

# Advances of Biotechnology in Quinoa Production: A Global Perspective

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#### Abstract

In the last 30 years, quinoa started to be tested and produced in more than 100 countries outside the Andes, its region of origin. Nowadays, quinoa is found in more than 120 countries around the globe. During this time, biotechnology has become an important tool for different areas of research in quinoa, especially with the use of genetic markers. Biotechnology applications in this underutilized grain started in the United States, and their use has been more intensive in countries where quinoa was recently introduced. Biotechnology benefitted the quinoa sector with numerous studies on the species evolution, responses to abiotic stress, and assisted methods for faster genetic improvement. The recent quinoa genome description enables an exponential development with the complementation from novel areas, techniques, and tools such as omics and bioinformatics. Despite this, biotechnology applications in the Andean countries

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have been more limited due to economic and politic contexts. Nevertheless, biotechnology has been used to characterize the rich Andean germplasm, improve conservation systems, and develop bioinput. In this sense, since biotechnology should keep providing solutions for food security under healthy, sustainable, and reasonable principles, its use can be highly recommended. Biotechnology has the great potential to accelerate conventional breeding processes commonly applied to this crop, as well as to generate alternative options to enhance the production system and as model to improve other crops. With an integrative view and collaboration between different countries, biotechnology can provide tangible benefits to different stakeholders.

#### **Keywords**

 $Chenopodium \cdot Genetic \ markers \cdot Genomics \cdot Endophytes \cdot Breeding \cdot Genetic \ improvement$ 

## 5.1 Introduction

For thousands of years, quinoa (*Chenopodium quinoa* Willd.) has been domesticated, produced, and concentrated in the Andean region, but it was not until the twentieth century that the qualities of this grain crop were rediscovered by the rest of the world. The recent interest for quinoa has triggered a fast dissemination of this crop around the globe. However, it is still considered an underutilized crop due to the limited application of technology and research compared to major crops.

Similarly, it is in the last centuries that modern biotechnology had an exponential development and expanded to include different new disciplines and novel technologies. Biotechnology represents an alternative for the improvement of agricultural systems and can enhance quinoa production as well.

In this context, the present chapter describes the advances of biotechnology in quinoa production. It starts with an overview of the production and presence of quinoa around the world and summarizes the state of the art of the use of biotechnology tools in this crop. The dynamics of quinoa production and the use of biotechnology differ in countries of traditional production and in countries of recent introduction. Therefore, the perspectives, challenges, and recommendations for biotechnology application are described under this consideration.

The description of the global dynamics of quinoa in parallel with the use of technology for its development is important to realize the achievements, future perspectives, and threats of biotechnology use in this crop. It also marks a different expansion path, compared with other New World crops that were disseminated to the rest of the world.

## 5.2 World Quinoa Production and Biotechnology Applications: State of the Art

#### 5.2.1 Brief Overview of Development

Different authors have described the production and dissemination of quinoa (Rojas et al. 2011; Bazile and Baudron 2014; Bazile et al. 2016; Alandia et al. 2020). Therefore, in this section, we provide a brief overview. Figure 5.1 summarizes the expansion of quinoa by illustrating its presence around the globe along time. A detailed description with the type of presence and areas of production with quinoa can be found in Alandia et al. (2020).

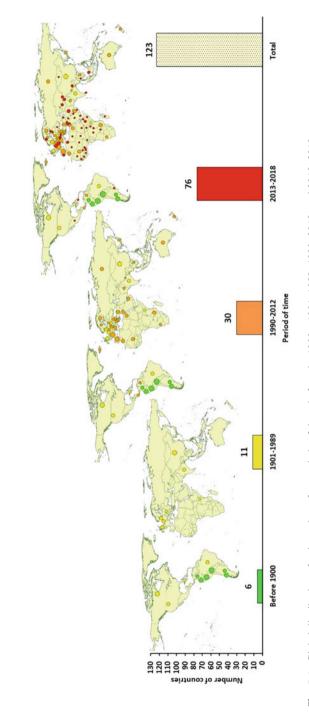
For thousands of years, the production remained concentrated in the Andes, its region of origin. Andean people used this grain as food, in religious contexts, and as part of its culture. Its production for food was tracked down to 3000 years BC (Bruno 2006; Planella et al. 2014). In the Hispanic period, because of the grain appearance, it was described by tellers and in communications to the Spanish crown as the millet or rice of the Incas (De la Vega 1609). In fact, before the twentieth century, quinoa was part of the cropping systems in only six countries in the world (in green in Fig. 5.1).

The rest of the world outside the Andean region started to rediscover quinoa driven by the development of new markets and research. The spread of this grain to other latitudes of the globe is reported for research back to 1935 (Bazile and Baudron 2014), but in reality, it was in North America where quinoa started to be produced and introduced to the market in the 1980s.

From there, different germplasm collections and breeding programs started to develop. In fact, from being only in six countries in the 1900s, quinoa is now present in more than 120 countries around the globe, both for research and for commercial production. It is in the last 30 years (from 1990 to 2018) that quinoa was introduced to 106 countries outside of its region of origin. A significant dissemination of this Andean grain took place with European research projects starting in the 1990s (in orange in Fig. 5.1). Thereafter, the second most significant spread of this grain took place with the International Year of Quinoa in 2013 that promoted this grain around the world (in red in Fig. 5.1). The main producers are still located in the Andean region, i.e. Peru, Bolivia, and Ecuador; these are followed now by the Netherlands, the United States, Canada, and Spain (Alandia et al. 2020).

## 5.2.2 Chronology in the Use of Biotechnology for Improving Cultivation

During the pre-Hispanic period, potato, maize, quinoa, and amaranth were important crops of the Andean civilization. The Spanish colony carried maize and potato through the Atlantic, and the global expansion of these two crops started. In contrast, quinoa and amaranth remained as underutilized crops (Butzer 1996). These two are





now an essential crop in the Andean region and were recently recognized by the rest of the world (Bazile et al. 2016).

The use and application of biotechnology started after the Green Revolution in the late 1960s with major crops such as wheat, rice, and maize (Pingali 2012). In quinoa, it started to be applied in the 1980s with the development of sterile male lines and the use of isozymes (Wilson 1988; Tamulonis 1989). This early application of biotechnology did not have a commercial purpose; it was oriented to understand phylogenetic relations between different *Chenopodium* and to demonstrate the potential of biotechnology for quinoa genetic improvement.

During the 1990s, the attention for molecular markers as random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) started. At that point, technology was expensive and was applied in the United States to quinoa originating from Peru, Bolivia, and Chile (Table 5.1). During this period, molecular markers also helped to screen and characterize quinoa accessions and material from interspecific and intergeneric crosses (Bonifacio 1995, 2004).

In the following decade, molecular characterization was used to differentiate domesticated and cultivated quinoa genotypes in the Bolivian Highlands (Rojas-Beltrán 2007). Andean and coastal quinoa genotypes were then used to elucidate *Chenopodium* domestication in North America (Maughan et al. 2006). Andean genetic resources were also used to develop simple sequence repeat (SSR) markers for quinoa to support germplasm characterization (Jarvis et al. 2008).

The most significant advances occurred in the twenty-first century where researchers used specific, more precise, and cost-effective molecular markers also at the RNA level. These tools were applied to characterize and identify potential genes for the genetic improvement of quinoa and further to understand abiotic stress tolerance (cold, salinity, and drought).

In the second decade of the 2000, studies in quinoa shed light on the complete genome of the plant (Yasui et al. 2016; Jarvis et al. 2017; Zou et al. 2017). This information can now be integrated with phenotyping, high-throughput sequencing, and other novel tools such as omics and bioinformatics for the identification of genes (Schmöckel et al. 2017).

The use of biotechnology tools in quinoa has evolved and generated extensive knowledge applicable to breeding as well as to the understanding of abiotic stress processes. This crop had a fast global expansion, and right now, the interest is concentrated on its genetic improvement. However, there is a difference in the use of biotechnology in the countries where it was originated compared with the countries of recent introduction as shown in Table 5.1 and Fig. 5.2.

#### 5.2.3 Quinoa Accessions and Biotechnology Applications

The dissemination of quinoa happened with the transportation of seed to different parts of the globe. During this process, its accessions started to be conserved at diverse institutions that are now reported in the Plant Genetic Resources for Food

Approach	During the 1980s	During the 1990s	After the 2000s
Use of vegetative part of the plant increases seed production	Development of sterile male lines from in vitro callus (Tamulonis 1989)		<ul> <li>Peruvian quinoa lines developed from in vitro vegetative (Ruiz 2002)</li> <li>In Brazil to increase stocks of hybrid seeds, a protocol is developed (Rocha 2011)</li> </ul>
Use of double haploid breeding			• In Peru, in vitro cultivation of anthers is carried out to obtain double haploid in quinoa with Rosada de Huancayo and Blanca de Hualhuas genotypes (Soplín 2009)
Use of biochemical and molecular markers	Use of isozymes to establish phylogenetic relationships between <i>Chenopodium</i> genera (Wilson 1988)	Fairbanks et al. (1993) used RAPD molecular markers to detect polymorphisms in quinoa	<ul> <li>Use of molecular markers for Andean germplasm characterization (Rojas-Beltrán 2007; Rodríguez and Isla 2009; Costa-Tártara et al. 2012; Morillo Coronado et al. 2017; Salazar et al. 2019)</li> <li>Use of fluorescence in situ hybridization (FISH) to quantify the number of RNA loci in quinoa (<i>C. berlandieri</i> var. zschackei and <i>C. berlandieri</i> spp. <i>nuttalliae</i> (Maughan et al. 2006))</li> <li>FISH is used to examine common ancestors between <i>C. quinoa</i>, <i>C. berlandieri</i>, and <i>C. album</i> (Sederberg 2008; Kolano et al. 2011)</li> <li>The evolution of polyploidy in quinoa was demonstrated at the chromosomal level using FISH (Kolano et al. 2012; Matanguihan et al. 2015)</li> </ul>

**Table 5.1** Key examples of biotechnology use in quinoa along time

Approach	During the 1980s	During the 1990s	After the 2000s
			• Hong et al. (2017) obtained complete chloroplast (cp) genomes of <i>C. quinoa</i> and <i>C. albun</i> by next-generation sequencing
Development		Bonifacio (1995,	RAPD was used by
of molecular markers		<ul> <li>2004) developed RAPD markers for screening interspecific and intergeneric crosses with <i>C. berlandieri</i>, ssp. <i>nuttalliae</i>, and <i>Atriplex</i> sp.</li> <li>Substantial genetic similarity was found between coastal and Andean ecotypes (Wilson 1988; Christensen et al. 2007)</li> </ul>	Ruas et al. (1999) and Del Castillo et al. (2007) to demonstrate the relationship between <i>C. quinoa</i> and related species of the Bolivian Highlands • Polymorphism was evaluated in six Peruvian and Bolivian commercial varieties with AFLP technology (Nolasco et al. 2013) • Mason et al. (2005) Fuentes et al. (2006), and Jarvis et al. (2006), and Jarvis et al. (2006), studied Chilean quinoa diversity with microsatellite markers (SSR markers) • Christensen et al. (2007) suggested a potential loss of genetic diversity of Chile commercial zones comparing Highlands and coastal ecotypes • Costa-Tártara et al. (2012), Rada (2015), and Morillo Coronado et al. (2017) studied genetic structure of cultivated quinoa from Northwest Argentina,
-			valley and Altiplano ecotypes, and Colombian accessions using RAM microsatellite markers
Genome sequencing and			• Mutagenesis in quinoa (Gomez-Pando and Eguiluz-de la Barra

#### Table 5.1 (continued)

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and Agriculture (PGRFA) global information system (FAO 2020). Figure 5.2 shows the 47 countries with gene banks holding accessions.

According to the official data from the PGRFA system, Bolivia has the biggest germplasm collection with almost 4000 accessions. Peru reports around 2000 accessions, and it is followed by the United Arab Emirates that hold 1306 accessions. Seven countries conserve from 100 to more than 900 accessions: Ecuador (910), Germany and Chile (more than 500), the United States (375), Japan and India (more than 150), and the United Kingdom (136). Moreover, 9 countries conserve up to 83 accessions (Mexico, Australia, Uruguay, Israel, Ethiopia, Colombia, Hungary, Argentina, and South Africa), and 12 countries (26% of the 47 countries) hold up to 15 accessions, and 16 countries (34% of the total) report up to 5 entries.

As previously described, molecular markers have been used since the 1990s. They were used mainly for breeding and phylogenetic studies and to characterize genetic resources in 14 countries. In the Andean region, markers were applied in Bolivia, Peru, Ecuador, Chile, Argentina, and Colombia. In countries of recent introduction, they were applied in the United States, the Netherlands, Belgium, Denmark, Italy, Saudi Arabia, China, and Japan (light blue circles in Fig. 5.2 which are covered by the genomics identifier in the last three countries of this list).

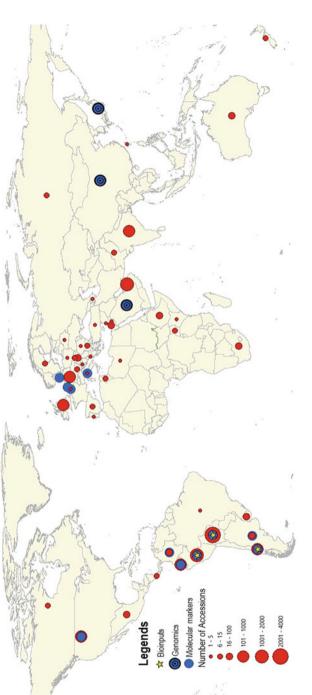


Fig. 5.2 Genebanks with quinoa accessions and biotechnology applications on this crop around the world. Red circles, gene banks and number of accessions held. Circles are scaled in proportion to the number of quinoa accessions conserved ("Number of accessions"); light blue circles, different studies using molecular markers in quinoa ("Molecular markers"); dark blue with black concentric circles, quinoa genome ("Genomics"); yellow star, research with endophytic microorganisms and bioindustrial applications addressed to quinoa production ("Bioinputs"). Identifiers can be superposed, as it is the case for Peru and Bolivia but also for Saudi Arabia, China, and Japan for the use of molecular markers and genomics. Sources: (FAO 2020) and references in Table 5.1

From the countries applying molecular markers, four of them (the Netherlands, Denmark, China, and Saudi Arabia) do not report local collections to the PGRFA system. Three countries have succeeded to obtain the genome sequencing, i.e., Japan, Saudi Arabia, and China (dark blue with black concentric circles in Fig. 5.2). Finally, biotechnology has also been used in Ecuador, Peru, Chile, and Bolivia for the identification of endophytic microorganisms to improve production (in yellow in Fig. 5.2). Until now, these countries constitute the only references for quinoa in this field.

#### 5.2.4 Examples of Biotechnology Uses in Underutilized Crops

Even when quinoa is now spread globally, it can be still considered an underutilized crop. The "underutilized" denomination is given to indigenous plant species grown locally, generally under low technology, linked to culture and traditions, and with an understudied potential for food security and new niche market development. Underutilized crops have been called differently according to the characteristics that authors want to highlight. Among other names, these species are also called neglected, orphan, or minor crops (Padulosi and Hoeschle-Zeledon 2004; Mayes et al. 2012; Tadele 2019).

In this section, we briefly describe some improvement advances in underutilized crops with successful biotechnology uses and with potential to be applied in quinoa. In general, the improvement of most underutilized crops has been done through conventional breeding techniques. These methods targeted traits such as plant architecture, crop cycle length, and tolerance to biotic and abiotic stressor reduction of antinutrient levels, among others (Esfeld et al. 2013; Tadele 2019; Gulisano et al. 2019).

The use of modern crop breeding techniques in orphan crops is recent and has been applied mainly in legumes (chickpea, cowpea, pigeon pea, lupins), grains (quinoa, amaranth, millet, teff, buckwheat), and few roots and tubers (e.g., manioc). Molecular-assisted approaches (genetic markers) have been applied in most of the abovementioned species for diversity characterization, phylogenetic studies, gene association, and mapping (Tadele 2019). There are reports of high-throughput techniques like TILLING (Targeting Induced Local Lesions in Genomes) in crops such as chickpea and teff. Omic tools were recently used for sequencing most of the species mentioned; in fact, most of their genomes are reported in 2017. Finally, genome editing (with the bacterial clustered regularly interspaced short palindromic repeats (CRIPSR)/Cas9 system) was practiced in manioc and ground cherry (Tadele 2019). Examples of advanced techniques used for breeding Andean grains are given in the table below.

Protocols for advanced breeding methods, such as speed breeding, have been developed (Ghosh et al. 2018). This method uses prolonged photoperiods under controlled conditions to accelerate plant growth rate and obtain more generations per year. Other genetic tools used in the Amaranthaceae have been genotyping by sequencing (GBS) and genome-wide association studies (GWAS) for phylogenetic

Breeding method/ technology	Andean grains	References
Speed breeding	Quinoa	Ghosh et al. (2018)
Marker-assisted breeding (GBS, GWAS)	Amaranth, quinoa	Lightfoot et al. (2017); Stetter and Schmid (2017); Joshi et al. (2018); Rodríguez et al. (2020)
High throughput (TILLING)	Quinoa	Mestanza et al. (2018)
Omics (genome, transcriptome, RNA sequencing)	Quinoa, amaranth	Jellen et al. (2013); Ruiz et al. (2019); Clouse et al. (2016); Lightfoot et al. (2017); Schmöckel et al. (2017); Zhang et al. (2020a, b)

 Table 5.2 Examples of advanced techniques used for breeding Andean grains

*Note: GBS* Genotype By Sequencing, *GWAS* Genome-Wide Association Study, *TILLING* Targeting Induced Local Lesions in Genomes

studies, gene association, and mapping (Lightfoot et al. 2017; Stetter and Schmid 2017; Joshi et al. 2018; Rodríguez et al. 2020). High-throughput TILLING was used in Regalona Baer quinoa cultivar to try to find mutants resistant to herbicides (Mestanza et al. 2018). Finally, different studies using novel areas such as omics have been applied in both quinoa and amaranth as described with some examples in Table 5.2.

Marker-assisted breeding (also known as marker-assisted selection) is a breeding technique that uses molecular markers (DNA fragments), associated with genes that are linked to targeted traits. With the use of markers, these genes can be traced, identified, and assembled for crop improvement. TILLING uses traditional mutation techniques or naturally occurring mutations (EcoTILLING), followed by high-throughput mutation detection. It is a reverse genetics method of relatively low costs. Successful examples have been obtained with teff and chickpea (Esfeld et al. 2013). Among the modern approaches that use biotechnology and molecular transgenic and non-transgenic techniques, marker-assisted breeding and TILLING have less restrictions to be used in underutilized crops and high potential to be applied in quinoa due to its non-transgenic nature.

## 5.3 Perspectives in Countries of Traditional Quinoa Production

#### 5.3.1 Biotechnology Use

Breeding programs in the Andean region were established as early as in the 1960s starting in Bolivia and Peru. The improvement in the region was primarily done through conventional breeding and interesting examples of participatory plant breeding methods. In general, biotechnology tools were used in the region to characterize the local genetic resources (Gomez-Pando 2015; Gomez-Pando et al. 2019; Danial et al. 2007; Rojas et al. 2015).

As described in previous sections, while numerous genotypes from Andean collections have been used to generate significant information and for the genetic improvement of quinoa with modern tools, most of these studies have been performed outside of the Andean region.

Examples of biotechnology applications can be found in the Andean countries (though in a smaller scale compared with the northern continents): in Bolivia for the characterization of germplasm and establishment of core collections (Rojas-Beltrán 2007; Veramendi et al. 2014); in Peru for phylogenetic studies (Nolasco et al. 2013) or the first studies with mutagenesis (Gomez-Pando and Eguiluz-de la Barra 2013); in Chile with AFLP markers to analyze and compare the genetic diversity of local germplasm and TILLING applied with next generation-sequencing (Rodríguez and Isla 2009; Mestanza et al. 2018); and in Ecuador, Argentina, and Colombia with microsatellite markers to analyze the level and structure of diversity of quinoa (Costa-Tártara et al. 2012; Morillo Coronado et al. 2017; Salazar et al. 2019).

### 5.3.2 Limitations of Current Technologies

In countries where quinoa is produced traditionally, the main limitations of biotechnologies to improve production are related to their application. The characteristics of the economies and policies in the Andean region limit the implementation of these techniques that require stable specialized staff with constant training, renovation, and update. In addition, these technologies need steady and well-equipped infrastructure constantly maintained and upgraded.

Unfortunately, in some countries where it is traditionally produced, research institutions can be significantly affected by the change of governments and policies. In addition, the applications of current technologies are mostly constrained by both insufficient investment and funding. Moreover, bureaucratic systems slow down innovation processes (Echeverría 1998; Rose Boom et al. 2006).

The reduced number of publications reporting the use of these technologies directly in the Andean region may reflect the abovementioned limitations. In fact, Andean countries have addressed the use of molecular tools to characterize the diversity of their quinoa germplasm, but there seems to be limited use of markers to assist processes of genetic improvement. So far, the majority of released cultivars resulted from conventional breeding processes (Apaza Mamani et al. 2013; Bonifacio et al. 2013, 2015).

Although with limitations, these technologies have been applied as a result of joined efforts between the governments, international cooperation, and public and private institutions. Countries with the richest quinoa diversity have been able to characterize their collections using molecular tools (Rojas-Beltrán 2007; Rojas et al. 2015; Gomez-Pando et al. 2019). Furthermore, other biotechnology uses in the region have been possible for innovation as described hereafter.

#### 5.3.3 New Biotechnological Tools

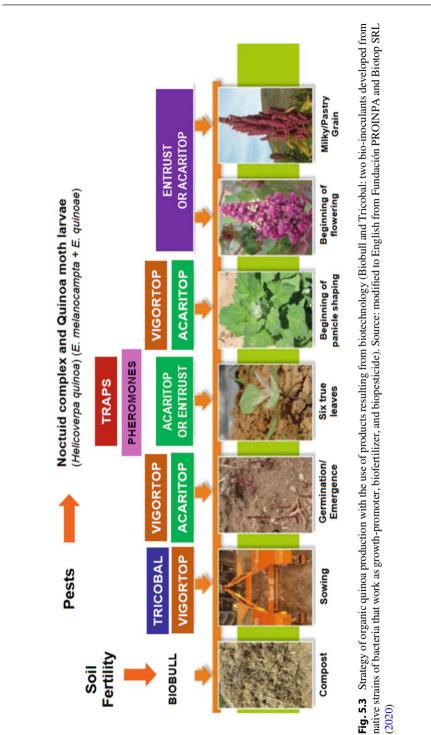
The main focus and application of biotechnology in the countries of traditional production has been the characterization of the local diversity. There have been interesting studies such as phylogenesis or the first reference of mutagenesis in quinoa, but innovative technologies have also been developed to support production. Bolivia is the second world producer. Production for export is mainly concentrated in the Southern Highlands, a region with extreme environmental conditions characterized with low annual temperatures and soils with low fertility (Alandia 2015). Pandey and Yarzábal (2019) have described the potential of plant growth-promoting microorganisms (PGPM) to improve soil fertility in tropical mountain regions.

In the 2000s, Bolivian researchers started to explore this area to enhance organic production concentrated in the Southern Highlands. With biotechnology tools and bioinformatics, they described diverse native strains of *Bacillus*, *Azotobacter*, *Pseudomonas*, *Rhizobium*, and *Flavobacterium* collected from farmer plots. The species found were adapted to extreme environmental conditions and had the capacity to fix nitrogen and solubilize phosphorus. *Entomopathogens* were also identified in these collections, highlighting the potential to develop bioinsecticides for organic production. Research continued with the identification of secondary metabolites promoting growth and increasing yields. Finally, all these efforts resulted in the formulation and development of bioproducts (Ortuño et al. 2013, 2014, 2017).

These researchers, together with innovative institutions, upscaled this technology and made different evaluations to validate it in farmer plots (Lino et al. 2019). The resulting products have been certified and included in a crop management strategy (Fig. 5.3) and are now commercialized and available to farmers producing under organic systems (Fundación PROINPA and Biotop SRL 2020).

Similar research was performed in Chile where an important array of fungal endophytes was found in rooting systems of desert areas (González-Teuber et al. 2017). Moreover, research performed in Peruvian communities by Chumpitaz-Segovia et al. (2020) characterized 51 strains of plant growth-promoting bacteria collected from the rhizosphere. From these, 73% had the capacity to grow at low temperatures and the potential to improve soil fertility for production. Pantoja and Juana (2015) demonstrated that two strains of bacteria BBAR001 (rhizobial) and BBAP001 (*Pseudomonas*) improved soil fertility in plots by solubilizing organic matter, total nitrogen, and phosphorus. Llanos Machaca (2017) used phosphate-solubilizing bacteria to promote the availability of phosphorus in the soil and had a positive effect during the seedling stage. Other bacteria, such as *Pseudomonas* (strain PQLMT18) and *Rhizobium* (strain DZ50), were used to enhance seed germination in soils with low fertility (Nina-Larico 2019).

Simple methods, such as organic amendments, can improve and promote the activity of microorganisms. Gomez-Montano et al. (2013) and Paco-Pérez and Guzmán-Vega (2019) showed that bacteria population and activity increased at the rhizosphere level in amended soil with lama manure.



Improving yields is a current challenge for organic production under traditional production systems and under extreme environmental conditions. Nevertheless, there is a good potential and interesting initiatives to improve plant responses with the use of endophytic microorganisms. Through biotechnology, native endophytes found in traditional farming systems have been isolated and are produced at the commercial level to improve organic production.

#### 5.3.4 Challenges and Recommendations

Since investment and funding are among the biggest limitations for research and innovation, the challenge in the Andean region is to build, maintain, and update capacities constantly and efficiently. To be effective with funding, countries should consider analysis and learning processes of their own experience as useful tools to adapt research systems to their context, needs, and also constant changes (Rose Boom et al. 2006). In this sense, when biotechnology is used to solve each country's problems and priorities without affecting biodiversity, governments should include it in serious plans supported by specialists. The recent COVID-19 pandemic is a clear example of a rapid change, and it is fundamental for institutions involved in research to respond and support with fast reactions to these circumstances. Optimist examples are the efforts in Bolivia with the recent national response plan to reactivate the agriculture sector to face COVID-19 effects, which suggests projects to develop bioinputs addressed to small farmers that practice organic agriculture (Gobierno del Estado Plurinacional de Bolivia 2020). Nevertheless, recent changes of government, can limit its application.

Having a rich array of genetic resources, the Andean region can use its diversity to adapt and respond to changes (from climate, market, policies, to mention some). Biotechnology has been a useful tool for the characterization of different accessions. The challenge is now to continue using this technology to improve plant productivity. It is fundamental to strengthen and link capable institutions and their human resources so they can continue applying novel tools. This will reduce the knowledge gap between traditional and new countries producing quinoa. In addition, the collaboration and partnerships between countries and institutions can interlock efforts to achieve successful products more efficiently. Different strategies adapted to each specific context can be used to improve the productivity. Good examples have been the complementation of breeding processes with participatory plant breeding (Rojas-Beltrán 2007; Danial et al. 2007) and the development of bioinputs using endophytic organisms as described above.

Results of molecular characterization of Andean germplasm should be used to achieve and enhance the diversification and quality of products, diets, market, demand, and certification. The use of the rich array of varieties and species from megadiverse systems can be positive not only for species such as quinoa but also for farmers' resilience and the diversification of their incomes. Moreover, successful applications of biotechnology should serve as an example for other underutilized crops in the region. Finally, in countries of traditional production, the integration of science and tradition becomes important in order to avoid the underestimation of any of these elements for agriculture development.

## 5.4 Perspectives in Countries of Recent Quinoa Production

## 5.4.1 Biotechnology Use

Experiments with quinoa outside the Andes were initiated in Kenya already in 1935, but this grain was first grown in the United States and Canada in the 1980s (Bazile et al. 2016). After the introduction of quinoa in the United Kingdom at the beginning of the 1980s, European collaborations were established, allowing the introduction of quinoa in Denmark, the Netherlands, Italy, and, to a minor extent, France. In 2008, the "Sustainable water use securing food production in dry areas of the Mediterranean region" (SWUP-MED) project brought together a number of partners from Italy, Portugal, the United Kingdom, the Netherlands, and Denmark, as well as several countries of the Mediterranean area (Turkey, Morocco, Egypt, the Syrian Arab Republic), generating a wave of expansion across Europe, the north of Africa, and the Middle East (Bazile and Baudron 2014).

Only in one year (2015), 20 countries were testing quinoa for the first time (Bazile et al. 2016; Murphy et al. 2016). Many countries, including China, the Netherlands, Denmark, and Germany, have now established extensive breeding programs for the development of varieties adapted to the specific environmental conditions. As for other crops, many genetic markers have been developed to assist its breeding efforts, including RAPD (Fairbanks et al. 1993; Ruas et al. 1999; Del Castillo et al. 2007), AFLP (Maughan et al. 2004; Rodríguez and Isla 2009), microsatellites or SSRs (Mason et al. 2005; Jarvis et al. 2008; Fuentes et al. 2009; Costa-Tártara et al. 2012), and single-nucleotide polymorphisms (SNPs) (Maughan et al. 2012; Jarvis et al. 2017). Chemical or physical mutagenesis and subsequent marker-assisted selection of desired traits have led to the generation of adapted varieties in several countries, but the rate of progress has been slow.

### 5.4.2 Limitations of Current Technologies

Most current breeding programs are based on marker-assisted selection. Compared to conventional breeding, breeding through marker-assisted selection allows for a faster generation of the desired variety. For instance, incorporation of one or a few genes into an adapted or elite variety is done by crossing this variety with another that contains the desired traits and subsequent backcrossing of the resulting progeny with the original elite variety. The use of DNA markers in backcrossing greatly increases the efficiency of selection. It does not rely on a visual phenotype, which may be particularly useful for traits that have laborious or time-consuming phenotypic screening procedures or are caused by gene variants that are inherited in a recessive manner, i.e., they require homozygosity to become apparent.

Marker-assisted breeding allows for elimination of most of the unwanted DNA incorporated in the first crossing line, thus reducing the chances of incorporating genes from the donor that might be negatively affecting the elite variety (Collard and Mackill 2008). In addition, marker-assisted breeding simplifies pyramiding processes (the combination of several genes into a single genotype). This is usually done by crossing a production variety with several other varieties with distinct desired traits in a consecutive manner. Pyramiding has been widely used for generation of varieties combining multiple disease resistance genes (Pedersen and Leath 1988; Kloppers and Pretorius 1997; Shanti et al. 2001; Pilet-Nayel et al. 2017; Mundt 2018). Selection of these varieties by conventional breeding is difficult because all genes give rise to the same phenotype, but this problem is eliminated when selection is directly based on genetic markers.

While marker-assisted breeding is much faster than conventional breeding, the need for crossing and backcrossing varieties still imposes a limitation in the speed of progress. In addition, the process is resource-demanding and requires qualified personnel, which precludes its use in developing countries where investments in research, technology, and training are more limited. Finally, marker-assisted breeding is heavily limited to varieties that can be effectively crossed.

Thanks to the advances in next-generation sequencing (NGS) technologies, the assembled genomes of two different varieties were recently published (Jarvis et al. 2017; Zou et al. 2017). Access to this information, together with the advances in bioinformatics and new genome editing technologies, opens up the possibility of facilitating the current breeding efforts.

#### 5.4.3 New Biotechnological Tools

A wealth of information has been generated in the past decades about the mechanism that has governed the domestication of our major crop species. For instance, several genes that are negative regulators of grain size have been identified in rice, including *GRAIN WIDTH AND WEIGHT2 (GW2)* (Song et al. 2007), *GRAIN INCOMPLETE FILLING1 (GIF1)* (Wang et al. 2008), *GRAIN SIZE3 (GS3)* (Fan et al. 2006; Gao et al. 2015), and *Protein Phosphatase with Kelch-Like repeat domain1 (OsPPKL1)* (Zhang et al. 2012). Resistance to powdery mildew in barley and wheat is related to *mlo* genes (Lyngkjær et al. 2000; Wang et al. 2014; Acevedo-Garcia et al. 2017). Plant height is usually controlled by genes involved in hormone signaling, such as *REDUCED HEIGHT (Rht)-B1* and *Rht-D1* in wheat (Flintham et al. 1997; Peng et al. 1999), *DWARF PLANT8 (Dwarf8)* and *Dwarf9* in maize (*Zea mays*) (Lawit et al. 2010), *SEMIDWARF-1(sd-1)* in rice (Peng et al. 1999; Spielmeyer et al. 2002; Monna 2002), or *sdw1/denso* in barley (Jia et al. 2009).

This information has been used together with bioinformatics tools to identify suitable targets in the quinoa genome that could potentially allow for marker-assisted breeding as well as gene editing (López-Marqués et al. 2020). Gene editing allows for precise creation of new variants of genes without inserting new DNA in the genome. In a standard gene editing strategy, enzymes that can cleave in any sequence within the genomic double-stranded DNA (nucleases) are introduced in cells (normally callus or protoplasts) of the plant of interest. These nonspecific nucleases are targeted to concrete positions in the genomic DNA using different strategies (described later in this section). Once the double-strand break is introduced at the desired position, the endogenous cellular machinery will try to repair it to prevent cell death, introducing changes in the genomic sequence. Finally, genetically modified cells that are devoid of the exogenous nucleases are selected for regeneration of whole plants. Two main reparation mechanisms exist in eukaryotic cells: non-homologous end joining and homology-directed repair. In non-homologous end joining, the DNA on each side of the double-strand break will simply be joined together by ligases. This process is highly error-prone, which results in the introduction of insertions or deletions (in-dels) in the repaired genomic DNA. By contrast, homology-directed repair uses a DNA sequence with homology to the edges of the double-strand break as a template for repair, for instance, another copy of the disrupted gene present in the genome. This mechanism thus allows for the introduction of new DNA in the region containing the double-strand break. In most plants, the most common cellular DNA repair mechanism is non-homologous end joining, and generation of in-dels in a gene of interest is the most widely used genetic engineering strategy. Precision breeding in this way has in recent years been carried out in a number of crops but still not in quinoa (López-Marqués et al. 2020).

The first technology for targeted gene editing in plants used zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (Gaj et al. 2013; Čermák et al. 2017). In these two technologies, the nonspecific nucleases used to generate the double-strand break are fused to sequence-specific DNA-binding domains (Joung and Sander 2012; Gaj et al. 2013). For TALEN strategies, the DNA-binding domain is an amino acid sequence designed in analogy to the TAL effectors of bacterial plant pathogens. A TAL effector contains a repeated 33-34 amino acid-long sequence that is highly conserved, except for 2 residues in the middle of the sequence that form the so-called repeat-variable diresidue (RVD) (Joung and Sander 2012). The amino acids in these two positions allow the recognition and binding of specific nucleotides in a DNA molecule. This way the DNA-binding modules of TALENs can be programmed to target any sequence of interest by designing RVDs with the desired amino acid combinations. Zinc fingers are DNA-binding domains present in a number of proteins in different organisms (Urnov et al. 2010; Hossain et al. 2015). These domains contain cysteine- and histidine-rich repeats that fold in a three-dimensional structure that allows binding to a specific DNA sequence composed of three nucleotides. Variations in the amino acid sequence of the zinc finger will generate slightly different folds and thus different DNA recognition sites. ZFNs have been used to modify a number of agriculturally relevant species, such as maize, soybean, rapeseed, rice, apple, and fig (reviewed in (Ran et al. 2017; Martínez-Fortún et al. 2017)). As an example, ZNF-assisted disruption of the IPK1 gene, which encodes an enzyme catalyzing the final step in phytate biosynthesis, in maize was used to generate plants with herbicide tolerance and altered levels of the phytate precursor inositol phosphate in developing seeds (Shukla et al. 2009). TALEN strategies have also been used to disrupt this gene (Liang et al. 2014), as well as many others in *Arabidopsis*, barley, *Brachypodium*, maize, tobacco, rice, soybean, tomato, and wheat (reviewed in (Malzahn et al. 2017)). However, both ZNFs and TALENs require the tedious task of designing protein modules for DNA-binding.

In the past decades, the adaptation of the bacterial clustered regularly interspaced short palindromic repeats (CRIPSR)/Cas9 system to plants has resulted in targeted engineering systems that are much easier to design (Chen et al. 2019). When bacteria are attacked by DNA viruses, a piece of the viral genetic material is introduced at a specific location in the bacterial genome. This genome location is organized in the form of regularly interspaced repeats (hence, the name CRIPSR), and its transcription is linked to that of nonspecific nucleases (CRISPR-associated nucleases, Cas). These nucleases form complexes with the RNA transcribed from the CRISPR locus, which contains a sequence complementary to that of the viral DNA. This way Cas proteins can recognize and cleave the genetic material of specific viruses based on sequence complementarity, thus conforming a type of bacterial immune system (Golubov 2016). In the adaptation of this system for genome editing, a single guide RNA (sgRNA) of approximately 20 nucleotides is designed to be complementary to a target genomic DNA sequence. This sgRNA forms a complex with the Cas9 protein, which generates double-stranded breaks that are subsequently repaired by the cellular machinery (Gaj et al. 2013). Due to the simplicity of designing an RNA sequence based on a known DNA sequence and the possibility of targeting several alleles of one home gene at the same time, the CRIPSR/Cas9 method has overtaken the field of targeted genome editing.

CRISPR/Cas9 has been used for targeting genes in a plethora of plants, including potato, petunia, wheat, sorghum, rice, barley, alfalfa, cabbage, soybean, cucumber, and grapevine (reviewed in (Liu et al. 2017)). In addition, several modifications of the system have been introduced to increase the efficiency of the process or for multiplex editing where several genes are targeted at the same time (Chen et al. 2019; Zhang et al. 2020a, b). For instance, four homologue MAP kinases were simultaneously targeted in rice using polycistronic RNA-sgRNA genes, which allow for expression of multiple sgRNAs from one DNA construct (Xie et al. 2015). In wheat, three different alleles of the MLO gene were simultaneously targeted to generate plants resistant to powdery mildew (Wang et al. 2014). Similarly, all alleles of a starch synthase could be disrupted using an optimized CRISPR system in potato (Johansen et al. 2019). Different genes can also be targeted at the same time by introducing multiple sgRNAs in the same transformation event. Such strategy has been successfully applied in wheat, cotton, tomato, and ground cherry (Xie et al. 2015; Gao et al. 2017; Lemmon et al. 2018; Wang et al. 2018; Zsögön et al. 2018). Lately, it has become possible not only to generate double-strand breaks but also to modify individual nucleotides using Cas9-assisted base editors (Zong et al. 2017; Li et al. 2018; Anzalone et al. 2019; Lin et al. 2020) and to generate plants that have become engineered without the need for incorporation of transgenes in the genome (Zhang et al. 2016; Liang et al. 2017; Andersson et al. 2018).

As an alternative to these new technologies, which might encounter legislation issues and consumer mistrust, due to their inherent genetically modified (GM) nature, advanced TILLING approaches could be used (Chen et al. 2014; Holme et al. 2019). In general, these techniques rely on the generation of random mutations in a genome by the use of mutagens, such as radiation or ethyl methanesulfonate (EMS), and the subsequent selection of the desired mutation. Initially, selection was based on phenotypic characterization. However, for polyploidy plants, the probability of finding a visible phenotype after a single round of mutagenesis is very low. Therefore, methods for identification of the desired mutation at the genome level were introduced. Such a screening strategy for identifying desired genetic variants has already been used in quinoa (Gomez-Pando and Eguiluz-de la Barra 2013; Mestanza et al. 2018). However, the generated mutant collections were relatively small, and further efforts to increase their size are required, if they are to be used for selecting specific mutations in selected genes. A large mutant collection combined with high-throughput PCR-based strategies for mutant selection will be the key for future improvement of quinoa using TILLING strategies (López-Marqués et al. 2020).

#### 5.4.4 Challenges and Recommendations

The first complete high-quality genomes were published in 2017 for a coastal Chilean quinoa accession (PI 614886) (Jarvis et al. 2017) and a Bolivian Real variety (Zou et al. 2017). This information is available online through public databases, such as the National Center for Biotechnology Information (NCBI) genome database (Bio Project no. PRJNA394587) and the Chenopodium DB at the King Abdullah University of Science and Technology (KAUST) in Saudi Arabia. In addition, a limited amount of gene expression data at different plant developmental stages and under salinity stress is available at the Sequence Read Archive at NCBI (Bio Project No. PRJNA394651 and PRJNA394652). Therefore, bioinformatics can be used for rational design of genetic engineering strategies aimed at improving quinoa. In addition, with the increased speed and affordability of high-throughput sequencing techniques, the amount of genetic resources is expected to expand exponentially in the coming years. While several South-American countries have their own biodiversity collections with limited accessibility, publically funded gene banks, such as IPK Gatersleben (Germany), accumulate extensive plant material including more than 400 individual quinoa accessions freely available for research purposes (Fig. 5.2).

While the use of new genome editing technologies in quinoa has not been reported yet, the fact that complex polyploidy genomes, such as hexaploid wheat, can be successfully targeted is encouraging. In contrast to wheat or barley, efficient transformation protocols do not exist for quinoa. However, quinoa can be transformed using *Agrobacterium*, and plants can be regenerated from calli (Komari 1990; Eisa et al. 2005; Telahigue and Toumi 2017; Shahin 2019). Increasing the transformation efficiency to levels adequate for genome editing might involve the use of boosters, as is the case for other species (Zuo et al. 2002; Deng et al. 2010). In addition, new technologies that allow for transformation of

plant meristems are developing, removing the need to work with calli (Maher et al. 2020).

Optimization of different elements of the CRISPR system might also be required. Thus, the efficiency of full allelic potato transformation increased substantially when a potato endogenous promoter was used to drive the expression of the sgRNA(s) of interest (Johansen et al. 2019), and codon optimization of Cas9 has been successfully tested in several species (Ma et al. 2015; Cui et al. 2019; Lin et al. 2020).

On the other hand, considering the current legislation, TILLING strategies might be a convenient tool for optimization of quinoa. The disadvantage of this type of technique resides in the need to grow quinoa for several generations in order for the mutation to become homozygous at all existing alleles. Moreover, pyramiding of desired traits will require extensive crossing and backcrossing of quinoa varieties.

## 5.5 Biotechnology for Quinoa Production: Benefits and Disadvantages

#### 5.5.1 Benefits

Biotechnology has a wide range of powerful tools that can be used to improve and facilitate quinoa breeding processes, as well as for the management, use, and conservation of quinoa genetic resources. In this section, we describe some of the benefits in relation to the technology that has been applied in this plant species.

Countries that hold in situ and ex situ quinoa diversity have been using these technologies to have a precise description of the genetic resources available. Andean countries have been able to describe their diversity and rationalize their conservation systems. The molecular characterization of quinoa diversity complements characterizations that use other quantitative and qualitative description methods (e.g., agromorphologic, chemical). It offers a genetic fingerprint of conserved accessions, which can be used to group and differentiate collections with high precision (e.g., to establish core collections). It is an effective tool which can be used to analyze an extensive number of individuals. It enables a better management for genetic resource conservation, also by reducing duplicated accessions and their related costs of maintenance in germplasm banks. Biotechnology offers a possibility to produce useful information that boosts quinoa uses and the diversification of products (e.g., for agroindustry, cosmetology) (Veramendi et al. 2014; Rojas et al. 2015).

Countries from the Andean region hold a high and rich diversity. However, the policy for conservation and germplasm exchange limit the improvement of quinoa in other latitudes (Jellen et al. 2013). Biotechnology has become a way to overcome these constraints for countries where it is recently introduced. The use of biotechnology enables the identification and characterization of microorganisms with the potential to enhance production. In a short period of time, it gives the possibility to obtain economic and effective bioproducts to enhance yields under challenging settings (organic, traditional, extreme environmental conditions). Moreover, omics

open the possibility to elucidate more on secondary metabolites with potential to improve quinoa production (Sarethy et al. 2019).

Genetic markers have shown to be useful tools to understand the genetics of complex traits and to develop and assist breeding programs in different parts of the world. Markers started to be used in the United States in 1993, and ever since, this country has explored diverse types of markers to accelerate breeding and to select targeted genotypes. Markers have been used for the detection of DNA polymorphisms, for the identification of true hybrids, and for studies and maps to describe the relationship between different *Chenopodium* spp. and ecotypes. The level of genetic diversity, evolution and studies of quinoa origin, and domestication were also facilitated with the use of genetic markers. These have also been used for the study of transcriptome changes, both at specific quinoa growing stages and under abiotic stress conditions (Jellen et al. 2013).

As previously described in this chapter, genomics have also started to be used in giving rise to the genome sequencing of an inbred line and quinoas from coastal and mountain regions. These high-quality sequencing enabled to describe phylogeny and evolution, as well as to identify genes and their functions. Genes related to saponin production, protein biosynthesis, and responses to salinity tolerance have been described. These tools open up the possibility of carrying out targeted breeding using marker-assisted selection and other strategies for the genetic improvement (Yasui et al. 2016; Jarvis et al. 2017; Zou et al. 2017). The application of omics in quinoa may expand the knowledge related to different structures, mechanisms, functions, pathways, and related genes, while bioinformatics may increase the precision and velocity of these studies (Muthamilarasan et al. 2019).

## 5.5.2 Disadvantages

Even though there are positive benefits from the use of biotechnology for quinoa development, there are also disadvantages from its use that we briefly describe in this section. In the case of traditional countries, a disadvantage is linked to intellectual property rights. Intellectual property systems are important because they reward the intellectual effort to develop new technologies and plant cultivars and protect breeders' interests. However, these systems do not protect Andean farmers' interests or include recognition for the people cultivating the plant materials used in the breeding innovation. For example, due to policy ambiguities, Bolivia has not ratified the Nagoya protocol<sup>1</sup> to avoid the appropriation or monopoly of natural processes, genetic resources, and biopiracy (Convention on Biological Diversity Secretariat 2020). An antecedent exemplifying these risks happened in 1992, when a patent was requested by the United States to protect the use of an Andean cytoplasmic male

<sup>&</sup>lt;sup>1</sup>International agreement for a fair and equitable share of benefits arising from the utilization of genetic resources.

sterile genotype (Apelawa) and the resulting hybrids. This case was controversial and contested by many sectors from the Andean region. The patent was not renovated due to the international pressure exerted (Risi et al. 2015). These aspects have been also discussed, and the main conclusions until now are the need to improve policy gaps (Bazile and Baudron 2014; Bazile et al. 2016; Chevarria-Lazo et al. 2015) and the close collaboration between breeders and traditional producing countries that can lead to tangible and agreed distribution of benefits (Alandia et al. 2020).

The development of new varieties through biotechnology requires high investments. Infrastructure, operation, research, and development costs are variable according to the country of implementation, the dimension, and the scope of the activities and methods used. The application of biotechnology in regions with development constraints can be limited. However, with time and the fast progress of biotechnology, useful tools such as genetic markers are getting more accessible and cost-efficient and are starting to be more used in minor crops (Jellen et al. 2013; Tadele 2019).

In addition, successful, stable, and homogeneous new cultivars could replace diverse production systems leading to monocropping or the loss of the rich quinoa diversity. Already the intensification of its production in the Andean region has shown to bring negative impacts to the environment that have been widely observed and discussed (Aroni et al. 2008; Jacobsen 2011, 2012; Reynolds et al. 2008; Winkel et al. 2012; Bedoya-Perales et al. 2018). In this case, productive cultivars could not only intensify agriculture but also reduce the existing diversity within the species and within the production system as it has been observed both in Peru and Bolivia. On the other hand, the development of new varieties with improved yields and desired traits for the market can bring positive impacts to farmers' economy provided they are addressed to sustainable production systems. Thus, with the quinoa boom, studies showed that farmers improved their quality of life with intensification but also increased inequality and reduced the use of their local agrobiodiversity (Astudillo and Aroni 2012; Avitabile 2015; Bedova-Perales et al. 2018; Núñez de Arco 2015). The use of high-yielding cultivars could change farmers' priorities, increase dependency and vulnerability, and compromise traditions, sustainability, resilience, and food sovereignty of small-scale farmers. New high-yielding cultivars may also have the potential to be more competitive and replace the Andean quinoa products in the global market (Altieri 2009; Ficiciyan et al. 2018).

The use of biotechnology in agriculture has been controversial when it has been related to the development and use of GM cultivars. In the case of quinoa, this technology has not been developed yet, but it is already considered. Genetic improvement through transgenic methods has to include a comprehensive biosafety assessment for precautionary risks to the health of humans and the environment in the resulting varieties, which are then subjected to strict regulations. These processes take long time and are usually a subject of strong debate. They also imply extra costs: besides the compliance costs for regulatory approval, extra costs also include the delaying process between approval and commercialization (Bairagi and Mohanty 2017; Smyth et al. 2017).

Biotechnology tools have the great advantage of being precise. In breeding, they offer the possibilities of targeting and using markers to reduce traits such as the saponin contents of grains. At present, one of the major breeding objectives to improve quinoa is to obtain saponin-free cultivars (Jarvis et al. 2017). However, there can be trade-offs since saponins play a role in plant defense systems (Francis et al. 2002; Troisi et al. 2015), and resulting plants could in return be more susceptible to disease and pest attacks.

There seems to be an incompatibility between technology and traditions. Unfortunately, this can restrict the genetic improvement (even if it is accomplished with non-transgenic methods) but also the recognition to the cultures that conserved this grain. Therefore, alternatives that make possible the co-existence of technology and tradition may be the way to overcome these constraints. It is fundamental that technology developers recognize that culture and tradition gave rise to the existing diversity and that Andean populations realize that rationally used technology can enhance their resilience. More communication, dialogue, and understanding are needed in order to solve these gaps.

## 5.6 Conclusions

Quinoa went through a rapid global dissemination. For thousands of years, this grain remained traditionally produced and conserved in the Andean region, and in only 30 years, it was introduced to 106 countries. Nowadays (2020), this grain can be found in more than 120 countries around the world.

The use of technology had different priorities and contexts of application, and this was also the case for biotechnology. While countries where this plant was recently introduced used biotechnology intensively to understand the species evolution, abiotic stress, and strategies for its genetic improvement, traditional countries mainly addressed it for germplasm characterization and interesting examples of bioinput development. Most biotechnology applications involved genetic markers. The recent release of the genome, complemented with different areas and tools in development, such as omics and bioinformatics, may give rise to a fast and efficient development of multiple cultivars.

Biotechnology can be used for development in different ways, and it should be recommended in each country when it proves to bring solutions for the local agriculture without harming the environment and human health. It has the potential to improve the productivity of this crop through the development of new cultivars and bioinputs. With biotechnology, a crop as resilient as quinoa has the potential to become a model to understand complex processes of tolerance in different crops. The areas of genomics and related omics are useful to understand and describe processes and pathways limiting production, which can be improved through marker-assisted selection or non-transgenic techniques such as TILLING.

With climate change, more attention is given to underutilized crops as a source of genetic resilience. As such, they are now called crops for the future. From being an underutilized crop, in a short period of time, quinoa has the potential to become a

global staple food. Tools and products of biotechnology have to be managed with responsibility to avoid processes of culture and agrobiodiversity loss.

Biotechnology is relevant for the management and development of new cultivars to face climate change and to contribute with high-quality products addressed to different markets. The use of these technologies should support food security more than the development of patents for seed companies to avoid a monopoly and appropriation of natural processes and genetic resources.

Higher impact of biotechnology use may be reached when it is integrated and complemented with other strategies, technologies, and species in order to improve quinoa production systems and support the diversification of products along the value chain. Multidisciplinary and multisectoral collaboration may expand possibilities to provide tangible ways to share the resulting benefits and to achieve a more sustainable future for all.

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