

# Chapter 8

## Origin of Genetic Variability and Improvement of Quinoa (*Chenopodium quinoa* Willd.)

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**Abstract** Quinoa is a pseudocereal having a very balanced composition of carbohydrates, fat, and protein. Various studies based on inheritance, molecular cytology, DNA markers, and single locus variability have established it as an allotetraploid ( $2n = 4x = 36$ ). It has been cultivated for 5 millennia in the Andes where it probably originated from its wild and weedy forms. Domestication process led to loss of many characters disadvantageous to farmers leading to narrowing of the genetic base. However, wide diversity based on plant color, seed color, types of branching and panicles, productivity, abiotic stress tolerance, and disease resistance still exists. This diversity is also reflected at the molecular level and is being used by the plant breeders worldwide to develop improved plant types with respect to uniformity, early maturity, seed yield, protein content, and reduced saponin content in the seeds.

**Keywords** *Chenopodium quinoa* · Origin · Domestication · Genetic variability · Improvement

### 8.1 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal and is one of the 250 species included in the genus *Chenopodium* (Amaranthaceae), commonly known as ‘goosefoot’ genus (Giusti 1970). The genus comprises herbaceous, suffrutescent, and arborescent perennials, although most species are colonizing annuals (Wilson 1990; Fuentes et al. 2012). Quinoa along with some leafy chenopods (*C. album* and *C. giganteum*) is very important to the food security of marginal farmers as they

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show adaptation to many abiotic and biotic stresses and their ability to grow with minimum inputs (Bhargava et al. 2003, 2006a; Jacobsen et al. 2003; IAEA 2004; Maughan et al. 2009; Bhargava and Srivastava 2013). It is an allotetraploid ( $2n = 36$ ) annual, self fertile crop as it shows disomic inheritance for most of the traits (Simmonds 1971; Risi and Galway 1984; Ward 2000; Maughan et al. 2004), two different non-transcribed sequence (NTS) classes in 5S rDNA spacer region (Maughan et al. 2006), two homoeologous *SOS1* loci (*cqSOSIA* and *cqSOSIB*) (Maughan et al. 2009), two distinct homoeologs of *GBSSI* gene (*GBSSIA* and *GBSSIB*) (Brown et al. 2014) and by the identification of two distinct subgenomes by FTL intron markers (Storchova et al. 2015).

The small seed of quinoa contains a balanced composition of carbohydrates, fat, and protein (Risi and Galway 1984; Coulter and Lorenz 1990; Chauhan et al. 1992). Moreover, the protein is not only higher (7.5–22.1 %) than major cereals (Tapia et al. 1979) but is also composed of 16 amino acids being rich in lysine, threonine, and methionine (Ruales and Nair 1992; Aubrecht and Biacs 2001; Gorinstein et al. 2002; Wright et al. 2002; Drzewiecki et al. 2003; Vega-Galvez et al. 2010; Stikic et al. 2012; Escuredo et al. 2014). The protein is of high quality containing much higher content of lysine than cereals and even milk and being devoid of gluten makes it suitable for celiac patients (Koziol 1992; Vega-Galvez et al. 2010; Stikic et al. 2012). Quinoa starch is present in the form of small granules of about 1–1.5  $\mu\text{m}$  in diameter and an average molar mass of  $11.3 \times 10^6$  g/mol (Tang et al. 2002; Wright et al. 2002; Tari et al. 2003; Lindeboom 2005). The small granules and high viscosity of quinoa starch make it useful for specialized industrial applications (Galwey et al. 1990) such as dusting starches in cosmetics and rubber tyre mold release agents (Bhargava et al. 2006a) and as biodegradable fillers in low-density polyethylene (LDPE) films (Ahamed et al. 1996b). Quinoa starch due to its unique mechanical properties can be utilized in the manufacture of carrier bags where tensile strength is important. Because of the freeze–thaw stability and resistance to retrogradation the quinoa starch paste is very suitable in the preparation of frozen and emulsion-type food products (Ahamed et al. 1996a; Bhargava et al. 2006a). It is also rich in essential vitamins and minerals, iron, and calcium (Risi and Galway 1984; Konishi et al. 2004). Quinoa in fact makes a fine example of a functional food being rich in antioxidants (Nisimba et al. 2008; Repo-Carrasco et al. 2003; Koziol 1992). Quinoa seeds contain a number of ecdysteroids beneficial to human health as they reduce glycemia of diabetic patients (Kumpun et al. 2011).

The balanced nutritional superiority of quinoa has been evaluated as a food by the National Research Council and the National Aeronautics and Space Administration (NASA) (Schlick and Bubenheim 1996) and has been recommended as a potential crop for NASA's Controlled Ecological Life Support System (CELSS), which aims to utilize plants to remove carbon dioxide from the atmosphere and generate food, oxygen, and water for the crew of long-term space missions (Schlick and Bubenheim 1996).

## 8.2 Brief History and Distribution

Quinoa has been recognized for centuries as an important food crop in the high Andes of South America (Tapia 1982). The name quinoa in the Quechua and Aymara languages means ‘Mother Grain’ and this crop occupied a place of prominence in the Inca Empire next only to maize (Cusack 1984). However, after the conquest of the region by the Spaniards in 1532 A.D. crops such as potato, faba beans, oats, and barley relegated quinoa to the background (Galwey 1995; Bhargava et al. 2006a). During the colonial period the cultivation of quinoa was discouraged, possibly because of its honored position in the Inca society and religion (Risi and Galwey 1989; Ruas et al. 1999). Quinoa’s religious significance for the Incas made it a less attractive crop to the Spanish. The status given to it as ‘Mother Grain’ and the ‘Grain of the Gods’ put it in direct conflict with the Catholic religion promoted by the Spanish Conquistadors who discouraged its production and consumption in the newly conquered territories. However, the frequent droughts in Andes once again necessitated the cultivation of quinoa as it showed nearly constant yields in severe conditions (Cusack 1984; Bhargava et al. 2006a). During the 1980s, the market for quinoa began expanding in Europe and North America mainly in the health-food sector and the demand was met partly by imports from South America and by the development of quinoa in new regions outside its center of origin.

In its native region, the major areas of current quinoa cultivation appear to extend southward from extreme southern Colombia through Ecuador, Peru, and Bolivia, with extensions into the Chilean altiplano (eastern Tarapaca) and northern Argentina (Jujuy and Salta) (Wilson 1990). According to Rojas (1998) the geographical distribution of quinoa in the region extends from 5°N in Southern Colombia, to 43°S in the Xth Region of Chile. During the last decade, Chile, Ecuador, Argentina, and Colombia have started extensive cultivation and research projects on quinoa, such as SICA (Agricultural Census and Information System) of the Agricultural Ministry of Ecuador; Quinuacoche CANOE program promoted by the Latin American Foundation in Colombia; Provincial Congress for Quinoa promoted by the Deputies Chamber of Salta, Argentina; Program of Encouragement for Business Design; and Innovation promoted by the Euro Chile Foundation (Taboada et al. 2011).

Quinoa was introduced in the 1970s in England and thereafter in Denmark. In 1993, a project titled ‘Quinoa-a multipurpose crop’ for EC’s agricultural diversification was initiated in the European Union (Jacobsen 2003). This led to setting up of laboratories in Scotland and France, and field trials in England, Denmark, the Netherlands, and Italy. Quinoa has been evaluated as a potential crop in Denmark, Poland, Sweden, Italy, and Greece (Iliadis et al. 1997; Gęsiński 2000; Bhargava and Srivastava 2013). It has also been successfully tested in North America and Africa and has been cultivated in the US since the early 1980s and commercially produced since the mid-1980s in the Colorado Rockies, especially in the San Luis Valley (Bhargava and Srivastava 2013). The North American Quinoa Producers Association

was formed in 1988 and a small processing plant was started for the crop produced in the area. Production has also been attempted in California, New Mexico, Oregon, and Washington. In Canada, cultivation is done in Saskatchewan and Manitoba most of which is organic (Bhargava and Srivastava 2013).

The Asian experiment on quinoa introduction has been quite impressive with the crop showing good adaptation and abundant yield in the Indian subcontinent. Quinoa was successfully introduced in India in the early 1990s and exhaustive field trials have proved its cultivation as an alternative winter crop for the North Indian Plains (Bhargava et al. 2007a). It was introduced in Pakistan in 2007 in the central Punjab to lessen the dependence of the common people on conventional crops (Munir et al. 2012). Field tests have been done in Japan in the climatic conditions of Southern Kanto District of Japan (Yamashita et al. 2007). Field tests in Kenya have shown seed yield up to 9 t/ha and biomass yield up to 15 t/ha indicating high seed yield comparable to that in the Andean region (Mujica et al. 2001). A partnership between the Danish Company Eghøjgaard and the Egyptian Natural Oil Company (NATOIL) has been constituted since the year 2007 for promoting quinoa in Egypt (Bhargava and Srivastava 2013). Quinoa was formally put in field trials in the Sinai Peninsula with 13 varieties and strains being tested in deserts of South Sinai governorate (near Nuwaiba city) which proved to be a success (Shams 2011). Recent introduction in Morocco has shown a high potential of adaptation in the country (Hirich et al. 2014).

### 8.3 Domestication

Quinoa is a part of a complex of interfertile New World wild, weedy, and domesticated ecotypes, variously listed as three or four separate taxa (Jellen et al. 2011). Quinoa has been cultivated for more than 5000 years in the Andes but was probably domesticated by ancient civilizations at different times and in different geographic zones, including Peru (5000 BC), Chile (3000 BC), and Bolivia (750 BC) (Tapia 1979; Kadereit et al. 2003). Ancient farmers in the Andean region of South America were the pioneers in domesticating quinoa from its wild or weedy forms until the current known types in a domestication/cultivation period of approximately 5000 years (Bazile et al. 2013). During this period, quinoa was subjected to diverse selection process for desirable traits for its cultivation and consumption by people belonging to different cultures and territories in South America like the Chibchas, Andaki, Inganos in southern Colombia; Aymara and Quechua in areas of Peru, Bolivia, and northern Chile; Diaguitas y Calchaquies in northern Argentina; and Mapuches in southern Chile (Mujica 2004). This process led to the loss of many undesirable characters present in the wild forms such as dehiscent seeds (seed shattering), seed dormancy, and thick seed coats, with simultaneous gain of useful characters such as larger and more starchy seeds, fewer and larger inflorescences, uniform maturity, and environmental adaptations (Galwey 1995; Bazile et al. 2013). This process has been continued by modern

breeding techniques for the development of best phenotypes in terms of yield and agronomic performance, which led to the narrowing of the genetic diversity further. However, wide diversity in plant and seed color, types of branching and panicles, as well as grain productivity, abiotic stress tolerance, and disease resistance can be observed in the field (Fuentes and Bhargava 2011; Ruiz-Carrasco et al. 2011).

Quinoa diversity, at a continental scale, has been associated with five main ecotypes viz. Highlands (Peru and Bolivia), Interandean valleys (Colombia, Ecuador and Peru), Salares (Bolivia, Chile and Argentina), Yungas (Bolivia), and Coastal/Lowlands (Chile); each of these is associated with subcentres of diversity that originated around Lake Titicaca (Risi and Galwey 1984). The recent genetic-based analyses have confirmed that quinoa has existed as two distinct germplasm pools: Andean highland quinoa with its associated weedy complex (ajara or ashpa quinoa, *C. quinoa* ssp. *milleanum* Aellen, also referred to as *C. quinoa* var. *melanospermum* Hunziker) and kinwa among the Mapuche people of the central and southern Chilean coastal/lowlands, representing in addition a second center of major quinoa diversity (Christensen et al. 2007; Fuentes et al. 2009; Jellen et al. 2011). The weedy *C. hircinum* from lowland Argentina can be considered as the third distinct germplasm pool, which may represent remnants of archaic quinoa cultivation in that part of South America (Wilson 1990).

Bazile et al. (2013) have recently differentiated 8 types of quinoa growing in the area of its origin. This classification is based on morphology, phenology, cultivation practices, and resistance to biotic and abiotic factors.

### **8.3.1 *Quinoas of the Altiplano (Northern Andean Highlands)***

Small plants of different colors adapted to the shore of the Titicaca Lake, having variable saponin content and 6 months of vegetative period with small to medium grain, less resistant to cold and drought, and sometimes adapted to grow in saline soils. Plants in the highlands have few branches and a unique panicle, with abundant foliage. These are moderately resistant to mildew, and are attacked by young plant cutters (*Feltia experta* Walker), Kona Kona (*Eurisacca quinoae* Povolny), and birds (Bazile et al. 2013). They usually require an annual rainfall between 700 and 800 mm and grow at an altitude of 3850 m, for example, Kancolla, Blanca de July, and Chullpi.

### **8.3.2 *Quinoas from the Salars (Southern Highlands)***

Cultivation is done on the flanks of the volcanos, in the middle of lava blocks and on the slopes where frosts are less frequent. Plants of the southern highlands are large, branched, having different colors, with large grains of 2.2–2.9 mm with high

saponin content, drought resistant, adapted to saline and sandy soils of the shore of salt lakes (called *Salares*), and adapted to high, dry, and cold conditions (Bertero et al. 2004; Mujica et al. 2010a; Bazile et al. 2013). The quinoas of this region correspond to a group of landraces from desert areas with low annual rainfall (between 150 and 350 mm) and an altitude of over 3800–4200 m, for example, Pandela, Utusaya, Toledo, and Achachino.

### **8.3.3 *Quinoas from Interandean Valleys***

Plants of this type are long, thick stemmed, branched with long vegetative period, have large to small grains of diverse colors, large leaves and inflorescences, susceptible to mildew, and have both high and low contents of saponin (Bazile et al. 2013). These grow between 2500 and 3200 m in areas having annual rainfall of 800–900 mm. These kinds of quinoas are usually called “Quinua” and grouped by their genetic and phenotypic characteristics (Medina et al. 2004), for example, Amarilla de Marangani, Blanca de Junín, Acostambo, Roja Coporaque, Nariño, etc.

### **8.3.4 *Quinoas from Arid Zones and Dry Condition (Eastern Highlands)***

Quinoas of these regions are small, with short vegetative period and present morphological, physiological, anatomical, biochemical, and phenological modifications to withstand drought stress (Mujica et al. 2010b). They grow in areas over 3900 m with an annual rainfall of 150–350 mm. Plants have small leaves, deep and highly branched root structure, small to medium sized grains, and high saponin content (Bazile et al. 2013). These quinoas are also called ‘*quinua*,’ for example, Antahuara, Ucha, Ccoyto, and Roja Ayauchana.

### **8.3.5 *Quinoas from High Altitudes and Cool Climate***

Plants are small with vivid colors like yellow, reddish, or purple in plants and grains and have small and compact glomerular panicles, bitter grain, high in protein content, cold resistant, with mechanisms of overcooling tolerance, and strong winds (Jacobsen et al. 2007; Bazile et al. 2013). They are resistant to ultraviolet radiation and sown over elevations of 4000 m and in areas with annual rainfall of 800 mm, for example, Huariponcho, Witulla, Kellu, Kancolla, and Roja.

### 8.3.6 *Quinoas from the Coastal Regions and Near the Sea*

This group has many types adapted to salty and sandy soils and grows in areas having average annual rainfall of about 500–650 mm (during 4–5 months) and have high evapotranspiration index (Bazile et al. 2010; Núñez et al. 2010). The plants are medium branched, with glomerular panicles and small leaves. All of them have small and hard grain and it is usually protected by perigonium which strongly adheres to the grain (Bazile et al. 2013). Plants are adapted to long days and are resistant to excess moisture with some having the ability to grow in over 2000 mm of annual rainfall, for example, Quinoa Blanca, Kinwamapuche, Lito, Faro and Islunga.

### 8.3.7 *Quinoas from Jungle and Tropical Zones*

These are tall quinoas having more branches, long vegetative period, large leaves, bright and intense colors, large and loose panicles, and small grains (Bazile et al. 2013). The category shows resistance to heat and evapotranspiration, and grows in areas having annual rainfall over 1500 mm, i.e., in Tupiza, Sandia, Puno, Ambo-Huánuco, and Lares-Cusco.

### 8.3.8 *Quinoa from High Rainfall and Humidity Zones*

These are tall, highly branched, resistant to heavy rainfall and poorly drained soils, high yielding, mildew resistant, and grow in areas having annual rainfall between 2000 and 3000 mm (Bazile et al. 2013), i.e., in Tupiza, Nariño, Sogamoso, Tunkahuan, Sogamoso, Mérida, Tupiza, and Amazonas.

## 8.4 Cytogenetics

The basic chromosome number in the genus *Chenopodium* is  $x = 8$  and  $x = 9$  (Kawatani and Ohno 1950, 1956). The number  $x = 8$  is restricted to section Ambrina (Uotila 1973) that contains *C. ambrosioides* ( $2n = 2x = 16$ ) as a representative member (Suzuka 1950; Giusti 1970). 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  $2n = 4x = 36$  (Palomino et al. 1990; Bhargava et al. 2006b). Gandarillas (1979) reported mixoploidy in *C. quinoa* with chromosome numbers of  $2n = 18$ ,  $2n = 27$ ,

$2n = 36$ , and  $2n = 45$ . Wang et al. (1993) studied the somatic chromosomes from root tips of nine taxa of five species of the genus *Chenopodium*. All the three cultivars of *C. quinoa* studied were reported to be tetraploid ( $2n = 36$ ).

Some workers have described certain conspicuous karyotypic features in some species of the genus (Tanaka and Tanaka 1980; Wang et al. 1993; Kolano et al. 2001). The detailed karyotype of quinoa was described by Catacora (1977) who inferred allopolyploidy based on chromosome arm length ratios, which arranges 36 chromosomes in nine groups. Bhargava et al. (2006b) studied seven accessions of *C. quinoa* and assigned them to 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. The symmetry index (TF%) on the basis of arm ratios varied from 43.9 % (PI 584524, most asymmetrical) to 47.4 % (CHEN 58/77, most symmetrical). All taxa were characterized by one satellite pair, the position of which varied according to its comparative size in the complement. The satellite pair was morphologically similar in all the accessions being median (m) or median-submedian (msm), and had the satellite on the short arm. The first chromosome in different complements was either m or msm with arm ratios varying between 1.18 (PI 510537) and 1.56 (CHEN 71/78), while 4th, 9th, and 18th pairs were the most conserved in being median (M or m) in the accessions studied. The greatest variability is observed in 10th and 13th pair with arm ratios ranging between 1.0–1.86 and 1.0–1.78, respectively. *C. berlandieri* subsp. *nuttalliae* also had only one SAT pair with the satellite on the short arm of 3rd pair which was msm. The first pair was msm and the 18th pair was median point (M) and as most of the accessions of *C. quinoa* there was no msm pair in the complement. The symmetry index was 44.1 % and the karyotype belonged to 1a class. The karyotype of *C. berlandieri* subsp. *nuttalliae* did not show any distinct differences and was basically similar to those of different accessions of *C. quinoa*. This was clear from karyotype formula, symmetry index, and one satellite pair of similar morphology as in *C. quinoa*. Similar results were obtained by Palomino et al. (2008) in *C. quinoa* and *C. berlandieri* where all the chromosomes were arranged in nine groups of four each with two pairs of satellite chromosomes in each complement. The accessions of *C. quinoa* studied showed only minor though consistent differences in their karyotypes, which is expected, as *C. quinoa* has a monophyletic origin from Andean crop/weed system (Wilson 1990). These minor differences in karyotypes due to chromosomal alterations are being maintained in quinoa due to predominantly self-pollinating behavior (Risi and Galwey 1984) and are also consistent with some degree of variability in morphological characters (Wilson 1988; Risi and Galwey 1984; Bhargava et al. 2007a), protein profiles (Bhargava et al. 2005), and RAPD profiles (Ruas et al. 1999). The karyotypic analysis has resulted in clearly identifiable 18 pairs thereby indicating allotetraploidy (Bhargava et al. 2006b). This is also supported by duplication of Lap loci (Wilson 1976), disomic inheritance of some characters (Simmonds 1971), and allelic segregation ratios of  $F_1$  and  $F_2$  which indicated disomic-digenic and tetrasomic inheritance in some traits (Ward 2000). The presence of one satellite pair has been corroborated by studies on fluorescent in situ hybridization with 45S rDNA showing two sites of hybridization on two



homologous chromosomes (Kolano et al. 2001). Both these rDNA loci are transcriptionally active which means that at least one such locus may have been lost (Kolano et al. 2001). Maughan et al. (2006) also observed one pair of 25S (NOR) rRNA pair and two pairs (terminal and interstitial) of 5S rRNA in *C. quinoa*. However, the number of NOR loci varied in related tetraploids where 2 NOR loci were identified in *C. berlandieri* subsp. *nutalliae* ‘Huauzontle’ and only one was observed in *C. berlandieri* subsp. *zschackei* and *C. berlandieri nutalliae* ‘Quelite.’ Similarly, *C. berlandieri* subsp. *zschackei* showed two 5S loci and *C. berlandieri* subsp. *nutalliae* ‘Huauzontle’ and ‘Quelite’ showed three 5S loci.

The presence of two subgenomes in quinoa has been demonstrated by FISH using two repetitive sequences, 12-P and 18-24J (Kolano et al. 2011). The specificity of 18-24J to one of the genomes was shown by strong signals on 18 chromosomes in the form of bands of differing intensities on chromosome arms while only minor signals on remaining 18 chromosomes in terminal and centromeric positions. Similar results were obtained with other tetraploids *C. berlandieri* subsp. *nutalliae* and *C. berlandieri* subsp. *berlandieri*. This sequence also hybridized with genomes of two diploid species *C. album* and *C. pallidicaule*. When simultaneous hybridization was done with 18-24J and rDNA sequences, one 35S rDNA locus and only one of the two 5S rDNA loci in *C. quinoa* and *C. berlandieri* subsp. *berlandieri* were located on chromosome pairs with 18-24J signals, while the second 5S rDNA locus was on chromosome pairs without 18-24J signals. In case of *C. berlandieri* subsp. *nutalliae* having three 5S rDNA loci, two pairs were located on chromosomes with 18-24J signals. These results, therefore, clearly show that the tetraploid species studied share at least one genome derived from a common ancestor (Kolano et al. 2011). The identification of the two genomes involved in the ancestry of *C. quinoa* has been done by the phylogenetic analysis of two flowering locus T-Like genes CrFTL1 and CrFTL2. One parent was shown to be related to North American *C. standleyanum*, *C. incanum* or any other related diploid (subgenome ‘A’) and the other parent belonging to the Eurasian species *C. suecicum*, *C. ficifolium*, or some related diploid species (subgenome ‘B’) (Storchova et al. 2015). In this respect, it is interesting to note that *C. quinoa* is inter-crossable with diploid cytotype of *C. album* occurring in North Indian Plains and the resulting triploid shows 18 II and 18I, therefore meaning that one of the genomes of *C. quinoa* is homologous with that of  $2x$  *C. album* (Pal and Ohri, unpublished). The close genetic relationship between *C. quinoa* and  $2x$  *C. album* has also been shown on the basis of RAPD and DAMD studied (Rana et al. 2010).

## 8.5 Genome Size

Bhargava et al. (2007b) found 4C DNA amounts ranging from 6.34 to 6.47 pg, showing a nonsignificant 1.02-fold variation in 21 accessions of *C. quinoa* by feulgen microdensitometry. Similarly, two accessions of related tetraploid species *C. berlandieri* subsp. *nutalliae* show 4C DNA amounts of 5.79 and 5.90 pg and

their average is 8.31 % less than the mean of 4C DNA values of 21 *C. quinoa* accessions. Palomino et al. (2008) obtained similar results by flow cytometry as *C. quinoa* cv. Barandales showed 2C value of 2.96 pg and six accessions of *C. berlandieri* subsp. *nuttalliae* varied from 2.96 to 3.04 pg. The above results have been supported by Kolano et al. (2012) who showed 2C values ranging from 2.9 to 3.0 pg in 20 accessions of *C. quinoa*. However, significantly lower 2C values of 2.66 pg have been obtained by Bennett and Smith (1991) by microdensitometry and 2.01 pg by flow cytometry (Stevens et al. 2006).

## 8.6 Interspecific Hybridization

Various attempts have been made to hybridize *C. quinoa* with related wild or cultivated tetraploids. Nelson (1968) produced artificial hybrids between *C. quinoa* and *C. quinoa* var. *melanospermum* and also confirmed the presence of natural hybrids. Heiser and Nelson (1974) produced F<sub>1</sub> hybrids between *C. quinoa* and *C. nuttalliae* or 'huahzontli' which, however, lacked pollen grains as male sterile parent was involved. The F<sub>1</sub> produced seed when backcrossed with the parents showing that two species are closely related. Remarkably, both the parents had light-colored fruits, while the F<sub>1</sub> had black fruit which was interpreted as due to genetic complementation showing thereby that light-colored fruit arose independently in Mexico and S. America. Wilson and Heiser (1979) showed very low pollen fertility (3 %) in hybrids between *C. quinoa* and *C. nuttalliae*, and the hybrids though are self-sterile produce seed when backcrossed with either parent. Similar results were obtained when *C. quinoa* was crossed with its N. American relative *C. berlandieri* (Wilson and Heiser 1979). Wilson (1980) obtained only one hybrid using male sterile *C. quinoa* with S. American diploid *C. petiolare*, out of 17 intersubsectional combinations. The hybrid showed developmental abnormalities and did not reach flowering. Intrasubsectional crosses of *C. quinoa* succeed not only with tetraploids *C. berlandieri* and *C. berlandieri* subsp. *nuttalliae* producing fertile hybrids, but also with diploid *C. neomexicanum* which is sterile due to triploidy. The hybrid between *C. quinoa* and *C. bushianum* is sterile but produced limited back-cross progeny (Wilson 1980).

Natural hybridization has been shown to occur freely between *C. quinoa*, when cultivated in N. America, with related wild species *C. berlandieri* as 30 % of the progeny of the latter was found to be F<sub>1</sub> crop/weed hybrids as confirmed by the presence of polymorphic quinoa isozyme alleles and morphologically intermediate leaves (Wilson and Manhart 1993).

## 8.7 Genetic Diversity Use of Molecular Markers

Random amplified polymorphic DNA (RAPD) markers were first used in quinoa by Fairbanks et al. (1993) who observed that 26 primers produced polymorphic markers among 16 randomly selected quinoa accessions. The RAPD markers were also used to identify genetic variation among 19 accessions of six species of the genus *Chenopodium* by Ruas et al. (1999). A total of 33 decamer primers generated 399 molecular markers with an average of 12 polymorphisms per primer, which grouped the germplasm collection into five different clusters. The results showed that wild and crop populations of *C. quinoa* shared a low level of molecular variation, without differentiation between sympatric domesticated and weedy populations. RAPD has also been used to study the hierarchical structure among ecotype populations of Highland and Interandean valleys in Bolivia (Del Castillo et al. 2007). The findings reported by scoring 38 selected bands from 10 RAPD primers on eight representative populations ( $n = 87$ ) directly sampled in farmers' fields, revealed a marked geographical effect on the populations' structure, and explained probably by climatic and orographic barriers present in the studied zone rather than to a distance effect. Thus, the population structure was related to the three major biogeographic zones present in Bolivia: northern and central highland, Interandean valley, and southern Salar. Interestingly, the intrapopulation genetic diversity was higher than expected, due basically to mainly autogamous reproduction, as well as the limited seed exchange among isolated regions considered. The genetic diversity was even higher than that reported in studies based on germplasm collections which suggested that germplasm collection under study may not be representative of the genetic variation of the quinoa complex and that further sampling for ex situ conservation will also have to take into account the hierarchical structure of the genetic variation (Del Castillo et al. 2007).

Rana et al. (2010) assessed the suitability and reliability of RAPD and directed amplification of minisatellite DNA (DAMD) markers to assess molecular diversity in 55 accessions belonging to 14 species of chenopods. A total of 242 polymorphic markers were generated from 12 random primers yielding optimum RAPD profiles, while four DAMD primers resulted in 107 polymorphic bands. The UPGMA tree showed two major clusters: the first cluster grouped all the accessions of *Chenopodium quinoa* and its related species *C. berlandieri* subsp. *nutalliae*, one *C. album* (4x) from Mexico and three north Indian 2x accessions of *C. album*, while the other cluster comprised mainly 6x accessions of *C. album* and *C. giganteum* in addition to *C. strictum*, *C. bushianum*, *C. opulifolium*, and *C. ficifolium* (Fig. 8.1). No genetic differentiation was observed with regard to the light- and dark-seeded quinoa accessions. The analysis allowed to assess intra- and interspecific variation within cultivated and noncultivated species in this large genus and to solve taxonomic problems either at or below the species level.

Mason et al. (2005) developed first large-scale SSR markers consisting of 208 polymorphic markers, which were validated and characterized in 31 cultivated quinoa accessions representing the main growing areas of South America. A total of

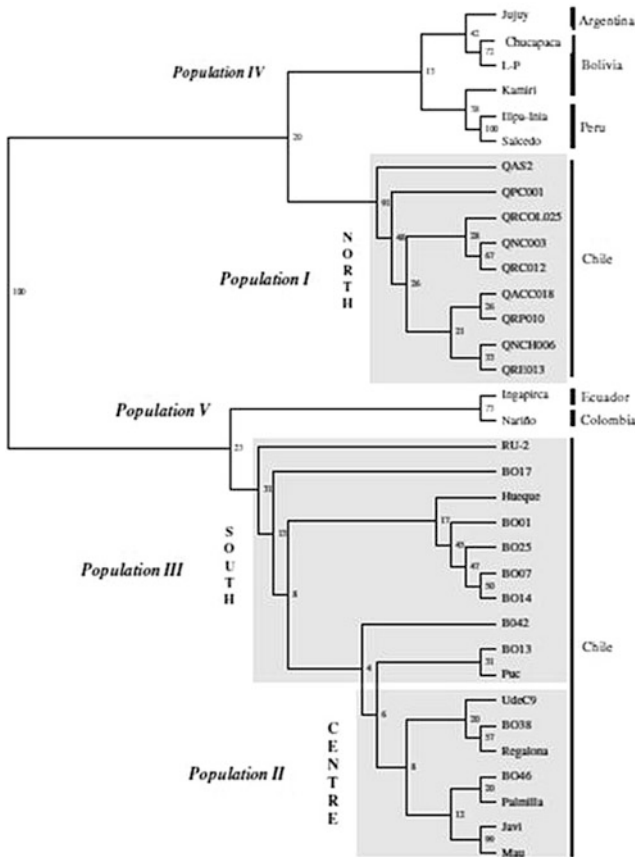


( $H \geq 0.70$ ). This set of SSR markers revealed the potential utility for molecular studies across related species of Chenopodioidae subfamily. The amplification of 202 of the 208 polymorphic SSR markers on a group consisting of two *C. berlandieri* subsp. *nuttalliae* (Huazontle), two *C. pallidicaule* (Canihua), and two accessions of *C. giganteum* (Khan chi) revealed that 67 % of the markers amplified successfully in all groups. The most notable PCR conservation was observed in *C. berlandieri* subsp. *nuttalliae*, with a 99.5 % of reproducible amplifications, of which 81 % were polymorphic.

The level of polymorphism and the genetic relationships were studied by means of molecular markers using the AFLPs and twenty morphological characters (Anabalon-Rodriguez and Isla 2009). Fourteen quinoa landraces from southern Chile, three landraces from highland from northern Chile, and a representation of *C. album* and *C. ambrosioides* were analyzed. The study reported 150 AFLP bands generated by three *EcoRI-MseI* primer combinations of which 130 were polymorphic.

Fuentes et al. (2009) characterized and quantified the genetic diversity within 28 Altiplano and 31 coastal Chilean accessions of quinoa using microsatellite markers. A total of 150 alleles were detected among the quinoa accession, ranging from 2 to 20 alleles per locus and an average 7.5 allele/locus. Both cluster (UPGMA) and principal component analyses separated the accessions into two discrete groups as also shown by isozymes analysis and morphological traits (Wilson 1988), 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 data obtained in the diversity analyses highlights the relationships within and among northern and southern Chilean quinoa accessions and provides a new set of easy 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 representative of Chile and the South American region to study the impact of farmers' seed exchanges and local production practices on the genetic structure and diversity of quinoa at national scale in Chile. The molecular analysis yielded 118 polymorphic markers for all quinoa accessions assessed, with a mean value of alleles per locus of 5.9. The  $H$  for all quinoa accessions 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 subdivided into five populations (Fig. 8.2). Population I contained nine accessions representative of the northern zone of Chile; population II seven accessions of the central zone; population III included nine accessions from the southern zone and only one from the central zone (B042); population IV contained six accessions from the highlands of Peru, Bolivia, and Argentina; and population V contained two accessions from Ecuador and Colombia. Thus, the genetic information allowed the detection of variation among and within the populations identified, which matches well with natural geographical–edaphic–climatic



**Fig. 8.2** 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)

constraints to the expansion of quinoa biodiversity. This grouping also correlates well with the social–linguistic context of ancient people inhabiting the Andes region, where agronomic and cultural traditions that have survived until the present time are very different.

In quinoa, the first source of SNP marker was reported by Coles et al. (2005) from an immature seed and floral expressed sequence tags (EST) libraries that were the first EST libraries developed for quinoa. EST sequences are partial sequences from transcribed cDNA sequences that reflect expressed genes in a given tissue type at a specific point of development. A total of 424 ESTs were found that corresponded to 331 sequences from the immature seed cDNA library and 83 sequences from the floral library, with an average length of 581 bp. The SNP studies yielded a total of 51 SNP markers in 20 EST sequences analyzed, consisting in 38 single-base changes and 13 insertions–deletions (Indels), with an average of 1 SNP per 462

base pairs (bp) and 1 Indel per 1812 bp. On inclusion of the *C. berlandieri* subsp. *jonesianum* accession, 81 additional SNPs were identified, bringing the total number of SNPs discovered to 132 (1 per 179 bp).

Maughan et al. (2012) reported a large-scale set of SNP markers and developed functional SNP assays for quinoa. 427 of 511 functional SNP markers were utilized to analyze a set of 113 quinoa accessions which showed MAF (minor allele frequency) values between 0.02 and 0.50. In this study, 90 % of the SNP loci were polymorphic and 46 % were reported to be highly polymorphic. The most frequent point mutation among all SNPs identified corresponded to transitions (A/G or C/T), being 1.6X higher than transversions (A/T, C/A, G/C, G/T). The phenetic analysis separated the accessions into two major groups viz. the Andean and lowland/coastal ecotypes. One interesting observation was the potential transferability of SNP markers to related species including four accessions of *C. berlandieri* (subsp. *nut-talliae*, var. *macrocalycium*, var. *boscianum*, and var. *zschackei*), two accessions of *C. hircinum*, and one accession of both *C. watsonii* and *C. ficifolium*. The two *C. hircinum* accessions and the four *C. berlandieri* accessions presented 81 and 79 % of successful amplification, respectively, which confirmed the close crop-weed sympatric relationship of these two tetraploid species with quinoa.

## 8.8 Genetic Linkage Maps

Maughan et al. (2004) reported the first quinoa genetic linkage map using AFLP, RAPD, and SSR markers. Selection of the mapping population was based on a preliminary genetic similarity analysis of four potential mapping parents. Breeding lines 'Ku-2' and '0654,' a Chilean lowland type and a Peruvian Altiplano type, respectively, showed a low similarity coefficient of 0.31 and were selected to form an F<sub>2</sub> mapping population. This map consisted of 35 genetic linkage groups containing a total of 230 AFLPs, 19 SSRs, and 6 RAPD markers, spanning 1020 cM with an average marker density of 4.0 cM per marker. This map provided a major breakthrough in the genetic dissection of agronomically important characteristics of quinoa, including seed saponin content, grain yield, maturity, and resistance to disease, frost, and drought.

Jarvis et al. (2008) reported the development of 216 new polymorphic SSR markers from libraries enriched for GA, CAA, and AAT repeats, as well as six SSR markers developed from bacterial artificial chromosome end sequences (BES-SSRs). Heterozygosity (H) values of the SSR markers ranged from 0.12 to 0.90, with an average of 0.57. A linkage map was constructed from a newly developed recombinant inbred line (RIL) population using these SSR markers. The linkage map also contained additional markers, including amplified fragment length polymorphisms (AFLPs), two 11S seed storage protein loci, and the nucleolar organizing region (NOR). The study culminated in the preparation of the first SSR-based map in quinoa and contained 275 markers, including 200 SSR. The map consisted of 38 linkage groups (LGs) covering 913 cM. Segregation distortion was

observed in the mapping population for several marker loci, indicating possible chromosomal region associated with selection or gametophytic lethality.

Maughan et al. (2012) prepared first SNP-based integrated linkage map combining data from two mapping populations from a large set of SNP loci. The map consisted of 29 linkage groups with 20 large linkage groups, spanning 1404 cM with a marker density of 3.1 cM per SNP marker. This linkage map was constructed employing a  $F_{2:8}$  RIL population from two advanced quinoa mapping populations (Pop1 and Pop39) sharing a common paternal parent (0654, Altiplano type) whose molecular data were combined to construct an integrated linkage map based on 128 individuals. This SNP-based map consisted of approximately twofold number of marker loci as well as spanned a greater genetic coverage than the previous reported maps.

## 8.9 Bacterial Artificial Chromosome Library

Stevens et al. (2006) constructed two separate quinoa BAC libraries using *Bam*HI (26,880 clones) and *Eco*RI (48,000 clones) restriction endonucleases from the “Real” quinoa type. Cloned inserts of *Bam*HI and *Eco*RI libraries averaged 113, 130 kb, respectively. The combined quinoa libraries represented about 9.0 di-haploid nuclear genome equivalents. An average of 12.2 positive clones per probe was identified with 13 quinoa single-copy ESTs as probes of the high-density arrayed blots, suggesting that the estimate of 9.0x coverage of the genome is conservative (Stevens et al. 2006). Furthermore, the probing of the library with partial sequence of the 11S globulin seed storage protein gene identified clones that represent two different 11S loci, which suggests the importance of BAC libraries in identifying and cloning the important genes (Stevens et al. 2006).

## 8.10 Characterization and Expression of Certain Genes

Quinoa is known for its ability to grow under harsh environmental conditions. Maughan et al. (2009) first described the molecular characterization of *Salt Overly Sensitive 1 (SOS1)* gene while studying the molecular basis of salt tolerance in quinoa. The complete genomic sequence of two homoeologous *SOS1* loci, *cqSOS1A* and *cqSOS1B*, were reported, which spanned 98,357 and 132,770 bp, respectively. The translation of *cqSOS1A* and *cqSOS1B* coding sequences yielded proteins of 1158 and 1161 amino acid, respectively. The comparison of these translated genomic sequences revealed a high degree of similarity with *SOS1* sequence from species belonging to the Caryophyllales order. Under saline conditions (450 mmol/L) relative gene expression of *SOS1* in roots was consistently 3–4 fold higher than in leaf tissue. The *SOS1* expression was more strongly up-regulated by salt stress in leaves as compared to the roots suggesting a



constitutive expression of *SOS1* genes in roots and an inducible expression in leaves under stress.

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

Ruiz-Carrasco et al. (2011) using similar approach 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, and between genotypes, by 300 mM NaCl.

## 8.11 Breeding System

Quinoa is gynomonoeious as it has hermaphroditic and female flowers arranged in dichasial cymes. Ten different types of inflorescences were classified in various accessions according to different proportions of hermaphroditic and female flowers and their arrangement on the dichasium (Bhargava et al. 2007c). Although predominantly autogamous, outcrossing occurs in quinoa over considerable distances. Out crossing occurs frequently up to a distance of 1 m and occasionally up to 20 m and its rates at different sowing distances in the Bolivian Altiplano ranging from 0.5 to 9.9 % (Gandarillas 1979). The average rate of outcrossing studied by Lescano (1980) in eight landraces and five varieties of quinoa was 5.8 %. A recent study on *C. quinoa* cv. Sajama gave the outcrossing rates as high as 17.36 % (Silvestri and Gil 2000). This is further corroborated by the absence of any inbreeding depression in characters like weight and height of the above-ground parts of the plant, development of inflorescence and seeds, or on the homogeneity of offsprings in six chenopod species studied (Dostalek 1987). However, some extreme cases of complete self-pollination through cleistogamy (Nelson 1968) and obligate outcrossing by self-incompatibility and male sterility (Nelson 1968; Gandarillas 1969; Simmonds 1971) are also reported. The rate of outcrossing is influenced by wind speed, the proportion of different flower types, and the extent of self-incompatibility prevalent in the plant (Risi and Galwey 1984). As a result of outcrossing, the quinoa landraces have a high level of heterogeneity and heterozygosity and more advanced cultivars are difficult to keep true to type by farmers who propagate their own seeds (Lindhout and Danial 2006).

## 8.12 Male Sterility

Male sterile lines are necessary for hybrid production (Wilson 1980; Risi and Galwey 1984; Fleming and Galwey 1995; Bhargava et al. 2006a). Both cytoplasmic and genic male sterilities have been reported in quinoa, though the latter is quite less frequent. Gandarillas (1969) reported a recessive nuclear gene controlling male sterility in some Bolivian quinoa lines which when crossed with male fertile line showed Mendelian ratio in  $F_1$  and  $F_2$ . However, some other male sterile line from Bolivia showed inconsistent Mendelian ratios which point toward cytoplasmic control (Gandarillas 1969). Rea (1969) also described a male sterile plant having yellowish white or light brown empty anthers but did not study the inheritance of this trait. The genetics of male sterility was studied by Simmonds (1971) in an unnamed quinoa of Bolivian origin and 3 loci: R (red plant) r (green plant); Ax (axil spot) ax (none); and Ms (hermaphrodite) ms (male sterile) were reported. The plants of genotype MsMs and Msms were fertile and showed breeding behavior as expected but that of the recessive msms showed that it carried an erratically expressed or transmitted cytoplasmic factor for male sterility.

A cultivar 'Apelawa' carrying normal and male sterile cytoplasm was isolated by Ward and Johnson (1993). The cross between male sterile and normal male fertile donors consistently produced male sterile offspring, while interspecific crosses between male sterile quinoa plants and *C. berlandieri* produced progenies having partial restoration of male fertility. However, plants of Bolivian cv. 'Amachuma' carrying a single nuclear recessive gene that in homozygous state produced anthers devoid of pollen grains were reported by Ward and Johnson (1994). The heterozygous plants at this locus were indistinguishable from homozygous male fertile ones and further segregation for male sterility followed a normal Mendelian single gene segregation pattern.

Normal hermaphrodite and male sterile quinoa plants were reported in an accession (PI 510536) in the USDA collection of quinoa by Ward (1998), wherein the male sterility was of cytoplasmic nature and was characterized by small shrunken anthers and the absence of pollen. A dominant nuclear allele that interacted with the male sterile cytoplasm to restore male fertility was also present in this accession.

## 8.13 Breeding and Genetic Improvement

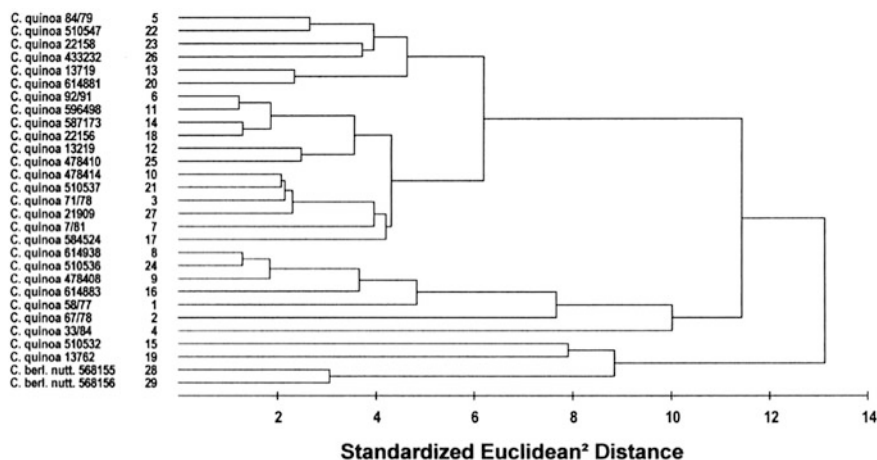
The basic objective of breeding in quinoa is the development of a variety with dwarf, non-branching, and uniformly early maturing plant type to facilitate mechanical harvesting (Jacobsen et al. 1996) and high grain yield with high protein and low saponin content (Bhargava et al. 2006a). Outside the Andean region, breeding research was initiated in Europe in the early 1980s with the objectives of adaptation to local climatic conditions and improvement with respect to uniformity,

early maturity, seed yield, protein content, and reduced saponin content in the seeds (Risi and Galwey 1989; Galwey 1993; Jacobsen and Stølen 1993; Jacobsen et al. 1994; Mastebroek and van Soest 1994; Limburg and Mastebroek 1997; Mastebroek and Limburg 1997). Breeding research in Denmark and Sweden was centered around improvement in the fodder quality (Carlsson 1980; Haaber 1991). Breeding research in the Netherlands started in 1986 when trials led to selection of some uniform lines adapted to the Western European climate (Mastebroek and Limburg 1997; Mastebroek et al. 2002). Quinoa was successfully acclimatized and established in India in the early 1990s and thereafter extensive field trials were carried out at the National Botanical Research Institute, India taking into account its importance both as grain and fodder crop (Bhargava et al. 2006a, 2007d).

However, hybridization in quinoa is cumbersome given self-pollinated nature small flowers making emasculation impossible. In spite of these difficulties, mass selection and hybridization have been practiced in quinoa (Risi and Galwey 1984). A practical approach can be the utilization of morphological markers to distinguish the hybrid from the parents.

## 8.14 Genetic Diversity

Quinoa displays ample genetic diversity for qualitative as well as quantitative traits, which allows obtaining a wide range of adaptability to agroecological conditions (Rodríguez and Isla 2009). Risi and Galwey (1989) assessed genetic diversity in 294 accessions of quinoa using PCA and canonical analysis. Ortiz et al. (1999) created a phenotypic distance matrix among 76 accessions from a Peruvian quinoa core collection. Rojas (2003) analyzed the genetic diversity in *C. quinoa* using three multivariate methods. Multiple group discriminant function analyses resulted in six statistically significant functions, which separated the different groups. The assessments showed that phenological variables such as initiation of the flowering and mid-bloom date were stronger discriminants as compared to the yield variables. Twenty-nine germplasm lines of *C. quinoa* and two of *C. berlandieri* subsp. *nuttalliae* were evaluated for 19 traits for cluster and PCA in the north Indian conditions (Bhargava et al. 2007e). Multivariate analysis showed that most of the variations were accounted for the first four PCs. Days to maturity, primary branches/plant, chlorophyll content, and seed yield/plant were the main traits that accounted for most variability in both PC1 and PC2. The germplasm lines were grouped into six clusters based on average linkage method (Fig. 8.3). The lines in cluster I were early maturing and high yielding but had low carotenoid content. Cluster II comprised lines having low seed quality but were higher in leaf quality components. Cluster III had highest seed yield and high values for protein and carotenoids. The lines in cluster IV matured earliest and had high seed protein, while cluster V had high seed yield, dry weight/plant, stem diameter, and maximum number of inflorescences. Cluster VI had low values for traits related to seed morphology and quality except for carotenoid content. This investigation also

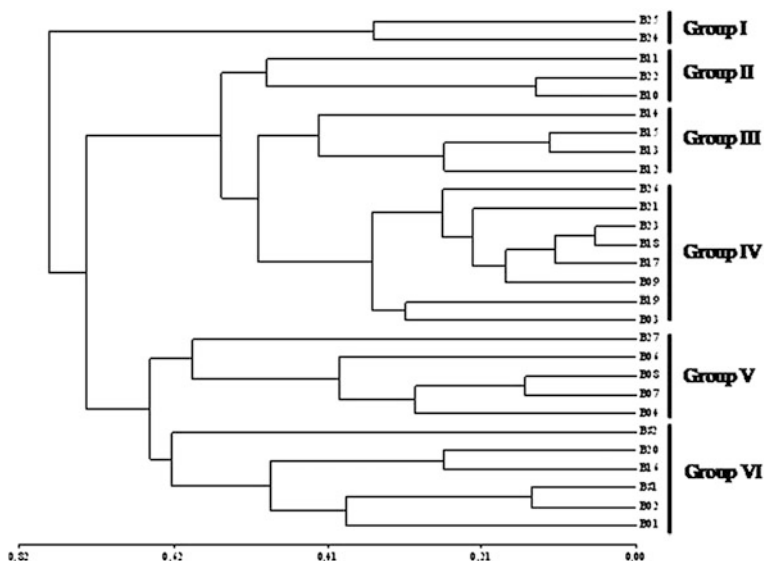


**Fig. 8.3** Dendrogram of 29 germplasm lines derived from average linkage method (Bhargava et al. 2007e)

clustered two lines of *C. berlandieri* subsp. *nuttalliae* separately from the quinoa line that is phylogenetically correct. The morphological diversity among 28 quinoa accessions from the Chilean highlands was assessed under desert lowland conditions using multivariate techniques to analyze measurements of 11 morphological descriptors (Fuentes and Bhargava 2011). The first four principal components accounted for 70 % of the total variation among the accessions. A correlation matrix involving the complement of the Pearson coefficient was used to construct a dendrogram using the UPGMA algorithm (Fig. 8.4). Cluster analysis allowed classification of the accessions into six discrete groups.

## 8.15 Correlation and Path Studies

The study of correlation is regarded as an important step in breeding programs since the information obtained is useful estimating the correlated response to directional selection for the formulation of selection indices. The path coefficient, also known as standardized partial regression coefficient, plays an important role in determining the degree of relationship between yield and yield components since it separates the direct and indirect effects of a correlation coefficient. Espinola and Gandarillas (1985) reported that inflorescence length was the most important component influencing grain yield in *C. quinoa*. Risi and Galwey (1989) reported strong correlation between plant height, stem diameter, inflorescence length, and inflorescence diameter with each other. However, the associations between the durations of the developmental phases were weak suggesting that there is a great scope for manipulation of the pattern of development through breeding. Ortiz et al. (1998) noted high correlation between stem and inflorescence color which



**Fig. 8.4** Cluster analysis for characterization of quinoa germplasm grown under lowland desert conditions (Fuentes and Bhargava 2011). Group I: Accessions having large inflorescences and fluctuating grain yields (GYs); group II: medium plant heights and medium GYs; group III: taller plants and low GYs (forage use); group IV: shorter plants with low GYs; group V: medium plant heights and medium-to-low GYs, and group VI: medium plant heights and medium-to-high GYs

confirmed the presence of partial common genetic control for pigmentation in the crop. Bhargava et al. (2003) calculated the correlation coefficients among various traits and their direct and indirect effects on grain yield in quinoa grown on normal and sodic soils. Stem diameter and dry weight/plant were positively correlated with grain yield on both soil types. It was concluded that selection of thick-stemmed plants with more number of inflorescences and high dry weight would be beneficial in breeding for high grain yield in quinoa on sodic soils. Spehar and Santos (2005) reported positive association of inflorescence length and diameter with grain yield, which indicated that the selection for these characters may result in more productive genotypes. Positive correlation was also observed between plant height and inflorescence length which suggested that high grain yield can be attained by selecting for stem/inflorescence ratio. In a more detailed study, the interrelationships among yield and yield components were elucidated in 27 germplasm lines of *C. quinoa* and two lines of *C. berlandieri* subsp. *nuttalliae* by Bhargava et al. (2007e). Significant correlation among branches/plant, inflorescence length, and inflorescence/plant pointed out that plants with good branching habit tend to develop a large number of long inflorescences. Inflorescence length was also positively associated with plant height indicating that lines with greater plant height also developed longer panicles, a fact also reported by Rojas (2003) and Ochoa and Peralta (1988). The nonsignificant correlation between seed yield and seed quality traits and low values of direct path would be beneficial for breeders since it would

not hinder attempts to breed lines with both greater grain yield and high seed quality. The path analysis revealed that 1000 seed weight had highest positive direct relationship with seed yield, followed by total chlorophyll and branches/plant. It was concluded that seed yield and seed protein were the only traits exhibiting high positive direct path and significant positive association with harvest index, indicating a true relationship among these traits (Bhargava et al. 2007e). Fuentes and Bhargava (2011) noticed high correlation between stem diameter and plant weight, stem diameter and plant height, plant weight and plant height, plant weight and inflorescence length, plant height and inflorescence length, and leaf length and leaf width. Harvest index showed negative association with stem diameter, plant height, inflorescence length, inflorescence width, and inflorescence branch number.

## 8.16 Genotype x Environment Interaction (GEI)

The interaction of cultivar with environmental factors is an important consideration for plant breeders. Plant breeders continuously strive to broaden the genetic base of a crop to prevent its vulnerability to the changing environments. A material when planted over different environments exhibits differential responses due to environmental variation (Bhargava et al. 2007d). Consistent performance across different sites and/or years is referred to as stability. A number of statistical models have been put forward for evaluating the yield stability of a genotype in yield trials (Finlay and Wilkinson 1963; Eberhart and Russel 1966; Tai 1971; Shukla 1972; Shafii and Price 1998). The knowledge of GEI and yield stability is important for breeding new cultivars with improved adaptation to the environmental constraints prevailing in the target environment (Bhargava et al. 2007c). Few studies are available on GEI and stability analysis in quinoa (Risi and Galwey 1991; Jacobsen et al. 1996; Jacobsen 1998; Bertero et al. 2004).

A study of the GEI by Risi and Galwey (1991) demonstrated that the GEI differed among the variables measured. Grain yield was strongly dependent on the variety, but micronutrient deficiency and weed competition affected the varieties differently.

The stability of various descriptive characters was studied over a 5-year period in 14 lines of quinoa to determine the most appropriate time in a breeding program when selection could be performed (Jacobsen et al. 1996). GEIs were significant for all characters, but for several traits of interest to the plant breeder, namely earliness, height, and inflorescence size, some of the best lines track the optimum response. The results pointed out that selection for height, inflorescence size, and developmental stage could be satisfactorily performed at an early stage of the breeding program. Potential parents were identified for use in the development of varieties suitable for North European conditions. Jacobsen et al. (1996) studied the stability of quantitative traits in 14 lines of *C. quinoa* and suggested that selection for height, inflorescence size, and developmental stage could be easily performed at an early stage of breeding program. Studies on developmental patterns of quinoa for North

European conditions were carried out in five groups of quinoa lines from different maturity classes in 3 years, and measured on five occasions between bud formation and seeds set (Jacobsen 1997). The seed types originating from Chile were found to be more adapted for growth under North European conditions.

Bertero et al. (2004) examined the size and nature of the GEI effects for grain yield, its physiological determinants, and grain size among 24 cultivars tested in 14 sites in a multi-environment trial across three continents. The G x E interaction to G component of variance ratio was 4:1 and 1:1 for grain yield and grain size, respectively. The clustering separated the cultivars from mid-altitude valleys of the northern Andes, northern Altiplano, southern Altiplano, and sea level. No single genotype group showed consistently superior grain yield across all environment groups. The genotype (G) and GEI effects observed for the duration of the crop cycle had a major influence on the average cultivar performance and on the form of GEIs observed for total above-ground biomass and grain yield. It was observed that good average performance and broad adaptation could come from the combination of medium-late maturity and high harvest index. Correlation analysis revealed no association between the average cultivar responses for grain yield and grain size (Bertero et al. 2004). Both observations indicated that simultaneous selection for grain yield and grain size can be expected from selection.

## 8.17 Conclusion

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. Many wild characters that were disadvantageous to the farmers such as seed shattering, seed dormancy, and thick seed coats were lost, while useful characters such as larger and more starchy seeds, fewer and larger inflorescences, uniform maturity, and environmental adaptations were preserved during domestication. However, wide diversity for plant color, seed color, types of branching and panicles, as well as grain productivity, abiotic stress tolerance, and disease resistance still exists despite narrowing of genetic base during domestication. This diversity is also reflected at the molecular level and is being used by the plant breeders worldwide to develop improved plant types with respect to uniformity, early maturity, seed yield, protein content, and reduced saponin content in the seeds.

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