomodulatory, antidiabetic, hepatoprotective, antiosteoporosis, hypolipidemic, antiviral, and antifungal actions (Mroczek 2015). Therefore, a thorough assessment of saponins was performed by high-resolution FTICR-MS in the full scan mode (**Figure 2.3**). Our experiment identified in total 12, out of which 11 had reported previously and Compound 8 was assigned as a novel saponin. The mass spectrum peak of novel saponin (3-O-HexA-SA 28-O-Hex) was spotted at m/z 837 $[M-H]^-$, 675 $[M-H-Hex]^-$, 499 $[M-H-Hex-HexA]^-$ or [SA-H].

The principal component analysis (PCA) has shown the four accessions (Cq-2, Cq-3, AZ-79, and AZ-129) were distantly located from those at the center of the bi-plot (**Figure 2.5A**). In addition, the first component of the PCA is well explained by the total saponins content and the second by the OA and HD. The general classification done by cluster analysis grouped these genotypes into two major clusters relating to high and low saponins content. The *C. quinoa* accessions grouped under low saponins content were further divided into three sub-groups, categorized into high, moderate, and low saponins. The grouping of the variables has been similarly described in evaluated quinoa varieties through total saponins and their aglycons (Medina-Meza et al. 2016). The cluster analysis showed that cluster 4 grouped those genotypes that had a high saponins content. Also, genotypes with a high percentage of PA were grouped into cluster 3. On the contrary, genotypes that had a considerably lower saponins content were grouped into cluster 5. Also, clusters 2 and 1 grouped those genotypes that had below-average PA contents and thus lower saponins. However, the results of multivariate analysis showed uninterpreted divergence among sub-groups of the major two groups without clear separation. The grouping of genotypes in two major clusters could be explained by the extant genetic similarity between highland and coastal-lowland *C.quinoa* genotypes (Christensen et al. 2007). Another study also

supported the existence of genetic diversity within and among the Chilean germplasm of different biomes, and such genetic diversity might be a key factor in the available variation in saponins content (Fuentes et al. 2009).

Besides triterpenoid saponins, our dataset provided detailed information related to the bioactive polyphenols content in Chilean *C. quinoa* genotypes. In the experiment, a total of sixteen different phenolic derivatives in their free and bound fractions of phenolics were determined in the studied genotypes. Such existence of phenolic derivatives in *C. quinoa* seeds is supported by earlier published articles (Han et al. 2019; Gómez-Caravaca et al. 2011). Our results show a 3-fold variation in total phenolics content in the studied seed extract that ranged from 35.51 mg/100 g to 93.23 mg/100 g of seed dry weight. In our study, 40% of evaluated genotypes had above-average total phenolics content. In our study, it was noticed that the Cáhuil accessions had an average of 47.25 mg/g of total phenolics content. This value is four times lower compared with that obtained by Vega Gálvez et al. (2018), who assessed the phenolic compounds in quinoa and reported 194 mg/100 g of total phenolics in Cáhuil ecotypes. Similarly, observed values for the central Chilean ecotype (FARO), the south Altiplano ecotype (Cancosa), and ecotypes from the Villarrica were two to four times lower than the reported ones in published literature by Vega Gálvez et al. (2018) and Sobota et al. (2020). The composition analyses in these references were done by the Folin-Ciocalteu method. As it was indicated by earlier studies, it can be inferred that a classical approach like the Folin-Ciocalteu assay leads to an overestimation of phenolic compounds because of the interference of non-phenolic substances (Melini and Melini 2021).

An interesting observation was found regarding variation in individual compounds, which was significant among the *C. quinoa* genotypes. In our study, the flavonoid glycosides were the most abundant free phenolics of the total free phenolics fraction for the studied genotypes (**Figure 3.4**). The present observation further supports the earlier report of Gómez-Caravaca et al. (2011) that is of the total free phenolic fraction the most abundant phenolic compounds are flavonoid glycosides. Also, our results show a 1.5-fold higher percentage of flavonoid glycosides in south Altiplano ecotypes compared to the central-southern ecotypes. Overall these results are similar to those obtained by Graf et al. (2016), who reported higher content of flavonoids in northern genotypes compared to those collected from the central regions of Chile. A thorough analysis of the bound phenolics fraction shows ferulic acid as a major compound of the bound phenolics fraction among all other phenolic acids (**Figure 3.4**). Earlier, several authors have also reported comparable results in *C. quinoa* (Han et al. 2019; Tang et al. 2015; Gómez-Caravaca et al. 2011). The free fractions of phenolics are reported as a significant contributor to the total phenolics content compared with the bound fractions (Li et al. 2021; Han et al. 2019; Vega Gálvez et al. 2018; Gómez-Caravaca et al. 2011). Our study confirms the greater contribution of free

phenolics to the total phenolics content. Such differences in the proportion of the contribution to the total phenolics could be possibly explained by the fact that free phenols are available on the outer surface of the seed pericarp, whereas bound phenols are more firmly attached to cell wall components.

A principal component analysis (PCA) showed that the distinct genotypes scattered distantly from those at the center of the bi-plot. Furthermore, a cluster analysis based on the PCA scores was done to create a dendrogram, whereby evaluated genotypes were classified into five separate groups related to the phenolic compounds content (**Figure 3.5**). The association of major groups of bioactive phytochemicals has been similarly described in Chilean germplasm through triterpenoid saponins and their aglycons (Pandya et al. 2021). The general classification provided by clustering showed that cluster 4 grouped those genotypes that had high total phenolics. Also, clusters 5 and 1 had a higher content of free fractions of phenolics, and therefore total phenolics compared to the remaining ones. In contrast, cluster 3 grouped the genotypes with low phenolics content. Interestingly, the genotypes belonging to cluster 2 were scattered in proximity to the central border of the second component of PCA, denoting an average of total phenolics in those genotypes. Although a multivariate analysis showed a clear classification, low phenolics genotypes showed variation within and among sub-groups. The interlinking of several genotypes within and among sub-groups reflects the existing shared alleles (Fuentes et al. 2009), consequently genetic similarity between highland and coastal-lowland quinoa genotypes (Christensen et al. 2007). Similar to our study, several other studies have also confirmed the extant genetic diversity within *C. quinoa* germplasm for various traits and such genetic variance could be a key reason for possible variation in phenolics content (Mizuno et al. 2020; Fuentes et al. 2009; Christensen et al. 2007).

1.3 Shoot and root growth variation pattern in *C. quinoa*

Our study postulated that *C. quinoa* exhibits variation in phenotypic traits that are related to shoot and root growth for genotypes grown in soil-filled rhizotrons. Available data based on plant morphology, and shoot-root growth certainly distinguished all studied genotypes, to some extent, with four genotypes (Cq-1, Cq-2, Cq-3, and AZ-97) showing vigorous shoot-root growth (**Figure 4.2**). Similar to our results, Alvarez-Flores et al. have reported clear differences in shoot and root-related growth annotated genetic differences among Chenopodium species in their studies (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b). The plant has to allocate biomass to leaves, stems, and roots in a way that optimizes the physiological activities of its different organs. Alvarez-Flores et al. (2014a) reported a biomass allocation to leaves, stems, and roots, with the major part (≥50%) located in leaves. In our study, the biomass allocation in plants during the vegetative phase was also allocated to leaves in a large proportion (65%), followed by stems and roots (**Figure 4.3**). Lower leaf area could have an impact on plant biomass and root growth since lateral root density can vary significantly depending on

how slowly leaves develop as leaves are the source of photosynthates and auxin to foster root growth (Smith and De Smet 2012; Boyer et al. 2010). Our results agree with such a statement and show higher leaf area in plants that had vigorous shoot-root growth. Similarly, a previously published article reported a larger leaf area in the genotypes that had high biomass production and vigorous rooting (Alvarez-Flores et al. 2014a; Alvarez-Flores et al. 2014b). Alvarez-Flores et al. (2014a) argued that the root system plays an important role and authors reported that high RA/LA in early growth stages suggests a balance between soil foraging and light capture. This is likely due to the equal or larger root area compared to the leaf area. Similarly, our data show higher CHA than LA during the vegetative phase, and a significant variation was observed in RA/LA among studied genotypes. In our study, most of the genotypes having vigorous growth had higher RA/LA compared to less vigorous genotypes. Overall, in our study Cq-1, Cq-2, Cq-3, and AZ-97 showed greater TRL values, which differs significantly from all other genotypes AZ-11, AZ-26, AZ-51, and AZ-62. An interesting observation was found related to the vertical root distribution of the root growth, which was different among substrate layers and genotypes (**Figure 4.5**). Our results show that most of the genotype having higher vertical root growth had greater CHA compared to those genotypes having less vertical growth and thus low CHA. In arid regions, fast vertical root growth is considered to be suitable for acquiring limited soil resources at depth rather than a dense root system in the upper soil layers (Alvarez-Flores et al. 2018; Alvarez-Flores et al. 2014b). Such rooting characteristics were noticed for the Cq-1, Cq-2, Cq-3, and AZ-97 which means these genotypes had greater rooting colonization at depth. Among these genotypes, data has shown that Cq-1 and Cq-3 have dense and deeper rooting patterns, which could lead to an advantage over other genotypes in both dry and wet habitats. It is possible that the success of certain breeding lines could be due to the fact that these are progenies of different parental lines that originated in a dry-habitat (Peru) and a wet-habitat (southern Chile) (Ruiz et al. 2017; Risi C and Galwey 1984). On the other hand, the remaining genotypes AZ-11, AZ-26, AZ-51, and AZ-62 had dense root development which seems advantageous for the soil conditions where soil moisture in superficial layers is more frequent. Our results are comparable with published articles by Alvarez-Flores et al. (2014a) and Alvarez-Flores et al. (2014b), who noticed less rooting foraging ability at depth for the quinoa genotypes originating from habitats with high-resource availability. Furthermore, in our study roots with >0.3 mm diameters were classified as the main root, while <0.3 mm as lateral roots, which is in agreement with the previously published data by Alvarez-Flores et al. (2014a).

The principal component analysis (PCA) placed more distinct genotypes distantly from those at the center of the bi-plot. Also, a cluster analysis classified all genotypes into three separate groups related to the shoot and root-related variables (**Figure 4.7**). The general classification by clustering showed that cluster 3 grouped those genotypes that had vigorous rooting patterns. In contrast, the least vigorous genotypes were grouped into cluster 1. However, the genotypes that had average values for the shoot-

root growth were grouped separately into cluster 2. Although most of the evaluated genotypes are collected from the same localities of central-southern Chile they fall into different sub-groups. Such genotype-dependent trait variation within the same geographic region owes to factors such as phenotypic and genetic heterogeneity of lines within a geographical region, the genetic architecture of accessions, and/or history of selection patterns for developmental traits (Fuentes et al. 2009; Christensen et al. 2007; Bhargava et al. 2007b).

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Supplementary or Supporting Materials

Table S1. Mean performance of 114 lines for agro-morphological traits in *C. quinoa.* In total 114 *C. quinoa* lines were grown under long-day conditions in the greenhouse (16h light, 22 ± 2 ºC). Agro-morphological traits were evaluated for their performance. The core dataset shows a mean ± standard deviation (SD) of measured traits for all the plant replicates of each genotype. TKW: Thousand Kernel Weight, NA: Data not available.

Quinoa line	Days to Flowering	Days to Maturity	Plant Height (cm)	Seed Yield (gm)	TKW (gm)	Panicle Shape
AZ-1	53.75 ± 5.18	134.75 ± 1.50	145.00 ± 3.37	19.05 ± 3.45	2.44 ± 0.09	Glomerulate
AZ-2	52.00 ± 2.30	126.50 ± 5.45	154.00 ± 4.97	26.49 ± 3.19	2.60 ± 0.07	Glomerulate
AZ-3	61.75 ± 5.90	149.00 ± 2.00	151.00 ± 1.63	17.88 ± 4.59	1.81 ± 0.09	Glomerulate
AZ-4	71.00 ± 2.30	150.50 ± 1.00	154.00 ± 2.94	16.77 ± 6.28	2.08 ± 0.70	Glomerulate
AZ-5	52.75 ± 3.40	127.00 ± 0.00	141.50 ± 2.08	16.23 ± 2.98	2.95 ± 0.25	Amarantiform
AZ-6	48.50 ± 7.04	122.25 ± 3.50	149.25 ± 6.55	25.89 ± 2.20	2.35 ± 0.29	Glomerulate
AZ-7	55.25 ± 3.50	138.00 ± 0.00	179.75 ± 5.91	21.56 ± 2.04	2.46 ± 0.20	Glomerulate
AZ-8	54.50 ± 5.44	140.25 ± 1.50	150.50 ± 5.45	18.79 ± 5.44	2.47 ± 0.38	Glomerulate
AZ-9	55.50 ± 6.35	145.00 ± 0.00	186.00 ± 6.06	18.93 ± 3.46	2.25 ± 0.17	Amarantiform
AZ-10	49.25 ± 5.90	122.75 ± 3.40	136.75 ± 4.79	26.13 ± 4.45	2.72 ± 0.19	Glomerulate
AZ-11	63.75 ± 2.63	149.25 ± 1.50	118.75 ± 1.71	12.19 ± 1.83	1.70 ± 0.40	Amarantiform
AZ-12	50.25 ± 4.71	128.00 ± 2.00	141.00 ± 3.92	26.24 ± 4.11	2.67 ± 0.18	Glomerulate
AZ-13	53.00 ± 7.11	128.25 ± 3.40	153.00 ± 3.37	16.00 ± 3.01	2.37 ± 0.27	Glomerulate
AZ-14	61.50 ± 5.44	149.00 ± 2.00	153.00 ± 4.08	15.72 ± 3.87	2.39 ± 0.20	Glomerulate
AZ-15	56.25 ± 1.50	148.00 ± 0.00	180.50 ± 6.61	19.45 ± 3.80	2.71 ± 0.20	Glomerulate
AZ-16	53.00 ± 2.00	131.00 ± 0.00	157.25 ± 5.06	19.97 ± 1.72	2.97 ± 0.27	Intermediate
AZ-17	NA	NA	NA	NA	NA	NA
AZ-18	59.75 ± 1.50	130.50 ± 1.73	158.00 ± 5.89	16.61 ± 3.56	2.27 ± 0.19	Glomerulate
AZ-19	42.50 ± 7.90	102.00 ± 2.00	123.00 ± 4.32	20.91 ± 5.23	2.54 ± 0.25	Glomerulate
AZ-20	59.75 ± 3.40	145.00 ± 0.00	144.75 ± 4.79	19.67 ± 2.04	2.22 ± 0.12	Glomerulate
AZ-21	55.50 ± 4.66	138.00 ± 0.00	149.50 ± 7.23	17.35 ± 2.27	2.31 ± 0.30	Glomerulate
AZ-22	50.25 ± 2.87	124.75 ± 1.50	145.25 ± 4.57	22.62 ± 5.81	2.72 ± 0.10	Glomerulate

AZ-23	47.50 ± 6.61	124.75 ± 1.50	134.75 ± 10.91	25.01 ± 5.58	2.37 ± 0.43	Glomerulate
AZ-24	55.50 ± 1.73	141.00 ± 0.00	156.75 ± 1.89	17.21 ± 1.60	2.06 ± 0.16	Glomerulate
AZ-25	59.00 ± 2.31	138.00 ± 0.00	152.50 ± 3.42	16.47 ± 3.13	2.19 ± 0.33	Glomerulate
AZ-26	57.00 ± 0.00	138.00 ± 0.00	159.00 ± 12.57	14.97 ± 3.44	2.12 ± 0.11	Glomerulate
AZ-27	57.25 ± 5.19	141.00 ± 0.00	161.00 ± 6.45	15.91 ± 3.14	2.20 ± 0.34	Glomerulate
AZ-29	59.75 ± 1.50	133.25 ± 0.73	152.00 ± 4.99	16.61 ± 3.56	2.27 ± 0.19	Glomerulate
AZ-30	53.50 ± 4.04	130.00 ± 2.00	129.50 ± 7.42	19.12 ± 4.26	2.19 ± 0.08	Glomerulate
AZ-31	53.00 ± 6.06	132.25 ± 3.50	147.25 ± 9.74	16.42 ± 2.48	2.30 ± 0.23	Glomerulate
AZ-32	53.00 ± 7.12	127.00 ± 0.00	149.75 ± 9.07	19.65 ± 2.38	2.67 ± 0.26	Glomerulate
AZ-33	49.50 ± 3.32	131.00 ± 0.00	139.50 ± 2.65	16.43 ± 3.42	2.08 ± 0.25	Intermediate
AZ-34	52.75 ± 3.40	127.25 ± 2.87	139.00 ± 2.16	16.12 ± 3.00	2.10 ± 0.38	Intermediate
AZ-35	51.00 ± 4.24	131.00 ± 0.00	174.75 ± 12.04	17.38 ± 1.73	2.68 ± 0.19	Glomerulate
AZ-36	51.75 ± 3.50	129.25 ± 3.50	161.25 ± 3.30	17.45 ± 4.31	2.72 ± 0.18	Glomerulate
AZ-37	52.75 ± 3.40	126.50 ± 3.32	155.75 ± 4.11	17.75 ± 4.35	2.54 ± 0.08	Intermediate
AZ-38	52.00 ± 2.31	137.00 ± 2.00	161.75 ± 7.27	20.29 ± 1.97	2.53 ± 0.21	Intermediate
AZ-39	57.25 ± 5.19	142.00 ± 0.00	164.75 ± 10.15	29.69 ± 6.91	3.18 ± 0.38	Glomerulate
AZ-40	55.25 ± 3.50	133.75 ± 1.50	157.25 ± 8.26	25.64 ± 1.26	2.78 ± 0.18	Glomerulate
AZ-41	55.75 ± 3.50	141.00 ± 2.00	171.50 ± 1.29	22.08 ± 6.88	2.22 ± 0.50	Glomerulate
AZ-42	58.00 ± 6.06	124.75 ± 1.50	162.75 ± 7.41	25.40 ± 4.52	2.58 ± 0.29	Glomerulate
AZ-43	49.25 ± 1.50	140.25 ± 1.50	155.00 ± 4.08	21.09 ± 2.53	2.37 ± 0.20	Glomerulate
AZ-44	54.50 ± 3.32	127.00 ± 0.00	145.75 ± 3.20	22.70 ± 2.17	2.79 ± 0.16	Glomerulate
AZ-45	50.25 ± 2.87	144.50 ± 1.73	153.75 ± 6.34	10.80 ± 3.47	2.58 ± 0.21	Glomerulate
AZ-46	59.75 ± 1.50	89.00 ± 0.00	137.25 ± 1.89	19.67 ± 4.06	2.92 ± 0.44	Glomerulate
AZ-47	39.75 ± 2.87	117.00 ± 0.00	145.50 ± 5.69	20.65 ± 0.57	2.74 ± 0.27	Glomerulate
AZ-48	46.00 ± 2.00	86.00 ± 2.00	126.50 ± 5.32	25.42 ± 1.66	2.82 ± 0.28	Glomerulate
AZ-49	37.00 ± 2.00	128.00 ± 2.00	149.00 ± 4.32	20.74 ± 2.24	2.40 ± 0.23	Glomerulate
AZ-50	49.50 ± 7.59	78.00 ± 0.00	123.75 ± 3.78	27.41 ± 2.40	3.08 ± 0.10	Glomerulate
AZ-51	39.75 ± 2.87	85.25 ± 2.87	121.25 ± 2.36	20.04 ± 5.97	2.56 ± 0.24	Intermediate
AZ-52	39.75 ± 2.87	81.00 ± 2.00	124.50 ± 4.44	19.12 ± 6.25	2.66 ± 0.18	Glomerulate

AZ-53	45.00 ± 2.31	110.75 ± 2.87	136.25 ± 4.92	16.62 ± 3.48	2.49 ± 0.19	Glomerulate
AZ-54	41.50 ± 11.36	89.75 ± 13.57	139.75 ± 14.50	20.25 ± 5.19	1.91 ± 0.22	Glomerulate
AZ-55	48.50 ± 1.73	118.25 ± 3.50	160.75 ± 4.50	15.27 ± 1.82	2.28 ± 0.21	Glomerulate
AZ-56	47.50 ± 3.32	119.50 ± 3.32	149.75 ± 6.70	16.05 ± 0.76	2.42 ± 0.19	Glomerulate
AZ-57	43.50 ± 4.04	112.25 ± 1.50	135.75 ± 6.55	22.68 ± 2.41	2.89 ± 0.21	Intermediate
AZ-58	43.25 ± 2.87	109.00 ± 2.00	151.75 ± 4.86	19.26 ± 5.90	2.96 ± 0.14	Glomerulate
AZ-59	51.00 ± 2.00	131.00 ± 0.00	149.00 ± 2.94	15.85 ± 1.43	2.21 ± 0.18	Glomerulate
AZ-60	55.75 ± 3.50	142.75 ± 1.50	164.00 ± 4.83	16.98 ± 2.72	2.71 ± 0.17	Glomerulate
AZ-61	44.25 ± 5.50	113.00 ± 0.00	148.50 ± 3.11	16.88 ± 5.94	2.31 ± 0.52	Glomerulate
AZ-62	34.50 ± 1.73	74.00 ± 2.00	111.00 ± 1.16	16.63 ± 1.01	2.10 ± 0.17	Glomerulate
AZ-63	52.75 ± 3.40	129.00 ± 2.31	156.50 ± 3.11	15.63 ± 2.92	2.06 ± 0.51	Intermediate
AZ-64	58.00 ± 8.04	141.00 ± 1.16	141.00 ± 3.92	14.01 ± 1.47	2.06 ± 0.32	Glomerulate
AZ-65	54.75 ± 5.91	139.50 ± 1.73	141.00 ± 8.29	17.66 ± 3.04	2.45 ± 0.31	Glomerulate
AZ-66	46.00 ± 2.00	113.25 ± 2.87	132.50 ± 5.92	20.26 ± 3.04	2.63 ± 0.39	Glomerulate
AZ-67	45.00 ± 2.31	114.00 ± 2.00	147.50 ± 2.52	16.80 ± 1.86	2.65 ± 0.32	Glomerulate
AZ-68	60.00 ± 2.00	141.75 ± 0.50	135.50 ± 2.52	16.95 ± 6.03	2.33 ± 0.12	Glomerulate
AZ-69	57.00 ± 0.00	145.00 ± 0.00	142.00 ± 4.08	13.50 ± 3.25	2.41 ± 0.08	Glomerulate
AZ-70	58.00 ± 7.44	142.00 ± 0.00	160.25 ± 2.99	22.76 ± 15.69	2.69 ± 0.51	Glomerulate
AZ-71	56.50 ± 3.32	145.00 ± 0.00	142.50 ± 3.87	20.73 ± 5.25	2.41 ± 0.10	Glomerulate
AZ-72	51.25 ± 3.40	129.00 ± 2.31	159.00 ± 6.48	19.45 ± 7.92	2.69 ± 0.52	Intermediate
AZ-73	47.00 ± 0.00	118.75 ± 3.50	139.00 ± 6.98	19.01 ± 3.10	2.45 ± 0.18	Intermediate
AZ-74	48.50 ± 1.73	124.00 ± 0.00	138.00 ± 4.24	20.81 ± 4.58	2.57 ± 0.18	Intermediate
AZ-76	45.00 ± 2.31	116.00 ± 2.00	152.25 ± 3.40	21.66 ± 6.49	2.71 ± 0.17	Glomerulate
AZ-77	49.25 ± 1.50	126.25 ± 1.50	160.50 ± 10.88	18.00 ± 2.87	2.68 ± 0.11	Glomerulate
AZ-78	46.00 ± 2.00	119.25 ± 1.50	148.50 ± 5.45	21.88 ± 1.80	2.58 ± 0.13	Glomerulate
AZ-79	50.50 ± 7.00	128.00 ± 2.00	127.00 ± 46.78	17.02 ± 2.93	2.54 ± 0.10	Glomerulate
AZ-80	NA	NA	NA	NA	NA	NA
AZ-81	46.00 ± 2.00	119.25 ± 1.50	143.75 ± 2.63	19.05 ± 2.85	2.73 ± 0.21	Glomerulate
AZ-82	45.00 ± 2.31	115.00 ± 2.31	129.75 ± 2.22	19.54 ± 2.70	2.70 ± 0.41	Glomerulate

AZ-83	46.00 ± 2.00	121.00 ± 1.16	136.25 ± 1.71	19.97 ± 4.86	2.12 ± 0.27	Glomerulate
AZ-84	62.75 ± 3.50	141.25 ± 0.50	162.25 ± 14.57	17.49 ± 5.07	2.15 ± 0.21	Glomerulate
AZ-85	52.75 ± 1.50	122.75 ± 1.50	145.25 ± 6.13	23.02 ± 6.31	2.77 ± 0.18	Intermediate
AZ-86	39.75 ± 5.19	88.50 ± 4.04	136.75 ± 5.25	24.15 ± 8.66	2.46 ± 0.30	Glomerulate
AZ-87	47.75 ± 1.50	121.00 ± 2.00	140.25 ± 8.06	18.70 ± 3.68	2.55 ± 0.15	Glomerulate
AZ-88	60.00 ± 7.12	139.50 ± 1.73	145.75 ± 5.50	16.68 ± 1.84	1.83 ± 0.18	Glomerulate
AZ-89	57.25 ± 2.87	138.00 ± 0.00	172.50 ± 7.42	20.74 ± 3.13	2.33 ± 0.48	Glomerulate
AZ-91	48.50 ± 1.73	124.00 ± 0.00	142.00 ± 5.35	20.03 ± 3.60	3.02 ± 0.05	Glomerulate
AZ-92	NA	NA	NA	NA	NA	NA
AZ-93	43.25 ± 5.19	111.50 ± 1.73	129.00 ± 3.56	21.14 ± 6.36	2.68 ± 0.17	Glomerulate
AZ-94	55.00 ± 0.00	140.00 ± 0.00	144.50 ± 6.14	15.97 ± 2.79	2.74 ± 0.18	Glomerulate
AZ-95	47.50 ± 3.32	123.00 ± 2.00	157.75 ± 3.30	19.64 ± 1.66	2.80 ± 0.10	Glomerulate
AZ-96	57.00 ± 2.31	146.00 ± 0.00	159.75 ± 5.38	11.60 ± 0.76	2.63 ± 0.08	Glomerulate
AZ-97	63.25 ± 4.57	144.25 ± 1.50	157.00 ± 2.45	21.57 ± 2.14	1.96 ± 0.07	Glomerulate
AZ-98	66.00 ± 0.00	146.00 ± 0.00	145.75 ± 7.09	11.43 ± 3.25	2.39 ± 0.21	Glomerulate
AZ-99	57.00 ± 2.31	146.00 ± 0.00	144.00 ± 5.10	13.48 ± 3.22	2.34 ± 0.17	Glomerulate
AZ-100	NA	NA	NA	NA	NA	NA
AZ-101	62.50 ± 7.00	146.00 ± 0.00	155.75 ± 6.40	11.22 ± 1.76	2.54 ± 0.18	Glomerulate
AZ-102	59.75 ± 1.50	145.25 ± 1.50	159.50 ± 2.52	15.10 ± 1.81	2.19 ± 0.07	Glomerulate
AZ-103	60.75 ± 3.50	120.25 ± 3.50	162.25 ± 4.27	13.52 ± 5.36	2.25 ± 0.14	Glomerulate
AZ-104	64.00 ± 2.31	145.25 ± 1.50	161.50 ± 5.75	8.59 ± 1.84	2.35 ± 0.37	Glomerulate
AZ-105	61.50 ± 5.00	132.00 ± 0.00	158.00 ± 3.37	19.05 ± 3.11	2.21 ± 0.34	Glomerulate
AZ-107	63.25 ± 5.50	147.25 ± 1.89	137.00 ± 3.56	13.80 ± 1.88	2.37 ± 0.11	Glomerulate
AZ-108	59.75 ± 4.57	146.00 ± 0.00	137.75 ± 3.40	12.73 ± 3.46	2.25 ± 0.16	Glomerulate
AZ-110	57.25 ± 1.50	143.75 ± 1.50	155.75 ± 1.26	18.99 ± 1.49	2.52 ± 0.18	Glomerulate
AZ-111	58.75 ± 4.72	143.00 ± 0.00	175.75 ± 2.87	12.24 ± 1.77	2.77 ± 0.40	Glomerulate
AZ-112	36.00 ± 4.40	87.50 ± 3.32	139.50 ± 1.92	17.89 ± 1.02	2.48 ± 0.26	Intermediate
AZ-113	54.25 ± 4.57	135.00 ± 1.41	150.75 ± 4.11	16.27 ± 4.65	2.46 ± 0.32	Glomerulate
AZ-114	63.25 ± 3.40	132.00 ± 0.00	136.00 ± 4.08	8.54 ± 2.17	1.93 ± 0.14	Glomerulate

AZ-115	56.25 ± 3.40	138.25 ± 1.50	137.00 ± 4.08	11.39 ± 2.26	2.48 ± 0.26	Glomerulate
AZ-129	NA	NA	NA	NA	NA	NA
Cq-1	NA	NA	NA	NA	NA	NA
Cq-2	NA	NA	NA	NA	NA	NA
Cq-3	NA	NA	NA	NA	NA	NA

Table S2. Total saponins content (mg/g) in *C. quinoa* lines. Table shows the total saponins content and individual sapogenins in *C. quinoa* seeds. Data are presented here as mean ± standard deviation (SD) values of independent sample extractions (n = 4). A one-way analysis of variance ANOVA was performed and p-value < 0.05 was considered as a statistically significant difference. A significant difference was found ($p < 0.001$) in relative saponins content. Significant codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 OA: oleanolic acid, HD: hederagenin, PA: phytolaccagenic acid.

Quinoa line	OA		HD		PA		Total Saponins	
AZ-1	3.01 ± 0.14	***	2.55 ± 0.13	***	ND		5.56 ± 0.24	***
AZ-2	NA		NA		NA		NA	
AZ-3	4.37 ± 0.13	***	2.99 ± 0.05	***	4.95 ± 0.22	***	12.32 ± 0.37	***
AZ-4	3.21 ± 0.10		2.98 ± 0.05	***	3.05 ± 0.14	***	9.25 ± 0.16	***
AZ-5	2.57 ± 0.36	**	2.83 ± 0.38	*	ND		5.41 ± 0.73	
AZ-6	0.99 ± 0.11	***	1.74 ± 0.31	***	2.81 ± 0.55		5.54 ± 0.94	
AZ-7	3.07 ± 0.46		2.35 ± 0.25	.	5.05 ± 0.53	***	10.47 ± 1.23	***
AZ-8	2.45 ± 0.62	***	2.76 ± 0.58	.	2.20 ± 0.48	***	7.41 ± 1.68	***
AZ-9	2.94 ± 0.21		1.76 ± 0.10	***	3.08 ± 0.25	***	7.79 ± 0.54	***
AZ-10	1.59 ± 0.12	***	1.34 ± 0.10	***	1.84 ± 0.14	***	4.78 ± 0.35	*
AZ-11	3.15 ± 0.05		4.01 ± 0.21	***	4.63 ± 0.18	***	11.80 ± 0.43	***
AZ-12	2.71 ± 0.26	*	2.34 ± 0.19	.	3.48 ± 0.14	***	8.55 ± 0.60	***
AZ-13	2.87 ± 0.17		1.20 ± 0.04	***	1.78 ± 0.10	***	5.86 ± 0.32	
AZ-14	2.00 ± 0.05	***	1.69 ± 0.03	***	3.04 ± 0.14	***	6.74 ± 0.16	**
AZ-15	2.66 ± 0.08	*	2.42 ± 0.06		3.81 ± 0.13	***	8.90 ± 0.27	***
AZ-16	2.15 ± 0.11	***	1.98 ± 0.07	***	3.00 ± 0.17	***	7.14 ± 0.32	***
AZ-17	0.99 ± 0.07	***	1.18 ± 0.06	***	2.24 ± 0.05	***	4.42 ± 0.18	**
AZ-18	1.71 ± 0.17	***	1.49 ± 0.10	***	2.02 ± 0.05	***	5.23 ± 0.23	
AZ-19	2.10 ± 0.11	***	1.96 ± 0.06	***	2.29 ± 0.10	***	6.35 ± 0.25	*
AZ-20	1.60 ± 0.08	***	2.42 ± 0.16		2.69 ± 0.14	***	6.72 ± 0.38	**
AZ-21	1.86 ± 0.07	***	2.02 ± 0.04	***	2.91 ± 0.03	***	6.80 ± 0.10	**
AZ-22	1.66 ± 0.14	***	1.64 ± 0.16	***	2.72 ± 0.29	***	6.03 ± 0.59	
AZ-23	1.84 ± 0.04	***	1.33 ± 0.15	***	2.15 ± 0.16	***	5.33 ± 0.32	
AZ-24	1.83 ± 0.06	***	1.37 ± 0.08	***	2.23 ± 0.27	***	5.45 ± 0.35	
AZ-25	3.94 ± 0.15	***	2.95 ± 0.12	***	4.42 ± 0.30	***	11.32 ± 0.53	***
AZ-26	2.61 ± 0.17	**	2.17 ± 0.13	***	4.62 ± 0.31	***	9.42 ± 0.62	***
AZ-27	2.23 ± 0.04	***	2.10 ± 0.02	***	3.80 ± 0.03	***	8.14 ± 0.05	***
AZ-29	3.54 ± 0.36	***	2.40 ± 0.26		4.53 ± 0.15	***	10.48 ± 0.78	***
AZ-30	3.18 ± 0.22		2.11 ± 0.09	***	3.99 ± 0.25	***	9.29 ± 0.57	***
AZ-31	1.63 ± 0.04	***	1.51 ± 0.08	***	2.05 ± 0.07	***	5.20 ± 0.18	
AZ-32	1.76 ± 0.17	***	1.51 ± 0.08	***	2.18 ± 0.13	***	5.46 ± 0.35	
AZ-33	1.98 ± 0.11	***	1.79 ± 0.04	***	2.08 ± 0.02	***	5.85 ± 0.13	
AZ-34	2.64 ± 0.21	**	2.09 ± 0.15	***	3.37 ± 0.42	***	8.11 ± 0.79	***
AZ-35	2.63 ± 0.03	**	2.12 ± 0.01	***	3.32 ± 0.11	***	8.09 ± 0.14	***
AZ-36	2.25 ± 0.22	***	1.75 ± 0.08	***	2.94 ± 0.29	***	6.95 ± 0.60	***
AZ-37	2.12 ± 0.07	***	1.92 ± 0.06	***	2.86 ± 0.04	***	6.91 ± 0.12	***
AZ-38	2.28 ± 0.07	***	2.53 ± 0.04		ND		4.82 ± 0.11	.
AZ-39	2.05 ± 0.01	***	2.03 ± 0.05	***	2.76 ± 0.07	***	6.85 ± 0.11	**
AZ-40	2.33 ± 0.06	***	2.68 ± 0.08	***	ND		5.01 ± 0.12	

AZ-41	2.05 ± 0.19	***	1.63 ± 0.05		2.45 ± 0.14	***	6.15 ± 0.36	
AZ-42	1.61 ± 0.06	***	1.44 ± 0.05	***	2.32 ± 0.17	***	5.38 ± 0.29	
AZ-43	2.29 ± 0.22	***	2.22 ± 0.18	***	ND		4.52 ± 0.41	**
AZ-44	1.88 ± 0.05	***	1.70 ± 0.07	**	2.76 ± 0.05	***	6.35 ± 0.18	*
AZ-45	1.76 ± 0.12	***	1.45 ± 0.08	***	2.38 ± 0.14	***	5.59 ± 0.35	
AZ-46	2.30 ± 0.04	***	3.38 ± 0.08	***	4.00 ± 0.09	***	9.69 ± 0.22	***
AZ-47	2.10 ± 0.30	***	1.73 ± 0.12	***	2.88 ± 0.12	***	6.72 ± 0.44	**
AZ-48	1.06 ± 0.01	***	2.65 ± 0.07	***	2.78 ± 0.07	***	6.49 ± 0.16	*
AZ-49	1.10 ± 0.01	***	2.89 ± 0.21	**	2.89 ± 0.36	***	6.88 ± 0.57	***
AZ-50	2.01 ± 0.09	***	1.87 ± 0.05		3.25 ± 0.06	***	7.14 ± 0.18	***
AZ-51	3.76 ± 0.88	***	3.00 ± 0.69	***	4.83 ± 1.22	***	11.60 ± 2.80	***
AZ-52	4.49 ± 0.06	***	3.24 ± 0.02	***	5.51 ± 0.08	***	13.25 ± 0.12	***
AZ-53	1.39 ± 0.04	***	1.55 ± 0.09	***	2.24 ± 0.06	***	5.19 ± 0.17	
AZ-54	1.65 ± 0.09	***	1.43 ± 0.04	***	2.08 ± 0.04	***	5.16 ± 0.18	
AZ-55	1.64 ± 0.06	***	1.79 ± 0.06	***	2.18 ± 0.05	***	5.61 ± 0.15	
AZ-56	1.31 ± 0.05	***	1.96 ± 0.09	***	2.24 ± 0.12	***	5.51 ± 0.25	
AZ-57	1.88 ± 0.03	***	2.04 ± 0.15	***	ND		3.92 ± 0.18	***
AZ-58	1.96 ± 0.01	***	1.75 ± 0.02	***	1.91 ± 0.05	***	5.63 ± 0.06	
AZ-59	1.89 ± 0.09	***	1.92 ± 0.04	***	2.44 ± 0.03	***	6.25 ± 0.11	·
AZ-60	2.03 ± 0.03	***	1.66 ± 0.04	***	2.72 ± 0.04	***	6.42 ± 0.10	*
AZ-61	2.34 ± 0.13	***	1.68 ± 0.09	***	2.32 ± 0.08	***	6.35 ± 0.25	*
AZ-62	1.66 ± 0.04	***	1.85 ± 0.07	***	1.83 ± 0.06	***	5.35 ± 0.17	
AZ-63	2.57 ± 0.02	**	1.70 ± 0.05	***	2.79 ± 0.03	***	7.06 ± 0.06	***
AZ-64	NA		NA		NA		NA	
AZ-65	2.42 ± 0.18	***	1.99 ± 0.17	***	2.45 ± 0.16	***	6.87 ± 0.51	***
AZ-66	1.77 ± 0.07	***	1.69 ± 0.05	***	2.35 ± 0.11	***	5.82 ± 0.23	
AZ-67	3.22 ± 0.51		1.80 ± 0.18	***	3.25 ± 0.43	***	8.28 ± 1.12	***
AZ-68	2.99 ± 0.10		1.92 ± 0.03	***	2.94 ± 0.08	***	7.85 ± 0.15	***
AZ-69	1.61 ± 0.02	***	1.36 ± 0.03	***	2.42 ± 0.14	***	5.40 ± 0.20	
AZ-70	1.26 ± 0.07	***	1.32 ± 0.05	***	2.35 ± 0.04	***	4.94 ± 0.16	
AZ-71	2.45 ± 0.06	***	1.54 ± 0.02	***	2.55 ± 0.04	***	6.54 ± 0.12	*
AZ-72	2.37 ± 0.09	***	1.52 ± 0.02	***	2.60 ± 0.07	***	6.50 ± 0.16	*
AZ-73	1.86 ± 0.11	***	1.34 ± 0.05	***	2.26 ± 0.03	***	5.47 ± 0.16	
AZ-74	2.05 ± 0.10	***	3.10 ± 0.16	***	ND		5.16 ± 0.26	
AZ-76	2.07 ± 0.14	***	3.30 ± 0.29	***	0.85 ± 0.00	***	6.24 ± 0.43	·
AZ-77	1.88 ± 0.15	***	1.30 ± 0.07	***	2.15 ± 0.05	***	5.34 ± 0.26	
AZ-78	1.53 ± 0.11	***	1.23 ± 0.04	***	1.73 ± 0.03	***	4.50 ± 0.19	**
AZ-79	0.96 ± 0.21	***	0.97 ± 0.21	***	2.79 ± 1.35	***	4.73 ± 1.78	*
AZ-80	2.42 ± 0.24	***	1.82 ± 0.11	***	2.65 ± 0.15	***	6.90 ± 0.51	***
AZ-81	2.60 ± 0.28	**	1.87 ± 0.14	***	2.25 ± 0.09	***	6.73 ± 0.52	**
AZ-82	1.06 ± 0.00	***	1.39 ± 0.02	***	1.90 ± 0.06	***	4.36 ± 0.08	**
AZ-83	1.54 ± 0.06	***	1.50 ± 0.11	***	2.57 ± 0.32	***	5.62 ± 0.49	
AZ-84	1.48 ± 0.19	***	1.20 ± 0.18	***	2.13 ± 0.54	***	4.82 ± 0.92	·
AZ-85	0.99 ± 0.05	***	1.46 ± 0.06	***	2.65 ± 0.10	***	5.12 ± 0.20	
AZ-86	1.02 ± 0.02	***	1.02 ± 0.01	***	2.69 ± 0.09	***	4.74 ± 0.13	*

AZ-87	0.78 ± 0.04	***	1.08 ± 0.00	***	3.16 ± 0.16	***	5.03 ± 0.12	
AZ-88	1.87 ± 0.02	***	2.18 ± 0.05	***	3.64 ± 0.17	***	7.70 ± 0.24	***
AZ-89	1.84 ± 0.24	***	1.72 ± 0.23	***	2.76 ± 0.44	***	6.33 ± 0.92	·
AZ-91	1.78 ± 0.04	***	1.12 ± 0.04	***	2.45 ± 0.09	***	5.35 ± 0.16	
AZ-92	NA		NA		NA		NA	
AZ-93	1.92 ± 0.03	***	1.10 ± 0.02	***	3.04 ± 0.16	***	6.07 ± 0.20	
AZ-94	2.46 ± 0.07	***	1.45 ± 0.02	***	1.89 ± 0.06	***	5.81 ± 0.11	
AZ-95	2.53 ± 0.07	***	0.88 ± 0.00	***	2.20 ± 0.08	***	5.63 ± 0.06	
AZ-96	2.91 ± 0.22		0.88 ± 0.02	***	2.13 ± 0.04	***	5.93 ± 0.29	
AZ-97	1.00 ± 0.05	***	1.31 ± 0.05	***	3.22 ± 0.11	***	5.54 ± 0.22	
AZ-98	1.56 ± 0.18	***	1.79 ± 0.17	***	3.43 ± 0.21	***	6.79 ± 0.50	
AZ-99	1.39 ± 0.25	***	1.03 ± 0.12	***	1.91 ± 0.36	***	4.33 ± 0.74	
AZ-100	NA		NA		NA		NA	
AZ-101	1.43 ± 0.25	***	1.26 ± 0.18	***	1.08 ± 0.10	***	3.78 ± 0.54	***
AZ-102	0.99 ± 0.11	***	0.89 ± 0.05	***	0.97 ± 0.04	***	2.85 ± 0.21	***
AZ-103	1.56 ± 0.20	***	0.95 ± 0.06	***	1.57 ± 0.17	***	4.08 ± 0.43	***
AZ-104	2.96 ± 0.35		1.94 ± 0.17	***	2.78 ± 0.30	***	7.69 ± 0.83	***
AZ-105	1.78 ± 0.37	***	2.04 ± 0.51	***	1.47 ± 0.27	***	5.30 ± 1.16	
AZ-107	2.69 ± 0.13	*	1.84 ± 0.05	***	3.05 ± 0.22	***	7.59 ± 0.40	***
AZ-108	2.80 ± 0.10		2.05 ± 0.04	***	2.81 ± 0.12	***	7.67 ± 0.25	***
AZ-110	2.37 ± 0.28	***	0.91 ± 0.06	***	3.06 ± 0.13	***	6.34 ± 0.46	*
AZ-111	3.84 ± 0.32	***	1.86 ± 0.13	***	2.66 ± 0.23	***	8.37 ± 0.68	***
AZ-112	2.47 ± 0.05	***	0.76 ± 0.01	***	2.11 ± 0.05	***	5.35 ± 0.12	
AZ-113	3.39 ± 0.14	**	1.79 ± 0.04	***	3.16 ± 0.17	***	8.35 ± 0.30	***
AZ-114	3.28 ± 0.13	*	1.95 ± 0.04	***	2.81 ± 0.08	***	8.05 ± 0.25	***
AZ-115	2.07 ± 0.12	***	2.52 ± 0.15		4.79 ± 0.39	***	9.39 ± 0.66	***
AZ-129	5.10 ± 0.08	***	3.68 ± 0.15	***	5.21 ± 0.27	***	14.00 ± 0.50	***
Cq-1	2.13 ± 0.24	*	2.28 ± 0.27		2.07 ± 0.25	***	6.49 ± 0.76	*
Cq-2	3.96 ± 0.02	*	3.31 ± 0.03	**	7.76 ± 0.11	***	15.04 ± 0.17	***
Cq-3	0.12 ± 0.00	***	0.05 ± 0.00	***	0.04 ± 0.00		0.22 ± 0.006	***

Table S3. **Tukey's – HSD multiple comparisons for saponins content.** Multiple comparison was carried out for saponins content to evaluate the significant differences. Different letters, small and capital, and letter combinations indicate significant differences among the *C. quinoa* genotypes. SEM is the standard error of the mean.

Quinoa line	Mean	SEM	Group
Cq-2	15.04301	0.086232	a
AZ-129	14.00729	0.252109	ab
AZ-52	13.25356	0.061921	ab
AZ-3	12.32097	0.188044	abc
AZ-11	11.80002	0.218069	abcd
AZ-51	11.60049	1.400407	abcde
AZ-25	11.32823	0.267662	abcde
AZ-29	10.48916	0.390508	bcdef
AZ-7	10.47728	0.617656	bcdefg
AZ-46	9.69409	0.112231	cdefgh
AZ-26	9.420545	0.310204	cdefghi
AZ-115	9.395139	0.332736	cdefghij
AZ-30	9.291495	0.285172	cdefghijk
AZ-4	9.249942	0.080017	cdefghijkl
AZ-15	8.906645	0.138964	defghijklm
AZ-12	8.551772	0.30433	efghijklmn
AZ-111	8.376476	0.340058	fghijklmno
AZ-113	8.357189	0.150995	fghijklmno
AZ-67	8.289419	0.564337	fghijklmnop
AZ-27	8.143056	0.025349	fghijklmnopq
AZ-34	8.111276	0.394906	fghijklmnopqr
AZ-35	8.093954	0.074104	fghijklmnopqr
AZ-114	8.053523	0.127838	fghijklmnopqr
AZ-68	7.859489	0.077819	fghijklmnopqrs
AZ-9	7.794394	0.274427	fghijklmnopqrs
AZ-88	7.706565	0.122289	ghijklmnopqrst
AZ-104	7.696932	0.418045	hijklmnopqrstu
AZ-108	7.67239	0.12883	hijklmnopqrstu
AZ-107	7.593334	0.201926	hijklmnopqrstuv
AZ-8	7.417461	0.840902	hijklmnopqrstuvw
AZ-50	7.144627	0.092807	hijklmnopqrstuvwx
AZ-16	7.143619	0.164276	hijklmnopqrstuvwx
AZ-63	7.06801	0.032752	ijklmnopqrstuvwxy
AZ-36	6.95475	0.300665	ijklmnopqrstuvwxyz
AZ-37	6.911229	0.060711	jklmnopqrstuvwxyzA
AZ-80	6.908392	0.255224	jklmnopqrstuvwxyzA
AZ-49	6.88429	0.285424	klmnopqrstuvwxyzA
AZ-65	6.87419	0.255177	klmnopqrstuvwxyzA
AZ-39	6.854671	0.05834	klmnopqrstuvwxyzA
AZ-21	6.803673	0.051158	lmnopqrstuvwxyzAB
AZ-98	6.792033	0.253215	mnopqrstuvwxyzAB

AZ-14	6.7416	0.083743	mnopqrstuvwxyzAB
AZ-81	6.732861	0.259839	mnopqrstuvwxyzAB
AZ-20	6.722011	0.194301	mnopqrstuvwxyzAB
AZ-47	6.719652	0.224148	mnopqrstuvwxyzAB
AZ-71	6.547472	0.063668	mnopqrstuvwxyzABC
AZ-72	6.503511	0.081821	nopqrstuvwxyzABC
AZ-48	6.496443	0.084155	nopqrstuvwxyzABC
Cq-1	6.496403	0.382012	nopqrstuvwxyzABC
AZ-60	6.425009	0.050915	nopqrstuvwxyzABC
AZ-19	6.358073	0.128722	nopqrstuvwxyzABC
AZ-61	6.355868	0.124868	nopqrstuvwxyzABC
AZ-44	6.353282	0.029387	nopqrstuvwxyzABC
AZ-110	6.347593	0.229898	nopqrstuvwxyzABC
AZ-89	6.335284	0.46285	nopqrstuvwxyzABC
AZ-59	6.256078	0.059655	nopqrstuvwxyzABCD
AZ-76	6.240691	0.219323	opqrstuvwxyzABCDE
AZ-41	6.153331	0.181129	opqrstuvwxyzABCDE
AZ-93	6.070646	0.103154	pqrstuvwxyzABCDEF
AZ-22	6.031523	0.295852	qrstuvwxyzABCDEFG
AZ-96	5.931692	0.147417	rstuvwxyzABCDEFGH
AZ-13	5.867038	0.160573	stuvwxyzABCDEFGH
AZ-33	5.858072	0.068394	stuvwxyzABCDEFGH
AZ-66	5.820114	0.117219	stuvwxyzABCDEFGH
AZ-94	5.818776	0.055331	stuvwxyzABCDEFGH
AZ-58	5.63775	0.03063	tuvwxyzABCDEFGHI
AZ-95	5.629688	0.034352	tuvwxyzABCDEFGHI
AZ-83	5.623982	0.249076	uvwxyzABCDEFGHI
AZ-55	5.619375	0.07892	uvwxyzABCDEFGHI
AZ-45	5.594212	0.178042	vwxyzABCDEFGHI
AZ-1	5.564102	0.120019	vwxyzABCDEFGHI
AZ-6	5.548225	0.473392	wxyzABCDEFGHI
AZ-97	5.542161	0.110766	vwxyzABCDEFGHI
AZ-56	5.517579	0.126249	wxyzABCDEFGHI
AZ-73	5.472562	0.080749	wxyzABCDEFGHIJ
AZ-32	5.464922	0.179211	wxyzABCDEFGHIJ
AZ-24	5.45157	0.177634	wxyzABCDEFGHIJ
AZ-5	5.413386	0.36931	wxyzABCDEFGHIJ
AZ-69	5.404513	0.103478	wxyzABCDEFGHIJ
AZ-42	5.380049	0.145934	wxyzABCDEFGHIJ
AZ-112	5.358347	0.062768	wxyzABCDEFGHIJ
AZ-62	5.358176	0.086516	wxyzABCDEFGHIJ
AZ-91	5.357895	0.082795	wxyzABCDEFGHIJ
AZ-77	5.341052	0.133745	wxyzABCDEFGHIJ
AZ-23	5.333431	0.164121	wxyzABCDEFGHIJ
AZ-105	5.308893	0.580436	xyzABCDEFGHIJK

AZ-18	5.237719	0.116556	xyzABCDEFGHIJK
AZ-31	5.208501	0.092832	xyzABCDEFGHIJK
AZ-53	5.196108	0.088562	xyzABCDEFGHIJK
AZ-54	5.167534	0.092352	yzABCDEFGHIJK
AZ-74	5.166952	0.134722	yzABCDEFGHIJK
AZ-85	5.123988	0.102174	zABCDEFGHIJK
AZ-87	5.038159	0.062303	zABCDEFGHIJK
AZ-40	5.019057	0.062891	ABCDEFGHIJK
AZ-70	4.940577	0.084208	BCDEFGHIJK
AZ-84	4.825833	0.461347	CDEFGHIJK
AZ-38	4.819712	0.055706	CDEFGHIJK
AZ-10	4.7871	0.179095	CDEFGHIJK
AZ-86	4.745504	0.068888	CDEFGHIJK
AZ-79	4.73064	0.892351	DEFGHIJK
AZ-43	4.525911	0.206006	EFGHIJK
AZ-78	4.503585	0.096793	EFGHIJK
AZ-17	4.426341	0.090418	FGHIJK
AZ-82	4.362027	0.041832	GHIJK
AZ-99	4.337887	0.372339	HIJK
AZ-103	4.088792	0.219066	IJK
AZ-57	3.92793	0.093483	JKL
AZ-101	3.781978	0.273797	KL
AZ-102	2.858134	0.10683	L
Cq-3	0.219794	0.003195	M

Table S4. Variance by genotypic effect. Variance explained by genotypic effect (Vg) was calculated according to the restricted maximum likelihood (REML) variance components. The table shows the significant genotypic effect represented by Vg, and the existence of genetic diversity is up to 94.5% within the tested population for saponin content. Mu and Sigma are the mean and the standard deviation of each variable. OA: oleanolic acid, HD: hederagenin, PA: phytolaccagenic acid.

Trait	Mu	Sigma	Min	Max	Vg
OA	2.184332	0.851878	0.119811	5.228009	95.526
HD	1.875622	0.690553	0.053093	4.182189	95.76
PA	2.626221	1.252596	0	7.874068	97.476
TS	6.686175	2.291514	0.214159	15.19523	94.561

Table S5. Phenolics content (mg/100 g) in *C. quinoa* seed. Table shows the free and bound fractions of phenolics and total phenolics content in *C. quinoa* seeds. Data are presented here as mean ± standard deviation values of independent sample extractions (n = 4). A one-way analysis of variance ANOVA was performed and p-value < 0.05 was considered as a statistically significant difference. A significant difference was found ($p < 0.001$) in relative phenolics content. Significant codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '

Quinoa line	Free phenolic fractions		Bound phenolics fractions		Total Phenolics	
AZ-1	53.29 ± 0.40	***	10.35 ± 0.39	***	63.64 ± 0.02	***
AZ-2	61.22 ± 0.56	***	10.79 ± 0.34		72.01 ± 0.22	***
AZ-3	46.64 ± 0.73	***	11.04 ± 0.21		57.68 ± 0.94	***
AZ-4	42.43 ± 0.41	***	11.48 ± 0.33		53.92 ± 0.73	***
AZ-5	54.23 ± 0.26		11.51 ± 0.31		65.74 ± 0.05	
AZ-6	51.58 ± 1.03		10.15 ± 0.46		61.73 ± 0.57	
AZ-7	60.29 ± 2.80	***	11.65 ± 0.33	.	71.94 ± 3.13	***
AZ-8	40.74 ± 2.17	***	9.03 ± 0.20	.	49.77 ± 2.37	***
AZ-9	43.98 ± 0.36	***	10.12 ± 0.11		54.10 ± 0.47	***
AZ-10	44.47 ± 1.57	***	9.51 ± 0.06		53.98 ± 1.63	*
AZ-11	40.50 ± 0.83	***	11.11 ± 0.22		51.61 ± 1.05	***
AZ-12	51.21 ± 2.52		9.98 ± 0.15		61.19 ± 2.67	
AZ-13	53.19 ± 0.98		22.37 ± 0.61	***	75.56 ± 1.59	***
AZ-14	49.76 ± 0.26	*	10.99 ± 0.08		60.76 ± 0.34	.
AZ-15	35.63 ± 1.15	***	11.47 ± 0.20		47.10 ± 1.36	***
AZ-16	44.55 ± 0.68	***	11.99 ± 0.49	*	56.54 ± 1.17	***
AZ-17	25.81 ± 0.88	***	15.15 ± 0.27	***	40.96 ± 0.61	***
AZ-18	23.83 ± 0.49	***	11.68 ± 0.50	.	35.51 ± 0.99	***
AZ-19	40.01 ± 0.97	***	11.39 ± 0.24		51.39 ± 0.73	***
AZ-20	35.26 ± 0.15	***	13.15 ± 0.33	***	48.41 ± 0.47	***
AZ-21	46.87 ± 0.00	***	11.89 ± 0.15	*	58.76 ± 0.14	***
AZ-22	45.54 ± 0.78	***	10.92 ± 0.11		56.45 ± 0.67	***
AZ-23	35.40 ± 0.10	***	9.72 ± 0.25		45.12 ± 0.15	***
AZ-24	43.84 ± 1.17	***	11.06 ± 0.47		54.91 ± 0.70	***
AZ-25	45.23 ± 0.31	***	10.39 ± 0.24		55.62 ± 0.55	***
AZ-26	47.37 ± 1.14	***	11.06 ± 0.57		58.43 ± 1.71	**
AZ-27	60.30 ± 3.14	***	14.92 ± 0.73	***	75.21 ± 2.41	***
AZ-29	50.30 ± 1.63	*	13.82 ± 0.79	***	64.11 ± 0.83	
AZ-30	60.06 ± 0.66	***	13.74 ± 0.93	***	73.80 ± 0.27	***
AZ-31	33.01 ± 1.26	***	10.69 ± 0.08		43.70 ± 1.34	***
AZ-32	39.91 ± 1.70	***	9.98 ± 1.26		49.89 ± 2.97	***
AZ-33	34.84 ± 0.04	***	14.58 ± 0.13	***	49.42 ± 0.09	***
AZ-34	54.43 ± 2.99		14.93 ± 0.79	***	69.37 ± 2.20	**
AZ-35	35.95 ± 0.63	***	15.79 ± 0.38	***	51.74 ± 1.01	***
AZ-36	35.53 ± 5.15	***	13.64 ± 0.62	***	49.18 ± 5.77	***
AZ-37	42.60 ± 0.57	***	14.05 ± 0.09	***	56.66 ± 0.66	***
AZ-38	45.82 ± 1.24	***	14.78 ± 0.19	***	60.60 ± 1.43	·
AZ-39	61.53 ± 1.76	***	16.53 ± 0.45	***	78.06 ± 2.21	**
AZ-40	39.63 ± 0.79	***	14.56 ± 0.80	***	54.18 ± 0.00	***

AZ-41	50.81 ± 1.82	.	15.73 ± 0.56	***	66.54 ± 2.38	·
AZ-42	49.94 ± 0.10	*	15.97 ± 0.65	***	65.90 ± 0.55	
AZ-43	31.44 ± 0.86	***	15.69 ± 0.23	***	47.13 ± 0.63	***
AZ-44	52.07 ± 1.56		15.00 ± 1.68	***	67.07 ± 3.24	*
AZ-45	44.64 ± 0.96	***	16.41 ± 0.06	***	61.06 ± 1.02	
AZ-46	43.55 ± 0.19	***	17.25 ± 0.26	***	60.81 ± 0.45	·
AZ-47	49.37 ± 4.02	**	15.89 ± 0.10	***	65.26 ± 3.92	
AZ-48	49.98 ± 2.08	*	15.58 ± 1.77	***	65.56 ± 3.84	
AZ-49	39.15 ± 0.43	***	14.46 ± 1.87	***	53.60 ± 1.45	***
AZ-50	42.12 ± 3.65	***	14.25 ± 0.04	***	56.37 ± 3.69	***
AZ-51	58.71 ± 2.12	***	13.25 ± 1.20	***	71.96 ± 3.31	***
AZ-52	40.88 ± 0.52	***	15.59 ± 0.61	***	56.47 ± 1.13	***
AZ-53	43.67 ± 0.29	***	18.19 ± 1.10	***	61.86 ± 0.80	
AZ-54	43.12 ± 0.65	***	15.12 ± 1.07	***	58.24 ± 1.73	**
AZ-55	42.35 ± 1.59	***	16.13 ± 1.25	***	58.48 ± 0.34	**
AZ-56	52.57 ± 1.34		15.70 ± 0.18	***	68.27 ± 1.16	**
AZ-57	36.96 ± 3.77	***	19.43 ± 0.62	***	56.39 ± 4.39	***
AZ-58	41.22 ± 0.52	***	15.23 ± 0.94	***	56.45 ± 0.42	***
AZ-59	39.80 ± 1.50	***	15.67 ± 0.02	***	55.47 ± 1.47	***
AZ-60	43.50 ± 1.78	***	15.14 ± 0.04	***	58.64 ± 1.81	**
AZ-61	44.55 ± 1.04	***	17.19 ± 0.03	***	61.73 ± 1.07	
AZ-62	29.90 ± 0.23	***	18.02 ± 0.29	***	47.92 ± 0.06	***
AZ-63	40.00 ± 0.67	***	15.98 ± 0.36	***	55.98 ± 0.31	***
AZ-64	35.02 ± 0.45	***	15.25 ± 0.40	***	50.27 ± 0.05	***
AZ-65	41.38 ± 0.25	***	16.83 ± 0.82	***	58.20 ± 1.07	***
AZ-66	46.39 ± 0.05	***	13.46 ± 1.50	***	59.85 ± 1.55	*
AZ-67	37.84 ± 0.48	***	14.86 ± 0.17	***	52.70 ± 0.30	***
AZ-68	35.12 ± 0.05	***	13.88 ± 0.13	**	49.00 ± 0.08	***
AZ-69	29.36 ± 0.74	***	12.88 ± 0.11	***	42.24 ± 0.85	***
AZ-70	36.33 ± 0.67	***	14.61 ± 0.26	***	50.95 ± 0.41	***
AZ-71	31.32 ± 0.59	***	14.14 ± 0.40	***	45.46 ± 0.19	***
AZ-72	27.45 ± 0.43	***	13.42 ± 0.01	***	40.87 ± 0.42	***
AZ-73	37.54 ± 1.22	***	17.85 ± 0.70	***	55.39 ± 1.92	***
AZ-74	39.83 ± 2.96	***	16.39 ± 0.14	***	56.21 ± 2.89	***
AZ-76	36.49 ± 0.01	***	16.13 ± 0.07	***	52.62 ± 0.09	***
AZ-77	33.12 ± 0.53	***	17.24 ± 0.31	***	50.36 ± 0.84	***
AZ-78	44.93 ± 0.97	***	16.87 ± 1.02	***	61.80 ± 1.99	
AZ-79	37.10 ± 0.53	***	15.74 ± 0.06	***	52.85 ± 0.60	***
AZ-80	28.47 ± 1.50	***	15.16 ± 0.10	***	43.63 ± 1.59	***
AZ-81	35.43 ± 0.65	***	16.48 ± 0.06	***	51.91 ± 0.70	***
AZ-82	34.57 ± 0.77	***	17.25 ± 0.49	***	51.82 ± 1.25	***
AZ-83	40.54 ± 0.54	***	17.81 ± 0.04	***	58.35 ± 0.50	**
AZ-84	26.38 ± 1.38	***	17.51 ± 0.25	***	43.89 ± 1.63	***
AZ-85	26.67 ± 0.08	***	16.57 ± 0.00	***	43.24 ± 0.08	***
AZ-86	37.27 ± 0.48	***	19.86 ± 0.05	***	57.13 ± 0.43	***

AZ-87	44.87 ± 0.74	***	18.60 ± 0.19	***	63.47 ± 0.55	
AZ-88	30.05 ± 0.25	***	15.42 ± 0.20	***	45.47 ± 0.45	***
AZ-89	38.26 ± 4.34	***	18.66 ± 0.13	***	56.93 ± 4.22	***
AZ-91	50.28 ± 0.47	*	19.46 ± 2.22	***	69.74 ± 2.69	***
AZ-92	NA	***	NA	***	NA	
AZ-93	41.22 ± 0.31	***	22.25 ± 1.28	***	63.47 ± 0.97	
AZ-94	57.77 ± 0.86	**	19.72 ± 0.33	***	77.49 ± 1.19	***
AZ-95	54.13 ± 0.63		33.05 ± 0.67	***	87.18 ± 0.05	***
AZ-96	60.84 ± 1.53	***	22.04 ± 0.54	***	82.89 ± 0.99	***
AZ-97	32.32 ± 1.58	***	21.43 ± 0.56	***	53.75 ± 2.14	***
AZ-98	26.03 ± 0.92	***	23.09 ± 1.47	***	49.12 ± 2.39	***
AZ-99	24.97 ± 0.41	***	18.53 ± 0.19	***	43.50 ± 0.22	***
AZ-100	NA	***	NA	***	NA	
AZ-101	38.10 ± 0.58	***	16.50 ± 0.29	***	54.60 ± 0.87	***
AZ-102	27.64 ± 0.02	***	15.40 ± 0.33	***	43.03 ± 0.35	***
AZ-103	24.00 ± 0.42	***	20.92 ± 0.26	***	44.92 ± 0.16	***
AZ-104	34.55 ± 0.73	***	20.13 ± 0.08	***	54.69 ± 0.81	***
AZ-105	39.35 ± 0.84	***	15.36 ± 1.02	***	54.71 ± 0.18	
AZ-107	36.68 ± 1.60	***	17.28 ± 1.47	***	53.96 ± 3.07	***
AZ-108	35.30 ± 0.24	***	17.48 ± 0.06	***	52.78 ± 0.19	***
AZ-110	56.65 ± 0.20	*	36.58 ± 3.10	***	93.23 ± 3.29	*
AZ-111	29.12 ± 0.37	***	15.41 ± 0.11	***	44.53 ± 0.48	***
AZ-112	48.02 ± 0.70	***	32.36 ± 2.12	***	80.38 ± 1.42	
AZ-113	31.31 ± 1.03	***	13.77 ± 0.04	***	45.09 ± 0.99	***
AZ-114	18.29 ± 0.11	***	18.13 ± 1.03	***	36.42 ± 1.14	***
AZ-115	62.27 ± 0.76	***	20.72 ± 0.42	***	82.99 ± 1.18	***
AZ-129	39.52 ± 0.32	***	27.28 ± 0.80	***	66.79 ± 1.12	·

Table S6. Tukey's – HSD multiple comparisons for phenolics content. Multiple comparison was carried out for phenolics content to evaluate the significant differences. Different letters, small and capital, and also letter combinations indicate significant differences among the *C. quinoa* genotypes.

Quinoa line	Mean of total phenolics	Group
AZ-110	93.22983	a
AZ-95	87.17887	b
AZ-115	82.9905	c
AZ-96	82.88524	c
AZ-112	80.37865	cd
AZ-39	78.06033	de
AZ-94	77.48487	de
AZ-13	75.55702	ef
AZ-27	75.21342	efg
AZ-30	73.80319	fg
AZ-2	72.01177	gh
AZ-51	71.956	gh
AZ-7	71.93701	gh
AZ-91	69.73767	hi
AZ-34	69.36536	hi
AZ-56	68.2674	ij
AZ-44	67.07299	ijk
AZ-129	66.79088	ijkl
AZ-41	66.53601	ijkl
AZ-42	65.90288	jkl
AZ-5	65.74014	jkl
AZ-48	65.56392	jkl
AZ-47	65.25482	jkl
AZ-29	64.11236	klm
AZ-1	63.64191	lmn
AZ-87	63.46929	lmn
AZ-93	63.46705	lmn
AZ-53	61.85943	mno
AZ-78	61.79592	mnop
AZ-61	61.73296	mnop
AZ-6	61.7266	mnopq
AZ-12	61.18842	mnopqr
AZ-45	61.05549	mnopqrs
AZ-46	60.80756	mnopqrs
AZ-14	60.75526	mnopqrs
AZ-38	60.60186	nopqrs
AZ-66	59.84657	opqrst
AZ-21	58.75804	opqrstu
AZ-60	58.63752	opqrstu
AZ-55	58.48027	opqrstu
AZ-26	58.42822	pqrstu

AZ-83	58.34946	qrstu
AZ-54	58.24188	rstuv
AZ-65	58.19942	rstuv
AZ-3	57.68249	stuvw
AZ-86	57.12863	tuvwx
AZ-89	56.9252	tuvwxy
AZ-37	56.65579	tuvwxy
AZ-16	56.54022	tuvwxy
AZ-52	56.47232	tuvwxy
AZ-22	56.45392	uvwxy
AZ-58	56.45309	uvwxy
AZ-57	56.39303	uvwxy
AZ-50	56.36905	uvwxy
AZ-74	56.21266	uvwxy
AZ-63	55.98056	uvwxyz
AZ-25	55.62015	uvwxyz
AZ-59	55.4715	uvwxyz
AZ-73	55.39096	uvwxyz
AZ-24	54.90791	vwxyzA
AZ-105	54.71002	wxyzAB
AZ-104	54.68513	wxyzAB
AZ-101	54.59629	wxyzAB
AZ-40	54.18043	xyzABC
AZ-9	54.09799	xyzABC
AZ-10	53.97499	xyzABC
AZ-107	53.95687	xyzABC
AZ-4	53.9167	xyzABC
AZ-97	53.75205	xyzABC
AZ-49	53.59938	yzABCD
AZ-79	52.84664	zABCDE
AZ-108	52.7747	zABCDEF
AZ-67	52.69803	zABCDEF
AZ-76	52.62313	zABCDEFG
AZ-81	51.90877	ABCDEFG
AZ-82	51.82193	ABCDEFG
AZ-35	51.74361	ABCDEFGH
AZ-11	51.60834	ABCDEFGH
AZ-19	51.38992	BCDEFGH
AZ-70	50.94574	CDEFGHI
AZ-77	50.35749	DEFGHIJ
AZ-64	50.27197	DEFGHIJ
AZ-32	49.88606	EFGHIJ
AZ-8	49.76812	EFGHIJ
AZ-33	49.41797	FGHIJ
AZ-36	49.17809	GHIJ

AZ-98	49.11803	GHIJ
AZ-68	48.99999	GHIJ
AZ-20	48.40739	HIJK
AZ-62	47.91763	IJKL
AZ-43	47.12669	JKLM
AZ-15	47.09704	JKLM
AZ-88	45.46851	KLMN
AZ-71	45.46205	KLMN
AZ-23	45.11911	KLMN
AZ-113	45.08812	KLMN
AZ-103	44.92076	LMN
AZ-111	44.53161	MN
AZ-84	43.88982	MNO
AZ-31	43.6996	NO
AZ-80	43.63056	NO
AZ-99	43.5028	NO
AZ-85	43.23764	NO
AZ-102	43.03049	NO
AZ-69	42.23851	NO
AZ-17	40.95686	O
AZ-72	40.86676	O
AZ-114	36.42125	P
AZ-18	35.51339	P

Table S7. Loading factors of variables in PCs in PCA. Principal component analysis (PCA) was carried out by considering both free and bound phenolics. The factor loadings on each component of the PCA show the contribution of variables to total phenolics variation among the studied population.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18	PC19
HA (free)	-0.03	0.10	-0.47	-0.18	-0.23	0.32	0.06	-0.41	0.40	-0.14	0.05	0.00	0.11	0.14	0.04	-0.45	0.01	0.02	0.01
VA (free)	-0.11	0.01	-0.43	0.20	-0.32	-0.24	0.36	0.01	-0.13	-0.05	-0.47	-0.17	-0.39	0.08	-0.08	0.18	0.02	0.05	0.02
SA (free)	0.11	0.02	-0.36	0.43	0.21	0.14	-0.26	0.30	0.08	-0.08	-0.38	0.05	0.51	-0.15	-0.08	0.00	0.00	0.00	0.00
CA (free)	0.07	0.03	-0.32	-0.53	0.24	-0.01	0.01	0.27	-0.06	-0.06	-0.11	0.17	0.00	0.16	0.58	0.24	0.04	0.09	0.03
FA (free)	0.04	0.05	-0.33	0.05	0.04	-0.76	-0.14	0.00	0.30	0.19	0.38	0.04	0.07	-0.07	-0.05	-0.01	0.02	0.04	0.01
Quercetin	-0.21	0.20	-0.26	0.13	0.21	0.19	-0.30	-0.17	-0.38	0.64	0.06	-0.07	-0.18	0.15	0.05	-0.10	0.00	0.01	0.00
Quer-C6-C6	-0.38	-0.02	0.16	-0.12	-0.09	0.08	-0.02	0.14	0.44	0.29	-0.09	-0.56	0.23	0.14	0.05	0.28	0.07	0.14	0.04
Quer-C6-A	-0.30	0.11	0.06	0.06	0.46	-0.10	0.33	-0.10	-0.05	-0.17	0.02	0.20	0.19	0.53	-0.29	-0.02	0.11	0.25	0.08
Quer-C5-C6	-0.33	-0.23	0.09	-0.10	-0.17	0.03	-0.22	0.23	0.21	0.16	-0.21	0.53	-0.24	-0.13	-0.14	-0.20	0.16	0.36	0.11
Quer-C6	-0.28	0.14	0.02	0.09	0.48	0.02	0.26	-0.26	0.20	-0.04	-0.09	-0.04	-0.18	-0.63	0.22	-0.02	0.01	0.02	0.01
Quer-C5-C6-C6	-0.31	0.19	-0.09	0.15	-0.21	0.05	-0.27	0.07	-0.29	-0.48	0.36	-0.18	0.00	-0.13	0.15	0.07	0.17	0.38	0.12
Free Phenolics	-0.44	0.03	-0.05	-0.02	-0.03	-0.02	-0.10	0.14	0.03	-0.15	0.04	0.11	-0.03	0.07	-0.01	0.02	-0.24	-0.27	-0.77
HA (bound)	-0.01	-0.41	-0.22	0.04	-0.01	0.24	-0.05	-0.37	0.05	0.02	0.20	0.24	0.03	-0.06	-0.16	0.67	0.08	-0.01	-0.03
VA (bound)	-0.10	-0.33	-0.12	0.19	-0.13	0.13	0.55	0.31	-0.15	0.25	0.34	0.05	0.23	-0.11	0.21	-0.20	0.22	-0.04	-0.08
SA (bound)	0.17	-0.27	-0.06	0.33	0.31	0.18	-0.10	0.26	0.34	-0.15	0.21	-0.18	-0.53	0.28	0.08	-0.07	0.02	0.00	-0.01

CA (bound)	0.00	-0.22	-0.25	-0.48	0.24	0.04	-0.02	0.19	-0.19	-0.08	0.06	-0.34	-0.06	-0.24	-0.55	-0.14	0.11	-0.02	-0.04
FA (bound)	-0.03	-0.42	0.12	0.03	0.08	-0.25	-0.23	-0.33	-0.13	-0.10	-0.26	-0.16	0.09	0.10	0.25	-0.19	0.54	-0.09	-0.19
Bound Phenolics	-0.05	-0.47	-0.01	0.02	0.06	-0.11	-0.02	-0.15	-0.15	-0.01	-0.05	-0.13	0.11	0.01	0.15	-0.15	-0.69	0.39	0.03
Total Phenolics	-0.41	-0.17	-0.05	-0.01	0.00	-0.07	-0.10	0.06	-0.04	-0.14	0.01	0.05	0.02	0.06	0.05	-0.05	-0.16	-0.63	0.57

Table S8. Variance by genotypic effect. Variance by genotypic effect (Vg) was calculated according to the restricted maximum likelihood (REML) variance components. The table shows the significant genotypic effect represented by Vg, and the existence of genetic diversity is up to 97.4% within the tested population for total phenolics content. Mu and Sigma are the mean and the standard deviation of each variable. HA: Hydroxybenzoic acid, VA: Vanillic acid, SA: Syringic acid, CA: Coumaric acid, FA: Ferulic acid, and Quer: Quercetin

Trait	Mu	sigma	Min	Max	Vg
HA (free)	0.592388	0.198319	0.198006	1.309528	94.254
VA (free)	2.107306	0.578495	0.967155	4.044008	97.344
SA (free)	0.074047	0.039782	0	0.263093	96.911
CA (free)	0.527335	1.040418	0.00281	9.90862	99.418
FA (free)	1.181418	0.423881	0.391423	2.727963	87.724
Quercetin	0.191008	0.113442	0.002923	0.515652	97.723
Quer-C6-C6	4.099636	1.646901	0.364768	11.49459	98.805
Quer-C6-A	3.236785	2.871954	0.196384	15.18767	98.547
Quer-C5-C6	7.971638	4.145332	1.706806	22.2887	98.91
Quer-C6	0.296524	0.268049	-0.00111	1.542875	99.033
Quer-C5-C6-C6	21.27081	4.373358	7.661806	32.14863	98.58
Free Phenolics	41.5489	9.72486	18.21315	62.81175	97.964
HA (bound)	1.292423	0.46281	0.5146	2.974102	95.429
VA (bound)	3.660862	1.331271	1.046432	7.632642	96.88
SA (bound)	0.161909	0.099703	0.015221	0.554955	98.038
CA (bound)	0.818578	0.64447	0.081537	4.796541	97.765
FA (bound)	9.833673	3.264481	5.138059	25.14741	98.37
Bound Phenolics	15.76745	4.590845	8.889862	38.76759	97.238
Total Phenolics	57.31634	10.88117	34.81036	95.55831	97.45

Table S9. Loading factors of variables in PCs in PCA. Principal component analysis (PCA) was carried out by using shoot- and root- related traits. The factor loadings on each component of the PCA show the contribution of variables to shoot-root trait variation among the studied *C. quinoa* accessions.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
SH	-0.243	-0.252	-0.118	0.098	-0.103	-0.473	0.163	-0.054	-0.545	0.456	0.066	0.003
SDM	-0.287	-0.181	0.028	0.101	0.001	-0.104	-0.068	-0.081	0.256	-0.322	0.199	0.070
SD	-0.151	0.210	-0.052	-0.706	-0.247	-0.350	0.051	-0.439	0.139	-0.095	-0.065	-0.009
LA	-0.288	-0.180	-0.071	0.016	-0.067	0.030	-0.129	0.171	0.105	0.034	-0.026	0.125
RDM	-0.295	-0.076	0.135	0.013	-0.034	0.051	-0.103	0.053	-0.456	-0.451	0.248	0.058
Root/Shoot	-0.223	0.180	0.394	-0.184	-0.077	0.297	-0.008	0.068	-0.245	0.182	0.013	-0.588
PRL	-0.229	0.108	-0.399	-0.087	0.0685	0.272	0.555	0.033	-0.230	-0.232	-0.055	0.238
LRL	-0.286	-0.159	-0.041	0.110	0.059	0.146	-0.275	-0.330	0.060	0.179	-0.230	0.227
TRL	-0.287	-0.152	-0.053	0.105	0.060	0.152	-0.251	-0.321	0.0513	0.168	-0.226	-0.055
RSD	-0.262	0.124	-0.234	-0.091	0.336	0.090	0.123	0.064	0.324	0.394	0.610	-0.080
RSW	-0.242	0.265	-0.024	-0.067	0.109	-0.460	-0.294	0.594	0.065	-0.016	-0.180	0.083
CHA	-0.284	0.068	-0.135	0.035	0.298	0.046	0.238	0.107	0.105	-0.075	-0.568	-0.294
LAR	0.092	-0.203	-0.530	-0.387	-0.190	0.329	-0.437	0.249	-0.155	0.015	0.015	-0.092
LMF	0.181	-0.407	0.001	-0.205	0.389	-0.139	0.044	-0.006	-0.033	-0.079	-0.023	-0.295
SMF	-0.050	0.393	-0.331	0.411	-0.448	-0.038	-0.053	-0.093	0.049	-0.022	0.039	-0.301
RMF	-0.227	0.171	0.399	-0.171	-0.074	0.268	-0.006	0.124	-0.006	0.154	-0.010	0.371
TPM	-0.291	-0.163	0.048	0.085	-0.005	-0.076	-0.075	-0.057	0.1268	-0.348	0.209	-0.299
RA/LA	0.085	0.467	-0.110	0.041	0.542	-0.036	-0.360	-0.295	-0.337	-0.102	0.079	0.055

Figure S1. Overview of the greenhouse experiment and phenotypic traits. *C. quinoa* germplasms were evaluated for agronomically important morphological and yield-related traits variation. plants from each line were monitored and measured for agr-morphological traits according to Sosa-Zuniga et al. (2017). A. seed germination (growth stage 0), B. stem and vegetative parts developments (growth stage 2-4), C. emergence of flower bud (growth stage 5), D. flowering (growth stage 6), E. fruit development and ripening (growth stage 7-8), F. Senescence (growth stage 9).

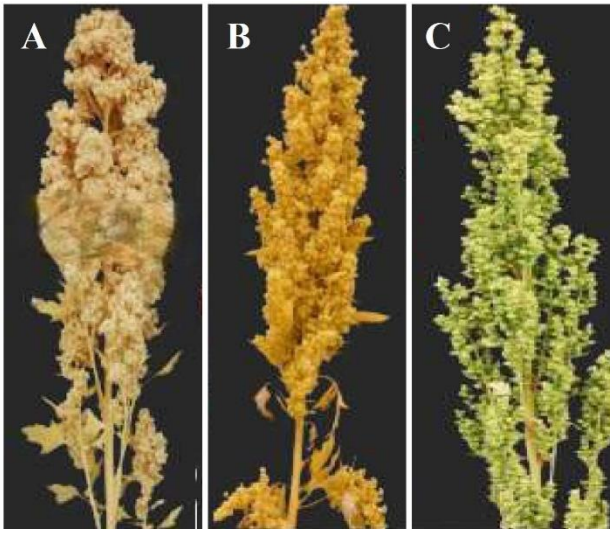

Figure S2. Shape and diversity of panicle structure. Three forms of panicle exist in *C. quinoa*. A. Glomerulated, B. Intermediate, and C. Amaranth- form.

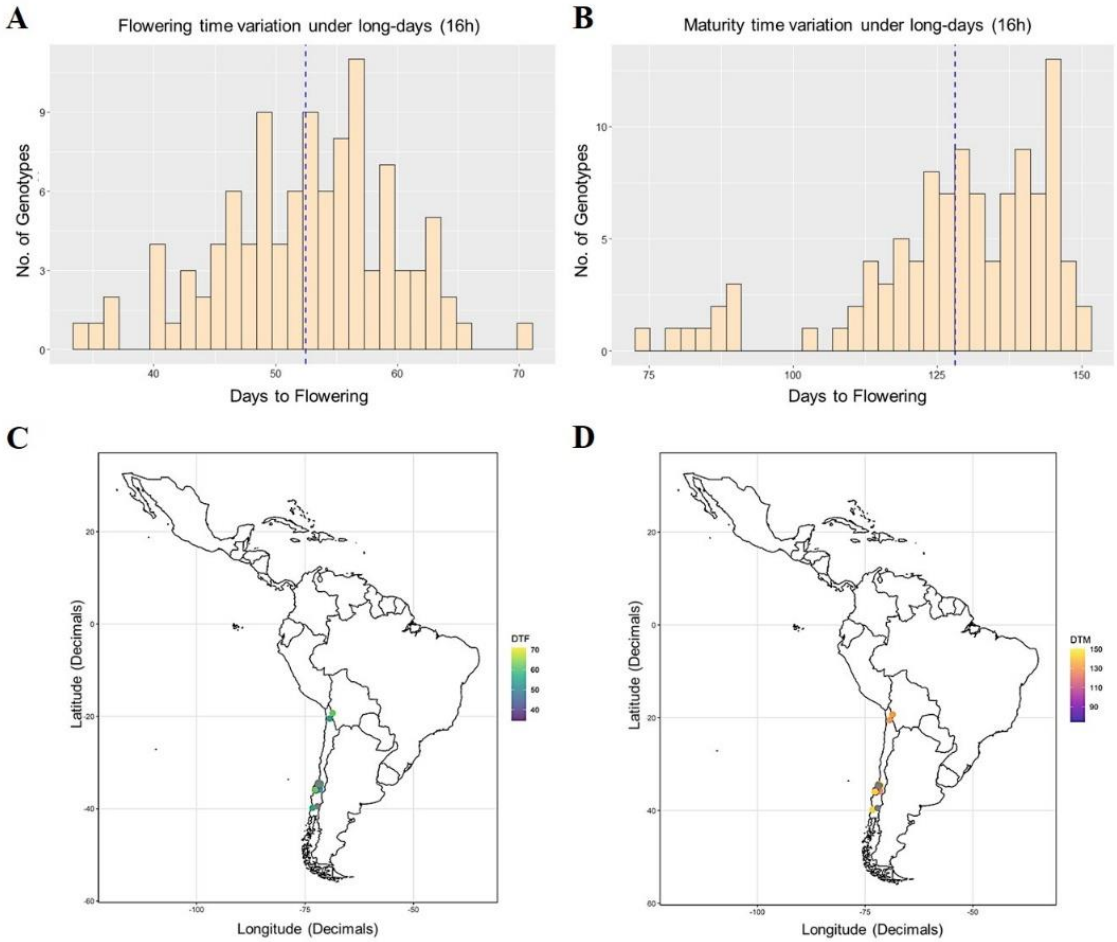

Figure S3. Flowering and maturity time variation and geograpgic origin of *C. quinoa* accession. and maturity time under long-days conditions (A and B), C and D shows the assignment of the *C. quinoa* accessions in relation to their origin and measured flowering time, as well as maturity time. Colored bars correspond to days to flowering and days to maturity under long-days. DTF: Days to flowering, DTM: Days to maturity.

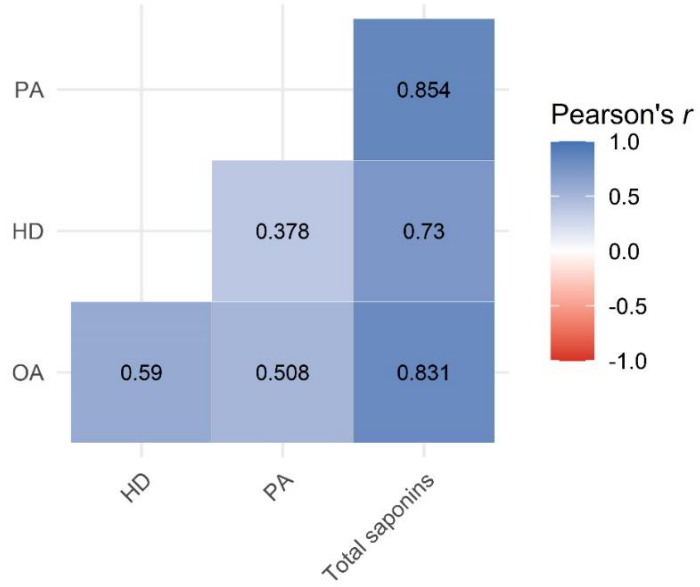

Figure S4 Pearson's correlation heat map of total saponins and individual sapogenins. Pearson's correlation matrix showing pairwise correlations between total saponins and individual sapogenins. The colored squares displays statistical significance and strength of the correlation apparent by color intensity. OA: oleanolic acid, HD: hederagenin, PA: phytolaccagenic acid.

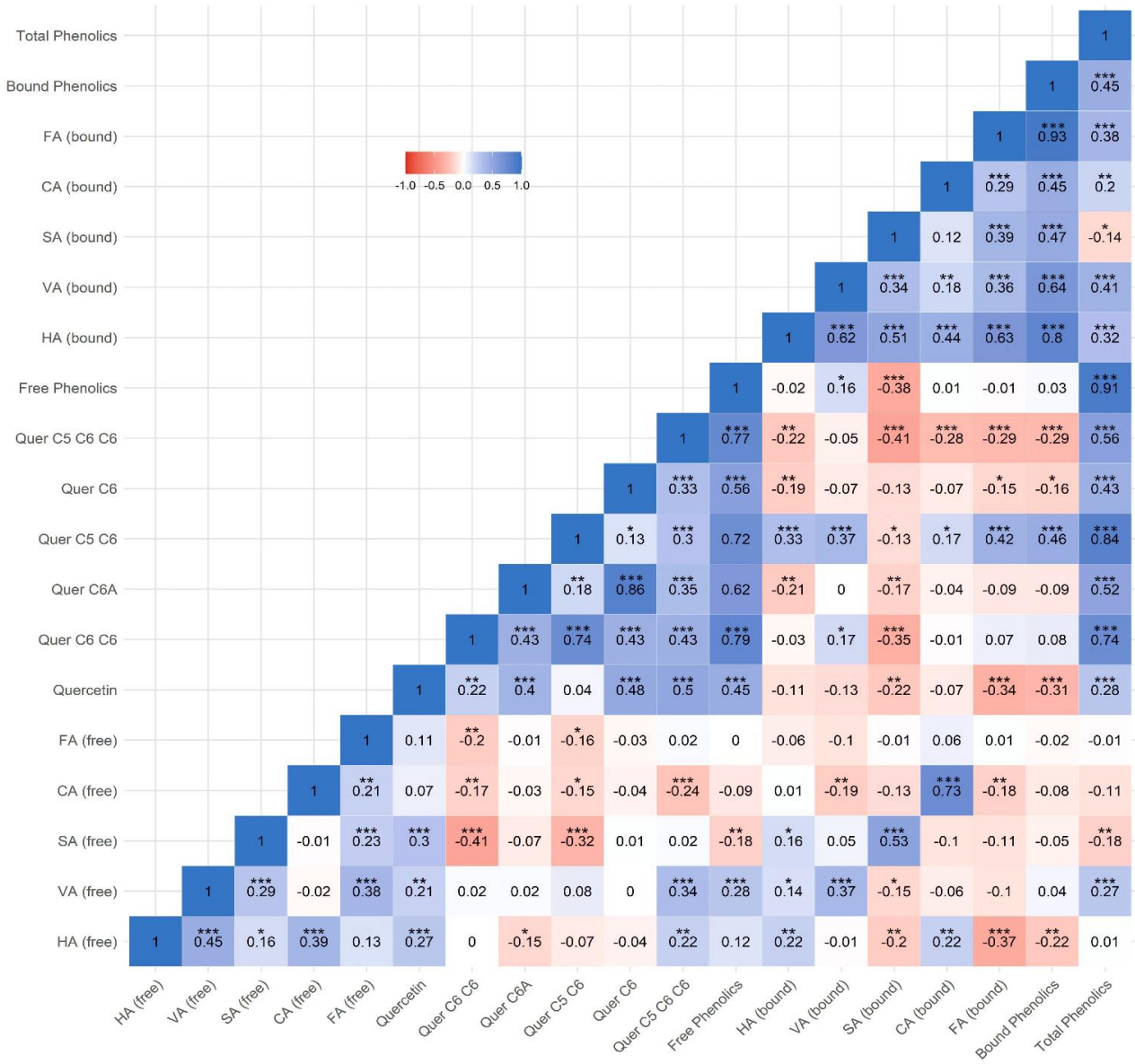

Figure S5. Pearson's correlation heat map of total phenolics and individual phenolic derivatives. Pearson's correlation matrix showing pairwise correlations between total phenolics and free and bound fractions of phenolics. The colored squares displays statistical significance and strength of the correlation apparent by color intensity. HA: Hydroxybenzoic acid, VA: Vanillic acid, SA: Syringic acid, CA: Coumaric acid, FA: Ferulic acid, and Quer: Quercetin.

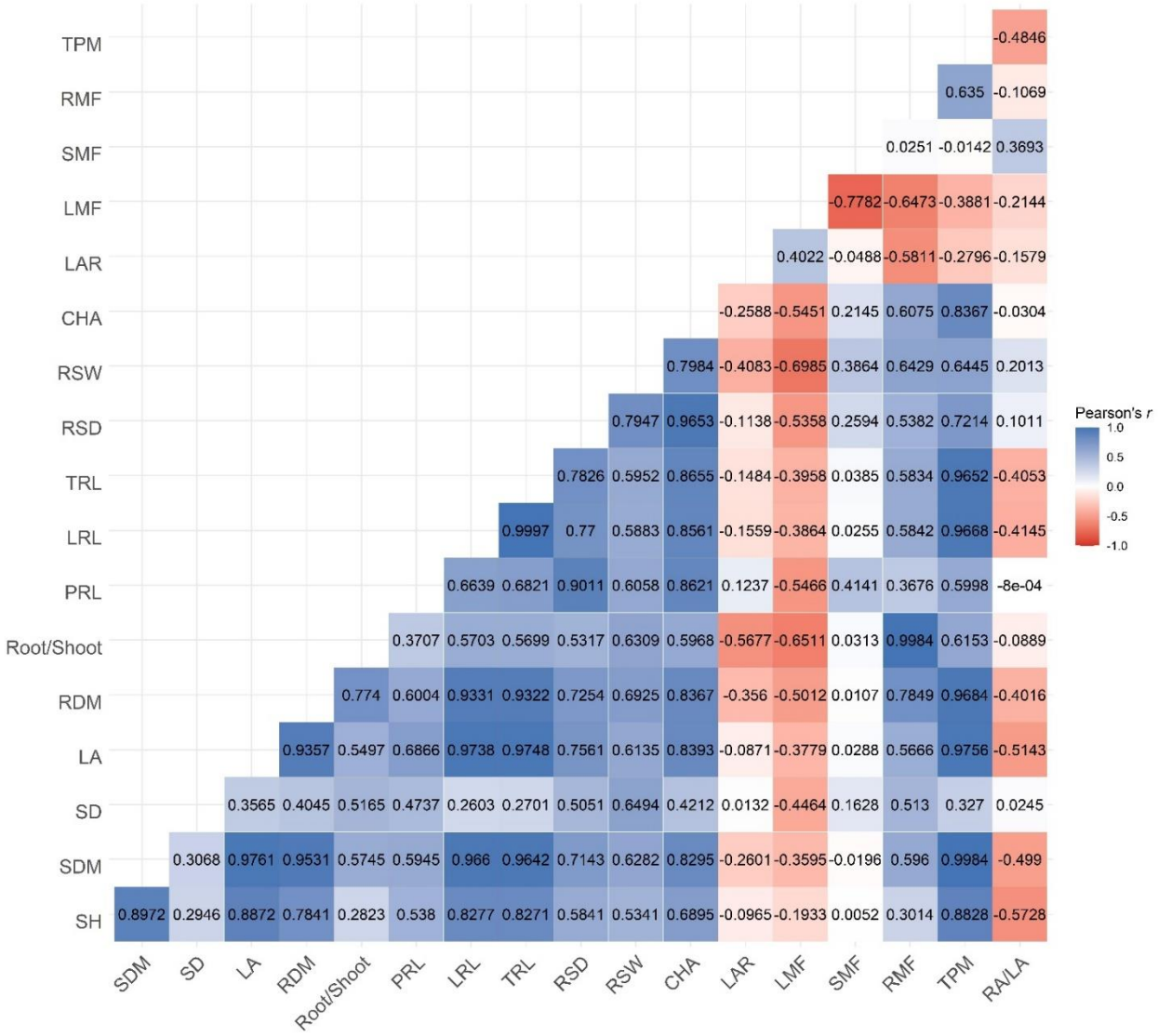

Figure S6. **Pearson's correlation heat map of the shoot- and root-related traits.** Pearson's correlation matrix shows pairwise correlations between root variables. The colored squares display statistical significance and the strength of the correlation is apparent by color intensity.

Publications, and Oral and Poster Presentations

Publications:

1) Pandya A, Thiele B, Zurita-Silva A, Usadel B, Fiorani FJA (2021). Determination and metabolite profiling of mixtures of triterpenoid saponins from seeds of Chilean quinoa (*Chenopodium quinoa*) germplasm. Agronomy 2021, 11 (9):1867 https://doi.org/10.3390/agronomy11091867

2) Pandya A, Thiele B, Köppchen S, Zurita-Silva A, Usadel B, Fiorani FJA. Characterization of bioactive phenolic compounds in seeds of Chilean quinoa (*Chenopodium quinoa*) germplasm. Agronomy 2023, 13(8), 2170; https://doi.org/10.3390/agronomy13082170

3) Pandya A, Siddiqui A, Sanmukh S, Khairnar K (2014) In-Silico Studies of Halophilic Archaeon DL31 Plasmids for Gene Annotation and Structure Prediction. IJERA 2014, 4 (1):01-12.

4) Siddiqui A, Ahmad M, Pandya A, Sanmukh S, Khairnar KJIJoCA (2014) Genome Annotation and Structure Predictions for Hypothetical Proteins in Agrobacterium Fabrum Str. C58 Plasmid At. IJCA 2014, 85 (1).

Oral and Poster presentations:

1) Archis Pandya, Andres Zurita-Silva, Fabio Fiorani, Björn Usadel, Björn Thiele. Determination and metabolite profiling of mixtures of triterpenoid saponins from Chilean quinoa (Chenopodium quinoa) germplasm. Plant 2030 status seminar. Annual meeting of plant research funded by the BMBF. March 2021.

2) Archis Pandya, Kathryn Dumschott, Björn Thiele, Andres Zurita, Nathalie Wuyts, Dalma Castillo, Christian Alfaro, Björn Usadel, Fabio Fiorani. Characterization and metabolite profiling of novel collection of quinoa germplasm for enhanced future breeding strategies. International Quinoa Research Symposium hosted by Washington state University. August 2020.

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Declaration

I, Archis K. Pandya, hereby declare that this thesis entitled ‘Genotype-dependent phenotypic variation for agronomic traits, seed composition, and root architecture of Chilean Quinoa (*Chenopodium quinoa* Willd.)’ submitted for the degree of Doctor of Philosophy (Ph.D.) is solely my own work, and has not been submitted in whole or in part for any other degree or diploma at any university or similar institution. I acknowledge the contributions of all individuals who provided assistance and support during the research and writing of this thesis. To the best of my knowledge, any sources of information used in this thesis have been duly acknowledged.

I also declare that this thesis has been written in accordance with the guidelines and regulations of the Forschungszentrum Jülich and the University of Bonn, and that all ethical considerations have been taken into account during the research process.

Archis K. Pandya