Characterisation of quinoa (*Chenopodium quinoa* Willd.) accessions for the saponin content in Mediterranean environment

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Abstract

Seeds of the Andean seed crop quinoa (*Chenopodium quinoa* Willd.) usually contain saponins in the seed coat. Saponins give a bitter taste sensation and are a serious antinutritional factor, therefore selection of sweet genotypes with a very low saponin content in the seeds is a main breeding goal. The objective of this work was to identify, within germplasm lines of quinoa, previously selected for production and quality traits, superior genotypes low in saponins. For this purpose the total saponin content was determined in seeds of eight lines of quinoa and one variety (cv. Regalona Baer) as a control, previously evaluated over a 2-year period in a Southern Italy environment. Significant variation for the saponin content was observed among the evaluated genotypes. The total saponin content ranged from 0.10 to 1.80%, with the Q12 genotype showing the lowest value, suggesting the possibility of selecting genotypes *sweet* to be used in subsequent genetic improvement programs. Based on these results, in fact, it was possible to identify, among the accessions previously selected, particularly suitable for growing in Mediterranean area, some genotypes with high yields of seed (2.5 tha^{-1}, on average), high protein (17%, on average) and fibres (13%, on average) and low content in saponins (0.57%, on average).

Introduction

Chenopodium quinoa Willd. (Amaranthaceae), a staple food of the Andean communities, commonly known as quinoa, is an emerging grain crop that is grown for its edible seeds.

Quinoa is of considerable scientific and commercial interest in many regions of the world because of the nutritional composition of their seeds with a high-quality proteins content (14-20%), fats and antioxidants, which are at least 5-fold higher than those of cereal flours (Koziol, 1992; Jacobsen *et al*., 2003; Bhargava *et al*., 2006).

Quinoa is also a good source of dietary fibre and contains relatively high quantities of vitamins and minerals, iron and calcium (Risi and Galwey, 1984). Furthermore, because of the absence of gluten proteins, quinoa can be used to produce gluten-free cereal-based products, and can thus be eaten by people who have celiac disease, as well as by those who are allergic to wheat (Gambus *et al*., 2002; Chillo *et al*., 2009). The genetic variability of quinoa is huge, with cultivars of quinoa being adapted to growth from sea level to an altitude of over 4000 meters and from cold, highland climates to subtropical conditions. This make it possible to select, adapt, and breed cultivars for a wide range of environmental conditions such as arid or humid areas, cold or hot environments, acidic or alkaline soils (Jacobsen, 2003). Therefore, several countries in all over the world started in the last years to promote researches for the development of quinoa cultivation. The main obstacle for introducing quinoa into Europe is the length of its growing season and its sensitivity to the photoperiod (Bertero, 2001; Casini, 2002). In these terms, the best results have been obtained with material that originated from Chile (Jacobsen, 1997), which is adapted to a photoperiod that is similar to that of temperate areas.

Quinoa is a complete and versatile food, that can be prepared in several ways: as whole grain or flour, for sweet or savory foods that are hot or cold, or even as a drink, all of which offer wide potential for future technological applications (pasta, bread, beer, breakfast cereals, biscuits, protein concentrates) (Tosi *et al*., 1996; Gambus *et al*., 2002). However, one factor that limits its widespread use is the bitter taste, caused by the presence of saponins, anti-nutritional compounds contained in the pericarp of seed of some quinoa varieties (Gómez-Caravaca *et al*., 2011).

Saponins are a large group of glycosides soluble in water, where they form solutions foaming. Present in all fractions of the plant, saponins have insecticidal, antibiotic, fungicidal, and pharmacological properties (Carlson *et al*., 2012; Vega-Gálvez *et al*., 2010), thus contributing to the plant's defence against pests and pathogens. However, most saponins have an intensely bitter flavour that reduces the organoleptic characteristics and the palatability of any products

with a their rich content. Moreover, all saponins are potentially toxic if ingested in large quantities, because of their haemolytic activity, and there is therefore a longstanding controversy about their functions in food.

Saponins are formed by sugar chains and a triterpenoid aglycone (sapogenin), each representing about 50% of the total weight of the molecule (Galwey *et al.*, 1990). Three main structures of sapogenins, oleanolic acid, hederagenin and phytolaccagenic acid, have been identified (Ridout *et al.*, 1991). In quinoa, over 20 different saponins have been described (Mizui *et al.*, 1988; Ridout *et al.*, 1991). These saponins derive from seven aglycones identified so far in the different parts of quinoa (flowers, fruits, seed coats and seeds) that in combination with the various sugar chains (arabinose, glucose and galactose) form the over 20 saponins until now discovered (Kuljanabhagavad *et al.*, 2008). Gas chromatography is the widely used method to quantify quinoa saponins (Ridout *et al.*, 1991).

Saponins are traditionally removed from quinoa by washing (moist method) the grain before cooking it, although mechanical dehulling by abrasion (dry method) is also effective (Mujica and Jacobsen, 1999). However, the moist method increases costs (due to drying the product) and grains may begin to germinate during the process, because quinoa has a very high germinative power. The dry method is cheaper than washing, but has the disadvantage that it does not eliminate all the saponin and if the efficiency is increased, some nutrients are lost (Wright *et al.*, 2002).

The amount of saponins present in the quinoa seeds depends on genoptype: it is higher in bitter-flavour varieties than in sweet, or low-saponins, varieties. For saponin content, considerable genetic variation is available and several sweet genotypes with low saponin content have been selected (Vela and Cabrera,1984). However, although some Ecuadorian and Bolivian quinoa cultivars have very low levels of grain saponin and do not require post-harvest processing, they are photoperiod sensitive, while those from Chilean origin, almost all from high-saponin lines, are early maturing and thus more suitable for European conditions (Johnson and Ward, 1993; Jacobsen *et al.*, 1996).

The selection criteria of genotype depend by the environment, the grain destination and use, the market demand (Mc Elhinny *et al.*, 2007). Nevertheless, high levels of saponin are considered a major impediment to the diffusion of the crop (Bhargava *et al.*, 2006) because they can affect the absorption and digestibility of nutrients (Maughan *et al.*, 2004). Consequently, the development of varieties with low or no saponin is one of the important breeding objectives for quinoa (Spehar and Rocha, 2010).

Saponin content depends on the developmental stage of the crop, being low during branching and high during flowering (Bhargava *et al.*, 2006). Drought reduces by 45% the accumulation of sapogenins in quinoa seeds, based on one study of severe water deficit conducted in Southern Europe (Gomez-Caravaca *et al.*, 2012), whereas salinity has the opposite effect (Soliz-Guerrero *et al.*, 2002; Pulvento *et al.*, 2012). More recently, however, a significant increase of saponins and other seed components has been reported in an arid location (irrigated) as opposed to a cold temperate climate (rainfed) site (Miranda *et al.*, 2012; Miranda *et al.*, 2013). Thus, the above data suggest that additional studies must be conducted to elucidate how the environmental and the genotypic effects influence the seed saponin levels (Zurita *et al.*, 2014).

The aim of this preliminary study was to identify, among superior genotypes for seed production and quality traits, those with reduced grain saponin content to be used in subsequent genetic improvement programs for developing highly yielding varieties with improved nutritional qualities.

Materials and methods

Plant materials

In this paper we analyse the quantitative/qualitative assessment of saponins, determined in 8 quinoa accessions from different origin, previously selected for productivity and quality traits and one commercial variety (Regalona Baer), of Chilean origin, used as a control.

Selection, development and planting of materials used have been previously described (De Santis *et al.*, 2011; 2014, 2016). Briefly, 25 germplasm lines of *Chenopodium quinoa*, 4 lines of *C. giganteum,* 1 line of *C. berlandieri* subsp. *nuttalliae*, from different geographical origins, were evaluated for seed yield and a range of morphological and quality traits, during the years 2009 and 2010, at the Cereal Research Centre in Foggia (southern Italy: 41° 28' N; 15 ° 34' E; 76 m a.s.l.). The soil was characterised by a clay-loam texture, with the following characteristics: 21% clay, 43% silt, 36% sand, pH 8 (in H_2O), 1.2 g kg^{-1} total N (Kjeldahl method), 15 mg kg^{-1} available P (Olsen method), 800 mg kg^{-1} exchangeable K (NH$_4$Ac), and 21 g kg^{-1} organic matter (Walkey-Black method). The climatic data recorded during both of these experimental years are shown in Figure 1.

Field trials were established in April in both years. The seeds were manually sown in polystyrene seed trays and seedlings were then transplanted to field plots, which consisted of 3 rows 1.0 m long and 50 cm apart, with 10 plants per row. The experimental design was a ran-
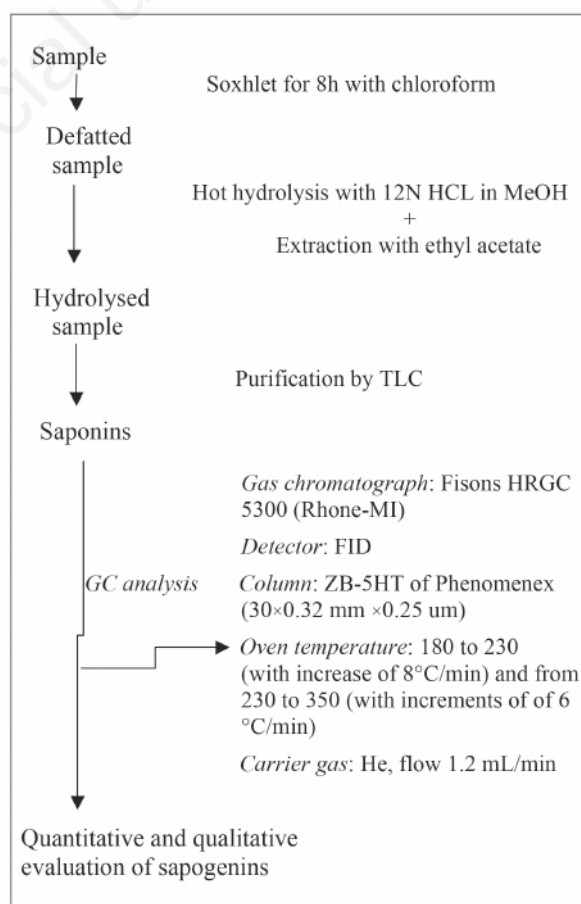

Figure 1. Analytical scheme for the extraction of saponins. TLC, thin layer chromatography; GC, gas chromatography.

domised complete block with three replications. Chemical fertilisers were applied both before and during the experimental period: in autumn, with 200 kg ha^{-1} of ammonium diphosphate (36 kg ha^{-1} of N, 92 kg ha^{-1} of P$_2$O$_5$), during vegetative growth (in May), with 200 kg ha^{-1} of ammonium nitrate (38 kg ha^{-1} of N). Irrigation was provided with three applications of 200 m^3 ha^{-1}, each) and weed control was managed by hand removal when required. The quinoa lines and cv. Regalona Baer were harvested at maturity (20th August 2009 and 15th July 2010).

Parameters estimated

Quinoa saponins were evaluated in terms of sapogenins (Gomez-Caravaca *et al.*, 2012) (Figure 1). A gas chromatographic procedure was applied for the evaluation of saponin aglycones (sapogenins) derived from the acid hydrolysis of samples (Ridout *et al.*, 1991).

Three major quinoa saponin aglycones were identified: oleanolic acid, hederagenin and phytolaccagenic acid, therefore values for the only these aglycones were reported.

Data analysis

For all the traits, statistical parameters of means, range, standard errors, variance and coefficients of variation were calculated over the two growing seasons for the whole collection by means of Graf Pad Instat 3 (Microsoft Software) statistics program.

Analyses of variance were computed separately for each year for all of the traits, and then combined over the years. The expected mean squares were based on a mixed linear model, with the genotypes having fixed effects and the years being random.

Results and discussion

The climatic characteristics of the trial site are typical of the Mediterranean climate, which is characterised by long dry summers and mild winters, with occasional frosts. Over the two years of the trial, the total rainfall was variable, particularly for its distribution during the growing season (Figure 2). The first year of the trial was wetter than the second year, with 341 mm of rain over the March to August period. Of this, 98.5 mm were concentrated in April and 65.2 mm in June, at the flowering stage. In contrast, the second year was relatively drier, with 201 mm of rain for the March-August period, with several not effective rainfall events during the growing season (Figure 2).

The differences between climatic conditions over these two seasons affected the saponin content of the evaluated quinoa accessions, resulting in a significant difference between the two seasons of research (1.10 and 0.92%, for 2009 and 2010, respectively).

The rainfall recorded in the first year, rainier than the second one, was concentrated in the last 10-day period of April (at the beginning of the growing season) and so it provided high level of air humidity and soil moisture. Moreover, the rainfall at June (65.2 mm) was concentrated in the second and third 10-day periods, when the crop was at early flowering stage, clearly affecting both flowering and seed-filling stages. The conditions wetter in the 2009 than in 2010, were favourable also for main pests (fungi, bacteria, insects); for this reason the metabolism of the crop was oriented in a higher synthesis of saponins in the first than in the second year. No significant interaction *year* × *genotype* was observed.

These results are in agreement with the study of Soliz-Guerrero *et al.* (2002), who reported that saponin content is affected by a soil-water deficit, to the extent that high water deficits promote low saponin contents and with Pulvento *et al.* (2012) who obtained, for a quinoa genotype (*Titicaca*), the highest saponin values in samples with full irrigation treatments.

The contrasting results of Miranda *et al*. (2012, 2013) who reported in Chile an increase of saponin content in an arid, but irrigated environment and a decrease, in rainy environment not for all the genotypes, suggest, in agreement with Zurita *et al.* (2014), that additional studies must be conducted to elucidate how the environmental and the genotypic effects influence the seed saponin levels.

The total saponin content from all the aglycones (Aglycone I-VII) in whole seeds of quinoa accessions and R. Baer variety ranged from 0.05 to 2.00%, indicating a large variability for this character (Table 1). The gas chromatographic analysis showed that all accessions, except the Q19 (1.80%) had values below the control Regalona Baer, considered a medium-bitter variety. Five genotypes were potentially *soft* values below 1%. The Q18, Q21, Q22 and Q29 accessions resulted equal, with an average of 0.57%, unlike the Q26, resulted in absolute, the accession with the lowest saponin content (0.01%).

From a qualitative point of view, confirmed also by the literature (Ridout *et al.*, 1991), three major quinoa saponin aglycones were identified: oleanolic acid (Aglycone I) (36-50% total), hederagenin (Aglycone II) (27-28%) and phytolaccagenic acid (Aglycone III) (21-36%).

In Table 2 were reported the results for seed production and some quality traits in the eight selected accessions. They, almost all of Chilean origin, were found to be the most productive (with values of more than 2 t ha^{-1} seed) and with high levels of proteins (range 16.0-17.8%) and fibres (9.4 and 14.6%) (De Santis *et al*., 2014) among the 30 accessions previously evaluated.

Among the 5 accessions with low saponins content emerged in this

Figure 2. Monthly climatic data during the two trial years (2009 and 2010), compared to long-term average (1953-2008).

Table 1. Means and significance for main sapogenins (Aglycone I-II-III) and total saponin content found in eight accessions and one variety (Regalona Baer) of quinoa (*Chenopodium quinoa* Willd.) in the two trial years (2009 and 2010).

Accession code	Origin	Aglycone I	Aglycone II	Aglycone III (mg kg^{-1})	Total saponins (I-VII) (%)
Q26	Chile	278[i]	126[f]	182[g]	0.12[f]
Q29	Chile	828[g]	837[d]	761[f]	0.54[e]
Q21	Bio-Bio (Chile)	758[h]	759[e]	827[e]	0.56[e]
Q18	Maule (Chile)	848f	758[e]	821[e]	0.56[e]
Q22	Chile	945[e]	841[d]	824[e]	0.69[d]
Q27	Chile	1831[d]	2597[a]	2213[d]	1.51[c]
Q12	Colorado (USA)	2566[c]	2546[b]	2490[c]	1.63[b]
Q19	Bio-Bio (Chile)	2709[b]	2423[c]	2632[b]	1.76[a]
R. Baer	Chile	2903[a]	2584[a]	2761[a]	1.77[a]
2009	-	1641[a]	1627[a]	1479[b]	1.10[a]
2010	-	1395[b]	1366[b]	1524[a]	0.92[b]

[a-i]Different letters indicate significant differences among genotypes and between years (Student-Newman-Keuls test, P≤0.05).

Table 2. Means and standard error for seed yield and seed quality traits in eight accessions and one variety (Regalona Baer) of quinoa (*Chenopodium quinoa* Willd.) combined over the two years.

Accession code	Origin	1000 Seed weight (g)	Harvest index	Seed yield (t ha^{-1})	Seed protein (%)	Seed total dietary fibre (%)	Seed total saponins (%)
Q19	Bio-Bio (Chile)	2.23	0.17	2.18	17.04	14.31	1.76
Q21	Bio-Bio (Chile)	2.58	0.24	2.30	16.39	9.35	0.56
Q12	Colorado (USA)	2.48	0.31	3.05	16.81	9.60	1.63
Q29	Chile	2.29	0.27	2.75	16.21	14.06	0.54
Q18	Maule (Chile)	2.60	0.25	3.01	16.65	11.85	0.56
Q26	Chile	2.30	0.17	2.15	15.98	9.51	0.12
Q22	Chile	1.94	0.17	2.01	17.77	14.14	0.69
Q27	Chile	2.46	0.18	2.87	15.96	14.65	1.51
R. Baer	Chile	2.20	0.26	2.65	16.03	11.54	1.77
	Mean±SE	2.34±0.07	0.22±0.01	2.55±1.24	16.54±0.18	11.90±0.35	1.01±0.08

SE, standard error.

research, those that also presented a high seed productivity and a combined good value for qualitative characteristics, are as follows: Q18 and Q29, with a productivity of 3.01 and 2.75 t ha^{-1}, respectively, and with a medium-high content of protein and fibres (16.5 and 13.0%, on average, respectively).

Conclusions

In the present study, the data for this two-seasons trial confirm or support the low potential saponin content for some of the selected quinoa accessions, however, this was strongly determined by the specific climatic conditions: higher saponins content in the rainy year and lower in the drier one. The large differences between climatic conditions over the two seasons of the trial allowed the assessment of plant behaviour under drought stress (2010), as a comparison to optimal conditions (2009), and hence to identify lines with good adaptability in the environment trials. Data revealed a wide genetic variability for the saponins content, suggesting the possibility of selecting genotypes *sweet* to be used in subsequent genetic improvement programs. Based on the results, in fact, it was possible to identify, among accessions pre-

viously selected, particularly suitable for growing in Mediterranean area, some genotypes with high yields of seed (2.5 t ha^{-1}, on average), high protein (17%, on average) and fibres (13%, on average) and with a very low content in saponins (0.57%, on average).

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