Agronomic performance for biodiesel production potential of *Brassica carinata* A. Braun in Mediterranean marginal areas

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Abstract

Brassicaceae are promising oil feedstock for cultivation in central-southern Italy. Therefore, a two-year investigation on *Brassica carinata* A. Braun (cv. CT 204) was carried out in three sites of Apulia region [Site 1, Alberobello - Murgia foreland; Site 2, Troia (Foggia) - Daunian sub-Apennines; Site 3, Monteroni (Lecce) - Area of Salento], and in one site of Basilicata region (Site 4, Hill of Matera). The aim was to identify site-specific management practices [by comparing minimum *vs* conventional tillage, low sowing density *vs* high sowing density; different levels of nitrogen (N) supply and organic fertilisers] in the four different marginal areas, to achieve optimum yield performance for biodiesel prospective production. The crop showed a good adaptability in the study sites, and the highest N level positively influenced the yield performance in Sites 1, 2 and 3. Moreover, the reduction of mechanical operations (minimum tillage) did not negatively influence crop production and seed oil content. The highest density of sowing

tested determined the best crop performance in Site 3, particularly showing the maximum seed oil content with the lowest N supply. Finally, in Site 4 the compost mixed with mineral N fertiliser as well as the sewage sludge from urban wastewater determined productive results comparable to those obtained with mineral fertiliser, evidencing that organic fertilisers could (partially or completely) substitute the mineral one for this crop in the study site. On the whole, seed yield and oil content showed a potential for biodiesel production of *Brassica carinata* cultivated with site-specific agronomic techniques in four different marginal areas of Southern Italy, suggesting it can be likely achieved the crop environmental adaptation.

Introduction

The European Directive has established that 20% of energy consumption should be represented by renewable energy sources by 2020 in each European member state (Fischer *et al*., 2010). Potential bioenergy feedstocks include materials such as herbaceous crops and agricultural/forest residues, and estimates of land potentials for feedstock production range from 20 to 60 Mha for the EU25 by 2020 or 2030 (EEA, 2007). However, despite bioenergy crops may compete for land and resources with food crops, some species could be grown in marginal lands with beneficial agro-environmental effects (Zegada-Lizarazu *et al*., 2010). Therefore, choosing the appropriate species and management approaches should be site-specific.

Cosentino *et al*. (2008) reported that from the 1990s onward attention has been particularly focused on Brassicaceae for biodiesel production. This is the case of Ethiopian mustard (*Brassica carinata* A. Braun), a native plant of the Ethiopian highlands with high erucic and linolenic acids in the extracted oil (Cardone *et al*., 2003). The crop has shown better adaptability and productivity than *B. napus* under unfavourable environmental conditions and low cropping systems, for its flowering earliness, resistance to lodging, large seed size and good shattering resistance, as well as both drought and heat tolerance (Pan *et al*. 2012; Stamigna *et al*., 2012).

According to Cardone *et al*. (2003), *B. carinata* is a promising oil feedstock for cultivation particularly in central-southern Italy. Moreover, as it was demonstrated in recent studies, residues deriving from biodiesel chain based on *B. carinata* crop can have interesting energy content, and their exploitation can improve the energy balance of biodiesel production (Del Gatto *et al*., 2015; Duca *et al*., 2015).

However, there is a need to identify the key management practices, such as tillage, seeding rate and nitrogen (N) supply, for achieving optimum seed yield and quality (Pan *et al*., 2012). To the best of our

knowledge, there are no scientific evidences about the effectiveness of *B. carinata* adaptation particularly in marginal areas of southern Italy, for biofuel potential production, as a sustainable alternative to fossil fuel. Therefore, this two-year research was aimed to identify the *best management practices* to be used for crop cultivation, assessing the productive performance of *B. carinata* in four different marginal areas under Mediterranean conditions. The selected study sites had the following different causes of marginality and semi-marginality: i) soils with medium-high slope, which could determine crop yield reduction; ii) absence of irrigation water; and iii) climate variability. Therefore, the farming techniques tested on *B. carinata* in the four sites were site-specific and oriented to minimise inputs and maximise yields.

Materials and methods

Study sites

A two-year rotation of *B. carinata* (cv. CT 204) with different cereal crops was carried out in Southern Italy. Field experiments were conducted in three sites of Apulia region [*Site 1*, Alberobello - Murgia foreland; *Site 2*, Troia (Foggia) - Daunian sub-Apennines; *Site 3*, Monteroni (Lecce) - Area of Salento] and in one site of Basilicata region (*Site 4*, Hill of Matera). The four experimental trials were conducted during 2010-2011 and 2011-2012 cropping seasons. The climate of the experimental sites is *accentuated thermo-Mediterranean*, as classified by the United Nations Educational Scientific and Cultural Organization-Food and Agriculture Organization (UNESCO-FAO, 1963), with winter temperatures that can fall below 0°C and summer temperatures that can rise above 40°C, and rainfall unevenly distributed during the year and mainly concentrated in winter months. The main characteristics of the study sites and their climatic characterisation during the field trials are reported hereafter.

Site 1, Alberobello - Murgia foreland

At Site 1, the field trial was carried out by the Research Unit for Agriculture in Dry Environments, Council for Agricultural Research and Economics (SCA-CREA) of Bari in a hilly area, located at Alberobello (BA) (40° 45' 35" N, 17° 12' 21" E, 428 m a.s.l.), which is characterised by a soil layer of 15-40 cm depth, resting on cracked and often emerging rocks. According to the Soil Taxonomy of the United States Department of Agriculture (USDA), the soil is a Typical Palexeralf clayey-skeletal, characterised by 35% rock fragment (>2 mm) and 20% slope (Soil Survey Staff, 1999).

During the first crop cycle, the total rainfall was 815 mm (data not shown), which was considerably higher than the long-term (30-year period) value (519 mm), and the wettest months were October, March and May. Monthly values of mean temperatures showed minimum value in December 2010 (–2.4°C), and maximum (32.2°C) value in June. During 2011-2012, the total rainfall was slightly lower (779 mm; data not shown) than in the first one, and the wettest months were November and February. Minimum temperature values were detected both in mid-January (–1.02°C) and February (–1.16°C) and the maximum temperature (35.3°C) was observed in June.

Site 2, Troia (Foggia) - Daunian sub-Apennines

At Site 2, the field trial was carried out by the Institute of Sciences of Food Production, National Research Council (ISPA-CNR) of Bari at Troia (Foggia) (41° 20' 21" N, 15° 24' 00" E, 300 m a.s.l.), in a hilly area of the Daunian sub-Apennines with 20-30% slope, on a soil with a clay-loam texture and rich in skeleton.

During the first crop cycle, the total rainfall was 595 mm (data not shown), being considerably higher than in the long-term period (383 mm). The wettest months were October, November and March. The temperature from mid-December to mid-March was equal to or below 0°C, with a minimum value of –7.8°C in December. The highest temperature (35.9°C) was observed in June. In 2011-2012, the total rainfall (336 mm) was notably lower both than in the first year and in the long-term period, and the wettest months were February and April. The January-February period was characterised by very low temperatures and snowfall that produced a snowpack of 20-50 cm, covering the crop for about 10 days. The minimum temperature was below 0°C in 22 days, with a minimum of –7.6°C in February. The highest temperature (41.8°C) was observed, again, in June.

Site 3, Monteroni (Lecce) - Area of Salento

At Site 3, the field trial was carried out by the Research Unit for the Identification and Study of High Value Crops in Dry Environments, Council for Agricultural Research and Economics (CAR-CREA) in the experimental farm of S. Anna at Monteroni (Lecce) (40° 33' 20" N, 18° 10' 27" E, 35 m a.s.l.), on a deep sand loamy soil. During the first crop cycle, the total rainfall was 302 mm (data not shown), which was slightly higher than the long-term value (293 mm), and the wettest months were January and March. The lowest mean temperature was observed in January (8.1°C) and the highest one in July (25.9°C). In the second crop cycle, the total rainfall was 390 mm (data not shown), which was higher than the long-term value, with February and July as wettest months. The lowest temperature was observed in January (7°C) and the maximum temperature value occurred in July (28.1°C).

Site 4, Hill of Matera

At Site 4, the field trial was carried out by SCA-CREA (Experimental Farm of Metaponto) at Matera (41° 27' N, 3° 04' E, 250 m a.s.l.), on a cereal farm typical of the study area. According to Soil Taxonomy USDA the soil of the field is a Vertisol of alluvial origin, classified as Typic Chromoxerert, Fine, Mesic (Soil Survey Staff, 1999). The experimental field is located in a marginal area, characterised by soils with high degree of slope and rainfall unevenly distributed during the year. During the first crop cycle, the total rainfall was 509 mm (data not shown), and the wettest months were November and March. Monthly values of mean temperatures showed minimum value in February 2011 (2.1°C), whereas the maximum temperature (31.4°C) was observed in June. In the second crop cycle the total rainfall (350 mm) was notably lower than in the long-term period (619 mm), and the wettest months were February and April. Monthly values of mean temperatures showed minimum value in January 2012 (–0.1°C), whereas the maximum temperature (35.3°C) was observed in June.

Experimental setup, treatments and measurements in each study site

The main agronomic information regarding experimental design, experimental treatments and crop measurements are reported below, for the four study sites. In each site, the crop was harvested when the seed moisture content was about 9%. At harvesting, samples of crop seeds were analysed for oil content with the European conventional methodology.

Site 1, Alberobello - Murgia foreland

In the autumn of 2010 (on soil left to set aside), minimum tillage was performed with two disk harrowings before sowing (at 10 cm soil depth, after rainfall, and at 5 cm), whereas in the autumn of 2011 soil was tilled after oats harvesting. In pre-sowing, fertilisers were distributed at a rate of 100 kg ha^{-1} both as P_2O_5 (triple superphosphate) and K_2O (potassium sulphate).

The crop was sown, in rows spaced 60 cm, on 8th October 2010 and 5th October 2011. The experimental design was a randomised block with three replications and elementary plots of 75 m². The following levels of N fertilisation were compared: i) unfertilised control (N0); ii) 50 kg N ha⁻¹ (N50); and iii) 100 kg N ha⁻¹ (N100). Nitrogen fertiliser (ammonium sulphate) was applied at the beginning of the growth season, at the stage of 10th-12th leaf. The crop was harvested on 28th June 2011 and 2nd July 2012 and the following parameters were determined: seed yield (t ha⁻¹), 1000 seed weight (g), number of siliques plant⁻¹, number of seeds silique⁻¹, plant height (cm) and total dry biomass (t ha⁻¹).

Site 2, Troia (Foggia) - Daunian sub-Apennines

In a split-plot experimental design with three replications and elementary plots of 100 m², the following tillage systems (main plot factor) were compared: i) conventional tillage (CT) and ii) minimum tillage (MT). The CT treatment included mouldboard plowing (40 cm depth) in late August and disk harrowing both at 10 cm, after rainfall, and at 5 cm before sowing to prepare seedbed. The MT treatment consisted of disk harrowing (5 cm depth) before sowing, simultaneously to the last CT tillage. In the sub-plots, three N (urea) levels were compared: i) 0 kg N ha⁻¹ as control (N0), ii) 50 kg N ha⁻¹ (N50), and iii) 100 kg N ha⁻¹ (N100). The fertiliser was applied splitting the rate (in the N50 and N100 treatments) as 60% at mid-December and 40% at mid-January.

The crop was sown in rows spaced 15 cm, on 20th October 2010 and 21st October 2011. A sampling area of 80 m² was harvested on 28th June 2011 and on 4th July 2012, with a combine harvester, and the following parameters were determined: seed yield (t ha⁻¹), 1000 seed weight (g), number of siliques plant⁻¹, number of seeds silique⁻¹, plant height (cm) and total dry biomass (t ha⁻¹).

Site 3, Monteroni (Lecce) - Area of Salento

In a split-plot experimental design with three replications and elementary plots of 20 m², the following three levels of N fertilisation (ammonium sulphate 21%) were compared as main plot factor: i) 0 kg N ha⁻¹ as a control (N0); ii) 60 kg N ha⁻¹ (N60); and iii) 120 kg N ha⁻¹ (N120). Moreover, in the sub-plots, two sowing densities were tested: i) low density (L) with 50 plants m⁻²; and ii) high density (H) with 100 plants m⁻². Minimum soil tillage was performed with disk harrowings (at 10 and 5 cm depth), simultaneously to a pre-sowing fertilisation with 120 kg ha⁻¹ P₂O₅. The crop was sown on 4th January 2011 and 7th December 2011 (average seed weight: 5 g 1000 seeds⁻¹), by using a mini seeder. The harvesting was done on 11th July 2011 and on 1sh August 2012, and the following parameters were determined: seed yield (t ha⁻¹), 1000 seed weight (g), number of siliques plant⁻¹, number of seeds silique⁻¹, and plant height (cm).

Site 4, Hill of Matera

The experimental design was a split-plot with three replications and elementary plots of 9 m². The following two methods of soil tillage (main plot factor) were compared: i) conventional tillage (CT), which was carried out with ripper at 50 cm of soil depth; and ii) minimum tillage (MT), with clod-smasher at 20 cm of soil depth. Furthermore, two disk harrowings to prepare seedbed were performed both in CT and MT plots. Different fertilising treatments were tested within each soil tillage treatment: i) mineral N fertiliser (urea 46% N), at 100 kg N ha⁻¹ (N100); ii) organic fertilisation with compost [2% N; Progeva srl, Laterza (TA), Italy], obtained from municipal solid wastes, at 100 kg N ha⁻¹ (100COMP); iii) compost mixed with mineral N fertiliser (MIX) (*i.e.*, 50 kg N ha⁻¹ as compost plus 50 kg N ha⁻¹ as mineral fertiliser); iv) sewage sludge from urban wastewater (SSW) at 100 kg N ha⁻¹. These treatments were supplied in early autumn, 1 month before sowing, and they were compared with an unfertilised control (N0). As regards compost, the total organic carbon content (266 g kg⁻¹) exceed-

ed the minimum value of 200 g kg⁻¹ established by the Italian Legislative Decree no. 75/2010 (Italian Regulation, 2010). Moreover, the copper (Cu) (125 mg kg⁻¹), zinc (Zn) (269 mg kg⁻¹), nickel (Ni) (6.15 mg kg⁻¹), and lead (Pb) (33.8 mg kg⁻¹) values were all lower than the established limits (*i.e.*, 230, 500, 100 and 140 mg kg⁻¹, respectively). Similarly, the values of the heavy metals (*i.e.*, Cu 280 mg kg⁻¹; Zn 1074 mg kg⁻¹; Ni 20.7 mg kg⁻¹; Pb 79.3 mg kg⁻¹), as main potentially toxic elements in the sewage sludge, were lower than the maximum permissible limits for land application (1750 mg kg⁻¹; 4000 mg kg⁻¹; 400 mg kg⁻¹; 1200 mg kg⁻¹, for Cu, Zn, Ni, Pb, respectively) fixed by the Directive 86/278/EEC (European Commission, 1986). The crop was sown in mid-November and harvested in the second half of June in both trial years. At harvesting, the following parameters were determined: seed yield (t ha⁻¹), 1000 seed weight (g), number of siliques plant⁻¹, number of seeds silique⁻¹, plant height (cm), total dry biomass (t ha⁻¹).

Statistical analysis

Data were submitted to the analysis of variance, and the differences between means were further analysed with the Duncan's multiple range test (P<0.05). The statistical analysis was carried out by using the STAT software 2012, release 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Site 1

In 2010-2011, 1000 seeds weight, plant height and total biomass were lower than in the second one (Table 1). In the first cropping cycle the N100 treatment determined the highest mean value of crop yield, being higher by 29.2% and 56.6% than N50 and N0 treatments, respectively. By contrast, the total biomass after application of the highest dose of N was comparable to that in N0 treatment plots, whereas N50 determined the highest value, which was significantly higher by 23.5% than in N100. In the second crop cycle, N100 showed both the highest crop yield and biomass values. In particular, the yield in N100 was significantly higher by 28.8% and 55.3% than N50 and N0, respectively, and the total biomass was significantly higher by 20.2% and 37.1%, respectively. In Figure 1 the seed oil content (%), divided by year and N fertiliser dose, is reported. The N50 and N100 treatments determined
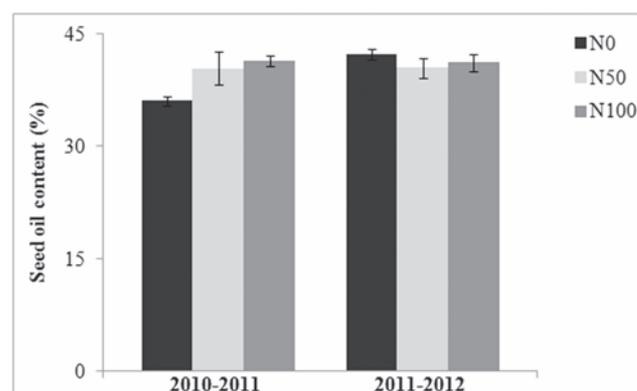
Figure 1. Seed oil content (%) in *Site 1* divided by year and nitrogen (N) fertiliser dose. Reported values are mean ± standard deviation.

comparable and significantly higher values than N0 in the first year, whereas in the second one there was an inverse trend even if the differences were observed in absolute value.

Site 2

The output of the analysis of variance revealed significant main effect of year for yield, 1000 seeds weight and siliques plant^{-1}. Main effect of tillage strategy was found for almost all parameters except siliques plant^{-1} and seeds silique^{-1}, whereas main effect of nitrogen level was found for all the parameters taken into consideration (Table 2). In addition, all the two-way interactions where not significant.

The N100 dose of fertiliser determined the highest values for all parameters, whereas the N50 determined intermediate values. In particular, crop yield was significantly higher in N100 by 84.6% and 33.3% than in N0 and N50, respectively.

In 2010-2011, no substantial differences were found for seed oil content among N fertiliser levels for each tillage strategy (Figure 2).

Moreover, no significant differences were found between CT and MT strategy. In 2011-2012, the highest oil content (38.1%) in absolute terms was determined by N0 in CT, whereas in MT the lowest value was found for N50 (34.9%). Again no significant differences were found between CT and MT strategies.

Site 3

The output of the analysis of variance revealed significant main effects of year except for 1000 seeds weight and seeds silique^{-1}, and except for 1000 seeds weight, seeds silique^{-1} and plant height as for density effect. No interactions between treatments were found (Table 3). The highest yield was found in the second cropping cycle (being higher by 18.0% than in the first one), which also showed the highest silique plant^{-1}. Among N levels, N120 determined yield significantly higher by 18.2% and 63.9% than N60 and N0 treatment, respectively, whereas it showed 1000 seeds weight comparable to N0 and significantly lower than in N60. The highest silique plant^{-1} and plant height values were found in

Table 1. Effects of year, and interactions with levels of N fertilisation, on crop performance at Site 1.

	Yield (t ha^{-1})	1000 seeds weight (g)	Silique plant^{-1} (n)	Seeds silique^{-1} (n)	Plant height (cm)	Total biomass (t ha^{-1})
Year						
2010-2011	1.42	5.2[b]	96.0	12.3	113b	2.52[b]
2011-2012	1.40	6.1a	96.3	11.2	129a	7.07[a]
	ns	**	ns	ns	*	*
Interactions (YxN)			**2010-2011**			
N level						
N0	1.13[c]	5.0	96.0	12.0	112	2.42[b]
N50	1.37[b]	5.6	79.1	11.8	108	2.84[a]
N100	1.77[a]	4.9	113	12.9	121	2.30[b]
Interactions (YxN)			**2011-2012**			
N level						
N0	1.12[c]	6.3	73.0	10.2	123	6.04[c]
N50	1.35[b]	6.0	102	11.3	132	6.89[b]
N100	1.74[a]	6.1	113	12.4	132	8.28[a]
	**	ns	ns	ns	ns	**

[a-c]The values in each column followed by a different letter are significantly different at P≤0.05 (Duncan). Significance of the F values: *, **Significant at the P<0.05 and 0.01, respectively; n.s., not significant. N0, 0 kg N ha^{-1}; N50, 50 kg N ha^{-1}; N100, 100 kg N ha^{-1}.

Table 2. Effects of treatments (tillage and levels of N fertilisation) on crop performance at Site 2.

	Yield (t ha^{-1})	1000 seeds weight (g)	Silique plant^{-1} (n)	Seeds silique^{-1} (n)	Plant height (cm)	Total biomass (t ha^{-1})
Year						
2010-2011	2.05	5.1	104	12.1	139	4.16
2011-2012	1.69	4.7	99.0	12.7	137	4.05
	*	*	*	ns	ns	ns
Tillage						
CT	1.68	4.7	101	12.4	136	3.76
MT	2.06	5.1	102	12.4	140	4.46
	*	*	ns	ns	*	*
N level						
N0	1.34[c]	4.3[c]	87.9[c]	11.8[c]	125[c]	2.89[c]
N50	1.83[b]	4.9[b]	106[b]	12.5[b]	141[b]	4.18[b]
N100	2.44[a]	5.5[a]	110[a]	13.0[a]	148[a]	5.27[a]
	**	**	**	*	**	**
Interactions	ns	ns	ns	ns	ns	ns

[a-c]Within treatments, the values in each column followed by a different letter are significantly different at P≤0.05 (Duncan). Significance of the F values: *, **, ***Significant at the P<0.05 and 0.01, respectively; n.s., not significant. CT, conventional tillage; MT, minimum tillage; N0, 0 kg N ha^{-1}; N50, 50 kg N ha^{-1}; N100, 100 kg N ha^{-1}.

N120, whereas this N dose determined seed silique^{-1} comparable to N60 and significantly higher by 7.3% compared to N0. As regards to crop density factor, the highest yield was determined by H treatment, being higher by 56.4% than using L strategy, despite a reduced number of silique plant^{-1}. In 2010-2011, in H plots, N60 treatment determined the highest oil content (33.7%), whereas in L plots N120 determined an oil content (34.2%) significantly higher than N60 (31.5%) (Figure 3). In 2011-2012, the highest oil content (45.5%) was determined by N0 in L, whereas the lowest one was found for N0 in H. Moreover, with H density, N60 treatment determined a value (37.8%) significantly higher both than N0 and N120, whereas with L density it produced the lowest value (35.7%).

Site 4

The output of the analysis of variance revealed significant main effect of year for all parameters. Main effect of tillage was found only for yield, whereas main effect of fertiliser was found for yield and total biomass. No interactions between treatments were found (Table 4).

The best crop performance was detected in the first year, showing the highest values of all tested parameters. As regards to tillage treatments, CT determined yield and total biomass significantly higher than

MT by 43.6% and 20.7%, respectively. Among fertiliser treatments, N100, MIX and SSW determined the highest yield values, whereas 100COMP and N0 showed the intermediate (and comparable to the previous three treatments) and the lowest results, respectively. A similar behaviour for N100, MIX and SSW was also observed for total biomass (but in this case with significant difference compared both to 100COMP and N0). SSW determined the highest absolute number of silique per plant, which was significantly higher by more than 100% than in N0, whereas the other fertilisers showed intermediate and comparable results among them. Finally, as for the plant height the N100 showed the highest absolute value, being statistically higher by 11% than in N0, but comparable to the other treatments.

Figure 4 reports seed oil content for each year of study, divided by N fertilisers, for each tillage strategy. In the first crop cycle, the CT strategy determined the highest absolute value combined with 100COMP (37.9%). The MT strategy determined higher oil content with N100 (37.7%) and SSW (38.2%), whereas 100COMP treatment showed the lowest in absolute value. In 2011-2012, in CT plots the highest oil content was found for MIX (43.9%) and the lowest one for SSW (41.4%), whereas in MT the highest value was determined by SSW (43.7%) and the lowest by N100 in absolute value (38.1%).

Table 3. Effects of treatments (N fertilisation levels and sowing density) on crop performance at Site 3.

	Yield (t ha^{-1})	1000 seeds weight (g)	Silique plant^{-1} (n)	Seeds silique^{-1} (n)	Plant height (cm)
Year					
2010-2011	1.39[b]	4.9	32.3[b]	14.3	148[a]
2011-2012	1.64[a]	5.0	36.1[a]	14.2	133[b]
	**	ns	**	ns	***
N level					
N0	1.11[c]	4.8[b]	29.3[c]	13.7[b]	130[c]
N60	1.54[b]	5.1[a]	34.4[b]	14.8[a]	145[b]
N120	1.82[a]	4.9[b]	38.1[a]	14.7[a]	152[a]
	***	*	***	*	***
Sowing density					
L	1.17[b]	5.0	39.4[a]	14.8	141
H	1.83[a]	4.9	28.5[b]	14.1	144
	***	ns	***	ns	ns
Interactions	ns	ns	ns	ns	ns

$^{a-c}$Within treatments, the values in each column followed by a different letter are significantly different at P≤0.05 (Duncan). Significance of the F values: *, **, ***Significant at the P<0.05, 0.01 and 0.001, respectively; n.s., not significant. N0, 0 kg N ha^{-1}; N60, 60 kg N ha^{-1}; N120, 120 kg N ha^{-1}; L, low density, with 50 plants m^2; H, high density, with 100 plants m^2.

A

B
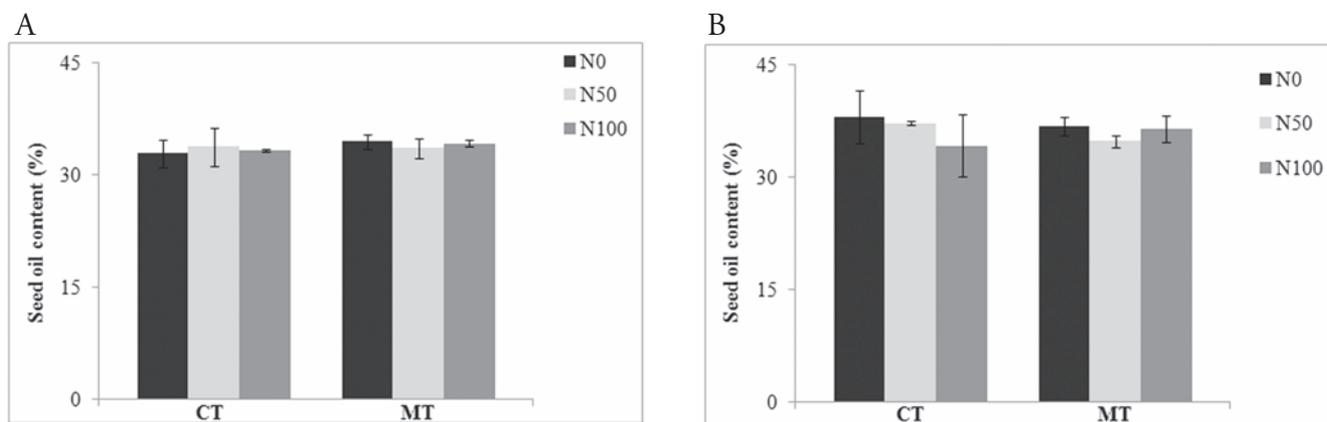
Figure 2. Seed oil content (%) in *Site 2* in (A) 2010-2011 and (B) 2011-2012, divided by nitrogen (N) fertiliser level for each tillage strategy. Reported values are mean ± standard deviation.

Table 4 Effects of treatments (tillage and fertilisers) on crop performance at Site 4.

	Yield (t ha^{-1})	1000 seeds weight (g)	Silique plant^{-1} (n)	Plant height (n)	Total biomass (t ha^{-1})
Year					
2010-2011	2.40[a]	4.6[a]	230[a]	155[a]	10.60[a]
2011-2012	0.84[b]	3.8[b]	87[b]	137[b]	6.61[b]
	***	***	***	***	***
Tillage					
CT	1.91[a]	4.2	172	148	9.41[a]
MT	1.33[b]	4.3	145	144	7.79[b]
	*	ns	ns	ns	ns
Fertilisers					
N100	1.84[a]	4.2	180[ab]	151[a]	9.25[a]
100COMP	1.48[ab]	4.4	156[ab]	147[ab]	7.56[b]
MIX	1.74[a]	4.2	160[ab]	146[ab]	9.33[a]
SSW	1.85[a]	4.3	201[a]	148[ab]	9.94[a]
N0	1.18[b]	4.2	97[b]	136[b]	6.90[b]
	*	ns	ns	ns	***
Interactions	ns	ns	ns	ns	ns

[a-c]Within treatments, the values in each column followed by a different letter are significantly different at P≤0.05 (Duncan). Significance of the F values: *, **, ***Significant at the P<0.05 and 0.001, respectively; n.s., non significant. CT, conventional tillage; MT, minimum tillage; N100, mineral N fertiliser at 100 kg N ha^{-1}; 100COMP, compost at 100 kg N ha^{-1}; MIX, compost mixed with mineral N fertiliser; SSW, sewage sludge from urban wastewater; N0, unfertilised control.
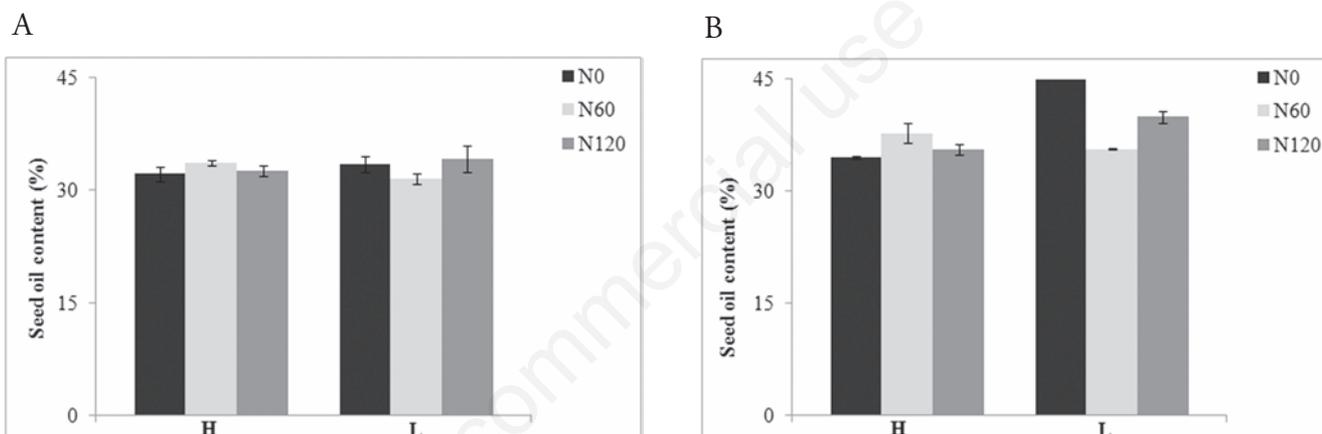
Figure 3. Seed oil content (%) in *Site 3* in (A) 2010-2011 and (B) 2011-2012, divided by nitrogen (N) fertiliser level for each sowing strategy. Reported values are mean ± standard deviation.
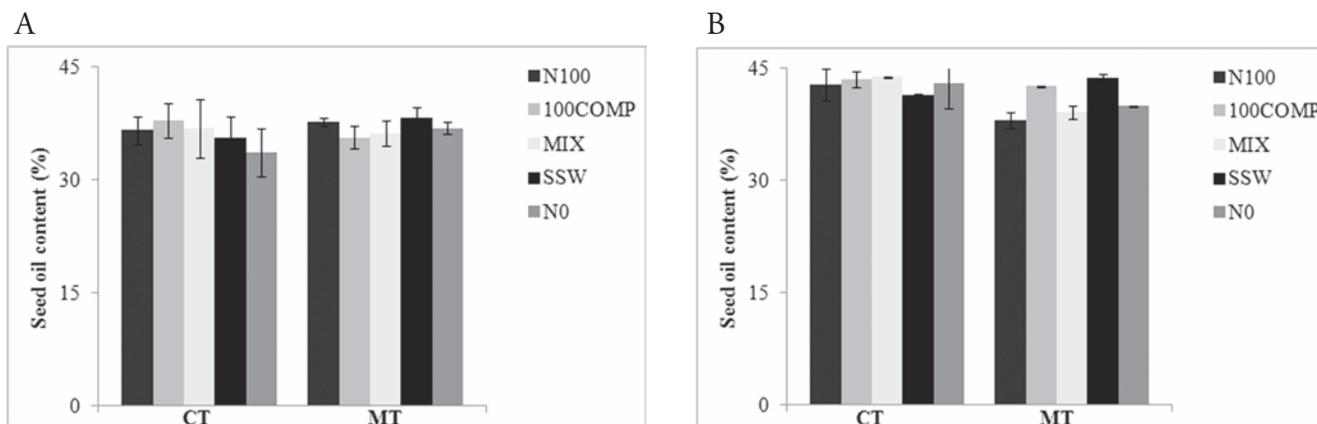
Figure 4. Seed oil content (%) in *Site 4* in (A) 2010-2011 and (B) 2011-2012, divided by nitrogen (N) fertilisers for each tillage strategy. Reported values are mean ± standard deviation.

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Discussion

In Site 1, the crop showed a good adaptability to this semi-marginal area and the highest nitrogen fertilisation (N100) positively influenced the yield, irrespective of the seasonal differences in the weather parameters. This N rate falls within the range indicated by Gan *et al*. (2007) who has shown that the amount of N fertiliser required for seed yields to plateau in various Brassica species ranged from about 100 to 160 kg N ha^{-1}, with the highest values reported for *B. juncea*. By contrast, other studies indicated that a rate was not identified in which *B. carinata* yields were maximised (Johnson *et al*., 2013). As regards to seed oil obtained for biodiesel potential production, in the first year (more rainy than the long-term period and milder than the second year) the N fertilisation positively influenced seed oil content. The oil content values were comparable with those observed in other studies for the same crop (Kaur and Sidhu, 2004; Johnson *et al*., 2013).

Among the four study sites, the best yield results were obtained by MT and N100 treatments in the Site 2. To define sustainable bioenergy chain, as well as to reduce the use of energy during the crop cultivation phase, it is fundamental the application of low external input cultivation systems. The tillage strategies in Site 2 were comparable between them, similarly to another study that found as the reduction of mechanical operations did not negatively influence *B. carinata* production and seed oil content (Lazzeri *et al*., 2009). This result would confirm the possibility of reducing production costs and environmental impacts for this crop. Despite the notable variability in weather conditions between the two trial years, it did not cause significant differences for half of tested parameters (seeds silique^{-1}, plant height and total biomass) in this site. Again, the N100 dose produced the best crop yield and oil production, confirming findings of Pramanik *et al*. (1996) who noted that the growth, seed and oil yields of the crop significantly improved with increasing N rates up to 100 kg N ha^{-1}, in different environmental conditions.

In Site 3, the most favourable weather conditions in the second year determined the highest production, which increased with the N level. This positive yield response to N rates was also reported by other authors for this crop in different environment (Kaur and Sidhu, 2004). Moreover, the best yield performance was found applying the high-density strategy, which likely hastens maturity and reduces the risk of crop failure. By contrast, Pan *et al*. (2012) reported that the maximum seed yield was produced in a wide range of plant population from 34 plants m^{-2} to 114 plants m^{-2}. As regards to seed oil percentage, in both years the N60 level reached the highest values with H strategy. Conversely, N120 determined better results with L than with H strategy. These outcomes would suggest that with high density of sowing it is possible/necessary to reduce the N fertilisation to obtain good results for oil. Finally, the obtained findings in Site 4 indicate the possibility to recycle, for *B. carinata* cultivation, both municipal solid waste and sewage sludge of urban wastewater, which are wastes so abundant in Mediterranean regions. These wastes present notable contents of organic matter and substantial quantities of nutrients, whereas main potential toxic elements (heavy metals) could be lower than the maximum permissible limits for land application fixed by current legislation, as in the organic materials used in our study. Therefore, their use in agriculture can contribute to closing the natural ecological cycles, without adversely affecting the environment (Diacono and Montemurro, 2011). In particular, MIX and SSW treatments determined productive results comparable to those obtained with mineral fertiliser. Therefore, organic fertilisers could (partially or completely) substitute the mineral fertiliser, in accordance to Montemurro *et al*. (2006) findings on other crops. In MT plots, the SSW also determined the highest oil content of seeds, whereas in CT there was more variability between years in the fertiliser effect on this parameter. This last result indicates (as in Site 1) that the agronomic management did not overcome the limit imposed by the genetic control of oil content parameter, as it was found also in other crops (Montemurro and De Giorgio, 2005). More specifically, this would confirm Zanetti *et al*. (2009) findings on *B. carinata*, indicating that the level of production inputs did not modify oil percentage, thus suggesting that this trait is more closely related to variety and to year. By contrast, Torabi *et al*. (2008) found that the tillage method significantly affected *B. napus* L. yield and oil content.

Conclusions

Information on proper agronomic strategies for energy crops is necessary to optimise their production, thus offering new perspectives to diversify farming activities and also cultivate marginal areas.

B. carinata is an oil-bearing crop that is characterised by rusticity and adaptability to different environmental conditions. In our study, among the tested agronomic practices, the positive influence on the crop productive results of high N fertilisation, high density of sowing, and organic fertilisers application was detected. In particular, it was showed that the reduction of mechanical operations did not negatively influence *B. carinata* production and seed oil content. Our results also suggest that with high density of sowing it is possible to reduce the N fertilisation, to obtain positive oil yield response. Therefore, considering seed yield and oil content, *B. carinata* could have a potential for biodiesel production in the study sites.

On the whole, in the four Mediterranean study sites of this research, the crop seemed to be self-adapted to specific agro-pedo-climatic conditions of each site, thus avoiding competition for land and resources with food crops in not-marginal areas. However, the main perspectives of the research regard the evaluation of the environmental impact of cultivation in other different marginal areas and alternative cropping systems.

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