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Low-input cultivation of camelina (*Camelina sativa* (L.) Crantz) in a Mediterranean semi-arid environment

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Abstract

The cultivation of oil crops for biofuel production has often been accused of not being environmentally sustainable due to the high inputs needed. To explore the effect of reduced input on productive and qualitative traits of camelina (*Camelina sativa* (L.) Crantz), a trial was carried out over a two-year period. This study analysed two different levels of input: a low input treatment (shallow non-inversion tillage and low fertilisation rate) and a high input treatment (deeper tillage and high fertilisation rate). Camelina was positively, even though to a limited extent, affected by high input treatment as highlighted by the increase in seed yield (from 1.8 to 2.0 t ha^{-1}), crop residues (from 4.8 to 5.2 t ha^{-1}), seed protein content (from 26.5 to 28.9%), seed oil content (from 41.5 to 43.4%) and oil yield (from 0.75 to 0.88 t ha^{-1}). So, from a sustainable point of view, we must consider negligible the effect of high input and satisfactory the performances of camelina in the low input regime. Low input management resulted in satisfactory yields in terms of both quantity and quality, results which were not very different from high input, indicating promising potential for conservation agriculture practices in camelina in a semi-arid environment.

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Introduction

The increasing need for energy and the simultaneous demand for energy less harmful to the environment has led policy and research towards renewable sources of energy. Solomon *et al.* (2007) in a technical report of the Intergovernmental Panel on Climate Change include as '*robust findings*' that '*Fossil fuel use, agriculture and land use have been the dominant cause of increases in greenhouse gases over the last 250 years*'. This process has generated interest in fuel coming from plants as possible alternative sources of energy, having far less impact on the environment than fossil fuel. Along with the well-known and most studied biodiesel oilseed crops (soybean, rapeseed, canola, *etc*.), some minor and less studied crops show potential for biofuel use. Among these, *Camelina sativa* (L.) Crantz, commonly known as camelina or false flax, is an interesting oilseed crop belonging to the *Brassicaceae* family, considered a valuable oil crop for the production of sustainable second-generation biofuel. It shows a high oil content of 35%-45% (Moser, 2010; Zanetti *et al.*, 2020), which is almost double that of soybean. Moreover, due to its high level of unsaturated fatty acids and the outstandingly high levels of α-linolenic acid (32-40% of total oil content), camelina is an interesting resource for animal feed too. Some authors (Fröhlich and Rice, 2005; Pecchia *et al.*, 2014) indicate that methyl ester obtained from camelina oil has properties similar to methyl ester obtained from rapeseed and that the fuel properties (cold flow properties, oxidative stability, kinematic viscosity, cetane number, *etc*.) of camelina-based biodiesel can be considered similar to those of soybean or canola-based biodiesel, which may suggest blending camelina oil with other esters or with diesel. Some authors consider camelina oil also suitable for jet fuel and high-value industrial lubricants (Fröhlich and Rice, 2005; Moser and Vaughn, 2010).

Camelina also shows valuable agronomic traits due to its minimal input requirements, its high compatibility with reduced tillage systems and its wide adaptability to marginal land with limited fertility and water availability (Putnam *et al.*, 1993; Vollmann *et al.*, 1996; Zubr and Matthaus, 2002; Iskandarov *et al.*, 2014). For these reasons, camelina can be easily included in crop rotation systems, being available both winter and spring types. Both types, having exceptionally low temperature tolerance, can be grown with autumn cycle (Masella *et al.*, 2014; Berti *et al.*, 2016; Righini *et al.*, 2019) under diverse climatic and soil conditions (Zubr, 2003), representing a valuable option from both economic and environmental points of view compared to other oilseed crops. In fact, in a multiyear and multi oilseed species trial including sunflower, safflower, soybean, rapeseed, mustard, flax, crambe, canola and camelina, this last emerged as the most economical crop and as the lowest input crop (Pilgeram *et al.*, 2007).

In this context, conservation agriculture practices with low energy inputs such as no-tillage or minimum tillage could furtherly optimise the energy and economic performance of camelina production in semi-arid areas of the Mediterranean environment (Chen *et al.*, 2015). Anyway, limited information is available regarding the performance of camelina grown under limited tillage practices (minimum tillage or no-tillage) (Gesch and Cermak, 2011; Berti *et al.*, 2016) and no literature, to our knowledge, is reported for semi-arid environments.

With this in mind, the present research analyses the combined effects of two different levels of input: a low input treatment with shallow tillage and low fertilisation rate, and a high input treatment with deeper tillage, plowing and high fertilisation rate.

Materials and methods

Plant material and field experimental design

The trial was conducted during the growing seasons 2007-2008 and 2008-2009 at the experimental farm of the University of Catania (Ispica, Southern Italy, 46 m a.s.l., 36°46' Lat N, 14°54' Long E). The soil is classified as a clay loam soil and contained high percentages of clay (38%) and sand (37%) with 0.91 g kg^{-1} of N, 42.5 mg kg^{-1} of available P_2O_5, and 363 mg kg^{-1} exchangeable K. The variety 'Calena' of camelina was sown in a silty-loam soil as an autumn crop under rotation with wheat on 13th and 10th November in 2007 and 2008, respectively. The seeding rate was 400 seeds per m^2 in rows 20 cm apart with a plot size of 200 m^2 (10×20 m) surface area. In a randomised complete block design with three replicates, we compared the effects of two levels of inputs on camelina: low (L) and high (H) inputs, differing in the intensity of soil tillage and fertiliser rates. Low input includes shallow non-inversion tillage with a ripping machine followed by harrowing and fertilisation with a low rate (32 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} and 35 kg K_2O ha^{-1}); High input includes conventional deeper tillage with plowing followed by harrowing and higher fertilisation rate (64 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} and 70 kg K_2O ha^{-1}). Nitrogen was applied as ammonium nitrate, phosphorus as super-phosphate and potassium as potassium sulphate. Plowing at the depth of 40 cm was conducted during summer in plots with high inputs. Ripping was carried out at a depth of 25 cm in low input plots. Harrowing was performed before sowing in both treatments. Phosphorus and potassium fertilisers were applied immediately before sowing, while nitrogen was applied both before sowing and during the vegetative stage. Weed management was carried out by hand and no irrigation was applied.

Field measurements

The main phenological phases of camelina were determined and are here reported for clarity according to the codes described by Martinelli and Galasso (2011): emergence of cotyledons (BBCH 09), beginning (BBCH 62) and end of flowering (BBCH 69), and seed maturation (BBCH 89). At seed maturity, when average seed moisture was lower than 10%, three sampling areas of 10 m^2 per plot were manually harvested, excluding outer-rows, to assess harvestable crop yield. The following parameters were surveyed: seed yield (t ha^{-1}), 1000 seed-weight (g), crop residues (t ha^{-1}), oil content (%). Oil yield (t ha^{-1}) was calculated by multiplying seed yield (t ha^{-1}) by oil content (%). Harvest was carried out in both years in the last ten days of May.

Daily precipitation and average, minimum, and maximum air temperatures were recorded in each growing season through an in-field meteorological station equipped with CR10X data logger (Campbell Scientific Inc., UT, USA).

Laboratory analysis

The protein content of camelina seed samples was determined adopting the Kjeldahl procedure, as described by AOAC Official Method 988.05 (AOAC, 1990). Crude protein was calculated by multiplying N content x 6.25. The oil was obtained by solvent extraction of camelina seeds, with n-hexane using the Soxhlet apparatus according to the procedure described by UNI EN ISO 659-2009.

The fatty acid composition was determined by gas chromatography (GC) of fatty acid methyl esters (FAMEs). The ester analysis was performed with an HRGC; Ega 2 (Carlo Erba Instruments) equipped with a flame ionization detector. FAMEs were identified adopting standards (Sigma-Aldrich GmbH, Steinheim, Germany) as a comparison and were quantified by measuring the area percentage of each peak.

Statistical analysis

Analysis of variance was performed to determine the effects of input level, year, and their interactions, using the excel statistical package DSAASTAT (Onofri, 2007). Means were compared by the Tuckey HSD test at 5% probability level.

Results and discussion

The main meteorological parameters are shown in Figure 1. In the 1st year, the mean temperature ((T min + T max)/2) was about 11.3°C, while the minimum and maximum values ranged from 1.8°C (in February) to 20.0°C (in May). Precipitation, recorded during the crop growing cycle, was 505 mm, mainly concentrated in November, December and March. The 2nd growing season resulted, on average, slightly warmer than the previous one (+1°C), with minimum and maximum equal to 2.8°C (in February) and 22.3°C (in May). Precipitation was 770 mm, an amount significantly higher than in the first year and higher than the long-term precipitation (405 mm), well distributed throughout the growing season.

The different thermal trends of the two studied years did not lead to differences in the growth of plants. The type of management techniques did not determine any difference in emergence, but affected the cycle duration (Figure 1). In both years the high input increased the length of the crop cycle when compared with low input treatment. On average of the two years, high input prolonged the development of camelina plants from sowing to flowering (+5 days), delayed the pod setting (+3 days) and maturation/harvest period (+4 days), effects that could be ascribed to the higher N rate (Leghari *et al.*, 2016).

The influence of agricultural management techniques on the productive performance of camelina was ascertained in each of the parameters investigated (Table 1). Seed yield (from 1.8 to 2.0 t ha–1), 1000-seed weight (from 1.3 to 1.5 g), and crop residues (from 4.8 to 5.2 t ha^{-1}) were positively, even though to a limited extent, affected by high inputs. Seed yield was also affected by the year of cultivation, the second year being more productive, even though producing seeds less rich in oil. This difference might be ascribed to the greater amount of precipitation that occurred in the second year. Seed yields agree with those obtained in other autumn sowing trials in the cooler environment: Masella *et al.* (2014) in a 2-year experiment a trial carried out in northern Italy found values ranging from 0.6 t ha^{-1} to 2.3 t ha^{-1}. Righini *et al.* (2019) obtained results ranged from 1.59 t ha^{-1} to 2.58 t ha^{-1}. Also, Campbell *et al.* (2013) in a multiyear and multisite experiment in Western Australia on camelina, found an average yield of 1.0 t ha^{-1}, with a peak of 2.4 t ha^{-1}. On the other hand, seed yield resulted higher compared to what was reported by Royo-Esnal and Valencia-Gredilla (2018) in a semi-arid Mediterranean environment and by Matteo *et al.* (2020) in the Po valley (Northern Italy). Contrasting pieces of information are reported in the literature for N effect on yield in camelina. Some studies indicated that camelina has a low response to N with requirements from 75 to 100 kg N ha^{-1} for maximum seed yield (Budin *et al.*, 1995; Zubr, 2003; Imbrea *et al.*, 2011), while other authors reported significative yield increment as the nitrogen increases up to 200 kg N ha^{-1} (Jiang *et al.*, 2013; Solis *et al.*, 2013; Jankowski *et al.*, 2019; Stolarski *et al.*, 2019). Afshar *et al.* (2016) reported that the yield response to N application further than 45 kg ha^{-1} was negligible in growing camelina, whereas Urbaniak *et al.* (2008), in a study on the effect of N rate on seed yields and oil quality on the cultivar Calena, showed how the yield increase was significant only at 60 kg N ha^{-1} and no further yield increase was found when the N application rate was increased to 80, 100, or 120 kg ha^{-1}. Bronson *et al.* (2019) reported that the optimum N rate for the highest seed production in camelina is 150 kg ha^{-1}.

The 1000-seed weight was negatively affected by low input treatment. Previous research concerning both camelina and other oilseed crops have demonstrated that the seed weight is genetically dependent, and much less influenced by the variation of nitrogen supply (Solis *et al.*, 2013; Agegnehu and Honermeier, 1997). This could be ascribed to the fact that the rate of nitrogen applied in the low input treatment (32 kg N ha^{-1}) was lower than the satisfactory nutrients threshold and consequently in a range that potentially could affect seed yield for nutritional stress. Anyway, it must also be stated that lower seed yield could also be related to the reduced tillage intensity. In fact, even though the magnitude of this effect can vary with climate, crop rotation, and soil type (Soane *et al.*, 2012). Cooper *et al.* (2016) and Pittelkow *et al.* (2014) proved how conservative agricultural practices could result in yield reductions of up to 10%, across several types of climate. Also, Afshar *et al.* (2016) found yield reduction up to 26% in no-till compared to conventional treatment. Moreover, the type of management significantly affected seed protein content. In the average of the two studied combined factors (nitrogen and tillage), the higher N supply resulted in higher protein content from 26.5 to 28.9%. The protein content was in the range of values reported by the literature (Ubraniak *et al.*, 2008; Campbell *et al.*, 2013), and in agreement with Malhi *et al.* (2014) and Jiang *et al.* (2013) who stated that the application of N increased the seed yield, protein content and protein yield. Also, oil content was affected by high input (+4%, from 41.5 to 43.4%) and year of cultivation, whereas the oil yield reached 0.87 t ha^{-1} when high input agronomic management was

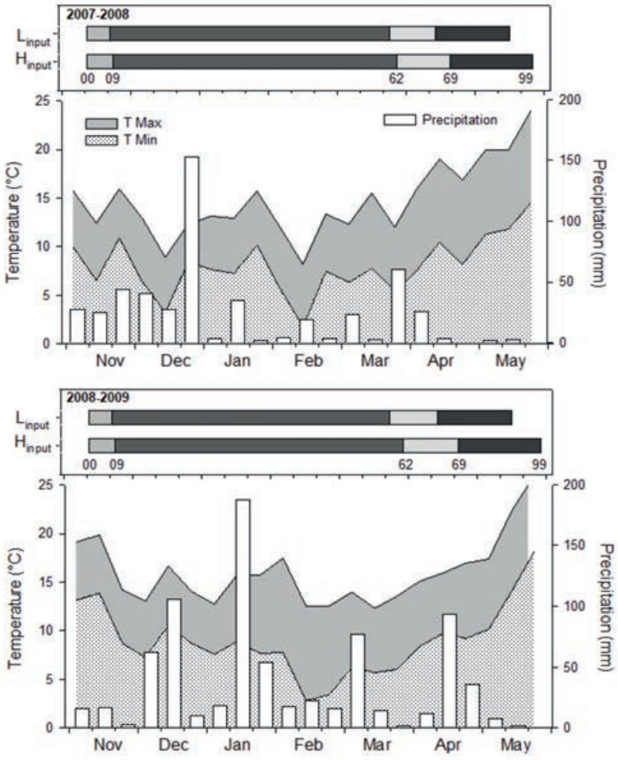

Figure 1. Meteorological trend and phenological growth stages of camelina recorded in the two-year trial Description of phenological growth stages according to the extended BBCH scale (Martinelli and Galasso, 2011): 00: Dry seed; 09: Emergence of cotyledons; 62: Flowering (20% of flowers open); 69: End of flowering/fruit set visible; 99: Harvested product.

Table 1. Agronomic and chemical parameters and P value of camelina in relation to year of cultivation and management regime.

		Yield	1000 seed weight	Straw yield	Protein	Oil	Oil yield
				P-value			
Year (Y)		0.010	0.012	0.002	0.056	0.002	0.115
Input (I)		0.014	0.000	0.015	0.000	0.001	0.004
Y×I		0.759	0.283	0.640	0.880	0.414	0.938
		(t ha^{-1})	(g)	(t ha^{-1})	(%)	(%)	(t ha^{-1})
Y	I	1.8^{b}	1.4^{b}	4.8^{b}	27.3^{a}	43.3^{a}	0.8^{a}
	II	2.0^{a}	1.5^{a}	5.2^{a}	28.1^{a}	41.6^{b}	0.8^{a}
I	L_{input}	1.8^{b}	1.3^{b}	4.8^{b}	26.5^{b}	41.5^{b}	0.8^{b}
	H_{input}	2.0^{a}	1.5^{a}	5.2^{a}	28.9^{a}	43.4^{a}	0.9^{a}

^{a,b}Different superscript letters in column indicate statistical significant difference ($P<0.05$)

practiced. The oil content agreed with that found by Righini *et al.* (2019) and similar to the level reported by Zubr (1997) for winter-sown camelina, but higher than that reported by several authors for camelina grown in Northern Italy (Pecchia *et al.*, 2014; Matteo *et al.*, 2020) and for spring-sown crops (Budin *et al.*, 1995; Agegnehu and Honermeier, 1997; Zubr, 2003). Righini *et al.* (2016) reported that camelina seed oil content can vary from 26% to 43% moving from south to north Europe. Oil yields agreed with the values reported by Vollmann *et al.* (2007) (values ranged from 641 to 983 kg ha^{-1}).

The ANOVA ascribed significant variations to α-linolenic and eicosenoic acid content in relation to both growing season and input, whereas erucic acid was affected only by input. The remaining fatty acids did not show any difference in relation to the different management techniques applied.

The fatty acid profile showed a clear prevalence of α-linolenic acid (31.3-34.1%), followed by linoleic acid (16.4-17.4%), oleic acid (16.7-17.1%), eicosenoic acid (15.2-15.9%) and a limited amount of palmitic acid (6.1-6.4%), erucic acid (2.9-3.9%) and stearic acid (2.6-2.9%) (Table 2). The poly-unsaturated fatty acids (linoleic and α-linolenic acid) were present up to 49%, the mono-unsaturated oleic, eicosenoic acid and erucic acid up to 36%, and the saturated fatty acid up to 9% of total fatty acids. The high content recorded for the unsaturated fatty acids (85% of total fatty acids) agreed with values reported by several authors (Zubr, 1997; Zadernowski *et al.*, 1999; Abramovic and Abram, 2005). The fatty acid profile resulted similar to that reported by Righini *et al.* (2019) for autumn sowing, except for the oleic acid, which had higher content in our trial (16.9% *vs* 12.9%).

The α-linolenic acid content in the first year (31.3%) was found to be lower than the second year (34.1%). On the contrary, the eicosenoic acid showed a higher level in the first year (15.9%) than in the second year (15.3%). Low input treatment showed, in both years, the highest content of eicosenoic and erucic acid. High input showed the highest content of α-linoleic acid. Similar results were reported in studies investigating the effect of N fertilisation on oil profile (Jiang *et al.*, 2013; Jankowski *et al.*, 2019). They showed that N fertilisation determined a significant increase in the proportion of linoleic acid and linolenic acid and a decrease in the proportion of oleic acid and eicosenoic acid.

The effect of agronomic management on oil profiles of *B. carinata* was reported by Zanetti *et al.* (2009) for oleic and linoleic fatty acids, which increased and decreased, respectively, with low input.

The main source of variations on fatty acid among all environmental factors is generally considered to be temperature. Some authors (Rodríguez-Rodríguez *et al.*, 2016; Zanetti *et al.*, 2017) reported that temperature during the seed filling stage has a primary role in determining the final FA composition in oilseed crops. Vollmann *et al.* (2007) partly explained the high levels of linolenic acid with low temperatures and high precipitations during the seed filling period. Results obtained by Righini *et al.* (2019) indicate that the kinetics of principal fatty acids in camelina seeds were significantly affected by temperature. They showed that high temperature affected positively linoleic acid and negatively linolenic acid. In our experiment, the thermal trend experienced by the plant from pod filling to ripening in both years can be considered comparable (0.5°C difference in the mean temperature), consequently some other variables could have affected the content of unsaturated fatty acid. Regarding this, it must be mentioned that management practices and other environmental factors (solar radiation, N availability and water supply) also act on the fatty acid synthesis pathway, and consequently on the final fatty acid content (Aguirrezábal *et al.*, 2009).

Conclusions

Good productive performances in camelina could be substantially maintained with low input management techniques. It should be noted that even when we planned the ‘high’ input treatment, in terms of tillage or fertilisation level, they were below the thresholds for conventional tillage or fertilisation intensity recommended as a standard for this crop. High input exhibited a slightly positive influence on the agronomic and, to a lesser extent, on the qualitative characteristics of camelina, although the lengthening of the crop cycle could represent a risk in environments characterised by an end-of-season drought, such as the Mediterranean environment.

On the other hand, low input management allowed yields close to high input in terms of both quantity and quality, indicating promising performances for conservation agriculture practices in camelina in semi-arid climates. When taking into account the environmental impact that the high input involves and its negligible effect, we should consider the low input as a sustainable effective management technique for producing camelina oil.

Table 2. Fatty acid profile (%) and P-value of camelina in relation to year of cultivation and management regime.

		Saturated fatty acids		Mono-unsaturated fatty acids			Poly-unsaturated fatty acids	
		Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Eicosenoic acid (C20:1)	Erucic acid (C22:1)	Linoleic acid (C18:2, n-6)	α-linolenic acid (C18:3, n-3)
					P-value			
Year (Y)		0.160	0.052	0.482	0.031	0.975	0.104	0.000
Input (I)		0.165	0.389	0.424	0.038	0.000	0.418	0.001
Y×I		0.966	0.807	0.554	0.034	0.734	0.684	0.128
					%			
Y	I	6.4	2.9	17.1	15.9^{a}	3.4	17.4	31.3^{b}
	II	6.1	2.6	16.7	15.3^{b}	3.4	16.4	34.1^{a}
I	L_{input}	6.1	2.8	17.1	15.9^{a}	3.9^{a}	17.1	31.5^{b}
	H_{input}	6.4	2.7	16.7	15.3^{b}	2.9^{b}	16.7	34.0^{a}

^{a,b}Different superscript letters in column indicate statistical significant difference ($P<0.05$)

Highlights

- Camelina showed high adaptability to conservation agriculture practices.
- Seed yield of 1.9 t ha^{-1} was obtained under reduced tillage and low fertilisation rate.
- High inputs (fertilisation and tillage) determined a 12-d longer crop cycle.
- α-linolenic, erucic and eicosenoic acids were affected by input levels.

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