# Seed yield of two new quinoa (*Chenopodium quinoa* Willd.) breeding lines as affected by sowing date in Central Italy

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#### ABSTRACT

Research on the introduction of quinoa in Italy is currently lacking. The present research was aimed at identifying the correct sowing period. Field experiment was consucted in Cesa, Tuscany, in 2017. Two new breeding lines coded as DISPAA-Q42 and DISPAA-Q47-CB were utilized. Three sowing dates (SD) were implemented: February 23; March 17 and April 27. Results showed that the most successful SD was February 23. A significant decrease in both seed yield and a delay in phenological phases, relating to plant maturation and flowering was associated with the sequential delay in SD in both lines. Results also showed a significant effect of lines on vield, true-leaf stage development, flower development and maturity. Only DISPAA-Q42 was considered suitable for cultivation in the Tuscan environment. DISPAA-Q47-CB was the more susceptible line, due to the sequential delay in SD and delayed plant maturation. No effect between lines was evident for protein and saponin content. The present study clearly shows the potential for the successful cultivation of quinoa in Central Italy, and highlights the necessity of taking into consideration both breeding lines and SD in order to accomplish this goal.

#### IZVLEČEK

#### VPLIV DATUMA SETVE NA PRIDELEK SEMENA DVEH NOVIH LINIJ KINOJE (*Chenopodium quinoa* Willd.) V OSREDNJI ITALIJI

Raziskav o uvajanju kinoje v Italiji trenutno ni. Namen te raziskave je bil ugotoviti primeren čas setve. V ta namen je bil leta 2017 izveden poljski poskus v Cesi, Toskana. Uporabljeni sta bili dve novi žlahtniteljski liniji kinoje, 'DISPAA-Q42' in 'DISPAA-Q47-CB'. Setev (SD) je bila opravljena v treh terminih: 23 februarja; 17 marca in 27 aprila. Rezultati so pokazali, da je bila najuspešnejša setev 23 februarja. Pri obeh linijah je bil pri kasnejših terminih setve opazen značilen upad pridelka in zastoj v fenoloških fazah kot sta cvetenje in zorjenje rastlin. Rezultati so pokazal značilni učinek linije na pridelek, razvoj pravih zelenih listov, cvetenje in zrelost. Samo linija DISPAA-Q42 se je izkazala primerna za gojenje v okoljskih razmerah Toskane. Linija DISPAA-Q47-CB je bila bolj občutljiva na kasnejšo setev zaradi zakasnelega zorjenja rastlin. Med obema linijama ni bilo nobenih razlik v vsebnosti beljakovin in saponinov. Raziskava jasno nakazuje potencial uspešnega gojenja kinoje v osrednji Italiji in poudarja potrebo po upoštevanju tako žlahtniteljskih linij kinoje kot časa setve za doseganje zastavljenih ciljev.

Ključne besede: osrednja Italija; *Chenopodium quinoa;* nove žlahtniteljske linije; kinoja; datum setve; Toskana

#### **1 INTRODUCTION**

The nutritional qualities of quinoa (*Chenopodium quinoa* Willd.), rich in both proteins and essential amino acids, together with its suitability for use by people with celiac disease, has resulted in an increased worldwide demand for food products. Among the world markets, the European market has registered the greatest increase. The Italian market for gluten-free products

currently ranks second in the world, with a shares of 13 % corresponding to an annual turnover of approximately 145 million Euros (Euromonitor International, 2015). Although there are no official data, it was estimated, in 2015, that Italy imported approximately 2.5 % of the world production in quinoa, an equivalent of 2500 t. In addition to the alimentary

Key words: Central Italy; *Chenopodium quinoa;* new breeding lines; quinoa; sowing date; Tuscany

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benefits (De Feo et al., 1997; Repo-Cardoso et al., 2003), the potential introduction of quinoa as an alternative crop has attracted the attention of farmers internationally, even within areas outside the geographical origin of this species. This is especially evident for temperate environments.

The concept of introducing quinoa in Italy originated in the early twentieth century, in view of the excellent nutritional properties already recognized (Racah, 1917; Anonymous, 1936; Maugini, 1936; Massa, 1936). However, the actual introduction of quinoa in Italy (approximately 500 ha) occurred more recently. Noteworthy, this introduction was performed in a disorganized manner, in that the preliminary phase of experimentation, necessary to identify both suitable agronomic varieties and cultivation techniques, was lacking. Initially, it was naively thought that it was merely sufficient to introduce the varieties in Italy. However, as could have been predicted scientifically (Christiansen et al., 2010; Bendevis et al., 2014), there were problems relating to photoperiod adaptation. The second phase of quinoa introduction in Italy involved the introduction of varieties established in Europe such as the 'Titicaca', 'Puno', 'Vikinga', 'Atlas', 'Pasto' and 'Rio Bamba'. Nonetheless, the biggest problems facing cultivation included the lack of adaptability to photoperiod, maturation difficulties, and a decrease in quality (Casini and Fabbrini, 2017). The introduction of quinoa in Italy could have had interesting prospects for farmers from the economic point of view. Farmers, due to the international quotations of common cereals, which are presently at minimum levels, are currently looking for valid alternatives.

Since the 1980s, various European countries have been conducting research on the cultivation of quinoa by exploiting the existing genetic variability (Jacobsen 1997, 2015). However, research in Italy has been limited (Casini, 1997, 2002; Casini and Proietti, 2002; Pulvento et al., 2010, 2012; De Santis et al., 2011, 2014, 2016; Lavini et al., 2014).

The first research project conducted in Central Italy (Tuscany) dates back to 1999, with the University of Florence as the national coordinator of the FAO-UNA-PERU project entitled "American and European test of

quinoa (Chenopodium quinoa)" (Mujica et al., 2001). The research stressed how photoperiod sensitivity rendered the genotypes derived from northern areas of the Andean Altiplano (mostly from Bolivia and Peru), unsuitable for introduction in the Mediterranean environments. Moreover, only few of the twenty five accessions reached physiological maturation, with the highest grain production attained by 'E-DK-4, BAER II' and '02-Embrapa' (2.8, 0.9 and 1.1 t ha<sup>-1</sup>, respectively). However, the results of the study were incomplete, and it was still necessary to address the problems facing the cultivation of quinoa. In fact, the identification of the most suitable sowing date is one of the most important agronomic aspects that needs to be taken into consideration for the successful cultivation of quinoa. The potential adaptation of this species to photoperiods, differing from that existing in the areas of origin, depends largely on an ecotype classification of varieties within the species. For example, the varieties of Chilean origin classified as "sea-level-type" are more easily adaptable to temperate environments, such as that of the Mediterranean areas (Wilson, 1990).

The only results published to date were those carried out in Italy (province of Caserta), whereby the period March-May was shown to be the most suitable sowing period (Pulvento et al., 2010; Lavini et al., 2014; De Santis et al., 2014). The only existing comparison between different sowing dates (Lavini et al., 2014), showed a considerable yield reduction of approximately 55 %, when the sowing date was delayed by one month in the period April-May. Therefore, it is evident that the potential for successful cultivation of quinoa in Italy necessitates further research.

The aim of the present study was to identify the most suitable sowing period for quinoa in the lowland areas of Central Italy. Moreover, the aim was also to assess whether two new varieties, selected on-site were suitable for cultivation and how this suitability may have been affected by sowing date. Suitability for cultivation was assessed, not only by examining effect of line and sowing period on the yield, but also on two biochemical parameters, namely protein and saponin content. Increased protein content is an important nutritional characteristic of quinoa, whereas reduced saponin content is a required technological aspect.

## 2 MATERIALS AND METHODS

The field experiment was carried out in Tuscany, Central Italy, in 2017 at the "Centro per il Collaudo ed il Trasferimento dell'Innovazione di Cesa (Arezzo)", 43° 18' N; 11° 47' E; 242 m a.s.l. The cultivation environment was comprised of a neutral, loamy-sandy soil. The principle physical and chemical characteristics of the soil were as follows: sand 36.0 %, loam 38.1 %, and clay 25.9 % respectively. The soil pH was 7.0. Total N was 0.110 % and P (Olsen) 13 ppm. Exchangeable Ca, Mg and K were 4123, 595 and 141 ppm,

respectively. Two new breeding lines, obtained by the University of Florence, in the same area of the experiment during 2010-2017, were used in the present research, and coded, ''DISPAA-Q42' and 'DISPAA-Q47-CB'. The lines were derived from two series of poly-crosses between Chilean "sea-level-type" genotypes that were selected based on photo-period adaptability, early-ripening and plant architecture according to the following ideotype defined by Donini (1997): maximum plant height of approximately 1.3 m, with no ramifications; early-ripening, and > 2.0 g mass of 1000 seeds. Based on previous observations (unpublished work), the autumn-winter sowing periods were not included due to serious damage induced by low temperatures. As a result, the sowing dates ranged from late winter to spring. Plots were arranged, according to a RCB split-plot design with three replicates. The size of the overall plot was 15.0 x 4.0 m, which constituted the main factor comprising line ('DISPAA-Q42' and 'DISPAA-Q47-CB'), while the subplots constituted three different sowing dates (SD) as follows: February 23; March 17 and April 27 (hereon referred to as first, second and third SD). Each subplot had a width of 2.0 m (four rows wide with 0.5 m row spacing) and a length of 5.0 m. The sampling area was comprised of the two central rows only. A seed quantity of 30 kg ha<sup>-1</sup> was used. In order to attain the correct planting density of 15 plants m<sup>-2</sup>, seedlings were thinned at the two-true leaf stage. Fertilizer treatment before seeding was as follows: 76 kg ha<sup>-1</sup> of N as ammonium nitrate, and 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as superphosphate. Plots were hand-weeded twice (35 and 55 Days After Emergence [DAE]) during the growth cycle. Due to the early onset of flea beetle (Chaetocnema tibialis (Illiger, 1807)), 10-15 DAE at all sowing dates, the seedlings were treated with the insecticide, deltamethrine (50 ml 100 l water<sup>-1</sup>). The following field measurements were recorded: emergence of the 2-, 4-, 6- and 10- true-leaf stages; early panicle appearance; full panicle appearance; early flowering; waxy maturation and maturation at 75 %. For the maturation stage, both total leaf loss and seed consistency were taken in consideration together with complete filling (nontranslucent endosperm).

Plant height was measured for each phenological stage, using a total of 10 plants per sample plot. Corresponding to the 10-true leaf stage, before the appearance and formation of the panicle, downy mildew (*Peronospora farinose* f. sp. *chenopodii* Fr.) was observed on the basal leaves of the plant. Sensitivity to the pathogen was estimated according the scale proposed by Inguilàn and Pantoja (2007). This scale takes into consideration the surface area percentage of the leaf showing disease symptoms. No specific treatment was applied.

The harvest was performed manually starting from July 7 to September 7, 2017. The duration of maturation was dependent on both the date of sowing and the line. As a result, the different plots of all replicates were harvested accordingly.

After drying the seeds to a standard humidity of 12 %, (airflow at 35 °C for 48 h), the yield calculations were performed. A sample from a seed batch was used to determine the mass of 1000 seeds. The saponin content was measured according to Koziol (1991). Total protein was determined from the N content (N x 6.25) using an Elemental Analyser EA FLASH 1112 of Therma Fisher Scientific. Climatic data was obtained from the meteorological station near the experimental site. Day records were provided by "Centro length Interdipartimentale di Bioclimatologia-CIBIC" (University of Florence). Cumulative Growing Degree Days (GDD) were recorded from the date of the first sowing period (February 23) to the last harvest period (September 9) with a  $T_z$  equal to 3 °C (Jacobsen and Bach, 1998) as follows:

T<sub>m</sub> is the daily mean temperature:

$$GDD = \sum_{days} (Tm - Tz)$$

Cumulative Total Solar Radiation (TSR) recorded during the trial was provided by the "Centro Funzionale Toscana" Regione which uses an ETG Agrometeotological Station. Differences between response variables were assessed with COSTAT 6.45 software. Statistical differences were tested at  $p \le 0.05$ ,  $p \le 0.01$  or  $p \le 0.001$ . The Tukey's HSD test was used to evidence significant differences between means and homogenous groups.

#### **3 RESULTS AND DISCUSSION**

Given that photoperiod and climatic conditions are imperative to the potential success of quinoa cultivation in Central Italy, it was important to consider this information during the experimental trial. The climatic data shown in Figure 1, indicated high temperatures recorded throughout the crop cycle. In particular, maximum temperatures exceeding 30 °C were recorded during mid-June to mid-September. Another noteworthy characteristic was the thermal variability, especially between June and August, where temperatures oscillated between 15 and 20 °C.



Figure 1: Temperature and rainfall recorded during the field experiment

The photoperiod and GDD trend are shown in Figure 2. From the first sowing date up until 200 DAE,

approximately 2700 °C were accumulated and photoperiod increased until 110 DAE.



Figure 2: Cumulative Growing Degree Days (GDD) and day-length recorded during the field experiment

The analysis of variance was conducted to verify whether line, sowing date and "line x sowing date" were factors influencing yield, as well as various phenological and biochemical parameters of relevance to this crop. Results (Table 1) highlight the significant effect of line on yield, true-leaf stage development, flower development and maturity. In contrast, no effect was shown for emergence date, waxy maturation, saponins and proteins content. The effect of sowing date was significant for all parameters analyzed with the exception of the emergence date (Table 1). Excluding the 10-true leaf stage and saponin content, the interaction "line x sowing date" produced significant effects for all variables considered.

Table 1: Analysis of variance.

Source of variation	DF	Yield	Plant height	Emergence	Two true	Four true	Six true leaves	Ten true	
					leaves	leaves		leaves	
Blocks	2	7.011	70.333	0.777	30.333*	7.444	8.444*	3.000	
Variety (V)	1	3.591**	953.388*	12.500	37.555*	144.512**	470.220***	107.556*	
Error	2	0.001	53.444	4.333	0.777	2.333	0.444	10.778	
Date of sowing (DS)	2	4.453**	11023.000**	80.111	312.333***	266.788***	843.111***	3710.333***	
DS x V	2	1.641**	1946.777**	3.000***	14.777**	110.334***	123.111***	5.444	
Error	8	0.055	147.555	15.555	4.222	15.566	20.445	24.889	
Source of variation	DF	First panicle	Panicle	Early	Waxy	Maturation	Saponins	Proteins	1000 seeds
		appearance		flowering	maturation				weight
Blocks	2	2.111	1.444	4.777	9.000	11.444	0.072	0.204	0.088
Variety (V)	1	72.000**	102.722*	186.889**	5.555	401.388*	0.049	1.069	0.103*
Error	2	1.000	5.444	0.777	1.444	8.777	0.050	0.308	0.007
Date of sowing (DS)	2	3468.122***	2268.778***	2277.445***	764.333***	5633.777***	0.687***	23.207***	0.275***
DS x V	2	22.334***	24.777**	25.444**	38.111**	43.111**	0.059	5.643***	0.217
Error	8	3.555	9.767	9.112	15.556	16.444	0.099	0.183	0.015

\*: significant at  $p \le 0.05$ ; \*\*: significant at  $p \le 0.01$ ; \*\*\*: significant at  $p \le 0.001$ .

Table 2 shows the number of days elapsing from the emergence date until the first appearance of the panicle, flowering, maturation and the respective duration of the photoperiod, besides GDD and the cumulative TSR. Generally, the number of days required for the appearance of the panicle and flowering date decreased significantly from the first to the third SD. In contrast, as regards maturation, the inverse trend was recorded.

Table 2: Main growth stages, day lenght, Growing Degree Days (GDD) and Total Solar Radiation (TSR) from emergence to flowering and from flowering to maturity.

Variety	Sowing date	First paniele	Flowering	Maturation	Day length	Cumulative GDD <sup>2</sup> from emergence	Cumulative TSR <sup>3</sup> from emergence	Day length from flowering	Cumulative GDD from flowering	Cumulative TSR from flowering
	ditte	(DAE) <sup>†</sup>	ditte	cure	to flowering	to flowering	to flowering	to maturation	to maturation	to maturation
		. /	(DAE)	(DAE)	(h)	(°C)	(Mj m <sup>-2</sup> )	(h)	(°C)	(Mj m <sup>-2</sup> )
DISPAA-Q42	February 23	79 b	94 ab	148 cd	11.3 - 15.2	907	1743.40	14.9 - 14.4	995	1679.78
	March 17	63 c	84 c	170 bc	12.6 - 15.1	1054	2058.35	14.6 - 13.5	1597	2275.38
	April 27	50 d	70 cd	196 a	14.1 - 14.6	1166	2095.12	14.0 - 12.2	2232	3554.80
DISPAA-Q47-CB	February 23	89 a	100 a	142 cd	11.3 - 15.2	1023	1934.64	15.0 -14.6	788	1289.33
	March 17	77 bc	94 ab	162 c	12.6 - 14.9	1231	2335.99	14.9 - 13.8	1282	1700.89
	April 27	60 cd	72 cd	180 b	14.1 - 14.6	1200	2162.61	14.1 - 12.6	1926	3119.12
Means followed by the	same letter(s) are n	ot different for $p \leq r$	0.05 according to 7	ukey test.	1111 1110	1200	2102.01	1111 1210	1720	5115.12

DAE: Days After Emergence

<sup>2</sup> GDD: Growing Degree Days. <sup>3</sup> TSR: Total Solar Radiadion.

Of note, for 'DISPAA-Q42', a significant difference in the number of DAE, culminating in the appearance of the panicle, was detected for each of the three respective sowing dates (ranging from 79 to 50 DAE). For 'DISPAA-Q47-CB', a significance difference was observed only for the first sowing date. Similarly, for both varieties, the number of DAE until the flowering date decreased significantly from the first to third SD, respectively. For 'DISPAA-Q47-CB', an increased number of days until flowering were required and differences in both temperature and solar radiation were also required. The same conditions of increasing photoperiod (11.3-15.2 h) for the first and third sowing periods, higher values of both GDD and cumulative TSR were required by 'DISPAA-Q47-CB' in comparison to that for 'DISPAA-Q42' (Table 2). The TSR requirement for the first and third SD was approximately 200-300 Mj m<sup>-2</sup> higher for 'DISPAA-Q47-CB'. These results confirmed those obtained in previous research (Bertero et al., 1999; Bertero, 2003; Hirich et al., 2014), showing that the response of quinoa to photoperiod is significantly affected by temperature. The current work corroborates the necessity of this type of preliminary research to identify both suitable agronomic varieties and cultivation techniques, which are lacking for the successful cultivation of quinoa in Central Italy.

Given that the two varieties vary in the level of precocity, maturation was attained under different photoperiod as well as GDD and TSR (Table 2). Corresponding to the first SD, plants were subjected to a constant photoperiod from flowering until maturation: 14.9-14.4 h for 'DISPAA-Q42' and 15.0-14.6 h for

'DISPAA-Q47-CB'. A decreasing photoperiod with a maximum difference of 1.5-1.8 h was evident for the first and third SD.

An increase in both GDD and TSR was necessary for the maturation of plants sown in March and April compared to plants sown in February. Varietal differences were also noted. For 'DISPAA-Q42', differences of 1237 °C and 1875 MJ m<sup>-2</sup> between SD1 and SD3 were required. In contrast, for 'DISPAA-Q47-CB', differences of 1138 °C and 1829 MJ m<sup>-2</sup> were required.

The total duration of the crop growth, expressed as days to ripening, is of utmost importance in attaining satisfactory seed yields. Delayed sowings can excessively prolong the life cycle of the plants, thereby either resulting in seed maturation after 150-180 DAE (Jacobsen, 1997) or by generating unripe seeds.

For the third SD, maturation occurred at 196 and 180 DAE for 'DISPAA-Q42' and 'DISPAA-Q47-CB', respectively, in comparison to 148 and 142 DAE at the first SD for 'DISPAA-Q42' and 'DISPAA-Q47-CB', respectively. This clearly shows the wastage in days associated with delaying the sowing date. Additionally, all phenological phases were strongly influenced by the sowing dates for both varieties (Figure 3).



**Figure 3:** Date of the main phenological phases according to lines and sowing date. Error bars represent the interval of the variability of the Tukey test (SD.q.<sub>95,2,8</sub>). If the bars do not overlap, the difference between averages is significant at  $P \le 0.05$ .

When comparing the first SD and remaining two dates, differences became significant at the 10-true leaf stage. Particularly evident was the wastage of days for 'DISPAA-Q42', (18-20 d) that tended to decrease progressively proceeding towards waxy maturation. From this stage, the attainment of full maturation was rapid for the first SD plants (54 d) and significantly longer for the third SD plants (124 d).

A similar response was observed in a different environment by de Vasconcelos et al. (2012). In the present experiment, plants of the late sowing date were exposed to long periods of high temperatures (>  $30 \circ$ C) and marked drought (37 mm in the period June-August). If these climatic conditions reduced the time intervals of the main phenological stages proceeding from the first to the third sowing age, then the delay in maturation could be attributed to the reduced growth of the plants, more specifically, of the leaves. This response of quinoa contributes to maintaining a water balance that allow plants to survive water deficit conditions (Claeys and Inze, 2013). A smaller foliar, or assimilatory surface, may have resulted in a decreased seed-filling rate, and consequently a delay in full maturation.

For 'DISPAA-Q47-CB', a similar trend was observed. However, evident differences were found between the first and third SD, for the developmental phases between the 10-true leaf stage and the beginning of flowering. This amounted to a wastage of 20 d.

Risi and Galwey (1989) reported that time differences from emergence to panicle formation constitutes the first response of the plants to change in photoperiod. In the present study, from emergence until panicle formation, significant time differences were evident for the different sowing dates. Passing from increasing photoperiod (11.3-15.2 h), at the time of the first SD, to a stationary photoperiod (14.1-14.6 h), at the time of the third SD, the appearance of the panicle was delayed by 29 days for both varieties. Similar trends were reported for Chilean sea-level-type accessions cultivated in temperate environments in Argentina with photoperiods similar to that of the present experiment (Bertero and Ruiz, 2008). Of interest, even within the period between flowering and the very first anthesis, these varieties were shown to be very sensitive to photoperiod and GDD.

The developmental trend in plant height, shown in Figure 4, was significantly different for both lines and sowing date. Plant height was not different for the first and second SD until the 6-true leaf stage (attaining a height of 40 cm). From this phase onwards, plant growth of the second SD underwent a progressive reduction, which was maintained until maturation, quantifiable in 10 cm and 28 cm for 'DISPAA-Q42' and 'DISPAA-Q47-CB', respectively. The latter line was shown to be more susceptible to the delayed sowing. Plant height development in plants sown in April was significantly stunted (Figure 4), attaining just 30 cm at maturation. The present results corroborate those of other authors (Risi and Galwey, 1991; Vasconcelos et al., 2012). Moreover, those authors also showed that an improved plant development was positively correlated to seed yield. This was also evident in the present study. The same figure shows that both varieties were affected by downy mildew from the 10-true leaf stage. Only the basal leaves were affected by the disease. According to the classification of Inguilàn and Pantojia (2007), corresponding to the state of resistance-tolerance to the pathogen, results of the present study showed a gradation of symptoms that ranged between 1 and 2 (1-25 % of basal leaves affected).



**Figure 4:** Trend of plant height according to line and sowing date. Error bars represent the interval of the variability of the Tukey test (SD.q.<sub>95,2,8</sub>). If the bars do not overlap, the difference between averages is significant at  $P \le 0.05$ . \*: numbers refers to the mildew susceptibility estimation according to Inguilàn and Pontoja (2007).

Figure 5 shows the seed yield of the two breeding lines. It is apparent that 'DISPAA-Q47-CB' is significantly less productive than 'DISPAA-Q42', with a maximum yield of 0.5 t ha<sup>-1</sup> recorded for plants sown in February. However, of interest, this line was less sensitive to the delay in sowing of 22 d (March) with a limited reduction in the yield, equivalent to 10 %. 'DISPAA-Q42' was clearly the more productive line. Yields amounted to 2.0 t ha<sup>-1</sup> for plants sown in February. However, seed yield was reduced by 25 % with the delay in sowing of 22 d (March). Both breeding lines

produced negligible yields for the third SD, in which maturation occurred over 180 DAE. The yields of the first two SD of 'DISPAA-Q42' can be considered to be of a good standard compared to other varieties obtained after spring sowing in Italy (Pulvento et al., 2010; Lavini et al., 2014). In the latter studies, using a slightly higher sowing density (20 plants m<sup>-2</sup>) and with cover nitrogen fertilization, the varieties, 'Titicaca' and 'Regalona', in addition to various genotypes of different origins, attained excellent yields of 2.3-3.6 t ha<sup>-1</sup>.



Figure 5: Seed yield of the varieties according to sowing date. Means within columns followed by same letter(s) are not different for  $P \le 0.05$  for the Tukey test

The significant decrease in production, associated with the sequential delay in sowing, can be ascribed to different factors. Above all, two factors appear relevant. Firstly, the growth of the plants (from emergence to flowering) sown in February and March occurred under conditions of increasing photoperiod 11.3-15.2 h and 12.6-14.9 h, respectively. Secondly, from flowering to the very first seed development in plants sown in March, high temperatures accompanied by low rainfall were registered. Negative effects on seed production attributable to climatic events were also found by Bertero (2003). The yield and plant height data at harvest confirmed the positive correlation highlighted by Vasconcelos et al. (2012). In our experiment, the correlation was significant ( $R^2 = 0,624^{**}$ ; Y = 49,81+106,87x-40,49x<sup>2</sup>).

Among the qualitative aspects of the seeds reported in Table 3, significant differences between the varieties were recorded for the mass of 1000 seeds. With the exception of saponins, the interaction "line x sowing date" generated significant differences at  $P \le 0.001$ . The mass of 1000 seeds was on average below 2.0 g and decreased by 17 % from the first to second SD.

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Source of variation	Saponin (mg g <sup>-1</sup> fresh mass)	Protein (%)	1000 seeds mass (g)
Variety (V)			
DISPAA-Q42	0.249	18.2	1.67
DISPAA-Q47-CB	0.353	17.7	1.52
f	ns	ns	*
Date of sowing (DS)			
February 23	0.166 b	16.1 c	1.72
March 17	0.577 a	17.8 b	1.64
April 17	0.1660 b	20.0 a	1.43
V x DS	ns	ns	***

Means followed by the same letter(s) are not different for P≤0.05 according to Tukey test.

\*: significant at p  $\leq$  0.05; \*\*: significant at p  $\leq$  0.01; \*\*\*: significant at p  $\leq$  0.001;

The data of the present study was similar to that reported by Isobe et al. (2016), providing confirmation that Chilean varieties classified as "sea-level-type" are extremely sensitive to planting delay, leading to a general decrease in seed yield and a significant reduction in the "mass of 1000 seeds.

The protein content is an important characteristic of quinoa from an alimentary perspective. In addition, saponin content is an important technological aspect and it is essential that the saponins are either completely or significantly reduced before removed commercialization of the product. A significant reduction in saponin content (-34.7 %) was only found in both varieties for the second SD. The saponin content was shown by De Santis et al. (2012), to be strongly influenced by environmental conditions. It could be hypothesized that this result was attributable to the high temperatures and low rainfall that occurred in the period immediately after flowering, similar to that observed by De Santis et al. (2011) for Italian environments. The average seed protein content increased significantly with the delay of sowing from 16.1 % to 20.0 %, and was significantly and positively correlated ( $R^2 = 0.928^{**}$ ) with the age of maturation.

As previously mentioned, saponin and protein content were unaffected by breeding line. However, given that only two varieties were utilized, more research is required in order to determine whether the selection for specific biochemical characteristics can be made from the best yielding varieties.

### **4 CONCLUSIONS**

At the end of a seven-year genetic improvement process, these results permitted us to evaluate the adaptability of two new quinoa lines to the environment of Central Italy at different sowing dates, spanning a period from the end of winter to early spring. Although the experimentation was conducted over the course of a single year, results showed that of the two varieties were obtained from poly-crosses between Chilean "sea-leveltype" lines, only one line 'DISPAA-Q42' can be considered suitable to the Tuscan environment with satisfactory yields. This study, therefore, highlights the importance of assessing varietal performance. Moreover, as anticipated photoperiod and radiation were important determinants of plant growth and yield.

The shortening of the phenological phases until the flowering in relation to photoperiod and increasing solar radiation, confirmed the research of Hirich et al. (2014). However, the present results are also in contrast with those of Hirich et al. (2014) and Jacobsen (1997), who claimed that the early maturation or early genotypes (bloom to anthesis) maintained the same trend throughout the reproductive cycle. The lack of adaptability of 'DISPAA-Q47-CB', as well as the reduced production of seed, also manifested itself in terms of a strong reduction in the growth of plants. The significant yield reduction, corresponding to the March sowing period can be ascribed to the high temperatures

and to the dry conditions occurring coinciding with bloom and anthesis. The spread of mildew was not evident, due to the hot and dry environmental conditions. The plants reacted to the presence of the mildew with an early filloptosis of the basal leaves affected. The potential repercussions of the fungus on the yield were not assessed by the present work. It was noted that at full formation of the panicle, 'DISPAA-Q47-CB' appeared more sensitive to the mildew compared to 'DISPAA-Q42'.

The accomplishment of the poly-crosses resulted in the production of at least one line, that appears to be well adapted to the environment of Central Italy notwithstanding the elevated average temperatures and prolonged drought that occurred between the complete emergence of the panicle and the milky maturation. Additionally, February was shown to be the most suitable sowing date.

Before reaching a definitive decision on the suitability of 'DISPAA-Q42', further experimentation is required to determine the performance in different environments and sowing densities. Based on small-scale experiments conducted this year (unpublished results) and from the literature (Risi and Galaway, 1991; Nurse et al., 2016), the above mentioned agronomic aspects significantly influence the date of maturation and seed production.

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