

Quinoa plant architecture: A key factor determining plant productivity and seed quality under long-term drought

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ABSTRACT

Chenopodium quinoa (quinoa) is an underutilized crop proposed as key to achieving food security within the current climatic context, where water scarcity in rainfed areas is becoming more frequent and severe, especially in Mediterranean regions. Thus, aiming at deepening our knowledge regarding the impact of water limitation on the quinoa seed nutritional quality, seeds obtained from primary and secondary panicles of four different European-adapted cultivars (F14, F15, F16, and Titicaca) growing under full irrigation and water-limiting conditions, were here analyzed. A set of parameters were evaluated in this work, including agronomical (such as yield, seed weight, seed area, and seed germination and viability rates) and nutritional (including the seed proximate composition, mineral content, and antioxidants) traits. Our results indicate that the morphological changes associated with drought stress affect secondary panicles' seed yield. This phenomenon was generally associated with an improvement in the nutritional quality of those seeds. However, cultivars such as F16, despite keeping total seed yield under low water availability, showed drought's detrimental effect on the seed nutritional quality. In contrast, cultivars like F15 and Titicaca reduced their seed yield under water-limiting conditions but increased their protein, iron, copper, calcium, manganese, and zinc contents, especially in secondary panicles. Therefore, the dichotomy between seed quantity and quality has to be considered in this crop under water stress scenarios, highlighting differences in sink strength along the plant panicles determining seed nutritional quality.

1. Introduction

Since the 1950s, Southern Europe has suffered an increase in the duration and intensity of drought (IPCC, 2012). These drought periods are expected to become more frequent, severe, and longer-lasting in Europe, and are expected to strongly affect Mediterranean areas (Forzieri et al., 2014). In the Mediterranean region, the climate is characterized by presenting warm to hot temperatures during the dry summer season, in which precipitations are scarce, especially in Southern areas (Lionello, 2012; Lloyd-Hughes and Saunders, 2002). This aspect has been a determinant for crop suitability in these areas, in which climate change impact has led to the development of water management optimization strategies, including the use of crop-rainfed agriculture,

together with plant breeding strategies that result in the selection of relevant physiological traits to develop water-use-efficient cultivars (Tramblay et al., 2020).

Chenopodium quinoa Willd., commonly known as quinoa, has been proposed as a suitable crop to be potentially established in many different regions of the world, out of its center of origin (the Andean Altiplano), including those that comprise the Mediterranean region (Bazile et al., 2016; Coccozza et al., 2013; Matías et al., 2021; Pulvento et al., 2010). Quinoa shows a large genetic diversity and has proved to be resilient to a wide variety of abiotic stressors (Jacobsen et al., 2003), which confers on this species huge potential adaptability to marginal environments, including areas suffering from drought episodes (Choukr-Allah et al., 2016; Jacobsen, 2003; Zou et al., 2017).

Abbreviations: WW, well-watered; WD, water deficit; PP, primary panicle; SP, secondary panicles; TFT, 2,3,5-triphenyl-2 H-tetrazolium chloride; FRAP, Ferric reducing antioxidant power assay; TFC, Total flavonoid content; QE, Quercetin equivalent; TPC, Total phenolic content; GAE, Gallic acid equivalent.

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Quinoa seeds are an exceptional source of nutrients. Their high nutritional value relies on the presence of high contents of proteins, ranging between 9% and 23% depending on the cultivar (Abugoch James, 2009; de Santis et al., 2016; Elsohaimy et al., 2015; Toapanta et al., 2016). Generally, the total protein content of quinoa seeds is higher than the content observed in common grains but lower than oilseeds and legumes (Abugoch James, 2009; Elsohaimy et al., 2015; Toapanta et al., 2016). Besides, quinoa proteins are of high quality presenting a good balance of essential amino acids, similar to the composition of the milk protein, casein (Repo-Carrasco et al., 2003), with high levels of lysine, tryptophan, and cysteine compared to other seeds and grains obtained from widely consumed commercial crops (Ruales and Nair, 1992). Also, despite not being considered an oilseed crop, quinoa presents an average oil content that varies between 5% and 7.2% (Vega-Gálvez et al., 2010), with a remarkable quality of the oil composition due to the high proportion of polyunsaturated fatty acids and a good ω -6/ ω -3 ratio (of around 6:1), which has been linked to multiple health benefits (Matías et al., 2022; Tang, Li, Zhang et al., 2015). Other quinoa seed features are the high amounts of fiber, B vitamins, vitamin E, minerals like magnesium (Mg), iron (Fe), potassium (K), manganese (Mn), copper (Cu), calcium (Ca), and phosphorus (P) (Navruz-Varli and Sanlier, 2016; Repo-Carrasco et al., 2003; Tang, Li, Chen et al., 2015), and the presence of several bioactive and antioxidant compounds like polyphenols, carotenoids, and tocopherols, which are considered the major contributors to the antioxidant capacity of quinoa seeds and are associated with reduced heart disease (de Santis et al., 2016; Hirose et al., 2010; Repo-Carrasco-Valencia et al., 2010; Repo-Carrasco-Valencia and Serna, 2011; Tang, Li, Chen et al., 2015).

Different abiotic stresses, including drought, have been proven to impact the phenological, morphological, physiological, and agronomical traits of quinoa plants. Among them, yield penalties are the most concerning effects of abiotic stresses for quinoa producers, especially drought and heat stress (Matías et al., 2021; Saddiq et al., 2021; Tovar et al., 2020; Valdivia-Cea et al., 2021). In line with this, interesting changes have been observed in quinoa development related to abiotic stress. For instance, both heat stress and prolonged water deficit (WD) cause a life cycle shortening, with flowering and seed maturation occurring earlier, impacting plant productivity (Maestro-Gaitán et al., 2022; Matías et al., 2021). Also, an altered plant architecture occurs in plants subjected to water stress, producing a lower number of branches and secondary panicles (SP) (Nguyen et al., 2021). Bennett et al. (2012) postulated that manipulation of plant architecture to obtain phenotypes with lower branching can result in plants with heavier seeds and altered nutrient content. Thus, plant architecture could be an important feature, especially because the growth habit of plants (number and distribution of branches along the plant (Bioversity International, FAO, PROINPA, 2013)) has recently been linked to water-stress tolerance of different quinoa cultivars, being the plants that show fewer branches (and hence, fewer SP) more tolerant to drought (Maestro-Gaitán et al., 2022). Furthermore, the seeds from SP mature later than those from primary panicles (PP) (Tovar et al., 2020), which could in turn affect the seed-filling process causing differential nutritional traits. Changes in plant architecture and osmotic tolerance mediated by molecular mechanisms including transcriptional factors and plant hormone regulation play key roles in drought stress tolerance (Otterbach et al., 2021; Zhu et al., 2022). Under stress, they act regulating source-sink relationships and nutrient remobilization (Etienne et al., 2018; Peleg et al., 2011) as well as essential adaptative responses such as leaf area, stomatal closure or the number of lateral branches, chlorophyll contents, photosynthetic activity and the leaf senescence process (Hejrák et al., 2015; Luo et al., 2012; Nguyen et al., 2021; Ricachenevsky et al., 2013; G. P. Wang et al., 2010).

Additionally, the environmental conditions have shown significant effects on the diverse nutritional traits of quinoa seeds (Gonzalez et al., 2012; Granado-Rodríguez, Aparicio et al., 2021; Prado et al., 2014; Pulvento et al., 2012; Reguera et al., 2018), although very few studies

have reported variations on the nutritional profile caused by long-term water stress or differential effects between seeds harvested from different branches along the plant (Tovar et al., 2022).

Thus, considering the impacts of water stress on plant development and sink-source relations (Maestro-Gaitán et al., 2022), the present study aimed to evaluate the effect of a prolonged WD on the nutritional composition of quinoa seeds comparing seeds from the PP (strong sinks) and SPs (weak sinks). For this purpose, we used four different quinoa cultivars showing different growth habits subjected to either full irrigation (WW) or WD (Maestro-Gaitán et al., 2022). Differences appeared among cultivars, water treatments, and PP and SP, highlighting, on one hand, the importance of the genotypic factor, the environment, and their interaction in determining the seed quality of quinoa; and, on the other, the significant effect of the panicle position in defining seed quality in quinoa under water stress.

2. Materials and methods

2.1. Experimental design

Seeds were harvested from four *Chenopodium quinoa* (quinoa) cultivars (F14, F15, F16, and Titicaca) grown in a greenhouse under two water irrigation treatments: control conditions (WW, full irrigation, maintaining soil water content (SWC)~70%) or long-term water deficit (WD, reduced irrigation, SWC~35%), using 25 plants per condition and cultivar. Plants were grown under natural light conditions supplemented with high-pressure sodium lamps (photoperiod ranging from 9 to 15 h of light), with temperatures ranging from 15 to 20 °C, and relative humidity between 50% and 60% as described by Maestro-Gaitán et al. (2022). F14, F15, and F16 seeds were provided by the company Algosur S.A. (Lebrija, Spain) and Titicaca seeds were supplied by the company Quinoa Quality (Copenhagen, Denmark). F15 and Titicaca plants represent the growth habit 3, being the most branched genotypes. F14 plants show a growth habit 2, and F16 plants present a growth habit 1, with fewer branches (Maestro-Gaitán et al., 2022).

2.2. Yield

Seeds were manually collected and cleaned. The bulk of seeds were collected from either the primary panicle (PP) or the secondary panicles (SP) of individual plants and then were weighed using an analytical balance (Sartorius M-Pact AX224, Sartorius Mechatronics, Göttingen, Germany). The PP was considered the dense bundle of seed-bearing peduncles at the top of the primary stem that grew vertically and were separated by short internodes, while SP grew obliquely, were more dispersed along the stem, and were separated by larger internodes (at least twice the length of the PP internodes) often with leaves growing in between. Panicles growing in branches were also classified as SP.

2.3. Seed weight and seed area

To determine seed weight, 100 seeds per replication were manually counted and weighed using an analytical balance. Three replications per cultivar and treatment were used.

To determine seed area, three replications of 50 seeds per replicate were used. The images utilized for seed area measurements were taken using an Olympus SZ61 stereomicroscope (Olympus Corporation, Shinjuku, Tokyo, Japan) and processed with the AnalySIS GetIT image software (analysis getIT 5.1, Olympus Corporation, Shinjuku, Tokyo, Japan). The seed area was calculated using the open-source software ImageJ (<http://rsbweb.nih.gov/ij/>).

2.4. Seed germination rate

Quinoa seeds were sterilized by soaking them for 2 min in ethanol

70% (v/v), followed by 2 min incubation in bleach 50% (v/v) with a drop of Tween-20®, and 5 consecutive rinses with dH₂O. After sterilization, the seeds were sown in Petri® dishes with a double layer of wet filter paper and then transferred to a growth chamber under darkness and a controlled temperature of 25 °C (three replicates per condition, each including 50 seeds). The germination rate was analyzed daily. Seeds were considered germinated when the radicle protrusion was longer than 2 mm.

2.5. Seed viability

Seed viability was analyzed following the tetrazolium method (2,3,5-triphenyl-2 H-tetrazolium chloride) (Granado-Rodríguez, Vilarino-Rodríguez et al., 2021). Briefly, for imbibing seeds, they were submerged in distilled water (dH₂O) at 30 °C for 1 h. After that, seeds were superficially and longitudinally cut along the embryo to facilitate the dying of the embryo tissues using 1% (v/v) tetrazolium chloride for 2 h at 30 °C. Seeds presenting more than 50% of the embryo stained, were considered viable seeds. One hundred seeds per replicate were used, with 3 replicates per cultivar and treatment.

2.6. Proximate composition

Nitrogen determination was obtained by Elemental Microanalyzer TruSpec Micro CHN (LECO Instruments, Madrid, Spain). Crude protein content was estimated using a nitrogen-to-protein conversion factor of 6.25 (Nascimento et al., 2014).

Ash content was calculated from the gravimetric method (UNE 050:1994). One g of the sample was placed in a previously calibrated clay capsule and was heated on a hot plate to 200 °C. Subsequently, when the residue no longer gives off vapor, the sample was put in the electric oven at 550 °C for 36 h. After that, the capsule was weighed, and ash content was expressed in % respect to the initial sample.

Fat was determined using a conventional Soxhlet system (920.39 Method, (AOAC, 2012)). The seeds were ground in a mechanical mill and 5 g of the sample was placed in a cellulose thimble (25 × 88 mm, Albet, Barcelona, Spain). The overall Soxhlet glassware was fitted to a distillation flask containing 70 mL of n-hexane and 2–3 boiling glass regulators. After extraction for 8 h, the solvent was released to a rotary-evaporator and the extracted oil was weighed. The values were expressed as % of fat with respect to the raw material.

Total fiber (TF) was determined according to the enzymatic-gravimetric method. The preparation of the samples for the analysis and TF determinations were carried out according to the method described by Lee et al. (1992), using α-amylase heat stable (Sigma-Aldrich, protease Alcalase 2.4 UA/g Novozymes, Bagsvaerd, Denmark) and amyloglucosidase solution from *Aspergillus niger*, (Sigma-Aldrich Chemical Co Ltd., St Louis, MO, USA). The results of TF were obtained by subtraction from ash and protein, calculating as previously described. The values were expressed in % of fiber with respect to the raw material.

A hundred seeds were manually counted and weighed using an analytical balance before (fresh) and after drying them in an oven for 17 h at 103 °C (to obtain the dry weight) (AOAC, 2000). Water content was then calculated following the equation:

$$\text{water content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100.$$

All the parameters mentioned above included at least 3 replicates per condition and cultivar.

After measuring the protein, fat, ash, fiber, and water contents of seeds, carbohydrate (CH) content was calculated following the equation: $\text{CH} = 100 - \text{protein}\% - \text{fat}\% - \text{ash}\% - \text{fiber}\% - \text{water}\%$.

2.7. Mineral contents

The mineral content was analyzed following the standardized official method from the Spanish Ministry of Agriculture (MAPA, 1995).

Phosphorus (P) content was determined using a spectrophotometer UV-VIS (Hitachi U-2810, Hitachi High-Technologies Co., Tokyo, Japan) measuring at 430 nm. Potassium (K) was determined by flame atomic emission spectroscopy (SpectrAA 110, Agilent (Agilent Technologies, Palo Alto, Calif., USA)). Calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe) contents were assessed using flame atomic absorption spectroscopy (AAS) (SpectrAA 110, Agilent (Agilent Technologies, Palo Alto, Calif., USA)) after mineralizing the samples with H₂O and HCl (35%, v/v).

2.8. Ferric reducing antioxidant power (FRAP) assay and total phenolic content (TPC), and flavonoid content (TFC) quantifications

The evaluation of the antioxidant capacity of quinoa seeds was performed following the protocols previously described by Granado-Rodríguez et al. (2022). Seed extracts were obtained by homogenizing 100 mg of seed flour in 1 mL of ice-cold methanol (95%, v/v), using three replicates per condition. Samples were vortexed for 2 min and stored under dark conditions at 4 °C for 48 h. Then, samples were centrifuged at 135000 rpm for 15 min. Supernatants were stored at −20 °C until the antioxidant analysis was performed.

2.8.1. Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was composed of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃·6 H₂O at a 10:1:1 ratio (v/v/v). Each sample volume of 20 µL was mixed with 180 µL of FRAP reagent in a 96-well microplate, and, after a 4 min incubation, absorbance was measured at 593 nm using a microplate reader (Lector Multi-ModalSynergy HTX, BioTek Instruments, Inc., Winooski, VT, USA). The antioxidant capacity was calculated using a calibration curve with iron (II) sulfate (FeSO₄). FRAP values were expressed as µmol of Fe²⁺/g of seed.

2.8.2. Total phenol content (TPC)

One hundred µL of the methanol seed extract (previously described) was mixed with 200 µL of Folin-Ciocalteu reagent (10%, v/v). After vortexing for 1 min, 800 µL of sodium carbonate (7.5%, v/v) was added, followed by 2 h incubation in darkness. Samples were then centrifuged to discard possible precipitates formed during the reaction. Two hundred µL of the supernatant was transferred to a 96-well microplate. Absorbance was measured at 765 nm using a microplate reader (Lector Multi-ModalSynergy HTX, BioTek Instruments, Inc., Winooski, VT, USA). Gallic acid with concentrations diluted in methanol 95% (v/v) ranging between 20 and 200 µg/mL was used as standard. TPC was expressed as mg of gallic acid equivalents per gram of seed (mg GAE/g).

2.8.3. Total flavonoid content (TFC)

Thirty µL of seed methanol extracts were added to a mixture consisting of 10 µL of sodium acetate (NaC₂H₃O₂) 1 M, 10 µL of aluminum chloride (AlCl₃) 10% v/v, and 250 µL of dH₂O. After 30 min incubation, absorbance was read at 415 nm using a microplate reader (Lector Multi-ModalSynergy HTX, BioTek Instruments, Inc., Winooski, VT, USA). Different concentrations of quercetin dissolved in ethanol (80%, v/v), ranging from 10 to 140 µg/mL, were used as a standard. TFC was expressed in mg of quercetin equivalents per gram of seed (mg QE/g).

2.9. Statistical analysis

To analyze the influence of the cultivar, the water treatment, and the panicle type on seed nutritional quality-related parameters, a three-way ANOVA was performed. Normality and homoscedasticity of the data was tested through Kolmogorov-Smirnov and Levene tests, respectively. For those variables in which normality and equal variances could be assumed, a one-way ANOVA test was performed, followed by a Tukey post-hoc test, to perform multiple comparisons at a probability level of 5% ($p < 0.05$). A Kruskal Wallis test by ranks was performed when data

did not present a normal distribution, and a Welch ANOVA test followed by a Games-Howell post-hoc test was performed when variances were not equal, both, at a probability level of 5% ($p < 0.05$). Student's t-test or U-Mann Whitney test were carried out for pair comparisons, when data distribution was or was not normal, respectively. A Pearson test was used to analyze correlations among variables for each cultivar. A Principal Component Analysis (PCA) was performed including all the parameters studied in order to reduce the variables affected by the factors analyzed in this study. The SPSS Statistics 26.0 (IBM SPSS Inc., New York, NY, USA) package was used for the statistical analyses. Correlograms were plotted using the corrplot package (v0.92) (Wei and Simko, 2021) running under R (v4.0.2) (R Core Team, 2021) in RStudio (1.4.1717) (RStudio Team, 2021).

3. Results

3.1. Yield

Under WW conditions, Titicaca was the cultivar showing the highest average total yield (PP+SP), 24.91 g/plant, compared to the other cultivars, whose yields ranged from 14.96 to 19.03 g/plant ($p = 0.024$) (Fig. 1). F16 maintained an average yield of 15.43 g/plant ($p = 0.849$) under WD, while the other cultivars showed significant yield penalties under stress conditions, with yields ranging from 11.41 to 13.94 g/plant. These reductions were mainly due to the impact of WD on the seed yield of the SP. In the cultivars F14, F15, and Titicaca, the SP contributed 57%, 63%, and 63%, respectively, to the total yield but their productivity was reduced under WD ($p = 0.004$, $p < 0.001$, and $p = 0.004$, respectively) dropping to 52%, 44%, and 44%, respectively (Fig. 1). Meanwhile, the yield of the PP did not show a significant impact according to the cultivar nor the water treatment ($p = 0.054$) and was not significantly reduced in any of the cultivars under WD conditions

($p = 0.221$, $p = 0.255$, $p = 1$, and $p = 0.351$, in F14, F15, F16, and Titicaca, respectively). Opposite to the other genotypes, F16 showed a seed yield concentrated in the PP ($\geq 63\%$ of total yield, Fig. 1) and no yield penalties were found in F16 PP or SP under WD conditions ($p = 0.511$).

3.2. Seed size

The seed area was significantly affected by the genotype ($p < 0.001$), the water treatment ($p = 0.001$), and the type of panicle ($p < 0.001$). The largest seeds belonged to the F16 cultivar, with areas ranging between 3.35 and 4.75 mm², while the smallest seeds were obtained from F15 plants and showed areas between 2.43 and 3.03 mm² (Fig. 2A, B). The seed area was larger in seeds from the PP and in seeds from WW plants, although the differences between water treatments were only significant in seeds from PP ($p = 0.004$) but not from SP ($p = 0.064$) (Fig. 2A, B).

The highest weight of 100 seeds was found in F16, ranging from 0.33 to 0.44 g, while the lowest in F15 seeds, varying from 0.22 to 0.28 g ($p < 0.001$). Overall, PP yielded heavier seeds than SP ($p < 0.001$), and no effect was associated to water treatment on seeds weight ($p = 0.109$) (Fig. 2C).

3.3. Germination rates and seed viability

The germination capacity after 1 day was very high in all the groups tested, ranging between 95% and 100% (Fig. 3A). Interestingly, genotype and water treatment did not significantly affect germination rate ($p = 0.126$ and $p = 0.212$, respectively), but seeds coming from the SP showed lower rates ($p = 0.045$).

The seed viability was not affected by the cultivar or water treatment ($p = 0.050$ and 0.071 , respectively). However, differences between

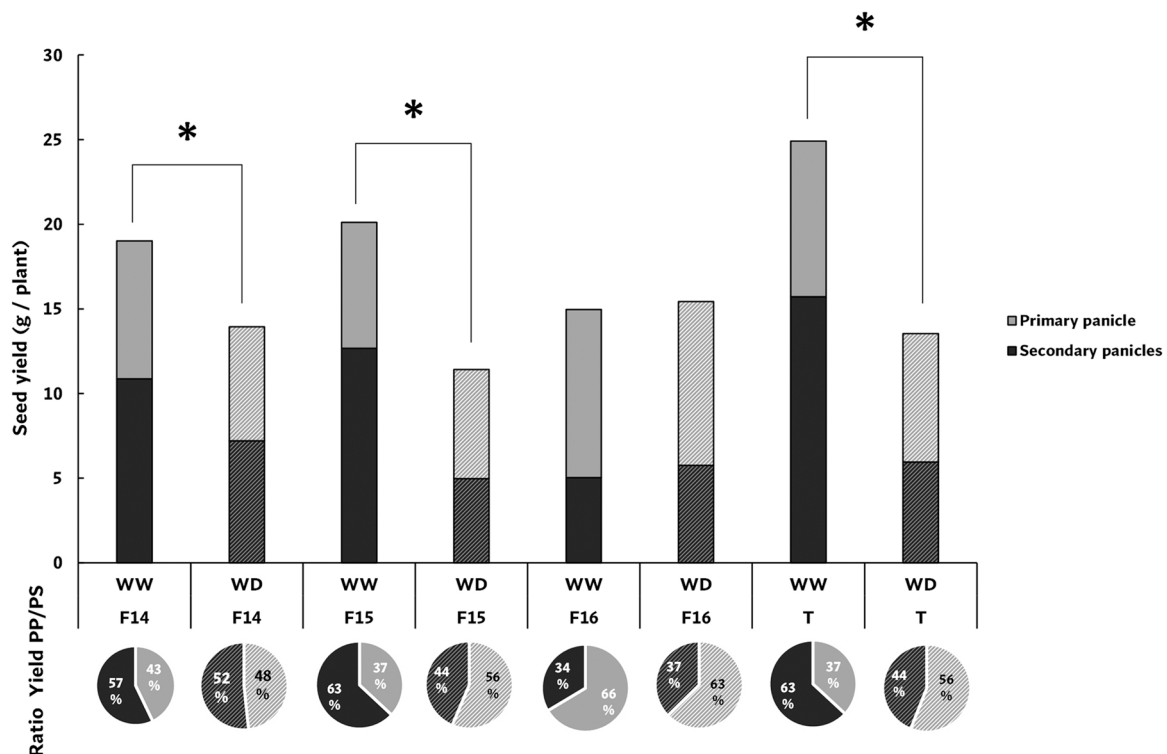


Fig. 1. Total seed yield of the four quinoa genotypes grown under two water treatments (WW or WD). Total seed yield (g of seeds/plant) is expressed as the seed yield provided by the primary panicle (PP) (dark columns) and secondary panicles (SP) (grey columns). Seed yield was determined at physiological maturity in the four genotypes (F14, F15, F16, and Titicaca (T)) that were grown under control water treatment (WW, solid columns) and water deficit treatment (WD, striped columns). Error bars represent the standard deviation (SD). U-Mann Whitney test was applied in the comparison between treatments in each cultivar for both panicle types. Asterisks (*) show statistically significant differences at a p -value < 0.05 .

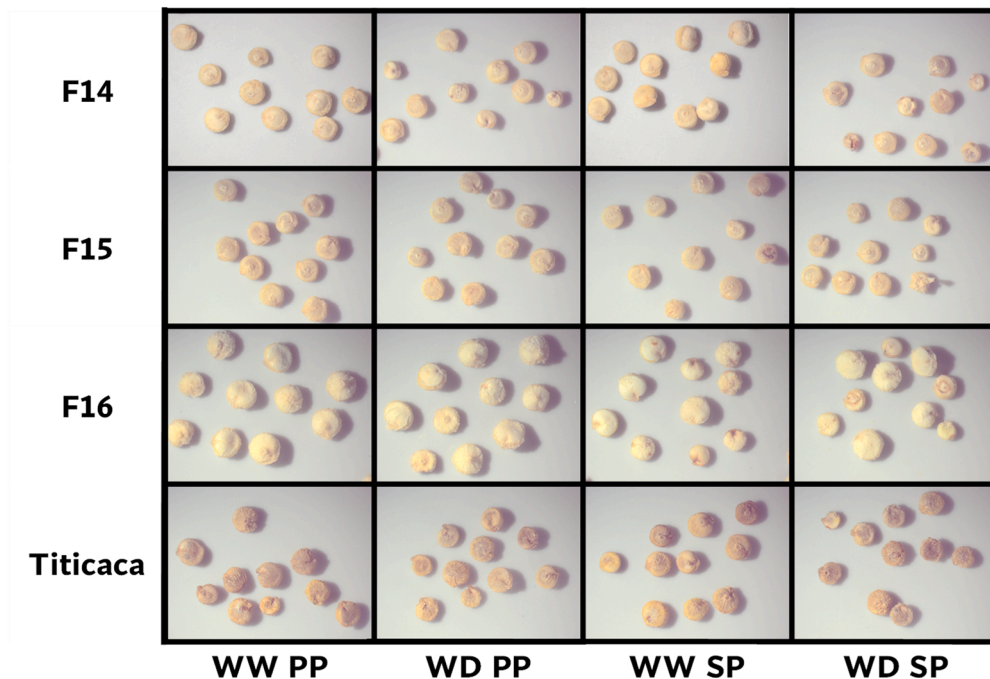
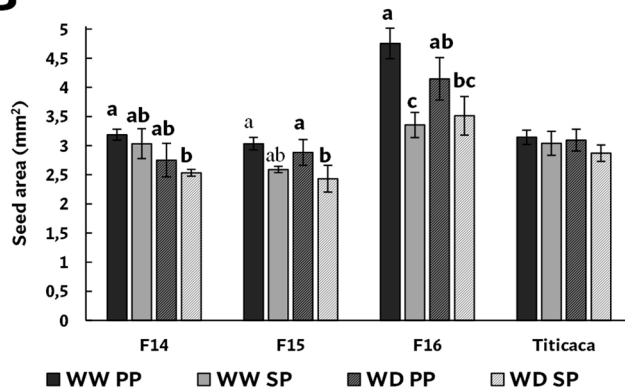
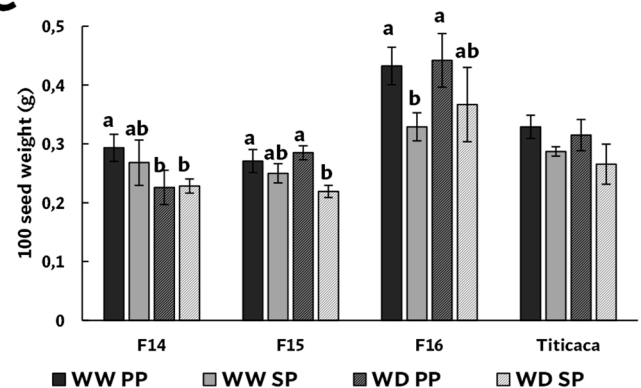
A**B****C**

Fig. 2. Seed area and 100 seed weight of the four quinoa genotypes grown under two water treatments (WW or WD). A) Images of the seeds obtained from the four quinoa genotypes studied (F14, F15, F16 and Titicaca) subjected to full irrigation (WW) or water deficit (WD). B) Seed area (mm²) and C) seed weight (g) of 100 seeds were determined for each cultivar, water treatment, and type of panicle (primary or secondary). Error bars represent the standard deviation (SD). Bars that do not share the same letters show statistically significant differences depending on the cultivar. A Welch ANOVA test followed by Games-Howell post hoc at a p -value < 0.05 was performed in F14 seed area, and a One-Way ANOVA followed by Tukey post hoc test at a p -value < 0.05 in F15, F16 and Titicaca seed area. A One-Way ANOVA followed by Tukey post hoc test at a p -value < 0.05 was used for the cultivars F14, F15 and F16, and a Welch ANOVA followed by Games-Howell post hoc test at a p -value < 0.05 for the cultivar Titicaca.

panicle types were remarkable ($p < 0.001$): seeds from PP presented a seed viability higher than 84%, while seeds from SP (except SP seeds from WW Titicaca, whose seed viability was 75%) did not exceed 60% (Fig. 3B).

3.4. Proximate composition analysis

The overall seed protein content was higher in F15 (17.63% and 20.31% in WW and WD, respectively) than in F16 (15.78% and 15.77% in WW and WD, respectively) or Titicaca (16.96% and 16.65%, respectively), showing a strong influence of the genotype in this parameter ($p < 0.001$). In addition, seed protein content also increased in plants growing under WD ($p = 0.004$) (Table 1), and the 3-Way ANOVA analysis showed that the panicle type was also determinant of

this parameter ($p < 0.001$), with higher protein contents in seeds coming from SP (Table 1). Furthermore, the influence of WD was only significant in the case of seeds from SP ($p = 0.001$), increasing F15 SP seed protein content from 17.67% to 21.73% ($p = 0.013$) (Table 1). On the contrary, seeds from the PP did not show significant differences depending on the cultivar or the water treatment ($p = 0.158$).

The fat content was higher in Titicaca ($p = 0.001$) than in F14 and F16 seeds, especially in SP ($p = 0.008$), with contents close to 5%. (Table 1). There was also a significant effect of the water treatment on the fat content ($p = 0.007$), with a reduction of 16–18% in WD, except for Titicaca seeds. However, this decrease was only significant in the PP ($p = 0.007$). The type of panicle did not show an overall effect on the fat content, except for F15 WW seeds, which showed higher contents in the PP ($p = 0.049$), and WW Titicaca seeds, with lower contents in the PP

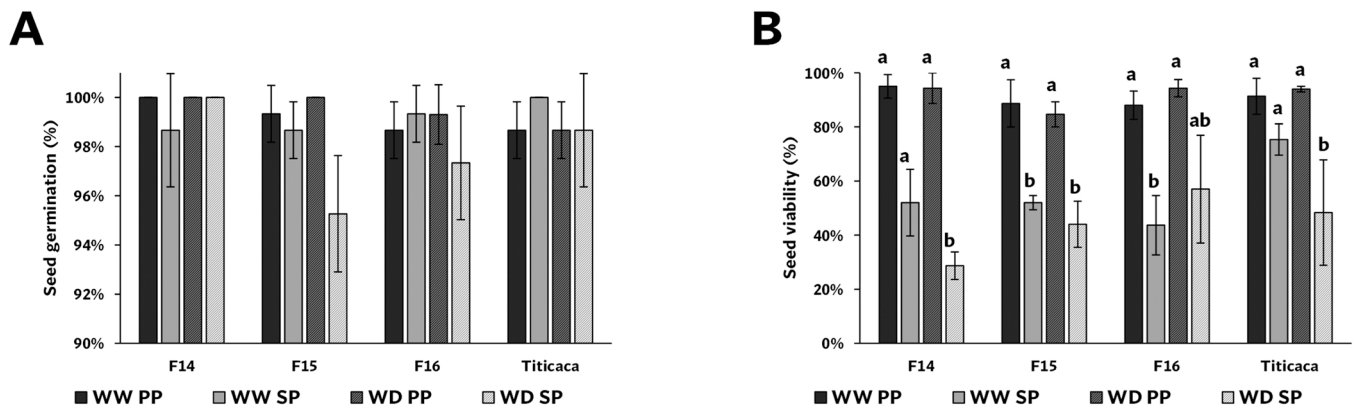


Fig. 3. Seed germination and seed viability of the four quinoa genotypes grown under two water treatments (WW or WD). A) Seed germination (%) 1 day after sowing (d.a.s) and B) seed viability (%) were calculated for each cultivar (F14, F15, F16 and Titicaca), water treatment and type of panicle (PP and SP). Error bars represent the standard deviation (SD). Bars that do not share the same letters show statistically significant differences among cultivars; Seed viability was analyzed following a Kruskal Wallis test at a p -value < 0.05 for the cultivars F14 and Titicaca, a One-Way ANOVA followed by Tukey post hoc test at a p -value < 0.05 for the cultivar F15 and a Welch ANOVA followed by Games-Howell post hoc test at a p -value < 0.05 for the cultivar F16.

Table 1

Proximate composition analysis in seeds obtained from four quinoa genotypes growing under full irrigation (WW) and water deficit conditions (WD). Data is presented as Mean \pm SD of protein, fats, ashes, fiber, and carbohydrates (CH) contents (%). A multiple comparison was performed by following a Welch ANOVA with Games Howell post-hoc at a p -value < 0.05 for %Protein content, %Fats, %Ashes and %Fiber and a Kruskal Wallis test at a p -value < 0.05 for %CH. In this case, lower case letters show differences, within each parameter, among cultivars, water treatment and panicle types. Capital letters represent differences among cultivars applying a Tukey post hoc test at a p -value < 0.05 for %Protein, %Fats, %Ashes and %Fiber and a Kruskal Wallis test at a p -value < 0.05 for %CH. Differences between panicle types or water treatments, which appear at the bottom of the Table, were analyzed at a p -value < 0.05 following a Tukey post hoc test for %Protein, %Fats, %Ashes and %Fiber and a U-Mann Whitney pairwise comparison for %CH.

Multiple comparisons															
Sample	% Protein			% Fats			% Ashes			%Fiber			% Carbohydrates		
F14 WW PP	15.50	abc	AB	5.43	ab	B	7.28	ab	AB	8.37	a	AB	49.67	bc	B
	± 2.19			± 0.52			± 3.00			± 0.42			± 4.92		
F14 WW SP	16.58	abc		4.33	ab		6.08	ab		10.01	ab		51.72	abc	
	± 2.29			± 1.80			± 1.70			± 1.62			± 5.23		
F14 WD PP	17.81	abc		3.36	ab		5.19	ab		8.16	ab		50.37	abc	
	± 0.89			± 1.01			± 2.89			± 0.90			± 5.27		
F14 WD SP	19.60	ab		4.30	ab		4.50	ab		9.45	ab		48.98	abc	
	± 0.22			± 0.45			± 1.09			± 1.13			± 0.63		
F15 WW PP	17.05	abc	A	5.50	a	AB	9.05	a	A	8.89	ab	B	47.92	bc	B
	± 1.55			± 0.24			± 0.63			± 0.98			± 1.07		
F15 WW SP	17.67	abc		4.60	ab		9.08	ab		8.73	ab		48.27	abc	
	± 1.36			± 0.51			± 1.34			± 0.71			± 1.45		
F15 WD PP	18.90	abc		4.66	ab		4.75	ab		6.98	ab		52.87	abc	
	± 1.87			± 1.09			± 1.76			± 1.77			± 1.61		
F15 WD SP	21.73	a		3.83	bc		6.88	a		9.42	a		45.64	c	
	± 0.93			± 0.29			± 0.46			± 0.10			± 1.12		
F16 WW PP	15.85	bc	C	4.45	ab	B	6.79	ab	B	9.26	a	A	51.48	abc	A
	± 0.79			± 0.51			± 0.30			± 0.43			± 0.98		
F16 WW SP	15.71	c		4.13	ab		4.29	ab		10.32	ab		52.81	abc	
	± 0.19			± 0.45			± 1.63			± 1.22			± 0.98		
F16 WD PP	15.79	c		3.52	b		6.01	a		10.20	a		52.51	abc	
	± 0.65			± 0.25			± 0.24			± 0.54			± 1.58		
F16 WD SP	15.75	c		3.51	ab		5.35	ab		9.13	ab		53.88	abc	
	± 0.66			± 0.76			± 2.06			± 0.94			± 1.62		
Titicaca WW PP	15.96	abc	BC	4.36	ab	A	1.57	b	B	8.59	a	B	57.13	a	A
	± 2.37			± 0.48			± 0.03			± 0.38			± 1.54		
Titicaca WW SP	17.96	abc		6.08	ab		3.99	ab		9.52	ab		49.98	abc	
	± 2.15			± 0.61			± 1.80			± 0.74			± 2.97		
Titicaca WD PP	16.83	bc		5.04	ac		9.04	ab		6.47	b		51.02	abc	
	± 0.7			± 0.22			± 1.36			± 0.27			± 1.62		
Titicaca WD SP	16.47	abc		5.14	ab		4.38	ab		7.77	ab		54.29	ab	
	± 1.31			± 1.10			± 2.36			± 1.53			± 1.04		
Pairwise comparisons															
Treatment	WW<WD			WW>WD			WW=WD			WW>WD			WW=WD		
Panicle	PP<SP			PP=PS			PP=PS			PP<SP			PP=SP		

($p = 0.019$) (Table 1).

The ash content varied with the cultivar ($p = 0.004$) and was generally higher in F15 seeds than in Titicaca, although particularly in the SP, the ash content was higher in F15 seeds than in F16 or Titicaca

($p = 0.006$) (Table 1). When focusing on the effect of water stress, it was not consistent throughout cultivars ($p = 0.604$), causing a 36% decrease in ashes from F15 seeds, and a 142% increase in ashes from Titicaca seeds. These changes were significant in seeds from the PP, but not in

seeds from the SP ($p > 0.055$) (Table 1), although ash content did not show a significant change between panicle types ($p = 0.195$).

The fiber content of quinoa seeds was influenced by the three factors analyzed, the genotype ($p = 0.001$), the water treatment ($p = 0.008$), and the type of panicle ($p = 0.002$) (Table 1). Therefore, the fiber content, which ranged between 6.47% and 10.32%, was generally higher in F16 seeds than in F15 or Titicaca; in seeds from WW plants than WD (PP); and in seeds from SP than the ones from PP (Table 1).

Lastly, the carbohydrate (CH) content of overall seeds ranged between 45.64% and 57.13%, but this was not significantly influenced by any of the factors analyzed (3-Way ANOVA $p = 0.079$). However, the CH content of seeds from SP did show a significant effect of the genotype, with higher contents found in F16 and Titicaca seeds than in F15, contrary to the patterns shown by the ash and protein contents in SP seeds (Table 1). Furthermore, in the PP, WD increased the CH content in F15 seeds ($p = 0.011$) and decreased in Titicaca seeds ($p = 0.009$).

3.5. Mineral content

All minerals measured, except potassium (K) and magnesium (Mg), fit into the genotype \times water treatment \times panicle type model and were significantly affected by, at least, the genotype factor (Table 2). In the case of zinc (Zn), F16 seeds showed the lowest content ($p = 0.001$), ranging between 21.38 and 32.51 $\mu\text{g/g}$, compared to the rest of the cultivars whose contents ranged between 27.34 and 41.45 $\mu\text{g/g}$ (Table 2). In the case of phosphorous (P), iron (Fe), and manganese (Mn) ($p < 0.001$), the lowest contents were also found in F16 seeds, ranging between 0.40% and 0.51% (P), 30.14 and 45.88 $\mu\text{g/g}$ (Fe), and 25.20 and 51.25 $\mu\text{g/g}$ (Mn). By contrast, the highest mineral contents were found in F15 seeds, which ranged between 0.58% and 0.81% (P), 69.98 and 102.19 $\mu\text{g/g}$ (Fe), and 69.00 and 102.18 $\mu\text{g/g}$ (Mn) (Table 2). In the case of copper (Cu), F15 seeds also showed the highest content ($p = 0.010$), ranging between 9.72 and 12.80 $\mu\text{g/g}$ (Table 2), while the lowest content was found in Titicaca seeds, ranging from 8.34 to 10.46 $\mu\text{g/g}$. In addition, Titicaca seeds also had the lowest content of calcium (Ca) ($p < 0.001$), with contents ranging between 0.04% and 0.24%, compared to the rest of the cultivars, which showed values between 0.08% and 0.39% (Table 2). On the other hand, the highest content of sodium (Na) ($p < 0.001$) was found in Titicaca, ranging from 90.57 to 317.55 $\mu\text{g/g}$ compared to the other cultivars, which ranged between 41.61 and 135.44 $\mu\text{g/g}$. Although the K content did not fit in the 3-Way ANOVA model ($p = 0.335$), there were significant differences when comparing genotypes, finding higher K contents in F15 than in F16 seeds (performing a pairwise comparison ($p = 0.026$), Table 2).

The panicle type constituted a significant factor for most minerals, including Ca, Fe, Cu, Mn, and Zn (with a $p = 0.032$ in Cu and $p < 0.001$ for the rest). Moreover, in all these minerals, contents were higher in seeds from SPs, except for Cu, which showed lower values in SP (Table 2). Meanwhile, when comparing between water treatments, a significant decrease under WD conditions of the overall Na content ($p < 0.001$) was observed together with a reduced Mn content in the cultivars F14 ($p = 0.001$) and F16 ($p = 0.031$), and Zn content in seeds from the PP ($p = 0.019$). On the other hand, WD caused an increase in the overall Fe content ($p < 0.001$) (Table 2).

3.6. Antioxidant capacity

The phenolic content was generally influenced by the genotype ($p < 0.001$), with higher contents found in Titicaca seeds, ranging from 0.56 to 1.08 mg GAE/g seed, compared to the contents of F15 and F16 seeds, which varied between 0.45 and 0.65 mg GAE/g seed (Fig. 4A). On the contrary, the water treatment ($p = 0.621$) and the type of panicle ($p = 0.167$) did not influence the total phenolic content. However, the phenolic contents of the SP seeds were higher in the case of F14 WD ($p = 0.049$), F16 WW ($p = 0.045$), and F16 WD seeds ($p = 0.033$); and the water stress increased the contents of phenols in Titicaca PP seeds

($p = 0.050$) and F14 SP seeds ($p = 0.039$) (Fig. 4A).

The flavonoid content was also influenced by the genotype ($p < 0.001$). However, it was not affected by the water treatment ($p = 0.203$), nor the type of panicle ($p = 0.176$) (Fig. 4B). F16 was the cultivar that yielded the lowest flavonoid content, ranging from 0.69 to 0.91 mg QE/g seed, compared to the rest of the cultivars, which showed contents ranging from 0.98 to 1.67 mg QE/g seed. The only significant difference found in TFC between panicle types was the higher content in Titicaca PP seeds under WD ($p = 0.623$) (Fig. 4B).

In line with the phenolic and flavonoid content results, the antioxidant capacity (FRAP) was not influenced by the water treatment ($p = 0.560$), nor the type of panicle ($p = 0.086$), but the genotype factor showed significant differences ($p < 0.001$). Titicaca was the cultivar whose seeds showed the highest FRAP values, ranging from 5.74 to 8.70 $\mu\text{mol of Fe}^{2+}/\text{g seed}$ while F16 was the cultivar showing the lowest activity (2.19 and 2.95 $\mu\text{mol of Fe}^{2+}/\text{g seed}$). Furthermore, F16 seeds under WD were the only ones that showed higher antioxidant activity in SP seeds than in PP seeds ($p = 0.035$) (Fig. 4C).

3.7. Correlations and principal components analysis (PCA)

High positive correlations were found between P and K seed contents ($r = 0.913$), and among those two minerals and Fe, Mn, and protein contents ($r > 0.586$) (Fig. 5). In addition, seed area negatively correlated with P, K, Fe, Mn, Zn, protein content and total flavonoids ($r < -0.544$), while it positively correlated with seed weight ($r = 0.947$) (Fig. 5). At the same time, the antioxidant parameters (antioxidant capacity (FRAP), TPC, and TFC) showed strong positive correlations with each other ($r > 0.796$) and with the Mg content, while negative correlations with fiber and Ca contents were found ($r < -0.498$) (Fig. 5).

The PCA resulted in 6 different principal components (PC) explaining 84.29% of the total variance. The PC1 explained 28.81% of the variance and was mainly represented by protein, P, K, Ca, Fe, Mn, Zn, and flavonoid contents, and negatively by the seed area, weight, and seed viability rate (Fig. 6). This new variable was notably influenced by the genotype, with F16 seeds showing the lowest PC1 values (between -1.74 and -0.73), F15 showing the highest (between 0.19 and 1.71), and F14 and Titicaca showing values close to 0 (between -0.44 and 0.73). Additionally, the type of panicle also influenced this variable with higher PC1 values found in SP, especially in F16, but not in F15 seeds. Importantly, F15 and F14 seeds showed an important effect of the water treatment on the PC1 values, with higher values shown under WD (Fig. 6).

The PC2 represented 18.37% of the variance and was mainly explained by the Na and Mg seed contents, the antioxidant activity (FRAP), TPC and TFC, and negatively by the Ca and fiber contents (Fig. 6). The genotype showed some influence in the PC2 values, being Titicaca seeds the ones showing higher PC2 values (from -0.10 to -2.51), F16 seeds showing negative values (between -1.22 and -0.34), and F14 and F15 seeds ranging around 0 (between -1.37 and 0.36). Besides, the water treatment also affected the PC2 values in F15 and F16 seeds, which showed higher values in the case of WW plants. Lastly, PC2 also yielded differences between panicle types within genotypes with Titicaca PP seeds being higher but lower values than SP seeds in the F15 cultivar (Fig. 6).

The PC3 represented 11.62% of the variance and was mainly comprised of fiber, Ca, Mg, Cu, and Zn contents, and negatively by the seed viability and germination rates. The genotype did not show significant differences in the PC3 values, but the seeds from SP showed higher PC3 compared to PP in all cultivars, while the water treatment only affected F16 and Titicaca seeds increasing the PC3 values in WW plants. The PC4 explained 9.1% of the variance and was mainly represented by the CH and Na contents and negatively by the ash contents, although the three factors studied did not significantly affect the PC4 values. The PC5, explaining 8.38% of the variance, represented by the seed yield, fat content, and germination rate, was not affected by the

Table 2

Mineral contents in seeds obtained from four quinoa genotypes growing under full irrigation (WW) and water deficit conditions (WD). Data is presented as Mean \pm SD. Units correspond to the percentage of seed weight (%) for P, K, Ca, and Mg, or as mg/Kg for Na, Fe, Cu, Mn, and Zn. A One-Way ANOVA was performed followed by a multiple comparison using Tukey post hoc at a p -value < 0.05 for the parameters %P, %K, %Ca, %Cu and %Zn. A Welch ANOVA followed by Games Howell post-hoc at a p -value < 0.05 was used for the parameters Fe (mg/Kg) and Mn (mg/Kg). A Kruskal Wallis test at a p -value < 0.05 for Na (mg/Kg) and %Mg. Lower case letters show differences, within each parameter, among cultivars, water treatment and panicle types. Capital letters represent differences among cultivars applying a Tukey post hoc test at a p -value < 0.05 . Differences between panicle types or water treatments, which appear at the bottom of the Table, were analyzed at a p -value < 0.05 following a Tukey post hoc test.

Multiple Comparison																														
Sample	P (%)			K (%)			Na (mg/Kg)			Ca (%)			Mg (%)			Fe (mg/Kg)			Cu (mg/Kg)			Mn (mg/Kg)			Zn (mg/Kg)					
F14 WW PP	0.49	bc	B	1.02	a	72.82	fg	B	0.14	cde	A	0.27	a	57.11	abcde	B	9.63	abc	AB	68.27	abc	B	27.34	bc	A					
	± 0.12			± 0.28		± 9.89			± 0.06			± 0.05		± 9.79			± 1.59			± 1.07			± 3.86							
F14 WW SP	0.59	abc		1.19	a	115.7	abcdef		0.35	ab		0.31 ± 0	a	61.28	bcd		8.01	c		84.60	ab		34.63	ab						
	± 0.07			± 0.24		± 31.5			± 0.11					± 5.77			± 1.28			± 3.87			± 1.38							
F14 WD PP	0.48	bc		1.03	a	61.75	fhg		0.18	bcde		0.27	a	75.65	c		11.15	abc		50.51	d		27.35	bc						
	± 0.15			± 0.28		± 14.79			± 0.03			± 0.03		± 0.94			± 2.31			± 3.08			± 6.24							
F14 WD SP	0.68	ab		1.31	a	87.91	cdef		0.37	ab		0.33	a	95.92	d		13.17	a		85.54	abcd		36.63	ab						
	± 0.09			± 0.18		± 10.52			± 0.03			± 0.08		± 0.08			± 2.79			± 11.52			± 7.31							
F15 WW PP	0.58	abc	A	1.17	a	41.61	h	B	0.34	ab	A	0.29	a	69.98	abcd	A	11.93	abc	A	69.00	abcde	A	31.68	abc	A					
	± 0.11			± 0.18		± 9.02			± 0.07			± 0.04		± 6.63			± 1.72			± 11.40			± 4.53							
F15 WW SP	0.69	ab		1.33	a	120.68	abcd		0.23	abcde		0.33	a	96.6	abc		12.57	ab		89.42	ab		37.09	ab						
	± 0.05			± 0.17		± 26.62			± 0.09			± 0.03		± 10.03			± 0.91			± 6.18			± 2.13							
F15 WD PP	0.81	a		1.54	a	85.31	cdefg		0.35	ab		0.20	a	77.08	abcde		9.72	abc		89.87	ab		28.13	bc						
	± 0.14			± 0.12		± 11.94			± 0.03			± 0.06		± 21.65			± 1.94			± 6.90			± 4.79							
F15 WD SP	0.67	abc		1.40	a	135.44	ab		0.39	a		0.32	a	102.19	abcde		12.80	a		102.18	a		41.45	a						
	± 0.03			± 0.13		± 11.94			± 0.01			± 0.03		± 18.58			± 0.83			± 8.59			± 3.33							
F16 WW PP	0.43 ± 0	bc	C	1.06	a	111.90	abc	B	0.25	abcd	A	0.34	a	34.52	de	C	10.24	abc	AB	41.16	cde	C	27.57	bc	B					
				± 0.42		± 5.07			± 0.07			± 0.13		± 2.05			± 1.28			± 6.60			± 0.71							
F16 WW SP	0.48	bc		1.02	a	83.6	efg		0.33	abc		0.30	a	45.88	abcde		10.98	abc		51.35	e		32.51	abc						
	± 0.03			± 0.03		± 6.53			± 0.1			± 0.03		± 13.59			± 1.92			± 9.76			± 3.81							
F16 WD PP	0.40	c		1.05	a	53.39	gh		0.08	de		0.22	a	30.14	e		8.77	abc		25.20	de		21.38	c						
	± 0.11			± 0.30		± 2.32			± 0.02			± 0.06		± 4.21			± 0.9			± 5.29			± 4.33							
F16 WD SP	0.51	bc		1.12	a	83.33	defg		0.38	a		0.27	a	40.58	de		12.22	abc		36.91	abcd		25.99	bc						
	± 0.01			± 0.10		± 8.57			± 0.12			± 0.01		± 5.4			± 0.31			± 2.86			± 2.79							
Titicaca WW PP	0.62	abc	AB	1.13	a	317.55	a	A	0.07	de	B	0.36	a	65.4	bcd	AB	10.39	abc	B	61.16	abcd	B	34.72	ab	A					
	± 0.01			± 0.07		± 52.17			± 0.01			± 0.04		± 6.12			± 0.75			± 7.29			± 2.89							
Titicaca WW SP	0.62	abc		1.13	a	108.04	abcde		0.21	abcde		0.35	a	51.39	cde		10.46	abc		62.57	abcde		33.42	abc						
	± 0.16			± 0.37		± 2.03			± 0.01			± 0.08		± 5.65			± 0.45			± 20.88			± 5.80							
Titicaca WD PP	0.57	abc		1.15	a	90.57	bcdefg		0.04	e		0.40	a	84.06	abcd		9.14	abc		57.33	cd		30.15	abc						
	± 0.04			± 0.15		± 11.73			± 0.02			± 0.19		± 10.24			± 1.25			± 5.01			± 1.38							
Titicaca WD SP	0.55	abc		1.14	a	92.80	bcdef		0.24	abcd		0.29	a	108.81	a		8.34	bc		81.89	ab		33.01	abc						
	± 0.11			± 0.34		± 2.32			± 0.04			± 0.07		± 0.37			± 0.22			± 2.94			± 4.63							
Pairwise Comparison																														
Treatment	WW = WD						WW > WD						WW = WD						WW < WD						WW = WD					
Panicle	PP = SP						PP = SP						PP < SP						PP < SP						PP > SP					

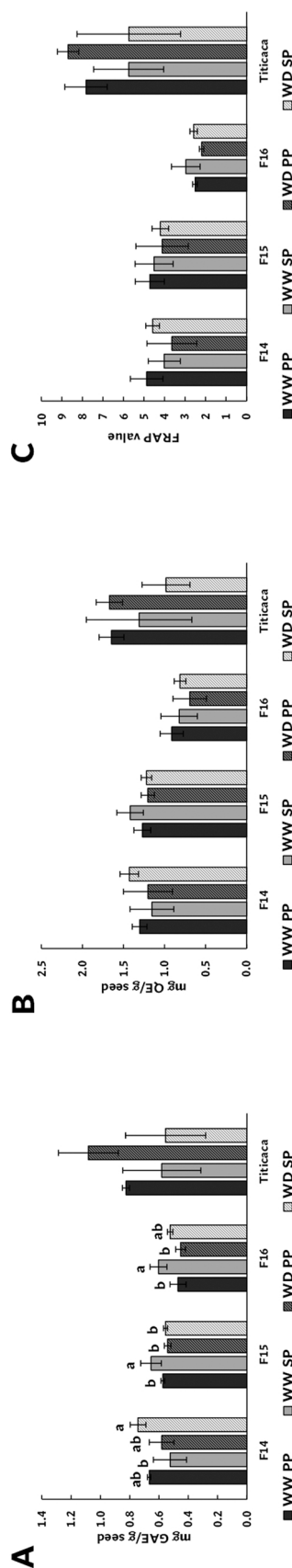


Fig. 4. Antioxidant capacity of seeds obtained from four quinoa genotypes grown under two water treatments, full irrigation (WW) or water deficit (WD). A) Total Polyphenol Content (TPC) expressed as mg of gallic acid equivalents (GAE) per g of seeds. Bars that do not share the same letters show statistically significant differences depending on the cultivar applying a Kruskal Wallis test at a p -value < 0.05 for the cultivar F15, and a One-Way ANOVA followed by Tukey post hoc at a p -value < 0.05 for the cultivars F14, F16 and Titicaca. B) Total Flavonoid Content (TFC) is expressed as mg of quercetin equivalents (QE) per g of seeds. A One-Way ANOVA followed by Tukey post hoc at a p -value < 0.05 was used for TFC statistical analysis. C) antioxidant capacity of the seed extracts was determined by FRAP assay and expressed as $\mu\text{mol of Fe}^{2+}$ per g of seed. Kruskal Wallis test at a p -value < 0.05 was used for the cultivar F15, and a One-Way ANOVA followed by Tukey post hoc at a p -value < 0.05 for the cultivars F14, F16 and Titicaca. Error bars represent the standard deviation (SD) for all the three parameters.

genotype nor the panicle type but did show higher values in WW seeds in all cultivars the PC6 represented 8.01% of the variance and was only significantly and negatively explained by the water content of the seeds. The type of panicle and water treatment did not consistently affect the PC6, but F14 seeds showed the lowest PC6 values compared to the rest of the cultivars.

4. Discussion

Quinoa is a plant species that presents a wide genetic diversity, which in turn results in great differences in plant morphology, adaptability to diverse environmental stresses, panicle and seed color, or seed quality and size, among other features (Rojas et al., 2015). Plant morphology in quinoa constitutes a very important trait for producers and farmers, since it can determine the type and scale of harvesting management (that will be discussed later in the text) (Gómez-Pando and Aguilar-Castellanos, 2016), whether the plant can be used for animal feed or human consumption (Rojas and Pinto, 2013), or even the tolerance to water stress, as was recently reported by Maestro-Gaitán et al. (2022). In the current study, the same genotypes as in Maestro-Gaitán et al., 2022 were used. They presented various patterns of branching, habit 2 in the case of F14, habit 3 in the case of F15 and Titicaca, and a simple habit or growth habit 1 in F16 (Bioversity International et al., 2013), with few branches in the higher part of the plant and a small number of leaves (Maestro-Gaitán et al., 2022). In that study, it was suggested that the lower number of leaves of the genotype F16 compared to the other genotypes contributed to its tolerance to long-term water stress, since the foliar surface was lower and prevented the higher water loss via transpiration. In the present study, it is proposed that the plant morphology and strategies against water deprivation of each genotype will impact the quality of their seeds. Thus, we separately evaluated the quality of seeds coming from primary panicles (PP, located in the apical part of the plant), and secondary panicles (SP, located in branches along the plant height) from plants grown under well-watered conditions (WW) or long-term water deficit (WD).

We found that the simple habit genotype, F16, was able to maintain seed yield under WD conditions while the other genotypes, which presented higher branching, reduced their SP yield under water stress (Fig. 1). There are two general explanations for the decreased in yield in crops growing under water stress. One reason is that photosynthesis could be inhibited due to stomatal closure as a response to water stress (Bewley et al., 2013; Maestro-Gaitán et al., 2022). This would result in the production of fewer photoassimilates, as primary carbon assimilation is reduced, producing a decrease in starch for seed filling (Y. Wang and Frei, 2011). The former study performed with the same cultivars showed that there was a significant inhibition of the photosynthetic activity in F14 and F15 leaves at seed filling stage (Maestro-Gaitán et al., 2022). However, this hypothesis does not explain why the yield loss mainly occurred in the SP. The other explanation states that the strategy of drought scape (by shortening the plant's lifecycle) results in earlier leaf senescence, which in turn abbreviates the seed-filling period (Eti-enne et al., 2018; Y. Wang and Frei, 2011). On the other hand, it should be considered that quinoa plants enter the seed-filling stage when the seeds of the PP initiate this process (Sosa-Zuniga et al., 2017), but the start of seed-filling in SP occurs days later (Tovar et al., 2020). Furthermore, the defoliation starts during the late stages of seed maturation in the PP and first affects the leaves from the lower part of the plant, continuing upwards until the higher leaves fall (Maestro-Gaitán et al., 2022; Sosa-Zuniga et al., 2017). Following this rationale, the senescence of the leaves at the lower parts of the plant coincides with the seed filling stage in the SP located in the branches at this height, while the seeds from the PP reached the maturation stage when the lower-leaves senescence reaches the upper leaves of the plant. This may explain why a reduction in yield of the SP under water stress was found for most genotypes, as all the genotypes tested showed a drought-scape strategy (Maestro-Gaitán et al., 2022) that could act reducing the seed

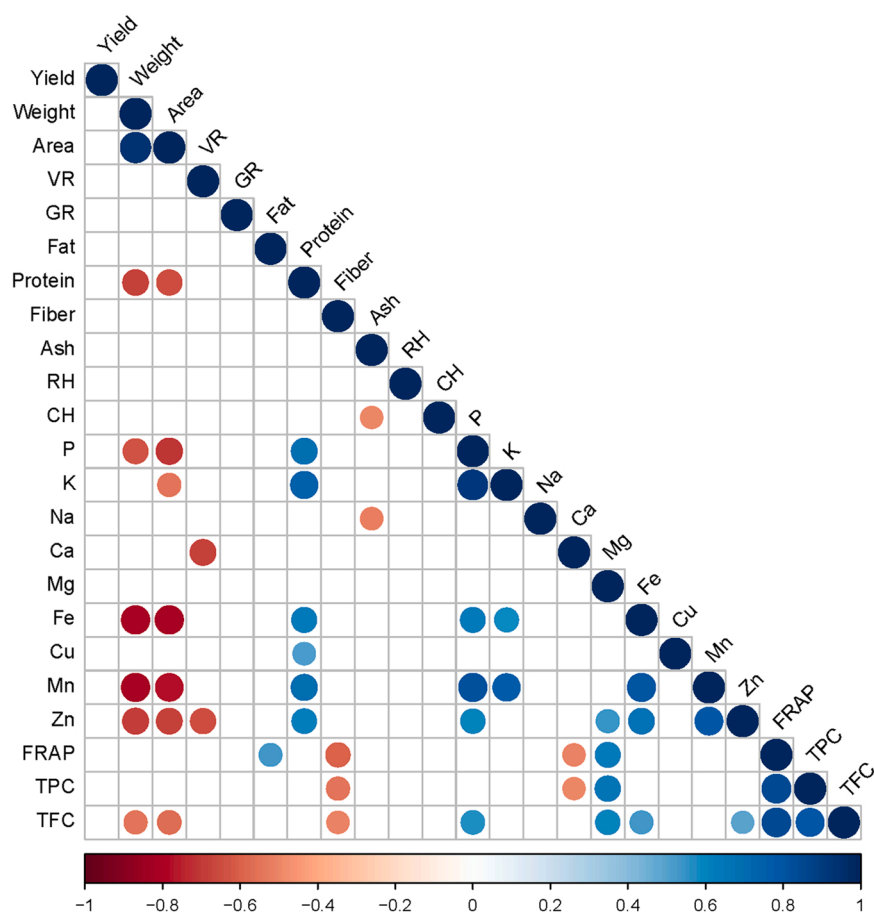


Fig. 5. Correlogram of different seed nutritional parameters determined in the four genotypes evaluated under full irrigation (WW) or water deficit (WD) conditions. Correlogram including the parameters determined in quinoa seeds harvested from primary (PP) or secondary panicles (SP) in the four cultivars grown under WW or WD conditions. Pearson's correlation coefficients (r) are shown when the correlations between variables are statistically significant ($p < 0.05$). Red cells indicate negative correlations and blue cells show positive correlations. The parameters considered in the correlogram were yield, seed weight, seed area, seed viability rate, germination rate (7 d. a.s), fat, protein, fiber, ash, relative humidity, carbohydrates, minerals (P, K, Na, Ca, Mg, Fe, Cu, Mn and Zn), and antioxidants (antioxidant capacity (FRAP), TPC and TFC).

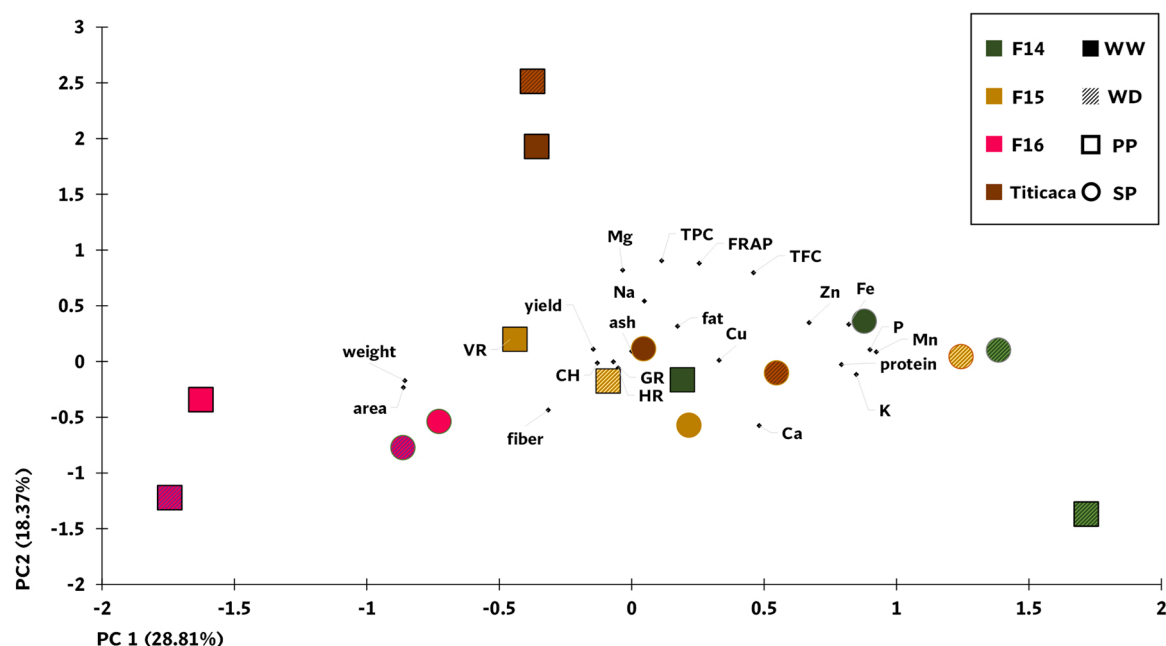


Fig. 6. Principal Component Analysis (PCA). Biplot of main components 1 and 2 for the seed yield and nutritional traits from primary (PP) or secondary panicles (SP) obtained from the four quinoa cultivars grown under full irrigation (WW) or water deficit (WD) conditions. Component 1 (X axis) was mainly explained by the seed protein, P, K, Ca, Fe, Mn, Zn, and flavonoid contents and, negatively, by the seed area, weight, and seed viability rate. Component 2 (Y axis) was explained by seed content of Na, Mg, total phenols (TPC) and flavonoids (TFC) and seed antioxidant activity (FRAP) and, negatively, by the seed fiber and Ca contents. T: Titicaca, WW: well-watered conditions, WD: water-deficit conditions, PP: primary panicle, SP: secondary panicles, VR: viability rate, GR: germination rate, TFC: total flavonoid content, TPC: total phenolic content, FRAP: ferric reducing antioxidant power (antioxidant capacity).

filling period of SPs. Only F16 remained unchanged under water limitation as F16 SPs were localized solely in the upper parts of the plant, close to the PP, extending SPs seed filling period compared to the other varieties analyzed (Maestro-Gaitán et al., 2022). Furthermore, the reduction in SPs yield in F14, F15, and Titicaca was caused by a reduction in seed number, since the seed size did not show significant changes (Figs. 1 and 2A, B).

The question of whether or how plant architecture can affect seed size and yield has been previously risen. Bennett et al. (2012) would argue that manipulation of plant architecture that results in plants with fewer branches reduces the number of sink organs, promoting apical dominance, which would result in improved allocation of nutrients and seed size, as it happens with the heavier seeds found in the lowly-branched genotype F16 (Fig. 2C). Meanwhile Guo et al. (2020) provides more examples of plants in which promoting more branched phenotypes improves grain yield. The latter explanation would fit with the higher yield found in the highly branched genotypes F15 and Titicaca under WW (Fig. 1), when more branches imply more source organs, as well as sinks, and the decrease in yield under WD, since the source strength would have been affected by the lower photosynthetic capacity of stressed plants (Maestro-Gaitán et al., 2022).

Going further, it is expected that these differences in sink strength, together with the desynchronization in seed maturation between panicle types and the effect of water stress will also impact the seed physiological and nutritional quality. These include important agronomical traits for farmers such as the germination capacity, essential for a successful crop establishment and to ensure high yields (Reed et al., 2022). In this study, the germination capacity was remarkably high and was not affected by the water treatment or the genotype, but the seed viability was reliant on the panicle type, with a decrease observed in SP seeds (Fig. 3). The tetrazolium assay measures seed vigor and it is highly correlated with the germination capacity of seeds (Ma et al., 2019). Also, the viability assay has also shown to be a good predictor of the germination capacity after months of storage (Granado-Rodríguez et al., 2022). Thus, this is the first report of a difference in seed vigor depending on the panicle type in quinoa, and, therefore, it would be recommended to be considered for farmers when selecting seeds for sowing.

Nutritionally, quinoa is widely renowned for its high protein content, at least higher than most of commercial cereal crops like wheat, barley, rice, maize, rye, or oats (Maradini Filho et al., 2017), and for possessing a complete amino acid profile, similar to casein (Repo-Carrasco et al., 2003). The seed protein content evaluated in the current study ranged between 15.50% and 21.73% (Table 1), which fits well into the range generally reported for quinoa (Angeli et al., 2020; Maradini Filho et al., 2017), and even exceeds it in the case of F15 SP seeds grown under WD (Table 1). The protein content showed an opposite pattern to the yield and the seed weight.

In the current work, at a single plant level, this trade-off was not enough to subvert the trend of protein yield, and the most productive plants, like Titicaca or F15 grown under WW, still yielded more protein (4.3 and 3.5 g per plant, respectively) than their WD counterparts or F16 under WW or WD conditions (2.3, 2.3, 2.4, and 2.4 g per plant, respectively). Thus, although a farmer would still produce more protein by growing high-yielding genotypes under optimal conditions, it is worth highlighting that the consumer's protein intake per serving (meaning, the amount of protein ingested per g of seeds) would be higher in the case of seeds harvested under WD conditions (Table 1).

This trade-off between protein content and yield had been observed previously in quinoa (Bascuñán-Godoy et al., 2016; Curti et al., 2018; Granado-Rodríguez, Aparicio et al., 2021; Granado-Rodríguez, Vilarino-Rodríguez et al., 2021; Reguera et al., 2018), and has been widely reported in other crops like wheat (Uauy et al., 2006; Zhao et al., 2009), maize (da Ge et al., 2010), and soybean (Rotundo and Westgate, 2009), especially when subjected to environmental stressors (Y. Wang and Frei, 2011). In those instances, the protein content increment was explained

through the process of early senescence shown in plants due to the shortening of their lifecycle under abiotic stresses, such as heat or drought (Y. Wang and Frei, 2011), and due to the regulatory action of transcription factors from the NAC family, such as *Gpc-B1* in wheat (Ricachenevsky et al., 2013). Leaf senescence triggers the catabolism of proteins that prompts nitrogen (N) remobilization, in the form of amino acids, to the sink tissues, i. e. to the seeds during the seed-filling stage (Etienne et al., 2018). Hence, the photosynthetic machinery is inhibited as the chloroplast proteins (i.e. Rubisco) are degraded, triggering the interruption of carbohydrate synthesis, whereas the translocation of N becomes more efficient, resulting in an overall decrease in seed biomass and the increase in the protein content per seed weight (Y. Wang and Frei, 2011). Thus, in the same way, that the yield is reduced in SPs seeds and under WD due to leaf senescence and the shortening of the seed-filling period, the protein content increases in these cases (Fig. 1, Table 1).

Furthermore, strong positive correlations were observed between the seed protein content and the P, K, Fe, Cu, Mn, and Zn contents (Fig. 5). In fact, Fe, Cu, Mn, and Zn contents were higher in SPs seeds and Fe content increased under WD, as occurred with the protein content (Tables 1, 2). Correlations between protein and these minerals had been previously observed in field and greenhouse studies in quinoa (Granado-Rodríguez, Aparicio et al., 2021; Granado-Rodríguez, Vilarino-Rodríguez et al., 2021), with the contents of P, Fe, Cu, Mn, and Zn increasing under heat stress conditions (Granado-Rodríguez, Aparicio et al., 2021; Matías et al., 2021; Tovar et al., 2022). Under drought stress, the increase in Ca, Mg, Zn, P, and Cu contents have also been observed together with the increment in seed protein content in crops like maize and wheat (da Ge et al., 2010; Zhao et al., 2009). The proposed explanation for the strong correlation between seed protein content and some of these minerals in a diversity of crops has been related with senescence (Etienne et al., 2018; Waters and Sankaran, 2011). In leaves, minerals like Cu, Zn, Fe, and Mn are found as part of diverse important proteins, including ferritin and chlorophyll. During senescence, these proteins are degraded to amino acids and peptides, but the micronutrients can be loaded to the phloem bound to chelators like nicotianamine (NA) or to protein ligands like the iron transport protein (ITP) (Curie et al., 2009; Krüger et al., 2002). Thus, the remobilization of N and these minerals to sink tissues would occur simultaneously. This hypothesis has been supported by the relation between the mineral content, the protein content, and the senescence-induced NAC transcription factors as the contents of protein, Zn, Fe, and Mn increased in lines carrying the *Gpc-B1* gene and decreased in *NAM-B1* knock-down mutant lines, another NAC transcription factor (Distelfeld et al., 2007; Waters et al., 2009).

Another significant effect of water stress observed in this study was the decrease in lipid content (Table 1). Even though this crop is not an oilseed crop, some authors have considered so because of its relatively elevated oil content of high quality (Abugoch James, 2009). Very few studies have investigated changes in seed lipid content in quinoa. Nonetheless, some have analyzed the effect of environmental conditions on the quinoa seed lipid fraction. For instance, heat stress has been reported to decrease the lipid content of quinoa seeds (Curti et al., 2020; Matías et al., 2021, 2022), similar to the decreasing pattern observed in both cereals, like maize and wheat (Ali et al., 2012; Zhao et al., 2009), and legumes, like lupine and soybean (I. S. Carvalho et al., 2004; Dornbos and Mullen, 1992). Nakagawa et al. (2018) linked this phenomenon to the overexpression of genes related to lipid degradation and the repression of lipid biosynthesis genes in soybean seeds under water stress. Furthermore, previous studies in quinoa seeds have found an opposite behavior between lipid and protein contents when plants are subjected to stress (Matías et al., 2021), also in other plant species such as cotton growing under drought (Li et al., 2022). C (and N) partitioning in seeds implies a complex metabolic network that determines the proportion of storage reserves in the form of lipids, proteins, or carbohydrates. The final allocation of C and N in the seed seems to be determined by the type and number of sources unloaded into this sink

tissue and the primary metabolic activity of the developing seed (Allen and Young, 2013; Li et al., 2022). In line with this, metabolic pathways such as seed glycolysis during seed maturation have been pointed out as key in determining the C destination (Meyer et al., 2012).

Multiple studies of seed composition under drought have reported reductions in the carbohydrate content (Y. Wang and Frei, 2011), showing decreases in both soluble sugars and starch (I. S. Carvalho et al., 2004; Yi et al., 2014). A study in quinoa showed that soluble sugars and starch contents in leaves were reduced due to a decrease in the photosynthetic activity under drought stress, which caused a slightly reduced starch content in seeds (Bascuñán-Godoy et al., 2016). In the current study, there was no reduction in seed carbohydrate content under WD, although the early senescence of lower leaves would have shortened the sugar and starch deposition in seeds (Y. Wang and Frei, 2011). However, there was probably a decrease in the translocation of carbohydrates in this experiment under WD, but this component was distributed among a lower number of seeds (Supplementary Fig. 1), thus keeping a similar relative content to WW seeds (Table 1).

Furthermore, quinoa is also known for the remarkable antioxidant capacity of its seeds, achieving similar values to other crops such as rice or soybean (Gorinstein et al., 2008), as well as higher values than cereals like barley, wheat, rye, and millet (Ragaei et al., 2006). Previous experiments described an increase in antioxidant capacity of quinoa seeds under abiotic stresses like salinity, heat stress, or drought (Aloisi et al., 2016; Fischer et al., 2013; Gómez-Caravaca et al., 2012; Ismail et al., 2016) in contrast to our results, which showed that the genotype was the only factor that influenced changes in this parameter (Fig. 4). Nevertheless, the abovementioned experiments assessed quinoa growth under field conditions, which implies a combination of various stresses affecting seed composition (Gómez-Caravaca et al., 2012; Granado-Rodríguez, Aparicio et al., 2021), or applied a higher intensity of water stress (severe stress) at certain developmental stages (grain filling stage) (Fischer et al., 2013). Here, we evaluated the effect of long-term drought applied at the early branching stage until plant harvesting, therefore, the absence of variation in the antioxidant capacity of the seeds between water treatments, which resembles the results found in other plant species such as cowpea (M. Carvalho et al., 2021), may imply that the antioxidant machinery is not always activated in quinoa to cope with stress. Nonetheless, it would be interesting to further explore if changes in the phenol and flavonoid profiles occur under water limitation. On the other hand, the intensity of drought that plants can tolerate, or resist, depends on the genotypic capability to cope with oxidative stress. Drought has been described to induce stomatal closure, thus negatively affecting the photosynthetic rate, and causing reactive oxygen species (ROS) overproduction (Hasanuzzaman et al., 2020). F16 presented tolerance to WD, as this genotype did not reduce the photosynthetic activity under these stress conditions (Maestro-Gaitán et al., 2022). This could be related to the fact that seeds from the F16 cultivar were the ones showing less antioxidant capacity (in terms of phenol, flavonoid y FRAP activity) since F16 was the least stressed cultivar under drought conditions (Maestro-Gaitán et al., 2022). Nevertheless, further experiments should be performed to evaluate if antioxidants and enzymes with antioxidant activity, as well as oxidative stress markers (Bakshi et al., 2019; Liné et al., 2021), change throughout long-term drought conditions in quinoa. These experiments could help in understanding if quinoa plants can acclimate to long-term stress exposure resulting in a stable antioxidant content (Fig. 4) and whether the genotypic-antioxidant capacity is always correlated (or not) with the vulnerability to water stress.

Nutritionally, F16 was the poorest cultivar among the genotypes here evaluated. It showed lower protein, fat, P, K, Na, Fe, Mn, Zn, phenolics, and flavonoid contents, and the lowest antioxidant capacity (Tables 1, 2, Fig. 4). Moreover, WD induced in this genotype a decrease in the fat, Na, Mn, and Zn contents, while protein and Fe contents remained unchangeable. Only seed fiber content remained higher in F16 among the cultivars analyzed (Table 1). Furthermore, its long lifecycle

could lead to yield losses in years in which the flowering stage coincides with higher temperatures or drought (Maestro-Gaitán et al., 2022; Matías et al., 2021), so a winter sowing date would be mandatory in Mediterranean areas. Nonetheless, F16 shows agronomically positive characteristics linked to morphological aspects, including large seeds (above 3.3 mm²). The simple habit shown by this genotype with few secondary panicles concentrated around the primary panicle (Maestro-Gaitán et al., 2022), facilitates harvesting allowing a higher-scale production (Gómez-Pando and Aguilar-Castellanos, 2016; Quiroga, 2015). Besides, the lower quantity of seeds coming from SP helps avoid yield losses due to the asynchronism of maturity between PP and SP (Ceccato et al., 2011; Mujica et al., 2013) and ensures a lower number of smaller seeds. Also, the water stress response shown by F16 could be considered an outstanding mechanism of this variety of special interest for areas destined for rainfed agriculture (Maestro-Gaitán et al., 2022).

On the contrary, the highly branched genotypes F15 and Titicaca (Maestro-Gaitán et al., 2022) stood out for their nutritional quality, showing the highest protein, P, K, Fe, Cu, Mn, and Zn contents in the case of F15 seeds, and the highest fat content and antioxidants capacity in Titicaca seeds (Tables 1, 2, Fig. 4). The high nutritional quality together with larger yields under WW conditions would make Titicaca an interesting genotype for breeders, despite its WD sensitivity and dark seed color. However, the highly branched phenotype and the desynchronization in the maturity of panicles types could cause difficulties for high-scale production machinery (Ceccato et al., 2011; Mujica et al., 2013). Thus, field trials with these genotypes evaluating yield and nutritional quality under different management conditions (such as irrigation or sowing density) would be interesting to test their potential exploitation. Either way, these genotypes would probably excel in low-scale production systems where the harvesting method can be adapted, and in local consumption markets that do not rely as much on seed size and color as on nutritional quality.

Therefore, it is evident that the inflorescence architecture is a determinant of its agronomical use affecting the nutritional potential of a crop as well. In this regard, the number of branches can also be affected by the genotype, the environmental cues, or by crop management aspects such as the sowing density (Rojas et al., 2011; Tovar et al., 2020). Higher sowing densities in different quinoa genotypes, including Titicaca, have been reported to reduce the number of branches while increasing the yield from the PP (Noulas et al., 2017; Rabbani et al., 2022; Risi and Galwey, 1991; Spehar and Rocha, 2009). Hence, as previously stated, it would be interesting to analyze the impact of sowing densities in highly branched but high-quality genotypes, like F15 or Titicaca, on seed yield and quality maintenance.

Overall, this work has explored variations in the inflorescence architecture and seed nutritional quality associated with different quinoa genotypes and water stress, as summarized in Fig. 7, which proposes a model highlighting the changes that occur in architecture and nutritional quality depending on the sensitivity to long-term water stress. The results revealed an interesting dichotomy between seed quantity and quality. While F16 was presented as a cost-effective option for producing stable and high-scale yields under drought (Maestro-Gaitán et al., 2022), it showed a poorer seed quality in contrast to the cultivars Titicaca and F15, which suffered seed yield losses under drought while producing more nutritious seeds. We must emphasize the importance of continuing genetic improvement programs in quinoa through the selection of varieties that are more tolerant to water stress without compromising crop productivity and nutritional quality, avoiding varieties with high-water demanding.

5. Conclusions

The drought-tolerant genotype F16 produced bigger seeds concentrated in the primary panicle, while most of the yield in the sensitive genotypes F14, F15, and Titicaca came from secondary panicles. These

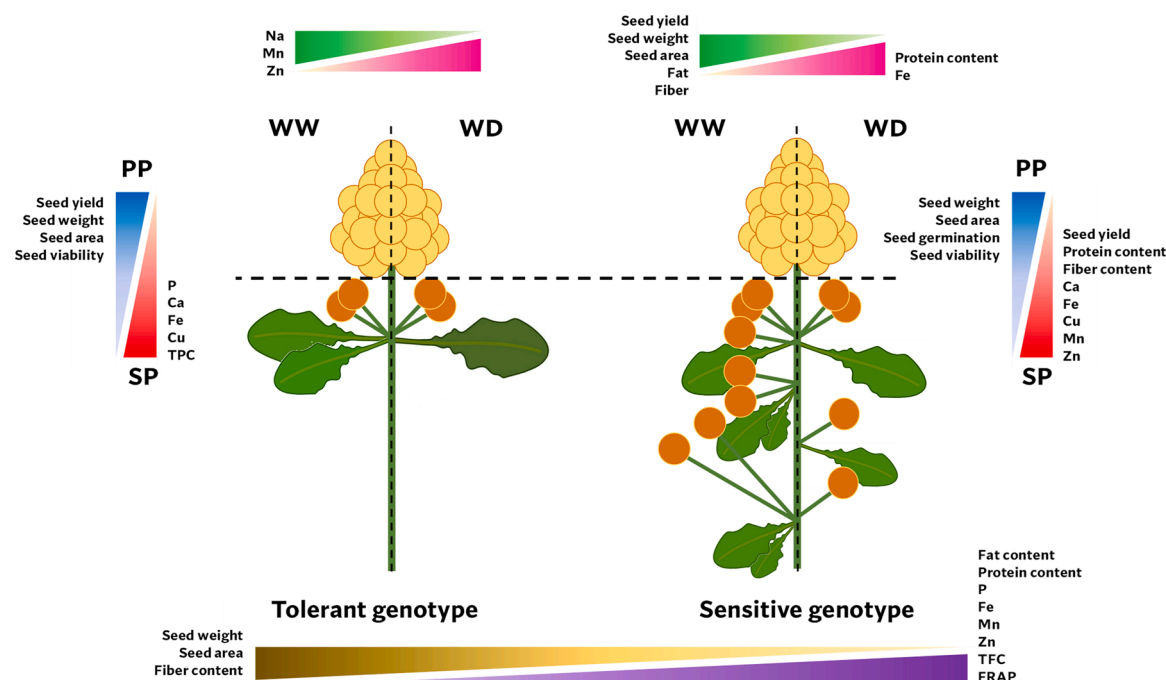


Fig. 7. Model of architectural and seed nutritional composition changes in quinoa inflorescences from tolerant and sensitive genotypes subjected to long-term water deficit (WD). This model highlights the differences that occur in plant inflorescence architecture and in the nutritional composition of seeds of a tolerant (left) and a sensitive (right) quinoa genotype when growing under well water conditions (WW) or long-term water deficit (WD) distinguishing the nutritional characterization between seeds coming from the primary (PP) or secondary (SP) panicles. The nutritional composition includes all the nutritional parameters that presented statistically significant changes which included seed yield, seed weight, seed area, seed viability, seed germination, fiber content, protein content, fat content, total phenol content (TPC), total flavonoid content (TFC), antioxidant capacity (FRAP) and mineral contents (including iron (Fe), calcium (Ca), Copper (Cu), Zinc (Zn), Manganese (Mn), Phosphorous (P) and Sodium (Na)).

secondary panicles produced smaller seeds with a lower germination capacity, but they were nutritionally richer than those from primary panicles, presenting higher contents of protein, iron, calcium, manganese, and zinc. In these panicles, drought-induced senescence resulted in a loss of yield coupled with an increase in protein and iron seed contents. Although F16 showed the most promising mechanism to counteract drought and possess an optimal inflorescence morphology, nutritionally, the seeds were poorer compared to the other genotypes here studied. Thus, we recommend further research on nutritionally rich genotypes such as F15 and Titicaca, to achieve drought-resistant genotypes able to endure the Mediterranean climate and rainfed agriculture without losing seed nutritional quality. Also, further research should be performed exploring the possibility of introducing an F16 drought response mechanism in other quinoa varieties with better nutritional profiles for irrigated conditions.

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CRediT authorship contribution statement

I. Maestro-Gaitá: Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration. **S. Granado-Rodríguez:** Methodology, Formal analysis, Investigation, Writing – original draft, Visualization; **L. Poza-Viejo:** Formal analysis, Writing – review & editing; **Matías, J.:** Methodology, Investigation, Writing – review & editing. **J. J. Pedroche:** Methodology, Investigation;

Cruz, V.: Investigation. **L. Bolaños.:** Writing – review & editing. **M. Reguera:** Conceptualization, Methodology, Validation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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