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Genetic Analysis of Quinoa Germplasms Reveal Genome Level Complexity

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ABSTRACT

The climate-resilient pseudocereal quinoa (Chenopodium quinoa Willd.) has enormous potential to enhance food and nutritional security in marginalized areas because of its nutritional richness, flexibility, and resistance to salinity and drought. The genetic diversity and molecular characterisation of eleven quinoa lines obtained from ICBA and other international institutes were investigated in this study. Polymorphism in 21 markers was found through genetic research using 40 InDel primers, which allowed accessions to be grouped into three groups according to characteristics including saponin concentration and yield potential. Important genetic resources were found to include saponin-free lines (Chen 254, D 11912, D 12406), genetically varied lines, and high-yielding lines (Co 407, BO 51). By establishing an indispensable molecular resource for quinoa breeding, our work speeds up the creation of sweet, high-yielding, and stress-tolerant varieties by facilitating marker-assisted selection (MAS). By combining genomics and traditional breeding techniques, quinoa will be able to spread into new agro-ecological zones, promoting sustainable agriculture and bettering public health, particularly in areas impacted by nutritional shortages and climate change.

Keywords: *Chenopodium quinoa, Molecular markers, Simple Sequence repeats.*

INTRODUCTION

Marginal and underdeveloped regions globally face challenges such as poor nutrition, low productivity, and economic vulnerability. Climate-resilient crops like quinoa offer promising solutions for enhancing agricultural sustainability in these environments. Native to the Andes, quinoa requires less water than conventional cereals and thrives in diverse Agro-climatic conditions (Zurita-Silva et al., 2014; Jacobsen, 2003; & Jacobsen et al., 2013).

Due to its outstanding nutritional profile—including high levels of protein, fiber, essential amino acids, vitamins, and minerals—quinoa has gained worldwide popularity in recent decades, making it a great option for health-conscious consumers and people with dietary limitations (Bhargava et al., 2006; James 2009; & Vega-Galvez et al., 2010). Quinoa is extremely beneficial for those who have celiac diseases or gluten intolerance because it is gluten-free and high in protein.

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Including all nine vital amino acids in its full protein profile (Repo-Carrasco et al., 2003; Vega-Galvez et al., 2010; Fuentes & Bhargava 2011; & Stikic et al., 2012). Quinoa, which was once farmed in Bolivia, Peru, and Chile, is now grown all over the world because of its flexibility. It is perfect for places with low annual precipitation (200 mm) and saline or poor soils. Its capacity to flourish in harsh conditions makes it a great plant for reclaiming deserted land and restoring productivity, thereby enhancing farmer livelihoods and food security in drought-prone regions (Mujica & Jacobsen 2006).

Through bioactive chemicals with antiviral, antibacterial, and anti-inflammatory effects, quinoa also improves public health. Quercetin, kaempferol, protocatechuic acid, vitexin, and other flavonoids have demonstrated the ability to strengthen immunity, which is particularly vital in the post-COVID age. The addition of flavonoid-rich foods like quinoa to diets may boost immunity and help prevent chronic illnesses (Lin et al., 2019).

Due to its low glycemic index, it has gained popularity as a health food, which has increased market demand. Quinoa seeds may be used in a variety of dishes, including baking, soups, cereals, and salads. Saponin, its main byproduct, is used in the chemical, pharmaceutical, and cosmetic industries. Quinoa has several advantages, but its growth is hampered by a small number of high-yielding varieties, the poor adaptability of traditional Andean cultivars in novel environments, and problems like seed cracking and bitter saponins in the seed coat.

These limitations are being addressed by current breeding techniques. Traditional breeding was centered on selection and crossing for characteristics such yield, disease resistance, and drought tolerance. However, the lengthy breeding cycles and intricate genome of quinoa provide difficulties. The quinoa genome sequence, which was made public in 2017, has helped newer techniques like genomic selection and marker-assisted selection, which can identify genes associated with important traits like nutritional content and stress tolerance, resulting in quicker and more accurate breeding (Jarvis et al., 2017).

Targeted and effective methods for enhancing quinoa characteristics like saponin concentration, drought tolerance, and disease resistance are provided by gene editing tools, particularly the CRISPR gene editing tool. The genetic modification of quinoa varieties still faces challenges in terms of public and regulatory approval. Quinoa is inherently resistant to drought and salinity, so breeding programs are concentrating on producing climate-resilient, sweet, and high-yielding types that can thrive in foreign climates, such as those found in Asia and Africa. In order to extend its cultivation into areas impacted by climate change, these initiatives are crucial. Breeding for enhanced seed quality—larger seed size, improved cooking characteristics, and lower saponin content—is also essential. Although saponins offer protection against pests, they must be eliminated before eating, which makes processing difficult. As a result, there is a great need for cultivars with low saponin content.

Public-private alliances are necessary to increase the output of superior quinoa cultivars in order to realize its full potential, particularly in low-income areas such the Middle East and North Africa, where quinoa can make a significant contribution to food and nutrition security. Seed companies, farmers, NGOs, and government institutions can all work together to multiply and distribute seeds in order to increase availability. Biochemical breeding is another avenue of investigation, focusing on identifying and improving the flavonoid level in quinoa in order to increase its health-promoting qualities. This aligns with contemporary nutritional objectives that take bioactive substances into account in addition to vitamins and minerals. These chemicals may be accurately measured using techniques like HPLC and GC-MS. For example, International Center for Biosaline Agriculture (ICBA) and the Max Planck Institute have utilized GC-MS

to make significant progress in saponin estimation.

Our continuous research on quinoa's adaptation to Asian settings includes: 1. Development of genomic tools to describe quinoa germplasm & 2. Genotypic description of a wide range of quinoa germplasm.

Quinoa breeding is changing to address nutritional and agricultural issues around the globe by utilizing traditional knowledge, contemporary genomics, and interdisciplinary teamwork. If innovation continues, quinoa may establish itself as a cornerstone of sustainable agriculture in harsh environments all over the world.

MATERIALS AND METHODS

Germplasm Materials

We have examined eleven distinct quinoa lines acquired from the ICBA in Dubai in order to investigate the possibilities of quinoa in severe growing conditions. Among these were two high-performing lines (ICBA-Q3 and ICBA-Q5), a purple-flowered variety from Colorado State University (Co-407), and BO-51, a line valued for its compact structure, vigor, and yield. Early-to-medium-maturing cultivars such as Chen-254, D12377 (with black seeds), D11912, D12401, and D12406 were popular for being saponin-free, which made them more palatable and less bitter. The Titicaca type was notable for its extremely early maturity. Before international biodiversity regulations went into effect, the majority of these materials were taken from the Andes.

Each of these quinoa lines exhibited unique features. For instance, D-12401 produced pink hermaphrodite blooms when cultivated in direct sunshine, whereas D-12377 was unusual in that it produced both brown and black seeds, suggesting that it may not be genetically stable. D-12047 was marked as inappropriate for further breeding efforts because it displayed evidence of segregation.

DNA Extraction

Using a modified-CTAB DNA extraction procedure, the we were able to identify the genetic basis of these characteristics and confirm the hybrid plants. They freeze-dried young leaves taken from 21-day-old seedlings rather than using liquid nitrogen. To isolate pure DNA, the powdered leaves were treated with particular chemicals and temperatures. Before being used in additional investigations, this DNA was then purified, stabilized, and subjected to a quality assessment (Cota-Sanchez et al., 2006).

Specific DNA fragments were amplified using the Polymerase Chain Reaction (PCR), a widely used technique in molecular biology. Targeted genes were replicated by cycling a precisely prepared mixture of DNA, primers, enzymes, and buffers through a series of heating and cooling phases. The procedures included an initial heating phase, alternating heating and cooling cycles for DNA replication, and a last stage to finish the process according to the Tenmykh protocol (Temnykh et al., 2000). The amplified DNA was tested using agarose gel electrophoresis to see the outcome as explained by Collard & Mackill (2009).

Diversity Analysis

Next, the DNA data was transformed into a binary format, where '1' represented the presence and '0' the absence of particular DNA bands. These patterns made it possible to pinpoint particular traits in particular kinds. Using a statistical technique called Jaccard's coefficient, the degree of genetic similarity between the lines was determined. The data were then used to create a family tree (dendrogram) that visually grouped similar types together through using the NTSYS-pc software (Version 2.02).

SSR Primer Resource Enrichment

In another aspect of the study, researchers looked for important molecular markers in quinoa's genome. They downloaded the entire genome sequence of the quinoa variety QQ74 and utilized specialized software to search for simple sequence repeats (SSRs), which are short, repeated DNA patterns frequently employed as genetic markers. Using an automated program called Primer3, primers, which are short DNA sequences that aid in the initiation of DNA replication, were designed to target these regions. To determine how

these markers might behave in quinoa and other related plant species, we conducted in silico PCR.

RESULTS

Genetic Analysis

We genotyped 11 quinoa accessions using 40 indel primers; 20 primers were polymorphic. Data were scored as binary (allele 1 = "1", allele 2 = "0", missing = "9"). A dendrogram grouped the accessions into three clusters: GP-I (High Yield): Co-407 and BO-51, GP-II (Diverse): D-12401, D-12377, D-12047, ICBA-Q5, Titicaca, ICBA-Q3 and GP-III (Saponin-Free): Chen-254, D-12406, D-11912. This clustering reflects both yield potential and consumer-friendly saponin traits (Figure 1-2).

SSR Motif Discovery

In the QQ74 genome, we found 587,245 SSRs (321,450 mono-; 31,438 di-; 41,220 tri-; 18,657 tetra-; 45,323 penta-; 16,785 hexa-nucleotide repeats). These included 3,558 unique motifs, plus 112,372 compound motifs (sizes 20–3,951 bp). A subset of 472 complementary SSRs ranged in frequency from 1 to 454,578 occurrences (Supplementary Table 1).

Primer Design and validation

A well-curated set of 496 SSR primer pairs has been developed to capture the rich diversity of microsatellite regions across the genome. From an initial 320,913 primer candidates, 496 high-quality primers were selected (GC 36–50%, Tm 57–59 °C, product size 250–300 bp) for downstream validation. These primers target different types of repeat motifs—including di-, tri-, tetra-, and hexa-nucleotide repeats—each carefully annotated with their sequence details, melting temperatures (Tm), GC content, and expected product sizes. Most primers exhibit Tm values between 57°C and 59°C with GC content ranging from about 36% to 50%, making them well-suited for consistent and efficient amplification. This primer collection provides a valuable resource for researchers working on genetic diversity studies, mapping, marker-assisted selection, and breeding programs, offering strong potential for high-resolution genotyping and genome-wide analysis (Figure 3).

SSR Marker validation

A total of 9 primers were amplified in the first round and some results were obtained. However, the results were not reproducible when repeated amplifications were carried out (Table 1, Figure 4).

DISCUSSION

In this study, 21 of the 40 InDel (Insertion-Deletion) primers tested revealed polymorphism, demonstrating a high level of genetic diversity between the quinoa genotypes examined (**Zhang et al., 2017**). This degree of polymorphism is essential for crop development because it offers a genetic basis for choosing and combining desirable features. Cluster analysis organized the genotypes into three distinct groups, each with distinct trait associations and breeding potential, revealing the genetic relationships.

Genotypes Co 407 and BO 51, which made up Group I, had certain alleles in common that were associated with superior yield potential. In breeding programs aimed at increasing productivity, particularly in areas where maximizing output is a top priority, these lines can be useful donor parents. The six genetically distinct lines that made up Group II reflected a wide range of genetic backgrounds. This kind of variety is quite advantageous for producing quinoa cultivars that are resistant to biotic and abiotic stressors like salinity, drought, and temperature extremes. Chen 254, D 12406, and D 11912 made up the third group, which was particularly remarkable for having seeds free of saponins. The lack of bitter saponins in these genotypes makes the grains acceptable for direct consumption without requiring significant post-harvest treatment, which is why they are highly desirable for their consumer-friendly features.

Along with the diversity study, a thorough genome-wide search for SSRs (Simple Sequence Repeats) was carried out, yielding the discovery of more than 500,000 SSR motifs in the quinoa genome. The outstanding genomic diversity and richness of quinoa, a crop well-known for its adaptability

and nutritional value, is emphasized by this wealth of SSRs. Despite the fact that many of these repeats are redundant or mononucleotide in nature, a sizable group of 3,558 distinct motifs—including compound, di-, tri-, and tetra-nucleotide repeats—was found. A panel of 496 primers with high SSR quality was selected from this pool. These primers are a powerful molecular toolbox for a variety of genetic applications, including evaluating genetic diversity, analyzing population structure, mapping key features, and supporting marker-assisted selection (MAS).

The following step is to experimentally validate these SSR primers using polymerase chain reaction (PCR) amplification and resolution via gel electrophoresis. The validated markers will then be used to genotype a larger and more varied group of quinoa germplasm. In addition to enhancing our knowledge of the genetic makeup of quinoa, this will make it possible to create comprehensive linkage maps and pinpoint markers that are strongly related to important agronomic characteristics, such as yield potential, stress tolerance, and low saponin concentration.

Combining these molecular methods with traditional breeding techniques provides a potent way to speed up genetic gains in quinoa. By meeting the dual goals of improving food security and encouraging climate-resilient agriculture, this integrated approach will aid in the development of better varieties that are high-yielding, nutritious, and able to thrive in marginal habitats. In particular, the discovery of stress-resilient and saponin-free varieties has great potential for broadening the production of quinoa to new agroecological areas and increasing the crop's appeal to both farmers and consumers.

Figures and Tables

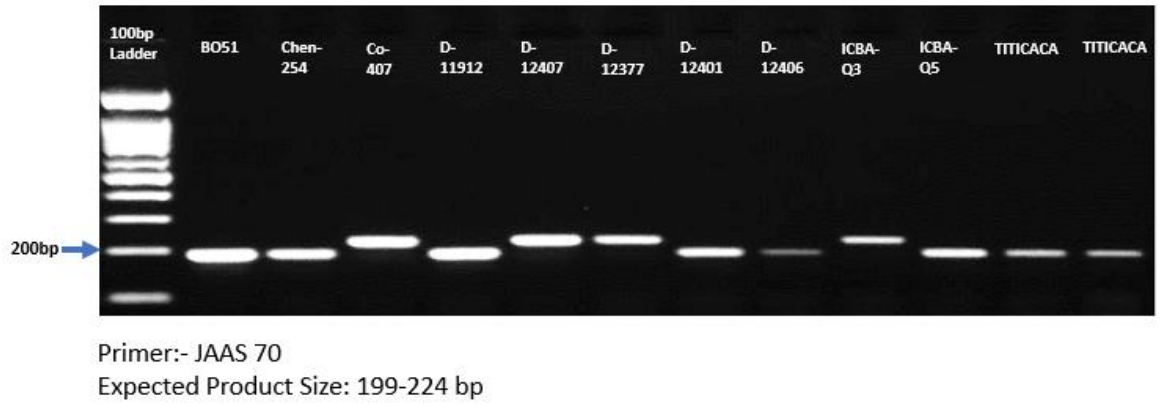

Figure 1: Figure presenting genotypic characterization of 11 lines using indel primer, JAAS70. The accession 'Titicaca' was kept twice as check in the last lane.

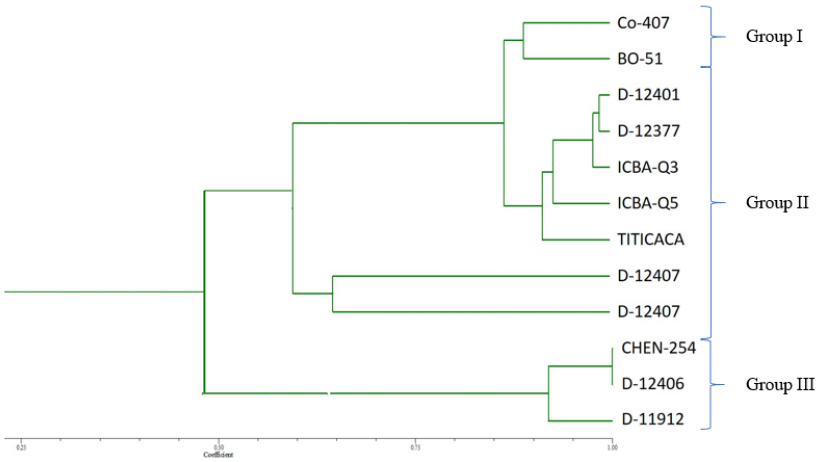

Figure 2: Genetic Diversity Results of *Chenopodium quinoa* accessions.

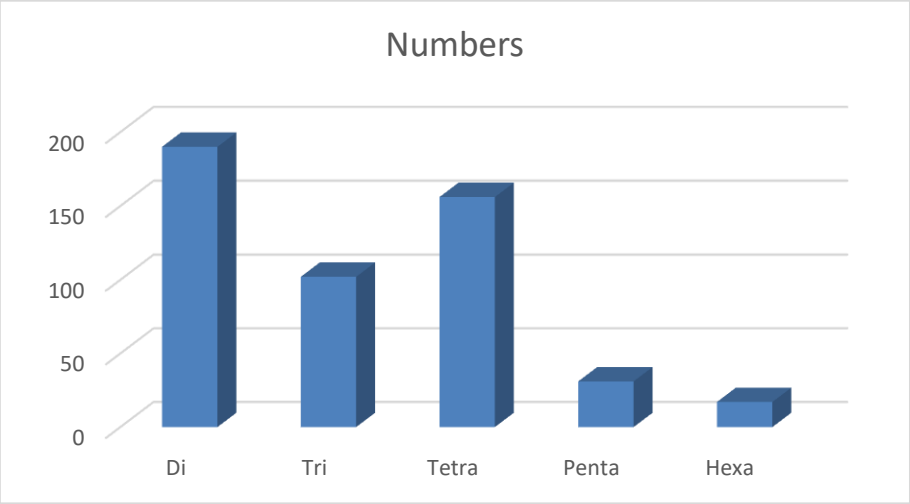

Figure 3: Graph presenting the number of different repeat motifs among *Chenopodium quinoa* specific SSR markers.

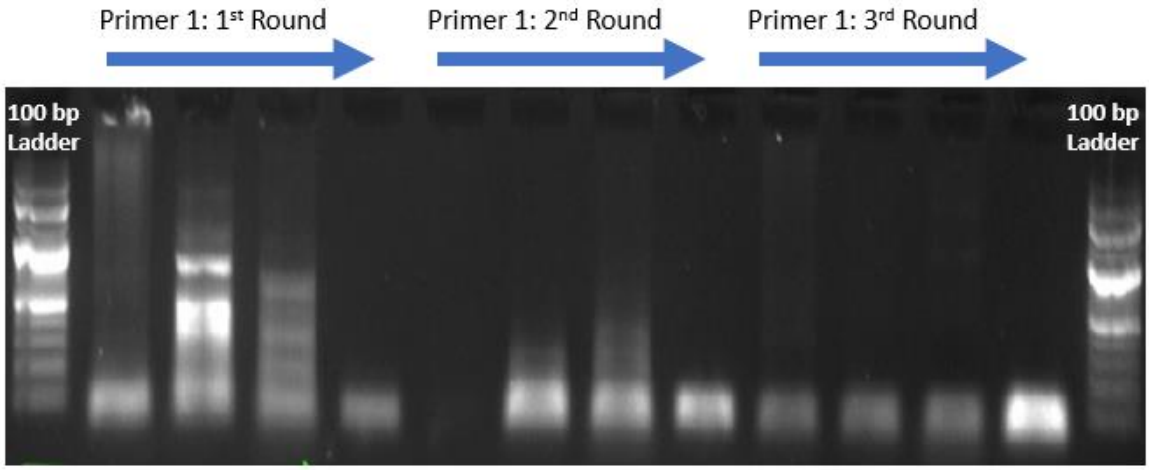

Figure 4: SSR Primer results not reproducible.

Table 1: Table presenting number of Di, Tri, Tetra, Penta and Hexa repeats among 496 SSR markers designed in this study. The product sizes, GC content and Annealing temperatures ranged similarly.

Repeat	Numbers	Product Size	GC content	Tm
Di	190	250 - 300	36 - 50	57 - 59
Tri	102	250 - 300	36 - 50	57 - 59
Tetra	156	250 - 300	36 - 50	57 - 59
Penta	31	250 - 299	36 - 50	57 - 59
Hexa	17	250 - 300	36 - 50	57 - 58.8

Supplementary Table 1: List of a set of 496 SSR primer pairs for amplification of microsatellite regions across the Chenopodium quinoa genome (Data not presented, it will be provided upon request)

CONCLUSION

This work demonstrates the great genetic diversity and molecular potential of quinoa for the development of high-yielding, saponin-free, climate-resilient cultivars. In marginal regions impacted by resource constraints and climate change, the produced SSR markers and identified genotypes provide useful tools for marker-assisted selection, promoting crop adaptation, nutritional security, and sustainable agriculture.

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Conflict of Interest:

There is no such evidence of conflict of interest.

Author Contribution:

All authors have participated in critically revising of the entire manuscript and approval of the final manuscript.

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