OPINION PAPER

Prospects for the accelerated improvement of the resilient crop quinoa

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Abstract

Crops tolerant to drought and salt stress may be developed by two approaches. First, major crops may be improved by introducing genes from tolerant plants. For example, many major crops have wild relatives that are more tolerant to drought and high salinity than the cultivated crops, and, once deciphered, the underlying resilience mechanisms could be genetically manipulated to produce crops with improved tolerance. Secondly, some minor (orphan) crops cultivated in marginal areas are already drought and salt tolerant. Improving the agronomic performance of these crops may be an effective way to increase crop and food diversity, and an alternative to engineering tolerance in major crops. Quinoa (Chenopodium quinoa Willd.), a nutritious minor crop that tolerates drought and salinity better than most other crops, is an ideal candidate for both of these approaches. Although quinoa has yet to reach its potential as a fully domesticated crop, breeding efforts to improve the plant have been limited. Molecular and genetic techniques combined with traditional breeding are likely to change this picture. Here we analyse protein-coding sequences in the quinoa genome that are orthologous to domestication genes in established crops. Mutating only a limited number of such genes by targeted mutagenesis appears to be a promising route for accelerating the improvement of quinoa and generating a nutritious high-yielding crop that can meet the future demand for food production in a changing climate.

Keywords: Chenopodium quinoa, drought tolerance, genome editing, molecular breeding, orphan crops, salt tolerance.

Introduction

The challenge of sustainable food production in the future

Plant production is facing unprecedented challenges. In 2050, the human population will exceed 10 billion (FAO, 2017), and the demand for staple crops and livestock will have increased by 60% (Springmann et al., 2018). Agricultural growth relies on productivity gains through increased crop yields, but, following the yield increases achieved during the Green Revolution, the percentage increase in yield has tended to stagnate or decline over
time (Lobell and Gourdji, 2012; Ray et al., 2012, 2013; Grassini et al., 2013). Climate change is predicted to drastically limit local plant production (Lobell and Gourdji, 2012). There is therefore an urgent need to develop crops that can tolerate abiotic stresses such as high temperatures, cold, frost, drought, soil salinization, and flooding. Drought and salt stress pose major challenges for agriculture because these adverse environmental factors prevent plants from realizing their full genetic potential. Non-optimal irrigation causes salinization of soils, and the shortage of high-quality irrigation water exacerbates problems caused by salinity. As a result, many of the arid regions that are presently cultivated may turn into marginal lands. To keep such lands productive, we will need resilient high-yielding crops that can replace current crops.

From a practical point of view, salt stress can be imposed more easily and precisely than drought stress in laboratory settings. Thus, most studies of drought tolerance have focused on salt stress, as plant responses to osmotic changes during both stress situations are closely related and the mechanisms overlap. Furthermore, as salinity imposes hyperosmotic stress on plants, salt-tolerant plants are also drought tolerant. However, genetically engineering salt-tolerant crops remains extremely challenging. As salt tolerance is a complex trait associated with multiple substrates [e.g. ion homeostasis, osmotic balance, and reactive oxygen species (ROS) regulation], each having a complex genetic basis, manipulating a single or a limited number of genes has so far failed to yield salt-tolerant crops (Ismail and Horie, 2017).

The next sustainable Green Revolution should utilize a wider diversity of crops, so that food production can benefit from a broader set of species, each adapted for specific marginal conditions (Jacobsen et al., 2013, 2015). This approach would involve the focused breeding of divergent variants of the main crops cultivated today and, concurrently, the domestication of neglected species, with a focus on resilient plants. Resilient plants include plants with high nutritional value that are able to thrive in suboptimal environments. The output will be sustainable agricultural systems adapted to harsh environments.

**Quinoa (Chenopodium quinoa) as a future major crop**

Quinoa (Fig. 1A) was originally domesticated in the Andean region of South America as early as 7000 years ago, and is adapted to the harsh climatic conditions of the Andean area (Pearsall, 1992). Due to its high genetic diversity and its adaptation to extremely harsh conditions in the highlands of the Andes, quinoa can be grown on marginal soils and is resilient to frost, drought, and salinity, and to large temperature variations between day and night (Jacobsen et al., 2005, 2007; Ruiz et al., 2014). In addition, the seeds are rich in minerals and vitamins and have exceptional nutritional qualities. Compared with conventional grains, quinoa seeds lack gluten, have a superior ratio of proteins, lipids, and carbohydrates, and have a higher content of essential amino acids (Zurita-Silva et al., 2014; Filho et al., 2017; Pereira et al., 2019). However, grain consumption is limited by saponins that accumulate in the seed coat as a defence mechanism against pests and pathogens, and must be removed before consumption (Filho et al., 2017; Jarvis et al., 2017). ‘Sweet’ varieties with reduced amounts of saponins are available but may be more vulnerable to certain pests and herbivores (Singh and Kaur, 2018; McCartney et al., 2019).

Quinoa is traditionally cultivated in South America, where several cultivars have been developed, and a few varieties have been introduced in North America, Europe, China, and the Middle East (Bazile et al., 2016a, b; Murphy et al., 2016; Jacobsen, 2017; Katwal and Bazile, 2020). Although >16 000 accessions of the genus Chenopodium exist (FAO, 2010), access to genetic resources for quinoa has thus far been limited, greatly hindering genetic studies and molecular marker-assisted breeding efforts (Zurita-Silva et al., 2014; Peterson et al., 2015; Murphy et al., 2016, 2018). However, in 2017, two high-quality genome drafts were published based on inbred lines of a coastal Chilean quinoa accession (PI 614886) (Jarvis et al., 2017) and a Bolivian Real variety (Zou et al., 2017). These genome sequences provide insights into the basis for the exceptional nutritional value of quinoa and open up the possibility of targeted breeding of new quinoa varieties.

**The molecular basis for salt/water stress tolerance of quinoa**

Salt bladders (Fig. 1B–E), cell structures homologous to epidermal hair cells consisting of an epidermal cell, a stalk cell, and an epidermal bladder cell, occur in many halophytes (naturally evolved salt-tolerant plants), including quinoa, and could be critical for...
their salt tolerance by serving as salt dumps (Shabala et al., 2014). The direct involvement of the bladder complexes in salt tolerance of quinoa was first suggested by Kiani-Pouya et al. (2017). Gentle removal of bladders neither initiated wound metabolism nor affected the physiology and biochemistry of control-grown plants, but did have a pronounced effect on salt-grown plants, resulting in a salt-sensitive phenotype.

Bioinformatic analysis of the RNA profile of quinoa epidermal bladder cells showed a small number of differentially expressed genes and insignificant changes in the transcript level of most transporter genes under salt exposure (Zou et al., 2017; Böhm et al., 2018). The same transcriptome analysis suggested that high abscisic acid (ABA) levels are required to maintain the cellular response to osmotic stress within the bladder cell and that ABA transporters may be used to import ABA from the leaf, or that ABA is produced in bladder cells for export into other plant tissues. Because of the relatively small number of significant changes in transcript levels under salt stress for most transporter genes, one could suggest that bladder cells are ‘constitutively active’ in salt sequestration and that the transcript level responses of transporters only play a minor role under salt stress. Nevertheless, this transcriptome analysis enabled the identification of candidate genes likely to be involved in salt tolerance and suggested a model for how salt is transported into bladder cells (Böhm et al., 2018). However, many halophytes do not use glands or external bladder cells to regulate their tissue ion concentrations (Flowers and Colmer, 2008), and direct measurements of the ion composition of quinoa bladder cells are lacking; thus, it remains to be confirmed whether these bladder cells serve as salt dumps.

The identification of transporters differentially expressed in the bladder cell transcriptome and functional electrophysiological testing of key bladder cell transporters in Xenopus laevis oocytes revealed that loading of Na⁺ and Cl⁻ into bladder cells is mediated by a set of tailored plasma- and vacuole membrane-based sodium-selective channel and Cl⁻-permeable transporters (Böhm et al., 2018). Two families of Na⁺ transport proteins are constitutively expressed in bladder cells and at high levels: HKT1-like Na⁺ transporters and NHX-like Na⁺ transporters. HKT1-like transporters mediate Na⁺ or K⁺/Na⁺ transport across the plasma membrane and have previously been identified in genetic screens for salt-tolerant crops (Hauser and Horie, 2010), including salt-tolerant accessions of barley (Hordeum vulgare), and groundcherry (Physalis pruinosa) and wild tomato (Solanum pimpinellifolium) (Palmgren et al., 2015; Østerberg et al., 2017). Strong support for this notion has come from the recent de novo domestication of wild tomato (Solanum pimpinellifolium) and groundcherry (Physalis pruinosa) (Leunom et al., 2018; Li et al., 2018; Zsögön et al., 2018). The general applicability of these findings remains to be tested in a wider range of plant species. Recent reviews have stressed that the accelerated improvement of resilient crops holds a huge potential for agriculture (Bailey-Serres et al., 2019; Eshed and Lippman, 2019).

In contrast to the bladder cell, nothing is known about the molecular nature and precise role of the stalk cell (Fig. 1E) which connects the epidermis cell with the bladder cell and serves as a transfer cell. No molecular picture of the transcellular ion transport of transfer cells exists so far either. To gain insight into the salt tolerance mechanism, it would be helpful to determine how stalk cells channel polar Na⁺, Cl⁻, and K⁺ as well as sugars and metabolites to supply the salt bladder with nutrients while compartmentalizing Na⁺ and Cl⁻.

Quinoa has also developed several other mechanisms that contribute to its high tolerance towards salt stress. In line with an increased K⁺ uptake under salt stress, quinoa can maintain high K⁺/Na⁺ ratios, which is a well-established indicator for salt tolerance (Maathuis and Amtmann, 1999; Shabala and Cuin, 2008; Hariadi et al., 2011). Na⁺ can also be compartmentalized in mesophyll vacuoles in old leaves and, when such leaves are shed, Na⁺ is also lost (Adolf et al., 2013; Bonales-Alatorre et al., 2013). The stomatal length was reduced in 114 quinoa accessions subjected to salt stress (Kiani-Pouya et al., 2019), suggesting that the design of the stomatal apparatus may also contribute to the water stress tolerance of quinoa (Hinojosa et al., 2019a; Kiani-Pouya et al., 2019).

### Target traits for improvement

Efforts in quinoa breeding have primarily been carried out by academic institutions, and the lack of private investment has greatly hindered progress. Compared with cereals, quinoa has fairly low yields, one reason being the extreme conditions under which it is grown in the high Andes. However, yield stability varies even under favourable conditions, which can lead to large gaps between potential and realized yields. There is also a need for extensive processing for saponin removal. Still, quinoa remains popular due to its high market value, worldwide demand, and abiotic stress tolerance. Therefore, efforts to convert quinoa into a major crop must aim to increase yield, achieve yield stability, and reduce the saponin content of the seed (Rao and Shahid, 2012; Choukr-Allah et al., 2016; Ruiz et al., 2017; Gamboa et al., 2018; Präger et al., 2018). Because quinoa displays a natural resilience to adverse environmental factors, breeding goals for quinoa require crop improvements that optimize productivity with minimum inputs (Zurita-Silva et al., 2014; Yabe and Iwata, 2020).

We have previously proposed that domestication arises from changes in just a few domestication genes and that these events can be mimicked by mutagenesis of homologous genes in wild species (Palmgren et al., 2015; Østerberg et al., 2017). Strong support for this notion has come from the recent de novo domestication of wild tomato (Solanum pimpinellifolium) and groundcherry (Physalis pruinosa) (Leunom et al., 2018; Li et al., 2018; Zsögön et al., 2018). The general applicability of these findings remains to be tested in a wider range of plant species. Recent reviews have stressed that the accelerated improvement of resilient crops holds a huge potential for agriculture (Bailey-Serres et al., 2019; Eshed and Lippman, 2019).
than those of widely grown cereals. Yield is a combination of many parameters including the number of seeds per plant, seed weight, and loss by seed shattering and pre-harvest sprouting (also called PHS). Beside parameters related to seed production, other factors, such as the number of plants per unit area, plant height, and variations in flowering time, also prevent quinoa from becoming a major food and feed source. In addition, most sweet quinoa varieties are extremely sensitive to mildew, resulting in large yield losses (Danielsen et al., 2000, 2003). Salt tolerance is likely to have an energetic cost for halophytes, as Na⁺ export diminishes the electrochemical gradient of H⁺ required for mineral uptake and turgor-driven processes in plants (Pedersen and Palmgren, 2017; Munns et al., 2020). As the expression of many salt tolerance genes in quinoa appears to be constitutive, energy loss may thus be a growth-limiting factor even when quinoa is grown in the absence of water stress. Thus, paradoxically, if quinoa is to compete with current crops on fertile soils, its resilience to environmental stress may become a barrier for its productivity.

**Seed size**

In rice, several genes have been associated with grain size control, including GRAINWIDTH AND WEIGHT2 (GW2), encoding a RING-type E3 ubiquitin ligase (Song et al., 2007), and GRAIN INCOMPLETE FILLING1 (GIF1), encoding a cell wall invertase required for carbon partitioning during early grain filling (Wang et al., 2008). GW2, an orthologue of DAZ2 in Arabidopsis thaliana (Xia et al., 2013), is a negative regulator of cell division, and GW2 loss-of-function mutants show increased cell numbers, resulting in a wider spikelet hull (Song et al., 2007). This increase in spikelet size accelerates the grain milking rate and results in increased yields due to enhanced grain width and weight. Of the three orthologues in wheat (TaGW2-A1, -B1, and -D1), at least TaGW2-B1 and -D1 influence grain width and length (Zhang et al., 2018). GIF1 is responsible for the smaller grain sizes in wild rice (Oryza rufipogon) (Wang et al., 2008). Cumulative mutations in the GIF1 gene have resulted in larger grains in domesticated rice cultivars. In addition, overexpression of the domesticated variant of GIF1 under the control of its native promoter results in increased grain size (Wang et al., 2008). In addition to GIF1, several other negative regulators of grain size have been described, such as GRAIN SIZE3 (GS3) or Protein Phosphatase with Kelch-Like repeat domain1 (OsPPKL1) (Fan et al., 2006; Zhang et al., 2012; Gao et al., 2015). While we only identified one orthologue of GIF1 (AUR620606205) (Table 1; Fig. 2I) in the quinoa genome, two homologues of DAZ2 are present (AUR62041781 and AUR62037970) (Table 1; Fig. 2A). Loss-of-function mutations of the GIF1 orthologue in quinoa would therefore be an obvious starting point for increasing seed size.

Combining loci for increased grain number and seed size in the same genetic background would provide a strategy for tailor-made crop improvement. In rice, the combination of loss-of-function mutations in GRAIN NUMBER 1A (Gn1a) and GRAIN SIZE 3 (GS3) is responsible for the heavy panicle phenotype of elite hybrid rice (S. Wang et al., 2018). The null gn1a allele is the determinant factor for heavy panicles through increased grain number, while gs3 is associated with increased grain size and weight (S. Wang et al., 2018). In Arabidopsis, the rice gn1a mutation can be mimicked by deletion of two homologous genes: AtCKX5, the orthologue of rice Gn1a; and AtCKX3 (Bartrina et al., 2011). The quinoa genome encodes two close homologues of AtCKX5 (AUR6203453 and AUR62014467) and another two of AtCKX3 (AUR62029062 and AUR62033955) (Table 1; Fig. 2L), which could be potential targets for improving seed yield in quinoa. In contrast, no homologues of the rice GS3 gene could be identified.

**Seed shattering**

Through evolution, plants have acquired different mechanisms that allow them to release their seed upon maturation. This ability is crucial for survival of plant species in the wild, but would cause enormous losses in agricultural production systems. Thus, domesticated crop plants are characterized by an inactivation of the seed spreading mechanisms present in wild plants. In general, domestication has yielded crops with thicker cell walls around the abscission areas, resulting in an inability of the seeds or fruits to dehisce from the mother tissue (Dong and Wang, 2015; Ballester and Ferrándiz, 2017).

A number of transcription factors from heavily populated protein families are involved in seed shattering, acting in multicomponent systems where the activity of one type of transcription factor is controlled by transcription factors belonging to other protein families. In rice, one such multicomponent system is formed by qSH1, SH4, and SHAT1. The coordinated action of these transcription factors is necessary for abscission zone development, with SHAT1 being the main player, while SH4 positively regulates SHAT1 activity and qSH1 affects the expression of the other two transcription factors (Hofmann, 2012; Zhou et al., 2012). In Arabidopsis, the redundant MADS-box transcription factors SHATTERPROOF1 (SHP1) and SHP2 are required for dehiscent zone differentiation and seed dispersal (Liljegren et al., 2000). Two homologues of SHP1/2 exist in quinoa (AUR62035850 and AUR62027653) (Table 1; Fig. 2G). However, these genes are phylogenetically closer to Arabidopsis AGAMOUS (At4g18960), which controls flower architecture (Yanofsky et al., 1990), and might have functions unrelated to seed shattering. In contrast, there is no homologue of SH4, despite the presence of two homologous genes for both SHAT1 (AUR62001901 and AUR6203911) (Table 1; Fig. 2B) and qSH1 (AUR62022770 and AUR62029222) (Table 1; Fig. 2E).

**Pre-harvest sprouting**

An important challenge when growing quinoa as a crop in countries with rainy summers, such as those in northern Europe, is pre-harvest sprouting (Ceccato et al., 2011). Early rain spells during crop dry-down will lead to germination in the panicle, reducing marketable yields and grain quality. This yield constraint has been studied in other crops, and possible solutions may be expanded to quinoa. Modulating grain dormancy is an effective strategy for controlling pre-harvest sprouting and designing crops that are better adapted to regional climates and post-harvest applications. In rice, endosperm sugar accumulation caused by mutation of PHS8/ISA1 leads to pre-harvest sprouting (Du et al., 2018). In wheat domestication, independent mis-splicing mutations
| Desired trait to modify | Genes involved in other species | Quinoa gene(s) | Subgenome | Gene chromosome coordinates (Phytozome v1.0) | % identity | Expression | Reference |
|-------------------------|--------------------------------|----------------|-----------|--------------------------------------------|-----------|-----------|-----------|
| Saponin biosynthesis    | TSAR1 (Medicago truncatula)   | AUR62017204    | B         | Chr16:68549573..68551812                   | 30.86     | Seeds     | Jarvis et al. (2017) |
|                         | TSAR2 (Medicago truncatula)   | AUR62017206    | B         | Chr16:68524854..68527010                   | 32.00     | Seeds     | Jarvis et al. (2017) |
| Seed size and number    | DA2 (Arabidopsis)/GW2 (Oryza) | AUR62041781    | B         | Chr17:39742130..39752168                   | 56.69/45.16| NA        | This work |
|                         | GIF1 (Oryza)                  | AUR62006205    | A         | Chr15:3135695..3137782                     | 56.66/45.57| NA        | This work |
|                         | CKX5 (Arabidopsis)/Gn1a (Oryza)| AUR62039523    | B         | Chr06:26006908..26013645                   | 68.64/67.93| NA        | This work |
| Seed shattering         | SHAT1 (Oryza)                 | AUR62010091    | A         | Chr07:69242892..69245843                   | 65.68/64.98| NA        | This work |
| Height                  | Rht-B1 (Triticum aestivum)    | AUR62013052    | A         | Chr15:4930835..4933952                     | 81.71      | Flowers   | Jarvis et al. (2017); Golicz et al. (2020) |
|                         | FT2 (Beta vulgaris)           | AUR62000271    | A         | Chr12:3192361..3196369                     | 82.12      | Leaves    | Jarvis et al. (2017); Golicz et al. (2020) |
| Early flowering         | FT1 (Beta vulgaris)           | AUR62010090    | A         | Chr14:14625033..14626940                   | 59.3       | NA        | This work |
|                         | TFL1 (Arabidopsis)            | AUR62004274    | B         | Chr01:117180795..117186698                 | 64.95      | NA        | Golicz et al. (2020)/ This work |
| Pre-harvest sprouting   | PIE1 (Arabidopsis)            | AUR62018509    | A         | Chr07:85323308..85337723                   | 60.55      | NA        | This work |
|                         | MFT (Arabidopsis)             | AUR62029959    | A         | Chr08:39671124..39679767                   | 73.41      | NA        | This work |
|                         | ELF4 (Arabidopsis)            | AUR62022878    | A         | Chr04:2907637..2908047                     | 47.27      | NA        | Golicz et al. (2020) |
|                         | MKK3 (Hordeum vulgare)        | AUR62015864    | B         | Chr05:956636..956737                       | 62.03      | NA        | This work |

Table 1. Targets for accelerated domestication of quinoa
in TaPHS1 led to resistance to pre-harvest sprouting (Liu et al., 2015). TaPHS1 is a homologue of MOTHER OF FT AND TFL1 (MFT), which encodes a phosphatidylethanolamine-binding protein that regulates seed germination in Arabidopsis (Xi et al., 2010). Through phylogenetic analysis, we identified a close homologue of MFT (AUR62029959) in quinoa (Table 1; Fig. 2H), suggesting that pre-harvest sprouting might be a relatively easy trait to improve in this plant. Nevertheless, another three quinoa proteins are relatively close phylogenetically to MFT (AUR62014698, AUR62012495, and AUR62014699) (Table 1; Fig. 2H), which might complicate the task due to functional redundancy.

Mitogen-activated Protein Kinase Kinase 3 (MKK3) is the causal gene of the major grain dormancy quantitative trait loci (QTLs) Osd2-4K (SD2) and PHS1 in barley and wheat, respectively (Nakamura et al., 2016; Torada et al., 2016). In rice, the MKKK62–MKK3–MAPK7/14 module controls seed dormancy via regulating OsMFT transcription (Mao et al., 2019). Exchange of the evolutionarily conserved amino acid N260 to T260 in MKK3 adapts barley to wet growth conditions in East Asia (Nakamura et al., 2016). Additionally, the semi–dominant ethylmethane sulfonate (EMS)–induced ERA48 allele of MKK3 (in which Glu365 is substituted with Lys) was shown to increase seed dormancy and thus pre-harvest sprouting tolerance in wheat (Martinez et al., 2020). The quinoa genome encodes three close homologues of MKK3 (AUR62015864, AUR62026127, and AUR62020359; Fig. 2K), and these are attractive targets for reducing pre-harvest sprouting.

**Plant height**

Lodging (bending over of the stems near ground level and stem breakage due to heavy panicles) is a common source of agricultural loss, due to the resulting difficulties in crop harvesting. This effect is more common with an increasing plant height. Thus, the so-called ‘Green Revolution’ genes in rice, barley, and wheat cause a decrease in plant height related to defects in the production or sensing of growth–controlling hormones (Hedden, 2003). REDUCED HEIGHT (Rht)-B1 and Rht-D1 in wheat and DWARF PLANT8 (Dw8) and Dwur9 in maize (Zea mays) are orthologues of Arabidopsis GIBBERELLIN INSENSITIVE (GAI) (Winkler and Freeling, 1994; Flintham et al., 1997; Peng et al., 1997, 1999; Fu et al., 2001; Lawit et al., 2010). Alteration of these genes results in defects in gibberellin sensing, and GAI expression in transgenic rice represses multiple gibberellin responses (Fu et al., 2001). In rice, the Green Revolution semi–dwarf (sd-1) phenotype is the result of a reduced content of active gibberellins caused by a defective biosynthetic enzyme (GA20ox2), in a similar manner to the sdr1/denso phenotype in barley (Peng et al., 1999; Monsa, 2002; Spielmeyer et al., 2002; Jia et al., 2009).

Because plant hormones are multifunctional, gibberellin–related dwarfing mutations cause pleiotropic phenotypes, including a higher seed yield due to altered nutrient partitioning and increased number of panicles per area (Peng et al., 1997; Zhang et al., 2017). In South America, quinoa plants can grow up to 3 m high (Apaza et al., 2015), making lodging a potential problem. In addition, plant height in quinoa is affected by environmental factors, and some studies have identified a negative association between plant height and seed yield for several cultivars (Malirio et al., 2017). Therefore, genes affecting plant height should be a target of any attempt aimed at increasing quinoa yields. Two homologues of wheat Rht-B1/Rht-D1 are present in the quinoa genome (AUR62039523 and AUR62014191) (Table 1; Fig. 2F), and these genes are also homologues of Arabidopsis RGA1, which encodes a transcription factor involved in gibberellin signal transduction (Silverstone et al., 1998). In contrast, no clear homologue of the gene encoding GA20ox2 could be identified.

### Flowering time

Production yields in quinoa are extremely sensitive to adverse weather conditions, generating a strong variation in flowering time amongst harvest seasons (Curti et al., 2016).

In Arabidopsis, flowering pathways are integrated by four main players: FLOWERING LOCUS C (FLC), SUPPRESSION OF CONSTANS OVEREXPRESSION1 (SOC1), FLOWERING LOCUS T (FT), and LEAFY (LFY) (van Dijk and Molenaar, 2017; Liu et al., 2020). Heterologous expression of the Arabidopsis FT gene in cassava (Manihot esculenta) improves flower development (Adyemo et al., 2017), and overexpression of the rice homologues RFT1 and Hd3a results in extremely early flowering (Kojima et al., 2002; Pasriga et al., 2019). Likewise, overexpression of LFY homologues in different plants resulted in early flowering phenotypes (Blázquez et al., 1997; Tang et al., 2016; Liu et al., 2017). Heterologous expression of SOC1 orthologues from different plant species in Arabidopsis soc1 plants rescues the late flowering phenotype of this mutant and results in early flowering in wild-type Arabidopsis (Lee et al., 2004; Lei et al., 2013; Fudge et al., 2018; Liu et al., 2020). In turn, FLC is a MADS-box transcription

### Table 1. Continued

| Desired trait to modify | Genes involved in other species | Quinoa gene(s) | Subgenome | Gene chromosome coordinates (Phytozome v1.0) | % identity | Expression | Reference |
|-------------------------|--------------------------------|----------------|-----------|--------------------------------------------|-----------|-----------|-----------|
| Heat stress             | PIF4 (Arabidopsis)             | No close homologue<sup>a</sup> |          | Chr16:76341712..76354887                   | 52.89     | NA        | This work |
|                         | HSFA1                          | AUR62018674    | B         | Chr13:2302837..2307436                     | 50.87     | NA        | This work |
|                         | (Arabidopsis)                  |                 |           |                                            |           |           | This work |
|                         | DREB2A                         | No close homologue<sup>b</sup> |          |                                            |           |           | This work |
|                         | AU6092073207                   |                 |           |                                            |           |           | This work |

NA, not available; ND, none detected;<sup>a</sup> 20 genes with E-scores <10<sup>-10</sup>.<sup>b</sup> 32 genes with E-scores <10<sup>-10</sup>.<sup>c</sup> 98 genes with E-scores <10<sup>-10</sup>.
factor that binds to the promoter of SOC1 and the first intron of FT, controlling their expression and repressing flowering (Helliwell et al., 2006; Searle et al., 2006). Consequently, null mutations in the FLC gene result in early flowering phenotypes (Michaels and Amasino, 1999). In addition, PHOTOPERIOD-DEPENDENT EARLY FLOWERING1 (PIE1) activates FLC expression, and mutations in the PIE1 gene result in early flowering due to the elimination of FLC-mediated flowering repression (Noh and Amasino, 2003).

SOC1 and FLC are also important coordinators of cold responses and flowering time in Arabidopsis. SOC1 attenuates the expression of a number of cold-responsive genes by repressing the promoters of CRT/DRE-binding factors (CBFs) (Seo et al., 2009). In turn, CBFs activate FLC expression, repressing flowering.
Although quinoa is quite resistant to cold temperatures (Jacobsen et al., 2005, 2007), low temperatures may result in delayed germination, and a reduction in growth and seed yield (Bertero et al., 2000; Jacobsen et al., 2005; Bois et al., 2006). Furthermore, flowering seems to be affected by the ability of the plant to reach the two-leaf stage, and temperature may affect the timing of this...
stage (Jacobsen et al., 2007). Therefore, homologues of SOC1 and FLC could be excellent targets for quinoa breeding.

Six homologues of Arabidopsis FT have been identified in quinoa (Table 1). Of these, only four are expressed at detectable levels (Golicz et al., 2020). In addition, several species, including Beta vulgaris, contain two orthologues of FT genes, FT1 and FT2. While BvFT2 promotes flowering, BvFT1 acts antagonistically, repressing flowering before vernalization (Dally et al., 2018). Of the four FT homologues expressed in quinoa, AUR62000271 and AUR62006619 are orthologues of BvFT2, making them the best targets to promote early flowering in quinoa through overexpression strategies. However, this could be a challenging task using current mutagenesis technologies.

The role of FT in flowering is mainly counteracted by the action of TERMINAL FLOWER 1 (TFL1), a close homologue belonging to the CENTRORADIALIS (CEN)-like subfamily of proteins. Indeed, FT and TFL1 have antagonistic roles in the regulation of flowering across different plant species (Seo et al., 2020). FT and TFL1 have antagonistic roles in the regulation of flowering genes, while members of the TFL1 protein family are involved in the transcriptional repression of genes activated by FT (Kaneko-Suzuki et al., 2018; Lee et al., 2019; Wu et al., 2019). In Arabidopsis, rice, and soybean (Glycine max), TFL1 loss-of-function mutations cause early flowering and the generation of a terminal inflorescence (Shannon and Meeks-Wagner, 1991; Liu et al., 2010; Repinski et al., 2012; Kaneko-Suzuki et al., 2018). At least in Arabidopsis and rice, FT proteins activate the expression of flowering genes, while members of the TFL1 protein family are involved in the transcriptional repression of genes activated by FT (Kaneko-Suzuki et al., 2018; Lee et al., 2019). If a similar mechanism operates in quinoa, generating TFL1 loss-of-function mutations might be a simple alternative to FT mutagenesis to achieve early flowering phenotypes. However, true orthologues, or even close homologues, of TFL1 have yet to be identified in the quinoa genome.

While SOC1 has four homologues in quinoa (Table 1, Fig. 2J) (Golicz et al., 2020) and PIE1 has two homologues (Table 1; Fig. 2D), a high-throughput genomic analysis failed to identify an orthologue of FLC (Golicz et al., 2020). Despite the presence of a putative FLC orthologue (AUR62005643) in the quinoa genome, its similarity to several MADS-box genes that differ from FLC casts doubt about the identity of this gene as a true FLC orthologue. In addition to these central players, other Arabidopsis flowering genes are homologue rich in quinoa. These include LFY and several members of the EARLY FLOWERING (ELF) family, including ELF3 and ELF4, which have three orthologues each in quinoa (Table 1). Considering the number of protein families involved in flowering control and the presence of multiple orthologues in quinoa, any attempt to promote early flowering should incorporate multiplex genome editing.

Heat tolerance

The optimal temperature for quinoa germination is ~20 °C (González et al., 2017; Mamedi et al., 2017). Heat stress has profound effects on plant growth and development, affecting both vegetative and reproductive processes. At the subcellular level, heat stress rapidly inhibits photosynthesis by changing the internal structure of the chloroplasts, inactivating Rubisco, reducing the abundance of photosynthetic pigments, and damaging PSII (Allakhverdiev et al., 2008; Sharkey and Zhang, 2010; B. Li et al., 2018). Deleterious effects on reproductive development include inhibition of gametophyte development, reduced pollen germination and pollen tube growth, disturbances in pollen tube guidance and fertilization, and early embryo abortion (Sage et al., 2015; B. Li et al., 2018). This is specifically true for quinoa; temperatures of >35 °C during anthesis significantly reduce quinoa grain yield (Isobe et al., 2012; Lesjak and Calderini, 2017; Hinojosa et al., 2019b), largely as a result of a reduction in pollen viability (Hinojosa et al., 2019b). Furthermore, heat alters phytohormone production and signalling (Abdelrahman et al., 2017) and induces transcriptomic reprogramming and metabolomic changes. Heat stress also results in an increased accumulation of ROS (Zandalinas et al., 2018), thereby affecting protein and membrane stability and causing organelle malfunctioning. In this context, the peroxisome biogenesis genes PEX11C and FIS1A were proposed to be sensitive biochemical markers to screen for heat stress tolerance in quinoa (Hinojosa et al., 2019a).

Upon sensing an elevated ambient temperature, plants initiate signal transduction networks that regulate the expression of a series of genes, including those encoding HEAT SHOCK PROTEINS (HSPs) and ROS-scavenging enzymes, to increase their thermotolerance (B. Li et al., 2018). This signalling relies on rapid changes in cytosolic calcium, ROS, and nitric oxide (NO) levels that alter HSP activity via post-translational modification. HSPs then act as molecular chaperones, preventing protein denaturation and aggregation (Ohama et al., 2016). Cumulative evidence suggests that various signalling pathways are integrated to regulate the abundance and/or transcriptional activity of the basic helix–loop–helix transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4), which forms part of the central regulatory hub mediating the diurnal growth of plants under normal and high temperature conditions (B. Li et al., 2018). Also, HSFA1s, a family of HEAT SHOCK FACTOR (HSF) proteins, have emerged as master transcription factors affecting plant heat shock responses (Liu et al., 2011; Yoshida et al., 2011). HSFA1 activation stimulates the expression of a number of transcription factors that participate in a critical transcriptional regulatory cascade underlying the acquisition of thermotolerance in plants (Dickinson et al., 2018). In addition to HSFs, the ERF/AP2 family transcription factor DREB2A also functions in heat shock-mediated transcriptional regulatory networks (B. Li et al., 2018). Knocking out DREB2A expression resulted in a heat stress-sensitive phenotype in Arabidopsis, and plants overexpressing a constitutively active form of DREB2A showed enhanced thermotolerance (Sakuma et al., 2006).

No obvious orthologues of PFI4 or DREB2A are present in the quinoa genome. In contrast, two close homologues of HSFA1 exist (AUR62018674 and AUR62007327) (Table 1; Fig. 2C). As for the early flowering phenotypes, acquisition of thermotolerance by genetic engineering of HSFA1 would require changes in cis-regions to increase gene expression, and might be difficult to achieve with current mutation technologies. In addition, a glasshouse-based screen of 112 quinoa genotypes and their subsequent field evaluation showed substantial genetic variability in their heat stress tolerance (Hinojosa et al., 2019b), with a clear difference between sea-level and high-altitude varieties. Therefore, genome-wide association study (GWAS) analysis and/or genome
sequencing of contrasting accessions may shed light on the molecular basis of differential heat tolerance in quinoa and suggest a strategy to incorporate this trait in high-yielding varieties.

Mildew tolerance

Downy mildew is a major cause of production loss in quinoa, with reductions of up to 99% in yield reported for susceptible cultivars (Daniehen et al., 2000, 2003). In quinoa, downy mildew is caused by *Peronospora variabilis*, and the resistance mechanisms to this disease are not yet understood. While it is generally assumed that bitter quinoa varieties with a high saponin content are less susceptible to microbial attack, there does not seem to be a correlation between downy mildew tolerance and saponin content in specific quinoa variants (Zurita-Silva et al., 2014). Further extensive research on the defence mechanisms of quinoa is needed to identify potential genetic targets for improved varieties, an approach that could be complemented with genetic assessments for resistance in planta.

Saponin content in seeds

Two beta helix-loop-helix transcription factors, AUR622017204 (TSARL1) and AUR62017206 (TSARL2), homologues of TSAR1 and TSAR2 in *Medicago truncatula* (Table 1), have been identified as controlling saponin biosynthesis in quinoa (Jarvis et al., 2017). Whereas TSARL2 is mainly expressed in roots, TSARL1 is expressed almost exclusively in seeds. Expression levels of TSARL1 are much lower in sweet quinoa varieties, most of which carry a single nucleotide polymorphism (SNP) in the last position of exon 3 of the TSARL1 gene. This SNP has been suggested to result in alternative splicing of the mRNA and generation of a premature stop codon. While not all sweet varieties of quinoa show this specific SNP, different mutations in the TSARL1 gene are present in all tested sweet varieties (Jarvis et al., 2017). In addition, sweet varieties have a thinner seed coat, which probably also contributes to their reduced saponin accumulation.

Methodological challenges for targeted breeding of quinoa

The advent of new breeding technologies, particularly CRISPR/Cas [clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein]-based systems, which allow the precise editing of several genes or alleles simultaneously, provides a promising platform for the targeted breeding of quinoa (Ma et al., 2015; Lowder et al., 2015; Qi et al., 2016; Čermák et al., 2017; Gao et al., 2017; Kim et al., 2017; W. Wang et al., 2018; Zhang et al., 2019; Zafar et al., 2020). However, transformation protocols are not well established in quinoa, complicating the delivery of the genome editing machinery. A full transformation procedure would require (i) delivery of transgenes into the cells; (ii) formation and selection of calli; and (iii) regeneration of full plants from callus tissue.

*Agrobacterium tumefaciens* has been used to transform quinoa cells in suspension cultures (Komari, 1990). This required the use of the highly virulent *Agrobacterium* strain A281. In addition, the binary plasmids used for the transformation had been modified to include a DNA fragment bearing an additional copy of the virB, virC, and virG virulence genes, generating a super-binary vector (Komari, 1990; Komari et al., 1996). While the efficiency of the transformation was suitable for delivery of a transgene into quinoa suspension cultures (10 positive calli out of $10^3$ transformed), it might be too low for implementation of a genome editing strategy.

Another important drawback of the transformation approach was the size of the super-binary plasmid. Due to the instability of this plasmid in *Escherichia coli*, the amount of DNA that can be additionally included in such a plasmid through regular cloning strategies is limited. Nevertheless, two T-DNA vectors can be co-transformed into *Agrobacterium* (Komari et al., 1996). In this type of approach, one T-DNA plasmid would contain the selection marker and the required virulence genes, while the other would contain the DNA construct of interest. About 25% of the co-transformed *Agrobacterium* cells contain both plasmids. After co-cultivation with *Agrobacterium*, transformed quinoa cells would need to be plated in an appropriate medium for callus development and selection. Optimized conditions for callus formation in quinoa have recently been described (Telahigue and Toumi, 2017; Shahin, 2019).

The final challenge in quinoa transformation is the regeneration of quinoa plants. Somatic embryogenesis from callus has already been described in quinoa (Eisa et al., 2005), and it does not seem to require more than transfer of the callus to hormone-free Murashige and Skoog (MS) medium. Thus, while successful transformation followed by regeneration has not been reported in quinoa to date, all the necessary steps have been previously tested. Therefore, it should be technically possible to establish an *Agrobacterium*-mediated transformation protocol for quinoa based on tissue culture and regeneration of transformed plants from callus.

To improve the transformation efficiency of quinoa, booster genes can be used. Boosters, such as *LEAFY COTYLEDON1* (Lotan et al., 1998), *Lec1* (Lowe et al., 2003), *LEAFY COTYLEDON2* (Stone et al., 2001), *WUSCHEL* (*WUS*) (Zao et al., 2002), and *BABY BOOM* (*BBM*) (Boutilier et al., 2002), stimulate the production of embryo-like structures or somatic embryos on numerous explants and also enhance regeneration in both monocot and dicot plant species (Srinivasan et al., 2007; Deng et al., 2009). The co-overexpression of maize *Bbm* and *Wis2* improves the transformation frequencies in sorghum (*Sorghum bicolor*) and sugarcane (*Saccharum officinarum*), which are recalcitrant to both biotic and *A. tumefaciens* transformations (Lowe et al., 2016).

To circumvent the need for inefficient and time-consuming tissue culture in quinoa transformation, *de novo* induction of gene-edited meristems could be an alternative approach. In this approach, boosters and gene editing reagents are co-delivered to somatic cells, and the transferred somatic cells are subsequently induced to meristems that produce shoots with targeted DNA modifications and gene edits (Maher et al., 2020). Transgenic shoots in tomato, potato, and grapevine (*Vitis vinifera*) have been generated using the *de novo* induction of meristems (Maher et al., 2020). Collectively, a highly efficient transformation and genome editing system could be established in quinoa with the help of boosters and the *de novo* induction of meristems.

A central challenge for genetic engineering of quinoa plants is the fact that quinoa is an allotetraploid containing A and B
Conclusions and prospects

The publication of high-quality genome data for quinoa has opened up the possibility of using targeted genome editing for adapting this plant to cultivation conditions in new geographic areas, and improving its agronomic performance. Apart from an increase in seed size and seed numbers, factors such as flowering time, resistance to pathogens, and adaptation to heat stress are important traits to modify in this context. While novel genome-editing technologies, such as CRISPR, could provide an efficient strategy for accelerating the generation of new varieties of this allotetraploid plant, some countries require that such plants be regulated according to GM legislation, which precludes the use of new varieties for commercialization. As an alternative, high-end TILLING technologies could be used for directed molecular breeding of quinoa. The end result would consistently be a nutritious high-yielding crop that is already adapted to a changing climate.

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Author contributions

RL-M and MP wrote the first draft of the manuscript. AN performed the BLAST searches and carried out the phylogenetic analyses. All other authors proposed targets for the BLAST searches and/or contributed to the writing of the final version of the manuscript.

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