

Article

Genotype and Seasonal Variation Affect Yield and Oil Quality of Safflower (*Carthamus tinctorius* L.) under Mediterranean Conditions

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Abstract: The adoption of climate-resilient and resource-use efficient crop species and varieties is a key adaptation action for farmers in the face of climate change. Safflower, an emerging oilseed crop, has been recognized for its high oil quality and its favorable agronomic traits such as drought and cold tolerance, making it particularly suitable to Mediterranean conditions. A 2-year field study was carried out to evaluate the effects of the genotype and growing season on the crop phenology, seed and oil production, macronutrient accumulation and partitioning, and fatty acid composition of spring-sown safflower grown under rainfed conditions. The experiment was conducted during the 2012 and 2013 growing seasons on an alluvial deep loam soil (Typic Xerofluvent) at the Centre for Agri-environmental Research “E. Avanzi” of the University of Pisa (Pisa, Central Italy). Higher seed yield and yield components (plant density, plant height, branching, number of capitula per plant and seeds per capitulum) were found in almost all genotypes when the seeds were sown in mid-March 2012 compared to in late April 2013. More favorable conditions in 2012, i.e., early sowing date, higher precipitation, and quite mild temperatures, led to a better seed and oil yield and greater aboveground biomass and nitrogen uptake, with the highest amounts being removed by straw. Greater seed yield was found to be associated with a greater plant height and a higher number of capitula per plant. Oil content was negatively affected by the higher temperatures and the lower amounts of precipitation that occurred during the 2012 growing season. Seasonal variation in fatty acid composition depended on the genotype. Lower precipitation and higher temperatures during 2013 favored oleic acid content in high linoleic acid genotypes and linoleic acid in medium to high oleic acid genotypes. Among the genotypes, the linoleic-type Sabina and the oleic-type Montola 2000 performed the best in both seasons. The results, besides identifying promising safflower genotypes for spring sowing in the Mediterranean region and for future breeding programs, pointed out the importance of early sowing to contrast unfavorable environmental conditions during seed-filling, thus ensuring higher yields.

Keywords: fatty acids; linoleic acid; oleic acid; crude protein; yield components; nitrogen and phosphorus accumulation and partitioning; agroecosystem diversification

1. Introduction

Adaptation to changing climate is currently one of the primary goals of agriculture. The use of suitable crop species and varieties and the diversification of cropping systems are key adaptive actions in response to weather challenges [1,2]. In this context, minor and “underutilized” oilseed crops have been making their way into our diets and production systems [3]. By introducing them into crop rotations, these species can reduce agricultural inputs (water, pesticides, fertilizers), alleviate biotic and abiotic stresses, and stabilize yields and economic returns due to their diverse traits and potential [4,5].

Currently, only three crops—rapeseed (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), and soybean (*Glycine max* (L.) Merr.)—dominate European oilseed production systems [6].

Safflower (*Carthamus tinctorius* L., Asteraceae) is a versatile minor oilseed crop that may offer several benefits to rainfed cereal-based cropping systems due to its tolerance to cold, drought, salinity, and its reduced needs for inputs [7–10]. This annual crop can be grown in arid, semi-arid, and rainfed conditions, thanks to its deep root system and xerophytic spine attributes [7,11]. Compared to sunflower, safflower has higher resistance to bird predation and diseases, greater weed competition, and can provide earlier soil cover when sown in autumn or spring, with reduced risk of N-leaching and soil erosion [12]. Safflower has recently received increased attention for all the reasons cited above as well as for the interesting properties of its oil for food and non-food uses [13,14].

Safflower oil is mostly made up of unsaturated linoleic (C18:2), oleic (C18:1), and linolenic (C18:3) fatty acids and of a low proportion of saturated fatty acids, with palmitic (C16:0) and stearic (C18:0) being the predominant ones [15]. Based on their seed oil composition, safflower varieties are grouped in either high linoleic or high oleic acid types, with the latter being able to replace sunflower and olive oil [16,17]. Safflower oil is also rich in tocopherols, making it a functional food that is particularly useful for its anti-cholesterol effects and cardiovascular protection [13,15,18]. Safflower oil can also be a source of natural polymers for industrial and pharmaceutical applications [18]. Moreover, the large amounts of safflower by-products such as seed meal may serve as feed rations for livestock [19] and for energy production [20], which further enhance the value chain of the crop.

Safflower yield and fatty acid composition, upon which the crop's suitability for nutritional, industrial, or pharmaceutical applications are dependent, are particularly affected by the genotype [21,22] and agronomic practices such as fertilization [23], irrigation [24], harvest date [25], and sowing date [26–28]. Likewise, genotypes and their interaction with environmental variables, mainly moisture and temperature, during seed maturation affect fatty acid synthesis and the proportions of oleic and linoleic acids in the seeds [17,29,30]. Several studies have shown that safflower can be grown as a winter crop in areas with mild temperatures or as a spring crop in cooler areas [27,31]. Safflower has shown great adaptability to the arid and semi-arid areas of the Mediterranean region and has the potential to be included in rotation with winter wheat or annual legumes [32,33]. In southern Italy, where mild weather occurs, safflower performance was better when sown in autumn rather than in spring [34]. When spring sowing is to be adopted, anticipated sowing may help the crop to extract the water stored in the soil during the winter and to minimize the negative impacts of heat and drought during safflower flowering and during seed filling periods [35].

The inclusion of safflower as alternative and drought-tolerant oilseed crop within the traditional rainfed cereal-based cropping systems, can be a reasonable choice that may help farmers to increase the efficiency of their food systems and to cope with challenging climate adversities and economic risks. Despite the numerous studies that have been conducted in Mediterranean areas [11,21,26,27,31], no agronomic evaluation of available oleic and linoleic-acid genotypes has been carried out in the north-Mediterranean climate of Central Italy. In the present work, six genotypes were compared across two growing seasons to evaluate their agronomic performance (seed and oil yield, yield components, nitrogen and phosphorus accumulation, and partitioning) and their seed quality characteristics (fatty acids composition and crude protein).

2. Materials and Methods

2.1. Experimental Setup

Six genotypes of different origins (Table 1) were tested in a two-year field experiment. Safflower seeds were kindly supplied by Prof. E. Alba (University of Basilicata).

Table 1. Main characteristics of safflower genotypes used in the study.

Genotype	Morphology	Type	Origin
Sabina	Spineless	Linoleic	Czech Republic
Boemondo	Spiny	Linoleic	Italy
Belisario	Spiny	Linoleic	Italy
Benno	Spiny	Linoleic	Italy
Roberto	Spiny	Linoleic	Italy
Montola 2000	Spiny	Oleic	USA

Field experiments were carried out during the 2012 and 2013 growing seasons at the Centre for Agri-environmental Research “E. Avanzi” of the University of Pisa (Pisa, Italy, 43°40' N; 10°19' E; 1 m elevation). Plots of the different genotypes were arranged in a randomized complete block design with four replications for each season. The plot area was 21 m² (7 m × 3 m). Safflower sowing occurred on 13 March 2012 and 18 April 2013. The plant density was 40 plant m⁻², with inter-row spacing of 0.5 m.

The morphology of the region was flat, and the soil was an alluvial deep loam, typical of the lower Arno River plain and classified as Typic Xerofluvent by the USDA system [36].

In both growing seasons, the physical and chemical characteristics of the soil, were evaluated at a 30 cm depth at the beginning of the experiment. Total nitrogen was evaluated using the macro-Kjeldahl digestion procedure [37]. Soil organic carbon (SOC) was determined using the modified Walkley–Black wet combustion method [38]. SOM was estimated by multiplying the SOC concentration by 1.724 [38]. The soil was characterized by a loamy texture and a medium level of total nitrogen and average content organic matter content (Table 2). Changes in the minimum, maximum, and mean air temperatures and in the total rainfall were recorded during the experiments by a meteorological station located near the experimental site.

Table 2. Physical and chemical characteristics of the soil at the experimental sites (0–0.3 m).

	2012	2013
Sand (%)	30.63 ± 3.66	35.70 ± 1.86
Silt (%)	41.28 ± 3.33	47.63 ± 2.39
Clay (%)	28.09 ± 2.86	16.67 ± 1.68
pH	7.92 ± 0.08	7.77 ± 0.03
Organic matter (%)	1.98 ± 0.67	2.02 ± 0.10
Total nitrogen (g kg ⁻¹)	1.37 ± 0.35	1.23 ± 0.04
Available phosphorus (mg kg ⁻¹)	6.62 ± 0.71	6.53 ± 0.67
Exchangeable potassium (mg kg ⁻¹)	56.61 ± 0.65	44.84 ± 0.58
Total CaCO ₃ (‰)	53.79 ± 3.89	57.38 ± 6.95
Active CaCO ₃ (%)	3.35 ± 0.50	3.69 ± 0.54
Electrical conductivity (mS cm ⁻¹)	0.64 ± 0.09	0.84 ± 0.06

In both years, winter wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) preceded safflower, assuming a rainfed cereal-based cropping system. An integrated management system was adopted with conventional tillage practices and mineral fertilization. Tillage was conducted at the end of the September of the year before, and it

consisted of medium-depth plowing at 30 cm. Plots were maintained under identical fertilizer regimes. Pre-sowing phosphorus (P) and potassium fertilization were performed at a rate of 50 kg ha⁻¹ P₂O₅ and K₂O each as triple superphosphate and potassium sulfate, respectively. Nitrogen (N) was applied at the rate of 80 kg ha⁻¹ after sowing as ammonium nitrate and was split into two applications: the first was applied when the crop was at the rosette stage, and the second one was applied during the stem elongation phase. Plants were grown without irrigation in both growing seasons. Plots were kept free of weeds by performing hand hoeing when necessary.

2.2. Plant Sampling and Measurements

Dates of emergence, stem elongation, flowering, and seed maturity (harvest) were recorded according to Flemmer et al. [39] for each genotype along the two growing seasons. Flowering was scored when 50% of the florets in a plot opened (BBCH scale code = 65). The date of seed maturity was recorded when 90% or more of the capitulum area turned yellow/brown (BBCH scale code = 89).

Cycle length was calculated as the number of days from sowing to harvest. The accumulated growing degree days (GDD) were calculated for each growing season above a base temperature of 10 °C and a maximum temperature of 30 °C as follows (1):

$$\text{GDD} = \sum_{S1}^{S2} (T_m - b_0) \quad (1)$$

where T_m was the mean daily temperature, b_0 was the base temperature, and $S1$ and $S2$ were the sowing and the harvest time. Seed harvest was carried out between 11–15 August and 18–21 August in 2012 and 2013, depending on the genotype.

When the seeds had fully ripened (seed moisture <12%, BBCH 89), the plants were manually harvested on a sampling area of 2 m² in the inner part of each plot by cutting the plants at ground level. Plants were separated thereafter into the straw (leaves and stems), capitula, and seeds for weight determination. A stationary threshing machine was used to separate the seeds from the capitula.

Plant density, plant height, and yield components (the number of branches per plant, the number of capitula per plant, the number of seeds per capitula, and the thousand-seed weight) were determined on a sub-sample of twenty plants from each harvested area. Thousand-seed weight (TSW) was assessed according to ISTA rules [40]. Plant samples were dried into a forced-draft oven at 40 °C until a constant dry weight was determined. The apparent harvest index (HI) was calculated as the dry seed yield/total above-ground dry biomass × 100. Concentrations of N and P in the plant samples were determined by the Kjeldahl method and the ammonium phosphomolybdate colorimetric test, respectively. Nutrient uptakes were calculated by multiplying the corresponding concentration by the dry yield of each plant part. For quality analyses, the dried samples were ground using a Retsch SM1 rotor mill to <297 µm and were used for the subsequent analyses.

2.3. Oil Content

The ether extract was determined using an Ankom^{XT10} extractor (Ankom Technology, Macedon, NY, USA) with petroleum ether as the solvent. Before the extraction, the samples were hydrolyzed. Samples were added to a filter bag with diatomaceous earth in this specific order: 0.5 g of diatomaceous earth, 0.5 g of minced sample, and 0.5 g of diatomaceous earth. Through a thermal welding machine, the bags were closed and then dipped in an acid bath of 5N HCl at 90 °C for one hour. The bags were rinsed for 15 min in distilled water at 90 °C. This step was repeated 4–5 times until the pH of the distilled water was 7. The filter bags were dried in an oven at 60 °C for three hours. Later, they were cooled in a dryer, and they were finally weighed. Oil yield was calculated by multiplying the dry seed yield by the oil content.

2.4. Fatty Acid Composition

Fatty acid (FA) composition was determined with direct esterification by following the method of Christie [41]. An internal standard along with 3 mL of methanolic HCl (10%) were added to 200 mg of finely ground seeds. Samples were then left to incubate at 50 °C overnight after vigorous shaking. After cooling to room temperature, 1 mL of n-hexane and 10 mL of 6% K₂CO₃ 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 was transferred to an amber vial to which 1 g of sodium sulfate (Na₂SO₄) was added to eliminate the water residues. Further washing was completed 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 was 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. A split/splitless injector with a split ratio of 1:40 was used. An aliquot of the sample (1 µL) was injected under the following GC conditions: the oven temperature started at 40 °C and was held at that level for 1 min; it was then increased to 163 °C at a rate of 2 °C/min and was held at that level for 10 min but it was increased once again 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 at a rate of 3 °C/min and was 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 FAs are reported as a percentage of the total FA.

2.5. Crude Protein Content

Crude protein content was obtained by multiplying the total nitrogen by 6.25.

2.6. Statistical Analysis

All the variables were subjected to the analysis of variance (ANOVA) using the statistical software CO-STAT Cohort V6.201 (2002). Factorial designs with year (Y) and genotype (G) as variability factors and their interactions (Y × G) were used. Comparison between means was performed using the least significant difference (LSD) when the ANOVA F-test per factor was significant at the 0.05 probability level. Linear correlation analysis was also performed to evaluate the relationships among the main biometric and productive parameters.

3. Results

3.1. Weather Conditions and Crop Phenology

The climate of the region is typical of the north Mediterranean area and is characterized by a long-term average annual rainfall of 941 mm year⁻¹, with most rainfall occurring in autumn and spring. The mean minimum temperature is 9.6 °C, and the mean maximum temperature is 20.0 °C. During summer (July–half August), a dry period generally occurs with low rainfall and high air temperatures.

Temperature and rainfall patterns at the site, during the two growing seasons (from March 2012 to August 2013), are shown in Figure 1. Rainfall distribution was not uniform across the years and growth stages of the plants.

In the 1st year, precipitation was mainly concentrated in April and May, reaching a total of 241 mm, while in the 2nd year, the plants received 158 mm of rain, the majority of which was received between the end of April and during the month of May.

Temperatures were almost similar in both years (Figure 1). The temperatures gradually increased from March to August, when a peak of almost 32 °C was reached during

safflower ripening and senescence. Although the temperature and rainfall patterns were similar in the two growing seasons, this matched with different stages of crop development. Comparing the rainfall distribution during the crop vegetative and the reproductive phases between the two years (Table 3), 2012 was characterized by greater water availability during the vegetative phase compared to the following year. This condition may have resulted in better leaf development and greater LAI (leaf area index), which, in turn, could have led to the predisposition of larger sources for assimilate production, which were translocated to the sink (seeds) afterwards. On the contrary, the second growing season (2013) showed higher mean air temperatures, both in the vegetative (+2 °C) and the reproductive phases (+1 °C) with lower rainfall, especially during the vegetative phase.

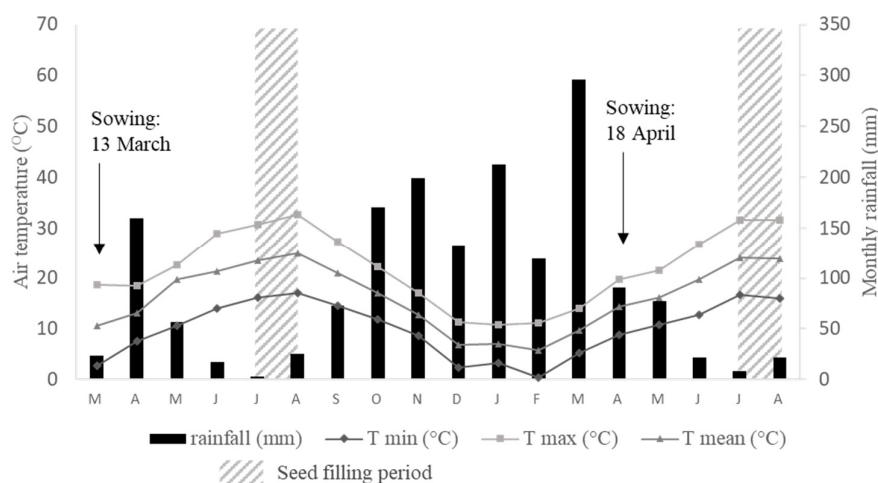


Figure 1. Monthly rainfall and mean, minimum, and maximum temperatures for the trial period with indications of sowing dates and reproduction stages susceptible to environmental stress (dashed grey bars).

Table 3. Temperatures and rainfall during vegetative and reproductive periods of each sowing date.

Sowing Date	Cycle	T _{min} (°C)	T _{max} (°C)	T _{mean} (°C)	Rainfall (mm)
13-March-2012	Vegetative	8.7–9.0	22.2–22.6	15.4–15.8	235.6–236.0
	Reproductive	16.3–16.4	31.2–31.3	23.8–23.9	4.2–5.2
18-April-2013	Vegetative	11.6–11.8	23.9–24.4	17.7–17.8	139.8–140.2
	Reproductive	16.9–17.0	32.3–32.4	24.6–24.7	13.0–17.4

In the 2nd growing season, when the seeds were sown in April, the safflower crops showed a shorter growth cycle compared to the previous year (with sowing accomplished in March), with an average cycle length of 139 days from sowing to harvest (Table 4). Inconsistent little differences were found amongst the genotypes regarding the length of their growth cycle in both growing seasons. The emergence of safflower happened as early as 12 to 15 days after sowing, independent of the sowing date. Safflower sown in mid-March 2012, flowered 106 days after sowing, but 10 days earlier with respect to those sown in late April 2013. Full flowering therefore corresponded to 25–29 June in 2012 and to 4–6 July in 2013. The seed filling period consequently coincided with the hot and the dry conditions of July–August, irrespective of the sowing time and season. Safflower genotypes needed on average of 1246 GDD to complete their cycle. Across both seasons, the Benno genotype accumulated the lowest GDD.

Table 4. Duration and cumulative growing degree days (GDD) required from sowing to emergence, stem elongation, 50% flowering, and maturity of six safflower genotypes across two growing seasons. Phenological stages according to Flemmer et al. [39].

Genotype	Duration (Days)				GDD (°C d)			
	Emergence	Stem Elongation	50% Flowering	Maturity	Emergence	Stem Elongation	50% Flowering	Maturity
2012								
Sabina	13	63	106	153	24	195	595	1222
Boemondo	13	65	105	153	24	201	578	1222
Belisario	14	63	108	155	26	195	625	1250
Benno	13	64	105	151	24	199	578	1194
Roberto	15	65	107	154	30	201	610	1237
Montola 2000	14	64	104	152	26	199	565	1207
Mean ± SD	14 ± 1	64 ± 1	106 ± 1	153 ± 1	26 ± 2	198 ± 2	592 ± 22	1222 ± 20
2013								
Sabina	12	50	78	123	67	308	603	1264
Boemondo	13	49	79	124	78	301	617	1278
Belisario	12	51	78	123	67	317	603	1264
Benno	14	50	77	122	88	308	591	1251
Roberto	12	52	79	125	62	326	617	1291
Montola 2000	13	51	78	124	78	317	603	1278
Mean ± SD	13 ± 1	51 ± 1	78 ± 1	124 ± 1	73 ± 10	313 ± 9	606 ± 10	1271 ± 14

3.2. Yield and Yield Components

Comparing the performance of the safflower genotypes in both sowing seasons, a significant interaction between the genotype and the growing year was found for seed yield, plant density, and thousand seed weight (Table 5). We noticed a drop in the seed yield of Sabina (−25%), Benno (−44.7%), and Montola 2000 (−23.1%) in 2013, while steady yields were obtained with the other genotypes. In 2012, early sown genotypes yielded between 1.8 and 2.7 Mg ha^{−1} compared to 1.3 and 2.0 Mg ha^{−1} in 2013. Despite this change, the harvest index was the same between the two seasons. In the first season, a better safflower performance was obtained, with few exceptions, which was also the case in terms of plant height (+29.9%), branching potential (+17.7%), number of capitula per plant (+30.4%), and number of seeds per capitulum (+34.5%), while the plant density remained largely constant. In contrast, in 2012, some genotypes, such as Sabina, Boemondo, and Montola 2000, were characterized by an increase in their thousand-seed weight. Among the genotypes, the observed differences were mostly due to the outperformance of Sabina over the other genotypes, which was averaged over the two years, in terms of plant density (40.5 plants m^{−2}), plant height (111.5 cm), and number of capitula per plant (17.4 capitula). Benno was able to produce plants with the highest branching, particularly in the 2nd year. Throughout both seasons and among genotypes, the TSW ranged between 32.7 and 44.5 g. No differences were seen among the genotypes for TSW in 2012. However, Benno and Roberto had the smallest seed weight in 2013. In addition, the kernel/hull weight ratio was calculated for each genotype. This characteristic can be useful to develop genotypes with higher oil and protein contents in safflower breeding programs. ~~Hull~~ Sabina had the lowest kernel percentage (41.8%) over the total seed weight and therefore the lowest kernel/hull ratio despite having a similar seed weight to the other genotypes. Instead, Boemondo showed the highest kernel/total seed weight percentage (53.2%) with respect to the other genotypes.

Table 5. Main biometric and productive characteristics (means $\pm$ SE) of six safflower genotypes sown in spring during two consecutive growing seasons. ***, significant at $p \leq 0.001$ level; **, significant at $p \leq 0.01$; *, significant at $p \leq 0.05$ level; n.s., not significant.

Variable	Year (Y)	Genotype (G)						Mean Year	Source of Variation		
		Sabina	Boemondo	Belisario	Benno	Roberto	Montola 2000		Y	G	Y $\times$ G
Seed yield (Mg DW ha ⁻¹)	2012	2.70 $\pm$ 0.14	1.83 $\pm$ 0.18	2.02 $\pm$ 0.33	2.28 $\pm$ 0.27	1.86 $\pm$ 0.18	2.34 $\pm$ 0.16	2.17	***	**	*
	2013	2.01 $\pm$ 0.09	1.84 $\pm$ 0.03	1.86 $\pm$ 0.13	1.26 $\pm$ 0.10	1.45 $\pm$ 0.05	1.80 $\pm$ 0.08	1.70			LSD = 0.475
	Mean genotype	2.36	1.84	1.94	1.77	1.66	2.07				
Harvest Index	2012	0.21 $\pm$ 0.02	0.14 $\pm$ 0.02	0.15 $\pm$ 0.02	0.15 $\pm$ 0.01	0.15 $\pm$ 0.02	0.16 $\pm$ 0.01	0.16	n.s.	n.s.	n.s.
	2013	0.18 $\pm$ 0.03	0.19 $\pm$ 0.04	0.17 $\pm$ 0.01	0.16 $\pm$ 0.03	0.15 $\pm$ 0.01	0.15 $\pm$ 0.01	0.17			
	Mean genotype	0.20	0.17	0.16	0.16	0.15	0.16				
Plants m ⁻²	2012	46.5 $\pm$ 3.6	29.5 $\pm$ 3.8	32.0 $\pm$ 2.9	32.5 $\pm$ 2.7	28.0 $\pm$ 3.9	28.0 $\pm$ 2.1	32.8	n.s.	**	*
	2013	34.5 $\pm$ 1.7	37.5 $\pm$ 2.0	32.3 $\pm$ 1.8	27.0 $\pm$ 4.4	35.5 $\pm$ 3.6	30.0 $\pm$ 1.8	32.8			LSD = 8.646
	Mean genotype	40.5	33.5	32.2	29.8	31.8	29.0				
Plant height (cm)	2012	128.3 $\pm$ 8.5	116.5 $\pm$ 3.8	116.5 $\pm$ 4.8	118.0 $\pm$ 3.2	114.3 $\pm$ 6.7	102.0 $\pm$ 2.5	115.9	***	**	n.s.
	2013	94.7 $\pm$ 2.5	77.7 $\pm$ 1.3	82.0 $\pm$ 5.3	76.0 $\pm$ 1.8	79.7 $\pm$ 7.8	77.7 $\pm$ 4.0	81.3		LSD = 9.935	
	Mean genotype	111.5	97.1	99.3	97.0	97.0	89.9				
Branch number per plant	2012	7.1 $\pm$ 0.2	7.2 $\pm$ 0.4	7.9 $\pm$ 0.2	8.2 $\pm$ 0.5	8.1 $\pm$ 0.3	8.2 $\pm$ 0.3	7.8	***	*	*
	2013	6.4 $\pm$ 0.2	6.5 $\pm$ 0.3	5.9 $\pm$ 0.2	7.3 $\pm$ 0.1	6.0 $\pm$ 0.2	6.3 $\pm$ 0.5	6.4			LSD = 0.863
	Mean genotype	6.8	6.9	6.9	7.8	7.1	7.3				
Capitula per plant	2012	19.04 $\pm$ 1.16	11.53 $\pm$ 0.66	13.36 $\pm$ 0.52	16.25 $\pm$ 1.44	12.93 $\pm$ 1.01	17.03 $\pm$ 0.68	15.02	***	***	n.s.
	2013	15.75 $\pm$ 1.02	8.83 $\pm$ 0.72	8.30 $\pm$ 0.57	9.86 $\pm$ 0.97	8.33 $\pm$ 0.91	11.90 $\pm$ 1.43	10.50		LSD = 2.175	
	Mean genotype	17.40	10.18	10.83	13.01	10.63	14.47				
Seeds per capitulum	2012	17.32 $\pm$ 2.10	18.67 $\pm$ 1.76	19.41 $\pm$ 2.37	17.23 $\pm$ 1.68	18.62 $\pm$ 1.31	18.23 $\pm$ 1.96	18.25	***	n.s.	n.s.
	2013	11.85 $\pm$ 0.95	12.57 $\pm$ 1.09	12.57 $\pm$ 1.38	11.81 $\pm$ 1.13	12.19 $\pm$ 1.27	10.79 $\pm$ 1.58	11.96			
	Mean genotype	14.59	15.62	15.99	14.52	15.41	14.51				
Thousand-seed weight (g)	2012	36.69 $\pm$ 0.25	37.26 $\pm$ 0.48	39.45 $\pm$ 1.58	36.42 $\pm$ 0.40	36.80 $\pm$ 0.57	34.84 $\pm$ 1.09	36.91	*	**	**
	2013	44.55 $\pm$ 0.90	44.04 $\pm$ 1.66	40.68 $\pm$ 2.39	32.76 $\pm$ 2.27	33.37 $\pm$ 2.98	40.53 $\pm$ 2.77	39.32			LSD = 4.926
	Mean genotype	40.62	40.65	40.07	34.59	35.09	37.69				
Kernel %	2012	43.47 $\pm$ 1.08	53.88 $\pm$ 6.36	49.70 $\pm$ 1.42	52.22 $\pm$ 1.91	53.59 $\pm$ 0.94	48.97 $\pm$ 4.18	50.31			*
	2013	40.15 $\pm$ 1.3	52.49 $\pm$ 1.28	51.35 $\pm$ 2.76	51.40 $\pm$ 3.31	51.76 $\pm$ 4.38	56.36 $\pm$ 0.33	50.59	n.s.	***	LSD = 4.280
	Mean genotype	41.81	53.19	50.53	51.81	52.68	52.67				
Kernel/Hull	2012	0.77 $\pm$ 0.03	1.17 $\pm$ 0.22	0.99 $\pm$ 0.06	1.09 $\pm$ 0.08	1.15 $\pm$ 0.04	0.96 $\pm$ 0.16	1.02			**
	2013	0.67 $\pm$ 0.04	1.11 $\pm$ 0.06	1.06 $\pm$ 0.12	1.06 $\pm$ 0.15	1.08 $\pm$ 0.20	1.29 $\pm$ 0.02	1.04	n.s.	***	LSD = 0.150
	Mean genotype	0.72	1.14	1.02	1.07	1.11	1.12				

3.3. Nitrogen and Phosphorus Uptake

With total biomass harvesting, safflower removed a greater quantity of N with respect to P, irrespective of growing season and genotype. Under the present conditions, safflower required 54.8 kg N and 9.0 kg P to produce 1 Mg dry seed yield in the spring crop (averaged over 2012 and 2013). With regard to nutrient partitioning within the plant, the contribution of the different plant organs (seed and straw) significantly varied in the function of the genotype and growing season (Table 6). As a general trend, straw (leaves + stem + remaining reproductive structures) was responsible of the major removal of both N (57.5% of the total plant uptake) and P (55.8%). Comparing the 2-year field trials (Table 6), we found that the nitrogen removed by seeds was affected by both the genotype and the growing year. Differently, the N removed by straw was significantly affected by the genotype $\times$ growing year interaction. In the second year, a decline in the N uptake by the seeds was observed. Sabina had the highest seed yield and, consequently, the highest seed N uptake, while Montola 2000 had the highest straw N removal. Analogous differences were seen for P uptake, but with much lower values.

Table 6. Nitrogen and phosphorus uptake (means $\pm$ SE) in seeds and straw of six safflower genotypes sown in spring during two consecutive growing seasons. ***, significant at $p \leq 0.001$ level; **, significant at $p \leq 0.01$; *, significant at $p \leq 0.05$ level; n.s., not significant.

Variable	Year (Y)	Genotype (G)						Mean Year	Source of Variation		
		Sabina	Boemondo	Belisario	Benno	Roberto	Montola 2000		Y	G	Y $\times$ G
Seed N uptake (kg ha ⁻¹)	2012	65.07 $\pm$ 9.40	51.24 $\pm$ 5.96	42.22 $\pm$ 9.79	39.22 $\pm$ 4.50	56.92 $\pm$ 2.27	51.01 $\pm$ 2.42	50.95	***	**	n.s.
	2013	45.63 $\pm$ 2.73	46.90 $\pm$ 0.15	44.71 $\pm$ 0.41	25.18 $\pm$ 0.16	37.90 $\pm$ 0.07	36.76 $\pm$ 0.80	39.51		LSD = 9.596	
	Mean genotype	55.35	49.07	43.47	32.20	47.41	43.89				
Straw N uptake (kg ha ⁻¹)	2012	57.06 $\pm$ 13.16	67.21 $\pm$ 12.94	47.13 $\pm$ 4.99	90.16 $\pm$ 18.39	50.24 $\pm$ 8.15	71.36 $\pm$ 12.61	63.86	n.s.	n.s.	*
	2013	66.17 $\pm$ 1.73	51.39 $\pm$ 0.97	53.75 $\pm$ 1.73	42.72 $\pm$ 0.11	55.9 $\pm$ 0.17	70.49 $\pm$ 1.27	56.74			LSD = 25.943
	Mean genotype	61.62	59.30	50.44	66.44	53.07	70.93				
Total N uptake (kg ha ⁻¹)	2012	122.13 $\pm$ 9.87	118.45 $\pm$ 7.35	89.35 $\pm$ 5.24	129.38 $\pm$ 9.67	107.16 $\pm$ 8.33	122.38 $\pm$ 10.29	114.8	***	***	***
	2013	111.8 $\pm$ 4.47	98.29 $\pm$ 5.83	98.46 $\pm$ 2.14	67.9 $\pm$ 3.27	93.79 $\pm$ 4.21	107.25 $\pm$ 2.08	96.25			LSD = 9.610
	Mean genotype	116.9	108.4	93.90	98.64	100.47	114.8				
Seed P uptake (kg ha ⁻¹)	2012	11.34 $\pm$ 0.27	6.59 $\pm$ 1.02	7.27 $\pm$ 1.11	5.7 $\pm$ 0.75	10.60 $\pm$ 1.22	9.13 $\pm$ 0.65	8.44	*	***	**
	2013	8.53 $\pm$ 0.58	8.41 $\pm$ 0.04	8.01 $\pm$ 0.03	4.99 $\pm$ 0.12	6.92 $\pm$ 0.08	7.59 $\pm$ 0.24	7.41			LSD = 1.929
	Mean genotype	9.94	7.50	7.64	5.35	8.76	8.36				
Straw P uptake (kg ha ⁻¹)	2012	4.08 $\pm$ 1.61	5.42 $\pm$ 0.51	9.86 $\pm$ 2.22	12.88 $\pm$ 2.24	5.34 $\pm$ 0.88	6.26 $\pm$ 1.20	7.31	***	*	***
	2013	11.02 $\pm$ 0.63	12.98 $\pm$ 0.12	9.18 $\pm$ 0.04	9.44 $\pm$ 0.17	9.86 $\pm$ 0.58	13.09 $\pm$ 0.36	10.93			LSD = 3.381
	Mean genotype	7.55	9.20	9.52	11.16	7.60	9.68				
Total P uptake (kg ha ⁻¹)	2012	15.42 $\pm$ 0.65	12.01 $\pm$ 0.44	17.14 $\pm$ 1.00	18.58 $\pm$ 0.97	15.95 $\pm$ 0.99	15.39 $\pm$ 0.72	15.75	***	***	***
	2013	19.55 $\pm$ 0.27	21.39 $\pm$ 0.49	17.19 $\pm$ 0.31	14.44 $\pm$ 0.21	16.79 $\pm$ 0.59	20.68 $\pm$ 0.16	18.34			LSD = 0.915
	Mean genotype	17.48	16.7	17.16	16.51	16.37	18.03				

3.4. Seed Oil Yield, Oil Composition and Crude Protein

The interaction between the growing season and the genotypes significantly affected the oil content, the oil yield, and the fatty acid composition of safflower sown in spring (Table 7). The oil content and oil yield followed the response of the yield and yield components that were described earlier. In 2013, the oil content decreased considerably in all the genotypes except for Montola 2000. Due to the drop in the oil content and seed yield, the oil yields of all the genotypes declined in 2013. Among the genotypes, Montola 2000 was able to preserve the oil content in both growing seasons, with the seeds comprising approximately 35.8% oil. The lowest producing genotypes were Sabina in 2012 (26.2%) and Roberto in 2013 (20.3%), depending on the growing season. Montola 2000 had the highest oil yields (620.5 and 838.2 kg ha⁻¹) due to the constant high oil content and the relatively high seed yield. A sharp fall in oil yield was seen for Roberto and Benno due to a severe decline in their oil content and seed yields as consequence of unfavorable thermal and rainfall conditions during the second growing season (Figure 1 and Table 3).

Oil of spring-sown safflower was composed of more than 90% of the unsaturated linoleic (C18:2n-6) and oleic fatty acids (C18:1n-9). As reported in Table 7, the decline in oil content in 2013 was accompanied by a decline in palmitic acid (C16:0), linolenic acid (C18:3n-3), and other minor fatty acids. For the remaining fatty acids, the response was genotype dependent. The environmental conditions of the second growing season favored the accumulation of stearic acid (C18:0) in the Sabina, Belisario, and Benno genotypes as well as in the oleic acid (C18:1n-9) and the linoleic acid (C18:2n-6) contents in most of the genotypes. When the performance of each genotype was compared, we found that Boemondo was the richest in palmitic acid, an important fatty acid for bio-based industries. Unsurprisingly, Montola 2000 was the richest in oleic acid (67.6–73.9%), as it is an oleic-acid type. The other genotypes had higher levels of linoleic acid, with 78.6% on average in Sabina and 61.4% on average in Roberto seeds.

The seed crude protein content also demonstrated different responses both among the genotypes and between the growing seasons. The statistical analysis showed no great differences between the seed protein contents in each genotype in both years apart from Boemondo and Roberto, which exhibited contrasting responses to the growing conditions. In both years, the crude protein was the highest in the Roberto seeds (16.3% to 19.1%) and was the lowest in the Benno seeds (10.7% to 12.5%).

Table 7. Differences in oil content (%), oil yield (kg ha^{−1}), fatty acid composition (% of total FA), and seed crude protein content (%) among six safflower genotypes sown in two consecutive growing seasons. ***, significant at $p \leq 0.001$ level; ** significant at $p \leq 0.01$; *, significant at $p \leq 0.05$ level; n.s., not significant.

Variable	Year (Y)	Genotype (G)						Source of Variation			
		Sabina	Boemondo	Belisario	Benno	Roberto	Montola 2000	Mean Year	Y	G	Y × G
Oil content (%)	2012	26.21 ± 0.50	28.73 ± 0.57	32.69 ± 0.22	33.36 ± 0.45	34.32 ± 0.24	35.82 ± 0.43	31.86	***	***	***
	2013	20.57 ± 0.21	26.79 ± 0.02	25.86 ± 0.03	21.90 ± 1.38	20.34 ± 1.45	34.47 ± 0.40	24.99			LSD = 1.90
	Mean genotype	23.39	27.76	29.28	27.63	27.33	35.15				
Oil yield (Kg ha ^{−1})	2012	707.7 ± 9.2	525.8 ± 12.4	660.3 ± 15.5	760.6 ± 19.2	638.3 ± 8.12	838.2 ± 23.0	688.5	***	***	***
	2013	413.5 ± 11.0	492.9 ± 8.0	481.0 ± 44.5	275.9 ± 34.2	294.9 ± 24.3	620.5 ± 26.8	429.8			LSD = 64.61
	Mean genotype	560.6	509.4	570.7	518.3	466.6	729.5				
C16:0a	2012	5.87 ± 0.03	6.20 ± 0.01	6.12 ± 0.01	6.00 ± 0.01	5.73 ± 0.02	4.74 ± 0.01	5.78	***	***	***
	2013	5.40 ± 0.01	5.59 ± 0.01	5.57 ± 0.01	5.66 ± 0.01	5.58 ± 0.01	5.27 ± 0.01	5.51			LSD = 0.034
	Mean genotype	5.63	5.89	5.84	5.83	5.65	5.00				
C18:0	2012	2.58 ± 0.03	2.31 ± 0.04	2.15 ± 0.17	1.98 ± 0.05	2.02 ± 0.05	1.85 ± 0.05	2.15	***	***	**
	2013	3.20 ± 0.02	2.36 ± 0.03	2.46 ± 0.15	2.27 ± 0.04	2.00 ± 0.03	1.99 ± 0.05	2.38			LSD = 0.223
	Mean genotype	2.89	2.34	2.31	2.13	2.01	1.92				
C18:1n-9	2012	9.20 ± 0.03	15.13 ± 0.07	12.22 ± 0.03	30.17 ± 0.03	30.38 ± 0.08	73.95 ± 0.07	28.51	***	***	***
	2013	14.67 ± 0.05	17.44 ± 0.06	15.47 ± 0.01	23.30 ± 0.05	30.29 ± 0.12	67.61 ± 0.07	28.13			LSD = 0.142
	Mean genotype	11.94	16.28	13.85	26.73	30.33	70.78				
C18:2n-6	2012	80.32 ± 0.04	74.33 ± 0.02	77.81 ± 0.06	59.96 ± 0.08	59.70 ± 0.05	17.04 ± 0.03	61.53	***	***	***
	2013	74.98 ± 0.05	73.16 ± 0.03	75.06 ± 0.07	67.64 ± 0.10	60.19 ± 0.05	23.36 ± 0.05	62.40			LSD = 0.134
	Mean genotype	77.65	73.75	76.44	63.80	59.94	20.20				
C18:3n-3	2012	0.27 ± 0.02	0.27 ± 0.01	0.28 ± 0.01	0.29 ± 0.03	0.30 ± 0.01	0.33 ± 0.01	0.29	***	n.s.	***
	2013	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.06 ± 0.01	0.11			LSD = 0.039
	Mean genotype	0.19	0.19	0.20	0.22	0.22	0.20				
Other FA	2012	1.76 ± 0.01	1.76 ± 0.02	1.42 ± 0.01	1.60 ± 0.03	1.87 ± 0.01	2.09 ± 0.01	1.75	***	***	***
	2013	1.64 ± 0.02	1.33 ± 0.03	1.31 ± 0.03	0.98 ± 0.01	1.80 ± 0.01	1.71 ± 0.01	1.46			LSD = 0.056
	Mean genotype	1.70	1.55	1.37	1.28	1.84	1.90				
Protein content (%)	2012	15.06 ± 0.57	13.63 ± 0.78	13.06 ± 0.72	10.75 ± 0.41	19.13 ± 0.63	13.63 ± 0.57	14.21	n.s.	***	*
	2013	14.19 ± 0.62	15.93 ± 0.74	15.03 ± 0.72	12.49 ± 1.64	16.33 ± 0.04	12.76 ± 0.98	14.46			LSD = 2.25
	Mean genotype	14.63	14.78	14.32	11.62	17.73	13.20				

3.5. Correlation among Parameters

Correlations were created to trace the relationships among the morphological and productive parameters of the safflower genotypes that were tested. The analysis, as seen in Table 8, showed that seed yield positively correlated with plant height and the number of capitula per plant. With an increase in plant height, the stem branching increased with a parallel increment in the number of capitula per plant, the number of seeds per plant, and the oil yield. No significant linear relationship was found between the thousand-seed weight (TSW) and the other yield components. An increase in safflower oil yield was considerably associated with an increase in seed yield and some yield components such as the number of branches per plant, capitula per branch, seeds per capitulum, and, above all, the seed oil content.

In addition, there were significant correlations between the yield and yield components with climatic variables along the vegetative and the reproductive growth stages (Table 9). Seed yield and yield components (plant height, number of branches, capitula per plant, and seeds per capitulum) decreased as temperatures and GDD increased and as precipitation decreased in the vegetative phase. The oil content and oil yield had demonstrated a similar association with the climatic variables during the vegetative period. The relationship between climatic variables and yield components varied somehow in the reproductive period, with precipitation having a negative effect on seed yield and its components, mainly in terms of the number of seeds per capitulum. No associations between climatic variables in neither growth phase and plant density, height, TSW, and kernel characteristics were found.

Table 8. Pearson's correlation coefficients (*r*) and their statistical significance (*P*) among pairs of variables related to the expression of growth and yield in six safflower genotypes under two growing seasons (13 March 2012 and 18 April 2013). ***, significant at $p \leq 0.001$ level; **, significant at $p \leq 0.01$; *, significant at $p \leq 0.05$ level; n.s., not significant.

		Seed Yield	Plant Density	Plant Height	Branches Number	Capitula per Plant	Seeds per Capitulum	TSW	Kernel %	Kernel/ Hull	Harvest Index	Oil Content	Oil Yield
Seed yield	r	1	0.52	0.72	0.38	0.85	0.55	0.16	-0.48	-0.49	0.47	0.42	0.82
	P		n.s.	**	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***
Plant density	r	1	0.24	-0.32	0.29	-0.05	0.24	-0.53	-0.52	0.80	-0.36	0.05	
	P		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	
Plant height	r	1	0.67	0.73	0.89	-0.16	-0.28	-0.30	0.03	0.42	0.66		
	P		*	**	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	
Branches number	r	1	0.57	0.81	-0.40	0.05	-0.00	-0.28	0.65	0.66			
	P		n.s.	**	n.s.	n.s.	n.s.	n.s.	*	*			
Capitula per plant	r	1	0.53	-0.05	-0.58	-0.58	0.30	0.36	0.72				
	P		n.s.	*	*	n.s.	n.s.	**					
Seeds per capitulum	r	1	-0.29	-0.01	-0.06	-0.20	0.59	0.69					
	P		n.s.	n.s.	n.s.	n.s.	*	*					
TSW	r	1	-0.24	-0.19	0.37	-0.05	-0.00						
	P		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.					
Kernel %	r	1	0.99	-0.65	0.44	0.00							
	P		***	**	n.s.	n.s.							
Kernel/Hull	r	1	-0.64	0.43	-0.02								
	P		*	n.s.	n.s.								
Harvest index	r	1	-0.37	0.01									
	P		n.s.	n.s.									
Oil content	r	1	0.86										
	P		***										
Oil yield	r	1											
P													

Table 9. Pearson's correlation coefficients (*r*) and their statistical significance (*P*) among yield and yield characteristics and climate variables in the vegetative and reproductive stage of six safflower genotypes grown across two years (2012–2013). ***, significant at $p \leq 0.001$ level; **, significant at $p \leq 0.01$; *, significant at $p \leq 0.05$ level; n.s., not significant.

[illegible]

4. Discussion

Six spring-sown safflower genotypes were evaluated across two seasons under rainfed conditions to verify their phenological development, yield, and seed quality performance in the specific climate conditions of Central Italy. In general, all six genotypes adequately adapted to the dry and hot conditions of the spring–summer period of the study area.

Safflower yield, according to literature, commonly ranges between 0.3 and 3 t ha⁻¹, depending on genotype, cultivation practices, and environmental conditions [21,28,42–44]. In our trials, the yields of the safflower genotypes fell within this range. However, differences in safflower performance arose between the two growing seasons due to differences in sowing time and environmental conditions. Plants sown in April 2013 had lower seed yields due to poorer establishment, shorter stems, less branching and fewer capitula and seed production.

The outperformance of the safflower genotypes that were grown in the first season can be ascribed to the higher amount of available water over the course of crop growth as well as to the earlier flowering (10 days earlier) compared to the crops that were grown in the subsequent season, which may have alleviated the abiotic stress during the critical reproductive phase. In fact, the total rainfall that was measured during the vegetative stage of the second season was about 100 mm less than that recorded during the first growing period. Furthermore, this season showed higher mean air temperatures, both in the vegetative (+2 °C) and the reproductive phase (+1 °C). These results corroborated several studies that showed how hot and dry conditions during the growing season may negatively influence the agronomic performance of safflower [44–46]. Despite safflower is considered to be tolerant to drought and heat, flowering remains the stage that is the most sensitive to environmental stress. In Mediterranean environments, this stage and the subsequent seed development occur during the hot and dry summer season, regardless of the sowing date [31]. These conditions can alter the photosynthesis and nutrient uptake of the plant. In such a situation, the aboveground biomass at anthesis can crucially define the final safflower yield. In this regard, it has been observed that, in safflower, the translocation and storage of pre-anthesis assimilates into the seed is a crucial physiological process during seed filling, especially under drought conditions, in order to obtain higher yields [31]. It has been reported that the translocation of dry matter and N accumulated during the vegetative stage may constitute around 65 to 72% of the final seed weight [31]. Therefore, the more dry matter and N content at the anthesis, the more translocation to seed during the filling period and the greater the yield. This aspect highlights the importance of a properly chosen sowing time, and indicates that it is not only environmental conditions per se that have positive effects on safflower. When sown earlier, safflower plants can benefit thereafter from a longer growing season [23]. In addition, environmental stress during early post-anthesis can reduce the amount of filled grain due to flower or developing embryo abortion [47].

Correlation analysis showed both plant height and the number of capitula per plant as significant determinants of seed yield. In fact, the number of capitula per plant was reported to be the most positively correlated characteristic to safflower yield [21]. Likewise, the greater stem reserves of taller plants might support seed filling better, and might extend this period that is crucial for yield formation [48].

The compared genotypes had similar seed attributes (TSW and hull proportion) that were indifferent to seasonal patterns. The safflower harvest index, one of the limitations of safflower, is low (18–24%) compared to other crops [48,49], which further binds the seed yield.

Data on safflower nutrient uptake and removal can be a valuable baseline for nutrient management to maximize yield while reducing the environmental impacts and the cost of fertilizers. In the present study, safflower needed between 44–65 kg of N and 6–12 kg of P to produce 1 Mg of dry seeds ha⁻¹ on average. These values were similar to the safflower

requirements that have been reported in the literature [48–50]. The N and P uptake partitioning patterns also showed considerable accumulation in safflower straw. Around 43–90 kg of N and 4–13 kg of P can be returned through safflower residues into the soil. These nutrients along with the carbon inputs can help sustain soil health for the next crops although via slow mineralization (C/N between 46 and 85 as found in La Bella et al. [21]).

Seed composition is also significantly influenced by genotypic and environmental factors such as in other oilseed crops [51,52]. In our study, safflower genotypes succeeded in producing fair contents of oil (between 20.3 and 35.8%). An oil concentration ranging from 17 to 43% and oil yields from 0.2 to 2.6 t ha⁻¹ have been reported in safflower genotypes grown in arid and semi-arid regions [21,29,30,44]. Besides yield, early sowings have been found to increase oil content in many genotypes [27,28]. In fact, seed development and maturation processes, i.e., seed filling processes and nutrient reserves accumulation, are highly susceptible to environmental changes, which thus being able to influence the qualitative and the quantitative traits of the final yield. In particular, drought and heat during seed development can hinder oil accumulation due to their deleterious effects on the enzymes involved in the conversion of carbohydrates to lipids [53]. In this regard, by delaying the sowing date to late April, a significant reduction in oil content occurred.

Similarly, Mohammadi et al. [44] observed, a decline in seed oil content across cultivars of safflower under drier conditions. This negative effect may be attributed to a decrease in the availability of carbohydrates for oil synthesis [53]. The decrease in oil content and yield components in different safflower cultivars has also been reported to be due to water deficiency [43,54], which may explain the higher oil content in the first growing season compared to in the second growing season. The outperformance of the high-oleic genotype Montola 2000 (34.5–35.8%) among the genotypes used in our study corresponded to values that have previously been reported in the literature [29,55]. Oil derived from conventional safflower varieties contains mainly palmitic acid (4–8%), stearic acid (2–3%), oleic acid (8–18%), and linoleic acid (73–84%) [15]. The high oleic acid types are characterized by higher oleic acid rather than linoleic acid, both of which are important fatty acids for oil quality due to their effects on oil stability and human health. However, the literature has reported different responses of singular fatty acids and their ratios to environmental stress, particularly moisture and temperature during seed maturation. Water scarcity was responsible for a decrease in the polyunsaturated fatty acid content under a late sowing date, which is mainly due to the decrease in the linoleic acid content, which coincides with some of our genotypes [52]. Others noticed a drop in the oleic acid content of 3 to 5% when safflower was grown in warm locations under dry conditions [51]. While confirming these results, Ashrafi and Razmjoo [56] attributed the decrease in oil content under drought conditions to a dramatic reduction in the saturated fatty acid contents. In high linoleic acid genotypes that have been subjected to water stress, an increase of between 1 and 2.6% in the oleic acid and a decrease in the linoleic acid (–1.7%) content was reported by Nazari et al. [57]. The same authors pointed out that under drought conditions, earlier plant maturity may shorten the grain filling period and therefore the time needed to convert oleic acid to linoleic acid. The change in oil composition shown by the reduction in the degree of unsaturated fatty acids following water stress may result from an inhibition of polyunsaturated fatty acids synthesis and denaturation activities [56]. The temperature between flowering and ripening might be the most important factor influencing fatty acid composition. It was reported that the linoleic acid content increases under low temperatures, while oleic acid increases under high temperatures [29]. High temperatures may inhibit the desaturase enzymes that convert oleic to linoleic acid [58], which explains why linoleic acids were lower when safflower was sown late in the spring.

The protein content is an important indicator of safflower seed quality because of its useful application in livestock feeds. It ranges between 10% and 22%, a finding that was also confirmed in the present study [21,59]. Inconsistent findings on crude protein across

the genotypes were obtained in this study; however, it has been reported that seed protein content has a contrasting response to environmental conditions. However, different responses due to genotype $\times$ environment interactions can be expected since the protein concentration is a quantitatively inherited trait [45].

5. Conclusions

This study showed that spring sowing of rainfed safflower can be adopted in the north Mediterranean climate of Central Italy as well as in areas that are characterized by similar pedo-climatic conditions. Despite the limited number of genotypes compared in the study, the good agronomic traits that were observed here make safflower a good candidate for larger-scale development, as it is able to increase the local production of vegetable oil and to provide protein-rich meals for livestock. The environmental factors influenced yield components, oil quality, and fatty acid composition, depending on the genotype used. In general, early sowing coupled with milder and wetter conditions seemed to be favorable for safflower production in the study area, as higher oil yield and aboveground biomass might be obtained. Montola 2000, an oleic acid-type genotype, and Sabina, a linoleic acid-type genotype, can guarantee the greatest oil yields. These well-adapted genotypes can also offer different opportunities for both food and the bio-based industries based on their oil quality. Further studies are needed to identify the more appropriate agronomic practices and new genotypes with superior morpho-agronomic features (i.e., wider range of temperature-responsive, water-use efficient genotypes) to optimize safflower production in the study area.

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