Article

Genotype-dependent variation of nutritional quality-related traits in quinoa seeds

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Abstract: Exploiting the relationship between the nutritional properties of seeds and the environment (E), genotype (G) and the GXE interaction, constitutes an essential analysis which contributes to broaden our knowledge regarding the control of the nutritional quality of seeds or of any other edible plant structure. This constitutes an important aspect when aiming at improving the nutritional characteristics properties of plant species, including those of *Chenopodium quinoa* Willd (quinoa) which is intended to be one of the main nutrient sources ensuring food security worldwide. This crop has gained popularity in the last decade achieving a fast-global expansion due to its excellent nutritional and agronomical properties together with the excellent adaptation shown to a wide diversity of agroclimatic conditions. Changes in the nutritional properties of quinoa seeds due to the influence exerted by the environment, the genotype, or their interaction, have been already described in previous works, but there is an important limitation in the analyses carried out, including the outcomes that can be translated into agronomical practices by which quality can be improved selecting the most adequate genotype. In here, several seed nutritional-related parameters from fifteen quinoa cultivars grown in a particular environmental context were analysed aiming at targeting compounds that can be determinants of seed quality varying with the genetic background. Important nutritional and agronomical differences were found among quinoa varieties highlighting the importance of choosing a proper genotype when cultivating quinoa.

Keywords: quinoa; genotype; nutritional traits; seed quality;

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a halophytic crop which belongs to the Amaranthaceae family. It can be adapted to a wide variety of agroecosystems and is resistant to stressful environmental conditions, showing tolerance to drought, frost and salinity [1], [2], possessing as well excellent nutritional properties [3], [1]. All these characteristics have resulted in a global expansion of quinoa cultivation during the past three decades [4] and, consequently, this explains why quinoa can be found from the Andean Altiplano, with harsh climatic conditions associated with high altitudes (reaching over 3500 m above sea level), to coastal areas. It can grow in different soils, extreme temperatures, and rainfalls, and shows tolerance to drought, frost and salinity [1], [2].

Quinoa was first domesticated by pre-Columbian cultures more than 7000 years ago, being one of their main sources of nutrients as a good substitute for the lack of animal

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protein. After the Spanish conquest, quinoa was highly rejected but maintained by indigenous farmers despite the introduction of Old-World species. These farmers domesticated the cultivars preserving the genetic diversity found currently in quinoa [5]. This genetic diversity can be divided into different ecotypes that include thousands of accessions (16,422) [6] that reflect the diffusion from the center of origin around Lake Titicaca [5].

Currently, quinoa is still the principal protein source in many areas of the Altiplano. The nutritional value of quinoa seeds was rediscovered during the last decades of the 20th century, leading to a renewal of its production [7]. This led to a boost of spreading its cultivation from very few countries growing this crop in the 80s to 123 countries in 2018 [4]. The success in its international acceptance has been possible mainly due to the nutritional characteristics of the seeds. They resemble cereal seeds due to the high starch content and overall morphology, which explains why quinoa is considered a pseudocereal. Quinoa seeds are gluten-free, have a low glycemic index, being low in sugar and calories but containing an excellent balance of essential amino acids, and high contents of fibre, lipids, carbohydrates, minerals and bioactive compounds such as vitamins (B2 and E), carotene, tocopherols and other molecules with antioxidant properties like flavonoids and other phenolic compounds [8], [9], [10], [11]. Furthermore, quinoa also shows a unique fibre, lipid (with a high ratio of omega-6: omega-3), micro- and macronutrient profiles (often higher than cereal-based products) that give quinoa seeds beneficial characteristics such as decreasing the risk of cancer, cardiovascular and inflammatory diseases, decreasing blood pressure, diabetes, development of hemorrhoids and weight control [12], improving intestinal health [9]. Due to all these characteristics, providing not only nutritional services but health benefits, quinoa is considered a “superfood of the future”.

Furthermore, quinoa is offered as a nutritious food for low-income countries and constitutes a crop able to grow on marginal lands (including those with limited rainfalls or poor soil quality) which are not suitable for other major crops [8]. This brings interesting opportunities for the agriculture of low-income countries and, generally, for those countries where agricultural water supply is or will be soon limited. These include Mediterranean countries where there is an urgent need to develop sustainable practices to mitigate the impacts of climate change and human pressure on soil resources [8] which is especially relevant within the current climate change and food security context [13]. Besides, it should be noted that quinoa is not only consumed by humans, as its different plant parts can be used as a nutritionally valuable forage crop, apt for feeding sheep, pigs, cattle, poultry and horses [14].

Importantly, it should be noted that quinoa exhibits a strong variability of cultivar-specific responses to environmental variation different environmental conditions have been described in quinoa for some seed quality-related parameters including seed size, protein or mineral contents depending on the specific genotype [15], [16], [17], [18], [19]. Thus, different cultivars of quinoa have shown substantial differences in the nutritional characteristics which also vary with the environment. What is still unclear is if the parameters that were evaluated are stable among cultivars at different locations or if steady correlations can be found between nutritional-related parameters. A recent work by Granado-Rodriguez et al. [20] showed that some quinoa cultivars, Titicaca and Vikinga, present better quality-related traits (including higher protein contents) despite not being the most productive when growing at the Northwestern part of Spain. In line with this, it is key to better understand the genetic and environmental factors determining the nutritional characteristics of quinoa selecting the best adapted genotypes for a particular cultivation environment in terms of yield potential, biotic and abiotic stress tolerance but also considering the different nutritional traits. This will be achieved through the use of conventional and molecular tools that will help unlocking the rich biodiversity and cultivation potential of this crop [21].

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Therefore, aiming at contributing to unravel genetic differences associated with different genotypes, in this study we analyzed a variety of nutritional-related parameters in fifteen different quinoa varieties with cultivation potential in the Southern region of Spain. Differences in the parameters analyzed were found among varieties supporting the presence of genetic determinants of nutritional quality in quinoa.

2. Results

2.1. Plant performance and physiological traits

In this study, seeds of fifteen different quinoa cultivars were sown on January 27th of 2018 and plants were harvested on either July 18th or August 1st aiming to analyze different nutritional traits under filed conditions (Supplementary Figure 1). Cultivars 'A-SE-03', 'A-SE-06', 'A-SE-07', 'A-SE-09', 'A-SE-12', 'A-SE-13', and 'A-SE-15' showed a 24 weeks-long life cycle and were harvested in July, while cultivars 'A-SE-01', 'A-SE-02', 'A-SE-04', 'A-SE-05', 'A-SE-08', 'A-SE-10', 'A-SE-11', and 'A-SE-14' presented longer life cycles of 26 weeks (Supplementary Figure 2). Total seed yield varied among cultivars, being A-SE-08 cv. the cultivar that presented the highest seed yield (4.7 t/ha) followed by A-SE-11 cv. and A-SE-05 cv. (with 3.5 and 3.4 t/ha, respectively), and A-SE-01 the cultivar with the lowest seed yield (0.96 t/ha). Precipitations along the growing season were concentrated in the first months of cultivation (February to April) coinciding with plant nascence and emergence (Supplementary Figure 1) while scarce precipitations were registered from flowering to harvesting time (May-July). Daily mean temperature varied from 8.7 °C (at sowing) to 25.7 °C and 30.7°C (at harvesting of short- and long-life-cycle cultivars, respectively) increasing progressively along the growing season (Supplementary Figure 1). Inflorescences started appearing in May. Temperatures higher than 25°C were registered (for all cultivars) at seed maturation stage.

Plant height also showed significant variations among cultivars (Figure 1). At early stages (82 d.a.s) A-SE-05 cv., A-SE-03 cv. and A-SE-06 cv. were the tallest, with 54 ± 5.76cm of plant length while at middle stages (100 d.a.s.), A-SE-03 cv. and A-SE-06 cv. were the highest with 130.4 + 8.94 cm and 130.4 ± 6.33 cm height, respectively. At latest stages (128 d.a.s), A-SE-07 cv. and A-SE-15 cv. were the tallest plants presenting 170.90 ± 22.87 cm and 163.40 ± 31.99 cm height, respectively. On the other hand, A-SE-04 cv. was the shortest cultivar throughout the time going from 14.9 ± 5.27 cm height at early stage, to 69.10 ± 11.74 cm height at middle stage and 117.30 ± 25.12 cm at the end of the life cycle. In line with this parameter, lodging was also evaluated in this study. Thus, it was observed that the cultivars A-SE-07 and A-SE-03 presented greater lodging resistance (3% of lodging plants), while the cultivar A-SE-12 showed great sensitivity to lodging with a 36% of affected plants.

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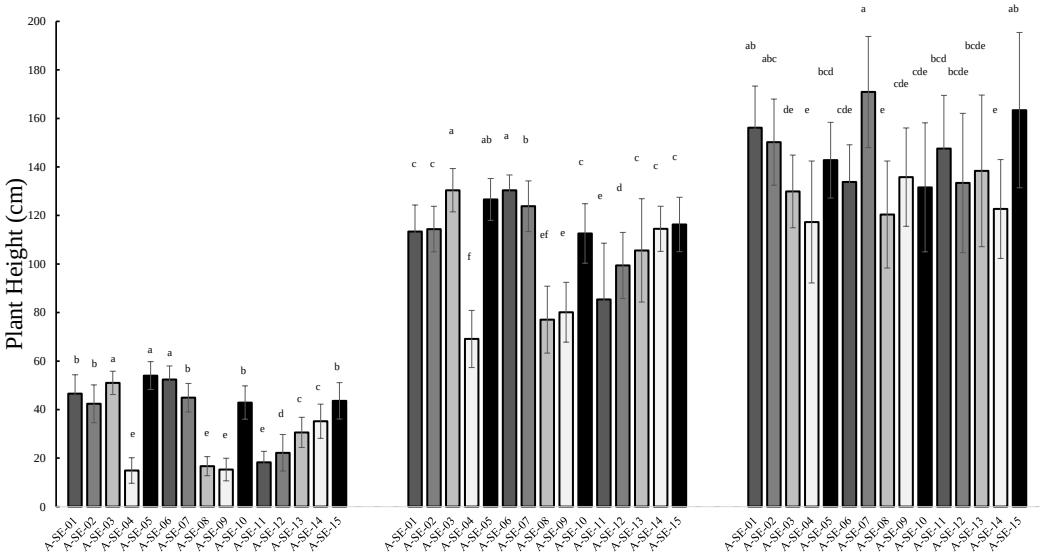

Figure 1. Plant height at 82, 100 and 182 d.a.s. Plant height (cm) was determined in the 15 cultivars analyzed. Error bars represent the standard deviation. Bars that do not share the same letters show statistically significant differences following the Krustall-Wallis at a *p-value <0.05* for 82 d.a.s. and 100 d.a.s. and ANOVA test and Tukey post-hoc test at a *p-value < 0.05* for 128 d.a.s.

Panicle length was determined in the different cultivars evaluated after 128 d.a.s.. Most of the cultivars showed panicle lengths between 30 and 40cm, with A-SE-01 cv. having the lowest values (21.70 ± 2.53cm) and A-SE-13 cv. showing the highest (40.40 ± 3.03cm) (Figure 2). In addition to the panicle length, the weight of those panicles was also measured (*data not shown*). The results pointed that having larger panicles usually correlated with heavier weights although some exceptions were observed (i.e. A-SE-02 cv. was 33.8 ± 3.67 cm length but presented the biggest weight, 3.20 kg/25 panicles).

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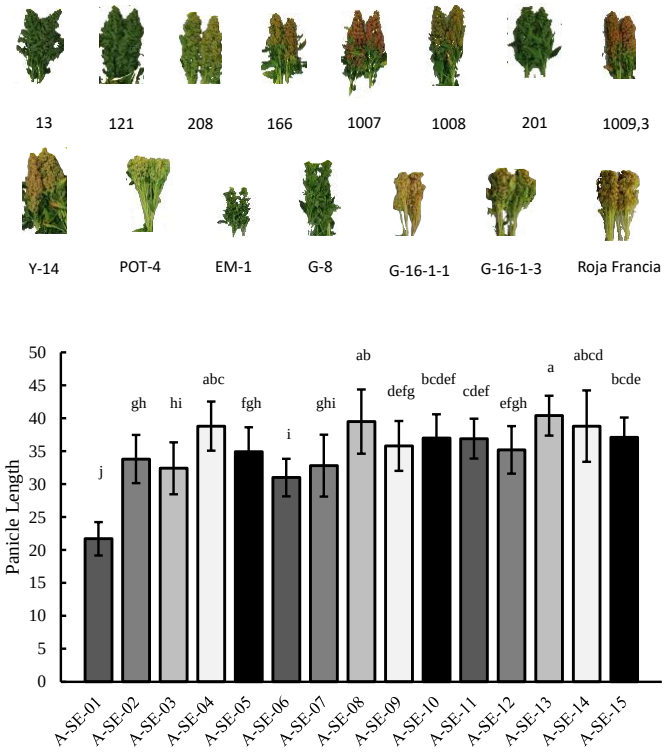

Figure 2. Panicle lenght. Panicle lenght (cm) was determined in the 15 cultivars analyzed. . Error bars represent the standard deviation. Bars that do not share the same letters show statistically significant differences following the Krustall-Wallis test at a *p-value* < *0.05*

Mildew incidence and severity were analysed throughout the experiment (at 82, 100 and 128 d.a.s.) (Figure 3). A-SE-03 cv. and A-SE-08 cv. were the less affected cultivars at early stages and A-SE-06 cv. and A-SE-12 cv. at later stages, meanwhile A-SE-09 cv., A-SE-10 cv., and A-SE-11 cv. were the most stricken ones in terms of severity, specially at 128 d.a.s.

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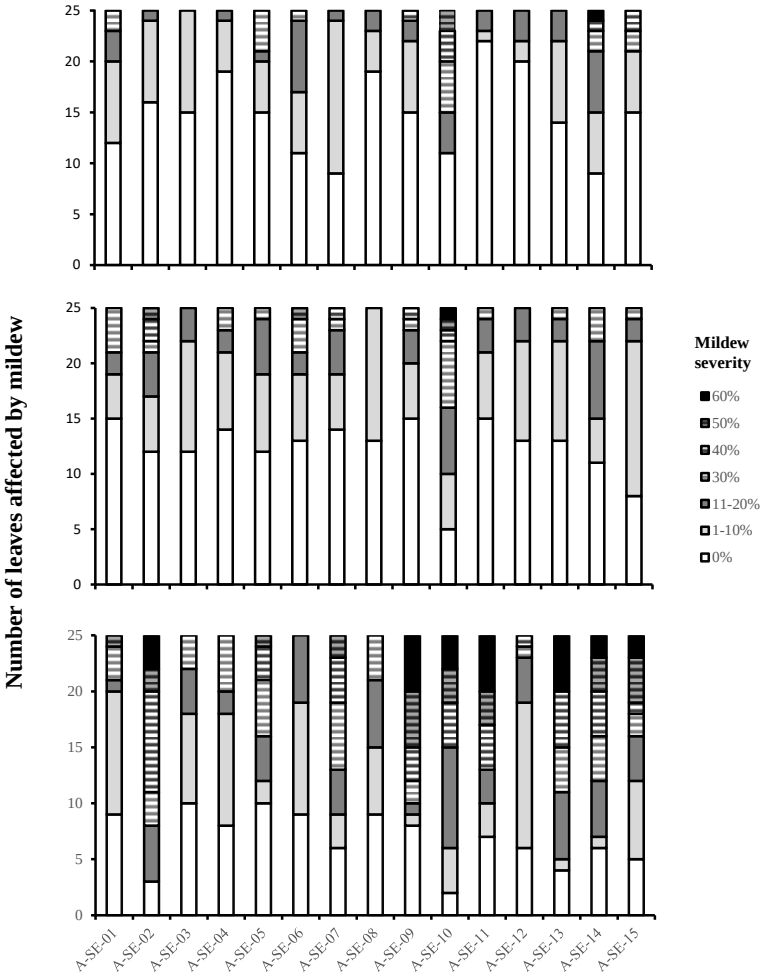

Figure 3. Mildew incidence and severity. Mildiu incidence and severity was determined as described in the Methods section. Different degree of severity was considered as the percentage (%) of leaf affected by the pathogen (leaf are converage of 0%, 1-10%, 11-20%, 30-, 40%, 50% or more than 60%). Mildiew incidence and severety were evaluated at different developmental stages: at 82 (upper panel), 100 (middle panel) and 128 d.a.s (bottom panel).

Seed weight showed an effect related to the cultivar (Figure 4A). The cultivars A-SE-05 cv., A-SE-13 cv. and A-SE-15 cv. presented the heaviest seeds while A-SE-04 cv., A-SE-10 cv., and A-SE-09 cv. showed lightest seed weights. Thus, in order from the heavier to the lighter seed weights, cultivars would be organized as follows: (A-SE-05 cv.=A-SE-13 cv.)>A-SE-15 cv.>(A-SE-06 cv.=A-SE-11 cv.)>(A-SE-02 cv.=A-SE-12 cv.=A-SE-14 cv.)>(A-SE-01 cv.=A-SE-07 cv.)>(A-SE-03 cv.=A-SE-08 cv.)>A-SE-09 cv.>(A-SE-04 cv.=A-SE-10 cv.)

Seed area showed high correlation with seed weight (Supp. Figure3). A-SE-05 cv., A-SE-03 cv., and A-SE-15 cv. presented the largest seeds while A-SE-04 cv., A-SE-08 cv., A-SE-09 cv., and A-SE-10 cv. had the narrowest seed areas (Figure 4B).

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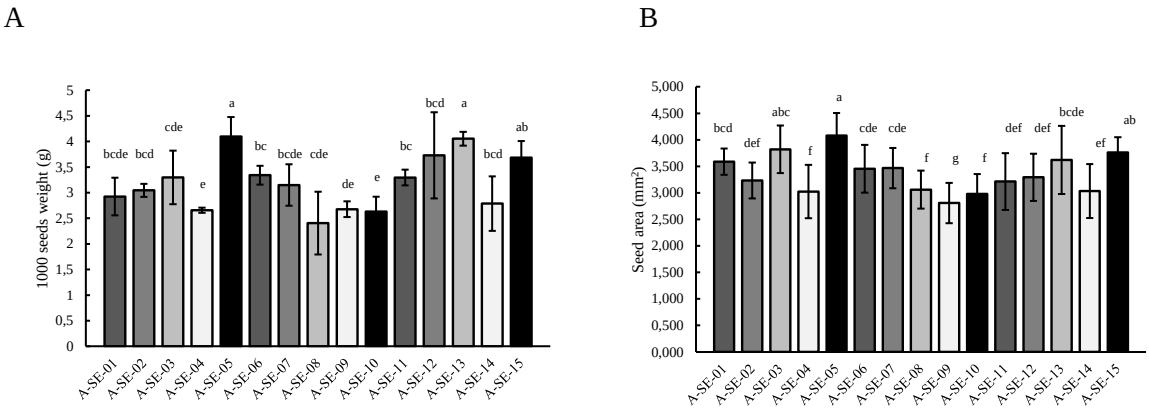
Figure 4. Seed weight and seed area. A) Seed weight (g) and B) area (mm) were determined among the different cultivars studied. Error bars represent the standard deviation. Bars that do not share the same letters show statistically significant differences following a Krustal-Wallis test by ranks for multiple comparisons at a *p-value* < *0.05*

2.2. Germination rates and seed viability



To evaluate the germination capacity of the seeds, germination rates were determined for all cultivars harvested (Figure 5A). Noticeably differences were found in the germination rates of the various cultivars analyzed. Thus, while A-SE-04 cv. and A-SE-15 cv. showed germination rates above 50% 3 d.a.s, reaching A-SE-04 cv. 80% germination rate 7 d.a.s (Figure 5B), A-SE-03 seeds were unable to germinate, and A-SE-06 cv. and A-SE-01 cv. did not overtake 20% germination rates 3 d.a.s. On the other hand, A-SE-12 cv., although showing a germination delay, were able to reach almost 50% in the germinating rates 7 d.a.s, being close to the A-SE-05 cv. germination rates at 7 d.a.s.

Seed viability was determined to complete the physiological analysis of the seeds (Figure5 C). For most of the cultivars, except for A-SE-15 cv., A-SE-04 cv., A-SE-03 cv. and A-SE-06 cv., seed viability showed no correlation with seed germination being, generally, severely reduced in most of the seeds tested.

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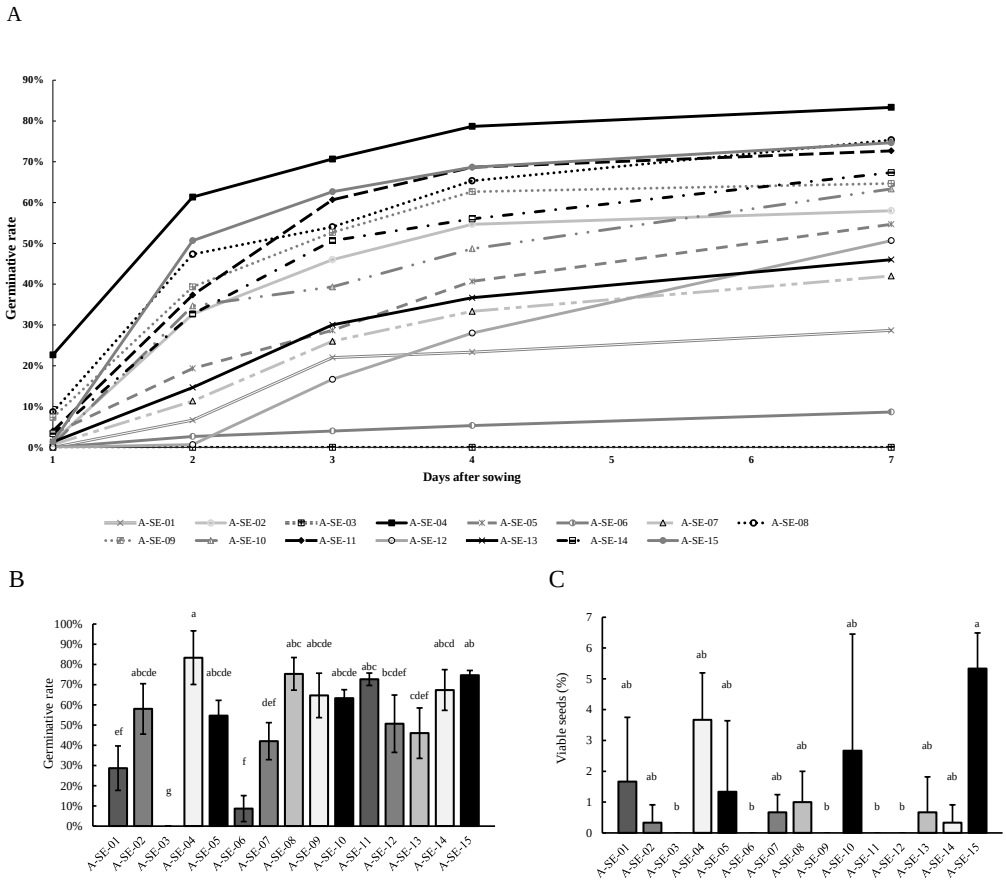

Figure 5. Germination rates (%) and seed viability. A) Time course of germination percentage (%) of quinoa seeds 1, 2, 3, 4, 5, 6 and 7 seven days aftert sowing (d.a.s.), B) Germination rate percentage (%) 7 d.a.s. and C) Percentage (%) of viable seeds. Error bars represent the standard deviation. Bars that do not share the same letters show statistically significant differences following Krustal-Wallis test by ranks for multiple comparisons at a *p-value* < *0.05*

2.3. Protein content.

Total protein contents in seeds revealed variations among cultivars (Figure 6). The cultivars A-SE-15 cv. and A-SE-02 cv. showed the highest contents, and, in contrast, A-SE-06 cv. and A-SE-03 cv. showed the lowest. A gradient in total protein content was found as follows: A-SE-15 cv.> (A-SE-02 cv.= A-SE-08 cv.=A-SE-12 cv.)>(A-SE-10 cv.=A-SE-11 cv.)>(A-SE-04 cv.=A-SE-13 cv.=A-SE-14 cv.)>(EM 1 cv.=A-SE-05 cv.=A-SE-07 cv.=A-SE-09 cv.)>A-SE-03 cv.>A-SE-06 cv.

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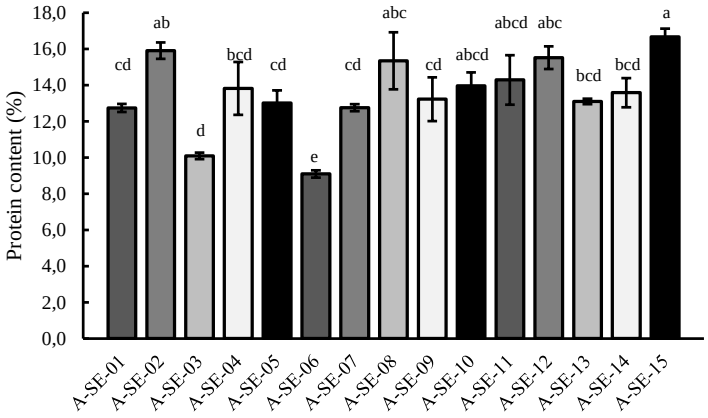

Figure 6. Seed protein content (%). Protein content was determined in seeds of the 15 cultivars evaluated. Error bars represent the standard deviation. Bars that do not share the same letters show statistically significant differences following the Krustal-Wallis test by ranks for multiple comparisons at a *p-value* < *0.05*

2.4. Mineral content.

The total contents (as %) of phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), (and as mg/Kg) sodium (Na), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in quinoa seeds were determined to analyze the effect of the genotype on this nutritional-related parameter (Table 1). Some mineral nutrients such as Mg did not show significant variation among genotypes or, as in the case of K, showed a small fluctuation. On the contrary, minerals such as Zn, showed a steeper variation, being, from the highest to the lowest, A-SE-12 cv. the one with a higher content followed by A-SE-15 cv.>A-SE-13 cv.>(A-SE-05 cv.=A-SE-08 cv.)>A-SE-07 cv.>A-SE-04 cv.>A-SE-02 cv.>(EM 1 cv.=A-SE-14 cv.)>A-SE-11 cv.>A-SE-06 cv.>A-SE-10 cv.>A-SE-03 cv.=A-SE-09 cv. Among cultivars, it should be noted that A-SE-12 cv. presented higher contents of P, Ca, Fe, and Zn, and intermediate levels of the rest of minerals, and A-SE-15 cv. presented the highest contents of P, Cu, and Zn, and the lowest of Ca, Na, and Fe. At the same time, A-SE-03 cv., A-SE-04 cv., and A-SE-06 cv., had higher contents of Ca and Na and lower of P and Cu.

Table 1. Mineral seed contents. Mean ±SD mineral contents are presented as percentage of seed weight (P, K, Ca, and Mg) or as mg/Kg (Na, Fe, Cu, Mn, and Zn). Statistical analysis following a Krustal-Wallis test by ranks (P, K, Mg, Na, Mn, Cu, Zn content) or a Welch's ANOVA with a Games-Howell post-hoc test (Ca and Fe contents) was performed. Different letters under each mineral content show statistically significant differences between samples.

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	Ash (%)	Nitrogen (%)	C/N ratio	P (%)	K (%)	Ca (%)	Mg (%)	Na (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
A-SE-01	3.48±0.02 b	2.04±0.04 cd	18.45±0.35 bc	0.29±0.00 bc	1.14±0.01 ab	0.30±0.02 abc	0.21±0.00 -	146.98±5.99 bcdefg	40.41±0.27 b	19.57±0.30 bcdef	9.30±1.42 bcde	29.18±0.34 efg
A-SE-02	3.22±0.10 abc	2.55±0.07 ab	14.81±0.56 de	0.27±0.00 bc	1.03±0.01 b	0.28±0.01 bc	0.19±0.01 -	116.63±7.66 gh	38.98±1.72 b	11.69±0.05 f	11.70±1.73 bc	29.56±0.44 ef
A-SE-03	3.47±0.07 abc	1.62±0.03 d	22.59±0.28 b	0.17±0.00 d	1.26±0.01 ab	0.38±0.02 a	0.19±0.00 -	233.42±55.08 ab	36.05±1.35 b	16.65±0.99 cdef	7.54±0.56 cdef	23.93±0.38 i
A-SE-04	3.03±0.33 abc	2.21±0.23 bcd	17.47±1.56 bcd	0.22±0.02 cd	0.98±0.12 ab	0.35±0.02 ab	0.20±0.02 -	279.7±37.63 a	32.85±3.45 bc	13.83±0.95 ef	7.86±1.21 bcdef	29.95±0.52 def
A-SE-05	3.66±0.27 abc	2.08±0.11 cd	18.21±0.92 bcd	0.27±0.02 bcd	1.24±0.10 ab	0.35±0.04 abcd	0.18±0.01 -	173.49±21.92 abcdef	29.73±2.74 bc	13.42±0.72 def	9.34±0.80 bcde	31.60±0.74 bcd
A-SE-06	3.11±0.17 abc	1.46±0.03 e	25.34±0.44 a	0.15±0.00 e	1.13±0.03 ab	0.36±0.12 abcdef	0.17±0.02 -	194.52±59.36 abcde	35.08±15.60 bc	14.32±6.60 cdef	9.40±3.17 bcdef	25.53±1.23 ghi
A-SE-07	3.18±0.09 bc	2.04±0.03 cd	18.40±0.45 bc	0.26±0.01 bcd	1.10±0.02 ab	0.18±0.02 def	0.19±0.00 -	125.43±14.68 efgh	42.71±3.77 abc	19.92±0.18 bcde	10.28±0.31 bc	30.85±0.22 cde
A-SE-08	3.43±0.36 abc	2.46±0.25 abc	15.65±1.65 bcde	0.28±0.03 bc	1.15±0.12 ab	0.21±0.02 cde	0.22±0.02 -	141.79±12.98 cdefgh	43.18±3.60 abc	22.90±0.38 ab	10.32±0.44 bcd	31.28±0.80 bcd
A-SE-09	3.50±0.47 abc	2.12±0.19 cd	17.90±1.50 bcd	0.26±0.03 bcd	1.16±0.16 ab	0.27±0.01 bcde	0.18±0.02 -	153.07±32.93 bcdefgh	39.54±4.68 abc	22.19±1.50 ab	7.08±0.53 def	23.80±0.25 i
A-SE-10	3.68±0.34 abc	2.24±0.12 abcd	16.95±0.99 bcde	0.24±0.04 bcd	1.22±0.17 ab	0.25±0.04 abcdef	0.19±0.03 -	191.24±21.87 abcd	47.12±8.56 abc	20.77±2.11 abcd	6.41±1.01 ef	25.32±1.36 hi
A-SE-11	3.62±0.63 abc	2.29±0.22 abcd	16.74±1.57 bcde	0.28±0.03 bcd	1.23±0.21 ab	0.24±0.03 abcdef	0.21±0.03 -	194.76±22.11 abc	39.56±6.24 abc	20.05±1.63 abcd	6.55±0.68 f	28.76±0.30 fgh
A-SE-12	3.26±0.03 c	2.48±0.10 abc	15.13±0.59 cde	0.31±0.01 b	1.12±0.01 ab	0.36±0.01 a	0.19±0.00 -	139.22±16.03 defgh	56.39±1.56 a	17.62±0.51 cdef	8.98±0.76 bcdef	35.23±0.21 a
A-SE-13	3.73±0.03 a	2.10±0.02 bcd	17.93±0.28 bcd	0.27±0.01 bcd	1.39±0.03 a	0.10±0.03 ef	0.18±0.01 -	121.64±15.62 fgh	35.17±3.55 bc	22.17±1.15 abc	19.52±0.60 a	32.45±0.58 bc
A-SE-14	3.34±0.21 abc	2.17±0.13 bcd	17.52±1.00 bcd	0.25±0.01 bcd	1.16±0.06 ab	0.10±0.02 f	0.20±0.01 -	125.35±15.11 fgh	33.19±2.41 bc	23.16±1.16 a	18.46±0.88 ab	29.37±0.97 efg
A-SE-15	3.19±0.08 bc	2.67±0.07 a	14.39±0.48 e	0.35±0.01 a	1.12±0.02 ab	0.09±0.01 f	0.21±0.01 -	100.87±8.96 h	25.10±1.29 c	20.34±0.50 abcd	19.75±0.96 a	34.09±0.81 b

2.5. Antioxidant capacity.

We evaluated the antioxidant capacity of the seeds by performing the FRAP assay and the quantification of total polyphenols (TPC) and flavonoids (TFC) contents (Figure 7). Among cultivars, A-SE-04 cv. followed by A-SE-15 cv. showed the highest antioxidant capacity, presenting high values of FRAP, TPC and TFC contents. On the contrary, A-SE-01 cv. together with A-SE-06 cv. the lowest. The other cultivars showed distinct patterns, presenting changes among the antioxidant-related parameters here evaluated. For instance, A-SE-10 cv. showed intermediate and high FRAP and TFC values, respectively, and low TPC levels.

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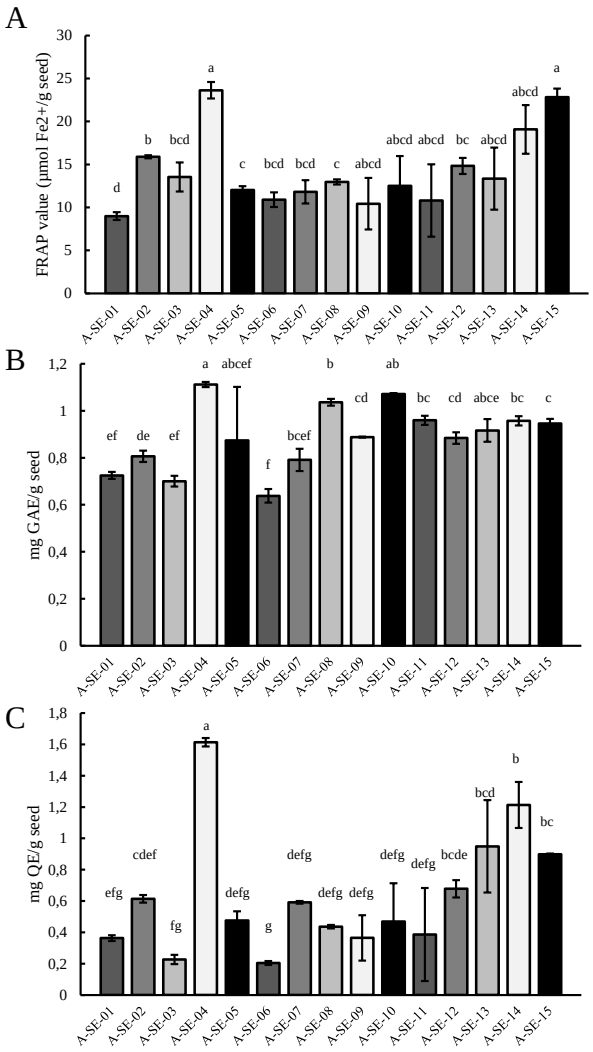

Figure 7. Antioxidant capacity of quinoa seeds. A) Antioxidant power of quinoa seeds was measured using the ferric reducing antioxidant power (FRAP) assay and is expressed as µmol of Fe2+per gram of seed. Statistical differences were analyzed through a Welch's ANOVA test followed by a Games-Howell post-hoc test. B) Total polyphenol content (TPC) is expressed as milligrams of gallic acid equivalents (GAE) per gram of seeds. The statistical analysis performed was a Welch's ANOVA test followed by a Games-Howell post-hoc test. C) Total flavonoid content (TFC) is expressed as milligrams of quercetin equivalents (QE) per gram of seeds. A Krustal-Wallis test by ranks was performed for multiple comparisons. Bars that do not share the same letters show statistically significant differences at a *p-value* < *0.05*. Error bars represent the standard deviation.

2.6. Saponin content

Saponin content was quantified in the cultivars studied (Figure 8). A-SE-06 was the cultivar showing the lowest saponin content while A-SE-10 was the cultivar with the highest saponin level. All of the cultivars exceed the limit of 0.11% established to classify quinoa varieties as sweet [22], however, none of them presented a content higher than 1%, which is usually overtaken by bitter quinoa seeds [23].

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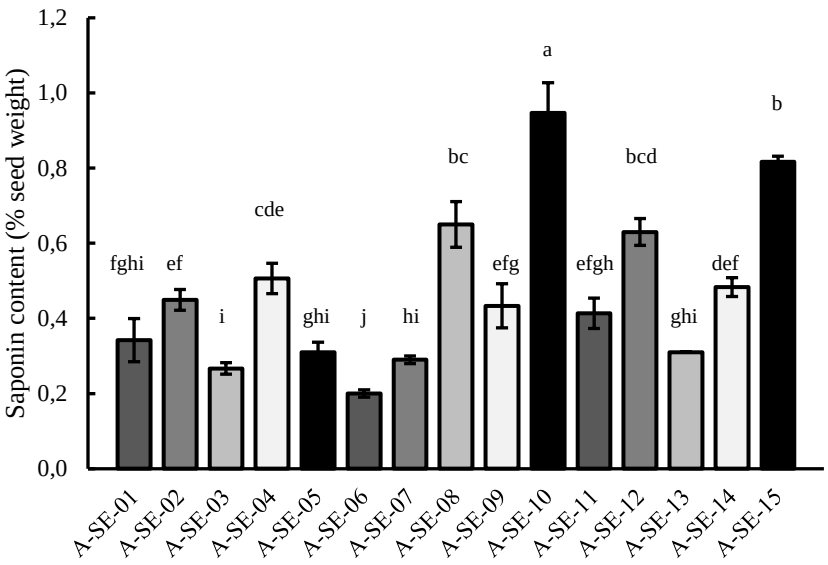

Figure 8. Saponin content. Saponin content was determined in seeds of the 15 cultivars evaluated. Error bars represent the standard deviation. Bars that do not share the same letters show statistically significant differences following the Krustal-Wallis test by at a *p-value* < *0.05*.

2.7. Path coefficient analysis

Path analysis was performed to define the direct and indirect contributions of each trait on seed yield. First, a predictive multiple linear regression model was performed following the stepwise method in order to find physiological traits with a direct effect on germination rates (Table 2) and yield (Table 3). As shown in Table 2, germination rates would be affected positively by the phenols (TPC) and P contents and indirectly by physiological parameters such as seed area or panicle biomass or biochemical properties of seeds such as protein or saponin content. On the other hand, yield would be explained in a negative way by the seed weight and panicle height, and positively by the panicle biomass and total biomass, meaning that these parameters may directly impact seed yield of the quinoa varieties here analyzed.

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Table 2 Direct effects of predictor variables of first-, second-, third-, and fourth order on germination rate, tolerance and variance inflation factor of the path analysis. Subsequent multiple linear regression analysis was performed. TPC: total phenol content. P: total phosphorous and Mg: total magnesium.

Response variable	Predictor varibles	Adjusted R^2	Direct effect	Tolerance	VIF
Germination	TPC	0,825	0,750	0,947	1,056
	P		0,390	0,947	1,056
TPC	Saponins	0,723	0,584	0,838	1,193
	Seed Area		-0,456	0,838	1,193
Saponins	Protein	0,876	0,731	0,781	1,280
	Panicle Biomass		-0,636	0,496	2,016
	Panicle Height		0,844	0,572	1,749
Protein	Mg	0,273	0,570	1	1
Panicle Biomass	Biomass	0,558	0,602	1	1
	Mildew damage (128 das)		0,521	1	1
P	Panicle Height	0,738	-0,371	0,901	1,110
	Protein		0,924	0,901	1,110

Table 3. Direct effects of predictor variables of first-, second-, third-, and fourth-order yield, tolerance and variance inflation factor of the path analysis. Subsequent multiple linear regression analysis was performed.

Response variable	Predictor varibles	Adjusted R^2	Direct effect	Tolerance	VIF
Yield	Biomass	0,880	0,481	0,624	1,603
	Panicle Biomass		0,469	0,522	1,914
	Seed Weight		-0,405	0,975	1,026
	Panicle Height		-0,102	0,631	1,584
Biomass	Plant Height (128 das)	0,013	-0,124	1	1
Seed Weight	Plant Height (128 das)	0,035	0,173	0,999	1,001
	Mildew damage (128 das)		-0,109	0,999	1,001
Panicle Height	Plant Height (128 das)	0,106	-0,194	0,999	1,001
	Mildew severity (128 das)		0,277	0,999	1,001
Panicle Biomass	Plant Height (128 das)	0,061	-0,121	0,999	1,001
	Mildew severity (128 das)		0,230	0,999	1,001

2.8. Principal components analysis (PCA)

A Pearson's correlation coefficient test was performed to analyze the correlation between variables (Supplementary figure 3) and a principal component analysis (PCA) to reduce the number of variables. This analysis identified five principal components that were able to explain 74.76% of the variance. Component 1, which contributes to 21.31% of the variance, was mainly explained by the protein and saponin contents and most minerals contents (P, Ca, Mg, Mn, Cu, and Zn contribute positively, and Ca and Na negatively), and by the germination rate, lodging, and plant height and mildew severity at 128d.a.s.

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For this new variable, A-SE-12 cv. and A-SE-15 cv. show high values while A-SE-03 cv. and A-SE-06 cv. present the lowest. There were correlations between most of these variables, but those between protein content and germinative rate (r=0.801), protein and P contents (r=0.846), P and Zn contents (r=0.728), and protein and saponin contents (r=0.695) were the strongest (Figure 9). Component 2 contributes to variance with a 18.30%, and comprises panicle length and biomass, plant dry weight, yield, germinative rate of seeds, and total phenolic content, and inversely plant height (at three time points) and seed area. Plant height at early stages (82 and 100 d. a. s.) correlates negatively with the final plant biomass, yield, germinative rate of seeds, and protein and phenolic contents, while the panicle height and weight at 128 d. a. s. correlates positively with these parameters. Strong correlations are found between the phenolic content and the germinative rate and panicle height (r=0.884 and r=0,780, respectively). For this component, there are high values in A-SE-08 cv. and A-SE-04 cv. and low in A-SE-01 cv. Component 3 explains a 12.19% of variance and comprises the viability rate, flavonoid contents, and antioxidant capacity, and inversely ash, K, and Fe contents. Both viability and germinative rate correlate with each other and with the antioxidant capacity and phenolic and flavonoid contents. There is also a strong correlation between ash and K content (r=0.851) since K is the main mineral present in quinoa (Table 1). A-SE-04 cv. and A-SE-15 cv. show high component 3 values and A-SE-09 cv., A-SE-11 cv., and A-SE-13 show low values. Area and seed weight, K and Cu content contribute positively to component 4 (explaining 11.96% of variance) and Fe and Ca contents negatively. Area and seed weight show a strong correlation (r=0.748). A-SE-13 cv. presents the highest and A-SE-01 cv., A-SE-09 cv., and A-SE-10 cv. the lowest values for component 4. Component 5 (11.00% of variance) comprises saponin content in seeds, panicle length and mildew severity at three stages. There is correlation between mildew severity at 82 and 100 d. a. s., but not with severity at 128 d. a. s., and saponin content and panicle height also show a strong correlation (r=0.655). A-SE-10 cv. showed high component 5 values while A-SE-12 cv. showed the lowest. Life cycle duration correlates with yield, the germinative rate of seeds, and their phenolic contents.

Plotting component 1 against component 2 reveals three clusters of cultivars (Figure 9). The first cluster is made up of A-SE-03 and A-SE-06 and is low for both component 1 and 2, which means they have taller plants at early stages but low yields, germinative capacity, and protein, P, saponin, and phenolic contents. These cultivars are also low for the component 3, and show the lowest viability rate, low antioxidant capacity but high ash content. The second cluster, comprising A-SE-01 cv., A-SE-02 cv., A-SE-07 cv., A-SE-12 cv., and A-SE-15 cv., has low component 2 values, especially A-SE-01 cv., so they are tall plants with smaller panicles and lower yields, but higher component 1 values than the first cluster, so generally they have higher protein, P, and Cu contents and lower Ca and Na contents than A-SE-03 cv. and A-SE-06. A-SE-05 cv., A-SE-08 cv., A-SE-09 cv., A-SE-10 cv., A-SE-11 cv., A-SE-13 cv., and A-SE-14 cv. comprise the third cluster, which has high component 2 values, having shorter plants and larger panicles, heavier plants, higher yields and germination rates, and higher protein and phenolic contents. A-SE-04 cv. and A-SE-15 cv. show the highest component 3 values, with the largest viability rates and FRAP values, but A-SE-04 cv. has lower component 1 values, with high Ca and Na contents and lower Cu and Zn contents, while A-SE-15 cv. has high component 1 values, and has high germination rates, and protein, P, Cu, and Zn contents and low Ca and Na contents.

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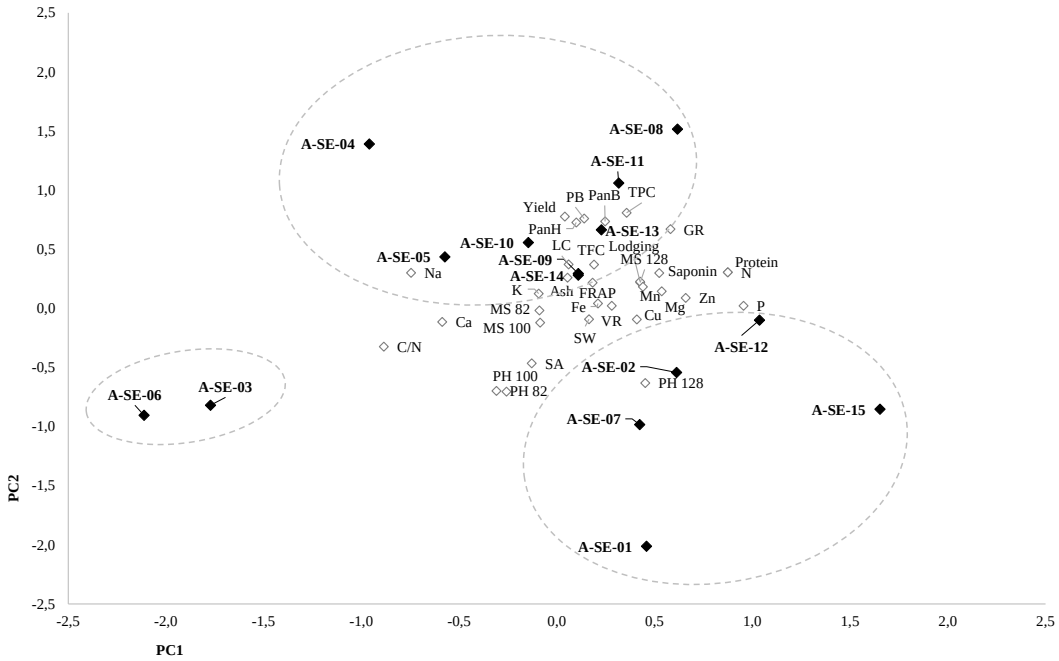

Figure 9. Principal components analysis (PCA). Biplot of main components 1 and 2 for the cultivars sown and for the variables tested. Component 1 (X axis) is contributed mainly by : protein and saponin contents, germination rate, minerals (P, Mg, Ca (-), Na(-), Mn, Cu, Zn), lodging, plant height 128 d.a.s., mildew 128 d.a.s. Component 2 (Y axis) includes germination rate, total phenolics content, plant height (-), plant biomass, panicle height and panicle weight (-).

3. Discussion

Quinoa is often compared to cereals and even considered a 'pseudocereal' due to the similarities in the composition and uses of their seeds [24]. Unlike cereals, quinoa seeds lack gluten, and have a great protein content, with a balanced amino acid profile, as well as high mineral contents and antioxidant power [25]. However, its yield potential in new cultivation areas is yet far to be reached, with levels similar to those of cereals such as wheat or rice before the Green Revolution [2]. The center of origin of quinoa is the Andean Altiplano [26], but in the last decades, quinoa has been introduced as an alternative emerging crop in more than 75 countries [5]. Along with its expansion, it has been observed that the establishment and adaptation of quinoa cultivars to these new agroclimatic contexts can result in changes in the nutritional properties of quinoa seeds which are associated with variations in the genotype (G), the environment (E) and their interaction (GXE) [14, 21]. Thus, there is yet much left for researchers and breeders to do to develop quinoa cultivars better adapted to specific locations presenting high yields while also maintaining or even improving the nutritional value of the seeds. In this study, we evaluated the physiological characteristics and different nutritional-related traits of seeds harvested from 15 different quinoa cultivars grown in southern Spain, aiming to expand our knowledge of the relation between yield and the nutritional quality of quinoa seeds and therefore, contributing to the selection of quinoa cultivars more appropriate for cultivation in a particular area of interest.

As previously described, the PCA revealed the existence of three clusters in which cultivars could be classified and showed distinct characteristics (Figure 9). At earlier stages of development (82 and 100 d. a. s.), plants from clusters 1 (A-SE-03 cv. and A-SE-06 cv.) and 2 (A-SE-01, A-SE-02 cv., A-SE-07 cv., and A-SE-15 cv.) were the tallest, but only those from cluster 2 kept taller at a later developmental stage (128 d. a. s., beginning of grain maturation) (Figure 1). Cluster 1 and cluster 2 plants also presented lower panicle lengths and biomass (Figure 2), and smaller seed yields. Seeds from these cultivars did

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not show higher nor lower seed weights or areas, and there was no correlation between these variables and yield (Supplementary Figure 3). From this, it can be assumed that plants from clusters 1 and 2, which invested more resources on growing at earlier stages of development, invested less resources on the development of the panicle and seed biomass. It should be noted that, for these plants, lower yields were not correlated with reduced seed weights, but with smaller panicles producing less seeds. This negative relation between plant height and seed yield and positive relation between panicle size (biomass and height) and yield (Table 3, Supplementary Figure 3) were previously reported in quinoa [27–29]. Furthermore, Gómez et al. [30] pointed the correlation between plant height and yield in quinoa and postulated that quinoa's low yield can be explained by its low sink capacity, and that an increase in reproductive partitioning, reducing plant height, could positively impact yield in this crop, as had happened previously to wheat and rice during the Green Revolution [31]. Therefore, plant height can be an important trait for breeding and further research analyzing endogenous factors that may control quinoa height and its relation with yield and lodging (i.e. phytohormones) should be considered in quinoa.

Yield was highly determined by different physiological traits including panicle size and plant biomass, but only correlated with two seed traits, that were the germination capacity and the phenolic content (Supplementary Figure 3). Intriguingly, previous works have observed correlations between yield and seed nutritional-related traits, like the positive correlation found between yield and the antioxidant capacity or the K content, or the negative correlation between yield and protein content or the amount of different amino acids [20,32,33]. However, these studies compared the nutritional profiles of quinoa seeds harvested from different cultivars but grown in different environmental conditions (with variations in the sowing date, the cultivation location and/or the year of cultivation), and the variations on the nutritional traits of the seeds were mainly determined by differences in the environmental conditions at seed filling stage, which affect both, yield and seed-quality traits [20]. In the present study, only the genetic factor was evaluated, so the lack of correlations between yield and seed nutritional-related traits suggests that there might be no link between them, and those relations are only relevant when introducing the cultivars to new environments where they are not yet adapted.

Downey mildew is one of the main diseases affecting quinoa on a global scale and it is caused by the fungus *Peronospora variabilis* Gäum [34]. Optimal conditions for mildew development are found at high humidity (>80% RH) and moderate temperatures (between 18°C and 22°C), but its expansion can be interrupted by long periods of sunny and dry conditions [34]. In this study, high RH was found in March, when most of the precipitations occurred and plants were still emerging or developing their first true leaves (Supplementary Figure 1). However, temperatures at that time were lower than 18°C so they were under the optimum for mildew development. Mildew produces chlorotic patches on leaves, which may result in premature defoliation by the plant as a defense mechanism. This reduction on photosynthetic area can lead to an atrophied development and smaller panicles, which in turn lowers seed yield [35]. When the infection occurs at early stages of development of quinoa, 20%-40% yield penalties have been estimated for mildew-resistant cultivars [36], and losses of up to 99% in susceptible cultivars [35]. However, the impact of mildew on well stablished mature plants are less important than abiotic stresses [37], since the defoliation caused by the disease and by the natural senescence of the plant overlap [34]. In the present study, mildew incidence and severity were limited at early stages (82 and 100 d. a. s.), with severities lower than 10% in most cultivars (Figure 3), but severity increased at a later stage (128 d. a. s.), being A-SE-02 cv. (cluster 2), A-SE-09 cv., A-SE-10 cv., A-SE-11 cv., and A-SE-13 cv. (cluster 3) the most affected ones, and A-SE-01 cv., A-SE-12 cv. (cluster 2), A-SE-03 cv., A-SE-06 (cluster 1), A-SE-04 cv., and A-SE-08 cv. (cluster 3) the least affected (Figure 3). Mildew severity did not correlate to yield (Supplementary Figure 3) nor to any seed nutritional traits (Supplementary Figure 3) [37], which suggests that the cultivars tested were resistant to mildew and did not suffer significant yield losses related to this disease.

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Saponins are considered as 'anti-nutrients' because of their negative effect on the bioavailability of minerals like Fe and Zn [38], together with the associated bitterness when they are present in substantial amounts in the seeds. Different breeding programs have been focused on the development of cultivars with very low seed saponin contents (sweet quinoa cultivars) [21]. Koziol [39] established the limit between sweet seed and bitter seed at 0.11% of seed weight, while Mastebroek et al. [40] considered sweet seeds those with saponin contents between 0.02% and 0.04% and bitter seeds those with contents between 0.47% and 1.13%. All of our samples fit the definition of bitter seed by Koziol [39], but only A-SE-04, A-SE-08, A-SE-10 cv. (cluster 3), A-SE-12 cv., and A-SE-15 cv. (cluster 2) would be considered bitter following the Mastebroek et al. [40] criterion, and all samples would be 'low-saponin' seeds according to Medina-Meza et al. [23]. In this regard, it should be pointed that sweet varieties are normally preferred since the elimination process of saponins is avoided. However, some farmers prefer bitter cultivars because saponins may confer resistance to biotic stresses [19, 37] Although saponins have been hypothesized to also give mildew resistance to quinoa [42] since they possess antifungal activities [43], no correlation has been found between seed saponin contents and mildew resistance [44] (Supplementary Figure 3).

Saponin is a highly genotype-dependent seed trait in quinoa [45], and no correlation to other seed traits had been found previously. In the present study, we found, for the first time to our knowledge, a correlation between saponin content with other seed quality-related traits like germination, protein content, and flavonoid content (Supplementary Figure 3, [42,43,44].

Germination capacity is an important seed characteristic since any genetic potential achieved through breeding efforts cannot be exploited if seed establishment in the field is not successful. In this study, most cultivars surpassed the 50% germination rate, but A-SE-13, A-SE-07, A-SE-01, and especially A-SE-03 and A-SE-06 (cluster 1), showed very low germination rates (Figures 5 and 9). Interestingly, it was found correlation between seed germination rates and the panicle's characteristics and seed yield of the mother plants [20] (Supplementary Figure 3), but germination rates were also influenced by nutritional traits of seeds. Both, the correlation and pathway analyses, showed a strong effect of the phenolic compounds and the P contents on the germination capacity of seeds (Supplementary Figure 3, Table 1). These results are in part supported by previous works, as it was already described a positive correlation between phenolic compounds and the germination capacity of quinoa seeds [20]. Furthermore, a stimulating effect of these compounds on the germination capacity has been reported as well in the close quinoa relative specie *Chenopodium album* L. [49]. On the other hand, P is present in quinoa seeds mainly as phytate [50], a form of P storage not bioavailable for many monogastric animal, including humans [47,48]. During germination, however, the phytase activity catalyzes the hydrolysis of the phytate [53], providing inorganic phosphate essential for the metabolism of the seed at the beginning of germination [54,55]. According to Nadeem et al., [56], a higher phytate content also means more hydrolysis and thus, higher phosphate available during germination, which may explain the correlation between P content and germination (Table 1, Supplementary Figure 3), since higher P contents in seeds are related to faster germination and better establishment of seeds in the field [57]. A strong positive correlation was also found between the germination capacity and protein content (Supplementary Figure 3), probably associated with the role that storage proteins play on germination [58].

In the present study, the protein content of seeds varied depending on the cultivar, with values ranging between 12.7% and 16.7% with the exception of two cultivars, the low-performing A-SE-03 and A-SE-06 (cluster 1). These two cultivars presented seed protein contents of 9%-10%, closer to the values found in cereals like maize and barley and lower than the values found in wheat [59]. The contents of the rest of cultivars fell within the range expected for quinoa seeds, with A-SE-02, A-SE-12, A-SE-15 (cluster 2), and A-SE-08 (cluster 3) exceeding the 15% [59]. However, it should be noted that the importance of the quinoa protein does not only rely on the quantity, but also on the quality, since it

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contains all amino acids which besides, are present in a proper balance, similar to the complete amino acid profile found in the cow milk and close to the ideal equilibrium recommended by the FAO for human consumption [60] [61].

The ash content ranged from 3.03% to 3.73% depending on the cultivar, although few significant differences were found among cultivars (Table 1) which were normal values for quinoa, but generally higher than those of cereals like wheat or rice [59,60]. The minerals that were present in higher amounts were K, P, Ca, and Mg, while Na, Fe, Zn, Mn, and Cu contents were the lowest (Table 1) [60]. All these minerals fell within the ranges previously reported for quinoa seeds [20,59], and some of them, like K, Ca, Mg, and Na, were higher than those found in cereals like maize, barley, rice, and wheat [61]. The high contents of Fe, Ca, and Mg are especially important since they are minerals less present in gluten-free products, and thus, quinoa seeds are an important source of these minerals for people with coeliac disease [60].

Noteworthy, the contents were significatively different among cultivars for all minerals except for Mg (Table 1). The variations in mineral contents in quinoa seeds had been previously reported to be cultivar-dependent, but they also respond to environmental differences during plant growth [20,33]. For instance, the cultivar A-SE-03 showed high Ca and Na, and low P and Zn contents, while A-SE15 cv. showed high P, Cu, and Zn contents and low Ca, Na, and Fe contents (Table 1). P content was high, but according to Konishi et al. [50], P is mostly found in quinoa seeds as phytic acid, which can form complexes with Fe, Zn, Mg, and Ca, reducing their bioavailability for human digestion [62]. Interestingly, [38] pointed out that, in fed experimental experiments with rats, there were no differences in Fe availability in quinoa supplemented diets compared to those supplemented with $FeSO_4$. Thus, further evaluation of the actual effect of quinoa seeds' phytic acid in Fe, Zn, Mg, and Ca availability should be carried out in order to elucidate which percentage of these minerals' contents is actually taken up during human digestion and if these contents reach the human nutritional requirements [63].

Antioxidants are of economic interest since they can minimize the rancidity and increase the shelf-life of food products [64], but they are also of nutritional interest due to their health-related benefits. Antioxidants have been found to reduce the risks of cancer and cardiovascular disease, and to present anti-inflammatory and anti-microbial activity [65,66]. Quinoa seeds are a good source of antioxidants, exceeding the antioxidant capacity of cereals [67]. This is due to their high contents of phenolic compounds, including flavonoids and vitamin E [68]. A correlation between phenolic compounds and flavonoids contents and the antioxidant capacity was expected (Supplementary Figure 3) [20,69]. However, other compounds may have an antioxidant role on quinoa seeds, such as ascorbic acid, phytic acid, sterols, carotenoids, saponins, and even some proteins, may also have radical scavenging potential [70,71]. The antioxidant capacity and phenolic compounds content are genotype-dependent in quinoa seeds [72], although they can also change depending on the environmental context [20,73,74]. In the present study, the antioxidant capacity, TPC, and TFC were comparable to those found in previous studies (Fig. 7) [20,66,72,74] and changed depending on the cultivar. For instance, the cultivars A-SE-04 and A-SE-15, and A-SE-04, A-SE-08, and A-SE-10 showed the highest levels of antioxidant capacity and TPC, respectively, while A-SE-01 presented the lowest antioxidant capacity and A-SE-03 and A-SE-06 the lowest TPC and TFC (Figure 7). These results correlated well with other seed-related traits like protein content and germination rates, and with seed yield (Supplementary Figure 3). This, together with the overall health benefits of antioxidants, make of TPC and TFC interesting traits for quinoa breeding programs.

4. Materials and Methods

4.1. Plant material, experimental design, and location

Field trials were carried out in a field experimental station located in Lebrija (Seville, Spain, 36.88°N, 6.13°W) in a clay-loam soil. Planting to harvesting dates took place from

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January to August of 2018. Fifteen different quinoa cultivars were used in this study, encoded as follows: 'A-SE-01', 'A-SE-02', 'A-SE-03', 'A-SE-04', 'A-SE-05', 'A-SE-06', 'A-SE-07', 'A-SE-08', 'A-SE-09', 'A-SE-10', 'A-SE-11', 'A-SE-12', 'A-SE-13', 'A-SE-14', and 'A-SE-15' and given by Algosur S.A. (Lebrija, Spain).

Each cultivar was sown on January 27th in two replicates of non-randomized plots, with dimensions of 4.5m x 266m, spacing between rows of 0.75m and 0.02m within rows. A drilling machine was used to sow with a density of seeds of $2kg.ha^{-1}$.

During the experiment, different measurements of physiological traits were taken. Plant height and downy mildew incidence and severity were measured at 82, 100, and 128 days after sowing (d.a.s), that corresponded to different developmental stages: fully emerged plants, panicle emergence, and beginning of seed ripening, respectively. Also, at 128 d.a.s., panicle length and weight of 25 plants per cultivar was measured. Plant harvesting took place when plants had naturally dried out at different time points: July 18th (172 days after sowing) for the cultivars 'A-SE-03' cv. 'A-SE-06' cv. , 'A-SE-07' cv., 'A-SE-09' cv., 'A-SE-12' cv., 'A-SE-13' cv., and 'A-SE-15' cv., and in August 1st (186 d.a.s.) for 'A-SE-01' cv., 'A-SE-02' cv., 'A-SE-04' cv., 'A-SE-05' cv., 'A-SE-08' cv., 'A-SE-10' cv., 'A-SE-11' cv., and 'A-SE-14' cv. Total seed yield was quantified from a 11.25 m^2 plot for each cultivar and the dry weight of 20 plants was measured.

Climatological data, including total precipitation, relative humidity (RH), and temperature was obtained daily from a local climatological station (Supplementary Figure 1). Sprinkle irrigation was supplemented at different developmental stages: at seed sowing (30 l/m^2),5 days after sowing (30 l/m^2), at the beginning of branching (30 l/m^2), at flowering (50 l/m^2) and during grain filling (50 l/m^2).

4.2. Seed weight and area.

Seeds were manually counted and weighed in an analytical balance. Seed area was analyzed using the open-source software ImageJ (http://rsbweb.nih.gov/ij/). Images 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).

4.3. Seed germination rate

Quinoa seeds were sterilized by soaking first in ethanol 70% for two minutes, next in bleach 50% with a droplet of Tween-20 for two minutes, and then rinsing several times in distilled water (H_2O). Sterilized seeds were sown on a double layer of filter paper wet with distilled water on Petri dishes and then transferred to a growth chamber under darkness and a controlled temperature of 23°C. Germination rate was counted daily for the first week after sowing. Seeds were considered as germinated when the radicle protrusion was longer than 2 mm.

4.4. Seed viability

Seed viability tests were performed using the tetrazolium method (2,3,5-triphenyl-2H-tetrazolium chloride). First, seeds were imbibed in distilled water at 30°C for an hour to facilitate longitudinal and superficial cuts of the embryo and to ensure a homogeneous dying of the seed tissues. After cutting, seeds were submerged in 1% tetrazolium chloride at 30°C for two hours. Seeds with more than 50% staining in the embryonic tissue were considered viable.

4.5. Saponin content

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To determine saponin content, 20 mL of 50 % ethanol was added to 1 g of powdered sample and left to macerate for 72 h at room temperature. Then, the extracts were filtered into 20 mL volumetric flasks. The samples were then filtered using a 0.45 µm nylon Filter-Lab syringe and analysed by HPLCDAD at 225 nm [75]. The results were expressed in g saponin $100g^{-1}$ of fresh weight.

4.6. Protein content

The protein content was determined according to AOAC Official Methods [76], using an elemental analyzer (Leco TruSpec) and considering a conversion factor of 6.25 [77].

4.7. Mineral content

The mineral content was analyzed following the official methods of analysis of the Spanish Ministry of Agriculture (MAPA, 1995). Phosphorus content was determined using a spectrophotometer UV-VIS (Hitachi U-2810) (yellow coloration, 430 nm). Potassium was determined through flame atomic emission spectroscopy. Calcium, magnesium, sodium, iron, copper, manganese, and zinc contents were assessed using flame atomic absorption spectroscopy (AAS) (SpectrAA 110, Agilent) after mineralizing the samples with H_2O and HCl (35%).

4.8. Ferric reducing antioxidant power (FRAP) assay.

To obtain total extracts, seeds were ground to a fine powder and 100 mg of the flour were homogenized in 1mL of an extraction buffer consisting of methanol (50%), acetic acid (1%) and distilled water (49%). These samples were vortexed for 2 minutes and kept in the dark at 4°C for 48 hours, before centrifugation for 15 minutes at 13500 rpm. The supernatants were stored at -20°C until their use in the FRAP and flavonoid content assays.

The antioxidant capacity of seeds was determined following the procedure described by Benzie and Strain [78]. The FRAP reagent consisted of a mix of 300 mM acetate buffer (pH 3.6), with 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1 (v/v/v). Twenty µL of sample extract and 180 µL of FRAP reagent were added into a 96-well microplate and, after 4 minutes, absorbance was read at 593nm using a microplate reader (Lector Multi-ModalSynergy HTX, BioTek Instruments, Inc., USA). The antioxidant capacity was calculated from a calibration curve obtained with iron (II) sulfate ($FeSO_4$). FRAP value was expressed as µmol of Fe^{2+} g^{-1} of seed.

4.9. Total phenol content (TPC)

Extracts were obtained homogenizing 100 mg seed flour in 1mL of ice-cold methanol (95%), vortexing, and centrifuging at 13500 rpm for 5 minutes after 48 hours kept in the dark and at 4°C.

The content of polyphenols was measured following the protocol described by Ainsworth and Gillespie [79]. Briefly, the mixture of 100 µL of sample extract or standard and 200µL of the Folin-Ciocalteu reagent 10% was vortexed for 1 minute before adding 800 µL of sodium carbonate 7.5% and was then incubated for 2 hours in the dark. The samples were centrifuged in order to eliminate precipitates. Absorbance was read at 765nm using a microplate reader (Lector Multi-ModalSynergy HTX, BioTek Instruments, Inc., USA). Concentrations of gallic acid between 20 $\mu g.mL^{-1}$ and 200 $\mu g.mL^{-1}$ in methanol (95%) were used as standard, and thus the TPC was expressed as mg of gallic acid equivalents per grams of quinoa seed (mg $GAE.g^{-1}$).

4.10. Total flavonoid content (TFC)

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Flavonoid content was determined following the procedure described by Valenzuela [80]. The same extracts as in the FRAP assay were used. Briefly, 30 µL of sample extract or standard, 10 µL of aluminum chloride ($AlCl_3$) 10%, 10 µL of sodium acetate (NaC2H3O2) 1M, and 250 µL of dH2O were mixed and incubated for 30 minutes. The absorbance was read at 415nm using a microplate reader (Lector Multi-ModalSynergy HTX, BioTek Instruments, Inc., USA). Quercetin dissolved in ethanol (80%) was used as standard with concentrations ranging from 10 $\mu g.mL^{-1}$ to 140 $\mu g.mL^{-1}$. The results were expressed in mg of quercetin equivalents per gram of quinoa seed (mg $QE.g^{-1}$).

4.11. Statistical analysis

To analyze the differences between cultivars different one-way ANOVA tests were performed. For variables where 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 one-way ANOVA on ranks (Krustal-Wallis test by ranks) was performed when data did not present a normal distribution, and a Welch's ANOVA test followed by a Games-Howell post-hoc test was performed when variances were not equal, both at a probability level of 5% ($p < 0.05$). Normality and equality of variances of the data were tested through a Kolmogorov-Smirnov's test and a Levene's, respectively. A sequential path analysis was performed to evaluate the specific contribution of different physiological traits to yield. This analysis allows ordering different variables as predictors of yield of first, second, or third-order [81]. For this purpose, a stepwise multiple linear regression procedure was used where variables that showed weak contribution ($p > 0.05$) to the dependent variable (yield) or high multicollinearity, were automatically dropped from the model. The variables entered into the model were considered as first-order predictors and the procedure was repeated using these variables as the response variable to identify traits that function as second-order predictors of yield. Tolerance and variance inflation factor (VIF) were used to measure the level of multicollinearity for each predictor trait, considering tolerance lower than 0.1 or VIF values higher than 10 as high levels of collinearity. Tolerance (1- R2i, where R2i is the coefficient of determination for the prediction of variable i by the predictor variables) is the amount of variance of the selected independent variable not explained by other independent variables. VIF (1/Tolerance) indicates the extent of effects of other independent variables on the variability of the selected independent variable. Principal component analysis was performed for plant parameters, like plant height at three stages, panicle length and biomass, plant biomass, mildew severity at different stages, resistance to lodging, life-cycle length and yield, and for seed parameters, like viability and germination rates, 1000 seeds' weight, seed area, saponin content, N and protein content and C-N ratio, FRAP value, phenols and flavonoids contents, and mineral contents. Correlations amongst variables were evaluated with a Pearson's correlation coefficient test. The SPSS Statistics 23.0 (SPSS Inc.) package was used for the statistical analyses.

5. Conclusions

Overall, this study reveals differences among cultivars for each physiological and seed nutritional-related trait analyzed, although there were similarities among some cultivars. For instance, A-SE-03 and A-SE-06, cluster together in the PCA, showed taller plants at early stages of development but shorter plants with smaller panicles and lower yields at maturity (Figures 1 and 9, Supplementary Figure 3). Regarding seed traits, they presented lower germination rates, and lower protein, P, phenols, flavonoids, and saponins contents (Figures 5, 7, 8 and 9, Table 1). The most promising cultivars for this agroclimatic context are those included in cluster 3, due to the higher yields, germination rates, and TPC (Figure 9). Yield is already the most important selection trait in breeding programs, since higher yields ensure higher productivity [21]. To keep an adequate germination rate is also an important agronomical trait in quinoa [82]. Also, TPC can be an interesting trait to explore in breeding programs, since polyphenols are bioactive compounds

that can improve shelf-life of the product and provide health benefits to the consumers [68]. Quinoa seeds are well known to be an excellent source of high-quality protein of non-animal origin. This is one of the main traits that makes quinoa a crop with a high nutritional quality, important for achieving food security locally and globally [83]. In line with this, also protein contents are relevant from a nutritional point of view. In this study, the higher protein contents were shown by A-SE-15 cv., A-SE-02 cv., A-SE-12 cv., and A-SE-08 cv. Thus, the cultivar A-SE-08 (cluster 3) not only presented a high protein content, but also higher yield, germination rate, P and phenolic contents, all of these making this cultivar one of the most promising ones for this particular agronomical area. Having all these traits positively correlated can greatly facilitate the development of a better adapted cultivar. However, it should be noted that saponin content was also higher in this cultivar (Figure 9). Saponin content is one of the main targets in breeding programs, as reducing the saponin content can improve nutritional quality and flavor [19,68]. Saponins are located in the external coat of the episperm, so they can be partially removed from seeds by using abrasive methods and/or washing with cold water [85]. However, these processes can be costly and time consuming [86]. Pulvento et al. [87] and Gómez-Caravaca et al. [48] have reported that agronomical management practices can lower the saponin content so it would be interesting to study these aspects when establishing this crop cultivation.

Therefore, the results here presented highlight the importance of considering the genotypic variation in quinoa when selecting improved quinoa varieties with better nutritional characteristics for new cultivation environments. Further studies are required to determine which exact parameters are genotype-variable and which ones show genotypic stability.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

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Data Availability Statement: The *data* that support the findings of this study are *available upon request.*

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