Ajit Varma *Editor*

Biology and Biotechnology of Quinoa

Super Grain for Food Security

Springer

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Preface

Quinoa, a vegan staple, has grown popularity globally. To be vegan means adopting a plant-based diet, which excludes animal foods like meat, poultry, seafood, eggs, dairy products, gelatin, etc. A vegan diet includes whole grains, vegetables, legumes, nuts, and fruits. The term vegan was conceptualized in 1944 by Donald Watson in Britain, who was deeply moved upon seeing frightened animals killed at a farm. Watson gave up consuming meat and dairy, advocating a plant-based diet, which was both compassionate and healthier. A vegan diet offers considerable health benefits, like a lower risk of developing type 2 diabetes, heart diseases, cholesterol, hypertension, and some cancers—a 2019 Lancet review found a plant-based diet could prevent 11 million deaths due to such diseases annually. Veganism also benefits the Earth's health—the UN Food and Agriculture Organization (FAO) finds the global livestock industry produces up to 14.5% of greenhouse gas emission, cattle farming for meat and milk producing 65% of these emissions. Surveys find switching to a vegetarian diet could reduce emissions by 33% while veganism could reduce emissions by 40%.

Mahatma Gandhi, who would certainly be vegan today, always questioned what he ate. This made him aware of cruelties involved in the production of milk, which he tried to stop consuming. The lack of alternative proteins, like soy products, at that time forced him back to milk, but he never lost a sense of guilt about it and was always mindful of the animals he depended on. Although he was drinking goat milk, he would never support the mass production of milk from animals fed with hormones and sometimes antibiotics to increase yields, penned up all their lives, drained by machines. The real impact of veganism lies in making us think about these issues. Where does the food we eat come from? How is it produced and does it have to be that way? Even if we don't want to drop animal foods entirely, we can limit our consumption to products sourced in relatively less cruel ways. Plant-based alternatives to meat, milk, and eggs help us limit animal consumption, and with many being delicious in their own right, in addition to having their health and environmental benefits, it is even easier. And when, as that menu scan shows us, so many options have their origins in India any way, it mirrors how easily vegan food can become an accepted part of our diet. It can be all too easy to set these reasons against each other, pointing out, for example, high cost of many vegan products or the way big food companies have jumped onto the trend. Or even how food products intrinsic to veganism, like tofu, can become suspect due to concerns

over how rainforests are being cleared for soybean farming. Vegans thus both end up arguing with themselves and with opponents who delight in trying to pick holes in their arguments.

Quinoa (*Chenopodium quinoa* Willd.) has gained recent popularity mainly due to its attractive nutritional profile and its ability to grow under extreme conditions, such as salinity, acidity, drought, flooding, and frost, as well as the functionality of its component. Starch is the main component of quinoa grain which constitutes up to 60% of the dry grain and plays a crucial role in the functional properties of quinoa. Quinoa starch granules are small, polygonal, and in the range of 0.5–3 μm, with unique physicochemical properties. These unique features have created research interest in the application of the quinoa starch for functional products such as stabilizer for creating Pickering emulsions. Chapter 15 summarizes the application of quinoa starch granules in native and modified forms as particles in the stabilization of Pickering emulsions.

In planning this volume, invitations for contributions were extended to leading national and international authorities working with symbiosis. The editors would like express sincere thanks and appreciation to each author for his/her work, patience, and attention to detail during the entire production process. It is presumed that the reviews, interpretation, and concepts proposed by the authors will stimulate further research, as the information presented tends to highlight both the need for further work in this challenging field and lack of agreement on some fundamental issues.

This book contains 20 independent chapters. The total number of contributing authors is 57. The editor is thankful to Dr. Sabine Schwarz, Executive Director, Life Science and Biomedical Books, Springer, Germany, Ms. Aakanksha Tyagi, Senior Editor, Life Sciences Books, Dr. Veena Perumal, Springer, India, and Dr. Naren Aggarwal, Editorial Director, Clinical Medicine, Biomedicine and Life Sciences (Asia), for their continued interest and active help.

We wish to acknowledge the help and support given to us by our students, faculty colleagues, family members, friends, and secretaries and thank them for their constant encouragement.

Noida, India Ajit Varma
July 25, 2020

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Quinoa's Spreading at Global Level: State of the Art, Trends, and Challenges

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Didier Bazile, Maria Cristina Biaggi, and Byron Jara

Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledonous herbaceous plant of the Amaranthaceae family. Its center of origin is in the Andes Region in South America. Across the region, an important biodiversity of the species has been maintained mainly for cultural reasons. The ancestral cultivation areas are found in the Southern Altiplano of Bolivia and the Puno Region in Peru, near Lake Titicaca (between 3650 and 4200 m above sea level).

Until 50 years ago, the production was exclusively located in South America. The United States started to produce in 1947, and Europe continued the expansion process in the 1970s. Since the 2000s, the cultivation of quinoa has expanded globally due to the recognition of its nutritional value and its ability to adapt to different geographic regions. Its consumption was promoted in many countries with food safety problems due to its high content of proteins, lipids, vitamins, and minerals together with an excellent balance of essential amino acids. In addition, it has the possibility of being cultivated in a wide diversity of agroecological environments, especially because it is tolerant to frost and drought and grows even in saline soils. In most countries, it is grown in small areas and with very little use of chemical inputs.

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The increase in the worldwide quinoa area is related to an increase in scientific knowledge, especially in crop agronomy for different environments. Currently, the main trend is the breeding of plant varieties adapted to specific conditions in countries with growing demand. Consumption focuses on pearlized and saponin-free grain and increasingly in processed products. A market niche for organically produced quinoa with a designation of origin is stable. The upward trend in quinoa consumption will continue in industrialized countries because it responds to a structural process associated with changes in eating habits.

Quinoa is a food of the future, not only because it is a crop to improve food security in some countries but also because of its ability to develop in regions of the world where climate change has weakened the conditions for agriculture. However, this increase in quinoa production at a global level requires efforts in protecting and preserving its biodiversity in situ in the Andean region.

Keywords

Quinoa · Agrobiodiversity · Cultivation · Trends · Global spreading · Andes · World

1.1 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an annual herbaceous plant belonging to the family Amaranthaceae, where the Chenopodioideae represents a subfamily with 26 genera. In the tribes of Atripliceae C. A. Mey. (Syn. *Chenopodieae* Dumort.), Fuentes-Bazan et al. (2012) have included here also the genus *Chenopodium* and related genera, such as *Chenopodiastrum*, *Lipandra*, and *Oxybasis*.

The species was domesticated in South America with a center of origin located near Titicaca Lake between Peru and Bolivia. Its high content of proteins, lipids, vitamins, and minerals and an excellent balance of essential amino acids (Hernández-Ledesma 2019) characterize the grain of quinoa.

The main production areas are in the Andean highlands, in the driest regions of the continent. During many years, Bolivia was the main exporter, but Peru nowadays is a strong competitor. In Bolivia, the largest areas of cultivation are concentrated in the Southern Altiplano (between 3650 and 4200 m above sea level), expanding to the Central Altiplano and other territories after the increase in demand for the grain in the three last recent decades. In Peru, quinoa crop is cultivated ancestrally in the Altiplano of the Puno Region and in recent decades with a significant growth of the surface toward the inter-Andean valleys and other zones of the country at low altitude. Like the potato tuber, quinoa crop was one of the main foods of the pre-Inca Andean peoples.

The special interest in Quinoa is favored because it is a crop with an extraordinary capacity to adapt to different geographical areas and a diversity of agroecological environments. It is a highly rustic plant with great tolerance to frost (Jacobsen et al. 2005), soil salinity (Hariadi et al. 2011), and drought (Razzaghi et al. 2011).

Consequently, regions with low food production, where soils are saline and water is scarce or of low quality, have begun cultivation with relative success in recent decades (Bazile et al. 2016b; Nanduri et al. 2019).

1.2 The Origin of Crop

Initially, the center of quinoa's genetic diversity was identified in the Southern Altiplano of Bolivia (Gandarillas 1979; Wilson 1988). In later years, Christensen et al. (2007) noted that the center of genetic diversity was located further north in the Central Altiplano area around Lake Titicaca between Peru and Bolivia at an altitude of 4000 m above sea level. These regions are mountainous and are characterized not only by high altitude but also by scarce and irregular rainfall, which means a limited agricultural productive capacity.

Molecular evidence suggests that genetic erosion—or loss of genetic diversity—was affected by at least four events (Fuentes et al. 2012). The first would have occurred in the initial polyploidization stage, when the two diploid ancestors of quinoa hybridized to generate tetraploid descendants in nature. The second occurred when quinoa was domesticated by Andean societies from its wild tetraploid relatives, in search of a plant suitable for agriculture, 7000 years ago (Bazile 2015; Jellen et al. 2015). Through long cycles of seed exchange and cultivation in new territories and climates, there was a wide range of morphological modifications, such as condensation of the inflorescence at the terminal end of the plant, increase in plant and seed size, loss of seed dispersal mechanisms, and high levels of pigmentation (Mujica et al. 2001). At this stage, quinoa became a central element of the agricultural and food systems of this region, being present in religious rituals and other daily aspects of Andean societies.

The third event of loss of genetic diversity began more than 500 years ago during the time of the Spanish conquest, when quinoa was culturally stigmatized as food for indigenous communities (Cusack 1984). European grains were imposed on colonized populations, and the food culture related to quinoa declined, considering it as food for animals and the poor. The central role of this plant within religious ceremonies and local daily life was what prevented its disappearance.

The recent history of quinoa suggests a fourth event caused by human migration from rural areas of the high Andes to urban centers and coca-growing regions of the eastern foothills, resulting in abandoned quinoa fields and loss of quinoa germplasm (Fuentes et al. 2012).

1.3 Cultivation and Expansion Worldwide

1.3.1 Quinoa's Global Spreading During the Last 30 Years

In the first stage, more than 5000 years ago, the limits of the geographical extension of quinoa were limited to only a few regions of the Andean countries. Since its domestication around Lake Titicaca, and during the course of human migrations, this was progressively adapted by farmers to other ecological and social contexts of cultivation, extending to new territories in Bolivia, Peru, Chile, Argentina, Ecuador, and Colombia along a latitudinal gradient that extends from 5°S to 30°S, although it is a truly common crop until 20°S.

Comparing the types of quinoa across the regions where it is grown allows for differentiation of ecotypes according to their agro-morphological characteristics and adaptation to the ecosystems. In this regard, Tapia (1996) was the first who proposed the differentiation of quinoa into five major groups according to their characteristics of adaptation to the different agroecological conditions in the Andes:

- The quinoa of the Northern Altiplano of Lake Titicaca (Peru and Bolivia), which has a short growth period and develops with rainfall that varies between 400 and 800 mm and minimum temperatures of 0 °C.
- The salt flats quinoa in the Southern Altiplano (Bolivia, northwest Argentina, and northern Chile), adapted to saline soils and having a larger grain size, are found in areas with less rainfall (250–400 mm) and minimum temperatures of −1 °C.
- The quinoa in the inter-Andean valleys (mainly in Peru and Ecuador), in mesothermal zones with rainfall varying between 700 and 1500 mm and minimum temperatures of 3 °C.
- Coastal or sea-level quinoa is a smaller, dark-grained plant grown in central and southern Chile with rainfall of 500–1500 mm and minimum temperatures of 5 °C.

The quinoa of the Yungas or subtropical zone, on the eastern slopes of the Andes in Bolivia, with rainfall that can reach more than 2000 mm and minimum temperatures of 11 °C.

During the second half of the twentieth century, the number of countries where quinoa is experimented or cultivated grew rapidly from the 7 that make up the Andean region (Argentina, Bolivia, Chile, Colombia, Ecuador, Peru, and Venezuela) to more than 100 in different climatic zones of the planet. Today in 2020, we identified more than 125 countries at global level. This worldwide expansion presents at least two main stages.

The first includes the main consuming and importing countries—the United States, Canada, France, the United Kingdom, Denmark, and the Netherlands—which sought to adapt the crop to their environments. The United States became interested in this grain as early as 1948 and in the early 1970s conducted experiments in southern Colorado with seeds of Chilean origin (Johnson and Croissant 1985). In Canada, it is grown on the plains of Saskatchewan and Ontario, which are traditionally grassland or cereal-producing areas. In this first stage is the introduction of

quinoa in Europe in 1978, also with germplasm from Chile (Universidad de Concepción in Chile) that was taken, selected, and tested in Cambridge (England) and in the Loire Valley (France) (Bazile and Baudron 2015). This Chilean germplasm plus the Andean germplasm collected in 1982 by Galwey and Risi generated the basis for the Cambridge University breeding program under the leadership of Nick Galwey (Fleming and Galwey 1995; Galwey 1989, 1993). From Cambridge, quinoa was distributed to Denmark, the Netherlands, and other European countries (Risi and Galway 1991).

A series of events spread the word about quinoa. For example, the International Board for Plant Genetic Resources, a member institution of the CGIAR (now *Bioversity International*), organized the first regional meeting on plant genetic resources of agricultural interest in the Andean Region in April 1981. Later, in 1993, a European Union project began field research with quinoa in England, Denmark, the Netherlands, and Italy, as well as laboratory tests in Scotland and France. Nevertheless, probably, the most important project in the 1990s, which explains the worldwide expansion of quinoa, is the one that started in 1996 with a shared coordination between the Danish International Development Agency (DANIDA) and the International Potato Center (CIP) in Peru. The aim of the project was to learn about the state of the art of quinoa and to carry out multiple experiments at a global level. Through this first international cooperation network, there were field trials in new countries such as Sweden, Poland, Czech Republic, Austria, Germany, Italy, and Greece (Iliadis et al. 1997). This initiative significantly increased the links between researchers and the number of research centers involved with the quinoa issue in developing and developed countries. It is from this period the research work carried out by Denmark and the Netherlands resulted in the first European variety of quinoa, Carmen, and continued with research aimed at reducing the saponin level of the grain.

The second stage of worldwide dissemination of quinoa began in recent years, where global climate changes were a reason for the advance of research in new countries. Quinoa is nowadays presented as a response to worsening environmental conditions, particularly in semiarid areas where intensive use of groundwater causes soil salinization. This is how the Asian continent enters the picture, with India and China (Bhargava et al. 2006; Xiu-shi et al. 2019) as well as Pakistan (Munir 2011). The Mediterranean region corresponds to the latest important step in the expansion of quinoa and links numerous partners from European Union countries (Italy, Portugal, the United Kingdom, the Netherlands, and Denmark) and Mediterranean countries (Turkey, Morocco, Egypt, Syria) (Benlhabib 2006; Pulvento et al. 2012).

In 2013, at the initiative of the Plurinational State of Bolivia, the International Year of Quinoa (IYQ) was celebrated with the aim of focusing world attention on the role of quinoa in food security and poverty eradication in support of the achievement of the Millennium Development Goals. The IYQ promoted awareness of the benefits and qualities of quinoa and a recognition of so-called underutilized species (NUS), which play a key role in food and health because of their high nutrient content and have characteristics that allow them to adapt to different ecological conditions (Bravo et al. 2010). The IYQ has had a positive impact, which is projected in an

Table 1.1 Number of Andean and non-Andean countries by first year of planting

First sowing date	<1935	1935–1954	1955–1974	1975–1994	1995–2012	2013–hoy
Andean countries	6	6	6	6	7	7
Outside Andes	0	2	2	13	38	118

Source. Adapted from Bazile et al. (2016a)

increase of research in numerous institutions around the world, in an extension of the areas of cultivation, and in an increase in consumption, especially in the United States, Europe, China, and India. The appearance on the market of numerous innovative products containing quinoa is one of the impacts of the IYQ, with the possibility that quinoa will become a food of the future.

Between 2013 and 2015, new countries received technical assistance from the FAO as part of the strategy to strengthen global food security through the cultivation of quinoa. Further, 26 countries benefited from 6 regional or local projects (Algeria, Egypt, Iraq, Iran, Mauritania, Sudan and Yemen/Djibouti, Ethiopia, Kenya, Somalia, Southern Sudan, Uganda and Zambia/Burkina Faso, Cameroon, Niger, Senegal, Chad, Guinea and Togo/Sri Lanka and Bhutan/Kyrgyzstan and Tajikistan/Morocco).

The following table lists the number of Andean and non-Andean countries by first year of planting (Table 1.1).

1.3.2 Current Status of the Production in Bolivia and Peru: The Two Main Producers at Global Level

Bolivia and Peru are the world's leading producers of quinoa. It is estimated that there are about 70,000 quinoa producers in Bolivia and another 60,000 in Peru, with production predominantly at the household level.

Peru, unlike Bolivia, decreased the areas of quinoa cultivation gradually in recent centuries after the appearance of new foods brought with the Spanish colony. However, it was maintained continuously in the Puno Altiplano in the ancestral systems or aynokas, in rotation with other crops, and preserved the quinoa and its wild relatives because of its nutritional, medicinal, and religious value (Mujica and Jacobsen 1988; Aguilar and Jacobsen 2006; Mujica 2008, 2011; Gómez Pando et al. 2014). It was also possible to find it in the inter-Andean valleys, where quinoa is planted in association with corn, beans, broad beans, and cucurbits. Although production in these areas used to be important, it was not sufficient to satisfy domestic demand, and, for this reason, Peru was for several decades the main buyer of Bolivian quinoa (Aroni et al. 2009). Currently, it has doubled the area with quinoa, reaching almost 70,000 ha by 2018.

In Bolivia, the area of quinoa remained stable over time. In the Southern Altiplano, the country's most traditional production area, it was planted on the mountain slopes so that it would be less exposed to night frost, and the plains

were used for grazing llamas and sheep, which are more resistant to the cold than crops (Pouteau et al. 2011). After the first quinoa boom of the 1980s, this was changed, and cultivation expanded with the introduction of machinery (Martz 2016; Kerssen 2015).

In both countries, the highest sales flows are recorded in the harvest months. However, due to fractional selling strategies, a part of the production reaches the market throughout the year. In general, producers in Peru and Bolivia sell quinoa at weekly fairs, and in some areas, the practice of barter is still alive, where the unit of measurement is the handful and it is exchanged for vegetables or bread. In these markets, quinoa is not standardized and farmers sell a favorable mix of quinoa varieties. Quinoa wholesalers, who handle large volumes and supply urban markets and agro-industries, meet at local fairs or larger markets, such as the Challapata fair in Bolivia or the Manco Capac market in Juliaca, Peru. These intermediaries buy at the weekly fairs or directly from the communities and, as in many other food chains, have some power in the negotiations.

In the 1990s, the certification of biological agriculture brought added value to this grain, and, more recently, the networks of the fair trade added an ethnic image to the product on the markets. These two processes allowed a better remuneration of the producers and at the same time signified a commitment of the consumer with the agroecological practices of the production.

Depending on the destination, a dichotomy of varieties between registered/improved and traditional varieties is maintained. However, with the recent boom in urban and international demand, agro-industries and exporters are trying to meet the demands of markets that generally require uniform and large grains. This encourages producers to plant certain improved varieties, and this may represent a risk to biodiversity if all export production follows this standardization dynamic.

In Bolivia, the most important production is the *quinoa real* of the Southern Altiplano (Departments of Oruro and Potosi). In its expansion and improvement of commercialization, the presence of the economic farmers' organizations has been fundamental, such as the *Central de Cooperativas Operación Tierra* (CECAOT) and the Bolivian National Association of Quinoa Producers (ANAPQUI), with the support of the *Confederación Sindical Única de Trabajadores Campesinos de Bolivia* (CSUTCB).

In Peru, the export boom began later in 2005 with the production of individual quinoa farmers who do not necessarily belong to a cooperative or association, as it is mainly the case in Bolivia. Faced with the commercial development of quinoa in nontraditional areas and abroad, Andean producers have initiated new ways of valorizing and protecting their products, such as the recognition of denominations of origin and solidarity trade. The diversity of original products, quality seals, alliances, and innovative institutional practices demonstrate the capacity for innovation of producers and the different actors in the quinoa value chain.

The destination markets for regional quinoa exports have changed in the last decade, both because of the emergence of new consumers and the new organization of existing ones. The United States has increased its importance as a destination market, accounting for 56% of imports from Bolivia, Ecuador, and Peru. Due to the

entry of new consumer countries in recent years, the European markets of Germany, France, and the Netherlands along with Japan were losing weight (in percentage) as purchasing countries. Although this occurs in the context of a general increase in the volume traded in the international market, in absolute terms, exports to Europe, especially, have also increased significantly even if the European production is always increasing year after year in different countries.

1.3.3 Current Outlook

For 20 years, Andean quinoa has been a highly dynamic product in world trade. Its consumption has increased globally, especially in the United States, Europe, China, and India. There are several reasons that explain the process, including the high nutritional quality of the grain, the propensity toward healthy eating patterns, the revaluation of ancestral cultures, and the fact that it is a product originated in small peasant farms and the mostly organic condition of the supply.

The "boom" of quinoa had its origin in the demand from North American and European countries for foods with specific dietary qualities and with an ecological origin. The growth of the markets for organic, natural, and family farming products is due to changes in consumption habits and to evidence linking the consumption of refined and ultra-processed foods with "modern" diseases such as diabetes, allergies, obesity, and cardiovascular diseases. This boom meant an increase in regional quinoa exports, with strong and sustained growth from the 1990s onward and an even greater boom since 2000.

Since 2012, there has been an increase in the area of production in the Andean countries and in the rest of the world. For example, Greece, India, Italy, Morocco, and Turkey have gone from having areas of less than 100 ha to crops of between 100 and 500 ha (only 5 years after the IYQ), and China has already reached 17,000 ha (2019) (Xiu-shi et al. 2019). Bolivia and Peru continued to increase the area of quinoa production after the IYQ, reaching maximum areas in Peru in 2018 and Bolivia in 2015, with 67,000 ha and 121,000 ha, respectively (FAO STAT 2020).

Quinoa production systems at the global level are generally characterized by small areas of cultivation (with an average of 2 ha), and, in general, the increase in the area of Quinoa at the global level is directly related to the increase in the number of farmers, both in the new producing countries and in those that started cultivation before the twenty-first century. Canada, France, and Turkey, which in 2012 had less than 100 producers, have moved to the stratum of 101–500 producers in 2018. Other examples are China and Colombia, which increased their number of producers to more than 1000 (5 years after IYQ). In the new producing countries, quinoa is added mostly as a crop for the diversification of production systems and in some cases as a replacement for another crop, which can be explained as an adaptation strategy for agriculture in these countries to the strong effects of climate change.

In these years, there have been significant advances in agricultural research with the development of new improved varieties and innovations for postharvest tasks

and the industrialization of the grain. The appearance on the market of many products containing quinoa after 2013 gives the prospect that this will become a food of the future, both for its high nutritional value and for its great genetic diversity that allows it to adapt to different agroecological conditions. However, this increase in quinoa production and consumption requires the protection and conservation of existing genetic biodiversity in situ in the Andes region and the need for specific mechanisms.

1.3.4 Who Is Doing Quinoa Research?

Universities and research centers are the main institutions related to quinoa expansion in new areas of production at the global level. There are mainly government research centers dedicated to genetic improvement and crop adaptation to new environments. Private companies and producer organizations are most linked to experiments with postharvest technology. In some areas, there are some key farmers dedicated to the improvement of the crop value chain from the seed selection stage to the search for alternatives to process quinoa.

For facilitating the dissemination of research results for sharing experiences, there are networks of experts made up of members of research centers and universities, local government staff, and farmers. In some cases, expertise is found in international agencies such as the FAO, and these people have to be connected with these scientific networks like the Global Collaborative Network on Quinoa (gcn-quinoa.org).

In the case of the Andean region, national and local governments are the other important participants in the promotion and research processes as well as several Non-Governmental Organizations (NGOs) and producer organizations. Farmers' organizations and NGOs were the first to develop the quinoa value chains in Bolivia during the 1980s and the 1990s, when government services appeared during the past 20 years, considering the importance of quinoa as an export product for the commercial balance of the country. It was the same in Peru with important programs managed by the two ministries of agriculture and economy, which are involved for supporting the development of the Peruvian quinoa value chain for the export markets.

After the IYQ in 2013, there was a real boost to quinoa in the Andean countries through the implementation of public policies toward the sector, with government investment in technological innovations and support to networks of experts and institutions for the improvement of quinoa production and commercialization, with emphasis on the sustainability of the crop. The main public policy of these countries is technical assistance to peasant family agriculture from both national and local governments, especially in areas where landowners had migrated and returned to their communities to cultivate the land.

As a result of the IYQ, the International Quinoa Centre (CIQ, *Centro International de la Quinoa* in Spanish) was established in August 2013 with headquarters in Oruro, Bolivia. Its objective is to develop scientific and applied research on quinoa

and related species and to implement appropriate technology to increase yield and sustainable production. Among its current activities is the systematization of local knowledge and know-how to face the extreme phenomena of climate change and the elaboration of resilience studies of production systems, with emphasis on the existing ancestral knowledge in the Central Highlands of Bolivia.

In the process of expanding sustainable quinoa production globally, the Global Collaboration Network on Quinoa (GCN-Quinoa) was established in 2015. This network seeks to create a collaborative space that facilitates exchanges between producers, processors, distributors, politicians, and all those involved in the development of quinoa at the global level. The main challenge of GCN-Quinoa is participatory plant-breeding program, where researchers and producers share knowledge of field experimentation in a diversity of agronomic systems and in a wide range of physical and environmental environments. It is currently made up of 300 researchers from 75 countries; Dr. Didier Bazile at CIRAD (France), the founder, assumes the facilitation.

1.3.5 Research Topics

The main research topic in the different countries is the adaptation of quinoa to new environments, and in the countries with more experience with the crop, there are today programs of genetic improvement. For example, China has had a breeding program since (1984), and this can be seen today in 18 certified varieties released (Xiu-shi et al. 2019; Guiying 2020). In the Netherlands, Wageningen University & Research (WUR) has developed sweet and short-cycle varieties adapted to European conditions. In Denmark, three varieties (Vikinga, Puno, and Titicaca) are registered, marketed, and disseminated at global level with good results in crop adaptation. In these two countries, the varieties are disseminated through the European catalogue and with the prerogatives of the Union for the Protection of New Varieties of Plants (UPOV) system. In the United States, several groups of researchers are dedicated to the breeding of quinoa varieties: currently, the Wild Garden Seed and Washington State University (WSU) companies have new materials in development, and the varieties can be shared through the seed network under open access (Open Seed Source Initiatives) (Chevarria-Lazo et al. 2015; Luby et al. 2016).

The planting of quinoa outside the Andean region faces difficulties for its production, such as heat during the flowering period that leads to irreversible stress (Hinojosa et al. 2019). The countries of the Mediterranean and the Middle East are very sensitive to this problem of temperature especially for cultivating quinoa as an alternative crop in marginal environments (Bazile et al. 2016b). In some cases, they are looking for alternative planting dates or new varieties that are more tolerant to high temperatures and drought, in addition to salinity stress (Rezzouk et al. 2020). Considering the high constraints increasing due to the effects of climate change, there are a number of new producing countries (Egypt, the United Arab Emirates, Iran, Kenya, Lebanon, Malawi, Morocco, etc.) that are working on the development of quinoa varieties adapted to the extreme environmental conditions of their

countries. This pathway in quinoa research will guide the next decade for new investigations.

1.4 Trends and Consequences

The global interest during the past 5 years after the IYQ shows four main trends for the next future. First, the expansion of cultivation areas outside the Andean region will increase with some new country producers able to produce enough for putting new volumes into the international market. Second, and for the moment, the global production is always in the hands of a greater number of producers, generally planting small areas and with family farming characteristics and practices. Third, the constant increase in global consumption, especially in Europe and the United States, will benefit the Andean countries because they will continue to be attracted to quinoa associated with cultural values and derived from organic and agroecological production. More of their trade links will be established over time with local organizations under fair trade agreements and mechanisms that will be maintained. Fourth, the increase in cultivation at global level is ongoing with an increase in scientific knowledge. The novelty of this new research is that it is developed and centered on environmental conditions and social and political issues, which are not linked to the Andean context.

The outlook for increased demand in the coming years continues to be favorable because it responds to processes of a more structural nature associated with trends and more general changes in consumption patterns for natural products at global level. These changes increasingly favor foods that have healthy nutritional characteristics and offer guarantees of health and safety. They are often linked to certain special nutritional characteristics (proteins, minerals, etc.), such as the fact that they are organic products or the expression of cultural traditions of recognized value.

The growth in the production and consumption of currently has many scientific institutions dedicated to basic and applied research at the global level. There is a clear interest in promoting and funding research from national governments, both in the Andean countries and in those with food insecurity or problems due to lack of productive land for other crops.

With the exception of areas where quinoa was seeded in large areas with commercial varieties and intensively in Peru and Bolivia, there are no significant changes in production systems between 2012 and 2018. In general, quinoa is produced on small plots in the new producer countries and with low use of agrochemicals and other inputs. The use of irrigation in the new producer countries and in the Andean countries that have intensified their agriculture appears to be the main innovation for ensuring the production in arid areas. In those countries, no conventional water and deficit hydric irrigation are two ways for adapting quinoa in these areas with water scarcity.

The main trend in the last 5 years is certainly the use of improved varieties. This is happening especially in the new producer countries because they have a very limited

access to the Andean genetic diversity of quinoa for cultivation. However, it is also a current trend in the Andean countries that have expanded their area and in production systems with more entrepreneurial characteristics. These agricultural systems with improved varieties have higher production costs.

To accompany the feasibility of these systems, they are looking for adding value to the quinoa grains, and consumption of processed quinoa has grown globally. The same may occur with other more specific uses, such as quinoa starch, quinoa flour, or quinoa in the form of milk. Many of these products currently have specific market "niches" in Europe and the United States, but it is likely that the consumption of "processed quinoa" will become more widespread in the coming years.

The IYQ has made quinoa in all its forms more accessible, both to people in the Andean region and to countries with food security problems. Five years after the IYQ, quinoa crop offers the opportunity to maintain and expand the spread and sustain its expansion dynamic. That is because quinoa could become one major food of the future, both because of its great genetic diversity that allows it to adapt to different agroecological conditions and because of its high nutritional value. However, this increase in quinoa production at the global level requires the protection and conservation of existing genetic biodiversity in situ in the Andean region, both of native cultivars (landraces) and of their crop wild relatives. Plant genetic resources have great value for the adaptive evolution of quinoa to different environments, where this crop is a priority for solving food security problems such as those triggered by climate change.

Certification of organic agriculture related to fair trade markets allowed a better remuneration of the producers, and at the same time, it means a commitment of the consumer with the agroecological practices of the production. However, if the crop continues to spread, there will be a need for international recognition of the Andean identity of quinoa's genetic resources and of the contribution of the peoples of the Andes to the conservation of their biological diversity and to the contribution of knowledge to its production.

Thinking globally about the sustainable use of quinoa biodiversity could facilitate a paradigm shift in agricultural models, taking more account of nutrition as an approach to agricultural development. Linking food security to the use of water and energy in agriculture, to aspects of health for farmers and consumers, and to the protection of biodiversity in agroecosystems for adaptation to climate change is probably the key for tomorrow agriculture where quinoa is a model crop (See Bazile 2020, Chapter 5).

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Taxonomy, Morphology, and Life Cycle of Quinoa

2

Ajit Varma and Aditi Jain

Abstract

The genus *Chenopodium*, originally a native of Peru and Bolivia, includes several species that are domesticated for human use, and some are even common, well-known weeds that have spread to different continents in a short span of time. Several *Chenopodium* species have the ability to survive and grow in harsh environmental conditions such as nutrient-depleted soils, water scarcity, saline soils, and even high-altitude mountains which are usually incapable of supporting vegetation due to extremely low temperatures and strong winds. This behaviour of *Chenopodium* suggests that it has exceptionally tolerant and multifunctional plant-stress response mechanisms incorporated stably in its genome. Some species are gaining popularity and demand due to their high protein content, low glycaemic index, and gluten-free compositions, which makes them important members of 'superfoods' in the human food diversity.

Keywords

Quinoa · Morphology · Habitat · Growth · Phenological growth · Life cycle

The genus *Chenopodium quinoa*, known as quinoa (Fig. 2.1), has recently been adopted by people worldwide as an alternative to animal-sourced protein food products because of the added benefits which are not provided by meat and poultry. Plant-based protein is becoming an important market as most of the teenagers and young adults are shifting towards 'vegan' diet plans, which offer negligible risks of allergies, hormonal imbalances, and even certain cancers.

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Fig. 2.1 *Chenopodium quinoa* plant (inserted with permission from open access, Baioumy et al. 2018)

The preferred common name of *Chenopodium quinoa* is quinoa, and internationally, it is also known as *quinua* (Spanish) and *Chenopode quinoa* (French) (CABI datasheet n.d.).

This chapter aims to introduce diverse morphological features that occur in large number of quinoa species, that allow members of this genus to exhibit some novel abilities to survive in varied climatic conditions. Different stages of the life cycle of a basic plant of *Chenopodium quinoa*, during which the growth of all vegetative and reproductive parts and organs occurs, must be understood in order to conduct research at different maturation stages of the plant, from sprouting to senescence.

2.1 Taxonomy of Quinoa (CABI Datasheet, 2020)

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Caryophyllales
- Family: Chenopodiaceae
- Genus: *Chenopodium*
- Species: *Chenopodium quinoa*

2.2 Morphological Features

Chenopodium quinoa is a temperate or subtropical plant that is cultivated in areas with temperature up to 35 °C. Based on several anatomical studies, scientist Zvietcovich (1976) concluded that it is in fact a C_3 plant. In certain parts of the world, it is also observed that a temperature above 35 °C caused sterility in this plant (Jacobsen and Stolen 1993).

2.2.1 Vegetative Morphology

The plant height ranges from 0.5 to 3 m from seedling stage to the fully developed stage, with an average of 1.0–1.5 m. Being an annual spring crop, it exhibits several degrees of branching, which is governed by many genetic and environmental factors (Jacobsen and Stolen 1993).

The basic colours in which Quinoa plant is found are green, purple, and red. There is a possibility for the green plants to turn white, yellow, orange, or red when reaching maturity (Koziol 1993; Jacobsen and Stolen 1993). The purple ones may stay as it is or change to yellow, but the red varieties remain red throughout their life. The pericarp may also range in colour from white, yellow, orange, and red to brown and black as well. Wild species are often observed to have black pericarp, and the spectrum of colours in different parts of the plant is controlled by various compositions, including antioxidants, pigments, etc. (Jacobsen and Stolen 1993).

2.2.1.1 Quinoa Plant Stem

The stem morphology is divided into two parts: one where the stem becomes cylindrical below the root neck and one where it becomes angular with alternating leaf positioning originating from four sides in turn below the root neck. It is erect and thick, and has a cutinized epidermis. Inside the stem, a fibreless mass, which is a cream-coloured marrow, is found. Before maturation and during the early stages of growth, this marrow is massive and soft, but as the plant comes close to maturation, it becomes hollow and spongy. The major length of the stem is achieved after a

minimum of 2 weeks, and the cortex is mostly firm and compact (Jacobsen and Stolen 1993).

The outer stem can be of various colours, mostly green, with axils that can be coloured but mostly occur in red (Gandarillas 1979b).

2.2.1.2 Branches

Originating from axils from each leaf on the stem, branches may largely vary in length, from a few centimetres to nearly the length of the main stem of the plant. The length depends upon the cultivar, conditions provided, minerals, and other environmental conditions (Fig. 2.2).

The two extreme types of the plants are classified as 'simple', which is the variety with no long branches, and 'branched'. Branching, during vegetation practices, is undesirable for grain production. Hence, to maximize the grain yield it is not preferred. They are minimized through various cultivation techniques (Jacobsen and Stolen 1993).

Fig. 2.2 Branched (left) and unbranched (right) plants of quinoa (inserted with permission, Jacobsen and Stolen 1993)

2.2.1.3 Quinoa Roots

Quinoa has a vigorous taproot system, which is deep rooting. Activities like seedling emergence, including root elongation and root hair production, occur quickly under adequate soil moisture conditions as there is no known variety of quinoa that exhibits seed dormancy. The taproot, below the root neck, divides to give rise to secondary and tertiary roots (Jacobsen and Stolen 1993). The reason for quinoa drought resistance and rare lodging is mainly the deep, branching root systems (Gandarillas 1979b).

2.2.1.4 Leaves

Quinoa plant leaves are thick, fleshy, broad, and smooth. The leaves are grooved on their upper part, thin, petiolate, and arranged alternatively. Leaf blade is ovatus in shape, obtususapix, breveaugustatus base, having sinuate margin.

The leaf blade is usually large, rhomboid, and triangular broad in the lower part, whereas they are small and lanceolate in the upper region. The edges of the leaves are sinuate. The blade is polymorphic in the same plant, rhomboid, triangular or lanceolate, flat or wavy, quite thick, fleshy, and tender. Rich amount of calcium oxalate is found in the leaves, which makes them appear gritty on the surface. Commonly the laminae are plane, but in certain varieties they may be undulating (Gomaa 2014) (Fig. 2.3).

The leaves possess various observable morphological adaptations that allow them to withstand water scarcity conditions during growth such as cuticular waxy coating, stomata protected by a thickened epidermis, and the presence of papillae on both sides (Jacobsen and Stolen 1993).

Most of the young leaves, stems, and flowers are covered on both sides by a number of white-, purple-, or red-coloured stellate papillae. Because of high calcium oxalate content of the leaves, the papillae act as hygroscopic agents, which aid in controlling excessive evapotranspiration to prevent water loss and support its retention during drought periods (Canahua 1977; Jacobsen and Stolen 1993).

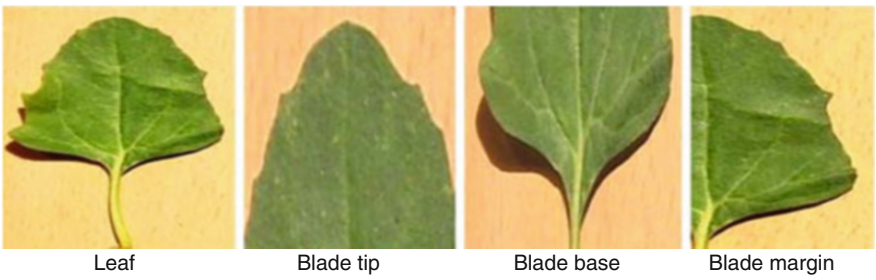

Fig. 2.3 Leaf morphology of quinoa (inserted with permission, Elham F. Gomaa 2014)

2.2.2 Floral Morphology of Quinoa Plant

2.2.2.1 Inflorescence

Inflorescence in the plant can be observed first, after about 7 weeks of sowing the seeds (Gomaa 2014). Inflorescence consists of a number of racemes (panicle), which originate from the top of the axils of the whole plant (Jacobsen and Stolen 1993).

Two types of inflorescence are known: glomerulate, where small groups of flowers (glomeruli) originate from tertiary axes, and amaranthiform, which has glomeruli originating mainly from secondary axes (Jacobsen and Stolen 1993) (Fig. 2.4).

2.2.2.2 Flowers

Chenopodium genus has incomplete flowers without petals. The flowers are hermaphrodite (self-fertilizing) or female. The hermaphrodite ones vary in size, ranging between 2 and 5 mm; occur with a 5-numbered perigonium, a pistil with an ellipsoid ovary, along with a two- or three-branched stigma surrounded by five stamens; and are two-lobed and four-loculed. The female flowers, which are 1–3 mm in size, contain only perigonium and pistil. The flowers can be with or without pedicels. Composed of five green sepals, the perigonium is surrounded by calcium oxalate crystals, and at the centre of each sepal, there is one small vascular bundle embedded in the ground tissue. The epidermal cells of sepals develop trichomes.

The filaments are relatively simple in structure, in which the parenchyma surrounds the vascular bundle and all of the anthers have four pollen sacs (Gomaa 2014).

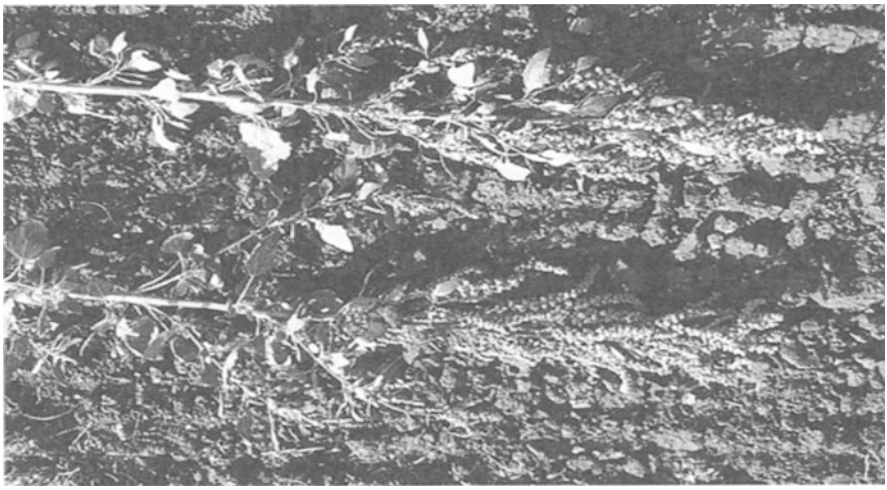

Fig. 2.4 Glomerulate (upper) and amaranthiform (lower) inflorescences of quinoa (inserted with permission, Jacobsen and Stolen 1993)

All flowers usually open within 12–15 days from the onset period of flowering (Gandarillas 1979a), and every flower stays open for about 5–13 days (Erquinigo 1970). Flowering is observed to start at the apex in each glomerulus.

Quinoa can reproduce through both self- and cross-pollination. The degree of cross-pollination varies and depends on several factors in different environments, but in the experiments conducted, it is found to be less than 10% (Gandarillas 1979a; Jacobsen and Stolen 1993).

2.2.2.3 Pollen Grain

Through light micrographs, the general shape of pollen grains is found to be small, circular, and polypantaporate and intectate (apparently pillate to clavate). Under scanning electron micrographs, these pollen grains can be observed to have pores on their surface. The pollens of quinoa are spheroidal in shape and pantoporate, and the surface is found to be flourished with minute grain spinules (Nowicke 1976; Gomaa 2014) (Fig. 2.5).

2.2.3 Fruit and Seed of Quinoa

2.2.3.1 Pericarp

The fruit is covered with a thin-layered pericarp and consists of an embryo composed of two cotyledons, with the plumule part in between and the radicle wrapping with a curvature at the micropylar end, as well as the seed, which envelopes the perisperm like a ring. The pericarp is double-layered, where the cells present in the outer layer are large and papillose in shape. The inner layer is somewhat discontinuous and the cells are tangentially stretched (Fig. 2.6).

The pericarp of the fruit contains saponins, which are compounds widely distributed throughout the plant kingdom, being reported in more than 500 species. Saponins may exert both negative effects, due to haemolytic activity and bitterness, and positive effects, due to cholesterol-binding ability. A haemolytic test showed that the saponin content in different lines of quinoa ranged from 0.02 to 0.51%

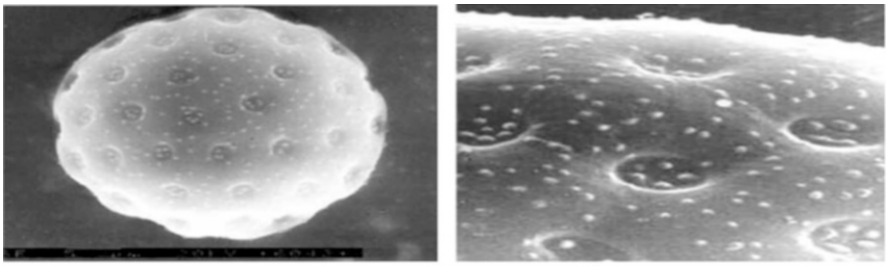

Fig. 2.5 Pollen grain surface sculpture appearance as shown by SEM. (**a**) Pollen grain. (3000×) (**b**) Portion of pollen showing pores and opercula. (10,000×) (inserted with permission, Elham F. Gomaa 2014)

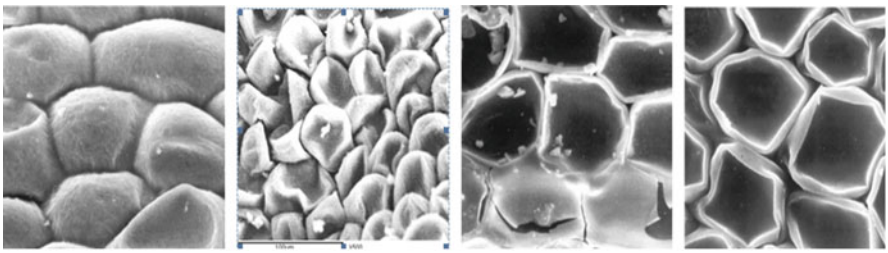

Fig. 2.6 Seed surface sculpture appearance of epicarp cell development as shown by SEM. (10,000×) (inserted with permission, Elham F. Gomaa 2014)

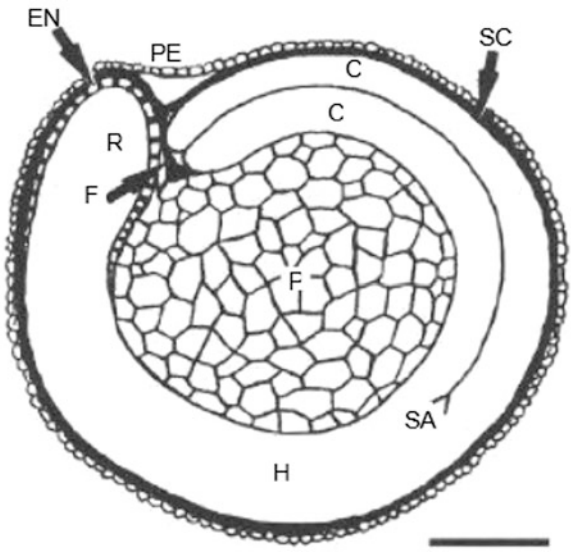

Fig. 2.7 Medial longitudinal section of quinoa seed showing the pericarp (PE), seed coat (SC), hypocotyl-radicle axis (H), cotyledons (C), endosperm (EN) (in the micropylar region only), radicle (R), funicle (F), shoot appendix (SA), and perisperm (P) (inserted with permission, Prego et al. 1998; Abugoch 2009)

(Jacobsen 1992). However, the pericarp, and hence the saponins, can be removed by abrasive dehulling of the seed (Jacobsen and Stolen 1993).

2.2.3.2 Perisperm

The main storage tissue of perisperm is made up of starch seeds or granules. It is polygonal in shape, with thin straight walls and with large aggregates of starch. It consists of non-living, uniform, and thin-walled cells. The cells are full of starch grains which are angular in shape. The nuclei and other cell organelles are not present at this stage (Prego et al. 1998) (Fig. 2.7).

2.2.3.3 Endosperm

Quinoa seed also has a cellular endosperm formed by different layers that completely surround the embryo, from which it is separated by a layer of air. In mature seeds, the endosperm can be located only in the micropylar region of the seeds and consists of one or two cell-layered tissues surrounding the hypocotyl-radicle axis of the embryo. A rectilinear vascular strand runs throughout the funiculus. The endosperm adheres to the seed coat from outside; it is a tissue that contains food reserves for the nourishment of the embryo. Gallardo et al. (1997) suggested that after the seed is hydrated, the cells of the endosperm enter into contact with the embryo, which rapidly consumes it during its growth. The ratio of cell constituents, i.e., the cytoplasm, endoplasmic reticulum, proplastids, and mitochondria is very small. Several protein and lipid bodies occupy most of the cytoplasmic region (Gomaa 2014; Prego et al. 1998) (Figs. 2.8, 2.9, 2.10, and 2.11).

Quinoa seeds may be of different shapes, like conical, cylindrical, or even ellipsoidal, and vary in size from large (2.2–2.6 mm), medium (1.8–2.1 mm), to small (<1.8 mm). Seed weight ranges from 2 to 6 mg, depending upon the variety and cultivar. Some seeds can exhibit a sharp or a rounded border, and this character can be used for classification systems. With a few exceptions, all quinoa cultivars usually have sharp borders, while seeds of the wild species have round borders (Jacobsen and Stolen 1993).

2.2.3.4 Embryo

The embryo of the quinoa seed consists of a hypocotyl-radicle axis and two cotyledons: in the axis, the meristem of the root appears with a root cap, and the apical meristems of the shoot are distinguishable. The embryo cells have thin primary cell walls, and the nuclei of each cell are round or lobed and are found at the centre of the cell. The cells also possess endoplasmic reticulum in the form of densely packed sheets of cisternae (Fig. 2.12). Protoplastids also occur in the protoderm and mesophyll regions of the cotyledons and in the ground meristem of

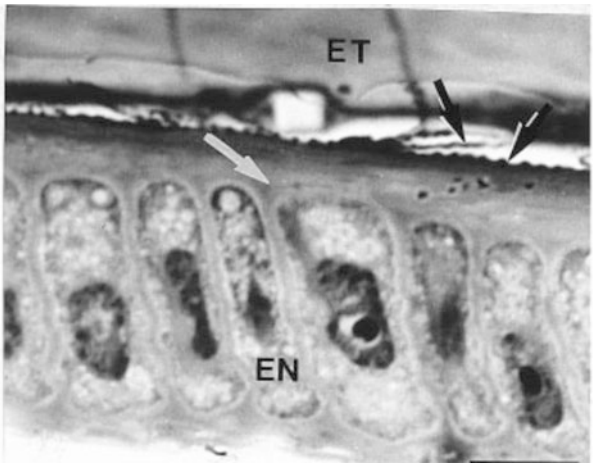

Fig. 2.8 Light microscopy of a section of the micropylar endosperm (EN). Endosperm cells have thick outer cell walls (white arrow). Cells of the exotesta (ET) with a crystal can be seen. Cells of the endotegmen show thickenings in their inner walls (black arrows). Section is stained with PAS and toluidine blue O. Bar = 10 μm (inserted with permission, Prego et al. 1998)

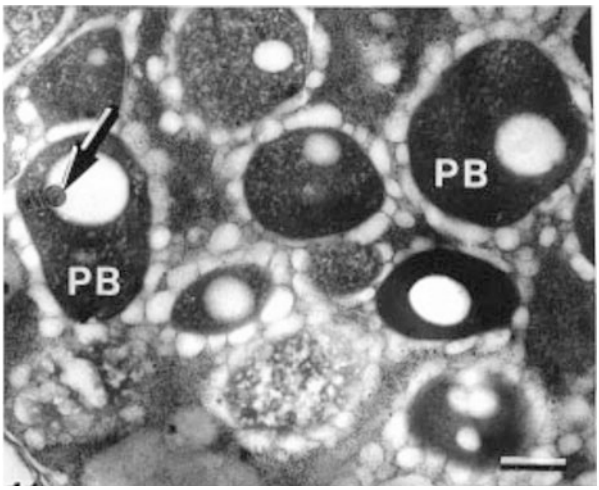

Fig. 2.9 Section of an endosperm cell showing protein bodies (PB); black arrow indicates a globoid crystal. Bar = 1 μm (inserted with permission, Prego et al. 1998)

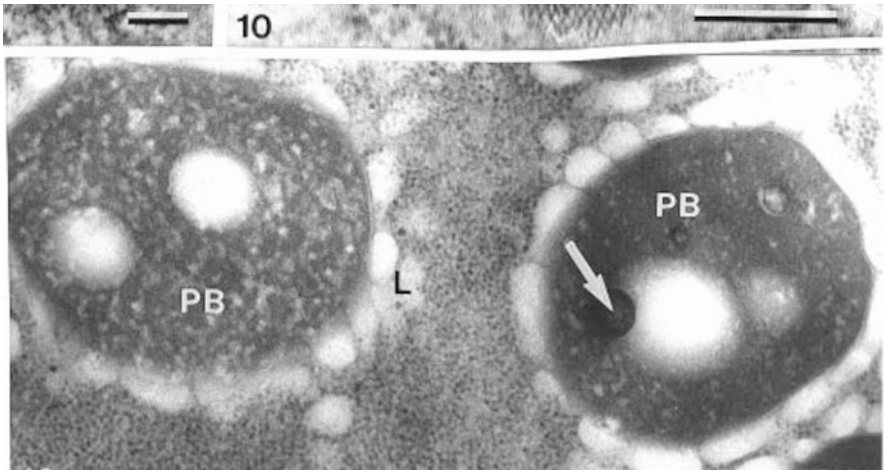

Fig. 2.10 An enlarged section of a cell of the endosperm showing lipid bodies (L) and two protein bodies (PB), one of them with a globoid crystal (white arrow). Bar = 1 μm (inserted with permission, Prego et al. 1998)

the axis; they contain clusters of dense particles of phytoferritin and, occasionally, starch grains. All of the embryo cells, including those of apical meristem, possess large amounts of protein and lipid bodies (Prego et al. 1998).

When it comes to storage, carbohydrates are mainly found in the perisperm, while other compounds, including proteins, minerals, and lipidic compounds, are localized mostly in the endosperm and embryo.

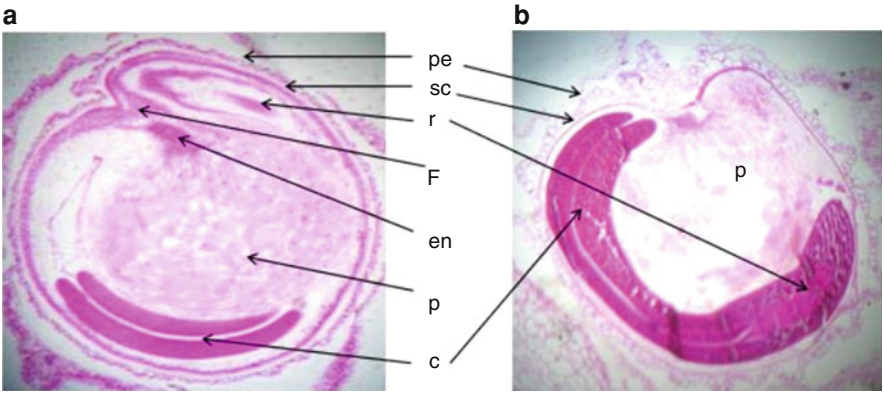

Fig. 2.11 Medial longitudinal section of quinoa seed showing the pericarp (pe), seed coat (sc), cotyledons (c), endosperm (en) (in the micropylar region only), radicle (r), funicle (f), and perisperm (p). Bar = 500 mm (inserted with permission, Elham F. Gomaa 2014)

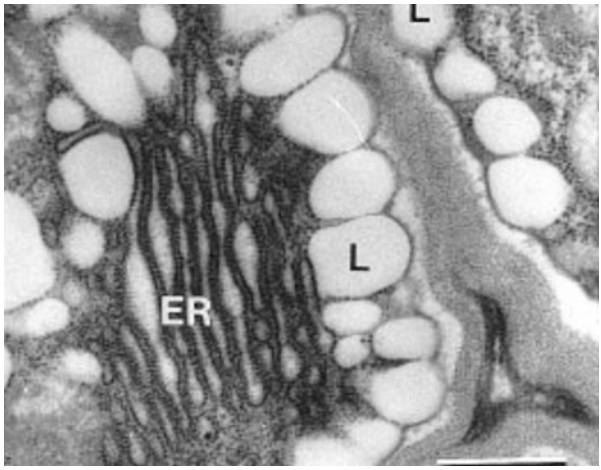

Fig. 2.12 Section of a cell of the ground meristem showing endoplasmic reticulum (ER) forming closely packed sheets of cisternae; L, lipid body. Bar = 0.5 μm (inserted with permission, Imelda Prego et al. 1998)

2.3 Favourable Habitats

Chenopodium quinoa is an Andean crop that has recently gained attention all around the world due to its nutrition-rich profile and striking ability to adapt to and withstand contrasting environments, including nutrient-deficient and saline soils, low moisture, and even drought-stressed marginal agro-ecosystems. In the last few decades, abiotic stresses have become more prominent due to unpredictable weather

patterns along with severe changes in the climatic conditions in different parts of the world.

Quinoa is widely cultivated from the sea level to 4000 m above the sea level. Traditionally, its cultivation is divided into five ecotypes based on geographic adaptation:

1. Valley—grown at 2000–3500 m.a.s.l. in Colombia, Ecuador, Peru, and Bolivia
2. Altiplano—grown at high altitudes of more than 3500 m.a.s.l. around Titicaca Lake on the border of Bolivia and Peru
3. Salares—grown in the salt flats of Bolivia and Chile and has a high tolerance to salinity
4. Sea level—grown in the low-altitude areas of southern and central Chile
5. Subtropical or Yungas—grown in the low-altitude, humid valleys of Bolivia and includes late flowering genotypes (Galwey et al. 1990)

Quinoa is adapted to a wide range of soils, including saline and drought-prone soils. However, the information regarding its tolerance and adaptation to other abiotic stresses, such as frost, UV-B irradiation, high air temperature, etc., is limited. It has been stated that these plants can tolerate light frost at any given stage of their growth, except when flowering (Hinojosa et al. 2018).

This crop can be easily grown, and optimally, it flourishes well in rich moist, well-drained sandy-loam to loamy-sand soils. It can tolerate pH range from 6 to 8.5 and moderate soil salinity. Plant parts above the soil are quite wind-resistant, and the crops are also observed to be drought-tolerant once properly established (Useful Tropical Plants Database n.d.).

Quinoa optimally prefers slight cool temperature of about 45°–50 °F. Germination usually takes place after 24 h of sowing when adequate amount of moisture is present in the soil, and seedlings are visible after 3–5 days.

Quinoa vegetation is day-length sensitive, and many cultivars fail to flower completely and properly when cultivated away from equatorial regions; however, those varieties coming from the south of its range in Chile are more likely to do well in Britain location-wise. Different varieties take different amounts of time, but on an average, it takes 90–220 days from sowing to harvest.

Yield harvest has been recorded as high as 5 tonnes per hectare in the Andes, which is comparable to wheat in that area (Useful Tropical Plants Database n.d.).The grains are not easily attacked by birds and animals due to high saponin content.

2.4 Phenological Growth and Life Cycle

2.4.1 Stage 0: Germination of Seed

Germination takes place as the cotyledons emerge from the soil, and several secondary stages begin to occur such as seed imbibition and radicle emergence. Germination is usually epigeal and is followed by the emergence of hypocotyls and cotyledons growing towards the surface.

2.4.2 Stage 1: Leaf Emergence and Development

In quinoa, it has been observed that leaves emerge in pairs, and a pair becomes visible when two leaf blades are separated from each other. This stage usually begins with full separation of cotyledons, followed by the appearance of photosynthetic leaves in the main shoot.

2.4.3 Stage 2: Development of Secondary Stems

Secondary stems can start developing before or after the emergence of inflorescence, depending upon the quinoa genotype.

2.4.4 Stage 3: Stem Elongation

Stem elongation in quinoa occurs simultaneously with leaf development, secondary shoot (secondary stem) development, as well as inflorescence emergence and flowering.

2.4.5 Stage 4: Development of Vegetative Parts

Vegetative parts begin to develop and increase in size.

2.4.6 Stage 5: Emergence of Inflorescence

It is usually noted to occur before the end of the leaf development stage at the shoot. Initially, inflorescence is not visible as it is covered by surrounding young leaves, but after leaf elongation, inflorescence can be visually spotted from above. At this stage, all the flowers are still closed.

2.4.7 Stage 6: Flowering

When anthers are extruded, flowering begins. Early end in flowering then follows anthesis, which starts with first senesced anthers of the main inflorescence. Inflorescence colour may change, depending upon the genotype, at this stage. When all anthers are senesced, anthesis is considered as complete.

2.4.8 Stage 7: Fruit Development

Thickening of the ovary indicates the development of fruit, followed by the occurrence of the first visible set of grains.

2.4.9 Stage 8: Ripening

Pericarp changes colour, from green to beige, red, or black. At this stage, the water content in achene varies and its texture is also modified.

2.4.10 Stage 9: Senescence

This stage addresses plant senescence and fruit ripening. Senescence is observed to start from the basal leaves, which then continues in the upward direction, while the stem still remains green in colour. The leaves then become dead and the stem changes its colour from yellow to brown. At the end, the whole plant becomes brown, dries up, and dies (Sosa-Zuniga et al. 2017) (Fig. 2.13, Table 2.1).

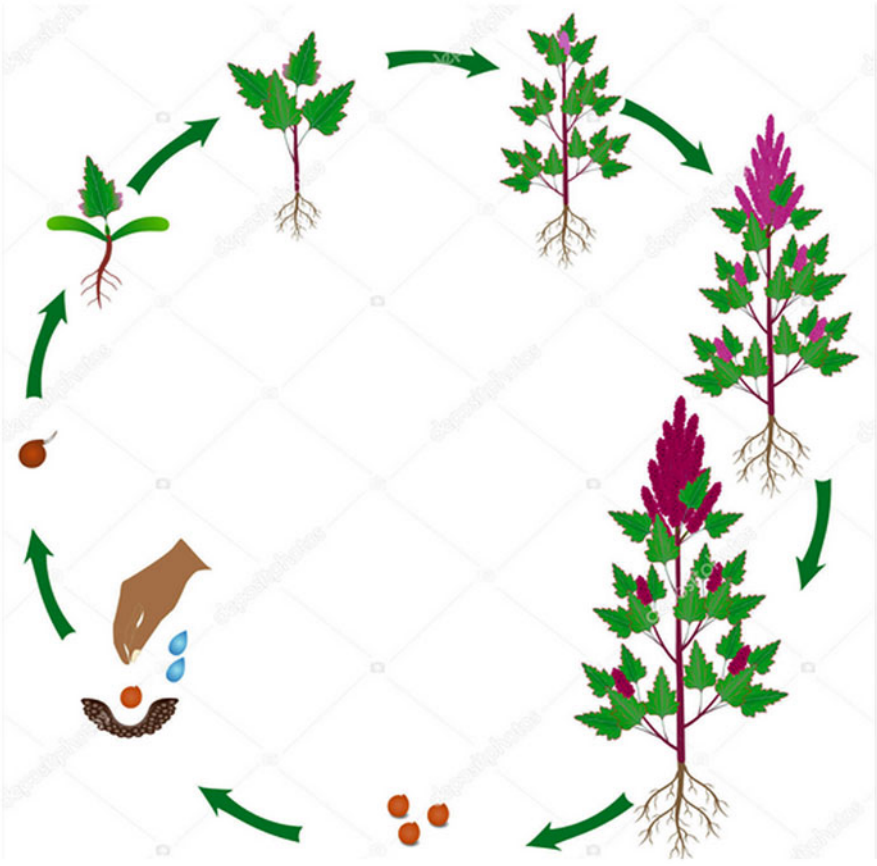

Fig. 2.13 Life cycle of quinoa (C.F.—ZAQzaq81, depositphotos.com)

Table 2.1 Description of the phenological growth stages of quinoa (*Chenopodium quinoa*) according to the extended BBCH scale (inserted with permission, Sosa-Zuniga et al. 2017)

BBCH code two-digit	Description
Principal growth stage 0: germination	
00	Dry seed
01	Initiation of seed imbibition
03	Seed imbibition completed
05	Radicle emergence from seed
07	Emergence of hypocotyl
08	Hypocotyl with cotyledons growing towards soil surface
09	Emergence of cotyledons through soil
Principal growth stage 1: leaf development	
10	Cotyledons fully emerged
11	First pair of leaves visible
12	Second pair of leaves visible
1.	Coding continues with the same scheme
19	Nine pair of leaves visible. If required, coding can continue following the same scheme
Principal growth stage 2: formation of side shoots	
20	Visible lateral buds or expanded leaves without lateral stems
21	One side shoot visible
22	Two side shoots visible
2.	Coding continues with the same scheme
29	Nine side shoots visible. If required, coding can continue following the same scheme
Principal growth stage 3: stem elongation (omitted)	
Principal growth stage 4: development of harvestable vegetative parts (omitted)	
Principal growth stage 5: inflorescence emergence	
50	Inflorescence present but still enclosed by leaves
51	Leaves surrounding inflorescence separated, inflorescence is visible from above
59	Inflorescence visible, but all the flowers are still closed
Principal growth stage 6: flowering	
60	Beginning of anthesis: main inflorescence flowers with first extruded anthers
67	Early end of anthesis: main inflorescence flowers with first senesced anthers
69	Complete anthesis: main inflorescence flowers with senesced anthers
Principal growth stage 7: fruit development	
70	Fruit set: ovary thickening and first visible grains in the main stem
Principal growth stage 8: ripening	
81	Milky grain, easily crushed with fingernails, liquid content and green pericarp
85	Thick grain, easily crushed with fingernails, white pasty content, green, beige, red or black pericarp
89	Ripe grain, difficult to crush with fingernails, dry content, the grain has a beige, red or black colour on its outside. Ready to harvest

(continued)

Table 2.1 (continued)

BBCH code two-digit	Description
Principal growth stage 9: senescence	
91	Only basal leaves are dry
93	Leaves of the first half portion of the plant, starting from the base, are dead
95	All leaves are dead, stem colour turns from yellow to brown
97	Plant dead and dry
99	Harvested product

2.5 Conclusion

It is very interesting to explore and study the intricate structure of this plant, and scientists are trying to figure out why it can withstand various environmental stresses while producing protein-rich grains. Quinoa has been of interest to researchers since the 1990s, and due to a sudden increase in its popularity, more detailed experiments are being conducted to further explore its genomic structure.

In the future it may become possible to combine and produce hybrid crop plants, with quinoa-like rich protein profile and other characters, that can be made available to the common population leading to satisfaction of the protein requirements in a healthy manner. Day by day, people are getting inclined towards vegan and vegetarian diets; owing to their large number of benefits shadowing the little drawbacks. Hence, this crop has great potential to become a staple diet for a large number of people.

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The Nutritional Applications of Quinoa Seeds

3

Okon Godwin Okon

Abstract

As global population increases, food crops, which at a particular time appeared to be neglected or lesser known, begins to gain recognition. *Chenopodium quinoa* (Willd.) (quinoa) belongs to the family Amaranthaceae; it is regarded as a pseudocereal that is a natural to the Andean regions and is adaptable to diverse soil types and climatic conditions. Its high nutritional composition has stirred up enormous attention from the scientific community. Quinoa is very rich in dietary fiber, proteins, vitamins, unsaturated fats, and minerals, having an astonishing equilibrium of very essential amino acids, similarly branded as being a gluten-free grain, which permits its usage in the nutrition of celiac patients. Saponins, protease, and phytic acid inhibitors are among the utmost antinutrients found in quinoa seeds. Saponins happen to be the most dominant and are present in the exterior coating of the seeds, where they are responsible for the seed's characteristic bitter taste. However, several researchers have tried to develop methods of saponin removal in seeds without altering the nutrient composition of the seeds significantly; this includes washing the seeds in cold water. Research shows that consumption of quinoa gave positive results against the antibodies of wheat proteins, thus suggesting that people with wheat protein allergies could consume quinoa. As a product of significance with regard to its mineral composition and phytochemicals, it is pertinent that more research should be carried out to come up with appropriate methods of propagation, distribution, and preparation to help solve the issue of malnutrition worldwide, especially in Africa and Asia where the production of food is constantly being threatened by environmental stress and global climate change.

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Keywords

Chenopodium quinoa · Glycemic index · Gluten · Protein · Quinoa · Saponin

3.1 Introduction

Chenopodium quinoa (Willd.) (quinoa) belongs to the family Amaranthaceae; it is regarded as a pseudocereal which is a natural to the Andean regions of Peru, Bolivia, Ecuador, and Chile and is adaptable to diverse soil types and climatic conditions. Its high nutritional composition has stirred up enormous attention from the scientific community. Quinoa is appropriately rich in dietary fiber, proteins, vitamins, unsaturated fats, and minerals, having an astonishing equilibrium of very essential amino acids, similarly branded as being a gluten-free grain, which permits its usage in the nutrition of celiac patients (Alvarez-Jubete et al. 2010a, b; Maradini-Filho et al. 2017). The cultivation of quinoa can be dated back to about 1000 years in the Andean region (Peru, Chile, Ecuador, and Bolivia) (Galvez Ranilla et al. 2009; Jancurová et al. 2009). In these regions, it is known by various local and common names, or it can simply or generally be referred to as "quinoa" (Vega-Gálvez et al. 2010). The Incas refers to quinoa as "a gift from the god" and "the mother of grains," which they use traditionally in treating various medical problems. Quinoa seeds can be consumed traditionally as cereal, cooked, roasted, sometimes supplemented to soups, and often fermented and made into beer or a local traditional drink by the Andes known as "chichi" (Vega-Gálvez et al. 2010; Bazile et al. 2014; Cooper 2015). Similar to spinach, quinoa leaves can be consumed in like manner (Oelke et al. 1992); young seedling sprouts are important components of salads (Schlick and Bubenheim 1996); quinoa whole plant stalks make up pig, poultry, and cattle feeds because of its excess nutritional value (Bhargava et al. 2006).

3.2 Nutritional Profile/Value of *Chenopodium quinoa* (Quinoa)

Quinoa possesses a very high level of protein which is very much comparable to those found in milk, and reports put its rate higher than values reportedly contained in other cereals, namely, maize, wheat, and rice; the National Aeronautics and Space Administration (NASA) deployed extensively the use of quinoa to meet the requirements for astronauts on missions in space because of the versatile nature of quinoa (Koziol 1992; Asao and Watanabe 2010; Cooper 2015).

Quinoa seeds are generally consumed in the same manner as other grains. It can be pulverized into flour and used to make bread, it is also cooked or supplemented in soups, and it can as well be fermented into drinks or beer. Note also that quinoa is a very decent source of minerals, proteins, dietary fiber, and polyunsaturated fats. Experts advise that although quinoa is endowed with all these nutrients, to achieve a decent inclusive nutrition, quinoa should be consumed as part of a balanced diet comprising several other food types. As regards the nutrition of quinoa, it is

equivalent with regard to energy to correspondingly other consumable foods like maize, wheat, or rice as presented in Table 3.1.

3.3 Protein/Amino Acid Composition of Quinoa

Proteins are considered as a key biological macromolecules which catalyzes several enzymatic reactions; it also serves as a structural element, an energy source, and a component in the synthesis of protein (Morrison and Laeger 2015; Lee et al. 2015). Quinoa is abundantly rich in proteins; this puts its worth among the finest sources of protein for human consumption. The biological protein value measures the quantity of protein absorbed from a food, which is then assimilated into human body proteins.

Quinoa protein content value is very high and ranges from about 13 to 17%, depending on the variety (Filho et al. 2015). Quinoa's biological value is very high, about 73%, comparable to that obtained in beef which is 74% and higher than those obtained in rice (56%), corn (36%), and wheat (49%) (Gordillo-Bastidas et al. 2016). According to the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), the protein obtained from quinoa can meet 100% of the day-to-day recommended ingestion of essential amino acids for humans (Reyes-Montaño et al. 2006; Jyoti and Chanu 2018). It is a well-known fact that amino acids make up proteins; eight of such amino acids are regarded as very essential for both children and adults. Table 3.2 shows the essential amino acid composition of quinoa seeds compared with that of other seeds.

3.4 Lipids/Fatty Acid Composition of Quinoa

Aside from possessing a good and high portion of biological quality of protein, quinoa also has an appropriate and remarkable composition of lipids. Quinoa seeds have an oil composition that varies between 2 and 10%, comprising very vital fatty acids like α-linolenic and linoleic acids (Jyoti and Chanu 2018); quinoa correspondingly has extraordinary concentrations of natural antioxidants, including α- and γ-tocopherol (Maradini-Filho et al. 2017), in an comestible proportion similar or higher than that found in rice, wheat, and corn (Jyoti and Chanu 2018); this makes quinoa a crop that can be consumed for its rich and readily available lipids (Navruz-Varli and Sanlier 2016).

The utmost essential fractions established in quinoa include diglycerides and triglycerides, which account for about 20 and 50%, respectively, for the neutral lipids found in quinoa seeds (Przybylski et al. 1994; Blanca 2019). When considering the total polar lipids in quinoa seeds, lysophosphatidylethanolamine and phosphatidylcholine make up 57% of the entire polar lipids; this makes them the most abundant (Blanca 2019). The fatty acids present in quinoa seeds are principally polyunsaturated and monounsaturated, making up about 55 and 27%, respectively; however, 12% of the entire fatty acids in quinoa is represented by saturated acids as shown in Table 3.3 (Blanca 2019).

Table 3.1 Comparative nutritional profile of quinoa seeds with that of other cereals

Food	Energy (Kcal)	Carbohydrate (g/100 g)	Protein (g/100 g)	Lipids (g/100 g)	Fiber (g/100 g)	Unsaturated fatty acids (g/100 g)	Essential amino acids (number)
Quinoa	368	64.12	14.1	1.92	2.8–10	1.61	10/10
Rice	365	79.95	6.6	0.21	0.3	0.12	9/10
Wheat	340	71.97	13.7	0.66	4.23	0.35	10/10
Maize	365	74.26	9.4	1.5	2.4	0.98	9/10

Sources: Gordillo-Bastidas et al. (2016); USDA (2015, 2017); Jyoti and Chanu (2018)

Table 3.2 Essential amino acid composition of quinoa seeds likened to other cereals

g/100 g edible portion	Quinoa	Maize	Wheat	Rice
Phenylalanine	0.59	0.46	0.68	0.35
Tryptophan	0.17	0.07	0.18	0.08
Threonine	0.45	0.35	0.37	0.24
Tyrosine	0.27	0.38	0.36	0.22
Isoleucine	0.50	0.34	0.53	0.29
Valine	0.59	0.48	0.59	0.40
Leucine	0.84	1.16	0.93	0.55
Arginine	1.09	0.47	0.48	0.55
Lysine	0.77	0.27	0.30	0.34
Histidine	0.41	0.29	0.32	0.16
Methionine	0.31	0.20	0.22	0.16
Alanine	0.59	0.71	0.43	0.38
Cysteine	0.20	0.17	0.29	0.14
Aspartic acid	1.13	0.66	0.62	0.62
Glycine	0.69	0.39	0.50	0.30
Glutamic acid	1.87	1.77	4.74	1.29
Proline	0.77	0.82	1.46	0.31
Serine	0.57	0.45	0.65	0.35

Sources: USDA (2018); Blanca (2019)

Table 3.3 Fatty acid composition of quinoa seeds likened to other cereals

g/100 g edible portion	Quinoa	Maize	Wheat	Rice	Sources
Linoleic/α-linolenic	5.8–13.8	–	14.5	nr	Beatriz and Suzana (2012); Bruni et al. (2001); Alvarez-Jubete et al. (2009).
Oleic acid	24.5–26.7	29.8	13.2	nr	
Linolenic acid	3.8–8.3	0.9	3.8	nr	
Eicosenoic acid	1.4	–	–	nr	
Myristic acid	0.1	0.2	–	nr	
9-Docosenoic acid	1.2–1.5	–	nd	nr	
Tetracosenoic acid	2.4–2.6	–	–	–	
Saturated	0.71	0.67	0.45	0.16	USDA (2018); Blanca (2019)
Monounsaturated	1.61	1.25	0.34	0.18	
Polyunsaturated	3.29	2.16	0.98	0.16	

nd not detected, *nr* not reported

3.5 Carbohydrate Composition of Quinoa

Reports have indicated that about 50–70% of biological energy obtained through diets are contributed by carbohydrates and its associated components (Beatriz and Suzana 2012). Based on the degree of polymerization, carbohydrates are classified into three major groups, namely, sugars (monosaccharides, disaccharides, polyols), oligosaccharides, and polysaccharides (starch and non-starch) (Copeland 2009; Beatriz and Suzana 2012).

Quinoa's carbohydrate composition is reported to be between 52 and 74% (dm) (Maradini-Filho 2017). This is still within the range of 58–64.2% of the dry weight also reported by Repo-Carrasco et al. (2003) and Blanca (2019). Quinoa seeds contain carbohydrate contents slightly lower than or similar to those found in maize, rice, and wheat (Table 3.1); however, its monosaccharide subunit composition can be preferably compared to similar quantity found in vegetables, fruits, and legumes (Maradini-Filho et al. 2017). Quinoa seed grains contain free disaccharides like sucrose (2.90 g/100 g dry weight) and maltose (1.40 g/100 g dry weight) and monosaccharides such as glucose (1.70 g/100 g dry weight) and fructose (0.20 g/100 g dry weight) (Repo-Carrasco et al. 2003; Blanca 2019). The starch quinoa is polygonal, which is much smaller in size in comparison to those found in other common cereals with a diameter of about 0.6–2.2 μm (Tari et al. 2003; Maradini-Filho et al. 2017; Maradini-Filho 2017). It can as well be used as thickeners in frozen food because of its rich contents of amylopectin, which gives it exceptional freeze-thaw stability (Tang et al. 2002; Maradini-Filho 2017).

3.6 Dietary Fiber Content of Quinoa

Quinoa has been reported to contain high dietary fiber. The percentage of plant food which is indigestible is referred to as "dietary fiber"; it aids proper digestion and prevents constipation. The dietary fiber composition of quinoa seeds is in close range with that found in other cereals; however, higher levels have been reported within embryos than the reported levels found within the perisperm (James 2009; Maradini-Filho et al. 2017; Maradini-Filho 2017). Lamothe et al. (2015), USDA (2018), and Blanca (2019) reported a scale of 7–10% of the total dietary fiber present in quinoa seeds. Repo-Carrasco-Valencia and Serna (2011) while studying about the four variations of quinoa reported a similar scale of dietary fiber in raw quinoa as 13.6–16.0 g/100 g dry weight. They also reported that most of the dietary fibers in the study were insoluble ranging from 12.0 to 14.4 g when matched to 1.4–1.6 g of soluble fiber per 100 g dry weight. Quinoa's dietary fiber contents just like its protein composition are usually higher than that of most of the other common grains but generally lower than that of legumes.

Quinoa is known to possess fiber content in high quantity, which aids in the relief of constipation. Improvement of food digestibility has been recounted as a function of the fiber content in quinoa, which is high and facilitates in the absorption of additional nutrients existent in quinoa (Ogungbenle 2003; Maradini-Filho et al.

2017; Maradini-Filho 2017). It also aids in the prevention of diseases of the heart as a consequence of its reduction effect on high blood pressure and diabetes (Shilpi et al. 2016). Shilpi et al. (2016) also reported that cholesterol is lowered by dietary fiber and glucose levels, ultimately leading to an abridged risk of the development of hemorrhoid and supporting weight loss.

3.7 Mineral Contents of Quinoa

Quinoa seeds, on average, have a high and better concentration of mineral nutrients than the majority of our common cereals, which is very essential for balanced diet to be maintained (Thoufeek et al. 1998). Quinoa seeds have abundant potassium, calcium, and phosphorus; magnesium, zinc, and iron can also be obtained in good quantities (Thoufeek et al. 1998) (Table 3.4). Quinoa seeds are also very rich in micronutrients as well; differences in values of macro- and micronutrients found in the seeds may be influenced by the variety of seeds and farming practices employed and also environmental conditions (Alvarez-Jubete et al. 2009; Jyoti and Chanu 2018). Quinoa seeds have calcium and potassium contents that can contribute to about 10% and 18–22% of the requirements needed by infants and adults, respectively (Comai et al. 2007; Abugoch 2009; Beatriz and Suzana 2012; Blanca 2019).

The deficiency of iron happens to be a major nutritional issue. Iron plays a lot of beneficial roles in the body of humans; it improves brain function and aids in the transport of oxygen from one cell to another (Shilpi et al. 2016). More so, just like any other food plant, quinoa seeds comprise some non-nutritive constituents that reduce mineral content and uptake. The exterior layer of quinoa seeds has a certain content of saponins that is responsible for its bitter taste, which is eliminated during treatment. Oxalate is also found in high concentration in quinoa seeds; this antioxidant is accountable for the reduction of uptake or absorption of minerals like magnesium and calcium when it binds to them (Siener et al. 2006).

3.8 Vitamin Composition of Quinoa

The Committee on Dietary Allowances postulated certain vitamin requirements which quinoa seeds satisfy effortlessly (Thoufeek et al. 1998). Quinoa seeds possess a very good portion of thiamine, B vitamin riboflavin, vitamin C, pantothenic acid, vitamin E, and folic acid (Table 3.5), even though the quantity of vitamin E seems to decline after processing (Koziol 1992). Folic acid aids decent emotional and mental health as it plays a vital role in proper brain functioning (Shilpi et al. 2016); similarly, riboflavin provides and increases brain energy metabolism and that of the muscle cells (Shilpi et al. 2016).

Table 3.4 Macro- and micronutrient composition of quinoa seeds likened to other cereals

mg/100 g edible portion	Macronutrients				Micronutrients			
	Potassium	Magnesium	Phosphorus	Calcium	Copper	Zinc	Iron	Manganese
Quinoa	563.0	197.0	383.7	148.7	0.6	4.4	4.6	2.0
Maize	287.0	127.0	292.6	17.1	0.3	2.9	2.7	0.5
Wheat	431.0	144.0	467.7	50.3	0.6	4.7	3.5	3.0
Rice	86.0	35.0	137.8	6.9	0.1	0.6	0.8	1.1
Sources	USDA (2018); Blanca (2019)	USDA (2018); Blanca (2019)	Koziol (1992); Shilpi et al. (2016)	Koziol (1992); Shilpi et al. (2016)	USDA (2018); Blanca (2019)	Koziol (1992); Shilpi et al. (2016)	USDA (2018); Blanca (2019)	USDA (2018); Blanca (2019)

Table 3.5 Vitamin composition of quinoa seeds likened to other cereals

	Vitamin C (mg/100 g)	Thiamine (B_1) (μg/100 g)	Riboflavin (B_2) (μg/100 g)	Folic acid (mg/kg DW)	Vitamin A (μg/100 g)	β-carotene (μg/100 g)	Pantothenic acid (μg/100 g)	Vitamin E (μg/1 g)
Quinoa	1.4	0.36	0.32	0.078	1.0	8.0	0.77	37.49–59.82
Maize	nr	0.39	0.20	0.026	11.0	nr	nr	nr
Wheat	nr	0.42	0.12	0.078	0.0	nr	0.94	nr
Rice	nr	0.07	0.05	0.02	nr	nr	1.34	nr
Sources	Gordillo-Bastidas et al. (2016)	USDA (2018); Blanca (2019)	USDA (2018); Blanca (2019)	Koziol (1992); Shilpi et al. (2016)	USDA (2018); Blanca (2019)	USDA (2018); Blanca (2019)	USDA (2018); Blanca (2019)	Gordillo-Bastidas et al. (2016)

nr not reported

3.9 Antinutritional Contents of Quinoa

Among the key and most commonly found antinutrients in quinoa seeds are saponins, phytic acid, and protease inhibitors (Thoufeek et al. 1998). The saponins found in quinoa seeds give the seeds its characteristic bitter taste, which occasionally results in gastric irritation (Gordillo-Bastidas et al. 2016). Saponin contents in quinoa seeds due to their varied nature (ranging from 0.1 to 5%) may be classified, agreeing to its free saponin levels, as either sweet (<0.11%) or bitter (>0.11%) quinoa (Gordillo-Bastidas et al. 2016). Chauhan et al. (1992) presented in their results that 34% of the total saponins are sited in the hulls of quinoa seeds and can be removed by dehulling.

3.9.1 Saponins

Saponins are largely spread among plant kingdom as one of the numerous key secondary metabolites found in plant seeds, roots, leaves, stems, and fruits. They are synthesized in plants basically for protection of the plants against harmful microorganisms and pest (Singh and Kaur 2018).

The high concentration of saponins in seeds of quinoa is responsible for its characteristic bitter taste, which occasionally results in gastric irritation. The saponin levels in quinoa seeds range from 0.1 to 5%. Quinoa seeds are oftentimes classified according to taste based on free saponins found in the seeds; it can be regarded as bitter when its free saponin level is >0.11% or sweet when its free saponin level is <0.11% (Gordillo-Bastidas et al. 2016). However, several researchers have tried to develop methods of saponin removal in quinoa seeds without altering the seeds' nutrient composition significantly; the predominantly used of all includes washing the seeds in water (cold) (Maradini-Filho et al. 2017; Gordillo-Bastidas et al. 2016).

3.9.2 Phytic Acid

In most plant tissues, phytic acid, which is regarded as a saturated cyclic acid, happens to be the primary storing form of phosphorus. Food substances have high contents of phytic acids; the minerals are rendered unavailable for metabolic processes because the acid binds the minerals (Fardet 2010; Gupta et al. 2015). When the phytic acid composition of quinoa seeds is compared to that of other cereals, it is observed that quinoa seeds hold a very low quantity of phytic acid, which ranges between 10.5 and 13.5 mg compared to wheat (390 mg), rice (60 mg), and corn (720 mg) (Vega-Gálvez et al. 2010).

3.10 Quinoa Seeds as Gluten-Free Diets

In 2013, a survey was carried out, and it was reported that in the United States alone, about one third of its population are trying to either minimize or avoid the intake of gluten (Shilpi et al. 2016). Gluten occurs as a complex of the proteins gliadin and glutenin (Zevallos et al. 2014); it is an ample constituent of most food substances which includes grains (Tovoli et al. 2015; Gordillo-Bastidas et al. 2016).

The occurrence of celiac disease, which is a genetic autoimmune disorder, happens when excessive consumption of diets rich in gluten results in injury of the small intestine (Shilpi et al. 2016). Estimate has it that this disease affects 1 out of every 100 people all over the world (Shilpi et al. 2016). Thus, celiac disease patients as a matter of urgency are required to consume gluten-free diets (Shilpi et al. 2016).

Several researchers have given a thought to the use of quinoa seeds as a very appropriate component for diets that are free from gluten (Shilpi et al. 2016). Quinoa seed's high composition of minerals and vitamins makes it a suitable candidate for any gluten-free healthy diet (Pellegrini and Agostoni 2014; Peñas et al. 2014); quinoa seeds are also rated as products free from gluten by the *Codex Alimentarius* nomenclature based on its gluten content (<20 mg/kg) (Zevallos et al. 2014).

3.11 Quinoa Seeds' Glycemic Index (GI)

Glycemic index may be regarded as a degree of how levels of sugar in blood are raised by food or the measure of how fast carbohydrates raise the levels of sugar in blood, which is usually on a measure of 0–100 usually after 2 h of consumption (Gordillo-Bastidas et al. 2016; Shilpi et al. 2016); glycemic points are classified as low (<55), moderate (56–69), and high (>70) (Gordillo-Bastidas et al. 2016; Shilpi et al. 2016). A major advantage of low glycemic foods includes the improvement of glucose and also lipid levels, also essential in weight control. Low glycemic foods also lead to reduction in the resistance to insulin and lower the risk of diabetes, cardiovascular diseases, and also cancers (Atkinson et al. 2008; Maki and Phillips 2015).

Quinoa seeds' glycemic index ranges from 35 to 53, which depends on the method and duration of cooking. Atkinson et al. (2008) reported that quinoa seeds of about 150 g cooked and then refrigerated before being reheated for about 1.5 min in a microwave maintained a glycemic index of 53; thus, if quinoa is overcooked, it will maintain a very low glycemic index.

3.12 Cooking, Applications, and Utilization of Quinoa Seeds

Recently, quinoa seeds, alongside its products, are readily available at many grocery stores, health food stores, and supermarkets worldwide. Even though some packaged quinoa found in supermarkets are already rinsed, it is important to always rinse quinoa seeds before preparation to remove saponins, the antinutrients that are

existent in the exterior coating of the seed, as this will get rid of the bitter taste. The seeds of quinoa are processed via several methods, which include drum drying, extrusion, and autoclaving (Gordillo-Bastidas et al. 2016).

Quinoa has over a hundred recipes. Firstly, the seeds of quinoa may be ground into flour and used to make cakes and biscuits. Generally quinoa in most cases is a very good substitute for rice in some recipes because it is high in essential minerals and vitamins; more so, it provides about 16% iron value that is required daily. It has also been reported that in meatloaf recipes, quinoa is often used as a suitable substitute for bread crumbs, which will give the bread a nutlike flavor and texture (Shilpi et al. 2016).

In the food industry, there have been several applications and usage of quinoa in the preparation and manufacturing of several food products such as breads, pasta, beverages, beer, breakfast cereals, bars, soups, diet supplements, sauces, cookies, snacks, muffins, etc. because quinoa possesses a very good oil and water holding capacity, which makes it very suitable for the formulation of human food and drinks (Jyoti and Chanu 2018).

3.13 Allergenicity Associated with Quinoa Seeds

Asao and Watanabe (2010) reported that the consumption of quinoa gave positive results against the antibodies of wheat proteins, thus suggesting that people with wheat protein allergies could consume quinoa. In contrast, Astier et al. (2009) reported a single instance in France where a 52-year-old man showed anaphylaxis to quinoa. He developed a general reaction that included dysphonia, dysphagia, angioedema, and urticarial after eating quinoa with bread and fish. Only quinoa out of all the food samples he ingested revealed immunoglobulin E (IgE) reactivity in his serum (Astier et al. 2009).

3.14 Conclusion and Future Perspective

Quinoa grains have been of interest to the people of the Andean regions of Peru, Chile, Ecuador, and Bolivia since the early 1970, because proteins are found in abundance in quinoa primarily, which is very useful in countries where quality and appropriate amount of protein are lacking. It also has a very high value of dietary fiber and also in polyunsaturated fatty acids, which has been reported to have the potentials of treating cardiovascular disease, obesity, and hypercholesterolemia (Jyoti and Chanu 2018). Most cereals are lacking when it comes to certain essential amino acids, which are present in abundance in quinoa seeds. Also, the natural quinoa possesses some natural antioxidants which preclude degenerative disorders. Saponin happens to be the foremost antinutrient aside from phytic acid, which is associated with quinoa. This saponin is similarly accountable for the bitter tastes in quinoa, in which the bitterness and the saponin levels can be reduced either by boiling, soaking, or washing.

With regard to the significance of quinoa in relation to its mineral composition and phytochemicals, it is pertinent that more research should be carried out to come up with appropriate methods of propagation, distribution, and preparation to help decipher the difficult issues of malnutrition worldwide, especially in Africa and Asia where the production of food is constantly being threatened by environmental stress and global climate change. By doing so, health conditions of several populace worldwide living below and within the poverty mark will be enhanced.

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Quinoa Genetics

4

Ogechi Ihuoma

Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal in the genus *Chenopodium* and is commonly known as "goosefoot." *C. quinoa* has a monophyletic origin from Andean crop/weed system and was first domesticated in the Altiplano region south of Lake Titicaca. Quinoa is predominantly an inbreeding species and an allotetraploid having a chromosome number of $2n = 4x = 36$; however, mixoploidy has been reported in *C. quinoa* with chromosome numbers of $2n = 18$, $2n = 27$, $2n = 36$, and $2n = 45$. Quinoa genome has been identified and confirmed to be divided into two subgenomes by several authors. Characters controlled by major genes in quinoa have been reported to exhibit simple disomic-monogenic inheritance. Few successes have been recorded for attempts to manually hybridize *C. quinoa* with either of its related wild or cultivated tetraploids, and this has hindered the creation of segregating generations large enough for genetic analysis. Genetic improvement of quinoa has so far received little attention unlike other major cereal crops which have been fully involved in modern plant breeding techniques and genetic research. A number of molecular markers (AFLP, SSR, and SNP) have been developed for quinoa and are being used today to enhance quinoa improvement programs.

Keywords

Quinoa · Inheritance · Genetic improvement · Cytogenetics · Genetic diversity

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4.1 Introduction

Quinoa (*Chenopodium quinoa* Wild.), commonly known as "goosefoot" (Giusti 1970), is a pseudocereal and one of the 250 species included in the genus *Chenopodium* (Amaranthaceae). Although most species of the genus are colonizing annuals, other habits such as herbaceous, suffrutescent, and arborescent perennials also exist (Wilson 1990; Fuentes et al. 2012). *C. quinoa*, *C. berlandieri* subsp. nuttalliae, and *C. album* are of economic importance as they are used as a leafy vegetable, grain, and forage (Risi and Galwey 1989a), while other *Chenopodium* species such as *C. ambrosioides*, *C. botrys*, and *C. murale* have been identified with various medicinal uses (Kirtikar and Basu 2001).

Quinoa has been cultivated for more than 5000 years in the Andes and was probably domesticated by ancient civilizations at different times and in different geographic zones (Bhargava and Ohri 2016). Many wild characters such as seed shattering, seed dormancy, and thick seed coats that were disadvantageous to the farmers were lost, while useful characters such as larger and starchier seeds, fewer and larger inflorescences, uniform maturity, and environmental adaptations were preserved during domestication (Bhargava and Ohri 2016).

Quinoa is a highly nutritious crop with remarkable agronomic adaptations to different adverse climatic conditions such as drought, high salinity, and frost (Ruiz et al. 2014, 2016), which makes it suitable for cultivation in countries that are susceptible to the effects of climate change. Due to its high level of adaptability, quinoa can survive in diverse environments such as lowlands, deserts, and areas over 4000 m above sea level (Jacobsen and Mujica 2003; Jacobsen et al. 2003, 2009; Maughan et al. 2009; Hariadi et al. 2010). Quinoa has the potential to become a sustainable food supply globally under rapidly changing climatic pattern shifts while at the same time ameliorating pressure on arable land (Ruiz et al. 2016) and is therefore considered as a climate change-resilient crop. Because of its resilience and nutritional benefits, quinoa, together with amaranth, has been described as "one of the grains of the 21st century" (Konishi 2002) that will play a key role in the provision of sustainable food in adverse environmental conditions resulting from climate change scenarios. It has exceptional capacity to grow in water-deficient soil due to its inherent low water requirement and the ability to resume its photosynthetic rate and maintain its leaf area after a period of drought (Galwey et al. 1989; Jensen et al. 2000; Jacobsen et al. 2003). Saponins are the main antinutritional compounds of quinoa, and they confer bitterness when present in the integuments of mature achenes. The developmental stage of the crop affects saponin content; it is low during branching and high during flowering (Bhargava et al. 2006a). Seed components have been reported to exhibit diversity with regard to the environment as considerable increase of saponins and other seed components has been reported in an arid location (irrigated) as opposed to a cold temperate climate (rain-fed) site (Miranda et al. 2012, 2013); this suggests that environment plays a major role in the expression of genes responsible for seed components. Development of varieties with little or no saponin is one of the vital breeding objectives (Spehar and Rocha 2010), and MAS combined with recently available linkage mapping can be valuable for

advanced genetic analysis of important agronomic traits (Mastebroek et al. 2000; Maughan et al. 2004, 2012).

Increasing but insufficient knowledge of quinoa genetics and its complex allotetraploid nature, together with its small flowers and self-pollination nature, makes emasculation, hybridization, and breeding difficult. The primary breeding objective is to develop a variety with a dwarf, non-branching, and uniformly early maturing plant type to aid mechanical harvesting (Jacobsen et al. 1996) and high grain yield with high protein and low saponin content (Bhargava et al. 2006a). However, for any breeding program to work, the genetics of the traits of interest should be well understood.

4.2 Genome Size

Studies on genome size of *C. quinoa* using Feulgen micro-densitometry found 4C DNA amounts ranging from 6.34 to 6.47 pg in 21 accessions, which showed a nonsignificant 1.02-fold (Bhargava et al. 2007a). Likewise, 4C DNA amounts of 5.79 and 5.90 pg were recorded in two accessions of related tetraploid species *C. berlandieri* subsp. nuttalliae, and their average is 8.31% less than the mean of 4C DNA values of the studied 21 accessions of *C. quinoa* (Bhargava et al. 2007a). Similar results have been obtained using flow cytometry in *C. quinoa* cv. Barandales, which showed 2C values of 2.96 pg. Also a range of 2.96–3.04 pg was obtained for six accessions of *C. berlandieri* subsp. nuttalliae (Palomino et al. 2008). The findings of Palomino et al. (2008) correspond to that of Kolano et al. (2012), who showed 2C values ranging from 2.9 to 3.0 pg in 20 *C. quinoa* accessions; however, significantly lower 2C values of 2.01 pg using flow cytometry (Stevens et al. 2006) and 2.66 pg (Bennett and Smith 1991) using micro-densitometry have been reported.

Kolano et al. (2011) demonstrated the occurrence of two subgenomes in quinoa by FISH using two repetitive sequences, 12-P and 18-24J. The specificity of 18-24J to one of the two subgenomes was revealed by strong signals on 18 chromosomes in the form of bands of differing intensities on chromosome arms, while only minor signals on the remaining 18 chromosomes occur in terminal and centromeric positions.

Two genomes involved in the ancestry of *C. quinoa* have also been recognized by Storchova et al. (2015) through phylogenetic analysis of two flowering locus T-like genes CrFTL1 and CrFTL2. One parent was assigned to subgenome "A" and was shown to be related to North American *C. standleyanum*, *C. incanum*, or any other related diploid, while the other parent belonging to the Eurasian species *C. suecicum*, *C. ficifolium*, or some related diploid species was assigned to subgenome "B."

4.3 Cytogenetics

The basic chromosome number in the genus *Chenopodium* is $x = 8$ and $x = 9$ (Kawatani and Ohno 1950, 1956). The number $x = 9$ is found in section *Chenopodia*, which has been further subdivided into three subsections, viz., Leiosperma, Cellulata, and Undata (Risi and Galwey 1984). Cytological studies have established that *C. quinoa* is a tetraploid having a chromosome number of $2n = 4x = 36$ (Palomino et al. 1990; Wang et al. 1993; Bhargava et al. 2006b); however, mixoploidy has been reported by Gandarillas (1979) in *C. quinoa* with chromosome numbers of $2n = 18$, $2n = 27$, $2n = 36$, and $2n = 45$. According to Nelson (1968), the basic chromosome number for the genus *Chenopodium* is $x = 9$, and this is in line with the high degree of self-fertility and low levels of inbreeding depression seen in the species.

The results of Ward (2000) are consistent with allotetraploidy. This suggests that functional alleles have been retained at some duplicate loci, and there is some association occurring between homologous chromosomes. Also, tetrasomic segregation ratios have been observed in a minority of families, which may be due to reciprocal fragment exchange between homologues (Ward 2000).

Bhargava et al. (2006b) divided the karyotypes of *C. quinoa* into two groups based on the ratio between the longest and the shortest chromosomes in the complement, which was <2.0 in 1a and >2.0 in 1b types of karyotypes. All taxa they studied were characterized by one satellite pair, the position of which varies according to its comparative size in the complement. The satellite pair was found to be morphologically similar in all the accessions, being median (m) or median-submedian (msm), and has the satellite on the short arm. The symmetry index (TF%) on the basis of arm ratios varies from 43.9% (most asymmetrical) to 47.4% (most symmetrical).

They further observed that the longest chromosome in different complements is either m or msm with arm ratios varying between 1.18 and 1.56, while 4th, 9th, and 18th pairs are the most conserved in being median (M or m) in all the accessions studied (Figs. 4.1a–e and 4.2a, b). The greatest variability was observed in 10th and 13th pairs with the arm ratio ranging between 1.0–1.86 and 1.0–1.78, respectively (Figs. 4.1a–e and 4.2a, b).

C. quinoa has a monophyletic origin from Andean crop/weed system (Wilson 1990); this was confirmed by Bhargava et al. (2006b) in the seven accessions of *C. quinoa* studied, which show only minor though consistent differences in their karyotypes. These minor differences in karyotypes due to chromosomal alterations (mainly pericentric inversions and translocations) are being maintained due to predominantly self-pollinating behavior (Risi and Galwey 1984), and this is consistent with some degree of variability in morphological characters (Risi and Galwey 1984; Wilson 1988a, b; Bhargava et al. 2007b), protein profiles (Bhargava et al. 2005), and RAPD profiles (Ruas et al. 1999). This implies that variation in morphological characters, karyotypic alterations, and protein and RAPD profiles is similar (Bhargava et al. 2006b).

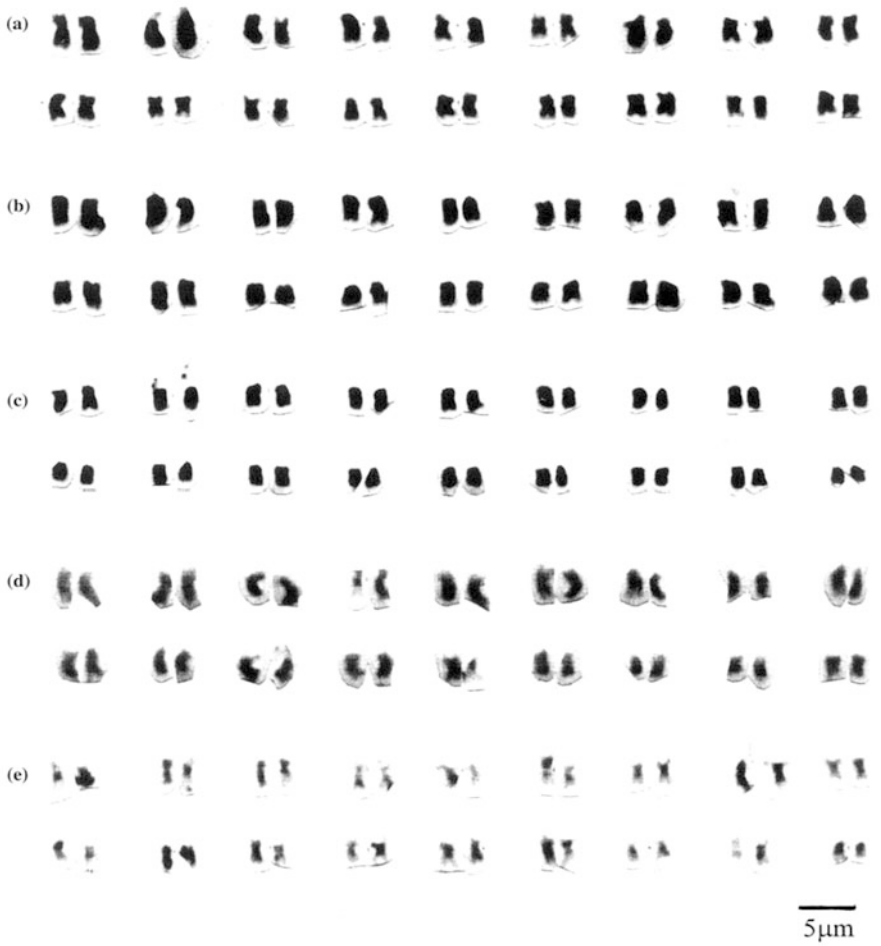

Fig. 4.1 Karyotypes of (**a**) *C. quinoa* PI 587173, (**b**) *C. quinoa* PI 584524, (**c**) *C. quinoa* PI 596498, (**d**) *C. quinoa* PI 510537, (**e**) *C. quinoa* CHEN 71/78 (Bhargava et al. 2006b)

In an earlier study by Catacora (1977), *C. quinoa* chromosomes could be arranged into nine groups of four homologues based on length and ratio between long and short arm. However, a more detailed analysis by Bhargava et al. (2006b) has resulted in clearly identifiable 18 pairs, thereby indicating allotetraploidy. This is also supported by duplication of Lap loci (Wilson 1976), disomic inheritance of some characters (Simmonds 1971), and allelic segregation ratios of F1 and F2, which indicated disomic-digenic and tetrasomic inheritance in some traits (Ward 2000).

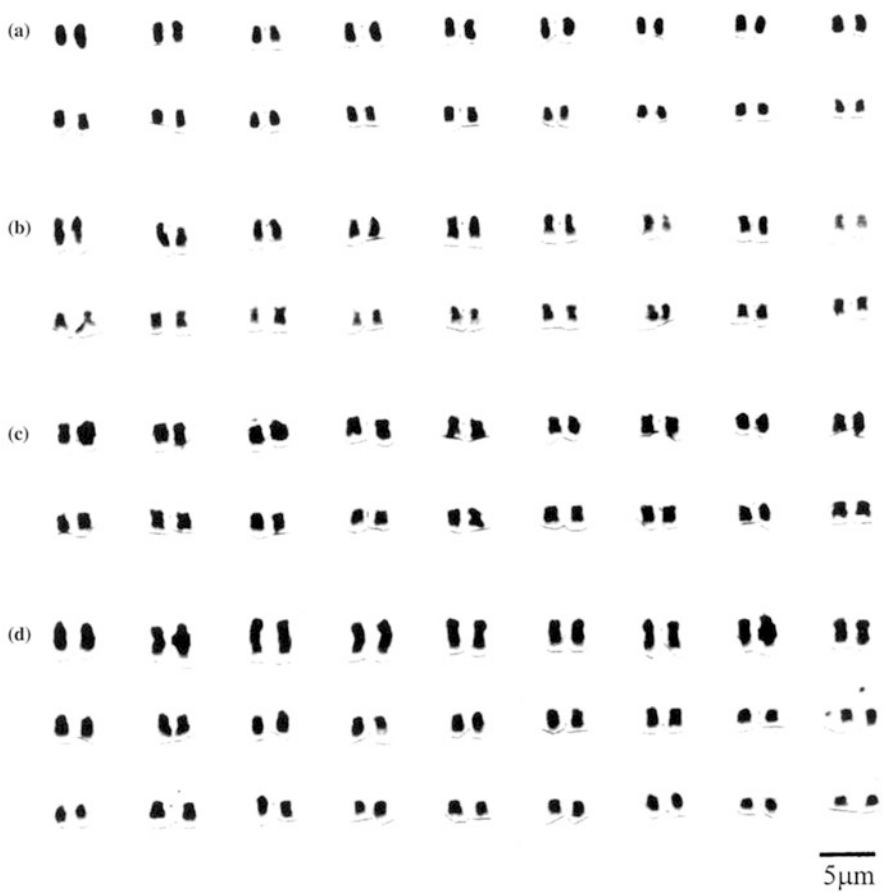

Fig. 4.2 Karyotypes of (**a**) *C. quinoa* CHEN 58/77, (**b**) *C. quinoa* CHEN 33/84, (**c**) *C. berlandieri* subsp. nuttalliae PI 568156, (**d**) *C. bushianum* 22,376 (Bhargava et al. 2006b)

4.4 Pattern of Trait Inheritance in Quinoa

An understanding of how alleles will segregate at loci controlling agronomically important traits is essential to quinoa breeders. Estimation of the heritability coefficient in the narrow sense (hr^2) is a very useful factor for breeders because one can predict the possibility of success with selection, as it reflects the proportion of phenotypic variation that can be inherited; that is to say that heritability coefficient measures the reliability of the phenotypic value as an pointer of genotypic value (Vasconcelos et al. 2012).

Characters controlled by major genes have been reported to exhibit simple disomic-monogenic inheritance (Simmonds 1971; Gandarillas 1979). This type of segregation has led to suggestions that quinoa possesses genes which suppress

pairing between homologous chromosomes, which to an extent places species as functionally diploid (Risi and Galwey 1984; Fleming and Galway 1995). The small size of the chromosome of quinoa makes study of the meiotic chromosome pairing difficult (Ward 2000). Meiotic chromosome configurations in both allo- and autotetraploid are often erratic, and several authors (Soltis and Reiseberg 1986; Krebs and Hancock 1989; Beaver and Iezzoni 1993) pointed out that allelic segregation analysis gives a more precise way of determining polyploidy type and whether inheritance is disomic or tetrasomic.

Homologous chromosome pairing from the same progenitor species in an allotetraploid would normally result in independent assortment at the duplicated loci and disomic inheritance. In autotetraploid, random association of the four homologous chromosomes and independent assortment at any one locus will result in tetrasomic inheritance (Ward 2000). However, segregation ratios which do not fit a simple disomic pattern have been observed in quinoa populations containing a fertility restoration gene (Ward 1998).

Ward (2000) carried out a research to use allelic segregation analysis of these traits: restoration of male fertility in CMS quinoa plants due to the presence of the *Frv* allele, with male fertile being dominant to male sterile (Ward 1998), red inflorescence and stem color due to the presence of the *R* allele with red being dominant to green (Gandarillas 1979), and pigmented axils due to the presence of the *Ax* allele, with pigmented being dominant to non-pigmented (Simmonds 1971) to determine the type of inheritance occurring in quinoa. The allelic segregation analysis was performed in a cross using male sterile plants as female parents produced F1 and F2 generations segregating for different single-gene morphological traits. The analysis revealed a range of F1 and F2 ratios indicative of both disomic-digenic and tetrasomic inheritance in two traits observed (Ward 2000). Also, distorted F2 ratios pointed to erratic multivalent formation at meiosis (Ward 2000). Certainly, tetrasomic segregation of "erratic multivalents" as observed by Ward (2000) would lead to distorted segregation ratios and may also contribute to the low levels of segregation distortion seen in the dataset studied by Maughan et al. (2004).

The level of bitterness of seeds (which is directly associated with saponin content of the seed) is quantitatively inherited (Risi 1986; Kenwright 1989). This has been confirmed by an earlier study by Gandarillas (1948), who observed a 3:1 segregation ratio for bitter versus sweet genotypes, suggesting that bitterness associated by saponin content is controlled by a single dominant gene.

Ward (2000) carried out an investigation in three cycles of pedigree selection with ten quinoa accessions and established that the action of a single dominant gene is an important part of the genetic variation regulating this trait. Due to the allotetraploid nature of the species, fixed heterozygosity at the locus controlling saponin content may also occur. Although identification of precise molecular markers of the dominant genetic locus could significantly accelerate breeding programs (Mastebroek et al. 2000) for selection of sweet genotypes, those efforts may be hindered if saponin content in leaves of bitter and sweet genotypes and their F2 progeny plants did not vary during the vegetative phase of plant development, signifying that the

sweet genotypes cannot be selected before anthesis, hence hampering the speed of a breeding program for this particular trait (Mastebroek et al. 2000).

In an attempt to reveal the genetic components of saponin biosynthesis, Reynolds (2009) reported the annotation of a large-scale EST collection from maturing seed tissues expressing saponins. Moreover, 39,366 unigenes, comprising of 16,728 contigs and 22,638 singletons, were assembled using Sanger and 454 GS-FLX pyrosequencing technologies. The identification of a set of candidate genes transcriptionally related with saponin biosynthesis included genes having homology to cytochrome P450s, cytochrome P450 monooxygenases, and glycosyltransferases was done using microarray analysis.

Plant color in quinoa, as reported by Fleming and Galway (1995), is governed by a single gene with three major alleles: red (R), dominant to purple (rP), which is dominant to green (r). Plant color-inflorescence phenotypes (R vs r) were determined for the parents and F1 and F2 populations.

4.5 Hybridization

Different attempts have been made to hybridize *C. quinoa* with either related wild or cultivated tetraploids by several researchers. In a study by Pal and Ohri (unpublished), *C. quinoa* was found to be inter-crossable with a diploid cytotype of *C. album* occurring in North Indian Plains. The resulting triploid shows 18II and 18I, which implies that one of the genomes of *C. quinoa* is homologous with that of 2x *C. album*. This close genetic relationship between *C. quinoa* and 2x *C. album* has been confirmed on the basis of RAPD and DAMD studies by Rana et al. (2010).

Nelson (1968) created artificial hybrids between *C. quinoa* and *C. quinoa* var. melanospermum and also confirmed the presence of natural hybrids. In another study, Heiser and Nelson (1974) produced F1 hybrids between *C. quinoa* and *C. nuttalliae* or "huauzontle," but these lacked pollen grains as male sterile parent was involved. The F1 hybrids, however, produced seed when backcrossed with the parents showing the close relatedness of the two species. Surprisingly, the F1 had black fruit, while both parents had a light-colored fruit, which was interpreted as a consequence of genetic complementation, thereby showing that light-colored fruit arose independently in Mexico and S. America.

C. quinoa cultivated in N. America has been shown to naturally hybridize freely with related wild species *C. berlandieri*; 30% of the progeny of *C. berlandieri* was found to be F1 crop/weed hybrids; this was confirmed by the presence of polymorphic quinoa isozyme alleles and morphologically intermediate leaves (Wilson and Manhart 1993).

Crosses between *C. berlandieri* subsp. nuttalliae and *C. quinoa/C. hircinum* (Andean complex) resulted in extremely low pollen stainability of 3–4% with no seed set after selfing, although the pollen stainability increases after backcrossing of the hybrid with *C. berlandieri* subsp. nuttalliae (Wilson and Heiser 1979). However, *C. berlandieri* subsp. zschackei of North American complex produces fertile hybrids in crosses with *C. quinoa/C. hircinum*, therefore showing closest affinity to the

Andean complex, and this can be a possible link between North and South American tetraploids (Wilson and Heiser 1979). A study by Bhargava et al. (2006b) seems to support this view point because of the close overall karyotypic similarity between *C. quinoa* and *C. berlandieri* subsp. nuttalliae. High sterility in F1 hybrids between these cultigens has been attributed to the accumulation of chromosomal differentiation following their origin and evolution in extensively separated geographical areas (Wilson 1980).

The karyotype of *C. bushianum* has marked differences in comparison with *C. quinoa* and *C. berlandieri* subsp. nuttalliae, with reference to number and morphology of satellite pairs and a very high ratio between longest and shortest chromosomes in the complement. This is reflected in its crossability relationships showing very low fertility and complete sterility of F1 hybrids (Bhargava et al. 2006b). The 2x types are cross compatible. However, 4x cytotype, which grows in Northern India, has an unusually asymmetrical karyotype as compared with those of diploid and hexaploid cytotypes (Bhargava et al. 2006b). This is reflected in complete crossing compatibility of 4x cytotype with 2x and 6x cytotypes of *C. Berlandieri* sp. nuttalliae and *C. quinoa*, respectively (Wilson 1980).

Owing to the small flower size and clustering of huge numbers of flowers on an inflorescence, there is difficulty of manual hybridization in quinoa, which has hindered the creation of segregating generations large enough for genetic analysis. Also, the existence of tetraploid segregations at some loci in quinoa makes breeding and genetic studies in the crop complex (Ward 2000). The occurrence of both disomic and tetrasomic segregations at the same locus is uncommon but could be explained by mutual exchange of fragments between homologous chromosomes. Although quinoa displays disomic inheritance for most qualitative traits (Ward 2000; Maughan et al. 2004; Fuentes and Bhargava 2011), combined modes of segregation could make genetic analyses and mapping of the quinoa genome very difficult (Ward 2000).

Despite these difficulties, mass selection and hybridization have been practiced in quinoa (Risi and Galwey 1984). A practical approach of effective selection can be the utilization of morphological markers to distinguish the hybrid from the parents (Bhargava and Ohri 2016).

4.6 Genetic Diversity of Morphological Traits of Quinoa

Genetic analysis studies are designed to determine the degree of heterogeneity among potential genotypes for selection to guarantee that only the best genotypes are selected in a breeding program (Silva et al. 2009); this is the same for quinoa which exhibits a high degree of heterogeneity, both within and among different geographic locations. The variability among cultivars reflects the heterogeneity of the genetic material, improves food security which is currently threatened by fluctuations in climatic conditions, and presents the possibility of identifying promising material for use in a plant breeding program (Ruiz et al. 2014).

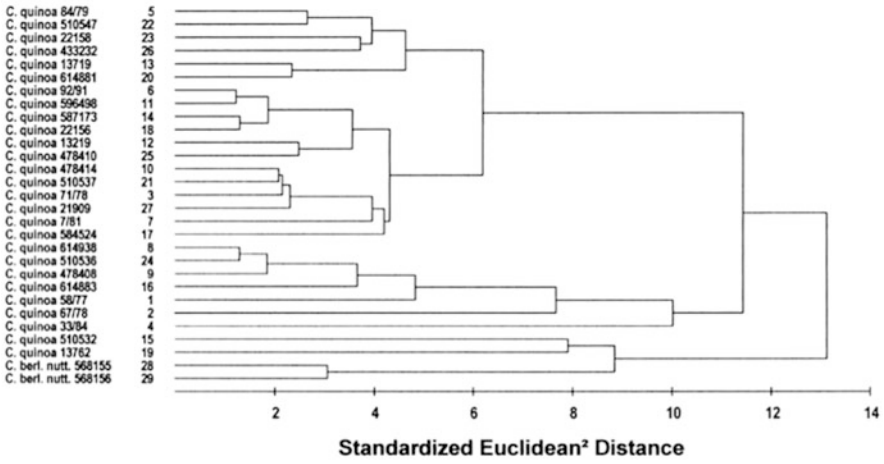

Fig. 4.3 Dendrogram of 29 germplasm lines derived from average linkage method (Bhargava et al. 2007b)

Quinoa exhibits ample genetic diversity for both qualitative and quantitative traits, which enables attaining a wide range of adaptability to various agroecological conditions (Rodriguez and Isla 2009). Gonzalez et al. (2012) showed that variability in the cultivation area of quinoa caused variation in yield and seed quality. High coefficients of heritability estimates are associated with a greater genetic variability, better selective accuracy (Cargnelutti Filho et al. 2009), and greater potential for success in selecting lineages with higher productivity of grain (Vasconcelos et al. 2016). Quinoa diversity, at a continental scale, has been associated with five main ecotypes, viz., Highlands (Peru and Bolivia), Inter-Andean valleys (Colombia, Ecuador, and Peru), Salares (Bolivia, Chile, and Argentina), Yungas (Bolivia), and Coastal/Lowlands (Chile), each of which is connected to subcenters of diversity that originated around Lake Titicaca (Risi and Galwey 1984).

Regardless of narrowing of genetic base during domestication, large genetic diversity still exists for plant color, seed color, and types of branching and panicles in addition to grain productivity, abiotic stress tolerance, and disease resistance (Bhargava and Ohri 2016). This diversity, which is also revealed at the molecular level, is being used by the quinoa breeders all over world to develop improved plant (Bhargava and Ohri 2016).

Bhargava et al. (2007b) studied the genetic variation of 19 traits among 29 germplasm lines of quinoa; the analysis of variance indicated the presence of high degree of morphological and qualitative variations among the lines studied, which showed that a vast amount of genetic variability existed in the quinoa germplasm lines. Cluster analysis (Fig. 4.1) grouped together lines that had greater genetic similarity but did not include lines from the same origin, indicating heterogeneity of the lines within a given geographical region (Fig. 4.3).

The germplasm lines were grouped into six clusters based on average linkage method. Cluster I grouped lines that are early maturing and high yielding but had low carotenoid content together. Cluster II comprised lines with higher leaf quality components but low seed quality. Cluster III lines had highest seed yield and high values for protein and carotenoids. The lines in cluster IV are early maturing and had high seed protein, while cluster V had high seed yield, dry weight/plant, stem diameter, and maximum number of inflorescences. Lines in cluster VI had low values for traits related to seed morphology and quality but not for carotenoid content. Also, the study clustered two lines of *C. berlandieri* subsp. nuttalliae separately from the quinoa line that is phylogenetically correct (Bhargava et al. 2007b).

Such genetic diversity of population within the same geographical region might be due to factors like heterogeneity, genetic architecture of population, history of selection, and/or developmental traits (Singh 1991), and the same has been reported by other researchers in different crop species (Ghafoor et al. 2001; Alemayehu and Becker 2002; Singh et al. 2004).

4.7 Genetic Improvement of Quinoa Using Mutation Induction

Genetic improvement of wild or cultivated plants requires variability, selection, and conservation of the characteristics of the types that are cultivated. Genetic improvement has, until recently, received limited attention unlike other major cereal crops which have benefited greatly from the modern plant breeding techniques and genetic research (Jarvis et al. 2008). However, emphasis has been mainly on its introduction to newer agroecological zones (Bhargava et al. 2007a), although initial reports on quinoa trials from Europe and Africa are encouraging (Mujica et al. 2001). There are several ways of improving crop performance, one of which is genetic improvement via mutation induction.

There are a lot of reports of improved morphological as well as physiological characteristics in cereals, grain legumes, fiber crops, oil seeds, vegetables, and ornamentals after mutation induction, and more than 2500 mutant varieties of more than 170 different species have been released and are available in the IAEA database (IAEA 2012). Of the various mutagens available, gamma ray is the preferred agent, and plant type and yield are the traits most commonly reported (Ahloowalia et al. 2004; Chopra 2005; Fu et al. 2008). Although the quality of many crops has been improved through mutation induction, few reports are available for quinoa.

Gomez-Pando and la Barra (2013) irradiated dry seeds (cv. Pasankalla) with gamma ray doses of 150 Gy, 250 Gy, and 350 Gy. They reported a delayed germination process in the M1 generation with increasing radiation dose. Also seedling height, root length, and leaf development were most reduced at 250 Gy, and at 350 Gy, no plants survived. In M2, the maximum spectrum of chlorophyll mutations corresponded to 150 Gy, while the maximum frequency was at 250 Gy. They also observed chlorophyll mutations with chlorina mutation being

predominant, followed by xantha. Changes were recorded for branch number, pedicel length, plant height, lifecycle duration, stem and foliage color, and leaf morphology at the two doses, with improvements in plant type.

Farmers are still using land races of quinoa with very long maturity period and very tall plants (Tapia 2000); the identified mutants with reduced life cycle could be beneficial, considering that some of the actual cultivars have long cycle reaching over 7 months in the field with the flowering and grain maturity time under adverse weather conditions (drought and frost), which significantly reduce the performance (Gomez-Pando and la Barra 2013). The same trend has also been reported for African yam bean (Ihuoma and Adesoye 2017) and *Jatropha curcas* (Dhakshanamoorthy et al. 2011), and suggestions are made on the use of such early maturing mutants to overcome some adverse field conditions that may arise during the flowering period such as pest attack. Gomez-Pando and la Barra (2013) also proposed that the identified mutants with reduced plant height will be very useful because they will decrease the high tendency of lodging and could improve the yield in similar way to that achieved in wheat (Rutger 1984; Sasaki et al. 2002; Zhou et al. 2007).

4.8 Molecular Marker Analyses

Molecular markers offer unique and valuable tools for evaluating and characterizing plant genetic diversity in a manner that is unaltered by the environment (Gupta and Varshney 2000). Genetic markers are very vital for germplasm conservation and core collection development (Diwan et al. 1995; Tanksley and McCouch 1997); it is also used in enhanced breeding applications such as marker-assisted selection (Staub et al. 1996).

The foremost molecular studies were focused on establishing genetic variability undomesticated quinoa and wild species (*C. hircinum* and wild quinoa ajara) using allozyme markers (Wilson 1988a, b). The results highlighted two distinctive groups on the basis of molecular information: a coastal type from southwestern Chile and an Andean type from northwestern Argentina to southern Colombia, suggesting the co-evolutionary relationship between domesticated and free-living populations of the Southern Highlands (Wilson 1988b). Similarly, Fairbanks et al. (1990) used protein-based approaches to characterize quinoa seed storage proteins as a valuable tool for cultivar identification and breeding programs for improved protein quantity and quality.

Fairbanks et al. (1993) were the first to use random amplified polymorphic DNA (RAPD) markers in quinoa, and they observed that 26 primers produced polymorphic markers among 16 randomly selected accessions. The RAPD markers were also used by Ruas et al. (1999) to identify genetic variation among 19 accessions of 6 species of the genus *Chenopodium*. The results showed that wild and cultivated populations of *C. quinoa* shared a low level of molecular variation, without delineation between sympatric domesticated and weedy populations. RAPD has also been used by Del Castillo et al. (2007) to study the hierarchical structure among gecotype

Table 4.1 Similarity matrix based on simple matching coefficients of potential quinoa parents for genetic linkage mapping

	Chucapaca	NL6	0654	Ku-2
Chucapaca	1.000			
NL6	0.245	1.000		
0654	0.576	0.327	1.000	
Ku-2	0.229	0.866	0.304	1.000

1 jump threshold = 5 (Kosambi mapping function) for all linkage groups (Maughan et al. 2004)

populations of Highlands and Inter-Andean valleys in Bolivia. The result revealed a marked geographical effect on the populations' structure and pointed out climatic and orographic barriers present in the studied zone contributed to the observed variations rather than to a distance effect. Hence, the population structure was associated with the three major biogeographic zones present in Bolivia, viz., Northern and Central Highlands, Inter-Andean valley, and southern Salar. The intra-population genetic diversity was higher than expected, basically due to autogamous reproduction, in addition to the limited seed exchange among isolated regions studied (Del Castillo et al. 2007).

Maughan et al. (2004) screened 60 RAPD primers in 4 mapping populations of quinoa; 6 (10%) created reproducible polymorphic markers and were included in linkage analysis for quinoa. One polymorphic band was scored from each of the six polymorphic RAPD primers, with an average of 3.8 prominent bands per RAPD reaction. One RAPD marker (O-F10), however, was scored in a co-dominant fashion, while the remaining five RAPD markers were scored as dominant markers. None of the RAPD markers deviated significantly ($P > 0.05$) from their expected segregation ratios.

The first step toward the development of genetic markers for quinoa was the development of a genetic linkage map by Maughan et al. (2004). The map was based primarily on amplified fragment length polymorphism (AFLP) and covered an estimated 60% of the genome. Eighty-eight (88) AFLP primer combinations were screened for polymorphism among four potential mapping parents ("Ku-2," "NL-6," "0654," and "Chucapaca"), representing two different ecotypes for quinoa: "Ku-2" and "NL-6" from the coastal region and "0654" and "Chucapaca" from the Altiplano region. A total of 597 polymorphic bands across the 4 potential parents were identified. The average number of bands identified for individual primer pairs ranged from 19 to 52, with an average of 6.8 polymorphic bands per primer combination. Moreover, 68 out of the 88 AFLP primer combinations screened for polymorphism between the mapping parents were polymorphic and highly reproducible based on duplicated samples. The similarity coefficients of the four potential parents for the linkage mapping (Table 4.1) ranged from 0.23 to 0.87 and the least genetic similarity between the Bolivian accession "Chucapaca" and the Chilean accession "Ku-2," while the highest similarity was between the two Chilean coastal accessions "NL-6" and "Ku-2." These findings supported the previous morphological and isozyme studies (Wilson 1988a, b; Risi and Galwey 1989a), which separated quinoa

germplasm into two distinct fundamental elements: Chilean coastal types and Andean Altiplano types.

The difficulties associated with AFLP marker technologies and the related transfer of this technology to developing world countries where quinoa is being cultivated have limited the use of AFLP markers to enhance quinoa improvement programs (Jarvis et al. 2008). This led to identification and characterization of more feasible SSR markers of quinoa which once developed can be used across different laboratories to determine genetic diversity in quinoa.

Maughan et al. (2004) screened 39 putative simple sequence repeat (SSR) loci previously identified from an SSR-enriched genomic library for polymorphism in 4 potential mapping population parents; they identified 21 SSR markers as polymorphic, while 13 produced simple monogenic banding patterns and were easily scored in a co-dominant fashion. A good number of the other SSR markers yielded amplification products with complex banding patterns that made scoring the marker in a co-dominant fashion difficult; however, a single, unambiguous, and clearly segregating band was scored in a dominant fashion. These complex banding patterns could be a result of the occurrence of duplicate chromosome regions (Rae et al. 2000), which may be the remains of quinoa's probable allotetraploid origin.

Mason et al. (2005) took the next step in quinoa marker development and characterized 208 SSR markers which were validated and characterized in 31 cultivated quinoa accessions, representing the main growing areas of South America. These SSR markers have been utilized to assess the genetic diversity among quinoa accessions within the USDA collection (Christensen et al. 2007).

Unfortunately, less than 10% of the 208 SSR markers identified by Mason et al. (2005) have been mapped genetically, and only 67 of these were considered highly polymorphic ($H > 0.7$), stressing the need for additional SSR marker development and genetic mapping. To this effect, Jarvis et al. (2008) developed a new set of polymorphic SSR markers to increase the number of SSR markers already available in quinoa and constructed a new genetic linkage map of quinoa based primarily on the SSR markers developed. From their result, out of the 402 SSRs tested, 54% (216) were polymorphic when tested on the screening panel of 7 quinoa accessions. An additional 4.7% (19) were polymorphic when the *C. berlandieri* accession was included in the analysis (interspecies polymorphism). The remaining 41.05% (165) primers were monomorphic or amplified poorly. In only 9 (2.2%) cases did a primer successfully amplify in quinoa but not in *C. berlandieri*, signifying that these two *Chenopodium* species share a high degree of DNA sequence homology.

All the 216 markers identified by Jarvis et al. (2008) were considered polymorphic (according to the recommendations of Ott (1992)), and 53 (25%) are considered highly polymorphic ($H \geq 0.70$), and H values ranged from 0.12 to 0.90, with an average value of 0.57.

Fuentes et al. (2009) genetically characterized Andean and Chilean germplasm to quantify the genetic diversity within 28 Altiplano and 31 coastal Chilean accessions of quinoa using microsatellite markers. Results of both cluster (UPGMA) and principal component analyses generated separated the accessions into two discrete groups, as was also revealed by isozyme analysis and morphological traits (Wilson

1988a, b), AFLP analysis (Pratt 2003), and microsatellites (Christensen et al. 2007). The first group contained quinoa accessions from the north (Andean highlands), and the second group consisted of accessions from the south (lowland or coastal). The result obtained in the diversity analyses emphasized the relationships both within and among northern and southern Chilean quinoa accessions and provides a new set of simple-to-use and highly informative genetic markers.

Fuentes et al. (2012) characterized 20 microsatellite genetic markers in a multi-origin set of 34 quinoa accessions to understand the impact of farmers' seed exchanges and local production practices on the genetic structure and diversity of quinoa on a national scale in Chile. The heritability for all quinoa accessions studied ranged between 0.12 (QGA17) and 0.87 (QAAT76) with a mean value of 0.65, which indicated the presence of wide genetic diversity in the quinoa samples and confirmed the highly informative quality of the markers used. The UPGMA analysis using the Jaccard coefficient identified two major groups which were further subdivided into five populations (Fig. 4.4). The genetic information obtained permitted the detection of variation among and within the populations identified, which corresponds to natural geographical-edaphic-climatic constraints to the expansion of biodiversity. This grouping also links with the social-linguistic context of ancient people inhabiting the Andes region, where agronomic and cultural traditions that have thrived until the current time are very different.

4.9 Abiotic Stress Tolerance and Associated Genetic Mechanisms

Quinoa has been found to tolerate several abiotic stresses such as differing soil pH, soil salinity, frost, and drought; this could be due extreme climatic conditions where quinoa evolved. Tolerance to these abiotic stresses is determined by complex mechanisms and polygenically inherited traits.

4.9.1 Soil pH and Frost

Quinoa can tolerate both highly acidic and basic soils with pH ranging between 4.8 and 9.5 due to its mycorrhizal associations, which also facilitates the acquisition of scarce nutrients (Urcelay et al. 2010). Since frosts are common in the Andes, the effects of temperature on germination, phenology, and growth have been the focus of several studies (Jacobsen et al. 2005, 2007). Several genotypes and cultivars from the Andean highlands of Bolivia that show varying degrees of responses to low temperatures have been identified (Bertero et al. 2004; Fuentes 2008). Quinoa can also tolerate freezing preceding the formation of flower buds (Bhargava et al. 2006a). It grows properly at temperatures of −5 °C and endures temperatures as low as −16 °C during the vegetative stage (Bois et al. 2006). During flowering, it tolerates −8 °C up to a period of 2 h (Jacobsen et al. 2007). However, details of the physiological and the genetic mechanisms responsible for the observed frost

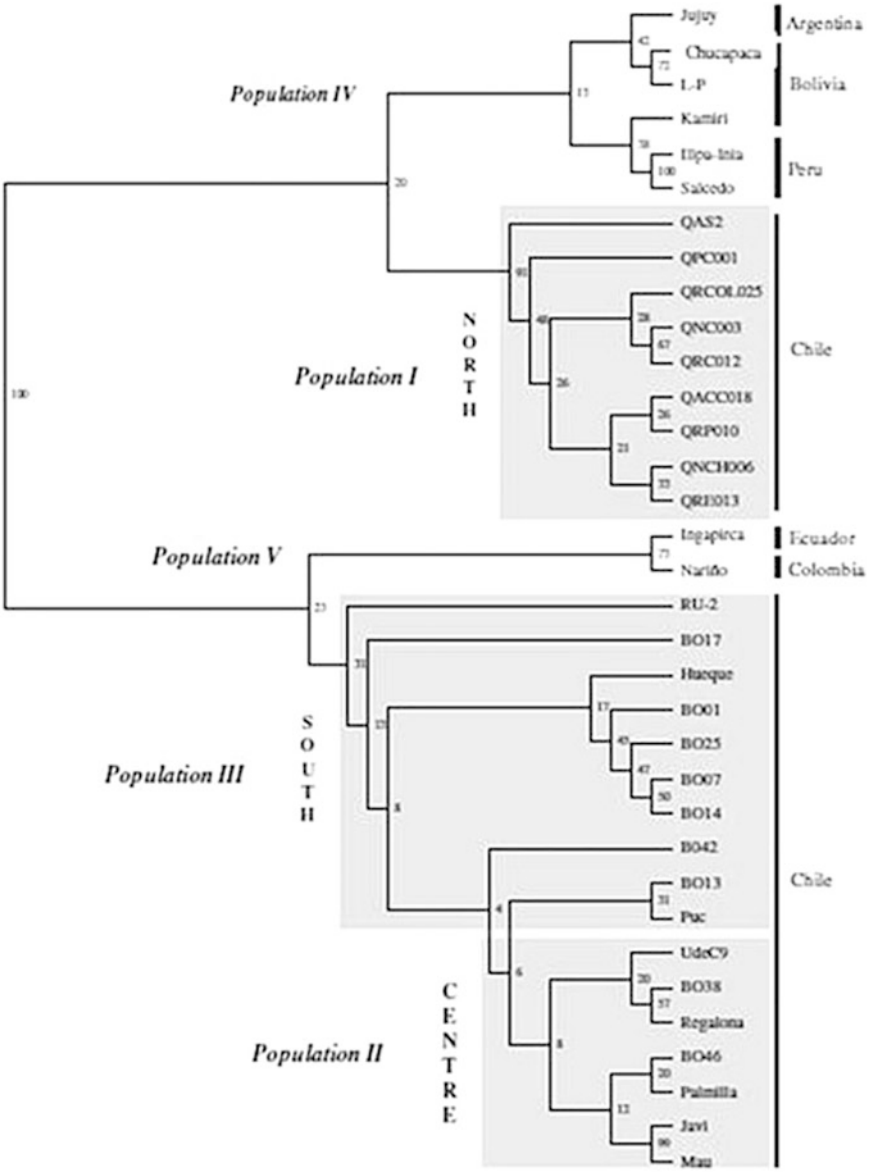

Fig. 4.4 UPGMA cladogram based on Jaccard's similarity coefficient of 34 quinoa accessions performed after 500 replicates for bootstrap test (percentage number between each node) (Fuentes et al. 2012)

resistance remain unknown (Jacobsen et al. 2007), but proline content and levels of soluble sugars such as sucrose might serve as markers of frost tolerance in quinoa breeding lines (Jacobsen et al. 2007).

4.9.2 Salinity

About 40% of quinoa genera are facultative halophytes; this advantageously places quinoa over glycophytes as it is known that it can thrive in saline conditions because of its capacity to avoid the harsh impacts of high salt accumulation, which will cause hyperosmotic stress in roots and other structures, thereby decreasing the plant's ability to absorb water efficiently (Adolf et al. 2012). Also, it has the ability to survive at salinity levels higher than that of seawater, and this makes it more suitable than some other halophytes under similar abiotic stress (Adolf et al. 2012). However, salinity increases the accumulation of sapogenins (Solíz-Guerrero et al. 2002; Pulvento et al. 2012); this implies that quinoa cultivated in areas with high soil salinity may tend to produce seeds that are bitter, a trait that is undesirable to many farmers.

A lot of the ~6000 global quinoa germplasm accessions have been subjected to salt treatments both in situ and field experiments, and they exhibited varying levels of tolerance both at germination and progressive developmental phases (Ruiz et al. 2014; Valencia-Chamorro 2003; Christiansen et al. 1999; Karyotis et al. 2003; Wilson et al. 2002). The significant characteristic of salinity tolerance has been thoroughly studied, particularly the physiological and the molecular mechanisms involved, the mechanisms specifically associated with salt ion accumulation in specialized tissues, and the adjustment of leaf water potential (Adolf et al. 2013). Quinoa species accumulate salt ions in its tissues by adjusting the water potential in its leaves; this allows the plant to sustain cell turgor and limit plant transpiration under saline conditions (Hariadi et al. 2010; Shabala et al. 2012). Other studies by Koyro and Eisa (2008) and Burrieza et al. (2012) suggest that dehydrin accumulation, subcellular localization, and phosphorylation state of mature seed embryos are related to high salt stress.

Genetic constituents related to salt tolerance exhibit additive effects, recessive or dominant relationships, and heterosis. Less than 25% of the salt-regulated genes that have been identified by Ma et al. (2006) are salt stress-specific. Adolf et al. (2012) in a review paper elucidated the mechanisms contributing to salt tolerance in quinoa to include efficient control of xylem Na^+ loading and Na^+ compartmentalization in leaf vacuoles, higher tolerance to reactive oxygen species (ROS), better K^+ retention, and an efficient control over stomatal development and aperture. Shabala and Mackay (2011) suggested that salinity tolerance may also be improved by pyramiding key genes regulating salinity tolerance, which is a very essential physiological trait, and quinoa might serve as a valuable donor of salt-tolerant genes to other crops. The large genetic variability for salinity tolerance in quinoa is a huge resource for the selection and breeding for higher tolerance; however, this poses challenges and opportunities for the future (Maughan et al. 2009; Gomez-Pando et al. 2010; Ruiz-Carrasco et al. 2011; Adolf et al. 2012).

While studying the molecular basis of salt tolerance in quinoa, Maughan et al. (2009) described the molecular characterization of Salt Overly Sensitive 1 (SOS1) gene. They reported a complete genomic sequence of two homologous SOS1 loci, cqSOS1A and cqSOS1B, which extended from 98,357 to 132,770 bp, respectively.

Relative gene expression of SOS1 in roots under saline conditions (450 m mol/L) was consistently three- to fourfold higher than in leaf tissue. A constitutive expression of SOS1 genes was observed in the roots, while an inducible expression occurred in leaves under stress; this is probably because the SOS1 expression was more strongly upregulated by salt stress in leaves as compared to the roots.

Similarly, Ruiz-Carrasco et al. (2011) reported gene expression analyses for two sodium transporter genes: CqSOS1 and CqNHX genes. Quantitative RT-PCR analyses of these genes revealed that their expression was differentially induced at the shoot and root level (as was reported by Maughan et al. (2009)) and between genotypes by 300 mM NaCl.

4.9.3 Drought

Quinoa has inherently low water requirements and is therefore highly drought-tolerant and responds to drought stress through drought escape, tolerance, and avoidance (Jacobsen et al. 1999; Jacobsen and Mujica 2003; Garcia et al. 2007). Other defensive mechanisms used by quinoa include tissue elasticity, low osmotic potential, decreased leaf area through dehiscence, and the presence of vesicular calcium oxalate and structurally with small and think-walled cells (Canahua 1977; Garcia 2003; Jacobsen et al. 2009; Abugoch et al. 2009). Drought reduces the accumulation of sapogenins by 45% in quinoa seeds, based on a study of severe water deficit conducted in Southern Europe (Gomez-Caravaca et al. 2012).

However, lack of understanding of the genetic behavior of such a complicated trait as well as biochemical constituents and anatomical attributes responsible for drought tolerance has led to the delay of plant breeders to incorporate drought stress tolerance into breeding programs (Al-Naggar et al. 2002a, b). Reports on heritability and genetic advance from selection for leaf anatomical traits of quinoa subjected to drought stress are scarce; this led to an investigation by Al-Naggar et al. (2017) on five genotypes of quinoa.

From their result, the effect of soil moisture content on leaf tissues had showed significant differences among the studied genotypes. The genotype CICA 17 (the most drought-tolerant) had the thickest leaf under well-watered condition (WW), moderate water stress condition (WS), severe water stress (SWS), and when all irrigation regimes were combined, while the thinnest leaf was shown by the genotype CO-407 and Ollague (drought-sensitive) under WS and when all irrigation regime conditions were combined. Heritability estimates in the broad sense for anatomical traits were very high in magnitude (>87.5%), except for the lower epidermis (41.18, 59.41, and 33.33) under WW, WS, and SWS, respectively, indicating that environment had minimal effect on the phenotype of most studied anatomical traits in the leaves of quinoa (Al-Naggar et al. 2017). The highest heritability estimate (100%) was shown by upper epidermis under severe water stress. The genetic advance (GA%) from selection was generally higher under moderate water stress (WS) for three anatomical traits (leaf thickness, lower epidermis, and palisade layer) and under well watering for two traits (upper epidermis and

spongy layer). GA ranged from 15.40% for the upper epidermis to 72.97% for palisade layer under SWS, from 52.66% for leaf thickness to 82.72% for palisade layer under water stress, and from 30.40% for leaf thickness to 87.12% for spongy layer under well watering (WW). Therefore, palisade and spongy layers under all environments were characterized by having high heritability accompanied by high values of expected genetic advance, especially under WS and SWS. Since efficiency of selection depends on the degree of heritable variability, higher heritability together with high expected genetic advance for the leaf anatomical traits studied should be quite valuable in future breeding programs for drought tolerance in quinoa (Al-Naggar et al. 2017).

Some authors (Blum 1988; Hefny 2007; Al-Naggar and Shehab-El-Deen 2012; Al-Naggar and Atta 2017; Al-Naggar et al. 2009, 2011, 2016a, b) opined that heritability and expected genetic advance is higher under stress than non-stress conditions and that selection should be practiced in the stressed environment to obtain higher genetic advance. However, another group of researchers found that heritability and GA from selection for grain yield is higher under non-stress than those under stress (Shabana et al. 1980; Atlin and Frey 1990; Banziger and Lafitte 1997; Worku 2005).

There is a need for further investigation on the type of gene action controlling the inheritance of drought tolerance traits to help plant breeders in tackling the physiologically and biochemically complex drought tolerance.

4.10 Conclusion

Quinoa cultivation constitutes an important opportunity to diversify low-input farming of growers in the Andes and elsewhere. Because of its well-documented tolerance to several abiotic stresses, such as drought, salinity, low soil fertility, and frost, this ancient crop could make vulnerable cropping systems much less unstable (McElhinny et al. 2007; Kitz et al. 2009; Razzaghi et al. 2012). Pivotal to achieving this aim are breeding programs focused on increasing yield potential, pyramiding of abiotic tolerances, and diminishing seed saponin levels to obtain sweet genotypes. Conventional as well as molecular tools should be utilized to unlock the rich biodiversity and potential of quinoa.

The wide range of environments in which quinoa can grow has a direct influence on its genetic diversity (Matanguihan et al. 2015). The adaptation of quinoa to vastly different climatic conditions over a long period of time may have contributed to its broad genetic diversity (Costa Tártara et al. 2012). Genetic variability has a spatial structure and distribution; this can be seen in quinoa as phenotypic and genetic diversity studies have shown that quinoa accessions are most often clustered according to their geographic origin (Risi and Galwey 1989a, b; Ortiz et al. 1998; Rojas et al. 2000; Del Castillo et al. 2007; Costa Tártara et al. 2012; Curti et al. 2012). Also, regardless of narrowing of genetic base during domestication, wide genetic diversity still exists for plant color, seed color, types of branching and panicles, as well as grain productivity, abiotic stress tolerance, and disease resistance

(Bhargava and Ohri 2016). This diversity, which is also reflected at the molecular level, is being used by the plant breeders all over world to develop improved plant (Bhargava and Ohri 2016).

The application of informative molecular markers has made it possible to reveal the genetic diversity of quinoa accessions. Seed exchanges and germplasm distribution have considerably affected the genetic diversity as well as genetic structure of quinoa (Costa Tártara et al. 2012). The results of genetic diversity studies of important agronomic traits in quinoa and associated genetics of such traits will greatly aid conservation efforts and, consequently, plant breeding programs.

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Advances of Biotechnology in Quinoa Production: A Global Perspective

5

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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 · Genetic markers · Genomics · Endophytes · Breeding · 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

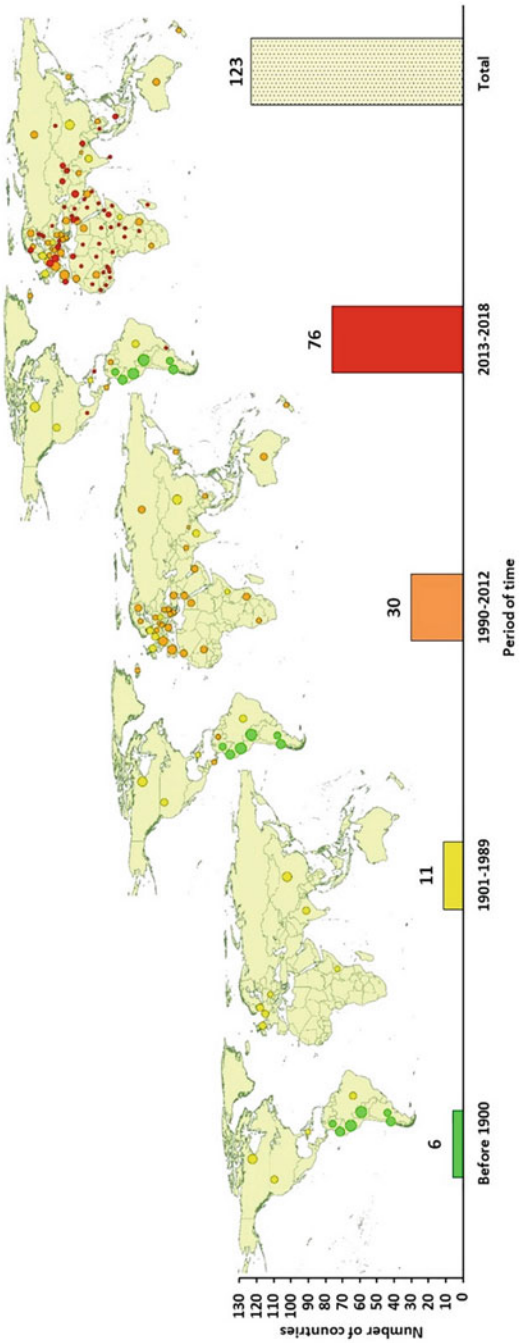

Fig. 5.1 Global distribution of quinoa along four periods of time: before the 1900s, 1901–1989, 1990–2012, and 2013–2018

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

Table 5.1 Key examples of biotechnology use in quinoa along time

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)		• Peruvian quinoa lines developed from in vitro vegetative (Ruiz 2002) • In Brazil to increase stocks of hybrid seeds, a protocol is developed (Rocha 2011)
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 *Chenopodium* genera (Wilson 1988)	• Fairbanks et al. (1993) used RAPD molecular markers to detect polymorphisms in quinoa	• 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) • Use of fluorescence in situ hybridization (FISH) to quantify the number of RNA loci in quinoa (*C. berlandieri* var. zschackei and *C. berlandieri* spp. *nuttalliae* (Maughan et al. 2006)) • FISH is used to examine common ancestors between *C. quinoa*, *C. berlandieri*, and *C. album* (Sederberg 2008; Kolano et al. 2011) • The evolution of polyploidy in quinoa was demonstrated at the chromosomal level using FISH (Kolano et al. 2012; Matanguihan et al. 2015)

(continued)

Table 5.1 (continued)

Approach	During the 1980s	During the 1990s	After the 2000s
			• Hong et al. (2017) obtained complete chloroplast (cp) genomes of *C. quinoa* and *C. album* by next-generation sequencing
Development of molecular markers		• Bonifacio (1995, 2004) developed RAPD markers for screening interspecific and intergeneric crosses with *C. berlandieri*, *C. berlandieri* ssp. *nuttalliae*, and *Atriplex* sp. • Substantial genetic similarity was found between coastal and Andean ecotypes (Wilson 1988; Christensen et al. 2007)	• RAPD was used by Ruas et al. (1999) and Del Castillo et al. (2007) to demonstrate the relationship between *C. quinoa* 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. (2008) 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

(continued)

Table 5.1 (continued)

Approach	During the 1980s	During the 1990s	After the 2000s
novel technologies			2013; Mestanza et al. 2018) • The Japanese team led by Yasui et al. (2016) reported an incomplete genome sequence in an inbred line of quinoa (Kd) • Jarvis et al. (2017) provided a complete genome of quinoa from a coastal Chilean quinoa accession • Zou et al. (2017) provided a complete genome of quinoa from a quinoa accession from the Highlands • Tolerance to salinity identified with genomics, high-throughput sequencing and bioinformatics in different *Chenopodium* accessions (Schmöckel et al. 2017)

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).

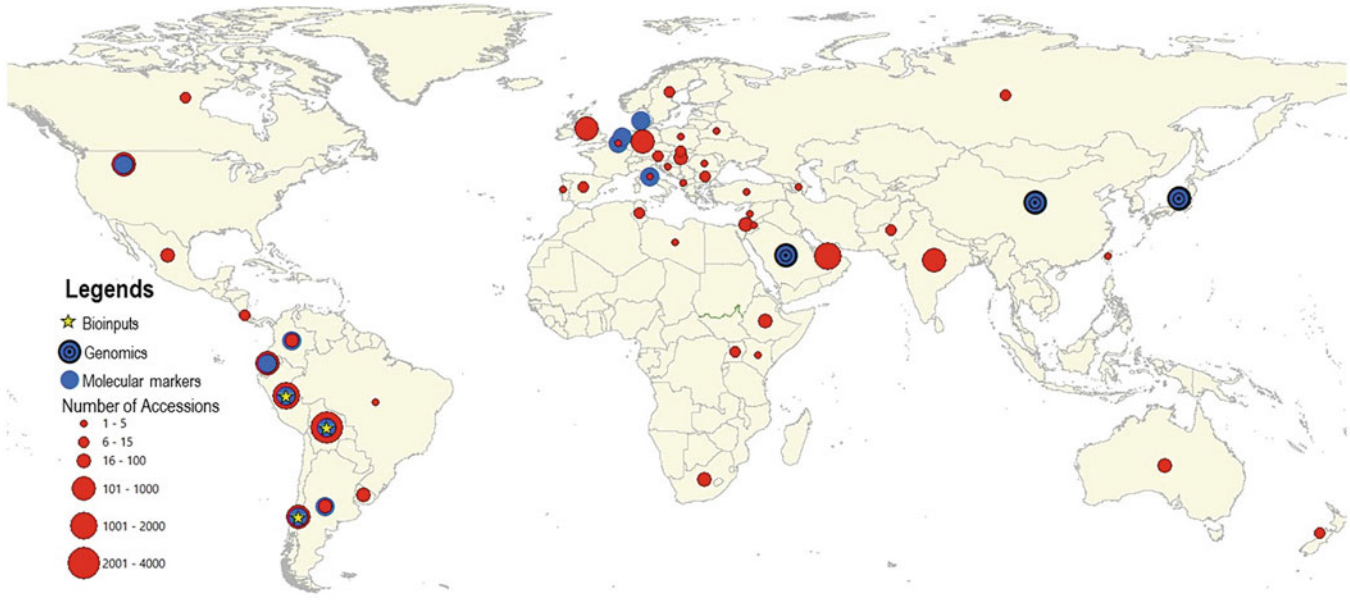

Fig. 5.2 Genebanks with quinoa accessions and biotechnology applications on this crop around the world. Red circles, gene banks and number of accessions held. Circles are scaled in proportion to the number of quinoa accessions conserved ("Number of accessions"); light blue circles, different studies 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

Table 5.2 Examples of advanced techniques used for breeding Andean grains

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)

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.

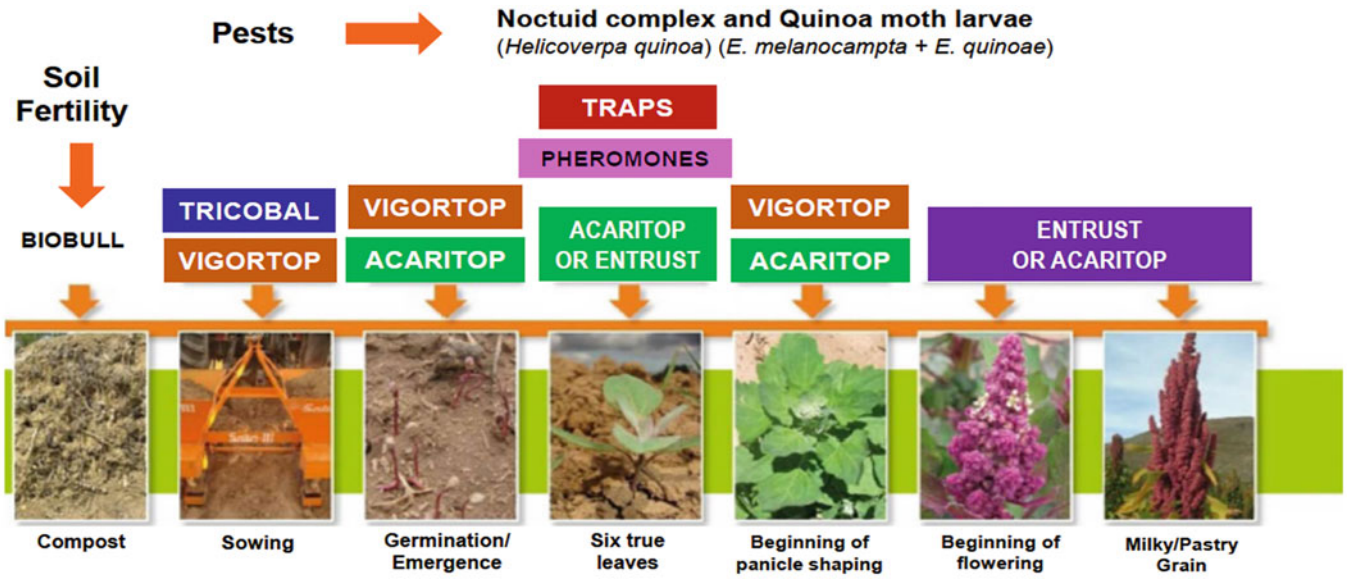

Fig. 5.3 Strategy of organic quinoa production with the use of products resulting from biotechnology (Biobull and Tricobal: two bio-inoculants developed from native strains of bacteria that work as growth-promoter, biofertilizer, and biopesticide). Source: modified to English from Fundación PROINPA and Biotop SRL (2020)

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. 2009; Yong 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 gene discovery to understand gene expression as well as 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[1] 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

[1] 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; Bedoya-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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Agronomic Manipulations for Cultivation of Quinoa (*Chenopodium quinoa* Willd.)

6

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a highly nutritious crop and has wider adaptability in varied agroecological environments including marginal lands. Other than the Andean region, the centre of origin of its cultivation is gaining popularity among farmers in countries like the United States, the United Kingdom, Denmark, India, Pakistan, and China, although production volume is meagre. The genetic and phenotypic variability of genotypes resulted in the elasticity of plant foliage. Because of the different growth habits and foliage coverage, it is cultivated under varying crop geometries at different sowing dates with variable plant population and density. Moreover, due to variations in native soil fertility and nutrient acquisition efficiency, the demand for fertilizers, specifically primary nutrients (N, P, and K), ranges from very low to very high. The yield or productivity is also substantially influenced by different agronomic interventions. Therefore, in this chapter, different agronomic manipulations for the cultivation of quinoa, viz. climate, soil, sowing time, sowing methods, crop

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geometry, varieties, nutrient management, weed management, water management, harvesting, storage, etc., have been discussed and elaborated. The improved agronomic practices would be helpful to achieve greater productivity and profitability.

Keywords

Agronomic interventions · Cultivation · Nutrients · Quinoa · Yield

6.1 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is considered to be native to the Andes Mountains of Bolivia, Chile, and Peru. Quinoa is also regarded as 'vegetable caviar' or 'Inca rice' and has been consumed for over 5000 years by people living on the mountain plateaus and in the valleys of Peru, Bolivia, Ecuador, and Chile. This crop has also been a staple food for the Inca people and remains an important food crop for their descendants, the Quechua and Aymara peoples who live in rural regions. Due to its high nutritional value and adaptability in marginal lands, quinoa is promoted in the Andes, and its demand in high-income countries is increasing at a faster rate. Consequently, production of quinoa has thundered enormously in the Andes region since 2010, with Peru serving as the main producer and exporter. In the Andes region, it is cultivated as a subsistence crop by smallholders and traditionally cultivated as a common staple. Moreover, in the Peruvian coastal region, it is increasingly produced by large-scale farms as a high-value export crop. Additionally, since 2014, cultivation of quinoa outside of the Andean region has been spreading in countries like the United States, the United Kingdom, and Denmark, although production volume is meagre. This changeover has occurred as a result of the release of improved varieties of quinoa, concentrating on higher yields and shorter maturity periods (Bonifacio et al. 2006; Ofstehage 2012; Bazile et al. 2016; Gamboa et al. 2018). Worldwide, it is cultivated in an area of 178,313 ha, yielding 158,920 tonnes and a productivity of 8912 kg/ha. Peru in South America is the largest producer (86,011 tonnes) with 54.12%, followed by Peru (70,763 tonnes) with 44.52% (FAOSTAT 2018). In India, quinoa was cultivated in an area of 440 ha with an average yield of 1053 tonnes (Rao 2015).

The growing period of quinoa varied between 70 and 200 days, and some germplasm lines/entries did not mature at some locations. Germplasm entries evaluated for maturity period of quinoa in America, Europe, and Africa reported that in Kenya, it matures in 65–98 days, and all cultivars matured with seed yield of 4000 kg/ha (Jacobsen 2003). In India, due to its wider adaptability and multifarious advantages, it can play a major role in future diversification of agriculture systems. Since quinoa is drought-, frost-, and salt-tolerant, it is considered suitable for growing in marginal lands (=underutilized, unproductive, waste, idle, abandoned, degraded lands). Further, it develops a highly specialized taproot system, which can penetrate up to the depth of 1.5 m underneath soil surface, extracts soil moisture

from the deeper layers of the soil, and can thrive well under drought conditions. Naturally, it is tolerant to varied abiotic stresses or natural environmental abnormalities. Likely, it is adapted to withstand frost, drought, extreme heat, and nutrient-deficient soils. It can survive on mountains with thin soil and high winds; it can be modified and grown to live in most types of soil and weather conditions (Jinger et al. 2017). More importantly, quinoa thrives well in low-nutrient or low-fertility soils. As a result of the Indian subcontinent having heavy population pressure with shrinking land holdings, it could be an alternative to easing the challenges of malnutrition and filling empty stomachs. Since this crop is gaining popularity worldwide, agronomic management practices for achieving better yields are being discussed in this chapter.

6.2 Climate

Quinoa requires short day lengths and cool temperatures for its growth. It requires a cool temperature for good vegetative growth. These plants are usually tolerant to light frosts. Normally, the acceptable mean temperature for growth is 15–20 °C, although it can thrive well at mean temperatures ranging from 10 to 25 °C (Jacobsen et al. 2003). However, temperature beyond 35 °C at the flowering stage tends to cause pollen sterility, flower abortion, or plant dormancy (Johnson and Croissant 1990). It flowers earlier when exposed to shorter day lengths. The successful cultivation in the Encalilla region of Argentina proved the suitability under desert-type (BWkaw) climate (according to the Köppen classification system). In Encalilla, Argentina, it is suitably cultivated with annual rainfall of 200 mm, of which 70% (∼150 mm) is received during the growing season (September–March), air temperature of 30.4 °C (maximum) and 11.1 °C (minimum), and relative humidity of 44.2% (minimum) and 54.2% (maximum). Further, it could withstand wind velocity ranging from 10 to 25 km/h, photosynthetic active radiation (at midday) values of 1403μ mol/m^2/s (under cloudy conditions) to 1993μ mol/m^2/s (under sunny conditions), and suitable day length at 9.8 h during early spring and 11.3 h during summer (Gonzalez et al. 2012).

Normally, quinoa is an extraordinary crop, capable of producing grains even under drought conditions. Phenological plasticity and resistance to climate constraints, the characteristics of quinoa, make it exceptionally adapted to the different arid climates. This is one of the few crops that can tolerate frost to a certain extent, but this depends largely on the duration of the frost, the quinoa variety, the phenological stage of the plant when frost occurs, the relative humidity, and the micro-location of the fields (Garcia et al. 2015).

6.3 Soil

The crop can be cultivated in soils with a textural class ranging from sandy to clay, although soils with good drainage are better for its growth and for achieving good harvest. In the continent of South America, it is preferably grown in marginal agricultural areas. These marginal areas are prone to drought and to having low to poor natural soil fertility. These soils also have poor or excessive drainage and are very acidic (pH of 4.8) to alkaline (8.5) in reactions. The crop can also grow well on sandy loam to loamy sand soils (Oelke et al. 1992). However, in Europe, it can be grown on a variety of soils including marginal soils with a pH range of 4.5–9.5 (Tapia 1982; Jacobsen and Stolen 1993). In central Greece, it thrives well from sandy to loamy soils under a wide range of soil pH (4.8–8.5). Furthermore, it is tolerant to saline soils and can be irrigated with water rich in salts (Jacobsen et al. 1999). Quinoa, being a facultative halophyte, can be grown in extreme saline soil conditions having high electrical conductivity up to 52 d S/m (Jacobsen et al. 2001).

6.4 Seedbed Preparation

Quinoa requires a level, well-drained seedbed in order to avoid waterlogging. Depending on the soil type and available soil moisture, the suitable depth of planting of seeds is at a depth of 1.25–2.50 cm (Oelke et al. 1992). However, the optimal depth of seeding is also advocated at 1–2 cm deep in a fine-structured, moist seedbed (Jacobsen et al. 2003). Further, in the Encalilla region of Argentina, central Greece, the sowing depth of 2–3 cm is general practice (Karyotis et al. 2003; Gonzalez et al. 2012). The small size of the seed makes it susceptible to both dehydration and waterlogging when planted too shallow or too deep.

Globally, various sowing techniques are employed for proper establishment of quinoa. Sowing of quinoa is done in a variety of ways. It can be sown in rows or groups or by broadcasting or transplanting. However, ridge planting (a ridge with furrow) provides favourable conditions to the roots and helps enhance grain yield. A field experiment was conducted at Faisalabad, Pakistan, on various types of sowing techniques, viz. flat sowing (75 × 15 cm), ridge sowing (75 × 15 cm), bed sowing (75 × 15 cm), and sowing in standing water (75 × 15 cm), wherein plant population was kept uniform throughout the sowing techniques. The sowing techniques substantially influenced yield and yield attributes of accessions. Bed sowing overtook the rest of the planting techniques and produced the highest main panicle weight, thousand grain weight, grain yield, and harvest index (35% and 67%, 21% and 26%, 75% and 64%, and 63% and 56%, respectively) during both years of experimentation compared with sowing in standing water (Ali et al. 2020). Overall, the trend of superiority of sowing techniques was in the following order: bed sowing < ridge sowing < flat sowing < sowing in standing water.

6.5 Sowing Time

Sowing is one of most imperative activities for establishment, as seedling emergence affects plant density and grain yield. In the Andes, the centre of origin is largely grown during the austral summer months (September to May), even though the sowing times markedly differ and, in some equatorial region cultivation, may go beyond that period (Garcia et al. 2015). The average sowing date in the Andes lies mostly between September and November, although in some minor areas close to the equator, the sowing date could extend until February (Garcia et al. 2015). The differences in sowing dates are ascribed to crop cycle lengths of individual varieties and local climatic conditions. Sowing time largely depends on climatic factors, seeds sown, location, variety, soil moisture, regional climatic variables, sowing depth, and other agronomic management variables. Further, greater genetic variability and an enormous number of cultivars/varieties/genotypes make it adaptable to growing from sea level to 4000 m above sea level, geographical coordinates ranging from 40°S to 2°N latitude, and growing conditions ranging from cold, highland climate to subtropical conditions worldwide (Hirich et al. 2014). Jacobsen et al. (2003) reported that a major constraint of growth in the northern parts of Europe and Canada and in high-altitude regions is the short growth season, since it requires a maximum developmental time of 150 days in order to attain seed harvestable status. Therefore, early maturity is one of the most significant traits of quinoa grown under these conditions. Wider sowing window led to good potential for increased production in southern Europe, the United States, and certain parts of Africa and Asia.

In order to investigate the effects of sowing date on performance, series of experiments were carried out in the south of Morocco by Hirich et al. (2014). They tested ten sowing dates, each at a 15-day interval starting from 1st November to 15th March, and revealed that sowing dates affected growth and productivity due to differences in temperature, precipitation, and radiation over the years. During the experiment, the highest seed yield and dry matter yield were attained when sown in November and early December. Early sowing in November to early December secured good plant development when low temperatures arrived in January and February and downy mildew appeared in March. The growing season length has been affected by accumulated radiation. In addition to abiotic factors (temperature, radiation, rainfall) affecting growth, biotic factors, such as downy mildew and weeds, affected the yield (Hirich et al. 2014). Field experiments conducted in semiarid regions of Telangana observed 15th October as normal date of sowing with 2001 kg/ha yield (Ramesh et al. 2017). Worldwide, based on the available research findings, different sowing times of quinoa are listed in Table 6.1.

6.6 Plant Population

Depending upon the types of genotypes and foliage, the row-to-row and plant-to-plant distance is optimized. The optimum crop geometry (row-to-row and plant-to-plant distance), recommended for several regions is entrusted in Table 6.2.

Table 6.1 Varying sowing time of quinoa over the region

Sowing time	Region	References
Mid-May	Rosemount, Minnesota	Robinson (1986)
Late April to mid-May	Southern Colorado	Robinson (1986)
15th October	Telangana, India	Ramesh et al. (2017)
Mid-September	Encalilla, Argentina	Gonzalez et al. (2012)
November to early December	Southern Morocco	Hirich et al. (2014)
First week of May	Western Greece	Kakaboukia et al. (2015)
First fortnight of March	Central Greece	Karyotis et al. (2003)
September to November	Andes, South America	Risi and Galwey (1984)
March–April	Cambridge, UK	Risi and Galwey (1991)
15 October to 15 November	Kota, India	Tetarwal et al. (2017)
Third week of November	Faisalabad, Pakistan	Ali et al. (2020)
Last week of May	Manitoba, Canada	Kostuik et al. (2014)
First week of November	Dubai, UAE	Rao and Shahid (2012)

Table 6.2 Crop geometry of quinoa in the different regions of the world

Crop geometry	Region	References
50–80 cm × 15 cm	Colorado, USA	Oelke et al. (1992)
15 × 10 cm	Telangana, India	Ramesh et al. (2017)
50 × 30 cm	Encalilla, Argentina	Gonzalez et al. (2012)
30 × 15 cm	Western Greece	Kakaboukia et al. (2015)
40–80 cm × 15 cm	Andes, South America	Risi and Galwey (1984)
75 × 15 cm	Faisalabad, Pakistan	Ali et al. (2020)
150 × 30 cm	Chile	Fuentes and Bhargava (2010)
50 × 25 cm	Dubai, UAE	Rao and Shahid (2012)

In Colorado State, USA, plant population of 320,000/ha appears to be optimal for achieving good yield. To achieve this plant population, a seed rate of 550–850 g/ha is sufficient. Under abnormal conditions, the seed rate could be enhanced. Unlikely, 10 kg seed/ha at a density of 150,000 plant/ha is being used in central and western Greece (Karyotis et al. 2003; Kakaboukia et al. 2015). In the Andes region of South America, a seed rate of 8–15 kg/ha is normally used (Risi and Galwey 1984). Better stands are obtained when seed is planted in a moist soil, instead of irrigating after planting prior to emergence. Moreover, it is revealed from the field experimentations carried out in the Great Britain that increasing plant density resulted in a slightly earlier maturity, enhanced seed yield, and less branching of plants. Field experiments conducted in the semiarid regions of Telangana observed crop geometry of 15 × 10 cm as optimum and achieved 2070 kg/ha yield (Ramesh et al. 2017).

While conducting experiments, Risi and Galwey (1984) at Cambridge, UK, reported that seed rate and crop geometry are eminently determined by varieties. They noted that Baer, a quinoa cultivar well adapted to temperate latitudes, produced highest grain yields, and it was achieved at a seed rate of 20 kg/ha at the dense rows spaced at 20 cm. Blanca de Junin, another cultivar commonly grown at lower

latitudes with a longer growing season and at lower plant population densities, is more phenotypically plastic and gave little increase in yield above a sowing rate of 10 kg seed/ha. However, Amarilla de Marangani, cultivar originating in a similar location, also showed a high degree of phenotypic plasticity but still gave increased yield up to a sowing rate of 30 kg seeds/ha. This response was surprising, and the high proportion of stunted plants at sowing rates above 20 kg seed/ha may mean that such crops are weak and susceptible to lodging. Henceforth, genotypic variations had substantial influence on the determination of seed rate and crop geometry to a larger extent.

6.7 Nutrient Management

Nutrient management is an important task to achieve good harvest. It responds well to nitrogen, phosphorus, and potassium fertilizers, although nutrient requirement of quinoa is high for nitrogen and calcium, moderate for phosphorous, and minimal for potassium (Mujica et al. 2001). The field experiments conducted worldwide envisage the importance of nutrients (Table 6.3), although limited research work has been

Table 6.3 Nutrient requirement of quinoa in the different regions of the world

Nutrient	Yield (kg/ha)	Region	References
50 kg N/ha	456	Dubai, UAE	Rao and Shahid (2012)
60 kg N/ha	1606	Bydgoszcz, Poland	Gęsiński (2008)
75 kg N/ha	6330	Cambridge, UK	Risi and Galwey (1991)
100 kg N/ha	1696	Telangana, India	Ramesh et al. (2017)
112 kg N/ha	1849	Manitoba, Canada	Kostuik et al. (2014)
120 kg N/ha	3495	Southern Germany	Erley et al. (2005)
120 kg N/ha	2500	Kota, India	Tetarwal et al. (2017)
140 kg N/ha	1545	Colorado, USA	Johnson and Croissant (1990)
200 kg N/ha	2600	Western Greece	Kakaboukia et al. (2015)
360 kg N/ha	1203	Wadi El Natrun, Egypt	Bhargava and Srivastava (2013)
40 kg P_2O_5/ha	1849	Manitoba, Canada	Kostuik et al. (2014)
50 kg P_2O_5/ha	1696	Telangana, India	Ramesh et al. (2017)
50 kg P_2O_5/ha	2300	Central Greece	Karyotis et al. (2003)
50 kg P_2O_5/ha	2500	Kota, India	Tetarwal et al. (2017)
50 kg P_2O_5/ha	456	Dubai, UAE	Rao and Shahid (2012)
53.5 kg P_2O_5/ha	6330	Cambridge, UK	Risi and Galwey (1991)
60 kg P_2O_5/ha	1606	Bydgoszcz, Poland	Gęsiński (2008)
11 kg K_2O/ha	1849	Manitoba, Canada	Kostuik et al. (2014)
50 kg K_2O/ha	1696	Telangana, India	Ramesh et al. (2017)
50 kg K_2O/ha	2500	Kota, Rajasthan	Tetarwal et al. (2017)
50 kg K_2O/ha	456	Dubai, UAE	Rao and Shahid (2012)
53.5 kg K_2O/ha	6330	Cambridge, UK	Risi and Galwey (1991)
100 kg K_2O/ha	1606	Bydgoszcz, Poland	Gęsiński (2008)

done on nutrient or fertilizer requirement. In Colorado, USA, the variety 'Linares' and others responded favourably to the application of nitrogen fertilizer. Research on nitrogen and phosphorus requirements conducted at Colorado State University observed that maximum yields are possible with the application of when 170–200 kg N/ha (Johnson 1990). However, yields declined due to higher levels of available nitrogen present as a result of slower maturity and more intense lodging. No effect on yield was observed when 34 kg/ha phosphorus in the form of phosphate acid was applied, in comparison to an untreated field plot. Depending upon native soil fertility status, soil types, crop varietal response to applied fertilizers, planting density, soil environment, nutrient acquisition from the soil, etc., the crop showed wider level of recommendations. It ranges from 50 to 360 kg N/ha, from 40 to 60 kg P_2O_5/ha, and from 11 to 100 kg K_2O/ha (Table 6.3).

As evident from the research experiments, the 15th October sown took up 87.8 kg N/ha, 23.4 kg P/ha, and 48.3 kg K/ha from the soil (Ramesh et al. 2017). Similarly, sown at the crop geometry of 15 × 10 cm took up 90.9 kg N/ha, 24.5 kg P/ha, and 50.0 kg K/ha from the soil (Ramesh et al. 2017). Application of nitrogen enhances grain yield as well as protein content in the quinoa seed. Quinoa responds substantially with the increasing nitrogen fertilization (Berti et al. 2000). In an experiment, Berti et al. (1997) applied 0–225 kg N/ha sown at sea level in Chile and found the highest yields (3555 kg/ha) at the highest fertilizer levels. The yield response to increasing N fertilization was quadratic in shape, and a slight decrease in nitrogen use efficiency was noted up to 225 kg N/ha. Unlikely, higher doses of phosphorus and potassium are known to enhance its vegetative growth. The application of 120 kg N/ha to quinoa in Southern Germany enhanced grain yield by 194% over the control or no nitrogen, and similarly, N uptake was recorded at 130.2 kg/ha (Erley et al. 2005).

The concerted experimental results reveal that it is needed to use organic matter in a ratio of at least 3 t/ha, if the crop is planted after a grain crop (corn or wheat on the coast and barley or oats in the mountains). The average fertilization required in these regions is at the rate of 80 kg N/ha and 40 kg P_2O_5/ha and zero potassium. Since potassium is easily available in the soils of the Andes and in South America, large quantities of potassium-retaining clays in the soils are available. On the coast, soil nutrients are scarce as the amount of organic matter is extremely low and soils are very sandy. Therefore, higher quantity of fertilizers is recommended in these regions. In general, the recommended rates of fertilization in coast are 240 N/ha, 200 kg P_2O_5/ha, and 80 kg K_2O/ha. In addition, depending upon availability, application of manure, compost, humus, or organic matter is also recommended (Garcia et al. 2015). The application of sheep and llama manure alone also enhances the yield. Moreover, combined application of organic and inorganic fertilizers had enhanced yield substantially and responded favourably, compared with the application of either fertilizer or manure alone (Rojas et al. 2004).

6.8 Water Management

Quinoa, being drought-resistant, traditionally is cultivated under rainfed conditions, even in semiarid and arid locations. However, low yields are recorded under rainfed conditions. Water requirement is not a fixed variable in any crop. Quinoa, being drought-tolerant, has a water requirement of 250–380 mm (precipitation and irrigation combined on sandy loam or loamy sand soils) and is very efficient in water use. In the Andean region, irrigation is not a normal practice in the farming systems. This plant, despite being a C_3 plant species, has drought mechanisms, viz. drought escape, tolerance, and avoidance (Jensen et al. 2000). Many of these mechanisms also help to make it tolerant or escape the effects of other abiotic stresses such as frost. Also, it avoids the negative effects of drought through a high root/shoot ratio, reduction in leaf area by leaf dropping, dynamic stomatal behaviour, and the presence of special vesicular glands of Ca-oxalate, which are small and thick-walled cells that preserve cell turgor even during severe water losses (Jensen et al. 2000). However, when drought occurs during sensitive phenological stages, such as emergence, flowering, and milky grain, yields can be severely reduced (Garcia et al. 2015).

Furthermore, in Colorado, crops planted in late April to mid-May did not usually need irrigation until mid-June, when the soil was nearing field capacity at the time of sowing. Further, irrigation till the two- or three-leaf stage is not desirable. Owing to the rainfall during the month of July in Colorado, there is sufficient moisture to lasts until August. Experiments conducted in Colorado on sandy loam soils using 128, 208, 307, and 375 mm of water produced grain yield of 1439 kg/ha with 208 mm of water including rainfall and irrigation (Flynn 1990). However, the study cannot be called conclusive since it was limited to a single location and soil type. Excessive irrigation after stand establishment usually produces tall, lanky plants with no yield improvement. Damping off and severe stunting of plants will occur with excessive irrigation in the seedling stages (Oelke et al. 1992). On the other hand, it is rarely grown under full irrigation, probably because it is not traditional and because it does not respond well to high irrigation due to the increased risk of downy mildew. Further, regardless of the growing location, irrigation at most critical stages, viz. crop establishment (emergence) and flowering, adds success of the crop and achieving good yield (Garcia et al. 2015). In western Greece, irrigating field using overhead sprinkler is recommended practice, and the total quantity of water used during cropping period is 180 mm (Kakaboukia et al. 2015).

In those regions where intraseasonal dry spells are of considerable importance, deficit irrigation approach has been widely examined as a treasured and sustainable production strategy (Garcia et al. 2003; Geerts et al. 2008). By limiting water applications to drought-sensitive growth stages, the practice aims to maximize water productivity and to stabilize, rather than maximize, yields (Geerts and Raes 2009). Experiments conducted in sandy soils at West Marrakesh (Morocco) and under rainfed condition in a farmer's field at Tnin Bou Chan showed that deficit irrigation (50 and 33% of full irrigation) affected performance and resulted in seed yield reduction with 15.8 and 30.1%, respectively, in the first season and with 15.2

and 41.5%, respectively, in the second season compared with full irrigation. Under rainfed conditions, seed yields were reduced by 62.1 and 59.3% during the first and second growing seasons, respectively, compared with full irrigation. CWP was maximized in the treatment, receiving 50% of full irrigation (Fghire et al. 2013; Choukr-Allah et al. 2016).

6.9 Improved Varieties

Quinoa is becoming increasingly popular, with an expanding number of commercially available varieties (Espíndola and Bonifacio 1996; Rojas-Beltran et al. 2007). The rising popularity inspired researchers throughout the world to breed varieties that are compatible and adaptable with local weather, photoperiod, agronomic management, and soil environment, which greatly differ from quinoa's original land, the Andean mountain region. Selection of suitable varieties is a primitive criterion of success or failure of a crop. Some of the important varieties suitable for cultivation are listed in Table 6.4.

6.10 Weed Management

Since quinoa plants grow slowly during the first 2 weeks after emergence, weed control in fields is difficult. Because of the initial slow growth, it faces competition from rapidly growing weeds during the initial phenological stages in the first

Table 6.4 Suitable varieties of quinoa and their yield level

Varieties/genotypes	Yield (kg/ha)	Specialty/adaptability	References
Cahuil	1950	Mix panicle colour	Johnson and McCamant (1988)
CO 407–78	1897	Yellow panicle colour	Johnson and Croissant (1990)
Baer	5140	Early maturity	Risi and Galwey (1991)
Regalona Baer	3420	Southern Italy	Pulvento et al. (2010)
Quillahuaman INIA	2800	White seeded	Apaza et al. (2015)
INIA 420–Negra Collana	3010	Black seeded	Apaza et al. (2015)
INIA 415–Pasankalla	3500	Red seeded	Apaza et al. (2015)
Chucapaca	2755	White seeded	Gonzalez et al. (2012)
Kancolla	2846	White seeded	Gonzalez et al. (2012)
RU–5	2300	Stay green	Karyotis et al. (2003)
Real	1354	Drought- and frost-resistant	Bertero et al. (2004)
PHX–01	2418	Lodging-resistant	Kostuik et al. (2014)
PHX–09	2046	Manitoba, Canada	Kostuik et al. (2014)

2 weeks after emergence. In commercial fields of southern Colorado, pigweed, kochia, lambsquarters, and sunflower have been the most common weeds. Wild mustard and sunflower can be a problem since it is not possible to separate them from quinoa seed. Competition from weeds is greater when it is planted later in the growing season. The number of kochia and lambsquarters can be reduced when field irrigation is followed by cultivation before seeding. Pigweed emerges too late in the growing season to depend on cultivation for weed control. Early planting may be the most effective means to control pigweed since the quinoa will have a good start in growth before the pigweed emerges (Oelke et al. 1992).

In western Greece, common weed species found in fields are *Amaranthus retroflexus* L. (redroot pigweed), *Portulaca oleracea* L. (common purslane), *Chenopodium album* L. (common lambsquarters), *Echinochloa crusgalli* L. Beauv. (barnyard grass), *Cynodon dactylon* L. Pers. (Bermuda grass), *Cyperus rotundus* L. (purple nutsedge), etc. The weed density and biomass are also affected by tillage system and fertilization or nutrient feeding. Conventional tillage has lower weed densities (105–116 plants/m^2) than those under minimum tillage (131–140 plants/m^2). Similarly, conventional tillage has lower total weed biomass (1072–1213 kg/ha) compared to minimum tillage (1340–1420 kg/ha) (Kakaboukia et al. 2015). Further, manure and nitrogen fertilization encourage weed density and biomass.

Hand hoeing is a common practice to control weeds. Presently, there are no effective herbicides to be used as chemical control of weeds. However, it has been observed that residues of imazaquin, an herbicide, cause phytotoxicity in seedlings (De Barros Santos et al. 2003). Moreover, there are also reports of evidence of allelopathic effect, and this allelopathic potential could probably be used as a component of integrated weed management (Bilalis et al. 2013).

Manual weeding includes drudgery and, therefore, it is least preferred. Contrary to this, chemical weed control is preferred due to the advantages of effective control of weeds, such as time-saving, labour-saving, and ultimately higher yield. Effective weed control has a major impact on grain yield. In Colorado, control of grasses increased yields from 640 kg/ha to 1822 kg/ha (Johnson 1990). In Colorado, studies on the application of pre-emergence herbicides, for instance, Dual, Furloe, Sutan, and Antor, not only exhibited control of grasses and broadleaf weeds but also resulted in decent crop safety. Post-emergent control was greatest for Poast, Tough, and Probe, with Tough and Probe at low application rates (Westra 1988). Furthermore, the application of Metamazide, Propachlor, Linuron, Propyzamide, and aloxium sodium in England had no negative effect on the reduction of plant stands of two cultivars (Galwey and Risi 1984).

6.11 Crop Rotations

In addition to sole crop, it can also be grown as a break crop in crop rotations as it is tolerant to diseases, insect pest attacks, as well as nematode attacks. Being tolerant to drought and salt, it could be a potential crop for drought-prone and salt-affected soils

Table 6.5 Quinoa-based crop rotations prevalent worldwide

Crop rotation	Region	References
Winter durum wheat	Western Greece	Kakaboukia et al. (2015)
Winter wheat	Central Greece	Karyotis et al. (2003)
Corn	South America	Garcia et al. (2015)
Barley	Andes	Garcia et al. (2015)
Oat	South America	Garcia et al. (2015)
Field pea	Peru	Aguilar and Jacobsen (2003)
Potato	Peru	Aguilar and Jacobsen (2003)

of Africa and Asia (Iqbal 2015). Rotating the crops in growing sequence helps to build soil fertility, breaks the disease cycle, and reduces insect breeding. Some of the quinoa-based common crop rotations being practiced worldwide are listed in Table 6.5.

6.12 Harvesting and Yield

The harvesting time in the South American Andes may extend from February to May, but April is the principal harvest month. Although optimum harvesting time is not a fixed time, it largely depends on the factors, viz. variety, growth habit, plant type, climatic conditions, soil type, humidity, prevailing temperature, etc. Knowing the readiness of harvesting time is a crucial factor for achieving good-quality produce. Typically, the leaves turn yellow or red, depending on the variety, and the grains can be seen in the panicle through the opening of the perigonium, also indicative of physiological maturity (Aroni 2005). Another way to test if the plant is ready for harvest is to tap the panicle with the hand. If the grains fall out, harvesting can be started (Garcia et al. 2015).

For the successful cultivation, the number of maturity days is a critical yet highly variable factor. In Colorado, days to maturity range from 90 to 125 days (Johnson and Croissant 1985), whereas days to maturity have ranged from 100 to 130 days for different varieties grown in Eastern Washington. In the state of Colorado, it matures in 90–125 days after planting. However, at high elevations, early maturing varieties are recommended because of the short growing season (Oelke et al. 1992). At maturity, quinoa has sorghum-like seed head, and harvesting commences when the seed can barely be dented with a fingernail, plants have dried and turned pale yellow or red colour, and leaves have dropped. Safe threshing of matured seed is desired at that time. In labour-intensive countries like India, harvesting manually using sickle is employed. However, in the industrialized countries like the United States, field drydown is usually acceptable, and plants are harvested effortlessly with a combined harvester. A sorghum header attachment is recommended, although platform headers can usually be used as well, without a large crop loss. Cylinder speed and air flow of combines are usually greatly reduced. Smaller screens are used with cereal grains due to the small size and lighter weight of seeds. Fanning mill and

gravity separator is usually necessary to remove trash from the seed after combining. Grain must be dry before storage. Quinoa stover contains little fibre and subsequently provides little crop residue. Rain during harvest poses to seed deterioration, and also mature seed could get germinated within 24 h after exposure to moisture.

Seed yield depends on the type of variety, physicochemical soil conditions, fertility status of soils, and climatic factors. Under neutral soil conditions, higher seed yield could be attained over saline-sodic conditions. Actual yield ranges from 500 to 1000 kg/ha of grain at farmers' level, even though yields of up to 5000 kg/ha can be achieved under suitable climatic conditions. On average, 5–10 tonnes of chaff per hectare could be harvested as a by-product and can be used as livestock feed or fodder (Garcia et al. 2015). On an average, 30–60 g of seed/plant could be harvested; however, in some rich soils, more than 170 g of seeds/plant could be harvested easily. In Mexico, the average yield of quinoa is 1–2 tonnes/ha (100–200 g/m^2), although yield up to 5 tonnes/ha (500 g/m^2) has also been harvested.

6.13 Drying and Storage

After sun drying of harvested bundles, threshing is carried out either manually or mechanically (Salas 2003). Natural or mechanized ventilation is used to remove impurities and dust. During storage, the seeds of quinoa should be dry (<10% moisture) and free from moist. Once the grain is cleaned, it is generally washed or toasted to remove saponin, a bitter substance in the pericarp that constitutes a chemical defence against the feeding activity of insects and animals. Prior to using quinoa in food processing, the saponins in the pericarp are removed by soaking them in water or by mechanical methods, such as with a rice polisher or a machine similar to those used to remove wheat bran. The use of chemicals like sodium hydroxide to make saponins soluble is effective but is not widely used. Saponin content is higher in varieties classified as 'bitter varieties' and lower in 'sweet varieties.' Processing costs are related to the saponin content of the variety (Garcia et al. 2015). Moreover, grain size and colour selection are also part of the postharvest process and criteria of consumer use as direct grain consumption and flour.

6.14 Conclusion

Wider adaptability to climatic conditions, soil environment, and crop management situations made it to thrive well and flourish under different agroecological zones worldwide. This crop requires less input and, therefore, it helps enhance input use efficiency. Additionally, crop management interventions, viz. sowing time, seed rate, crop geometry, nutrient management, water management, weed management, varietal selection, harvesting, storage, etc., have substantial influence on the grain yield. Quinoa, being a new crop for introduction in some countries, recommended agronomic package of practices needs to be developed as per the local growing environments and agroecological situations. Further, advantages in terms of human

health, nutritive animal feed, drought tolerance, salt tolerance, low water requirement, low incidence of insects and pests, poor crop weed completions, and so on will open up the avenues for commercial cultivation in the near future.

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Symbiotic Native Microorganisms of Quinoa in the Bolivian Altiplano

7

Noel Ortuño, José A. Castillo, and Mayra Claros

Abstract

Quinoa evolved in the highland area of Bolivia and other Andean countries in an extreme zone, with altitudes between 2800 and 4000 m above sea level, semiarid climates with low precipitation (250 mm/year), temperatures from −3 to 21 °C, soils with less than 1% organic matter, and a weak structure without aggregates. At the same time, quinoa co-evolved with symbiotic microorganisms, which provide different environmental services. Microorganisms were isolated from different parts of the quinoa plants, including grains, leaves, roots, and the rhizoplane and rhizosphere, which were molecularly identified. We mainly found filamentous fungi and bacteria of the *Bacillus* genus and other genera. These microorganisms were analyzed to understand the functional relationship with the plant, determining the production capacity of phytohormones, the recycling of nutrients, and the suppression of soil pathogens. The effect of the metabolites generated by symbiotic filamentous fungi was also analyzed. Taken together, the present study revealed that quinoa harbors a large number of diverse cultivable symbiotic bacteria and fungi that also serve as new sources of beneficial microorganisms and bioactive metabolites.

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Microbial diversity · Quinoa symbionts · PGPR in quinoa

7.1 Introduction

Quinoa has co-evolved in the Bolivian Andes with the ancestral man, domesticated by ancient civilizations such as the Tiwanaku, before the Aymara and Inca cultures. Until the early 1980s of the last century, the Andean peoples cultivated it due to its advantages in adapting to altitudes between 2800 and 4000 m above sea level, semiarid climates with low precipitation (250 mm/year), soils with less than 1% organic matter, and weak structure without aggregates. Quinoa has a wide diversity of native varieties adapted to specific areas of the Central, North, and South Highlands and valleys. In the Southern Altiplano, it is the main food crop for the inhabitants of these areas, being the only crop that thrives there and thus becoming a monoculture (Bonifacio et al. 2014).

Currently, its growing demand in international markets and high prices have stimulated the expansion of the crop, which implies changes in the production process and new technological needs. In turn, climate change, rapidly eroding soils, population growth, and poor agricultural practices have led to desertification, resulting in soils with low vegetation cover, very low content of organic matter, and low fertility (Bonifacio et al. 2014). These problems have caused drastic reductions in crop production, reducing farmers' income; however, quinoa is still a desirable crop to produce (Ortuño et al. 2013).

In recent years, there has been a growing interest in beneficial soil microorganisms, in promoting plant growth, and in preventing parasitism in plants by pathogens. Such microorganisms can be symbiotic or free-living (Benizri et al. 2001; Bacilio-Jiménez et al. 2003). Another group, the endophytes, which are a specific group of microorganisms (bacteria, actinomycetes, fungi) that are found internally associated with various plant tissues, occurs in crops of agricultural importance (Surette and Sturtz 2003). Existing among these, the so-called plant growth-promoting bacteria (PGPB) have a versatile metabolism with the ability to use various substrates released by the plant for their development and have short reproduction times, high mobility, and a great capacity to colonize the plants. In addition, roots produce secondary metabolites that regulate plant growth and rhizospheric microbial populations (Kapulnik 2002). However, these microorganisms are also affected by environmental stress that can be lessened by acclimatization to their host plant. This symbiosis could represent a greater ecological advantage that depends on plant cultivar and the co-evolution with the microbial species (Sturz and Nowak 2000).

The effect of PGPBs on plant growth and development is due to mechanisms of direct and indirect action. Among the former is the production of hormones such as auxins, gibberellins, cytokinins, and ethylene, the production of organic acids, nitrogen fixation, solubilization of phosphate and other nutrients, and their

mobilization. Within the second mechanism, there is protection against pathogens by antagonistic interactions, production of antibiotics, the release of enzymes such as chitinases and glucanases, in addition to the induction of systemic resistance in the plant to viruses, bacteria, and pathogenic fungi (Pal et al. 2000). Among the microorganisms, there are groups of soil bacteria, such as *Azotobacter* sp., *Azospirillum* sp., *Azoarcus* sp., *Klebsiella* sp., *Bacillus* sp., *Pseudomonas* sp., and *Rhizobium* sp., that have a versatile metabolism and the ability to use various substrates released by the plant for their development (Kapulnik 2002).

These antecedents demonstrate the need to study the associations of microorganisms with plants to know the benefits that microorganisms provide to the growth and production of crops. In the agricultural soils of the Andes, microbial communities belonging to a wide variety of genera and species also co-evolved with quinoa. This symbiont biota, strict or optional, is associated with different environmental services. The microbial diversity associated with quinoa is largely unknown; however, some studies have provided valuable information on the microorganisms that interact with various organs of quinoa, indicating that diversity is extraordinary (Ortuño et al. 2013). This chapter presents some results from the study of native microorganisms that co-evolved with quinoa in the Bolivian Altiplano. Likewise, an analysis of the environmental services offered by these microorganisms is made, which includes nitrogen fixation, phosphate solubilization, and the production of phytohormones such as indole acetic acid (IAA). These benefits are useful for improving production yields, reducing pest damage, and reducing the deterioration process of Andean soils.

7.2 Identification of Native Quinoa Microorganisms by Molecular Means

Endophytic microorganisms were isolated from the plant's phyllosphere, rhizoplane, and rhizosphere. The quinoa plants were obtained from the Northern, Central, and Southern Altiplano of Bolivia. These isolated microorganisms were identified by molecular techniques.

7.2.1 Identification Using Local DNA Sequence Alignment and Comparison to DNA Sequence Databases

Molecular methods offer advantages over conventional methods (phenotypic tests, the use of taxonomic keys, and biochemical methods) since they are based on DNA sequences and comparison of the sequences with those deposited in the databases (NCBI Resource Coordinators 2017). For this purpose, the program BLAST (Basic Local Alignment Search Tool) (Altschul et al. 1990) is used, which calculates the statistical significance of the coincidences between the query sequence and sequences housed in databases. When the sequence of the microorganism of interest and the sequence(s) identified by BLAST in the database present identity of >97%,

then they can be considered as the same species (Lozupone and Knight 2009). Sometimes, the discriminating power of the sequences is not high enough to identify the specimens at the species level (Srinivasan et al. 2015; Johnson et al. 2019). For this reason, additional genes are used, which adds specificity and reliability to the identification (Kolbert and Persing 1999). This strategy was used to identify several native fungi associated with the roots of quinoa (see below).

In this way, molecular methods constitute a significant advance in the identification of specimens extracted from nature since they are fast and accurate and do not require the participation of specialists in some specific groups of fungi, actinomycetes, and others, an aspect that is generally not within the reach of most institutions in developing countries. Since all living beings including viruses have genetic material (DNA or RNA), this technology is universal and guarantees a reliable result (Ansorge 2009; Loman et al. 2012).

Additionally, to add greater confidence to the result, it is suggested to make a phylogenetic inference using the DNA sequences of the microorganisms under study and the sequences of related organisms that the BLAST program found. A simple neighbor-joining tree or a more sophisticated one such as one inferred using the maximum likelihood method is sufficient to recognize the phylogenetic relationship with related microorganisms. There are several computer programs that facilitate the reconstruction of the phylogenetic past of the species (viz., MEGA 6, Tamura et al. 2013; PAUP * 4, Swofford 2002; and others).

7.2.2 Identification Using DNA Barcode

The use of DNA barcodes has been presented as a tool for taxonomic identification of specimens and as a method for discovering unknown species. The DNA barcode uses short DNA sequences, obtained from standard parts of the genome of microorganisms, in the same way that a supermarket scanner distinguishes products using the black stripes of the universal product code. The difference with the simple application of BLAST is that the DNA barcodes use a database of standardized DNA sequences and ad hoc designs (Hebert et al. 2003a), as well as a standard region of some marker genes. For example, a fragment of the mitochondrial gene cytochrome C oxidase subunit 1 (COI) of approximately 648 bp is normally used for the identification of higher organisms (Hebert et al. 2003b). However, the inter-genomic region of ribosomal genes (internal transcribed spacer or ITS) is also widely used, especially to identify fungi (Schoch et al. 2012). In the case of bacteria, the gene encoding the small subunit (16S) of ribosomal RNA has been widely used in studies for the identification of larger categories in prokaryotes, but it does not provide sufficient information for identification at the species level (Zeigler 2003); therefore, other genomic regions (genes encoding essential proteins) have been used to achieve this end. The genes encoding housekeeping proteins are recognized for providing superior resolution and are therefore useful for the identification of bacteria at the species level (Hill et al. 2004).

The Reference DNA Sequence Database is the most valuable resource in DNA barcode identification. The National Center for Biotechnology Information houses the largest and most comprehensive database of DNA sequences (genes and non-coding regions) and proteins. To identify the specimens, the first step involves culturing the microorganism starting from a single cell (spore, conidium, etc.). Once the microorganism has grown, the DNA is extracted through standardized procedures and then in vitro amplification of specific DNA fragments using primers defined for the region of interest. The sequence is then determined and analyzed using bioinformatics tools such as BLAST or others that have been intentionally designed to identify a particular group of microorganisms.

7.2.3 Identification of Native Microorganisms in the Cultivation of Quinoa in Bolivia

Bacteria isolated from the different organs of quinoa plants have been identified following the procedure indicated above. The bacterial region chosen to obtain the sequence constitutes most of the gene that codes for 16S ribosomal RNA. The primers used for amplification and sequencing were 27A/C and 1488 (Lane 1991). Table 7.1 summarizes the identification of some bacteria isolated from plant roots using only the 16S ribosomal RNA gene sequence. As mentioned above, the use of the 16S ribosomal RNA gene for identification carries a low discriminatory power since it was only possible to differentiate the isolates at the genus level. The sequences were obtained by the conventional or Sanger technique and then compared using BLAST against the general database (GenBank) and fungi (mainly *Trichoderma*) using DNA barcode (Druzhinina et al. 2005).

In the case of beneficial fungi (*Trichoderma*) associated with quinoa, four genetic markers have been identified according to the DNA barcode scheme constructed for the identification of *Hypocrea/Trichoderma* isolates. The genes used are ITS [internal transcribed spacer 1 and 2 (ITS1 and ITS2)] of the RNA gene group and the *tef1* gene that encodes the EF-1 alpha protein (translation elongation factor 1 alpha), which contains three phylogenetic markers: the fourth and fifth intron (large and small, respectively) and the sixth exon. The bioinformatics tools used were a specific multilocus database of phylogenetic markers and the *Trich*OKEY (Druzhinina et al. 2005), *Trich*oMARK, and *Trich*oBLAST (Kopchinskiy et al. 2005) programs. The results expressed in Table 7.2 indicate that the barcode strategy allows identifying natural isolates of *Trichoderma* to the species level.

Marker genes ITS1-2 and *tef1* allow the identification of some isolates such as *T. asperellum* and *T. asperelloides*, respectively. In this sense, the ITS region is highly conserved between both species, and the single nucleotide difference in the ITS2 sequence makes it impossible to distinguish between *T. asperellum* and *T. asperelloides*. Both species cannot be distinguished by their phenotype, biology, or biogeography (Samuels et al. 2010); however, they can be distinguished by markers of the *tef1* gene. In the case of *T. harzianum*, this species has been divided

Table 7.1 Native bacterial isolates identified by sequencing the gene encoding 16S ribosomal RNA and the BLAST program

Genus or species	Place of extraction
Bacillus sp. (*B. cereus* or *B. thuringiensis*)	Rhizoplane
Bacillus sp. (*B. cereus*)	Endophyte roots
Bacillus sp. (*B. subtilis* or *B. amyloliquefaciens*)	Endophyte roots
B. licheniformis	Endophyte leaves and stem
B. horikoshii	Endophyte roots
B. atrophaeus	Endophyte roots
Paenibacillus sp.	Endophyte leaves
B. aryabhattai	Endophyte roots
B. megaterium	Endophyte leaves
P. odorifer	Endophyte leaves
Pseudomonas sp.	Endophyte roots
Bacillus sp. (*B. pumilus*, *B. safensis*, or *B. altitudinis*)	Endophyte roots
Rhizobium sp. or *Phyllobacterium* sp.	Rhizoplane
Agrobacterium tumefaciens or *Rhizobium*	Rhizosphere
Pseudomonas sp. or *Azospirillum* sp.	Endophyte root
Rhizobium leguminosarum	Rhizosphere
Rhizobium sp.	Rhizosphere
Agrobacterium tumefaciens	Rhizoplane
Flavobacterium johnsoniae	Rhizoplane
Rhizobium rhizogenes	Rhizosphere
Agrobacterium larrymoorei	Roots
Agrobacterium or *Rhizobium* sp.	Roots
Variovorax sp. (*V. paradoxus*)	*Chenopodium pallidicaule* root endophyte

into several phylogenetic species, including *T. harzianum* sensu stricto, and other new species such as *T. afroharzianum* and *T. pseudoharzianum* complex.

7.3 Environmental Services of Native Symbiotic Microorganisms of Quinoa

Microbial biodiversity provides ecological services to agricultural systems, including the recycling of nutrients, regulating populations of undesirable organisms, promoting plant growth, and detoxifying harmful chemicals from the soil. The determining factor of the microbial diversity of the soil is related to the complexity of the microbial interactions in it, including the interactions between microorganisms and soil and microorganisms and plants (Garbeva et al. 2004).

Numerous studies show that different agricultural works modify biodiversity and alter the structure of soil microbiological communities (Kennedy and Smith 1995; García de Salamone and Monzón de Asconegui 2008). Therefore, it is important to

Table 7.2 Native isolates of the Bolivian Andean zone of *Trichoderma* identified following the "DNA barcode" strategy designed specifically for the taxonomy, identification, and characterization of *Trichoderma/Hypocrea* species

	Gen *tef1*		
ITS1 and ITS2	Intron 4 (large)	Intron 5 (short)	Exon6 (large)
na	*T. afroharzianum*	*T. afroharzianum*	*T. afroharzianum*
T. asperellum	*T. asperelloides*	*T. asperelloides*	*T. asperelloides*
T. asperellum	*T. asperelloides*	*T. asperelloides*	*T. asperelloides*
T. asperellum	*T. asperelloides*	*T. asperelloides*	*T. asperelloides*
T. asperellum	*T. asperelloides*	*T. asperelloides*	*T. asperelloides*
T. koningiopsis	*T. koningiopsis*	na	na
T. koningiopsis	*T. koningiopsis*	na	na
T. harzianum	*T. afroharzianum*	*T. afroharzianum*	*T. afroharzianum*
T. harzianum	*T. afroharzianum*	*T. afroharzianum*	*T. afroharzianum*
T. harzianum	*T. pseudoharzianum*	*T. pseudoharzianum*	*T. pseudoharzianum*
T. asperellum	*T. asperelloides*	*T. asperelloides*	*T. asperelloides*
na	*T. asperelloides*	*T. asperelloides*	*T. asperelloides*
T. koningiopsis	*T. koningiopsis*	na	na
T. harzianum	*T. pseudoharzianum*	*T. pseudoharzianum*	*T. pseudoharzianum*
T. harzianum	*T. pseudoharzianum*	*T. pseudoharzianum*	*T. pseudoharzianum*
T. harzianum	*T. pseudoharzianum*	*T. pseudoharzianum*	*T. pseudoharzianum*
T. harzianum	*T. pseudoharzianum*	*T. pseudoharzianum*	*T. pseudoharzianum*
T. harzianum	*T. pseudoharzianum*	*T. pseudoharzianum*	*T. pseudoharzianum*
T. harzianum	*T. pseudoharzianum*	na	NA

na not analyzed

analyze the natural advantages of the symbiosis between microbes and plants grown in their natural habitat, in order to detect opportunities for improvement in crop productivity based on the functionality of the microorganisms. This type of study allows the development of biotechnological lines of production contributing to healthy agriculture.

Microorganisms are capable of stimulating the production of auxins and other phytohormones in plants, thanks to the secretion of various secondary metabolites (Kapulnik 2002). Likewise, the same microorganisms can produce IAA within their secondary metabolism, which indicates that this auxin is an important characteristic to be able to evaluate possible PGPB's bacteria (CIP 2008).

On the other hand, the ability to colonize the roots is an essential condition for a bacterium to be considered PGPB. This capacity constitutes a first step and a crucial feature for the selection of microbial inoculum to be used as biofertilizers, biopesticides, phytostimulators, or bioremediators (Lugtenberg et al. 2001). It is also the first indication that microorganisms are capable of achieving the expected beneficial effect. In addition to the colonization capacity, the success in the use of plant growth-promoting microorganisms resides in the study of compatible and specific strains to the various crops and the prevailing environmental conditions (Peña and Reyes 2007).

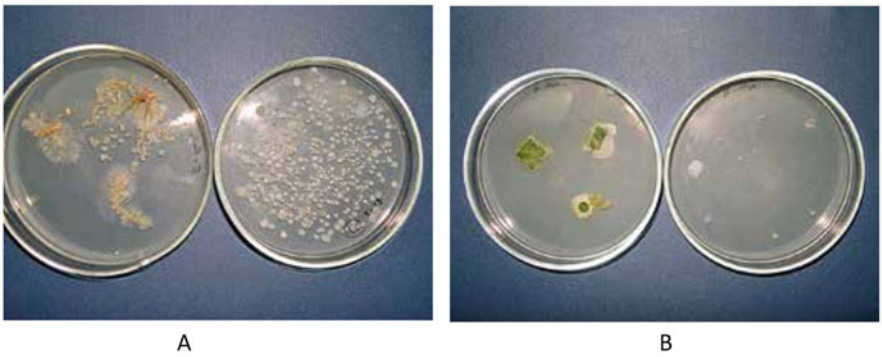

Fig. 7.1 Bacterial colonies isolated from (**a**) roots and (**b**) leaves

Many of the microorganisms that are characterized as PGPBs are isolated either from soil or as endophytes. Endophytes are a specific group of microorganisms (bacteria, actinomycetes, and fungi) that are found in different types of plant tissues, including seeds, fruits, leaves, tubers, stems, etc. Endophytes present a high occurrence in crops of agricultural importance and high relevance in their production systems. These are capable of inducing resistance to both biotic and abiotic stresses in inoculated plants or reducing the development of diseases (Surette and Sturtz 2003).

Thus, soil microorganisms have several beneficial functions that can be used in favor of agricultural production. Therefore, exploring the microbial diversity of the soil of quinoa production plots is essential to know the functional aptitudes to improve crop yields and promote the sustainable production of the agricultural systems of the Altiplano.

For this, samples of plants were taken from ten organic quinoa production plots located in three rural communities of the southern Altiplano of Bolivia. Specifically, the areas of Salinas de Garci Mendoza, Challapata, and Quillacas in the department of Oruro also in the department of Potosí in the communities of Chacala, Mañica, and Llica were chosen (Fig. 7.1). Similarly, superficially associated and endophytic microorganisms were isolated from different plant organs (stem, leaves, and grains) (Fig. 7.2).

From 1489 bacterial isolates, 235 were detected as nitrogen fixers, which are free-living, so these strains do not require the plant to carry out the nitrogen fixation process. These bacterial isolates subsequently entered a more specific selection and identification program at the species level according to the molecular procedure described above. At the same time, 456 strains were selected as phosphorus solubilizers, some having been identified at the species level. Finally, the isolates were qualitatively analyzed regarding their ability to produce the phytohormone IAA. 789 isolates tested positive for IAA production (Fig. 7.3).

Complementarily, endophytic bacteria were analyzed in the quinoa seeds, and it was established that these contained bacteria from the phylum Firmicutes, which

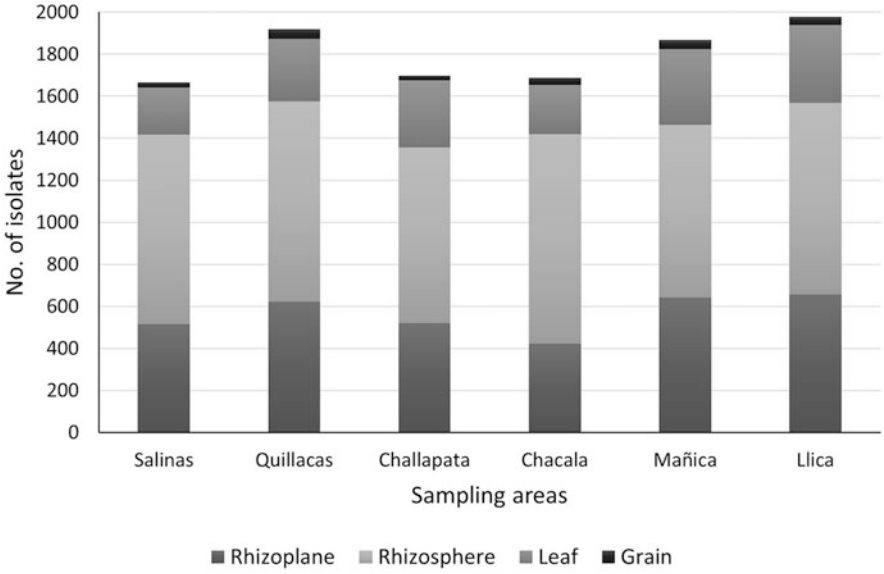

Fig. 7.2 Isolations of microbial populations associated with different organs of the quinoa plant, sampled from different communities of the Bolivian Altiplano

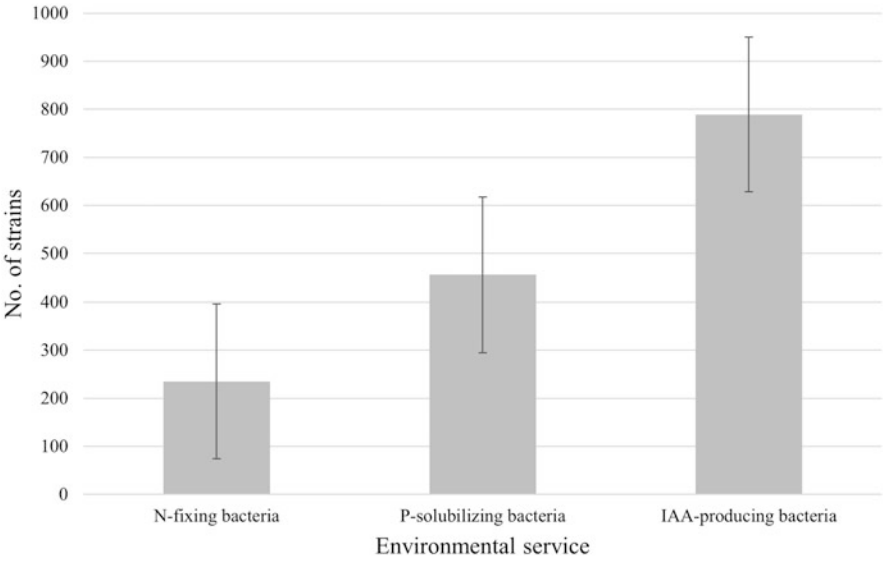

Fig. 7.3 Bacteria isolated from quinoa characterized by their environmental service

were identified as *Bacillus* sp., *B. horikoshii*, *B. firmus*, *B. subtilis*, and *Paenibacillus* sp. Thus, it was shown that quinoa seeds travel with their symbionts and potentially can help them to adapt to new environments, constituting a means of dispersal of endophytes.

7.4 Native Bacterial and Nitrogen Fixation in Quinoa Plants

Nitrogen is known to be one of the most demanded nutrients by the plants, but its low availability in soils is frequently a limiting factor in the production of various crops of economic importance (Sylvia 1999). Many microorganisms can fix the atmospheric nitrogen and provide it to the plant, which uses it for the synthesis of new proteins, nucleic acids, and other molecules. Bacteria that have the ability to fix nitrogen are called diazotrophic bacteria and are of two types: those that are obligate symbionts and need a plant to live (rhizobia) and those that are free-living. Within the group of free-living diazotrophic bacteria are species such as *Azotobacter diazotrophicus*, *Herbaspirilum* sp., and *Azoarcus* sp. (Kapulnik 2002).

The presence of diazotrophic bacteria associated with quinoa was evaluated. For this purpose, 21 bacterial strains (endophytic and rhizospheric bacteria) were evaluated using the Burk and the NFB selective media. Bacteria that grow in this medium can fix gaseous nitrogen (N_2) to produce NH4+ (ammonium), which is used for amino acid biosynthesis (Dion and Magallon 2009; Cárdenas et al. 2010). Using this technique, the 21 strains were evaluated, of which 8 isolates were determined as nitrogen fixers. Some of these strains were named 101J (unidentified), 102J (unidentified), 103J (unidentified), as rhizoplane strains, 10M (*Bacillus atrophaeus*), 2A (*Paenibacillus* sp.), 2C (*Paenibacillus* sp.), BV54 (*Paenibacillus polymyxa*.), and BV39 (*Bacillus megaterium*) isolated from the phyllosphere.

The application of quinoa-associated diazotrophic bacteria to quinoa plants increased crop yields. This was observed in inoculating quinoa plants with strains 101J, 103J, and BV54 and commercial products as controls (Graminante and Azozim, those containing *Azospirillum* sp., a nitrogen-fixing bacterial species) under greenhouse conditions. The inoculated plants showed a higher grain yield compared to the uninoculated control (T0). Also, Graminante was statistically superior to the control treatment. In addition, other complementary variables were analyzed (plant height, panicle diameter, foliage weight, root weight, root length), and it was established that the most prominent nitrogen-fixing bacteria were *Paenibacillus* sp. and *Azospirillum* sp. (Fig. 7.4) (Gutierréz et al. 2018).

This shows that quinoa has symbionts related to nitrogen fixation and that quinoa plants require nitrogen like any other crop for normal growth. In addition, this shows that there are microorganisms that co-evolved with this plant, adapting to ecologically extreme conditions, such as the Bolivian Altiplano.

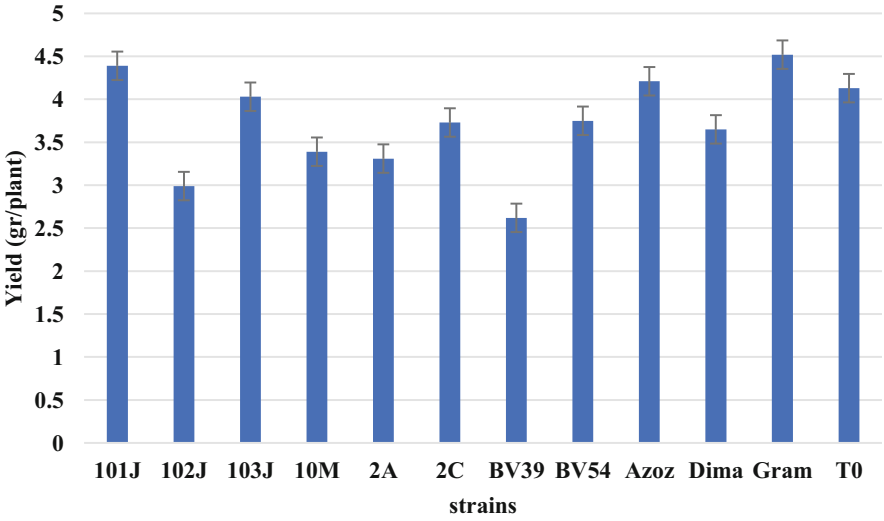

Fig. 7.4 Effect of strains of nitrogen-fixing bacteria on the yield (g/plant) in quinoa plants

7.5 Native Phosphate-Solubilizing Bacteria in Quinoa

Another essential chemical element in plant nutrition is phosphorus. However, phosphorus is usually found as phosphate rock that is poorly assimilated by the plant. For this reason, phosphorus must be previously treated for proper use by plants. Microorganisms can make phosphorus available to plants through a process called solubilization. The process of solubilization of mineral phosphorus is carried out with the release of organic acids by microorganisms. This causes the oxidation of the minerals that trap phosphorus in the soil, thus increasing its solubility. The process of solubilization of organic phosphorus is the result of the production of phosphatase enzymes, responsible for the dephosphorylation of phosphate groups attached to organic matter, and phytases, which catalyze a hydrolysis process, which in turn releases two enzymes, phosphatases and CP lyases, which break the bond between carbon and phosphorus of organophosphate compounds (Fernández et al. 2005).

The solubilization of insoluble mineral phosphates is the result of the secretion of organic acids such as lactic, oxalic, and citric by microorganisms. The means used to establish the solubilizing phosphate activity in vitro was the development of clear areas around the microbial colonies, which indicate a shift in the pH of the medium due to the synthesis of the acids mentioned above (Metha and Nautiyal 2001). Bacteria of the genus *Bacillus* are the most studied group that shows this property. *Bacillus* members have mechanisms of action that make them suitable for the formulation of viable and stable bioproducts enriched in these bacteria. Among these mechanisms are the ability to form spores that survive and remain

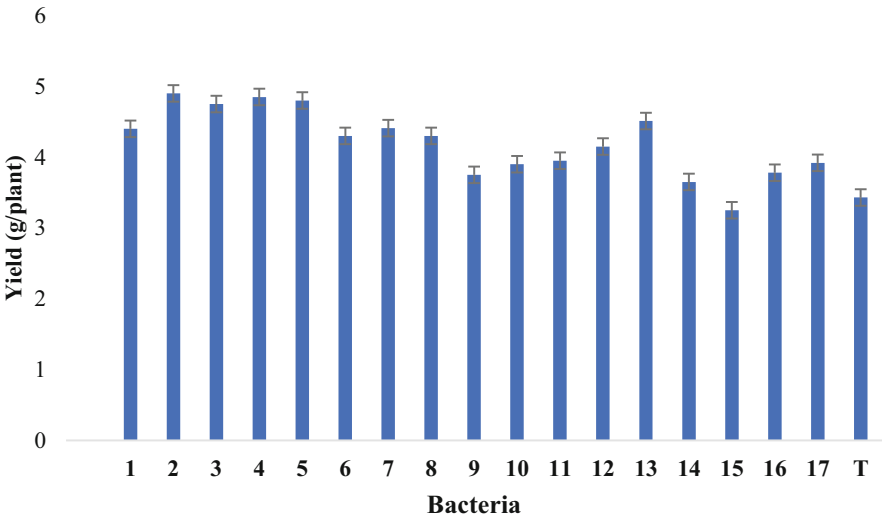

Fig. 7.5 Effect of *Bacillus* strains on the yield of quinoa plants (Arévalo 2015)

Table 7.3 Bacteria selected as phosphorus solubilizers

Strains	Species	Additional treatment
2	*Paenibacillus polymyxa*	Phosphate rock
3	*B. simplex*	Phosphate rock
4	*Paenibacillus*	Phosphate rock
5	*B. megaterium*	Super phosphate

metabolically active under adverse conditions, competition for space and nutrients (Handelsman and Stab 1996), and a proven effect on plant growth promotion (Kloepper et al. 2004). Phosphate-solubilizing bacteria such as *Bacillus megaterium* and *Pseudomonas fluorescens* produce phosphatases and organic acids that solubilize organic and inorganic forms of phosphorus not available in soil solution (Metha and Nautiyal 2001).

In the case of quinoa, 69 bacterial strains of the genus *Bacillus* were analyzed, which were shown in the NBRIP culture medium (Dion and Magallon 2009), using phosphate rock as a source of phosphorus. Eighteen strains were selected by their ability to solubilize and assimilate phosphorus. The selected bacteria were inoculated to quinoa plants in tests designed to compare the bacterial strains with phosphate rock and with superphosphate under controlled greenhouse conditions. Plants inoculated with the bacteria presented higher values in all agronomic variables (height of the plant, panicle diameter, foliage weight, root weight, root length, grain yield) compared with superphosphate, except in plant height, in which the results of treatments with superphosphate are greater than obtained in the phosphate rock treatments (Fig. 7.5 and Table 7.3).

Of all the endophytic native bacterial strains isolated from quinoa plants and analyzed by their property of solubilizing phosphate rock, four were selected as the most efficient strains: 4, 6, 3, and 11 (Table 7.3).

7.6 Wild Plants Supplying Growth-Promoting Bacteria for the Sustainable Production of Quinoa

In an agricultural production system, the soil microorganisms may get adapted to the plants inhabiting the same agroecosystem, so common symbionts can co-exist between wild plants and domesticated crops. These microorganisms could potentially be used for the healthy production of crops without harmful effects on the environment.

We collected different wild plants from the central and southern Altiplano coinciding in the areas where quinoa is cultivated. As in the other tests, bacterial strains were isolated from the following wild species: tola (*Parastrephia lepidophyla*), garbancillo (*Astragalus nitidiflorus*), paja brava (*Stipa ichu*), yareta (*Azorella compacta*), lampaya (*Lampayo castellanii*), kila kila (*Lupinus mutabilis*), and wild quinoa (*Chenopodium quinoa*) (Table 7.4).

From these plants, 85 endophytic strains were isolated, of which 65 belonged to the *Bacillus* genus. As in the previously described tests, some of these strains were inoculated to quinoa plants. After an analysis of all the parameters (plant height, panicle diameter, foliage weight, root weight, grain yield), six wild strains were the most outstanding in promoting plant growth, having an effect similar to *Bacillus* subtilis, whose effect as a growth promoter is well known (Fig. 7.6). This shows that

Table 7.4 Wild species and the localities from where they were sampled

Zone or region	Collection sites	Locality	Wild species collected
Central Altiplano	Oruro	Rancho Grande	Tola, paja brava, quinoa
Central Altiplano	Oruro	Crucero Belen	Tola, garbancillo, quinoa, wild potato
Southern Altiplano	Potosí	Tica Tica	Tola and wild tarwi
Southern Altiplano	Potosí	Galerias	Quinoa
Southern Altiplano	Potosí	Challa	Quinoa
Southern Altiplano	Uyuni	Chacala	Garbancillo, yareta, and tola
Southern Altiplano	Challapata	Tacagua	Tola, quinoa, wild tarwi and native legume
Southern Altiplano	Uyuni	Colchani	Lampaya
Southern Altiplano	Uyuni	Chaquilla	Tola and paja brava

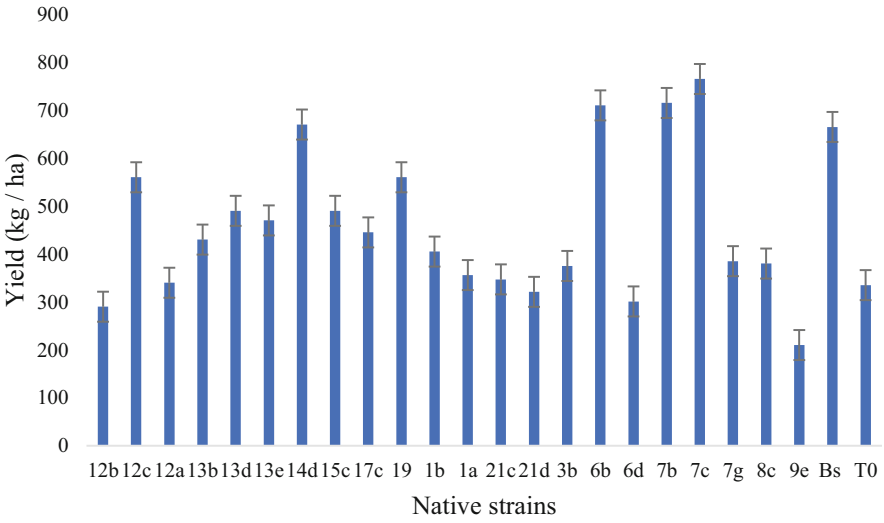

Fig. 7.6 Effect of bacterial strains isolated from wild plants on grain yield in quinoa plants

the strains isolated from wild plants are beneficial microorganisms for quinoa growth and that the interaction of microorganisms from wild and cultivated species may contribute to the perpetuation of these strains in the ecosystem.

7.7 Native *Trichoderma* of Quinoa in the Control of Soil Pathogens

Using the same strategy described in the *Trichoderma* confrontations (see below), it was determined that this fungus is a potential suppressor of soil pathogens. Dual cultures were performed: *Rhizoctonia-Trichoderma* and *Fusarium-Trichoderma* in culture medium for fungi, observing that after 4–8 days of incubation, *Trichoderma* did not allow the growth of pathogenic fungi (Fig. 7.7).

Trichoderma sp. was not used as a biological control agent in the cultivation of quinoa, because fungi soil diseases are not frequent in quinoa areas of the Bolivian Altiplano. However, this fungus is reported, in other crops, as effective control of pathogenic soil fungi (Rodríguez 2002; Harman 2006). Different strains of *Trichoderma* are generally present in all types of agricultural soils and ecosystems, and their versatility, adaptation, and easy manipulation have allowed them to be used for more than 70 years as biological control (Bae et al. 2011; Mulaw et al. 2010; Ortuño et al. 2013; Mukherjee et al. 2013; Gupta et al. 2014). Several of them are effective antagonists of pathogenic fungi, which is why they are used as biological control agents for *Sclerotium rolfsii*, *Rhizoctonia solani*, and different species of *Fusarium* and other pathogenic fungi that cause diseases in the stems and roots of a wide variety of crops (Hoyos-Carbajal et al. 2008; Verma et al. 2007).

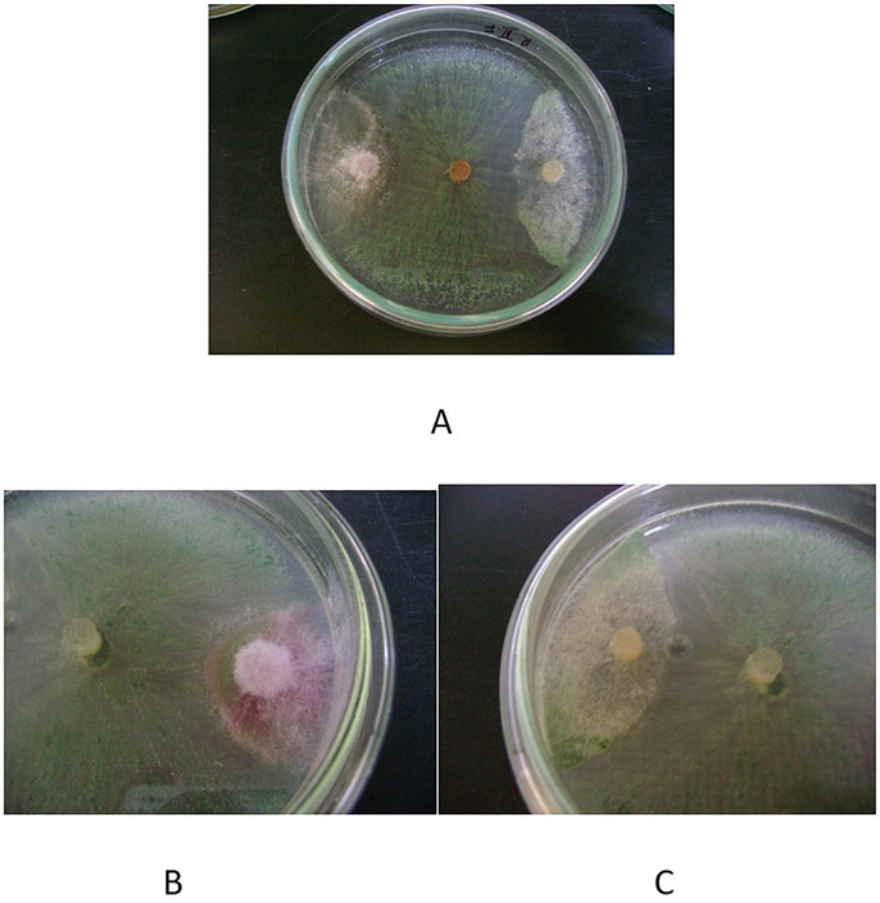

Fig. 7.7 (**a**) Development of *Trichoderma harzianum* colonies suppressing (green colony) soil pathogens. (**b**) Suppression of *Fusarium* (pink colony). (**c**) Suppression of *Rhizoctonia* (brown colony)

7.8 Native *Trichoderma* as a Growth Promoter and Solubilizer of Phosphates

Trichoderma has significant functional variability in soil biological activity. Thus, we have strains isolated from the rhizosphere of quinoa that are growth promoters and phosphate solubilizers, which shows their biological versatility in the soil ecosystem of these arid zones.

To demonstrate the solubilizing capacity of phosphates, 43 strains were evaluated in NBRIP medium (Nautiyal 1999), selecting 4 strains as positive due to the presence of a translucent halo around the colony in the culture medium that is an

indicator of solubilization of phosphate. Subsequently, these were inoculated to quinoa plants together with a source of phosphate. Results show the ability of these *Trichoderma* strains to solubilize phosphates, and their effect as growth promoters were evidenced (Table 7.5). Thus, strains TR-22 and TR-21 were more efficient in solubilizing phosphates compared to when the source of phosphorus was not used. On the other hand, the strains TR-9 and TR-8, although they solubilize phosphorus, are more effective as growth promoters, because higher percentages of grain yield were observed when only the fungus inoculum was used.

7.9 Effect of Quinoa *Trichoderma* Metabolites on Other Crops

Many organic substances are capable of regulating plant growth because they affect the physiology of plants and their morphological processes at very low concentrations. Within the term "PGPF" (plant growth-promoting fungi), a large part of the growth-promoting activity falls on the synthesis of phytohormones and other compounds that are released to the medium where they exert a specific function in plants (Dobbelaere et al. 2003).

To determine the effects of these compounds, as growth promoters, we isolated different strains of *Trichoderma* (Table 7.6) since it represents a high potential for agricultural biotechnological development. This fungal species belongs to the Deuteromycetes subdivision that is characterized by not having, or not presenting, a specific sexual state. There are more than 30 species of this microorganism, all with

Table 7.5 Percentage of yield compared with the control (without inoculum) in dry grain of quinoa

	Panoja dry weight	
Source of P	Inoculum	% yield
Superphosphate	TR-22	37.73
Without P source	TR-22	24.31
Superphosphate	TR-21	29.69
Without P source	TR-21	16.16
Superphosphate	TR-9	10.23
Without P source	TR-9	30.07
Superphosphate	TR-8	17.38
Without P source	TR-8	29.68
Superphosphate	Without inoculum	0.0[a]

[a]It is the base value on which the increases in percentages were calculated

Table 7.6 Promising native fungi identified that provide environmental services in the Bolivian Altiplano

Harvesting area	Cultivation	Mushroom	Environmental service
Altiplano	Quinoa and wild species	*Trichoderma asperellum, Trichoderma koningiopsis*, *Trichoderma harzianum*	Pathogen suppressor and growth promoter

beneficial effects for agriculture. This fungus is widely distributed in the world and is presented in different areas and habitats, especially in those that contain organic matter or decomposing plant debris, also in crop residues, especially those that are attacked by other fungi (Kubicek and Harman 2002).

Several members of the genus *Trichoderma* generate low molecular weight metabolites (<3000 Da) and volatile substances, which are released and capable of travelling significant distances through the edaphic system and the rhizosphere. This is the case of 6-pentyl-α-pyrone (6PP), which generates structural changes at the cellular level, such as vacuolation, granulation, cytoplasmic disintegration, and cell lysis in *Fusarium* (Schuster and Schmoll 2010). On the other hand, high molecular weight and polar metabolites including peptaibols can exhibit their activity by direct contact between *Trichoderma* species and their antagonist organisms (Kubicek and Harman 2002).

The effect of these secondary metabolites in the quinoa-*Trichoderma* interaction was accidentally observed when dual confrontations were made to determine that the native strains isolated from quinoa were compatible with each other. For this, 35 different strains of *Trichoderma* were analyzed, making 105 confrontations in the PDA medium. Most were compatible, so they could live together. However, 20 clashes formed a yellowish film between them (Fig. 7.8). Later it was found that this film or halo was a substance that contained IAA among other compounds. These metabolites were applied to lettuce and radish crops, having proven an effect of plant growth promotion (Ortuño et al. 2016). For this, the halo produced when facing *Trichoderma* isolates was inoculated to lettuce and radish plants in independent experiments. Different parameters of plant growth were evaluated such as plant height, foliage weight, root weight, root length, and yield (Ortuño et al. 2016).The results indicate that there was a statistically significant difference in the weight of the foliage of the lettuce plants (Figs. 7.9 and 7.10), the best treatments being the confrontations of the *T. koningiopsis* species with *T. asperellum* and the individual cultivar *T. asperellum* (strains 2 and 7).

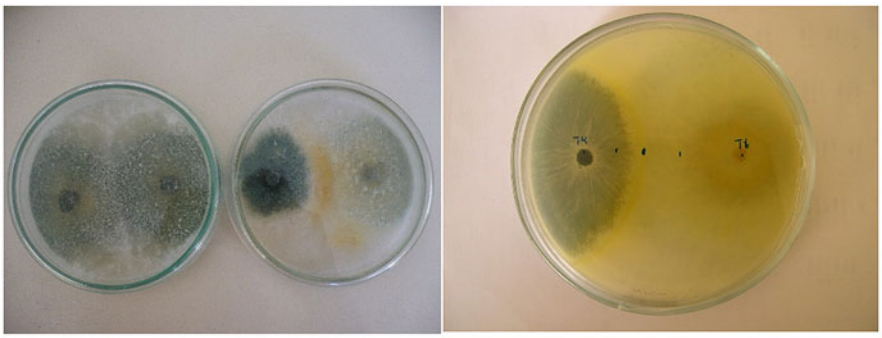

Fig. 7.8 Strains of *Trichoderma* sp. compatible (**a**). Generation of secondary metabolites in yellow color (**b**)

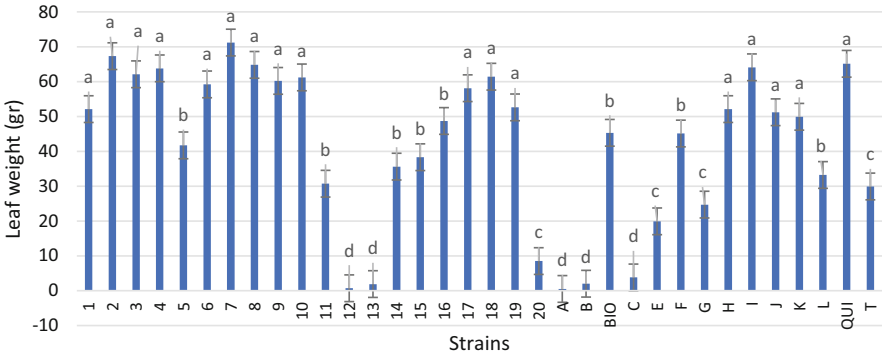

Fig. 7.9 Variation of the effect of metabolites on the yield (foliage weight) of lettuce concerning the control (T)

Fig. 7.10 Effect of *Trichoderma* metabolites on the development of lettuce plants

This effect demonstrates the potential representing the secondary metabolites of *Trichoderma*, constituting a promising, simple, and accessible technology for quinoa producers to ensure the sustainability of this crop in the Bolivian Andes.

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Root Analysis of Quinoa Plant

8

Saumya Singh, Aditi Jain, and Ajit Varma

Abstract

Different *Chenopodium* species have a specialized herringbone root system. They have a deep taproot with intense lateral branches. This root topology helps quinoa species to survive through adverse of situations. A plant's root system depends on both its genetic composition and environmental conditions. These variations in plant root architecture due to soil environmental conditions (physical and biological) are important factors that help plants to sustain through adverse conditions. This factor makes study of root architecture an important aspect of science. Study of root architecture can be done through several methods, one of which being through software such as WinRHIZO (described in this chapter). Further, microbial communities are an important aspect that determines root architecture of plants. To have a better understanding about quinoa's adaptation to adverse climatic conditions, this chapter also reviews some of the previous researches carried out to study microbial communities closely associated with quinoa in natural habitats. These communities are governed on the large part by fungi living in the rhizosphere as epiphytes and endophytes of the roots systems and then a few bacterial species that occur as seed endophytes in majority of quinoa seeds produced. Therefore, we can conclude that specialized root architecture of quinoa along with diverse rhizospheric microbial community is responsible for its sustenance under different abiotic stresses.

Keywords

Quinoa · Herringbone · Root system architecture · WinRHIZO · Root topology · Root endophytes · Seed endophytes · Rhizosphere · Abiotic stress · Bioproducts

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8.1 Introduction: Why Do We Study Root System?

Plant roots form the connection between the plant body and nutrient source, which is responsible for the life and being of the plant. They form the base on which all of the major processes and biochemical reactions depend and pave way for several beneficial as well as pathogenic interactions with microorganisms, parasites, arthropods, and nematodes. Roots form the channel through which a plant is able to absorb and assimilate nutrients from its surroundings for preparing its food and releasing gases into the atmosphere. Roots help the plant in the accumulation of water, nutrients, and hormones from the rhizosphere and also provide mechanical support to the plant (Merrill et al. 2002). Roots are also found to contribute in disease resistance, important microbial interactions, supply of C and N, and improving the quality of soil organic matter (Sainju et al. 2005a, b). The root network contributes to about 10–20% of the plant weight. Most of the root growth is genetically controlled; however, external environmental conditions also influence their architecture and their efficiency to trap nutrients. Root endophytic microbial interactions play an important role in plant processes as they impart important secondary metabolites, enzymes, ions, and even antibiotics, which in turn help the plant to thrive and overcome harsh environmental conditions and pathogen attacks. This kind of a relationship is often mutualistic as the microorganisms benefit from the plant in terms of favourable habitat, shelter, constant supply of C for energy, water, and enzymes that help it to flourish abundantly. It is important to study the different characteristics of roots to understand the underlying mechanisms on which the life of the vegetation depends. Such studies further help in molecular analysis and biotechnological manipulations that can help in transferring a favourable trait from a donor plant to a lacking receiver plant variety or genus. Such experiments might result in the development of species with the ability to grow under extremely unfavourable conditions such as snow, rainfall, drought, and deserts.

8.2 Quinoa Root System

Various studies involving different species of *Chenopodium* have revealed a herringbone topology in root system branching. Quinoa plant consists of an extensive root system with a central taproot. It extends up to 30 cm under field conditions, with several lateral branches. According to this classification, there exist a main axis and primary laterals. This branching system favours plants in efficient acquisition of nutrients from a poor habitat. It has been found theoretically and experimentally that this type topology decreases root competition within the plants and thus increases nutrient use efficiency of plants. These root characteristics make quinoa a promising candidate for agriculture under different abiotic stresses such as water scarcity and sandy soil. Under abiotic stresses, quinoa develops a tendency of profligate root elongation with thicker roots. These thicker roots with extensive network of external links travel deep inside the soil for better exploration (Alvarez-Flores et al. 2014).

It has been observed that under scarce nutrient availability, plants tend to develop more herringbone-like root system. However, herringbone root system is considered as an expensive root system. Under nutrient scarcity, this root system limits growth, which compensates with the cost of the root system. Therefore, any other species that have the characteristics to grow on infertile or arid soil should tend to develop herringbone root system. Fitter et al. (1991) performed experiments with different plant species and found that under low nutrient supply, plants tend to develop herringbone system, irrespective of being dicots or grasses. As a characteristic of this root system, plants tend to increase link lengths. This root system limits the competition among the roots of the same plant as well as among roots of the neighbouring plants. This helps to improve exploitation of limited water and nutrients available in soil. Furthermore, quinoa ecotypes growing under dry conditions tend to develop strong and deep main root axis to explore deep soil layers for water availability.

8.2.1 Case Study: Difference in Root Architecture of Quinoa Ecotypes Helps to Survive Drought Conditions

Alvarez-Flores (2012) performed an experiment with two contrast ecotypes of quinoa, i.e. Salare ecotype from the southern dry Altiplano of Bolivia with the annual precipitation of 150–300 mm and the coastal ecotype from the humid coastal lowlands of Chile with annual precipitation of more than 1200 mm. Two ecotypes differed in their morpho-physiological traits such as photosynthesis and transpiration, leaf morphology, leaf pigment content, stomatal movement, leaf water potential, and root lengths as well as in their pedoclimatic conditions, which include combined effects of temperature, water content, and aeration.

Both ecotypes were grown under water-limiting and water-available conditions. It was found that both ecotypes revealed a herringbone pattern of root architecture. Indeed, as previously stated, this root pattern helped in minimizing neighbouring competition under scarce water conditions. Constant examination of root system architecture of the two ecotypes led to better understanding of the importance of herringbone pattern under drought conditions. Under non-limiting conditions, Salare ecotype developed deeper primary root faster than the coastal ecotype. It was observed that until the first 6 weeks after seed germination, only primary roots developed in both ecotypes. It was only after the sixth week when growth of primary roots halted and development of other root system was accelerated due to ramification and elongation of the lateral roots (Fig. 8.1). In the same experiment, it was observed that under water-deficit conditions, Salare ecotype developed deeper roots as compared to the coastal ecotype (Zurita-Silva et al. 2015). All these observations were possible due to root system architecture analysis.

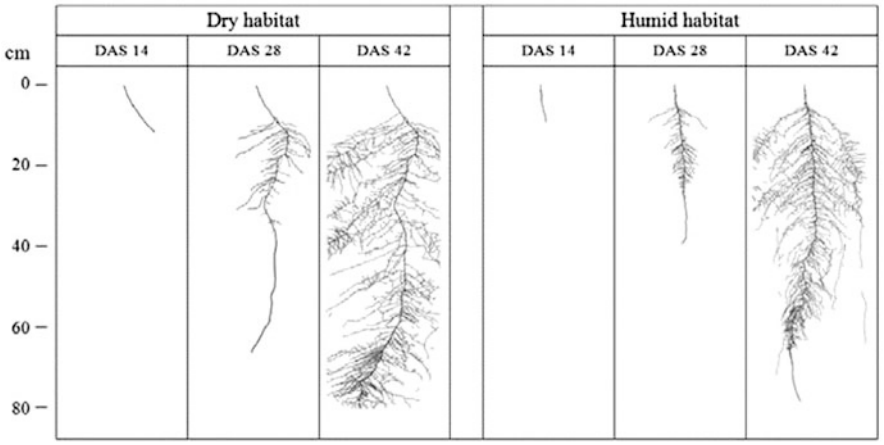

Fig. 8.1 Study of root architecture of two different quinoa ecotypes at different time intervals (Zurita-Silva et al. 2015)

8.3 Drought Mechanism in Quinoa

Researchers have found that flowering and milk grain stages in quinoa are most sensitive to water scarcity. In order to withstand drought conditions, plants adopt any of the following three mechanisms: (1) morphological strategy (escape, avoidance, or phenotypic flexibility), (2) physiological strategy (antioxidants, stabilization of cell membrane, osmotic adjustments), and (3) molecular strategy (aquaporins, osmoprotectants).

Among all the different strategies, root system architecture of quinoa has been found to play an important role in drought management. Quinoa roots have been found to exhibit faster root elongation as compared to its closely related relatives. It has more abundant and longer external branching of the roots in order to improve its foraging capacity. Under water scarcity, quinoa tends to develop extended taproots with longer, coarser, and more abundant root segments than it does under wet conditions.

Furthermore, it has been found that quinoa roots inhabit several endophytic fungi and bacteria. In addition to different molecular aspects, these endophytic microorganisms tend to alter root architecture of plants. The alterations in root architecture thus produced support plants indirectly to survive under dry conditions (Hussin et al. 2017).

8.4 Root System Architecture

The overall spatial configuration of roots of different order and age comprises of root system architecture (RSA). RSA as a whole describes the different aspects of root structure and shape, collectively. Root structure defines the assembly and features of various segments of roots. Various plant developmental processes control root structure, i.e. its expansion, direction of growth, senescence of old roots, and development of new ones, whereas root shape defines spatial distribution of roots and its functional properties such as nutrient uptake, anchorage, and plant hydraulic. Any small variation in RSA of a plant could be responsible for its adaptation to its normal or extreme environment. That is to say, plant adapts to its environment by causing variations in its RSA, such as promotion or inhibition of primary root growth, through growth of lateral roots or through formation of root hairs (Lynch 1995). Since nutrients are heterogeneously distributed in the soil, variations in RSA lead to a significant difference in uptake. Variation in the spatial arrangement of roots is responsible for differential uptake of nutrients from the rhizosphere.

However, it is important to note that RSA do not directly control the nutrient and water supply to plants. Nutrient uptake is governed by various cellular and mechanical mechanisms such as cellular transporters and apoplastic transport. Similarly, water transport is basically governed by local water potential, xylem diameter, etc., but despite these facts, RSA indirectly affects nutrient and water transport in plants.

8.5 Study of Root System Architecture

Earlier, visual and manual methods were employed to study root system architecture of a plant. These methods proved to be very tedious, labour- and time-intensive, and more prone to errors. Sometimes it was not even possible for the researchers to measure root architecture. One of the early developed methods to measure total root length was line intersect (LI) method. This method was developed by Newman in 1966 (Delory et al. 2017). In this method, uprooted and washed plant roots were randomly placed in a tray of known area. Newman developed an equation where he estimated the total root length of a sample by counting the total number of intersections made by the root placed in a tray and randomly oriented straight lines, of known lengths, placed in a tray. Later, 2D image analysis method was developed for studying variability in plant roots. Different softwares were developed for easy computation of scanned root images. One such software developed was WinRHIZO.

8.6 Method for Root Analysis Through WinRHIZO

WinRHIZO is an image analysis software designed for the measurement of root as a whole unit in different forms. It can be used to analyse root morphology and architecture along with its topology and colour. This is a user-friendly and

cost-effective computer program which is combined with an image scanner component. The program is currently available in four versions (Basic, Reg, Pro, *Arabidopsis*), differing in the features offered (https://regent.qc.ca/assets/winrhizo_about.html).

8.6.1 Image Acquisition by Root Scanner

Freshly uprooted and clean roots are gently placed over the glass scanner or Regent's water-proof tray. If Regent's tray is being used, the roots are placed in a thin layer of water. It is easy to position the roots in tray. The roots may overlap at some points during the scanning process. Image can be directly captured using WinRHIZO software, and can be stored in the desired format. The software then takes few seconds to analyse the image. Some analyses, such as nodule counting, colour assessment, and topology need interaction of the user before proceeding further. The software then saves the data obtained, which can be easily read and used further as desired (Fig. 8.2).

The software helps the user to analyse different aspects of root system in detail. The characteristics of root system can be analysed as follows:

Root Morphology The software facilitates automatic measurement of total length, average diameter, total area, number of tips, forks and crossings, and manual counting system for nodules. Further, it divides plant roots into different classes and subclasses based on their root diameter, surface area, projected area, and colour. The software makes it possible for the user to differentiate the entire root as a function of colour. It makes it possible for the user to measure root parameters of a specific colour or group of colours.

Link Analysis Measurements The software provides for the global analysis for the entire root image. It measures the total number of links, average link length,

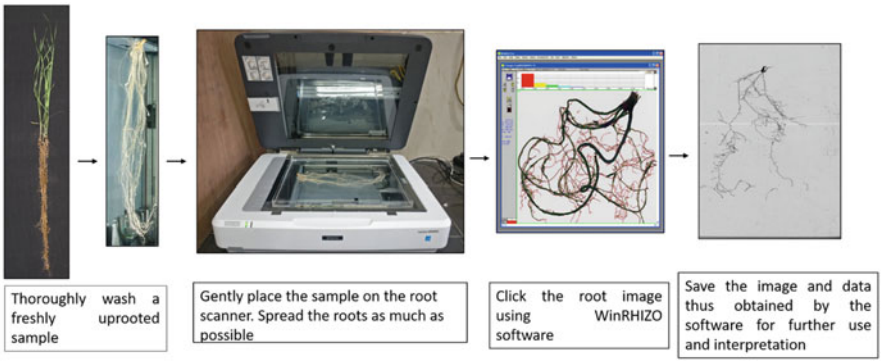

Fig. 8.2 Schematic representation of steps involved in roost system architecture analysis through WinRHIZO

diameter, area, volume, and branching angle. Link analysis is a study of basic morphology and connectivity of root segments. The software can perform this analysis for complete or incomplete root segment.

Root Topology The software measures external path length and altitude, globally for the entire root system. It also favours analysis of magnitude, path length, altitude, and structured connectivity for a single link. The user has to make sure that the root system to be analysed is not very dense and can be monitored visually. If not so, the data thus recorded would not be reliable.

8.7 Importance of Root Analysis

Roots are majorly responsible for the acquisition of water and nutrients for plants. In addition, it is important to notice that around half of the food made by the plant is spent in the maintenance of root system. Therefore, it is important to monitor, how efficiently the roots can use water and nutrients in order to be as energy-efficient as possible for the plants.

The study of root functioning in different agricultural plants is necessary to plan sustainable cultivation patterns, maintain soil fertility and composition, and reduce the amount and cost of nutrient and water input without jeopardizing the nutritional quality of the produce. Root analysis helps the cultivators to predict changes in growth patterns, which provide them with sufficient time to adjust their practices to earn maximum benefits and profits. With proper use of resources, financial and external investments can be reduced, which will ultimately lead to more profit and better environmental protection.

About half of the food prepared by the plant through photosynthesis is directed and spent up in the roots for accumulating nutrients and water from the surrounding soil. Efficiency in root functioning directly affects plant yield and the composition of rhizospheric microbiota. Roots are important soil carbon pools and are thus being studied for their effects in various ecosystems. This acts to fight important phenomena to prevent climate change. The carbon added to the soil by the roots remains there for longer durations of time than that obtained from the decomposition of organic matter.

Root analysis helps in studying and reducing both biotic and abiotic stresses that affect plant growth. They give direct indication of plant health and give enough strength to cope up with the adverse situations by adjusting their growth patterns. Roots of the plant affect the soil profile just as much as the soil affects the root growth. Root exudates, composition of microorganisms in the rhizosphere, excretion of secondary metabolites, root respiration and gas exchange, root temperature regulation, water activity, secretion of plant hormones, nutrient regulation, conversion of compounds into easily assimilated forms, etc. are some of the important processes through which plant roots can affect the quality and biomass in the soil.

The following are the root analyses associated with different fields of agriculture and science (Trimble 2019):

- Agronomy: It helps in reducing biotic and abiotic stresses, which in turn helps to improve crop health and productivity. It helps in the identification of different symbiosis, nutrient, and water-use efficiency.
- Soil Science: Roots tend to modify chemical and physical nature of rhizosphere. Roots respire, form symbiotic association with different microorganisms, and secrete different exudates which vary under different circumstances. These root activities tend to change under different biotic and abiotic stress conditions. Therefore, root analysis becomes important.
- Ecophysiology: Under this field, researchers are concerned about the variations in functional, molecular, and physiological mechanisms of root systems when they interact with different microorganisms or with different roots.
- Climatology: Roots are a major source of environmental carbon pool. Root carbon has been found to be very important for the prevention of major changes in climate, and thus root carbon pool should be protected.
- Hydrology: Water availability is an important factor that controls plant root architecture. Root system varies greatly with the status of water in natural ecosystems. Researchers have found major differences in plant root system architectures grown in different ecosystems such as drought or wetlands.

Root analysis can help in identifying crop specific and beneficial microbial species for agro-biochemical formulations. One such example is by Ortuño Castro et al. (2013), in which they designed and performed an experiment where they aimed to isolate microorganisms surrounding the quinoa plants, and then formulated the beneficial ones to be used as bioproducts for increasing the quinoa produce in fields. Many different bacterial and fungal species were isolated, and out of them, several showed plant growth-promoting functions such as phosphate solubilization, nitrogen fixation, nutrient cycling, production of growth hormones, antibiotic production, and activities such as those of biocontrol agents. After proper greenhouse testing and analysis of the effects of these microorganisms, they were first cultivated on a suitable growth media. Then, a large-scale production of these microbial formulations was carried out. The aim was set to use simple inexpensive culture media to manage costs. After thorough testing, plant fields in natural environment were inoculated with these formulations and observed for growth changes (Ortuño Castro et al., 2013). The results showed positive effects in plant productivity. This is an excellent example of industrial microbiological processing for the use of naturally occurring microorganisms as fertilizers and biocontrol agents.

8.8 Root Endophytic Fungi of *Chenopodium quinoa*

A variety of endosymbiotic microorganisms, including mycorrhizae, bacteria, and endophytic microorganisms, are associated with each plant variety and affect its growth and nutrition in a certain direction. Certain endophytic fungi associated with roots are commonly observed to occur in angiosperms for which a comparatively high rate of colonization has been observed (González-Teuber et al. 2017).

Endophytic microorganisms are ubiquitous and colonize different parts and tissues of the plant without any signs of the disease and live as natural inhabitants of the organ.

The plant-microbe interactions are governed by several important ecological aspects, including host colonization patterns, mechanisms of nutrient uptake and transmission, production of secondary metabolites, type of biodiversity, energy source, and waste production. Endophytic fungi have shown to be important in providing *Chenopodium quinoa* with the ability to survive under stressful conditions. For conducting this study, González-Teuber et al. (2017) performed an experiment to investigate the diversity of fungal endophytes associated with the roots of *Chenopodium quinoa* growing near the salt lake of Atacama Desert, Chile. Although desert conditions provide one of the most challenging habitats for the growth of plants, quinoa still managed to survive in such a condition along with high salt stress. The Atacama Desert is considered to be one of the driest regions of the world with low water availability, high temperature, and irradiance. For this, he collected six quinoa plants from the experimental site, washed the roots thoroughly under running tap water to remove mud and soil debris, and performed surface sterilization. Small sections of the roots were cut and cultivated on PDA plates at room temperature for about 3–4 weeks and were observed for fungal growth. Pure fungal isolates were obtained, classified and grouped based on morphological characteristics. Only three pure isolates of fungi belonging to the same genus were considered for DNA extraction by growing isolated pure cultures and performing molecular extraction and identification techniques. Genomic DNA was then isolated, species were identified using the primers ITS1-F-KYO1 (CTHGGTCATTTAGAGGAASTAA) and ITS4 (TCCTCCGCTTATTGATATGC), and ITS region was amplified using the PCR technique. Purification and sequencing of PCR products were carried out by Macrogen in South Korea. Sequence alignment and preparation of phylogenetic tree were done using various bioinformatics tools and software.

It was observed that the roots of the sample plant were colonized by a large diversity of fungal endophytes and the community was mainly dominated by the genus *Penicillium* (59% of total culturable community), *Fusarium* and *Phoma* (<15%), and others (1–5%). According to the obtained phylogenetic tree, a total of 11 noticeable genera were obtained, which included *Penicillium*, *Alternaria*, *Fusarium*, *Rhinocladiella*, *Cadophora*, *Phoma*, *Bartalinia*, *Neonectria*, *Sarocladium*, *Coniochaeta*, and *Plectosphaerella*.

Penicillium, *Phoma*, and *Fusarium* have earlier been found to be associated with roots of many plant species, and occur to play important beneficial roles in plant's tolerance against abiotic stresses and plant growth (González-Teuber et al. 2017).

In deserted areas, plant survival under conditions of extreme water deficit and high temperature depends upon several integrated morphological and physiological responses that either help in preventing water loss or help the plant to tolerate such extremities. Plant cell architecture, transport mechanisms, root growth and biomass adjustments, water-use efficiency (WUE), decrease in stomatal conductance and transpiration, etc. are some of the factors that help plants survive in arid areas, but

one factor that has been extensively studied and has been proven to play very important roles in survival under such conditions is microbial associations. Association of the plant with symbiotic fungi is a beneficial mechanism to regulate water stress tolerance (Malinowski et al. 1997; Redman et al. 2001; Rodriguez et al. 2004; Rodriguez and Redman 2008).

In order to study in details the effects of the root endophytic fungi in response to drought conditions in *Chenopodium quinoa*, González-Teuber et al. (2018) conducted another experiment in which they collected seeds of *Chenopodium quinoa* plants growing around the village of Socaire.

A total of four plant groups were established, in which group 1 was inoculated with laboratory pure culture of *Penicillium minioluteum* (fungal endophyte which was found to be present as a common inhabitant in quinoa roots in his previous experiment) in a formulated solution having the concentration of 1×10^7 spores mL^{-1} and normal irrigation with sterile water, the second group plants were inoculated with the endophyte without any irrigation intended to induce drought stress conditions, and the other two groups were cultivated without the endophyte inoculation under irrigation and drought conditions, respectively, all under sterile conditions to prevent any contamination, only to study the effects of the fungal endophyte on plant growth in various conditions. All of the plants were supplemented with nutrients in the form of Murashige and Skoog (MS) nutrient solution and sterile vermiculite to support growth. The temperature of the laboratory was kept normal and maintained between 17 and 23 °C.

After careful observations over several weeks, it was found that the fungal endophyte helped the plant to tackle with drought stress conditions by increasing root growth, vigour, and development, and the positive effects were mainly below ground. Root biomass was significantly increased, and root-to-shoot ratio was improved by inoculation of this fungus. Such an increase in root growth enabled the plants to reach and proliferate deep into the soils for nutrition in such an arid climate. Decrease in shoot biomass was also observed in inoculated plants, which ultimately lead to reduction in water loss from leaves through evaporation. It was concluded that the relationship between the plant and the endophyte grew positive only under stress conditions and no such increase or adjustment in biomass took place under normal conditions (González-Teuber et al. 2018).

Urcelay et al. (2010) after experimentation published in their research paper that *Chenopodium quinoa* exhibited no mycorrhizal associations and showed no signs of colonization by dark septate endophytes in their roots. The family of Chenopodiaceae was already considered as non-mycorrhizal, and it was further confirmed by their work. Moreover, they observed that the roots of quinoa plants were infected with significant population of *Olpidium* sp. (*Chytridiomycota*), which are normally considered as harmless root pathogens but have been found to be responsible for the transmission of several plant viruses, which can lead to some of the severe plant diseases.

8.9 Root Endophytic Bacteria of *Chenopodium quinoa*

Noel Ortuño Castro et al. (2014) proceeded with a set of experiments with plants from the Bolivian Altiplano to study much of the bacterial species that were associated with cultivation of quinoa. In the research experiment, molecular identification revealed that *Bacillus aryabhattai*, *Bacillus horikoshii*, *B. megaterium*, *B. pumilus*, *Paenibacillus odorifer, Pseudomonas* sp., *B. subtilis*, and *Azotobacter* sp. were present in quinoa roots as endophytes (Fig. 8.3). These endophytes imparted several benefits to the plant, such as activation of jasmonic acid cycle by *B. subtilis* that induces plant resistance against pathogen attack, *Azotobacter* sp., *Pseudomonas* sp., and *Paenibacillus* acting as nitrogen fixers, production of the phytohormone indole acetic acid (IAA) by *Bacillus aryabhattai* and *Bacillus horikoshii*, and solubilisation of phosphorous by *Bacillus pumilus* and *Pseudomonas* sp.

Bacillus thuringiensis was also isolated as a root endophyte in quinoa which acted as an entomopathogen against *Lepidoptera* and several other insect species.

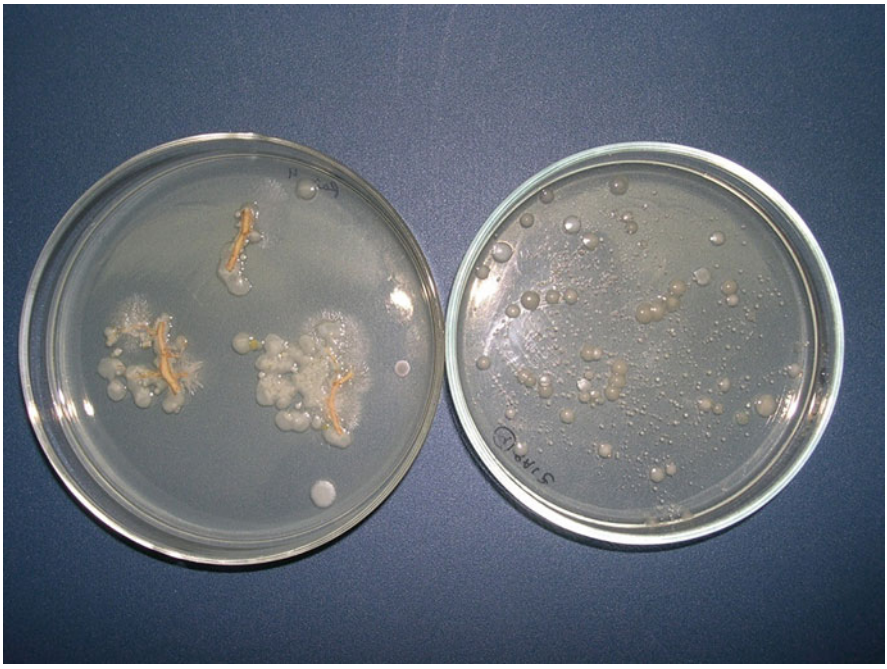

Fig. 8.3 Endophytic bacterial colonies isolated from the roots of *Chenopodium quinoa* (C.F.—Ortuño Castro et al. (2014))

8.10 Conclusions and Future Prospects

A plant's root system, along with its rhizospheric community, is responsible for the survival of the plant. Quinoa's herringbone root system accompanied by its versatile microbial community helps the plant to survive through the adverse climatic conditions it grows in. Therefore, the study of both root system architecture and their native microbial community is important to have a better understanding of what helps plants to survive through various biotic and abiotic stresses. Hence, careful observations, detailed study of root system architecture, and microbial interactions are important in order to explore the gap in the present-day understanding of the subject and to delve into new possibilities and sustainable approaches to prevent crop diseases, minimize yield losses, and promise significant profits to the hard-working cultivators all around the globe.

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9 Influence of Biotic and Abiotic Stresses on Quinoa Cultivation: Insights into Microbe-Assisted Stress Tolerance

Shyamalina Haldar, Alka Kumari, Anupama Ghosh, and Abhrajyoti Ghosh

Abstract

Chenopodium quinoa Willd. (quinoa) is a pseudo-grain serving as a staple dietary food in South America due to its high nutritional values. However, the high genetic diversity of this crop determines its high potential of adaptability to contrasting environments, including nutrient-poor soil, drought, heavy metals, fluctuating temperatures, and UV-B light irradiance. Despite enormous studies on the influences of abiotic stresses and the ability to combat the stresses by the plant itself, the role of the plant-associated microbiome in stress tolerance of quinoa has not been elucidated. Therefore, this chapter aims to provide a deep insight into the (1) abiotic stresses which are challenging for the growth and yield of quinoa, (2) role of microbiota in assuaging the effects of stresses by comparing with other grain crops, and (3) formulation of methods to use this potential of the microbiome for better yield of high quality of quinoa.

Keywords

Quinoa · Abiotic stress · Microbiome · Tolerance

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9.1 Introduction

True cereals and pseudo-cereals, presently sustaining no less than two-thirds of the world's food system, are regarded as important markers of human life and culture throughout the history. Despite the difference in plant biology, both are grouped based on their mutual usage as edible starch. The key crops were farmed through ancient civilizations about four millennia ago, and efforts were taken to transform them into better qualities to feed the increasing global population. In contrast, minor (underutilized/orphan/neglected) crops have remained unnoticed until the present century. In late 2000, as the science became aware of the gluten-free crops, these cereals such as *Amaranthus*, *Eragrostis tef*, and *Chenopodium quinoa* came into notice, and the exploration of genetics of these plants began from the last 20 years with the publishing of complete genome sequence of the quinoa very recently (Cheng 2018). Quinoa (*Chenopodium quinoa*), a flowering plant and a pseudo-grain belonging to amaranth (Chenopodiaceae) family, is an annual herb originated in the Andean region of northwestern South America (Food and Agriculture Organization 2011; Fuentes et al. 2008). It is cultivated as a food crop as the seeds are rich in protein, vitamin B, dietary fiber, and minerals. Although quinoa seeds found utilization in animal feed formulation about 5.2–7000 years ago, human consumption began only about 3000–4000 years ago in the Lake Titicaca Basin of Peru and Bolivia (Kolata Alan 2009). For that reason, the quinoa cultivation has attracted attention in more than 70 countries including Europe, Kenya, India, and the USA, with its prices also being tripled between 2006 and 2013. The global production of quinoa recorded was 146,735 tons in 2017, led by Peru and Bolivia, contributing to 99% together (FAOSTAT 2018). The easy adaptability of quinoa in extreme and adverse environmental conditions of saline soil, nutrient-poor soil, and drought-stressed environments makes this crop an important global food crop (Hinojosa et al. 2018). This variable resilience of quinoa is possible due to its natural variability in different traits, including life cycle duration, inflorescence type, seed color and size, saponin content, and nutritional value. Based on geographical adaptation, quinoa is classified into five ecotypes as a valley, altiplano, salares, sea-level, and subtropical/Yungas (Hinojosa et al. 2018). The health benefits of quinoa include its anti-inflammatory and antioxidant activities contributed by the lipophilic (e.g., fatty acids, tocopherols, and carotenoids) and hydrophilic (e.g., phenolics, betacyanins) nutrients, thereby lowering the risk of the oxidative stress-related diseases including diabetes, cardiovascular disease, obesity, and cancer (Tang and Tsao 2017). The combination of essential amino acids, along with the absence of gluten, makes it an important daily fiber for celiac patients (Fuentes et al. 2008). This is considered as an oil crop due to the significant content of omega-6 and vitamin E (Abugoch James 2009). However, with high cost combined with poor knowledge on the nutritional attributes, seeds are not extensively consumed globally, thereby warranting further research to upsurge insight about this crop and to establish its benefits (Fuentes et al. 2008). Due to high protein content, versatile yet easy preparation, and a high potential for increased yields, it has been selected as an experimental crop in the Controlled Ecological Life Support System for long-duration human-occupied space

flights developed by NASA (Greg and David 1993). Also, a total number of 193 secondary metabolites have been identified from quinoa with diverse physiological functions and biological activities, indicating that it is a golden grain (Lin et al. 2019). The diverse use of quinoa is attributed to its various functional characteristics, including emulsifying, foaming, gelation, water-holding capacity (WHC), and solubility. Quinoa also produces a wide range of phytohormones which also add to human nutrition (Vega-Gálvez et al. 2010). Taken altogether, it is a promising alternative cultivar (Abugoch James 2009). Though the genetic constitution of quinoa is well established, limited resources hinder the genetic development of this plant (Jarvis et al. 2017). To promote the ability for the betterment of human lives, both the awareness and access to quinoa's health value are an absolute necessity. To comprehend this, the two strategies to be adopted are (1) to expand the cultivation of this crop to different countries affected by climate change and desertification and (2) to propagate the knowledge regarding biodiversity, nutritional values, uses, and methods of sustainable farming of quinoa. Therefore, a comprehensive study on the physiological characteristics and the mechanisms of biological responses to versatile environment will not only help to gain a better understanding of the biology of this crop but will also aid to develop a broader insight of how to develop genetically modified version and also to correlate the findings with the other similar or dissimilar plants.

9.2 Biotic Stress

Being sessile, the plants encounter a wide range of environmental stresses, including both biotic and abiotic types. The abiotic stresses mostly include non-living factors like temperature, sunlight, drought, and salinity, while the biotic stresses involve living organisms.

In plants, biotic stress is caused by the attack of bacteria, viruses, fungi, arachnids, nematodes, insects, and weeds. Upon infecting plants, they create a negative impact on plant growth and production. Agents of biotic stress induce stress by depriving nutrients to the host plant, which is an extreme condition that leads to the death of plants. The type and extent of biotic stress depend on the climate of growing area and genomic makeup of plant species, which provides resistance to a particular kind of stress. The study of biotic stress has historical importance, unlike the abiotic stress that got attention lately due to global warming that leads to climate change. There are numerous cases when biotic stresses (diseases) have destroyed crop production, leading to famine in those areas, e.g., coffee rust in Brazil (Rogers 2004), potato blight in Ireland, and maize leaf blight in the USA (Ullstrup 1972). In India, the Great Bengal Famine in 1943 is an example of a disease disaster when the rice crop was infected by fungus that leads to brown spot disease in rice (Padmanabhan 1973). These diseases not only affect crop production and crop economy but also resulted in death of millions and moreover migration of individuals to distant regions. A survey related to biotic stress states that diseases reduce 10–15% of global food production,

leaving about 800 million people ill-fed (Christou and Twyman 2004; Onaga and Wydra 2016).

Pathogens An array of bacteria, fungus, and virus that infect plants causes considerable damages to all crops. Nevertheless, the severity of infection depends on the genetic composition and geographical distribution of crops. For example, an estimate of 40% loss in the yield of grains was reported for finger millet due to the blast disease caused by the fungus, *Magnaporthe oryzae*, while 70% loss in tuber yield was found in cassava due to the cassava mosaic disease (Lule et al. 2014; Fargette et al. 1988).

9.2.1 Agents of Biotic Stress in Plants

9.2.1.1 Bacteria

More than 200 bacterial species, mostly belonging to *Agrobacterium*, *Erwinia*, *Dickeya*, *Pseudomonas*, *Pectobacterium*, *Ralstonia*, *Xylella*, and *Xanthomonas* genera, have been identified as plant pathogens causing diseases in plants (Considine and Considine 1995; Mansfield et al. 2012; Buttimer et al. 2017).

Pseudomonas syringae *P. syringae* belongs to *Gammaproteobacteria* class. *P. syringae* is divided into more than 50 pathovars. The strain of different pathovars classically reveals a narrow range of hosts except for *P. syringae* pv. where it has been reported to infect more than 80 plant species. Bacteria infect plants through the stomatal opening. *P. syringae* opt type III secretion system to release its effector molecules. Strain *P. syringae* pv. tomato causes a disease known as bacterial speck in tomato (Buttimer et al. 2017).

Ralstonia solanacearum It is soilborne and one of the most destructive phytopathogens. Bacteria infect the plants by entering through roots, followed by colonization in the xylem. It can infect up to 200 different plants from 50 different families (Denny 2007). They infect and cause disease such as brown rot of the potato or bacterial wilt in banana, tobacco, tomato, etc. (Sanchez Perez et al. 2008).

Xanthomonas This is one of the most abundant pathogenic genera; it has a wide range of hosts and can infect around 400 different plant species. Several important crops such as banana, tomato, pepper, rice, and citrus fruits can be infected by it. Globally *X. campestris* pv. *Vesicatoria* is the causative agent for bacterial spot diseases in tomato and pepper (Bouzar et al. 2004). This disease is responsible for 50% loss in the yield of tomatoes.

Erwinia amylovora This is the causative agent of fire blight disease. It enters the plants through the opening, such as wounds. Once infected, it circulates in the plant using the intracellular space of parenchyma. In the latter stages, it reaches to the xylem (Buttimer et al. 2017). This destructive disease occurs in the Rosaceae family.

It severely affects the production of apples and pears. The disease has been reported in approximately 40 countries across Europe, North America, Middle East, and the Pacific Rim (Bonn and van der Zwet 2000).

Xylella fastidiosa *X. fastidiosa* comes under class *Gammaproteobacteria*. This pathogen is xylem restricted and infects plants with the help of insect vectors. Insects also help in its distribution. The movement of nutrients and water is blocked in the vascular system through the formation of biofilm aggregates (Chatterjee et al. 2008).

Dickeya *and* Pectobacterium Both pathogenic bacteria cause soft rot *Enterobacteriaceae*. By degrading the cell wall, they attack the plant. About 10 monocot and 11 dicot families are infected by the genera *Dickeya*, while *Pectobacterium* is found to infect 11 monocot and 16 dicot families (Ma et al. 2007). Different subspecies of *Pectobacterium* have been found to infect potato globally.

Bacterial Disease

Being resistant to bacterial infection, negligible reports of bacterial infection of quinoa have been documented. However, Alandia et al. (1979) and Pańka et al. (2004) reported the occurrence of bacterial blight disease in quinoa. The causal agent of blight disease is *Pseudomonas* bacteria.

9.2.1.2 Fungi

Fungi are the most ecologically and economically assorted threatening plant pathogens (Doehlemann et al. 2017). Fungi use different strategies to get food from the plants by establishing both beneficial and harmful interactions with the plants. Pathogenic fungus is broadly grouped into two phyla *Ascomycota* and *Basidiomycota*. Based on its mode of pathogenesis, the fungus is divided into three groups: biotrophic pathogens are fungal pathogens that infect the plant and live on and utilize living tissues for food (biotrophs), whereas necrotrophic pathogens kill the host plants and get nutrients (necrotrophs). In addition to the above two groups, hemibiotrophic fungal pathogens infect plants as biotrophs and then shift to necrotrophs.

Biotrophic Pathogens Even being an economically most devastating group of fungal pathogens, they cause mild phenotypic appearance. Biotrophic pathogens have either obligate or non-obligate life cycle. Obligate biotrophs are the causative agents of powdery mildew (*Ascomycota*) and rust (*Basidiomycota*) diseases. Downy mildew and white rusts are also obligate parasites, while facultative biotrophs include the smuts (*Basidiomycota*, *Ustilaginales*) and certain species of *Claviceps* (*Ascomycota*, *Clavicipitaceae*). For infecting host plant, biotrophic pathogens germinate on a leaf of host, and developing hyphae enter the leaf through the stomatal pore and grow in mesophyll space. The invading hypha again differentiates into substomatal vesicles, which produce primary hyphae. Another mode of infection also occurs where ascomycete (order *Erysiphales*) conidia germinate on the leaf

surface and penetrate the epidermal cells with the aid of appressoria (Spanu et al. 2010; Doehlemann et al. 2017).

Necrotrophic Pathogens Necrotrophic fungal pathogens feed on dead plant tissue. Broadly necrotrophs are divided into two categories, one which attack and kill healthy plants and the other secondary necrotrophic-like pathogens, which are saprophytic and feed on dead plant tissue but may occasionally infect plants that have been previously weakened, e.g., by other pathogens, injury, or abiotic effects (Doehlemann et al. 2017). For killing and colonization in plants, necrotrophic fungus releases toxins. Based on the released toxin, the fungus has been grouped to narrow-host-range and broad-host-range species (Mengiste 2012). Based on the production of host-specific toxins, necrotrophic fungus is categorized into broad host range and specific host. *C. carbonum*, *C. heterostrophus*, and *C. victoriae* are few examples of *Cochliobolus* pathogenic fungal having host-specific toxins (Wolpert et al. 2002). Broad-host-range necrotrophs lack host-specific toxins. They can infect a wide range of plant species. *B. cinerea* and *S. sclerotiorum* are examples of this group. Both fungi have considerably broader host ranges than most plant pathogens (200–400 plant species) (Doehlemann et al. 2017).

Hemibiotrophs

Broadly, hemibiotrophic pathogens are those fungal pathogens that are having biotrophic and necrotrophic lifestyles. Hemibiotrophic pathogens have a variable length of a biotrophic phase before shifting to the necrotrophic phase (O'Connell et al. 2012). The presence of actual biotrophic organs such as filament, haustoria, etc. indicates hemibiotrophs lead to the correct biotrophic phase. During the early biotrophic phase, fungal pathogens also secrete effectors to suppress the plant defense rather than toxins to kill a plant. However, during the transition from biotrophic to necrotrophic phase, the fungus undergoes a massive developmental change. The following are a few examples of it. The rice blast fungus *M. oryzae* and anthracnose in maize by *C. graminicola* and *Colletotrichum* cause anthracnose diseases in more than 600 dicot (strawberries, bean, tomato, cucurbits) and monocot plant species and include severe pathogens of important crops (Doehlemann et al. 2017). Other examples of hemibiotrophs are *Fusarium* (Ma et al. 2013), *Verticillium* (Fradin and Thomma 2006), *Mycosphaerella* (Churchill 2011), and others.

Fungal Disease of Quinoa

Downy Mildew The downy mildew disease is one of the devastating fungal infections of quinoa crop. It drastically reduces quinoa production by 35–90% worldwide (Danielsen et al. 2000, 2003; Danielsen and Munk 2004). The causative agent of downy mildew is a biotrophic fungus, *Peronospora variabilis*, which is the most significant environmental threat to quinoa cultivation (Danielsen et al. 2003). Fungal pathogen requires high humidity and temperature as the ideal conditions to grow. *Peronospora farinosa*, the oomycete from *Peronosporaceae* family (*Peronosporales* order), infects globally the different members of the Chenopodiaceae family, including spinach, beet, and quinoa (Danielsen et al.

2003). Downy mildew-infected plants show a variety of phenotypes, including yellow chlorotic lesions above the leaf surface, whereas below the leaf surface, they show gray, slimy mycelial sporulation, and in severe conditions, it results in premature leaf loss (Jancurová et al. 2009). However, the severity of infection and pathogen generated phenotype depends on many factors such as the genotype of quinoa crop, biological age and stage of quinoa crop, strain of infected pathogens, and geographical location and environmental condition (Danielsen et al. 2003).

Leaf Spot *Ascochyta hyalospora* fungus is the causative agent for leaf spot of quinoa, a seed-borne disease (Jancurová et al. 2009). The symptom of leaf spot infection appeared as spots of light color with brown edge, which eventually turned necrotic. At the later stage, due to necrosis, the black color appeared in pycnidia (Alandia et al. 1979; Danielsen et al. 2003). In the advance stage of infection, leaves become dry and fall off.

Brown Stalk Rot Otazu and Salas (1977) first time reported the brown stalk rot disease in quinoa caused by *Phoma exigua* var. *foveata* (Foister) in Peru. In the following years, globally, quinoa grown farmers have been reported infection of this disease (Alandia et al. 1979). *Phoma exigua* is a soilborne pathogen that grows in low temperatures and high humidity conditions. *Phoma exigua* also causes gangrene disease in potato (Otazu and Salas 1977; Danielsen et al. 2003). Brown stalk rot infection includes small spots with a watery appearance on the upper third part of the stalk. In the advance stage of the watery disease, lesions expand to cover most of the stalk, and inflorescence and pycnidia become visible (Danielsen et al. 2003).

Black Rot The pathogens of black rot of quinoa root have not been identified, and it is believed that it is a soilborne disease caused by fungi, nematodes, and bacteria. The symptom of the disease includes black rot of quinoa root, which supplies less water and nutrient from root to stems and leaves, resulting in yellow leaves or withered death. However, the effect of the disease can be overcome by increasing soil permeability (Li et al. 2017).

***Rhizoctonia* Damping-Off and *Fusarium* Wilt** Damping-off and wilt disease of quinoa were reported in the year 1998 at the International Potato Center (Barboza et al. 2000). Isolated pathogens were cultured in the laboratory and able to reproduce the phenotype.

Seed Rot and Damping-Off Seed rot and damping-off are caused by *Sclerotium rolfsii* Sacc. *Sclerotium rolfsii* Sacc. is a soilborne fungal pathogen. In this crop, it was first reported in California (Beckman and Finch 1980). During its pathogenesis in quinoa pre- and post-emergent damping-off of seedlings, the phenotype was noticed.

Similarly, in quinoa, damping-off phenotype was also noticed due to infection of *Pythium zingiberum*. *Pythium zingiberum* is a parasitic oomycete fungal pathogen. They also cause rhizome rot in ginger (Ikeda and Ichitani 1985).

Table 9.1 Bacterial, fungal, viral, and nematode diseases of quinoa and their causal organism (Adopted from, Bhargava et al. 2006)

S. no.	Disease	Causal organism	References
1.	Damping-off	Fungi *Sclerotium rolfsii*	Danielsen et al. (2003)
2.	Stalk rot	Fungi *Phoma exigua* var. *foveata*	Alandia et al. (1979); Danielsen et al. (2003)
3.	Downy mildew	Fungi *Peronospora farinosa*	Danielsen et al. (2000); Danielsen and Munk (2004)
4.	Stem gothic spot	Fungi *Phoma cava* sp.	Alandia et al. (1979)
5.	Gray mold	Fungi *Botrytis cinerea*	Johanson (1983)
6.	Leaf spot	Fungi *Ascochyta hyalospora*	Danielsen et al. (2003)
7.	Stem rot	Fungi *Choanephora cucurbitarum*	Sun et al. (2018)
8.	Bacterial blight	Bacteria *Pseudomonas* sp.	Alandia et al. (1979), Pańka et al. (2004)
9.	Chlorotic mosaic virus	*Chenopodium mosaic virus*	Alandia et al. (1979); Tomlinson et al. (1981)
10.	False nodule	Nematode *Nacobbus* spp., *Thecavermiculatus* spp.	Alandia et al. (1979); Franco (2003)

Gray Mold The first report of quinoa infected by gray mold came from Cambridge. According to literature, Baer variety of quinoa was infected by *Botrytis cinerea* (Li et al. 2017). *Botrytis cinerea* is a necrotrophic fungus. *Botrytis cinerea* infects stems and inflorescences of mature quinoa. As the central infection axis of the inflorescence gets feeble and soft, the secondary axes are liable to collapse (Johanson 1983). Apart from these reports, few more fungal diseases have been reported that are summarized in Table 9.1.

9.2.1.3 Virus

Viruses are microscopic obligate pathogens. To complete its life cycle, it depends on host machinery. The virus infects a wide range of plants and crops and causes enormous losses globally, both qualitatively and quantitatively. Roughly, worldwide more than $30 billion crop loss due to viral infection was estimated per year (Sastry and Zitter 2014). Based on the genetic material, plant viruses are grouped into different families as:

Caulimoviridae They have double-stranded circular DNA as genetic material. The virus causes chlorosis, mosaic, stunting, and other phenotypes when infected to plants. Cauliflower mosaic virus (CaMV) comes under this and is one of the most common viruses of the *Brassica* family (Sutic et al. 1999). *Caulimovirus* has a restricted range of hosts. It can infect Solanaceae and Cruciferae family (Chenault and Melcher 1993).

Geminiviridae This family constitutes one of the largest, most diverse, and economically important families of plant viruses. A total number of 360 species belonging to 9 genera have been identified from this family. The various disease symptoms associated with plants infected with this family include yellow mosaic, leaf curling, yellow mottle, stunting, bright yellow mosaic, streaks, and low yields (Zerbini et al. 2017).

Potyviridae One-third of plant virus belongs to the *Potyviridae* family (Riechmann et al. 1992). Diseases associated with this family include papaya ring spot virus, bean mosaic virus, etc. This virus can infect papaya, bean, tomato, potato, etc.

Luteoviridae Luteus means "yellow" in Latin. The virus of this family gives a yellowing phenotype. Its host is restricted to solanaceous family including tomato, *Datura*, potato, etc.

Closteroviridae *Closterovirus*, or beet yellows viral group, is a genus of viruses that causes yellowing and necrosis that affect the phloem tissue. The beet yellows virus (the type species) and Citrus tristeza virus are important plant diseases.

Bromoviridae This family of the virus has a tripartite (+) strand of RNA as genetic material. Cucumber mosaic virus and alfalfa mosaic virus are examples of the disease caused by the virus of this family.

Comoviridae Two single RNA strands act as genetic material for the virus of this family. This is one of the common viral infections of *Vigna radiata*. The infected leaf has a puckered appearance. *Datura*, *Glycine max*, tobacco, and pea are their suitable hosts.

Tombusviridae The virus of this family has single-stranded positive RNA as genetic material. Artichoke mottled crinkle virus, petunia asteroid mosaic virus, and tomato bushy stunt virus are the few examples of diseases spread by the members of this family.

Along with the family mentioned above, there are few more viral families which infect plants along with animals and fungus and function as an agent of biotic stress.

In quinoa, little is known about the viral infection. There is a report of chlorotic mosaic virus infection causes by the *Chenopodium* mosaic virus in the quinoa crop (Alandia et al. 1979; Tomlinson et al. 1981). *Chenopodium* mosaic is a seed-borne mosaic virus.

9.2.1.4 Nematodes

Numerous species of nematodes are infectious and cause a series of diseases in foliage plants, vegetable crops, cereals, fruit and nut trees, etc. Burrowing (*Radopholus similis*), Cyst (*Heterodera* and *Globodera*), Root lesion (*Pratylenchus*), Spiral (*Helicotylenchus*), Bulb and stem (*Ditylenchus dipsaci*),

Pine wilt disease (*Bursaphelenchus xylophilus*), Root-knot (*Meloidogyne*), Dagger (*Xiphinema*), etc. are a few examples nematode mediated diseases in plants (Williamson and Hussey 1996; Stirling 2018). Nematode-mediated disease of quinoa has been mentioned in Table 9.1.

9.2.1.5 Insects

Dipterous and lepidopterous stem borers are the main group of insect pests that contribute in 30–60% crop losses in Africa (Oerke 2006). Insects act as a vector for spreading viral and bacterial disease among plants. Aphids, bugs, leafhoppers, plant hoppers, whiteflies, treehoppers, mealybugs, flower thrips, maggots, beetles, honey bee, ant, leaf miner flies, and psyllids are the examples of insect vectors which infect plants directly or by acting as a vehicle for the viral and bacterial disease (Weintraub and Beanland 2006).

Insect Pathogens

A wide range of insect pests has been reported in the native cultivation area of the quinoa crop. Insect pests cause 8–40% loss of the crop due to infection (Tapia et al. 1979; Bhargava et al. 2006). The most economically significant and endemic insect pest attack belongs to quinoa moth (*Meyrick* sp., *Eurysacca melanocampta*) and *ticona* complex (*Spodoptera*, *Heliothis*, *Feltia*, and *Copitarsia turbata*) group. The type of the pest-insect, stage of pathogens, and quinoa plant parts affected due to infection were summarized in the tabular form in Table 9.2 (Bhargava et al. 2006).

9.2.2 Abiotic Stress

One of the primary reasons for worldwide crop losses resulting in the reduction of yields by more than 50% is the abiotic stress. The principal abiotic stresses that have been studied include drought, high salinity, waterlogging, excess heat, frost, heavy metals, and ultraviolet-B light (UV-B) irradiance.

9.2.2.1 Drought

Drought is defined as the inadequate moisture content in the soil, thereby reducing the growth of the crops. Quinoa, which can grow in arid and semiarid regions of South America, is an exciting model plant to study the mechanisms of the drought tolerance for the breeding of quinoa varieties with improved drought tolerance (Fuentes and Bhargava 2011).

The milk grain and the flowering stages are the most drought-sensitive stages of quinoa (Geerts et al. 2008a). At an early growing stage, though, this is insensitive to water stress; at a later stage, it becomes drought-resistant through the high rate of photosynthesis and development of specific leaf areas and larger root systems (Jensen et al. 2000; Geerts et al. 2008b). Even a slow growth rate in certain varieties helps it to avoid drought stress. The increased concentration of abscisic acid (ABA) in the xylem of the roots and the shoots of the different varieties of has been reported as the primary mechanism for drought tolerance (Hinojosa et al. 2018). Also, the

Table 9.2 Insects and pests and their infective stages causing diseases on various plant parts of quinoa

Type	Causal organism	Stage	Plant part affected	References
Leaf miner	*Lyriomiza brasiliensis*	Larva	Leaf	Tapia et al. (1979)
Leaf sticker, *Kconakcona*	*Eurysacca* spp.	Larva	Inflorescence, stored grain	Galwey et al. (1989); Rasmussen et al. (2003)
Cutworm	*Feltia experta*, *Spodoptera* spp.	Caterpillar	Stem, leaf	Zanabria and Mujica (1977); Rasmussen et al. (2003)
Looper	*Perizoma sordescens*	Caterpillar	Stem, leaf, seed, inflorescence	Zanabria and Mujica (1977)
Leaf and inflorescence caterpillar	*Hymenia recurvalis*, *Pachyzancla bipunctales*	Caterpillar	Leaf, inflorescence	Tapia et al. (1979)
Defoliating insect	*Epitrix subcrinita*, *Epicauta* spp.	Adult	Leaf, inflorescence	Zanabria and Mujica (1977); Tapia et al. (1979)
Piercing and cutting insect	*Macrosiphum* spp., *Myzus persicae*, *Bergallia* spp., *Frankliniella tuberosi*	Adult	Whole plant	Tapia et al. (1979)

production of reactive oxygen species (ROS) scavengers, accumulation of osmolytes by ornithine and raffinose pathways as antioxidant defenses, and buildup of soluble sugars and proline that maintain the osmotic potential through maintenance of turgor pressure in the stomata, fast stomatal closure, regulation of cellular water deficiency, and ratio of root to shoot increasing the efficiency of water use have been identified as the response mechanisms to water stress (Hinojosa et al. 2018). The investigations of the root architecture revealed that the high rate of elongation, enhanced growth of the taproots, abundance, and long external branching and plentiful root segments are the probable causes for their adaptability to the drought conditions (Alvarez-Flores et al. 2013). RNA sequencing using quinoa root tissue identified 462 differentially expressed contigs and 27 putative genes with regulatory functions. However, most of the features of the proteins remained uncharacterized; few (AUR62041909 and AUR62015321) have been identified to have roles in flavonoid synthesis, disease response, and lignification (Davin and Lewis 2000). The expressions of 13 *hsp70* genes under drought conditions were observed in quinoa plants with 6 being downregulated at the initiation and the recovery phases of the drought stress. The genes CqNCED3a and CqNCDE3b were found to be upregulated during drought stress in quinoa and were linked to ABA biosynthesis (Morales et al. 2017).

9.2.2.2 Salinity

During the last two decades, the response of quinoa plants to salinity has been extensively studied which has shown better tolerance to salinity (salt concentration of 150 mM NaCl to as much as 750 mM NaCl) than other food crops and vegetable crops, thereby identifying it as a facultative halophyte crop (Hinojosa et al. 2018). However, these salt tolerance levels are genotype-dependent, with 100–200 mM NaCl being the optimal salinity conditions for quinoa growth. The salt concentrations above 250 mM NaCl delay the commencement of the germination of quinoa seeds by changing the activity of the invertase enzyme and the metabolism of soluble sugar. The concentration of sugar has been found to increase or decrease in the cotyledons and the roots based on the genotype (Hinojosa et al. 2018). Experiments revealed that increasing the water salinity from 100 to 400 mM NaCl decreased seed yield by 72% and the net assimilation rate of photosynthesis by 48%. However, the augmented atmospheric CO_2 (540 ppm) alleviated the effect of high salinity by moderating the limited impact of the stomata on photosynthesis and, therefore, inhibiting oxidative stress (Dinneny 2015). However, seed priming and the use of plant growth-promoting rhizobacteria are novel techniques used to alleviate this salinity stress. It has been successfully shown that the negative impacts of salinity were reduced in the presence of *Enterobacter* and *Bacillus* isolates.

Similarly, saponin and paclobutrazol, a gibberellic acid biosynthesis inhibitor, showed a high yield of quinoa under high salt conditions (Gómez et al. 2011). The non-enzymatic antioxidant rutin, as well as choline (the metabolic precursor of glycine betaine), also helps in the osmotic adjustment during salinity stress. Due to the antioxidant activity, tyrosine-derived betalains are involved in salt stress tolerance (Hinojosa et al. 2018).

The mechanism for salt tolerance has been attributed to the high ABA synthesis in roots, consequently transported through the xylem to the leaves where it regulates the conductance of the stomata by closing the pores, subsequently reducing the uptake of both water and CO_2 and inhibiting photosynthesis (Hinojosa et al. 2018). Na+ sequestration in the leaf vacuoles along with the maintenance of low cytosolic Na+ levels, Na+ loading in the xylem tissues, higher ROS tolerance, better K+ retention, and lowering of slow and fast activities in the tonoplast channels along with high rate of H+ pumping in the mesophyll tissues are the principal mechanisms to combat salinity stress (Hinojosa et al. 2018). Epidermal bladder cells in the leaves help in the confiscation of Na+, the retention of K+, and the storage of metabolites like proline and phenolics that together control the ionic concentrations in the young quinoa plants. Genes related to energy input and ABA biosynthesis were found to be highly expressed in the bladder cells as compared to the lamina. A positive correlation between the density of the stomata and the tolerance to the salinity has been reported indicating that the stomatal density and size could be playing a crucial role in enhancing water-use efficiency in quinoa under high salt environments (Hinojosa et al. 2018). The activities of the enzymes like ascorbate peroxidase, catalase, peroxidase, and superoxide dismutase in quinoa plants are found to increase, while the activity of mitogen-activated protein kinase (MAPK) was found to decrease under saline conditions. Even K+ and H+ fluxes were found to be generated in

quinoa roots resulting in the activation of H+-ATPase in the plasma membrane under high NaCl (Hinojosa et al. 2018). Though protein content remained unaltered, seven out of ten essential amino acids, polyphenol content, and essential minerals like iron were found to increase in quinoa seeds along with the reduction in saponin content and seed fibers under salt stress. However, the findings were found to be plant genotype-dependent. Recent studies have evaluated the expressions of different genes related to salinity stress, of which the two important ones reported are (1) Salt Overly Sensitive 1 (SOS1), encoding a Na+/H+ antiport at the plasma membrane of the epidermal root cells helping in the extrusion of Na+ out of the cell, and (2) tonoplast-localized Na+/H+ exchanger 1 (NHX1), which helps in accumulation of Na+ inside the vacuole (19). Both genes were found to be upregulated in the leaves but not in roots. In addition to ABA and proline, polyamine synthesis genes, ABA receptor, and transportation genes were also found to be expressed in quinoa under salt stress. A total of 1413 genes were reported to be differentially expressed in response to salt stress in quinoa, of which 15 new candidate genes were reported between the varieties (Ruiz et al. 2016; Zou et al. 2017).

9.2.2.3 Temperature

The various genotypes can withstand a broad range of temperatures from −8 to 35 °C, along with relative humidity values ranging between 40 and 88% depending on the phenological stages (Jacobsen et al. 2005). However, still, a reduction in yield is observed under high temperature conditions during flowering and seed germination stages, thereby forming an intensive blockade to the worldwide expansion of the cultivation. The varieties from the dry cold regions have been found to have higher temperature sensitivity than those from hot and humid climatic zones. Withstanding the frost temperatures has been found to vary according to the plant genotypes and developmental stages, with the flowering stage being the most sensitive to the frost temperatures. The resistance to the formation of ice by maintaining a high content of soluble sugar and the amino acid, proline, is the response mechanism to the frost temperatures. The variation in the activities of the enzymes like invertase, sucrose synthase, and sucrose-6-phosphate synthase in cotyledons and embryonic axes during the development of the seeds along with the sucrose-starch partitioning in the cotyledons is markedly observed under frost temperatures (Rosa et al. 2009).

The seed germination is directly correlated to the temperature with the optimum temperature of germination being 30–35 °C and maximum germination temperature being at 50 °C, while the base temperature is 3 °C. However, the germination temperature has been found to vary for different genotypes and indicates that quinoa can withstand an extended range of temperatures, though above 35 °C, the plant is found to produce inflorescence without seeds. A night temperature between 20 and 22 °C (~4 °C higher than ambient night air temperature) was reported to decrease (1) seed yield by 23–31%, (2) biomass, and (3) seed numbers, keeping the protein content of the seed and the harvest index unaffected (Lesjak and Calderini 2017). The stomatal conductance, photosynthetic rates, chlorophyll fluorescence, and content index and anions and cations from the xylem sap were found to increase at high

temperatures (Hinojosa et al. 2018). Contrastingly, an increase in temperature decreased the viability of the pollens without affecting the seed sets or pollen structure (Hinojosa et al. 2019). These changes could be attributed to the differential expression of heat shock transcription factors (CqHsfs), and among 23 such CqHsfs, 4 were found to be upregulated with CqHsfs-3, CqHsfs-4, CqHsfs-9, and CqHsfs-10 having the highest expressions at 6 h and 12 h, respectively (Tashi et al. 2018).

9.2.2.4 UV Radiation

Experiments on the effects of UV radiation, particularly UV-B, on plants have been extensively performed at high altitudes of South America where the exposure to UV is the highest. Variations in responses were observed based on the genotype with an upsurge in flavonoid content of the leaves and a decrease in the height of the plants and the size of the leaves (Palenque et al. 1997). However, dramatic effects of UV radiation have been observed on plant morphology (plant height, stem diameter, leaf number, and specific leaf area), thylakoid organization, pigment synthesis, and metabolic and ultrastructural characteristics of seedlings (Bhargava et al. 2008). The amounts of UV-B-absorbing compounds including total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, protective pigments, epidermal lignin, and soluble sugars (glucose, fructose, and sucrose) were found to increase in all the quinoa genotypes. However, the distribution pattern varied between the leaves and the cotyledons, indicating the plasticity of the metabolic processes in the quinoa plants. All these data indicate that the quinoa plants have their adaptation mechanisms toward the different dosage of UV radiation (Hinojosa et al. 2018).

9.2.2.5 Heavy Metal

A total number of 17 quinoa accessions were found to accrue a high quantity of most of the heavy metals, including cadmium, chromium, nickel, and zinc. However, the amount of absorbed metals varied between genotypes. Though molecular mechanisms of accumulation of heavy metals in quinoa plants have not been elucidated, the effects on oxidative stress with the generation of high hydrogen peroxide and proline contents along with the enhanced tyrosine aminotransferase activity have been observed in these varieties (Bhargava et al. 2008).

9.3 Biotic Stress with Abiotic Stress

In nature, numerous times, plants were exposed to multiple stresses at a time rather than single stress as we study in the laboratory. During exposure to various stresses together, the ability of plants to identify and respond to combined stresses is essential when the individual stresses could generate a negative effect on the growth and reproduction of plants (Suzuki et al. 2014). Variations in climatic conditions also increase the host range of pathogens with higher chances of development of virulent strains (Garrett et al. 2006; Ramegowda and Senthil-Kumar 2015). The abiotic stresses, if present concurrently, decrease/increase the susceptibility of the host toward the pathogens through modulation of signaling molecules (Atkinson and

Urwin 2012). Under single stress, significant biomass reduction was observed that got further elevated in the presence of combined stresses.

9.3.1 Negative Interaction

Temperature plays as a crucial additive abiotic stress to decide the severity of the pathogen attack. Increased temperature suppresses the resistivity in tobacco and pepper to tobacco mosaic virus (TMV) and tomato spotted wilt virus (TSWV), respectively (Kiraly et al. 2007; Zhu et al. 2010; Moury et al. 1998). Sharma et al. (2007) reported the increased infection of spot blotch (caused by *Cochliobolus sativus*) in wheat with increased night temperature (Sharma et al. 2007). High temperature also suppresses the basal and R gene-mediated resistance in *Arabidopsis* and tobacco against *Pseudomonas syringae*. It was also observed that induction of R gene against potato virus and TMV also gets delayed in the presence of high temperature. Similarly, cold temperature (stress) was found to inhibit gene silencing and potent plant defense against viral pathogens by altering siRNA synthesis (Szittya et al. 2003).

9.3.2 Positive Interaction

However, a combination of stresses not always impacts negatively to plant. In spring wheat (*Triticum aestivum*), high temperature stress enhanced resistance to stripe rust (caused by *Puccinia striiformis*) (Carter et al. 2009). Similarly, salinity stress in barley has been shown to increase resistance toward powdery mildew (caused by *Blumeria graminis*) although resistance depends on the strength of salinity (Wiese et al. 2004). Increased salinity restricts the growth of pathogens through developing ion and osmotic toxicity. In rice, nematode infection reduces the effect of drought stress (Atkinson and Urwin 2012).

9.4 The Mechanism in Plants to Combat Biotic Stress

In responding to the attack of various pathogens and pests, plants have evolved an intricate and complex defense system. Broadly, plant defense has been divided into innate and induced immunity (Kiraly et al. 2007). An innate defense in the plant is non-specific to the pathogen (general resistance). Innate immunity in plants includes morphological and structural barriers such as cuticles, wax layer, cell walls, cell membrane, trichomes, thorns, etc. and chemical compounds such as phenolic and nitrogenous compound, glucosinolates, steroids, saponins, terpenoids, secondary metabolites, and proteolytic enzymes (Freeman and Beattie 2008; Reina-Pinto and Yephremov 2009; Bednarek 2012). These components of innate immunity provide resistance to pathogens by protecting the plant from their attack and invasion.

9.4.1 Induced Immunity

In plants, induced immunity is a two-layered defense response. It is classified as effector-triggered immunity (ETI) and microbial/pathogen-associated molecular pattern-triggered immunity (MTI/PTI). The plasma membrane-bound pattern recognition receptors (PRRs) recognize microbe associated molecular patterns (MAMPs) and initiate the microbial/pathogen-associated molecular pattern-triggered immunity (MTI/PTI) (Dodds and Rathjen 2010; Beck et al. 2012; Muthamilarasan and Prasad 2013). MAMPs are conserved characteristic molecules of pathogens. MAMPs include an array of different molecules such as ergosterol, cold shock protein, oligogalacturonides, Pep-13, bacterial flagellin, xylanase, lipopolysaccharides, etc. (Galletti et al. 2008; Granado et al. 1995; Brunner et al. 2002; Beliën et al. 2006; Dow et al. 2000; Muthamilarasan and Prasad 2013). Plant pattern-recognition receptors (PRRs) belong to either receptor-like proteins (RLPs) or the receptor-like kinase (RLK) families. While RLK receptors have an extracellular domain, a transmembrane domain, and a cytosolic kinase domain, RLPs lack the cytosolic kinase domain (Nürnberger and Kemmerling 2009). Activated PRRs induce defense responses by activating downstream signaling cascade in the cytosol (Hammond-Kosack and Jones 1996). During the infection process, pathogens synthesize different lytic enzymes to overcome the architectural barriers of a cell such as cell wall. As a result, different digested products such as cell wall fragments, cutin monomers, and peptides (Darvill and Albersheim 1984; Kauss et al. 1999; Boller 2005; Huffaker et al. 2006) are generated. These products represent the damage-associated molecular patterns (DAMPs). Similar to MAMPs, the DAMPs also stimulate innate immunity.

In consequence of plant's PTI-based combat strategy, adapted pathogens secrete effector proteins that manipulate plant's PTI defense system. Plants have evolved an intricate defense system that is targeted to the pathogen effectors and is referred to as effector-triggered immunity (ETI) in plants. The primary components of plant ETI involve a class of proteins belonging to nucleotide-binding leucine-rich repeat (NB-LRR) family. NB-LRR family proteins are encoded by plant "R" genes. In general, PTI and ETI defense responses exploit similar signaling cascades, but ETI yields a faster and quantitatively stronger response. ETI results in hypersensitive response that is marked by localized cell deaths. The hypersensitive response thus restricts the infection to its original site and prevents it from spreading to the adjacent tissues.

9.4.2 Phytohormone Regulated Induced Immunity

Phytohormones play a crucial role in regulating plant growth, development, and defense. The network of phytohormones is essential in both PTI- and ETI-based defense. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are the plant hormones that play significant roles in plant defense. For instance, *Arabidopsis* and tobacco plants become hypersusceptible to virulent pathogens, when bacterial *Nah*

G gene encoding a salicylate hydroxylase was overexpressed. Overexpression of salicylate hydroxylase resulted in reduced accumulation of salicylic acid (SA) and hence increased the susceptibility of the host plants toward pathogen infection (Halim et al. 2006). In *Arabidopsis*, NDR1 and EDS1, the two components of the "R" signaling cascades, act upstream of SA biosynthesis. NPR1 on the other hand regulates the downstream pathway relative to SA signaling. Similar function is mediated by WRKY45 in rice (Century et al. 1995; Shah et al. 1999; Inoue et al. 2013). SA also increases resistance in tobacco and potato against tobacco mosaic virus and potato virus (Vlot et al. 2009). SA has been found to establish systemic acquired resistance (SAR) through the induction of pathogenesis-related (PR) proteins. It also contributes to the HR-associated resistance through the production of reactive oxygen species (ROS), thereby resulting in cell death (Delaney et al. 1994; Mur et al. 1997, 2006; Shirasu et al. 1997).

JA has been associated with responses to both abiotic and biotic stresses (Wasternack and Hause 2002). Studies in *Arabidopsis* with altered jasmonic acid biosynthesis pathways have shown reduced resistance against soilborne necrotrophic pathogens such as *Botrytis cinerea* and *Alternaria brassicicola* (Halim et al. 2006; Penninckx et al. 1998; Thomma et al. 1998). A burst of JA synthesis/accumulation has also been found near insect wounds in plants (Halim et al. 2006; Bari and Jones 2009). JA is a key signal in the SA-independent induced systemic resistance elicited by rhizosphere biocontrol bacteria (Pieterse et al. 1996; Van Loon et al. 1998; Mur et al. 2006). Volatile jasmonates also exhibit a role in defense against herbivorous adult insects and larvae by attracting predators and parasitoids (Birkett et al. 2000). According to a recent study by Xu and Brosché (2014), an *Arabidopsis* mutant requires JA and ethylene signaling for inducing SA-mediated resistance. Similar observations have been noticed by other groups working in *Arabidopsis* defense against stress (Tuominen et al. 2004; Zander et al. 2014). Along with these hormones, abscisic acid (ABA), gibberellic acid (GA), auxin, and peptide hormones also play a role in defense mechanisms in plants (Bari and Jones 2009).

In quinoa, however, orthologs of *Arabidopsis* genes (AtCAT2 and AtEP3) involved in the salicylic acid defense response pathway show no change in expression. Nevertheless, quinoa orthologs of the *Arabidopsis* genes (AtWRKY33 and AtHSP90) were found to be significantly upregulated in plants infected with *Peronospora variabilis*. This observation indicates the contribution of the jasmonic acid pathway in the defense against *P. variabilis* (Rollano-Peñaloza et al. 2019). Poque et al. (2018) identified a stable interaction between a putative *C. quinoa* CA gene (CqCA1) and the viral protein of zucchini yellow mosaic virus (ZYMV) through yeast two-hybrid assay. This CqCA1 protein shows high similarity with the SABP AtCA1 of *Arabidopsis*, demonstrating a plausible role of this protein in viral defense through modulation of SA-mediated defense response (Poque et al. 2018).

9.4.3 Metal Ion-Based Induced Defense

Insect attacks in plants elicit an influx of Ca^{2+} ions into the cytosol. This Ca^{2+} ion influx activates calcium-binding proteins such as calmodulin. The calmodulins in turn activate other Ca^{2+}-dependent protein kinases. Ca^{2+} ions also activate mitogen-activated protein kinase (MAPK)-based pathway, some of which are involved in inducing plant defense against environmental stresses. Overexpression of the *Arabidopsis* IQD1 gene (calmodulin-binding transcriptional regulator) leads to induction of resistance in plants against herbivore kinase activity. Pathogenic attacks activate superoxide-generating NOXs (Lamb and Dixon 1997; Bolwell and Wojtaszek 1997). However, high K^+ concentration in crops limits the occurrence of the disease (Prabhu et al. 2007).

9.5 Microbial-Assisted Remediation in Quinoa and *Amaranthus*

Studies have isolated various microorganisms from quinoa from the stressful environments (arid/semiarid or high saline regions), and their effects have been studied on the growth of quinoa. In a study by Cao et al., an actinomycete strain, identified as *Streptomyces pactum* Act12, isolated from the low-temperature, high-altitude, arid environment of Qinghai-Tibet Plateau, able to promote the growth of cucumber, ginseng, and cotton, was inoculated with *Amaranthus hypochondriacus* L. in Cd-treated soil (Cao et al. 2016). However, plant height, root length, and biomass of the plants were found to increase, and it was attributed to the detoxification and resistance to Cd in the plants on the one hand and synthesis of plant growth-promoting molecules by the bacteria. The oxidative stress produced by Cd in the plants was improved in the presence of Act12 through the enhancement of antioxidant activity and glutathione content together with the reduction of malondialdehyde (MDA, a product of plasma membrane damage due to lipid peroxidation) content in leaf tissue.

Pitzschke observed the abilities of relaxation of cell wall and generation of the elicitor responses (superoxide accumulation and cellulolytic and pectinolytic activities) by the endophytes isolated from the seeds in saline and drought conditions (Pitzschke 2016). The reports showed the ability of the endophytes to initiate cell expansion, movement across cell walls, generation of impairment-associated molecular patterns, and stimulation of MAPK in plants. This indicates that bacteria assist in the sprouting and develop a primed state upon the rehydration of the seeds instantly. Transfer into non-native crops appears both desirable and feasible. This can be well applied for the priming of seeds that are directly grown in coastal zones and have to withstand high salinity stress. The seed-borne endophytes contribute toward the phenotypic characteristics and the germination of seeds. These endophytes were identified as *Bacillus.* The persistence of *Bacillus* as quinoa seed endophytes is due to its ability to form spores under extreme conditions and thereby to remain inhabited inside the seeds for a considerable time. Also, the seeds create an

enriching propagating source for those bacteria, and a highly compatible association is formed between the bacteria and the host plant. The bacterial superoxide, along with the plant growth-promoting substances, aids in overcoming the most subtle developmental stages of quinoa plants. This association also prevents pathogenic species and thereby increases plant survival and vitality (Pitzschke 2016).

Diverse bacterial taxa, including *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*, have been reported as seed endophytes like other crops (Truyens et al. 2015). These endophytes have displayed a wide range of phenotypic properties, including amylase production, high motility, and high capability to withstand osmotic pressure. The bacteria degrade the starch with the amylase and thereby can soften the seed coat and enter into the perisperm. It has been proved by the presence of numerous dead cells of *Bacillus amyloliquefaciens* (Lopez-Fernandez and Maldonado 2013). The high motility of the bacteria helps in their entry into the seeds before hardening, followed by the formation of spores to endure the osmotic pressure built up due to the high starch content and dehydration during maturation of seeds.

Additionally, these endophytes can also tolerate high concentrations of NaCl and $CdCl_2$, thereby helping the seeds to overcome the adverse stresses. This also demands to investigate quinoa-endophyte association for bioremediation (Pitzschke 2016). Interestingly, the quinoa endophytes form metabolically active participants that travel along with the plants, thereby creating variations in the microbe-associated molecular patterns (MAMPs) and activating diverse host receptors. MAPK signaling pathway initiated by this MAMP is also responsible for the maintenance of low counts of stomata in the quinoa plants during drought stress (Wang et al. 2007; Pitzschke 2015).

Potential roles of isolated root endophytic fungi in the development of tolerance toward abiotic stresses in plants from the desert ecosystems of chili were studied by González-Teuber (González-Teuber et al. 2017). This study identified *Ascomycota* as the only fungal group with *Penicillium*, *Phoma*, and *Fusarium* species as the predominant ones. Similar findings were also observed from the roots of the Andean and Bolivian-Andean desert quinoa varieties (Ortuño et al. 2014; Pitzschke 2016; Urcelay et al. 2011). The Andean varieties showed the presence of dark septate endophytes and the mycorrhizal fungi in definite ratios, indicating a functional relationship between these two symbionts (Urcelay et al. 2011). These findings suggested that fitness and adaptative strategies in the extreme arid systems are attributed to the presence of these endophytic fungi.

A study of the effects of inoculation of *Amaranthus cruentus* with the *Burkholderia* in the presence of elevated CO_2 showed an increase in plant biomass, which enhanced its ability to uptake cesium (Tang et al. 2011). This study demonstrated that the microbial inoculation in the presence of high CO_2 not only enhances plant growth but also augments the uptake and thereby aids in the removal of radionuclides from the soil paving the way for CO_2^- and microbe-assisted phytoextraction. Similar plant growth promotion and better uptake of cadmium were observed in the presence of *Rahnella* for *Amaranthus hypochondriacus* and *Amaranthus mangostanus*, strengthening the hypothesis of using the *Amaranthus*

rhizosphere isolated bacterial species for phytoextraction in heavy metal-contaminated soils (Yuan et al. 2014).

9.6 Conclusion

Quinoa by itself shows a significant tolerance level to various environmental stresses be it biotic or abiotic in nature. A part of this tolerance is attributed to the genetic makeup of the plant. For instance, the increased accumulation of abscisic acid in the xylem of roots and shoots of some of the varieties accounts for the enhanced tolerance of the plants to drought stress. Besides, genetic factors that regulate the accumulation of various other osmolytes to maintain the osmotic potential of plant under stress conditions have also been reported. However, in addition to these genetic factors, use of plant growth-promoting rhizobacteria (PGPR) to enhance tolerance to varied environmental stresses is being actively investigated in recent times. *Streptomyces* species in particular are actively present in the quinoa rhizosphere and exhibit both heavy metal remediation and plant growth-promoting activities. Besides PGPR, positive influence of endophytes belonging to *Bacillus* has also been evidenced in rehydration of seeds under saline and drought conditions. Taken together, quinoa poses as an excellent choice of next-generation staple that has a potential to sustain the changing environmental condition yet maintaining the nutritional values. However, further extensive studies in relation to the stress tolerance mechanisms of the plant will open up avenues for transformation of the other crop varieties with such tolerance potentials.

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Physiology of Quinoa in Saline Conditions

10

Hugo Bosque and Juan Pablo Rodríguez

Abstract

Salinity is affecting many regions in the world, and common crop plants are not capable of growing under these conditions. First, to introduce new plants to other ecosystems, it is necessary to understand how they perform under salinity conditions. Some plants can grow under high salinity conditions as halophytes. Quinoa, an Andean native crop, is known as a facultative halophyte because can grow up to 18 d S m^{-1}, a high level of salinity, but can tolerate and perform without having a decrease in seed yield and biomass with salinity up to 6 d S m^{-1}.

Quinoa is compared with other plants due to its capacity to withstand saline conditions. Throughout this chapter, the physiological aspects under salinity conditions are depicted and how salinity can affect the absorption of macro- and microelements and high salinity can increase the availability of some elements such as Fe and decrease the availability of microelements. Content of Fe is important in seeds, and that is why quinoa is recommended for marginal, semiarid, and arid regions with soils affected by salinity to grow.

Another advantage of why Quinoa can tolerate salinity is due to particular cells found in the leaves. Roles and functions of epidermal bladder cells (EBCs) located in the leaves are important in quinoa for tolerance to salinity. The EBCs play an important function to quinoa and its environment to regulate tolerance to salinity and high temperature.

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At molecular and genetic level, it is discussed the recent genes discovered in quinoa genotypes that make up to quinoa ready to tolerate salinity. The main advantages of quinoa tolerating salinity are due to the huge quinoa gene pool with many genotypes, particularly "Salares" genotypes tolerant to salinity.

Keywords

Andean grain · Facultative halophyte · Biosaline solution · Sustainable saline plant solution · Saline tolerance

10.1 Introduction

Saline conditions, both soil and water, have always been a significant problem in crop production, because they directly influence the dynamics of water and nutrients in the plant organism, the physiological and metabolic behavior, and indirectly the physical and chemical and even biological properties of the soil, which results in the negative effect on the growth and development of plants in general.

As this situation significantly affects food production, a number of investigations have been generated in all around the world, particularly in those regions with soil salinity problems. Indeed, this aspect is not only limited to traditional, arid, semiarid, and coastal areas but also to those areas that, having adequate conditions in their soils, may suffer salinization phenomena, due to poor management of irrigation and fertilizers or to the use of water with high levels of salinity.

Due to the high economic and social costs of attempts for reclaiming saline soils, many investigations have focused on the use of plant species considered tolerant to salinity. These researches show that this strategy is the most appropriate from the economic point of view, also, with the understanding that global climate change will expend millions of hectares with this problem.

In this way, a race against time is faced, so that humanity has plant genetic resources specially adapted to the new production conditions, mainly salinity and droughts, which will take place in many parts of the world, where particularly glycophytes will no longer have opportunity to contribute to the provision of food.

Moreover, halophytes and facultative halophytes, with food production potential, have a particular interest for many countries and their scientists, giving global importance to this group of plants, which many of them are already considered the food of the future for the humankind.

In this way, the *Chenopodium quinoa* Wild, which is available, and because of its exceptional nutritional quality (Rodríguez et al. 2020), versatility to be adapted to extreme environments such as droughts, frosts, high temperatures, wide latitudinal and altitudinal distribution, have made to this crop, native from Los Andes, and it has captured worldwide interest in the last 20 years. In consequence, facing research its unique treatment in the world of science and technology implies a unique opportunity for those of us who are dedicated to research and academia.

This chapter deals with the plant physiology in saline conditions, understanding that this discipline is of particular interest, when we treat the behavior of an organism under stress conditions, mainly referring to salinity and its consequences on the general metabolism of this crop and others under these conditions.

10.1.1 Saline Conditions

A salt-affected soil is defined as one that has been adversely affected, to the extent that it is no longer suitable for the growth of most crops, by the presence or action of soluble salts. This group of soils includes both saline and sodic soils (Sparks 2003; Evangelou 1994). In general, saline soil is considered with a specific electrical conductance of saturated extract (EC_e) greater than 4 d S cm^{-1} at 25 °C and percentage of Na^+ ions less than 15 concerning the total exchange bases and the pH generally less than 8.5 (O'geen 2018; Ibañez 2008), which is equivalent to approximately 40 mM NaCl, and generates an osmotic potential of approximately 0.2 MPa (Razzaghi et al. 2011), while a saline-sodic soil has CE_e higher than 4 d S cm^{-1} at 25 °C, exchangeable sodium more significant than 15%, sodium index higher than 13, and pH less than 8.5 due to the high electrolyte concentration causing flocculation of soil particles.

Usually, the presence of salts in agricultural soils comes from different sources, like native, irrigation water, fertilizers, groundwater, and others. However, the primary source of salts in soil and waters is the ever-continuing geochemical weathering of rocks that form the upper strata of the earth's continental crust (Tanji 1990). When we refer to saline conditions, we indirectly refer to the reduction of the soil water potential, that is, the higher concentration of salts in the soil solution will be a factor of water deficit due to osmotic effect for plants. The effect on the physical, chemical, and biological properties of the soil is highly significant, due to the ionic composition that occurs when this situation is reached or when the process occurs naturally.

It is well known that saline soils generally have standard physical properties, but as it is mentioned by Warrence et al. (2003), the soil solution salinity can have a flocculating effect on soils, causing fine particles to bind together into aggregates. Elevated salt concentration in the soil solution will promote clay particle aggregation. The net result of this aggregation is that voids between the soil aggregates will be relatively more significant than in non-flocculated soil, the soil will remain more permeable, and the soil will be less likely to become or remain waterlogged upon wetting. The same authors remark that the relationship between soil salinity and its flocculating effects, and soil ESP (exchangeable sodium percentage) and its dispersive effects, dictates whether or not a soil will stay aggregated or become dispersed under various salinity and sodicity combinations.

The excess of sodium in the soil is harmful because it promotes the soil particle dispersion, which at the same time causes the aggregate destruction. This phenomenon causes the clays to close the soil pores and crusting problems, which means that when the soil is moisturized, the particles swell and block the water infiltration. As

mentioned by Evangelou (1994), many processes and conditions in the soil environment are highly dependent on colloid dispersion or flocculation. Such processes or conditions include erosion, water suspension of solids, soil structure, and hydraulic conductivity, among many others.

Related to the chemistry of salt-affected soils, Tanji (1990) mentions that the essential cations are Na^+, $Ca2^+$, $Mg2^+$, and, to a lesser extent, K^+. The significant anions are Cl^-, SO_4^{2-}, HCO_3^-, NO_3^-, and, at high pH, CO_3^{2-}. These interactions occur rapidly enough that we can neglect kinetics and assume chemical equilibrium conditions to prevail in soil chemistry. In some instances, K^+ and NO_3^- may contribute to salinity, and when the pH is greater than 9, CO_3^{2-} becomes an important anion (Dudley 1994).

As Orsag (2020) summarizes, each of these salts has specific characteristics in their properties when they are present in saline soils. The most soluble salt is NaCl, and it is the most frequent in saline soils by this property. It affects the solubility of other salts by the effect of a common ion. The $MgCl_2$ also has high solubility and is very hygroscopic and can absorb water from the air that dissolves the crystals of these salts. The KCl is a salt with properties analogous to those of NaCl, and it is scarce in saline soils because K^+ can form internal sphere complexes on the surface of some clays, where it is retained. The Na_2SO_4 is frequent in saline soils and presents notable characteristics, its molecular composition varies with humidity, and its solubility varies significantly with temperature, which affects precipitates from other salts. The $MgSO_4$ is a frequent constituent and highly soluble and never accumulates in soils in pure form but in combination with other soluble salts. The presence of Na_2CO_3 and $NaHCO_3$ indicates special physicochemical conditions, linked to alkalinization processes, which leads to soils with very high pH.

On the other hand, $CaCO_3$ is a very poorly soluble salt, and they form firmly cemented, waterproof, and impenetrable soil horizons, while $MgCO_3$ is much more soluble than $CaCO_3$; however, accumulations of this salt rarely occur in soils, due to the adsorption of Mg by clays. In most cases, $CaCO_3$ and $MgCO_3$ are present together. $CaSO_4.2H_2O$ has very low solubility, and nitrates ($NaNO_3$ and KNO_3) are highly soluble salts. However, they do not accumulate in large amounts in soils, where they rarely exceed 0.05%.

Regarding the biological properties of the soils, it should be noted that every organism has specific requirements on temperature, moisture, pH, aeration, and organic matter content as an energy source for its proliferation, among the other factors. On the other hand, it is well known that the microorganisms play a significant role in the dynamics of nutrients and other aspects to give natural fertility to soils. In this way, salinity precisely does not provide these appropriate conditions, so the effects will be more negative as the salt concentrations increase steadily.

In saline conditions, the loss of biological activity of soils is associated with the decline of food supplies for soil microflora necessary for ecosystem functioning (Pankova et al. 2018). High concentrations of soluble salts affect microbes via two primary mechanisms: osmotic effect and specific ion effects. Soluble salts increase the osmotic potential (more negative) of the soil water, drawing water out of cells which may kill microbes and roots through plasmolysis (Yan et al. 2015). Then

come biological degradations in saline conditions, mainly because of the reduction of the contents of organic matter and humus and the population of its living organisms. The reason for organic matter reduction is because of the increase in soil pH above 8.5 which promotes the increase in the solubility of organic matter and consequently its washing (Orsag 2010).

10.1.2 Halophytes and Facultative Halophytes

Through the scientific literature, when referring to quinoa, it was indiscriminately referred to as a halophyte (Cai and Gao 2020; Eisa Sayed et al. 2017; Panuccio et al. 2014; Hirich et al. 2014; Koyro and Eisa 2008) or facultative halophyte (Causin et al. 2020; Pitzschke 2016). However, it should be clarified that it is a facultative halophyte, mainly in its place of origin, the Andean region of South America, since there are quinoa crops in saline and non-saline soils. However, some ecotypes have better performance in saline conditions and vice versa.

The halophytes correspond to a group of plants that occupy a famous line within the plant taxonomy. Rozema (1995) refers that the halophytes are plant species with a set of ecological and physiological characteristics allowing growth and reproduction in a saline environment. The occurrence of halophytes on saline soils has often led to the assumption that these halophytic plant species have a (physiological) requirement for salt, sodium, and chloride. In niches of halophytic species, high levels of salt in soil and water often play an essential role. For halophytes, functional traits, those plant attributes that significantly influence establishment and survival, include any mechanisms that contribute to their tolerance of high soil or water salinity as well as other abiotic stresses of their habitats, such as drought or flooding (Flowers and Muscolo 2015).

The definition of halophytes has had significant historical development, and they are manifold. In this regard, Grigore et al. (2014) recount the evolution of this definition since 1892, referring to Grazier who indicates that a halophyte is a plant containing a large quantity of common salt in its composition and which thrives best in salty places, until 2009, referring to Quinn, who says that the halophytes are plants that are tolerant of excess salt.

Nikalje et al. (2019) say that halophytes grow in saline soils and are suitable for saline agriculture. Most of the halophyte plants respond similarly to biochemical and physicochemical stress by salinity. Halophytes are more prepared to tolerate salinity than glycophytes. Halophytes could have stress memory to salinity when pretreatment/priming is used in seeds.

Halophytes can survive under harsh conditions because of their developed adaptation mechanisms, not only toward salinity but also to other abiotic stresses. These tolerance mechanisms help plants to tolerate a wide range of abiotic stresses. Tolerance mechanism involved in abiotic stress includes ion toxicity, water stress, oxidative burst, osmotic adjustment, and homeostasis. Interaction between salinity and other abiotic stresses leads to physiological and biochemical amplification of

specific traits, which are usually associated with salinity, such as proline accumulation and antioxidant capability (Nikalje et al. 2019).

While we still have much to learn about these salt-tolerant plants, more than one mechanism operates to generate tolerance—hence the difficulties in engineering tolerance in more salt-sensitive species. Consequently, it is crucial to understand tolerance mechanisms operating at various levels, molecular, physiological, and ecological, to develop an understanding of what is involved in being a halophyte. The long-term aim of such research is to be able to utilize knowledge of halophytes for improvement of the performance of crops in salt-affected soils. However, halophytes are not only valuable as scientific models but also have potential as crops in saline agriculture (Flowers and Muscolo 2015).

10.2 Water Relations in Saline Conditions

Since the last century, dynamics and nature of water-soil and the plant-atmosphere have received much attention, mainly for the development of several concepts to explain the movement of water throughout this complex system. As Kramer and Boyer (1995) summarize, in the twentieth century, emphasis has been placed on osmosis, water potential, water balance, relative water content, plant water potential, and soil-plant-atmosphere continuum, and then research emphasis has had shifted from the whole plant to the cellular and molecular level.

Under saline conditions, the primary importance is related to the influence of electrical conductivity (EC_e) of the soil, as a consequence of the concentration of salts, on the behavior of plants in the uptake of water and specific ions, which will have in its physiological and biochemical effects. In this way, quinoa has been a plant widely investigated to test these effects.

Aly et al. (2018) have found that this plant was able to complete its life cycle and produced economic yield at the salinity of 6.3 d S m^{-1} (EC_e) with 100% of field capacity, while the use of saline water of 38.1 d S m^{-1} and higher caused the quinoa plant's permanent wilting. Nguyen (2016) also confirmed that at a high salt concentration as much as 8 d S m^{-1} NaCl, most of the studied quinoa still produced acceptable yield. Ebrahim et al. (2018) reported that their results showed that the tested cultivars were successfully grown and produced seed yield under high salinity in soil equal to 44% of that present in seawater level. Peterson and Murphy (2015) mentioned that quinoa cultivars decline 73.7% in growth at 32 d S m^{-1} compared with the no-salt control. Eisa Sayed et al. (2017) say that these plants can be grown and yielded successfully in salt-affected soils (17.9 d S m^{-1}), where most if not all of the traditional crops cannot grow, although the yield was reduced. Derbali et al. (2020) mentioned that the tested varieties were able to survive under high salt level (500 mM NaCl) more than seawater salinity (with electrical conductivity of 35 d S m^{-1} as 400 mM NaCl).

In the same way, Yan et al. (2020) have found that at less than 200 mM NaCl, the electrical conductivity reached 18.6 d S m^{-1}, and the water potential of the treatment solutions ranged from −0.22 to −0.89 MPa. Talebnejad and Sepaskhah (2016)

reported that the mean value of water potential decrease from −1.53 to −3.09 MPa by increasing water salinity from 10 to 40 d S m^{-1}. With these results, we can conclude that quinoa can still give some yield at high salinity levels and can survive at maximum salinity levels as 35 and 40 d S m^{-1}.

This behavior is related to the water uptake rate. In this sense, Hirich et al. (2014) showed that the increasing levels of irrigation water salinity significantly depressed the water uptake, in which, in terms of total water uptake, there was 25% reduction by using saline water with 10 d S m^{-1} and 52% when irrigating with 30 d S m^{-1} compared to the irrigation with freshwater. Razzaghi et al. (2011) showed that the continuous addition of the salt solutions decreased the soil water osmotic potential and then inhibited plant water uptake, causing the reduction of the total soil water potential. It means that increasing salinity stress and decreasing water availability for plants will have direct effects on physiological behavior. To test water relations in plants and plant water status have been used mainly the leaf water potential, stomatal conductance (or resistance), and transpiration rate, and some findings in these topics are presented in the following paragraphs.

Cocozza et al. (2012) reported that the plant water relations were relatively more sensitive to salt stress concerning water limitation, showing lower values of potential water components. Stomatal conductance decreased as leaf water potential became more negative with a steep drop at leaf water potential between −0.8 and −1.2 MPa, and stomatal conductance decreased with diminishing turgor pressure, with a steep drop between 0.6 and 0.3 MPa. Razzaghi et al. (2011) mentioned that the variation in stomatal conductance in saline-stressed quinoa declined steadily, and Causin et al. (2020) found that in environments with moderate to high salinity, both the decrease in water potential and the accumulation of Na^+ and Cl^- ions can constitute stress factors.

In the same way, Bosque Sanchez et al. (2003) indicate that *Chenopodium quinoa* has amphistomatous leaves and anomocytic stomata with randomly orientated pore, and there is a general tendency in saline-stressed plants to have higher stomatal resistances because they experience a reduction of transpiration rate, which helps the plants to survive the water deficit. Morales (2009) reported that the stomatal conductance measurement of salt-treated plants was also significantly lower than their corresponding controls. Related to these parameters, Saleem et al. (2017) showed that the transpiration rates were found to be decreased in plants with increasing salinity up to 200 mM salinity level. These results suggest high plasticity of quinoa for tolerance to increasing salinity stress, which means increasing soil water deficit. Saleem et al. (2017) also reported that transpiration rates were found to be decreased in two ecotypes of quinoa plants with increasing salinity, up to 200 mM salinity level. Killi and Haworth (2017) also found that supplied saline water (300 mmol NaCl equivalent to 60% of the salinity of seawater) induced reductions in stomatal and mesophyll conductance. Talebnejad and Sepaskhah (2016) concluded that a decrease in transpiration rates accompanied salt-induced photosynthesis inhibition, but also with improved intrinsic water use efficiency. Derbali et al. (2020) mentioned that the maintenance of shoot and root hydration under moderate salinity was undoubtedly due to the control of transpiration rate which remained almost constant,

even at high salinity (300 and 500 mM NaCl). These results allow us to conclude that quinoa, with salinity levels as high as 500 mM (50 d S m^{-1}), reduces its transpiration rate but still maintains its metabolic functions.

The stomatal resistance and water potential on quinoa plants are related to some other features. Bosque Sanchez et al. (2003) found out that the salt is not active in the metabolism of the leaves and it is "compartmentalized" in the tissues and organelles. The most elaborated adaptation to salinity is the formation of salt-excreting structures or the salt glands (bladders) on both leaf surfaces and in the stem, inflorescences, and panicles. Ruiz et al. (2015), say that its essential influence in plant water retention and they may also be useful for reducing water loss and UV-induced damage to the photosynthetic apparatus by acting as a "secondary epidermis." Hinojosa et al. (2018) refer that the epidermal bladder cells are modified epidermal hairs and classified as trichomes, along with glandular hairs, thorns, and surface glands. These glands are shaped like gigantic balloons, with a diameter around 10 times bigger than epidermal cells, and can sequester 1000-fold more Na^+ compared with regular leaf cell vacuoles. Talebnejad and Sepaskhah (2016) say that these structures are involved in compartmentalizing salt, thereby excluding it from the other leaf tissues from the underlying photosynthetically active mesophyll. Kiani-Pouya et al. (2019) emphasize on the presence of epidermal bladder cells (EBCs) in halophytes that allows a considerable amount of Na^+ being accumulated, away from the metabolically active mesophyll cells.

Further salinity stress significantly increases bladder density in all the quinoa plants, and at a maximum amount, it increased by more than 3.5-fold, complementing that bladder diameter remained unchanged under saline condition. Shabala et al. (2012) remark that the EBC density differs dramatically with leafage, being most dense in young juvenile leaves; hence, it can be envisaged that it may be attributed not only to the differential amounts of accumulated organic osmolytes but also to the difference in epidermal bladder cell densities. Kiani-Pouya et al. (2017) found the direct evidence for a role of epidermal bladder cells in salt tolerance in halophytes and attribute this to (1) a vital role of these cells as a salt dump for external sequestration of sodium, (2) improved K^+ retention in leaf mesophyll, and (3) the bladders as a storage space for several metabolites known to modulate plant ionic relations. Complementing these assumptions, Orsini et al. (2011) say that the density of epidermal bladder cells on the leaf surface remained unaffected up to 600 mM NaCl. Derbali et al. (2020) reported that the high ability to maintain shoot water content and biomass production under moderate salinity (100 mM NaCl) together with Na^+ accumulation offers quinoa a new character as "salt-includer halophyte."

Regarding the behavior of the stomata, Kiani-Pouya et al. (2019) mentioned that salinity stress significantly affected stomata characteristics, saying that a large genetic variability was found for the stomata density among accessions, ranging from 67 to 159% in relative terms. The relative length of stomata declined by 3–43% in salt-grown plants, which implies that quinoa plants manage to reduce stomatal gas exchange under saline condition by minimizing the size of the pores. Salt-tolerant plants had a negative correlation between salinity tolerance index and stomatal

length under saline condition. In this way, Orsini et al. (2011) mentioned that transpiration and stomatal conductance were decreased at the highest salinity levels tested, consistent with reduced stomatal density and size. Talebnejad and Sepaskhah (2016) indicated that salt-induced closure of stomata resulted in photosynthesis rate reduction and, consequently, reduced dry matter accumulation, concluding that stomatal closure in quinoa occurred when the leaf water potential fell below about −1.0 MPa. Increasing water salinity from 30 to 40 d S m^{-1} resulted in a significant decrease in transpiration rate.

Waqas et al. (2017) reported that salinity stress induced a significant reduction in leaf stomata characteristics on adaxial and abaxial leaf surfaces of quinoa plants. The exposure to salinity stresses markedly reduced stomata density by 33 and 35% on adaxial and abaxial surfaces of the leaf, respectively, relative to the untreated control. The stomata aperture also diminished when quinoa plants were exposed, and nonetheless, stomata aperture was reduced by 35.61 and 36.21% on both surfaces of the leaf under salinity stress. Morales (2009) mentioned that the stress response commonly observed in green plants decreases stomatal apertures to limit water loss. Becker et al. (2017) reported that plants increased their number of stomata in response to salt stress but reduced their size on both sides of the leaf, which gives to a hypothesis that this morphological plasticity improves the partition of water and CO_2 resulting in maintenance of photosynthesis in quinoa under adverse environmental conditions. Cocozza et al. (2012) said that the salt-irrigated plants showed a severe drop in leaf water potential, resulting in stomatal closure through interactive effects of soil water availability and salt excess to control the loss of turgor in leaves. Ruiz et al. (2015) said that the observed reduction in stomatal conductance in halophyte leaves is assumed to be essential for better water use efficiency. It may originate from both physiological, e.g., control over stomatal aperture, and morphological, e.g., stomatal density and size, adaptive responses to salinity. Decreased stomatal conductance under saline conditions is regulated by reversible and rapid regulation of the opening and closing of the stomatal pore via ion fluxes in and out of guard cells. Furthermore, Hinojosa et al. (2018) said that their study found a strong positive correlation between stomatal density and plant salinity tolerance and determine that the stomatal density and size could be a key mechanism for optimizing water use efficiency under saline conditions.

Another feature in these aspects is the osmotic adjustment, which is regarded as a critical adaptation of plants to salinity because it helps to maintain turgor and cell volume, and there is a wealth of evidence linking exclusion of salt from the leaf with salt tolerance (Volkmar et al. 1997). Related to this statement, Cocozza et al. (2012) mention that the ability of leaves to adjust osmotically and thereby decrease the value of turgor pressure at full turgor did not appear to be present in different trends between plants experiencing water deficits and salinity conditions, probably because the concurrent increase in tissue elasticity resulted in a larger symplast volume at full turgor. Muscolo et al. (2016) narrated that in most plants, especially halophytes, the solute content of cells at high salinity is higher than in non-saline conditions, mainly due to the accumulation of ions (e.g., Na^+ and Cl^-) and organic solutes, showing that the Cl^- concentration was more than enough to contribute to osmotic adjustment.

Complementarily, Hariadi et al. (2011) observed that quinoa possesses a very efficient system to adjust osmotically for abrupt increases in NaCl stress. Up to 95% of osmotic adjustment in old leaves and between 80 and 85% of osmotic adjustment in young leaves were achieved employing an accumulation of inorganic ions (Na^+, K^+, and Cl^-) at these NaCl levels. Also, Ruiz et al. (2015) reported that the accumulated ions, mainly Na^+, Cl^-, and K^+, are supposedly used for osmotic adjustment, thus facilitating water uptake and transport and presumably lowering the metabolic cost required for the production of organic osmolytes, and this appears to be the case also for quinoa. In the same way, Shabala et al. (2012) found that plant's ability to maintain positive growth under extreme saline condition was indicative of the fact that a full osmotic adjustment in the shoot tissues was achieved by the accumulation of Na^+, Cl^-, and K^+ in old leaves. Moreno et al. (2017) have found that the seed hydropriming and osmopriming caused significant improvements in germination velocity and uniformity with solutions of low water potential of *C. quinoa.*

Furthermore, Orsini et al. (2011) reported the importance of inorganic ions for osmotic adjustment, the plant's ability to maintain K^+ levels, and the involvement of putrescine efflux in maintaining ionic balance under high salinity conditions. Conversely, ion excretion and proline appear to play a minor role. This physiological mechanism is significant for quinoa crops, mainly in saline environments. Complementarily, related to other organic compounds, Delatorre-Herrera et al. (2019) found the osmotic stimulus increased the concentrations of proline, glycine betaine, sucrose, fructose, glucose, and trehalose two- to sevenfold compared to a low salinity conditions.

Regarding the relative water content, Cocozza et al. (2012) observed that the effects of salinity and drought resulted in strict dependencies between relative water content and potential water components, showing that regulating cellular water deficit and volume is a powerful mechanism for conserving cellular hydration under stress. Riaz et al. (2019) mention that the relative water content was significantly decreased at higher salinity levels. Complementarily, Parvez et al. (2020) reported that the relative water contents of leaves were negatively influenced by increasing levels of salinity (300 mM NaCl). Riaz et al. (2019) found that the relative water content was not decreased at lower salt level (100 mM) and was somewhat increased. Stefanov et al. (2020) complemented that large amounts of Na^+ limit the uptake of K^+ from the plants, which is a reason for the disruption of the stomatal regulation and results in irreversible changes in the transpiration flow and a loss of cellular water content. Higher accumulation of Cl^- in plants leads to a significant reduction in the growth and the efficient use of water in plants.

In the end, when we are talking about plant water relations, we focus on water use efficiency. Saleem et al. (2017) said that the intrinsic water use efficiency ($_i$WUE) significantly increased following salinity treatment, showing that their results revealed that $_i$WUE was increased to 58.45% and 37.85% at 20 and 10 d S m^{-1} NaCl treatments, respectively, as compared to non-saline condition. Related to this statement, Shabala et al. (2012) reported the changes in stomatal density as a mechanism contributing to the improved WUE. Saleem et al. (2017) showed that another interesting observation was the phenomenon of salinity-induced reduction in

stomatal density observed in quinoa leaves. It is in contrast to the consensus that, as the cell growth rate is reduced under saline conditions, cells become smaller in size, and this resulted in the more significant number of cells per surface area (i.e., increased cell density).

On the contrary, they believe that such changes may represent a fundamental mechanism by which plant may optimize the WUE under saline conditions. A substantial amount of water evaporated from the leaf surface may bypass stomata and occur through the cuticle. The decrease in stomata density may be a direct result of leaf succulence and an increase in the size of pavement cells. It could be suggested that, by doing this, plants not only improve WUE for the reasons discussed above but also provide additional space for efficient Na^+ sequestration in the leaf epidermis.

As a conclusion for this topic, we refer to Iqbal et al. (2017) who observed that the large quinoa genetic variability in salinity tolerance opened new avenues to explore it further in different salt-affected field conditions. Also, Bonales-Alatorre et al. (2013a, b) from their results suggested that multiple mechanisms contribute toward genotypic differences in salinity tolerance. These include (1) a higher rate of Na^+ exclusion from leaf mesophyll; (2) maintenance of low cytosolic Na^+ levels; (3) better K^+ retention in the leaf mesophyll; (4) a high rate of H^+ pumping, which increases the ability of mesophyll cells to restore their membrane potential; and (5) the ability to reduce the activity of slow tonoplast and fast tonoplast channels under saline conditions. These mechanisms appear to be highly orchestrated, thus enabling the remarkable overall salinity tolerance of quinoa species.

10.3 Nutrient Dynamics on Saline Conditions

Whether in the soil or the plant, due to salinity, the dynamics of mineral nutrients are positively or negatively affected, what is called synergism and ionic antagonism between the different nutrients, since they are assimilated in ionic form from the soil, and also into the plant itself, there will be some interactions. Under this consideration, the most conspicuous in saline conditions is the accumulation of Na^+ and Cl^- in plants, above normal levels, and that indirectly affects the relative concentration of other nutrients. It should also be remembered that sodium is not an essential nutrient. However, most of the research carried out has focused on the dynamics of these two ions and the effect they have on the other nutrients and on the plants.

In regard to this consideration, Aly et al. (2018) observed that salinity induced a significant increase of Na^+ and Cl^- concentrations, while it reduced the Mg_2^+ and Ca_2^+ in stems, leaves, seed's coating, and seeds. Kiani-Pouya et al. (2019) have found that under saline condition showed higher K^+, indicating that the uptake of this ion was stimulated under this condition. Also, Koyro and Eisa (2008) found that there were high concentrations of the potential cations K, Ca, and Mg in the pericarp and additionally of the potential anions S and P in the seed interior (perisperm, cotyledons, and hypocotyl). Besides this apparent gradient between potentially toxic (Na and Cl) and primarily needed elements (K, Mg, Ca, P, and S) across the seed coat, there was also a visible change in the distribution of elements in the embryo.

The concentrations of all essential nutrients decreased NaCl-related in the hypocotyl, whereas they were stable (Mg and Ca) or significantly elevated (K, P, S) in the cotyledons. In this way, Cai and Gao (2020) indicated that leaf osmoregulation, K^+ retention, Na^+ exclusion, and ion homeostasis are the main physiological mechanisms conferring salinity tolerance. Complementarily, Eisa Sayed et al. (2017) observed that soil salinity led to a significant decrease of Ca^{++} and Zn^{++} contents in the seed. The higher ash content in seeds under saline conditions was due to the increase of Na^+, K^+, P_3^-, and Fe^{++} concentrations. Iron increases in quinoa seeds produced under high saline conditions given quinoa a distinctive value for human consumption. This assumption is complemented by Guarino et al. (2020) who also mentioned that the treatment with NaCl alone enhanced the Fe accumulation.

Talebnejad and Sepaskhah (2016) pointed out that increasing water salinity from the lowest value (10 d S m^{-1}) to the highest value (40 d S m^{-1}) resulted in about 4.6-fold increases in plant Na^+ concentration that is almost proportional to an increase in salinity level. At the highest salinity (40 d S m^{-1}), foliar injury symptoms were observed on quinoa leaves that occurred due to the toxic effect of excess Na^+ and Cl^- accumulation in plant. Related to C^{2+}, their results showed that plant Ca_2^+ concentration variation was in line with plant Na^+ concentration, saying that increasing water salinity from the lowest value (10 d S m^{-1}) to the highest value (40 d S m^{-1}) resulted in about 2.1-fold increases in plant Ca_2^+ concentration. Increasing water salinity resulted in a significant increase in plant Cl^- concentration, finalizing that the increasing water salinity resulted in a significant decrease in K^+/Na^+ ratio. In regard to these results, Hariadi et al. (2011) found a robust correlation between $NaCl^-$-induced K^+ and $NaCl^-$-induced H^+ fluxes that was observed in quinoa root, suggesting that rapid $NaCl^-$-induced activation of H^+-ATPase is needed to restore otherwise depolarized membrane potential and prevent further K^+ leak from the cytosol. Muscolo et al. (2016) have observed a significant accumulation of Na^+ and no Cl^- in shoots. Orsini et al. (2011) also have observed that the tissue contents of Na^+ and Cl^- increased dramatically with salt treatment but resulted in only a 50% increase in Na^+ from 150 to 750 mM NaCl. Internal K^+ was unaffected up to 450 mM NaCl but increased at the highest salinity levels tested. Sun et al. (2017) suggested that salinity tolerance in quinoa is achieved by a faster removal of Na^+ from the cytosol and a high K^+ concentration in roots and shoots under salinity, resulting in a high K^+/Na^+ ratio, and that a higher proton pump activity drives this mechanism. Parvez et al. (2020) found that the concentration of Na^+ in the shoot and root of quinoa increased with increasing levels of salinity. The lowest amount of K^+ was noted in plants that received an elevated amount of Na^+. Intriguingly, Na^+ and K^+ ions have a very similar ionic radius and hydration energy. Resultant concentration of Na^+ ions is increased, which decreases the concentration of essential nutrients such as K^+. Due to these essential physiological roles of K^+ in plant cells, the maintenance of the proper amount of K^+ under salt stress is very crucial.

Derbali et al. (2020) suggested that some varieties of quinoa possess a high capacity to limit the sodium accumulation in shoot via the control of Na^+ uptake from the medium. Generally, excessive accumulation of Na^+ leads to a nutritional

imbalance, usually associated with the restriction of nutrient (K^+, Mg^{2+}, and Ca^{2+}) uptake. It is also the same in the case of quinoa which showed a significant decrease in leaf K^+, Mg^{2+}, and Ca^{2+} under salt treatment. Their data also showed a high K^+ and Mg^{2+} efficiency under high salinity (300 and 500 mM NaCl). A decrease in K^+ content under high salinity can be related to its replacement by Na^+ for a function in osmoregulation. In addition to this statement, Derbali et al. (2020) have shown that low level of sodium toxicity was accompanied by high K^+ and Na^+ selectivity and high K^+ and Mg^{2+} efficiency under high salinity. Complementarily, Muscolo et al. (2016) stated that the significant amount of SO_4^{2-} and NH_4^+ could be the result of accelerated protein catabolism generally activated in high-stress conditions. The deleterious effects of the accumulation of sulfate in seedlings were sufficient to cause metabolic disorders such as chlorophyll decrease and consequent seedling growth depression. Ruffino et al. (2010) mentioned that salt stress considers exerting both osmotic and ionic effects. The content of Na^+ and Cl^- in cotyledons of *C. quinoa* significantly increased in the presence of 250 mM NaCl, whereas K^+ content showed a slighter decrease. As expected, the endogenous Na^+ concentration in cotyledons of *C. quinoa* was significantly higher in the presence of salt. By contrast, K+ concentration decreased slightly under salinity. They conclude that the results confirm the hypothesis that the high adaptability to soil salinity that was growing quinoa seedlings exhibit, is a consequence of better metabolic control than non-halophytic species, based on cotyledon's functionality, of ion absorption, osmolyte accumulation, and osmotic adjustment.

On the other hand, Panuccio et al. (2014) showed that in the presence of KCl and $CaCl_2$, the total ionic concentration gradually decreased with increasing concentrations of salts. Different salts caused a different distribution of cations and anions between the root and shoot. The highest accumulation of anions was observed with $CaCl_2$ and KCl but with a different trend. In $CaCl_2$, the anions increased in a concentration-dependent manner; in contrast, increasing KCl concentrations lowered the anion percentage. El Sebai et al. (2016) showed that all salinity levels resulted in a gradual reduction in N, P, and K percentage. Isobe et al. (2019) showed that increasing the exchangeable K content in soil by the application of KCl has contributed to the increased accumulation of K. Thus, the lower exchangeable K content in the soil led to lower plant K content. Kaya and Aydemir (2020) mentioned that the highest Ca and K nutritional element values were found, respectively, as 12.73 and 33.43 g kg^{-1} dry matter in Heloud cultivar. Peterson and Murphy (2015) say that at 32 d S m^{-1}, quinoa exhibited higher tolerance to Na_2SO_4 applications than to NaCl. Substantial variation was found in salinity tolerance among the quinoa cultivars. Wilson et al. (2002), in the examination of ion ratios, indicated that K^+/Na^+ ratio decreased with increasing salinity. A similar observation was also made for the Ca^{2+}/Na^+ ratios. In quinoa, leaf K^+ levels measured at 19 d S m^{-1} had decreased by only 7% compared with controls. Stem K^+ levels were not significantly affected. Adolf et al. (2012) say that in quinoa the key traits seem to be an efficient control of Na^+ sequestration in leaf vacuoles, xylem Na^+ loading, higher tolerance, better K^+ retention, and efficient control over stomatal development and aperture.

Related to the overall K^+/Na^+ ratio, Aly et al. (2018) say that this was also reduced in plant shoots with salinity stress. Although Na^+ increase was very high, the K^+/Na^+ ratio never fell below 1. P, Zn, Mn, and Cu increased in plant tissue even at high salinity. The seed coat limited the passage of possibly toxic Na^+ and Cl^- to the seed interior, as high Na^+ and Cl^- concentrations were found in the seed coat. Riaz et al. (2019) reported that the Na^+ concentrations in shoot and root were increased with an increase in salinity levels, whereas the K^+ concentrations of plants (shoot and root) decreased as a result of increasing NaCl, giving the ratios of K^+/Na^+ in plants decreased considerably with an increase in the salt stress. Saleem et al. (2017) stated that the highest salinity level is linked to salt stress, which might be due to more absorption of K^+ by the roots at increased Na^+ level. As the salinity level increased, the selective transport of K^+ decreased significantly from root to leaves.

Related to the carbon dynamics, Hussain et al. (2018) showed that salinity decreased the C concentration at both levels (10 and 20 d S m^{-1}) as compared to control. The Na concentration increased more (and was higher), and the C concentration decreased more (and was lower) as in any other part of the seed interior. They concluded that quinoa genotypes differ in foliar $\delta^{13}C$ and $\delta^{15}N$ isotope composition, which reflected complex interactions of salinity and plant carbon and nitrogen metabolisms; then this result can be interpreted as an evidence for the substitution of carbohydrates with NaCl. Koyro and Eisa (2008) say that the change in C concentration mirrored the one of oxygen independent of the salinity treatment.

As a summary of all the research results described in this section, we must point out that, when it is related to mineral nutrients as macronutrients N, P, and K, and secondary Ca, Mg, and S, and micronutrients Fe, Zn, Cu, B, Cl, Mn, and Mo. From this set, in saline conditions, we observed a significant accumulation of Na^+ and Cl^- ions, which will directly or indirectly influence the absorption of all these nutrients. If Na^+ or Cl^- is absorbed, composition of elements such as N, P, K, Ca, Mg, and S can decrease. There are stability or balanced concentration and excellent absorption of Na^+ that would favor the absorption of these elements because the Na^+ ion is excluded as a mechanism of tolerance to salinity. In any case, it is observed that the quinoa plant has the capacity for homeostasis, which makes this species an exceptional plant crop of adapting to extreme saline conditions and salinity itself. Something interesting about this analysis is that, at higher salinity, there is more significant absorption of Fe, a very positive aspect when it comes to the nutritional quality of quinoa, apart from its already known qualities regarding the content of proteins and antioxidants. Undoubtedly, more research will continue to be carried out in this subject, which in the long run will allow us to have a clearer picture of the dynamics of nutrients, mainly under stress conditions, not only saline but others, mainly droughts.

10.4 Photosynthesis in Saline Conditions

Everything we have seen so far will result in the metabolic process itself, that is, both photosynthesis and the respiratory process will be affected differently; it is understood that in general salinity is considered a stress condition. One of the essential factors for the photosynthetic process is water, and salinity causes a deficit of this essential element. On the other hand, the gas exchange in the leaves is considered necessary, which is sometimes affected because the plant, as a preventive way against salinity, causes stomata to close or at least increases the stomatal resistance, and also affects the chlorophyll content. In general, it will be observed that salinity will negatively affect cellular metabolism, which we briefly describe in some research results on quinoa in saline conditions. Stefanov et al. (2020) emphasized it must be borne in mind that salt-induced changes in photosynthesis are directly related to total plant yield, and it is essential to understand how this process is affected by high salt concentrations. Thus, we summarize some research results in saline stress effects in the photosynthetic process.

As is reported by Saleem et al. (2017), they found a severe decline in photosynthesis activity, and it was correlated with a significant reduction in stomatal conductance and high levels of Na^+ accumulation in leaf tissues, which also sharply decrease the photosynthetic capacity of plants. Hussain et al. (2018) have found that the ratio of intercellular to ambient CO_2 concentration was significantly less after treatment with 20 and 10 d S m^{-1} as compared to control, indicating the closing of stomata and inhibition of CO_2. Killi and Haworth (2017) found that quinoa may not be a suitable crop for areas subject to intense salt stress or irrigation with a concentration of saline water equivalent to a 300 mmol NaCl solution. Talebnejad and Sepaskhah (2016) found that increasing water salinity from 10 to 40 d S m^{-1} resulted in 12% and 45% decrease of photosynthetic rate, respectively. Zelm et al. (2020) say that there is also an ionic effect or at least a stomatal closure-independent effect of sodium on photosynthesis, and the Na^+ influences photosynthesis by disrupting the proton motive force and chloroplast function and by interfering with CO_2-fixing enzymes. Bonales-Alatorre et al. (2013a, b) concluded that the negative control of tonoplast channel activity in old leaves reduces Na^+ leak, thus enabling efficient sequestration of Na^+ to their vacuoles, which enables optimal photosynthetic performance, conferring salinity tolerance in quinoa species. Derbali et al. (2020) demonstrated that under high salt treatment (500 mM NaCl), the most resistant variety achieved a high stomatal conductance, leading thus to high levels of both intercellular CO_2 concentration and net photosynthesis. Killi and Haworth (2017) showed that salt stress-induced short-term diffusive but also longer-term metabolic limitations to CO_2 assimilation in quinoa. The deleterious effect of salinity was apparent in impaired Rubisco carboxylase activity, RuBP regeneration, and PSII performance.

Regarding the photosynthetic pigments, the chlorophylls (a and b) and carotenoids are the primary photosynthetic pigments because they directly play mainly in the photosynthetic process. Changes in their content will affect the photosynthetic rate directly. El Sebai et al. (2016) reported that quinoa plants

irrigated with saline water (4000 and 8000 mg L^{-1}) caused significant gradual decreases in chlorophyll a, chlorophyll b, carotenoid, and total pigment contents. In this way, Riaz et al. (2019) also found that at lower salinities, 100–200 mM NaCl, the chlorophyll content was higher compared with control treatment but decreased at 400 mM NaCl. Waqas et al. (2017) mentioned that salinity affects decreasing chlorophyll a and b and carotenoid content. Rangani et al. (2016) also reported that the reduction in chlorophyll contents at higher salinity level might be due to the degradation of chlorophyll structure. Parvez et al. (2020) observed that chlorophyll contents, chlorophyll a, chlorophyll b, and total chlorophyll decreased with increasing levels of salinity (300 mM NaCl). Ruffino et al. (2010) reported that the total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid concentrations on a dry weight basis were significantly lower in salt-treated samples than in control cotyledons. Under salinity, the content of chlorophyll b was reduced faster than in control cotyledons. Chlorophyll a/b ratio in salt-treated cotyledons showed a progressive increase until the end of the experiment, whereas in control cotyledons, the chlorophyll a/b ratio did not show significant variations during the experimental period. Qureshi and Worku (2020) reported that the chlorophyll content of five genotypes of quinoa tolerated salinity stress up to 10 d S m^{-1} but decreased significantly at higher salinity levels. The highest chlorophyll content was recorded at salinity levels of 0–5 d S m^{-1}. The results indicate a decreasing trend of chlorophyll content with increasing salinity stress. In the same way, Ruiz et al. (2016) found that their data support the inherent potential of the salares landrace R49 to tolerate salinity insofar as it was the only one of the three genotypes analyzed not to exhibit decreased photosynthetic pigment concentrations.

On the other hand, the advent of "stress meters" has allowed the rapid determination of some stress conditions on plants, the fluorescence quenching parameters, especially the ratio of variable to maximal fluorescence, Fv/Fm (Hovenden and Seppelt 1995). Bosque Sanchez et al. (2003) have shown that the salt-treated plants, in general, had slightly higher Fv/Fm ratios than the control. It indicated that salt-stressed plants have better protection to photoinhibition at the level of the reaction center of PSII. Killi and Haworth (2017) reported that analysis of the chlorophyll fluorescence transient suggests that electron transport was impaired throughout PSII in salt-stressed quinoa. Salt stress degraded and damaged the pigment-protein complexes of the thylakoid membrane, likely inducing oxidative stress. Manaa et al. (2019), say that a high resistance of quinoa photosynthetic machinery under moderate salinity as assessed by the high stability of PSI and PSII functions, and by maintenance of functional chloroplast ultrastructure. Indeed, the high PSII efficiency largely maintained under moderate salinity could be associated with the following features: (1) high PSII connectivity and donor side intactness (maintained the fluorescence at K and J step of the induction curve (Fk/Fj) ratio and energy transfer between PSII and antennae) and (2) maintenance of maximal photochemical efficiency (Fv/Fm) and whole primary photochemical reactions. However, under high salinity, both PSI and PSII activities were impaired differentially, and the swelling of thylakoids and disappearance of grana observed under 300 mM NaCl may cause the

marginal decrease of maximal fluorescence (Fm) and the decrease in the maximal photochemical efficiency (Fv/Fm), leading to the downregulation of PSII activity.

In this topic, given the scope of the chapter, many other aspects that determine the photosynthetic process have not been explored, that is, the structure and physiology of the photosynthetic apparatus, as well as other factors such as lipid metabolism, proteins, and other organic compounds such as sugars that play a fundamental role in this process. It has focused on the photosynthetic rate and the content of photosynthetic pigments, considering that these are the most significant and that they are directly affected by the salinity of soils and water. It is well known that the essential factors for photosynthesis to take place are the quantity and quality of light, the concentration of CO_2, the content of photosynthetic pigments, the ambient temperature, and even the natural state of the plant organism.

This clarification indicated that in conditions of saline stress, quinoa plants tend to close the stomata to minimize the rate of transpiration and reduce gas exchange, that is, absorption of CO_2, which affects negatively on the capture of light energy by the photosynthetic apparatus, which results in a negative effect on the entire photosynthetic process and therefore on the final yield of the crop. The reduction in the absorption of water by salinity is the determining factor, as this element is crucial for all metabolic processes at the cellular level. It is also worth mentioning that the behavior of the content of photosynthetic pigments is essential since, in most cases, at high salinity levels, there is a degradation of these molecules.

With this conclusion, the question is what happens with quinoa in saline conditions and why so many researchers around the world are looking for salinity tolerance in quinoa these last years? Taking this into account, Shabala et al. (2012) reported that a lack of any detrimental effects of salinity on chlorophyll content it is reasonable to suggest that all accumulated Na^+ was safely sequestered in vacuoles, regardless of the leaf position. These changes include preferential accumulation of Na^+ in old versus young leaves; better osmoprotection of young developing leaves against associated oxidative stress; a significant reduction in the number of stomata per leaf area, as well as a concomitant decrease in the number of pavement cells; and a reduction in measured stomatal conductance that was less pronounced in salt-tolerant varieties. Collectively, these traits contribute to the remarkable salinity tolerance of quinoa, a species that can complete its life cycle in NaCl concentrations equivalent to seawater.

It was concluded that the vast diversity of quinoa is an incredible pool to be able to have appropriate genetic material for extreme conditions of stress, in this case, the stress of salinity.

10.5 Abscisic Acid: The Plant Stress Hormone

Plant hormones play vital roles in the ability of plants to acclimatize to varying environments by mediating growth, development, and nutrient allocation. Hormones move through specific pathways to regulatory sites where they respond to stress at the deficient concentration (Fahad et al. 2014). In several plant species are observed

marked and often rapid changes at the hormonal levels in response to stresses, including osmotic and water stress, anaerobiosis, nutrients, and temperature extremes (Naqvi 1994).

In this feature of saline stress conditions, the role of abscisic acid (ABA) is well known; as Taiz and Zeiger (2002) mention, it inhibits growth and stomatal opening, mainly when the plant is under environmental stress. Hartung and Davies (1994) referring to this hormone mention that when abscisic acid is applied externally to plants, their water relations are improved. ABA reduces water loss and promotes water uptake into roots and helps plants to cope with a range of environmental stresses. Examples of such changes are the restricted growth of shoots, reduction in leaf surface area, stimulation of root extents, lateral root growth, and root hair development. Thus, under these remarks, there are research on ABA and its role in quinoa.

Askari-Khorasgani and Pessarakli (2020), when dealing with this hormone, in their recently published work entitled "Phytohormone Homeostasis and Crosstalk Effects in Response to Osmotic Stress, In Passarakli M. (Ed.) Handbook and Crop Stress," extensively explained the role of ABA, whose content was summarized as follows: ABA induces stomatal closure and, therein, activates guard cell anion channels in a calcium-dependent as well as calcium-independent manner. ABA triggers the release of anions and K^+ from guard cells. The decrease in guard cell osmotic pressure and volume results in stomatal closure, reducing the transpirational loss of water from the leaf. The role of ABA on stomatal closure in stress conditions could be originated either from its biosynthesis in the roots or directly from guard cells. Throughout, the ABA contents of the guard cell apoplast, but not the guard cell symplast, were convincingly correlated with stomatal aperture size, identifying an external locus for ABA perception under stress conditions. ABA accumulates in the guard cell apoplast by evaporation from the guard cell wall, so the ABA signal in the xylem is amplified maximally at high transpiration rates. Leaf ABA and ABA in the xylem stream entering the leaf account for changes in the stomatal conductance. On receiving a stress signal, ABA formation starts in vascular tissues and is released from the biosynthesis site into other cells through ATP-dependent transporters. The pH of the xylem sap and water relations can modify the leaf ABA signal.

Additionally, the limitations in stomatal opening imposed by high concentrations of ABA can rapidly and completely reverse by lowering the leaf temperature, and there are also precise interactions between the effect of ABA and CO_2 on stomata (apoplast of the guard cells), intercellular CO_2, and also evaporative demand. ABA, both endogenous synthesized and exogenous application, affect plant water status, water uptake, and growth in different ways such as hydraulic water conductivity both in roots and leaves, root system architecture, and aquaporin (AQP) activity. Despite contradictions, the central tendency is toward a positive effect of ABA on hydraulic conductivity, AQP activity, maintenance of primary root growth with less lateral roots, and plant recovery after rehydration, resulting in higher productivity. ABA can be removed from or released into the transpiration stream before reaching guard cells, depending on the membrane pH gradients around the ABA transport

pathway. Changes in transpiration rate can influence the local accumulation of ABA in guard cell walls, while the metabolism of ABA can prevent its buildup.

After having this explanation regarding ABA on stress conditions, we will outline some research results on quinoa on this topic. According to Razzaghi et al. (2011), the xylem ABA concentration in the shoot was two to nine times higher than in the root in saline conditions, while Cocozza et al. (2012) reported that in 2-year experiment, the ABA concentration in leaves was significantly higher in 2010 than in 2009, and marked differences have been observed between days of monitoring in both years. Ruiz et al. (2015) reported that the decreased stomatal conductance under saline conditions is regulated by a reversible and rapid regulation of the opening and closing of the stomatal pore via ion fluxes in and out of guard cells. The process in the stomata is under the control of abscisic acid (ABA), concluding that the first increases in ABA and decreased leaf and soil water potential are indicative of osmotic stress caused by salinity.

As salinity is synonymous with water deficit, we can compare with studies in drought stress, where Jacobsen et al. (2009) reported that root-originated ABA plays a role in stomata performance during soil drying. ABA regulation seems to be one of the mechanisms utilized by quinoa when facing drought inducing a decrease of turgor of stomata guard cells. Gamez et al. (2019) reported that the lower stomatal opening and transpiration rates were also associated with higher leaf ABA concentration values detected in Rainbow cultivar. They have found negative logarithmic relationships between stomatal conductance and leaf ABA concentration in two quinoa varieties (Rainbow and Illpa). These moderate-to-medium values suggest that, in addition to ABA signaling, other causes for stomatal closure under drought such as hydraulic regulation may play a role. Fahad et al. (2014) found that ABA acts as a mediator in plant responses to many stresses, including salt stress, and the functional analysis of cytokinin receptor mutants shows that cytokinin receptors of *Arabidopsis* act as negative regulators in ABA signaling and osmotic stress response.

10.6 Molecular and Cellular Response in Saline Conditions

Salinity in soil and water presents constraints when the resources of freshwater are reducing in the world. Many crop plants that we consume are glycophytes, which grow with freshwater. However, some plants can grow under saline irrigation known as halophytes (Volkmar et al. 1997). The reaction at the cellular and molecular level is different between them. Amaranthaceae (botanical family) has many plant species that can grow under saline conditions such as *Swaeda foliosa*, *Suaeda fruticosa*, *Salicornia europaea*, *Atriplex cordobensis*, *A. nummularia*, and the facultative *Chenopodium quinoa*. Recently, quinoa is a well-promoted Andean crop and introduced in many parts of the world due to its capacity to tolerate salinity in irrigation and soil content.

Salinity effect in plants affects principally at the cellular level; however, quinoa tolerates salinity due to specific cells located in the epidermis of leaves. These cells

are the epidermal bladder cells (EBCs) that help quinoa to balance salt between the environment and the plant.

Salinity is a significant factor that can affect strongly the productivity in crops (Shabala et al. 2016; Bohm et al. 2018). However, some plants can tolerate salinity from low to high concentration of salt. This type of salinity-tolerant plants can become an alternative to produce food, seed, and biomass. Several studies confirmed the potential of quinoa to grow from emergence stage to grain setting under saline conditions. *Arabidopsis* plant has been used as a model plant to understand salinity adaptation at the cellular and molecular level (Hasegawa et al. 2000; Yun 2005). Quinoa due to its high genetic variability can offer many genotypes to grow under saline conditions, low water requirement, seed size, and growth type. However, *Chenopodium quinoa* at the cellular and molecular level becomes an extraordinary Andean plant model to salinity tolerance. Quinoa is a facultative halophyte that can tolerate high levels of salinity up to 50 Mm (50 d S m^{-1}) (Adolf et al. 2012; Becker et al. 2017).

At the molecular level, epidermal bladder cells (EBCs) are external structures that are of interest of quinoa due to capacity to tolerate salt. Kiani-Pouya et al. (2017) gave direct evidence on the role of EBC to salt tolerance and salt dump for external sequestration of sodium in quinoa. Besides, quinoa becomes a good model plant to explain salt mechanisms at the cellular and molecular levels (Ruiz et al. 2017; Bohm et al. 2018). When salt enters and is accumulated in the vacuole, the Na^+ and Cl^- are required to cross the membranes and be transported to bladder cytoplasm (Bohm et al. 2018).

Bohm et al. (2018) demonstrated by RNA-seq analysis of leaf and bladder samples a small number (83) of genes showing responses to salt. It is suggested that bladder cells are responsible for salt sequestration. Quinoa has two genes expressed in the root CqHKT1.1 and the leaves CqHKT1.2. The bladder in quinoa leaves has a specific function to allow Na^+ inside salt dumpers. This gene in quinoa allows to supervise enough amount of Na^+ and to avoid overloading the cytoplasm (Bohm et al. 2018).

Quinoa bladders in the leaves contain CIC-type proteins as an ortholog of AtC1C-c, which has the function to accumulate chloride in EBC vacuoles (Bohm et al. 2018). At the cellular level, the osmolytes have a role in protecting the cytosolic metabolism from the toxic effect of NaCl. Proline an essential amino acid produced in the leaves has a role in supporting the leaves to tolerate salt stress conditions (Bohm et al. 2018). Bohm et al. (2018) demonstrated with their study that bladder in quinoa leaves functions as salt dumper which helps tolerate salt.

Morales et al. (2017) used a Chilean "Salares" quinoa ecotype. RNA-seq analysis of R49 genotype compared drought and control irrigation conditions. 104.8 million reads were obtained with 54 M reads for the control and 51 M reads for drought condition. Expression pattern for canonical drought responses such as ABA biosynthesis and other genes induced by qPCR was assessed, and it suggests the novelty of R49 drought responses. R49 genotype had the best performance on physiological parameters and the highest tolerance to drought.

Zou et al. (2017) generated a high-quality genome draft using an inbred line of the quinoa cultivar "Real." The genome was highly repetitive (64.5% repeat content) and contained 54,438 protein-coding genes and 192 microRNA genes, with more than 99.3% having orthologous genes from glycophylic species. Stress tolerance in quinoa is associated with genes involved in ion and nutrient transport, ABA homeostasis and signaling, and enhanced basal-level ABA responses.

Ruiz et al. (2019) used quinoa to clear up on salt tolerance mechanisms at the transcriptomic level. RNA-seq analysis of genotype R49 at an early vegetative stage compared high salinity (300 mM NaCl) and control (freshwater) conditions in a time-course pot experiment. A total of 2416 differentially expressed genes (DEGs) were identified based on the treatment and time of sampling. A total number of upregulated and downregulated genes were 945 for salt-treated and 1471 for control plant. Besides, the genes are involved in biological processes like oxidation-reduction, response to stress and response to ABA, and cell wall organization. These genes in this quinoa genotype have the role of "stress-anticipatory preparedness" to salinity tolerance.

Shi and Gu (2020) performed a reference-guided assembly and compared gene expression in quinoa roots treated with 300 mM NaCl for 0, 0.5, 2, and 24 h of two contrasting genotypes, salt-tolerant (ST) and salt-sensitive (SS), under salt stress. One hundred seventeen DEGs were common of both genotypes, identified as core salt-responsive genes, including some transcription factor members, like MYB, WRKY, and NAC, and some plant hormone signal transduction-related genes, like PYL, PP2C, and TIFY10A, that play a role in the adaptation to salt conditions. Twenty-one DEGs by quantitative real-time PCR (qRT-PCR) were detected and confirmed the reliability of the RNA-seq results—candidate genes involved in salt tolerance as DEGs in ST genotype.

Quinoa is a facultative halophyte plant and is considered halophyte at laboratory perspective for its tolerance to salinity and other metals in the soils. Guarino et al. (2020) demonstrated with real-time RT-qPCR analysis on gene transporters for sulfate, iron, and phosphate and phytochelatin, metallothionein, glutathione synthetase, dehydrin, Hsp70, and enzymes responsible for the biosynthesis of proline (P5CS), glycine betaine (BADH), tocopherols (TAT), and phenolic compounds (PAL). The analysis showed that genes were affected by Cr(III), Cr(III)+, and NaCl. Changes in sulfur and phosphorus allocation are related to quinoa and its ability to tolerate Cr through the activation of stress-protective molecules. With this study, Guarino et al. (2020) demonstrated that quinoa could be an essential alternative to grow in soils affected by chromium and moderate saline conditions.

10.7 Conclusions

In conclusion, there is a shred of clear evidence that quinoa has high adaptability to salinity. Halophyte plant species grow in extreme salinity conditions. Nevertheless, many of them do not have the same profile as the quinoa. *Chenopodium quinoa* is a facultative halophyte that can grow and produce an adequate seed yield at 6 d S m^{-1}

without the effect of salinity; however, salinity irrigation up to 18 d S m^{-1} decreases seed yield.

Chlorophyll content in leaves is affected when saline conditions are high and environmental temperature is warm. However, quinoa can withstand these conditions. Therefore, quinoa is recommended for cultivation in semi-arid to arid ecosystems, when precipitation is lower and soils are affected by salinity.

High salinity affects the absorption of macro- and microelements essential for the nutrition of the quinoa plant but can increase the mobilization of Fe, an important element, particularly in the seed.

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Mechanisms of Salinity Tolerance in Quinoa 11

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Abstract

This chapter summarizes the current knowledge of mechanisms of salinity tolerance in *Chenopodium quinoa*. Quinoa utilizes Na^+ as a cheap osmoticum for osmotic adjustment, actively taking up Na^+ but ensuring its efficient sequestration away from metabolically active compartments. Internal and external Na^+ sequestrations are among the tissue tolerance mechanisms employed by quinoa. The internal Na^+ sequestration is mediated by three complementary mechanisms: (1) active Na^+ loading into vacuole by NHX Na^+/H^+ antiporters; (2) active H^+ pumping by the tonoplast-based H^+-ATPases and H^+-PPases to provide sufficient energy for NHX operation; and (3) preventing Na^+ back-leak into the cytosol by efficient control of Na^+-permeable tonoplast channels. The external sequestration of Na^+ relies on Na^+ loading into epidermal bladder cells (EBCs), with

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CqHKT1.2 and CqSOS1 transporters playing a key role in this process. Quinoa also adapts to hypersaline soil conditions via efficient stomata patterning and operation, thus increasing its water use efficiency. At the root level, SOS1-mediated Na^+ loading into the xylem was suggested as a key mechanism operating in quinoa. SOS1 operates in the removal of Na^+ out of the root cells with higher *CqSOS1* transcript levels found in salt-tolerant accessions. Furthermore, to deal with K^+ leakage that resulted from saline conditions, quinoa roots possess strategies to efficiently regulate K^+ loss via voltage- and ROS-activated K^+-permeable channels.

Keywords

Osmotic adjustment · Tissue tolerance · Sodium sequestration · Potassium retention · Reactive oxygen species · Stomata · Xylem loading · Epidermal bladder cells

11.1 Introduction

In 2050, the human population will exceed ten billion prompting a need to increase food production by 60% (Lopez-Marques et al. 2020). This goal must be achieved under conditions of diminishing resource (arable land, nutrients, freshwater) availability and significant impact of abiotic stresses. One of these stresses is soil salinity. Currently, 4.04 billion people, or 52% of world population, live in salt-affected countries (Liu et al. 2020). It is broadly accepted that in the near future, marginal dry lands will have to be used for food production; these cannot be made productive without irrigation. The shortage of high-quality irrigation water, however, will further exacerbate problems caused by salinity. Salinity currently destroys 3 ha of arable land every minute, as between 2 and 6 tons of salt is added to 1 hectare of arable land with irrigation water (Liu et al. 2020). At the same time, three major staple crops (rice, wheat, and maize) that are responsible for over 50% of humans' calorie consumption are highly sensitive to salinity. In this context, quinoa is an ideal species to fill this niche.

Quinoa (*Chenopodium quinoa*) is a halophytic species from the Amaranthaceae plant family that ticks all the boxes as a long-term strategic solution to deal with this problem. Quinoa originates from the Andean region of South America, where the natural habitat (both soil and climatic conditions) is harsh. As a result, quinoa plants have developed a remarkable abiotic stress tolerance. This also includes an ability to perform well on soil-affected land. The optimal growth and yield of quinoa are observed at salinities with EC around 8–15 d S/m (Adolf et al. 2013; Hariadi et al. 2011), conditions that will cause over 50% reduction in wheat yield and kill most of the rice plants. Thus, it is ideally suited as an alternative cash crop to be grown on marginal saline lands. In addition, plant can be used as a "blueprint" for targeting key traits conferring its superior salinity stress tolerance, to be incorporated in major staple crops. This chapter summarizes major features behind superior ability to deal with salinity stress.

11.2 Osmotic Adjustment

The presence of significant amounts of NaCl in the rhizosphere creates osmotic and water stress in plants. Plants need to adjust their internal turgor pressure to the increased external osmolality to overcome osmotic stress imposed by this salinity stress and to continue expansion growth of root and shoot tissues. In this context, Na^+ level below the toxicity threshold can have a beneficial role in plants' osmotic adjustment. Osmotic adjustment in plants can be achieved by accumulating a diverse range of molecules in the cytoplasm which can be categorized into two major components as organic osmolytes and inorganic ions (Hariadi et al. 2011; Shabala and Mackay 2011).

The relative contribution of organic versus inorganic osmolytes in tolerance to environmental stresses in halophytes is still highly disputed in the literature (Hariadi et al. 2011). Most glycophytes (crop species) rely on de novo synthesis of organic osmolytes, and their levels are increased dramatically under salinity (Chen and Murata 2002). However, production of organic osmolytes comes with an extremely high carbon cost to the organism (Munns and Gilliham 2015). As a result, halophytic plants utilize instead inorganic ions such as Na^+ and Cl^- as cheap osmoticums (Flowers and Colmer 2008; Shabala 2013). The above strategy does not exclude the role of compatible solutes in plant adaptation to salinity; it is simply not confined to merely osmotic adjustment (Shabala et al. 2012). Multiple roles have been proposed as functions of these compounds that include their roles in maintaining membrane integrity, protecting the structure of proteins, operating as low-molecular-weight chaperones, protecting the photosynthetic machinery, and scavenging of reactive oxygen species (Glenn et al. 2010; Smirnoff and Cumbes 1989). Physiologically low concentrations of organic osmolytes have been also shown to be highly efficient regulators of both tonoplast (Pottosin et al. 2014) and plasma membrane (Cuin and Shabala 2005) ion channels, thus optimizing intracellular ion homeostasis under saline conditions. Therefore, the accumulation of organic and inorganic osmolytes in a given plant, e.g. halophytic species such as quinoa, depends on how plant balances the carbon cost of osmotic adjustment and the osmoprotective role of organic osmolytes (Shabala et al. 2012). This plant is not an exception and utilizes Na^+ as a cheap osmoticum for osmotic adjustment, actively taking up Na^+ but ensuring that this ion does not interfere with cell metabolism. The model calculations demonstrated that more than 95% of cell osmotic adjustment in old leaves and 80–85% of osmotic adjustment in young leaves were gained through accumulation of inorganic ions such as Na^+, K^+, and Cl^- in plants exposed to salinity stress ranging from 0 to 500 mM NaCl (Hariadi et al. 2011). This finding shows that osmotic adjustment in both young and old leaves in quinoa is almost achieved by inorganic osmolytes.

While the energetic benefit of inorganic osmolyte strategy for osmotic adjustment is obvious, it should also be noted that detrimental effects of high levels of cytosolic Na^+ are equally harmful for both halophytes and glycophytes (Flowers and Colmer 2008). For this reason, halophytic plants should have an efficient sequestration ability for Na^+ in the vacuoles of their leaves, to prevent negative consequences of

high Na^+ accumulation in the shoot. Additionally, to maintain the turgor pressure within the cell, this increased content of Na^+ in the vacuole must be accompanied by a concurrent increase in the cytosolic osmolality. Higher level of osmolality then is gained either through an increased content of cytosolic K^+ or by accumulating compatible solutes in this compartment (Shabala et al. 2012). Consistent with this notion, increased levels of soluble sugars, proline, and glycine betaine have been reported in salt-exposed quinoa plants (Ruffino et al. 2009).

Furthermore, it has also been shown that the contents of organic and inorganic osmolytes in quinoa depend on the age of plant as well as physiological competence of a specific tissue (Hariadi et al. 2011). Young leaves that have small vacuole as compared to old leaves (Kim 2006) have consistently higher concentration of K^+ while keeping low Na^+ levels at various salt concentrations (Hariadi et al. 2011). This results in much higher sap K^+/Na^+ ratio in young leaves which is in agreement with evidence of maintaining high K^+/Na^+ ratio for salt tolerance (Shabala and Cuin 2008). Moreover, much higher osmolality is required to drive the leaf expansion growth in young leaves (Shabala et al. 2012). It has long been argued that protecting young developing leaves from detrimental effects of Na^+ is a primary attribution of Na^+ sequestration at whole plant level (Munns 2002) which also appears to be the case for quinoa.

11.3 Tissue Tolerance Mechanisms

11.3.1 Internal Na^+ Sequestration

Vacuolar Na^+ sequestration is a physiological hallmark and a key determinant of the "tissue tolerance", e.g. a capacity of tissues to function while containing a high internal Na^+ and Cl^- concentration (Munns et al. 2016). Three major components contribute to this trait. These include (Bonales-Alatorre et al. 2013a; Shabala et al. 2020) (1) active Na^+ loading into vacuole by Na^+/H^+ antiporters (encoded by NHX gene); (2) providing sufficient energy for NHX operation by active H^+ pumping by tonoplast-based H^+-ATPases and H^+-PPases; and (3) preventing Na^+ back-leak into the cytosol by efficient control of Na^+-permeable tonoplast channels.

Tonoplast antiporters are constitutive in halophytes (Barkla et al. 1995; Glenn et al. 2010), whereas they must be activated by NaCl in salt-tolerant glycophytes (Garbarino and Dupont 1988), while in salt-sensitive plants, their expression levels are extremely low and not salt-inducible (Apse et al. 1999; Zhang and Blumwald 2001). NHX operation in halophytes is also regulated at posttranslational level, with many reports of stimulation of Na^+/H^+ exchanger activity in halophyte species including *Mesembryanthemum* (Barkla et al. 1995; Vera-Estrella et al. 2005), *Salicornia* (Parks et al. 2002), and *Atriplex* (Hamada et al. 2001). It can be envisaged, therefore, that a similar scenario may be applicable for quinoa. Also, overexpression of Na^+/H^+ antiporter from halophyte species was shown to confer salt tolerance in glycophytes (Li et al. 2007; Liu et al. 2017). Similarly, both the expression levels and hydrolytic activity of V-ATPase (Barkla et al. 2002; Debez

et al. 2006; Wang et al. 2007) are increased upon salinity exposure in halophytes. This is also a case for quinoa (Bose et al. 2015).

Efficient control of tonoplast Na^+ leak channels is equally important for vacuolar Na^+ sequestration but, until recently, received much less attention. Two types of Na^+-permeable channels, namely, slow (SV)- and fast (FV)-activating ion channels, are present at the tonoplast (Bonales-Alatorre et al. 2013b; Hedrich et al. 2018). SV channels are encoded by TPC1 gene and permeable to both mono- and divalent cations, while FV channels (of no known molecular identity) are permeable to monovalent cations only (Pottosin and Dobrovinskaya 2014). Both FV and SV channels are ubiquitous and abundant (several hundred active copies per vacuole) (Demidchik et al. 2018). The model calculations show that if each cell opened only one SV channel at a specific time, the back-leak fraction would range from 30 to 100% (Shabala et al. 2020). Thus, to avoid the futile movement of Na^+ into and out of the tonoplast, plants can afford to open only a very small percentage (about 0.1%) of all tonoplast channels. This implies highly efficient control of SV and FV channel gating. This is indeed a case for halophytes. It was shown that the plants are capable to reduce the number of open SV and FV channels by several folds, when grown under saline conditions (Bonales-Alatorre et al. 2013a, b). The specific details behind this phenomenon remain to be elucidated. SV channels are known to be efficiently blocked by low millimolar-range concentrations of choline in mesophyll vacuoles (Pottosin et al. 2014), and rapid salinity-induced increase in the levels of choline and its immediate precursor phosphocholine in leaf tissues has long been reported (Summers and Weretilnyk 1993). It was argued that this blockage is essential to rapidly sequester Na^+ in leaf vacuoles to achieve rapid osmotic adjustment and maintain shoot turgor (and, hence, growth) at initial stages of salt stress (Pottosin et al. 2014). SV channels are also efficiently blocked by polyamines (PA; Pottosin et al. 2014). Salinity stress-induced transcriptional activation of elevation of genes involved in PA biosynthesis and a massive increase in the amount of free PA in plant tissues are well documented (Alcazar et al. 2010; Shabala and Pottosin 2014) and, among other things, may be causally related to control of tonoplast Na^+ leak.

A recent bioinformatic analysis of the evolution and structure of TPC1 (slow vacuolar) channels has shown that most of TPC1s were fairly conserved in the key species of evolutionarily important lineages (Shabala et al. 2020). However, protein alignment and logo analysis demonstrated that TPC1s in the halophytes are distinct as compared to those in other plant and algal species. CqTPC1 showed significant loss of amino acid residues at transmembrane domains 6, 7, and 9 and lacked pore area 2, and S2–S3 were predicted as having only one TM region. While the functional significance of these structural changes remains to be studied, this data is consistent with the notion that these functional losses may lead to the reduced responsiveness of TPC1s to cytosolic changes (e.g. Ca^{2+}, pH, Na^+) in halophytes.

11.3.2 External Na^+ Sequestration

One of the special anatomical features of halophytic plants such as quinoa is a presence of epidermal bladder cells (EBCs). EBCs as balloon-like objects are present on all the plant external surfaces such as abaxial and adaxial sides of leaves, stems, and flowers that are able to sequester excess salt from mesophyll tissue. Various roles have been proposed for EBCs including an external storage site for toxic ions such as Na^+ and Cl^-, a storage site for water and different metabolic compounds, as well as protecting plant from UV (Kiani-Pouya et al. 2017).

Our previous investigation revealed that Na^+ sequestration into EBCs is an efficient strategy contributing to salt tolerance in quinoa where we demonstrated that compromising this ability by the gentle mechanical removal of EBCs resulted in a salt-sensitive phenotype (Kiani-Pouya et al. 2017). Also, by screening a large number of quinoa accessions under saline conditions, we showed that both salt-sensitive and salt-tolerant groups had the same leaf Na^+ concentration where salt-sensitive and salt-tolerant plants had on average 1649μmol/g DW and 1700μmol/g DW Na^+ content, respectively, suggesting that both plant groups had similar ability in preventing Na^+ entry to the shoot (Kiani-Pouya et al. 2019). On the other hand, salt-sensitive plants showed a negative association between leaf Na^+ concentration and salinity tolerance which suggests this group failed to deal with high Na^+ content that resulted in a lower biomass production. Given that salt-tolerant accessions had a higher bladder density and larger EBCs, that means they had higher external capacity for storage of salt. Altogether, these results are fully consistent with the role of EBCs as salt dumpers for Na^+ sequestration away from the cytosol.

Given the fact that EBCs are external structures which act as an extra reservoir for toxic Na^+, they required to have all the necessary metabolic pathways in place, e.g. they need a source of energy for their activities. Recently, a working model

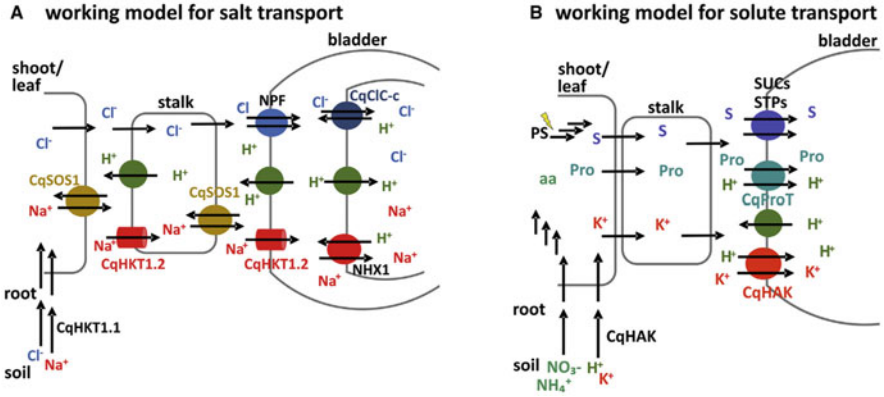

Fig. 11.1 A working model proposed for the bladder transport systems. (**a**) The transport of Na^+ by CqHKT1.2 and CqSOS1 transporters from the soil into the EBC illustrated by the red and yellow pathway. (**b**) The solute transport from leaves to EBCs is required to provide energy and osmotic balance in the EBCs. Based on Bohm et al. (2018). See text for more details

(Fig. 11.1) of delivery of Na^+ and Cl^- to EBCs has been proposed (Bohm et al. 2018). Based on this model, Na^+ is transported via CqHKT1.2 and CqSOS1 from the soil into the vacuole of EBC as the final destination of salt (Fig. 11.1a). Also, to provide energy and osmotic balance in the EBCs, the solute is transported from leaves to EBCs (Fig. 11.1b). Details of the involved transporters are discussed below.

Toxic ions need to pass through the plasma membrane and accumulate in the vacuole of EBC. Thus, certain vacuolar membrane transporters are required to enable this process. There is confirming information revealing that genes such as *HKT1* and *SOS1* have a key role in Na^+ transport across the plasma membrane (Qiu et al. 2002; Shi et al. 2000; Waters et al. 2013). It has been shown that EBC has a very low level of *SOS1* transcripts compared to the leaf, while there is a high expression level of *HKT1* in EBC (Bohm et al. 2018). In the working model proposed for Na^+ transport into the EBC (Fig. 11.1a), CqHKT1.2 and CqSOS1 transporters facilitate transfer of Na^+ from the soil into the EBC. Two co-orthologs of *AtHKT1*, namely, *CqHKT1.1* and *CqHKT1.2*, were found in both leaves and EBCs (Bohm et al. 2018). While CqHKT1.1 channel is present in quinoa root and transports Na^+ into the plant, CqHKT1.2 on the other hand is present in both stalk cell and EBC and mediates a one-way Na^+ transport from mesophyll into stalk cell, and then that is directed to EBC due to its electrophysiological properties where the voltage-dependent Na^+ channel is responsible for loading this ion into the EBC (Bohm et al. 2018). The model also suggests that Cl^- could be loaded into cytosol of EBC by plasma membrane-located NPF transporter and then loaded into the vacuole of EBC via tonoplast-located CqClC-c transporter. It has been proposed that CqClC-c acts as a Cl^-/H^+ antiporter and uses the proton motive force (PMF) to compartmentalize Cl^- into the EBC vacuole (Bohm et al. 2018). Furthermore, the transport of Na^+ by CqSOS1 needs the PMF, which is established by H^+-ATPases.

The transport of salt from leaves into EBC requires energy. A recent transcriptomic investigation comparing bladder-bearing leaves with those that did not have EBCs revealed a low expression of photosystem II (PSII)-related genes in EBCs (Zou et al. 2017). Also, Bohm et al. (2018) reported a relatively few chloroplasts in EBCs. Thus, due to the low photosynthetic activity of EBCs, these cells are dependent on the external energy source which is provided from the leaf. In this context, sugar transporters such as SUCs and SWEETs can mediate this requirement. Additionally, under saline condition, the salt concentration within EBCs can reach up to 1 M (Shabala et al. 2014), and hence, salt gradient must be balanced between the EBC apoplast and cytoplasm on one side and cytoplasm and vacuole on the other. Given that compatible osmoprotectant plays a key role in protecting the cytoplasmic metabolism from the toxic ions (Shabala and Mackay 2011), the transportation of Na^+ and Cl^- across the cytoplasmic layer of the EBC into the vacuole is likely buffered by the de novo synthesis of osmolytes or uptake of them from external sources (Bohm et al. 2018).

Proline as an organic osmolyte has a primary role in balancing the osmotic adjustment and also providing shield against the toxic effects of ions such as Na^+ and Cl^- (Daum et al. 2010). As it has shown in Fig. 11.1b, nitrogen from the soil is

metabolized in the leaf which results in producing compatible osmolytes such as proline. This organic osmolyte then is transported to the sink tissue by the Pro/H^+ symporter CqProT (Fig. 11.1b). Upregulation of a CqProT-type proline transporter has been reported which shows that proline is transported from surrounding leaf cells into EBC (Bohm et al. 2018). In agreement with this result, a metabolomic analysis has shown an increase in proline content in bladder-bearing leaves of quinoa under saline conditions (Kiani-Pouya et al. 2017).

CqProT transporter also has a role in GABA transportation that is a stress-related compound and has effects on plant channel activity (Shabala et al. 2014). Higher content of GABA in EBC could mediate the rate of toxic ions loading into EBC (Kiani-Pouya et al. 2017), and therefore, it could be proposed that CqProT has a dual role of providing proline as an osmolyte and controlling ion transport by GABA (Ramesh et al. 2015).

Besides proline, K^+ as the primary inorganic osmolyte plays a key role in many metabolic functions in plants such as cytoplasmic homeostasis and maintaining cell turgor under saline conditions (Tada et al. 2014). In light with this, a salt-induced increase in K^+ concentration has been reported in EBC of quinoa (Kiani-Pouya et al. 2017). This increase in K^+ in EBC is mediated by operation of K^+/H^+ symporter CqHAK (Fig. 11.1b).

11.3.3 Potassium Retention in Leaf Mesophyll

Potassium is a major cationic inorganic nutrient in plants (Shabala and Pottosin 2014) and is essential for maintaining membrane potential and appropriate osmotic adjustment as well as maintaining activities of many cytoplasmic enzymes (Wu et al. 2015). Therefore, delivery of sufficient quantities of K^+ to the shoot and K^+ retention in leaf mesophyll are needed for plants to maintain cell metabolism in photosynthetically active tissues.

Leaf mesophyll is central to photosynthesis performance in plant, and thus, biomass gain in plant and its performance under environmental stresses such as salinity to a large extent depend on efficiency of photosynthetic machinery (Wu et al. 2015). For this reason, K^+ homeostasis in leaf mesophyll may be essential for better performance of photosynthesis (Cakmak 2005) where reduction in CO_2 assimilation rate has been reported for K^+-deficient crop plants such as barley (Degl'Innocenti et al. 2009) and cotton (Bednarz and Oosterhuis 1999). Given the importance of maintaining high shoot K^+/Na^+ ratio as a fundamental feature of salt-tolerant crops (Shabala and Mackay 2011), K^+ homeostasis is a key component in salinity tolerance in both halophyte and glycophyte species (Volkov and Amtmann 2006). Wu et al. (2015) by screening of 46 barley genotypes reported a significant correlation between K^+ retention ability in mesophyll and salt tolerance indicating the important role of K^+ in salinity tolerance. Our large-scale screening of 114 accessions of quinoa also demonstrated that, with an exception of a few accessions, leaf K^+ concentration of plants grown under 400 mM salt was increased compared to non-saline plants (Kiani-Pouya et al. 2019). In this study, the relative K^+

concentration was increased up to 258% in accessions grown under 400 mM salt indicating that K^+ uptake was stimulated under saline condition. Halophytes like quinoa use K^+ to balance cytosolic osmotic potential and match one in the vacuole, to prevent water movement between these compartments.

It has been argued that the higher capacity of K^+ retention in halophytes may be associated with the superior Na^+ sequestration ability into vacuoles in these plants that prevents depolarization of the plasma membrane and the resultant K^+ leakage (Shabala et al. 2012). On other hand, higher K^+ retention is essential to reduce the formation of ROS (Hafsi et al. 2010) which in turn could restrict K^+ leakage induced by ROS-activated non-selective cation channels (Bose et al. 2014a). This capability would operate in a positive feedback manner that finally reduces detrimental effects of salt stress on cell metabolism.

Under saline condition, massive K^+ leakage from the cytosol of root and leaf tissues occurs (Shabala et al. 2006). At the same time, in vitro experiments on isolated chloroplasts suggested that maximum efficiency of operation of PSII was achieved in the presence of 150 mM K^+ in the bath solution (Percey et al. 2016). This value is consistent with reported values for cytosolic K^+ concentration (Britto and Kronzucker 2008) and also in a good agreement with those believed to be optimal for ribosome function and enzymatic activity in the stoma (Jin et al. 2011). The depletion of cytosolic K^+ pool may trigger programmed cell death in plant tissues through activation of enzymes associated with protein catabolism (Demidchik et al. 2010). Under these circumstances, efficient K^+ retention ability has main contribution to salinity tolerance. Comparative analysis of NaCl-induced K^+ fluxes from leaf mesophyll between quinoa and bean species showed that its magnitude was 30–40% less in the former (Percey et al. 2016). It was estimated that exposure of the broad bean mesophyll to 100 mM NaCl for 3 days has resulted in the intracellular K^+ content dropping from 150 to 59 mM (Percey et al. 2014), compromising PSII activity.

11.3.4 ROS Detoxification

ROS are a by-product of different metabolic pathways in plants (Foyer and Shigeoka 2011) and thus produced in numerous subcellular compartments. However, chloroplasts, peroxisomes, and mitochondria are known as the main sites of ROS production particularly during abiotic stresses (You and Chan 2015). In chloroplasts, the photosynthetic photosystem I and II reaction centres are the main sites for production of ROS. Peroxisomes are also producing large quantity of ROS such as H_2O_2 through processes such as photorespiration glycolate oxidase reaction and fatty acid β-oxidation (Foyer and Noctor 2003). Mitochondria are another important site of ROS generation in plants that have significantly lower level of ROS production than chloroplasts and peroxisomes (Foyer and Noctor 2003). In this subcellular organelle, complexes I and III in the electron transport chain are the primary ROS generation sites (Sweetlove and Foyer 2004). In addition to these sites, ROS are also

generated through NADPH oxidases at the apoplastic space, cell wall-associated peroxidases, and oxalate oxidases (Sagi and Fluhr 2006).

When produced in small quantities, ROS plays a key role in regulating plant growth, development, and defence pathways (Foyer and Shigeoka 2011). ROS acts as signalling molecules that can control various biological processes such as growth, cell cycle, programmed cell death, and hormone signalling, as well as biotic and abiotic stresses (Bose et al. 2014a). The amount of ROS produced and used for signalling purposes is strictly controlled by numerous enzymatic and non-enzymatic antioxidant systems. However, when plants are exposed to salinity, the balance between ROS production and scavenging is broken, and excessive ROS accumulation occurs. Under such stress conditions, various ROS species such as O_2, H_2O_2, O_2, and HO·may be generated (Apel and Hirt 2004). These various ROS species operate upstream of the key membrane transporters and, therefore, control adaptive responses to stress in plants such as ABA-induced closure of stomata (Wang and Song 2008) and xylem ion loading(Garcia-Mata et al. 2010).

The H_2O_2 signalling kinetics in halophytic plants is much faster than traditional plants (Bose et al. 2014a). For instance, the accumulation of H_2O_2 induced by salinity stress reached its maximum amount after 4 h of commencement of salt stress in the leaves of halophytic species *Cakile maritima*, while in the leaves of glycophyte *Arabidopsis thaliana*, this was gained at 72 h after application of salinity stress (Ellouzi et al. 2011). Similar results with other plant species have led to conclusion that the higher levels of H_2O_2 upon salinity stress are required to trigger salinity stress signalling and adaptive response in halophytes (Shabala et al. 2015).

In the case of quinoa, it has been shown that exogenously applied H_2O_2 as priming agent resulted in earlier emergence and increased drought tolerance in this plant (Iqbal et al. 2018). The results of this study also demonstrated that exogenously applied H_2O_2 led to higher photosynthetic factors such as stomatal conductance and CO_2 assimilation rate as well as higher chlorophyll content index, and proline and total soluble sugar contents, under drought stress conditions. It was proposed that exogenous application of H_2O_2 enhances quinoa performance under stressful conditions through mechanisms such as higher accumulation of osmolytes and improved enzymatic antioxidant activities (Iqbal et al. 2018).

Similar to other plants, when ROS are generated in excess in quinoa, the detoxification machinery that contains both enzymatic and non-enzymatic antioxidants is activated, to effectively scavenge toxic ROS enabling plants to survive under saline conditions (Ruffino et al. 2009). This capability might be one of the salt-responsible characteristics in quinoa where it has been reported that when this plant is exposed to salt stress, the level of enzymatic and non-enzymatic antioxidant systems against cytotoxic ROS has been increased (Aloisi et al. 2016; Panuccio et al. 2014). Furthermore, an efficient antioxidant mechanism shown by the activities of antioxidant enzymes has been reported in quinoa where it was activated by salinity stress during the germination and early seedling growth (Panuccio et al. 2014). The results of this investigation have shown that the total antioxidant capacity was always higher in salt-grown quinoa plants than those grown under non-saline conditions. It has also been demonstrated that the extent of oxidative stress damage

to the photosynthetic machinery was correlated with the leaf age where the damage is much less in young leaves than in old leaves (Shabala et al. 2012). This effect was attributed to the difference in a relative fraction of the organic osmolyte pool, whereas this difference was 1.5-fold in plants grown at non-saline conditions and was sixfold under 400 mM NaCl salt stress (Shabala et al. 2012). This difference might be attributed to the action of organic osmolytes and molecular chaperons in protecting PSII against oxidative damage (Bose et al. 2014a).

Four major groups of organic osmolytes are known: amino acids, sugars, polyols, and quaternary amines (Bohnert et al. 1995). Several of them may act as molecular chaperons to protect PSII against oxidative damage, while others could directly scavenge ROS (Smirnoff and Cumbes 1989). All of these four classes of organic osmolytes are present in tissues (Ruffino et al. 2009). Exogenously applied physiologically relevant concentrations of glycine betaine significantly ameliorated or fully mitigated the damaging impacts of oxidative stress on maximum photochemical efficiency of PSII (Shabala et al. 2012). Furthermore, glycine betaine content in quinoa shoot tissues was increased approximately by 40% under saline conditions (Ruffino et al. 2009), which is suggested to play a key role in enhancing catalase activity and in activating the H_2O_2-inducible protective mechanism (Hayashi et al. 1997). Based on the complexity of enzymatic and non-enzymatic scavenging system (Foyer and Noctor 2003), it is suggested that compatible solutes be fully accountable for the oxidative damage protection in young leaves (Shabala et al. 2012).

11.4 Stomata Patterning and Operation

11.4.1 Control of Stomata Development and Density

As stomata mediate all plant gas exchange, their operation needs to be balanced to maximize CO_2 uptake for photo-assimilation on one hand and to minimize water loss through transpiration on the other hand (Chaves et al. 2016; Hetherington and Woodward 2003). Morphological and anatomical features of stomata such as shape, size, and density vary broadly across plant species. These diverse stomata characteristics influence stomatal operation which in turn affects photosynthesis capacity, stomatal conductance, and water use efficiency (WUE) in plants (Bertolino et al. 2019; Lawson and Vialet-Chabrand 2019).

Stomatal development plays an important role in plant performance under various environmental conditions (Hetherington and Woodward 2003). In this context, salinity stress as an environmental factor also negatively affects stomatal parameters through either osmotic stress or toxic level of Na^+ in the cytosol (Shabala et al. 2013). Given that halophytes are capable of optimizing their stomata performance under saline conditions (Hedrich and Shabala 2018), their special strategies in stomata operation under salinity stress are of a particular interest. However, the mechanisms behind this superiority have not been properly elucidated. A comparative study between halophyte *Thellungiella halophila* and *Arabidopsis* as its glycophyte counterpart demonstrated that salt stress increased stomata density in

Thellungiella by twofold (Inan et al. 2004). Our current study of exploring varietal differences in a salinity tolerance of quinoa has shown that stomata density remained unchanged between salt stress and control conditions, while the stomata length reduced between 3 and 43% among accessions (Kiani-Pouya et al. 2019).

Stomatal pores are responsible for about 95% of total water loss in plants (Hedrich and Shabala 2018). Transpirational water loss by stomata, on the other hand, is regulated through stomata parameters including density, structure, and aperture (Bertolino et al. 2019; Hetherington and Woodward 2003; Lawson and Vialet-Chabrand 2019), and it has shown that plants control water loss via stomata length but not by stomata density. While stomata density was not altered between control and 400 mM salt-grown plants, stomata length (as a proxy for stomata aperture) decreased on average by 30% in all employed accession (Kiani-Pouya et al. 2019). This investigation also showed that salt-tolerant plants employed a different stomatal strategy to cope with salt stress than salt-sensitive counterparts. There was a negative association between salinity tolerance index (defined as dry weight under saline condition divided by dry weight under non-saline condition) and stomata length in the salt-tolerant group suggesting that tolerant plants decreased their guard cell aperture as a mechanism to manage water loss. Results further confirmed that salt-tolerant plants were able to increase their stomata density as a compensation strategy for declined stomata length (Kiani-Pouya et al. 2019). As a result of this balancing mechanism, the gas exchange is efficiently controlled in a way that it balanced leaf water loss and CO_2 assimilation under saline condition enabling plants to better deal with salt stress.

11.4.2 Stomata Operation

Although significant advances have been made in our understanding of stomatal function and the signalling pathways that control guard cell operation in glycophytes (Bertolino et al. 2019; Chaves et al. 2016), much less is known about stomata operation in halophytes (Hedrich and Shabala 2018). Thus, the question of how environmental factors and particularly salt stress modulate stomatal development pathway in these plants needs more studies.

Similar to other plant species, salinity stress reduces photosynthetic activity through declining stomatal conductance that eventually leads to decreasing transpiration rate and assimilation rate of CO_2 (Orsini et al. 2011). The results revealed that by increasing the salinity levels which results in decreasing water potential, the stomatal conductance values significantly decreased accessions; however, there were significant genetic variations among employed accessions (Shabala et al. 2013). This observed salinity-induced reduction in stomatal conductance in quinoa may represent a primary strategy by which salt-tolerant quinoa plants optimize WUE under salinity stress as similar observations have also been reported in several investigations (Kiani-Pouya et al. 2019; Orsini et al. 2011; Shabala et al. 2013). Our investigation has also shown that salt-tolerant plants were able to regulate stomatal characteristics such as stomata density and degree of stomata aperture to

cope with salt stress (Kiani-Pouya et al. 2019), and as a result of this efficient balancing mechanism, plants are able to have superior performance under saline conditions.

One of the subjects that have been a matter of debates in regard with stomatal operation in halophytes is the role of Na^+ in stomatal movements in these plants. In this regard, two possible stomatal adaptation strategies have been proposed. Based on the first proposed mechanism, halophytes are able to utilize Na^+ instead of K^+ in stomatal opening and closure (Kerstiens et al. 2002). As a case study of this strategy, it was demonstrated that when *Suaeda maritima* plants were exposed to salt stress, Na^+ was determined as a main cation in the guard cells, while when the stomata were closed, there was a lower concentration of Na^+ in the guard cells suggesting the ability of substitution of Na^+ for K^+ in the stomatal movement in this plant (Kerstiens et al. 2002). The second adaptation mechanism suggests that halophytes have efficient strategy to restrict Na^+ delivery into guard cells, and therefore, K^+ plays a key role in stomatal movement (Robinson et al. 1997). Based on this strategy, there is similarity of stomatal function between halophytes and traditional plants where both groups use K^+ as a major cation in stomatal opening and closure. Our results demonstrated that quinoa may use the first strategy and utilize Na^+ instead of K^+ in stomatal opening and closure (Rasouli et al., unpublished). The effect of Na^+ and K^+ on stomatal movement on epidermis strips revealed that stomata in this species were able to function normally in the presence of Na^+, while that was not the case for other plants such as sugar beet and sea beet where the stomata of these plants were less opened compared to non-saline conditions (Rasouli et al., unpublished). Furthermore, the results also showed it is likely in quinoa that maintaining higher K^+ concentration under salinity stress was involved with faster stomatal movement. In agreement with this finding, it has also been shown that K^+ selective transporters in guard cells were significantly overexpressed in salt-stressed quinoa plants (Rasouli et al., unpublished).

11.5 Root-Related Traits

11.5.1 Na^+ Exclusion from Uptake

Restriction of Na^+ delivery into the plant tissues is a hallmark of salinity tolerance in all glycophyte and halophyte species (Munns and Tester 2008; Tester and Davenport 2003). Quinoa plants with higher Na^+ exclusion capability had higher salinity tolerance (Kiani-Pouya et al. 2019).

Membrane transporters play an important role in balanced Na^+ uptake in shoot and root tissues (Shabala and Mackay 2011). SOS1 transporter which operates as Na^+/H^+ antiporter and is present at the plasma membrane of root apex is a primary mechanism for removing Na^+ out of the cell (Deinlein et al. 2014). Tester and Davenport (2003) argued that in thermodynamic point of view, the unidirectional Na^+ influx is a passive process and thus is not well controlled. For this reason, the fundamental mechanism for Na^+ exclusion is the prevention of Na^+ build-up in the

root and its loading into the xylem. In *Arabidopsis*, *SOS1* is mainly expressed in the root apex (Shi et al. 2000) and is the only characterized transporter that exports Na^+ from the cytosol to the apoplast. Accordingly, it has been proposed that Na^+ loading to the xylem in halophytes is an active process that needed upregulation of *SOS1* at the xylem parenchyma tissue (Shabala and Mackay 2011). In this context, loss of *SOS1* function in *Eutrema salsugineum*, a halophytic relative of *Arabidopsis*, resulted in a salt-sensitive phenotype in this plant (Oh et al. 2009).

SOS1-mediated Na^+ loading into the xylem was also suggested as a key mechanism operating in quinoa (Adolf et al. 2013). Two homologues of *SOS1* gene, *CqSOS1A* and *CqSOS1B*, that were upregulated in leaf when plants are grown under saline condition showed no increase at expression levels in the root (Maughan et al. 2009). Also, the transcript levels of *CqNHX1* were upregulated under salinity stress in both root and shoot (Ruiz-Carrasco et al. 2011).

As discussed earlier, EBCs are main components of salinity tolerance, and plants that have higher EBC density and larger bladders are able to efficiently sequester salt away from metabolically active cellular compartments in the leaf (Kiani-Pouya et al. 2019). However, in the absence of such a possibility where the large external Na^+ storage is not available, Na^+ exclusion at the root level and hence maintain leaves with low Na^+ content has been demonstrated act as primary compensation mechanism (Kiani-Pouya et al. 2020). The results of our study revealed that quinoa accessions with low EBC volume had efficient Na^+ sequestration mechanism at the root level, assessed based on their superior ability for net Na^+ efflux from the root epidermis compared with two other accessions having higher EBC volume (Kiani-Pouya et al. 2020). This study demonstrated operation of an active Na^+ efflux system to remove the Na^+ out of the root cells of accessions with low EBC volume, and Na^+/H^+ exchanger encoded by *SOS1* gene was suggested as the most suitable candidates for this role. This finding was confirmed through gene expression analysis where salinity exposure resulted in a fivefold overexpression of *CqSOS1* transcript levels (Kiani-Pouya et al. 2020). The results also were consistent with the notion that the SOS1 as a plasma membrane Na^+ efflux transporter operates in the removal of Na^+ out of the root cells with higher *CqSOS1* transcript levels found in salt-tolerant accessions. This finding is in agreement with the earlier investigations showing operation of such exchangers in roots of various plant species including wheat (Feki et al. 2014), *Arabidopsis* (Ullah et al. 2016), and barley (Wu et al. 2019).

11.5.2 Root K^+ Retention

K^+ retention in roots has been characterized as a key salinity tolerance trait (Shabala and Cuin 2008) where strong association between K^+ retention ability in root and salinity tolerance has been reported in many crop plants (Wu 2018). Salt-induced K^+ efflux occurs due to depolarization of the plasma membrane caused by transfer of the positive Na^+ charge. Although salinity stress significantly depolarizes the plasma membrane in each plant species, the magnitude of depolarization, however, is different among plants (Bose et al. 2013; Jayakannan et al. 2013). For example,

salt-induced depolarization was significantly lower in the roots of quinoa than *Arabidopsis* root (Bose et al. 2015). Also, the results demonstrated a linear association between the peak of K^+ loss and the respective membrane potential where the smaller salt-induced depolarization was responsible for the lower K^+ efflux from the roots of halophytes (Bose et al. 2015).

Most of the salt-induced K^+ loss is mediated through depolarization-activated K^+ (GORK) channels (Jayakannan et al. 2013). GORK channel permeability is under the strict control of the plasma membrane H^+-ATPase activity, responsible to maintain membrane negativity under salinity stress. Furthermore, K^+ efflux may also occur by non-selective cation (NSCCs) channels as a result of generated ROS under saline conditions (Bose et al. 2014b). In quinoa, pharmacological evidence has shown that K^+ loss induced by salt stress in the roots of this plant was mediated predominantly through the voltage-gated channels regulated by the plasma membrane H^+-ATPase (Bose et al. 2015).

To deal with K^+ leakage resulting from saline conditions, roots must possess strategies to efficiently regulate K^+ loss via voltage- and ROS-activated K^+-permeable channels. Pre-treating roots of quinoa with H^+-ATPase inhibitor vanadate resulted in a substantial reduction of the extent of H^+ extrusion and increased K^+ loss induced by salinity stress (Bose et al. 2015), pointing out at the essentiality of maintenance of negative membrane potential (MP) as a component of tissue tolerance mechanism. Importantly, the resting MP values of halophytic quinoa and *Atriplex* species were substantially more negative (by 10–15 mV) than were in *Arabidopsis*, suggesting constitutively higher rate of H^+-ATPase pump operation.

The magnitude of NaCl-induced K^+ efflux from quinoa root differed between contrasting varieties and negatively correlated with their salinity tolerance (Kiani-Pouya et al. 2020). We have also shown that NaCl-induced K^+ efflux was smaller in accessions lacking external salt storage capacity (e.g. having smaller density/EBC volume), suggesting the existence of the compensation mechanism. Given the fact that K^+ leakage may stimulate programmed cell death (Shabala 2009), plant's K^+ retention ability may be a primary component of the tissue tolerance mechanism.

11.5.3 Control of Xylem Ion Loading

Roots take ions (such as Na^+) and then transport them to shoots via xylem loading. Control of this process plays a primary role in plant overall salinity tolerance. Shabala (2013) suggested that a fast loading of Na^+ into the xylem at the early growth stages could be an ideal strategy to rapidly achieve full osmotic adjustment and maintain the normal growth under saline condition. Supporting evidence for this concept was provided (Shabala et al. 2013; Zarei et al. 2020). Comparative kinetics of xylem Na^+ and K^+ loading between quinoa and glycophyte plants (pea and beans) has shown that even in the absence of the salinity stress, quinoa had high initial Na^+ contents in the leaf which also was matched by sevenfold higher xylem sap Na^+ concentration (Zarei et al. 2020). This study further confirmed that quinoa as a halophytic plant had different strategy than traditional counterpart plants. While

salinity stress caused a rapid but transient increase in the xylem sap Na^+ levels in quinoa, this increase has occurred with much delays in glycophytic plants (Zarei et al. 2020). The rationale behind this was that traditional species tend to re-absorb Na^+ back into the stele, hence decreasing the Na^+ loading to xylem at the early growth stages of salt exposure. Quinoa, on the other hand, was capable to release Na^+ even in the presence of high levels of Na^+ in the xylem. Such a mechanism was also reported for other salt-tolerant plants. In barley, it has been demonstrated that transgenic plants overexpressing *HvHKT2;1* gene contained higher concentration of Na^+ in the xylem and had better performance under saline condition than wild-type barley plants (Mian et al. 2011). This superior performance could be due to the reliance on Na^+ transport to the shoot for osmotic adjustment.

It is noteworthy to mention that once quinoa has achieved the required Na^+ content at early growth stage, this plant then reduces the rate of Na^+ loading to xylem to the minimum required levels to control cell turgor in new tissues which is not the case for traditional plants where they lack an ability to limit xylem Na^+ loading (Bose et al. 2014b; Zarei et al. 2020).

11.6 Conclusions and Prospects

Quinoa is a semi-domesticated crop and is currently used both as a cash commodity and a convenient model species to understand mechanism of salinity tolerance that could be potentially incorporated in traditional major crops species. This could be done by either molecular means or via the marker-assisted selection, via the pyramiding approach (Shabala 2013; Shabala et al. 2016; Witcombe et al. 2008; Yeo and Flowers 1986). For practical purposes, the number of genes should be limited to some reasonable numbers. In this context, genes conferring internal (vacuolar) and external (trichomes) Na^+ sequestration should receive a major attention. Cytosolic K^+ retention and stomata development/operation are equally important to deal with the osmotic component of the salt stress. However, all the above transport processes come with significant carbon cost to plants. Both Na^+ sequestration and cytosolic K^+ retention rely on operation of H^+-ATPases, which are required to establish electrochemical gradients at the plasma and tonoplast membranes (Munns et al. 2020; Pedersen and Palmgren 2017). As the expression of salt tolerance genes appears to be constitutive, energy loss may thus be a growth-limiting factor even when the plant is grown in the absence of salinity or water stress (Lopez-Marques et al. 2020). Similarly, incorporating these traits into crops may also come with a yield penalty under non-stress conditions (e.g. a classical trade-off between productivity and tolerance). This conundrum may be possibly resolved by substituting H^+-ATPases by the functional H^+-PPases at the plasma membrane (Shabala et al. 2016). The feasibility of this approach should be tested in future studies.

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Bioactive Compounds in Quinoa (*Chenopodium quinoa*) and Kañiwa (*Chenopodium pallidicaule*)

12

Ritva Repo-Carrasco-Valencia and Julio Mauricio Vidaurre-Ruiz

Abstract

Quinoa (*Chenopodium quinoa* Willd.) and kañiwa (*Chenopodium pallidicaule*) are very nutritious crops native to the Andean region of South America. Both grains contain good-quality proteins and micronutrients such as iron, calcium and vitamins. Their fat content is relatively high, making them a source of essential fatty acids and tocopherols. Kañiwa has a particularly high dietary fibre content, thus being beneficial for human health. Quinoa and kañiwa seeds are major sources of flavonoids which consist mainly of glycosides of the flavonols kaempferol and quercetin. Processing Andean grains can lead to changes in the content of beneficial bioactive compounds; precautions must thus be taken to avoid losses. The traditional way to process Andean grains is cooking and roasting. It has been reported that it is possible to maintain the level of phenolic compounds in quinoa after cooking, if the cooking water is not discarded. Heat treatments can cause the release of phenolic compounds from the grain matrix, making them more bioavailable. During the milling of Andean grains, the bran fraction should be collected and used in food products because the majority of bioactive compounds are concentrated in this fraction.

Keywords

Pseudocereals · Native crops · Phenolic compounds

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12.1 Introduction

The Andean area of South America is an important centre of domestication of edible crops. The great diversity of landscapes and agro-ecological zones is due to the variation in climate and altitude (1500–4200 m above sea level). In this area, it is very difficult to find flat, fertile and well-watered soil. The people in Andean areas have cultivated their crops for centuries on small plots on mountainsides rising thousands of metres. At the time of the Spanish invasion, the Incas cultivated and used almost as many species of crops as the farmers in Asia and Europe. It has been estimated that Andean native peoples domesticated as many as 70 separate crop species (National Research Council 1989). The cultivation of many of these plants was reduced dramatically after the arrival of the Europeans. Until recently, these plants have been neglected and have not received major scientific or commercial interest.

Quinoa (*Chenopodium quinoa* Willd.) is a seed crop of the Amaranthaceae family. It was a very important crop for the ancient cultures of Peru and Bolivia. Nowadays, quinoa is cultivated mainly in the Andean region from Colombia to the north of Argentina, with Peru and Bolivia as the most important producers. There are different varieties and landraces of quinoa, which have adapted to distinct environmental conditions, for example, to high plateau, Andean valley and coastal areas. The seeds of quinoa are small, about 2 mm in diameter, and can be of various colours: white, cream, purple, yellow, red or black (Fig. 12.1a, b).

Kañiwa (*Chenopodium pallidicaule* Aellen) is a relative of quinoa, and it was considered a variety until 1929 when it was classified as a different species (Gade 1970). Kañiwa grows under very harsh environmental conditions, mainly in the Peruvian and Bolivian *Altiplano*, and is more resistant than quinoa to frost. In its native area, the temperature average is less than 10 °C, and frost can occur for at least 9 months of the year. For highland farmers, kañiwa is very important because it is the only crop that can resist during the frost. The most intensive production of kañiwa occurs in the southern Andes of Peru and Bolivia in the surroundings of Lake Titicaca. Kañiwa is a small plant and its seeds are smaller (approx. 1 mm) than those of quinoa. They are usually grey or brown, but there are some coloured ecotypes as well (Fig. 12.1c, d).

12.2 Nutritional Composition and Bioactive Compounds

Table 12.1 shows the proximate composition of quinoa and kañiwa seeds. The protein content of quinoa samples ranges between 13 and 17.9%, the black variety having the highest values. In the case of kañiwa, these values are between 14.4 and 16.9%. The protein of both grains is of good biological quality because of the balanced essential amino acid composition, especially because of the high lysine content. Quinoa protein is higher not only in lysine but in another important essential amino acid, methionine, than any other cereal (Arendt and Zannini 2013). These amino acids are especially important for vegetarian or vegan diets because they are

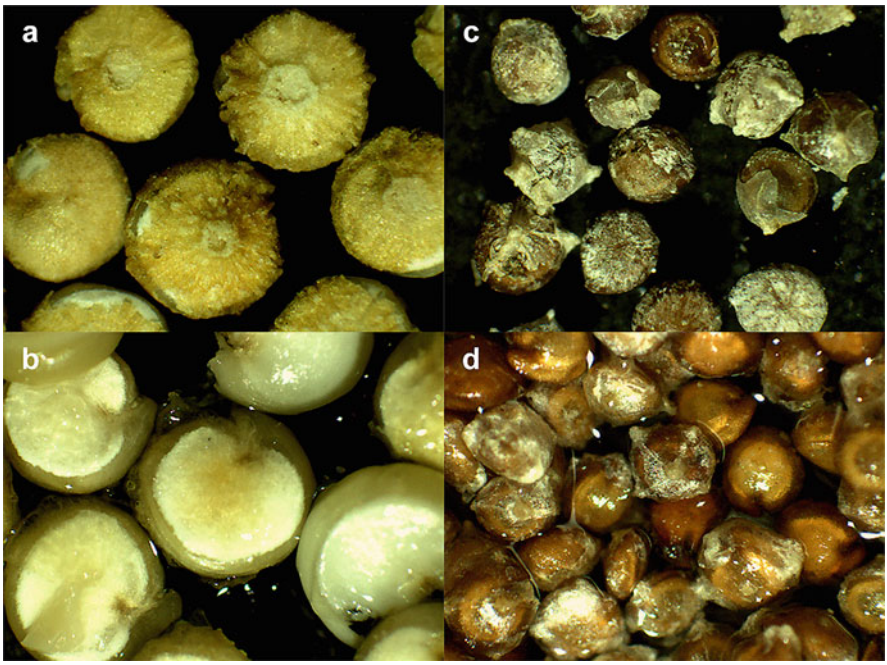

Fig. 12.1 Quinoa grain Amarilla Sacaca variety: (**a**) raw and (**b**) washed. Kañiwa grain Cupi variety: (**c**) raw and (**d**) washed (R. Repo-Carrasco-Valencia Unpublished)

limiting amino acids in vegetable proteins: cereals are limiting in lysine and legumes in methionine. Thus, quinoa and kañiwa can be used as a complement in products based on cereal and legume proteins.

Bioactive peptides in Andean grains have been studied by Chirinos et al. (2018) and Vilcacundo et al. (2017). These peptides show antidiabetic, antioxidant and antihypertensive properties in vitro. These results indicate that seed protein is a potential source of bioactive peptides that could be used as raw material in the nutraceutical and functional food markets.

The fat content of all the grains is between 5.7 and 7.3%, and it is mainly located in the embryo. The oil content in Andean grains is considerably higher than that in common cereal grains (6–7% versus 2–4%, respectively). The oil in Andean grains is of high nutritional quality, containing the essential fatty acids linoleic and linolenic acids in adequate proportions. A diet with a high n-6/n-3 ratio (linoleic/linolenic acid ratio) can promote many chronic diseases, such as cardiovascular disease, cancer and osteoporosis, as well as inflammatory and autoimmune diseases. An increased n-3 fatty acid intake in diet reduces the biological markers associated with the above-mentioned diseases. The current n-6/n-3 ratio in Western countries has been estimated to be in the range 14:1–20:1 and is far from the recommended levels of 5:1–10:1. Quinoa's n-6/n-3 ratio, at 6.2, falls within the recommended

Table 12.1 Proximate composition of quinoa and kañiwa varieties

Grain	Moisture (g/100 g dw)	Fat (g/100 g dw)	Protein (g/100 g dw)	Crude fibre (g/100 g dw)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)
Black quinoa[a]	10.9	7.0	17.9	4.2	3.1	72.1
White quinoa Kancolla variety[a]	11.6	6.8	13.0	2.3	3.2	76.9
White quinoa INIA Salcedo variety[a]	10.6	7.3	14.4	2.5	2.6	75.7
Kañiwa, commercial sample[a]	11.2	8.9	16.9	4.9	3.8	70.2
Kañiwa Cupi variety[b]	10.4	5.7	14.4	11.2	5.0	63.6
Kañiwa Ramis variety[b]	11.8	7.0	14.9	8.2	4.3	65.6

[a]Data from Repo-Carrasco-Valencia et al. (2019)
[b]Data from Repo-Carrasco-Valencia et al. (2009). *dw* dry weight

Table 12.2 Dietary fibre in Andean grains

Sample	TDF (g/100 g)	IDF (g/100 g)	SDF (g/100 g)
Black quinoa/Negra Collana	18.06	16.10	1.95
Quinoa/white grain from highlands	12.35	11.12	1.22
Quinoa/white grain from coast	8.35	6.43	1.91
Quinoa/Kancolla	9.94	7.81	2.14
Quinoa/INIA Salcedo	12.33	9.38	2.95
Kañiwa/commercial	20.85	18.29	2.56

Data from Repo-Carrasco-Valencia et al. (2019). *TDF* total dietary fibre, *IDF* insoluble dietary fibre, *SDF* soluble dietary fibre

values (Alvarez-Jubete et al. 2009). Quinoa and kañiwa could serve as raw materials to produce healthy edible oils.

The total carbohydrate content in common cereals and Andean grains is similar at about 60–75%, starch being the main carbohydrate in all of these grains. The starch found in Andean grains has some very interesting chemical and rheological properties, which could have industrial applications, for example, in gluten-free baking (Vidaurre-Ruiz et al. 2020).

Andean grains are important sources of dietary fibre as can be seen in Table 12.2. Kañiwa is especially high in this compound. The highest dietary fibre content is found in black quinoa. The fibre is mainly insoluble, as is common in all grains. Varietal differences in dietary fibre content are common in grains. This variation

Table 12.3 Pentosan content in Andean grains

Sample	Dry matter	Pentosans % dw
Quinoa/Negra Collana	90.23	1.91
Quinoa/white grain from highlands	90.24	2.20
Quinoa/white grain from coast	90.82	1.85
Quinoa/Kancolla	88.48	1.87
Quinoa/INIA Salcedo	88.85	1.70
Kañiwa/commercial	89.55	2.35

Data from Repo-Carrasco-Valencia et al. (2019). *dw* dry weight

may be related to environmental conditions, such as soil nutrient content and water availability. Interactions between the genotype and environment may occur, resulting in different impacts on the concentrations of components (Shewry 2009).

Pentosans are one of the important soluble dietary fibre components in cereals. They consist mainly of the pentosan sugars L-arabinose and D-xylose. Pentosans decrease the absorption of lipid and cholesterol in the human body and can help to prevent cardiovascular diseases. They have a positive effect on food processing as well, for example, on dough rheological characteristics and in macaroni processing. Table 12.3 presents the pentosan content in Andean grains.

Quinoa and kañiwa are particularly good sources of tocopherols (Repo-Carrasco et al. 2003). Tocopherols are compounds with high antioxidant capacity and other important physiological functions; some of them have the function of vitamin E. Tocopherols exist as four different isomers with antioxidant power, that is, in decreasing order, $\delta > \gamma > \beta > \alpha$. Both grains have α-tocopherols and γ-tocopherols, γ-tocopherols being the main compounds. Black quinoa and kañiwa seem to be the best sources of these compounds (Repo-Carrasco-Valencia et al. 2019). The tocopherol content in quinoa and kañiwa is superior to that in common cereals.

Schoenlechner et al. (2010) analysed the folate content in quinoa and its products. They found that the content of this vitamin was 0.13 μg/100 g, about ten times as much as in wheat. The bran fractions contained on average 124% of total folate, while only 57% on average was present in the flour fractions. Repo-Carrasco-Valencia et al. (2019) analysed folic acid in quinoa and kañiwa samples and found 20–47 μg/100 g in different varieties.

Quinoa and kañiwa are good sources of some important minerals such as calcium, magnesium and iron (Kozioł 1992; Repo-Carrasco-Valencia et al. 2019). According to Nascimento et al. (2014), 100 g of quinoa could contribute more than 50% of the dietary reference intake of copper, iron, manganese, magnesium and phosphorus established by the Institute of Medicine (IOM) of the U.S. National Academies.

Like most grains, quinoa contains phytic acid. Phytate forms complexes with multivalent metal ions such as iron, calcium, magnesium and zinc, reducing their bioavailability. According to Ruales and Nair (1993), the phytic acid content in quinoa seeds is about 1% of the dry matter. Scrubbing and washing reduce the phytic acid content of the seeds by about 30%. These authors detected neither protease inhibitors nor tannins in grains.

Repo-Carrasco et al. (2010) studied the effect of wet and dry processing on the iron, calcium and zinc content of raw and processed seeds. Regarding zinc and calcium, quinoa grains contained the highest levels of both minerals. There was a significant decrease in iron content during the cooking process in all samples. Wet processing procedures in general cause a loss of dry matter and iron. Cooking reduced the zinc content in quinoa and kañiwa. Roasting negatively affected the calcium content in quinoa but not in kañiwa.

Quinoa contains saponins which are glycosylated secondary metabolites found in many plants. Saponins are soluble in methanol or water. They produce stable foams in aqueous solutions and haemolyse red blood cells (Ruales and Nair 1993). Seed contains three or four different sapogenins, oleanolic acid, hederagenin, phytolaccagenic acid and sometimes deoxyphytolaccagenic acid, depending on the variety (Ridout et al. 1991; Cuadrado et al. 1995). Glucose, arabinose and occasionally galactose are the sugars bound to the sapogenins. Saponins are considered to be antinutritional compounds; however, they have some interesting health-promoting properties such as reducing serum cholesterol levels, possessing anti-inflammatory and antioxidant activity and exhibiting insecticidal, antibiotic and fungicidal properties.

Polyphenol compounds have been extensively researched for health-promoting properties such as their role in the prevention of degenerative diseases which include cancer and cardiovascular disease. The most important phenolic compounds in cereals are phenolic acids, alkylresorcinols and flavonoids. These phytochemicals in whole grains are complementary to those in fruits and vegetables when consumed together. Quinoa and kañiwa seeds are abundant sources of flavonoids, which consist mainly of glycosides of the flavonols kaempferol and quercetin (Peñarrieta et al. 2008; Alvarez-Jubete et al. 2010b). Kañiwa is exceptionally rich in resorcinols, compounds not very common in plants. Of the major cereals, resorcinols have been reported to be present in high levels in wheat, rye and triticale and in low amounts in barley, millet and maize. Cereal alkylresorcinols (ARs) have been reported to have anticancer and antimicrobial effects, as well as the ability to inhibit some metabolic enzymes in vitro. ARs have also been reported to have antioxidant activity (Ross et al. 2003).

Repo-Carrasco et al. (2010) studied the profile of phenolic compounds in quinoa and kañiwa varieties. The flavonoid content of *Chenopodium* species was exceptionally high, varying from 36.2 to 144.3 mg/100 g. The predominant flavonoids in quinoa samples were quercetin and kaempferol, while in some varieties, myricetin and isorhamnetin were also found. Kañiwa samples contained mostly quercetin and isorhamnetin with smaller amounts of myricetin, kaempferol and rhamnetin in some varieties. Both in phenolic acid and flavonoid contents, much variation was found between different samples (varieties). Berries have been considered as an excellent source of flavonols, especially quercetin and myricetin. When compared on a dry weight basis, the flavonoid content in berries and *Chenopodium* samples are of the same magnitude.

Pilco-Quesada et al. (2020) studied the effect of germination and kilning on the phenolic compound content in quinoa and amaranth. Altogether, 21 phenolic

compounds, mainly hydroxybenzoic acids, hydroxycinnamic acids and flavonols, were identified in the samples. The main flavonols were quercetin and kaempferol. Hydroxycinnamic acids identified from seeds mainly contained derivatives of coumaric and ferulic acids. Hemalatha et al. (2016) and Tang et al. (2015) quantified hydroxybenzoic acid derivatives from the extracts of (white, red and black) quinoa grains at high levels; these included gallic acid, *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, vanillic acid and vanillic acid 4-glucoside. Abderrahim et al. (2012) studied the effect of germination on total phenolic compounds, total antioxidant capacity, Maillard reaction products and oxidative stress markers in kañiwa. They found that germination can enhance the total antioxidant capacity in kañiwa. Based on these studies, germination can improve the nutritional composition of Andean native grains by increasing the total phenolic compound content. The results encourage the application of germinated and kilned quinoa as potential ingredients for the development of innovative and nutritious products.

Plant sterols (phytosterols) are another group of biologically active components present in pseudocereal lipids. Phytosterols, which cannot be absorbed in the human intestine, have a very similar structure to cholesterol and inhibit intestinal cholesterol absorption, thereby lowering plasma total and low-density lipoprotein (LDL) cholesterol levels (Alvarez-Jubete et al. 2010a). Phytosterols have also shown antiviral and anti-tumour activity (Li and Zhang 2001). Black quinoa and kañiwa seem to be interesting sources of phytosterols (Repo-Carrasco-Valencia et al. 2019).

12.3 Effect of Processing on the Phenolic Compounds

The ways of consuming quinoa and kañiwa are very varied, and they can be consumed in the form of cooked whole grain or as flour to prepare snacks, bakery products, noodles, stews, desserts, drinks and soups (Repo-Carrasco-Valencia and Vidaurre-Ruiz 2019). As is known, the processing of food for consumption causes certain changes in the food matrix, and this can promote the increase or degradation of phenolic compounds.

12.3.1 Washing and Desaponification

The first stage of the processing to which the quinoa and kañiwa grains are subjected consists of the elimination of saponin and some waste such as dirt and sticks; this process can be carried out by using water or abrasion. Washing seeds with water for 15 min has been reported to increase the content of total phenolic compounds by 19.6% (116.77 $\pm$ 4.80 mg GAE/100 g dw) (Nickel et al. 2016), basically due to the release of bound bioactive compounds, which may be present in a good proportion in quinoa (Tang et al. 2016b). Although washing can be considered promising to increase the content of bioactive compounds, this process is often inefficient because a lot of water is wasted when it is necessary to eliminate the saponin from some bitter varieties of quinoa such as the *Amarilla sacaca* variety.

The pearling process (<15.89%) has also been reported to be appropriate for removing saponin from the grain and for maintaining its phytochemicals. With this percentage of abrasion, a 23.8% loss of total phenolic compounds (152.70 ± 1.20 mg GAE/100 g dw) and a 22.9% loss of flavonoids (173.10 ± 5.27 mg CE/100 g DW) are achieved (Han et al. 2019). Similar results have been reported by Gómez-Caravaca et al. (2014), who reported a small reduction of total free phenolic compounds, from 261.04 to 204.87 mg/100 g, and bound phenolic compounds, from 16.46 to 10.66 mg/100 g, when grains are pearled by 30%. The individual phenolic compounds that are lost the most during the pearling process are gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid and vanillin (Han et al. 2019), although there is an increase in *p*-coumaric acid and certain flavonoids such as kaempferol, which can be present in the inner layers of the grain (Gómez-Caravaca et al. 2014).

12.3.2 Drying

After the washing process, an alternative to remove water from the grain is drying with hot air. It has been reported that drying of grains at high temperatures such as 60, 70 and 80 °C, respectively, with an air velocity of 2.0 ± 0.2 m/s, produces a notable loss of phenolic compounds; however, this does not affect the antioxidant capacity, since high antioxidant capacity has been reported when seeds are dried at 80 °C (Miranda et al. 2010). This effect may be related to the generation and accumulation of different antioxidant compounds which could show greater antioxidant capacity. Multari et al. (2018) expanded the research on the phenolic compounds present in grains (cultivated in Finland) after the drying process at temperatures of 40, 50, 60 and 70 °C, finding that the heat caused the degradation of free phenolic acids. The authors reported that the drying process carried out at 70 °C allowed the greatest recovery of total phenolic compounds, showing the presence of gallic acid at that process temperature.

12.3.3 Cooking

Another alternative in the processing of quinoa is that after washing, it is cooked so that the grain can be consumed. Dini et al. (2010) reported significant losses of total phenolic compounds and flavonoids when it is cooked in a 1:10 ratio (grains/water) for 20 min. The authors reported phenolic compound losses of 62.8% for sweet quinoa (28.7 ± 2.8 mg GAE/10 g dw) and 31.2% for bitter quinoa (59.4 ± 23.0 mg GAE/10 g dw); flavonoid compound losses were 77.8% for sweet quinoa (1.8 ± 0.7 mg CE/10 g dw) and 54.7% for bitter quinoa (6.3 ± 1.5 mg CE/10 g dw).

Nickel et al. (2016) reported that cooking grains for 11 min at atmospheric pressure, in a 1:3 ratio (grains/water), can increase total phenolic compounds by 13.5% (110.65 ± 3.43 mg GAE/100 g dw) and that cooking in a pressure cooker for

6 min can increase the total phenolic compound content in quinoa by up to 30.8% (127.54 ± 7.22 mg GAE/100 g dw).

A lower raw material/water ratio (1:1) during cooking has been reported by Rocchetti et al. (2019); the authors reported that it is possible to maintain the level of total phenolic compounds after cooking for 15 min (70.3 ± 2.5 mg GAE/100 g dw), being mainly flavonoids, phenolic acids and other polyphenols such as alkylphenols and hydroxyphenylpropenes, responsible for the differences in phenolic profile during cooking. These authors postulated that heat treatment is responsible for the solubilization of some flavonoids in the cooking water.

12.3.4 Germination and Malting

Germination has proven to be an efficient and economical process to increase the phenolic compound content in grains (Alvarez-Jubete et al. 2010b; Abderrahim et al. 2012). An increase in total phenolic compounds of more than 100% (147.2 mg GAE/100 g dw) has been reported when sprouted at 10 °C for 84 h. Specifically, a more than 50% increase in flavonoids such as kaempferol glycosides (56 μmol/100 g) and quercetin (66.6 μmol/100 g) has been reported compared to non-germinated grain (Alvarez-Jubete et al. 2010b). Paucar-Menacho et al. (2018) reported that the optimum temperature and germination time are 20 °C and 42 h, respectively. Under these conditions, the total phenolic compound content can almost double. Specifically, the researchers identified the formation of *trans*-*p*-coumaroylhexoside acid, *trans*-feruloyl hexoside acid isomers and sinapoylhexoside acid, as well as reported the most notable increase in flavonoid compounds such as kaempferol-*O*-dirhamnosyl-galactopyranose and quercetin-*O*-glucuronide.

Among the commercial varieties, it has been reported that red quinoa (Pasankalla variety) and black quinoa (Collana variety) would be promising for the germination process because they show a 49% increase in total phenolic compounds and 18% increase in flavonoids (Aguilar et al. 2019). In a recent publication, Pilco-Quesada et al. (2020) reported that germination of Chullpi quinoa at 22 °C for 72 h increases phenolic compounds such as coumaric acid (1346.4 μg/g dw) and kaempferol-deoxyhexosido-deoxyhexosido-hexoside (725.8 μg/g dw) by more than 100%. Likewise, it has been reported that when the Real Quinoa variety from Bolivia is germinated at 20 °C for 72 h, the most notable increase in phenolic compounds was of *p*-coumaric and vanillic acids.

The formation of phenolic compounds during germination can be explained by the release of bound phenolic compounds, which can be released during the early stages of germination, basically due to the breakdown of proteins and carbohydrates in the cell wall. Likewise, the synthesis of new phenolic compounds occurs with increased germination time, with glucose being the main precursor (Gan et al. 2017).

12.3.5 Milling

The milling of desaponified grain is an alternative process for converting it into flour and its subsequent application in different processed foods such as bakery products and desserts (Repo-Carrasco-Valencia and Vidaurre-Ruiz 2019).

It has been reported that of the fractions obtained during the milling, the bran fraction has the highest content of total phenolic compounds (4.29 mg of ferulic acid equivalents/g) and flavonoids (2.35 mg of catechin equivalents/g), while milled grain has the lowest content of total phenolic compounds (1.92 mg of ferulic acid equivalents/g) and flavonoids (1.09 mg of catechin equivalents/g). The loss of bioactive compounds is close to 30–40% after the milling process; however, it has been reported to be much less than the losses of bioactive compounds from wheat and barley (Hemalatha et al. 2016).

The degree of milling of grain is also a factor that affects the phenolic compound content in the finished product. Han et al. (2019) have reported that there is a linear relationship between the increase in degree of milling (DOM) and the loss of total phenolics and flavonoids in both free and bound forms. With a DOM of 27.23% (the most aggressive), the content of free phenolic compounds can decrease by up to 31.5% (111.51 mg GAE/100 g dw) and that of bound phenolic compounds by 31.1% (25.85 mg GAE/100 g dw). Similarly, the free flavonoid content can be affected, decreasing by up to 35.7% (95.19 mg CE/100 g dw); in the case of bound flavonoids, it can decrease by up to 52.4% (36.48 mg CE/100 g dw). Compared to the loss of phenolic compounds after grinding other grains with a similar DOM, grain shows less loss after the process. This could indicate that phenolic compounds are distributed in a more balanced way in the quinoa grain than in other cereals (Gómez-Caravaca et al. 2014; Hemalatha et al. 2016; Han et al. 2019).

12.3.6 Fermentation

Fermentation, like germination, is a low-cost, low-energy processing method that can improve the functional aspect of Andean grains. Recent research has shown that fermentation for 72 h with *Lactobacillus* (*L. reuteri* and *L. plantarum*) significantly increases the total phenolic compound content, showing an increase of almost 2.6 times in comparison to the initial content (12–32 mg GAE/g dw) (Ayyash et al. 2019). The fermentation time is an important factor in achieving the increase in phenolic compounds, since the longer the fermentation time, the more hydrolysis of the so-called non-extractable polyphenols in the food matrix is promoted. Rocchetti et al. (2019) fermented grains with *L. paracasei* and *Pediococcus pentosaceus* and with a blend of both strains, finding that after 24 h of fermentation, a slight increase in total phenolic compounds was evident in the samples fermented with *P. pentosaceus* and *P. pentosaceus* + *L. paracasei* (70.9 and 74.8 mg GAE/100 g dw, respectively). Through metabolic analysis, the authors determined that the process of fermenting seeds with *L. paracasei* showed a maximum increase in phenolic compounds (fold change, FC = 1.5), improving the content of phenolic

acids and tyrosols, probably the result of hydrolysis of the linked polyphenols of higher molecular weight.

Yeast (*Saccharomyces cerevisiae*) used for brewing and baking has also been used for the fermentation of grains. In the research carried out by Carciochi et al. (2016), fermentation for 24 h produced a 55% increase in the content of total phenolic compounds, specifically a significant increase in *p*-OH-benzoic acid, vanillic acid and *p*-coumaric acid. The fungus *Rhizopus oligosporus* has also been used for the fermentation; Starzyńska-Janiszewska et al. (2016) performed a prolonged tempe-type fermentation of white, red and black quinoa for 30 and 40 h. The authors found that long-term fermentation increased the content of soluble phenols such as vanillic acid, protocatechuic acid and rutin.

It is clear that the increase in phenolic compound content during the fermentation process can be mediated by microbial enzymes, which can induce the breakdown of the grain's cell wall structure and/or hydrolyse the esterified or insoluble phenolics; it is also possible that the microorganisms metabolize new phenolic compounds (Carciochi et al. 2016; Starzyńska-Janiszewska et al. 2016; Ayyash et al. 2019).

12.3.7 Phenolic Compounds in Products Made with Quinoa and Kañiwa

The inclusion of previously processed grains in the development of products with functional characteristics has gained interest in recent years (Pérez et al. 2016; Ludena Urquizo et al. 2017; Repo-Carrasco-Valencia and Vidaurre-Ruiz 2019). It has been reported that as the level of flour in food increases, the content of bioactive compounds, such as total phenolic compounds and flavonoids, increases.

Chlopicka et al. (2012) showed that replacing 30% of wheat flour with quinoa flour produces breads with 2.54 mg GAE/g of total phenolic compounds and 28.7 mg CE/g dw of flavonoids. Proportional amounts of total phenolic compounds have been reported by Xu et al. (2019), who found that the replacement of 0, 5, 10 and 15%, respectively, of wheat flour by quinoa flour increases the total phenolic compound values from 0.95 to 1.63 mg GAE/g in doughs and 0.63 to 1.01 mg GAE/g in breads. The authors noted that the loss of total phenolic compounds after baking was in the range of 30–37%. Alvarez-Jubete et al. (2010b) showed lower total phenolic compound values in gluten-free breads based on potato starch and with 50% substitution for quinoa flour; the total phenolic compound content after baking was 0.307 mg GAE/g. The authors also pointed out that losses of phenolic compounds are evident after baking and indicate that the presence of simple phenolics was not detected, but it was possible to detect flavonoids such as quercetin glycosides and kaempferol glycosides. In a recent investigation, Ballester-Sánchez et al. (2019) indicated that the replacement of 25% of wheat flour by quinoa flour (white, red and black organic quinoa Real) doubles the total phenolic compound content in breads (20.70–24.12 mg GAE/g dm), hydrolysable polyphenols (17.34–20.23 mg GAE/g dm) being found at a higher proportion in breads than extractable polyphenols (3.35–3.89 mg GAE/g dm).

Quinoa sprouts have recently been used to produce a dairy substitute and later in yogurt making. Joy Ujiroghene et al. (2019) reported that the maximum content of total phenolic compounds and flavonoids in yogurt produced with 100% fermented sprouted quinoa (Mengli 2 variety) was 276.9 mg GAE/100 g and 560.1 mg quercetin/100 g, respectively, while for yogurt produced with 50% v/v UHT milk/fermented sprouted quinoa milk, it was 144.7 mg GAE/100 g and 439.9 mg quercetin/100 g, respectively. The authors pointed out that phenolic compounds such as epicatechin, gallic acid, kaempferol, kaempferol-3-glucorhamnoside, kaempferol-3-*O*-rutinoside, quercetin, 7-hydroxycoumarin, methyl vanillate, morin, ferulic acids, dihydroartemisinic acid, artemisinic acid, gentisic acid, azelaic acid and protocatechuic acid were found in yogurts, as well as flavonoids such as kaempferol and quercetin.

Extruded quinoa products are also a source of phenolic compounds. Repo-Carrasco-Valencia and Serna (2011) reported that the total phenolic compound content in extrudates made from four varieties of quinoa (La Molina 89, Kcancolla, Blanca de Juli and Sajama) was between 1.66 and 3.28 mg GAE/g. Similar results have been reported by Kowalski et al. (2016) who reported that extrudates made with the Cherry Vanilla quinoa variety contain 11.81–20.22 mg GAE/g.

The inclusion of quinoa and kañiwa in pasta has also been investigated. Recently, Bustos et al. (2019) have reported that replacing 20% of wheat flour with kañiwa flour allowed them to obtain functional pasta with satisfactory cooking quality and good nutritional characteristics. Likewise, the phenolic compounds in a gluten-free commercial pasta after the cooking process have been analysed, and it has been reported that the free phenolic content after cooking does not decrease significantly compared to uncooked pasta (19.27 mg GAE/g); however, the authors showed a loss of bound phenolic compounds, which affects the antioxidant capacity of the pasta (Rocchetti et al. 2017). The authors stated that the lignans, followed by stilbenes and flavonoids, decreased during the pasta cooking process. However, phenolic acids and other phenolic compounds showed greater stability.

12.4 Effect of Processing on Antioxidant Activity

Reactive oxygen species (ROS) are known to be free radicals produced by endogenous oxidation-reduction (REDOX) reactions (Orona-Tamayo et al. 2019). When there is an imbalance in organism's ROS production, oxidative stress occurs, which can cause cell damage and macromolecular damage such as ageing, cancer, hypertension, neurodegenerative disorders and heart disease (Tang and Tsao 2017; Orona-Tamayo et al. 2019). Andean grains are sources of bioactive compounds such as phenols, carotenoids and bioactive peptides that show antioxidant activity. These compounds are capable of donating or receiving electrons to neutralize free radicals (Repo-Carrasco-Valencia et al. 2009; Tang and Tsao 2017; Multari et al. 2018; Ayyash et al. 2019).

The most widely used chemical methods for evaluating the antioxidant activity of Andean grains are the DPPH free radical test, oxygen radical absorbance capacity

(ORAC), Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and ABTS radical scavenging assay (Peñarrieta et al. 2008; Tang and Tsao 2017; Liu 2019). Various investigations have correlated the phenolic compound content in Andean grains with antioxidant activity (Hemalatha et al. 2016; Pellegrini et al. 2017; Liu et al. 2020); in the same way, the antioxidant activity of Andean grains has been successfully correlated with the seeds' unsaturated fatty acid content, total carotenoid index and total tocopherol index (Tang et al. 2016a; Tang and Tsao 2017).

During the processing, certain changes may occur in the grains that can favour or harm their antioxidant capacity. A summary of the investigations carried out so far on how the different processes such as washing, drying, grinding, cooking, germination and fermentation can affect the antioxidant activity is shown in Table 12.4.

It is clear that the optimization of parameters such as time, temperature, type of microorganism used or technology used to process the grains plays an important role in preserving the antioxidant activity of Andean grains; however, much more research is needed regarding kañiwa which is still scarcely studied.

12.5 Health Benefits

In vitro studies have shown that many of the bioactive components of quinoa and kañiwa (phenolics, bioactive peptides, carotenoids, saponins, betalains, fatty acids, tocopherols and carotenoids) have beneficial effects on human health, showing different properties such as an antioxidant (Pellegrini et al. 2017; Šťastná et al. 2019), anti-inflammatory (Tang et al. 2016b; Capraro et al. 2020; Liu et al. 2020), antihypertensive (Chirinos et al. 2018), anti-carcinogenic (Paśko et al. 2019; Liu et al. 2020) and antidiabetic effects (Tang et al. 2016b).

However, scarce research has been done in vivo, with animals, or in clinical trials with people. Within animal studies, it has been possible to determine that quinoa intake has a positive association with weight loss, as well as being effective in improving the blood glucose response and keeping plasma free fatty acids (FFA) (Mithila and Khanum 2015). The quinoa diet has also been reported to decrease the mass of adipose tissue and significantly reduce the expression of inflammatory adipokines (Foucault et al. 2012). Paśko et al. (2010) pointed out that feeding rats with seeds for 5 weeks effectively reduces total serum cholesterol, LDL and triglycerides. Likewise, a significant reduction in the blood glucose level and total plasma protein level was evident.

The effect of intake of sprouted and fermented seeds on physical and biochemical parameters in Wistar rats has also been investigated; according to the research carried out by de Oliveira Lopes et al. (2019), they showed that diets with sprouted and fermented seeds reduce the glycaemic index of diets with high levels of simple carbohydrates. They also reduce glucose and lipid levels in the blood and the accumulation of epididymal adipose tissue. Products processed with quinoa have also been investigated with in vivo models. In a recent investigation, Carrizo et al. (2020) made pasta bio-enriched with B2, B9 and minerals using sourdough

Table 12.4 Effect of different types of process on the antioxidant activity of quinoa and kañiwa

Transformation process	Evaluation	Method	Antioxidant activity	Units	Findings	References
Milling	Influence of the degree of milling (DOM) of quinoa grains (0%, 8.45%, 15.9%, 21.17%, 27.23%)	ORAC	8.48–14.05	mg TE/g dw	As DOM increases, the antioxidant capacity of quinoa grains decreases. A high DOM is detrimental to the retention of quinoa's antioxidant activity	
		FRAP	116.54–196.43	mg TE/100 g dw		
	Different fractions of grinding quinoa (hulls, dehulled grain, milled grain and bran)	DPPH	9.84–31.90	IC_{50} (μg/mL)	Bran fractions and hulls exhibited stronger radical scavenging than milled quinoa grain	
		FRAP	3.85–10.15	mmol Fe^{2+} equiv/100 g		
Washing	Influence of manual rubbing of quinoa grains in running water for 15 min	DPPH	4830.72	IC_{50} (μg/mL)	Antioxidant capacity increased slightly compared to unwashed grains	
		DPPH	30.96	mg TE/100 g dw		
		FRAP	15.05	mg TE/100 g dw		
Drying	Influence of hot air drying of quinoa grains at 40, 50, 60, 70 and 80 °C	DPPH	≈2300–3200	IC_{50} (μg/mL)	High antioxidant capacity when quinoa grains are dried at 40, 50 and 80 °C instead of 60 and 70 °C	
Cooking	Effect of cooking bitter and sweet quinoa grains	DPPH	19.9–35.7	μmol TE/10 g	Bitter quinoa grains had a higher antioxidant capacity than sweet quinoa grains. Cooking caused a significant loss of antioxidant capacity	Dini et al. (2010)
		FRAP	12.4–47.7	μmol TE/10 g		

	Effect of cooking quinoa at atmospheric pressure	DPPH	3409.47	IC_{50} (μg/mL)	Cooking at atmospheric pressure increased antioxidant capacity due to the release of conjugated and individual phenolic compounds	Nickel et al. (2016)
		DPPH	32.13	mg TE/100 g dw		
		FRAP	17.44	mg TE/100 g dw		
	Effect of cooking quinoa grains	FRAP	178.5	μmol GAE/g dw	Increase in the elimination of FRAP and ORAC radicals in quinoa seeds after the cooking process due to alteration of the matrix and consequent release of bioactive compounds	Rocchetti et al. (2019)
		ORAC	14161.8	μmol TE/g dw		
Germination	Influence of germination time (78, 72 and 96 h) of kañiwa seeds	ABTS	100.94	mmol Trolox/kg dw	The optimal germination time to increase the antioxidant capacity of kañiwa was 72 h	Abderrahim et al. (2012)
	Optimization of germination time and temperature of quinoa seeds	ORAC	315.8–1410.42	mg TE/100 g dw	The best germination conditions were at 20 °C for 42 h, which caused a 130% increase in the antioxidant activity of sprouted quinoa compared to quinoa seeds	Paucar-Menacho et al. (2018)
Malting	Influence of malting on three varieties of quinoa	DPPH	22.9–58.1	% inhibition	The % inhibition of DPPH increased up to 34% for the Pasankalla Roja variety and 40% for the Negra Collana variety	Aguilar et al. (2019)
	Optimization of roasting temperature of sprouted quinoa seeds	DPPH	40.68–53.04	μmol TE/100 g	Maximum antioxidant activity was obtained by roasting sprouted quinoa seeds at 145 °C for 30 min	Carciochi et al. (2016)

(continued)

Table 12.4 (continued)

Transformation process	Evaluation	Method	Antioxidant activity	Units	Findings	References
Fermentation	Influence of fermentation time (0, 24, 48 and 72 h) of quinoa grains with three *Lactobacillus* strains on antioxidant capacity	DPPH	≈25–65	% inhibition	Grains fermented for 72 h with *Lactobacillus reuteri* K777 and *Lactobacillus plantarum* K779 had the highest % inhibition of DPPH and ABTS	Ayyash et al. (2019)
		ABTS	≈12–73	% inhibition		
	Effect of fermentation of quinoa seeds with lactic acid bacteria (*Lactobacillus paracasei* and *Pediococcus pentosaceus*)	ORAC	5967.7–7010.7	mg TE/100 g dw	The elimination of ORAC radicals from fermented quinoa seeds was higher than in raw seeds, with higher values reported by fermentation with *P. pentosaceus* and with the blend of *P. pentosaceus* + *L. paracasei*	Rocchetti et al. (2019)
	Effect of quinoa seed fermentation with baker's and brewer's yeasts (*Saccharomyces cerevisiae*)	DPPH	≈350–390	μmol TE/100 g	Fermentation with both *S. cerevisiae* strains showed increases in antioxidant activity compared to raw quinoa: 43% and 33% for DPPH, 22% and 27% for ABTS + and 51% and 50% for FRAP for baker's and brewer's yeast, respectively	Carciochi et al. (2016)
		ABTS	≈450–470	μmol TE/100 g		
		FRAP	≈190–200	μmol TE/100 g		
	Effect of prolonged tempe-type fermentation on white, red and black quinoas	DPPH	1.42–2.48	μmol TE/g dw	Quinoa fermented for 40 h had a more favourable antioxidant potential than standard products. Reducing power increased on average by 30% (red and white seeds) and 18% (black seeds), compared to fermentation for 30 h	Starzyńska-Janiszewska et al. (2016)
		ABTS	8.13–21.80	μmol TE/g dw		

(*L. plantarum* CRL 2107 + *L. plantarum* CRL 1964) and found that consumption of the pasta increased the levels of B2 and B9 in the blood of mice. Also, they had higher concentrations of minerals, haemoglobin and haematocrit compared to the group of mice that were deficient.

Intake of quinoa by obese diabetic mice improves or decreases the conditions associated with type 2 diabetes improving liver steatosis, plasma lipids and the state of inflammatory-oxidative stress in this type of animal (Noratto et al. 2019). It has recently been reported that a mixture of quinoa flour and sorghum flour has a high antioxidant capacity in vivo, which would be related to the high content of phenolic compounds (Medina Martinez et al. 2020).

There have been very few clinical trials assessing the effects of quinoa consumption. Ruales et al. (2002) found that twice-a-day intake of 100 g of porridge made from quinoa by preschool children (5 years old) for 15 days can increase the levels of growth factor similar to plasma insulin (IGF-1). This means that the consumption of quinoa promotes growth in children. Farinazzi-Machado et al. (2012) investigated the effects of consuming quinoa cereal bars (twice a day, for 30 days) in 22 students. The authors found that the consumption had beneficial effects in part of the studied population since the levels of total cholesterol, triglycerides and LDL-c were reduced. De Carvalho et al. (2014) carried out a prospective, double-blind study for 4 weeks with 35 overweight postmenopausal women who consumed 25 g of flakes or cornflakes daily. The results found indicate that the consumption of flakes manages to reduce total cholesterol and LDL cholesterol (LDL-c) and increase GSH, showing a possible beneficial effect of quinoa flakes.

12.6 Conclusions

Quinoa and kañiwa are relatively rich in protein and fat, with significant differences in nutritional composition among the different varieties of these two grains. The oil in Andean grains is of high nutritional quality, containing the essential fatty acids linoleic and linolenic acids in adequate proportions. Kañiwa oil is particularly notable because it is very rich in phytosterols, tocopherols and unsaturated fatty acids. Andean grains contain flavonoids, a type of phenolic compound with significant antioxidant activity. Plant sterols, phytosterols, are another group of biologically active components found in pseudocereal lipids. Black quinoa and kañiwa are interesting sources of these compounds. During the processing, certain changes may occur in the grains that can favour or harm their nutritional value. It is clear that the optimization of processing parameters such as time, temperature, type of microorganism used or technology used to process the grains plays an important role in preserving the nutritional value and content of bioactive compounds of Andean grains; however, much more research is needed especially regarding kañiwa which is still scarcely studied. In general, these Andean native grains are very rich in health-promoting compounds, and future studies should evaluate the bioavailability of these compounds.

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Protocol for Seed Surface Sterilization and In Vitro Cultivation

13

Ajit Varma and Aditi Jain

Abstract

Quinoa is a pseudocereal which is growing popularity day by day in the scientific world due to its rich nutrient profile and easy cultivation. Several scientists are taking interest in these novel grains in order to study as well as to learn the mechanisms underlying its tolerance to diverse stress conditions. In order to prepare fresh grains for work in the laboratory, it is essential to ensure sterility from contaminating microorganisms so that no interference can be caused during observations. In the field, several millions of microbes come in contact with the grains and other parts of the plant which live either as symbionts, parasites, and even pathogens. These microbes exist in nature within high concentrations in soil and sometimes even help and support the plant growth by establishing symbiotic relationships. Parasites and pathogens, on the other hand, possess the ability to destroy the whole field. It is necessary to take various precautionary measures to prevent their entry in the vegetation through various physical and sometimes chemical and biological practices. This chapter also discusses in depth the composition of two culture media that can be used in laboratory to cultivate this crop which are Murashige and Skoog (MS) media and Gamborg's B5 media for tissue culture operations, aseptic cultivation, and practices that need to be undertaken for maintenance of plant cell cultures in vitro.

Keywords

Quinoa · Endophytes · Surface sterilization · Tissue culture · Nutrients · Growth media · Aseptic cultivation

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13.1 Commonly Found Endophytic Microorganisms in Quinoa Seeds

A plant growing in vivo comes in contact with a number of microorganisms, out of which some are beneficial for the growth of the plant as they participate in various beneficial interactions, whereas there are also such microorganisms that harm the plant by showing parasitic or pathogenic activity.

The highest concentration of microbes around the plant is found in the rhizosphere region, which is the part of soil surrounding the roots of the plant. During various stages of development, quinoa seeds may also get contaminated by root endophytic microorganisms, mainly bacteria and fungi, which can act as contaminants while cultivating such seeds in vitro.

There are some common species of microbes studied to be found routinely as endophytic residents of quinoa seeds. These include *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus methylotrophicus*, and *Bacillus tequilensis* for bacteria (Pitzschke 2016), and for fungi, the common contaminants are members of genera *Trichoderma*, *Penicillium*, *Aspergillus*, *Fusarium*, *Ulocladium*, *Mucor*, *Rhizopus*, *Alternaria*, *Absidia*, *Cladosporium*, *Eurotium*, *Drechslera*, *Epicoccum*, *Monascus,* and *Peronospora* (Pappier et al. 2008). Fungal contaminants, unlike bacteria, are mainly found around roots and as root endophytes.

13.2 Seed Surface Sterilization of Quinoa Seeds for Plant Tissue Culture Techniques

Most of the losses in microbiological experiments are due to contamination by unwanted microbes including bacteria, fungi, yeasts, and viruses (Arab et al. 2014). Contamination in experiment samples can be sourced from outside unfiltered air, explant surface, external/internal plants, insufficiently sterilized culture media, unsterilized glassware or plasticware, and inaccuracy of the worker in maintaining aseptic environment (Arab et al. 2014; Bhojwani and Dantu 2013).

An effective sterilant is a chemical which is cheap, nontoxic, effective, applicable, and easily available (Bhojwani and Dantu 2013). Different chemicals are used to achieve sterilization of different explant materials in tissue culture, with mild chemicals for delicate ones and strong ones for others. An effective sterilant for a particular plant material is the one which is effective enough to get rid of all of the contaminating microorganisms and can even be used at its highest possible concentration, if required, without causing harm to the plant material or the surrounding environment (Arab et al. 2014; Jafari et al. 2016).

For plant tissue culture, sterile and aseptic environment is the first and foremost requirement for an experiment to take place. For studying quinoa characteristics and interactions in vitro, it is necessary to carry out surface sterilization of seeds as they are obtained fresh, directly from agricultural fields. In order to achieve this, certain steps are undertaken which eliminate the contaminants while maintaining the seed nutritional quality and germination potential, also known as seed 'viability'.

Although there are several methods and techniques to remove surface contamination from seeds for microbiological experimentation, there are certain series of steps that are routinely undertaken by many scientists and are found to promise fairly high and even 100% germination rate. When brought to a laboratory, it is important to rinse the seeds with sterile distilled water to remove dust particles and other impurities. Then, depending upon the choice of chemical used for surface sterilization, seeds are immersed in the concentrate for a strict period of time and the monitored over the coming days for their growth.

A variety of surface sterilants, including sodium hypochlorite (NaOCl), bromine water (Br_2), ethanol (C_2H_5OH), calcium hypochlorite ($Ca(ClO)_2$), hydrogen peroxide (H_2O_2), mercuric chloride ($HgCl_2$), silver nitrate ($AgNO_3$), and even certain antibiotics and antifungal agents, are used to achieve surface sterilization. However, sodium hypochlorite is the most commonly and routinely used agent as it has been proved to be excellent at eliminating almost all types of bacteria, fungi, and even viruses (Bhojwani and Dantu 2013). Due to being relatively safe to be used by humans and its strong oxidizing properties, it effectively reacts with amino acids, nucleic acids, amines, and amides of microorganisms (Jafari et al. 2016). Increasing the concentration of sodium hypochlorite in the treatment solution with longer immersion times corresponds to a significant reduction in the contamination, but on the other hand, with stronger and longer exposure, negative effects are seen on seed germination rate. Because seeds are very small and delicate, it is important to test and find out the best possible set of chemical concentration and exposure time to ensure 100% germination rate (Hesami et al. 2018). In experiments, quinoa seeds are surface sterilized by giving them a treatment with 10% $Ca(ClO)_2$ for 20 min (Burnouf-Radosevich and Paupardin 1985) or 20% NaOCl for 5 min (Hesami et al. 2018).

When seeds are treated with sodium hypochlorite for longer immersion times (15 min), the seed germination rate decreases significantly (56.67%) in the experiment conducted by Hesami et al. (2018). Also, with an increase in the concentration of NaOCl, the seed germination rate is yet again decreased (Hesami et al. 2018). After the germination of such treated seeds in an in vitro culture, seedling growth and development and the viability of the tissues were also negatively affected by high concentrations of NaOCl (Jafari et al. 2016). For seeds, in vitro surface sterilization requires treatment with the lowest concentration of sterilant applicable, for the shortest amount of time. After treatment with an appropriate concentration of a sterilant, seeds are again rinsed with sterile distilled water several times to remove any chemical residue.

13.3 Tissue Culture Media Preparation Protocol

For healthy plant growth and maintenance, a medium should be used which contains all the required nutrients. It largely consist of macronutrients which are the nutrients required by the plant in large quantities, micronutrients which are required by the plants in comparatively lower quantities, amino acids for cellular growth, vitamins,

nitrogen supplement, carbon source for energy, growth regulators (if required), and a solidifying agent.

13.3.1 Macroelements

Besides oxygen, hydrogen, and carbon, nutrients required in fairly large quantities by the plants for germination, growth, and morphogenesis include nitrogen, phosphorus, sulphur, potassium, magnesium, and calcium. A minimum of 30–60 mM of inorganic nitrogen is mandatory for healthy plant cell development. Most of the media complexes contain potassium in the form of nitrate or chloride salts where it is required in concentration of about 20–30 mM for normal growth (Saad and Elshahed 2012).

13.3.2 Microelements

Some of the crucial micronutrients for plant cell tissue culture are zinc, manganese, cobalt, copper, boron, iron, and molybdenum. The most important one is iron, which is required in tiny amounts but is very necessary as it is required by the plant cells to produce the pigment chlorophyll from the process of photosynthesis. Iron also acts as a catalyst in many enzymatic reactions that take place in the plant metabolism. Iron is usually added in media in citrate or tartrate salt form, but there are certain challenges due to difficulty in dissolving it, and sometimes precipitation also occurs which may degrade the quality of the nutrient. To overcome this, it is advised to prepare the iron stock containing iron-EDTA chelate (FeEDTA) (Steiner and van Winden 1970) separately from the minor stock, which is usually kept in a dark-coloured glass bottle away from sunlight or any other kind of light. Cobalt and iodine are also listed in a few culture media, but their active function in the plant growth as to why they are necessary has yet not been defined. In a standard media, cobalt and copper are added in a concentration of about 0.1–0.15 μM, iron and molybdenum in 1.0 μM, iodine in 3–5 μM, zinc in 5–30 μM, manganese in 15–90 μM in the form of manganese salts, and boron in 20–100 μM range (Torres 1989; Saad and Elshahed 2012).

13.3.3 Energy Source: The Carbon

As a carbon source, normally sucrose is used; however, other carbohydrates may also be used depending upon the plant's ability to assimilate them. Often, carbon source is used at a concentration of 2–5%. Other than sucrose and glucose, maltose, lactose, starch, and galactose have also been experimented upon, but they all were found to be less effective. Fructose has also been used, but it is also found to be less effective than the earlier ones, as during growth, plant first finds the need of glucose and then requires fructose. It is advised to use autoclaved sucrose than filter sterilized

sucrose in a medium as heat hydrolyses the sugar into more simpler and easily utilizable form. sugars. In an experiment, it was also reported that sucrose acts as a morphogenetic inducer which leads to the formation of axillary buds and promotes branching of adventitious roots (Vinterhalter and Vinterhalter 1997).

13.3.4 Vitamins

Plants are able to synthesize some of the required vitamins on their own but need other vitamins to be supplemented for their growth and development. Vitamins also play an important role as catalysts in various metabolic and biochemical processes; hence they are crucial for sustenance and cellular growth of the plants. Commonly used vitamins in the cell and tissue culture media include thiamine (vitamin B_1), nicotinic acid (B_3), and pyridoxine (B_6).

Thiamine Thiamine can be found in various organs of the plant including leaves, roots, flowers, shoot, fruits, seeds, tubers, and even bulbs. Thiamine takes part in the primary regulatory system of plants (Bocobza and Aharoni 2014) and acts as a cofactor in the form of thiamine pyrophosphate (TPP) in various important metabolic activities such as acetyl-CoA synthesis, citric acid cycle, and synthesis of amino acids, and also finds use in the Calvin cycle (Du et al. 2011). It also aids in plant protection by acting as a response molecule against various biotic and abiotic stresses (Subki et al. 2018).

Nicotinic acid Nicotinic acid in its amide form constitutes Nicotinamide Adenine Dinucleotide (NAD) and Nicotinamide Adenine Dinucleotide Phosphate (NADP), which are the pyridine nucleotide coenzymes and are found in their functional moiety. This acid plays an important role in both primary and secondary metabolic reactions of higher plants including forming the building units of several simple pyridine compounds such as trigonelline, nicotine, anabasine, and ricinine and also forming other more complex alkaloids found in different parts of the plant (Barz 1985).

Pyridoxine Pyridoxine acts as a coenzyme for several metabolic enzymes and has been recently proved to be functioning as an antioxidant. It exists in different types of natural forms known as vitamers and is mainly synthesized in plants through de novo biosynthetic pathway. One form of pyridoxine, known as vitamer PMP (pyridoxal monophosphate), is found to channel the information to plant cells of their ammonium content which is utilized for protein biosynthesis. When enough ammonium is present, further synthesis of nitrate is prevented to conserve energy and prevent toxic effects of overaccumulation of compounds. In such as case, PMP probably informs the plant about its ammonium content in order to regulate its synthesis as reported by Maite Colinas of the Department of Botany and Plant Biology, University of Geneva, 1211 Geneva, Switzerland (Colinas et al. 2016).

These vitamins are crucial for plant growth and may act as growth-limiting factors if not supplemented in desired concentrations, whereas other vitamins such as biotin, folic acid, ascorbic acid, pantothenic acid, tocopherol (vitamin E), riboflavin, and *p*-aminobenzoic acid are added in some culture media but are not necessarily mandatory for plant growth and thus do not act as growth-limiting factors. In simpler or basal media, their use can be generally avoided without any hindrance to plant growth.

13.3.5 Myo-inositol

Myo-inositol or simply inositol is a sugar-like carbohydrate and is important for normal plant growth and development. It is often supplemented in plant tissue culture media as it is believed to play an important role in cell division and storage of phosphate in seeds as it breaks down into ascorbic acid and pectin, and incorporates into phosphoinositides and phosphatidyl-inositol. In the polysaccharide production of plant cell walls, oxidized form of inositol is very important (Loewus and Murthy 2000). Inositol also contributes to plant protection against salt stresses (Loewus and Murthy 2000; Bohnert et al. 1995) by preventing oxidative damage to cellular structures by reactive oxidizers such as hydrogen peroxide and also aids to control water pressure inside the cells. Inositol also takes active part in molecular signalling pathway known as the phosphoinositide (PI) pathway (Munnik et al. 1998). This pathway is crucial to many plant responses such as the tendency of roots to go downwards in response to gravitational pull (Perera et al. 1999) and pressure changes in leaf pores that control the process of wilting in different environments (Cote and Crain 1993). Another important role is in storage and transport of auxin, which is an important plant hormone that controls plant cell division and elongation (Bandurski 1979; Lebanonturf 2013).

13.3.6 Amino Acids

Some of the required amino acids are synthesized by plants themselves, but certain amino acids need to be supplemented in the media in order to promote establishment of cell cultures and protoplasts. They act as nitrogen sources which are readily bioavailable and are easily assimilated by plants for cell division and tissue formation. Amino acids in the form of casein hydrolysate, L-glutamine, L-asparagine, and adenine are routinely used by researchers as readily available nitrogen source in culture media. They also take up on the crucial role of forming proteins, important enzymes taking part in biochemical reactions, as well as in structural components.

13.3.7 Undefined Supplements

Some of the organic, naturally occurring mixtures are added to culture media as undefined supplements, because their exact composition is unknown, but they are found to support healthy growth in plants. Extracts such as potato extract, corn steep

liquor (Fox and Miller 1959), ground banana and tomato juice (Larue 1949), protein hydrolysates, coconut milk (Van Overbeek et al. 1941, 1942; Gautheret 1942), yeast extract (Robbins 1922; White 1934), orange juice, etc. have been tested for their effects on plant growth and are now routinely added to plant culture media (George et al. 2008).

13.3.8 Solidifying Agents

In culture media, a solidifying agent is added to provide a solid base to which a plant can adhere to and can stay in an upright position, so its root system can be easily and readily observed and examined. The hardness of the media also directly influences the growth of plant cells as the harder the media, the less will be the water movement and hence the slower the nutrient supply. The softer the media will be, the more will be the water activity, and more readily nutrients will reach the plant. Quinoa is a plant with fairly high nutrient requirement for in vitro cultures; hence 0.6% agar is used, instead of a standard 0.8%. Other than agar, agarose and gellan gum are also used (IAEA 2004).

Agar is obtained from seaweed and is the most commonly used gelling agent as it a polysaccharide which has several benefits over others and is therefore a more successful gelling agent. It easily melts at a temperature range of 60–100 °C and solidifies firmly at about 45 °C, forming a stable gel at all feasible incubation temperatures without rupturing or melting away. Also, it is not digested by the plant and does not react with other ingredients of the media which might cause a disturbance in nutrient bioavailability and transport. It is advised to use plant tissue culture grade pure agar powder while preparing the media, as certain agar may contain contaminants such as Ca, Fe, Cu, Mg, Ba, Si, Co, Cl, and N which can lower the functionality of agar (Pierik 1997).

13.3.9 Plant Hormones and Growth Regulators

Growth hormones may be supplemented in desired concentrations according to their use in order to perform different experiments that include callus induction, root and shoot elongation, tropism, and study of apical dominance. There are mainly four groups of growth regulators, namely, auxins, gibberellins, cytokinins, and abscisic acid. In simple plant cultivation involving the study of plant-microbe interaction, addition of hormones is not necessary as they may mask the effects a microbe is exerting on the plant growth and may cause disturbances. They are used in different plant tissue culture experiments where growth regulation is desired without an interaction with a foreign microbe or compound.

Auxins Commonly used auxin compounds include indole-3-acetic acid (IAA) which is the naturally occurring form of auxin in plants, indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthaleneacetic acid (NAA). Several other synthetic auxin formulations such as 4-chlorophenoxy acetic acid or

p-chloro-phenoxy acetic acid (4-CPA, pCPA), 2,4,5-trichloro-phenoxy acetic acid (2,4,5-T), 3,6-dichloro-2-methoxy benzoic acid (dicamba), and 4-amino-3,5,6-trichloro-picolinic acid (picloram) are also used in certain media (Torres 1989). Auxins are mainly added to induce cell elongation, shoot elongation, stimulation of callus production, formation of plantlet from callus, rooting, somatic embryogenesis and to stimulate growth from shoot apices and shoot stem cultures. It is advised to use freshly prepared IAA solution while preparing the media. It can be stored at 4 °C for no longer than a week.

Cytokinins Commonly added cytokinin compounds in plant tissue culture media include BAP (6-benzylaminopurine), 2iP (6-dimethylaminopurine), kinetin (*N*-2-furanylmethyl-1H-purine-6-amine), zeatin (6-4-hydroxy-3-methyl-*trans*-2-butenylaminopurine), and TDZ (thidiazuron-*N*-phenyl-N′-(1,2,3-thiadiazol-5-yl) urea). Zeatin is a naturally occurring cytokinin which is most effective in plant growth. 2iP is also a naturally occurring cytokinin but is found in lower amounts than zeatin. Cytokinins play several important roles in plant growth and development including induction of cell proliferation, differentiation of callus into plant shoot when applied with auxins, embryogenesis, maintenance of shoot apical meristem proliferation and functioning, affecting root apical meristem growth, vascular cambium development, radial plant growth, promoting the outgrowth of dormant axillary buds, root elongation, root nodule formation, negative regulation of lateral root formation, etc. Cytokinin compounds are relatively stable in culture media and can be desiccated and stored for longer periods of time at −20 °C. It is recommended to add a few drops of 1 N NaOH or 1 N NHCl if it is difficult to dissolve the cytokinin compound in a media preparation (Werner and Schmülling 2009; Werner et al. 2001).

Gibberellins There are over 20 different compounds of gibberellins, but not all of them are biologically active and as effective as GA_3 which is the most common and easily assimilated form of this plant hormone. Gibberellins are mainly involved in enhancing callus growth (Lance et al. 1976; Vasil and Thorpe 1998) and elongation of dwarf plants (Phinney 1985); are applied for controlling bolting and flowering of long-day plants (Jung et al. 2020), breaking the dormancy (Finkelstein et al. 2008; Bentsink and Koornneef 2008), inducing parthenocarpy, fruit ripening, accelerating the growth leading to early seed production, increasing stem length; and are also used as a substitute for cold treatment for desired results (Yamaguchi 2008). Gibberellic acids are mainly biosynthesized from trans-geranylgeranyl diphosphate (GGDP) in the growing organs of higher plants by methylerythritol phosphate (MEP) pathway (Hedden 2002; Hedden and Thomas 2012).

Abscisic acid It is a type of plant hormone that is mainly secreted during times of environmental stresses and can be supplemented in media to inhibit or stimulate callus growth depending upon certain plant species. During external stresses such as cold, drought, heat, and high salinity, this hormone is rapidly accumulated helping the plant to adapt and survive under such harsh conditions. This ‘stress hormone’

also plays important roles throughout the life of the plant in various processes such as embryo development, seed maturation, seed dormancy and germination, enhancing shoot proliferation, establishment of the seedling, inducing vegetative growth and development, root growth, controlling stomatal pore opening and closure to prevent transpiration and evaporation, plant flowering, pathogen response regulation, and plant senescence. It is transported through the vascular tissues in plants and coordinates between root and shoot development functions (Li et al. 2017).

Utilization of plant hormones in plant tissue culture media should be carefully monitored as they are comparatively expensive ingredients and are required in minute quantities. A minor increase than the desired quantity or an overdose can lead to deleterious results and may inhibit the plant mechanisms vital for life sustenance. For in vitro cultivation of quinoa, Murashige and Skoog (MS) basal media without any growth regulators can be used. Quinoa seeds can be grown on both half MS media (half concentration) with 5% sucrose and standard full concentration MS media. Better shoot and root growth as well as early sprouting was observed in standard MS composition with 0.6% agar.

It is preferred to prepare the culture media in a microbiological laboratory equipped with all of the required tools, devices, and safety measures that can be undertaken in case of any mishandling of ingredients. The compartment should be constructed in such a way so as to provide ease in cleaning, adequate space for work, proper ventilation and lighting. Properly set up systems of water sterilization (autoclave) and deionization (distillation unit) are mandatory as only autoclaved double-distilled water is to be used for stock preparation. The devices required for media and stock preparation include accurately calibrated analytical weighing balance; refrigerators: one maintained at 4 °C for storage of prepared media plates and cultures and another one at −20 °C for storage of plant hormones, vitamins, and other heat-sensitive material for longer durations; hot plate with magnetic stirrer to melt and mix the ingredients; pH meter; autoclave for media sterilization; membrane filter; heater or oven; Bunsen burner and laminar airflow. Required chemicals, properly cleaned and dried glassware, and tools must be arranged before starting the experiment. It has been widely established that mistakes that occur in tissue culture practices mainly originate from inaccurate media preparation, faulty use of ingredients and dirty glassware, so care should be taken and work should be meticulously monitored.

Appropriate personal protective equipment (PPE) is mandatory while working in a laboratory which includes lab coats, protective gloves, eye protection, and face masks.

13.4 Media Composition of Murashige and Skoog Media (MS Media) (Table 13.1)

Table 13.1 MS media composition (Murashige, T. and Skoog, F. 1962)

Salts	Amount (mg/L)
Stock 1 (major salts)	
Ammonium nitrate, NH_4NO_3	16,500.00
Potassium nitrate, KNO_3	1900.00
Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$	370.00
Potassium dihydrogen ortho-phosphate, KH_2PO_4	170.00
Calcium chloride, $CaCl_2.2H_2O$	440.00
Stock 2 (minor salts)	
Potassium iodide, KI	0.83
Boric acid, H_3BO_3	6.20
Manganese sulphate, $MnSO_4.4H_2O$	22.30
Zinc sulphate, $ZnSO_4.7H_2O$	8.60
Sodium molybdate, $Na_2MoO_4.2H_2O$	0.25
Cupric chloride, $CuSO_4.5H_2O$	0.025
Cobalt chloride, $CoCl_2$	0.025
Stock 3 (iron source)	
Ferrous sulphate, $FeSO_4.7H_2O$	27.80
Disodium EDTA, $Na_2.EDTA.2H_2O$	37.30
Stock 4 (vitamins)	
Glycine	2.00
Myo-inositol	100.00
Nicotinic acid	0.50
Pyridoxine HCl	0.50
Thiamine HCl	0.10

Source: Murashige and Skoog (1962, 2008)

13.4.1 Nutrient Stock Solutions for MS Medium

13.4.1.1 Macroelement Stock (MS- I) in 1000 mL (Table 13.2)

Table 13.2 Macroelement stock composition

S. no.	Chemicals	Quantity in gm
		for 20× solution
1	KNO_3	38.0
2	NH_4NO_3	33.0
3	$KHPO_4$	3.4
4	$MgSO_4.7H_2O$	7.4
	Vol. mL per litre	50.0

Take 500 mL double glass-distilled water in a beaker; weigh, add, and keep on dissolving each salt sequentially (dissolve by magnetic stirring); and finally make up the volume to 1000 mL by again adding double glass-distilled water. Store at 4 °C.

13.4.1.2 Calcium Stock (MS-II) in 1000 mL (Table 13.3)

Table 13.3 Calcium stock composition

S. no.	Chemicals	Quantity in gm
		for 20× solution
1.	$CaCl_2.2H_2O$	8.8
	Vol. mL per litre	50.0

It is recommended to prepare calcium stock separately, instead of adding it to the macro stock as calcium salts may sometimes result in precipitation. Take 500 mL double glass-distilled water in a beaker, weigh and add $CaCl_2.2H_2O$ and keep on dissolving (dissolve by magnetic stirring), and finally make up the volume to 1000 mL by adding double glass-distilled water. Store at 4 °C.

13.4.1.3 Microelement Stock (MS-III) in 1000 mL (Table 13.4)

Table 13.4 Microelement stock composition

S. no.	Chemicals	Quantity in mg
		for 40× solution
1	H_3BO_3	248
2	$MnSO_4.H_2O$	675.6
3	$ZnSO_4.7H_2O$	344
4	KI	33.2
5	$Na_2MoO_4.2H_2O$	10
6	$CuSO_4.5H_20$	1
7	$CoCl_2.6H_20$	1
	Vol. mL per litre	25.0

Take 500 mL double glass-distilled water in a beaker; weigh, add, and keep on dissolving each salt sequentially in the mentioned order (dissolve by magnetic stirring); and finally make up the volume to 1000 mL by adding more double glass-distilled water. Store at 4 °C.

13.4.1.4 MS Iron-EDTA Stock (MS-IV) in 1000 mL (Table 13.5)

Table 13.5 Iron-EDTA stock composition

S. no.	Chemicals	Quantity in gm
		for 20× solution
1.	$Na_2EDTA.2H_2O$	0.745
2.	$FeSO_4.7H_2O$	0.557
	Vol. mL per litre	50.0

Take 1000 mL double glass-distilled water in amber-coloured bottle, and warm the water near boiling. Now weigh and add $Na_2EDTA.2H_2O$ while stirring under magnetic stirrer; after $Na_2EDTA.2H_2O$ has been dissolved, add the mentioned amount of $FeSO_4.7H_2O$ gradually. Store at 4 °C.

13.4.1.5 MS Vitamin Stock (MS-V) in 1000 mL (Table 13.6)

Table 13.6 Vitamin stock composition

S. no.	Chemicals	Quantity in mg
		for 20× solution
1	Myo-inositol	2000
2	Glycine	40
3	Thiamine HCl	2
4	Niacin or nicotinic acid	10
5	Pyridoxine HCl	10
	Vol. mL per litre	50.0

Take 500 mL double glass-distilled water in a beaker; weigh, add, and keep on dissolving each salt in a sequential manner; and finally make up the volume to 1000 mL. Store at 4 °C. Vitamin stock is prone to microbial contamination. Therefore, always check the stock solution for turbidity or visible microbial growth before use.

As better practice, Myo-inositol powder is added separately in the final media mix even though it is a vitamin. So it is suggested to not add Myo-inositol to the vitamin stock solution. Here, it's composition is added in case a researcher decides to add it along with the vitamin stock solution.

13.4.1.6 Preparation of 1 L MS Basal Medium (Table 13.7)

Table 13.7 Stock solution composition to make 1 L of MS basal media

S. no.	Stocks	Volume to be added (mL)
1	Macro MS-I (20×)	50
2	Calcium MS-II (20×)	50
3	Micro MS-III (40×)	25
4	Iron MS-IV (20×)	50
5	Vitamins MS-V (20×)	50
6	Myo-inositol	100 mg
7	Sucrose	30 g
8	Agar	8 g

Add stock components, followed by sucrose and mix well. Check and adjust the pH for 5.8–5.9 before making up the volume to 1000 mL by adding double glass-distilled water. Add agar as gelling agent.

A convenient and widely adopted method for tissue culture media preparation is the preparation of concentrated stock solutions and storing them at 4 °C for multiple uses. Every time the medium needs to be prepared, a certain amount of stock solution can be diluted and used without having to go through the entire process of collecting and weighing the chemicals again, saving both time and energy. Iron stock should be prepared separately as iron-EDTA complex. Vitamin stock solution must be stored at −20 °C for longer periods of time or can be placed in a refrigerator at 4 °C for about 2 months only.

Other media suitable for tissue culture and plant cell maintenance is Gamborg's B5 (Gamborg et al. 1968, 1976; Gamborg and Wetter 1975) media whose composition is as follows:

13.5 Media Composition of Gamborg's B5 Media

13.5.1 Macroelement stock composition (Table 13.8)

Table 13.8 B5 media macroelement stock composition

Chemical ingredients	Formula	mg/L
Ammonium sulphate	$(NH_4)_2SO_4$	134.000
Calcium chloride	$CaCl_2.2H_2O$	150.000
Potassium nitrate	KNO_3	2500.000
Magnesium sulphate	$MgSO_4.7H_2O$	250.000
Sodium phosphate monobasic	$NaH_2PO_4.H_2O$	150.000

13.5.2 Microelements (Table 13.9)

Table 13.9 B5 media microelement stock composition

Chemical ingredients	Formula	mg/L
Boric acid	H_3BO_3	03.000
Cobalt chloride hexahydrate	$CoCl_2.6H_2O$	00.025
Copper sulphate pentahydrate	$CuSO_4.5H_2O$	00.025
EDTA disodium salt dehydrate	Na_2-EDTA	37.300
Ferrous sulphate heptahydrate	$FeSO_4.7H_2O$	27.800
Manganese sulphate monohydrate	$MnSO_4.4H_2O$	10.000
Molybdic acid (sodium salt)	$Na_2MoO_4.2H_2O$	00.250
Potassium iodide	KI	00.750
Zinc sulphate heptahydrate	$ZnSO_4.7H_2O$	02.000

13.5.3 Vitamins (Table 13.10)

Table 13.10 B5 media vitamin stock composition

Chemical ingredients	Formula	mg/L
Myo-inositol Chemical ingredients, formula, mg/L	$C_6H_{12}O_6$	100.000
Nicotinic acid (free acid)	$C_6H_5NO_2$	001.000
Pyridoxine HCl	$C_8H_{12}CINO_2$	001.000
Thiamine hydrochloride	$C_{12}H_{17}N_4OS$	010.000

13.5.4 Carbohydrate (Table 13.11)

Table13.11 Carbohydrate composition

Chemical ingredients	Formula	mg/L
Sucrose	$C_{12}H_{22}O_{11}$	20.000 g/L

Unlike MS media, it is advised to prepare B5 media freshly, and preparation of stocks is a personal preference as concentrated solutions may sometimes lead to precipitation of salts. So, it is important to store the stocks carefully and handle them gently. Before addition of any solidifying agent, appropriate pH of both the media needs to be ensured; otherwise, the media might not solidify properly as acidic pH leads to reduced gelation forming a semi-solid, broken, or flowing gel without a firm shape and alkaline pH will lead to the formation of a hardened gel. Double glass-distilled tissue culture grade water should be used as lower grade of tap water might precipitate the salts and lead to improper gelation. After pH adjustments using 1 N NaOH/HCl, gelling agent such as agar should be properly measured and added slowly. The ingredients should be added in 2/3 of the total volume of water required,

and after they are completely dissolved, the volume should be made up to 1 L. Heat the mixture slightly on a hot plate to dissolve the gelling agent completely. Sterilization should be done using autoclave by providing wet heat at 121 °C, at 15 psi for 15 min after the mentioned pressure is attained.

13.6 Aseptic Cultivation and Maintenance

Cultivation of quinoa under controlled environmental conditions and maintenance of plants require careful attention and precision. Quinoa seeds are incubated at 25 °C and ideally given 12–16 h photoperiod to germinate under artificial light on culture media (Hesami et al. 2018).

It is advised to cultivate quinoa plants on media in a jam bottle instead of a Petri plate for two reasons: In a Petri plate, several water droplets develop on the inside of Petri plate cap as a result of condensation, which then fall and get accumulated on the media surface and disturb the plant's adherence to culture media. Moreover, high amount of water may decay the plant and roots causing serious damage. In a jam bottle, such a problem does not take place, and it becomes easy to maintain a plant.

The second reason is shoot development. In a jam bottle, shoot development, leaf growth, colour, stem vigour, etc. can be observed clearly, whereas in a Petri plate, due to lack of space, the shoot does not develop to its full potential with congested leaves overlapping each other. Also, because the stem does not stay in an upright position in a Petri plate, it curls into a coiled shape looking like a tendril which is not characteristic to the agricultural grain-producing crops.

The plant should be subcultured frequently onto freshly prepared media under aseptic conditions to provide adequate nutrients to allow the plant to grow. Care should be taken not to break or rip off the roots and root hair as they are very thin and delicate when the plant is young and may sometimes stick onto the agar surface making it difficult to pick up the whole plant at once for subculturing.

13.7 Conclusions and Future Prospects

In vitro experiments are central to understanding the plant systems at microlevel which pave way for the development of a large number of techniques to improve crop produce in agricultural fields. With such advances, even the crops with naturally low yield can be manipulated to increase yield productions, given that all of its nutritional and physical characteristics are preserved without any losses.

Improved varieties of different crops such as wheat, rice, barley, etc. are cultivated worldwide to satisfy food requirements of the population. However, quinoa has not been explored much on a genetic level, and it was only around the 1990s when scientists started to gain interest in this novel grain. Apart from nutritional composition and natural microbial interactions, there is a large scientific area around this grain crop which is still unexplored. Because the seed is already rich in amino acid content and is able to grow in low-nutrient soils, it is an ideal candidate

for experimentation to produce different varieties with variation in grain number, seed germination, improved taste, etc. Working with quinoa in laboratories is relatively easy as it grows well on MS basal media without the requirement of growth hormones and is easily maintained in subcultures. The composition of media for sufficient growth of the plant should be decided beforehand, and application of desired plant growth hormones must be regulated. Preparation of the media takes time and precision, so it is advised to prepare stocks days before starting an experiment, and freshly prepared media should be used for cultures.

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Quinoa Starch Granules as Emulsion Stabilizers

14

Ali Marefati and Marilyn Rayner

Abstract

Quinoa (*Chenopodium quinoa* Willd.) has gained recent popularity mainly due to its attractive nutritional profile and its ability to grow under extreme conditions such as salinity, acidity, drought, flooding, and frost as well as the functionality of its component. Starch is the main component of quinoa grain which constitutes up to 60% of the dry grain and plays a crucial role in the functional properties of quinoa. Quinoa starch granules are small, polygonal, and in the range of 0.5–3 μm, with unique physicochemical properties. These unique features have created research interest in the application of the quinoa starch for functional products such as stabilizer for creating Pickering emulsions. This chapter summarizes the application of starch granules in native and modified forms as particles in the stabilization of Pickering emulsions.

Keywords

Quinoa · Starch granules · Pickering emulsions · Stabilizers · Modification · Formulation · Stability · Encapsulation

14.1 Quinoa Starch Granules

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal originating from the Andes in South America, which has been cultivated for thousands of years and constituted an important component in the diet of the Inca civilization mainly in Bolivia and Peru (Li et al. 2016; Lindeboom et al. 2005). Recently it has attracted interest for its

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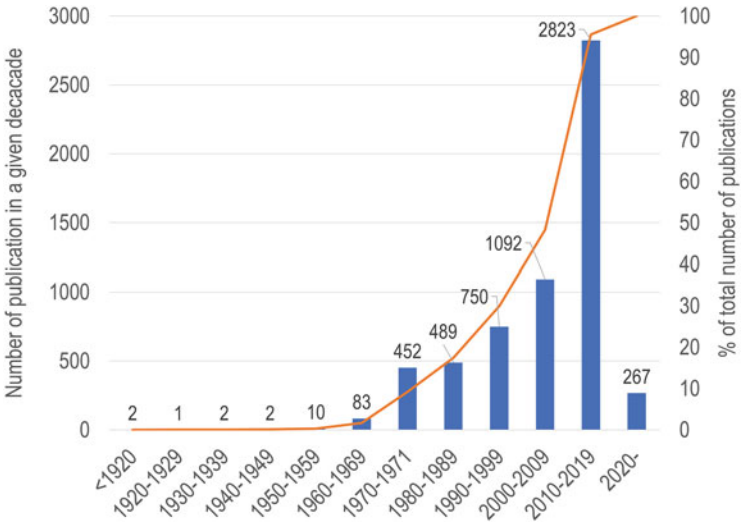

Fig. 14.1 Bibliographic analysis of publications on quinoa data obtained on June 30, 2020, in the Web of Science

unique characteristics including high nutritional value due to the quality of protein and fatty acids and its ability to grow under extreme conditions such as salinity, acidity, drought, flooding, and frost (Gonzalez et al. 1989; Li et al. 2016; Przybylski et al. 1994). The oldest publication mentioning quinoa found in the Web of Science database is from an 1876 publication in the Bulletin of the Entomological Society of France, referring to a beetle destroying an introduced plant, *Chenopodium quinoa*, from the Peruvian Andes. Since then, more than 5900 additional publications can be found on the topic, and the majority of which have been published in the past 20 years. Of the 5972 records found containing "quinoa," 540 also included "starch," and 79 included "emulsion." The bibliometric study of published publications on quinoa is presented in Fig. 14.1.

Starch is the major component of the seed which comprises up to 70% of the dry matter (Li and Zhu 2018; Lindeboom et al. 2005; Mundigler 1998). The starch is present in the form of small polygonal and unimodal granules with a narrow particle size distribution with a range of 0.5–3 μm in diameter and a mean diameter of 1.5 μm as can be seen in Fig. 14.2 (Atwell et al. 1983; Lindeboom et al. 2005; Lorenz 1990; Tang et al. 2002). The amylose content is reported to vary ranging between 3.5 and 27% (Inouchi et al. 1999; Lindeboom et al. 2005; Qian and Kuhn 1999; Tang et al. 2002). These unique features have created research interest in the application of the starch for functional products such as stabilizer for creating Pickering emulsions.

In addition to nutritional values, starch has attracted technological interest for the development of emulsion formulations for food, pharmaceutical, and cosmetic products. This chapter tries to have an overlook of starch granules as stabilizer for the formation of Pickering emulsions.

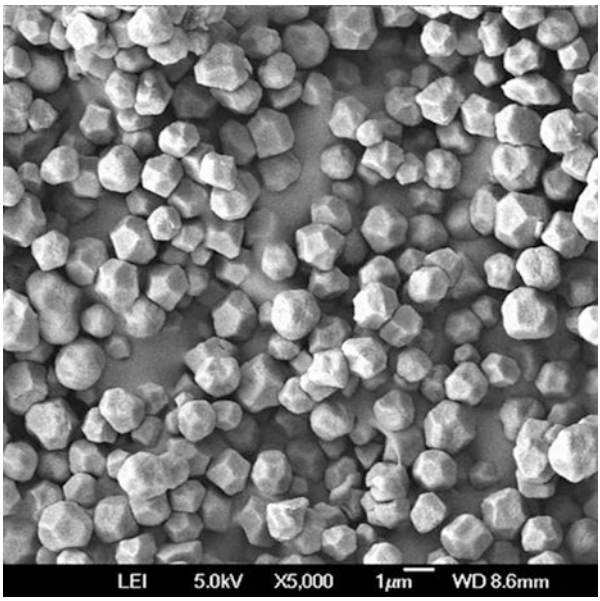

Fig. 14.2 Scanning electron microscopy image of quinoa starch granule, reprinted by permission from Marefati et al. (2017a)

14.2 Emulsions and Pickering Emulsions

Many of the food, pharmaceutical and cosmetic formulations are based on emulsions. Emulsions are often described as mixtures of at least two immiscible liquid phases (usually oil and water), with one phase dispersed in the other phase as spherical droplets. Due to the nature of the different phases that are constituting emulsions (commonly oil and water) and the large interfacial area between the dispersed and continuous phases, emulsions are thermodynamically unstable systems; thus, there is a reduction in free energy if the dispersed phase coalesces, minimizing the interfacial area (Marefati et al. 2017b). Emulsifiers are used to prevent coalescence by decreasing the interfacial tension between the phases and increasing the steric hindrances and/or electrostatic repulsion between the droplets. Surfactants (e.g., monoglycerides, polysorbates, and lecithin) and amphiphilic biopolymer emulsifiers (e.g., whey protein, caseinate, and modified starches) are common examples of emulsifiers. Since the stability of emulsions is important for the chemical, food, pharmaceutical, and cosmetic industries, there is a constant search for new and innovative emulsifiers that can improve the stability of emulsions. As a result, emulsions stabilized by colloidal particles, known as Pickering emulsions, with high stability have been vastly exploited by researchers (Binks 2002).

Solid colloidal particles can be absorbed at liquid-liquid interfaces, thus stabilizing emulsions. This phenomenon that was first described independently by Ramsden (1904) and a few years later by Pickering (1907) is named after the second scientist. This type of emulsions has regained research interest due to their long-term

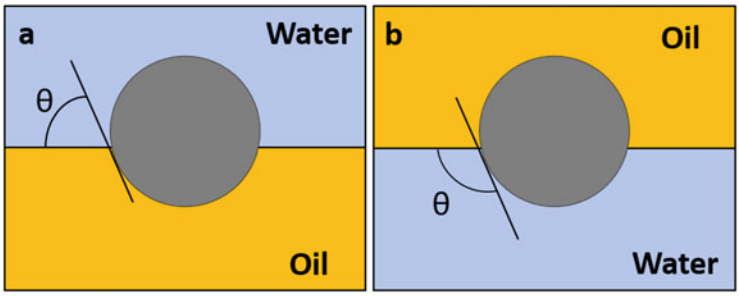

Fig. 14.3 Schematic representation of contact angle: (**a**) when lower than 90°, oil-in-water emulsions are formed, and (**b**) when higher than 90°, water-in-oil emulsions are formed

stability during the past couple of decades (Sjöö et al. 2015a, b). Despite similarities, the fundamental stabilization mechanisms of Pickering-type emulsions are different from conventional emulsifiers. The adsorption of particles at the oil-water interfaces is achieved by dual wettability of particles toward both phases, something that is characterized by contact angle (Θ) as illustrated in Fig. 14.3. Once absorbed at the interface, the particle will cause the interface to bend toward the phase that has a lower affinity for the particle. As a result of this, the more hydrophilic particles are more suitable for oil-in-water (O/W) emulsions, while more hydrophobic particles are more suitable for water-in-oil emulsions (W/O) as can be seen in Fig. 14.3 (Berton-Carabin and Schroën 2015).

Compared to the conventional emulsions stabilized by surfactants, Pickering emulsions are known to possess merits such as long-term stability due to higher stability toward coalescence and Ostwald ripening (Aveyard et al. 2003; Yusoff and Murray 2011). The higher stability of Pickering emulsions is due to the large particle sizes (>10 nm) resulting in (1) high energy of detachment, (2) prevention of close contact of droplets by providing steric hindrance, and (3) interfacial pressure that is formed at the interface between the two neighboring particles impedes mass transfer (Dickinson 2010; Matos et al. 2017; Schröder et al. 2017). The energy of detachment (ΔG) can be calculated using the following equation:

$$\Delta G = r^2 \pi \Upsilon_{OW} |1 - \mathrm{Cos}\Theta|^2 \tag{14.1}$$

where r is the particle radius (m), Υ_{OW} is the interfacial tension between the oil and water (N/m), and Θ is the particle-oil-water contact angle (Binks 2002). Therefore, the large particle sizes result in the formation of a thick and irreversibly adsorbed barrier that protects emulsions against destabilization providing that the contact angle is optimum.

In addition to large particle sizes and the attributes they bring about in stabilization of Pickering emulsions, particle-particle interactions participate in the stability of Pickering emulsions in three main ways:

(a) Formation of capillary forces due to particle-particle interactions between two adjacent particles on the same droplet interface results in the formation of interfacial pressure that prevents mass transfer across the interface (Matos et al. 2017; Schröder et al. 2017).
(b) Formation of bridges between droplets as a result of the interactions of particles that are adsorbed at the interface of neighboring droplets can create clusters or aggregates in the form of three-dimensional networks of emulsion droplets that support the stability of the emulsions (Lam et al. 2014; Schröder et al. 2017).
(c) Creation of a gel-like structure as a result of the interparticle interactions between the excess of particles in the continuous phase which leads to the formations of networks of aggregated particles that can increase the apparent viscosity of the emulsions and thereby, decrease the rate of gravitational separation (Dickinson 2010).

Pickering particles have shown to have several advantages over conventional stabilizers such as lower toxicity, lower irritation to the skin, lower negative impact on gut health, and, finally, lower contamination for the environment (Chassaing et al. 2015; Marefati et al. 2017a; Qi et al. 2014; Wahlgren et al. 2013). Furthermore, the application of Pickering particles can eliminate the common problems associated with surfactants including air entrapment, foaming, irritancy, and interaction with living matter (Frelichowska et al. 2009, 2010). Traditionally, inorganic or synthetic particles such as latex, silica, and clay have been used as Pickering emulsifiers. As a result, the number of particles that are bio-accessible and biocompatible is still limited which, in turn, limits the applicability of Pickering emulsions within the food and pharmaceutical industries (Aveyard et al. 2003). From the number of available colloidal particles that are proposed for stabilization of Pickering emulsions, only a few are acceptable in the food industry. These food-based Pickering particles include carbohydrate-based, protein-based, and lipid-based particles (Berton-Carabin and Schroën 2015). Recently, there has been a new demand toward formulations based on natural and renewable ingredients which makes the application of natural colloidal particles more desirable (Berton-Carabin and Schroën 2015; Sarkar et al. 2016; Sjöö et al. 2015a, b). Starch is one of the best candidates to be used as colloidal particle for stabilization of Pickering emulsions.

14.3 Starch Granules as a Stabilizer for Pickering Emulsions

Following cellulose, starch is the most abundant biopolymer found in the nature that is synthesized and stored in the form of granules in different plants. In addition to being abundant, starch is tasteless, colorless, odorless, non-allergic, and inexpensive. Starch is deposited by plants in the form of granules. Depending on the botanical source, starch granules are different in shape, size, and composition which affects physicochemical properties as well as functional properties of starches. Concerning the size, starch granules are classified into (1) large granules (30–100 μm) such as potato and canna; (2) medium granules (5–30 μm) such as barley, maize, sorghum,

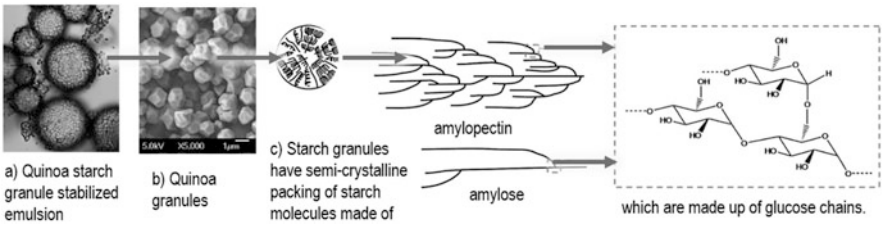

Fig. 14.4 Starch granules as particles for stabilization of Pickering emulsions

and tapioca; (3) small granules (2–10 μm) including rice, oat, and buckwheat; and, finally, extremely small granules (0.3–2 μm) including quinoa, amaranth, cow cockle, and pigweed (Hall and Sayre 1971; Jane et al. 1994; Marefati et al. 2017b). Different types of starches have been used in the formulation of Pickering emulsions in previous studies (Marefati et al. 2017b; Saari et al. 2016; Simsek et al. 2015; Timgren et al. 2013). The starches included in these studies were rice, maize, potato, wheat, quinoa, amaranth, tapioca, barley, and oat. Among different types of starches, small starch granules can provide more surface coverage per unit of mass, and therefore they are more suitable for stabilization of Pickering emulsions. The relative amount of particles required to stabilize a given droplet size can be estimated using the theoretical maximum coverage (Γ_M in mg m^{-2}) in the following equation:

$$\Gamma_M = \rho_{sg} \frac{2}{3} d_{sg} \times 10^6 \tag{14.2}$$

where ρ_{sg} is the starch density (≈1500 kg m^{-3}), d_{sg} is the surface mean diameter of the starch granule (d_{32}), and φ is the packing density. The assumptions are the starch particles are identical and spherical and are attached at the oil-water interface at a contact angle of 90° with an interfacial packing fraction $\varphi \approx 0.9$, i.e., hexagonal close packing (Rayner et al. 2012a, b).

Since starch is hydrophilic and adsorbed particles at the interface will bend the interface toward the phase with the lower affinity, starch is suitable for the formulation of oil-in-water (O/W) Pickering emulsions (Fig. 14.4). In other words, the dispersibility of starch granules in water makes them a suitable candidate for the formation of oil-in-water (O/W) emulsions. This property makes starch granules more suitable for the food industry, where most emulsions have an aqueous continuous phase. In addition to being attractive due to the superior stability of their resulting emulsions, application of micron-sized starch particles eliminates the negative public impression on nano-sized ingredients such as silica and crystalline cellulose (Ali et al. 2015).

Although native starch granules have successfully been used for the development of Pickering emulsions (Li et al. 2013; Marefati et al. 2017b; Timgren et al. 2013), the emulsifying capacity has shown to improve by physical or chemical modification (Abdul Hadi et al. 2020a, b; Marefati et al. 2018, 2017b; Rayner et al. 2012a, b; Simsek et al. 2015; Timgren et al. 2011). Among all varieties of starches tested,

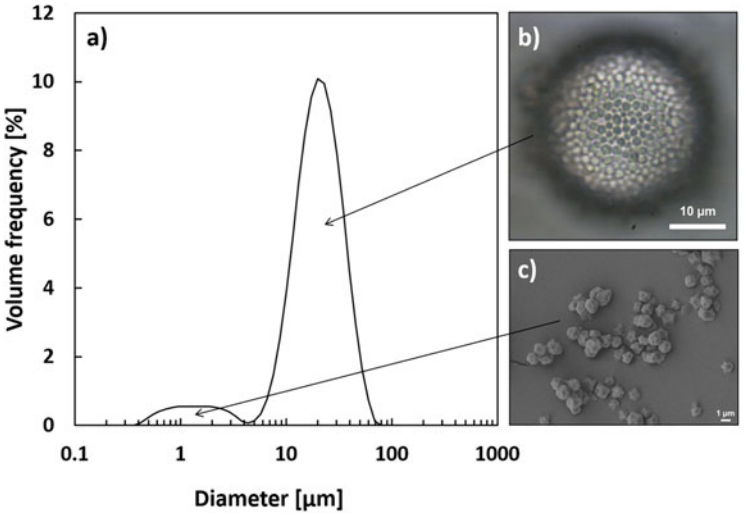

Fig. 14.5 (**a**) Particle size distribution of quinoa starch granule-stabilized Pickering emulsions, (**b**) microscopy image of a droplet where individual starch granules can be seen, and (**c**) SEM image of quinoa starch granules

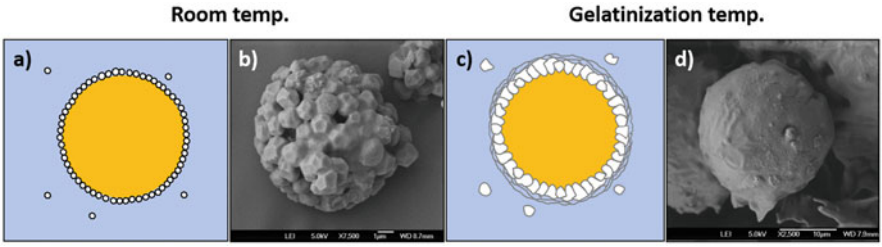

Fig. 14.6 Schematic representation and SEM image of non-heat-treated (NHT) quinoa starch-stabilized Pickering emulsion (**a**, **b**) and schematic representation and SEM image of heat-treated (HT) quinoa starch-stabilized Pickering emulsion (**c**, **d**)

quinoa (Fig. 14.5) has shown to be the best candidates for stabilization of Pickering emulsions followed by rice, with a large margin (Marefati et al. 2017b; Simsek et al. 2015; Timgren et al. 2013). The higher emulsifying capacity of quinoa starch granules is discussed in the following section.

Due to the special physicochemical properties of starch, under certain conditions of heat and moisture, starch can be gelatinized. During gelatinization, starch absorbs water and swells. The swelling of starch results in an increase in the granule sizes to 3.8 µm, compared to the original size of the granules with 1.8 µm (Marefati et al. 2017a, b). Gelatinization is an important feature of starch that provides its unique exceptional functional properties. Application of a careful heat treatment on starch granule-stabilized Pickering emulsions causes in situ partial gelatinization of starch at the oil-water interface (Fig. 14.6). The gelatinized layer of starch at the oil-water

interface forms a cohesive barrier around the droplets that leads to improved functional properties of the interfacial barrier resulting in higher stability during storage, process, encapsulation, and digestion stability of starch granule-stabilized Pickering emulsions (Marefati et al. 2013, 2015, 2017a, b; Sjöö et al. 2015a, b; Timgren et al. 2011).

During gelatinization, the starch granules swell and partially merge together which if happens to the neighboring starch granules on the same droplet can lead to the formation of a cohesive layer of gelatinized starch on the surface of the droplets. If gelatinization happens to the starch granules that are adsobed on the neighboring droplets, it can lead to the formation matrices composed of three-dimensional networks of gelatinized starch where the oil droplets are entrapped in (Marefati et al. 2013, 2015). To achieve individually existing emulsion droplets, heat treatment procedure should be accompanied by a moderate mixing as was reported by Marefati et al. (2017a) and can be seen in Fig. 14.7.

14.4 Isolation and Modification of Quinoa Starch

14.4.1 Isolation of Quinoa Starch

The isolation of starch varies depending on its botanical origin. Due to the unique and inimitable protein composition of quinoa starch, the isolation of starch is rather more complicated; however, unlike rice starch, the isolation procedure for quinoa starch is not extensively industrialized. Isolation of quinoa starch has been previously performed using alkali or enzymatic procedures to remove the protein impurities (Fig. 14.8). In a study using alkali procedure by Rayner et al. (2012a, b), quinoa seeds were soaked in distilled water in cold storage before milling into a smooth pulp using a blender (Philips HR7625, the Netherlands). The mixture was then filtered by cheesecloth and rinsed with distilled water. The starch slurry was allowed to settle and the supernatant was removed. The sediment layer was re-dispersed in water and centrifuged at 3000 × g for 10 min, and the supernatant and the gray layer on the top of the sediment layer which included proteins and fibrous material were removed. Thereafter, the remaining proteins and impurities were removed by washing the starch with alkali solution (0.3% NaOH) for two times and centrifuged as explained earlier, and the supernatant and the top layer including impurities were removed. The alkali was then neutralized using citric acid (pH 4.5) and then washed with distilled water for two times and then centrifuged. The compact starch layer at the bottom of the centrifuge tubes was scraped off and spread on trays to dry at room temperature (Fig. 14.8a). In another method using an enzymatic procedure for removal of protein impurities by Marefati et al. (2017b), the grains were dry-milled, and the flour was suspended in water and mixed. Thereafter, enzymatic hydrolysis was used to improve protein separation using a commercially available enzyme (Alcalase 2.4 L FG, Novozymes A/S, Bagsvaerd, Denmark) followed by mixing with a screw loop mixer (type 50, DMT, Germany) and a high-pressure homogenizer. The starch and fiber were then separated by sieving.

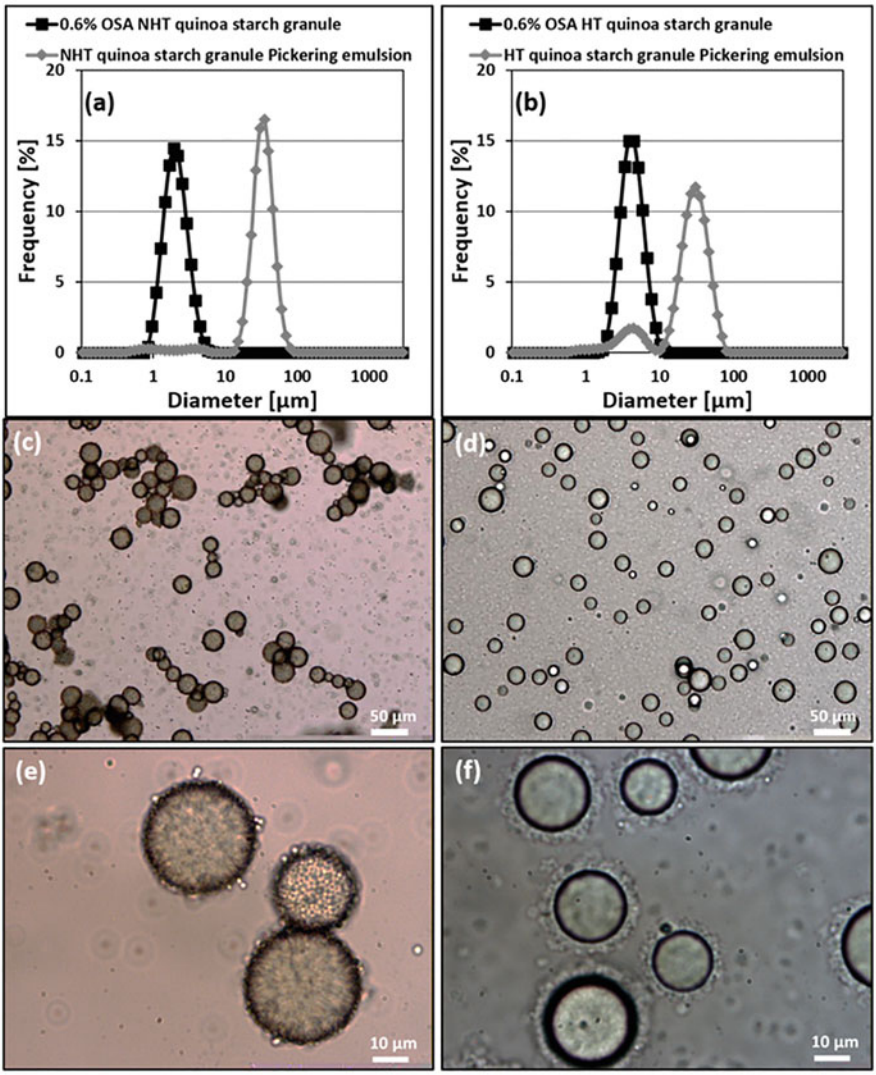

Fig. 14.7 Particle size distribution of quinoa starch granules and emulsion in non-heat-treated (NHT) and heat-treated (HT) forms (**a**, **b**) and light microscopy images of non-heat-treated and heat-treated emulsions (**c**–**f**), reprinted with permission from Marefati et al. (2017a)

Thereafter, the proteins were removed in two steps, first using a decanter and then by centrifugation and manual scraping. Finally, the starch was dried using a spray drier (type Minor Production, Niro A/S, Denmark) at an input and output temperatures of 180–80 °C, respectively (Fig. 14.8b). Since enzymes act selectively and specifically on proteins, the enzymatic method leads to a minimal (if any) degradation of starch granules and can be used to remove the production of a high volume of alkaline

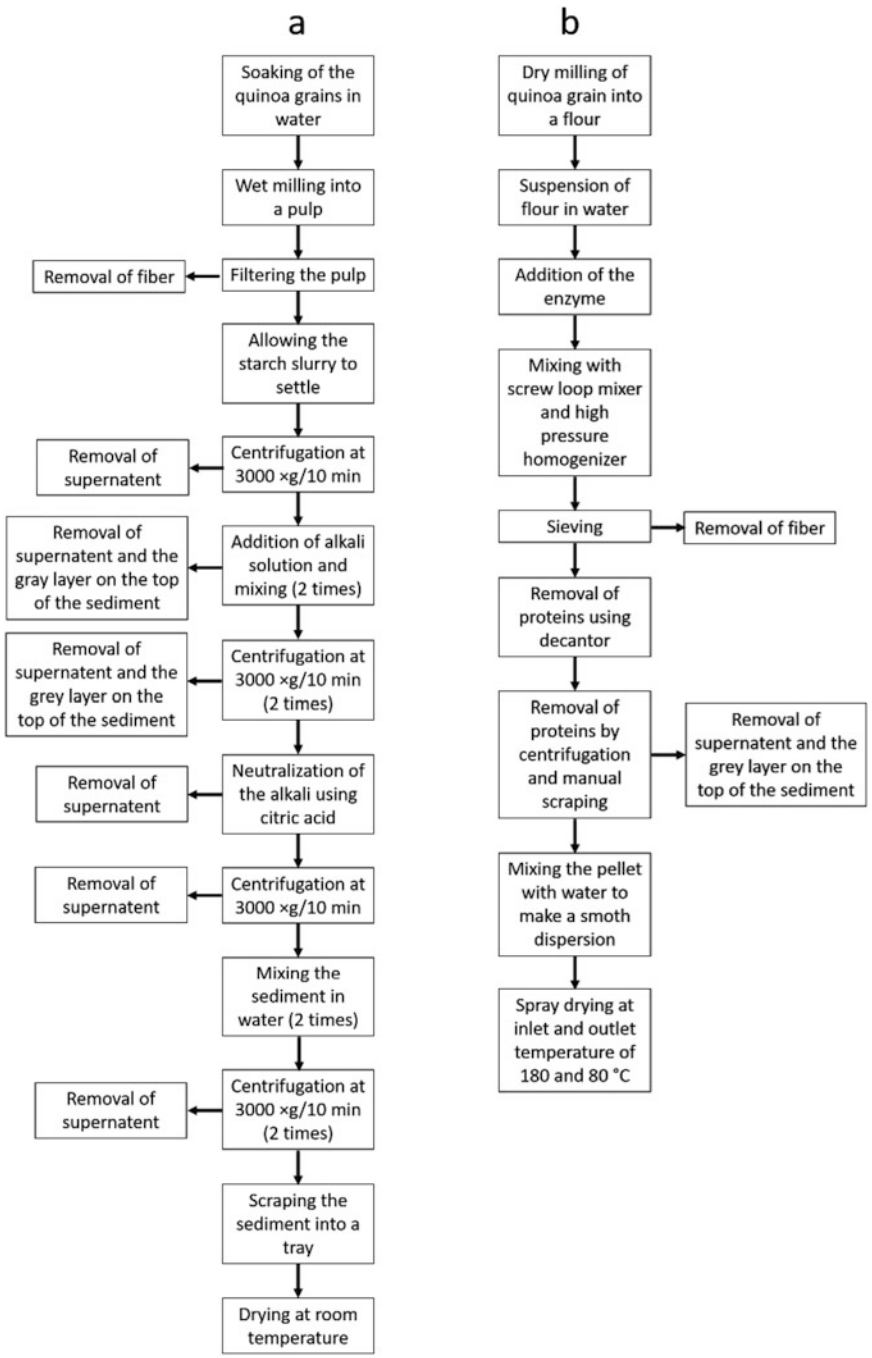

Fig. 14.8 Flowchart of (**a**) alkali and (**b**) enzymatic methods for isolation of quinoa starch

waste. Still, the extent of the effect of isolation methods on the physicochemical properties of quinoa starch, such as morphological properties, pasting, and thermal and functional properties, needs to be investigated further.

14.4.2 Modification of Starch

In some applications, the properties of starch are not optimal, and therefore, modification is used to improve the functional properties of starches. Such modification can provide a wide range of novel and value-added starch-based material with new functional properties for industrial applications. To improve the functional properties of native starch, it can be modified using physical, chemical, or enzymatic methods. These procedures involve alteration of morphology, surface proteins, or cross-linking, esterification, etherification, oxidation, etc. Although quinoa starch granules have shown to have an emulsifying capacity even in native form, the hydrophobicity can be improved by physical or chemical modification. From all modification techniques used for modification of starches, physical modification by dry heat treatment and chemical modification by esterification have been used to improve the emulsifying capacity of the quinoa starch granules.

14.4.2.1 Physical Modification of Starch

Thermal modification or heat treatment has been used to enhance surface hydrophobicity of starch granules which acts by modification of surface proteins of starch (Madivala et al. 2009; Rayner et al. 2012a, b; Seguchi 1984). Heat treatment can optimize the emulsifying capacity of starch. In this method, the starch powder is heated at 120 °C for different lengths of time (30, 60, 90, 120, and 150 min, respectively). The starch is then cooled down to the room temperature before being used as a Pickering emulsifier.

14.4.2.2 Chemical Modification of Starch

The modification of starch granules with hydrophobic groups including alkenylsuccinic anhydride, octenyl succinic anhydride (OSA), dodecyl succinic anhydride (DDSA), and fatty acid anhydrides to increase the emulsifying capacity of starch granules has been used frequently (Abdul Hadi et al. 2020a, b; Li et al. 2019a; Marefati et al. 2017b; Rayner et al. 2012a, b). OSA modification is commonly used to increase the hydrophobicity of starch. The chemical modification of starch by OSA is achieved in a mild alkali environment. The starch powder is first mixed with water to prepare a starch slurry, and depending on the method, the pH is set to 7.6 (Rayner et al. 2012a, b) or 8.2–8.4 (Marefati et al. 2017b) using HCl or NaOH (1 M). Then, the desired amount of OSA is divided into four portions added to the starch slurry at 15-min time intervals. During this time, the pH is maintained at 7.6 using an automated titration machine, and when the pH is constant for at least 15 min, the modification is considered complete. The mixture is then centrifuged at 3000 × g for 10 min, and the supernatant is discarded. The starch will then be washed with distilled water twice by the addition of water and centrifugation as

explained earlier followed by a wash with citric acid (pH 4.5) to neutralize the remainder of alkali and another two times with distilled water. Finally, the OSA-modified starch is spread on trays and left to be dried at room temperature for at least 48 h (Fig. 14.9a). The degree of modification is commonly quantified by titration method (Rayner et al. 2012a, b; Timgren et al. 2013); however, nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR), or even direct stoichiometric methods have also been proposed (Abdul Hadi et al. 2020a, b; Sweedman et al. 2013).

In addition to OSA, quinoa starch has been modified using acid anhydrides with different chain lengths of short-chain fatty acids (SCFA) to improve its emulsifying capacity (Abdul Hadi et al. 2020a, b). The process for SCFA modification of starch is also in an alkali solution and similar to that of OSA as can be seen in Fig. 14.9b (Abdul hadi et al. 2020b).

Compared to other starches, the reaction efficiency of quinoa starch granules to chemical modification with OSA is higher (Marefati et al. 2017b). This could be due to larger available surfaces for modification of starch granules both due to the smaller granular sizes and the presence of cracks and pores as was shown by measurements of specific surface area and pore size distributions (Marefati et al. 2018). In this study, measurements of specific surface area and pore size distributions of starch granules were carried out using the Brunauer-Emmett-Teller (BET) adsorption isotherm equation, and the results showed that compared to the surface area calculated by surface mean diameter (Sauter diameter or d_{32}), the surfaces measured by BET measurements were 40% larger for starch granules due to cracks and pores. In contrast, the surface area measured by BET for rice was around 20% smaller than calculated surface area using the surface mean diameter which indicated the absence of pores and cracks and presence of aggregated granules. The absence of pores in rice has been indicated in another study by Cai et al. (2015).

14.5 Application of Quinoa Starch Granules as Pickering Emulsifiers

Starches with smaller granular sizes have shown to have better performance in the stabilization of Pickering emulsions. The higher emulsifying capacity of starch granules is due to larger surface coverage provided by smaller granules at a given mass of starch particles. The specific surface area of the starch granules (S) can be calculated using the following equation where ρ is the density of starch (1500 kg/m^3) and D_{32} is the surface-weighted mean diameter of starch granules:

$$S = \frac{6}{\rho D_{32}} \tag{14.3}$$

Marefati et al. (2018) have calculated the specific surface area for rice, quinoa, and amaranth starch granules as presented in Table 14.1. We have calculated and added the specific surface area of corn, tapioca, potato, and wheat starches based on

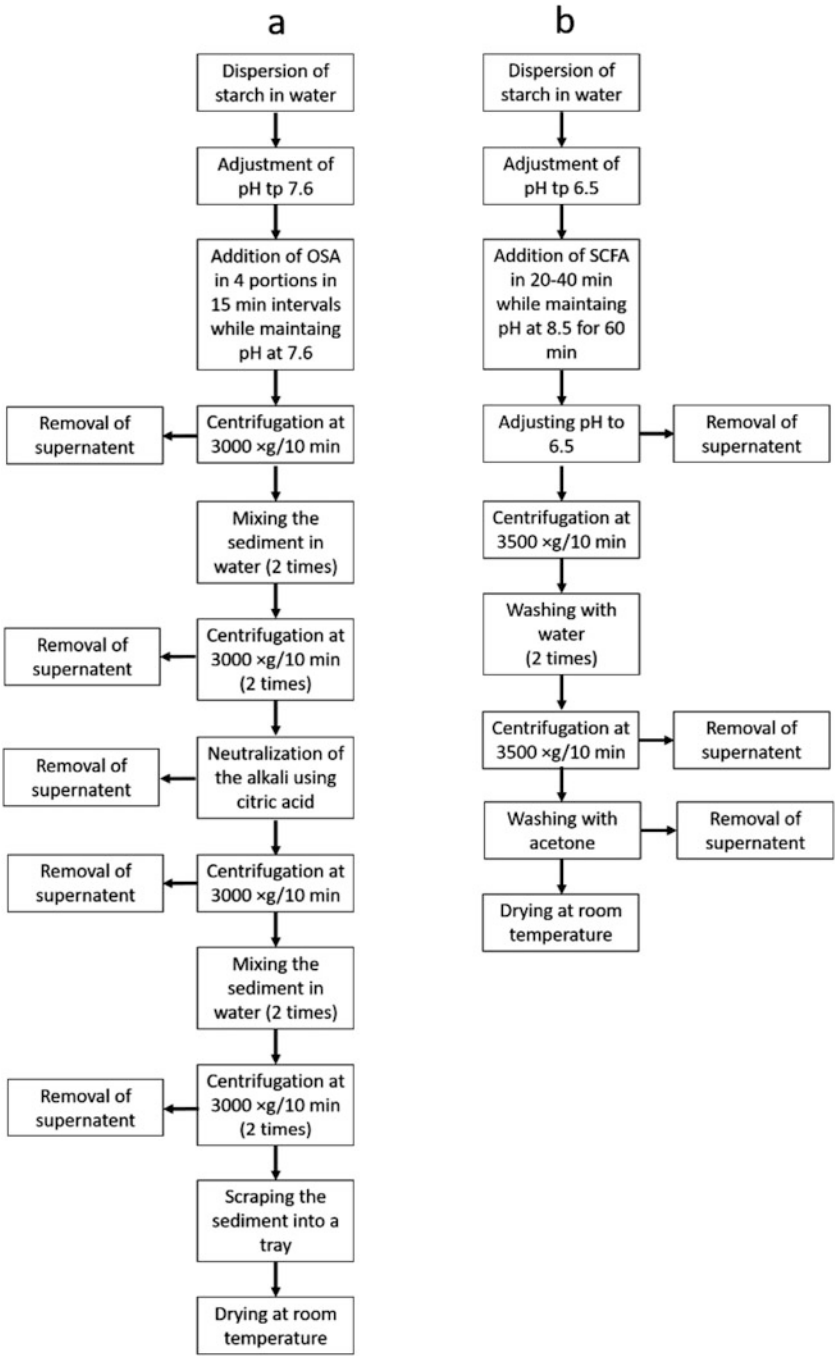

Fig. 14.9 Modification process for quinoa starch granules: (**a**) modification with OSA and (**b**) modification with SCFA

Table 14.1 The specific surface area of starch granules based on mean surface diameter: data for rice, quinoa, and amaranth, from Marefati et al. (2018), and data for corn, tapioca, potato, and wheat calculated based on d_{32} from Simsek et al. (2015)

Starch type	D_{32} [μm]	S [m^2/g]
Rice	3.29	1.22
Quinoa	2.02	1.98
Amaranth	1.40	2.86
Corn	7.80	0.51
Tapioca	7.59	0.53
Potato	21.58	0.19
Wheat	10.25	0.39

Eq. 14.3 and the surface-weighted diameter from Simsek et al. (2015), and the results are presented in Table 14.1.

According to Eq. 14.3 and Table 14.1, the order of surface coverage provided by these starches is directly correlated to the surface mean diameter as follows: amaranth > quinoa > rice > tapioca > corn > wheat > potato. Besides, amaranth, quinoa, and rice starch granules that can provide larger surfaces to cover belong to extremely small and small granular starches as it was pointed out earlier and were the only starches that could produce emulsions. However, despite the larger specific surface area of amaranth, quinoa produced better emulsions as was reported previously (Marefati et al. 2018, 2017b). Therefore, it seems that in addition to the specific surface area, other factors are involved in the emulsifying capacity of starch granules. Marefati et al. (2017b) discussed that the higher emulsifying capacity of starch granules was due to the higher levels of surface proteins that can act as hydrophobic domains and are necessary for optimized wettability of particle at the oil-water interface.

14.6 Applications of Native Starch

Unlike other types of starch that need to be modified to be able to act as emulsion stabilizers, native quinoa starch has shown to be able to stabilize emulsions. The emulsifying capacity of native quinoa starch granules has been attributed to the higher levels of protein in quinoa starch compared to starches from other sources (Marefati et al. 2017a). Since the majority of proteins are on the surface of the starch granules as was described by Baldwin (2001), the protein molecules can provide hydrophobic domains to enhance the wettability of the starch granules for adsorption at the oil-water interfaces. The effect of the protein on the emulsifying capacity has been further investigated by Kierulf et al. (2020) where they investigated starches with varying levels of proteins and established a positive relationship between protein level and emulsifying quality. Timgren et al. (2013) showed that native starch granules can stabilize emulsion droplets at concentrations as low as 100 mg/mL oil; however, the volume-weighted mean diameter (D_{43}) of droplets achieved in

this way was 370 μm and much larger than a more recent study by Marefati et al. (2018) with the D_{43} size of 74 μm using the same formulation parameters. When a higher amount of starch granules was used (200 mg/mL), due to the higher surface coverage provided by the extra amounts of starch, the average droplet size dropped to around 33 μm.

14.7 Applications of Modified Starch

To improve and diversify the functional properties of starch, quinoa starch granules have been modified using physical and chemical modifications. Some aspects of these modified starches are discussed in the following sections.

14.7.1 Applications of Physically Modified Starch

In addition to chemical modification, as explained earlier, quinoa starch granules can be modified using physical treatments such as heat. Heat treatment modifies the surface proteins of starch granules and thereby, their wettability, making them more hydrophobic for adoption at the oil-water interface of emulsion droplets. Rayner et al. (2012a, b) investigated the effect of different lengths of time of heat treatment (120 °C for 30, 60, 90, 120, or 150 min) to improve the surface hydrophobicity of starch granules and compared the microstructure of emulsions stabilized using dry heat-treated starch granules with native and OSA-modified starches. The results showed that only heat treatment for 150 min at 120 °C improved the emulsifying capacity of starch compared to native counterparts which interestingly in their case did not have emulsifying capacity. As can be seen in Fig. 14.10, increasing the length of thermal treatment resulted in improving the hydrophobicity of starch granules which was accompanied by a higher degree of adsorption of starch granules to the surface as was indicated by smaller peak representing free starch which, in turn, gave rise to the emergence of the peak representing emulsion droplets.

These results also indicated that heat treatment increased the particle size of starch granules significantly that could be due to limited aggregation. This study also showed that the emulsifying capacity of heat-treated quinoa starch granules was lower than OSA-modified starch granules.

14.7.2 Applications of Chemically Modified Starch

The most widely used modification is esterification with acid anhydride such as octenyl succinic anhydride (OSA) under mild alkali condition as explained earlier. The OSA-modified starch with a degree of modification up to 3% based on the dry weight of starch is approved food ingredient (E1450) and pharmaceutical excipient with no limit on application (Bhosale and Singhal 2006; Timgren et al. 2011). Other chemical modification methods include esterification with short-chain fatty acid

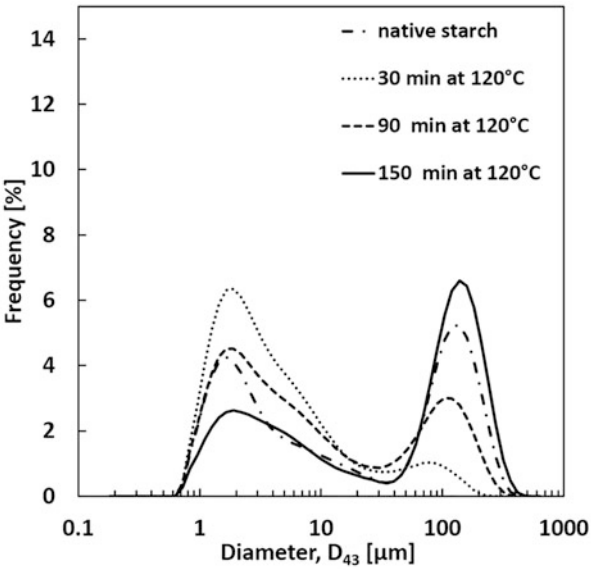

Fig. 14.10 The effect of heat treatment on increasing the emulsifying capacity of quinoa starch granules, from Rayner et al. (2012a)

(SCFA) anhydride or phthalic anhydride (Abdul Hadi et al. 2020a, b; Tan et al. 2014). In addition, modification of starch granules using dodecyl succinic anhydride has been studied (Li et al. 2019a, b). From the data presented in a study of modification with short-chain fatty acids with different chain length (acetic acid C = 2, propionic acid C = 3, and butyric acid C = 4), it was shown that increasing the chain length results in increasing the hydrophobicity of starch which in turn resulted in better affinity or optimized wettability for starch at the oil-water interface (Abdul Hadi et al. 2020a, b). However, at the highest modification level of butyrylated starch, the emulsion size distribution decreased, which was presumably due to aggregation of starch granules which in turn provided lower surface coverage. Comparison of these results with the result of quinoa starch granules modified by OSA, with 9-carbon chain length, showed that the longer chain of OSA could improve the emulsifying capacity of starch granules even more. Li et al. (2019a, b) proposed that compared to OSA with 9-carbon chain, dodecyl succinic anhydride (DDSA) with 12-carbon chain could improve the hydrophobicity of starch granules even further and DDSA-modified starches have higher hydrophobicity at a lower level of modification. The structures of common hydrophobic groups used in the modification of starch granules are presented in Fig. 14.11.

14.8 Formulations of Pickering Emulsions Using Starch Granules

Quinoa starch granules provide highly compatible systems concerning technological formulation criteria. Pickering emulsions stabilized by starch granules have shown to be stable over different preparation and processing conditions such as a large

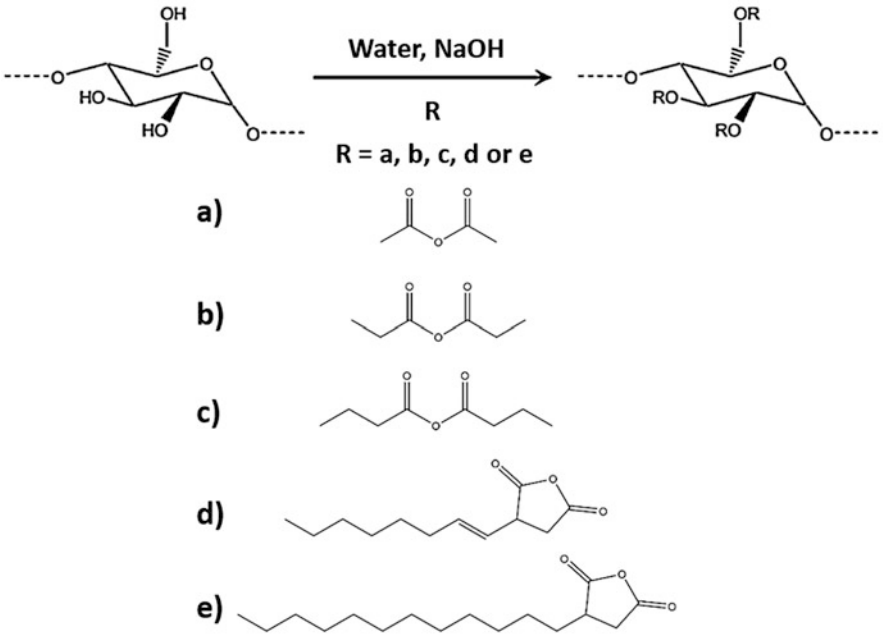

Fig. 14.11 Chemical modification process and the chemical structure of common hydrophobic groups used in the modification of quinoa starch granules: (**a**) acetic anhydride, (**b**) propionic anhydride, (**c**) butyric anhydride, (**d**) octenyl succinic anhydride, and (**e**) dodecyl succinic anhydride, adopted with permission from Sweedman et al. (2013)

range of pH and salt concentration, as well as within a large range of oil and starch content (Li et al. 2019b; Marku et al. 2012; Rayner et al. 2012a, b). Furthermore, it has been shown that these formulations are stable against harsh conditions during common processing procedures in the food industry such as shear, freeze-thaw cycling, and freeze-drying (Marefati et al. 2013, 2015, 2018). Lastly, starch granules have shown to be stable over several years of storage without an indication of coalescence (Ali et al. 2015; Marefati and Rayner 2020).

A large number of research work has been done on Pickering emulsions stabilized by starch granules, and several papers have been published. In these works, formulation parameters have been varied, and subsequent changes resulting from a wide range of formulation parameters have been discussed. Pickering emulsions produced in this way were mostly in the form of simple oil-in-water emulsions (Timgren et al. 2011); however, double water-in-oil-in-water emulsions have also been produced and investigated using starch granules (Lin et al. 2019; Marefati et al. 2015; Matos et al. 2013). Regardless of formulation parameters, in all cases, Pickering emulsions were prepared and performed successfully. Some of these reports are summarized below:

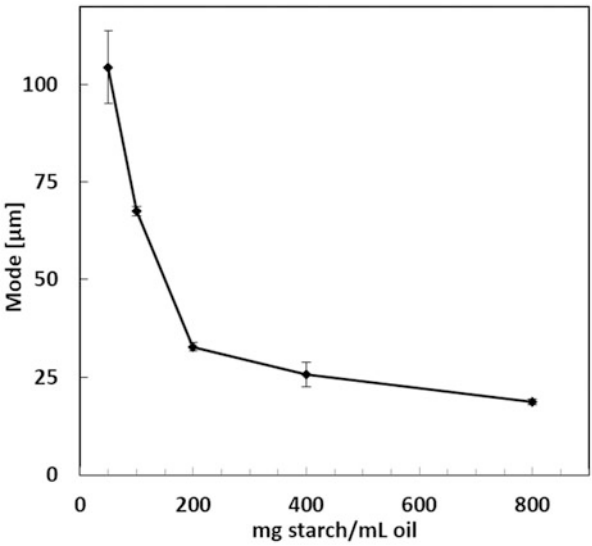

Fig. 14.12 Particle size (mode of D_{43}) for 3.0% OSA-modified quinoa starch granule-stabilized Pickering emulsions with varying amount of starch, reprinted with permission from Marefati et al. (2017b)

14.8.1 Formulation with Varying Starch Concentration

Formulation of Pickering emulsions stabilized by starch granules has been conducted with varying the concentration of OSA-modified starch granules (Lin et al. 2019; Marefati et al. 2017b; Marku et al. 2012; Rayner et al. 2012a, b) or SCFA-modified starch granules (Abdul Hadi et al. 2020a, b). Regardless of the type of hydrophobic modification, it was shown that increasing the amount of starch while keeping all the other parameters at a constant level can result in decreasing the size of the oil droplets. A study on OSA-modified starch granule showed that increasing the concentration of starch from 50 oil to 1000 mg/mL oil will result in a decrease in the droplet size (D_{43}) from 102 to 34 µm (Fig. 14.12). The decreased droplet sizes as a result of an increase in particle concentration are due to higher surface coverage that additional amount of particles can cover during emulsification (Marefati and Rayner 2020; Marefati et al. 2017b; Marku et al. 2012; Rayner et al. 2014, 2012a, b). Similar results were observed by Lin et al. (2019), when the percentage of OSA-modified starch granule used in the stabilization of double emulsion increased from 1 to 8% (Fig. 14.13). Likewise, when SCFA-modified starches were used, the droplet sizes decreased with increasing starch mass, as can be seen in Fig. 14.14.

14.8.2 Formulations with Varying Modification Level

Pickering emulsions have been formulated with starch granules in native and different modification levels. Several studies have been conducted to evaluate the emulsifying capacity of modified starch at different levels (Abdul Hadi et al. 2020a;

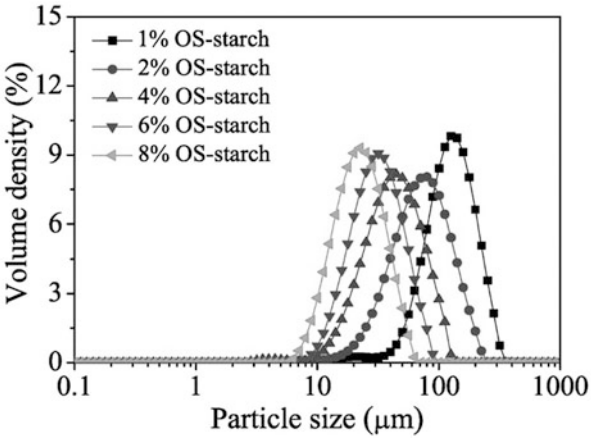

Fig. 14.13 The effect of increasing the amount of OSA-modified quinoa starch granules on the size distribution of Pickering emulsions, reprinted with permission from Lin et al. (2019)

Li et al. 2019a, b; Marefati et al. 2018; Marefati et al. 2017b; Rayner et al. 2012a, b). Regardless of the type of modification, increasing modification level has shown to optimize the starch granule wettability which, in turn, increases the interfacial curvature and thereby decreases the droplet size of the emulsions and results in emulsions with increased stability. Marefati et al. (2018) showed that increasing OSA modification level from the native to 3% (at the intervals of 0.6, i.e., 0, 0.6, 1.2, 1.8, 2.4, and 3%) decreased the mode of D_{43} from 85 to 59 μm for emulsions with 20% oil fraction and starch concentration of 100 mg/mL which can be seen in Fig. 14.15 where the main peak representing the emulsions' droplets shifted toward smaller sizes. Their results also indicated that increasing the modification level resulted in a decrease in the amount of unbound or free starch which can be seen as a decrease in the height (volume frequency) of the minor peak in Fig. 14.15.

Another study by Rayner et al. (2012a) on the emulsifying capacity of starch at native form and different OSA modification levels (i.e., 1.95, 3.21, and 4.66%), showed that the emulsions droplet sizes decreased from the native state to 1.95 and 3.21% modification levels were used and increased when 4.66% modified starch was used. The weaker performance of the starch at the highest modification level could be due to aggregation of starch particles at the 4.66% modification level which resulted in the provision of a lower surface coverage per unit of mass. This conclusion can be confirmed by comparing the volume-weighted mean diameter (D_{43}) of the starch granules which increased from 2.51 to around 3.97 μm despite the similar mode of D_{43} which was around 1.7 μm. The rheological properties of these emulsions showed that increasing the modification level to 4.66% increased the elastic modulus meaning that the gel-like structure was reduced to a more liquid-like behavior (Rayner et al. 2012a, b).

A study by Li et al. (2019b) showed that increasing the degree of substitution (DS) with OSA not only resulted in decreasing the droplet sizes but also decreased the amount of unbound starch in the emulsion systems (Fig. 14.16). These results showed that at the highest DS (0.0286), no free starch was observed.

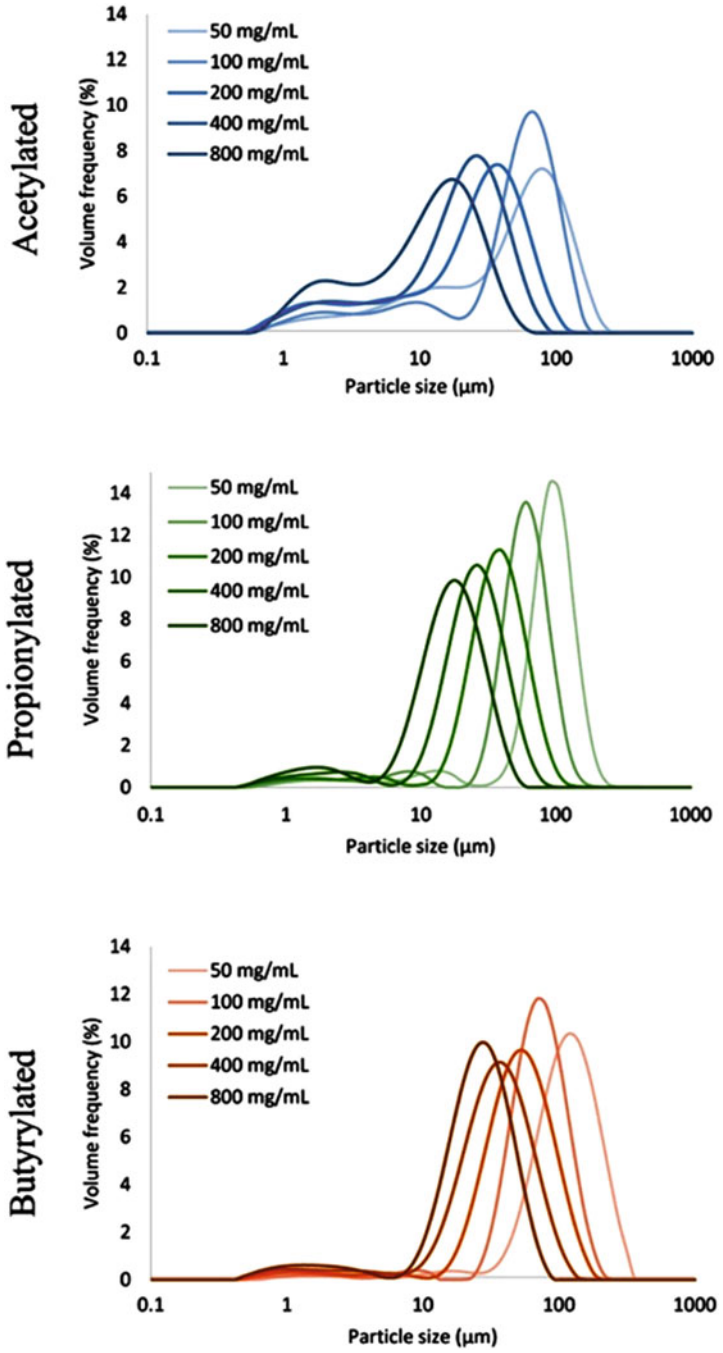

Fig. 14.14 Particle size distribution of acetylated, propionylated, and butyrylated quinoa starch granule-stabilized emulsions with 50, 100, 200, 400, and 800 mg starch/mL of oil, reprinted with permission from Abdul Hadi et al. (2020a)

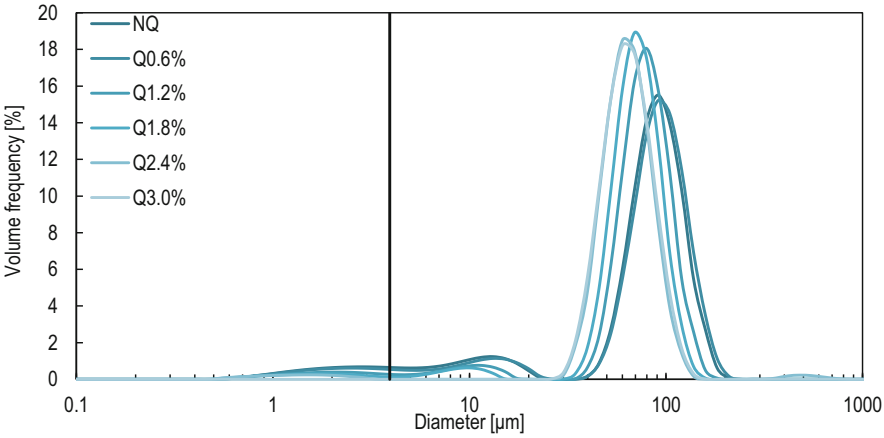

Fig. 14.15 Particle size distribution of Pickering emulsions stabilized by quinoa starch granules in native form and varying OSA modification levels (0.6, 1.2, 1.8, 2.4, and 3.0%, respectively); the vertical line represents the diameter of starch granules (mode of D_{43}), adopted with permission from Marefati et al. (2018)

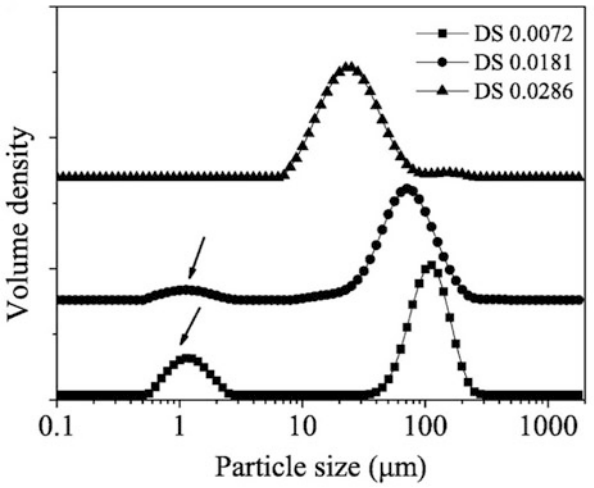

Fig. 14.16 The effect of increasing OSA modification level on the size distribution of quinoa starch granule-stabilized Pickering emulsions, reprinted with permission from Li et al. (2019b)

14.8.3 Formulations with Varying Oil Type and Concentration

Different types of oil such as liquid paraffin, Miglyol, or shea butter which are composed of hydrocarbons, medium-chain triglyceride, or long-chain triglycerides, respectively, and with different melting points were used in the formulation of

quinoa starch granule Pickering emulsions (Marefati et al. 2013; Marku et al. 2012). Marku et al. (2012) discussed that compared to the liquid paraffin and Miglyol with similar droplet size, the emulsions prepared with the solid shea nut butter had higher droplet size, viscosity, and yield stress.

Ali et al. (2015) have also reported that starch granules are compatible with most common oil phases used in the cosmetic industry regardless of the physical properties or the degree of polarity. In their study, they used non-polar oil (dimethicone), medium polar oils (caprylic/capric triglyceride, isostearyl isostearate, isopropyl palmitate), or polar oils (sweet almond oil and coconut oil) where a droplet size range of 28–41 μm was acquired, and all resulted in homogeneous emulsion systems except some creaming that was observed for dimethicone possibly due to the low density of the oil phase.

The concentration of oil used in these studies was varied from 5 to 90% (S. Li et al. 2020; Marefati et al. 2017b; Marku et al. 2012). In a systematic study of the effect of the oil content on the microstructure of Pickering emulsions stabilized by starch granules, Marku et al. (2012) used 12.5, 16.6, 25.0, and 33.3% v/v oil, with the starch-to-oil content being constant at 214 mg starch per mL oil. The results showed that there were no significant changes in the droplet sizes and the droplet sizes remained in the range of approximately 36–37 μm when the oil fraction was increased.

Marku et al. (2012) showed that formulation with 84% oil did not yield in an emulsion; however, an emulsion was possible to make using 70% oil fraction but with some free oil on the top of the emulsion layer, while no creaming or free oil was observed for 56% oil fraction and these emulsions remained stable for 8 weeks.

In another study, Li et al. (2020) investigated the effect of increasing the (corn) oil fraction from 10 to 90% using OSA-modified starch granules and successfully produced emulsion with up to 70% oil fraction with no obvious change after 31 days of storage. They showed that the emulsions formulated in the range of 30–60% oil fraction were "self-standing and immobile" upon the inversion of the glass tubes, and therefore, they called them "emulsion gels" (Fig. 14.17). These emulsions showed to have viscoelastic behavior imparting solid fat functionality to liquid oils, and they can be utilized as a template for the formulation of functional oils and delivery of bioactive compounds. However, their result also indicated some oiling-off at 70%. Their observation implied that increasing the oil fraction above 70% will result in phase inversion. These results are in line with the previous observations of Binks (2002) on silicon-based colloidal particles where the phase inversion has happened in the range of 65–70% oil fraction.

These results showed that the rheological properties of Pickering emulsions are highly dependent on the oil fraction. The rheological properties of the emulsion indicated that these emulsions had viscoelastic behavior. Moreover, the addition of the oil fraction from 20 to 60% resulted in a gradual increase of G' and G'' representing the presence of an enhanced network between droplets (Li et al. 2020). Finally, all emulsions represented a shear thinning behavior in the range tested (from 0.01 to 1000 s^{-1}) which was due to the breakup of droplet aggregates under the applied shear.

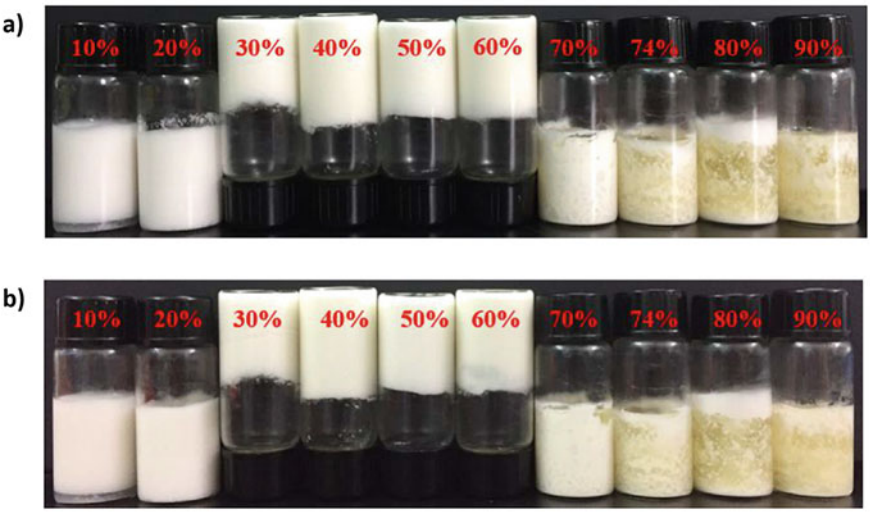

Fig. 14.17 Pickering emulsions formulated with increasing oil fraction from 10 to 90%: (**a**) freshly made and (**b**) after 31 days of storage, reprinted with permission from Li et al. (2020)

14.8.4 Formulation with Varying pH Levels

Although the effect of pH on quinoa starch granule-stabilized Pickering emulsions has not been studied systematically, and most studies have been conducted around neutral pH values, the results of gastric digestions showed that decreasing pH to 1.2 did not negatively affect the droplet sizes (Lin et al. 2019; Marefati et al. 2017a).

14.8.5 Formulations with Varying Salt Concentrations

Various salt (NaCl) concentrations were used in the continuous phase in a range of 0–2 M, and the effect of salt concentration on droplet size of Pickering emulsions stabilized by quinoa starch granules modified at three different OSA levels of 1.95, 3.21, and 4.66% was evaluated (Rayner et al. 2012a, b). The results showed that increasing salt concentration did not significantly affect the droplet size of emulsions except for the highest level of modification where an increasing trend for the droplet sizes was observed. It was shown that despite the increase in droplet sizes, the addition of the salt did not affect the viscoelastic properties of the emulsions.

14.8.6 Formulations with Different Preservatives

It was reported that Pickering emulsions stabilized by quinoa starch granules are compatible with common preservatives in the industry where all emulsions showed similar droplet sizes (Ali et al. 2015). These preservatives include caprylyl glycol,

ethylparaben, methylparaben, lactic acid, phenoxyethanol, sodium benzoate, and potassium sorbate. These results showed that except for caprylyl glycol which showed some creaming (due to surface activity), formulation of emulsions with other preservatives resulted in homogeneous systems.

The sensorial evaluation was concocted on topical formulations based on starch granule-stabilized Pickering emulsions and were compared to commercially available formulations with respect to texture and stickiness before application and spreadability, stickiness, greasiness, shine, residual coating, and absorbability after application of the cream (Ali et al. 2015). These results showed that compared to the commercial replica, the sensorial properties of Pickering emulsions were not only acceptable, but also in some cases, Pickering formulations performed better particularly with respect to stickiness and greasiness.

14.8.7 Formulation with Different Homogenization Techniques

Quinoa starch-stabilized Pickering emulsions have been prepared using high-shear mixing (Timgren et al. 2011) or high-pressure homogenizer (Marefati and Rayner 2020). Regardless of the homogenization technique used, these formulations have shown to be extremely stable over a long period of time from 1 week to 2 years for emulsions produced by the rotor-stator high-shear mixer and to 8 years for emulsions produced by a high-pressure homogenizer. Despite being used in a study for long-term storage stability, the effect of different pressures on the droplet size distribution of these formulations and a systematic comparison of high-pressure homogenization with high-shear homogenization have not been studied. Other possibilities can be the application of ultrasonic transducer or micro-fluidizer that has not been yet used in the preparation of Pickering emulsions stabilized by quinoa starch granules.

14.9 Stability of Starch Granule-Stabilized Pickering Emulsions

Compared to traditional emulsions, Pickering emulsions are known to have higher stability. The higher stability of Pickering emulsions stabilized by quinoa starch granules is discussed in the following sections under storage, process, and encapsulation stability.

14.9.1 Storage Stability

As discussed earlier in this chapter, Pickering emulsions are known to have advantages over conventional emulsions. The most important advantage of Pickering emulsions over surfactant-stabilized emulsions is improved and long-term stability against coalescence. Storage stability of the Pickering emulsions has been frequently studied in the literature, for instance, 1-week stability test (Marku et al. 2012), 2-month stability study (Wang et al. 2017), hundred-day storage (Song

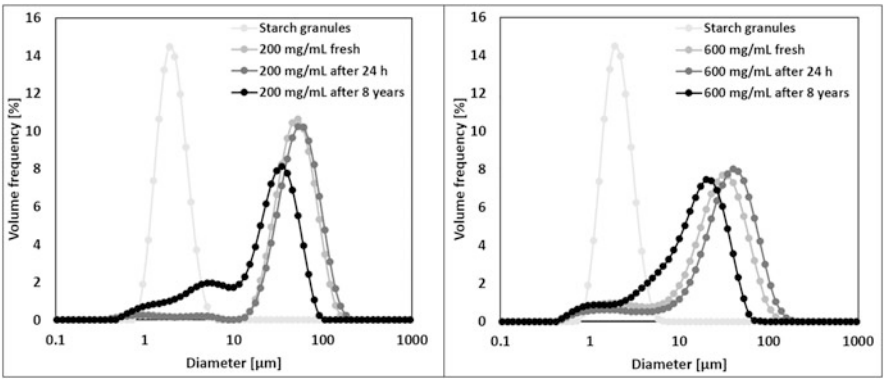

Fig. 14.18 Particle size distribution of the starch granules and Pickering emulsions stabilized using two different concentrations of starch, 200 mg/mL of oil (on the left) and 600 mg/mL oil (on the right), during the storage period of 8 years, $n = 6$, re-plotted from Marefati and Rayner (2020)

et al. 2014), or even 2-year stability investigation (Timgren et al. 2013). A more recent study has been conducted by Marefati and Rayner (2020) on Pickering emulsions formulated by two different concentrations of starch (i.e., 200 and 600 mg/mL oil) using high-pressure homogenizer that showed that starch granule-stabilized Pickering emulsions showed remarkable storage stability over 8-year period cold storage with no indication of coalescence and without the addition of preservative (Fig. 14.18). The sizes measured by the particle size analyzer showed a decrease over time which was due to the partial dissociation of networks of aggregated droplets that are formed because of particle-particle interactions.

14.9.2 Accelerated Stability

Marefati et al. (2018) used starch granules in native form and OSA-modified quinoa starch at defined modification intervals to prepare Pickering emulsions which were then subjected to accelerated stability test using centrifugation method developed previously (Smith and Mitchell 1976; Tcholakova et al. 2002, 2006) with some modification, and the results were compared to rice and amaranth starch granule-stabilized Pickering emulsions. In this study, when the emulsions are subjected to the centrifugal acceleration (Fig. 14.19) at a defined intensity and time, due to their relatively lower density, they moved toward the axis of rotation (Z). Primarily, emulsion droplets form a cream layer and due to buoyancy forces are forced to one another, and depending on the stability of the emulsions as the centrifugal force increases, the emulsion droplets are pressed tighter and tighter together, and eventually, the interfacial layer surrounding and stabilizing the droplets will rupture releasing a layer of oil on the top of the emulsion column in the tube

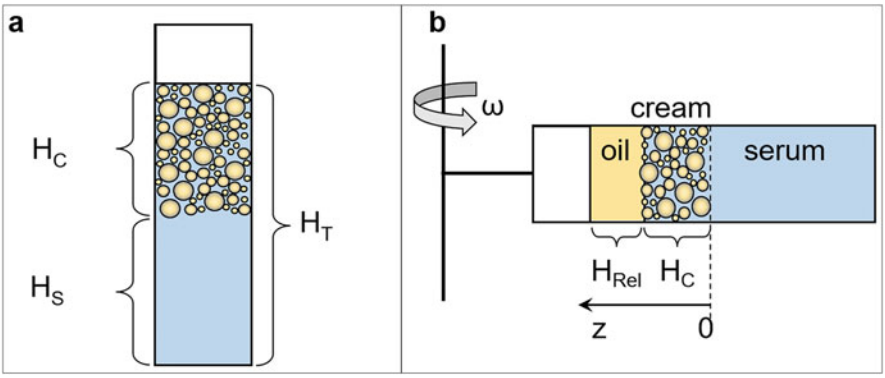

Fig. 14.19 (**a**) Schematic representation of an emulsion sample used for estimation of emulsion index and (**b**) schematic representation of the gravitational stability test and the respective thickness of the released oil, emulsion, and serum layers, reprinted with permission from Marefati et al. (2018)

(Fig. 14.19). After centrifugation, the height of the oil released (H_{Rel}) and cream layer (H_C) in relation to the total height of the emulsion samples (H_T) is measured accurately using a static multiple light scattering (Turbiscan, Formulation Co., France), and the emulsion indices (EI%) are calculated and compared within the same sample before and after centrifugation as well as among different starches with different modification and different sources. The emulsion index (EI%) is then calculated from the equation below:

$$\mathrm{EI}\% = \frac{H_C}{H_T} \times 100 \tag{14.4}$$

The results showed that the emulsion indices (EI%) were shown to be the highest for quinoa compared to rice and amaranth in both initial and after accelerated stability test which was due to formation of smaller droplets and presence of more adsorbed starch granules in the system that was reflected as a larger cream layer (H_C). In addition, increasing the level of modification resulted in increasing the height of the cream layer (H_C), which was due to the formation of smaller droplets and the presence of more adsorbed starch granules in the system, and decreasing the number of free starch granules. Furthermore, the increased hydrophobicity caused by increasing the modification level resulted in higher stability of the emulsions during centrifugation (Figs. 14.20 and 14.21). These results showed that compared to emulsions prepared by rice and amaranth, the stability of emulsions prepared by quinoa starch granules was higher, and at the higher modification levels for quinoa (2.4 and 3%), no oiling-off was observed after centrifugation at 5250 × g for 10 min.

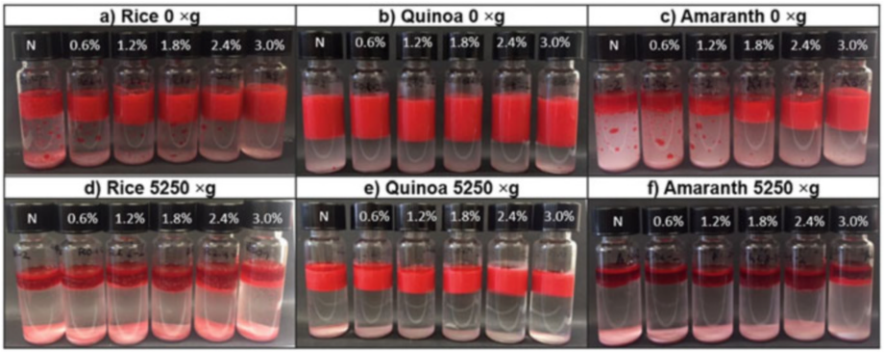

Fig. 14.20 Representation of Pickering emulsions stabilized by rice (**a**, **d**), quinoa (**b**, **e**), and (**c**, **f**) amaranth starch granules in native and varying modification levels, before (**a**–**c**) and after (**d**–**f**) accelerated stability test (5250 × g), reprinted with permission from Marefati et al. (2018)

14.9.3 Process Stability

Emulsion formulations may undergo different processes during their preparation. These processes may alter the emulsions which could result in their destabilization. These processes include heating, freezing, and drying. Freezing and freeze-drying are commonly used to increase the shelf-life of food and pharmaceutical products.

14.9.4 Freeze-Thaw Stability

Freezing is a process that is frequently used to increase the shelf-life of products. During freezing, the water content of products is frozen, and thereby the activities of microorganisms and chemical reactions that can lead to deterioration of the quality of the emulsion products are attenuated. Moreover, freezing is the precursor of freeze-drying which is another process to increase emulsions' shelf-life. During the freezing process, either or both of the water and oil phases of the emulsions crystalize, and therefore, freezing can cause destabilization of the droplets in some ways: (1) Ice formation may disturb hydration of emulsifier by water, (2) ice formation can result in droplet-droplet interaction, (3) elevation of the concentration of solutes may change pH and ionic strength, (4) penetration of ice crystals may rupture the interfacial layer which can make oil-oil contact in oil-in-water emulsions possible, and (5) penetration of the oil crystals from one droplet into another one can result in partial coalescence (Ghosh et al. 2006; Magnusson et al. 2011; McClements 2004; Palanuwech and Coupland 2003; Thanasukarn et al. 2004). Therefore, cryo-protectants such as sucrose and maltodextrin are used to prevent emulsion destabilization during the freezing process (Thanasukarn et al. 2004). Since the rate of coalescence during freezing has been correlated with the thickness of stabilizer, another way to prevent destabilization of emulsions is by increasing the thickness of the interfacial layer (Boode et al. 1991; Boode and Walstra 1993; Palanuwech and

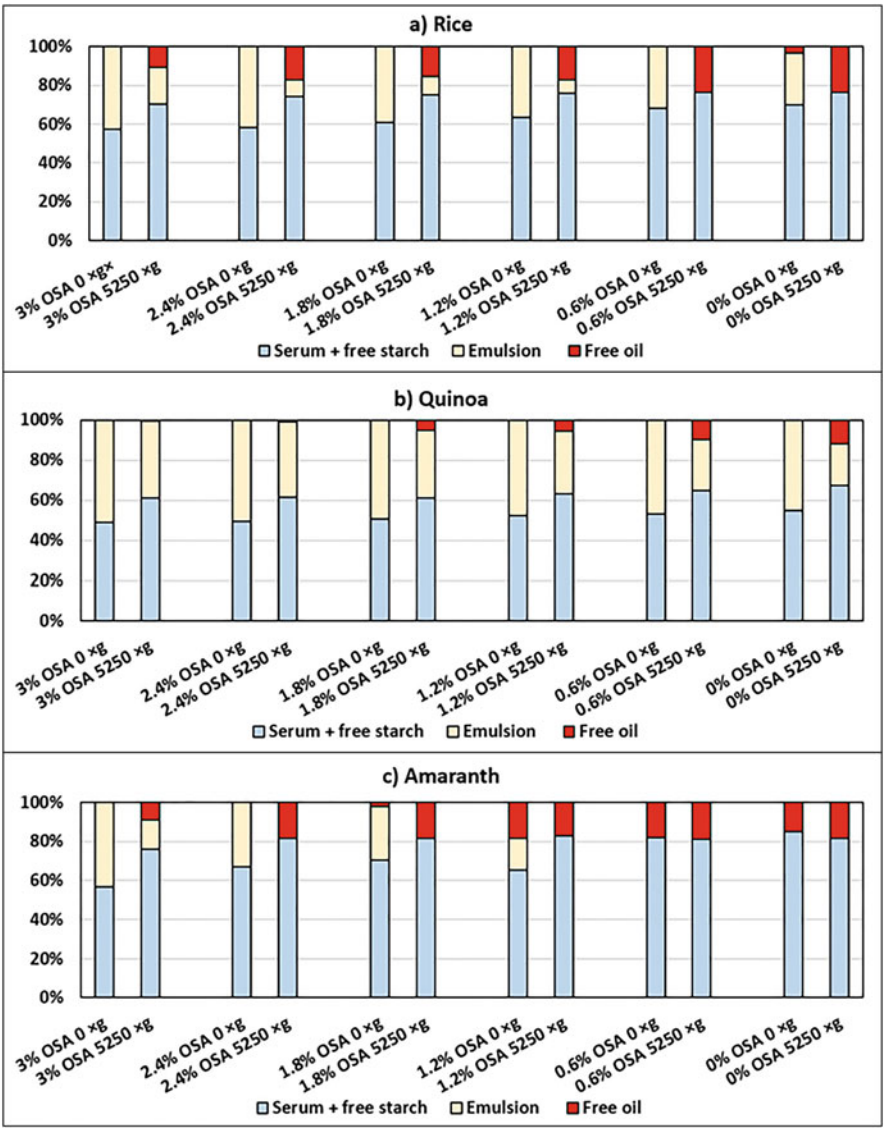

Fig. 14.21 Representation of emulsions before and after the accelerated stability test (5250 × g) for Pickering emulsions stabilized by rice (**a**), quinoa (**b**), amaranth (**c**) starch granules, where the blue part represents the serum (H_S) phase, the yellow part represents the cream phase (H_C) and the red part represents the free oil (H_{Rel}) as measured by multiple light scattering, $n = 2$, reprinted with permission from Marefati et al. (2018)

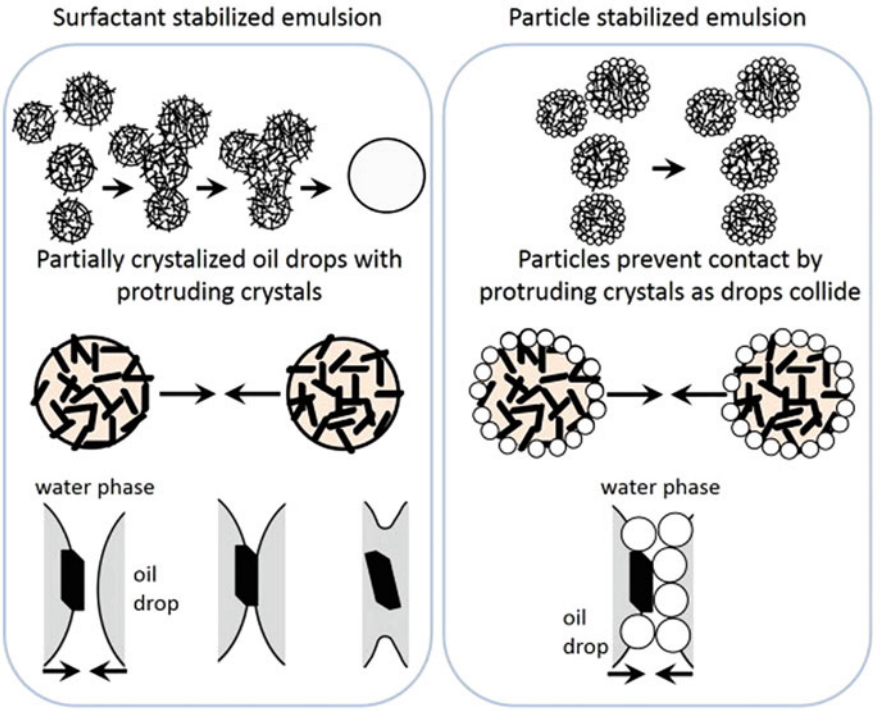

Fig. 14.22 The mechanism proposed for freeze-thaw stability of particle-stabilized emulsions where the large particles prevent crystal protrusions and eventual coalescence upon thawing, reprinted with permission from Rayner et al. (2014)

Coupland 2003; Thanasukarn et al. 2004). Due to the large particle sizes, Pickering emulsions can provide a thick barrier and avoid crystal penetration and oil-oil contact during the freezing process as can be seen in Fig. 14.22 (Aveyard et al. 2003; Rayner et al. 2014). Relatively large sizes of the starch granules compared to ice crystals and the high energy required to desorb a micron-sized particle from the oil-water interface make Pickering emulsions stabilized by quinoa starch granules a suitable formulation approach for preservation of the stability of emulsions.

14.9.5 Freeze-Drying Stability

Drying or dehydration is another process that is frequently used to increase shelf-life, improve the application, or facilitate the transportation of emulsion-based products (Adelmann et al. 2012; Marefati et al. 2013). Freeze-drying is a type of drying that is known to impose the lowest degree of damage to the sensitive structure of complex materials, and therefore, it is useful for the preservation of the heat-sensitive material such as enzymes and microorganisms, among others (Liu et al. 2008). During the

freeze-drying process, the solvent content of the materials is frozen at low temperature and then is sublimated directly from the frozen state into vapor at low pressure. As freezing is a precursor to the freeze-drying process, the same destabilizing mechanisms due to the formation of crystals in water and oil phases are likely to ensue.

In addition to those destabilizing mechanisms mentioned for freezing, the drying step may cause instability due to the disruption of the interfacial properties. Several approaches have been applied to preserve the stability of emulsions during the freeze-drying process. A common way is the addition of solid hydrophilic carriers such as lactose, glucose, maltodextrin, and cellulose (Adelmann et al. 2012; Mezzenga and Ulrich 2010). To avoid carrier compounds, reinforcement of the interfacial layer through layer-by-layer (LBL) deposition of polyelectrolytes and cross-linking of protein-stabilized interfaces have been proposed (Guzey and McClements 2006; Marefati et al. 2013; Mun et al. 2008). Similar to freezing, large particle sizes of Pickering emulsifiers can protect emulsions during freeze-drying. Compared to surfactant-stabilized emulsions, Pickering formulations demonstrated controlled evaporation of the continuous phase (Aveyard et al. 2003).

Previous studies of the feasibility of the development of re-dispersible oil-filled powders from OSA-modified quinoa starch granule Pickering emulsion by freeze-drying showed that powders with at least 70% oil content were achieved (Marefati et al. 2013, 2015). These studies showed that formulations, where the oil phase was solid at the freeze-drying temperature, were more stable during the freezing and freeze-drying processes. Besides, the formulation where the starch granules at the interface were gelatinized was shown to have higher stability during freezing and freeze-drying even when the oil phase was at liquid state (Fig. 14.23).

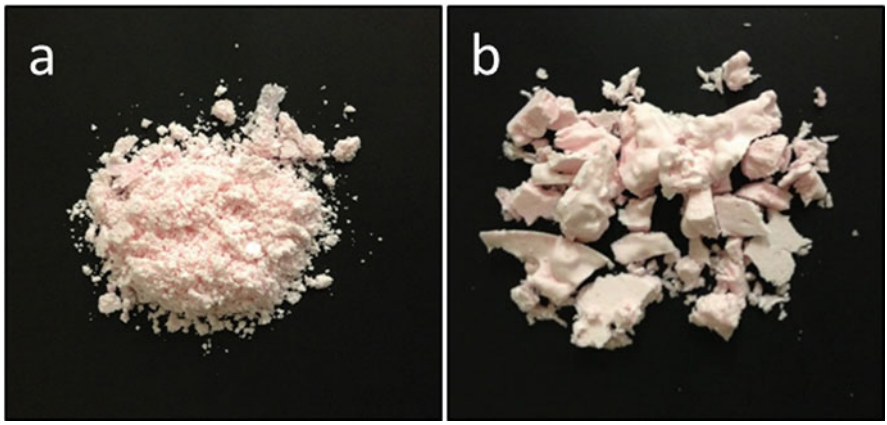

Fig. 14.23 Freeze-dried Pickering double emulsions with encapsulated carmine using solid shea nut oil (melting point 33 °C): (**a**) non-heat-treated freeze-dried double emulsion and (**b**) heat-treated freeze-dried double emulsion on the right, reprinted with permission from Marefati et al. (2015)

14.10 Encapsulation of Bioactive Compounds

In many applications, bioactive compounds are incorporated in another phase not only to protect them but also to develop formulations for controlled release and targeted delivery of bioactive compounds to specific locations in the human gastrointestinal tract. The incorporation of bioactive compounds in another phase is known as encapsulation (Gibbs et al. 1999). Emulsions have been commonly used for encapsulation of bioactive compounds to protect, modulate, and increase the bioavailability of bioactive compounds in food and pharmaceutical products. In addition to improved and long-term stability against coalescence, Pickering emulsions have demonstrated other advantages in encapsulation, controlled and targeted release of active substances due to gel-like network formation (Frelichowska et al. 2010; Li et al. 2020). Unlike conventional emulsions stabilized by surfactants and biopolymers, due to the higher physical stability and rigidity of the interfacial layer, Pickering emulsions can control and prolong the release of bioactive compounds. Additionally, the bioactive compounds encapsulated in Pickering emulsions are less likely to be exposed to environmental stress such as light and oxygen due to the surrounding interfacial network (Li et al. 2020; Marefati et al. 2017a, b). The nature of encapsulated bioactive compound is dependent on the formulation of Pickering emulsion and can be hydrophilic or hydrophobic for oil-in-water (O/W) or water-in-oil-in-water (W/O/W) emulsions, respectively (Marefati et al. 2015, 2017a).

14.10.1 Encapsulation of Hydrophobic Bioactive Compounds

Since starch granules are suitable for oil-in-water (O/W) emulsions, encapsulation of hydrophobic compounds can be readily achieved by dissolving hydrophobic compounds into the oil phase. A previous study on the encapsulation of a model hydrophobic bioactive compound, curcumin, showed that the initial encapsulation efficiency (EE%) of curcumin using quinoa starch granule Pickering emulsion is >79% (Marefati et al. 2017a). The encapsulation stability (ES%) was evaluated in excess of water was just >38% after 24-h storage; however, when samples were heat-treated (HT) to induce gelatinization of starch at the oil-water interface, the encapsulation stability was >73% after 24 h. The higher storage stability of the heat-treated samples was due to the formation of a thick and cohesive barrier of gelatinized starch at the interface compared to the porous interfacial layer when the starch was not heat-treated (NHT). As was pointed out in another study on Pickering emulsions, the porous interface as a result of the gaps between Pickering particles may enhance the release of the encapsulated curcumin (Tikekar et al. 2013).

In addition, Marefati et al. (2017a, b) evaluated the encapsulation stability during in vitro gastrointestinal digestion for non-heat-treated and heat-treated quinoa starch granule-stabilized Pickering emulsions. This study showed that during oral digestion, the droplet sizes decreased for non-heat-treated samples significantly which could be due to disaggregation of emulsion droplets as a result of mixing during digestion. Mixing can separate the droplet aggregates which results in separation of

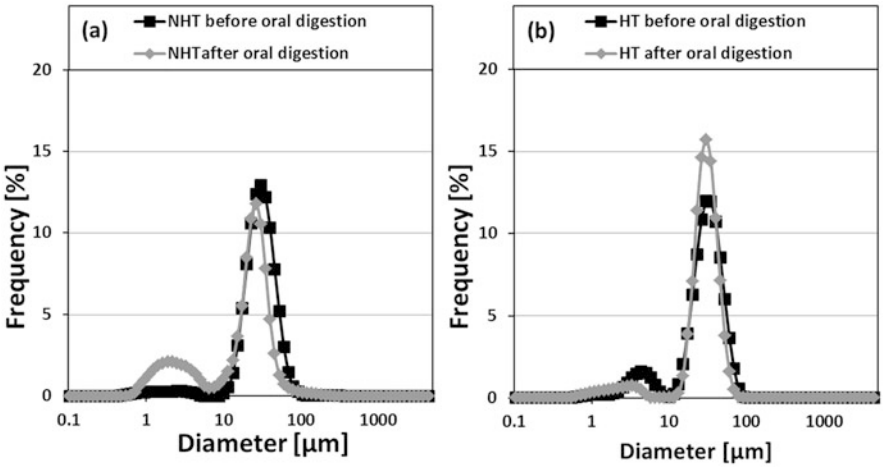

Fig. 14.24 Particle size distribution (D_{43}) for (**a**) non-heat-treated (NHT) and (**b**) heat-treated (HT) emulsions before and after simulated oral digestion (5 min), $n = 6$, reprinted with permission from Marefati et al. (2017a)

droplets and also release of free starch that is entrapped in the droplet aggregates which can be seen as the appearance of a minor peak at around 2 μm. However, the heat-treated samples did not show a significant difference in droplet sizes during oral digestion, while the small peak representing free gelatinized starch showed a decline in size and quantity of free starch, the gelatinized starch at the interface could protect the heat-treated emulsions from destabilization. In addition, compared to non-heat-treated samples with approximately 70% encapsulation stability, heat-treated samples could retain over 95% of encapsulated curcumin (Fig. 14.24).

During in vitro gastric digestion, non-heat-treated emulsions faced a slight decrease in the droplet size distribution which could be due to disaggregation of emulsion droplets as a result of the mixing during gastric digestion (Fig. 14.25). Similar to the oral digestion, the appearance of a minor peak accompanied the droplet size reduction during gastric digestion of non-heat-treated emulsion. However, the size of the droplets of heat-treated emulsions remained unchanged. Gastric digestion had a small impact on the encapsulation stability of curcumin with 86.2% and 82.4% for non-heat-treated and heat-treated samples, respectively. The low extent of the impact of the gastric digestion on these emulsions was correlated to the ineffectiveness of the gastric conditions (i.e., protease and acidic pH) on the quinoa starch granule-stabilized emulsions. Similar findings were observed in another study by Lin et al. (2019).

In order to separate the effect of the digestive enzymes from that of the bile salts, the in vitro intestinal digestion was performed with and without the addition of bile salts. During intestinal digestion without bile salts, some droplet destabilization was observed at 30 min for non-heat-treated emulsions, while it took 120 min for heat-treated samples to record changes (Fig. 14.26a, b). When the intestinal digestion was

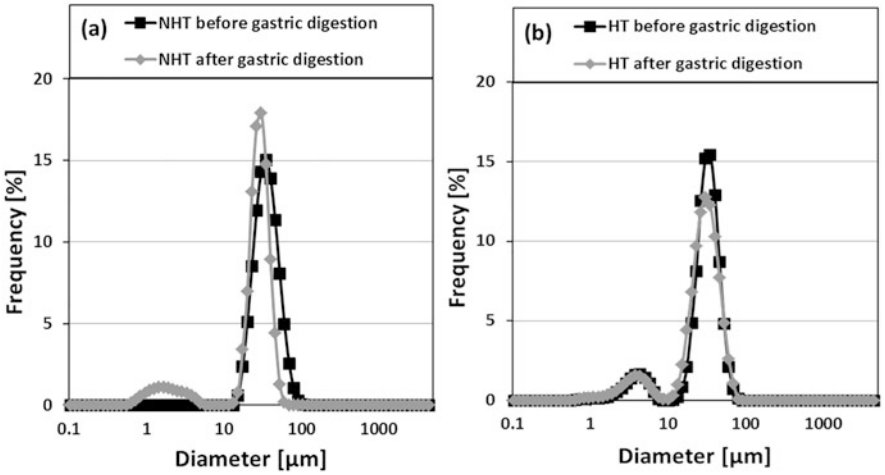

Fig. 14.25 Particle size distribution (D_{43}) for (**a**) non-heat-treated (NHT) and (**b**) heat-treated (HT) emulsions before and after simulated gastric digestion (120 min), $n = 6$, reprinted with permission from Marefati et al. (2017a)

performed with bile salts, the destabilization was faster; however, unlike non-heat-treated samples where the bile salts can facilitate lipid-lipase interaction through the gaps between the particles, the changes in the microstructure of heat-treated samples did not happen until 30 min (Fig. 14.26c, d). The higher stability of the heat-treated samples is due to the lower efficiency of the bile salts to provide the interaction between lipolytic enzyme and oil droplets as a result of the fused barrier created by the partial gelatinization of starch making it difficult for the bile salt to displace the starch granules from the oil-water interface. This gelatinized barrier seemed to be able to remain encircling the droplets and provide a barrier to mass transport. Despite the higher susceptibility of the gelatinized starch toward amylolytic activity present in the pancreatin mix in the intestinal digestion, it seems that the partial gelatinization introduced to the starch granules at the interface of the droplets did not completely distort the starch crystalline structure, and it was hypothesized that the inner side of the granules toward the oil droplets has retained their crystallinity. The encapsulation stability of emulsions after the intestinal digestion displayed that while just over 40% of the encapsulation curcumin remained in the non-heat-treated emulsions, heat-treated emulsions could retain over 86% of encapsulated curcumin. Comparing the results of the encapsulation stability of curcumin in quinoa starch granule Pickering emulsions toward in vitro digestion with silica-stabilized Pickering emulsions (Tikekar et al. 2013) showed a similarity to the non-heat-treated systems, while heat-treated systems provided higher encapsulation stability.

In another study, Li et al. (2020) investigated the stability of lutein as a hydrophobic bioactive compound in starch granule Pickering emulsions during a 31-day storage. Their results showed that encapsulation of lutein in starch granule-stabilized Pickering emulsions was positively correlated to the oil content which provided

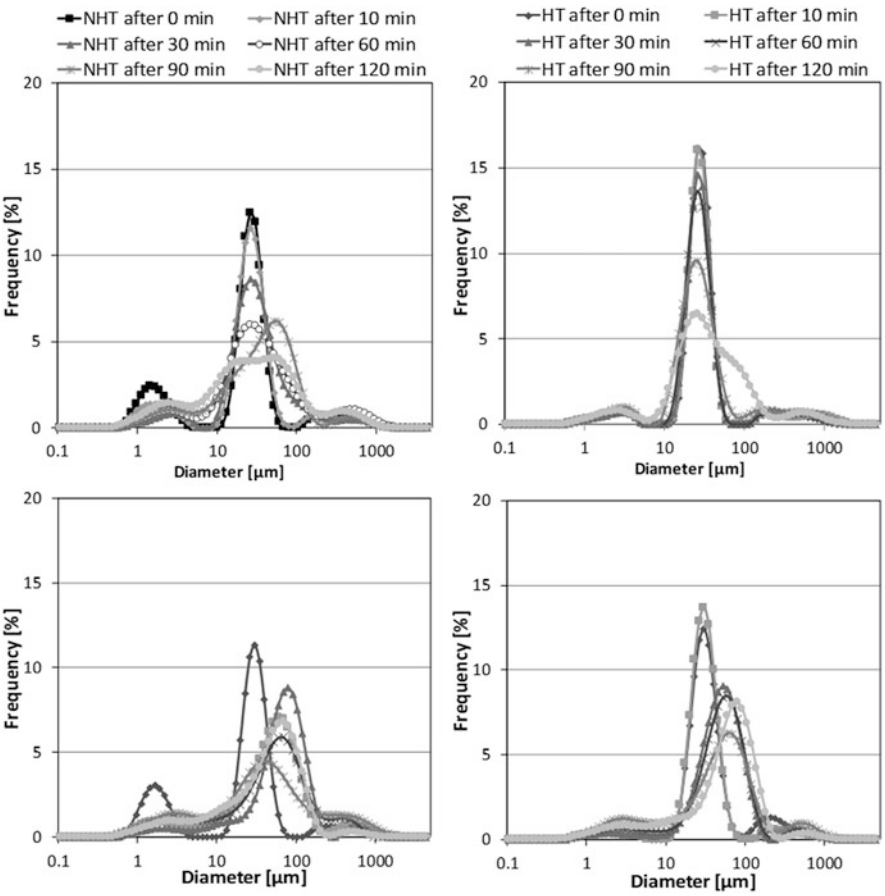

Fig. 14.26 Particle size distribution (D_{43}) for non-heat-treated (NHT) and heat-treated (HT) emulsions before and after simulated intestinal digestion (2 h): (**a**) and (**b**) without bile salts and (**c**) and (**d**) with bile salts, $n = 6$, reprinted with permission from Marefati et al. (2017a)

higher encapsulation stability of >55% after 31 days, compared to nano-emulsions emulsified by corn fiber gum with 40–60% encapsulation stability after 7 days.

14.10.2 Encapsulation of Hydrophilic Bioactive Compounds

Encapsulation of hydrophilic compounds in quinoa starch Pickering emulsion formulation can be achieved through preparation of double water-in-oil-in-water (W_1/O/W_2) emulsion by the dissolution of the hydrophilic compound into an aqueous phase (W_1) which will be then included into an oil phase (O) before being emulsified into a second aqueous phase (W_2). Since their discovery by Seifriz (1925), double emulsions have been frequently used due to the possibility for the entrapment of

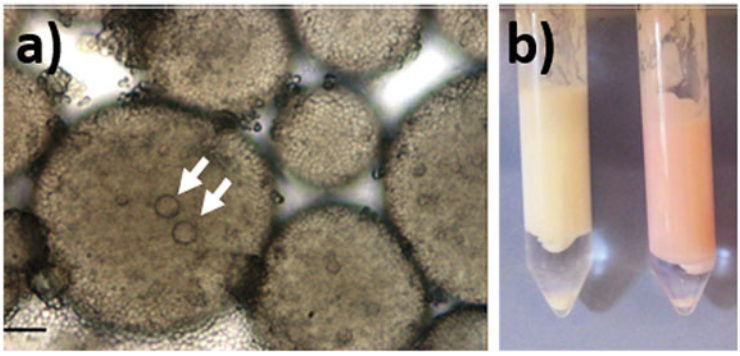

Fig. 14.27 Micrograph of double $W_1/O/W_2$ emulsions encapsulated with carmine with 50× objective magnification; the white arrows show the inner water droplets, and the scale bar represents 10 μm; a representative sample of double emulsions without encapsulated substance (left) and with encapsulated carmine (right) is also included in the figure, adopted with permission from Matos et al. (2013)

desired material in the inner phase which can provide versatile opportunities for food, cosmetic, and pharmaceutical products for encapsulation and delivery of bioactive compounds. Examples of these bioactive compounds are vitamins, minerals, flavoring agents, antioxidants, and even probiotic bacteria (Bonnet et al. 2009; Dickinson 2011; Giroux et al. 2013; Marefati et al. 2015; Muschiolik 2007; O'Regan and Mulvihill 2010; Pimentel-González et al. 2009; Rodríguez-Huezo et al. 2014; Shima et al. 2006; van der Graaf et al. 2005).

Previous studies on encapsulation of hydrophilic marker (carmine) using Pickering emulsion formulated with starch granules as secondary emulsifier showed the feasibility of encapsulation of double emulsions with >97% initial encapsulation efficiency (EE%) which remained >90% after 3-week storage (Fig. 14.27). These results also showed that these formulations had high encapsulation stability of the hydrophilic compound used during freeze-thaw cycling (>98%) and freeze-drying (>97%) (Marefati et al. 2015; Matos et al. 2013).

In a study on the encapsulation of anthocyanin using quinoa starch granule-stabilized Pickering emulsions, it was demonstrated that the formulation could be prepared with approximately 96% encapsulation efficiency (Lin et al. 2019). These results also showed that the encapsulation efficiency was positively correlated to the polyglycerol polyricinoleate (PGPR) concentration and negatively correlated to the internal aqueous phase fraction. However, increasing the amount of starch, despite lowering the droplet sizes from 130 to 25 μm which increased the interacting surface of the secondary emulsion droplets to the aqueous continuous phase, did not induce a significant change to the encapsulation efficiency of anthocyanins maintaining an encapsulation efficiency of >95%. When the volume fraction of the external aqueous phase increased, the encapsulation efficiency showed an increasing trend at first reaching a value of approximately 97% followed by a decrease. The storage stability results showed that the encapsulation rate decrease to 80 and 60% after 3 and 7 days,

respectively. Moreover, the encapsulation properties during in vitro gastrointestinal digestion showed that these formulations could retain over 86% of encapsulated anthocyanins after 60 min of gastric digestion, while the anthocyanin retention decreased significantly during the intestinal digestion reaching approximately 38% during 120 min. The higher encapsulation stability of these emulsions during in vitro gastric digestion compared to protein-stabilized emulsions was due to the fact that starch granule-stabilized Pickering emulsions were less likely to be affected by simulated gastric digestion due to acid and protease resistance of starch granules which is in line with the previous study by Marefati et al. (2017a), where gastric digestion did not induce drastic changes to microstructure of emulsions. On the other hand, the higher deficit in encapsulation stability during in vitro intestinal digestion was due to the higher extent of destruction imposed on the microstructure of emulsion stabilized by starch granules as a result of the action of enzymes present in the pancreatin. The amylase and lipase present in the intestinal pancreatin can hydrolyze starch and lipids respectively and result in the release of the major part of the anthocyanins. These results showed that formulation of double emulsions stabilized by quinoa starch granules can be used for protection of the encapsulated bioactive compounds during gastric digestion and providing a controlled release in the small intestine.

14.11 Future Perspective

In terms of future perspectives, what is limiting the widespread use of starch granules as Pickering emulsion stabilizers is their commercial availability. Despite the fact that the starch industry, in general, is well established, the isolation of small granular starches can be more difficult on a large scale. The reason is that many of the existing starch isolation processes use separation technology that relies on Stokes' velocity of particles to separate in a gravitational field (continuous centrifugal separators, hydrocyclones, and decanters). This velocity is proportional to the square of the particle radius; thus, the smaller the particles, the slower they settle. This means that in many current starch processing systems where the starch granules are on the size of 10 s of microns, quinoa starch will be lost. Therefore, new industrial starch isolation processes need to be specifically developed using approaches suitable for the small size starch granules.

Another factor, beyond the technical that has perhaps limited its wider spread of use, is the cost of the quinoa itself. In 2010, the global production of quinoa was 80 k metric tons with an average price of 2.96 USD per kg; by 2014, the production had more than doubled reaching 194 k metric tons with an average price of 6.74 USD per kg. By 2019, the production was still 159 k metric tons, but the price has dropped to 3.58 USD per kg (FAO 2020a, b). The cost of quinoa as a raw material is significant and has increased, meaning that applications of using starch granules must provide additional value beyond that of just being able to stabilize emulsion droplets. Many of the formulations discussed above are adding this value, for example:

- The possibility to create emulsions with moderately large droplet sizes that exhibit outstanding long-term coalescence stability, even in cases of poorly covered interfaces after several years of storage or after being subjected to centrifugation or freeze-thaw cycling. These types of systems could also be used as liquid-liquid contactors for environmental and catalyst applications.
- The capacity encapsulating ingredients by creating dense adsorbed layers. The resulting encapsulating ability of starch-based Pickering emulsions has many potential applications in food products including taste-masking of healthy yet poor-tasting ingredients, as well as protecting sensitive compounds from various types of degradation during processing and storage such as oxidation or reactions with other ingredients.

These properties arise from the remarkably high energy of detachment of micron-sized starch granules and their partial dual wettability for both the oil and water phases, the absence of small molecular weight emulsifiers in the formulation, as well as the possibility to change particle properties in situ to enhance barrier properties at the oil-water interface. Topical formulations stabilized by starch granules can significantly decrease or even eliminate the use of synthetic surfactants. Due to increased regulations and market demands for more clean labeled products, formulators have been making great efforts to reduce surfactant usage in general and to replace synthetic surface-active agents with more biologically and environmentally friendly alternatives in particular. This, together with the fact that starch granules are made by plants, thus, renewable and biodegradable, is in an ideal position to be the subject of further academic research interest, as well as to see wider industrial uses in a variety of food, pharmaceutical, and technical applications.

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Quinoa Fermentation and Dry Roasting to Improve Nutritional Quality and Sensory Properties

15

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Abstract

Quinoa is a pseudocereal that has gained more attention in the last decades, due to its outstanding nutritional value. Quinoa has a very good protein quality and content, with a complete amino acid profile; it is also rich in minerals and bioactive compounds. However, quinoa, like other cereals and legumes, has phytate which inhibits the absorption of essential minerals. High content of phytate is usually associated with vegetarian diets and diets of rural areas of developing countries. Such diets may lead to mineral deficiencies. Fermentation of quinoa has been shown to be a very effective method for reducing the phytate content and therefore increasing the bioavailability of essential divalent minerals such as iron, calcium and zinc. Fermentation has also been investigated for its effect on improving the antioxidant capacity and content of phenolic compounds, which are considered health-promoting molecules. In addition, this chapter also presents information on the organoleptic changes that occur during quinoa fermentation, which in some cases were shown to be negative. Successful research has been done on the use of dry toasting, either before or after fermentation, to improve the sensory properties of the fermented quinoa. Fermented quinoa, besides having the attributes of being nutritionally adequate, safe and healthy, should also have good sensory properties, which are indispensable for its broad acceptability.

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Quinoa · Fermentation · Roasting · Phenolic compounds

15.1 Introduction

Quinoa (*Chenopodium quinoa Willd.*) is an Andean pseudocereal; it was called 'the mother grain' by the Incas; their argument was that quinoa is a very resistant crop with the ability to grow in arid and salty soils, like those found in the Andes of Bolivia and Chile. Moreover, it grows in conditions of water scarcity and has high resistance to extreme temperatures as low as −4 °C–38 °C (Jacobsen et al. 2003; Ruiz et al. 2014). Quinoa has a balanced nutrient profile characterized by good protein quantity and quality, which is in fact generally superior to those of cereal grains such as wheat and rice. It contains essential amino acids (i.e. lysine, methionine and threonine) that are particularly low in vegetal protein sources (Vilcacundo and Hernández-Ledesma 2017). Quinoa's protein content has been reported to be between 13.1 and 16.7%, which is comparable to soy and milk protein (Vilcacundo and Hernández-Ledesma 2017). The reported protein quality for quinoa is 81–90%, close to that of casein which is 100%. Its protein digestibility is 83% also close to that of casein (91%) (D'Amico et al. 2017). Quinoa grains contain 7.8–14% of fibre (higher than rice 0.4%, wheat 2.7% and corn 1.7%), especially insoluble fibre; about 78% of its fibre content is insoluble and 22% soluble (Alvarez-Jubete et al. 2010). Quinoa is considered a good source of many micronutrients such as riboflavin, thiamine, folate and α- and β-tocopherol (Alvarez-Jubete et al. 2010; Repo-Carrasco-Valencia and Serna 2011). More importantly, seed has more Ca, Fe, Mn, Mg, Cu and K than other cereals (Konishi et al. 2004; Ruales and Nair 1993). Moreover, quinoa also contains a high amount of natural antioxidants with health-promoting properties, including saponins, phytosterols, phenolics, flavonoids, tocopherols and bioactive peptides (Alvarez-Jubete et al. 2010; Montemurro et al. 2019; Navruz-Varli and Sanlier 2016; Vega-Gálvez et al. 2010). Furthermore, there is a special interest on quinoa as gluten-free grain for people who are affected by gluten intolerances and coeliac disease (Jacobsen et al. 2003).

Quinoa has, however, some anti-nutritional compounds such saponins, which are responsible for the bitter taste and may be toxic in high concentrations; some saponins were found to form complexes with iron, thus reducing its bioavailability (Ruales and Nair 1993). Saponins are located on the outer layers of the grains, and can be removed by washing the grains thoroughly with water or by polishing the grains (Ruales and Nair 1993). Another compound found in seed is phytic acid, commonly found as salt phytates; it is negatively charged with 1–6 phosphate groups, which act as strong chelators of divalent cations. Thus, it inhibits the absorption of essential minerals such as zinc, iron and calcium by making them immersed in insoluble complexes (Weaver and Kannan 2002). Quinoa has also polyphenols, which are antioxidants, but they also have the ability to form insoluble complexes with divalent minerals, reducing their bioavailability (Petry et al. 2010;

Sandberg 2002). On the other hand, polyphenols have antioxidant properties that have been widely studied. Polyphenols have shown to have health-promoting effects that are beyond modulation of oxidative stress (Scalbert et al. 2005).

Besides the nutritional quality, many health benefits are also reported, such as considerably positive effects on metabolic, cardiovascular and gastrointestinal health mostly studied in experimental in vivo models (mice, rats) (Noratto et al. 2019) but also few studies in humans (Bastidas et al. 2016; Navruz-Varli and Sanlier 2016; Noratto et al. 2019).

There is a growing trend on health-conscious consumers, who show preference towards more nutritious foods and value-added products from which they can get more than just taste but also good nutrients and possible health benefits. Innovations in food industry are increasing the opportunities for consumers to supplement or replace common cereal grains (rice, wheat, corn) with higher nutritional value options such as quinoa, and products derived. This highlights the importance of research on processing, with a special attention for populations that need to follow gluten-free diets, to maintain a good lifestyle without complications. There is agreement on the fact that development of new food products with improved nutritional quality and health-promoting effects is pursued for industries and demanded by consumers. Quinoa has become one of the prime alternative grains that motivates further research towards using conventional and innovative food processing methods to reduce its anti-nutritional compounds and improve its palatability.

15.2 Fermentation

Fermentation is an ancient food processing method which was first used in the East and Southeast Asian regions as an effective method for food preservation. Its use was mainly to change or improve sensory properties such as flavour, taste, colour and overall acceptability. Nowadays, fermentation of cereals and pseudocereals is getting more importance due to the interesting nutritional and sensorial changes that can be achieved in the food matrix. Furthermore, modification made by microflora during the fermentation process may release bioactive ingredients that are beneficial to human health.

Fermentation is a metabolic process, subjected to the effect of microbial enzymes (i.e. amylases, proteases and lipases), which liberates energy by biochemical transformation of carbohydrates, proteins and lipids into products with particular tastes, aromas and textures that are many times preferred by the consumers (Caplice and Fitzgerald 1999). In addition, the conditions achieved by fermentation ($pH < 4.5$) are essential to ensure the microbiological safety and shelf life of the product.

Currently, fermentation of cereals and pseudocereals is increasing in importance; during such fermentations there is a production of compounds that transform the organoleptic characteristics (i.e. aroma, texture, flavour) of the product. In addition, it has been reported that through fermentation, an improvement on nutritional properties can be achieved, which would in turn have a positive effect on human

health (Bourdichon et al. 2012). Different authors have shown that cereals and pseudocereals, rich in nutrients, are particularly good medium for microbial fermentations. For example, quinoa is rich in polysaccharides, which are used during fermentation as a source of carbon and energy by the microorganisms. Seeds, besides carbohydrates, also contain other growth factors such as vitamins and minerals, which create a good environment for microorganism growth (Montemurro et al. 2019). Cereals and pseudocereals contain indigenous microbiota, lactic acid bacteria (LAB), mould and enterobacteria that compete for nutrients during fermentation; thus, it is very common to add starter culture in fermentation, to ensure that desirable bacteria are grown above the least desirable. During fermentation conditions such as pH, temperature, water, salt concentration and composition of the food matrix can be modified in order to favour the growth of desirable bacteria (Castro-Alba et al. 2019b, c; Rollan et al. 2019; Salovaara and Gänzle 2011).

Lactic acid fermentation, carried out by laboratory, is one of the preferred processes for fermentation of cereals and pseudocereals; it was shown that through this method, the nutritional and functional qualities of foods can be improved in various ways. It was reported that seeds decrease mineral inhibitors such as phytates and tannins (Castro-Alba et al. 2019b, c; Rollan et al. 2019), and increase the antioxidant capacity and total phenolic content (Rocchetti et al. 2019; Rollan et al. 2019). These changes can promote a positive effect on the nutritional status, immune system and overall health of the consumers.

The group of microorganisms that have been used to ferment and preserve foods since ancient times includes microorganisms from *Lactobacillus*, *Pediococcus*, *Streptococcus* and *Leuconostoc* genera; they are generally recognized as safe (GRAS). During fermentation, catabolism is undertaken *via* two main routes: homo- and heterofermentative (Axelsson et al. 1989). Microorganisms produce lactic acid from hexoses, and since they lack functional haem-linked electron transport chains and Krebs cycle, they obtain the needed energy through substrate phosphorylation (Caplice and Fitzgerald 1999).

The ones classified as homofermenters are *Pediococcus*, *Lactococcus*, *Streptococcus* and some lactobacilli. They produce lactic acid as the major end product of glucose fermentation. Heterofermenters such as *Weissella*, *Leuconostoc* and some lactobacilli produce, via the hexose monophosphate pathway, equimolar amounts of lactate, CO_2 and ethanol from glucose (Caplice and Fitzgerald 1999).

Various strains of microorganisms have been used for seed fermentation; one of the most common fermentation products is sourdough bread, for which the following LAB strains have been used, *L. plantarum*, *L. brevis*, *Lc lactis*, *Leuc. mesenteroides*, *Leuc. citreum* and *E. casseliflavus* (Ruiz Rodríguez et al. 2016). Rizzello et al. (2016) have reported that strains such as *L. plantarum*, *P. pentosaceus and L. rossiae* are good inocula for fermentation, based on the best acidification, release of free amino acids and growth capability (Rizzello et al. 2016). The number of cereal products obtained by LAB fermentation is quite ample; however, the number of fermented products is more limited and slowly increasing. Table 15.1 shows a summary of fermented products obtained with different types of fermentative microorganisms.

Table 15.1 Microorganisms commonly used to obtain fermented quinoa products

Product	Microorganisms used in fermentation	Country	Reference
Fermented quinoa flour	*L. plantarum 299v*	Sweden-Bolivia	Castro-Alba et al. (2019b); Castro-Alba et al. (2019c)
Sourdough bread	*L. plantarum*, E. faecium, *E. mundtii*, *W. cibaria*, L. rhamnosus, *Lc. lactis*	Argentina	Ruiz Rodríguez et al. (2016)
Fermented quinoa beverage	*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactococcus lactis*	Finland	Ludena Urquizo et al. (2017)
Quinoa suspension-soup	*Lactobacillus plantarum 1*	Colombia	Bolívar-Monsalve et al. (2018)
Mixed pseudocereal sourdough	*Yeast and LAB*	Spain	Carbó et al. (2020)
Pasta with fermented quinoa	*L. plantarum CRL2107*, *CRL 1964 Lc. mesenteroides CRL 2131*, *L. rhamnosus CRL 1963*, *CRL 1984 and CRL 1983*	Argentina	Carrizo et al. (2020)
Sourdough bread	*Lactobacillus plantarum JCM1149 and LP 1*	Colombia	Ceballos-González et al. (2018)
Fermented quinoa slurry	*L. plantarum CRL 778*	Argentina	Dallagnol et al. (2013)
Sourdough bread	*Autochthonous isolated lactic acid bacteria from quinoa*	Italy	Rizzello et al. (2016)
Cooked and fermented quinoa	*Isolated bacteria from quinoa Lactobacillus paracasei A1 2.6 and Pediococcus pentosaceus GS·B*	Italy	Rocchetti et al. (2019)
Solid-state fermented quinoa	*Aspergillus oryzae*, *Rhizopus oligosporus and Neurospora intermedia*	Poland	Starzyńska-Janiszewska et al. (2019)

15.2.1 Fermentation Effect on Phytate Content and Mineral Bioavailability

Even though quinoa has an outstanding nutritional profile, it also has compounds such as phytates that can inhibit the bioavailability of essential minerals. This is particularly important in rural areas in developing countries, where the common is monotonous and plant-based diets containing little or no animal products. This problem also concerns to vegetarian and vegan diets, which are trendier in developed countries. In such diets, bioavailability of minerals is low, due to the lack of meat products and the presence of phytate. Quinoa grains contain phytates in a range of 8.44–22.8 g/kg (Castro-Alba et al. 2019a; Lazarte et al. 2015; Tang et al. 2015). It is reported that phytate in cereals is located in the aleurone layers adherent to bran

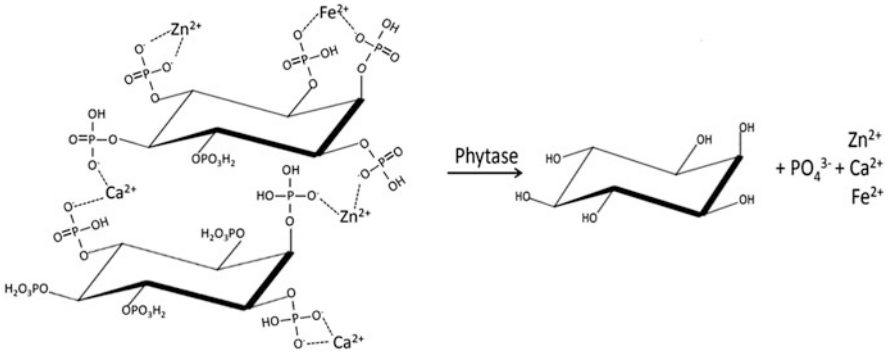

Fig. 15.1 Enzymatic hydrolysis of phytate (adapted from Lazarte 2014)

fraction; however, in quinoa, phytate is evenly distributed in the endosperm (Ruales and Nair 1993).

Phytate (myo-inositol hexakisphosphate, IP6) is the main phosphorus storage compound in pseudocereals and cereals; it is a strong chelator that binds divalent mineral cations to form insoluble compounds (Weaver and Kannan 2002). The resulting complexes are difficult for humans to hydrolyse during gastrointestinal digestion, thus making the minerals less available for absorption. Phytate forms insoluble complexes with nutritionally important essential minerals such as zinc, iron and calcium, therefore having an adverse health effect, particularly in populations that follow plant-based diets (Sandberg 2002; Troesch et al. 2013; Weaver and Kannan 2002). The stability of complexes formed by phytic acid and divalent minerals is influenced by pH, the number of phosphate groups in the phytate ring and the molar concentrations of phytate and mineral present in the food matrix. Thus, the more phytate in the food, the least bioavailable are the divalent essential minerals. Also, in the food matrix are found enzymes such as phytases, which are a subgroup of acid phosphatases. Phytase has the ability to hydrolyse phytates (IP5, IP6) and inorganic phosphate (Pi) (Sandberg and Andlid 2002), through hydrolysis of phytate. During hydrolysis, the phosphate groups are removed and thus divalent minerals are released, and their solubility increases making them more bioavailable for absorption during gastrointestinal digestion (Sandberg and Andlid 2002). This is one of the main drivers to investigate different quinoa processing techniques to achieve a significant reduction of phytate content.

Fermentation has been successfully used to reduce the phytate content of cereals and pseudocereals, including quinoa. The principle by which the phytate content is reduced during fermentation is enzymatic hydrolysis taking place during the fermentation (Fig. 15.1). The pH and temperature of fermentation can activate the endogenous phytase in the food matrix and initiate phytate hydrolysis, where ions of divalent minerals will be set free of the phosphate groups, thus being more available for absorption and utilization in the body (Sandberg 2002; Sandberg and Andlid 2002). Phytase can also be secreted by microorganisms to the food matrix during fermentation or in in vitro experiments. Some microorganisms showed to efficiently

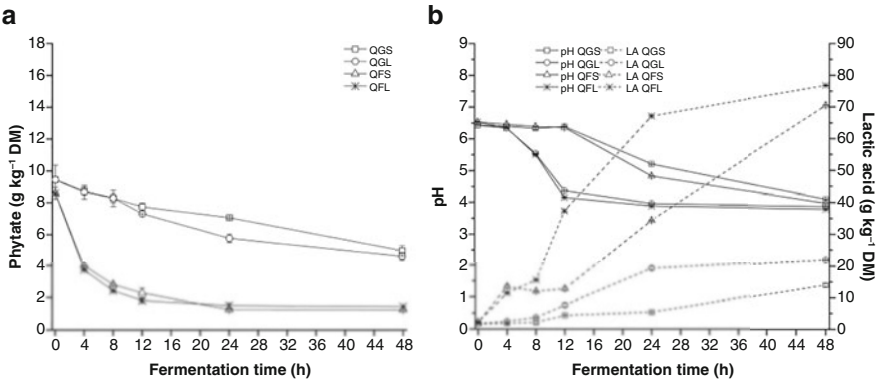

Fig. 15.2 Fermentation of quinoa grains and flour, spontaneous and with *L. plantarum 299v* (*Lp*) degradation during fermentation of quinoa. (**a**) Phytate reduction and (**b**) changes of pH and lactic acid content. *QFS* quinoa flour spontaneous, *QFL* quinoa flour with *Lp*;,*QGS* quinoa grains spontaneous, *QGL* quinoa grains with *L*p (Castro-Alba 2019)

degrade phytate as a source of phosphorus and energy required for growth (Sandberg and Andlid 2002). Some bacteria showed to be able to degrade phytate during growth by producing extracellular phytases, for example, *E. coli*, *Bacillus subtilis*, *Pseudomonas* spp. and *Klebsiella terrigena* have been able to manifest phytase activity (Greiner and Alminger 1999; Greiner et al. 2001).

Quinoa fermentation has been investigated by various researchers from different countries in South America and Europe. Castro-Alba et al. (2019b) reported the degradation of phytate in quinoa grains and flour fermented spontaneously and with *Lactobacillus plantarum 299v*. Their findings (Fig. 15.2a) showed that phytate content was reduced by 47–51% in grains during fermentation, while a phytate reduction of up to 83–85% was achieved when flour was fermented. The authors argued that fermentation of flour is more effective than grains since phytate is not only located in the seed coat but in the entire food matrix. Quinoa flour also presents an increased surface area than the grains, which allow for an easier diffusion of water and nutrients, and probably more contact of phytase and phytate for the enzymatic hydrolysis (Castro-Alba et al. 2019b). The mechanism by which phytate is reduced during fermentation is the activation of endogenous phytase at the fermentation conditions (pH range 4–5) which leads to enzymatic hydrolysis of phytate. Castro-Alba et al. (2019b) reported a reduction of pH from 6.2 to 3.82 and an increase of lactic acid from 14.1 to 75.2 g/kg (Fig. 15.2b); such conditions are reported to be favourable for activation of endogenous phytase activity. It is also reported that quinoa fermented with selected starters of LAB had a phytase activity of 2.75 times higher than phytase activity in raw flour, which may support the theories of phytate reduction during fermentation (Rizzello et al. 2016). Another hypothesis is that during fermentation, certain microbial strains are capable of exhibiting phytase activity; this was shown in various studies of fermentation of cereals (Cizeikiene et al. 2015; De Angelis et al. 2003). However, there is scarce information on phytase

activity of autochthonous quinoa LAB. *Lactobacillus plantarum* CRL2106 is one of the few strains isolated from quinoa that showed phytase activity (730 ± 25 U/mL) (Carrizo et al. 2016).

To further elucidate the differences between spontaneous fermentation and fermentation with added bacteria *Lactobacillus plantarum 299v*, Castro-Alba et al. (2019b) analysed the kinetics of phytate degradation in these fermentations. The degradation rate of phytate was suggested to follow a first-order reaction, proportional to the available phytate for degradation. It was shown that the addition of inoculum *L. plantarum* induced a faster phytate degradation, demonstrated by the degradation rate constants (K_{phy}), $-0.25\ h^{-1}$ for fermentation with inoculum and $-0.16\ h^{-1}$ for spontaneous. The use of *L. plantarum 299v* resulted in a faster pH reduction and a higher production of lactic acid than in a spontaneous fermentation; these conditions may have facilitated the faster phytate degradation during fermentation. Table 15.2 shows a summary of studies of fermentation; it presents the microorganisms used, fermentation parameters and yield of phytate reduction.

15.2.2 Fermentation Effect on Phenolic Compounds and Total Antioxidant Capacity

Seeds are reported to contain phenolic compounds such as flavonoids (i.e. glycosidic forms of the flavonoids, kaempferol and quercetin) and phenolic acids (Alvarez-Jubete et al. 2010; Rocchetti et al. 2017). Bound phenolics are especially important for their ability to establish complexes with the food matrix components. Later on, phenolic compounds are released during gastrointestinal digestion, making them available for bacterial microflora and promoting antioxidant environment (Rocchetti et al. 2018). Phenolic compounds have gained greater importance in the last years as health-promoting compounds; evidence supports their positive effect on the prevention of osteoporosis and cardiovascular diseases; it was also suggested that they have a positive role in the prevention of diabetes and neurodegenerative diseases (Scalbert et al. 2005).

It has been reported that fermentation has a significant effect on increasing the total phenolic compounds (TCP) and antioxidant capacity. It was explained that the changes on bioactive compounds, such as polyphenols and other antioxidants, during fermentation of cereals and pseudocereals are due to the metabolic activity of the microorganisms (Đorđević et al. 2010; Katina et al. 2007a).

Quinoa fermentation performed with two different strains of *S. cerevisiae* showed an increase of TCP levels by about 55% with respect to raw quinoa grains (Carciochi et al. 2016). TCP was also reported to be twofold higher sourdough bread as compared to raw quinoa (Rizzello et al. 2016). During fermentation, various microbial enzymes may prompt cell structure breakdown and hydrolyse insoluble and esterified phenolic compounds, thus facilitating their release into the food matrix (Đorđević et al. 2010). Enzymes such as esterase were also reported to be activated during sourdough fermentation conditions; this enzyme has the ability to hydrolyse complex phenolic compounds and their glycosylated structures into corresponding

Table 15.2 Parameters of quinoa fermentation and phytate reduction

Fermented product	Microorganisms	Fermentation conditions	Phytate reduction	End pH	Increase of mineral solubility[a]	Reference
Quinoa flour	*Lactobacillus plantarum v299*	Suspension of quinoa flour in water 1:2 30 °C, 24 h	83–85%	3.82	Iron 3.5-fold Zinc 4-fold Ca 3.5-fold	Castro-Alba et al. (2019b)
Quinoa flour	*Lactobacillus plantarum v299*	Suspension quinoa flour in water 1:2 30 °C, 4 and 10 h	72–73%	4.27–4.29		Castro-Alba et al. (2019c)
Cooked fermented quinoa	*Lactobacillus plantarum*	Suspension of quinoa flour in water 1:10 30 °C, 16–18 h	88%	<3.8	Iron 5- to 8-fold	Valencia et al. (1999)
Pasta made of fermented quinoa	*L. plantarum CRL2107*, *CRL 1964* *Lc. mesenteroides CRL 2131*, *L. rhamnosus CRL 1963*, *CRL 1984 and CRL 1983*	Sourdough quinoa flour:cellular microorganism suspension (2:1) 30 °C, 24 h	51%	4.05–4.12		Carrizo et al. (2020)

[a]Mineral solubility or bioaccessibility measured in in vitro studies of gastrointestinal digestion

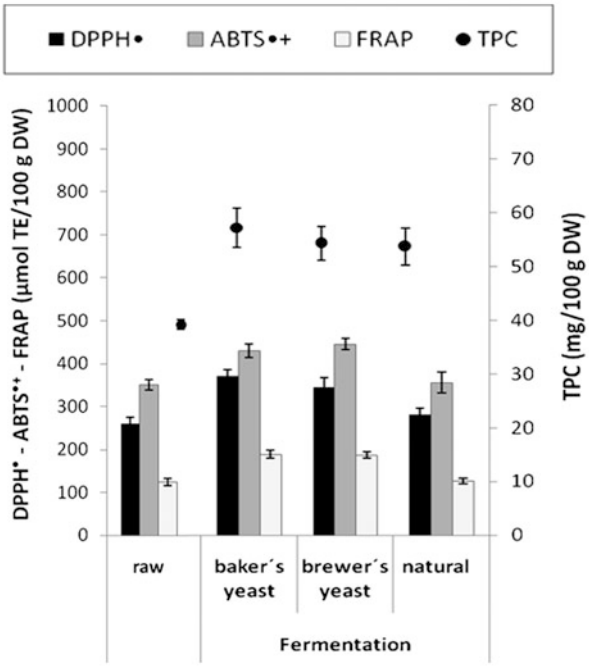

Fig. 15.3 Total phenolic content (TCP) and antioxidant capacity (DPPH•, ABTS•+ and FRAP) values of raw and processed quinoa seeds (Carciochi et al. 2016). (*Fermentation baker's yeast = Saccharomyces cerevisiae NBRC 2375, brewer's yeast = Saccharomyces cerevisiae NBRC 1951) and natural fermentation = spontaneous

phenolic acids (Nionelli et al. 2014; Rizzello et al. 2016). However, other authors have reported that there were not significant changes in TCP in cooked and fermented quinoa with isolated bacteria (*P. pentosaceus*, *L. paracasei*) (Rocchetti et al. 2019). It seems that prior treatments, such cooking may affect the changes in TCP; another important factor may be the type of bacteria strains used for fermentation.

Antioxidant capacity has also been reported to increase during fermentation. Antioxidant capacity can be measured by different methods such as DPPH (radical scavenging activity), ABTS (radical scavenging assay), FRAP (ferric-reducing ability power) and ORAC (radical scavenging). In the study presented by Carciochi et al. (2016), the results of DPPH•, ABTS and FRAP showed similar trends in the antioxidant activity of quinoa grains before and after fermentation (Fig. 15.3). In experiments where seed was fermented with two strains of *S. cerevisiae*, DPPH showed a significant increase of 33 and 43%, ABTS increased by 22 and 27% and FRAP increased by 50 and 51%. In the case of spontaneous fermentation, the changes in antioxidant capacity were not significant. A 72% increase in antioxidant activity (DPPH•) was also reported for quinoa sourdough bread, fermented with bacteria (Rizzello et al. 2016). Other authors have analysed the changes in antioxidant capacity as FRAP and ORAC in cooked fermented quinoa with *P. pentosaceus* and *L. paracasei*; interestingly their results after fermentation showed that FRAP decreased to not-detectable levels, while ORAC showed a significant increase after fermentation (Rocchetti et al. 2019). The authors discussed the results indicating that

the increase in antioxidant capacity may be affected by various factors such as the pH, temperature, fermentation time, microorganism species, aerobic conditions as well as solvent and water ratios during extraction. Further studies on the effect of fermentation on TCP and antioxidant capacity using other type of microorganisms and varying fermentation conditions would add valuable information to this field.

Fermentation also showed an effect on the phenolic profile. In raw quinoa, four free phenolic acids were identified (*p*-hydroxybenzoic, *p*-coumaric, ferulic and vanillic acids), and two flavonoids (kaempferol and quercetin) (Carciochi et al. 2016; Tang et al. 2015). A significant increase on the content of phenolic acids (i.e. *p*-OH-benzoic acid, vanillic acid, and *p*-coumaric acid) was observed after fermentation of seeds with two strains of *S. cerevisiae* (Table 15.3). It was argued that the increase on the content of phenolic acids was due to hydrolytic enzymes, produced by bacteria, capable of releasing insoluble bound-phenolic acids and/or conjugated phenolic acids from the grains (Carciochi et al. 2016). This mechanism of increased phenolic acids during fermentation has also been reported for other food matrices, for example, rye (Katina et al. 2007b) and wheat bran fermentation (Moore et al. 2007). Another theory backing up these changes is that the reduction of pH (4.9–5.5) during fermentation may be optimum for activation of endogenous or microbial enzymes that degrade the cell wall and facilitate the release of phenolic acids (Carciochi et al. 2016; Katina et al. 2007b). On the other hand, the same authors reported that the content of flavonoid compounds (quercetin and kaempferol) was decreased to not-detectable levels after the fermentation (Carciochi et al. 2016). This decrease was attributed to the more acidity conditions reached during fermentation pH <4 (3.92) (Carciochi et al. 2016). Low pH conditions were reported for other food matrices to significantly reduce the activity of cell wall-degrading enzymes, thus interfering in the release of insoluble-bound phenolic compounds (Hur et al. 2014). The magnitude of changes in the content of phenolic compounds will depend upon the microorganisms used as starter culture, as well as the end pH of the fermentation (Svensson et al. 2010). Further studies appear to be worthwhile to elucidate the different mechanisms that lead to variations on phenolic compounds during fermentation.

15.3 Animal and Human Studies on the Nutritional and Health Benefits

The mineral bioavailability of fermented flour was investigated in an animal study; iron in the liver and zinc content in the femur of rats were used as indicators of iron and zinc bioavailability, respectively (Castro-Alba 2019). The author found that the concentration of iron in the liver of rats fed fermented seed increased by 55% compared to rats fed unfermented flour. Similarly the zinc in the femur increased by 54% for rats fed the fermented diet (Castro-Alba 2019). It was discussed that the mechanism by which the iron bioavailability is increased in fermented foods is due to the phytate reduction, which is related to the increased solubility of minerals in the small intestine, facilitating then their absorption (Sandberg 2002; Schlemmer et al.

Table 15.3 Effect of fermentation on major phenolic compounds and pH of quinoa seeds (Carciochi et al. 2016)

Quinoa flour	Phenolic compounds (% area relative to raw grain)						pH
	p-OH-benzoic acid	Vanillic acid	*p*-coumaric acid	Ferulic acid	Quercetin	Kaemferol	
Raw	100	100	100	100	100	100	6.75
Fermentation							
S cerevisiae (baker's yeast)	831	142	1002	235	n.d.	n.d.	5.50
S. cerevisiae (baker's yeast)	868	343	813	61	n.d.	n.d.	4.96
Natural	n.d	n.d.	145	14	n.d.	n.d.	3.92

n.d. not detected, *ud.* not determined

2009). Moreover, fermented seed has shown higher content of organic acids, such as lactic acid (Castro-Alba 2019; Castro-Alba et al. 2019b, c; Valencia et al. 1999), which could in turn improve the absorption of iron in the small intestine (Bering et al. 2006). In another animal study, Carrizo et al. (2020) reported an increase in vitamin B2 (riboflavin) and B_9 (folate) content in mice fed pasta made of fermented quinoa compared to animals feed control pasta made of unfermented flour. In addition it was shown that fermentation increases resistant starch and delays gastric emptying by organic acids produced during fermentation; this condition tends to reduce the glycaemic index (GI) of fermented cereals and pseudocereals (Östman et al. 2005; Scazzina et al. 2009). In this regard, it was found in an animal study that rats fed diets with fermented nutrition showed a decrease on blood glucose and lipid levels, indicating that fermentation potentiates the ability to reduce the GI (de Oliveira Lopes et al. 2019). Other researchers have associated the presence of phytochemicals and bioactive compounds with its glucose-lowering effect (Paśko et al. 2010). There is a huge potential for further investigation on this topic; in general low GI diets have shown to be healthier and effective in the prevention and control of obesity, diabetes and cardiovascular diseases (Brand-Miller et al. 2003). While some studies have already been conducted on the positive effects of quinoa on reducing glucose and cholesterol and preventing obesity, diabetes and cardiovascular diseases (Bastidas et al. 2016; Navruz-Varli and Sanlier 2016; Noratto et al. 2019; Paśko et al. 2010; Ruiz et al. 2017), it remains relevant to further investigate the health effects of fermented seeds.

15.4 Dry Roasting

It is quiet documented that fermentation of cereals may positively influence sensory properties of end products due to the production of flavour-enhancing compounds (Rollan et al. 2019). However, during fermentation of pseudocereals, it has been challenging to achieve a good palatability and acceptable sensory properties, attributed to the production of off-flavour compounds from sulphur amino acids (Di Renzo et al. 2018). Within all microorganisms, it was found that dough fermented with microbial strains had better palatability and sensory properties that can be further improved (Corsetti and Settanni 2007; Montemurro et al. 2019). Dry roasting has been investigated for some authors as a process to improve the sensory properties of fermented products.

Dry roasting is a heating process that has been used since ancient times to cook and enhance flavour of raw quinoa. Factors such as particle size, temperature and time of process should be taken into account to obtain an appetizing dry-roasted food product. Seeds can be dry roasted as whole grains or as a flour. Grain's size ranges between 1.0 and 2.6 mm (Bertero et al. 2004), being this size small enough to dry roasting in a tray in an oven or in frying pan on the stove. Temperature is an important parameter to be controlled during dry roasting process. Temperatures between 120 °C and 200 °C have been used for dry roasting (Brady et al. 2007; Castro-Alba et al. 2019c; Nickel et al. 2016). The temperature of the process is

directly related to the development of flavour compounds. A quinoa sample that is treated at different temperatures develops different flavour profiles. The dry roasting time is interlinked with the temperature. Usually high dry roasting temperature is applied together with short dry roasting time, or vice versa.

It has been reported that there are different effects of dry roasting on antinutrient and mineral inhibitors present in grains. Regarding saponin content, Brady et al. (2007) reported that the chemical profile of flour changed after dry roasting at 200 °C for 10 min. They suggested that dry roasting resulted in degradation of saponins due to the increasing in the content of a triterpenoid structure, an aglycon of the major saponin present. On the other side, Nickel et al. (2016) found that there was not a significant change in saponin content of washed quinoa grains heat-treated at 200 °C for 10 min.

Phytate content can be also diminished by heat treatments. Castro-Alba (2019) showed that dry roasting had a significant effect on phytate content of grains. The grains were subjected to a heating treatment at 120 °C for 5 min resulting in a 20% phytate degradation from the initial levels, which can have a positive influence on improving the bioavailability of divalent minerals.

Phenolic compounds can also be affected in their composition after dry roasting. Nickel et al. (2016) reported that the heat treatment at 200 °C for 15 min significantly decreased the content of total phenolic compounds in washed grains, and they suggested this reduction was mainly due to the high temperature used during this treatment. It was also shown that the antioxidant activity was decreased, mainly because this activity is directly correlated with the total phenolic content. Conversely, it was shown that the total phenolic and total flavonoid content increased due to increase in dry roasting temperature, while the processing time had a minor but significant effect (Carciochi et al. 2016). As a result of the increase in these compounds, the antioxidant activity of dry-roasted quinoa grains was increased between 78 and 135% compared to non-dry-roasted grains.

15.5 Sensory Properties of Roasted Seeds

To improve the flavour and colour of fermented quinoa, Castro-Alba (2019) have performed dry roasting at different stages of their process. In a process, grains before lactic acid fermentation were dry roasted at 120 °C for 5 min, and in another process flour, after fermentation, was dry roasted at 120 °C for 3 min. In these processes, the temperature and the pH range (4.28–6.70) were appropriate parameters to develop flavour and colour compounds through the Maillard reaction, which occurs between amino acids and sugars (Fayle and Gerrard 2002). Quinoa has a high content of amino acids, e.g. lysine (5.6–6.0 g/100 g protein) (Repo-Carrasco et al. 2003), which is the main amino acid involved in the Maillard reaction. Moreover, the sugar content of raw grains is increased after lactic acid fermentation due to starch hydrolysis during this process (Dallagnol et al. 2013).

The typical flavour compounds formed during dry roasting are aldehydes, pyrazines, pyrroles and furfurals. Alkylpyrazines, acylpyridines, furans, furanones

and pyranones are products of the Maillard reaction. Acylpyridines, which are regarded as unpleasant, are also formed during this reaction (Van Boekel 2006). It was also reported that the degradation of saponin content during dry roasting may have a positive influence on the sensory properties of quinoa. Less saponin content in quinoa grains may result in a more palatable quinoa product (Brady et al. 2007).

Colour development during the Maillard reaction is related to the formation of 5-hydroxymethylfurfural (HMF), which is a precursor to the formation of brown polymers called melanoidins (Parisi and Luo 2018). In this regard, it was reported that brown polymers were formed when grains were dry roasted at 130 °C (Carciochi et al. 2016). Castro-Alba (2019) reported that colour development during dry roasting of fermented flour was faster than in quinoa grains, attributed to the higher content of free amino acids and sugars as well as the pH range (4.0–5.0) which favoured the formation of colour compounds.

The acceptability of dry-roasted fermented seeds was evaluated by including it in different food products. It has been reported that the acceptability of porridges prepared with fermented flour that was dry roasted was comparable to the acceptability of a porridge prepared with dry-roasted flour (Castro-Alba et al. 2019c). Furthermore, there was no significant difference in the sensory properties of the products if dry roasting was conducted before or after fermentation; the products obtained similar colour, odour/aroma, taste, aftertaste and texture scores. The authors mention that this similarity is due to the fact that the dry roasting of grains develops flavour compounds and reduces the formation of off-flavour compounds during fermentation, as well as the reduction of volatile off-flavour compounds and formation of more flavour compounds during dry roasting of fermented flour.

The influence of roasting time on the sensory properties was also investigated; grains were roasted for 15, 30 and 45 min at 177 °C, where the sensory scores for appearance, colour, flavour, texture and overall acceptability were decreasing as the roasting time increased (Rothschild et al. 2015). Thus, to obtain acceptable sensory properties of quinoa products, it is important to find the most appropriate combination of time and temperature.

15.6 Conclusion

Quinoa is an interesting alternative to cereals, e.g. as gluten-free crop, which provides high amount of important essential minerals such as zinc, iron and calcium. However, it also contains phytates that inhibit the bioavailability of these minerals. Fermentation is an effective processing alternative to improve nutritional properties, e.g. reduce the phytate content and increase the bioavailability of limiting minerals. Moreover, fermentation has also shown an increase in the content of important polyphenols which could have positive health implications. Regrettably, off-flavours appear when it is fermented, which reduce the sensory properties. The addition of a well-balanced dry roasting process proved to significantly improve the taste of the final fermented product. In food industry, despite the nutritious and healthiness of food products, good sensory properties remain an essential requisite

for broad acceptability of products. Therefore, further research on nutritious food development should be hand by hand with research on additional processes to improve the sensory properties of fermented products.

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Climate Change: Challenge of Introducing Quinoa in Southeast European Agriculture

16

Zorica Jovanovic, Radmila Stikic, and Sven-Erik Jacobsen

Abstract

The aim of this chapter is to describe the potential effects of climate changes in Southeast European (SEE) countries, and the implications on agricultural production. Adaptation measures to mitigate these effects could be to introduce new crops tolerant to various stress factors, such as drought, saline soils, and varying temperatures. Quinoa is a plant that has great potential for growing in such unfavorable conditions. In the presented review, we explain the origin, importance, and application of quinoa in agriculture with special emphasis on its nutritional and health significance as well as the mechanisms of resistance to stress factors. The opportunities for quinoa breeding in SEE are presented on the basis of data from Greece, Romania, Serbia, North Macedonia, and Turkey, varying depending on local agroclimatic conditions. The nutritional composition of the quinoa seeds is of very high value also when grown under rain-fed conditions in Serbia. There were good results from adding quinoa to wheat bread. Conclusions are that although the quinoa market in SEE is not as large as in other European countries, it is growing very intensively, and the food industry is developing new quinoa products. Thus, the prospects for future quinoa production in SEE countries are promising.

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Climate change · Quinoa as an alternative crop · Stress resilient crop · Nutritional and health benefits · Prospects for quinoa in Southeast European agriculture

16.1 Introduction

Climate change is one of the most important threats facing the world today. The predictions of the Working Group of the Intergovernmental Panel on Climate Change are that global mean temperatures will continue to rise together with greenhouse gas emissions until the end of the twenty-first century (IPCC 2013). Global temperatures will be 1.5 °C higher in the period 2081–2100 compared to the period between 1850 and 1900. Atmospheric concentrations of carbon dioxide increased from a pre-industrial value of 278 ppm and reached 403 ppm in 2017 (WMO 2019). Also the frequency, duration, and magnitude of hot extremes along with heat stress are expected to increase, and changes in average precipitation will exhibit spatial variation. Annual runoff is projected to decline in parts of southern Europe, the Middle East, and southern Africa, with an increase in northern latitudes expected by the end of the twenty-first century.

Agriculture is especially dependent on climate factors, and it is expected that climate change will limit agricultural production and food security. Increases in temperature and extreme weather events (heat waves, storms, flooding effects) as well as reduced water reserves are expected to be more frequent in the future. According to Wiebe et al. (2019), projections at the global scale in 2050 indicated that yields of major crops (cereals, oilseeds, and sugar crops) will decline by 5–7%, while food prices will increase by 10–15%, relative to proposed levels in the absence of climate change.

Therefore, predictions are that climate variability will have significant impacts on crop production and food security at both global and local levels. The United Nations predictions are that the world population will reach 9.8 billion in 2050 and 11.2 billion in 2100, which means that food sustainability in a stressful environment will become one of the most important future challenges. Increased demand for food due to population growth and changes in global food consumption patterns will increase pressure toward more sustainable agricultural production and adaptation, and mitigation measures (Campbell et al. 2016; Wheeler and von Braun 2013).

In response to climate change, the adaptive capacity of the agricultural sector must be increased. According to EEA report (2019), this should include a number of adaptation measures at national, regional, and farm level. Measures at the national and regional levels primarily involve farmers and measures to raise their awareness of these changes and provide appropriate advice that they can apply to their farms. Integrating adaptation into farm advices includes risk management insurance against climate, improving irrigation efficiency and infrastructure, and flood management prevention. Adaptation measures at the farm level depend on the specific climate impact, economic situation, farm size, cultural background, and farmer education.

These measures are numerous, including the use of appropriate agronomic methodology (altering sowing and harvesting time, use of new crops, crop rotation, improved irrigation and fertilization techniques, variation in cropping schemes, etc.) (Jacobsen et al. 2013, 2015; Raza et al. 2019).

Measures to cope with extreme climate condition effects on crops (especially drought and high temperature) also include different genetic and molecular approaches as genome targeting selection (genetically engineered plants for stress tolerance, stress-resistant genotypes). One of the possible approaches is also to introduce in agricultural production ancient crops resistant to various stress factors, such as quinoa.

Quinoa (*Chenopodium quinoa* Willd.) belongs to the Amaranthaceae family, originating from the Andean region of South America. It has recently expanded all over the world (Bazile et al. 2016). The crop currently is in focus due to its high potential for becoming a new food (Ruiz et al. 2014) and its high tolerance to various abiotic stress factors (Nanduri et al. 2019), including frost (Jacobsen et al. 2005, 2007), drought (Hirich et al. 2014a, b; Jacobsen et al. 2009; Razzaghi et al. 2012a, b, 2015), salinity (Adolf et al. 2012, 2013; Becker et al. 2017; Bonales-Alatorre et al. 2013; García et al. 2003, 2007; Ismail et al. 2016; Iqbal et al. 2019; Jacobsen 2003, 2017; Lavini et al. 2016; Panuccio et al. 2014; Riccardi et al. 2014; Shabala et al. 2013; Sun et al. 2017; Yang et al. 2016b, 2017), heat (Yang et al. 2016a), as well as its exceptional nutritional value of seeds (Repo-Carrasco et al. 2003) and vegetative parts.

According to FAO (2013a), the advantage of quinoa is that it can be considered a multifunctional agricultural crop that can be used in human and animal nutrition as well as in medical or industrial applications. The seeds and leaves can be used for human food as different products (bread, pastries, sauces, soups, noodles, desserts, etc.). For animal feed, the whole plant can be used as green forage. The potential medical use of quinoa is for wound healing, reduction of swelling, soothing pain, etc., as well as for other industrial uses (saponins for shampoos, detergents, toothpastes, pesticides, etc.). The nutritional and health-promoting values of quinoa seeds and leaves are the result of a high content of minerals, vitamins, proteins, and other important multiple bioactive compounds (Hernández-Ledesma 2019). The nutritional value of quinoa and its health-beneficial aspect (especially as a gluten-free culture) have attracted the attention of many consumers of healthy diets, so the world and European markets for quinoa as "superfood" are growing significantly. Thus, quinoa is recognized as one of the crops with an important role in ensuring future food security, also demonstrated by the FAO designated year 2013 as the "Year of Quinoa" (Bazile et al. 2015).

This chapter reviews the effects of climate change on crop production in Southeast Europe (SEE) with the focus on the possibility to cultivate quinoa in the region as a stress-resistant crop. The special emphasis is on the possibility for introducing quinoa production in Serbia as a SEE country with agro-meteorological characteristics of agriculture similar to other SEE countries.

16.2 Climate Change Projection in Southeast Europe

The projections are that climate changes in Europe such as global warming as a result of elevated anthropogenic greenhouse gas emissions, and uneven distribution of rainfall, will affect the decisions to be taken in agricultural production. Future climate scenarios indicated the probability for increasing mean temperatures (1.5–2 °C) across Europe with characteristic pattern leading to a substantial increase of temperatures in North Europe during the winter, and in Southeast Europe and Mediterranean regions during the summer (IPCC 2018). Along with temperatures, an increase in water availability is also predicted in central and northern Europe, while water shortages in southern Europe, particularly in Italy, Greece, Portugal, Spain, and Turkey (Bisselink et al. 2018).

As a result of current climate change, rising temperatures, and lack of precipitation, droughts are predicted to occur in Europe by the end of the twenty-first century. However, there are two different possible scenarios about this climate phenomenon. According to the moderate climate scenario (RCP 4.5), frequency and intensity of drought will rise in the Mediterranean, western Europe, and Northern Scandinavia, whereas severe climate scenario (RCP 8.5) predicted the appearance of intensive droughts in all Europe. Taking into account both scenarios, drought frequency will increase during spring and summer (especially in southern Europe) and decrease in winter over northern and western Europe (Spinoni et al. 2018).

Projected climate changes for 2071–2100, compared to 1971–2000, based on the average of a multi-model ensemble with RCP 8.5 scenario indicated that annual average temperatures will increase over eastern and northern Europe as well as southern Europe (EEA 2015). However, annual precipitation is generally projected to increase in northern Europe and to decrease in southern Europe and highlighted the differences between wet and dry regions (Fig. 16.1). The increased risk of climate change will affect not only Mediterranean countries but also Southeast European countries. Southeast Europe or Southeastern Europe (SEE) is a geographical region of Europe, consisting primarily of the Balkan Peninsula. There are overlapping and conflicting definitions as to where exactly Southeastern Europe begins or ends or how it relates to other regions of the European continent. States and territories that are usually included in the SEE region are Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Montenegro, North Macedonia, Romania, Serbia, as well as East Thrace as European part of Turkey (Fig. 16.1).

Climatic conditions in the SEE countries range from temperate continental climate (Serbia, lowlands and mountains areas of Bosnia and Herzegovina, Croatia, the northern parts of Montenegro and North Macedonia, Greece, Romania, Bulgaria, and European part of Turkey) to Mediterranean and sub-Mediterranean climate (Albania, the southern part of Montenegro and North Macedonia, and coastal areas of Croatia, Bosnia, and Herzegovina, Montenegro, Greece, and Bulgaria).

Cheval et al. (2017) evaluated past and projected variability of the air temperature, precipitation, evapotranspiration, and aridity in SEE throughout 1961–2050 periods. The data were aggregated from three regional models (RegCM3, ALADIN-Climate, and Promes) at 25-km spatial resolution. Their study confirmed that the

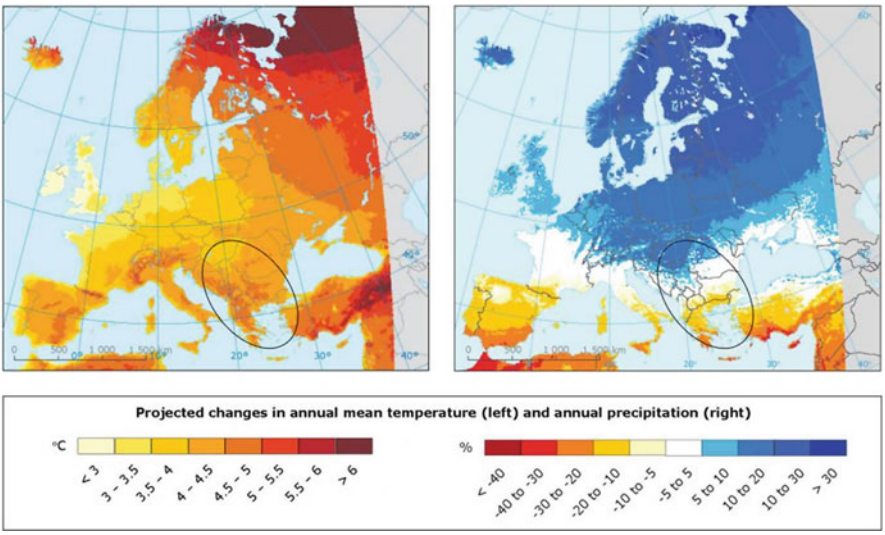

Fig. 16.1 Projected temperature and precipitation changes in Europe for the period 2071–2100 based on an ensemble of regional climate model simulations (modified from EEA 2015). The marked sections on the maps indicate the region of Southeast Europe

Southeast Europe is warming, with the important qualitative shifts toward more aridity will occur over Pannonian Plan, in the proximity of the Black Sea and in Eastern part of the Balkan Peninsula. In general, water is one of the most of important climate change factors in the SEE region because the availability of water resources (for municipal, industrial, and irrigation purposes), forestry and agriculture, biodiversity, and human health is declining.

Greece is particularly vulnerable to climate change. Its climate varies from Mediterranean, with mild and humid winters in the southern lowlands and island regions, to cold winters with heavy snowfall in mountainous regions in the central and northern regions. Greece also has a very long coastline of 16,300 km, of which around 1000 km are areas highly vulnerable to climate change compared to other regions. The World Bank Climate Change Knowledge Portal (n.d.) projection for Greece by 2050 is that mean annual temperature will rise by 2.3 °C. Similar forecasts are for Montenegro (mean annual temperature will rise by 2.4 °C, annual precipitation will fall by −35.2 mm, and total annual hot days of temperature above 35 °C will rise by 2.2 days) and for North Macedonia.

The USAID (2017) has also proposed a Climate Change Risk Profile for others of the SEE countries by 2050 year. Climate scenarios for Albania include intense temperature increase (2.4 °C–3.1 °C) from June to August; decreased annual precipitation (less than 10%), with the greatest decrease from June to September; an increase in precipitation that falls as rain instead of snow, potentially reducing snowfall; an increase in intensive episodes of rain; and floods along coastlines with a

rise from 48 to 60 cm sea level by the year 2100. For Serbia projected changes are an increase in average annual temperature of 1.5–2.2 °C; decrease in average annual precipitation of 1.1–3.5%, with the largest reductions in July and August; an increase in the number of dry days by 11–18%, and 21–31% increase in total annual precipitation on extreme rainfall days. Bosnia and Herzegovina and Croatia are also highly vulnerable to the impacts of climate change, especially in the coast and coastal zones.

The further prediction for global warming in Serbia, as a Southeast European country, is more serious with an increase in mean temperature over 2.5 °C according to the moderate climate scenario (RCP 4.5) and over 5 °C under the severe scenario (RCP 8.5). Extended periods of drought combined with heat waves and low precipitation, especially in summer, indicate that there is a trend of warming in Serbia, which is particularly pronounced in the central and southern parts of the country (Vuković et al. 2018).

16.3 Climate Change Impacts on Agriculture

Agriculture is a very vulnerable sector to climate change. It can be directly influenced by changes in crop growth and phenology (due to significant increases in CO_2 levels and temperature), but also by the more frequent appearance of extreme events (heat waves, frost, droughts, floods, hail storms, reduced water resources) and increasing the risk of plant diseases and pests. Also, the indirect impact on agricultural production will have a significant social and economic effect on global level, as increasing occurrence of extreme climate events will negatively affect trade sector, farmer income and agribusiness sector, food chain supply, or food security (EEA 2017; FAO 2016).

To mitigate the effects of climate change on agriculture, the FAO introduce the climate-smart agriculture (CSA) concept in the most vulnerable regions and countries. The CSA system is associated with actions to address the specific needs of local farms by incorporating technologies that are adapted to increase agricultural productivity and income and to reduce greenhouse gas emissions, where possible (FAO 2013b; Lipper et al. 2014).

Europe's agriculture is also vulnerable to climate change. Considering a wide range of variable climatic conditions across Europe, as well as the types and uses of land and vegetation, it is expected that these differences could have significant and different impacts on crop production and diversification (Blanco et al. 2017). The climate changes are predicted to be less negative on crop productivity in northern Europe than in southern part where is expected to decline. Changes in crop phenology will result from faster crop development (shorter crop growing cycles) and the negative effects of high temperatures and water deficits on yield, especially during the reproduction phase (flowering and grain filling) when crops are particularly sensitive to adverse factors (Olesen et al. 2011).

As a result of climate change in Europe, a continuous trend of change in the agroclimatic zone of eastern Europe (especially continental) in relation to northern

Europe has been observed (Ceglar et al. 2019). This will lead to a decrease in crop-specific cultivation (mainly due to high temperatures and frequent droughts) in some regions of Southeastern Europe and the Mediterranean, while the northern European regions become more favorable areas for crops originating from the warm season (King et al. 2018). On the basis of such forecast, an increase in the yield of rain-fed crops in central and northern Europe is expected, while for southern Europe, an opposite trend and a decrease in crop yield are expected for the period 2021–2050 (Ciscar et al. 2018).

Climate change risk presents a potential threat for European agriculture, since some projections indicated up to 16% loss in agriculture income by 2050, with large regional variations (IPCC 2019). A study of the potential impact of climate change on yields has indicated that the most negative effect will be on the yield of dominant crops (maize, wheat, barley, soybeans) as it will be reduced by 6.3–21.2% in western and southern Europe (Ray et al. 2019). Also, currently, extreme weather events (heat waves, storms, flooding) and especially high temperature and drought have caused yield declines in southern Europe. At the end of the twenty-first century, Greece should expect a long-term negative impact on cereals, especially wheat and barley (Mavromatis 2015). Climate problems also occur in northern Europe, for example, in 2018 there was a drought, because there was no precipitation during the summer for 3 months.

The impact of climate change on agriculture is also a challenge for the economy of Southeast Europe, especially in the Western Balkan region, given the important role of agriculture and its contribution to gross domestic product (GDP). The contribution of agriculture to GDP in SEE countries varies from 3.00% in Croatia to 18.44% in Albania in 2018 (The Global Economy n.d.). Global warming together with extreme events (very frequent and intense droughts and floods) predicted for all Southeast European countries could significantly reduce crop yields, especially maize (OECD 2018). Under these conditions, fungal diseases and pests are also expected to increase, which will further reduce crop yield and quality.

The negative impact of climate change on crop production and agriculture in Serbia is similar to the change in other SEE countries. Droughts in Serbia are most prevalent in the eastern and northern part of country, and the Vojvodina region, as the most important agricultural area. It is expected that drought up to the end of the twenty-first century will have significant negative effects on the yield of both winter and summer crops (FAO 2018). In Serbia, most of the agricultural production takes place under rain-fed conditions, so the expected climate change (drought and high temperatures) will greatly reduce the yield of different crops, especially in the summer months. Predictions of regional climate models for the period up to the end of the twenty-first century and for maize as a strategically important crop for Serbian agriculture and mainly grown in rain-fed conditions are that its yield will decrease by 52% due to high temperatures and reduced rainfall during the summer months (Mihailović et al. 2015). Extreme events (high temperature, heavy rainfall, etc.) can also reduce the production of fruits and cereals that are the most important agricultural products in terms of production areas and economic output (USAID

2017). It is expected that the impact of climate change on agriculture in other SEE countries will be similar to the effect on Serbia.

Projected changes in the impact of climate change on crops under the SRES scenarios (A2 and B1) indicate that in 2030, wheat yields will increase by 21–22% in Greece and by 7–13% in other parts of southern Europe, while maize yields decline in the Balkans and Southeast Europe (Greece, 4%, others, 2–7%), and this trend will further continue until 2050 (Supit et al. 2012). Although climate scenarios generally predict the adverse impact of climate change on agriculture, there are many sources of uncertainty that should be considered when interpreting the results for specific countries and regions. These predictions should take into account changes in CO_2 concentration, precipitation, and temperature, as well as soil quality characteristics and management, and type of crop traditionally grown in certain regions. Some climate models such as those of Donatelli et al. (2012) could have significant practical application in 27 EU Member States. They would enable the identification of areas where adaptation, like those simulated, may be run autonomously by farmers growing wheat, rapeseed, and sunflower. Given that the examined crops and conditions expected in Greece are similar to those of neighboring countries, such as Italy, Spain, southern France, and Cyprus, as well as some regions of the Balkan Peninsula and Turkey (Georgopoulou et al. 2017), these findings may provide useful insights into the implementation of similar adaptation measures in these countries.

Adaptation measures to mitigate the reduction in yields caused by climate change include the cultivation or testing of the use of species that are resistant to various abiotic stress factors but are underutilized globally. One of these species is quinoa which is native and traditional from the Andean region of South America. The increasing interest in quinoa cultivation in the world is associated with its exceptional nutritional and health-beneficial properties and its ability to withstand abiotic stresses.

16.4 Quinoa as Promising Alternative Crop for Climate Changes

Quinoa (*Chenopodium quinoa* Willd.) is an Andean grain crop belonging to the Amaranthaceae family and originated from South America (the Andean region—Peru, Bolivia, Chile, Argentina), where it has been traditionally cultivated for more than 7000 years as a native food (Jacobsen et al. 2003). The Andean region with diverse agroecological conditions related to geographic positions (from coastal and inland saline soils, arid and semiarid land to highlands with harsh conditions) has created the opportunity for developing a large biodiversity of quinoa. Quinoa can be divided into main five ecotypes associated with a specific area in this region (Bazile and Baudron 2015; Fuentes et al. 2012). Due to its broad genetic variability and ability to adapt to different biotic and abiotic stress factors, quinoa has a huge potential to spread widely all over the world. There are 16,422 accessions of quinoa, and its wild relatives in gene banks are distributed in 30 countries worldwide (Rojas et al. 2015). The FAO together with the Universidad Nacional del Altiplano de Puno and CIP organized a worldwide test of quinoa which provided valuable results, and

induced an interest for the crop in many countries (Izquierdo et al. 2003; Jacobsen 2003), later followed up by other tests, mainly in Africa and Central Asia (Bazile et al. 2016).

16.4.1 Nutritional Importance and Health Characteristics

Quinoa was known by the Incas as the "mother of grains," and according to the legend, the Incan armies during their war periods consumed an energy-rich mixture of quinoa and fat known as "war balls." Quinoa is also called "grano de oro" (**golden grain**) by the local people, and the name refers to the shiny golden appearance of the seeds and also to the high nutritional value of this crop.

Pericarp of quinoa seed contains saponins, plant glycosides that create a bitter taste for seeds. They are water-soluble compounds that can be easily removed by washing, soaking, quinoa boiling, or mechanical abrasion (Ruiz et al. 2017b). The amount of saponins present depends on the variety of quinoa. Jarvis et al.'s (2017) results indicated that the content of saponin in seeds is correlated with seed coat thickness, with the bitter lines having significantly thicker seed coats than sweet lines. The same research group has identified one of the genes that they believe controls saponin production in quinoa, which would make it easier to develop saponin-free varieties.

The nutritional value of quinoa is high due to its high protein content, but especially their high quality. In addition quinoa contains several important minerals, vitamins, and other important multiple bioactive compounds (Gordillo-Bastidas et al. 2016; Maradini-Filho 2017; Repo-Carrasco et al. 2003; Stikic et al. 2012).

The exceptional nutritional quality is based on a high content of proteins (13.1%–16.7%) with the essential amino acids (lysine, methionine, threonine, and tryptophan) that are scarce in cereals and legumes (Vilcacundo and Hernández-Ledesma 2017). This makes quinoa a superior crop compared to cereals, such as wheat and rice. The protein content is similar to eggs (13–14%), but much higher than in cow and human milk (3.50 and 1.80, respectively), indicating that quinoa could be an ideal food for humans and animals (FAO 2011). Carbohydrates are a major component of quinoa seeds (59.9–74.7%), both primary starch and also individual sugars (maltose, galactose, ribose). Together with the high content of dietary fiber (7.0–11.7%), quinoa is an ideal source of energy (Vilcacundo and Hernández-Ledesma 2017). Seeds are also rich source of lipid components (5.5–7.4%) with high quality of essential fatty acids (linoleic and alpha-linolenic acids), and other lipophilic phytochemicals (including carotenoids). Quinoa seeds also have a higher mineral content than other cereals (Ca, Fe, K, Mg, P, Zn, Mn), and some of them, such as K, Ca, and Mg, are in bioavailable forms, and their content is adequate for a balanced diet (Vega-Galvez et al. 2010).

Important components are vitamins, and levels of riboflavin (B2), pyridoxine (B6), vitamin E (tocopherol), and folic acid are higher than in wheat and rice. Also, high levels of vitamin C found in quinoa seeds (4.0–16.4%) along with vitamin E

have a significant role as a powerful antioxidant against oxidative stress (Vilcacundo and Hernández-Ledesma 2017).

Quinoa is also used for people with celiac disease (allergy to gluten), as a suitable replacement for the cereals wheat, rye, and barley, which all contain gluten (Peñas et al. 2014). Because of all these excellent characteristics, quinoa is considered a "golden grain," and the NASA has integrated it into the diet of astronauts (Arneja et al. 2015).

In addition to its exceptional nutritive characteristics, quinoa has a very good health characteristic and is defined as "natural functional food." Its antioxidant and anti-inflammatory compounds provide a beneficial effect on human health in preventing the risk of various serious diseases such as a diabetes 2, cardiovascular disease, and cancer (Navruz-Varli and Sanlier 2016; Tang and Tsao 2017).

The beneficial effects on health are primarily based on the high content of antioxidants (including polyphenols, flavonoids), and other important multiple bioactive compound (Lutz and Bascuñán-Godoy 2017; Repo-Carrasco-Valencia et al. 2010). Quinoa seeds contain a variety of hydrophilic (e.g., polyphenols and betalains) and lipophilic components (polyunsaturated fatty acids, carotenoids, and tocopherols) with high antioxidant activities that contribute to reducing the risks of oxidative stress related to different diseases (Abderrahim et al. 2015; Tang et al. 2015). Important phytochemicals in quinoa seeds are phytosterols and phytoecdysteroids. Various studies have shown that that phytosterols as a lipophilic compound could have a hypocholesterolemic effect in humans while some bioactive phytoecdysteroids from quinoa seeds significantly lower blood glucose and have antidiabetic properties (Graf et al. 2014). Recent results have also shown that saponins, as a component of quinoa seed pericarp, also have a wide range of biological activities relevant to human health, including antifungal, antiviral, anticancer, hypocholesterolemic, hypoglycemic, antithrombotic, diuretic, and anti-inflammatory activities (Graf et al. 2015).

In some species of *Chenopodium*, bioactive compounds with antioxidant and cytotoxic properties were extracted from various plant parts (Nowak et al. 2016). Although there is limited literature on the cytotoxic activity of quinoa, recent results have shown that extracts from quinoa leaves or seeds can have a cytotoxic effect on various types of cancer in humans, including liver and breast (Hu et al. 2017) and cervical carcinoma (Paśko et al. 2019). High content of phenolics as well as high antioxidant activity contributed to the effect of quinoa leaf extract against prostate cancer (Gawlik-Dziki et al. 2013). Also, various bioactive quinoa polysaccharides exhibit in vitro significant antioxidant, immunomodulatory, and anticancer effect (Yao et al. 2014). Currently, in vitro gastrointestinal digestion study indicates that bioactive peptides released from quinoa seed proteins have chemopreventive potential that act as an anticancer compound (Vilcacundo et al. 2018). Our latest results show that seed extracts of Puno and Titicaca cultivars grown in Serbia in 2018 contain significant amounts of phenolic and flavonoid components and exhibit strong antioxidant activity and potential anticancer activity against the human colorectal cancer cell line HCT-116 (Stikić et al. 2020). All the previously mentioned positive effects of quinoa nutrient components on human health support the

fact that quinoa exhibits great potential that can be used as a food ingredient or as a drug component to modify the human immune system against serious diseases. Saponins in pericarp of quinoa seeds are also useful for protecting crops against microbial infection and insect and bird herbivory (Graf et al. 2015).

16.4.2 Quinoa as a Stress-Resilient Crop

Quinoa, which originates from the Andean region with harsh climatic conditions, is exposed to temperatures from −4 to 38 °C, humidity from 40 to 88%, poor soil quality, and rain-fed conditions (FAO 2011; Jacobsen 2011). Due to such different environmental conditions, quinoa is a well-adapted culture to most of these abiotic stress factors, including low temperatures, frost, drought, soil salinity, wind, and hail (Hinojosa et al. 2018; Jacobsen 2011).

The most common abiotic stress factors are drought and salinity, which are widely presented and have a significant impact on crop growth and productivity. Knowledge of tolerance mechanisms is important for drought and salt mitigation through different approaches: introducing tolerant genotypes as well as land and water management strategies to increase water productivity in drought- and salt-prone regions.

Quinoa is well adapted to drought conditions, thanks to a variety of mechanisms including drought escape, avoidance, and tolerance (Jacobsen et al. 2003; Zurita-Silva et al. 2015). As one of the earliest approaches in crop stress physiology, drought escape is based on faster plant development and early maturity (before stress becomes serious).

Quinoa drought avoidance mechanisms include different morpho-anatomical and physiological changes in order to reduce water loss by transpiration and increase water uptake *via* the root system. Morpho-anatomical changes include both changes at leaf level (small leaf area, cells with thick wall, leaf dropping, epidermal cell bladder) and root level (deep and dense root system) (Jacobsen et al. 2003; Jensen et al. 2000). Physiological responses are based on the control of stomatal conductance in order to maintain leaf water potential and photosynthesis, where an elevated ABA concentration in leaves and xylem could make an important contribution to the stomatal response, which is largely genotype dependent (Jacobsen et al. 2009; Razzaghi et al. 2011; Sun et al. 2014).

Drought tolerance in quinoa is achieved by different mechanisms, from tissue elasticity and osmotic adaptation based on the accumulation of osmolytes (soluble sugars, proline) and inorganic ions (Jacobsen et al. 2003; Jensen et al. 2000), higher stomatal control and maintenance of photosynthetic activity (González et al. 2011), activation of antioxidant enzymes (Fgire et al. 2013), and gene expression of stress proteins (osmoprotectants, HSP) as well as associated with ABA biosynthesis (Liu et al. 2018; Morales et al. 2017).

A drought tolerance study of varieties of quinoa of different origin highlighted the importance of genotypic differences, as Danish (Titicaca) variety was more sensitive

to progressive droughts than varieties originating in Bolivia (as Achachino) (Sun et al. 2014).

During quinoa development, one of the most vulnerable stages of drought, as well as critical for determining yield, is the flowering and milk grain phase when water supply is important. The later stages are less sensitive, creating the opportunity for water management to apply a reduced amount of irrigation water by applying different irrigation strategies as deficient irrigation techniques. Several field studies at different locations in the Bolivian region of the Altiplano have demonstrated that deficit irrigation techniques can result in good quinoa yields and high crop productivity (Geerts et al. 2008, 2009). Experiments with the Danish cultivar Titicaca have shown a tolerance to soil drying during the seed-filling phase and that the use of deficit irrigation can maintain quinoa yield and improve water productivity and save water for irrigation (Razzaghi et al. 2012b). The application of different deficit irrigation techniques in the experiment with the same cultivar showed that alternating root zone drying in combination with high temperatures induces a better adaptive response related to growth and biomass and an increase in WUE (water-use efficiency) compared to deficit irrigation (Yang et al. 2016a). Successful application of deficit irrigation techniques to maintain quinoa yield and at the same time save water for irrigation is important for drought-prone and semiarid areas where drying and rewetting events occur occasionally, and may be more frequent depending on predicted climate change.

The experiment of Ahmadi et al. (2019) done with the newly released quinoa cultivar (cv. K5) showed that a vigorous root system extending up to 1.2 m of soil helps the quinoa to increase water-use efficiency from irrigated soil. By using a suitable cultivar and adjusting the appropriate planting density and managing the water saving of irrigation, quinoa as a "super crop" has the potential to grow successfully and produce yield even in hot and semiarid regions.

Recent results also suggested that different soil applications could improve the response of quinoa to drought. A study in which N fertilization was applied in drought conditions showed a positive impact on quinoa yield and physiology and highlighted the role of N remobilization in sustaining seed yield under drought stress (Alandia et al. 2016). Also, various organic amendments added to the soil (compost and acidified biochar) together with applied deficit irrigation techniques can improve quinoa growth and yield quality, which is related to the biochemical attributes of quinoa seeds in drought conditions (Hirich et al. 2014a, b).

Salt stress is a very common abiotic factor, and quinoa is well adapted to varying levels of soil salinity, as a facultative halophyte. There are several mechanisms by which quinoa adapt to the saline environment, and most of them are similar to the reaction to drought. Quinoa response to salinity includes morpho-anatomical properties (stomatal density and epidermal salt bladders) as well as physiological and metabolic reactions such as stomatal regulation and photosynthesis, osmoregulation, K^+ retention and Na^+ loading, transport and storage, and gene expression of membrane transporters (Ruiz et al. 2015). The main response against osmotic and ionic stress under salinity is osmotic adaptation based on the primary accumulation of salt ions (Na^+, K^+, Cl^-) in tissues, as well as organic osmolytes that regulate the

leaf water status and maintain the cell turgor (Hariadi et al. 2011; Jacobsen and Mujica 2003; Shabala et al. 2012). Similar to the drought response, salt stress also triggered an antioxidant response in quinoa by increased activities of superoxide dismutase, catalase, ascorbate peroxidase (Amjad et al. 2015), and molecular, ABA-related response (Ruiz et al. 2017c).

Quinoa shows a high tolerance to soil salinity, but with significant varietal differences. Some varieties can grow in salt concentrations similar to those in seawater or even higher (Adolf et al. 2013; Jacobsen et al. 2003). Such a response made it possible to use quinoa for cultivation not only in the saline area but also in regions where it is possible to use saline water for irrigation (Mediterranean and similar regions).

Drought and salt stress interactions are often presented in some regions, and the quinoa response to these adverse factors depends on the type and intensity of stress. The experiments with cv. Titicaca indicated that the interaction of severe salinity and water deficit did not adversely affect the total dry matter production but increased the water productivity of dry matter (Razzaghi et al. 2012a). A similar effect with the same cultivar was observed in a field study in Southern Italy, where saltwater irrigation together with drought stress did not significantly reduce quinoa yield (Pulvento et al. 2012). Investigation of the ecophysiological characteristics of quinoa cultivation in field conditions in Southern Italy has shown good resistance to drought and salt stress through stomatal reactions and osmotic adaptations, which play a central role in maintaining plant growth and preserving crop yield (Cocozza et al. 2012). On the contrary, the results of a field trial where saline water was use for quinoa irrigation in Adana, Turkey, showed that the interaction of salinity and drought stress (induced by different deficit irrigation techniques) significantly reduced crop grain and biomass yields. However, salinity stress alone did not significantly affect grain and biomass yield (Yazar et al. 2015a).

Along with high tolerance to adverse abiotic factors, quinoa can also mitigate ecosystem changes as a consequence of global warming and increased anthropogenic activities, such as desalination and phytoremediation (Jaikishun et al. 2019). Various studies have shown that quinoa is also suitable for cleaning polluted soil by phytoextraction of heavy metals such as Ni, Cr, Cd, Fe, Cu, Zn, and Cr (Bhargava et al. 2008; Ruiz et al. 2017a). Quinoa also has the ability to hyperaccumulate Pb in various plant organs, and despite this its concentration in seed remains within safe limits recommended for human use (Haseeb et al. 2018), which highlighted the role of quinoa as a superior crop.

16.5 Challenges and Opportunities for Growing Quinoa in SEE Region

With the increasing impact of climate change on traditional agricultural crops, it is very important to introduce into agricultural production plants that are grown as specific representatives of biodiversity in particular localities, including SEE. Introducing quinoa as an ancient plant resistant to different climatic effects is the

best example of this approach. The great genetic potential of quinoa, its resistance to various climatic factors, and significant nutrient and health-beneficial effects have enabled quinoa production to expand from the South American region to other parts of the world.

The largest quantities of quinoa seeds used for food are still imported from South America (especially Bolivia and Peru), but also many other countries, including the USA, Canada, and some European countries (France, Spain, Denmark, Italy), aspire to enter the world market by commercially cultivating and exporting quinoa, and some small companies are breeding and selecting new cultivars. Knowledge of the quinoa genome is of particular importance for the breeding of new quinoa cultivars and successful selection for growing quinoa in particular regions. Recently the genetic structure of quinoa was mapped, allowing genetic modification, which may prove crucial for increasing the productivity of quinoa crops and explaining its resistance to stress (Jarvis et al. 2017).

Since quinoa is a new food crop for cultivation in many countries including SEE, it is a challenge to encourage and educate farmers and agricultural companies to introduce quinoa as an alternative stress-resistant crop in their field programs. In order to successfully cultivate quinoa in a specific locality and region, in addition to selecting a specific variety, a number of agronomic studies have to be carried out that would allow for optimal yield and income. These include analyses of soil fertility; sowing time and plant density; fertilization and irrigation needs; morphological traits and phenological stages; control of pests, diseases, and weeds; and identification of seed maturity and harvest time. The lack of modern and appropriate machinery for sowing or harvesting very small quinoa seeds may be a limiting factor. Therefore, the cost of labor in some countries can increase the cost of quinoa production. All this requires the advancement of cultural practices and technologies for the cultivation of quinoa in a specific locality. The choice of quinoa varieties must also take into account the effects of climatic conditions in a given region and stress factors. The appearance of drought during flowering or grain filling phase could significantly reduce yields in many crops, but due to a higher drought tolerance of quinoa compared to other crops, these negative effects would be less expressed (Zurita-Silva et al. 2015).

According to Jacobsen (2017) in Europe, there are nine registered cultivars, that is, five from the Netherland (Carmen, Atlas, Pasto, Riobamba, and Red Carina), three from Denmark (Titicaca, Puno, and Vikinga), and one from France (Jessie). Präger et al.'s (2018) results have shown that of these European varieties, four are suitable for cultivation in southwestern Germany (Puno, Titicaca, Jessie, Zeno) with regard to grain yield, thousand kernel weight, saponin and protein contents, crude fat content, amino acid profile, and fatty acid profile.

Similarly, to the other part of Europe, experiments aimed at testing the opportunities for quinoa cultivation in SEE countries have shown that there is great potential to expand organized quinoa cultivation. Cultivation of quinoa was tested in different regions of Greece (Iliadis et al. 2001; Karyotis et al. 2003; Noulas et al. 2017), Serbia (Glamoclija et al. 2010; Stikic et al. 2012), North Macedonia (Bosev et al. 2007), Romania (Szilagyi and Jornsgard 2014), and in some regions in

Turkey (Geren 2015; Tan and Temel 2018; Yazar and Ince Kaya 2014; Yazar et al. 2015a, b).

Danish quinoa cultivars are well adapted to northern Europe with average yields ranging from 1 to 3 t/ha (Jacobsen 2017) but have also shown the best adaptation and suitability to local climatic conditions in most Southeast European countries. In studies with numerous quinoa varieties of different origins (including European and Latin American) growing in lowlands in central Greece with characteristic warm and dry climates, the average grain yield was about 1–1.5 t/ha, with some varieties having high protein levels (15–18.5%) and mineral content. Among them, the Danish varieties had a seed yield of 1.3 t/ha (Noulas et al. 2017). The coastal parts of Greece have characteristics of the Mediterranean climate. Recent results have confirmed the great potential for quinoa production in the Mediterranean region, which is characterized by highly variable climates with hot, dry summers, but also cold and rainy winters (Bilalis et al. 2019; Jacobsen et al. 2012; Jacobsen 2014). Depending on the variety, planting time, and specific field conditions and treatments, quinoa grain yields in other countries with Mediterranean conditions, such as Italy, are in the range between 1.5 and 3.4 t/ha (Lavini et al. 2014; Pulvento et al. 2010, 2012). In Romanian agro-climates, Danish quinoa breeding materials showed great potential for commercial cultivation, where Jacobsen 2 and Mixed Jacobsen had the highest seed yields (2.96 and 2.53 t/ha, respectively) and the harvest index than the other quinoa tested (Szilagyi and Jornsgard 2014).

Although there are no available data for quinoa cultivation in the European part of Turkey, cultivation in other parts in this country where the climate is similar to other Southeast European countries has been successful. Testing of the Titicaca variety in several field trials in Adana, part of Turkey, with a characteristic Mediterranean climate showed that seed yields varied from 1.69 to 2.12 t/ha (Yazar et al. 2015b). Also, testing the salt stress effect showed that grain yields were slightly reduced by saline irrigation compared to freshwater irrigation, with both yields ranging from 1.87 to 1.96 t/ha. A similar difference was observed for biomass yield, and these responses suggest a good adaptation of the Titicaca quinoa cultivar under these agroecological conditions (Yazar et al. 2015a). The good potential for quinoa as an alternative crop was also confirmed by a field study in the lowlands of the Eastern Anatolia region of Turkey (Tan and Temel 2018). In this study, a large number of cultivars were tested, and, as a result, there was a great variation in yield**.** Grain yields, of up to 4 t/ha, have been reported for the same quinoa cultivar, which is higher than in other studies conducted in different regions of Turkey. Also, it has been shown that variations in the yield were due to the differences in the locality in which the plants were grown.

The high tolerance of quinoa to arid conditions has also been tested in North Macedonia to identify the possibility of growing it as a new alternative crop. In field experiments with the Titicaca and Puno varieties at the Ovče Pole (as a particularly arid region in North Macedonia), an average yield of about 0.70 t/ha was observed in irrigated fields, twice as high as in rain-fed conditions (Bosev et al. 2007). Seeds from irrigated field have slightly higher protein and oil content than seeds from rain-fed conditions.

Table 16.1 Chemical characteristics and mineral composition of the quinoa seeds (cv. Puno). The values are expressed on the dry weight basis (modified from Stikic et al. 2012)

Content (%)	Quinoa whole seeds
Protein	17.41
Oil	4.79
Crude fiber	10.32
Ash	7.06
Starch	49.55
P (g kg^{-1})	2.40
Ca (g kg^{-1})	4.50
K (g kg^{-1})	9.52
Mg (g kg^{-1})	1.50
Fe (mg kg^{-1})	49.63
Cu (mg kg^{-1})	2.89
Zn (mg kg^{-1})	18.70
Mn (mg kg^{-1})	19.43

Table 16.2 Amino acid profile (g 100 g^{-1} protein) in purified Puno quinoa seeds (modified from Stikic et al. 2012 and Präger et al. 2018)

	References	
Essential amino acid	Stikic et al. (2012)	Präger et al. (2018)[a]
Thr	3.03	3.23
His	2.64	2.18
Tyr	3.63	2.34
Val	5.34	3.90
Met	2.16	1.65
Lys	3.91	4.47
Ile	5.00	3.20
Leu	8.29	5.48
Phe	4.69	3.52

[a]Data represent 2-year mean values

Danish cultivar Puno was the first for testing the possibility to grow in Serbian agroclimatic conditions during 2009 year (Stikic et al. 2012). Even in rain-fed and fertilizer-free conditions, seed yields of up to 1721 t/ha were obtained, while the quality of the seeds was extraordinarily good (Table 16.1), with high protein content and content of minerals.

The content of essential amino acids was also very high (Table 16.2) when compared to the results of Präger et al. (2018). These differences can be primarily attributed to specific agroecological conditions of growing Puno cultivar in these two experiments.

In addition to the lowland regions and growing plants at fertile chernozem soil, testing of quinoa cultivar Puno as an alternative grain was also done in the hilly and mountainous regions of Serbia. These results demonstrated that quinoa could be successfully grown in these conditions with a yield varying between 0.69 and 0.83 t/ha depending on locations and climatic conditions in 2009 and 2010 (Glamoclija

et al. 2010). In the mountainous areas of Serbia, there are conditions for organic cultivation of different plants (especially fruits), so that with improved agro-technology for increasing yield, quinoa could be grown there even as organic culture. Quinoa in these areas could also be used to feed livestock, which is of particular importance because livestock farming is one of the basic agricultural activities in mountainous region in Serbia, and similarly in other SEE countries.

To test the possibility of growing different cultivars of quinoa in Serbia, the Titicaca cultivar was also included. These results did not show significant differences between investigated cultivars (unpublished data). Comparison between macro- and microstructures of grain (done by Raman and FTIR spectroscopy) also showed no significant differences in structure between Puno and Titicaca seeds as well as in their biochemical composition (crude protein and starch content). These results indicate that Raman spectroscopy as a relatively simple and inexpensive "in vivo" method is very useful for localization, quantification, and structural identification of stored reserves inside the seeds of different genotypes of quinoa (Czekus et al. 2019).

The market for quinoa is increasingly growing because a lot of consumers in Europe, as well as in SEE, recognized the need for healthy food and diet. Especially because both the FAO and EFSA (European Food Safety Authority) have proposed that quinoa, due to its favorable nutritional balance, be used to improve the nutrition of the world population, especially in less developed countries. The use of quinoa could be a challenge for an increasing bakery market. However, because of its low baking quality, which is due to the lack of gluten, quinoa flour can only partially substitute wheat flour in bread making or other baked products.

Our results have shown that wheat bread supplemented with quinoa seeds cultivated in Serbia could enable the development of a number of new baking products with increased nutritional values (Stikic et al. 2012). The nutritional value of wheat breads produced with the addition of 20% of Puno seeds had a much higher content of protein, oil, and fiber than wheat bread (Table 16.3). Additionally, sensory characteristics of evaluated quinoa breads were excellent. Also, the implementation of quinoa together with a buckwheat seeds at a 40% level increased the content of protein and fiber in supplemented bread (Demin et al. 2013). The addition of quinoa and buckwheat seeds also affected the rheological characteristics of dough and improved sensory characteristics of supplemented breads. Further studies showed that wheat flour supplemented with a mixture of quinoa, buckwheat, and pumpkin seeds was used to make a new type of bread and that the bread thus formed resulted in an increase in the protein, oil, and crude fiber content of the control, wheat bread (Table 16.3). Supplemented bread also had higher energy value, specific volume, and good sensory characteristics as aromatic odor and taste (Milovanović et al. 2014).

Results of Jaldani et al. (2018) also confirmed good nutritional and digestibility properties of quinoa flour, which makes it a suitable option for enrichment of bread formulation. Similarly, investigations of Ballester-Sánchez et al. (2019) confirmed that inclusion of flour obtained from three quinoa types (white, red, and black) improved the quality of the bakery products with respect to fatty acids such as

Table 16.3 Chemical characteristics of wheat breads and breads produced with the addition of purified quinoa seeds and other supplements. The values are expressed on the dry weight basis

	References	
	Stikic et al. (2012)	
Content (%)	Wheat bread	Bread + 20% quinoa
Protein	11.89	13.83
Oil	0.98	1.90
Crude fiber	0.60	1.71
Ash	2.98	2.60
Starch	70.25	67.36
	Demin et al. (2013)	
Content (%)	Wheat bread	Bread + 20% quinoa + 20% buckwheat
Protein	13.06	15.47
Oil	0.25	2.12
Crude fiber	0.50	0.91
Ash	3.98	3.35
Starch	71.45	67.60
	Milovanović et al. (2014)	
Content (%)	Wheat bread	Bread + 15% quinoa + 15% buckwheat + 10% pumpkin seed
Protein	11.21	17.27
Oil	0.85	4.69
Crude fiber	4.7	9.29
Ash	2.56	2.07
Starch	67.39	59.70

linoleic and linolenic acids, dietary fiber, Fe and Zn, protein quality, and a reduced glycemic index.

The quinoa market is largely driven by the increasing use in the food industry leading to the creation of new quinoa products. In the European and SEE markets, and especially in health food stores, there is an increase in number of food products, such as quinoa bakery, soups, sweets, pasta, noodles, breakfast cereals, baby food, etc. Because quinoa is regarded an alternative to meat, the price is very competitive. However, quinoa seeds in Southeast European countries are mainly imported and sold in health food stores, so the growth of quinoa market is still limited. Also, high quinoa prices, compared to other similar crops, limit its purchase from low-income households, as is the case in many Southeast European countries. If quinoa were grown commercially in the countries of Southeast Europe as an alternative and drought-resistant crop, its price will be lower and its consumption would increase. It is especially important that in addition to the agricultural market, the markets of the cosmetics, pharmaceutical, and pharmaceutical industries also use the nutritional value of quinoa, which will also affect the expansion of the quinoa cultivation area. Therefore, the cultivation of quinoa in the future could become economically very attractive for SEE region.

However, there are many different agroecological zones in the countries of SEE region, so it takes time to test the appropriate agro-technologies for quinoa cultivation, especially the time and rate of irrigation and fertilization, and to find the most suitable quinoa varieties for a particular region that will produce a good and stable yield. Training of farmers and other food producers in the successful cultivation of quinoa should also be organized, and efforts should be stepped up to promote and popularize quinoa production. This is still not done enough in the SEE region, although social networks, especially the Internet, are very intensively promoting the use of quinoa and its products as nutritionally valuable and health food.

The increasing emphasis on nutritional and health-promoting effects of quinoa, especially online, and the increasing presence in the market of quinoa seeds and its food products (both in classic and health food stores) have encouraged some local farmers in Serbia and Croatia to try to grow quinoa in an organized manner.

16.6 Conclusion and Future Prospects

In Europe, the southeastern region is particularly vulnerable to climate change. Climate change is projected to cause global warming in this region, resulting in increased temperatures, periods of drought, and the occurrence of increasingly frequent extreme climate change effects (heat waves, drought, floods, and storms). In such conditions, the agricultural production is very threatened. This is of particular importance because in many SEE countries, agriculture makes a significant contribution to their overall economy. Therefore, the challenge for the whole SEE region in the coming years will be to ensure that agricultural production is maintained at a level that will ensure optimal agriculture and food safety and sustainability.

Climate change responses include increasing the agricultural sector's adaptive capacity in the future and implementing appropriate measures at regional, national, and farm level. Adaptation measures at farm level are numerous, including the application of appropriate agronomic methodology (changing planting and harvesting times, use of stress-resistant crops and varieties, crop rotation, improved irrigation, fertilization techniques, etc.).

One of the possible approaches is also to introduce in agricultural production ancient crops resistant to stress factors, such as quinoa. Thanks to their unique properties (stress resistance, nutritional and health-giving characteristics) and the great interest of consumers, production of quinoa is expanding in the world. However, there is still no organized production of quinoa in the SEE countries, but the number of experiments in which it is being tested is increasing significantly. Testing the conditions of quinoa cultivation in individual localities (agrotechnical measures, especially nutrition and irrigation, as well as the selection of suitable varieties) will allow the area of its organized cultivation to extend to the specific SEE region and localities. Also, other necessary measures include the training of farmers and the agroindustry for whom quinoa is a new crop. State institutions could also assist with

incentive measures for quinoa cultivation and the formation of quinoa producer associations.

Although the quinoa market in SEE countries is not as large as in other European countries, it is growing very intensively so that the food industry develops still more quinoa products. This will also lead to increasing economic effects, so the prospects for future quinoa production in SEE countries are promising.

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Recent Advances in the Application of Biotechnology for Improving the Production of Secondary Metabolites from Quinoa

17

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Abstract

Quinoa (*Chenopodium quinoa*) has been identified as a unique plant with several benefits that could solve several challenges facing mankind. The application of some recent advances in biotechnological techniques could help toward enhancing the production of important metabolites and nutritional attributes and improve the quality of several products that could be derived from quinoa. It is a source of

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excellent antioxidant activity along with high values of amino acids, carbohydrates, fatty acids, minerals, phenolic compounds, and saponins. Some of these metabolites possess biotechnological relevance in the production of pharmaceutical, insecticidal, biopesticidal, and nematocidal products. This chapter provides detailed information on the utilization of in vitro tissue culturing for effective production of essential metabolites, while the application of somatic embryogenesis methodology has been identified as significant instrument for effective production of virus-free plants. Furthermore, detailed information on the application of metabolomics together with hyphenated analytical and spectroscopic methodology which included gas chromatography coupled to mass spectrometry, liquid chromatography, and nuclear magnetic resonance spectrometry is provided. Relevance of synthetic biology, informatics, computational biology, and bioinformatics together with nanotechnology on how they could improve some bioactive constituents derived from quinoa plants was also highlighted.

Keywords

Quinoa · Bioactive compounds · Pharmaceutical constituents · Active metabolites · Biotechnology · Insecticidal · Pesticidal · Nematocidal · Abiotic stress

17.1 Introduction

Quinoa (*Chenopodium quinoa*) is a nutritional diet source across the world and is mainly used as staple food instead of wheat and barley. It is mostly grown in Andean region, Himalayan mountain regions, and mountain regions of Central Africa (Jacobsen and Risi 2001). Cultivation of plant started in ancient days in the Andeans including Bolivia, Chile, Peru, and Columbia (Valencia-Chamorro 2003). Quinoa is a different species, but it generally contains saponins, glycosides, and bitter triterpenoid present in high concentration in seed coat (Antúnez de Mayolo 1981). Quinoa contains lysine, cysteine, and methionine when compared to other cereals and grains, and it is also rich in beneficial fatty acids and tocopherols (Repo-Carrasco et al. 2003).

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Apart from the various dietary benefits of quinoa, it also has health benefits for children, old aged, anemic, lactose-intolerant persons, and people suffering from celiac disease. These health benefits are reported to be due to the high protein content in combination with high concentrations of vitamins, fibers, fatty acids, and minerals. This shows that quinoa holds a better position in terms of human health and nutrition as compared to other cereals and grains (Navruz-Varli and Sanlier 2016). Quinoa-derived saponins possess bioactive potentials to ameliorate or prevent the following conditions such as cancer, viral infection, thrombotic disorder, inflammation, and fungal infection (Graf et al. 2015).

The main bioactive components of quinoa plant include saponins, flavones, phytosterols, and phytoecdysteroids. In this review article, the different processes and their effects on bioactive compounds of quinoa plant samples and their utilization for bringing improvement in the field of metabolic engineering will be discussed. These processes or techniques are being widely used throughout the world to obtain health-benefitting compounds of interest from quinoa by proving favorable conditions for plant growth.

Quinoa is an Andean region crop, attaining attention globally due to its nutritional and functional properties with diverse growing conditions (Vega-Gálvez et al. 2010; Celik and Tuncil 2020). It is also part of the "golden grains" also used by astronauts of the NASA and National Academy of Sciences USA establishing its important role as healthy food (Carrasco and Soto 2010). The Food and Agriculture Organization in its 37th meeting announced the year 2013 to be the international quinoa year because of food security it gives to distress countries (Arneja et al. 2015).

There are 250 species of quinoa (Bhargava et al. 2006) with seeds that are usually oval-shaped, flat with color variations (Jacobsen et al. 2000). Quinoa is able to withstand all harsh conditions and produce grains containing higher levels of protein and minerals (Karyotis et al. 2003;Koyro and Eisa 2008). It is currently grown in Colorado, Denmark, England, France, Holland, Italy, Nevada, and Sweden. The foremost quinoa-producing countries are Bolivia, Ecuador, and Peru. Quinoa in contrasting cereals possesses starchy dicot seed, well known as pseudo-cereal (Arneja et al. 2015).

Quinoa possesses wide essential varieties of amino acids, minerals, different fatty acids, carbohydrates, dietary fibers, and different health bioactive compounds as phytochemicals like betalains, saponins, and polyphenols shown in Table 17.1 (Wang et al. 2015; Celik and Tuncil 2020). Quinoa has been highlighted as the grains of the twenty-first century (Adolf et al. 2013).

A detailed data on the use of biotechnological techniques that could improve the quality of metabolites produced from quinoa, which could be used for resolving several problems combating mankind, is provided. Moreover, several techniques used for the characterization of these active metabolites derived from quinoa were also highlighted.

On the basis of environmental conditions, quinoa plants are generally characterized. Currently, almost 1800 varieties and 120 species of quinoa plants are available (Bhargava and Srivastava 2013). There is also a wide variation in the color of seeds which may be gray, orange, red, yellow, pink green, purple, or black

Table 17.1 Relevant bioactive constituents present in quinoa

Quinoa components		
Amino acids	Histidine, isoleucine, leucine, methionine + cysteine, threonine, phenylalanine + tyrosine, valine, lysine, tryptophan	
Bioactive compounds	Betalains	Amaranthin, isoamaranthin, betanin, dopaxanthin, betaxanthin
	Saponins	Oleanolic acid, hederagenin, phytolaccagenic acid
	Polyphenols	1-*O*-galloyl-β-D-glucose, benzoic acid, ferulic acid kaempferol, quercetin, quercetin-glucuronide, rutin, protocatechuic acid 4-*O*-glucoside, vanillic acid, vanillic glucoside
Carbohydrates	Starch; amylose and amylopectin	
Dietary fiber	Soluble and insoluble fiber	
Fatty acids	Myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), behenic acid (C22:0), 9-docosenoic acid (C22:1w9), tetracosanoic acid (C24:0), tetracosenoic acid (C24:1)	
Minerals	Copper, iron, lead, magnesium, manganese, mercury, nickel, phosphorus, potassium, selenium, sodium, zinc	
Vitamins	Vitamin A, vitamin E (α-tocopherol), vitamin C (L-ascorbic acid), folic acid, riboflavin, thiamin, niacin	

(Vega-Gálvez et al. 2010). Quinoa is conventionally classified based on seed color into three types: red quinoa, black quinoa, and white quinoa. The variation in seed color is due to the existence of phenolic and saponin content in pericarp (Celik and Tuncil 2020). On the ecological adaptation base, there are five different types of quinoa recognized (Repo-Carrasco-Valencia 2011). Some biotechnological techniques that could be utilized for enhancing the nutritional and biological activities of the chemical constituents of quinoa plant will be elucidated in this chapter.

17.2 Pharmaceutical Constituents Derived from Quinoa

Different quinoa varieties have been discovered to possesses flavonoids and phenolic acids in the range of almost 36.1–144.2 and 16.6–59.6 mg/100 g of grain correspondingly (Repo-Carrasco-Valencia et al. 2010). Brend et al. (2012) concluded flavonoid and phenolic acid concentration depends on the type of quinoa seeds. For example, red quinoa contains 90% more flavonoids, 50% more phenolic content, and antioxidant property, three times more than yellow quinoa (Brend et al. 2012). Several studies have established anticarcinogenic, antiallergic, anti-inflammatory, antiviral, antidiabetic, lipase-inhibitory, anti-obesity, and hypocholesterolemic properties of phenolic acids because of their powerful antioxidant effects (Repo-Carrasco-Valencia et al. 2010; Carciochi et al. 2014; Zhang et al. 2015). Predominantly, saponins are derivative of hederagenin, oleanolic acid, serjanic acid, and phytolaccagenic acid with sugar moieties (Zhu et al. 2001). The bioactive functions of saponins include anticarcinogenic, anti-inflammatory,

antithrombotic, diuretic, and hypoglycemic (Madl et al. 2006). Bile acid and dietary interaction is helpful in bile acid excretion in feces (Francis et al. 2002).

Pharmacologically it has been identified that saponins possess the capability to enhance the intestinal permeability and motility necessary for the absorptive function of the intestine (Oakenfull and Sidhu 1990). Man et al. (2010) reviewed anticarcinogenic properties of quinoa-extracted saponins (Man et al. 2010). Flavonoids like apigenin, genistein, quercetin, and phytoecdysteroids are famous bioactive compounds having anticancer features (Ren et al. 2003; Graf et al. 2014). Among all the Poaceae cereal crops, this contains phytoecdysteroids (Graf et al. 2016). Anti-inflammatory and weight control properties of flakes were evaluated in 35 postmenopausal women that are overweight (De Carvalho et al. 2014). Furthermore, several in vivo studies have established that quinoa saponins could help in reducing obesity and weight gain (Improta and Kellems 2001;Graf et al. 2014). Mithila and Khanum (2015) established that taking quinoa cholecystokinins might increase postprandial which might led to satiety state and cause lesser food intake (Mithila and Khanum 2015). Likewise, Foucault et al. (2012) perceived that lipid storage gene expression was declined in fatty diet rats taking also quinoa extract supplemented with 20-hydroxyecdysone (Foucault et al. 2012).

Betalains are natural plant pigments giving black, red, and yellow colors to different varieties and structurally derived from aromatic nitrogen-containing indole. Moreover, it has been discovered that betalains consist of the following which entails amaranthin, betacyanin, isoamaranthin, and isobetanin (Tang et al. 2015). Recently, betalains possess antidiabetic, hypolipidemic, antiradical, chemopreventive, diuretic, hepatoprotective, and joint pain-relieving effects (Khan 2016).

Phytic acid is another pharmaceutical compound present in epicarp of seed (Gani et al. 2012). Extensive literature presents the bioactive effects of phytic acid like on diabetes, urolithiasis, various types of cancers, and CVDs broadly studied by numerous researchers (Rickard and Thompson 1997;Oatway et al. 2001, Bastidas et al. 2016). The hypocholesterolemic and antioxidant effect is obtained by decreasing Zn to Cu ratio and binding with Zn (Goufo and Trindade 2014). Several alkaloids have been investigated in quinoa like pyridine, tropane, and piperidine. Numerous alkaloid extracts at smaller doses have displayed sundry pharmacological properties like anti-arrhythmic, analgesic, anticholinergic, antibiotic, antihypertensive, antimalarial, and antitussive effects (Roberts 2013; Cushnie et al. 2014; Knölker 2016; Hashmi et al. 2018). Srikanth and Chen (2016) and Bai et al. (2015) described that trypsin inhibitors are effective in apoptosis, blood clotting, and inflammation. But quinoa contains very little amount of trypsin inhibitors as compared to beans, lentils, and soybeans (Bai et al. 2015; Srikanth and Chen 2016).

17.3 Bioactive Compounds Present in Quinoa Which Could Enhance Human Health

General bioactive compounds found in quinoa include saponins, phytosterols, phytoecdysteroids, isoflavones, peptides, polysaccharides, phenolic acids, and flavone glycosides. About 20 different phenolic compounds are found in seed coat of

quinoa plant which exist either freely or in conjugated form. These phenolic acids are generally seen to be composed of derivatives of ferulic and vanillic acids and also glycosides of quercetin, flavonoids, and kaempferol (Tang et al. 2015, 2016). These phenolics are stated to exhibit antioxidant function, and along with that, they also displayed pancreatic lipase and α-glycosidase-suppressive effects (Tang et al. 2015).

Seed extract rich in saponins has been reported to suppress inflammatory processes. It inhibits the release of inflammatory cytokines and eventually decreases nitric oxide concentration in the cell line derived from macrophages (Yao et al. 2014a, b). Moreover, they also have a suppressive effect on adipogenesis (Yao et al. 2015).

Phytosterols, one of the important lipophilics, are present in quinoa; these compete for intestinal absorption of cholesterol due to structural similarity of phytosterols with cholesterol. This eventually reduces the fabrication of atherogenic lipoprotein in the liver and intestine, thus helping to lower serum cholesterol level (Ho and Pal 2005). One of the important polyhydroxylated steroids, polyecdyteroids, is also present in this plant. These carry a structural relationship with insect-molting hormones due to which they play role in defense mechanism of the plant. Furthermore, phytoecdysteroids also have an inhibitory effect on osteoporotic events and ameliorate obesity and diabetes (Graf et al. 2014).

17.4 Production of Active Compounds with Enhanced Pharmaceuticals from Quinoa

Usually, bioactive compounds from quinoa are not eco-friendly and may be harmful to microorganisms, aves, and pests. They are also of great benefit to humans. Quinoa can serve as antidiabetic (Graf et al. 2014), immune-regulatory (Yao et al. 2014a, b), inflammation-lowering (Yao et al. 2014a, b), antitumor (Hu et al. 2017), cytotoxic (Kuljanabhagavad and Wink 2009), antimicrobial agents (Miranda et al. 2014), and also as adjuvant (Estrada et al. 1998).

Tang et al. (2016) established that quinoa-derived phenolic acids exist in conjugated form and sometimes in free state. Research has shown that the percentage configuration of conjugated phenolic metabolites from quinoa is usually similar to that found in the free phenolic metabolites in quinoa plant. Then they proposed that the chromatographic analysis of the phenol extract from the plant could reveal the free state of the phenol acid present in the plant.

Phenolics from plant are in confined state and are usually not affected by stress from their immediate environment (Gómez-Caravaca et al. 2012). One of the analogues of phenolic acids is benzoic acid. Benzoic acid derivatives are gallic acid (Jacobsen 2003; Suttiarporn et al. 2015), protocatechuic acid (Hu et al. 2017), syringic acid (Miranda et al. 2014), vanillic acid (Yao et al. 2014a, b), and among others. Researchers found the acids to be greatly deposited in the leaves and seeds (Tang et al. 2016; Gawlik-Dziki et al. 2013). Metabolites from the equivalents of benzoic acids have been establish to have reduced microbial infection (Cho et al. 1998), antioxidant (Ti et al. 2014), and anti-pesticide (Abou-Zaid et al. 2001)

properties. Ruiz et al. (2014) demonstrated that some secondary metabolites of quinoa fibers have the ability to shield quinoa from pathogenic microorganisms.

Terpenoids are made up of monoterpenoids and triterpenoids synthesized naturally from metabolism of isoprenoid. Monoterpenoids serve as allele chemicals, while triterpenoids, located in quinoa seed, coat the exterior of the plant. They are usually of a bitter and harsh taste. These organoleptic properties offer protection from aves and pests (Sun et al. 2009). Monoterpenoids are essential oils found in quinoa (Dembitsky et al. 2008). Triterpenoids, with their aglycones and glycosides, are also known to prevent pests and microorganisms that could harm the survival of quinoa plant (Ruiz et al. 2014). Saponins from quinoa are also considered as a metabolite that is bitter (De Simone et al. 1990; Mastebroek et al. 2000).

Madl et al. (2006) documented tetracycles and/or pentacycles as quinoa triterpenoids and are known to occur in the primary structures of quinoa plant. The derivatives of saponin are known to have five-ring building blocks synthesized naturally from β-amyrin in plants (Kim et al. 2008; Kuljanabhagavad and Wink 2009). Oleanolic acid was found to comprise majorly of aglycone (Burnouf-Radosevich and Delfel 1984).

Phytoecdysteroids are bioactive compounds present in quinoa, and studies have revealed that they are effective offering protection for plant against pest and pathogen insects and they have also demonstrated great therapeutic activity in mammals. Quinoa lipids have seven sterols that have been recognized which are Δ^7-campesterol, Δ^5-avenasterol (Saeki et al. 2000), campesterol (Cincin et al. 2015), cholesterol (Carmona et al. 2019), β-sitosterol (Lutz et al. 2013), and Δ^7-stigmasterol (Choo et al. 2002; Montoya et al. 2018).

17.5 Genetic Transformation of Tissue Cultures and Their Role in the Investigation of Biosynthesis Pathway Regulating the Production of Active Metabolites Available in Quinoa

Henry (1998) reported an in vitro propagation method of producing plants that are virus-free. The application of cell cultures could help in the selection of cell lines that can be improved, and also this method could serve as a beneficial method because the method has a large scale for rapid collection. Cell, organ, and tissue cultures of plants were extensively used for traditional techniques of crop production (Basu et al. 2002; Pauk et al. 2002; Borsani et al. 2003; Cherian and Reddy 2002, 2003; Zair et al. 2003).

Tocopherols, which are also known as vitamin E, are lipophilic antioxidants, vital nutritional ingredients. Among the tocopherols that exist, α-tocopherol is the greatest noteworthy vitamin E form existing in the tissues of green plants and has the maximum vitamin E properties. Synthetic α-tocopherol, which is a racemic mixture of eight diverse stereoisomers, is usually less active than α-tocopherol, the natural system (Basu et al. 2002; Pauk et al. 2002). Tyrosine aminotransferase (TAT) could speeds up the reversible feedback of tyrosine + 2-oxoglutarate ↔ 4-hydroxyphenylpyruvate + glutamate. Tyrosine is mostly produced from

4-hydroxyphenylpyruvate by TAT in bacteria, but these enzymes have been found to play a significant contribution in plant production (Buchanan et al. 2000), conversion of tyrosine to the metabolite for the production of plastoquinone, tocopherols, rosmarinic acid, and benzylisoquinoline alkaloids (Henry 1998; Borsani et al. 2003; Compton et al. 2004).

17.6 Significance of Biotechnology for Enhanced Food Security in Quinoa

Bhargava et al. (2006), Jancurova et al. (2009), and Inglett et al. (2015) documented that starch form is best in treating people with diabetes, cardiovascular diseases, and obesity. This is possible because of the little glycemic index found in quinoa. The starch found is also very useful for food production purposes (Araujo-Farro et al. 2010; Inglett et al. 2015). By comparison, starch granules are lesser with 3.0μm than maize which has 23μm starch granules and wheat which has 40μm; this property has made this recognized and endorsed for food (Valencia-Chamorro 2004; Valcárcel-Yamani and da Silva Lannes 2012; Rayner et al. 2012). The cloudy appearance of quinoa starch explains its usefulness in emulsion foods, while its resilient attribute to deterioration explains its usefulness in frozen food (Bhargava et al. 2006; Harra et al. 2011; Escuredo et al. 2014). Jancurova et al. (2009) reported on albumin and globulin which are primary proteins found in quinoa plant, and this protein constitutes 77% and 7% prolamins. Researchers described that the balanced quinoa protein amino acid profile is because of lysine and histidine in the plant. Quinoa can be a healthier substitute for protein source particularly in persons living in developing communities and countries (Comai et al. 2007).

Owing to the findings of quinoa being a cereal plant with no trace of gluten, it could be as well recommended for celiac disease patient and individuals intolerant to wheat. Studies of Delatorre-Herrera et al. (2010) have shown that gluten-free is very essential for enhancement of physical features (Thomas and Gausling 2000; Tongsawang and Sdoodee 2008; Delatorre-Herrera et al. 2010; Fghire et al. 2015).

Spaghetti fabrication to ensure that it is gluten-free also determined the parameters that could alter the quality, texture, and viscosity of their product. Their spaghetti had quality physical properties when compared with wheat-fabricated spaghetti (Tongsawang and Sdoodee 2008). Quinoa is applied in making pasta and pastries. These formulated food substances are usually consumed by people in need of the gluten-free food substances (Valcárcel-Yamani and da Silva Lannes 2012).

17.7 Insecticidal and Biopesticidal Compounds from Quinoa

Among the production losing factors in horticulture and agriculture departments, insects are key factor. Almost 25–30% production loss occurs on an average and sometimes exceeds to total loss (De Geyter et al. 2007a, b). Diverse species

possessed settled resistance against insecticides, indicating an extraordinary demand for advance insecticide objects (De et al. 2014). Many scientists and academia attracted more toward most effective plant compounds as natural insecticides (Isman and Grieneisen 2014). In this scenario, the interesting groups of molecules are plant secondary metabolites: the saponins, steroidal or triterpenoid molecules (Wink 2003).

Triterpenoid saponins are present in different legumes like spinach, soybeans, sugar beet, peas, tea, and quinoa (De Geyter et al. 2007a, b). These secondary metabolites possess ecological and physiological role against insects, birds, and various microorganisms (Izhaki 2002). The seeds are of important value because of better quality protein (Jancurova et al. 2009). The external part of the seed coat contains bitter saponins, and so seeds required special treatment for usage (Van Raamsdonk et al. 2010). Saponins possess numerous biological properties like membrane permeabilizing, antioxidant, immune stimulant, hemolytic, anti-inflammatory, and anticarcinogenic and affect food intake, as well as animal growth and reproduction (Sparg et al. 2004; Avato et al. 2006; Tava and Avato 2006). Saponins can be used as pesticides, fungicides, and molluscicides and against some viruses and bacteria (Duke et al. 2010). Many studies reported that plant herbivore insects fed less amount of saponin-containing food as compared to normal food (Agerbirk et al. 2003). Saponins decreased food motility across the gut of insect, hence decreasing digestion of food through inhibition of gastric enzyme secretions (De Geyter et al. 2007a, b). Starvation and disruption in digestion are two factors responsible for reducing insect growth (Narayanan 2004). Adel et al. (2000) also explained through experiment that the larvae lost weight before pupation as compared to control (De Geyter et al. 2007a).

Saponins are used as natural insecticides (Chaieb 2010). At sublethal dose, saponins prevent crop damage by lowering the food intake of insects (Francis et al. 2002). The instant effects of saponins on insects not only give protection to plants from insect pest damage but also provide protection against insect-mediated transmitted diseases. Exogenic usage of saponins in the form of powders and solutions on fields is available as natural insecticides in Asian region (De Geyter et al. 2007a, b). Among the secondary metabolites, flavonoids perform a vital role for plants against herbivores and feeding insects (War et al. 2012; Bartwal et al. 2013). The US Environmental Protection Agency has registered *Chenopodium quinoa* as biopesticide (Jiang et al. 2018). The ecdysteroids also known as phytoecdysteroids are the steroid-type quinoa seed metabolites. Other names of ecdysteroids are 20-hydroxyecdysone, kancollosterone, and makisterone and mostly prime in quinoa amounts fluctuating between 450 and 1300μg (Valoy et al. 2015). The ecdysteroids are important structurally due to their resemblance with insect steroid hormones (Dinan 2001). These steroid hormones are responsible for the regulation of physiological and biochemical processes linked with insect reproduction, embryo maturing, growth, and metamorphosis. These compounds control pest and prevent their outbreaks (Thummel and Chory 2002). The quinoa seeds have antiherbivore compounds like caffeic acid, cinnamic acid, gallic acid, hesperidin, kaempferol, morin, neohesperidin, orientin, quercetin, glycosides, rutin, and vitexin

(Paśko et al. 2008; Dini et al. 2010). Rutin serves as phagostimulant, while kaempferol is genotoxic acting as feeding restrictive and disturbs the emergence of aphids (Petersen and Simmonds 2003). The glucose ester of vanillic acid affects greatly progenies of aphids, while tannins reduce the tastiness of plant tissues due to their astringent effects or by forming protein complexes deactivate digestive enzymes. Similarly phytic acid plays its role by chelating various ions, thus interfering with insect diet (Paśko et al. 2008; Misra 2009; Terwel et al. 2011; Valoy et al. 2015).

17.8 Nematocidal Compounds from Quinoa

Similarly flavonoids relating to physiological and feeding behavior have restrictive effects against certain soil herbivorous nematodes (De Geyter et al. 2007a, b). Various families of plant kingdom possess phytoecdysteroids responsible for preventing against different nonspecialist herbivores and nematodes. These compounds become lethal and disturb molting of various nematodes (Soriano et al. 2004;Walters 2017). Numerous flavonoids are considered as antiviral, antibacterial, and antifungal and effective not only for plant pathogens but also against for animals and humans pathogens (Friedman 2007). Kaempferol along with its derivatives exhibits an antibacterial action against *Candida glabrata* and other bacteria (Saleem et al. 2010; Graikou et al. 2011). Quinoa possesses almost eight quercetin derivatives (Hirose et al. 2010). Quercetin, kaempferol, and myricetin represented as restraints against *Meloidogyne incognita* and *Radopholus similis* (Patra 2012). Quercetin suppressed the germination of *Neurospora crassa* (Treutter 2006).

17.9 The Application of Quinoa as a Biotechnological Tool When Applied as a Biopesticide

Research has shown that quinoa has long been used as biochemical pesticide and fungicide. The active component in the production of this biopesticide is saponins of *Chenopodium quinoa*, which was used on pieces of potato seed, bean, pea, and wheat seeds and for tomato seedlings before transplant (Taylor and Parker 2002). Researchers used pathogenic fungi, viral plant diseases, and bacteria as their organisms of interest in the work.

Davidson (2012) and the UN reported that the reason for the application of quinoa in the diet was because there is a global protein crisis. They added that the diet of humans is composed of excess unreasonable proteins. As the population is inflated, the use of large land mass in the production of animal protein was neither promising nor normal. They said that to have a more maintainable progress, a shift to food rich in plant protein is necessary, which would result in the eradication of the protein crisis, production of nitrogenous waste, and alleviation of adverse weather conditions (Davidson 2012). Mineral composition of plant is keen to the percentage

and accessibility of soil mineral available for the plant (Vega-Gálvez et al. 2010). Numerous vitamins are present in quinoa; however, the vitamin C concentration declines when the plant has been stored for too. The work of Fischer et al. (2013) reveals similar reduction in vitamin E due to excessive water in flowering stage.

Due to food quality problems, it has gained recognition for its ability to thrive in harsh environmental conditions. The availability of plant has reduced and brought solution to the problem of food sovereignty and security in vulnerable areas (FAO 2011). Valencia-Chamorro (2003) investigated the upgraded financial and societal advantages of quinoa cultivation which prompted haste for use of more farming lands.

17.10 Application of Quinoa as a Biotechnological Tool for the Management of Pests

Crops are in interdependent relationships with microbes in their normal environments, and they include mycorrhizal fungi or fungal endophytes. These symbiotic microorganisms exhibit tremendous activities that are eco-friendly, are suitable, and support plant evolutionary processes (Cicatelli et al. 2010). Some microorganisms have been found in quinoa (Claros et al. 2010; De Vos et al. 2013; Francesco et al. 2011).

17.11 Prevention of Abiotic Stress

Thomas and Gausling (2000) reported that plants have developed a lot of devices to deal with the incidence of water-inadequate conditions and that one of the most shared mechanisms involves stomata closure, which causes reduced loss of water and regulates plant water potential (Fghire et al. 2015). Tahi et al. (2007) used leaf extract potential to signify water-conserving action by plant leaves which was demonstrated to decrease quinoa leaf water capacity (Fghire et al. 2015; Tahi et al. 2007). There is disturbance in the conductance of the plant stomata by moisture pressure, which could lead to the decline of photosynthesis and the availability of CO_2 needed by the plant (Liu and Stützel 2002). Nevertheless, the quinoa leaves may not be the principal pointer of water stress. Undoubtedly dehydrated roots can also produce chemical signals bringing about stomata closure (Tahi et al. 2007). During research it was recognized that a signal was emitted from the roots to the leaves which is typically preferred in the dryness of the soil; this reaches the leaves by water transpiration, bringing about stomata closure. Grant et al. (2012) identified abscisic acid, signals in the plant which was said to have been synthesized in the roots in response to the soil dehydration. The stomata respond otherwise depending on the time of the drought and also the type of soil in which the plant is grown (Jensen et al. 2000). Jensen et al. (2000) suggested stomata conductance decrease could be a good indicator to the water stress and reduction in leaf water potentials. During water shortage, the stomata close to reduce water losses (Tahi et al. 2007;

Jensen et al. 2000). Stomata closure and performance of the photosynthesis depend on the harshness of water deficit (Tsonev et al. 2014).

Findings have shown that the water status of the sheet fuses with stomata conductance and transpiration and a good relationship is often experimented between leaf water potential and stomata conductance under water shortage (Giorio et al. 1999). Nevertheless, more authors have reported a large heterogeneity at the relationship among stomata conductance and water potential (Fernández et al. 1997). Stomata response to other ecological features may be accountable for this scattering (Aasamaa and Sõber 2011). Indeed, the exact relationship seems to be principally dependent on the species studied, the stage of the water shortage, growing conditions, and timing of the measures (Tongsawang and Sdoodee 2008; Ashraf and Harris 2013). Chaves and Oliveira (2004) revealed that the improvement in plants grown under narrow moisture situations was seriously thwarted (Chaves and Oliveira 2004). Decreased mass and leaf part were largely perceived by Ashraf and Harris (2013). Martínez et al. (2009) described quinoa as offering a lot of benefits to farmers especially during dry season (Martínez et al. 2009). De Vos et al. (2013) concluded that it's better for humans to depend on quinoa for farm land recultivation and remediation after it has been affected by salt. Quinoa is a unique crop, which is valuable in the study of devices embraced by plants to adapt to increase saline and drought (Adolf et al. 2013; Francesco et al. 2011; Pulvento et al. 2012; Ruiz-Carrasco et al. 2011; Shabala et al. 2013). Quinoa is known to have the ability to survive in hostile soil and weather settings and also suitable for several reasons in achieving food security. Pulvento et al. (2012) and Razzaghi et al. (2012) documented that a decrease of about half of water supply by irrigation did not alter the performance (Pulvento et al. 2012; Razzaghi et al. 2012). This plant was discovered to germinate adequately in hostile soils, though it may produce low yield when compared to it being planted in suitable climatic conditions. The researchers added that this effect could be moderated by the application of composted organic matter (Martínez et al. 2009).

17.12 Application of In Vitro Tissue Culturing of *Chenopodium quinoa* Using Biotechnological Techniques for Producing Essential Metabolites and Healthy Plants

It has been discovered that one of the critical factors for production of quinoa as a typical example of grain crop during large production is majorly affected by seed-borne diseases. Therefore, the use of somatic embryogenesis methodology has been acknowledged as significant instrument for operative plant production devoid of viruses. This method gives opportunity for fruitful large-scale cultivation of genetically modified plants which enhanced their capability to study their influence on plants emanating from the breeding process. Eisa et al.'s (2005) methodology was launched from the cell cultures and calluses of *Chenopodium quinoa*.

Burnouf-Radosevich and Paupardin (1985) performed a protocol for vegetative cultivation of two different varieties. The mature plant and the shoot tips of seedlings

were encouraged for generation of numerous shoots by axillary branching in a B5 medium altered reducing sucrose (10 g/L) and enhancing the level of the following phosphate (315 mg/L) salts and nitrate (2700 mg/L), together with glycine. The altered medium was added to naphthalene acetic acid (0.018 mg/L) as well as benzyl adenine (0.22 mg/L). These circumstances enable the growth rate to double shoots' quantity per culture when compared to when cultivated on non-modified B5 medium. There was typical deficiency of external growth substances which enhanced root elongation and rooting when compared to the addition of gibberellic acid and auxin. The rooted plants transferred to the greenhouse became matured within 2 months.

Cosac et al. (2016) performed an experiment involving rational and systematic utilization of extinct taxons, beginning from the old seeds derived from the soil in the herbarium to establish germinative attributes to conserve biodiversity. The species used include *Chenopodium* spp. and *Achillea* spp. It was observed that numerous methodologies for in vitro propagation of different species were established. Generation of callus in plant micropropagation was a major reason for numerous compositions of nutritive medium.

Shahin (2019) performed an experiment which focuses on callus induction and improved ancillary metabolic products from explants of *Chenopodium quinoa.* The maximum performance of seed growth was observed from seed cultivated in MS medium with full strength in the full-strength MS medium (100%). The maximum callus generation derived from seedling explant was derived from addition of 2 mg/L 2,4-D + 0.05 mg/L Kin to MS. It was observed that the callus of explants cultivated on MS media with 2 mg/L 2,4-D + 0.05 mg/L Kin demonstrated maximum significant tocopherol content with 22μg/g fresh wt followed by seedling on half-strength MS medium. Furthermore, the authors also performed enzymatic and protein extraction evaluation of tyrosine aminotransferase. The result obtained from the enzymatic experiment indicated that the level of enzyme activity present in the leaves was twofold greater than these observed in the seeds, while the callus culture activity was about onefold lower than the leaf extracts.

It has been observed that betalains belong to water-soluble pigments of plants containing hydrophilic attribute possessing bioactive capability. Betalains have been identified as a typical example of edible sources of betalains derived from grain crops such as *Chenopodium quinoa* Willd, which possess numerous colors which include yellow, red, and violet grains which was impacted by the presence of these pigments. In view of the aforementioned, Henarejos-Escudero et al. (2018) produced callus cultures from numerous plant varieties. It was observed that there was presence of steady callus lines which showed generation of coloration when cultures on Murashige and Skoog medium in the presence of regulator of plant growth 6-benzylaminopurine (8.88μM) with decrease in the level of nitrogen sources to 5.91 mM. The structural elucidation and the detection of the numerous pigments were carried out by ESI-MS/MS and HPLC-DAD which shows the presence of individual pigment cell lines. The result obtained indicated that vulgaxanthin I and phyllocactin are the major pigments available in these plant species. Their study

validates the significance of *Chenopodium quinoa* culture cell line in the generation of specific compounds of significant nutritional values.

17.13 Application of Metabolomics and Machine Learning Technology

The presence of pharmacological active constituents present in quinoa plant which could be of biotechnological importance could be detected using the molecular markers which might be generic or metabolic. The generic option proffers a unique solution that permits the identification of various species, genera, and plant diversities. Applying DNA markers as a diagnostic apparatus could also help in identification of useful components of plants. The application of metabolomics together with hyphenated analytical and spectroscopic methodology which includes liquid chromatography also offers interesting results. The application of numerous metabolomics techniques also help in the identification of numerous biotechnological techniques in plants. Metabolomics profiling also ensures detection of numerous plant varieties and crop cultivars.

Moreover, the application of adaptive binning of the nuclear magnetic resonance data together with evaluation of quantitative variation among lines for individual bins and detection of the major genomic regions evaluating the metabolomics profile variability of specific compounds of interests such as secondary metabolic products and sucrose-derived metabolites, together with some undetected compounds, could be linked to one or more genetic loci. Moreover, the synergetic effects of these metabolomics analytical tools could also validate the presence of multiple loci throughout the genome that could influence the presence of numerous compounds mainly through a network of interactions, where individual loci may affect more than one compound available in plant of special interest with high nutritional and medical significance such as quinoa (Erban et al. 2019; Adetunji et al. 2018a, 2019a, b).

17.14 Application of Nanotechnology for the Improvement of Active Metabolites

Nanotechnology has been recognized as a sustainable technology that could be utilized for the bioengineering of specific metabolites of greater interests which could be utilized for resolving numerous nutritional challenges, food insecurities, and environmental challenges and management of human, plant, and animal diseases. The application of nanotechnology through the synthesis of biogenic nanoparticles derived from eco-friendly, novel, and active biological constituents from quinoa could also be responsible for their biological activities. Therefore, the application of synthetic biology, informatics, computational biology, and bioinformatics together with nanotechnology could also give a better insight on some other unique application of these nanoparticles derived from quinoa plants (Adetunji et al. 2019a, b; Adetunji and Ugbenyen 2019).

17.15 Conclusion and Future Recommendations

This chapter has shown that quinoa possesses more nutritional values than most of the cereals and grains. In present-day world, latest techniques are being developed, and further research is continued to obtain such methods which can help in making a better source of food and getting more health beneficial compounds. Various processes of metabolic engineering have played a role in this context so far. More quantity of compounds of interest from these plants can be obtained, and their production can be improved by using the applications of metabolic engineering like fermentation and germination, growth under saline conditions, and air-drying and dehydration procedures. Fermentation and germination have proven to be the advantageous processes to produce energy-rich ingredients with excellent antioxidant activity and health-improving effects. Furthermore, clinical trials and advanced scientific investigations are necessary to increase quinoa bioactivities and medicinal applications.

The application of techniques that could establish the structures of relevant metabolites with higher biotechnological techniques was also discussed (Abubaka et al. 2019; Adetunji 2008; Adetunji et al. 2011a, b, c, 2012, 2013a, b, 2014, 2017, 2018b, c, d, 2019c; Adetunji and Olaleye 2011). Also, the relevance of synthetic biology, informatics, computational biology, and bioinformatics together with nanotechnology on how they could improve some bioactive constituents derived from quinoa plants was also highlighted.

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Quinoa, A Model Crop for Tomorrow's Agriculture

18

Didier Bazile

Abstract

The worldwide interest in cultivating quinoa (*Chenopodium quinoa*, Willd.) is mainly due to the plant's hardiness and its strong nutritional potential. It is one of the main foods of the Andean people before the Incas. The expansion of the crop that was until now considered relatively minor because it was geographically limited to a few Andean countries has raised several issues. The effort to promote the crop is part of a broader FAO (FAO: Food and Agriculture Organization of the United Nations) (UN) strategy to promote traditional or forgotten crops as a means to combat hunger and promote healthy eating. With experiments and field trials being conducted on every continent, it is well on its way of becoming a major crop for world agriculture and food. In February 2013, FAO Director-General José Graziano da Silva said, during the official launch of the International Year of Quinoa at New York UN Headquarters, that this can play an important role in eradicating hunger, malnutrition, and poverty. Many countries not only in South America are increasing production. This chapter addresses the key question of how it serves for increasing access to nutritious food. The issue is not only because of its nutritional value but also because smallholder farmers always currently produce the most part of quinoa internationally consumed. As the world faces the challenge of increasing the production of quality food to feed a growing population under climate changes, it offers a valuable alternative food source if we preserve practices that shape its characteristics. Thinking globally about the sustainable use, biodiversity could facilitate a paradigm shift in agricultural models, taking more account of nutrition as an approach to a broad

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agricultural development. Linking food nutrition and security to the use of water and energy in agriculture, to aspects of health for farmers and consumers, and to the protection of biodiversity in agroecosystems for adapting farming systems to climate change, that is probably the key for tomorrow's agriculture where quinoa appears as a model crop.

Keywords

Quinoa · Nexus · Agriculture · Biodiversity · Alimentation · Health · Energy · Water

18.1 Introduction

The quinoa (*Chenopodium quinoa* Willd.) is a plant native to the Andes cultivated for millennia. The current high genetic diversity of the cultivated species is based on the selection of farmers' varieties conducted over time by generations of farmers. It is estimated today that more than 6000 accessions are conserved in gene banks in Andean countries. This has allowed the cultivation of the species to be adapted to different environments in Latin America by responding to local agroecologies.

Quinoa is a very hardy plant capable of withstanding drought, salinity, frost, solar radiation, etc., as long as the farmer is able to sow the variety in such a way that is appropriate to his local environment. This is why five major ecotypes of quinoa have differentiated over time (Fuentes et al. 2012). Today, they are present in Andean agriculture and are associated in different ways with other crops depending on the environment (Alandia et al. 2019). Altitude, temperature, and precipitation are three major elements to consider to understand its insertion in the cropping systems taking into account its development cycle. These will be determining factors to define the potential areas where quinoa can be introduced in new agricultures elsewhere in the world. Today, its cultivation is present in 123 countries on all continents (Alandia et al. 2020).

The main question we raise here is: how quinoa can be a factor in the sustainability of world agriculture? A large part of the answer lies in the richness of its genetic diversity and how we will be able to value it while conserving it dynamically (Louafi et al. 2013). To conserve this heritage, we hypothesize that it relies on the uses and in particular food. Quinoa is internationally considered as one of the main superfoods of the planet. It has an exceptional nutritional composition, which is why it can contribute to global nutritional and food security. Understanding the role that quinoa can play as a functional food makes us switch to its role for human health. However, if we want to offer a healthy food, it is necessary to produce seeds without pesticides, which is facilitated by its hardiness: plant health and ecosystem health will then be better taken into account, the health of farmers preserved and the health of consumers improved.

Understanding the overlapping of these elements for the production of quinoa will require a systems approach through complex systems. We will then have to

integrate into our framework the water and energy dimensions, both necessary for production, transformation, transport, and final consumption. Approaching quinoa, from folk to fork, requires us to distance ourselves from the agricultural production, to understand all the actors involved in the whole process from seed to plate and their objectives, and to place them in the context of more encompassing issues, which is made possible by the use of Nexus to build a new logical analysis framework.

Thinking of quinoa's future perspectives is necessary for preserving quinoa genetic diversity that is the base for being able to adapt quinoa to marginal environments, to feed malnourished people in developed and developing countries, and to generate incomes for small-scale farmers while preserving all essential ecosystem services for the benefit of humanity.

18.2 Quinoa Crop in Agriculture

Quinoa (*Chenopodium quinoa* Willd.) is a cultivated plant native to the Andean region (Galwey 1992; Gandarillas et al. 2014; Jacobsen 2003). The main center of origin of the species is considered the shores of Lake Titicaca, at more than 3800 m above sea level, where it was first domesticated about 7000 years ago (Bazile 2015). It is difficult to date precisely its domestication, but the archaeological sources have shown that the leaves and seeds of wild quinoa were used as food even before it was agriculturally domesticated (Planella et al. 2015). As is the case in any domestication process from a wild relative, it has undergone a wide range of morphological changes as a result of human activities of plant selection and farmers' breeding process. Plants today present a more compact inflorescence at the tip of the plant, an increase in the size of the stem and seeds, loss of seed dispersal mechanisms, and high pigmentation levels (Bazile et al. 2013).

18.2.1 Agroecology of Quinoas in the Andes

Over the course of human migrations, quinoa has been gradually adapted by farmers to other ecological and social farming contexts by spreading to what are now Bolivia, Peru, Chile, Argentina, Ecuador, and Colombia (Bazile 2009). Even though quinoa grows along a latitudinal range extending from 5° S to 30° S, the plant is most commonly found north of 20° S (Bazile and Negrete-Sepulveda 2009). Today, small farmers mainly working with their communities produce almost all of quinoa. We distinguish five major ecotypes of quinoa (Fuentes et al. 2012) from the first characterizations (Tapia 1997), each associated with a particular Andean agroecosystem defined according to altitude and rainfall gradients. Each quinoa group presents specific characteristics due to differences in the conditions of environments and associated agricultural practices (Fagandini Ruiz et al. 2020).

Around Lake Titicaca (mean altitude of 3800 m above sea level), the *quinoa of the Altiplano* has white grains, and plants are more colored at a higher altitude. These

Altiplano quinoas are grown under varying conditions, characterized by low precipitation (400–600 mm per year) and favorable temperature conditions (6–17 °C).

The quinoas of the salars (salt flats) are mainly found in southern Bolivia with some extensions into northern Chile and northern Argentina. These high-altitude desert quinoas can withstand extreme xerophytic conditions, with often less than 200 mm of annual rainfall, up to 250 frost days in a year and winter temperatures that drop below −20 °C. Given this extreme dryness, farmers have developed specific strategies to use the residual water that accumulates in the soils of the Southern Altiplano, which acts as a bowl without discharge to the sea. A very early preparation of the soil allows good infiltration of water and prevents it from runoff or evaporation. The field thus absorbs water for a year before being planted with quinoa. Quinoa is the only crop that can withstand the climatic and soil conditions of the salars. The farmers have selected varieties with stems and seeds both having a wide range of shapes and colors. However, after processing to remove the saponin, all these quinoa varieties appear as large white grains, which is what the consumer sees in supermarket packs (e.g., quinoa real).

The quinoas of the inter-Andean valleys, often considered a separate group, can, however, be divided into those from arid valleys (e.g., Junín) and those from humid valleys (e.g., Cajamarca). A distinction can also be made between varieties that benefit from certain forms of irrigation (as in Urubamba, Peru, and Cochabamba, Bolivia) and those that grow in rainfed conditions (as in Huaraz, in the Mantaro Valley, and in Ayacucho and Abancay, Peru). These quinoas grow most often in valleys between 2500 and 3600 m of altitude, with rainfall up to or even exceeding 1000 mm per year. Temperatures are warmer than at higher altitudes.

The quinoas of the yungas (humid forest valleys) in Bolivia grow in subtropical conditions. This small group of quinoas has been adapted to high-altitude wetlands located between 1500 and 2000 m above sea level. On maturity, the plants exhibit a characteristic orange stem. Their adaptation to subtropical climates allows them to adapt to high precipitation and heat (annual mean temperatures above 20 °C).

The quinoas of sea level are characteristic from central and southern Chile. They can be found ranging from fields on the Pacific coast, in the region of Bernardo O'Higgins (Nunez and Bazile 2009), to the south of Temuco, in Valdivia and even on Chiloé Island. Their altitudinal limit is around 1500 m, which explains why these quinoas are also found in the Andean foothills, especially in the gardens of the Mapuche women (Bazile and Thomet 2015). Over this region's north-south span, quinoa grows under an annual rainfall ranging from 400 mm to more than 2000 mm in the Villarica-Pucón area. These quinoas have smaller grains than do the others, often beige in color.

The model of quinoa's evolutionary dynamics shows the differentiation of the five Andean ecotypes: (a) inter-Andean valleys (Ecuador, Colombia); (b) yungas (Bolivia); (c) Altiplano (Peru, Bolivia, Argentina); (d) salars (Chile, Bolivia); and (e) sea level (south-central Chile) (Fuentes et al. 2012). These ecotypes are closely linked to agroecological conditions that put the evidence that agriculture and biodiversity are going together.

18.2.2 Quinoa Adaptation for Crop Diversification Worldwide

The cultivation of quinoa really started to come out of the Andes in the 1990s. The emergence of consumption in the northern hemisphere in the 1970s and 1980s generated a strong interest in the nutritional value of these grains (Bazile 2015). The first variety of quinoa selected in the USA appeared in the mid-1980s in Colorado. Nevertheless, it is really only during the 1990s that the rise of quinoa had its first boom because of the increasing world consumption. This fact was the condition for initiating the first wave of worldwide trials of the species.

Quinoa was then offering a new alternative path for the diversification of cropping systems outside of the Andean region, and production was increasing progressively. In southern Europe, the gradual abandonment of cotton and tobacco cultivation found in quinoa a species with strong development potential and growing markets. Italy, Greece, and Spain were immediately candidates for testing the crop under their agroecological conditions. Galwey (1992) working closely with J. Risi from Peru developed together the basis for understanding the main elements needed for adapting quinoa in temperate environments (Risi and Galwey 1991a). They were the firsts to develop quinoa-breeding programs in Europe, especially in the UK.

The trials carried out in England, even if they have not been very visible outside a circle of specialists, are nevertheless those that have served as a basis for the future development of culture in Europe, and certainly on a global level. Jacobsen (1998), like the researchers in the Netherlands, was able to continue the approach initiated by Risi and Galwey (1991b) to understand the genotype-environment relationship of Andean quinoa varieties in order to adapt it elsewhere, taking into account the effects of latitude between northern and southern Europe were essential parameters for developing varieties adapted to temperate conditions.

From then on, at the end of the 1990s, the first quinoa boom had already led to its worldwide recognition as a superfood among the consumers interested in the health and nutritional aspects of their diet. This is why researchers involved in plant-breeding programs in different parts of the world including in the Andes converged to describe the strong worldwide potential for quinoa (Bhargava et al. 2006; Jacobsen 2003; Mujica et al. 2003).

Jacobsen et al. (2003) explained why the resistance of quinoa to adverse abiotic factors is the key for a wide expansion of the crop. Considering the characteristics of the species in Andean agroecosystems, they considered that: "There is no crop other than quinoa that resists the combination of adverse factors, present at various times, intervals, and intensities in the Andes. The perception that quinoa has a pronounced resistance to drought, and other factors that affect crop yield under the harsh conditions of the high Andes has been confirmed. All drought-mediating mechanisms are found in the species, although not all mechanisms are present in all genotypes. Quinoa escapes drought principally through early maturity, an important trait in areas where drought risk is likely toward the end of the growing season (terminal drought). Quinoa may tolerate drought through growth plasticity, low osmotic potential, and tissue elasticity, and it may avoid drought through a deep, dense root system, through reduction in leaf area by leaf dropping, through

generation of papillae containing calcium oxalate, and through stomatal behavior. The improved knowledge of the mechanisms of resistance of quinoa to adverse abiotic factors will help develop techniques for overcoming the constraints imposed by harsh environments existing not only in the Andes but in other regions worldwide. Quinoa has the potential for providing a nutritious food staple to countries in arid and semiarid regions." Based on scientific evidences from their research during the 1990s, these authors considered all the issues that are today the baseline of quinoa spreading worldwide.

The main point they did not integrate into their framework were issues about how to achieve sustainability in agricultural systems. The power of quinoa to conquer the world has undoubtedly masked the limits and the risks that must be considered today in order not to fall into the drifts of an industrial production-oriented agricultural system when one has a species such as quinoa with so many advantages to build a new, more sustainable agricultural and food model.

18.2.3 Perspectives on Sustainability for Food and Agriculture

Species diversification in cropping systems was initially a solution to substitute high value-added species whose markets were shrinking. Then, the irregularity of production of certain cereals also led some farmers to look for alternatives. The recent accentuation of the effects of climate change in agriculture may further increase this craze for quinoa, considered it as a hardy and very water-efficient plant (Ruiz et al. 2014). The environmental footprint of human activities also leads us today to look differently at our agricultural choices, in particular the place of animal husbandry in our diet. The quality of proteins means that its cultivation also responds to a concern for diversification to increase the proportion of vegetable proteins cultivated (De Ron et al. 2017).

The perspectives raised by the climatic deterioration to come, widely underlined in the latest reports of the IPCC and IPBES experts, show to what extent agriculture, and our production-oriented agricultural models, is called into question in the current situation and its probable evolution over the next century. Global quinoa production is expected to increase in the future. This ancient grain is now there, but it can also help us rethink the sustainability of our agricultural and food systems. Access to the biodiversity of quinoa preserved by generations of farmers is an essential factor in the adaptation of the species to new environments, but also as a base material to pursue plant-breeding programs (Bazile 2016; Bazile et al. 2016a). Beyond biodiversity as a source and input in the design of new multispecies cropping systems will be a major key to respond to global changes. Finally, while quinoa production will only be sustainable if the grain is consumed, the nutritional value and functional properties of quinoa mean that the grain has assets well beyond food and is often referred to as a healthy food. The danger of the expansion of quinoa cultivation is however due to the current craze for its nutritional qualities which today define its commercial value, because they also determine its weakness if its other assets of hardiness and low water requirements are not sufficiently taken into account. Energy

and water are two pillars of the future of agricultural and food sustainability on which quinoa can also rely for further development. Water scarcity, water and soil salinity, and energy cost for producing food are key elements that could be central for quinoa future (Bazile et al. 2016a, b).

All the issues perceived in the development of quinoa culture worldwide are also essential to understand and integrate to accompany the ongoing changes in quinoa culture in the Andes (Garcia et al. 2015).

18.3 Quinoa, Crop Wild Relatives, and Biodiversity

18.3.1 Quinoa Agrobiodiversity

Biodiversity in agriculture exists only because it was created and it is maintained by human practices that preserve local varieties (landraces) in a multiplicity of production systems and agrarian landscapes. In this sense, the destiny of men and plants is closely linked, and exploring the dynamics of quinoa will necessarily lead us to reflect on the relationship man has with biodiversity and on the relationships between men when they attempt to access this biodiversity and make use of it.

This approach through agrobiodiversity (or agricultural biodiversity) allows us to address several intersecting dynamics, both in time and space, in order to try to discern the actors involved and the resources concerned. This thinking about agrobiodiversity may assist us in determining whether eating quinoa helps in maintaining its biodiversity and contributes to improving the living conditions of mainly small-scale and poor producers, and whether we can contribute to global food security by promoting a healthy agricultural model through our consumption choices. In that way, quinoa biodiversity will not be a pillar of the Nexus but at the heart of the Nexus.

The problems of agricultural production, product flows, and local territorial identities—with the presence of some endemic indigenous species—the existence of biodiversity hotspots linked to globalization, and discourses of local communities and NGOs advocating the defense of the environment all refer us to an analysis in which the elements are dependent on the representations of human society. The concept of biodiversity is drifting slowly away from its ecological foundations to encompass economic and social issues and, in particular, the ownership of living things.

Access to seeds is essential for a farmer, without which there can be no agricultural production. If he is able to use a wide range of varieties, there is the possibility of making different choices depending on climatic and soil conditions, technical constraints, and own personal preferences or to fulfill a specific market demand or even meet a family request. However, unlike the so-called "wild" biodiversity, the biodiversity of cultivated species exists only because of human activities. It was the very first farmers who domesticated certain species near their settlements. When farmers began collecting seeds for reproducing the plants of the next generation, they were able to select certain traits of character depending on the geographic, social, or

cultural environments. The multiplicity of conditions and orientations of varietal selection by a wide range of human groups forms the basis of the diversity of cultivated plants. The fact that it is man who created this diversity makes him responsible for its reproduction and maintenance (Bazile et al. 2013). In fact, agrobiodiversity requires active and continuous human intervention to maintain the existing cultivated diversity.

In this context, the case of quinoa is very interesting because it is faced by several challenges related to the current global expansion of its cultivation (Bazile and Baudron 2015). Seed exchanges during human migrations have enriched the original genetic diversity of the species, leading to five distinct major ecotypes in the world. Varietal selection undertaken in the extreme environmental conditions of the Altiplano, the original and still the main center of quinoa production, has conferred the traits for hardiness and adaptability to the plant. Agrarian Andean communities still grow quinoa today according to agroecological practices that are called "traditional." This has led consumers in global markets to recognize traditionally grown quinoa as a healthy product. But the rapid expansion of the area under quinoa cultivation on all the continents is taking place at the same time as its insertion into so-called "conventional" agricultural models which rely on chemical fertilizers and pesticides. Intensifying its cultivation is a source of many potential conflicts, within and outside the Andean zones (land issues, standardization of practices, market competition, etc.).

18.3.2 Quinoa Genetic Resources

Quinoa (*Chenopodium quinoa* Willd.) is present in all Andean Cordillera under different altitudes and associated climates. Worldwide, there are more than 16,000 accessions conserved in national gene banks for the species or its wild relatives. Rojas et al. (2015) estimated that more than 50 gene banks distributed in more than 30 countries had accessions in their collections. It is considered that the Andean countries still concentrate most of the genetic diversity (88%) in their gene banks. Nearly 6000 peasant varieties are reported to exist between Peru and Bolivia.

Bazile et al. (2016a) question the availability of this quinoa genetic diversity to adapt the crop to other environments outside the Andes. For centuries, since its domestication on the shores of Lake Titicaca, farmers have traveled with seeds or exchanged them with other farmers. The movement of seeds between neighbors, between farmers, or with people passing through has allowed quinoa to travel in space as human groups moved. The agroecological diversity within the new spaces of agricultural practices and customs has made the selection criteria and practices evolve in order to increase the genetic diversity of the quinoa species. At that time, there were no legal restrictions on the circulation of seeds between states, except for customary rules defined within local societies (rituals, marriages, etc.).

Rojas et al. (2015) have identified in 2014 that non-Andean countries also have their own quinoa collections. Following prospecting missions, as was the case in the 1970s and 1980s, some countries set up these collections. The main objective of

these collections was to have genetic material of their own to initiate plant-breeding programs. Bazile and Baudron (2015) counted more than 25 gene banks of this type around the world, and today, considering the worldwide expansion of cultivation, the number can easily be doubled.

Chevarria-Lazo et al. (2015) analyzed the challenges of the regulation systems for the exchange of quinoa genetic resources. The genetic resources of quinoa are still mostly located in the Andean countries which conserve them both in situ (in farmers' fields) and ex situ (in gene banks). The governments of these countries do not wish to be dispossessed of the benefits related to the use of these genetic resources. A general debate on the contribution of Andean genetic resources to the expansion of cultivation is necessary to collectively establish a fair and equitable sharing of the benefits that could result (Bazile 2016).

There are several main problems with the global seed regulatory system. Firstly, no Andean state can claim ownership of quinoa genetic resources because state boundaries do not overlap with the boundaries of natural ranges of genetic diversity. Secondly, since quinoa has been able to pursue its diversification by increasing its genetic diversity through human migrations, is it necessary given our current legislation, and reasonable, to stop this evolutionary dynamic that links people and plants around agriculture (Bazile 2014)? Thirdly, since part of the genetic resources of quinoa is already found outside the Andes in a legal context (collection established before 1992) to develop new varieties, what cooperation can be put in place so that Andean countries contribute but also benefit from the research work conducted around the world?

18.3.3 Quinoa's CWR, Gene Pools, and Territorial Biodiversity

The major challenge for tomorrow's agriculture may well lie in its ability to evolve to cope with the effects of global changes. This capacity of agricultural systems will depend on crop resilience, which in turn depends on the level of genetic diversity maintained and used in cropping systems. Maintaining the current level of genetic diversity is a strong challenge for which all users must work together at a global level.

Beyond this potential resulting from the intrinsic genetic diversity, the crop evolves in an open environment with close links with other close species, its wild relatives. The characterization of the phylogenetic link with these wild relatives in an evolutionary dynamic will allow us to improve our knowledge on the major traits and its evolutionary potential (Maughan et al. 2004).

Castañeda-Álvarez et al. (2016) demonstrate in their paper the importance of conserving the wild relatives of cultivated species for world agriculture and food. Quinoa, like many other neglected or underutilized species, remains traditionally cultivated in an environment where related species can easily interbreed with the crop (Katoch 2020). Properly identifying the geographical distribution of these quinoas' wild relatives is a challenge in order to be able to locate if they are within

Table 18.1 Content of essential amino acids (EAA) in quinoa in comparison with the values recommended by the FAO (in grams per 100 g of protein)

	FAO recommendations	Quinoa
Isoleucine	3.0	4.9
Leucine	6.1	6.6
Lysine	4.8	6.0
Methionine	2.3	5.3
Phenylalanine	4.1	6.9
Threonine	2.5	3.7

the cultivated plot, nearby (for pollen exchange), or under other agroecology or remote geographical areas (Fagandini Ruiz et al. 2020).

Understanding the organization of the cropping space and the consideration of different sub-agroecologies by Andean farmers will facilitate the characterization and conservation of quinoa genetic resources. Landscape agronomy is a new discipline that can contribute to a better understanding of cropping environments. The conservation of the biodiversity and its wild relatives will undoubtedly benefit from advances in this new approach to systemic agronomy. Quinoa's CWR may be explored, and characterized, in that way for then being integrated as new candidate genes in quinoa-breeding programs.

18.4 Alimentation

Farmers in the Andean countries first domesticated quinoa about 7000 years ago on the shores of Lake Titicaca. It was the staple of the diets of pre-Columbian civilizations. However, unlike other species such as beans, potatoes, or corn, which Spaniards brought back to Europe from Central and South America, this was not accorded any importance by the colonizers. Various reasons explain this lack of interest, including the saponin content of the coating of its seeds, which makes it unsuitable for human consumption without prior dehulling, and the fact that flour, which contains no gluten (today, a virtue), cannot be baked into bread.

In parallel, the Spanish conquistadors in the sixteenth century imposed a cereal-based European diet as part of their efforts to subjugate local populations, and, consequently, the strong nutritional potential was first ignored and then forgotten. It was not until the 1970s that vegetarian consumers in the countries of the North sparked renewed interest in quinoa, primarily because of its protein content, characterized by the presence of all the essential amino acids (EAA) (Kozioł 1992).

Research by NASA, the US space agency, to select crop species for extraterrestrial missions singled out quinoa by highlighting its richness in and balanced composition of all essential amino acids (Alandia et al. 2011). It emphasized in particular the presence of lysine, an essential amino acid usually absent in most crops. The landmark NASA study by Greg Schlick and David L. Bubenheim (1996) emphatically recognized quinoa's qualities and suitability for expansion to new cultivation regions: quinoa's EAA levels match perfectly with human requirements as outlined by the Food and Agriculture Organization (Table 18.1).

While the public knows quinoa almost exclusively as a grain for consumption in human food, other uses of the crop exist. Humans do not consume only the grains of quinoa. They also consume the tender leaves, up to the time the panicle starts developing (before 60 days). The leaves' protein content can reach 33% of the dry matter. In rare cases, the tender panicles are also consumed, as is the case for huauzontle (*Chenopodium berlandieri* ssp. *nuttalliae*), a quinoa cousin grown in Mexico.

Quinoa is unique in that it is a seed eaten in a manner similar to a grain. The grains can be processed, just like cereals, to form complete flour that is raw or toasted (after roasting grains), flakes, semolina, and instant powder, which can be used to prepare many dishes or drinks. Quinoa can be also used to supplement cereal flours by adding between 10 and 30% of quinoa for bread, up to 40% for pastry, and 70% for biscuits. Grains are the only ones that can today meet a growing international demand for gluten-free products.

Quinoa is also used sometimes as a supplement in animal feed. The entire plant can be used as green fodder. Crop residues are incorporated into the feed of cattle, sheep, pigs, horses, and poultry. Various trials are taking place in Chile and Bolivia for processing quinoa into silage in order to maintain a high nutritional value in the processed quinoa, equivalent to basic green fodder.

18.4.1 Quinoa's High Nutritional Composition

The nutritional content is well described in many publications so only key elements are reported here. It contains all ten essential amino acids, and the protein content of quinoa varies from 12 to 19.5% depending on varieties and growing conditions. It is a good source of unsaturated fatty acids, dietary fiber vitamins, minerals, and other bioactive compounds (betaine, carotenoids, isoflavones, polyphenols). It has glucose, fructose, sucrose, and maltose. The starch content of quinoa varies from 58.1 to 64.2%. Quinoa may also be used in various foods such as pasta, bread, cookies, and baby food. Regarding its nutrition, it is comparable or superior in energy to similarly eaten foods such as beans, maize, rice, and wheat. In addition, quinoa is internationally recognized as a good source of quality protein, dietary fiber, polyunsaturated fats, and minerals. While it is a good source of many nutrients, it is important to consume it as a part of a balanced meal with many other food types to obtain good overall nutrition.

Proteins are made up of amino acids, of which eight are considered essential for both children and adults in human nutrition. When compared to the FAO's recommended essential amino acid scoring pattern, quinoa exceeds the recommendation for all eight essential amino acids. In contrast to quinoa, most grains are low in the essential amino acid lysine, while most legumes are low in sulfuric amino acids methionine and cysteine (Kozioł 1992; Nowak et al. 2016; Repo-Carrasco et al. 2003; Vega-Gálvez et al. 2010).

The average protein content of quinoa (16.3%) should be compared to that of rice (6.7%) which is the staple food of the main gluten-free diets as it is present in a high

proportion in the majority of food products already processed (Mota et al. 2016). It is becoming increasingly urgent that the nutritional composition of foodstuffs should guide the labeling of products to a greater extent and then be reflected in the production conditions that influence these compositions.

The nutritional quality of the protein of the seed of quinoa can be confirmed due to the presence of high levels of essential amino acids that are not always present in most cereals and legumes. But the partial characterization showed that the crude extract and its fractions have anti-nutritional factors. These factors were confirmed during the digestibility evaluation, which demonstrated that the adequate time treatment of quinoa seeds results in more efficient activity of the digestive enzymes. Further studies on this subject could focus on the mechanism involved in protein digestion. Therefore, the seeds of quinoa, if adequately prepared, demonstrate to have proteins suitable for human consumption, containing significant amounts of essential amino acids and a high index of absorption in the body, which makes it a great alternative to fight starvation worldwide (da Silva et al. 2015).

18.4.2 Quinoa Functional Food

On average quinoa is a better source of minerals than most grains. It is especially a good source of iron, magnesium, and zinc when compared to the daily mineral recommendations. A lack of iron is often one of the most common nutrition deficiencies. However, like all plant foods, it does contain certain nonnutritive components that can reduce its mineral content and absorption. Most notable are its saponins, which are found on the outer layer of the quinoa seed and are usually removed during processing to remove their bitter taste. This is also high in the compound oxalate, which can bind to minerals such as calcium and magnesium, reducing their absorption in the body.

Dietary fiber is the indigestible portion of plant foods, and it is important for good digestion and to prevent against constipation. Quinoa oil showed an interesting content of Ω3 fatty acids. It contains more fat (6.3 g) per 100 g dry weight than beans (1.1 g), maize (4.7 g), rice (2.2 g), and wheat (2.3 g). Fat is an important source of calories, and aids in the absorption of fat-soluble vitamins. Of quinoa's total fat content, over 50% comes from essential polyunsaturated fatty acids linoleic (omega-6) and linolenic (omega-3) acids. Linoleic and linolenic acids are considered essential fatty acids because the body cannot produce them. Quinoa's fatty acids have been shown to maintain their quality because of quinoa's naturally high value of vitamin E, which acts as a natural antioxidant.

The types of fiber found in flour may improve the digestibility and absorption in the large intestine. In general, a diet high in soluble fiber may help in the treatment of cardiovascular disease and type 2 diabetes by normalizing blood cholesterol, glucose, and insulin levels. Insoluble fiber, on the other hand, may help maintain adequate bowel function. A diet high in fiber is also associated with a lower risk of colon cancer. Finally, even if they contain no calories, a high intake of total fiber

would provide a greater feeling of satiety. Due to its composition, quinoa has a medium glycemic index, making it a grain of choice for people with diabetes.

Quinoa contains about 15% protein, and its amino acid composition is better balanced than that of most other grains, such as millet, sorghum, rice, wheat, and corn. Protein is used primarily to form, repair, and maintain healthy tissues, such as the skin, muscle, and bone. It is also used to form digestive enzymes and hormones. The Food and Agriculture Organization of the United Nations has observed that proteins, with or without saponins, would be equivalent in quality to those contained in whole milk powder. However, this does not mean that quinoa can replace milk and dairy products in a balanced diet. Quinoa and milk come from two different food groups, each providing specific essential nutrients to the body. Quinoa has a low percentage of prolamins (a kind of protein), which indicates that it is gluten-free and therefore interesting for people who suffer from gluten intolerance, known as celiac disease.

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules and thus reduce the damage caused by free radicals. A recent study showed that *pseudocereals* such as quinoa had high antioxidant activity. In addition, quinoa also contains isoflavones such as daidzein and genistein. Isoflavones are phytoestrogens, molecules of plant origin that act in the body much like the estrogens naturally produced by the body. Estrogens play a role in the regulation of the menstrual cycle, pregnancy, and breastfeeding, in addition to helping prevent bone demineralization and maintain healthy blood vessels. This raises the question of whether phytoestrogens can mimic the effect of estrogen. The effects are promising, but other studies have yet to confirm these hypotheses.

One *alicament* is a food combining the concept of food and drug. Much work using the first development on that issue (Asao and Watanabe 2010; Filho et al. 2017; Fuentes and Paredes-Gónzalez 2013; Graf et al. 2015; James 2009; Pellegrini et al. 2018; Repo-Carrasco-Valencia and Serna 2011) is currently being done to clarify how quinoa should be considered more than a food in itself but as an *alicament* or strong functional food.

18.4.3 Quinoa as a Part of the Diet

In order for quinoa production to successfully continue to increase, it has to face a number of challenges, both in the Andean region and in the rest of the world. Nutrition as an approach to agricultural development is one of them.

It is a crop that has tremendous potential to play a role in contributing to global food security. It is particularly high in nutrients, such as amino acids and certain minerals. But there are considerable varietal and environmental differences in the content of nutrients, bioactive compounds, and saponins in quinoa, which are important from an agricultural and nutritional point of view. Therefore, in an attempt to increase awareness and use of the different varieties of quinoa, greater emphasis needs to be placed on the production and communication of relevant knowledge at and below the crop species level (Nowak et al. 2016).

Considering the importance of quinoa proteins and essential amino acid content, Craine and Murphy (2020) said that "effective breeding strategies for improving quinoa protein quality should focus on identifying limiting amino acids, the factors that influence amino acid content, and increasing the content of limiting amino acids to improve PDCAAS.[1] Moreover, increased lysine and sulfur amino acid content are important targets, because these amino acids are limiting in most common cereals (e.g., wheat and maize), in addition to leucine content. Future work must be context specific with respect to germplasm adapted to the target production environment and culture."

Quinoa is a plant with a thousand virtues as it is often stressed. However, it is not useless to recall the importance of agrobiodiversity in food security, as each food never covers all the needs. How do the nutritional content and quality of the quinoa being produced help balance diets? At the macroeconomic level, an international approach may be useful in countering ongoing controversies over food security in the Andean countries. But from a practical and real point of view, it is at the household level that in-depth studies for monitoring quinoa consumption deserve to be undertaken. Only then will it be possible to understand how quinoa is inserted into the diets of mainly rural people and the impact it has on these diets. Quinoa's contributions can be judged directly by assessing local consumption-based products, or indirectly through the additional income quinoa generates which allows producers to buy foods to diversify or supplement their diets. The stability of food security depends on the frequency of food supplies. If quinoa is to be introduced with a goal of food security of populations, this aspect and existing diets have to be considered in order to overcome existing deficits at the right time (Bazile 2015).

Re-evaluating agricultural and food production not only through the goods they provide but also through ecosystem services—both received from and provided to the system—will require scientific advances in the medium term. Increased recourse in agricultural systems to biological and ecological regulations that one seeks to use to enhance performance requires more than the convergence of ecology and the agricultural sciences. From a theoretical and conceptual point of view, to undertake this paradigm shift, the expected transitions and recompositions of agricultural systems require not only the integration of knowledge between ecology and agronomy but also a renewed link to biology, the earth sciences, and the humanities and social sciences.

18.5 Plant Health, Ecosystem Health, Human Health, and Planet Health

How to ensure that food and nutritional security incorporates health-related aspects to a greater extent? The growing interest in agroecology is based on a paradigm shift: going from "producing more" to "producing better." Agroecological innovations

[1] PDCAAS: Protein Digestibility Corrected Amino Acid Score.

consist mainly of reductions in chemical inputs that lead to lower costs for producers, better soil health, pest control by seeking a balance through better use of biodiversity, and, finally, reduced health hazards for the producers (given the toxicity of pesticides).

Food labeling does not yet sufficiently highlight the dangers accruing from pesticide applications on crops. Only organic certifications guarantee that harmful chemicals have not been applied. Yet, consumers would like to know the level of potentially hazardous materials present in all products they ingest. For example, the nitrate levels in bottled water are clearly printed on the labels. A better knowledge of its nutritional content and potential health risks would be a major advantage in popularizing quinoa. Indeed, the mere mention of absence of gluten (or risk of allergy) will reassure and attract consumers with celiac disease.

In addition to its ability to grow on very poor soils (Jaikishun et al. 2019), quinoa is an extractive or purifying crop. Thus, given its fixative properties, planting in soils with heavy metals will purify the soil. The downside of this property is that in case the profile of the soil is being grown is not properly known or monitored, there is a risk of fixing elements harmful to human health if the final product is destined for consumption as healthy food (Navruz-Varli and Sanlier 2016; Vilcacundo and Hernández-Ledesma 2017).

The agroecological practices that have allowed it to grow in extreme conditions rely on the intrinsic crop genetic diversity that has evolved to adapt the species to these harsh environments. These practices in low-input systems protect the human health of farmers who do not use harmful pesticides as much as consumers who benefit from harvesting healthy produce. This shows that plant health, human health, and ecosystem health are intimately linked to form a whole. Thinking about the complexity in our modes of production and consumption would be a real step forward for planet health dimension in a transition for agroecology.

18.6 Energy and Water in the Quinoa Sustainable Value Chain

The global population is on the path of an increasing trajectory with a simultaneous decline in arable land resources through salinization and desertification that have resulted mainly from climate change and other anthropogenic activities. Rising temperatures will cause changes in the entire ecosystem, resulting in significant alterations in global climate paradigms and a threat to food security. Focusing on how the highly resilient quinoa crop can sustainably mitigate some of the detrimental impacts, such as starvation, and support or provide ecosystem services, in comparison, compared with the traditional staple food crops, quinoa has remarkable tolerance to abiotic stresses and is highly nutritious, with a unique balance and higher amounts of nutrients, and can therefore be an important crop for food security and nutritional adequacy. This crop has the potential to ameliorate global challenges with respect to the increase in global population, effects of climate change, desalinization, and phytoremediation, satisfy nutrient deficiency, and alleviate poverty.

The vast majority of quinoa is still produced today in the Andes in agricultural systems with very low levels of chemical inputs. The work is mainly done manually except for soil preparation and sowing which are increasingly mechanized. All steps from sowing to harvesting are still done by hand. This mode of agriculture with agroecological practices by default relies on family or local community labor. This family agriculture consumes very little fossil fuel energy for mechanization, which remains reduced, or for the production of chemical inputs (Bazile et al. 2019).

The development of cultivation in other contexts requires much more energy for the different stages of its production. Indeed, whether quinoa is produced in organic agriculture or in conventional chemical agriculture, in both cases, the use of mechanization is almost generalized at all stages of the technical itinerary. This minimal energy consumption in this case is in addition to the energy related to the use of chemical fertilizers sometimes used in large quantities. Finally, the interest of growing in arid zones as an alternative to local crops usually requires irrigation water, which generates a new expenditure of energy, most often fossil fuels (Bazile 2015; Bazile et al. 2016a, b).

The comparison of the modes of producing quinoa remains important and incomplete to date. However, it would make it possible to highlight the beneficial effects for the environment and, in especially for the climate, of organic production under certain conditions. This is why it is urgent, in the context of a production located mainly in Peru and Bolivia, but with exports to several countries on all continents, to be able to scientifically determine the environmental footprint of different methods of production according to geographical and social contexts. Cancino-Espinoza et al. (2018) proposed a first framework for life cycle assessment for specific situations of organic agriculture in Peru. This first work, even if it remains incomplete, marks the importance of this need in the current context of global changes. The implementation of a large-scale multinational study is now necessary for a local crop sold on a global market. Indeed, beyond the energy expenses related to production, the cost of transport must be integrated into the general thinking.

Production and transportation are the two pillars of energy expenditure that we must associate with the transformation and distribution of the final product. The outer part of the quinoa grain contains saponins that must be removed to prevent this toxicity and make it safe for human consumption. The current debates on the plant breeding of sweet varieties (without saponin) are interesting to address the issue of natural resistances induced by saponins against pests and diseases. This faculty will allow the farmer to limit his treatments and applications of pesticides (reducing his energy cost), but the fact of having to remove the saponin afterward before consumption of the grains generates again an energy cost.

Only a holistic view of the agricultural and food system from the producer to the consumer, through a complete life cycle analysis, will allow us to compare organic or conventional quinoa produced in different regions of the world. Today the debate on local consumption and short circuits (with reduction of intermediaries but distances that can remain far) remains open and deserves to be addressed because there are obvious contradictions in each model that must be understood in order to

overcome them (Michel et al. 2020). Producing an organic quinoa by default on the Bolivian Altiplano does not necessarily have a higher environmental footprint for a French consumer than a conventional European quinoa produced less than 200 km from the final consumer.

18.7 Water

Globally, agriculture consumes 70% of the water extracted for human consumption. This is why the global food system is a major source of degradation of lands, forests, fish stocks, and water. To counter the effects of climate change on agricultural production, researchers are pursuing several avenues to find solutions for adapting agriculture to global changes. A major effort is on saving water, not only through solutions to limit water losses by using more efficient technologies but also through recourse to agricultural biodiversity in the form of research on species (and varieties) and on combinations of species that can use water more efficiently, especially when its supply is limited (Rosa et al. 2020).

Chouchane et al. (2020) showed the limits that humanity faces today to feed a growing population despite increasingly severe constraints on water resources. The adaptation of societies to water scarcity will require major changes in our conception of cropping systems and their organization in space. The choice of the types of agricultural production (cropping systems with adequate species and varieties) will have to be quickly rethought in the context of the current global changes so as not to further deteriorate the situation by posing an additional risk to the food security of populations. Rethinking crop irrigation schemes with a view to long-term management of resources appears indispensable today, knowing that many regions still take too little account of this and increase their already high existing vulnerabilities. Knowing that some of these countries are among the poorest countries in the world and have very high population growth rates, not addressing today the consequences of agricultural systems that are subsidized can jeopardize the survival of populations in the medium term and generate large-scale political instability in these regions.

Many authors have already developed research on efficiency in water use as an alternative crop in marginal environmental conditions (Jacobsen et al. 1999). And then, they can consider deficit hydric in irrigation as a good option for limiting water consumption in agriculture (Ali et al. 2019; Bazile et al. 2016a, b; Razzaghi et al. 2020). Recent works have also put their focus on the rusticity of quinoa species. The plant can tolerate high concentration of salinity (Ruiz et al. 2016) and continue producing grains (Aloisi et al. 2016; Iqbal et al. 2020; Ruiz et al. 2014). The capacity of quinoa to tolerate salt opens new windows for tomorrow research considering the large area of soils degraded and unavailable for agriculture due to high salinity levels (Iqbal et al. 2020; Yazar et al. 2015). This consideration allows thinking about nonconventional water and especially saline water for irrigating quinoa in arid areas (Choukr-allah et al. 2016). That can provide high nutritious foods in regions with high levels of malnutrition.

18.8 Conclusions and Recommendations

Quinoa is an Andean grain with high nutritional quality and that has recently been qualified as a miracle food (McDonell 2015). The grain contains high-quality proteins including all the essential amino acids and many other important nutrients for considering it as a functional food. We need to preserve its nutritional content through agricultural practices that sustain these qualities and attributes. Today quinoa values are still the result of small-scale farmers' practices using a wide genetic diversity of quinoa. However, its modes of cultivation are changing as it spreads to all continents.

The expansion of cultivation, in its multiple dimensions, can serve us as a model for studying an ecological transition in which agricultural biodiversity will become increasingly important. Analyzing the changes underway, while being actors in these very processes of change, requires recourse to special and committed support based on multidisciplinary approaches if we really want quinoa to serve as an example of an ecological transition toward a world agriculture that takes agricultural biodiversity in all its dimensions into account.

The Nexus concept could be mobilized for developing a holistic framework considering simultaneously food and agriculture with health, water, energy, and biodiversity. This is the challenge we have if we want that quinoa serve as a crop model for tomorrow's agriculture under a context of global changes.

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Quinoa, The Next Biotech Plant: Food Security and Environmental and Health Hot Spots

19

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Abstract

The world population has been stated to increase drastically to nine billion in the year 2020. Therefore, there is a need to search for sustainable solution that could help in mitigating several challenges facing mankind which includes food insecurity and health and environmental hazards. The utilization of quinoa plant as a sustainable biotechnology solution will be a preferred solution to all these highlighted challenges. This chapter provides a general overview on the uses as a next generational plant that could solve food insecurity, health challenges, and maintenance of cleaner environment. Moreover, recent advances in the application of as a depository of pharmacoactive constituents (protein, dietary fiber, vitamins, minerals, essential amino acids, betacyanins, betaxanthins, and flavonoids) and their diverse application in the treatment of several diseases such as diabetes and glycemic index, immune-regulatory activity, hepatoprotective, antioxidant activities.

Keywords

Food security · Environmental · Health · Biotechnology · World population · Sustainable solutions

19.1 Introduction

Quinoa, also known as *Chenopodium quinoa*, belongs to the Amaranthaceae family. The plant has close resemblance with spinach and beetroot in terms of edibility, nutritional value (Paniagua Bermejo et al. 2020; Martínez-Villaluenga et al. 2020), health prospects, and appearance and has been continuously grown for over 8000 years. This small herbaceous pseudo-cereal plant is traced to Latin America and popular in the Andes region (an area geographically characterized by plateaus and mountains) where it is consumed as one of the common staple foods (Kierulf et al. 2020). Its relevance in the food scale and placement within the Latin Americas earned it the code name "mother grain" by the Incas of the ancient Inca Empire (Ecuador, Peru, Chile).

Though the little annual flowering plant has its origin in Andes region as stated earlier, it has presently become a widely grown crop cultivated in the Americas, Europe, Asia, and Africa, thus making it an intercontinental grain crop with geographical spread across more than 50 countries (Alonso-Miravalles et al. 2020; Solaesa et al. 2020; Wieme et al. 2020). The crop appearance varies from one geographical area to another, and about 120 varieties have been identified. It is submitted that it's potential to adapting to various environments accounts for its diversity in appearance. As an annual crop, quinoa has some remarkable characteristics. It is resistant to drought, frost, and terrains prone to wind. The seeds are self-preserved owing to their saponin coating (Navarro Del Hierro et al. 2020) that renders the seeds inedible to birds. Its average life is 155 days from

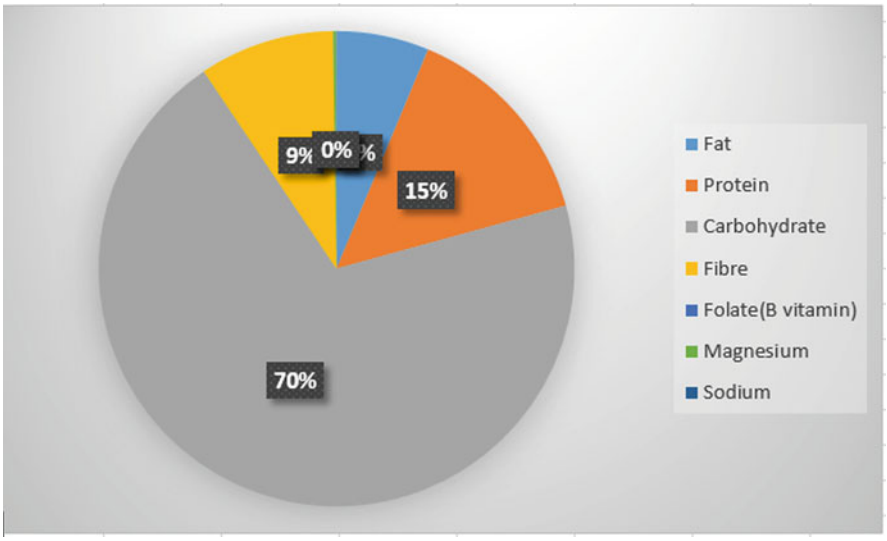

Fig. 19.1 Major nutritional content of cooked quinoa per cup

planting to harvesting with its peak at 220 days, while some early maturing strains could take just 90 days from planting to harvest.

In respect of its edibility to humans, mature seeds are edible after removal of the superficial saponin coating through 1–2-h soaking and subsequent washing. The cleansed seeds provide sweet savory meal comparable to rice. Common recipes include salad, lemon chicken soup, breakfast porridge, black bean tacos, and other local cuisines (Sammells 2019; Linares-García et al. 2019).

Apart from providing nourishing meals, seed is used as an ingredient in food production (Väkeväinen et al. 2020; Solaesa et al. 2020; Alonso-Miravalles et al. 2020) and nonfood products (Romano et al. 2020; Golicz et al. 2020; Jiang et al. 2020; Alonso-Miravalles et al. 2020). The major nutritional content is shown in Fig. 19.1. A cup of seed when cooked yields from 120 to 180 kcal owing to its high protein (Roa-Acosta et al. 2020) and carbohydrate content, thereby providing sufficient dietary requirements for the end consumer.

In addition to the major nutrients, vital biochemical components derived from quinoa include lysine, manganese, iron, copper, thiamin and vitamin B6, phosphorus, zinc, etc. Medicinally, it is a treasure crop. Studies have documented the benefits of the plant in the following health issues: prevention of osteoporosis (Remedios 2016), cardiovascular health (Edwards et al. 2007), dietary serving containing oleic acid, and mono-saturated fat constituting 25 and 8% omega-3 fatty acid and alpha-linolenic acid. These two fatty acids are heart-modulating agents.

Some of these include anticarcinogenic properties (Gawlik-Dziki et al. 2013; Nussgruber 2016), anti-oxidative properties (Obaroakpo et al. 2020), skin protection and healing, antihypertensive agent, antidiabetic agent (Martínez-Villaluenga et al.

2020), prevention of anemia (Gawlik-Dziki et al. 2013), improves general metabolism, regulate appetite and prevent weight gain, and anti-inflammatory properties.

Economically, it is a desirable and highly demanded food grain owing to its health benefits. It is one of the crops that exhibit remarkable export value. Flowing from the foregoing, the crop is celebrated as one of the prominent economic crops that could be harnessed to provide an all-encompassing socioeconomic well-being of every society across the globe in the face of the impending global food shortage.

19.2 Quinoa for Management of Diseases

19.2.1 Antihypertensive Effects

Zheng et al. (2019) recently demonstrated the hypotensive potential of quinoa through the suppression of ACE. The cardiovascular preventive effect is further supported by its moderate lipid content and its triglyceride-lowering ability (Navarro-Perez et al. 2017). ACE is known to be involved in the pathobiology of cardiovascular diseases globally (World Health Organization 2013). ACE is the enzyme responsible for the production of angiotensin II with significant vascular constricting capabilities (Hernández-Ledesma et al. 2011). Previously, it has been reported to have a bioactive peptide such as dipeptidyl-peptidase IV inhibitory peptide, and likewise an antioxidant peptide has been discovered from quinoa protein isolate and globulin (Nongonierma et al. 2015; Obaroakpo et al. 2019).

19.2.2 Anti-obesity Function

Obesity has attained epidemic proportions in the world. Obesity has been associated with metabolic syndrome. Metabolic syndrome is termed a constellation of pathologies, such as abdominal fat accumulation, low HDL cholesterol, elevated triglycerides, elevated fasting glycemia, and hypertension (Alberti and Zimmet 1998). Numerous studies have suggested that with its very rich phytochemicals, it could be a major source of nutritional supplement for the management/treatment of diabetes, obesity, metabolic disorders, and nonalcoholic fatty liver disease (Vilcacundo and Hernandez-Ledesma 2017).

Foucault et al. (2012) have reported that seed extract possesses the ability to decrease adipose tissue expansion. Obesity-lowering action has been associated with upsurge in energy utilization, modification in lipid generation, and reduction in fat reabsorption (Foucault et al. 2014). Hence, it can be a biomolecular raw material for drug development for prevention and management of obesity and its complications. The active compound which is responsible for decreased adipose tissue mass and reduction in fat storage gene manifestation is 20-hydroxyecdysone (20E) (Foucault et al. 2012). This is because treated mice had related effects on lipid metabolism signifying that 20E may play a significant contribution to the obesity-lowering function of quinoa extract.

Furthermore, a study by Navarro-Perez et al. (2017) reported that eating of seeds resulted in low circulating triglycerides and elevated HDL cholesterol with subsequent reduced incidence of metabolic syndrome. Likewise, the consumption led to reduction in lipid accumulation (triglycerides, total and LDL cholesterol) and oxidative stress biomarkers (De Carvalho et al. 2014).

Exceptional phytochemical blends inside make it one of the richest phenolic compounds. Several experimental studies have documented that consumption enhanced health protection especially against chronic metabolic diseases (Pasko et al. 2010). Also, in human intake, it has been associated with reduced body mass index and glycated hemoglobin levels (Abellan Ruiz et al. 2017), reduction in impaired lipid metabolism (Farinazzi-Machado et al. 2012), and also postmenopausal women (De Carvalho et al. 2014). Recently, Noratto and coworkers demonstrated in animal model of diabetes and obesity that intake ameliorated obesity and T2DM-associated complications such NAFLD, dyslipidemia, inflammation, and impaired anti-oxidative defenses (Noratto et al. 2019).

19.2.3 Hepato-protective Effect

Quinoa has been documented to possess antioxidants, rich phytochemicals (Tsai et al. 2011), and anti-inflammatory and hepato-protective characteristics (Hong et al. 2016). Subsequently, a bioactive flavonoid called rutin (vitamin P) from red quinoa was suggested to mediate hepato-protective capabilities (Nafees et al. 2015). Rutin is known to suppress inflammation (Sikder et al. 2014) and oxidative injury (Nafees et al. 2015; Saxena et al. 2017) and possess anticancer and hepato-protective properties (Hafez et al. 2015). Interestingly, Lin et al. (2019) documented that ethanol extract of bran with increased rutin concentration provides significant hepato-protection and anti-fibrotic effect through inhibition of pro-inflammatory cytokines. In addition, the assertion that it is hepato-protective was further supported by the report of another study that depicted hepato-protective effects of seed (*Chenopodium quinoa*) against liver injury caused by CCl4 through reduction of aspartate aminotransaminase and alanine transferase activities alongside increased antioxidant (superoxide dismutase and glutathione) levels (Saxena et al. 2017).

19.2.4 Gut Dysbiosis and Colitis

Gut microbiome has gained attention in recent times. Gut microbiota is a unique player in the maintenance of host health. Furthermore, gut microbiota derived metabolites like propionate, butyrate, and acetate from breakdown of indigestible carbohydrate. Prebiotic activity has been documented in an in vitro study because of its rich fiber content through the enhancement of commensal microbial organism development and the increased generation of microbial metabolites (Gullon et al. 2016). In addition, quinoa-derived polysaccharides have been reported to have

immune-regulatory activity (Hu et al. 2017). Therefore, it can be inferred that seeds may have positive effects on gut health.

Dysbiosis is the alteration of the gut microbial homeostasis causing disruptive influences in the host-microbiome cross talk. Dysbiosis, therefore, is associated with the pathobiology of metabolic and gastrointestinal disorders (Frank et al. 2007; Sartor 2008; Lopetuso et al. 2014). Liu et al. (2018) demonstrated that intake restored gut microbiota homeostasis and ameliorated detrimental signs of ulcerative colitis resulting from dextran sulfate sodium as signified by decreased body weight loss, less histomorphological colon damage, and reduced inflammation, suggesting that consumption of quinoa may be a novel dietary intervention for the maintenance of the gut health because of its rich fiber, polyunsaturated fatty acids, protein, and abundant phytochemical contents. Hence, plant will offer protection against inflammation-related disease conditions (Tang and Tsao 2017; Ren et al. 2017). Furthermore, the ability to improve intestinal health is supported by the finding of inhibitory effect on colitis-induced expansion of *Proteobacteria* which has been implicated in gut dysbiosis (Shin et al. 2015).

19.2.5 Diabetes and Glycemic Index

Diets with low glycemic index are a better and efficient approach in treating and managing obesity and other noncommunicable ailments. Quinoa has been reported to be associated with hypoglycemic effect due to its low glycemic index (Onwulata et al. 2008). Hence, its consumption is beneficial for diabetes and obese patients. Reports exist that diets with low glycemic index are associated with positive physiological effects such as reduction in glucose and insulin responses; low cholesterol responses; reduced body weight, adiposity, and colonic cancer; and decreased T2DM threat (Augustin et al. 2015; Sacks et al. 2014). Pasko et al. (2010) reported that seed consumption led to reduced glucose compared to consumption of standard feeds. Interestingly, quinoa-induced glucose-lowering effects are associated with rich phytochemical constituents and high fiber nature of seeds (Berti et al. 2004; Pasko et al. 2010).

Importantly, high glycemic index diet intake causes more postprandial hunger due to insulin-mediated reduction in glycemia, resulting in extended and constant excess eating, regardless of the restored normoglycemia. Furthermore, elevated fat accumulation was reported in rodents consuming high glycemic index diet (Lerer-Metezger et al. 1996). The high glycemic index diet resulted in cardinal features of obesity (such as fat accumulation, increased adiposity, elevated glycemia, dyslipidemia, and elevated fat accumulation in the epididymis) in rodents that consumed them, e.g., amylopectin (Sacks et al. 2014; Lopes et al. 2019).

19.3 Application in the Environment

Quinoa is an aspiring and multipurpose plant with auspicious potential that has been of significant assistance in enhancing the demand of food and the assuagement of poverty globally in light of the surge of the present growth in global population and the impacts of global warming. It has great economic and environmental importance. Several studies have revealed that this is a special kind of plant with great potential (Konishi 2002; Ruiz et al. 2014, 2016; Jaikishun et al. 2019). Jaikishun et al. (2019) in their study reported that quinoa has all it takes to assist in the mitigation of the present environmental challenges that have been faced globally. In this regard, it is believed that it will be of great help in mitigating the impacts of climate change, removal of salt and other minerals that could be harmful from water (desalinization), and nourishment of nutrient deficiency and in the assuagement of poverty generally.

Several studies have revealed that quinoa is a climate change-resilient plant (Jacobsen et al. 2009; Ruiz et al. 2014; Jaikishun et al. 2019). It is a gluten-free and enormous nourishing cereal plant that originated from South America region of the world with amazing agricultural resilience against diverse antagonistic environmental situations, which qualifies it to be appropriate for cultivation in other regions of the world that is susceptible to the detrimental influences of environmental alteration (Jaikishun et al. 2019).

Quinoa has great ability to acclimate and grows in the utmost life-threatening and challenging weather conditions like high temperature (drought), frost, and high saline (Ruiz et al. 2014, 2016). Accordingly, it has phenomenal adaptation level; this accounts for why it can subsist in swamps or lowlands, deserts, and regions with very high elevation above sea level greater than 4000 m (Jacobsen and Mujica 2003; Jacobsen et al. 2003, 2009; Hariadi et al. 2010; Maughan et al. 2009).

The United Nations professed the year 2003 as "Year of the Quinoa" due to its auspicious and nutritive potentials. Ever since then there has been a lunge for the upsurge of production. Consequently, several countries have now been involved in its production and valuation (Jacobsen and Mujica 2003; Jacobsen et al. 2003; Ruiz et al. 2016). According to Ruiz et al. (2016), the plant has all it takes to become a global sustainable food supply with respect to rapidly fluctuating climatic model alterations even though concurrently enriching pressure on cultivable land. In affirmation of this fact, Konishi (2002) reported that in comparing its resilience and nutritional reimbursements, quinoa is now been described as "one of the cereal plants of the present epoch" which performed significant function in delivering sustainable food in antagonistic climatic situations ensuing from environmental alterations. Consequently, as a result of its resilience and nutritional reimbursements, the National Aeronautics and Space Administration (NASA) data recommended it as the diet for astronauts on astronomical board missions (Jaikishun et al. 2019).

19.4 Application for Climate Change Resilience

Apparently, over the years there has been continuous change in the climate globally. Consequently, it is believed that these changes would incessantly intensify in the years to come with much consequences (Ukhurebor and Umukoro 2018; Ukhurebor and Azi 2019). These changes in the climate could be as a consequence of natural or man-made actions. Presumably, several studies have shown that man-made actions are contributing more than the natural actions (Ukhurebor and Abiodun 2018). Climate change is now a threat not just to humans but also to all living organisms including plants following the report of the Intergovernmental Panel on Climate Change (IPCC 2014). The main consequence of changes in the climate system resulted in what is known as global warming. Global warming is presently contributing detrimental influences agronomically affecting food security as well as the existence of an increasing population globally (Field et al. 2014; Ukhurebor and Abiodun 2018).

Several reports highlighted quinoa as a significant sustainable food source but responds resiliently to climate changes and global population growth (Jaikishun et al. 2019). Studies have it that these plants can survive coarse difficult terrains for several millennia, because of their ability to germinate in the plateaus, swamps, and valleys and even in challenging weather that stands imperiled life existence (Jaikishun et al. 2019). It has an extraordinary ability to survive in soil with deficient of water as a result of its innate little need for water along with capacity to continue photosynthesis at a perturbed rate as well as the ability to sustain its leaf parts after a period of water deficiency (Jacobsen et al. 2009; Jensen et al. 2000).

According to the IPCC (2014), the unswerving impacts of global warming are but not limited to the following:

1. **Flooding**: This is to do with excess of water to the environment which could result in enormous environmental menaces. There are several research reports about this as one of the few plants with the capacity to resist very low-temperature conditions. This according to them is as a result of its special mechanism to avert impairment by very low temperatures resulting from flooding or other severe climatic scenarios (Bois et al. 2006; Jacobsen et al. 2003; Vera-Hernández et al. 2018; Jaikishun et al. 2019).
2. **Soil salinization**: This is the addition of salt and other minerals that could be harmful to soil water. Several studies have shown that quinoa can tolerate high level of salinity, and this necessitates for its cultivation globally (Metternicht and Zinck 2003; Gomez-Pando et al. 2010; Morales et al. 2011; Orsini et al. 2011; Qadir et al. 2014; Razzaghi et al. 2015; Jaikishun et al. 2019).
3. **Drought**: This is to do with the deficiency of water which would lead to the immense vegetation dilapidation as well as the dilapidation of the structure of the soil. Several studies have also shown that it survives in drought via its anticipatory mechanisms (Abugoch et al. 2009; Jacobsen et al. 2009; Jensen et al. 2000; Yang et al. 2016; Jaikishun et al. 2019). Accordingly, this accounts for its production in susceptible regions and strengthens its repossession.

Although the ideal temperature that is most promising for planting ranges from about 15 to 20 °C, studies have shown that some varieties still survive extremes of ranging from −8 to 38 °C (Bazile et al. 2016a, b). However, studies have shown that most plants would be cruelly obstructed by high temperature, soil moisture variations, evapotranspiration, as well as life-threatening weather scenarios (IPCC 2014). This in no doubt will have great impacts on plant yields. Consequently, global warming poses solemn threat to food security globally especially in regions that are unswervingly pretentious by their impacts.

Studies have shown that quinoa stands at the zenith as a tool that would challenge these climate inconsistencies and subsist with considerable yields in these antagonistic scenarios as a result of its extraordinary level of adaptations to these inconsistencies (IPCC 2014; Panta et al. 2014).

In accordance with the FAO (FAO 2018), there is a belief that in eclectic ecological habitats, the nature of quinoa as reported by Valencia-Chamorro (2003) could be a contributing factor for its exceptional and extraordinary adaptation potentials.

Quinoa possesses idiosyncratic climatic and edaphic features, as well as its suitable nourishing composition, this according to Miranda et al. (2012), again validates the strength in surviving diverse weather scenarios, and this ultimately impact its edaphic situations. Accordingly, Yang et al. (2016) reported that these attributes offer optimistic suggestions about the capacity of quinoa to resist detrimental impact of diverse climatic scenarios globally.

19.5 Effect of Quinoa in Phytoremediation, Treatment of Environmental Pollution, and Eco-restoration of Heavily Polluted Soil

The accretion of salt, heavy metal, petroleum, and other contaminants in the soil is becoming a severe issue especially for the agricultural cultivation due to the fact that it limits agricultural productivity and yields. These soil contaminants contribute significantly to changes in the climate conditions as well as in soil qualitative dilapidation which could be an impediment to global food security and sustainability if not properly contained (Hariadi et al. 2010; Halford et al. 2015; Mickelbart et al. 2015).

Besides, high amount of salt (salinization) is disturbing in terms of its frequency, and the way productive lands are now becoming deserts (desertification) in some regions is a validation that plant coverage and biodiversity would inexorably be reduced as a result of the habitat forfeiture ensuing from climate change, inadequate loyalty to land use acts, and the raise of industries as well as other anthropogenic events (Jaikishun et al. 2019).

The reduction of plant coverage would in due course lead to the reduction of the global change of carbon dioxide to oxygen. The study of Hariadi et al. (2010) suggested that quinoa could be a competent means for removing salt from the soil (desalinization) in regions that are vastly pretentious and could increasingly be of

assistance for the cultivation of other salt-penetrating plants. Evidently, Bhargava et al. (2008) reported that some varieties have high acquisitive capacity for metals like nickel, chromium, and cadmium, hence making them auspicious entrants as phytoremediators to decontaminate soils that have been contaminated. Record has it that quinoa has the potential to excerpt more of these contaminants in the soil compared to other halophytic plants because of its ability to accumulate well-suited organic solutes, humidity, and its extraordinary adaptation features. The fact that it can subsist in these contaminated soils makes it appropriate contenders for soil decontamination (Hariadi et al. 2010; Zorrig et al. 2012; Song and Wang 2014; Ado et al. 2016). However, according to recent study by Jaikishun et al. (2019), there are limited studies on the decontamination potentials via phyto-desalinization and phytoremediation. It is therefore recommended that more studies need to be invigorated in order to authenticate, establish, and evaluate the decontaminating potential of various varieties.

19.6 A Resolving Tool for Food Insecurity

This plant is one of the widely commercial crops that can tolerate abiotic pressures from climatic influences. Its wide commercialization shows its prospective and promising usage for future agricultural management and ecological biosecurity as well as in fighting malnourishment globally. Choukr-Allah et al. (2016) looked at prospective nutritional as well as food security of quinoa plant. The authors stated that the different cultivars have worldwide interest that has been selected for positive characteristics, nutritional value, H_2O utilization efficiency, and salinity tolerance. The authors recounted that the ability of the plant to withstand these high ecological stresses lies on the rigorous nature of the plant. The health and nutritional values were also highlighted. In conclusion, the authors stated that more work and awareness are necessary to enhance production, nutritional values, and market need using postharvest techniques to sustain its utilization globally and food security at large.

The issues of climate change and its threat to biodiversity are of a great concern in the production of crop. However, for food substance, many stress resistance crops are cultivated in order to combat eco-stress environment like increased aridity and salinity. In view of this, Ruiz et al. (2014) reported on how the issues weather and global warming impact security of food and utilizing quinoa as an option to combat the impact as regards to sustainability and biodiversity survival. The authors stressed that species of high biodiversity and economic values as well as good genomic traits are used to retain the stride of food manufacturing and human population growth and that quinoa has an enormous diversity and agroecological features. In conclusion, they propose a graphical model that incorporated cultural identity, conservation of biodiversity, and food security using quinoa as a promising tool of eco-restoration.

Zikankuba and James (2017) tested and evaluated the nutritional and food security of plant in Tanzania. The authors reported that nutritional and food insecurity as a result of climate change has affected population growth in Tanzania. The report from the characterized result from their study showed that the constituents of

ash, dietary fiber, fat, and protein in quinoa were 3.8, 3.8, 10 and 23%, respectively. The elemental constituents are Zn, Ca, and Fe (44, 132, 1487 mg/100 g correspondingly). They also stated other functions of seeds in the addition to or substitution of some food formulae, the utilization of flour in different confectioneries, for making detergents, shampoos, etc. In conclusion, they recommend it as a first-class plant for national nutrition and food security because of the economic and ecological importance it portends.

Bazile et al. (2016a, b) did a preliminary investigation which evaluated the possibility of utilizing *Chenopodium quinoa* Willd. in contending with environmental stressors like salinity, aridity, cold, and drought. They recounted that quinoa is present as a major protein option possessing a huge variety crosswise the Andean region. Seven countries (Yemen, Mauritania, Lebanon, Iran, Iraq, Egypt, and Algeria) were assessed from 2013 to 2015. The results from their pilot study showed that out of 22 genotypic stocks tested, Q12 and Q18 from landraces showed more adaptation to environmental stresses, and Q21 and Q26 were screened to have satisfactory and stable levels of produce >1 t. ha^{-1} each at various sites of field trials. Findings from their study showed that the stability of production was considered prominence especially in harsh climatic conditions. The authors in conclusion recommend *Chenopodium quinoa* Willd. as the first-class candidate among other stocks to offset food security under any environment conditions for the sustenance and availability as staple food across the globe because it can grow in various harsh abiotic ecosystems.

Katwal and Bazile (2020) tested and evaluated, in a field study, the efficacy in harsh mountainous region of Bhutanese as climate buoyant plant with the aim of providing food security and safety. About ten varieties of quinoa assortments were utilized in this study from 2016 to 2017. The findings from their results revealed successful growth in two different mountainous ecological agricultural zones from 0.61 to 2.68 t. ha^{-1}. Quinoa growth and production was also observed in lesser elevated platform from 1.59 to 2.98 t. ha^{-1}. These yields were driven by certain genetically enhanced features in the mountainous ecological agricultural zones that elicited the maturity of the crops expressively from 92 to 197 days, with the maturation of genotypic traits in the lesser elevated platforms where the range of temperature was higher at the initial growing season. The authors opined that the cultivation of quinoa in mountainous region of Bhutanese has successively outweighed temporary and adapted to the old-style cropping methods used in planting potato, rice, and maize in that region. This will alternatively and rapidly promote a substitute for food-insecure crops that cannot grow in harsh climatic conditions in the community of Bhutan. In summary, the authors suggested the utilization and consumption as well as cultivation because it has high nutrient values and can enhance nutritional deficiencies in growing children and serves as best alternative for future assurance of sustainable food outlet.

Salinity and drought are majorly the environmental factors limiting plant growth, development, and production, consequence of climate change. To curb this problem for the sustenance of food security and safety, plant of resistance and highly genetic vigorous crops is the best option. In this context, Yazar and Kaya (2014) evaluated

the ability of quinoa to resist arid and saline ecosystem for possible agricultural production. The authors recounted that this plant possesses the capacity to thrive in poor environments and also adapt or tolerate physical ecological stress like drought and salinity. More so, the plant has high nutritional values, devoid from gluten and nontoxic, which makes it one of the most recent best crops for human consumption. This study demonstrated that it was able to tolerate high salty environment of concentration 40 ds m^{-1} in the root zone. The authors, in conclusion, proposed this as a perfect substitute for borderline agricultural areas because of its adaptability to certain limiting ecological factors, more so that different cultivars can be planted in different biotic community for their economic value, nutritional constituents, and food security purposes.

Chandra et al. (2018) did a review of the importance of *Amaranthus* spp. and *Chenopodium quinoa* in providing food security at the times of need. The authors reported that rapid growth of the population of India has caused the dire need for food security and that these plant species have high nutritional values and tend to supply energy as well as macronutrients. Other health benefits of the plants, such as in the treatment of lactose-intolerant individuals, celiac and diabetic patients, and high alertness in athletes and for growing children and adult groups, were reported. They also stated that the pseudo-grains can alleviate food security as well as provide food for the hungry teeming population in Indian. They concluded by stating that awareness is the only sole aim in meeting the social and economic livelihoods of the farmers and populace of the country and the global community at large.

Shams (2018), in a field study, assessed a novel genetic composition that can adapt to sandy-soil settings in agricultural land in Egypt. Nine genotypes sourced from six Peruvian stocks (Rosada de Huancayo, Salcedo INIA, Kancolla, Blanca de Junin, *Amarilla sacaca*, and *Amarilla marangani*) and three novel accessions (QS17-2, QS16, and QS14) were assessed and compared to three standard replications. This was proven successfully good in terms of yield production in the sandy soil. One of the novel genotypes QS17-2 was able to survive between 115 and 120 days of the field trials because of environmental stress and was taken or chosen as a short accession stock. Growth variations of the four assortments (Rosada de Huancayo, Salcedo INIA, Kancolla, and Blanca de Junin) and the two other attainments (QS16 and QS14) were quite moderate. The varieties *Amarilla marangani* and *Amarilla sacaca* were considered to have the extended period of genotypes as well accession QS17-2 when compared to genotypes of other grain yield. However, the protein concentration of the plant was observed to be 13.60% (QS17-2), but the smallest concentration was 10.75% (Blanca de Junin stock). In addition, the variety Salcedo INIA had the least saponin concentration of 0.07%, while accession QS16 had the highest concentration of 0.22%. In conclusion, the authors suggested that crop can restock gap where grain/cereals can grow competitively and successfully with high effectiveness to farmers of the range of small scale in sandy-soil settings.

Khan et al. (2019) did a critical evaluation on the beneficial and harmful effects of *Chenopodium* and *Amaranthus* weeds in the background as food security in rustic areas. The authors stated that food insecurity is a certainty for over 100 million

individuals globally. The awareness of these pseudo crops has caused an atmosphere of ambiguity among farmers in rustic region of the world. As a result of this, the world's food security has increased and has sharply elicited the agricultural industries. These pseudo-plants are rich with different mineral and vitamin contents as well as rich protein concentrations. In Ethiopia, the high incidence of food insecurity is due to intense aridity and low rainfall that have led to low moisture content in the soil in lowland rustic regions. The awareness of the pseudo crops has led to an alternative and crop boost in their production. More detailed evaluation of these pseudo crops that looked more on the economic and ecophysiological importance was highlighted. Further screening of the harmful and beneficial influence of these pseudo crops was discussed.

Climate change has affected the agricultural sector of Pakistan tremendously. Production of crops is strictly based on the irrigation method of farming in order to balance the moisture contents of the soil for plant survival. Farmers are seeking basic alternative to curb this menace. In the light of this, Mazhar and Bajwa (2017), in a review, evaluated the need for reinforcing the supply of quinoa in the agricultural sector of Pakistan in order to combat harsh environmental conditions that might impede the large scale of economic viable crops. The authors stated that different initial tests and screening were done in different agricultural ecological locations to ascertain the effectiveness of the study. Based on the findings of the nutritional, economical, and environment resilient toward stressors, the crop has been recommended as a first-class tier for large-scale production in the aspects of oil production, feed, and food for humans and animals. However, there is a clarion call to establish and explore it for both export and domestic markets as well as to link quinoa agriculturalists for the sustainable fabrication both for subsistence and large-scale purposes. In conclusion the authors recommend the safety of food as a primordial procedure to regulatory agencies, farmers, stakeholders, and researchers for the production and consumption of quinoa as a sustainable means for food security.

19.7 Conclusion and Recommendation

This chapter has provided comprehensive information on the relevance of quinoa plant as a sustainable biotechnology solution that could proffer solution to some of the selected challenges facing mankind discussed during this review, etc. There is a need to apply genetic engineering for the mass production of new breeds that could enhance the production of more effective pharmacoactive constituents. There is a need for an interdisciplinary collaboration between all scientists from diverse field to work together on how to increase the mass production of these plants (Abubaka et al. 2019; Adetunji 2008; Adetunji et al. 2011a, b, c, 2012, 2013a, b, 2014, 2017, 2018a, b, c, 2019; Adetunji and Olaleye 2011). Moreover, there is a need to perform a structural elucidation of the most pharmacologically active constituents present in quinoa plants so as to maximize their benefits in different sectors such as food, environment, and health. The application of synthetic biology, bioinformatics,

computational biology, and nanotechnology will also improve the application of the pharmacoactive constituent present in quinoa plants.

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Quinoa: From Farm to Traditional Healing, Food Application, and Phytopharmacology

20

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Abstract

Quinoa plant has been recognized as a well-balanced pseudo-cereal which has been identified as an excellent grain due to the presence of excellent constituents like high gluten-free proteins, several minerals, and polyunsaturated fatty acids.

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This wonderful plant has been recognized to possess several pharmacoactive constituents that could be utilized for the management of several diseases. Furthermore, it is a beneficial pseudo-cereal in celiac patients because it is gluten-free and can easily be digested by celiac patients as well as portends the capability to decrease the threat of heart diseases as well as very strong anti-hypercholesterolemic activity. Therefore, this chapter intends to highlight several pharmacological constituents available in quinoa plant which could be utilized in the treatment of several diseases. Also, more emphasis was laid on the nutritional benefits of quinoa plant as a depository of essential nutrients which could help in the maintenance of the well-being of mankind.

Keywords

Quinoa · Cereals · Cholesterol · Celiac disease · Hypercholesterolemia · Cardiovascular disease

20.1 Introduction

Quinoa belongs to the Chenopodiaceae family and had been cultivated for 7000 years. It is a dicotyledonous plant which flowers twice in a year (Jacobsen 2003). Quinoa is regarded as a pseudo-cereal due to the characteristics of its grain (Vega-Galvez et al. 2010). The seed of this plant is mostly consumed by humans. The seed is, however, consumed after the elimination of the seed coats or hull which is rich in saponins. The seed is either consumed whole or processed to flour which can be used in the formulation of bread and pastry. The leaves and stems are used as animal feed (Vega-Galvez et al. 2010; Repo-Carrasco et al. 2003).

The composition of quinoa includes dietary fiber, minerals in significant quantity, vitamins, unsaturated fatty acids, proteins, and essential amino acid; it can be said to be a complete food. In 2013, the UN named it "The International Year of Quinoa." It is presently been cultivated in most part of the world (Suttiarporn et al. 2015; Kim et al. 2018). The bioactive compounds of quinoa such as terpenoids, flavonoids, steroids, and nitrogen-containing compounds have diverse function such as the prevention of the harmful effect of microorganisms, insects, and birds. Some beneficial effect on human includes as anticancer (Hu et al. 2017), antimicrobial (Miranda et al. 2014), antidiabetic (Graf et al. 2014), and adjuvant activities (Estrada et al. 1998).

Quinoa plant is a focus of attention due to its low glycemic index and high protein (gluten-free protein) value as compared to other cereals of the same species (Chauhan et al. 1992). In modern age, eating of cereals has gone beyond breakfast to lunch, brunch, or dinner due to the presence of excellent constituents comprising of carbohydrates of low glycemic index and high protein ratio (12.9–16.5%) greater than barley, oats, rice, etc. that provide immense energy to the consumer (Freitas and Moretti 2006). Quinoa is rich in calcium and vitamins that include vitamin E,

thiamine (0.4 mg per 100 g), niacin (1.06 mg/100 g), folic acid (23.6 g), and riboflavins (0.39 mg per 100 g). Quinoa is beneficial for celiac disease patients (Abugoch James 2009). Quinoa is consumed in the form of grains, flakes, and wheat all over the world. Its wheat is also used in making energy bars for the diabetic patients who need good amount of carbohydrates with low glycemic index (Abugoch et al. 2008). Seed-extracted oil contains 89% unsaturated fatty acids and 54% polyunsaturated fatty acids which are more health friendly than other plant/seed oils. The essential fatty acids present are very good for general health as they are protective against heart diseases due to their antihyperlipidemic activity. These polyunsaturated fatty acids are protected by antioxidant present in quinoa in the form of vitamin E. They also help to boost immunity and fight against inflammation (Kim et al. 2006) In a study where individuals were given quinoa cereal bars daily for 1 month, after a month it was observed that consuming those cereal bars led to improved lipid metabolism along with improved gluco-metabolic regulation and reduction in body weight and blood pressure in most individuals which shows that quinoa may be useful for the management of cardiovascular diseases (Farinazzi-Machado et al. 2012). As we all know that celiac disease is a serious problem in which there is gluten intolerance in individuals who have gluten sensitivity when they take gluten proteins from barley, wheat, etc., it causes inflammation in the small intestine. These patients can take quinoa as an alternative because it does not have gluten protein (Leite Olivera 2008). Quinoa consumption in humans has been demonstrated to improve gut health and lipid metabolism in celiac disease patients, but there was a little decrease in total cholesterol (Zevallos et al. 2014). The dietary fibers present in quinoa are soluble and insoluble dietary fibers, and in comparison with wheat, seeds have a greater fiber, and this is largely responsible for the hypocholesterolemic effect of quinoa. Dietary fibers can lower cholesterol uptake from diets by binding to bile acids, which in turn enhances cholesterol breakdown resulting in the generation of potent gut metabolites from the breakdown of the indigestible fibers, thereby lowering hepatic cholesterol metabolism (Repo-Carrasco-Valencia and Serna 2011). Therefore, we can say that quinoa has the capability to allocate nutrition and therapeutic benefits to thousands of malnourished people worldwide (Ruales et al. 2002).

Phytopharmacology attributes, relevant phytochemicals, and significant nutritional constituents present in quinoa plant will be highlighted in this chapter.

Sinapinic acid (9), rosmarinic acid (10), and their analogues are typical examples of cinnamic acid derivatives (Fig. 20.2). Tang and Tsao (2017) reported ferulic acid and its derivatives to be extremely plentiful bound phenolic acid in quinoa seeds. Ferulic and sinapinic acids have been reported to have adverse phytotoxic effects on the seedling of cucumber (Gerig and Blum 1991; Hung et al. 2000). Quinoa-based phenolic compounds extracted from diverse species of quinoa possess some bioactive effects like antidiabetic (Hunyadi et al. 2012), allelopathic (Slimen et al. 2017), and antimicrobial (Cho et al. 1998) activities.

20.2 Energy and Nutritional Value

About half of the human nutritional need for protein and energy are gotten from grains. Some important food grains in the world include rice, oat, rye, wheat, barley, and wheat. Quinoa is rich in lipid, minerals, and protein which show its superiority over other grains.

20.2.1 Proteins

The protein content of quinoa has been reported to be 15% on the average. Quinoa protein content is comparable to wheat but somewhat higher than the protein content of rye, corn, barley, rice, and sorghum (USDA 2015). Quinoa protein is majorly made of globulins (37%) and albumins (35%). It consists of low prolamins, whose concentration depends on the species of quinoa under consideration (Abugoch James 2009). Vega-Galvez and co-workers demonstrated that the protein content of quinoa is similar to that in milk (casein) as it has some vital essential amino acids (Vega-Galvez et al. 2010).

The Food and Agriculture Organization (FAO) reported quinoa to consist of basically all amino acid necessary for healthy living, including lysine's which is deficient in most cereal grains (Maradini Filho et al. 2015). Protein digestibility or its bioavailability increases with cooking and depends on the species of quinoa. Seed is not only rich in protein, it also has a high content of protein and nonprotein tryptophan which is metabolized by the body readily (Comai 2007). This aids in the utilization of amino acid in the brain cells, hence influencing serotonin neurotransmitter synthesis.

20.2.2 Carbohydrates and Fiber

Starch is the major carbohydrate as it constitutes about 52–69%. The dietary fiber and soluble fiber are within the range of 7–9.7% and 1.3–6.1%. The sugar content is about 3% and mainly composed of the D-ribose and D-galactose (Abugoch James 2009). Quinoa is ideal for use as thickeners in soups, sauces, and flours due to its perfect physicochemical characteristics, such as its stability at low temperature, low gelling points, perfect freeze-thaw stability, and resistance to retrogradation. Its ability to resist retrogradation makes it an ideal fat mimetic substitute (Vega-Galvez et al. 2010). Quinoa starch amylose is about 22% which is low compared to those reported for corn and wheat but higher than those present in barley and rice. Report by Tang et al. (2002) further affirms its resistance to retrogradation processes.

20.2.3 Lipids

Quinoa is an alternative oily seed because of its lipid content. Its oil is rich in alpha-linolenic and linoleic acid ranging from 2.0 to 9.5%. Typical antioxidants such as α- and β-tocopherol are also in high concentration. The oil content is quite higher than those in maize and many other grains. Its oil content is however 19% lower than soybean. Oil oleic, linolenic, and α-linoleic acids are at similar level with those of corn and soybeans. Several studies have reported these values to be close to 88% of the overall fatty acid of the seeds (Maradini Filho et al. 2015; Abugoch James 2009). 10% of its overall fatty acid content is palmitic acid. Repo-Carrasco et al. (2003) reported the oleic (19.7–29.5%) and α-linolenic (8.7–11.7%) to make up huge percentage of the overall fatty acid content in quinoa, which is comparable to which is obtainable in soybean composition.

20.2.4 Vitamins

The USDA (2015) reported quinoa to be rich in minerals and vitamins. Although there is a dearth of knowledge on the vitamins of the seeds, it has, however, been established that it has a higher concentration of folic acid and pyridoxine (B6). These vitamins have been reported to meet the daily needs of human adult. Abugoch reported that consumption of 100 g of quinoa supplies the human adult and human child about 40% riboflavin needs. Though its niacin content doesn't meet the human needs, it, however, is an important source for diet. Oat and barley have lower thiamine level than quinoa, while its folic acid, riboflavin, and pyridoxine are greater than the ones in rye, wheat, and rice. Several studies have reported its vitamin E content to be higher than those in wheat (Abugoch James 2009; Alvarez-Jubete et al. 2010). The ascorbic acid concentration in quinoa is within the range of 0–0.63 mg/100 g. Data on vitamin content can be misleading at times because they were reported in dry weight basis while they are majorly consumed in the weight basis form. Likewise, the processing method in which the sweet and quinoa go through can result in changes in the raw materials' vitamin level (Koziol 1992).

20.2.5 Minerals

Quinoa contains ash (3.4%) which is higher than those of other grains such as the rice grain (0.5%) and wheat (1.8%). It contains 0.26% magnesium in comparison with 0.16 and 0.14% in wheat and 0.14%. It is a fairly well-balanced diet since its potassium, magnesium, and calcium are present in biologically appropriate proportion (Vega-Galvez et al. 2010; Repo-Carrasco et al. 2003). It has very rich iron content than many other traditional cereals previously listed in this study. The presence of phytic and saponin can, however, affect the bioavailability of these minerals to some degree. Studies using animal model have revealed that the iron content of quinoa diet is comparable to ferrous sulfate (Koziol 1992; Ruales and Nair

1993). There are still needs for study using the human model on the mineral availability.

20.3 Beneficial Effects on Human Health

Quinoa consumption is beneficial health-wise to consumers prone to some health issues such as diabetes, anemia, lactose intolerance, dyslipidemia, osteoporosis, celiac diseases, and obesity. Its gluten-free content, therapeutic characteristics, and significant nutritional value give it nutraceutical functionality. The presence of antioxidants, fiber, vitamins, phytochemicals, and minerals gives quinoa its nutritional and therapeutic advantage over other plants (Vega-Galvez et al. 2010; Bhargava et al. 2006).

20.3.1 Health Benefits of Consuming in Humans

There is, however, insufficient report as regards the health role in which quinoa consumption plays in human health. There was an increase in plasma insulin-like growth factor 1 (IGF-1) levels in kids who were feed 100 g quinoa daily. In a study by Ruales et al. (2002), fortification by quinoa enhanced baby food with numerous nutritive substances like proteins, minerals, and vitamins serving as a means of preventing malnourishment in growing children. Daily consumption of 50 g quinoa for 6 weeks did not significantly affect gastrointestinal parameters. Triglyceride, total cholesterol, HDL, and LDL levels were significantly lowered (Zevallos et al. 2014). A diet supplemented with quinoa was well accepted by celiac patient and did not complicate the illness. The findings of this study conclude that consumption of about 50 g of quinoa over 6 weeks had a positive effect in the health status of celiac disease patients. In another study by Farinazzi-Machado et al. (2012), there was reduction in circulating lipids in a group of 22 students who were daily fed quinoa candies or 30 days. These students were aged 18 and 45 years old. There was also insignificant decrease in blood pressure, blood glucose, and body weight. In another study, noteworthy reductions in vitamin E, serum triacylglycerol, and TARS concentration were recorded for postmenopausal women who were overweight and consumed about 25 g of cornflakes and quinoa flakes successively for 4 weeks. There was, however, significant GSH increase as opposed to LDL and total cholesterol decrease. These researchers (De Carvalho et al. 2014) concluded that consumption of flakes reduces blood fat levels. In a study by Sanchez (2012), diabetic individuals consumed more grains but less flour than those with no diabetes. The anabolic/catabolic hormonal status, body composition, and catabolism indicator were not affected in men who did three times a week resistant exercise (Wilborn et al. 2006).

20.3.2 Antidiabetic, Anti-obesity, and Blood Fat-Reducing Effects of Quinoa

There was significantly improved glucose and lipid metabolism in rodents fed with quinoa seeds. Fructose, however, was noticed to decrease significantly in the control experiment. Seeds were affirmed to reduce the negative fructose effect on glucose and lipid levels (Pasko et al. 2010). The bioactive role of 20-hydroxyacdysone (20HE) in recent studies demonstrated potent preventive and therapeutic management of postmenopausal disorders with metabolic syndrome (Foucault et al. 2014). Glucose levels and adiposity were reduced with increasing insulin sensitivity in a 13-week administration of 10 mg/20HE IN C57B1/6J rats that are hyperglycemic obese (Kizelsztein et al. 2009). Wang and co-workers (2011) documented decreased fat accumulation in muscles and the body weight of rats fed a diet high in fats in a 12-week period. Foucault et al. (2011) revealed that rats given a diet high in fat were reported to experience reduction in the adipose tissue development without a change in the weight gain in the case of supplementation with quinoa. It fortified with a low standard or high-fat diet enriched with 20HE supplement and fed mice for 3-weeks experiences reduction in its weight and adipose tissues. Reduction in gene expression responsible for lipid storage was linked to the effect observed in the adipose tissue effect (Foucault et al. 2011).

20.3.3 Positive Health Implications

Despite the diverse bioactive compounds which had been derived from quinoa, there are still limited studies of the application or effect of these compounds to either humans or animals. Reduction in the level of lipid peroxidation in blood and reduced oxidative injury due to enhanced antioxidant effects (Pasko et al. 2010) suggest that quinoa has some kind of protective effect against oxidative stress (Farinazzi-Machado et al. 2012). These researchers revealed that 3-week administration of a 20HE-enriched extract of seeds resulted in decline in adiposity and no significant alteration in body weight of mice placed on a high-fat diet. Gene expression linked to lipid storage was associated with the adipose tissue-specific effect. There are limited experimented human trials on the evaluation of the benefits on humans. The insulin in the blood plasma, i.e., growth factor (IGF-1) levels of Ecuadorian boys feed 100 g seeds twice a day, was augmented significantly though quinoa is a known food product which is capable of preventing malnutrition due to its balance nutritional components (Ruales et al. 2002). There are also reported studies which demonstrated that diet which was fortified with quinoa modulated metabolic and cardiovascular parameters in both healthy and obese subjects (Zevallos et al. 2014; Navarro-Perez et al. 2017; Li et al. 2018a). The safety of quinoa consumption was evaluated by Berti et al. (2005) using celiac patients. A decline in the total cholesterol, total glucose, HDL, and LDL was also reported when 50 g of quinoa was consumed on a daily basis for 6 weeks. Gastrointestinal and histological parameters were other factors that declined after the experimenter time.

20.4 Bioactive Compounds

Aside from the quinoa being a nutritive and gluten-free diet, it also an ideal meal for consumers referred to as high risk such as the elderly, children, lactose-intolerant patient, patients who are diabetic, and the obese. Patients with celiac disease, dyslipidemia, and anemia are also included in this evaluation. These positive effects have been correlated with the presence of vital nutritional constituents and phytochemicals which marks quinoa's nutraceutical seeds.

20.4.1 Saponins

Saponins are a group of compounds which can be referred to as secondary metabolites. They are majorly found in diverse part of a plant including its roots, leaves, seeds, fruits, and stems. They help some plant in preventing invading insects, birds, and microorganisms (Singh and Kaur 2018).

Though saponins are known for their unfavorable taste, they are known to have diverse biological functions which include anti-inflammatory, hypoglycemic, hypocholesterolemic, antiviral, anticancer, antiviral, diuretic, and antifungal effects (Vega-Galvez et al. 2010). Saponins are bitter substances that need to be removed from the quinoa seeds before consumption. Also, a special breed of quinoa has been developed which has lower saponin amount.

There is the presence of a triterpenoid aglycone also referred to as a sapogenin and certain sugar chains in its structure. These sugars are categorized based on the number of sugar chains present. This category can be categorized as a mono-, di-, or a tridesmosidic. External seed coat possesses huge concentration index of saponin (Güclü-Üstündag and Mazza 2007). Saponins are made up of a complex mixture of triterpene glycosides which is a derivative of serjanic acid, hederagenin, 3b,23,30-trihydroxyolean-12-en-28-oic acid, as well as oleanolic acid. Arabinose, glucose, and galactose are major carbohydrates existing in the saponin, while carbohydrate present in lower quantity includes xylose and glucuronic acid (Kuljanabhagavad et al. 2008). About 87 saponins have been elucidated from crude extract by Madl and co-workers (Madl et al. 2006), while Kuljanabhagavad et al. (2008) and Ruiz et al. (2017) reported 20 saponin derivatives. Jarvis and co-workers identified the transcription factor which controls the triterpenoid seed saponin synthesis (Jarvis et al. 2017). This will improve the accuracy for the selection of quinoa varieties known as the sweet varieties with minimal saponin content. It is necessary to remove the saponin content in the seeds as it is a nutritional factor which affects its digestibility and palatability. Saponins can be removed with two basic methods. The wet method involves rubbing or washing in water at 4 °C, while the dry method involves toasting and removal of the outer layer. Extensive reports exist on diverse biological roles of saponins in spite of its indigestible characteristics. Some of these biological activities include anti-inflammatory, antimicrobial, antiviral, antioxidant, hypocholesterolemic, immune-stimulatory, mineral absorption was modulatory, anti-thrombotic and vitamin absorption modulatory (Graf et al. 2015).

Woldemichael and Wink (2001) showed that saponins from quinoa have inhibitory effect on the *Candida albicans* growth. *Botrytis cinerea* was also reported to be inhibited by the quinoa saponins in a study by Stuardo and San Martin (2008). The alkaline treatment of the extracts of saponin inhibited the growth of mycelia and germination of conidial (Stuardo and San 2008). Bidesmosidic saponins and their aglycone derivative have been reported to exert some form of toxicity effect on the carcinoma cells of Caco-2 cells. In a study by Yao et al. (2014a, b), crude extracts of saponin were shown to reduce nitric acid and inflammation-related cytokines stimulated by lipopolysaccharide.

Yao et al. (2015) showed that the cell capability and adipocyte proliferation were suppressed by quinoa saponins. These researchers concluded that saponins are bioactive compounds of natural origin effective against the modulation of adipose tissue and adipogenesis suppression. Even though there is a dearth of knowledge on saponin biological activities, these compounds were demonstrated to enhance immunological responses in mice (Verza et al. 2012).

20.4.2 Phytosterols

About 118 mg phytosterols are contained in 100 g of the seeds of quinoa. Stigmasterol, campesterol, β-sitosterol, and brassicasterol are some major phytosterol components present in the quinoa seeds (Villacr'es et al. 2013). Ryan et al. (2007) estimated β-sitosterol, campesterol, and stigmasterol to be 63.7 mg/100 g, 15.6 mg/100 g, and 3.2 mg/100 g, respectively. These figures documented actually exceed those reported for millet, rye, and barley. Phytosterols have some structural similarity to cholesterols because they are lipophilic in nature. Reductions in cholesterol in humans have been correlated to phytosterols from prior meta-analyses, intervention studies, and epidemiological evidence (Graf et al. 2015; Marangoni and Poli 2010). Ho and Pal (2005) in their study observed that phytosterol contends with the gut cholesterol intake site, thereby reducing the generation of atherosclerotic lipids. Ryan et al. (2007) demonstrated the protective effects of phytosterols against cancer, oxidative injury, and inflammation.

20.4.3 Phytoecdysteroids

Quinoa has the highest content of phytoecdysteroids out of the plants consumed by humans. Aside from their known effect such as pest control, they also display metabolic as well as pharmacological properties in mammals according to reports by Foucault et al. (2011) and Dinan (2009). The overall quinoa content of phytoecdysteroid was reported to be about 138–570 mg/g. The 20-hydroxyecdysone (20HE) is the most common ecdysteroids which are commonly found in several plants like quinoa. Graf et al. (2016) reported 20HE to constitute about 62–90% of the total phytoecdysteroids present in the seeds.

20.4.4 Betalains

Betalains are classified under the order Caryophyllales, and they can be said to be nitrogenous plant pigments. Based on the chemical nature of their structure, betalains can be subdivided into the yellow-orange betaxanthins or the red-violet betacyanins. The US FDA (Gengatharan et al. 2015) and European Union (additive E-162) have approved the use of betalain extracts as colorants in food and pharmaceutical products. There has been increasing application of betalains in functional food formulation in recent times (Gandía-Herrero et al. 2016), largely because of significant anti-lipidemic, antioxidant, anticancer, and antimicrobial effects of betalains. Quinoa is rich in betalains and a promising crop for betalain extraction though this is still under investigation. In a study by Repo-Carrasco-Valencia et al. (2010), betacyanins were not detected in quinoa seeds of some Peruvian Altiplano's varieties even though Abderrahim et al. (2015) detected betalain content ranging from 0.15 to 6.10 mg/100 in seeds from the same region.

In red and black varieties, betacyanin and isobetacyanin contents were documented by Tang et al. (2015); however, the study did not provide quantitative data (Tang et al. 2016). Aguilar-Tuesta et al. (2018) also demonstrated that betacyanins as well as betaxanthins had been elucidated from 29 varieties of Peruvian quinoa. The grain extract was observed to be rich in antioxidant activities. They concluded that these quinoa varieties are possibly sourced for extracting bioactive betalains. The microencapsulation of maltodextrin having saponin in lower concentration and betacyanin might give this product unique nutraceutical and functional properties.

20.4.5 Phenolic Compounds

Phenolic is not a major plant derivative having a chemical structure which is highly stable and has a minimum of an aromatic hydrocarbon ring with hydroxyl group (s) attached. In summary, phenolic compounds can be classified into phenolic acids, lignans, flavonoids, stilbenoids, coumarins, phenols, phenylpropanoids, quinines, and xanthones. Gomez-Caravaca et al. (2011) reported a total quinoa polyphenol content of between 0.46 and 1.84 mg/g dry weights. The most abundant phenolics are the flavonol glycosides (Dini et al. 2004). They are majorly made up of the kaempferol and quercetin derivatives with an individual concentration range of approximately 0.84 mg/g dry weight (Hirose et al. 2010; Pasko et al. 2008).

Flavonoids present inside seeds consist majorly of orientin, vitexin, rutin, morin, hesperidin, and neohesperidin in the range of 1.08 mg/g dry weight, 0.71 mg/g dry weight, 0.36 mg/g dry weight, 88.9μg/g dry weight, 1.86μg/g dry weight, and 1.93μg/g dry weight, respectively (Gorinstein et al. 2008). Acid as well as alkaline hydrolysis has been reported to result in the identification of phenolic compounds, both free and conjugated forms. These include the p-coumaric, ferulic, 4-hydroxybenzoic, vanillic, protocatechuic, and their derivatives. Ferulic and vanillic acids were the two bounds extractable elucidated. Isoflavones were

elucidated from the seed varieties of ten different quinoas by Rice-Evans and co-workers (Rice-Evans et al. 1997). Genistein and daidzein were two identified isoflavones with concentration range of 0.5–4.1μg/g and 7.0–20.5μg/100 g, respectively. A tannin content (0.05%) is comparable to values obtained for amaranth. This value was, however, higher than those for soybean (0.034%) and rice (0.035%) (Tang et al. 2015).

Antioxidant properties of seeds have been investigated by numerous studies, and it has been related to quantities of phenolic compounds it possesses (Pasko et al. 2008; Laus et al. 2012). Results obtained were in comparison with legumes and pseudo-cereals (Alvarez-Jubete et al. 2010; Tang et al. 2015; Abderrahim et al. 2015). Non-phenolic compounds have also been documented to be associated with the antioxidant effect of this seed (Nsimba et al. 2008). Pro-inflammatory cytokines are downregulated in the cells of the colonic epithelial Caco-2 (Hemalatha et al. 2016). This helps to improve gastrointestinal health and avert obesity caused by induced inflammation in mice. The milled fraction of whole grain has been classified as potent antioxidant (Lutz et al. 2013). These researchers also described the bound phenolic to inhibit pancreatic lipase activities and the inhibitory effect on α-glucosidase. This study suggests quinoa to having the possible capacity of preventing hyperglycemia and complications associated with it (Yang et al. 2008).

20.4.6 Polysaccharides

There has been increased study on roles of natural source polysaccharide as free radical scavengers, promoters of natural killer cells, lipid oxidation agents, interleukins, and macrophages (Song et al. 2010; Cordeiro et al. 2012). More research attention has been directed toward polysaccharide constituent in the fiber fraction. Polysaccharides rich in pectic were characterized from the quinoa seeds by Yao et al. (2014a, b). Isolated and purified polysaccharides have been reported to show improved immune-regulatory and antioxidant activities (Hu et al. 2017). Esatbeyoglu et al. (2014) reported that novel fraction of polysaccharide which comprises glucose and galactose shows that significant radical scavenging activity was extracted. This fraction showed improved antioxidant properties and likewise inhibits the production of nitric oxide in the macrophages. They further affirmed some cytotoxic effects of this polysaccharide against cancer cells of human (SMMC 7721) and the cancer of the breast (MCF-7 cells) and no effect on normal cells.

20.4.7 Bioactive Proteins and Peptides

Aside from its nutritional properties which have been widely reported, protein component of quinoa possesses bioactive attributes. Aluko and Monu (2003) reported that the total cholesterol increased in the liver and plasma was inhibited when fraction rich in protein was administered to experimental mice. This is possible through the suppression of bile acid reabsorption in the gut and regulation of the

catabolism and production of cholesterol. Though there is still scarce information, quinoa protein has been extensively studied and affirmed as bioactive peptides sourced by several researchers. Hydrolysates of protein having an antioxidant and enzyme-inhibitory activities of angiotensin were first reported by Aluko and Monu (2003). This novel protein hydrolysate was reportedly obtained using the alcalase enzyme. Likewise, the papain hydrolysates of protein fractions also are affirmed to have inhibitory and antioxidant effects (Nongonierma et al. 2015). This shows that quinoa proteins possess antidiabetic effect which makes them a potential functional ingredient. Several protein peptides have been identified in gastrointestinal digest (Vilcacundo et al. 2018; Li et al. 2018a, b) with fermented dough (Vilcacundo et al. 2018). Its multifunctional characteristics including chemopreventive, antioxidant, and antidiabetic properties show that the derived protein peptides have potentials as functional food ingredients which can be used to prevent the chronic disease pathogenesis in association with diabetes, hypertension, and oxidative stress. These peptide mechanism and its bioactivities still need further studies using animal or human models.

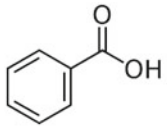
20.5 Bioactive and Nutraceutical of Quinoa

20.5.1 Phenolic Acids and Their Biological Activities or Functions

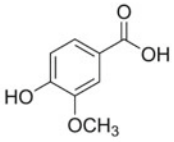
Approximately 29 analogues of phenolic acid were reported in previous studies. This can be categorized with respect to their structural features into the cinnamic acid analogues and benzoic acid. The derivative of phenolic acid exists either in the conjugated or free forms. Chemical (acid and alkaline) as well as enzymatic treatments can result in the discharge of phenolic acids from its conjugated forms. Tang and co-workers reported the discharge of 19 phenolic acids from quinoa residue (Tang et al. 2016). They observed bound phenolic acid derivative not to be affected by stress factor resulting from the environment. Higher phenolic contents also showed stronger antioxidant activities and inhibition of α-glucosidase and pancreatic lipase activities.

20.5.2 Biological Functions of Benzoic and Cinnamic Acid Analogues

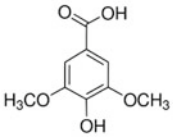
Sixteen analogues of benzoic have been identified. Examples of benzoic acid derivatives and their analogues include benzoic acid (1), vanillic acid (2), syringic acid (3), gallic acid (4), and protocatechuic acid (5) (Fig. 20.1). Though there are no reports of the elucidation of the benzoic acid analogues of quinoa, however, the metabolites from the species of other plant possess antioxidant (Ti et al. 2014),



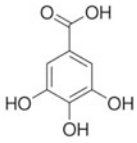
1. Benzoic acid



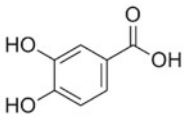
2. Vanillic acid



3. Syringic acid

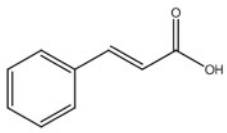


4. Gallic acid

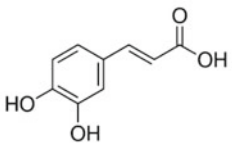


5. Protocatechuic acid

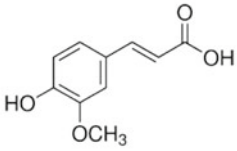
Fig. 20.1 Analogues of benzoic acid isolated from quinoa



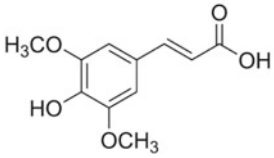
6. Cinnamic acid



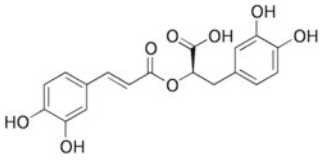
7. Caffeic acid



8. Ferulic acid



9. Sinapic acid



10. Rosmarinic acid

Fig. 20.2 Analogues of cinnamic acid isolated from quinoa

antimicrobial (Cho et al. 1998; Hung et al. 2000), allelopathic (Slimen et al. 2017), and antifeedant (Abou-Zaid et al. 2001) activities.

A total of 13 cinnamic acid analogues have been elucidated. These derivatives of coumaric acid include cinnamic acid (6), caffeic acid (7), and ferulic acid (8) (Fig. 20.2).

20.5.3 Biological Functions of Flavonoids and Their Derivatives

Flavonoids can be described as molecules with 15-carbon skeleton and 2-benzene ring connected via a heterocyclic pyrene ring (Kumar and Pandey 2013). Glycoside and aglycones are also constituents present in them. Quercetin (11) and kaempferol (12) are typical examples of flavonoid aglycones. Genistein (13), daidzein (14), acacetin (15), and myricetin (16) (Fig. 20.3) are other typical examples. Flavonoids can be classified according to their structural features into flavanones (or dihydroflavones) (17), flavones (18), flavanols (19), and isoflavones (20) (Fig. 20.4). Flavonoids have deterrent effects against the physiological and

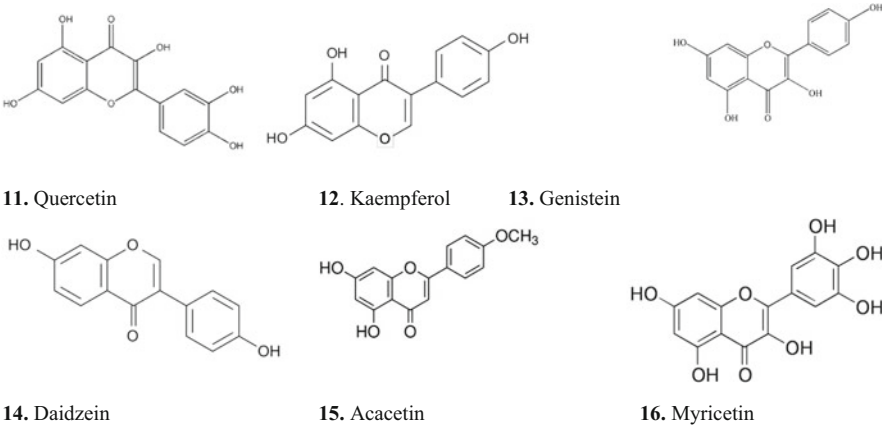


Fig. 20.3 Analogues of flavonoid aglycones isolated from quinoa

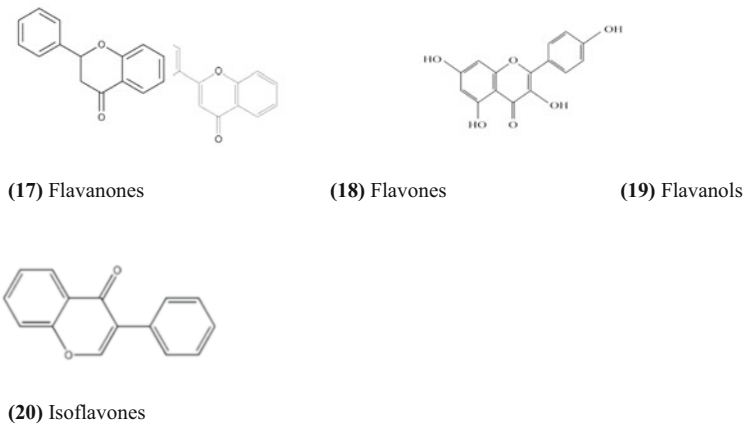


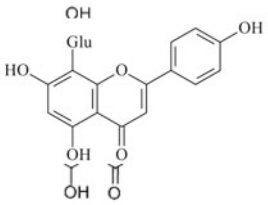
Fig. 20.4 Structures of the analogues of flavonoid aglycones isolated from quinoa

feeding behavior of herbivores and insects (Harborne and Williams 2000; Wuyts et al. 2006).

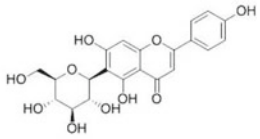
Examples of flavones which have been elucidated from include orientin (21), acacetin (15), vitexin (22), and isovitexin (23). The sprouts are richer in flavones than any other part of the plant. Vitexin and isovitexin are present in sprouts grown in darkness, while isovitexin is present in only those grown in daylight.

Arora et al. (1998) documented that myricetin had higher antioxidant capabilities than kaempferol. Likewise, Rice-Evans et al. (1995) described compounds lacking the ortho-dihydroxy substitution as having lower antioxidant potentials than others with the substituent ortho-dihydroxy substitution. Flavonoid derivative, such as quercetin, had the most potent antioxidant properties. Gawlik-Dziki et al. (2013) reported the flavonoid derivatives kaempferol and isorhamnetin to be most abundant in the leaves. It was also reported to consist of rutin in a larger amount. While two quercetin 3-glycosides showed strong antioxidant activity, four kaempferol 3-glycosides displayed reasonable antioxidant action. This suggests quinoa to be an essential source of free radical inhibitors (Zhu et al. 2001).

Several flavonoids have been categorized based on some biological activities such as antiviral, antifungal, and antibacterial (Fig. 20.4). These categorizations are mainly not just for its action against pathogens of plant origins but also against animal and human pathogens. Kaempferol, a typical flavonoid, possesses antibacterial activity against *Candida glabrata* as well as against Gram-negative and Gram-positive bacteria (Bloor 1995; Zhu et al. 2001) (Figs. 20.5 and 20.6).



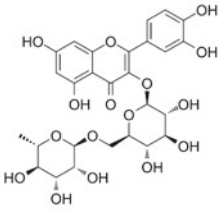
(21) Orientin



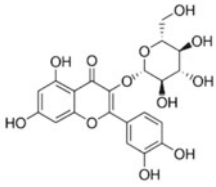
(22) Vitexin

(23) Isovitexin

Fig. 20.5 Structures of the analogues of flavones isolated from quinoa

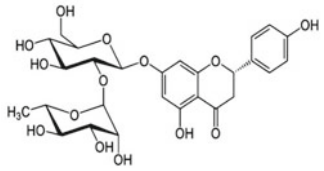


(24) Rutin

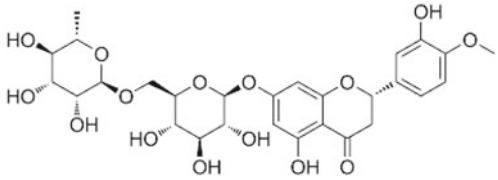


(25) Quercetin-3-glucoside

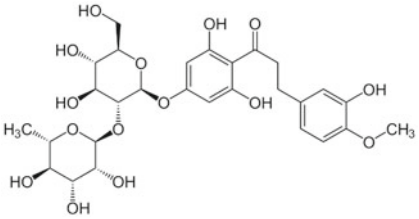
Fig. 20.6 Structures of quercetin derivatives isolated from quinoa



(26) Naringin



(27) Hesperidin



(28) Neohesperidin

Fig. 20.7 Typical flavanones identified in quinoa

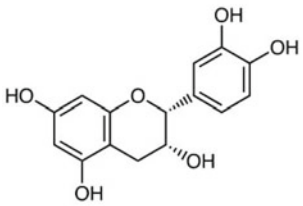
Other identified quercetin derivatives include rutin (24), myricetin (16), and quercetin-3-glucoside (25). Quercetin inhibits the growth of *Meloidogyne incognita* and *Radopholus similis* (Wuyts et al. 2006), while quercetin-3-glucoside and rutin of *P. banksiana* inhibit *Lymantria dispar* development (Wuyts et al. 2006). It likewise results in increased mortality of *L. dispar*. Parvez et al. (2004) observed that the fungus, *Neurospora crassa* spore germination, and *Arabidopsis thaliana* are inhibited by quercetin.

Naringin (26), hesperidin (27), and neohesperidin (28) (Fig. 20.7) are typical flavanones extracted from quinoa. Pasko et al. (2008) reported neohesperidin and hesperidin to be present in the growing sprouts of the quinoa seeds. These flavanones exhibited diverse biological effects such as anti-inflammatory (Parhiz et al. 2015), antioxidant (Al-Ashaal and El-Sheltawy 2011), neuroprotective (Carmona et al. 2019), and antifungal (Salas et al. 2011) activities.

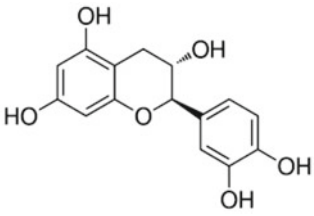
Typical examples of flavanols, namely, epicatechin (29), catechin (30), and epigallocatechin (31) (Fig. 20.8), have been elucidated from the seeds of quinoa. Antimutagenic and anti-oxidative (Fukumoto and Mazza 2000; Huang and Frankel 1997) are basic biological activities shown by these plant seeds.

About five derivatives of isoflavones have been elucidated. These include genistein (32), biochanin (33), prunetin (34), daidzein (35), and puerarin (36) (Fig. 20.9). They have been reported to have positive effect on *Radopholus* (Wuyts et al. 2006). Isoflavones have been linked to the reduction in cancer risk factors due to their estrogenic constituents. When they are metabolized to active compounds by gut microorganism such as genistein and daidzein, their action is enhanced.

(29) Epicatechin



(30) Catechin



(31) Epigallocatechin

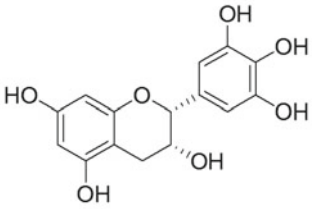
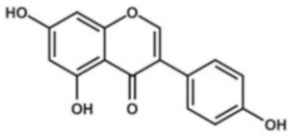


Fig. 20.8 Typical flavanols identified in quinoa

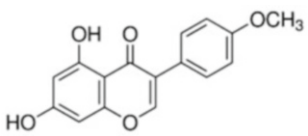
20.5.4 Terpenoids and Their Biological Activities or Functions

Two typical quinoa terpenoids synthesized through isoprenoid metabolic pathway are the monoterpenoids and triterpenoids. The monoterpenoids mostly function as allelochemicals in quinoa, while triterpenoids have a characteristic taste which is distasting to insects and birds (Vega-Galvez et al. 2010). It also possesses detergent properties. Vaccines from the saponins of quinoa are produced commercially (Sun et al. 2009).

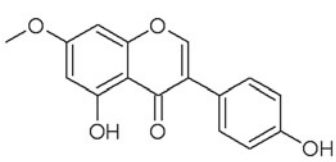
Dembitsky et al. (2008) in their study identified 15 monoterpenoids in quinoa essential oils. Gomez-Caravaca et al. (2011) identified pentose bioside, an iridoid glycoside extracted from the quinoa flour. Yoshitomi et al. (2016) elucidated γ-terpinene from rice. This was proven to have some antibacterial effect on *Xanthomonas oryzae* pv. oryzae (Xoo).



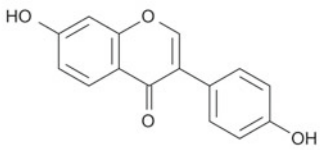
(32) Genistein



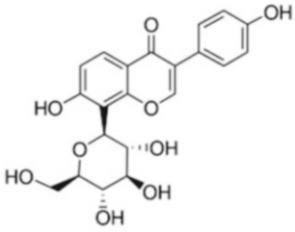
(33) Biochanin



(34) Prunetin



(35) Daidzein



(36) Puerarin

Fig. 20.9 Isoflavone derivatives identified in quinoa

20.6 Traditional Uses

Locally quinoa is utilized in South America as a phytomedicine and dietary supplement (Vega-Galvez et al. 2010; Bhargava and Srivastava 2013). Seeds are made into several food products similar to other commonly consumed food such as rice or baked goods (Bhargava et al. 2006). Just like spinach, quinoa leaves had been eaten likewise, while germinated seedlings of quinoa had been used in the supplementation of salads (Oelke et al. 1992). They have reported production of beer, also known as "chicha," from the sprouted seeds (Healy 2001; FAO 2014). It is used as an alternative feed for livestock due to its rich nutritional compounds (Bhargava et al. 2006). Several researchers have reported the uses for medical purposes such as wound and fracture treatment and in the digestion improvement (Bhargava et al. 2006; FAO 2014). Some other studies have identified quinoa as ideal for the formulation of food that can be said to be endurance promoting and invigorating and promotion of wellness (FAO 2014). The Andean farmers have formulated an

energy-sustaining food known as "llipta," which is prepared from quinoa stem ashes mixed with cocoa leaves (Martindale 3rd 1894). War bar, which is a combination of quinoa and fat, was utilized by the Incan armies to sustain their strength in the battle over the Andes Mountains (Small 2013).

20.7 Anti-Hypercholesterolemic Effect (In Vivo)

Cholesterol is an important structural component of cell membrane in animal cells; it is necessary for several metabolic processes occurring in our body, i.e., production of many hormones, vitamin D, and enzymes needed for digesting food. But, high cholesterol levels (more than needed by the body) can cause serious health problems like cardiovascular diseases. As discussed earlier, the seeds of plant are known to have hypocholesterolemic activity. And this has been shown in many studies. Cholesterol-lowering activities of isolates were evaluated by Tako and colleagues. They extracted and isolated fractions of protein from seeds. Mice were used for this purpose and fed on 0.5% cholesterol diet distributed among three groups containing 0, 2.5, and 5% of QP-quinoa protein for 30 days. After a month it was observed that this diet outstandingly averted the elevation in plasma cholesterol levels (Takao et al. 2005).

Another study was conducted in 2010 by Pawel Pasko and his team. They investigated the effect of seeds on lipid and glucose metabolism in 24 male Wistar rats with a mean weight of 245 g. Rats were kept in a moderate humid atmosphere in cages each containing three rats. After the experiment it was observed that the quinoa diet productively lowered circulating lipids, glucose, and total protein. Quinoa seeds can decrease or prevent most of hazardous fructose-induced hyperlipidemia and hyperglycemia (Pasko et al. 2010).

A probable double-masked study was organized in 2013 by De Carvalho. This study was conducted on 35 obese women. Half of the women were given 25 g of quinoa flakes (QF), and the others were given 25 g of corn flakes (CF) daily. After 30 days, a reduction in circulating cholesterol was observed only in women fed with quinoa flakes (Arneja et al. 2015).

Mona S. Halaby et al. (2017) along with her mates designed a study to observe protective influence of quinoa seeds on hypercholesterolemia in rodents. Rats were first given hypercholesterolemic diet, and then they were fed on quinoa seed powder to evaluate the potential effect against hypercholesterolemia. Hypercholesterolemia was induced by adding 2% cholesterol in daily diet. The blood samples after centrifugation were used for the biochemical analysis of serum, i.e., total cholesterol, triglycerides, uric acid, urea, and creatinine. All the biological parameters were recorded. It was concluded from the results that the group of rats fed on diet with 2% cholesterol were at major risk of hypercholesterolemia. The diet with 30 and 40% seed powder improved the body weight, reduced blood cholesterol and lipid profile, and also improved liver and kidney functions as compared to the groups who were not fed with seed powder. In short, diet with 40% quinoa seed powder reduced the detrimental effect of hypercholesterolemia (Halaby et al. 2017).

Quinoa seeds has been demonstarted to possess hypocholesterolemic effects in male rats. Thirty-two normal adult male rats (weighing approx. 150 g) were taken for this purpose. Hypercholesterolemia was induced in the rat population by adding 2% cholesterol in their basal diet for almost 2 weeks. After 2 months, rats were fasted for overnight, and next day they were sacrificed to take blood samples for biochemical analysis. Total cholesterol, triglycerides, LDL, and HDL were analyzed. The rats were also tested for liver and kidney functions. From results it was concluded that rats fed with 35 and 45% of quinoa seed powder showed a decrease in total serum cholesterol level as compared to the controlled group and their liver and kidney functions were also improved. Quinoa seed powder was demonstrated to significantly reduce the adverse effects of hypercholesterolemia (Alghamdi 2018).

20.8 Technological Innovations in Quinoa Processing and Applications

Technological innovation which reduces cook time and creates packaged food products which are ready to eat or concentrated form of phytochemicals can improve the healthy food lace with beneficial phytochemicals. Due to the increasing acceptance of quinoa as healthy food, there have been increasing studies and new food product development from these seeds. Increased publications on studies involving this plant buttress this fact. Research has focused majorly on how it can be formulated to food component that has not just improved nutritional constituents but therapeutic effect on human health. Some aspects of human health taken into consideration include (1) drug absorption, (2) weight loss, (3) fitness enhancement, (4) sports performance, (5) hair and skincare, and celiac disease.

20.9 Food Processing, Packaging, and Formulation

Saponins have been extracted from the plant through the use of steam that is superheated (Thomas 1995). The solid-state fermentation process has been employed in the production of tempe (Penaloza et al. 1992). This product was reported to consist all of the essential amino acids. The level of estrogenic compounds was estimated to be less than in soy-based tempe prepared traditionally (Penaloza et al. 1992).

Thermostability and smaller mass of the starch granules confer to it the characteristics of use in the production of thickeners and malted beverages and in the packaging of frozen foods (Bhargava et al. 2006). Pagno et al. (2015) suggested foodborne pathogens such as *Staphylococcus aureus* and *Escherichia coli*, two common pathogens of food, can be prevented with biofilms made from nanoparticles of gold materials integrated with the starch. Likewise, maltodextrins derived from starch hydrolysis have been applied to use as a substitute of fat/cream substitute and as a delivery medium for nutrient of better quality (Singer et al. 1990; Enrione et al. 2013). Wheat-based bread was reportedly prepared from 10% flour. The loaf volume

was unchanged while the nutritional quality was significantly improved. In comparison with bread made from potato starch, quinoa bread had better protein, minerals, fiber, antioxidants, polyphenol, and vitamins with better crumb structure and bread volume (Valcárcel-Yamani and Caetano da Silva Lannes 2012). Studies by Elgeti and co-workers also show that bread texture and loaf volume were improved when quinoa flour was substituted with corn or rice flour (Elgeti et al. 2014). There is, however, the need to conduct further studies on quinoa and its compositions especially those that are utilized for pasta as well as bread productions.

20.10 Conclusion and Future Recommendations

This chapter entails comprehensive information on the pharmacologically active metabolites and nutrient profiles available in the quinoa plant. The benefits and demerits of the quinoa plant are highlighted in detail along with the medicinal significance of the active constituents present in quinoa such as anti-hypercholesterolemic activity and its activity against cardiovascular diseases and many others. The nutritional profile and benefits of the plant which contains a well-balanced nutritionally important pseudo-cereal are discussed. An important health benefit of quinoa is that it can be used by patients of celiac disease as it has gluten-free proteins. Moreover, there is a need to establish some other nutritional attributes of quinoa plants via further studies using cell culture and whole animals. Furthermore, there is a need to create more awareness on the nutritional importance when compared to other cereals having high gluten proteins and glycemic index which is not health friendly. This study shows that quinoa plants and numerous plants with several medicinal benefits entail several beneficial metabolites that could help in the achievement of sustainable development goals (Abubaka et al. 2019; Adetunji 2008; Adetunji et al. 2011a, b, c, 2012, 2013a, b, 2014, 2017, 2018a, b, c, 2019; Adetunji and Olaleye 2011). Therefore, there is a need to search for more pharmacological metabolites that could perform more biological activities after they have been subjected to more *in vitro* and *in vivo* biological activities.

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