Running title: *environmental determinants of fatty acids composition in quinoa*

Oil quality in sea level quinoa as determined by cultivar-specific responses to temperature and radiation conditions

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Abstract

BACKGROUND

There is renewed interest in quinoa as a potential source of vegetable oils; however, there is no knowledge about how environmental conditions affect its fatty acids composition, a critical indicator of its oil quality. The fatty acid concentrations of four cultivars adapted to temperate environments were compared at three sowing dates in order to evaluate the effect of environmental conditions during the seed-filling period on the variation in oil quality.

RESULTS

The interaction between cultivar and sowing date was the main source of variation explaining the changes in the lipid content and fatty acid concentrations in quinoa. Most of the variation in the concentration of unsaturated fatty acids was attributed to the temperature and solar radiation during the seed-filling period; cultivar-specific responses to photo-thermal conditions were observed among the sea level quinoa cultivars evaluated.

CONCLUSION

Lipid content and concentration of fatty acids in quinoa is affected by sowing date. This effect is exerted through changes in temperature and solar radiation conditions. Therefore, this managing practice can be used to achieve quinoa oil with different qualities.

Keywords: *Chenopodium quinoa* Willd.; Lipid content; Unsaturated fatty acids; Sowing dates; Seed-filling period.

INTRODUCTION

In oilseed crops, the fatty acids composition determines its oil quality [1]. Changes in the fatty acid composition have been associated to the temperature conditions during the seed-filling period, because of their regulation in the activity of desaturase enzymes [2,3]. It has been demonstrated that the level of fatty acid unsaturation is inversely associated with growth temperature during seed development [3]. As a rule, a decrease in the unsaturation level occurs when the temperature is high during seed development [2-4]. However, oilseed crops showed specific-responses to temperature as this condition weakly affected the proportion of unsaturated fatty acids in safflower (*Carthamus tinctorius*) and castor bean (*Ricinus communis*), moderately in soybean (*Glycine max*), rapeseed (*Brassica napus*) and maize (*Zea mays*) and highly in sunflower (*Helianthus annuus*) [4].

Recently quinoa (*Chenopodium quinoa* Willd.), an underutilized Andean crop, drew worldwide attention due to their nutritive value as well as their high potential yields [5]. This pseudocereal has a lipid content ranging from 48.7 to 97.0 g kg^{-1} in its whole seeds and yield between 80-400 kg of oil ha^{-1}, which highlight it as a new potential oilseed crop [6]. Besides, the high content of oleic, linoleic and α-linolenic acids (unsaturated fatty acids) and a closer to an optimal omega-6/omega-3 ratio, are the facts supporting the oil quality of this crop [7]. Quinoa cultivars and accessions from the Andean and sea level gene pools show variation in their fatty acids composition [6]. However, studies addressing the contribution of environmental conditions to changes in the fatty acid composition of quinoa are lacking. We hypothesized that variation in the

fatty acid composition are related to temperatures during seed development. In this sense, quinoa seeds with a low proportion of unsaturated fatty acids could be achieved if their seed-filling periods are matched to high temperatures.

MATERIALS AND METHODS

Sowing dates experiments were conducted at a low elevation enviroment (Faculty of Agronomy of the University of Buenos Aires). Temperature and solar radiation values were obtained from a weather station (Li-COR 1200; Lincoln, NE, USA) located in the experimental field. The experiment was a split-plot with the sowing dates as main plots and cultivars as sub-plots layout in a randomized complete block design with three replicates. Sowing dates were July 2 (winter), October 10 (early spring) and November 15 (mid spring) whereas the sea level quinoa cultivars were NL-6, CO-407, Salto de Agua and 2-Want. Plots were five rows wide by 3 m long with an area of 7.5 m^2. In order to complement natural rainfall and avoid water deficit and mineral restrictions, plots received supplementary irrigation during the growing season and received fertilization at sowing (20 kg P and 18 N kg ha^{-1}) and one urea application (totaling 100 kg N ha^{-1}) 30 days after emergence. The duration of the seed-filling period is defined as the time (days) between the end of anthesis and physiological maturity (when seeds from the main panicle become resistant when pressed).

Raw quinoa seeds were assessed for their total lipid content according to the AOAC official method 930.09 [8]. The lipid extraction was performed by using a mixture of chloroform-methanol (2:1, v/v), 10% of methanolic BF3 was added to the chloroform

phase to obtain fatty acid methyl esters. Free fatty acids were analyzed in a Hewlett Packard 6890 gas chromatograph with a flame ionization detector; a capillary column of 30 m × 0.25 mm and 0.1 mm film thickness (Chrompack CP SIL 88) [9]. The concentrations of the main fatty acids in relation to the content of total lipids in whole seeds were calculated for each cultivar and sowing date, which were expressed as g kg^{-1}. The unsaturated/saturated fatty acid ratio (hereafter, UFA/SFA) was also calculated. Although they were not determined in this study, other long chain fatty acids are presented in quinoa [10]. Nevertheless, their concentrations are lower than those of oleic, linoleic and α-linolenic acids [7,10].

Linear models were applied to evaluate the main and interaction effects for lipid content and fatty acid concentrations through analysis of variance. First, we fitted a linear model considering the cultivars and sowing dates and cultivar × sowing date as main and interaction effects, respectively. In the second stage, the environmental conditions (temperature and solar radiation) during the seed-filling period were used to fit a linear model. The *post-hoc* DGC multiple comparison test [11] was used to determine differences among treatment means when significant differences were detected. The DGC multiple comparison test is named for the authors Di Rienzo, Guzman and Casanoves and its main advantage resides in avoiding the overlapping among groups of treatment means. All statistical analyses were conducted using the Infostat® software [12].

RESULTS AND DISCUSSION

Variation in sowing dates exposed the seed-filling period of sea-level quinoa cultivars to different temperature and solar radiation conditions as shown in Figure 1. The seed-filling period was exposed to a maximum, mean and minimum temperatures and solar radiation values of 24.0, 18.4, 13.7 °C and 21.1 MJ m^{-1} day^{-1} during the winter sowing date, whereas to 28.9, 25.0, 19.3 °C and 23.3 MJ m^{-1} day^{-1} in the mid-spring sowing date (Figure 1). A significant variation in the lipid content and the values for all the fatty acids was observed among cultivars and sowing dates (Table 1). Most of this variation was accounted by the cultivar-by-sowing date (C × S) interaction effect (Table S1). However, the ratio among mean squares for cultivar (C) and C × S interaction effects ranged from 1.93:1 for the stearic fatty acid to 20.36:1 for the oleic fatty acid (Table S1). This higher magnitude of C compared to C × S support the notion, at least partially, that a selection of sea level-quinoa cultivars could be adopted to maximize specific combinations of lipid content and fatty acid concentration.

Our results contrast with a study recently conducted in Germany in which only the genotype effects accounted most of the variation in the fat content and the fatty acid concentration [13]. The contrasting results could be due to the cultivars and/or to the explored growing conditions. Large differences in temperature and solar radiation conditions were observed among sowing dates in our experimental site (Figure 1), whereas the variability among years in German environmental conditions during the seed-filling period were unable to induce variation in the lipid content and the fatty acid concentration [13].

We hypothesized that temperatures during seed development affect the fatty acid composition in quinoa determining its oil quality. Although, a significant cultivar-by-temperature interaction effects accounted the variation in the concentration of all fatty acid and the total lipid content. The interaction of cultivar-by-solar radiation conditions also affected both traits in sea-level quinoa cultivars (Table 2). This result is consistent with the response displayed by other oilseed crops such as sunflower, maize, soybean and rapeseed [4]. Besides, both factors seem to have additive effects in the quality of quinoa oil since a similar pattern of response for total lipid and fatty acids was observed when increasing the temperatures and solar radiation conditions from winter to mid-spring sowing dates, respectively (Figure 2). It should be taken into account; however, that this similarity could be due to the covariation of temperatures and solar radiation conditions along with sowing dates (Figure 1). Future experiments aimed at isolating the effects of temperature and solar radiation conditions are needed to establish their relative contribution in the fatty acid composition of quinoa seeds.

The photo-thermal conditions during the seed-filling period in our low elevation experimental site induced changes in the lipid content that were accompanied by a significant variation in the fatty acid concentration (Figure 2). However, the general decrease in the lipid content did not affect identically the major unsaturated fatty acids among cultivars. A decrease in the oleic and α-linolenic concentration was observed for almost all cultivars, whereas the linoleic concentration remains unchanged for some cultivars (e.g. cultivars CO-407 and Salto de Agua) or even increased in others (e.g. cultivars 2-Want and NL-6) (Figure 2). This result suggests that cultivar-specific

responses to photo-thermal conditions during the seed-filling period are involved in quinoa as it was reported among sunflower hybrids and oilseed rape cultivars [4,14]. Future studies should evaluate the underlying mechanism which explains the differential thermal stability of desaturases enzymes in sea level quinoa cultivars in response to their fatty acid composition.

CONCLUSIONS

The results of the present study support the notion that cultivar-specific responses for total lipid content and fatty acid composition to photo-thermal conditions during the seed-filling period were involved in sea level quinoa. Accordingly, oils with differential quality could be achieved in quinoa by appropriately selecting cultivars and growing conditions during the seed-filling period.

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Table 1. Lipid content and fatty acids composition (g kg^{-1}whole seeds) for sea level quinoa cultivars and sowing dates. Values are mean plus/minus standard error, *n*= 3. Different letters within columns indicate statistical differences after DGC multiple comparison test.

Cultivar	Sowing date	Lipid content	Saturated fatty acids			Unsaturated fatty acids				UFA/SFA†
			Myristic (C14:0)	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1 *w*-9)	Linoleic (C18:2 *w*-6)	γ-Linolenic (C18:3 *w*-6)	α-Linolenic (C18:3 *w*-3)	
CO407	W‡	60.87 ± 2.19[c]	0.15 ± 0.01[c]	7.05 ± 0.25[c]	0.60 ± 0.02[d]	13.71 ± 0.49[c]	34.98 ± 1.26[b]	0.59 ± 0.02[c]	4.39 ± 0.16[c]	6.68
	ES‡	47.53 ± 0.95[a]	0.09 ± 0.01[a]	5.65 ± 0.11[b]	0.52 ± 0.01[c]	8.57 ± 0.17[a]	29.15 ± 0.58[a]	0.14 ± 0.01[a]	3.39 ± 0.07[b]	6.57

	MS‡	53.77 ± 0.81[b]	0.13 ± 0.01[b]	5.89 ± 0.08[b]	0.46 ± 0.01[b]	9.30 ± 0.14[a]	34.06 ± 0.51[b]	0.53 ± 0.01[b]	3.35 ± 0.05[b]	7.27
NL6	W	66.0 ± 1.0[c]	0.16 ± 0.01[c]	6.66 ± 0.10[c]	0.47 ± 0.01[b]	15.97 ± 0.24[d]	36.37 ± 0.55[b]	0.87 ± 0.01[f]	5.07 ± 0.08[e]	7.98
	ES	49.73 ± 0.73[a]	0.17 ± 0.01[d]	5.19 ± 0.07[a]	0.48 ± 0.01[b]	12.13 ± 0.18[b]	28.32 ± 0.42[a]	0.66 ± 0.01[d]	2.76 ± 0.04[a]	7.49
	MS	63.83 ± 0.60[c]	0.18 ± 0.01[d]	6.53 ± 0.06[c]	0.35 ± 0.01[a]	12.49 ± 0.12[b]	38.98 ± 0.37[c]	0.84 ± 0.01[f]	4.41 ± 0.04[c]	8.01
Salto de Agua	W	67.57 ± 1.50[c]	0.23 ± 0.01[f]	7.58 ± 0.16[d]	0.48 ± 0.01[b]	14.30 ± 0.32[c]	40.27 ± 0.89[c]	0.76 ± 0.02[e]	4.70 ± 0.10[d]	7.23
	ES	48.67 ± 0.79[a]	0.25 ± 0.01[g]	6.19 ± 0.10[b]	0.45 ± 0.01[b]	9.51 ± 0.15[a]	28.93 ± 0.47[a]	0.51 ± 0.01[b]	2.82 ± 0.05[a]	6.06
	MS	64.50 ± 1.27[c]	0.14 ± 0.01[c]	6.71 ± 0.13[c]	0.40 ± 0.01[a]	13.02 ± 0.26[b]	40.70 ± 0.79[c]	0.83 ± 0.02[f]	2.69 ± 0.05[a]	7.88

2 Want	W	72.40 ± 2.12[d]	0.21 ± 0.01[e]	8.45 ± 0.24[e]	1.76 ± 0.05[e]	18.04 ± 0.53[e]	39.22 ± 1.15[c]	0.81 ± 0.02[f]	4.09 ± 0.12[c]	5.96
	ES	66.83 ± 3.06[c]	0.21 ± 0.01[e]	7.37 ± 0.33[d]	0.62 ± 0.03[d]	14.86 ± 0.68[d]	39.45 ± 1.81[c]	0.75 ± 0.03[e]	3.56 ± 0.16[b]	7.14
	MS	72.07 ± 1.55[d]	0.28 ± 0.01[h]	6.73 ± 0.14[c]	0.56 ±0.01[c]	15.35 ± 0.33[d]	43.99 ± 0.95[d]	0.94 ± 0.02[g]	4.21 ± 0.09[c]	8.51

[†]UFA: unsaturated fatty acids; SFA: saturated fatty acids

[‡]W: winter; ES: early spring; MS: mid spring

Table 2. Analysis of variance for lipid content and fatty acids concentration (g kg^{-1} whole seeds) for cultivars, temperature and solar radiation conditions during seed-filling period. Values are mean squares.

Sources of variation	Df†	Lipid content	Myristic	Palmitic	Stearic	Oleic	Linoleic	γ-Linolenic	α-Linolenic
Cultivar (C)	3	417.02**	0.02**	3.75**	0.60**	49.09**	107.70**	0.30**	0.80**
Temp./SR‡	2	599.7**	$1.20e^{-4}$	5.68**	0.51**	56.77**	211.02**	0.26**	6.29**
C × Temp./SR	6	35.26**	0.01**	0.57**	0.31**	2.41**	22.14**	0.02**	1.05**
Block	4	4.76	$4.80e^{-5}$	0.06	$1.20e^{-3}$	0.27	1.52	$5.80e^{-4}$	0.02

†Df: degrees of freedom.

‡Temp: temperature; SR: solar radiation.

*Significant at $P< 0.05$; ** significant at $P< 0.01$.

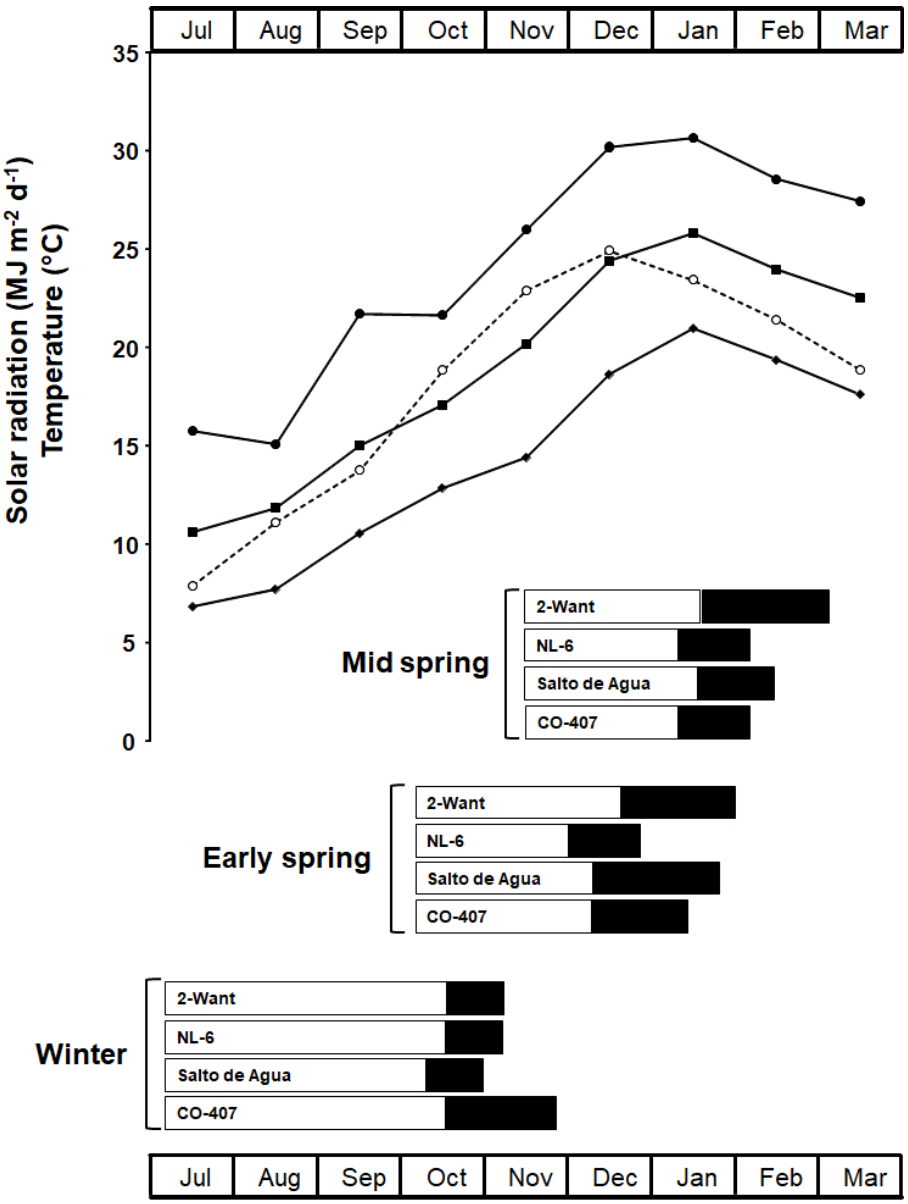

Figure 1. Mean monthly conditions for temperature (ºC) and solar radiation (MJ m^{-2} d^{-1}) for the sowing date experiments. Minimum (♦), mean (■), maximum (●) temperatures, and solar radiation (○). Durations from emergence to the end of flowering (white horizontal bars) and from the end of flowering to physiological maturity (black horizontal bars) for four cultivars sown at three dates: winter (July 2), early spring (October 10) and mid-spring (November 15) are presented.

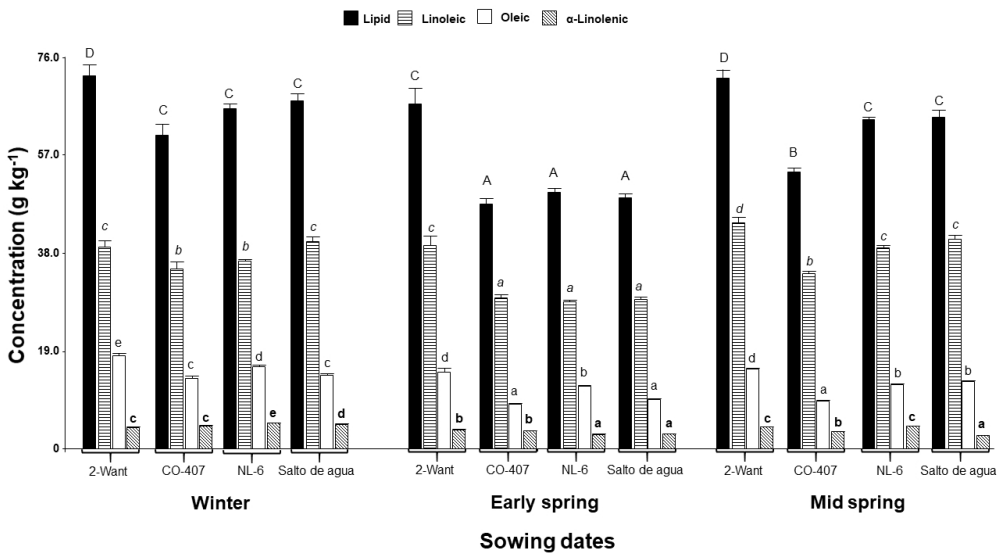

Figure 2 Lipid content and fatty acids concentration for sea level quinoa cultivars for winter (mean temperature 18.4 ºC and solar radiation 21.1 MJ m^{-2} d^{-1}, respectively), early (25 ºC and 24.5 MJ m^{-2} d^{-1}) and mid-spring (25 ºC and 23.3 MJ m^{-2} d^{-1}) sowing dates. Bars are means plus standard errors. Different letters among bars indicate statistical differences after DGC multiple comparison test. Uppercase letters: lipid content, italics lowercase letters: linoleic acid, lowercase letters: oleic acid and bold lowercase letters: α-linolenic acid