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ORIGINAL ARTICLE

Water deficit stress-induced changes in carbon and nitrogen partitioning in *Chenopodium quinoa* Willd.

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Abstract

Main conclusion **Water deficit stress followed by re-watering during grain filling resulted in the induction of the ornithine pathway and in changes in Quinoa grain quality.**

The genetic diversity of *Chenopodium quinoa* Willd. (Quinoa) is accompanied by an outstanding environmental adaptability and high nutritional properties of the grains. However, little is known about the biochemical and physiological mechanisms associated with the abiotic stress tolerance of Quinoa. Here, we characterized carbon and nitrogen metabolic changes in Quinoa leaves and grains in response to water deficit stress analyzing their impact on the grain quality of two lowland ecotypes (Faro and BO78). Differences in the stress recovery response were found between genotypes including changes in the activity of nitrogen assimilation-associated enzymes that resulted in differences in grain quality. Both genotypes showed a common strategy to overcome water stress including the stress-induced synthesis of reactive oxygen species scavengers and osmolytes. Particularly, water deficit stress induced the stimulation of the ornithine and raffinose pathways. Our results would suggest that the regulation of C- and N partitioning in Quinoa during grain filling could be used for the improvement of the grain quality without altering grain yields.

Keywords C and N partitioning · Grain nutritional quality · Ornithine pathway · Quinoa · ROS scavengers · Stress recovery · Source and sink interactions · Water deficit stress

Abbreviations

GDH	Glutamate dehydrogenase
GS	Glutamine synthetase
MDA	Malondialdehyde
ROS	Reactive oxygen species

Introduction

Quinoa (*Chenopodium quinoa* Willd), a member of the Amaranthaceae family (Alvarez-Jubete et al. 2010) is widely cultivated in South America, mainly in the arid and semi-arid areas of the Andean region (Martínez et al. 2009; Ruiz et al. 2014). This crop is well adapted to different environmental conditions including water scarcity (Martínez et al. 2009; Ruiz et al. 2014), low temperatures (Jacobsen et al. 2007; Rosa et al. 2009), salinity (Hariadi et al. 2011; Razzaghi et al. 2012b, 2015) and poor soils (Aguilar and Jacobsen 2003; Bois et al. 2006). Because of the high nutritive value of Quinoa seeds and the great adaptability of the plant to stress environments, it has been considered an important crop with the potential of contributing to food security worldwide (FAO 2011).

L. Bascuñán-Godoy and M. Reguera contributed equally to this work.

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The different Chilean Quinoa varieties have been grouped into two broad categories: "Andean" and "Lowland" (Fuentes et al. 2009, 2012; Zurita-Silva et al. 2014). Previous work demonstrated that several Andean Quinoa varieties are drought tolerant during their reproductive stage (Razzaghi et al. 2011, 2012a, b). On the other hand, although it has been reported that lowland Chilean Quinoa varieties have exceptional yields under extreme water deficit conditions (Martínez et al. 2009; Ruiz et al. 2014), reports describing the mechanisms associated with this response are scanty.

The maintenance of carbon (C) and nitrogen (N) assimilation during water deficit stress and the interaction between C- and N metabolism play important roles ensuring plant growth and development and maintenance of source/sink relationships, minimizing stress-induced yield losses (Coruzzi and Zhou 2001; Reguera et al. 2013). Many C- and N-related compounds have been associated with an increased water stress tolerance in plants including soluble sugars, free proline, anthocyanins, glycine betaines and soluble proteins (Monreal et al. 2007).

In addition to the osmoregulatory properties of these metabolites, active photoprotective roles have been proposed including their action as scavengers of reactive oxygen species ROS (Kalamaki et al. 2009; Szabados and Savoure 2010; An et al. 2013; Minocha et al. 2014).

Aiming to better understand the biological mechanisms that control stress tolerance in lowland Quinoa plants, we analyzed the effects of water stress during grain filling on C and N partitioning in two lowland genotypes naturally adapted to different climatic conditions and identified key metabolic pathways that are associated with the stress response and stress recovery of this high valuable crop.

Materials and methods

Plant material and growth conditions

Two lowland genotypes with similar morphological and phenological characteristics, but from different geographical and climatic areas of Chile were used in this work. Faro seeds (Central Chile genotype, latitude 34.65° and longitude 71.91°) were obtained from the Cooperative Las Nieves, Chile. BO78 seeds (south of Chile genotype, latitude 38.51° and longitude 71.42°) were obtained from the National Seed Bank collection at Vicuña, Chile (INIA-Intihuasi).

Plants were grown in 10 L pots. Two plants were planted per pot containing a mixture of 80 % sand and 20 % peat. Greenhouse conditions were kept at 1200 µmol photons m^{-2} s^{-1} of light intensity, 12 h day length at 26/20 °C day/night temperature and 60 % relative humidity.

Seeds were germinated directly in the soil. During the experiment, plants were irrigated daily to field capacity and fertilized with a solution of 50 % N:P:K (20:10:20, w/v) and 50 % ammonium sulfate (total of 0.5 g nitrogen) every week until panicle initiation. Leaf relative water content (RWC) was determined as previously described (Reguera et al. 2013). Water stress treatments were carried out during grain filling period (2 weeks after flowering) by stopping the irrigation until visual stress symptoms appeared (leaf wilting and curling that appeared 6 days after water stress initiation). Field capacity (FC) was measured considering the initial pot weight (5 kg) and the weight of the pots after adding water until draining and waiting for 1 h to ensure the downward water movement decrease. Soil water content (SWC) was determined every 2 days during the stress period as the amount of water retained in the pot (% of water holding capacity). SWC achieved 40 % at the end of the stress episode. Recovery was measured after 3 days of re-watering (see Suppl. Fig. S1).

Metabolite profiling

Metabolites were analyzed from fully expanded *C. quinoa* leaves grown under control, water deficit stress and re-watering conditions. Leaves were collected and immediately frozen in liquid N. The grinded samples were freeze-dried and their metabolic profile was analyzed at the West Coast Metabolomics Center at UC Davis (Davis, CA, USA) following the protocol described in Botanga et al. (2012). Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities using the Fiehn lab libraries (Kind and Fiehn 2006).

Starch content

Starch quantification in leaves and grains was determined as described by Smith and Zeeman (2006). The release of glucose was determined at 340 nm using a Glucose Assay (GAHK20, Sigma–Aldrich, St. Louis, MO, USA) with a spectrophotometer (Beckman Coulter DU-640, Brea, CA, USA). Starch content was calculated as 90 % of glucose content.

Nitrate (NO_3^-) and ammonia (NH_4^+) contents

Grinded leaf freeze-dried tissue (0.01 g/sample) was used to determine NH_4^+ and NO_3^- contents. NH_4^+ was determined according to Forster (1995). Absorbance was measured at 660 nm in a spectrophotometer (Beckman). NO_3^- was determined by reducing nitrate to nitrite using

vanadium (III) chloride (VCl_3) followed by the addition of sulfanilamide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NEDD) (Doane and Horwáth 2003). Subsequent colorimetric nitrite (NO_2^-) analysis was measured at 540 nm in a spectrophotometer (Beckman). NO_2^- analysis was performed without the use of VCl_3 and after substraction of the nitrite contents determined for NO_3^- analysis. Standard curves were obtained using KNO_3 as a standard.

Proline assay

Proline (Pro) was determined using the rapid method developed by Bates et al. (1973). The absorbance was measured at 520 nm in a spectrophotometer (Beckman).

Amino acid quantification

Free amino acids were extracted from 0.1 g of freeze-dried tissue as described previously by Hacham et al. (2002). Determination of free amino acids was carried out on a Hitachi L-8900 amino acid analyzer (Minato-ku, Tokyo, Japan).

Enzyme assays

Sucrose phosphate synthase (SPS), cell wall, cytosolic and vacuolar invertases, nitrate reductase (NR), glutamate dehydrogenase (GDH) and glutamine synthase (GS) activities were determined following the protocols described in Reguera et al. (2013).

Lipid peroxidation

Lipid peroxidation was determined in vitro by estimating the formation of malondialdehyde (MDA) according to the method described by Ortega-Villasante et al. (2005). Leaf frozen tissue (0.1–0.2 g) was homogenized in 1 mL of TCA–TBA–HCl reagent (15 %, w/v) trichloroacetic acid, 0.37 % (w/v) 2-thiobarbituric acid, 0.25 M HCl, and 0.01 % butylated hydroxytoluene. After homogenization, samples were incubated at 90 °C for 30 min and centrifuged at 12,000*g* for 10 min. Absorbance was measured at 535 and 600 nm in a spectrophotometer (Beckman).

Anthocyanin content

Total anthocyanin in leaves and grains of *C. quinoa* was determined following the method described by Neff and Chory (1998) with minor modifications. Absorbance at 530 and 657 nm was measured in a spectrophotometer (Beckman). Anthocyanin content was estimated using the equation $A_{530} - 0.25A_{657}$ (Mancinelli 1984).

Grain yield and N content

Grain yield was determined as the total grain weight per plant. Nitrogen (N) content in grains was determined by grinding and oven-drying overnight at 80 °C. One hundred milligram was used to quantify N by Kjeldahl (1883). The nitrogen harvest index was determined as the percent of N contents of grain and the whole dry shoot weight (Derkx et al. 2012).

Statistical analysis

Three-way ANOVA (level of significance $P < 0.05$) was applied to calculate statistically significant differences including the following factors: genotype, condition (control or water deficit stress) and treatments (6 days of water stress or re-watering). Significant differences were also determined by two-way ANOVA analysis ($P < 0.05$). Fisher and *t* Student's tests were used to identify means with significant differences. Statistical analyses were performed using the STATISTICA 6.0 software.

Results

Changes in carbon- and nitrogen metabolism of *Chenopodium quinoa* under water deficit stress and re-watering conditions

To evaluate the biochemical response of Quinoa to water deficit stress (at a leaf RWC of 33 %, Suppl. Fig. S1) and stress recovery after re-watering, we analyzed the effects of water deficit on source/sink relationships by determining relative contents of key metabolites and the activities of enzymes that participate in C- and N metabolism regulation.

The relative metabolite content was calculated as the ratio of each metabolite to its control sample for each of the conditions (stress and re-watering, Fig. 1). In both genotypes, a marked decrease in glucose (Glc), glucose 6-phosphate (Glc-6-P), fructose (Fru), fructose-6-phosphate (Fru-6-P), glycerate 3-phosphate (3-PG) and starch contents were observed with the stress. The only carbohydrate that significantly increased its content (almost three times with respect to control conditions) was raffinose. After re-watering, raffinose was restored to control conditions in both genotypes but BO78 showed significantly higher contents of Fru while Faro showed a steep increase of 50 % in Glc (Fig. 1b).

No changes in sucrose (Suc) or Suc-related enzyme activities were found during stress (Table 1; Fig. 2a–d). In both genotypes, tricarboxylic acid (TCA) cycle related metabolites increased with stress, with the exception of

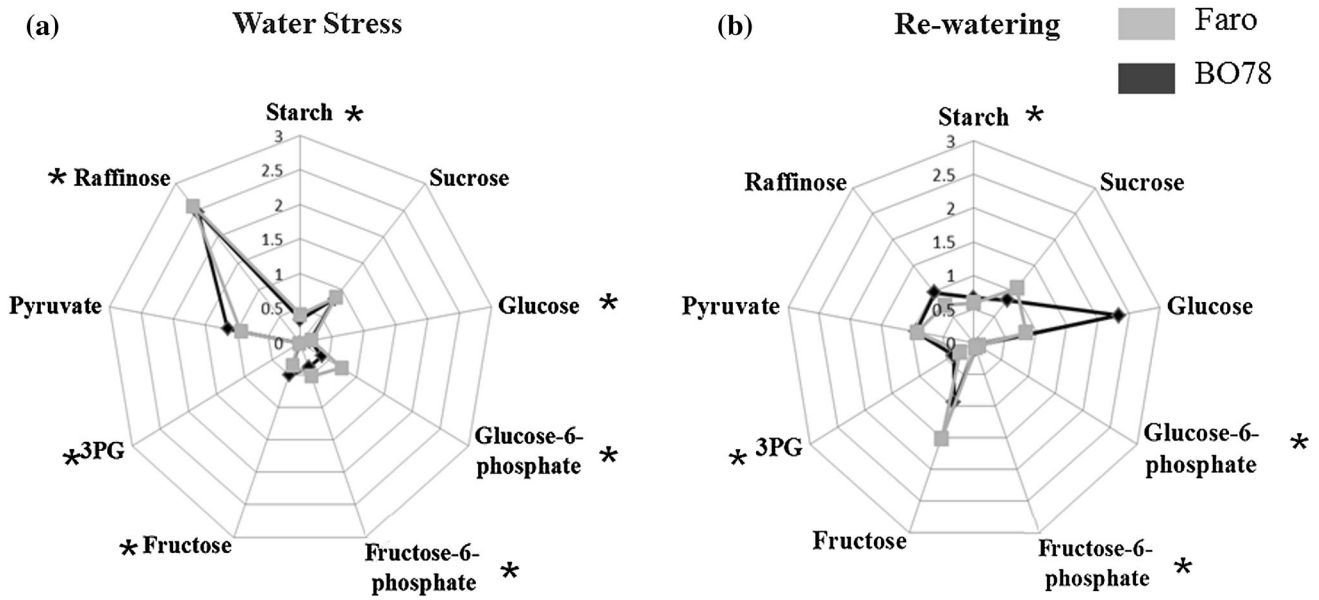

Fig. 1 Carbohydrate content changes in response to water stress and re-watering in *C. quinoa* genotypes Faro and BO78. *Radar charts* showing changes in the relative contents of sugar metabolites in two lowland quinoa genotypes (in *gray,* Faro and in *black,* BO78) subjected to water stress (**a**) and 3 days after re-watering (**b**). Relative contents were calculated as the ratio of the relative content for each metabolite to their control sample. 3PG, glycerate 3-phosphate; Values are mean ± SE ($n = 6$). *Asterisks* indicate significant differences respect to controls ($P \leq 0.05$)

Table 1 Changes in C-associated metabolites in two genotypes of lowland *C. quinoa* subjected to water deficit stress followed by re-watering

	Water stress				Re-watering			
	Control		Stress		Control		Stress	
	Faro	BO78	Faro	BO78	Faro	BO78	Faro	BO78
Starch[a]	**70 ± 10**	**56 ± 5**	23 ± 3	23 ± 2.3	71 ± 7	69 ± 10	**48 ± 6**	**41 ± 5**
Sucrose	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.4	0.5 ± 0.2	0.3 ± 0.2	0.6 ± 0.1	0.4 ± 0.2	0.7 ± 0.2
Glycolysis (relative intensity)								
Glucose	**6 ± 1**	**5 ± 2**	0.3 ± 0.6	1.0 ± 0.5	5.5 ± 0.5	4.3 ± 1.0	**8.4 ± 0.5**[a]	4.7 ± 0.5
Glc6P	**10 ± 2**	**12 ± 4**	0.1 ± 0.2	0.3 ± 0.7	6.0 ± 0.8	8.0 ± 2.3	4.5 ± 0.7	2.5 ± 1.9
Fructose Fru6P	**2 ± 1**	**2 ± 1**	1.5 ± 1.2	0.0 ± 0.2	2.1 ± 0.8	1.5 ± 0.6	1.3 ± 0.5	**2.7 ± 0.3**[a]
Others	**12 ± 3**	**11 ± 4**	0.1 ± 0.1	0.3 ± 0.4	**8.5 ± 0.4**	**8.3 ± 2.0**	3.6 ± 0.9	2.8 ± 1.7
Gal-6-P	**7 ± 2**	**8 ± 2**	0.1 ± 0.2	0.1 ± 0.3	4.3 ± 0.7	5.1 ± 1.2	2.6 ± 0.7	2.7 ± 1.1
Raffinose	3 ± 2	5 ± 2	**9.1 ± 3.1**	**13 ± 3.8**	2.1 ± 0.2	3.3 ± 3.0	2.4 ± 1.5	2.8 ± 1.6
Myo-inositol	1.0 ± 0.1	0.6 ± 0.2	**1.4 ± 0.2**	**1.5 ± 0.5**	0.2 ± 0.5	0.5 ± 0.4	0.1 ± 0.3	0.4 ± 0.1
TCA cycle (relative intensity)								
Pyruvate	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.1 ± 0.2	0.4 ± 0.1	0.1 ± 0.3	0.7 ± 0.3	0.4 ± 0.1
Citrate	0.4 ± 0.1	0.3 ± 0.1	**1.5 ± 0.7**	**1.7 ± 0.2**	0.4 ± 0.1	0.7 ± 0.5	0.1 ± 0.1	0.1 ± 0.3
Aconitic acid	0.3 ± 0.3	0.3 ± 0.3	**3.8 ± 1.8**	**2.3 ± 1.0**	1.3 ± 0.7	0.7 ± 0.4	0.5 ± 0.2	0.3 ± 0.4
Iso citric acid	0.1 ± 0.2	0.3 ± 0.3	**2.3 ± 1.3**	**1.7 ± 0.5**	0.3 ± 0.3	0.1 ± 0.4	0.4 ± 0.2	0.2 ± 0.1
α-Ketoglutarate	**28 ± 4**	**18 ± 3**	0.6 ± 0.3	0.4 ± 0.3	**49 ± 13**	**15 ± 5**	3.8 ± 5.0	0.15 ± 0.04
Succinic acid	1.7 ± 0.6	1.5 ± 0.2	0.9 ± 0.4	0.6 ± 0.7	1.2 ± 0.3	1.2 ± 0.3	1.3 ± 0.5	0.1 ± 0.2
Fumarate	0.2 ± 0.2	0.9 ± 2.0	**1.9 ± 0.0**	**1.5 ± 0.4**	0.2 ± 0.3	1.1 ± 0.6	0.4 ± 0.1	0.4 ± 0.1
Malate	**1.4 ± 0.1**	**1.4 ± 0.3**	0.6 ± 0.4	0.4 ± 0.2	1.1 ± 0.3	1.0 ± 0.2	0.8 ± 0.1	0.7 ± 0.2

Relative contents of C-related metabolites were analyzed in *C quinoa* leaves grown under control, 3 days of water deficit stress conditions and 3 days after re-watering. Values are mean ± SE ($n = 6$). Data were analyzed using one-way ANOVA followed by Student's *t* test. *Bold font* indicates significant differences by treatment ($P \leq 0.05$). *Asterisks* indicate significant differences by genotype ($P \leq 0.05$)

[a] Starch (μg g^{-1} DW)

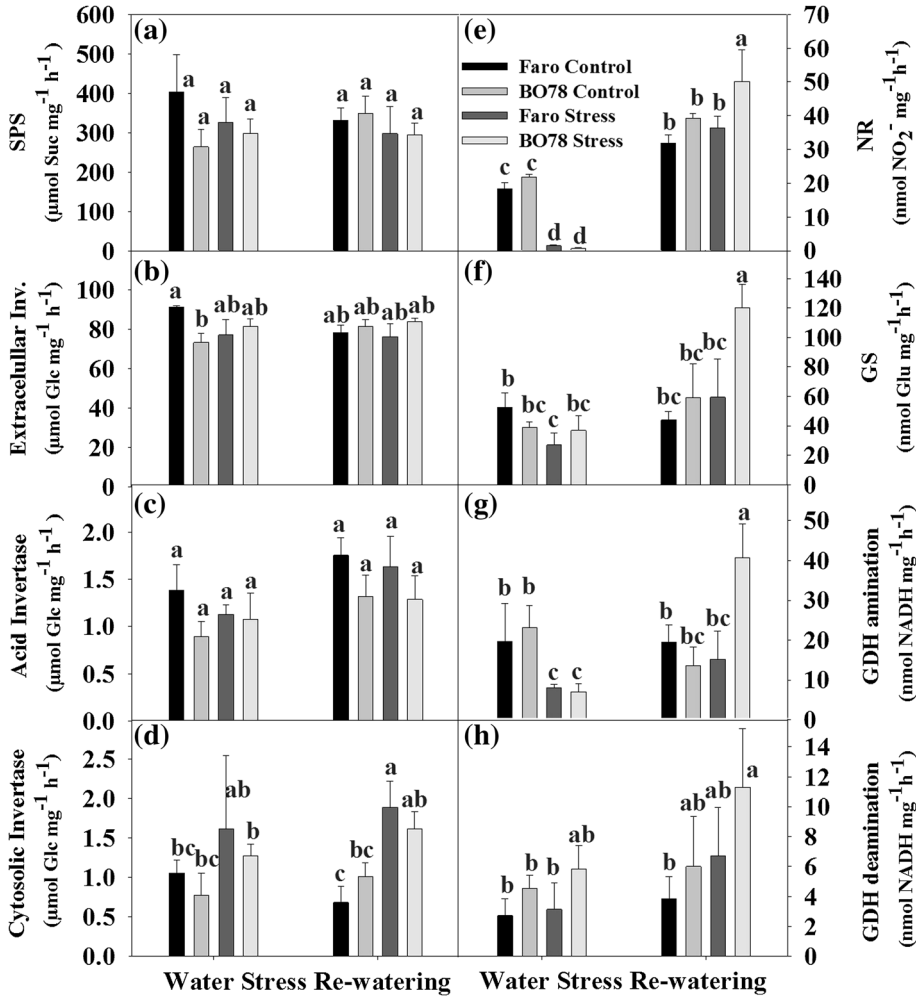

Fig. 2 Changes on C- and N-associated enzymatic activities in leaves of *C. quinoa* Faro and BO78 after water deficit stress and re-watering. Sucrose phosphate synthase (SPS, **a**), extracelullar invertase (**b**), acid invertase (**c**), cytosolic invertase (**d**), nitrate reductase (NR, **e**), glutamine synthase (GS, **f**), glutamate dehydrogenase (GDH) in the amination direction (**g**) and glutamate dehydrogenase in the deamination direction (**h**). Enzymatic activities are expressed as moles of metabolite generated/consumed per milligram of protein per unit of time. Additional details are provided in "Materials and methods". *Glc* glucose, *Glu* glutamate. Values are mean ± SE ($n = 6$). *Different letters* represent significant differences among genotypes, treatments (control and stress) and conditions (water stress and re-watering) (Fisher LSD test; $P \leq 0.05$) using three-way ANOVA

2-oxoglutarate, malate and succinate (Table 1). 2-Oxoglutarate contents decreased with stress in both genotypes while succinate remained unchanged (Table 1). After re-watering, starch, Glc and Fru remained low (Table 1), but TCA-related metabolites (except 2-oxoglutarate) returned to control levels (Table 1).

Total protein contents in *C. quinoa* were reduced with the stress and increased upon recovery (Fig. 3a). This decrease correlated well with an increased NO_3^- and NH_4^+ contents (Fig. 3b, c). While the increase in NO_3^- could be associated with the marked stress-induced decrease in nitrate reductase (NR) activity (Fig. 2e), NH_4^+ increase is more likely associated with the enhancement in protein degradation and N re-assimilation processes (Reguera et al. 2013). No changes in deamination activities of glutamate dehydrogenase (GDH) during stress or after re-watering (Figs. 2h and 3) were observed. On the contrary, changes in the GDH amination activities decreased in both genotypes with stress and decreased or increased in Faro or BO78, respectively, after re-watering (Fig. 2g). Thus, the reduced GDH amination activity could contribute to the accumulation of NH_4^+.

Increased photorespiration under stress, supported by the higher compensation points seen in both genotypes under stress (Suppl. Table S1), and/or the higher activities of phenylalanine ammonia lyase might also be responsible for the higher NH_4^+ contents seen under stress in both genotypes (Fig. 3 and Suppl. Table S2).

The role of glutamine synthetase–glutamate synthase (GS-GOGAT) pathway in detoxifying NH_4^+ has been well established (Masclaux-Daubresse et al. 2006). The total glutamine synthase (GS) activity remained constant in B078 during stress, but was reduced in Faro (Figs. 2f and 8). Faro recovered its GS activity during re-watering while a marked increase in GS activity was seen in B078 (Fig. 3f) and this effect was well correlated with higher glutamine (Gln) contents in B078 (Fig. 4 and Suppl. Table S2).

Water deficit stress induced changes in amino acid contents in both genotypes, being Gln and glutamic acid (Glu) the most abundant (Suppl. Table S2 and Fig. 4).

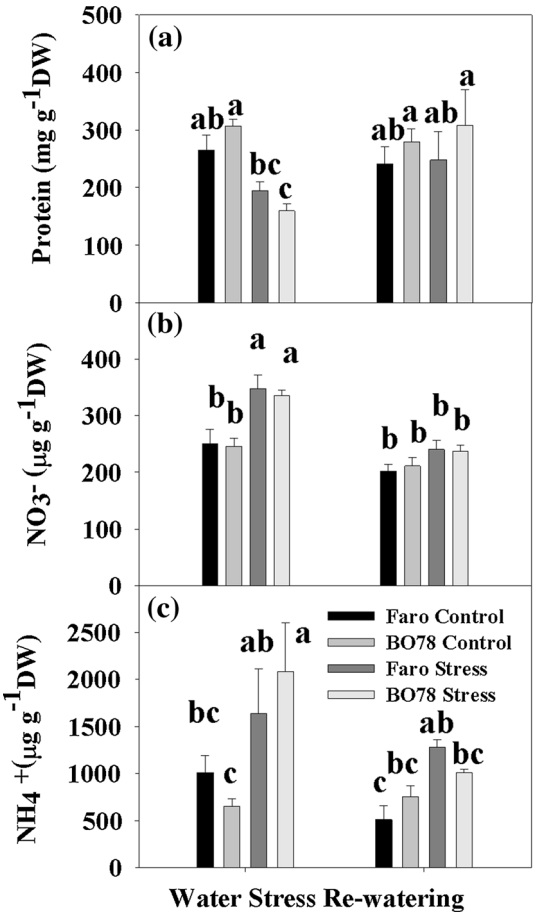

Fig. 3 Total protein, NO_3^- and NH_4^+ contents in *C. quinoa* Faro and BO78 leaf samples. Total protein content (**a**), NO_3^-(**b**) and NH_4^+(-**c**) contents were analyzed in *C quinoa* leaf samples after 6 days of water deficit stress and 3 days following re-watering (see "Materials and methods"). Values are mean ± SE ($n = 6$). *Different letters* show statistical differences using three way ANOVA considering genotypes, conditions (control and water stress) and treatments (during stress and re-watering) as factors (Fisher LSD test; $P \leq 0.05$)

Phenylalanine (Phe), arginine (Arg), histidine (His), tyrosine (Tyr), lysine (Lys), methionine (Met), leucine (Leu) and cysteine (Cys) contents increased in both genotypes while isoleucine (Ile), tryptophan (Trp) and threonine (Thr) increases were seen only during stress in Faro leaves. Both genotypes displayed a reduction in serine (Ser) and aspartic acid (Asp) contents under stress (Suppl. Table S2).

Faro and B078 responded differently to re-watering after the stress episode. In Faro, only Arg contents remained higher and Thr, Glu and Asp were lower than in plants grown under control conditions (Fig. 4d). B078 displayed high contents of Arg, Phe, His, Met, Thr, Trp, Tyr, Leu, Lys, Gln, Asp, Glu and Ser after re-watering (Fig. 5e, f). The altered amino acid content of B078 was well correlated with the higher NR, GS and GDH amination activities seen after re-watering (Fig. 2e–g).

Lipid peroxidation and antioxidants

Lipid peroxidation, estimated as the malondialdehyde (MDA), increased significantly with stress in both genotypes (Fig. 5a). BO78 showed higher lipid peroxidation than Faro and remained unchanged after re-watering. In Faro, peroxidation levels decreased significantly 3 days after re-watering (Fig. 5a).

Urea, ornithine, citrulline and Pro contents increased during stress and these levels were maintained after re-watering (Fig. 5). Faro maintained higher α-tocopherol contents than B078 under stress and under control conditions and these high levels remained unchanged after re-watering (Fig. 5b). Ascorbic acid and anthocyanins increased significantly under water stress in both genotypes and remained constant after re-watering (Fig. 5c, d). On the other hand, trigonelline contents were higher in Faro during re-watering (Fig. 5e).

Effects of water stress in grain yield and grain quality in *Chenopodium quinoa*

Total grain yield was analyzed at the end of the Quinoa life cycle. The stress-induced alteration of primary C- and N metabolism in plants subjected to water stress often results in the acceleration of nutrient remobilization during grain filling with the concomitant grain yield penalties (Yang and Zhang 2006; Reguera et al. 2013). In the Quinoa genotypes analyzed in this study, no detrimental effects of the water deficit stress on grain yields were observed (Fig. 6a).

We also analyzed anthocyanins, total N, total protein contents and the nitrogen-harvest index (NHI) of Quinoa grains (Fig. 6b, e, f). Under control conditions, total nitrogen and total protein contents were higher in BO78 than in Faro (Fig. 6c, e). Faro did not show changes in grain total N or protein content harvested from stressed plants. On the other hand, a 17 and 35 % increases in N and protein contents, respectively, were seen in grains from B078 plants after stress (Fig. 6). The NHI was significantly higher in BO78 after stress, while no changes were found in Faro (Fig. 6). Following a stress episode, BO78 grains displayed increased anthocyanin contents (Fig. 6c) and a slight decrease in starch content (Fig. 6d).

Both varieties displayed an increase in amino acid contents after stress (Fig. 7). The highest amino acid contents in Faro grains were Gln = Glu > alanine (Ala) > Arg, while B078 stored Glu > Ala > valine (Val) > Met = Gly = Glu > Phe. A large increase in Gln content after stress was seen in B078 (15 µmol/g) versus Faro (5 µmol/g). Also, notably differences were detected with other amino acids; while Thr was only detected in Faro grains and remained unchanged during stress. Cys was higher in BO78 than in Faro. In addition, Gly and Lys

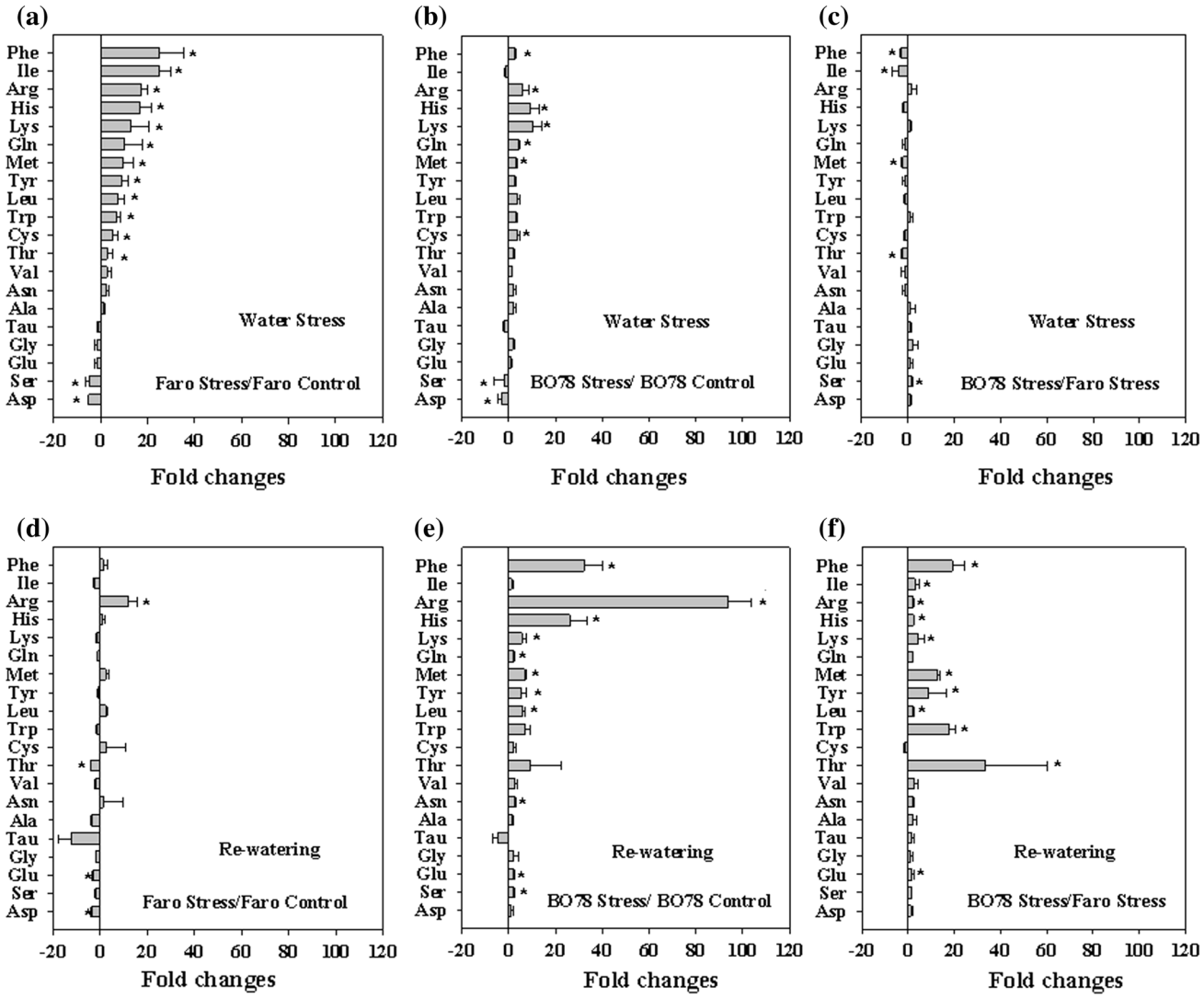

Fig. 4 Leaf amino acid content fold change in response to water deficit stress in *C. quinoa*. Amino acid content fold change of leaf samples during stress (**a**, **b**) and following re-watering (**d**, **e**), in Faro (**a**, **d**) and BO78 (**b**, **e**). **c**, **f** Amino acid fold change comparing genotypes (BO78 compared to Faro) during water deficit stress (**c**) and following re-watering (**f**). *Asterisks* show significant differences between treatments or genotypes ($P \leq 0.05$) using one-way ANOVA followed by Tukey *t* test

contents increased only in BO78 grains from plants subjected to stress.

Discussion

The agronomic characteristics of Quinoa have gained worldwide attention because of its high grain nutritional value (Pasko et al. 2009, 2010) and the significant tolerance to abiotic stresses such as drought (Gonzalez et al. 2011) or salinity (Shabala et al. 2013; Razzaghi et al. 2015). However, little is known about the biochemical and physiological mechanisms responsible for the environmental adaptability and stress tolerance of Quinoa. Here, we aimed at characterizing key metabolic pathways that operate during water deficit stress and their influence on the grain quality of two different Quinoa lowland genotypes.

We analyzed the effects of water stress on C- and N metabolism in Quinoa leaves and grains. C and N metabolic re-adjustments play critical roles in the increased resistance of plants to water deficit stress (Reguera et al. 2013). This decline in photosynthesis during stress was accompanied by a decrease in several glycolysis-related metabolites (Fig. 1; Table 1; Suppl. Fig. S2; Suppl. Table S1). Although no differences were observed in leaf sucrose content or sucrose phosphate synthase (SPS) activity (Fig. 2), starch contents decreased and this decrease was well correlated with increments in raffinose contents during the stress. Changes in the photoassimilate patterns (mainly starch, Suc, Glc and Fru) are characteristic

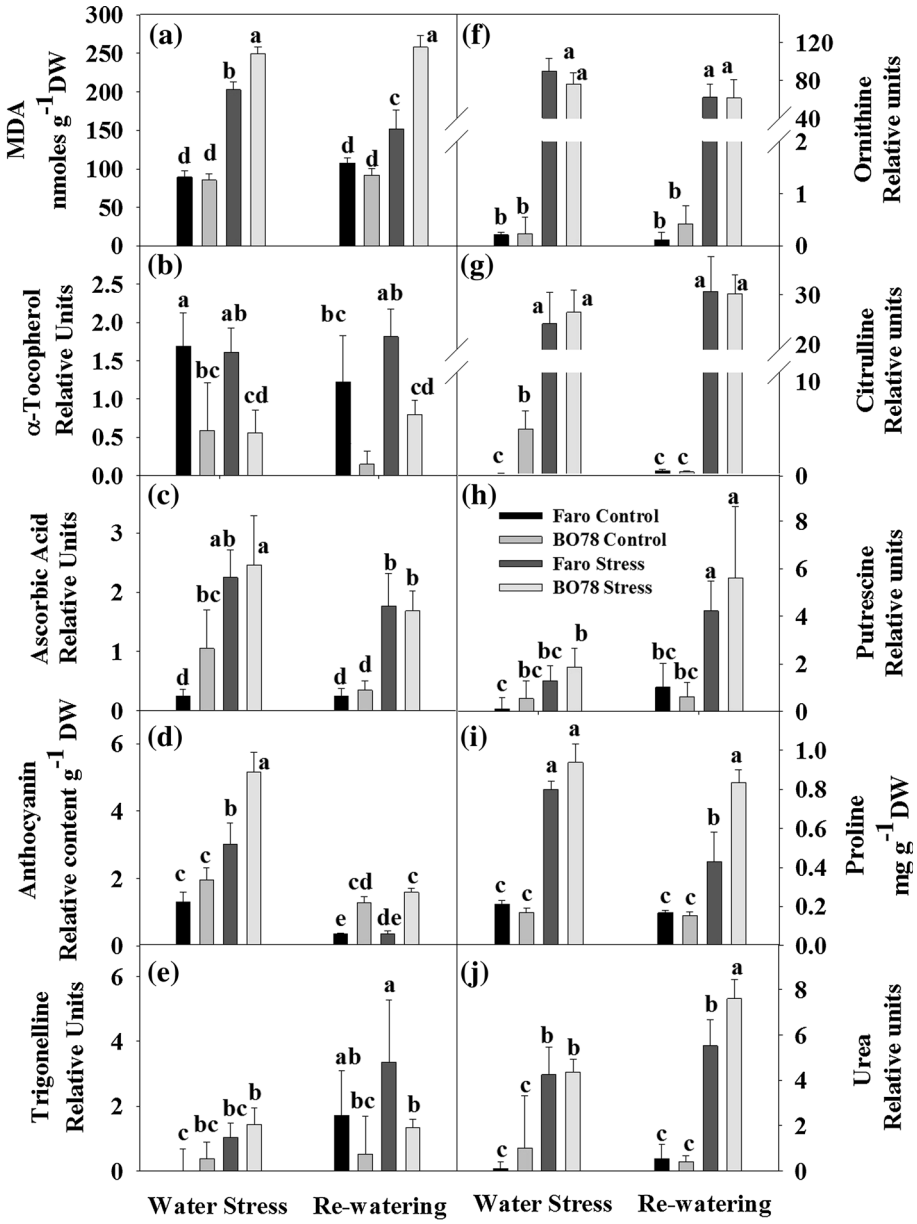

Fig. 5 Antioxidant-related metabolite content in *C. quinoa* Faro and BO78. MDA to asses lipid oxidation (**a**), and antioxidant related metabolites involved in the Halliwell-Assada and ornithine pathways are shown, including α-tocopherol (**b**), ascorbic acid (**c**), anthocyanin (**d**), trigonelline (**e**), ornithine (**f**), citrulline (**g**), putrescine (**h**), proline (**i**) and urea (**j**). Values are mean ± SE ($n = 6$). *Different letters* show statistical differences using three way ANOVA considering genotypes, treatments (control and water stress) and conditions (stress and re-watering) as factors (Fisher LSD test; $P \leq 0.05$)

plant water stress responses (Pinheiro and Chaves 2010) and raffinose has been associated with osmoregulatory functions acting as well as a ROS scavenger during stress (Nishizawa et al. 2008). It has been postulated that raffinose functions as a carbon reservoir that could contribute to a faster recovery of Quinoa after stress (Karner et al. 2004). Interestingly, myo-inositol, one of the substrates in the raffinose synthesis pathway increased in both genotypes subjected to stress. Similar to raffinose, myo-inositol could have osmoregulatory (Ishitani et al. 1996) and antioxidant properties (Shao et al. 2008; Duan et al. 2012).

A significant increase in anthocyanin content was detected in leaves of both genotypes after stress, but only BO78 showed increased anthocyanin contents in grains harvested from stressed plants (Fig. 7b). Anthocyanins act as photoprotectants by masking photosynthetic pigments and quenching free radicals (Carletti et al. 2013), and they have been identified as important components of Quinoa grains due to their high nutritional value and health benefits (Pasko et al. 2009; Alvarez-Suarez et al. 2014). The induced synthesis and accumulation of anthocyanins with the stress during grain filling could be an important functional trait for grain nutritional quality of Quinoa.

A stress-induced stimulation of the ornithine–citrulline pathway was observed in both genotypes, resulting in the increase of ornithine-related metabolite contents (Fig. 8). The ornithine cycle plays an important role in the assimilation of ammonia in different organisms including animals,

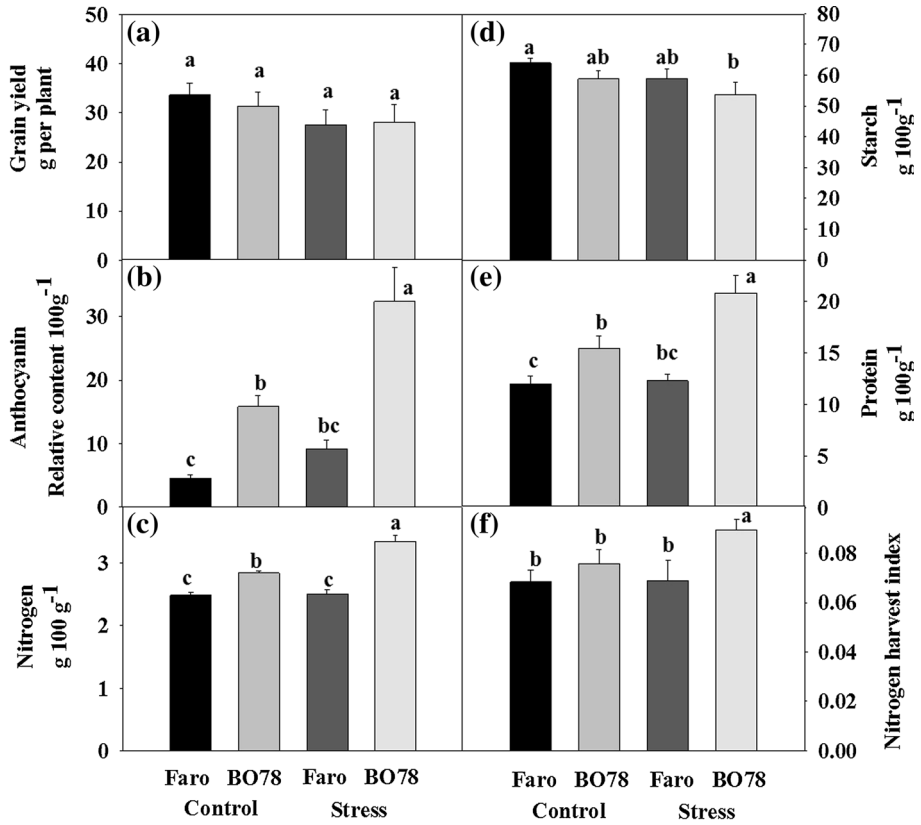

Fig. 6 Effects of water deficit stress on grain yield and grain quality in *C. quinoa* Faro and BO78. Grain yield (**a**), anthocyanin in grains (**b**), total nitrogen (**c**), starch (**d**) protein content (**e**) and nitrogen harvest index (**f**). Values are mean ± SE ($n = 6$). *Different letters* show statistical differences using three way ANOVA considering genotypes, treatments (control and water stress) and conditions (stress and re-watering) as factors (Fisher LSD test; $P \leq 0.05$)

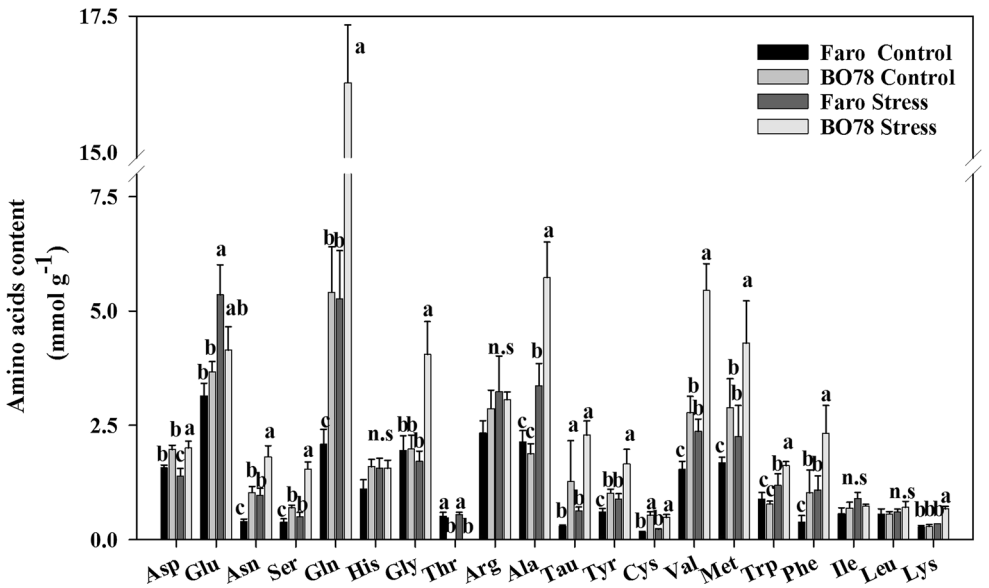

Fig. 7 Changes on free amino acids associated with water stress in grains of two genotypes of *C. quinoa.* Amino acid contents are expressed in millimole per gram of DW grain. *Asp* aspartate, *Glu* glutamate, *Asn* asparagine, *Ser* serine, *Gln* glutamine, *His* histidine, *Gly* glycine, *Thr* threonine, *Arg* arginine, *Ala* alanine, *Tau* taurine, *Tyr* tyrosine, *Cys* cysteine, *Val* valine, *Met* methionine, *Trp* tryptophan, *Phe* phenylalanine, *Ile* isoleucine, *Leu* leucine and *Lys* lysine. *Different letters* show statistical differences using three way ANOVA considering genotypes, treatments (control and water deficit stress) and conditions (stress and re-watering) as factors (Fisher LSD test; $P \leq 005$)

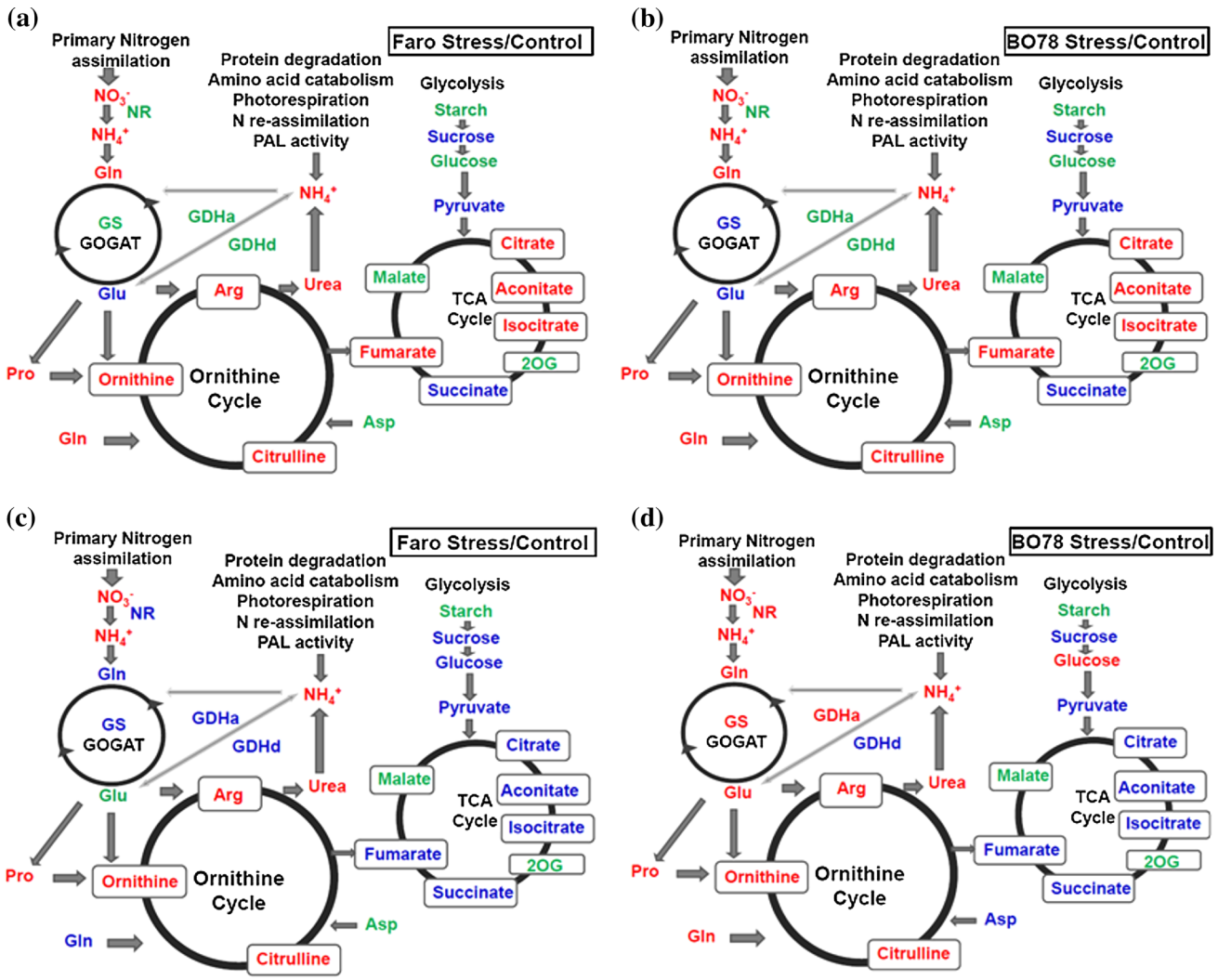

Fig. 8 Schematic representation of C- and N metabolic changes in *C. quinoa* Faro and BO78 in response to water deficit stress and after re-watering. Overview of the main metabolic pathways presenting significant differences during water stress and 3 days after re-watering in *C. quinoa* plants (primary nitrogen assimilation, GS-GOGAT, the ornithine and TCA cycles). *Red*, *green* and *blue colors* indicate higher, lower and unchanged metabolite content/enzymatic activity, respectively, when compared to control plants during water deficit stress (**a**, **b**) or after re-watering (**c**, **d**). *NR* nitrate reductase, *GDHa* glutamate dehydrogenase amination, *GDHd* glutamate dehydrogenase deamination, *GS2* glutamine synthase, *GOGAT* glutamate synthase, *2OG* 2-oxoglutarate, *Gln* glutamine, *Glu* glutamate, *Arg* arginine, *Pro* proline, *Asp* aspartate, NO_3^- nitrate and NH_4^+ ammonia. One-way ANOVA followed by Student's *t* test ($P < 0.05$) was performed using the values (metabolite content or enzyme activity) obtained from six different plants ($n = 6$) per genotype/condition for each of the treatments (water stress and re-watering)

plants and microorganisms (Berg et al. 2002; Linka and Weber 2005; Allen et al. 2011). Although the mechanisms associated with conferring stress tolerance by the ornithine pathway are not yet well understood in plants, the impact of this cycle in proline and polyamine metabolism and the correlation between the induction of the ornithine pathway and stress tolerance, suggests role(s) of the pathway in counteracting stress effects due to osmoregulatory and ROS-scavenging functions (An et al. 2013; Cvikrova et al. 2013). For example, stressed cashew plants showed an increase of δ-ornithine amino transferase (OAT) activity resulting in increased Pro contents (da Rocha et al. 2012). Similarly, elevated Pro contents were observed in rice *OsOAT*-overexpressing plants, that also showed an increased tolerance to oxidative and drought stress (You et al. 2012). In *Arabidopsis 35S::SlNAGS1* transgenic plants an increase of ornithine contents was positively correlated with a higher tolerance to stress (Kalamaki et al. 2009). The accumulation of citrulline and arginine, as C skeleton donors of the ornithine cycle, in drought-tolerant wild watermelon plants during water stress, has been associated with ROS scavenging functions (Yokota et al. 2002). Despite all the evidence, further investigation of the role of the ornithine cycle in the plant stress response(s) is required.

Although a decrease in Pro contents after stress relief was found in some plant species (Gorai et al. 2015), the Pro content recovery occur gradually (in a period of days) as has been previously shown in *Medicago sativa* plants subjected to salinity stress (Miller et al. 2005). Also, in species such as *Periploca sepium*, it was observed that elevated Pro contents after re-watering could act conferring antioxidant capacity (An et al. 2013). Interestingly, salt and water stress applied in Quinoa plants grown in the field demonstrated the involvement of osmolytes such as proline contributing to prevent ROS generation through osmotic adjustment during stress (Razzaghi et al. 2015). We observed a similar response in Quinoa, suggesting that the maintenance of higher Pro, and other antioxidant-related metabolite contents after re-watering, can enhance the antioxidant capacity of the plants, helping in the recovery from the stress episode.

Few differences were observed during the stress response between genotypes. Only α-tocopherol showed higher contents in Faro during stress when compared to BO78. α-Tocopherol is an antioxidant found in thylakoids that acts deactivating photosynthesis-derived ROS, preventing the propagation of lipid peroxidation (Miret and Munné-Bosch 2015). The higher α-tocopherol contents observed in Faro could confer a higher photoprotective capacity in Faro what was well correlated with the lower MDA generation and the reestablishment of maximum rate of electron transport (J_{max}) in Faro (Suppl. Table S1). This metabolic effect, together the differences observed between genotypes during re-watering, including differences in enzymatic activities (such as NR, GS and GDHa) or in amino acid contents (Phe, His, Tyr, Trp, Met, Leu, Thr and Glu), points to a distinct response to stress between genotypes. Interestingly, these differences were well correlated with differences in grain composition (quality). Nonetheless, a similar stress tolerance was observed (in terms of yield) in both genotypes. Further studies should be performed to determine if these metabolic differences could lead to differences in tolerance when these genotypes are subjected to a different kind of stress (i.e., long-term drought or salinity stress).

An overall increase in leaf amino acid contents was observed during stress in both genotypes (Fig. 5a, b), and the changes were larger in BO78 after re-watering (Fig. 5a, b). In leaves, water deficit stress induced increments in Arg, GABA and Pro contents in both genotypes (Suppl. Table S2). Pro and GABA have been associated with water stress and also with the recovery of plants from stress (Shelp et al. 1999; An et al. 2013). In leaves, the decrease in Glu during water stress was accompanied by the accumulation of Gln (Suppl. Table S2). Gln, together with Asp and Glu, have been shown to be the main N-related metabolites that are translocated from sources to sinks (Masclaux-Daubresse et al. 2010). The increase in Gln contents in leaves was paralleled by an increase of Gln in grains (Fig. 8). Consistently, Gln seems to be the preferable transported form of N in Quinoa (Varisi et al. 2008). Both genotypes display an increase in grain contents of Phe, Val, Tryp and Met with the stress episode, indicating a stress effect on the nutritional properties of Quinoa grains.

The increase in raffinose and myo-inositol contents during stress, together with the increased ascorbate, citrulline, ornithine, proline and anthocyanins, indicated an induction of antioxidant metabolism in Quinoa that might play pivotal roles during the stress response and stress recovery. Interestingly, while the major biochemical changes in Faro and BO78 leaves occurred during water deficit stress episode, in BO78 larger changes occurred during re-watering. This response of BO78 leaves was well correlated with the relative high contents in essential amino acids, anthocyanin, nitrogen and protein contents of BO78 grains.

Conclusions

In conclusion, our results provide insights into the role of water deficit stress in the regulation of C- and N partitioning in Quinoa plants as schematically presented in Fig. 8. Although differences in the stress recovery response were found between the two genotypes evaluated, both genotypes showed a common strategy to overcome water deficit stress by displaying a fast recovery from stress, inducing the synthesis of ROS scavengers and osmolytes, especially those related to the ornithine cycle. Source to sink remobilization processes are regulated by stress and senescence (Hörtensteiner and Feller 2002; Yang et al. 2003) and the increase in nutrient remobilization and the induction of antioxidant metabolism driven by water deficit stress could be used for the improvement of the nutritional quality of Quinoa grains with no alterations in grain yield.

Author contribution statement Conceived and designed the experiments: LBG, MR and EB. Performed the experiments: LBG, MR and YMAT. Analyzed the data: LBG and MR. Contributed reagents/materials/analysis tools: LBG, MR and EB. Wrote the paper: LBG, MR, YMAT and EB. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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