



# Saponins of Quinoa: Structure, Function and Opportunities

# 8

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## Abstract

In quinoa, saponins are found predominantly on the outside of the seeds. Saponins are triterpenoid glucosides that have diverse structures. Over 90 different saponins have been identified in quinoa seed hulls. They are bitter in taste and produce foam, which make them undesirable for human consumption. Their function in quinoa seeds is poorly understood. In this chapter, we provide an overview of these diverse compounds, their structure, and biosynthesis. We provide an overview of techniques for detection and quantification of saponins. We discuss their potential biological roles, from antifungal and anti-herbivory activity to their impact on germination and stress tolerance. Finally, we explore traditional and commercial methods for saponin removal from grain prior to consumption, breeding programs to reduce or alter saponin content, and the use of

saponin waste as a novel bioproduct. While there is still much more to investigate regarding quinoa saponins, this work summarises the current knowledge and provides a basis for future studies.

## 8.1 *Chenopodium Quinoa* Contains Saponins

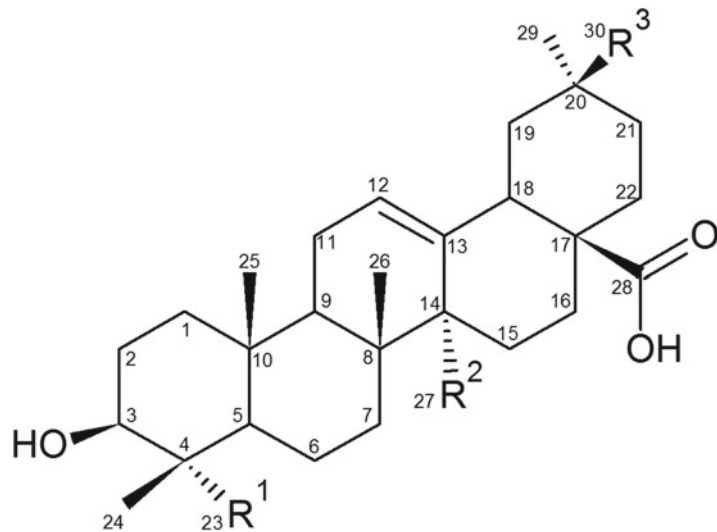
Saponins are a broad class of bitter-tasting and foaming compounds that are found in many species including *Chenopodium quinoa* Willd. (quinoa) seeds. They are amphipathic glycosides occurring in two major classes of the plant kingdom, Magnoliopsida (dicotyledon) and Liliopsida (monocotyledon) (Vincken et al. 2007), as well as a number in marine animals such as sea cucumbers (Holothuroidea) (van Dyck et al. 2010) and starfish (Asteroidea) (Liu et al. 2008). Most known saponins are plant-derived secondary metabolites, displaying a wide range of activities, like defense against fungi, microbes, insects and molluscs (Abdel-Gawad et al. 1999; Sindambiwe et al. 1998; Sparg et al. 2004; Woldemichael and Wink 2001). Recent studies on the biological activity of saponins suggest saponins have beneficial properties to health, such as anticarcinogenic and anti-inflammatory properties (Ismail et al. 2018; Man et al. 2010). The name saponin originates from the Latin word “sapo”, which reflects their ability to produce soap-like foams in aqueous solutions. This is due to their structure of one or more

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**Fig. 8.1** Triterpene aglycone backbone



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
Oleanolic acid (OA) (I)	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>
Hederagenin (HED) (II)	CH <sub>2</sub> OH	CH <sub>3</sub>	CH <sub>3</sub>
Phytolaccagenic acid (PA) (III)	CH <sub>2</sub> OH	CH <sub>3</sub>	COOCH <sub>3</sub>
Serjanic acid (SA) (IV)	CH <sub>3</sub>	CH <sub>3</sub>	COOCH <sub>3</sub>
3β-Hydroxy-23-oxo-olean-12-en-28-oic acid (V)	CHO	CH <sub>3</sub>	CH <sub>3</sub>
3β-Hydroxy-27-oxo-olean-12-en-28-oic acid (VI)	CH <sub>3</sub>	CHO	CH <sub>3</sub>
3β, 23α, 30β-Trihydroxy-olean-12-en-28-oic acid (VII)	CH <sub>2</sub> OH	CH <sub>3</sub>	CH <sub>2</sub> OH

hydrophilic glycoside moieties combined with a lipophilic aglycone. Saponins are divided into two major classes depending on the aglycone backbone: triterpenoid or steroid glycosides (Abe et al. 1993).

Saponins in quinoa are mainly triterpene glycosides (Kuljanabhagavad et al. 2008; Madl et al. 2006; Woldemichael and Wink 2001), consisting of a pentacyclic C<sub>30</sub> skeleton (sapogenin, Fig. 8.1). They exist as a mixture of derivatives of oleanolic acid (OA), hederagenin (HED), phytolaccagenic acid (PA), serjanic acid (SA) as the main aglycones, and others like 3β-hydroxy-23-oxo-olean-12-en-28-oic acid, 3β-hydroxy-27-oxo-olean-12-en-28-oic acid, and 3β, 23α, 30β-trihydroxy-olean-12-en-28-oic acid, decorated with hydroxyl- and carboxyl groups at C<sub>3</sub> and C<sub>28</sub>. The major sugars are arabinose, glucose, and galactose; less common are glucuronic acid and xylose (Dini et al. 2001a, b, 2002; Kuljanabhagavad et al. 2008; Madl et al.

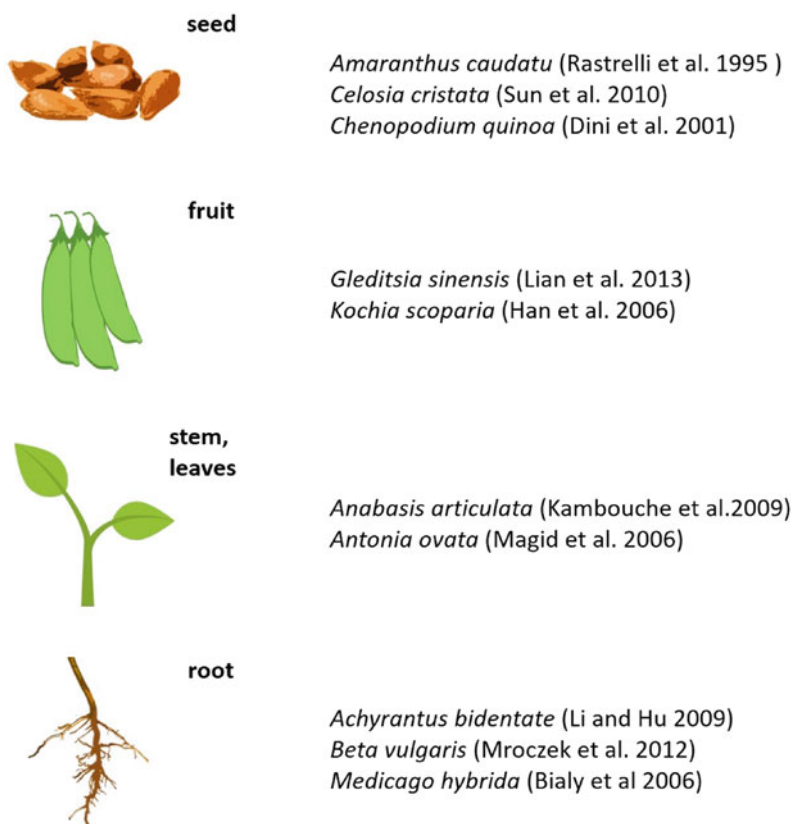
2006; Mastebroek et al. 2000; Mizui et al. 1988, 1990; Woldemichael and Wink 2001; Zhu et al. 2002). Further, the number of sugar chains on the aglycone characterizes the saponins as mono-, bi- or tri-desmosidic (Meyer et al. 1990). The triterpene saponins in quinoa contain mainly three carbohydrate units; one at C<sub>28</sub> and two units at C<sub>3</sub>, where they can be linear or branched (Fig. 8.1.) (Kuljanabhagavad and Wink 2009).

## 8.2 Saponins Accumulate Predominantly in the Seeds of Quinoa

Saponins have been found in different organs and tissues of plants (Fig. 8.2). In many plants, roots are the major storage and synthesis organ, e.g. *Beta vulgaris* (beet), *Glycyrrhiza glabra* (licorice), and *Panax ginseng* (ginseng) (Cheok et al. 2014).

**Fig. 8.2** Plant tissues

containing triterpene saponins in other plant species. The pictures in the graphic are all licensed under CC BY-SA 4.0 or CC0 and modified by Sophie Otterbach

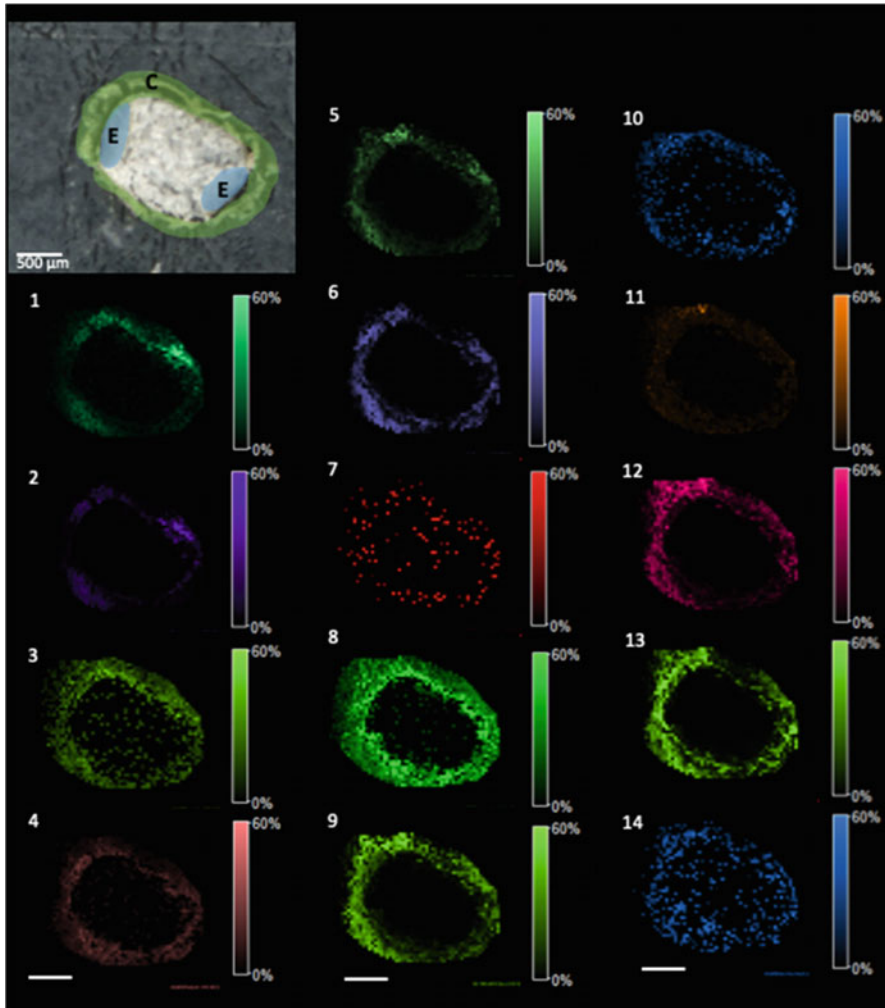


In the medicinal plant *Achyranthus bidentate* it has been demonstrated that the root is the main storage tissue of triterpene saponins, but the leaves seem to be the biosynthetic active site and the vascular bundle the transport organ (Li and Hu 2009). Additionally, saponins can also be stored at multiple storage sites, as in the case of *Medicago truncatula*, where the highest amounts of triterpene saponins were detected in the roots, followed by leaves and seeds (Huhman et al. 2005).

In quinoa, the triterpene saponins accumulate predominantly in the seeds. Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) analyses suggest that saponins are localized in the outer layers of the seeds, more specifically in the external layer, the pericarp and the two seed coat layers (Figs. 8.3 and 8.4). The pericarp appears as a friable layer consisting mostly of saponins (Fig. 8.4) (Jarvis et al. 2017; Koyro and Eisa 2008; Prego et al. 1998;

Varriano-Marston and Defrancisco 1984). Moreover, saponins have been identified in different tissues of quinoa, including flowers and fruits, and to a lesser extent in leaves (Kuljan-abhagavad et al. 2008; Mastebroek et al. 2000). In leaves the saponin content is produced from the onset of flowering and increases until the end of the seed ripening, appearing in the later stages of the plant development (Mastebroek et al. 2000). Measurements in shoots of quinoa showed a similar peak in saponin concentration coinciding with the beginning of the blooming, while the content was lowest in the branching stage and during grain filling (Soliz et al. 2002). Saponins make up to 4% (w/w) of the seed mass (Jarvis et al. 2017).

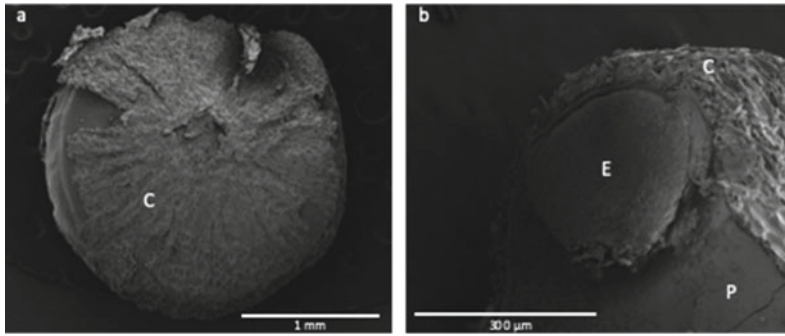
In quinoa, accessions with naturally low amounts of saponins can be found (Mastebroek et al. 2000). These accessions are classified as 'sweet' if the saponin levels are under 0.11% of the dry weight and as 'bitter' if above (Koziol



ID	Saponin	Formula	Calculated	Observed	Error (ppm)
1	PA(unknown) +Na	[C36H56O10+Na]+	671.37657	671.37080	-8.6
2	PA(unknown) +K	[C36H56O10+K]+	687.35051	687.34390	-9.6
3	AG487(Pent) +Na	[C41H64O14+Na]+	803.41883	803.41090	-9.9
4	AG487(Pent) +K	[C41H64O14+K]+	819.39277	819.38620	-8.0
5	PA(Pent) +Na	[C42H66O15+Na]+	833.42939	833.42941	0.0
6	PA +K	[C42H66O15+K]+	849.40333	849.40366	0.4
7	OA(Pent-Hex)b +Na	[C47H76O17+Na]+	935.49747	935.49550	-2.1
8	OA(Pent-Hex)b +K	[C47H76O17+K]+	951.47141	951.47800	6.9
9	PA(Hex-Pent)x2 +Na	[C48H76O20+Na]+	995.48222	995.48150	-0.7
10	AG515(HexA-Hex-Pent) +Na	[C47H68O22+Na]+	1007.40945	1007.40000	-9.4
11	Hed(Hex-Hex-Pent) +K	[C53H86O23+K]+	1129.51915	1129.51100	-7.2
12	PA((Hex-Pent-Hex))/(Hex-Hex-Pent)b +Na	[C54H86O25+Na]+	1157.53504	1157.53340	-1.4
13	PA((Hex-Pent-Hex))/(Hex-Hex-Pent)b +K	[C54H86O25+K]+	1173.50898	1173.50820	-0.7
14	AG489(Hex-Hex-HexA-) +K	[C54H86O26+K]+	1173.52995	1173.52580	-3.5

**Fig. 8.3 Saponins are predominantly located in the pericarp in quinoa.**

Imaging mass spectrometry analyses (analyses performed exactly as described in Jarvis et al. (2017)) detected 14 different saponins, which are predominantly located in the seed coat (C) and not in the embryo (E). Scale bar indicates 500  $\mu\text{m}$



**Fig. 8.4 Scanning Electron Microscopy picture of quinoa seed.**

Scanning Electron Microscopy (SEM) picture of (a) a bitter seed containing saponins and (b) a cross-section. Perisperm (P), embryo (E) and seed coat (C) are indicated

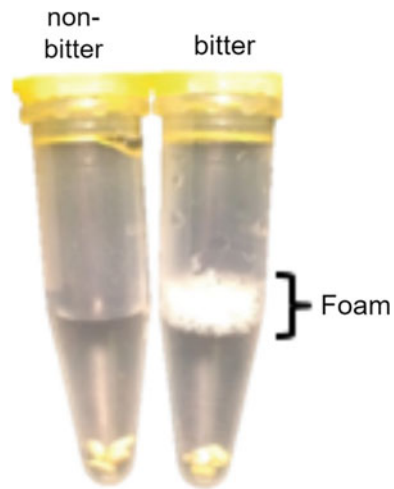
1992). It appears that bitter and sweet accessions show a similar saponin content in the leaves, with hederagenin as the major saponin. Oleanolic acid is the main saponogenin in seeds (Mastebroek et al. 2000).

Congruent with the notion that saponins predominantly accumulate in the seed coat, it was found that the seed coat of bitter seeds is significantly thicker than that of sweet seeds. However, the origin of the saponins in quinoa seeds remains unclear and has not been addressed in the literature thus far.

### 8.3 Methods to Detect and Quantify Saponins

Saponins are a large group of compounds with a great natural diversity of their structures. The aglycone varieties are multiplied by their composition of sugar chains, a number of sugar moieties, and the branching patterns. In plant extracts, saponins occur in a mixture of structurally related forms with similar polarities, which complicates the separation.

Detection methods for saponins can be classified into quantitative and qualitative methods. The first semi-quantitative determination methods of saponins in plant materials were predominantly based on gravimetry or on the exploitation of some of their chemical and biological features (Van Atta et al. 1961). The well-known foaming property of saponins in water and the building of stable froth makes it easy to



**Fig. 8.5 Afrosimetric method (foaming test) to estimate saponin content in quinoa.**

Here, five seeds are placed in 1.5 mL microcentrifuge tube with 0.5 mL of water and shaken vigorously until a stable foam can be measured

measure saponin content (Fig. 8.5). A standardized method to determine saponin content in the seeds is the afrosimetric method (Koziol 1991), which is fast but with some considerable disadvantages. The method underestimates high levels of grain saponin and may be affected by other surfactants in the plant material. Additionally, saponins with one or two-branched sugar chains do not form stable froth and, conversely, some plants may not contain saponins and yet produce a froth (Oleszek 2002).

A biological feature of the saponins is its ability to cause membrane perturbation, also referred to as hemolytic activity. This property

can be used in a semi-quantitative erythrocyte-assay, where saponins cause lysis of the cells. It is thought that saponins can form complexes with membrane sterols of erythrocytes, causing an increase in permeability and a subsequent loss of hemoglobin (Baumann et al. 2000). Saponins differ in their hemolytic activity, depending on the structure and on the hemolytic assay used. Furthermore, quantitation of saponins can also be performed with other biological methods including the growth of sensitive fungus colonies on saponin containing plants (Shany et al. 1970; Zimmer et al. 2010).

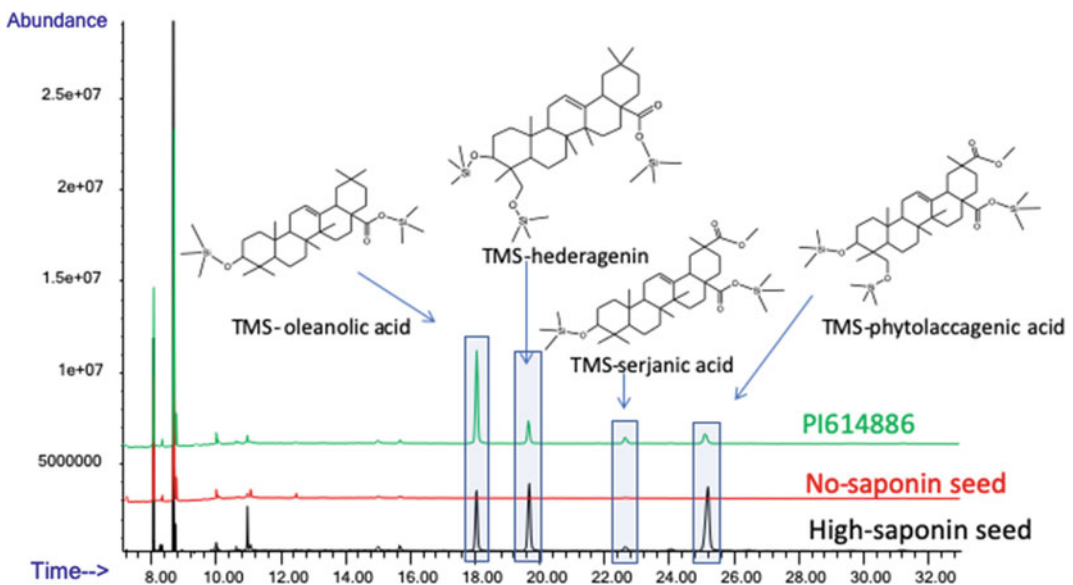
These tests are best to be seen as an approximate of the total saponin content, they are simple tests and good for a rough comparison of saponin content, for example in breeding experiments. However, standardization with saponins is necessary to quantify the glucosides.

Non-biological methods to determine the quantity of saponins in plant material are spectrophotometry and chromatography. Thin-layer chromatography (TLC) in one- or two-dimensional modes is an efficient tool for

separation of saponins and has been used to determine saponin content of quinoa seeds in breeding programs (Dini et al. 2002; Ng et al. 1994). However, the simultaneous analysis of saponin standards on a plate is complicated but necessary to minimize variations of the method. Nowadays, TLC is only used as a supportive separation technique for saponin analysis.

TLC-colorimetry uses colorimetric determination of saponins using crude plant extracts. The TLC is the means of compound separation: the bands are scraped off the plate, extracted with alcohol, and treated with a colorant. The typically used reagents, Ehrlich and vanillin, are prone to react with the hydroxyl group at C3 of bile acids or sterols (Nakajima 1976). This means that also sterols are detected, which can result in misleading information of the saponin character in crude plant extracts.

Gas chromatography (GC) is a semi-quantitative method, which separates the saponins into their aglycones and sugar moieties through acid hydrolysis (Fig. 8.6). This means that the large mixture of saponins present in the



**Fig. 8.6** Detection of saponins using Gas Chromatography-Mass Spectrometry (GC-MS).

Overlaid chromatograms from extracts of seeds with no detectable saponins (red), high amount of saponins (green), and accessions PI614886 (QQ74) also with high saponin content. Four main saponin aglycones were detected. Procedure as described in Jarvis et al. (2017). All samples were extracted in methanol-water, hydrolyzed, and derivatized with trimethylsilyl (TMS) agent (removing the sugar groups).

seeds is broken down to only their aglycone backbones. This will allow estimation of the total amount of saponins based on the backbone, but not identify the individual saponins. In addition, under hydrolysis conditions, several artifacts can be formed which makes GC-analysis complicated. However, the method has been successfully employed in combination with mass spectrometry (MS) or flame ionizing detector (FID) to profile saponins in quinoa (Madl et al. 2006; Medina-Meza et al. 2016).

Separation of individual saponins is complicated and time-consuming, due to their similar polarities in plant extracts. A cascade of separation techniques (such as TLC, column chromatography, flash chromatography, Sephadex chromatography, high-pressure LC (HPLC)) is used to isolate individual saponins. Recent studies used liquid chromatography (LC) methods with mass spectrometry (LC-MS) to identify and quantify individual saponins.

The lack of chromophores prevents UV detection, therefore, wavelengths of 200-210 nm are applied for HPLC methods. These wavelengths are not ideal, as other components in the plant sample may overlap. The development of new methods like liquid chromatography-electrospray mass-spectrometry (LC-ESI-MS) has improved saponin determination. Additions like tandem mass spectrometry (MS/MS) support identification of structural information from complex triterpene saponin samples as (Madl et al. 2006) showed using nano-HPLC electrospray ionization multistage tandem mass spectrometry (nLC-ESI-MS/MS). This method allowed detection of over 80 saponins in crude extracts of quinoa seed hull. However, new technologies, such as nuclear magnetic resonance (NMR) should enable to elucidate saponin structures in the future.

Those spectroscopic methods are quite time-consuming and resource expensive, a simple and non-invasive method is needed to detect saponin content in seeds. Near-infrared spectroscopy (NIRS) is frequently used for grain quality analysis due to its speed, low cost, and lack of requirement for sample preparation. NIRS has been optimized to detect protein, carbohydrate,

lipid, ash, moisture content, and amino acids in quinoa seeds (Ferreira et al. 2015). The method may also be applied to measure concentrations of fiber, fatty acids, vitamins, and minerals, though the determination of secondary metabolites has not been successfully used in quinoa seeds yet (Bittner et al. 2017; Czekus et al. 2019; Graf et al. 2015).

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## 8.4 Role of Saponins in Quinoa

Many plant species, from multiple plant families, have developed the ability to produce saponins, regardless of their geographical region or climate zone and growth habit. The fact that saponins have different concentrations in plant organs and tissues, and that these concentrations are affected by developmental factors, suggests that there are differential synthesis and regulation depending on biological activities. Due to the often toxic nature of saponins, especially to fungi, insects, and molluscs, saponins are generally considered to be plant defense compounds; however, their role is not yet fully understood.

### 8.4.1 Toxicity and Biological Activity

The biological activity of saponins is closely linked to their chemical structure, which determines polarity, hydrophobicity, and acidity. The cytotoxicity and hemolytic activity of saponins are well known and based on the aglycone and the linked sugar chains (Oda et al. 2000). Several studies suggest that mono-desmosidic saponins (C3) are more active hemolytically toward erythrocytes than bi-desmosidic saponins (Takagi et al. 1986; Voutquenne et al. 2002; Wang et al. 2007; Woldemichael and Wink 2001). However, reports show that the hemolytic activities and the cytotoxicity of saponins are not necessarily linked to each other, as the underlying mechanism seems to be different (Gauthier et al. 2009).

Although the exact mechanism of the hemolytic property is not yet clearly understood, it was found to be correlated with their amphiphilic properties. One hypothesis is that saponins

interact with plasmatic membrane sterols and form insoluble complexes, those may cause curvature and evoke pore-like structures or result in sterol extraction via vesiculation, which reduces the integrity of the membrane (Baumann et al. 2000). An alternative model describes a prior step to complex formation with sterols, saponins may migrate toward sphingolipid/sterol-enriched membrane domains and interfere with specific domains (Lin and Wang 2010). However, these properties differ for each individual saponin.

#### 8.4.1.1 Antifungal Activity of Saponins

In recent years, there has been an increasing amount of research on the antifungal activity of various saponins (De Lucca et al. 2006; Kuljan-abhagavad et al. 2008; Ribeiro et al. 2013; Sindambiwe et al. 1998; Stuardo and San Martín 2008), which could be used as natural fungicides in organic agriculture.

The main triterpene saponin avenacin A-1 in *Avena spp.* (oats) is predominantly localized in the epidermal cells of the root tips and released into the surrounding soil, where it shows its antifungal potential by providing resistance to phytopathogenic fungi (Carter et al. 1999; Crombie and Crombie 1986).

Total saponin content extracted from quinoa showed inhibition of the growth of the fungal pathogen *Candida albicans* at a concentration of 50 µg/mL. However, no individually tested saponins displayed a similar effect toward the fungi, suggesting a possible synergistic effect between all saponins or a different compound exhibiting the antifungal activity (Woldemichael and Wink 2001).

In another study, alkali-treated saponins showed the best results against the fungus *Botrytis cinerea*, an important disease of grapes. The mycelial growth and conidial germination were significantly inhibited at 5 mg saponins/mL, whereas untreated quinoa extract showed only minimal activity against *B. cinera*. The antifungal activity in alkali-treated saponins arises from membrane disruption, probably due to the higher number of hydrophobic saponin derivatives, which may have a higher affinity toward the

sterols molecules in the membrane (Stuardo and San Martín 2008). San Martín et al. (2008) obtained similar results testing alkali-treated saponins from quinoa hulls of their molluscicidal activity and argues that the newly formed hydrophobic compounds are responsible for the membrane disturbance.

The first commercial biochemical pesticide/fungicide based on quinoa saponins was released in the Early twenty first century based on the patent of Dutchshen and Danyluk (2004). “Heads Up”<sup>®</sup> plant protectant is based on the triterpene bi-desmosidic glycosides of quinoa seeds extract (HeadsUp Plant Protectant Ltd., Kamsack, Canada). The product is applied as suspension directly on tuber (e.g. potato seed pieces), legume (e.g. bean, pea), and cereal (e.g. wheat) seeds, as a root dip or foliar spray on tomato seedlings to prevent fungal or bacterial growth, and viral plant diseases (USEPA 2002). Although, the company campaigns with proof of vigour improvement of plants (applied to seeds) by initiating the plants own defense mechanism, little research is available on the efficacy of the product. Research on potato brown leaf spot disease shows no effect of “Heads Up”<sup>®</sup>; however, a tuber yield improvement was observed compared to other commercial products (Soleimani and Kirk 2012).

#### 8.4.1.2 Anti-herbivory and Antinutritional Activity of Quinoa Saponins

Due to the potentially toxic/hemolytic properties and perceived bitterness of saponins, these compounds are generally assumed to play a role in plant defense, particularly as antifeedants and predation deterrents. Interestingly, despite the hemolytic properties of saponins, there is little evidence to support for direct toxicity of saponins when consumed normally by non-aquatic animals, although saponins can be effective piscicidal agents by damage respiratory epithelia in fish (Francis et al. 2002; Roy et al. 1990).

Animal feed produced from high saponin species such as alfalfa (Sen et al. 1998), soya, and legumes (Cheeke and Carlsson 1978) can impact animal growth; however, the mode of



action is still unclear. The antimicrobial activity of saponins may affect gut microbial communities, especially in ruminants (Francis et al. 2002). Additional antinutritional effects may be due to saponins reducing protein digestibility, through the formation of stable protein-saponin complexes (Potter et al. 1993) and reducing the absorption of nutrients (Gee et al. 1997). However, the differing saponin compositions and concentrations in different plant species make comparisons related to the role of quinoa saponins difficult. It is suggested that the major antinutritional activity of saponins is the result of food aversion due to the perceived bitterness (Fleming and Galwey 1995).

As for quinoa specifically, there has been limited animal feeding trials to investigate the effect of saponins (Martens et al. 2012). In rats, quinoa meal had an expected negative effect on growth (Cheeke and Carlsson 1978), while sweet (low-saponin) quinoa was shown to have little effect (Grant et al. 1995). Similarly, quinoa containing meal showed reduced growth performance in poultry and pigs (Carlson et al. 2012; Jeroch et al. 1993). In addition, in feeding trials of broiler chickens with feed containing: unwashed, polished, or washed quinoa grain, chicks fed unwashed grains had significantly lower survival (Improta and Kellem 2001). Due to the unpalatability of unwashed grain, quinoa seed saponins are traditionally thought to be a bird deterrent, although evidence for this in control studies is limited. In field trials in Saudi Arabia with saponin containing bitter and low saponin sweet accessions of quinoa, a strong preference for birds feeding on the sweet accessions was observed (personal observation).

Similarly, although saponins are suggested to be involved in deterring insect predation (Dowd et al. 2011; Städler et al. 1992), few studies have examined this in quinoa, with one study showing increased insect activity in high-saponin accessions compared to low-saponin accessions (Yábar et al. 2002). Further studies are needed to confirm the anti-herbivory and anti-nutritional role of saponins in quinoa directly.

## 8.4.2 Germination

The localization of saponins on the outside of the seeds may play a role in plant development and seed germination. *Avena spp.* (oats) release saponins into the rhizosphere where they might serve as allelopathic agents, suppressing the growth of adjoining plants (Carter et al. 1999). In crop rotation of *Medicago sativa* (alfalfa) with cotton, a 50% reduced emergence of cotton seeds was observed (Mishustin 1955). This effect was verified with in vitro assays of extracted alfalfa saponins, which showed a detrimental effect on cottonseed germination (Pedersen 1965). However, on wheat seeds, the alfalfa saponins had no effect, but the growth of the seedlings was indirectly influenced by inhibition of the root growth (Oleszek and Jurzysta 1987). Stimulated germination was observed of barley seeds (*Hordeum vulgare*) and broomrape (*Orobanche minor*) when treated with the triterpene saponin soyasapogenol B (Evidente et al. 2011; Macías et al. 1997). However, similar effects have not been reported for quinoa and their saponins and allow only room for speculations on their role in germination processes.

## 8.4.3 Stress Tolerance

Abiotic stresses often negatively influence plant growth; drought and salinity are two major factors limiting crop growth and production. Quinoa is considered a drought and salt-tolerant plant (Jacobsen et al. 2003). Whether saponins play a role in conveying resistance to abiotic stresses is not well understood and only a few studies focus on secondary metabolites.

The influence of different irrigation levels was evaluated on bioactive compounds in quinoa seeds. The plants were tested under different saline-water and non-saline-water irrigation treatments (Gómez-Caravaca et al. 2012). Interestingly, the saponin content in the seeds decreased by 45% under drought conditions compared with non-saline watered conditions and by 50% under drought conditions compared with

saline-water irrigation. The total saponin content is 35% higher in plants watered with saline-water than with non-saline water. This could be interpreted as a stress response, but further studies are needed to understand saponin production under saline conditions (Gómez-Caravaca et al. 2012; Pulvento et al. 2012). Quinoa seeds contain higher levels of saponins when watered normally. This observation suggests, that reduced irrigation for quinoa plants could be beneficial to reduce the saponin levels. As to why saponin production is reduced under drought stress is still unclear; however, we can speculate that increased saponins under normal watering conditions may protect seeds from fungal pathogens that may not be present in water-limited conditions during grain filling; or possibly that the synthesis of saponins is too energetically expensive to produce under stress conditions.

Seed priming has been shown to be beneficial in adapting plants to abiotic stresses. This strategy can invoke cross-tolerance in seeds when previously exposed to abiotic stresses. (Bradford et al. 1990). In a priming experiment with quinoa seeds *cv. Titicaca* showed that salt stress tolerance was positively influenced when treated with saponins. A concentration of 10-25% saponins alleviated the negative effect of the salt stress during seed germination. Despite, the salt tolerance of mature quinoa plants, at the germination stage, they are prone to the salty surroundings and less likely to establish. Priming with saponins (15%) seems to improve the germination rate under salt stress by shortening the metabolic phase and weakening the pericarp, acting as biostimulant (Yang et al. 2018). These results demonstrate a fruitful avenue for future research in salt stress tolerance for other crops.

#### 8.4.4 Uses of Saponins

Saponins derived from a large variety of plants have been traditionally used for a wide range of purposes, from soaps and detergents for washing (such as from *Yucca schidigera* (Oleszek and Hamed 2010), toxins for fishing (such as from *Serjania lethalis* (Teixeira et al. 1984)), and are

present in many traditional medicines. Industrially, the foaming properties of saponins have been used for the production of detergents, shampoos, foaming agents for beer and fire-extinguishers (Güçlü-Üstündağ and Mazza 2007; Zurita-Silva et al. 2014).

The biochemical activity of saponins, especially the hemolytic activities make them potential organic fungicides, insecticides, and antimicrobial agents. Saponins from quinoa have been registered for use as a biochemical pesticide and fungicide for treatment of tuber, legume, and cereal seeds, for root and for foliar application of tomato seedlings (USEPA 2002) due to their apparent antifungal and anti-insect properties as previously discussed.

The increased intestinal permeability mediated by saponins may also aid drug absorption and assist with reduction of cholesterol (Jacobsen 2003); however, the role of saponins from quinoa for cholesterol reduction is still under debate as the hypocholesterolemic effect was seen with desaponified grain meal in rat studies (Takao et al. 2005).

Additionally, a wide range of plant-derived saponins have been linked to anti-cancer activity (Man et al. 2013; Rao and Sung 1995), with some quinoa derived saponins were shown to induce apoptosis of cancer cells in cell-cultures (Kuljanabhadgavad et al. 2008; Kuljanabhadgavad and Wink 2009). Saponins may also serve as immunological adjuvants or immunostimulators (Campbell and Peerbaye 1992; Kenarova et al. 1990; reviewed in Rajput et al. 2007), such as from *Quillaja saponaria* (Kensil et al. 1991). Similar uses have been suggested for quinoa derived saponins and shown promise in mice immunization studies, where bulk quinoa saponins used as an adjunct increased systemic and mucosal antibodies, possibly by increasing permeability of antigens (Estrada et al. 1998).

For these reasons, there are exciting new prospects for the use of quinoa saponins. However, further study is required to determine the safety and beneficial effects, either nutritionally or medicinally, if any. More investigation into the use of saponins from quinoa is needed to exploit these interesting compounds.

## 8.5 Removal of Saponins from Quinoa Seeds

The bitter taste of unprocessed quinoa seeds and potential anti-nutritional properties of saponins necessitates the removal of these compounds before consumption. As a large proportion of saponins are located in the seed coat (34%) (Chauhan et al. 1992; Jarvis et al. 2017) the majority of saponins can be removed through milling to reduce this layer. Dehulling and washing decreases saponin content by up to 72% (Chauhan et al. 1999; Gee et al. 1993; Ruales and Nair 1993) although even following these processes some saponins remain in the seed. Other

forms of processing such as alkaline washing and heat treatment can also help in removal or debittering of quinoa seeds by degradation of saponins (Gee et al. 1993; Zhu et al. 2002); however, these processes individually reduce saponin content to a lesser extent than with dehulling (Gee et al. 1993).

For traditional consumers, quinoa seeds are generally stored unprocessed, likely to help improve storage life due to the anti-herbivory and antifungal properties of saponins. Directly prior to consumption, an intensive process of roasting, hand milling, and repeated washing is used to remove saponins (reviewed in Quiroga Ledezma 2015) (Fig. 8.7



**Fig. 8.7** Traditional quinoa processing by hand in Bolivia.

Traditionally, harvested quinoa seeds are threshed and separated from chaff by hand for storage. Prior to consumption, several laborious rounds of washing and hand milling are required to remove saponins before cooking. Photo credit: G. Fiene, KAUST. Community of Aroma, Bolivia (2019)

As quinoa has rapidly gained in popularity in the last decades, worldwide production has increased from approximately 80 mT in 2010 to nearly 200 mT at the peak in 2015 (FAO 2019). In response, improvements in processing grain to dehull and remove saponins have been made to allow increased availability to consumers.

Depending on the variety (and starting grain saponin content), either washing (wet) or mechanical milling (dry) methods can be used (Bacigalupo and Tapia 1990; Quiroga Ledezma 2015). Generally, a combined wet-dry method for saponin removal, which removes saponins and maintains seed quality, is used to comply with market expectations. This process is similar to traditional methods where grain is first milled to remove the hull and majority of saponins, several washing steps to extract remaining saponins, followed by centrifugation and a final drying heat treatment to reduce the water content of the grain prior to storage. The use of water required for processing is estimated at  $>5 \text{ m}^3/\text{t}$  of grain (Quiroga Ledezma 2015) and is contaminated with potentially bio-active saponins which may pose an environmental hazard if not disposed of responsibly (Jiang et al. 2018).

Although progress has been made to increase the efficiency of quinoa grain processing and reducing water usage, further improvements can be made. Some “water-less” quinoa processing machines are available on the market (such as from Schule Mühlenbau GmbH, Germany), which operate primarily by dehulling to remove the majority of saponins, or through non-aqueous extractions (Muir et al. 2008), although their uptake of these methods is still limited.

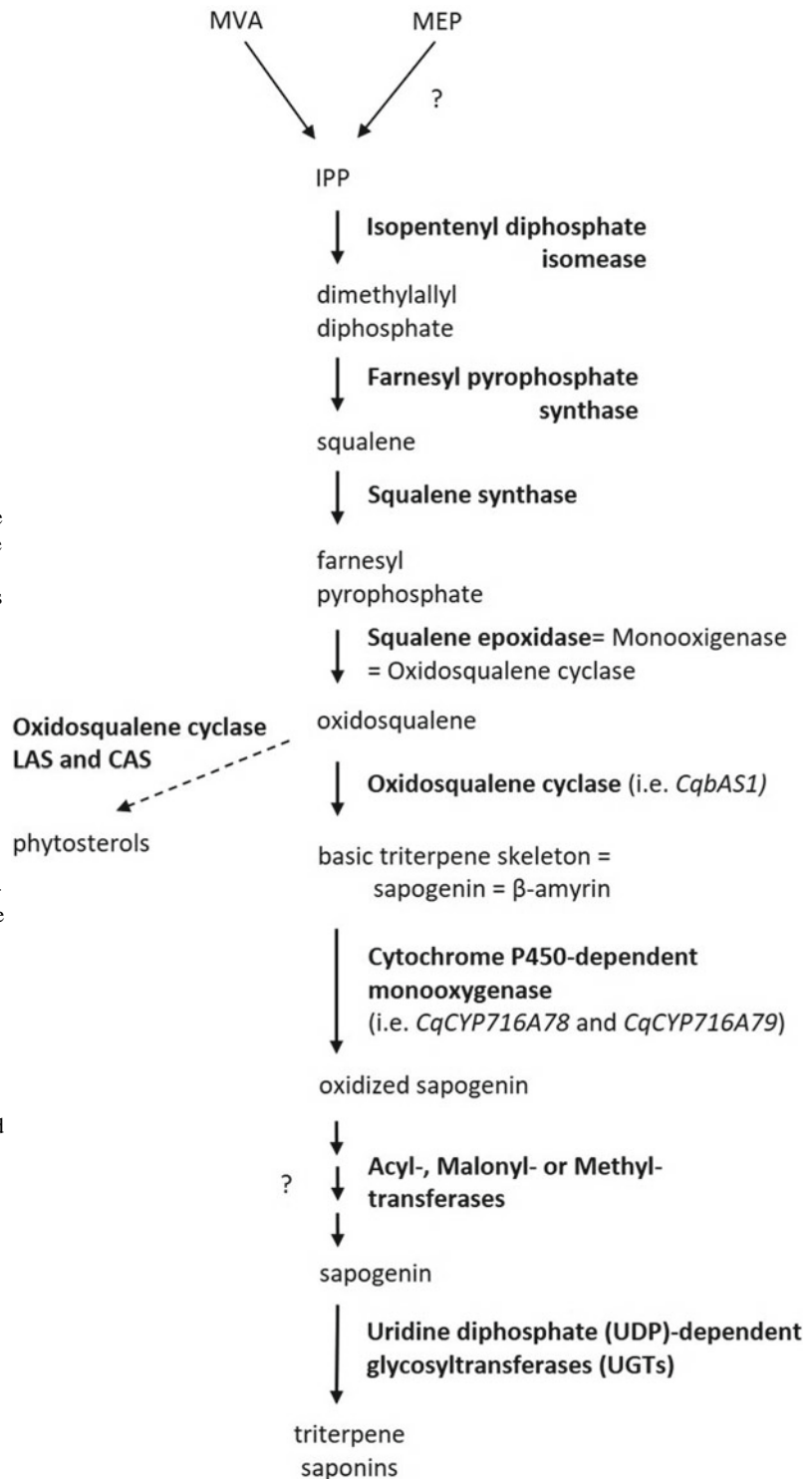
Further research into the efficient and inexpensive removal of saponins is required to make quinoa a more widely consumed crop. Additionally, the purification of interesting bioactive compounds from saponins mixtures is required before these compounds can be used in widespread industrial and medicinal products.

## 8.6 Regulation of Synthesis

Triterpene saponins in quinoa derive from the precursor isopentyl pyrophosphate (IPP, isoprenoid), resulting from the cytosolic mevalonate (MVA) pathway (Fig. 8.8). These precursors are shared with the phytosterol and steroidal saponin synthesis pathway. The oxidation of oxidosqualene is the first diversifying step in the triterpenoid biosynthesis (Abe et al. 1993; Kalinowska et al. 2005). Oxidosqualene is formed through the condensation of two units of IPP with one unit of its allylic isomer dimethylallyl pyrophosphate (DMAPP) yielding in farnesyl pyrophosphate (FPP). Subsequently, two units of FPP are condensed to squalene (30 carbon atoms) (Moses et al. 2013) and further epoxidized to 2,3-oxidosqualene catalyzed by the 2,3-oxidosqualene cyclase (OSC) to a tetra or pentacyclic structure to form dammarenes, tirucallanes and phytosterols, or the oleananes, ursanes, lupanes, and taraxasteranes, respectively (Augustin et al. 2011; Phillips et al. 2006). In quinoa, the oleanane types are the common saponin types, those result from the precursor  $\beta$ -amyryn which is cyclized by a specific  $\beta$ -amyryn synthase (*CqbASI*) from 2,3-oxidosqualene. The following oxidation step facilitated by the quinoa-specific cytochrome P450-dependent monooxygenases CYP716A78 and CYP716A79 oxidize  $\beta$ -amyryn at C28 to form sapogenin. In the subsequent reactions, the sapogenins are further modified by acyl-, malonyl- or methyltransferases (Fiallos-Jurado et al. 2016; Thimmappa et al. 2014). The probable last step to form saponins is promoted by enzymes belonging to the family of uridine diphosphate glycosyltransferases (UGTs) to glycosylate the sapogenin at C3 and/or C28 (ester linkage) (Augustin et al. 2011).

The seed saponin content in quinoa seeds varies with accession, can be influenced by environmental factors and is therefore considered

**Fig. 8.8** Pathway for saponin biosynthesis in quinoa. The triterpenoids are built from isopentenyl diphosphate (IPP), which is supplied from the cytosolic mevalonic acid (MVA) pathway and/or potentially the plastidial methylerythritol phosphate pathway (MEP). The contribution of the MEP pathway to the IPP pool leading to triterpenoid saponins is still being investigated, as denoted by the “?”. The cyclization of oxidosqualene is the first diversifying step in triterpenoid biosynthesis. The biodiversity of oxidosqualene cyclases commits the production of phytohormones or triterpenoid saponins. For instance, the  $\beta$ -amyrin synthase in quinoa produces (i.e. *CqbAS1*)  $\beta$ -amyrin, which is the sapogenin for oleanolic saponins. The quinoa specific cytochrome P450 monooxygenases (i.e. *CYP716A78* and *CYP716A79*) oxidize  $\beta$ -amyrin to sapogenin (Fiallos-Jurado *et al.* 2016). A diverse number of acyl-, malonyl, and/or methyltransferases are involved for further decorations, however, the degree of involvement and order is unknown and indicated by “?”. The probable last step is promoted by Uridine diphosphate (UDP)-dependent glycosyltransferases (UGTs) to produce a large variety of saponins



a quantitative trait (Kenwright 1989; Risi 1986). However, crossing experiments of bitter and sweet quinoa varieties showed an F<sub>2</sub>-segregation of 3:1, suggesting that bitterness is determined by a single dominant locus (Gandarillas 1948; Jarvis et al. 2017; Ward 2002). Recently, a reference genome sequence of quinoa has been published offering new insights into the regulation mechanism of saponins. By means of linkage mapping and bulk segregant analysis (BSA) in two populations of crossing events of sweet and bitter varieties (Kurmi (sweet) × 0654 (bitter), and Atlas (sweet) × Carina Red (bitter)) the same region on chromosome *CqB16* was identified to contain the locus conferring bitterness. In this region, two neighboring genes were striking: *AUR62017204* (*TSAR-like 1*, *TSARL1*) and *AUR62017206* (*TSAR-like 2*, *TSARL2*), annotated as a basic helix–loop–helix (bHLH) transcription factors similar to the class IVa bHLH genes (Jarvis et al. 2017). In *Medicago truncatula* were two transcription factors of the same class reported to regulate triterpenoid biosynthesis, *triterpene saponin biosynthesis activating regulator 1* (*TSAR1*) and *TSAR2*. Overexpression of these transcription factors in hairy roots showed an increase of the transcript levels of triterpene biosynthetic genes and accumulation of triterpene saponins (Mertens et al. 2016).

Expression analysis in quinoa showed root tissue-specific expression of *TSARL2*, while *TSARL1* was mainly expressed in flowers and immature seeds. The DNA binding motif for TSAR of *M. truncatula* was found upstream of several saponin biosynthetic pathway genes in quinoa. Those genes were significantly down-regulated in sweet accessions, correlating with the lower expression levels of *TSARL1*. Comparing the transcripts of *TSARL1* in sweet and bitter accessions revealed a spliced alternative in the sweet accessions, showing a single nucleotide polymorphism (SNP) that alters the intron/exon splicing boundary. The spliced alternative of *TSARL1* in sweet varieties results in a truncated protein predicted to be compromised to form homodimers and/or to bind DNA and is therefore likely restricted in transcription regulation. Further analysis of *TSARL1* in sweet varieties

showed multiple, independent gene mutations which co-segregate with the sweet phenotype, suggesting that this gene regulates the presence and absence of saponins in quinoa seeds.

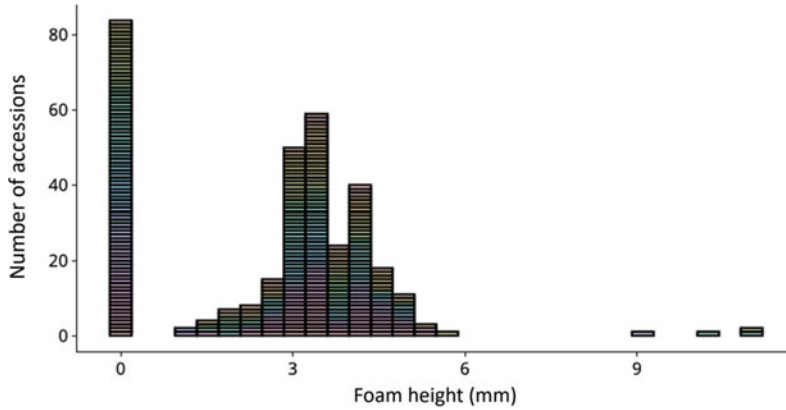
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## 8.7 Perspectives in Quinoa Breeding for Altered Saponin Content

Although increased yield remains the highest priority for the majority of quinoa breeding programs, both commercial and indigenous communities also target “sweetness” or reduced seed saponin content (McElhinny et al. 2007). Reduction of saponin content in quinoa grain would greatly reduce the labor and resources used for processing grain for consumption. The use of aqueous methods for saponin removal produces a significant amount of saponin contaminated wastewater which is potentially hazardous to the waterways due to the general piscicidal properties of saponins, if not properly disposed of. Additionally, the quantities of water required for saponin removal limits industrial grain processing, especially in traditional quinoa growing areas in South America where access to good quality water is scarce. The drought tolerance of quinoa is contrasted by the water usage required for processing. Some saponin-free or at least very low saponin varieties do exist (Koziol 1991, 1992) so there is scope for development of reduced saponin lines (Fig. 8.9).

Complete removal of beneficial saponins in quinoa, however, may result in increased insecticide usage, bird predation, and possibly reduce shelf-life of unprocessed quinoa due to fungal contamination.

Further study is required to identify particular toxic, anti-nutritional, or bitter saponins to target breeding removal of these compounds, as well as simple, high-throughput methods for assessing saponin diversity in quinoa grain. Removal of particularly pH- or heat-stable saponins may also improve processability, reducing the need for heat-treatment or alkaline washing processes, while retaining some of the beneficial saponins.



**Fig. 8.9** Histogram indicating saponin content in 334 accessions of *Chenopodium quinoa*. Foam height generated by vigorous shaking of 5 seeds in 0.5 mL water in a

1.5 mL microcentrifuge tube was measured as an indication for saponin content in 334 *Chenopodium quinoa* accessions

Additionally, the wide-spread industrial, food additive and medicinal uses of saponins from other species warrant further investigation of the uses for quinoa saponins for these purposes. Development of products using quinoa saponins would turn current waste into valuable by-products.

With recent advances in genome editing in plants, such as via CRISPR technology (Li et al. 2015), there is scope for selectively altering saponin biosynthesis in quinoa. Targeting of key saponin biosynthesis and regulatory genes, previously described in this chapter, such as replicating the SNP leading to inactivated or altered expression of *TSARL1* in sweet varieties would be one such approach for rapid breeding for reduced saponin content.

However, to our knowledge, there is yet no established protocols for the stable transformation or whole plant regeneration of quinoa, although there are several works describing callus induction and transient transformation (Eisa et al. 2005; Hesami and Daneshvar 2016; Komari 1990). The transformation of related species such as amaranth (Li et al. 2012; Yaroshko et al. 2019), sugar beet (Dhalluin et al. 1992; Yang et al. 2005), and spinach (Al-Khayri 1995; Naderi et al. 2012) indicates that transformation of quinoa is similarly possible. With some effort and the large diversity of quinoa germplasm available, it is likely that transformation tractable varieties can be identified.

In the short term, the use of virus-induced gene silencing (VIGS), such as recently done by Adhikary and colleagues (Adhikary et al. 2019) in *Amaranth spp.*, may be of use to rapidly screen for gene candidates useful for quinoa breeding, however, how amenable the VIGS system is to altering gene activity in developing seeds is yet to be tested.

## 8.8 Conclusion

Saponins are a diverse range of bitter-tasting, biologically active secondary compounds, generally considered to be bio-protectants. Natively, the role of saponins in quinoa is likely to serve a similar role, particularly due to their concentration in seed coats as a deterrent to bird predation and limiting fungal growth. However, further research into the role of saponins in quinoa, especially into their impact on abiotic stress tolerance is required. With recent advances in quinoa genomics, there are good prospects for molecular plant breeding and gene editing for altering saponin content and diversity. Such developments will benefit both basic research into the role saponins play in quinoa and related *Chenopodium* species, as well as further the development of this currently underutilized crop, particularly in the production of sweet varieties that require less post-harvest processing.

Investigation into potential uses of quinoa saponins, currently being disposed of as a waste-byproduct of quinoa processing is also required, to help maximize the value and limit the impacts of quinoa processing.

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