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# Performance and Potentiality of Camelina (*Camelina sativa* L. Crantz) Genotypes in Response to Sowing Date under Mediterranean Environment

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**Abstract:** Given the growing interest for camelina, as a multipurpose oilseed crop, seven cultivars and two sowing times were compared to characterize camelina's production potential in the rainfed agroecosystems of Central Italy. A split-plot design, with sowing date as main plot (autumn and spring) and cultivar (V1, V2, V3, V4, V5, V6, and CELINE) as subplot, was adopted over two growing seasons (2017–2019). Phenology, yield and yield components, protein and oil content, and fatty acid profile were evaluated. Going from autumn to spring sowing, a significant reduction was observed in the number of days (139 vs. 54 days) and GDD (642 vs. 466 °C d) from emergence to beginning of flowering, with more consistent variations among cultivars. V1 and V2 were the earlier ones both in spring and autumn sowing. Autumn sowing increased seed yield (+18.0%), TSW (+4.1%), number of siliques per plant (+47.2%), contents of α-linolenic, eicosenoic, erucic and eicosadienoic acids, and polyunsaturated to saturated fatty acid ratio. Regarding genotype, V3 showed the best seed and oil yield in autumn, whereas V1 and CELINE were the best performing in spring. Finally, TSW and number of siliques per plant were the key yield components for camelina. Results identify, in relation to sowing date, the most suitable cultivars for the tested environment, in terms of earliness and quanti-qualitative traits.

**Keywords:** crop diversification; yield components; siliques; oil; PUFA; α-linolenic; ω-3; eicosenoic; crude protein

## 1. Introduction

Camelina (*Camelina sativa* (L.) Crantz) or false flax, is an ancient minor oilseed crop belonging to the Brassicaceae family that has recently gained an increasing attention due to its good agronomic features and its promising industrial and healthy food applications [1]. Camelina has been cultivated in Europe for centuries as a source of edible oil and used in folk medicine before being replaced with more productive oil crops in the 1950s [2,3]. Nowadays, camelina is mainly used as oilseed crop for biodiesel production, mainly in aviation fuel, thanks to its ability to reduce greenhouse gas emissions [4–6].

Several agronomic characteristics have conferred advantages to camelina, over other oilseed crops, contributing to its recent widespread expansion. It has, in fact, low agricultural input requirements, and a good tolerance and resistance to cold and drought, as well as to diseases that commonly affect oilseed Brassica crops [7–9]. Camelina can also be grown profitably on poor and marginal lands and it is considered to be suitable for 96% of the marginal lands in the Mediterranean regions, where high valued crops may not be viable economically [10]. Camelina well adapts to a wide range of environments and different sowing times, being a rapidly maturing short-season species, with both spring and winter forms. These features make camelina suitable to replace fallow periods

especially in rainfed cereal based-crop rotations [11,12] and to succeed as cover or cash crop, in double or relay cropping thus improving crop yield and energy balance [12,13]. As annual winter crop, camelina provides a number of agroecological benefits such as protection from soil erosion, prevention of nitrates leaching and run-off [14], weed control [15], and provision of feed for pollinators [16]. The high potential of camelina for both industrial and nutritional applications is attributed to the distinctive fatty acid composition of its oil, which is predominantly unsaturated, with α-linolenic (18:3) and linoleic (18:2) acids having the highest contents [2,17]. Due to the richness of camelina oil in α-linolenic acid, i.e., up to 40% of total seed dry matter, and its role in regulating the ω−6/ω−3 balance of human diet, camelina oil can be recommended as a dietary supplement [18]. Being an essential ω-3, α-linolenic acid has beneficial health effects on humans [19,20]. In addition, due to its unique fatty acid composition, camelina oil is attractive for the biorefinery industry for the recovery of oil derivatives, bioactive molecules, and biopolymers [21,22]. Based on this, camelina may find wide applications in coatings, i.e., varnishes, paints, in cosmetics, and pharmaceutical products as well as lubricants, adhesives, and packing materials [23–25]. The presence of eicosenoic acid (11–19%) and tocopherols in relatively large amounts, and the low content of antinutritional erucic acid and glucosinolates, are additional distinctive differences from commonly used vegetable oils [18,26]. By-products of camelina oil extraction, like press cake may serve as feed, as well by providing essential fatty acids and a protein-rich ingredient in animal rations which gives camelina cultivation an added value and increases its economic viability [27,28]. All these seed quality features are important characteristics for marketing and processing of the crop in competition to other oilseeds. However, there is a wide range in most seed quality parameters attributable to both genetic and environmental conditions, resulting in unpredictable performances in different growing environments [29].

In light of the current situation, where the sustainable intensification and diversification of cropping systems are recommended and bio-based economy is fast-growing, camelina, thanks to its agronomic attributes and seed quality features, seemed promising to be introduced in Mediterranean agroecosystems. In such conditions, where cereal-based cropping systems are prevailing, crop diversification with camelina as winter crop, is seen as an option to mitigate the negative climate change impacts (mainly low and/or erratic rainfalls and intensive summer drought), to preserve agricultural productivity, to increase resilience, and, at the same time, provide income to farmers.

In the evaluation of feasible introduction of camelina under environmental conditions of the Mediterranean region, a proper evaluation of agricultural management, including sowing date and varietal choice, is mandatory. Temperature and soil water are the most important environmental conditions affecting camelina seed yield and quality. Camelina seed yield can be limited by water shortage or lower precipitations and a heat stress during the reproductive phase [23,30]. Yield decline with spring sowing, caused by fewer siliques per plant, decreased seed weight, and reduced branching, was documented in previous studies on camelina [31,32]. On the other hand, camelina sown in autumn accumulated larger biomass, benefiting from a longer season and cooler temperatures, but translocated less photosynthates to the seed [31]. In the Mediterranean climatic conditions, with autumn sowing the crop meets relatively milder temperatures during seed filling in comparison with spring sowing, thus promoting polyunsaturated fatty acids (PUFAs) production and a higher high α-linolenic acid (ALA) content [6,33,34]. Obour et al. [23] observed a strong decrease in PUFA content when, during seed filling, temperatures fall above 25 °C. Drought and heat during seed development greatly also affect the oil content, most likely due to deleterious effect of heat on the enzymes involved in the conversion of carbohydrates to lipids [34–36].

With camelina making its way through industrial oil crops, more genetic resources, with improved agronomic and seed quality characteristics, are being available allowing for a widespread exploitation. Genotypic adaptation and cultivar selection are also key management components in successful camelina cultivation. The suitable varieties for Mediterranean area should be characterized by earliness, drought resistance, high productivity, and quality to better exploit the water available in the soil and escape heat stress at flowering and seed fill, which could reduce camelina yields and oil quality [36].

Although several studies have been conducted in different environments, the performance of multiple camelina varieties under Mediterranean conditions has not been fully explored. Therefore, the objective of the study was to determine the best performing varieties and the optimum sowing date suitable for developing site-specific recommendations for camelina cultivation in Central Italy. Consequently, seven camelina varieties and two sowing dates (autumn and spring) were compared for two consecutive growing seasons through a field experiment carried out in Arno river plain (Tuscany coastal area, Central Italy). The environmental and genotypic effects were assessed on plant growth, seed and oil yield, yield components, protein and oil seed content, and fatty acid profile.

## 2. Materials and Methods

### *2.1. Site Characteristics*

Seven camelina genotypes were compared in two sowing dates, autumn and spring, in a two-year field experiment (2017–2019) at the Experimental Centre of the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa, San Piero a Grado, Pisa, Italy (46°67′ N latitude; 10°31′ E longitude, altitude 1 m a.s.l. and 0% slope). The climate is typical of the North-Mediterranean area, characterized by a long-term average annual rainfall of 907 mm, with most rainfall occurring in spring and fall, and an average annual temperature of 15.5 °C. The area is characterized by flat land, and by a deep silt–loam soil, originated from alluvial sediments and classified as Typic Xerofluvent based on the USDA taxonomy [37]. Physical and chemical characteristics of the soil, performed in soil samples collected at 0–30cm depth at the beginning of experiment and in both years of cultivation, are reported in Table 1. Soil pH determination was performed on a 1:2.5 soil:water suspension following McLean procedure [38]. Electrical conductivity was measured at 20 °C by using a GLP-31 Crison conductimeter (52.93 electrode) (Montepaone s.r.l., San Mauro Torinese, Torino, Italy). Total nitrogen was evaluated using the macro-Kjeldahl digestion procedure [39], available phosphorus by colorimetric analysis using the Olsen method [40] and the cation exchange capacity following Mehlich method [41]. Nitrate ($NO_3^-$–N) concentration was performed by ion-exchange chromatography (Dionex ICS 45001; AS4A column). The exchangeable macronutrients, such as K and Mg were determined using the Thomas method [42], while total soil S concentration was determined after wet digestion according to Izza et al. [43]. Soil organic matter was calculated by multiplying by 1.724 the soil organic carbon concentration, measured using the modified Walkley–Black wet combustion method [44]. For total $CaCO_3$ evaluation, Dreimanis [45] method was used, while the ammonium oxalate-titration method was used for active $CaCO_3$ determination. The soil C/N ratio was computed as the quotient of organic C and N concentrations. For each year, a separate undisturbed soil core was collected by a metal cylinder (5 cm height) in each plot at 10 cm depth increments to a depth of 30 cm to determine bulk density (BD). BD was obtained by dividing the mass of the oven-dried sample at 105 °C for 48 h by the volume of the probe (100 $cm^3$) [46].

Daily minimum and maximum air temperature and rainfall during the period of the study were collected from a meteorological station close to the experimental site (less than 500 m) (Figure 1).

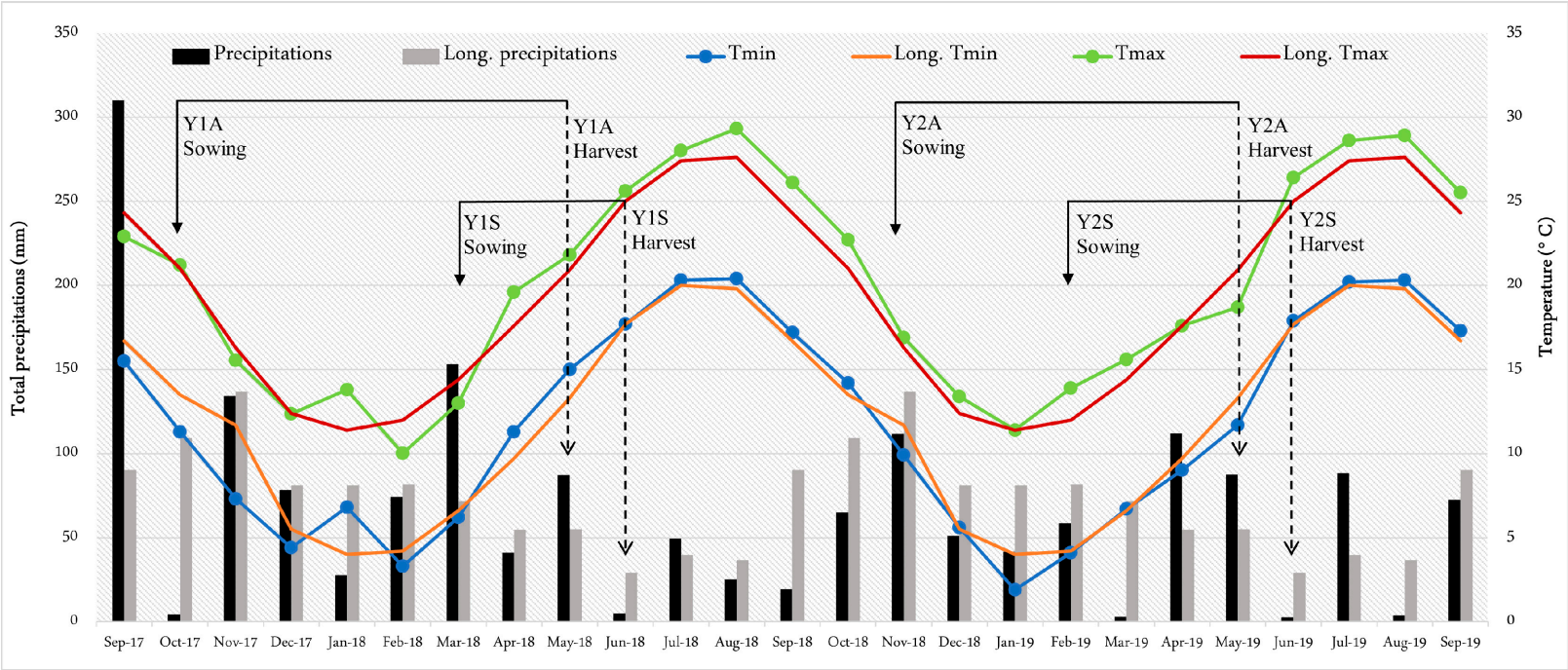


**Figure 1.** Monthly precipitations and mean minimum and maximum air temperatures (°C) during the study period, compared to long-term (2001–2019) mean values. Y1A and Y2A for the Autumn-sown crops and Y1S and Y2S for the Spring-sown crops in the 1st and 2nd year of field trial.

**Table 1.** Soil characteristics (0–30 cm depth) at the start of the experiment.

| | 2017–2018 | 2018–2019 |
|---|---|---|
| Sand (%) | 50.97 | 45.86 |
| Silt (%) | 31.39 | 39.00 |
| Clay (%) | 17.64 | 15.14 |
| pH | 7.9 | 8.0 |
| Electric conductivity (mS/cm) | 0.044 | 0.097 |
| Total N (g $kg^{-1}$) | 1.18 | 1.27 |
| $NO_3^-$-N (mg $kg^{-1}$) | 11.08 | 12.70 |
| Available P Olsen (mg $kg^{-1}$) | 5.48 | 9.09 |
| Exchangeable K (mg $kg^{-1}$) | 86.4 | 83.8 |
| Exchangeable Mg (mg $kg^{-1}$) | 172.5 | 169.7 |
| Total S (g $kg^{-1}$) | 0.3 | 0.3 |
| Organic matter (%) | 1.91 | 1.98 |
| Total $CaCO_3$ (%) | 6.57 | 6.34 |
| Active $CaCO_3$ (%) | 2.87 | 3.03 |
| C/N | 9.41 | 9.04 |
| Cation-exchange capacity (meq/100 g) | 9.22 | 9.04 |
| Bulk density (g $cm^{-3}$) | 1.38 | 1.35 |

### 2.2. Experimental Setup

A field plot experiment was set up, by adopting a split-plot design with sowing times (Autumn and Spring, as A and S) as main plots and cultivar as subplots (6 m × 3 m size), replicated four times in two years (2017–2018 as Y1; 2018–2019 as Y2). The seven genotypes, compared in this study were V1, V2, V3, V4, and V5, obtained from Camelina Company España (Camino de la Carrera, 11-11, 28140 Fuente el Saz, Madrid, Spain), beside an Italian variety named V6 (belonging to DAFE collection), and the commercial CELINE (Limagrain, Saint-Beauzire, France). These cultivars were chosen because they have different precocity, are commercially available and suitable for Mediterranean climate.

In both years of cultivation, winter wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) Preceded camelina, assuming a rainfed cereal-based cropping system. An integrated management system was adopted with conventional tillage practices and mineral fertilization. Fertilizer rate was calculated according to nutrients levels in the soil and in compliance with the disciplinary for integrated agricultural production. Pre-planting phosphorus and potassium fertilizations were performed at a rate of 80 kg $ha^{-1}$ $P_2O_5$ by triple superphosphate and 50 kg $ha^{-1}$ $K_2O$ by potassium sulfate. Nitrogen (as ammonium nitrate) was applied, at the rate of 50 kg N $ha^{-1}$, when the crop was at the rosette stage. Before camelina sowing, the soil was moldboard ploughed at 0.30 m followed by a disk and rotary harrowing to incorporate the fertilizers and to prepare the seedbed. The seeding rate, in each year, was around 6.5 kg $ha^{-1}$, considering percent seed germination as well as 1000-seed weight (TSW), in order to reach a target of 500 plants $m^{-2}$ for all camelina genotypes. Sowing was performed by a Wintersteiger plot drill on 0.15 m spaced rows and a depth of 0.01 m on 18 October 2017 and 20 November 2018 for autumn sowing and on 28 March 2018 and 26 February 2019 for spring sowing.

No chemical treatments for pests and pathogens were necessary during the whole experiment, and weeds were controlled by hand weeding. Downy mildew caused by *Peronospora parasitica* (Pers.: Fr.) Fr. (synonym *Peronospora camelinae* Gäum.), has been observed on both camelina spring crops after periods of warm/wet weather. The incidence and severity of these attacks were low, and no significant effect was observed on both siliqua development and seed yield.

### 2.3. Sampling and Measurements

The phenological growth stages of camelina, including beginning of stem elongation (BBCH 31), beginning (BBCH 60) and end of flowering (BBCH 69) and maturation (BBCH 89) were determined during each growing season using the extended BBCH scale described by Martinelli and Galasso (2011) [47]. In addition, number of days and accumulated growing degree days (GDD) between two or

more growth stages were calculated using daily maximum air temperature ($T_{max}$), daily minimum air temperature ($T_{min}$) and base temperature ($T_{base}$) of 5°C [35], as follows (1):

$$GDD = \Sigma[(T_{max} + T_{min})/2 - T_{base}] \quad (1)$$

Camelina plants were harvested manually sampling 6 rows from the central portion of each plot (~5.0 $m^2$), at seed full ripening, when more than 90% of siliques were dried and turned brown and most seed was reddish-brown in color (seed moisture ≤12%, BBCH 89). Harvest date of autumn-sown camelina occurred between 17 and 21 May 2018 and between 16 and 22 May 2019. Spring-sown camelina was harvested on 25 and 14 June, in 2018 and 2019, respectively.

Plant density, plant height, above-ground biomass (stalk, leaves, and empty siliques), seed yield and yield components (number of siliques, number of seeds per silique, 1000-seeds weight) have been evaluated. To assess seed yield, the plants were threshed by a fixed machine, using sieves suitable for small seeds. Fresh weight was measured, and plants subsequently allowed to dry into a ventilated oven (40 °C) for dry weight determination and evaluated for their moisture content. The crop harvest index, which indicates the efficiency of photosynthates translocation to harvestable products, in this case the seed, was calculated by dividing dry seed weight by dry weight of total aboveground crop biomass at harvest. Thousand seed weight was assessed at the Seed Research and Testing Laboratory of DAFE, on representative seed samples deriving from each plot according to the international rules for seed testing [48].

Seed oil and protein contents were determined on representative samples from each plot. Oil content was measured following the AOAC (Official Methods of Analysis) procedure for ether extract level, using ANKOM model XT10 extractor (ANKOM Technology, Macedon, NY, USA). Fatty acids (FA) profile was determined with a direct esterification following the method of Christie [49]. Briefly, internal standard along with 3 mL of methanolic HCl (10%) were added to 200 mg of finely ground seeds. Samples were then left for incubation at 50 °C overnight after vigorous shaking. After cooling to room temperature, 1 mL of n-hexane and 10 mL of 6% $K_2CO_3$ were added. The mix was then vortexed and centrifuged at 5000 rpm and 4 °C for 10 min. The organic phase was removed from the top layer and transferred to an amber vial to which 1 g of sodium sulfate ($Na_2SO_4$) was added to eliminate water residues. Further washing was done with 1 mL of hexane before centrifuging again at 5000 rpm and 4 °C for 10 min. The supernatant was then transferred to a new vial and dried under Nitrogen flux. Prior to analysis, 1 mL of n-hexane was added to the sample. A GC2010 Shimadzu gas chromatograph (Shimadzu, Columbia, MD, USA) equipped with a flame-ionization detector and a high polar fused-silica capillary column (Chrompack CP-Sil88 Varian, 152 Middelburg, the Netherlands; 100 m, 0.25 mm i.d.; film thickness 0.20 μm) was used for the analysis. Hydrogen was used as the carrier gas at a flow of 1 mL $min^{-1}$. Split/splitless injector was used with a split ratio of 1:40. An aliquot of the sample (1 microL) was injected under the following GC conditions: the oven temperature started at 40 °C and held at that level for 1 min; it was then increased to 163 °C at a rate of 2 °C /min, and held at that level for 10 min, before being once again increased to 180 °C at 1.5 °C /min and held for 7 min, and then to 187 °C at a rate of 2 °C /min; finally the temperature was increased to 220 °C with a rate of 3 °C /min and held for 25 min. The injector temperature was set at 270 °C and the detector temperature was set at 300 °C. Individual FA methyl esters were identified by comparison with a standard mixture of 52 Component FAME Mix (Nu- Chek Prep Inc., Elysian, MN, USA). Individual FA are reported as a percentage of the total FA. Saturated fatty acids (SFA) were calculated as the sum of saturated fatty acids.

Seed crude protein content was calculated by multiplying the total nitrogen percentage by 6.25 and total N content was determined by means of the mini-Kjeldahl method on dry seed samples ground to 0.5 mm size by a laboratory mill (Grindomix GM 200, 247 Retsch, Retsch Italia, Pedrengo (BG), Italy).

### *2.4. Statistical Analyses*

All statistical analyses were performed with "RStudio" statistical software [50]. A three-way analysis of variance was done using "lmerTest" package to test the effects of genotype (G), sowing time (ST), and year (Y) as well as their interactions on the measured parameters, considering the three factors as fixed factors and the replication as random one. A visual inspection of model residuals was also done to check for model assumptions, which did not show any significant departures from normality and variance homogeneity. Only for plant density, number of siliques and number of seeds per silique, generalized linear models were used using Poisson distribution. Comparison among the levels of dependent variables was achieved via Tukey's HSD test at $p \leq 0.05$ level. Data are presented as lsmean ± standard error.

## 3. Results

### *3.1. Weather and Phenological Stages*

Weather conditions registered over the course of the study are presented in Figure 1. In both years, cumulative precipitations received by winter crops were less than the long-term average. Monthly rainfall was below long-term average in October and January of 2017–2018 season and the whole 2018–2019 winter. Cumulative rainfall from autumn sowing to harvest varied between the two years, being considerably lower in 2018–19 (571 mm in Y1A and 352 mm in Y2A). However, the amount of rainfall received in both years by winter crops exceeded the requirements of camelina [51]. Total amount of precipitations during the growth of both spring crops (Y1S and Y2S) was significant lower compared with winter crops, but almost consistent with long-term trend, although their distribution showed some deviations particularly in March of both years. The cumulative rainfall from spring sowing to harvest differed between the two years, with Y1S receiving 28% less rain than Y2S (146 mm vs. 203 mm in Y1S and Y2S respectively). Rain distribution differed between the vegetative and reproductive phase with lower amounts of rainfall between start of flowering till seed maturity in Y1S crop (Table 2).

**Table 2.** Length (days), growing degree days (GDD) (°C d) and cumulative rainfall (mm) corresponding to vegetative and reproductive cycles of camelina in autumn (Y1A and Y2A) and spring sowing (Y1S and Y2S) in both years of cultivation (Y1 and Y2). Data are averaged over all genotypes.

| Sowing Time | | Cycle Length (days) | | GDD (°C d) | | Cumulative Rainfall (mm) | |
|---|---|---|---|---|---|---|---|
| | | Vegetative † | Reproductive †† | Vegetative | Reproductive | Vegetative | Reproductive |
| Y1A | 18 October 2017 | 154 | 58 | 762 | 580 | 442.8 | 128.5 |
| Y1S | 28 March 2018 | 46 | 43 | 491 | 663 | 93.4 | 52.6 |
| Y2A | 20 November 2018 | 124 | 56 | 522 | 479 | 181.7 | 170.0 |
| Y2S | 26 February 2019 | 61 | 46 | 442 | 563 | 109.0 | 94.0 |

† Vegetative cycle corresponds to the period from sowing till start of flowering. †† Reproductive cycle corresponds to the period from start of flowering till seed maturity.

The long-term average annual temperatures referring to camelina growing period (October till June), are 9.4 °C and 16.8 °C as the minimum and maximum temperatures respectively, with a minimum of 4 °C in January and a maximum of 25 °C in June. In the first year, minimum temperatures were lower than the long-term ones, especially during seed germination, seedling emergence and initial leaf development (October till December 2017). The mean minimum temperature reached 3.3 °C in winter 2018 and autumn-sown crop experienced snow event and cold temperature from 26 February to 2 March 2018. After this period, warmer spring temperatures were registered in April and May 2018 compared to the long-term data.

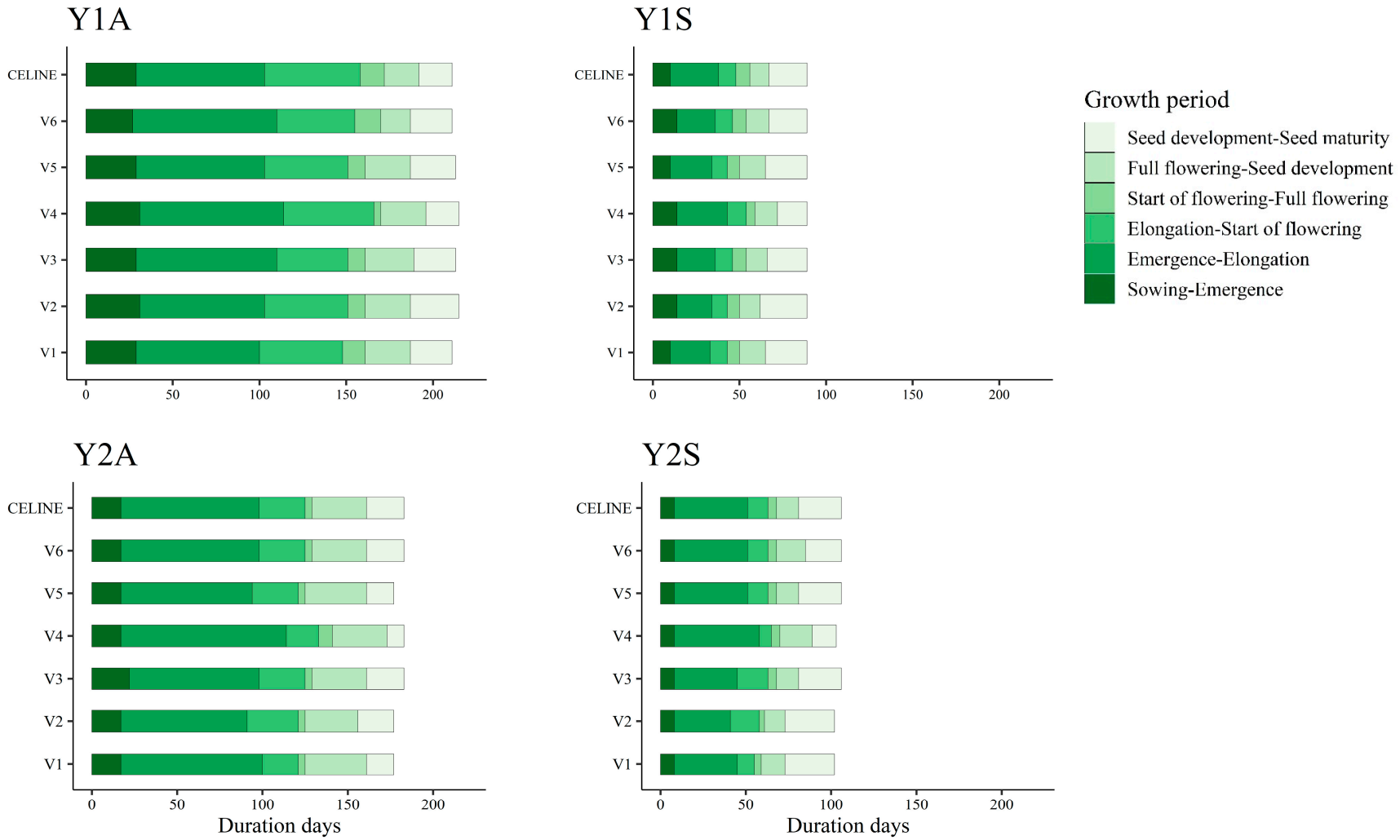


**Figure 2.** Duration in days of the main phenological growth stages of the seven camelina genotypes sown in autumn 2017 (Y1A) and 2018 (Y2A) and in spring 2018 (Y1S) and 2019 (Y2S).

Sowing time and genotype affected the number of days and GDD accumulated from sowing to flowering (Table 2 and Tables S1 and S2 in the Supplementary Materials). Across the year, the length of this stage decreased as sowing was delayed, passing from 139 days, as mean value over year, for autumn sowing, to 54 days for spring one (Table 2). Minor differences were observed for the GDD accumulated from sowing to flowering between the two sowing dates (642 vs. 467 GDD for autumn and spring sowing, respectively), while more consistent variations have been detected among genotypes. In general, V1 and V2 were the earlier ones, starting flowering after having accumulated respectively 420 and 436 GDD in spring sowing, and 615 and 624 GDD in autumn sowing. V4 instead was characterized by a longer vegetative cycle, in comparison with the other cultivars, in both sowing times and in both years of trials, needing more GDD to flower. Camelina genotypes completed their growing cycle in 197 and 98 days, as mean values, when sown in autumn and spring, respectively (Figure 2). Despite this obvious reduction in the number of days to complete the crop cycle, the GDD accumulated to reach maturity seemed only slightly influenced by the sowing period (1172 and 1082 in autumn- and spring-sown crops respectively), which make thermal time a useful predictor of camelina maturity and harvest time. The values of GDD and the growing cycle length were in the range of those found by other studies, carried out in Europe, Canada, and Central US [33,35,52].

### *3.2. Seed Yield and Yield Components*

Table 3 shows the F-test results of genotype (G), sowing time (ST), year (Y), and their reciprocal interaction effects on camelina seed yield and yield components. The interaction of the three variables was significant for seed yield, above-ground biomass yield (as stalk, leaves, and empty siliques at harvest), and all seed yield components, except for the number of seeds per silique, 1000-seed weight, and plant height. This discloses the important influence of the seasonal variation on the different responses of camelina genotypes to sowing time due to variability in the amount and distribution of rainfall and to temperature fluctuations. Seed yield differed between years, with greater yields in 2018–2019 growing season, likely related to relatively wetter and mild spring during Y2A and Y2S crops reproductive cycle. Highest yields were also observed with autumn sowing, even if only in the second year a remarkable increase in both seed and biomass yields were detected (+33% and +53% respectively) (Table 4). This is especially evident for V6 and V3. In particular the latter when sown in autumn, showed a yield more than double compared to that obtained with spring sowing. On the contrary, in the first year, the only significant differences in seed yield were observed in spring sowing among V1, CELINE, and V4, with similar values (around 2 Mg ha$^{-1}$) for the first ones and the lowest for this last genotype (Table 4). The yield reached its highest value of 3.4 Mg ha$^{-1}$ in the second year with V3 winter crop and the lowest one (1.1 Mg ha$^{-1}$) in V4 spring crop in the first year. Compared with earlier works on camelina both in Europe and the US [1,33], the varieties tested herein showed from acceptable to particularly high production as V3. In a previous work [53] carried out in different environments of the Columbia Plateau region (USA), CELINE was among the highest productive variety tested in the study, able to produce more than 3.5 Mg ha$^{-1}$ as spring variety. In our case, CELINE sown in spring, gave a stable yield of around 1.9 t ha$^{-1}$, while a greater variability (from 1.5 to 2.1 t ha$^{-1}$) was observed for this genotype when sown in autumn.

The evaluation of biometric characteristics showed higher number of siliques per plant (+45.3%) in autumn sowing in comparison with spring one (Table 3). This most evident for all varieties in the autumn of the first year of cultivation, exception given for V5 which did not show significant differences between the two sowing dates in both years (Table 4). In the second year, only V1, V3, and V6 produced, in autumn, a significant greater number of siliques per plant (Table 4). Conversely, the number of seeds per silique did not vary in response to Y and ST variability factors, nor to their reciprocal interaction, exception given for the effect of variety, for which slight differences have been observed (Table 3). Camelina 1000-seed weight (TSW) varied between 0.7 and 1.3 g being within the range of many tested camelina genotypes (0.8–1.8 g) [2,29,33]. Sowing time influenced camelina TSW, but this depended on the year, being higher for camelina sown in autumn with respect to spring,

only in the first year (Figure 3A). The earlier autumn sowing allowed the plants to intercept and absorb more solar radiation for longer growing period, but also prevented drought stress during seed filling. The absence of differences between sowing times in the following year was due to an increase in TSW in Y2S crop, probably due to earlier sowing and longer growing period than Y1S crop. In addition, our results showed that TSW is strongly depended on genotype; in both years and sowing times, V3, V1, and V5 produced the seed with greatest weight, while V4 and V6 the smallest ones (Table 3). The increase in TSW due to genetic characteristics was higher than that due to sowing time (+4.1%). It is known as the optimal sowing time in camelina varies with environments and locations. Autumn sowing (October–November) was proven to be better in Mediterranean climates due to the sensitivity of camelina to temperature over 25 °C [31]. In fact, camelina seed yield can be limited by water shortage or lower precipitation and a heat stress during the reproductive phase [23,30]. Yield decline with spring sowing, caused by fewer siliques per plant, decreased seed weight, and reduced branching, was documented in previous studies on camelina [31,32].

The tallest plants were obtained in autumn sowing for all camelina varieties. This is in agreement with Berti et al. [31] and Angelini et al. [54] who indicated that late sowing led to a reduced plant height in camelina, planted in Chile and in Pisa, Italy (same site of this experiment), respectively. Genetic characteristics had limited effect on this parameter with V4 having the tallest plants (103.5 cm) and V1 and V2 the shortest (around 88 cm) (Table 3). The interaction between ST and G, reported in Figure 3C, highlights the greater and significant differences between the cultivar in the earlier sowing for this trait. The increase in plant height with autumn sowing did not cause lodging, except to V6 and CELINE during seed ripening (beginning of May 2019). In these two cultivars, lodging interested respectively 25% and 70% of the plants with a rate of 4/5 (rating on 1–9 scale; 1 = completely erect; 9 = completely flat).

A significant higher plant density was registered when camelina was sown in spring (+30%) (Table 3), except for V5, for which a denser plant stand in autumn sowing (+33%) was observed. CELINE in spring 2018, and V1 in spring 2019 reached the highest number of plants, with 464.8 and 468.8 plants $m^{-2}$, respectively. V6 and V3, with both the highest seed yields and siliques per plant, were characterized by the lowest crop establishment (Table 4). Probably, they compensated the low plant density developing a more pronounced branching. Interestingly, V5 seemed to be the outperforming variety in autumn but the least one in spring (Table 3). All camelina varieties showed a major plant density in the second year, with the exception of V6 in autumn and CELINE in spring. A low level of emergence and poor crop establishment of camelina was already found in other studies [55,56], but the performance of the varieties tested in this study was better than those obtained previously, for both sowing times. Camelina survival has been reported to be susceptible to dry conditions [31] and waterlogging [7], besides frost damage and winterkill [56]. In our experiment, the frequent rainy events that occurred during November/half of December of the first growing season caused waterlogging to camelina during emergence and the first rosette stages, with a consequent reduction of plant density.

The highest above-ground biomass yield, similarly to seed yield, was found in crops sown in autumn (+36%) (Table 3). This trait was significantly influenced by both GxST and STxY. In the first year, significant differences among varieties were observed only in spring-sown crops, with the highest biomass produced by CELINE (Table 3). Sowing time, genotype and year had significant effects on the harvest index (HI). The detected values fell within the wide range reported for camelina (15 to 40%) [7,57]. In general, sowing in spring increased camelina HI despite the lower yields (Table 3). Disproportionate changes between seed and biomass production between the two sowing times resulted in this difference. Camelina crop sown in autumn accumulated larger biomass, thanks to a longer season and cooler temperatures, but translocated less photosynthates to the seed, as previously observed by Berti et al. [31].

Among the varieties, V3 had the highest HI (0.26) and V4 the lowest one (0.20). Plant height can play an important role in defining harvest index. In our study, an increase of plant height generally determined a decrease of HI. This in particularly evident for V4, which showed the tallest plants in

autumn showing, at the same time, the lowest HI. Furthermore, HI was also higher in the first year of the study, in comparison with the second one, due to a greater reduction of above-ground biomass (−34%) with respect to seed yield (−20%).

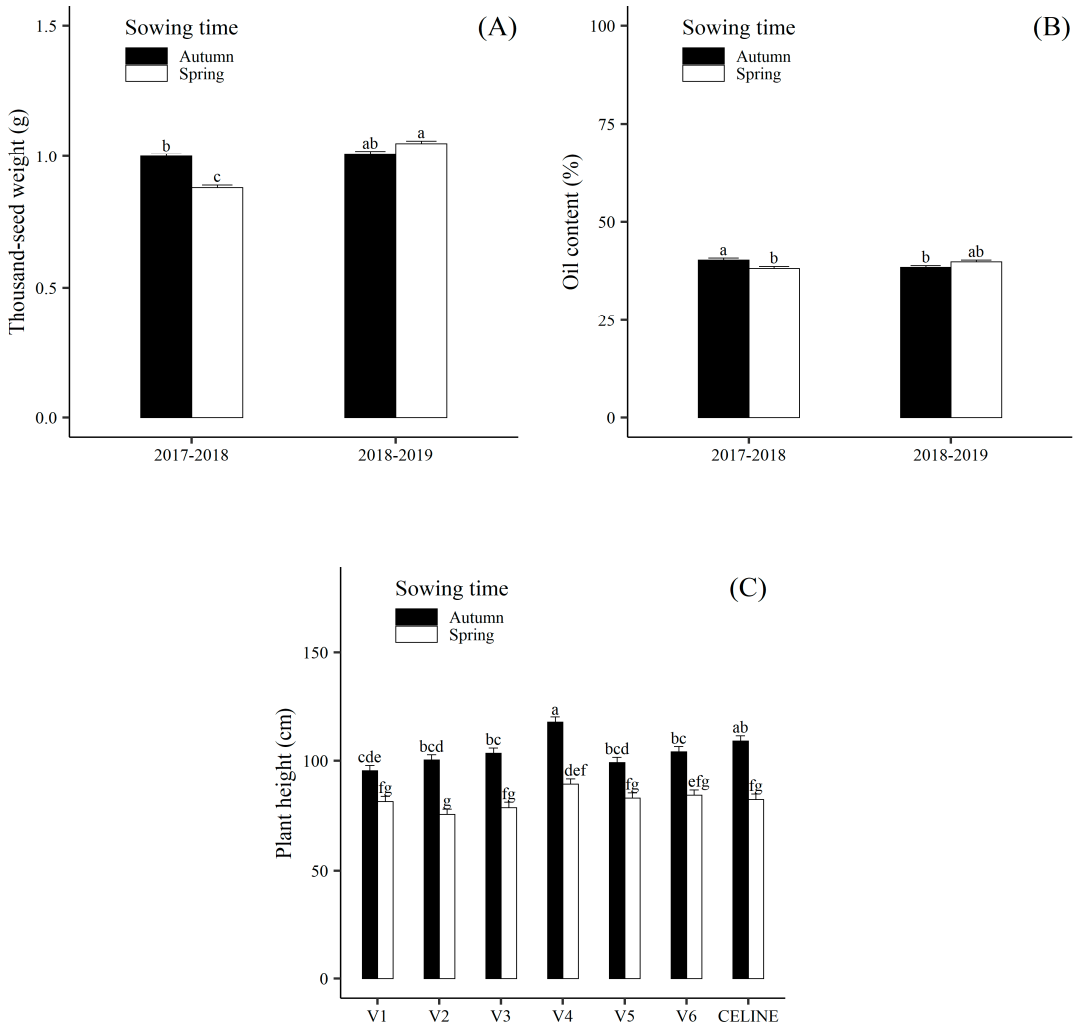


**Figure 3.** Effect of sowing time and year interaction (ST × Y) on camelina thousand-seed weight (**A**) and oil content (**B**), and effect of genotype × sowing time interaction (G × ST) on plant height (**C**). Means followed by the same letter are not significantly different at $p \leq 0.05$ based on Tukey's HSD test.

**Table 3.** Main effects of year (Y), sowing time (ST), and genotype (G) and their interaction on camelina production traits and *p*-values of the analysis of variance.

| Term | | Seed Yield | No. Siliques Plant$^{-1}$ † | No. Seeds Silique$^{-1}$ † | 1000-Seed Weight | Plant Height | Plant Density † | Above-Ground Biomass †† | HI ††† | Oil Content | Oil Yield |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (Mg ha$^{-1}$) | | | (g) | (cm) | (No. m$^{-2}$ ) | (Mg ha$^{-1}$) | | (% dry Weight) | (Mg ha$^{-1}$) |
| Year | 2017–2018 | 1.57 ± 0.06 $^{b}$ | 137.5 ± 1.6 $^{a}$ | 8.4 ± 0.4 $^{a}$ | 0.94 ± 0.01 $^{b}$ | 90.4 ± 0.9 $^{b}$ | 221.0 ± 3.7 $^{b}$ | 4.74 ± 0.10 $^{b}$ | 0.25 ± 0.003 $^{a}$ | 39.3 ± 0.4 $^{a}$ | 0.65 ± 0.02 $^{b}$ |
| | 2018–2019 | 1.95 ± 0.06 $^{a}$ | 139.2 ±1.6 $^{a}$ | 7.4 ± 0.4 $^{a}$ | 1.03 ± 0.01 $^{a}$ | 96.1 ± 0.9 $^{a}$ | 288.9 ± 4.6 $^{a}$ | 7.19 ± 0.10 $^{a}$ | 0.21 ± 0.003 $^{b}$ | 39.2 ± 0.4 $^{a}$ | 0.76 ± 0.02 $^{a}$ |
| Sowing time | Autumn | 1.90 ± 0.06 $^{a}$ | 167.8 ± 1.7 $^{a}$ | 8.1 ± 0.4 $^{a}$ | 1.01 ± 0.01 $^{a}$ | 104.2 ± 0.9 $^{a}$ | 220.7 ± 3.7 $^{b}$ | 6.88 ± 0.10 $^{a}$ | 0.22 ± 0.003 $^{b}$ | 39.4 ± 0.4 $^{a}$ | 0.77 ± 0.02 $^{a}$ |
| | Spring | 1.61 ± 0.06 $^{b}$ | 114.0 ± 1.4 $^{b}$ | 7.6 ± 0.4 $^{a}$ | 0.97 ± 0.01 $^{b}$ | 82.2 ± 0.9 $^{b}$ | 289.4 ± 4.6 $^{a}$ | 5.05 ± 0.10 $^{b}$ | 0.24 ± 0.003 $^{a}$ | 39.1 ± 0.4 $^{a}$ | 0.65 ± 0.02 $^{b}$ |
| Genotype | V1 | 1.86 ± 0.09 $^{ab}$ | 122.9 ± 2.8 $^{de}$ | 7.7 ± 0.7 $^{ab}$ | 1.13 ± 0.02 $^{ab}$ | 88.6 ± 1.7 $^{c}$ | 290.9 ±5.9 $^{a}$ | 5.72 ± 0.20 $^{ab}$ | 0.25 ± 0.01 $^{a}$ | 40.3 ± 0.6 $^{a}$ | 0.77 ± 0.03 $^{ab}$ |
| | V2 | 1.62 ± 0.09 $^{bc}$ | 150.6 ± 3.1$^{b}$ | 6.9 ± 0.7 $^{b}$ | 0.90 ± 0.02 $^{cd}$ | 88.0 ± 1.7 $^{c}$ | 248.6 ± 5.3 $^{bc}$ | 5.22 ± 0.20 $^{b}$ | 0.24 ± 0.01 $^{ab}$ | 39.7 ± 0.6 $^{a}$ | 0.66 ± 0.03 $^{bc}$ |
| | V3 | 2.08 ± 0.09 $^{a}$ | 140.2 ± 3.1 $^{bc}$ | 7.7 ± 0.7 $^{b}$ | 1.17 ± 0.02 $^{a}$ | 91.2 ± 1.7 $^{bc}$ | 224.5 ± 4.9 $^{d}$ | 5.95 ± 0.20 $^{ab}$ | 0.26 ± 0.01 $^{a}$ | 38.7 ± 0.6 $^{a}$ | 0.83 ± 0.03 $^{a}$ |
| | V4 | 1.44 ± 0.09 $^{c}$ | 113.9 ± 2.7 $^{e}$ | 10.2 ± 0.8 $^{a}$ | 0.86 ± 0.02 $^{de}$ | 103.5 ± 1.7 $^{a}$ | 261.6 ± 5.5 $^{b}$ | 6.06 ± 0.20 $^{ab}$ | 0.20 ± 0.01 $^{c}$ | 38.9 ± 0.6 $^{a}$ | 0.58 ± 0.03 $^{c}$ |
| | V5 | 1.75 ± 0.09 $^{b}$ | 128.8 ± 2.8 $^{cd}$ | 8.4 ± 0.7 $^{ab}$ | 1.10 ± 0.02 $^{b}$ | 91.1 ± 1.7 $^{bc}$ | 235.2 ± 5.1 $^{cd}$ | 6.35± 0.20 $^{a}$ | 0.22 ± 0.01 $^{b}$ | 38.6 ± 0.6 $^{a}$ | 0.69 ± 0.03 $^{bc}$ |
| | V6 | 1.70 ± 0.09 $^{bc}$ | 189.6 ± 3.5 $^{a}$ | 7.6 ± 0.7 $^{ab}$ | 0.82 ± 0.02 $^{e}$ | 94.3 ± 1.7 $^{bc}$ | 221.5 ± 4.9 $^{d}$ | 6.02 ± 0.20 $^{ab}$ | 0.22 ± 0.01 $^{b}$ | 39.1 ± 0.6 $^{a}$ | 0.69 ± 0.03 $^{bc}$ |
| | CELINE | 1.84 ± 0.09 $^{ab}$ | 134.5 ± 2.9 $^{cd}$ | 7.0 ± 0.7 $^{b}$ | 0.95 ± 0.02 $^{c}$ | 95.8 ± 1.7 $^{b}$ | 297.7 ± 6.1$^{a}$ | 6.43 ± 0.20 $^{a}$ | 0.22 ± 0.01 $^{b}$ | 39.5 ± 0.6 $^{a}$ | 0.74 ± 0.03 $^{ab}$ |
| G | | <0.001 | <0.0001 | 0.02 | <0.0001 | <0.001 | <0.0001 | <0.001 | <0.001 | 0.35 | <0.001 |
| ST | | <0.001 | <0.0001 | 0.33 | 0.001 | <0.001 | <0.0001 | <0.001 | 0.002 | 0.43 | <0.001 |
| Y | | <0.001 | 0.95 | 0.08 | <0.0001 | <0.001 | <0.0001 | <0.001 | 0.002 | 0.80 | <0.001 |
| G × ST | | <0.001 | <0.0001 | 0.98 | 0.34 | 0.02 | <0.0001 | <0.001 | <0.001 | 0.48 | <0.001 |
| G × Y | | 0.17 | <0.0001 | 0.54 | 0.28 | 0.44 | <0.0001 | 0.02 | 0.06 | 0.06 | 0.10 |
| ST × Y | | <0.001 | <0.0001 | 0.82 | <0.0001 | 0.09 | 0.002 | <0.001 | 0.90 | <0.001 | <0.001 |
| G × ST × Y | | <0.001 | <0.0001 | 0.97 | 0.06 | 0.29 | <0.0001 | 0.001 | <0.001 | 0.06 | <0.001 |

† Back-transformed data. †† Above-ground biomass (stalk, leaves and empty siliques). ††† Harvest index is the ratio of grain yield to total plant dry mass. Means followed by the same letter are not significantly different at $p \leq 0.05$ based on Tukey's HSD test.

**Table 4.** The interaction effects of genotype and sowing time for each growing season on camelina yield and yield components.

| Genotype | Sowing Time | Seed Yield (Mg ha$^{-1}$) | | No. Siliques Plant$^{-1}$ | | Plant Density (No. m$^{-2}$) | | Above-Ground Biomass (Mg ha$^{-1}$) | | HI | | Oil Yield (Mg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2017–2018 | 2018–2019 | 2017–2018 | 2018–2019 | 2017–2018 | 2018–2019 | 2017–2018 | 2018–2019 | 2017–2018 | 2018–2019 | 2017–2018 | 2018–2019 |
| V1 | Autumn | 1.6 ± 0.2 $^{ab}$ | 2.0 ± 0.2 $^{bc}$ | 170.7 ± 6.5 $^{bc}$ | 150.7 ± 6.5 $^{c}$ | 198.1 ± 7.5 $^{ef}$ | 283.6 ± 9.3 $^{def}$ | 4.8 ± 0.4 $^{ab}$ | 7.2 ± 0.4 $^{bc}$ | 0.25 ± 0.01$^{a}$ | 0.22 ± 9.3 $^{abc}$ | 0.64 ± 0.06 $^{ab}$ | 0.81 ± 0.06 $^{bc}$ |
| | Spring | 2.0 ± 0.2 $^{a}$ | 1.8 ± 0.2 $^{bc}$ | 110.7 ± 5.3 $^{fgh}$ | 80.0 ± 4.5 $^{g}$ | 271.6 ± 9.1 $^{bc}$ | 468.8 ± 12.6 $^{a}$ | 5.0 ± 0.4 $^{ab}$ | 5.9 ± 0.4 $^{cd}$ | 0.29 ± 0.01$^{a}$ | 0.23 ± 0.01 $^{abc}$ | 0.79 ± 0.06 $^{a}$ | 0.76 ± 0.06 $^{bc}$ |
| V2 | Autumn | 1.4 ± 0.2 $^{ab}$ | 2.0 ± 0.2 $^{bc}$ | 192.7 ± 6.9 $^{ab}$ | 138.7 ± 5.9 $^{cd}$ | 158.4 ± 6.7 $^{g}$ | 254.9 ± 8.7 $^{fg}$ | 4.5 ± 0.4 $^{ab}$ | 7.2 ± 0.4 $^{bc}$ | 0.25 ± 0.01$^{a}$ | 0.21 ± 0.01 $^{abcd}$ | 0.59 ± 0.06 $^{ab}$ | 0.74 ± 0.06 $^{bc}$ |
| | Spring | 1.5 ± 0.2 $^{ab}$ | 1.6 ± 0.2 $^{c}$ | 121.7 ± 5.5 $^{efg}$ | 158.0 ± 6.3 $^{c}$ | 288.4 ± 9.4 $^{b}$ | 327.8 ± 10.1 $^{bc}$ | 3.7 ± 0.4 $^{b}$ | 5.5 ± 0.4 $^{cd}$ | 0.28 ± 0.01$^{a}$ | 0.23 ± 0.01 $^{abc}$ | 0.59 ± 0.06 $^{ab}$ | 0.64 ± 0.06 $^{c}$ |
| V3 | Autumn | 1.9 ± 0.2 $^{a}$ | 3.4 ± 0.2 $^{a}$ | 188.0 ± 6.8 $^{ab}$ | 224.5 ± 7.5 $^{b}$ | 173.2 ± 7.0 $^{fg}$ | 217.9 ± 8.0 $^{g}$ | 4.8 ± 0.4 $^{ab}$ | 10.2 ± 0.4 $^{a}$ | 0.28 ± 0.01$^{a}$ | 0.25 ± 0.01 $^{a}$ | 0.74 ± 0.06 $^{a}$ | 1.35 ± 0.06 $^{a}$ |
| | Spring | 1.5 ± 0.2 $^{ab}$ | 1.4 ± 0.2 $^{c}$ | 98.0 ± 4.9 $^{gh}$ | 93.5 ± 4.8 $^{fg}$ | 251.4 ± 8.7 $^{bc}$ | 267.9 ± 9.0 $^{ef}$ | 3.9 ± 0.4 $^{b}$ | 4.8 ± 0.4 $^{d}$ | 0.28 ± 0.01$^{a}$ | 0.23 ± 0.01 $^{abc}$ | 0.57 ± 0.06 $^{ab}$ | 0.56 ± 0.06 $^{c}$ |
| V4 | Autumn | 1.4 ± 0.2 $^{ab}$ | 1.5 ± 0.2 $^{c}$ | 156.0 ± 6.2 $^{cd}$ | 110.7 ± 5.3 $^{ef}$ | 194.9 ± 7.5 $^{ef}$ | 298.6 ± 9.6 $^{bcde}$ | 5.4 ± 0.4 $^{ab}$ | 8.6 ± 0.4 $^{ab}$ | 0.21 ± 0.01$^{a}$ | 0.15 ± 0.01 $^{e}$ | 0.58 ± 0.06 $^{ab}$ | 0.59 ± 0.06 $^{c}$ |
| | Spring | 1.1 ± 0.2 $^{b}$ | 1.7 ± 0.2 $^{bc}$ | 73.7 ± 4.3 $^{i}$ | 132.0 ± 5.7 $^{cde}$ | 243.4 ± 8.5 $^{cd}$ | 330.6 ± 10.2 $^{bc}$ | 3.7 ± 0.4 $^{b}$ | 6.5 ± 0.4 $^{cd}$ | 0.22 ± 0.01$^{a}$ | 0.21 ± 0.01 $^{bcd}$ | 0.38 ± 0.06 $^{b}$ | 0.70 ± 0.06 $^{bc}$ |
| V5 | Autumn | 1.5 ± 0.2 $^{ab}$ | 2.1 ± 0.2 $^{bc}$ | 134.0 ± 5.8 $^{def}$ | 130.7 ± 5.7 $^{cde}$ | 241.4 ± 8.5 $^{cd}$ | 304.5 ± 9.7 $^{bcde}$ | 5.5 ± 0.4 $^{ab}$ | 9.9 ± 0.4 $^{a}$ | 0.22 ± 0.01$^{a}$ | 0.18 ± 0.01 $^{de}$ | 0.65 ± 0.06 $^{ab}$ | 0.75 ± 0.06 $^{bc}$ |
| | Spring | 1.6 ± 0.2 $^{ab}$ | 1.8 ± 0.2 $^{bc}$ | 132.0 ± 5.7 $^{def}$ | 119.0 ± 5.4 $^{de}$ | 183.2 ± 7.2 $^{efg}$ | 227.2 ± 8.2 $^{gh}$ | 4.4 ± 0.4 $^{ab}$ | 5.1 ± 0.4 $^{cd}$ | 0.26 ± 0.01$^{a}$ | 0.24 ± 0.01 $^{ab}$ | 0.60 ± 0.06 $^{ab}$ | 0.68 ± 0.06 $^{c}$ |
| V6 | Autumn | 1.6 ± 0.2 $^{ab}$ | 2.4 ± 0.2 $^{b}$ | 206.5 ± 7.2$^{a}$ | 283.5 ± 8.4 $^{a}$ | 168.4 ± 6.9 $^{fg}$ | 199.9 ± 7.6 $^{h}$ | 5.3 ± 0.4 $^{ab}$ | 8.8 ± 0.4 $^{ab}$ | 0.23 ± 0.01$^{a}$ | 0.21 ± 0.01 $^{abcd}$ | 0.63 ± 0.06 $^{ab}$ | 0.97 ± 0.06 $^{b}$ |
| | Spring | 1.4 ± 0.2 $^{ab}$ | 1.4 ± 0.2 $^{c}$ | 141.0 ± 5.9 $^{de}$ | 156.5 ± 6.3 $^{c}$ | 209.9 ± 7.8 $^{de}$ | 340.6 ± 10.4 $^{b}$ | 3.9 ± 0.4 $^{b}$ | 6.0 ± 0.4 $^{cd}$ | 0.26 ± 0.01$^{a}$ | 0.19 ± 0.01 $^{cde}$ | 0.53 ± 0.06 $^{ab}$ | 0.54 ± 0.06 $^{c}$ |
| CELINE | Autumn | 1.5 ± 0.2 $^{ab}$ | 2.1 ± 0.2 $^{bc}$ | 193.0 ± 6.9 $^{ab}$ | 141.7 ± 5.9 $^{cd}$ | 180.2 ± 7.2 $^{efg}$ | 292.4 ± 9.5 $^{cdef}$ | 5.4 ± 0.4 $^{ab}$ | 8.7 ± 0.4 $^{ab}$ | 0.22 ± 0.01$^{a}$ | 0.19 ± 0.01 $^{cd}$ | 0.61 ± 0.06 $^{ab}$ | 0.81 ± 0.06 $^{bc}$ |
| | Spring | 1.9 ± 0.2 $^{a}$ | 1.9 ± 0.2 $^{bc}$ | 92.2 ± 4.8 $^{hi}$ | 129.7 ± 5.7 $^{cde}$ | 464.8 ± 12.6 $^{a}$ | 320.6 ± 10.0 $^{bcd}$ | 6.0 ± 0.4 $^{a}$ | 5.7 ± 0.4 $^{cd}$ | 0.24 ± 0.01$^{a}$ | 0.25 ± 0.01 $^{ab}$ | 0.72 ± 0.06 $^{a}$ | 0.77 ± 0.06 $^{bc}$ |

Means within each column followed by the same letter are not significantly different at $p \leq 0.05$ based on Tukey's HSD test.

### *3.3. Oil Yield and Seed Quality*

The statistical analysis showed no effect of genotype on the seed oil content with mean values among varieties ranging from 38.6 to 40.3% of seed dry matter (Table 3). These values fell in the range of the expected oil content that goes typically between 30 and 45% [22,31,35] but could be considered fairly high with respect to previous studies on other genotypes cultivated in Italy, for which values of 27 to 37% have been observed [58,59]. A significant interaction between sowing time and year revealed an increase in oil content with autumn sowing (40.4%) with respect to spring (38.3%), but only in the first season (Figure 3B). In fact, during 2019, the oil content decreased with autumn sowing, reaching levels similar to those observed for the spring crop. Climatic conditions over the two growing seasons had prevalent effects over genetic characteristics on oil content as already found in another study [33]. It is known that, in oilseed crops, heat during seed development greatly affect the conversion of carbohydrates to lipids and may explain herein the differences noticed in oil content [34–36]. At the same time, in brassicas water deficit during flowering and silique development was responsible of a decrease (3 to 12%) in oil content [60,61].

In our case, in March 2019 during flowering and seed set of autumn-sown crop, higher temperatures and drier conditions occurred, which may have been responsible of the 2% decrease in oil concentration with respect to Y1A.

Given the subtle differences in seed oil content, oil yield of camelina was mostly affected by seed yield and followed the same trend. In this case, the interaction was significant for all three variables and the effect of genotypes depended on year and sowing time, contrary to the oil content (Table 3). In the first growing season, oil yield was almost similar between the different genotypes and sowing times although V1 in spring (0.79 Mg ha$^{-1}$) and V3 (0.74 Mg ha$^{-1}$) in autumn tended to have the highest oil yields (Table 4). V3 showed in the second year the highest oil yield when sown in autumn (1.35 Mg ha$^{-1}$). The increase in oil yield registered for V3, CELINE, and V6 in autumn and for V3 in spring of the second year with respect to the 1st year, were in line with the increase in their seed yields. In all cases, the oil yield in our study was in the range of the oil content obtained in previous reports [29,33,36].

In addition to its agronomic advantages, camelina is characterized by an exceptional oil composition suitable for many industrial uses such as for cosmetic, nutraceutical and the retrieval of green polymers, in addition to biofuel. As shown in Tables 5 and 6, camelina oil is mostly unsaturated with polyunsaturated fatty acids amounting to about 60%. Linolenic (C18:3), linoleic (C18:2), eicosenoic (C20:1), and oleic (C18:1) acids are the predominant ones. All three variables (G, ST, and Y) had significant main effects on the FAs of camelina seed oil. Most of them were also affected by the interaction between sowing date and year. Moreover, the genotype had a substantial effect on the oil profile which seemed, on the other hand, to be less affected by the changes in climatic conditions (Y × G not significant).

In general, autumn sowing increased the content of the polyunsaturated α-linolenic (C18:3) and eicosadienoic (C20:2) acids, and the monounsaturated eicosenoic (C20:1) and erucic (C22:1) acids. As expected, over 31% of polyunsaturated acids were α-linolenic acid (31–39%), which is uncommon in vegetable sources [18]. The amount of erucic acid in camelina oil was affected only by sowing date and year. However, the interaction between year and sowing time showed significant differences between the two sowing times only in the first year (4.3 ± 0.1 vs. 3.5 ± 0.1% in Y1A and Y1S respectively and 2.8 ± 0.1 vs. 2.6 ± 0.1% in Y2A and Y2S, respectively). The content of erucic acid in the tested varieties was anyway below the maximum concentration allowed for edible use (5%). Higher amount was obtained in the first year for both seasons. Camelina is also rich in eicosenoic acid (15.7%), which is affected, to lesser extent, by sowing time and genotypes (Table 5). Eicosenoic acid can be a valuable source of medium chain FA for the bio-based industry which nowadays, are not produced in Europe being totally derived from palm and coconut oils [22].

**Table 5.** Major fatty acids content (% of total FA) in camelina seeds in response to genotype (G), sowing time (ST) and year (Y).

| Term | | C18:3 | C18:2 | C20:1 | C18:1 | C16:0 | C22:1 | C18:0 | C20:2 | C20:0 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % | | | | | | | | |
| Year | 2017–2018 | 34.62 ± 0.35 $^{b}$ | 18.37 ± 0.07 $^{a}$ | 14.84 ± 0.12 $^{b}$ | 14.33 ± 0.21 $^{a}$ | 5.13 ± 0.02 $^{a}$ | 3.90 ± 0.11 $^{a}$ | 2.07 ± 0.02 $^{a}$ | 2.02 ± 0.01 $^{b}$ | 1.33 ± 0.02 $^{a}$ |
| | 2018–2019 | 35.82 ± 0.35 $^{a}$ | 17.16 ± 0.07 $^{b}$ | 16.54 ± 0.12 $^{a}$ | 12.02 ± 0.21 $^{b}$ | 4.86 ± 0.02 $^{b}$ | 2.72 ± 0.11 $^{b}$ | 2.06 ± 0.02 $^{a}$ | 2.12 ± 0.01 $^{a}$ | 1.29 ± 0.02 $^{b}$ |
| Sowing time | Autumn | 36.68 ± 0.35 $^{a}$ | 16.92 ± 0.07 $^{b}$ | 16.25 ± 0.12 $^{a}$ | 11.76 ± 0.21 $^{b}$ | 4.85 ± 0.02 $^{b}$ | 3.58 ± 0.11 $^{a}$ | 2.06 ± 0.02 $^{a}$ | 2.22 ± 0.01 $^{a}$ | 1.29 ± 0.02 $^{b}$ |
| | Spring | 33.76 ± 0.35 $^{b}$ | 18.60 ± 0.07 $^{a}$ | 15.13 ± 0.12 $^{b}$ | 14.59 ± 0.21 $^{a}$ | 5.14 ± 0.02 $^{a}$ | 3.05 ± 0.11 $^{b}$ | 2.07 ± 0.02 $^{a}$ | 1.93 ± 0.01 $^{b}$ | 1.32 ± 0.02 $^{a}$ |
| Genotype | V1 | 35.17 ± 0.42 $^{ab}$ | 17.10 ± 0.13 $^{cd}$ | 16.54 ± 0.21$^{a}$ | 12.45 ± 0.26 $^{c}$ | 5.04 ± 0.03 $^{ab}$ | 3.54 ± 0.16 $^{a}$ | 1.99 ± 0.03 $^{cd}$ | 2.16 ± 0.02 $^{b}$ | 1.41 ± 0.03 $^{a}$ |
| | V2 | 36.02 ± 0.42 $^{a}$ | 16.26 ± 0.13 $^{e}$ | 15.69 ± 0.21 $^{abc}$ | 14.16 ± 0.26 $^{a}$ | 4.83 ± 0.03 $^{d}$ | 3.16 ± 0.16 $^{a}$ | 2.16 ± 0.03 $^{ab}$ | 1.83 ± 0.02 $^{d}$ | 1.30 ± 0.03 $^{bc}$ |
| | V3 | 35.44 ± 0.42 $^{a}$ | 18.46 ± 0.13 $^{b}$ | 15.79 ± 0.21 $^{abc}$ | 11.59 ± 0.26 $^{d}$ | 5.17 ± 0.03 $^{a}$ | 3.52 ± 0.16 $^{a}$ | 1.95 ± 0.03 $^{d}$ | 2.30 ± 0.02 $^{a}$ | 1.38 ± 0.03 $^{ab}$ |
| | V4 | 33.97 ± 0.42 $^{b}$ | 19.89 ± 0.13 $^{a}$ | 15.00 ± 0.21$^{c}$ | 12.86 ± 0.26 $^{bc}$ | 4.89 ± 0.03 $^{cd}$ | 3.27 ± 0.16 $^{a}$ | 1.93 ± 0.03 $^{d}$ | 2.32 ± 0.02 $^{a}$ | 1.25 ± 0.03 $^{cd}$ |
| | V5 | 35.20 ± 0.42 $^{ab}$ | 16.59 ± 0.13 $^{de}$ | 16.01 ± 0.21 $^{ab}$ | 13.60 ± 0.26 $^{ab}$ | 4.99 ± 0.03 $^{bc}$ | 3.34 ± 0.16 $^{a}$ | 2.13 ± 0.03 $^{ab}$ | 1.86 ± 0.02 $^{d}$ | 1.42 ± 0.03 $^{a}$ |
| | V6 | 35.37 ± 0.42 $^{a}$ | 17.63 ± 0.13 $^{c}$ | 15.60 ± 0.21 $^{abc}$ | 14.01 ± 0.26 $^{a}$ | 4.98 ± 0.03 $^{bc}$ | 3.27 ± 0.16 $^{a}$ | 2.21± 0.03 $^{a}$ | 1.98 ± 0.02 $^{c}$ | 1.25 ± 0.03 $^{cd}$ |
| | CELINE | 35.37 ± 0.42 $^{a}$ | 18.41 ± 0.13 $^{b}$ | 15.19 ± 0.21 $^{bc}$ | 13.50 ± 0.26 $^{ab}$ | 5.10 ± 0.03 $^{ab}$ | 3.10 ± 0.16 $^{a}$ | 2.09 ± 0.03 $^{bc}$ | 2.05 ± 0.02 $^{c}$ | 1.15 ± 0.03 $^{d}$ |
| G | | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.28 | <0.001 | <0.001 | <0.001 |
| ST | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.56 | <0.001 | 0.05 |
| Y | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.42 | <0.001 | 0.04 |
| G × ST | | 0.49 | <0.001 | 0.81 | 0.10 | 0.06 | 0.61 | 0.05 | 0.72 | 0.75 |
| G × Y | | 0.07 | 0.02 | 0.04 | 0.62 | 0.67 | 0.48 | 0.01 | 0.56 | 0.20 |
| ST × Y | | 0.34 | 0.01 | 0.77 | <0.001 | <0.001 | 0.009 | 0.45 | <0.001 | 0.003 |
| G × ST × Y | | 0.23 | 0.003 | 0.49 | 0.19 | 0.36 | 0.82 | 0.45 | 0.15 | 0.38 |

Means followed by the same letter are not significantly different at $p \leq 0.05$ based on Tukey's HSD test.

**Table 6.** Characteristics of camelina seed oil and protein content as affected by genotype, sowing time, and year.

| Term | | SFA † | MUFA †† | PUFA ††† | PUFA/SFA | ω-3 | ω-6/ω-3 | Crude protein |
|---|---|---|---|---|---|---|---|---|
| | | | | | % | | | |
| Year | 2017–2018 | 9.07 ± 0.04 $^{a}$ | 30.1 ± 0.1 $^{b}$ | 60.1 ± 0.3 $^{a}$ | 6.64 ± 0.04 $^{b}$ | 39.5 ± 0.3 $^{a}$ | 0.53 ± 0.01 $^{a}$ | 20.7 ± 0.4 $^{b}$ |
| | 2018–2019 | 8.51 ± 0.04 $^{b}$ | 33.1 ± 0.1 $^{a}$ | 57.6 ± 0.3 $^{b}$ | 6.77 ± 0.04 $^{a}$ | 38.2 ± 0.3 $^{b}$ | 0.51 ± 0.01 $^{b}$ | 22.1 ± 0.4 $^{a}$ |
| Sowing time | Autumn | 8.62 ± 0.04 $^{b}$ | 30.7 ± 0.1 $^{b}$ | 59.9 ± 0.3 $^{a}$ | 6.96 ± 0.04 $^{a}$ | 40.6 ± 0.3 $^{a}$ | 0.47 ± 0.01 $^{b}$ | 21.0 ± 0.4 $^{b}$ |
| | Spring | 8.97 ± 0.04 $^{a}$ | 32.5 ± 0.1 $^{a}$ | 57.7 ± 0.3 $^{b}$ | 6.45 ± 0.04 $^{b}$ | 37.1 ± 0.3 $^{b}$ | 0.56 ± 0.01 $^{a}$ | 21.8 ± 0.4 $^{a}$ |
| Genotype | V1 | 8.83 ± 0.07 $^{ab}$ | 31.8 ± 0.3 $^{ab}$ | 58.5 ± 0.5 $^{abc}$ | 6.64 ± 0.07 $^{bc}$ | 39.1 ± 0.5 $^{ab}$ | 0.50 ± 0.01 $^{d}$ | 20.7 ± 0.5 $^{a}$ |
| | V2 | 8.66 ± 0.07 $^{bc}$ | 32.6 ± 0.3 $^{a}$ | 57.8 ± 0.5 $^{bc}$ | 6.69 ± 0.07 $^{bc}$ | 39.5 ± 0.5 $^{a}$ | 0.46 ± 0.01 $^{e}$ | 21.9 ± 0.5 $^{a}$ |
| | V3 | 8.96 ± 0.07 $^{ab}$ | 30.1 ± 0.3 $^{c}$ | 59.9 ± 0.5 $^{a}$ | 6.69 ± 0.07 $^{bc}$ | 38.9 ± 0.5 $^{ab}$ | 0.54 ± 0.01 $^{b}$ | 21.3 ± 0.5 $^{a}$ |
| | V4 | 8.50 ± 0.07 $^{c}$ | 30.7 ± 0.3 $^{bc}$ | 59.9 ± 0.5 $^{a}$ | 7.05 ± 0.07 $^{a}$ | 37.5 ± 0.5 $^{b}$ | 0.60 ± 0.01 $^{a}$ | 21.1 ± 0.5 $^{a}$ |
| | V5 | 8.99 ± 0.07 $^{a}$ | 32.4 ± 0.3 $^{a}$ | 57.4 ± 0.5 $^{c}$ | 6.40 ± 0.07 $^{c}$ | 38.8 ± 0.5 $^{ab}$ | 0.48 ± 0.01 $^{de}$ | 21.4 ± 0.5 $^{a}$ |
| | V6 | 8.85 ± 0.07 $^{ab}$ | 32.3 ± 0.3 $^{a}$ | 58.8 ± 0.5 $^{abc}$ | 6.65 ± 0.07 $^{bc}$ | 39.0 ± 0.5 $^{ab}$ | 0.51 ± 0.01 $^{cd}$ | 21.6 ± 0.5 $^{a}$ |
| | CELINE | 8.76 ± 0.07 $^{abc}$ | 31.5 ± 0.3 $^{ab}$ | 59.6 ± 0.5 $^{ab}$ | 6.80 ± 0.07 $^{ab}$ | 39.0 ± 0.5 $^{ab}$ | 0.53 ± 0.01 $^{bc}$ | 22.0 ± 0.5 $^{a}$ |
| G | | <0.001 | <0.001 | <0.001 | <0.001 | 0.03 | <0.001 | 0.25 |
| ST | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.01 |
| Y | | <0.001 | <0.001 | <0.001 | 0.01 | <0.001 | 0.02 | <0.001 |
| G × ST | | 0.07 | 0.52 | 0.46 | 0.38 | 0.82 | 0.02 | 0.67 |
| G × Y | | 0.13 | 0.05 | 0.50 | 0.23 | 0.15 | 0.02 | 0.70 |
| ST × Y | | <0.001 | 0.001 | 0.35 | 0.07 | 0.89 | 0.08 | 0.01 |
| G × ST × Y | | 0.37 | 0.28 | 0.76 | 0.90 | 0.39 | 0.03 | 0.38 |

† Saturated fatty acids. †† Monounsaturated fatty acids. ††† Polyunsaturated fatty acids. Means followed by the same letter are not significantly different at $p \leq 0.05$ based on Tukey's HSD test.

Delayed sowing favored the accumulation of linoleic acid in both growing seasons (+10%). There was also an increase in the content of oleic acid. The relationship between temperature and fatty acids accumulation was in line with the findings of previous works that have shown a rise in oleic (C18:1) and linoleic acids (C18:2) at elevated temperatures and an increase in α-linolenic (C18:3) and eicosenoic acid (C20:1) at lower temperatures [30,34,36]. Saturated FAs represented a smaller amount of the total FAs in camelina and were made up mainly of palmitic (C16:0), stearic (C18:0), and arachidic acids (C20:0). Both palmitic and arachidic acids were increased by a delayed sowing (Table 6).

Like other oilseed crops, also in camelina, climatic conditions and genotypic characteristics are the main factors that influence the oil FA profile. High temperatures during flowering and seed ripening interfere with the enzymes responsible of the metabolism of the polyunsaturated fatty acids (PUFA) and decrease their content [29,62], which explain the 2% more PUFA with autumn sowing (Table 6). Likewise, camelina grown in autumn had more ω-3 (+3.5%), mainly as α-linolenic acid and, consequently, a greater PUFA/SFA and a lower ω-6/ω-3 ratio (Table 6). Such results demonstrated that camelina grown in autumn has more beneficial health properties, as raising the dietary PUFA/SFA ratio, has been recommended for the prevention of cardiovascular diseases [20]. On the contrary, camelina varieties grown in spring had higher content of SFA and MUFA (monounsaturated fatty acids), which make their oil more stable. Among the varieties, V4 had simultaneously the highest content of PUFA, the highest ratios of both PUFA/SFA and ω-6/ω-3, which make this oil attractive for food and feed purposes. On the other hand, V2 had the opposite behavior, i.e., a low level of monounsaturated fatty acids and a ratio of polyunsaturated over saturated acids of nearly 7. This oil displays excellent emollient properties for the preparation of natural cosmetic products [63].

Seed protein, feature of great importance for animal feed, varied between 21% and 22%, and this slight variation was the result of the interaction of sowing time and year (Table 6). According to statistical analysis, in the 1st year of trial, seeds from spring sowing were characterized by slightly higher crude protein content (21.5 ± 0.4 vs. 19.9 ± 0.4%) while in the second year it was similar between the two sowing times (21.1 ± 0.4%). According to Singer et al. [64], the diversion of carbon to protein increases with temperature, conversely to oil content. Thus, the increase reported herein could be ascribed to the rise in temperature during flowering and seed setting (May–June) in spring sowing.

### *3.4. Relationships between Environmental Factors, Seed Yield, Yield Components and Oil Content*

To better understand the relationship between seed yield and yield components, as well as between them and environmental conditions, correlations were performed (Table S3 in Supplementary Materials). The analysis showed that number of siliques per plant and TSW are the key yield components associated positively with camelina seed yield, as already found by Berti et al. [31]. The seed yield was positively correlated to seed production per plant, to above-ground biomass production and plant height as well. The higher the precipitations, the higher plant height, number of siliques per plants well as the seed oil content. No relationship was noticed between seed yield and precipitations, despite earlier studies finding positive correlation between both [65]. The rainfall distribution, with excessive precipitation in autumn, negatively affected plant density, as observed in camelina stands in autumn of each year. Camelina can counterbalance the lower plant density with a greater number of siliques per plant. Plant density was, in fact, negatively correlated to number of siliques per plant. This character is known to increase at low plant density in relation to the adaptive plasticity of brassicas to sustain crop productivity [66,67]. More branching is produced to compensate lower plant density as shown by Hossain et al. [55]. This was further corroborated by the absence of a clear relationship between camelina yield and the number of plants per unit area. A negative correlation has been also observed between HI and plant height to confirm previously reported findings [31].

There was also a fair negative relationship between oil content and camelina above-ground biomass. No clear relationship was noticed between seed oil content and seed yield, but higher oil content was obtained by crops with a greater number of GDD and precipitations accumulated from sowing to harvest. The oil yield per hectare strongly followed the seed yield per hectare and

was positively correlated to the number of siliques per plant, seed production per plant, TSW and above-ground biomass yield. The analysis demonstrated the inverse relationship between protein and oil content, reported earlier and observed in other studies [33,57], and confirmed that delayed sowings reduce seed oil content but simultaneously increase seed protein content.

## 4. Conclusions

The results of this study showed that all camelina varieties here tested are well adapted to Mediterranean conditions, with a strong dependence of crop yield and seed quality on genotype and sowing time. Even though satisfactory yields may be obtained when certain cultivars are sown in spring, camelina seemed more promising for autumn sowing (October–November), due to greater seed yield and better seed qualitative traits (high ω-3 and eicosenoic acids). These findings highlight that camelina can represent an alternative winter oilseed crop able to contribute to the diversification of traditional rainfed cereal-based cropping systems thus increasing their sustainability, in particular in less-favored agricultural areas. Camelina, has confirmed to be a flexible crop that can be included as winter or spring crop, in several crop rotational schemes.

Among the investigated cultivars, V3 proved to be, in the tested environment, the best performing cultivar in autumn sowing for its superior oil yield and seed size, while both CELINE and V1 can be successfully grown as short season cultivars, for spring periods. The low seed yields in camelina with respect to other oilseed crops (sunflower, linseed, and soybean), may be compensated by the high value of camelina oil and the valorization of the by-products of oil extraction like seed cake, thus guaranteeing increased income for farmers. The present study confirmed the high camelina's versatility due to distinctive qualities of its oil over other vegetable oils, which are particularly affected by environmental variables mainly temperature and precipitation. The richness of autumn-sown camelina cultivars mainly in ω-3 make them attractive to industrial purposes, especially for a healthy alternative cooking oil as well as for pharmaceutical and cosmetic applications.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4395/10/12/1929/s1, Table S1. Main phenological growth stages of the seven camelina genotypes sown in autumn 2017 (Y1A) and 2018 (Y2A) expressed as growing degree days (GDD) with the corresponding cumulative rainfall (mm). Table S2. Main phenological growth stages of the seven camelina genotypes sown in spring 2017 (Y1S) and 2019 (Y2S) expressed as growing degree days (GDD) with the corresponding cumulative rainfall (mm). Table S3. Pearson's product-moment correlation coefficients and their *p*-values for environmental and agronomic traits of camelina.

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