

# Adaptability and Sustainable Management of High-Erucic *Brassicaceae* in Mediterranean Environment

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## 1. Introduction

The use of high-erucic acid oils is currently receiving increasing attention, due to the great interest in chemical compounds derived from “green feedstock”. At world level, the production of these raw materials is constantly growing, and a real niche market has progressively been created (Mosca & Boatto, 1994). This scenario will allow greater substitution of chemicals with “green” compounds, and the introduction of industrial oil-crops may lead to further expansion of the green market. Alternative uses of crops for non-food purposes may be an interesting source of profit for farmers, as is happening for high-erucic acid oils. The current demand for these oils is still limited: at world level, it is nearly 20,000 tonnes of erucic acid, corresponding to about 57,000 tonnes of oils, used for deriving erucamide and various others chemical compounds (Figure 1).

Erucic acid is an unsaturated fatty acid (C22:1) with a large number of applications in the chemical industry because it confers desirable technological characteristics, such as high lubricity, cold stability and fire resistance, on oils and derived compounds.

Erucic acid is mainly transformed into erucamide, a slip agent for plastic film production. There are also other important derived compounds, such as behenic, brassilic and pelargonic acids, obtained through chemical reactions (e.g., hydrogenation, ozonolysis), which have interesting and innovative uses in the manufacture of chemicals, lubricants and detergents (Cardone et al., 2003; Gunstone & Hamilton, 2001; Taylor et al., 2010).

The actual world need for seeds containing erucic acid is not very large, about 100,000-120,000 tonnes, but the positive trend observed in the last few years should allow significant extension of these cultivations. This implies that studies on the adaptation of species containing erucic acid in various environments are really essential.

*Brassicaceae* are the most interesting botanical Family for producing erucic acid, due to the large number of suitable species and varieties, providing on their own the whole amount of erucic needed worldwide. The content of erucic acid ranges greatly, with high inter- and intra-specific variations (Table 1): within the same species, variations may be very large,

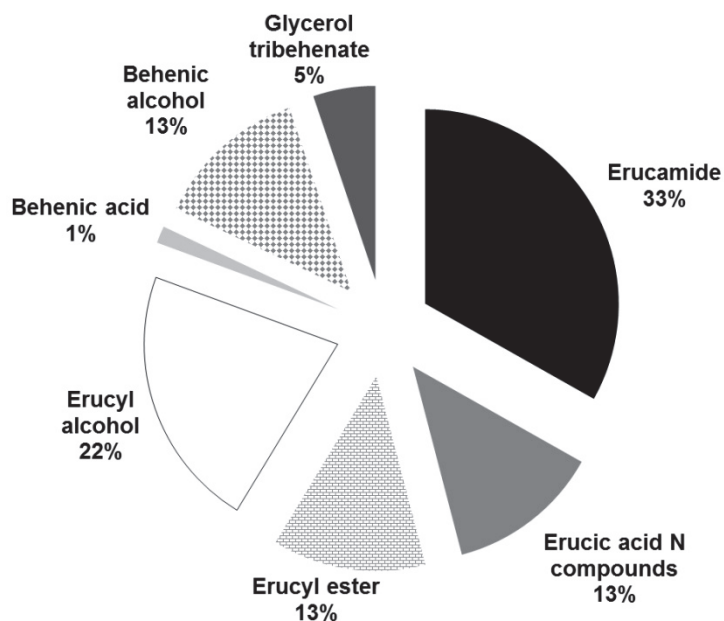


Fig. 1. World production of chemical compounds derived from erucic acid, expressed as percentages of total amount (Source: Gunstone & Hamilton, 2001).

Species	Oil (%)	Erucic acid (%)
<i>Brassica carinata</i> A. Braun	37–51	35–48
<i>Brassica juncea</i> (L.) Czern	35–45	18–49
<i>Brassica napus</i> L. var. <i>oleifera</i> Metzger (HEAR)	35–45	45–54
<i>Crambe abyssinica</i> Hochst ex R.E. Fries	30–35	55–60
<i>Eruca sativa</i> Mill.	28–30	34–47
<i>Sinapis alba</i> L.	25–30	33–51

Table 1. Variations in oil and erucic acid contents in various *Brassicaceae* species (Source: Mosca, 1998).

even higher than 30%, as for *Brassica juncea*. This probably indicates that efficiency in erucic acid accumulation may be greatly improved by adequate screening of genotypes, but also that important genetic resources may be used for breeding purposes. In this regard, an amount of 66% of erucic acid in oil must be considered, at least in rapeseed, as the current theoretical limit of accumulation (Renard et al., 1994).

For some of these species (e.g., *Brassica napus* var. *oleifera*, *Crambe abyssinica*) yield potential and environmental adaptation have been sufficiently studied in southern Europe (Lazzeri et al., 2009; Zanetti et al., 2006 a), although there is lack of information on some others, such as *Brassica juncea* and *B. carinata*.

## 1.1 Species of interest

In this review, four *Brassicaceae* species are considered as the most promising for producing erucic acid, i.e., *Brassica napus* var. *oleifera*, *Crambe abyssinica*, *Brassica juncea* and *B. carinata*. Although *B. napus* var. *oleifera* and *Crambe abyssinica* are the most frequently cultivated crops for producing erucic acid, because of their high and stable yields, the introduction of new species may be an opportunity to satisfy further future needs and perhaps to extend the cultivation basin of erucic acid.

The genotypes of winter *Brassica napus* var. *oleifera* are commonly defined HEAR (High Erucic Acid Rapeseed). HEAR – among the sources of erucic acid – is the most widely cultivated at world level (~30,000 ha in the world, 20,000 of which only in the UK) (Meakin, 2007). HEAR is characterised by an elevated content of erucic acid in oil, commonly over 48% (Meakin, 2007). This species can constantly provide high seed yields (De Mastro & Bona, 1998) – about 3.5–4.5 t DM ha<sup>-1</sup> with 35–45% of oil – although new recently released hybrids may even reach 50% of oil (Zanetti et al., 2009). The most valuable characteristic of winter HEAR is high tolerance to low temperatures, down to -13°C (Auld et al., 1984), thus allowing early autumn sowing, long crop cycle and potentially high seed yields, even in northern Italy. With respect to the other species considered in this review, HEAR has undergone massive breeding, and the market has now made available not only open pollinated varieties but also some new CHH (Composite Hybrid Hybrid) hybrids which may reach greater yields, especially under high input management (Zanetti et al., 2006 b).

*Crambe abyssinica* (crambe) is one of the species richest in erucic acid (~55% of oil), but its limited cold resistance only allows almost exclusively spring sowing in north Italy and higher latitudes, with lower yield potentials (Zanetti et al., 2008). This crop may be an interesting source of erucic acid only in those environments where autumn or early spring sowings are possible. In locations with mild winters, some drawbacks may also be encountered, a marked reduction in yield being possible in cases of sudden cold spells late in the season.

Compared with the other considered species, crambe has some morpho-physiological peculiarities, such as seeds enclosed in hulls (Figure 2) – they normally remain on the seed after harvest – smaller plants, and lower oil content (~35%).

Compared with the above-cited high-erucic species, *Brassica juncea* (Indian mustard) owns peculiar morphological traits, such as taller plants, smaller seeds and elongated leaves (Figure 3). Its cultivation is widespread, especially in its native geographic area (India and Pakistan), due to some interesting agronomic characteristics (e.g., drought resistance) and the low incidence of pod shattering, which can significantly reduce yield losses. Unluckily, its resistance to cold, which is extremely differentiated among varieties, is generally quite low, although some genotypes tested can tolerate down to -5°C and provide interesting yield results. At this time in Europe there are no available commercial varieties of Indian mustard, since its cultivation is widespread only in native regions. Great interest in this species in last few decades has emerged in Canada, due to its better adaptability to spring sowing in dry environments with respect to conventional canola (Getinet et al., 1996).

Among new *Brassicaceae* suitable for cultivation in southern Europe, *Brassica carinata* (Ethiopian mustard) (Figure 4) may be considered the most promising, because it provides good seed yield (2.5–3.6 t ha<sup>-1</sup>) and has several favourable characteristics, such as low bird predation and good tolerance to pests, diseases (i.e., blackleg and *Alternaria* leaf spot)



Fig. 2. Seeds of *Crambe abyssinica*, enclosed in hulls, at seed filling stage.



Fig. 3. Experimental plot with *Brassica juncea* in spring sowing (University of Padova experimental farm).



Fig. 4. *Brassica carinata* plants at flowering, characterised by simultaneous presence of white and yellow flowers.

(Bayeh & Gebre Medhin, 1992; Gugel et al., 1990; Yitbarek, 1992) and drought (Monti et al., 2009). This species has been studied since the early 1980's, showing interesting performances in semi-arid temperate climates (Fereris et al., 1983; Hiruy et al., 1983). Nevertheless, its poor tolerance to cold should be carefully taken into account in order to choose the more appropriate sowing time, especially at northern latitudes, where the possibility of early frost but also of temperature fluctuations (below and above 0°C) can significantly compromise crop success (Lazzeri et al., 2009).

Within this framework, in order to achieve high and stable production of erucic acid, it is essential to identify the most productive genotypes, among available species, for each environment. In this report, seed and oil productions of some high-erucic *Brassicaceae* species, derived from field tests set in northern Italy, are presented in response to different agronomic input management. In particular, responses of *B. napus* HEAR, *B. carinata* and *Crambe abyssinica* are discussed in relation to their results obtained from multi-scale trials (years and locations).

## 2. Materials and methods

In this review, the results from three separate field trials, set in different years and locations, are reported and discussed. Accordingly, the three experiments are described separately.

### 2.1 HEAR adaptation to reducing agricultural inputs

The trial was carried out for 2 years during growing seasons 2006-07 and 2007-08 (autumn sowing) in Legnaro (45°21'N, 11°58'E, NE Italy) at the experimental farm of the University

of Padova, following a split-plot experimental design with 3 replicates. Aiming at mimicking farm-scale cultivation as closely as possible, plot size was set at around 400 m<sup>2</sup> surface area and agricultural practices were applied by means of farm-scale technologies. Three commercial genotypes of *B. napus* HEAR – two open pollinated varieties: Maplus (NPZ Lembke, Germany) and Hearty (Monsanto, France), and one CHH (Composite Hybrid Hybrid) Marcant (NPZ Lembke, Germany) – were sown on 27 and 28 September respectively in 2006 and 2007 in a silty-loam soil (pH 8.4; 2.5% organic matter; 0.1% total N) by a cereal seeder. The seed dose was 4.5 kg ha<sup>-1</sup>, roughly corresponding to a density of 100 seeds per m<sup>2</sup> for all varieties. Two contrasting levels of inputs, high and low, were tested, involving soil tillage, fertilisation and weed management (Table 2).

	High input	Low input
<b>Soil tillage</b>	Ploughing to 0.35 m depth + grubbing + harrowing	Disc-harrowing + rotary tillage
<b>Weed management</b>	Metazachlor 1,250 g ha <sup>-1</sup> (pre-emergence)	Mechanical weeding (one autumn passage)
<b>Inter-row distance</b>	0.30 m	0.45 m
<b>Pre-sowing fertilisation (kg ha<sup>-1</sup>)</b>	N = 30 P <sub>2</sub> O <sub>5</sub> = 90 K <sub>2</sub> O = 90	N = 0 P <sub>2</sub> O <sub>5</sub> = 50 K <sub>2</sub> O = 50

Table 2. Characteristics of two contrasting agricultural managements for HEAR rapeseed.

For proper mechanical weed control, the inter-row distance was increased from 0.3 to 0.45 m in the low input regime. After pre-sowing fertilisation (30 kg ha<sup>-1</sup> N, for high input only), spring nitrogen supply was calculated following the method proposed by CETIOM (Centre Technique Interprofessionnel des Oléagineux Métropolitains, 1998), called “Réglette Azote”. In brief, it recommends increasing doses of N for rising potential yields (i.e., revealed by higher shoot biomass at the end of winter); for the same value of expected yield, the lower the shoot biomass, the higher the amount of N. According to this approach, about at the end of January, the shoot biomass of 1 m<sup>2</sup> for each plot (2 replicates per plot) was sampled and weighed (fresh green matter). Biomass values were related to potential yields of 3 and 4 t ha<sup>-1</sup>, chosen respectively for low and high input managements, to calculate the N dose. The resulting amount of N, applied as ammonium sulphate in mid-February, ranged between 0-20 kg ha<sup>-1</sup> in the first year and 0-60 kg ha<sup>-1</sup> in the second for low input, and between 35-50 kg ha<sup>-1</sup> (first year) and 80-130 kg ha<sup>-1</sup> (second year) for high input, depending on genotype (Table 3).

Genotype	Input level	2006-07	2007-08
		N (kg ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )
Maplus	High	50	130
	Low	0	60
Hearty	High	50	110
	Low	20	40
Marcant	High	35	80
	Low	0	0

Table 3. Amount of spring nitrogen in two years of trial, calculated by following “Réglette Azote” method: experimental site was considered to have deep soil rich in organic matter.



During stem elongation and flowering stages, at about 7-day intervals, the N status of the crop was checked by determining the shoot nitrogen content (Kjeldahl method). Harvesting took place about in mid-June in 2007 (Hearty and Marcant June 5, Maplus June 11), and later in 2008 ( June 19, all genotypes), by a combine harvester equipped with a wheat-cutting bar. Seed oil content was measured by the Soxtec-Tecator equipment (FOSS Analytical, Höganäs, Sweden) on 2 g of milled seeds, with diethyl ether as solvent.

The profiles of fatty acids were determined on methylated oil (preparation of methyl esters according to International Standard ISO 5509) (Bondioli & Della Bella, 2002) by gas-chromatography (GC 1000, DANI Instruments, Milan - Italy). Helium was used as carrier gas and a capillary column (SUPELCO SP<sup>TM</sup>-2380, 30 m long, 0.25 mm outer diameter, 0.20 µm film) to separate fatty acids. The oven was set at an initial temperature of 180°C and maintained for 5 min; later, 240°C was reached by progressive increases (+5°C per minute) and maintained for 8 min, so that each analysis lasted 25 min. The gas chromatograph had a split/splitless (SL/IN) injector (250°C) with a split rate of 100:1 and a flame ionization detector (FID) (275°C).

## 2.2 *Brassica carinata* response to N fertilization in multi-locality trials set in NE-Italy

In order to test the possible introduction of *B. carinata* in NE Italy, extensive field trials were set during 2006-2009 in two different locations, both in the Veneto region (NE Italy). The two sites were Legnaro (45°21'N, 11°58'E), at the University of Padova experimental farm, and Rosolina (45°04'N, 12°14'E), at the experimental research center of the Veneto region called "Pò di Tramontana". The sites were only 35 km apart, but they were characterised by different soil type, climatic conditions, and agronomic management (Tab. 4). The N fertilisation strategy was differently applied in the two sites; in the soil at Rosolina,

Localities	LEGNARO	ROSOLINA
Years	2006-2008	2007-2009
Soil type	Silt-loam	Sandy-loam
Mean annual temperature	10.2°C	14.2°C
Mean annual precipitation	820 mm	639 mm
Soil tillage	Subsoiling or cultivator + disk harrowing + rotary harrowing	Spading + rotary harrowing
Pre-sowing fertilisation (kg ha <sup>-1</sup> )	30-90-90 N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O	30-90-90 N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O
Sowing date	End of September	End of September
Inter-row distance	0.45 m	0.45 m
Weed management	Mechanical	Chemical
Sowing density	4.5 kg	4.5 kg
Spring N fertilisation (kg ha <sup>-1</sup> )	3 doses: 0 N 100N Reglette dose	2 doses: Low: 70÷100 N High: 100÷140 N
Harvesting date	Mid-June	Mid-June

Table 4. Most important protocol details of experiments in Legnaro and Rosolina with *Brassica carinata*.

characterised by a medium content of OM (1.7%), three fixed not limiting N (70, 100, 140 kg ha<sup>-1</sup> N) doses were compared. In Legnaro, the three N doses tested were: i) unfertilised control (0N), ii) a high dose (100 N), and iii) an intermediate dose (*Reglette*).

The aim of the trials was to assess the adaptability of *Brassica carinata* to extensive cultivation in NE Italy. For reliable results, the trials were set on a field scale (~1 ha, each plot) adopting farm-scale technologies. The experiment lasted two years (2006-08 in Legnaro, 2007-09 in Rosolina) and the varieties BRK1 (Eurogen, Italy) and ISCI 7 (Triumph, Italy) were cultivated in Legnaro and Rosolina, respectively.

After harvest, seed yield, oil and protein were revealed and compared between cultivars and localities.

### 2.3 Introduction of newly released genotypes of *Crambe abyssinica* in southern European environment

The aim of the study was to test the possibility of introducing *Crambe abyssinica* as a new spring crop for large-scale erucic acid production in north Italy. The experiment was carried out at Legnaro, following a completely randomised block design, with 3 replicates. For a 2-year period (2006 and 2007) three commercial varieties of *Crambe*, i.e., Mario (Triumph, Italy), and Nebula and Galactica (Springdale Farm, UK) were compared in large plots (~1,000 m<sup>2</sup>). In both years, sowing took place in early spring (March 30 2006, March 15 2007) and harvesting at the beginning of summer (July 5 2006, June 30 2007). Only limited amounts of fertilisers were applied just before sowing (30, 90 and 90 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively). Inter-row distance was 0.23 m and the amount of seeds 18 kg ha<sup>-1</sup>. This high seed dose was necessary to compensate the lower germination rate of available seeds (Lazzeri, 1998). There was no need to control weeds or pests during crop cycle.

In 2008, when Galactica and Nebula were no longer available on the market, an experiment was set up to assess the most suitable sowing date. The cv. Mario was cultivated in big strips (~3,500 m<sup>2</sup>) at two sowing times: late March (March 20) (suggested as optimal) and about the end of April (April 28) (late sowing), adopting the same seed density (15 kg ha<sup>-1</sup>), and tillage system and fertilisation (30, 90 and 90 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively). Harvest was on July 3 for optimal sowing time and three weeks later (July 24) for delayed sowing.

In both experiments seed yield, oil and seed weight were evaluated at the end of the crop cycle for all tested genotypes.

## 3. Results and discussion

### 3.1 HEAR adaptation to reducing agricultural inputs

HEAR genotypes of *Brassica napus* turned out to be suitable for agricultural input reduction. Reduced tillage may conveniently be adopted for these new cultivars, characterised by high, fast germination. In addition, the great vitality, vigour and rapid growth re-start after winter also compensate possible reduced plant density after severe frosts. These positive traits are even more evident in hybrids, which have more vigour than open pollinated varieties. The aim of this study was to compare the adaptability of different genetic types of HEAR, open pollinated varieties vs. hybrids, in response to low input management.



The weather conditions of the two experimental seasons did not greatly differ, presenting almost the same amount of precipitation (~550 mm, during crop cycle) and similar temperatures. The only appreciable difference regarded the distribution of rain during crop cycle: the first year (2006/07) was characterised by severe drought stress during the blooming period, with only 5 mm rain in April; after two days of abundant rain at the beginning of May, the rest of the cycle was characterised by the absence of precipitation, leading to anticipated maturity, nearly two weeks with respect to the common harvest time for that environment. Instead, the second year was characterised by appropriate rain distribution over crop cycle, a fact that did not lead to any final yield increase, which was unexpectedly significantly higher in the first year ( $P < 0.05$ , data not shown). Anticipation of phenological phases in the first year allowed the crop to accumulate higher and earlier aboveground biomass, probably the reason for the higher yield performance.

Analysing the effects of input reduction on main productive parameters (i.e., seed yield, oil and protein content) it emerged that cost reduction may be extremely profitable, especially if the best genotype is chosen correctly. The application of low input regimes did not lead to any significant yield reduction ( $P < 0.05$ ) nor of oil content (Table 5), although seed proteins were reduced, probably because of lower N fertilisation. Considering seed yield, Hearty and Marcant showed the same values under high and low inputs (3.54 vs. 3.57 t DM ha<sup>-1</sup>, high and low, respectively, for Hearty; 3.38 t DM ha<sup>-1</sup> in both regimes for Marcant); whereas in Maplus a limited, but not significant, yield increase in response to high input was measured (3.48 vs. 3.36 t DM ha<sup>-1</sup>, for high and low input, respectively).

	Yield (t DM ha <sup>-1</sup> )			Oil content (% DM)			Protein content (% DM)		
	H	L	Mean	H	L	Mean	H	L	Mean
<b>Maplus</b>	3.48	3.36	3.42	46.1 b	47.9 a	47.0	23.8 a	22.2 b	23.0 a
<b>Hearty</b>	3.54	3.57	3.56	47.1 ab	46.8 ab	47.0	22.2 b	22.0 b	22.1 b
<b>Marcant</b>	3.38	3.38	3.38	47.0 ab	47.7 ab	47.3	22.7 ab	21.9 b	22.3 ab
<b>Mean</b>	3.47	3.44		46.7	47.5		22.9 a	22.1 b	

Table 5. Performance of HEAR genotypes at Legnaro (2-year means), under high (H) and low (L) input managements. Letters: statistically significant differences for multiple comparisons ('genotype × input level' interaction). Bold letters: significant differences among grand means for each variable (main effects: input level and genotype) ( $P < 0.05$ , Duncan's test).

As expected, seed oil content was even more stable than seed yield, in response to intensified management but our genotypes were able to reach very high values (47.1% DM, general mean) compared with older varieties. Only Maplus appeared sensitive to input intensification, reaching significantly higher seed yield when high inputs were applied ( $P < 0.05$ ). Instead, seed protein content benefited by intensification (Tab. 5) (high vs. low input: 22.9 vs. 22.1%,  $P < 0.05$ ), due to higher N availability, as widely reported in the literature for rapeseed and other crops (Zhao-Hui et al., 2008).

One of the most important qualitative traits of HEAR production - fatty acid profile of extracted oils - appeared to be mainly under genetic control rather than influenced by agronomic management. The fraction of erucic acid in oils was only slightly higher in low input conditions (49.3 vs. 48.9% DM, low vs. high input, respectively), but differences were not statically significant (Figure 5).

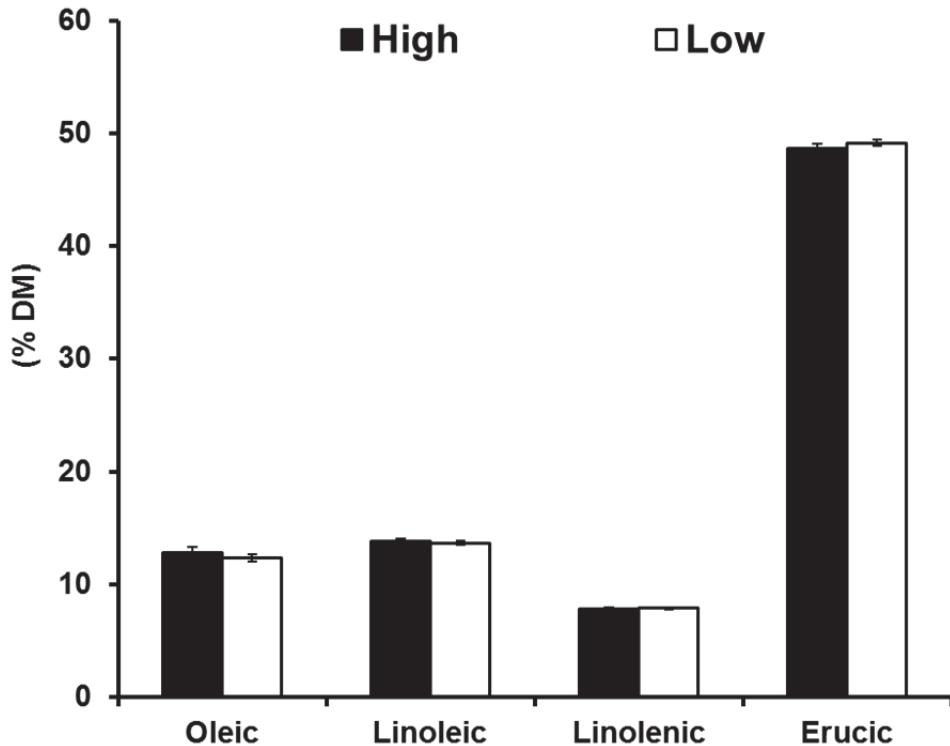


Fig. 5. Oil composition as % of main fatty acids in HEAR rapeseed cultivated under high and low input management (mean of three varieties). Vertical bars: standard error.

Analysing the oil composition of each cultivar separately (main effect genotype) (Figure 6) some significant differences emerged for oleic, linoleic and erucic acids. Anyway, the mean erucic acid content in our study was very high, always exceeding 47%. Among genotypes, Hearty was the richest and Maplus the poorest ( $P < 0.05$ ) (Figure 6).

It was also particularly interesting to see how Hearty oil composition was completely indifferent to input application, without any significant changes for the fatty acids in question (i.e., oleic, linoleic, linolenic and erucic acids; data not shown). In the hybrid Marcant, oil composition was found particularly interesting because of its high erucic and oleic contents and lower degree of PUFA (Poly Unsaturated Fatty Acids), normally related to higher oil stability (Zanetti et al., 2009). This peculiar characteristic of Marcant may be favourably exploited by the oil extraction industries which normally look for this kind of oil – stable and very rich in erucic acid.

We conclude that input reduction may be recommended for HEAR rapeseed without compromising yield, occasionally allowing the seed oil percentage and erucic acid accumulation to increase.

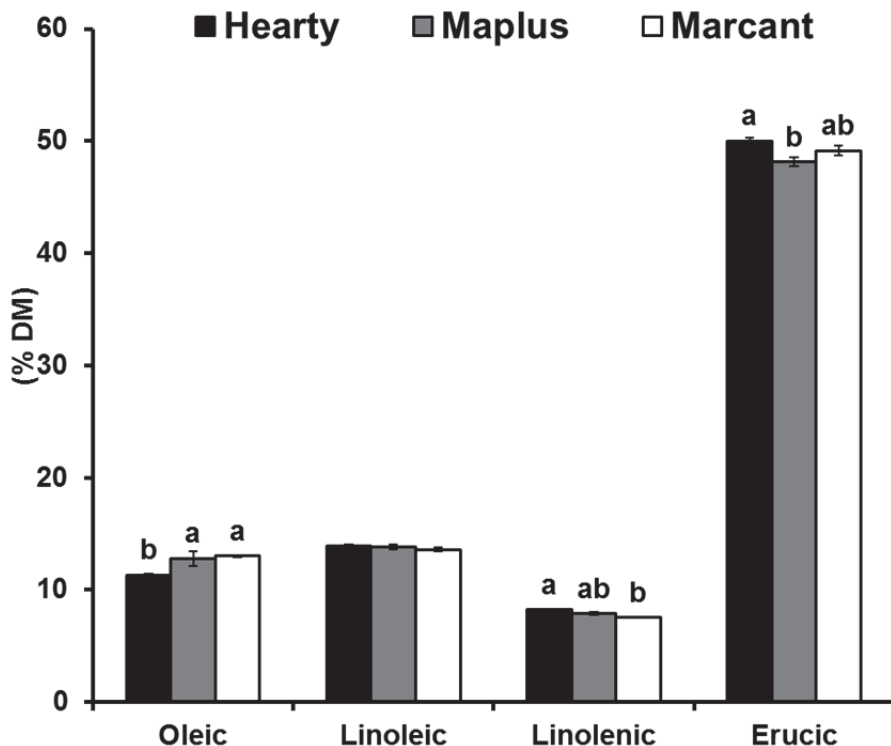


Fig. 6. Oil composition for main fatty acids in HEAR rapeseed cultivated under two input levels (main effect: cultivar). Vertical bars: standard error. Letters: statistically different values ( $P < 0.05$ , Duncan's test), within same fatty acid for different genotypes.

### 3.2 *Brassica carinata* response to N fertilisation in multi-locality trials set in NE Italy

*Brassica carinata* turned out to be only partially adapted to the environmental conditions of NE Italy, since mild winters may induce early stem elongation, thus exposing the crop to severe damage during late frosts. For this reason, ascertaining the optimal sowing date for each cultivar is essential, since great variability among genotypes and localities was observed in our experiments. Our trials aimed at verifying whether the cultivation of *B. carinata* is feasible in north Italy and whether the cultivation technique used for oilseed rape is also suitable for this newly introduced oil crop.

Yield results were quite different between localities and, within the same site, between years, indicating that genetic breeding is not yet sufficient to achieve adequate stability, with the aim of providing a stable productive range for farmers.

The soil conditions at Legnaro appeared to be better for Ethiopian mustard than at Rosolina, since all production parameters considered, i.e., seed yield, oil content, and seed proteins, were significantly higher. However, the better adaptation of cv. BRK 1 (at Legnaro) than ISCI 7 to autumn sowing cannot be excluded.

The two localities differed mainly in soil type (Legnaro vs. Rosolina: silt-loam vs. sandy-loam) than in climatic conditions (Table 6. Source: ARPAV). In both localities, some climatic differences emerged between years; in Legnaro, the total amount of precipitation was similar between years, but distribution during the season was different. The first year (2006/07) was characterised by severe drought during blooming, as happened in the HEAR experiments (see above), and only 5 mm of rain fell in April; temperatures did not differ greatly between years, but both minimum and maximum temperatures during the second season (2007/08) were higher. In Rosolina, the 2007/08 season was characterised by a lack of 150 mm of water compared with both the historical mean and the Legnaro site; temperatures ranged within normal reference values for the locality (min 6.4°C; max 14.7°C). The second year in Rosolina (2008/09) was characterised by abundant precipitation, almost double that of the first season, together with higher temperatures (+1°C for both min and max).

Localities	Year	Rainfall (mm)	Mean minimum T° (°C)	Mean maximum T° (°C)
Legnaro	2006/07	588	7.2	17.2
	2007/08	548	5.7	14.8
	Mean 1992-05	593	5.4	14.9
Rosolina	2007/08	370	6.4	14.7
	2008/09	790	7.5	15.4
	Mean 1992-05	523	6.3	14.5

Table 6. Most significant climatic data from two experimental sites during two growing seasons (Oct-June) of *Brassica carinata* (Source: ARPAV)

The effect of N fertilisation was limited in this oil crop, although some influences were observed at Rosolina only. *B. carinata* seems to be a more rustic crop than *B. napus*, and a reasonable reduction of N supply would be possible to reduce cultivation costs, at least in the more fertile soil of Legnaro. In Rosolina, the effect of N fertilisation was evident in all parameters, positively influencing seed yield and protein content, but with negative effects on oil accumulation.

At Legnaro, the performance of BRK1 was quite satisfactory in terms of seed yield, oil and protein production, while in Rosolina, results fluctuated among years and N rates (Table 7). The abundant fraction of sand in the soil at Rosolina probably makes precise N management difficult to be defined.

Unexpectedly, the parameter most influenced by N fertilisation was seed oil content, which also showed an unstable response across years.

Although *B. carinata* reached higher yield at Legnaro, it was not significantly different from that at Rosolina (Legnaro and Rosolina: 2.25 vs. 1.99 t DM ha<sup>-1</sup>). Significant differences emerged for oil content, which was higher in Rosolina ( $P < 0.05$ ), and for seed protein

	LEGNARO					ROSOLINA					<i>P</i> value Main effect site
	0 N	Reglette	100 N	<i>P</i> value	Mean	70 N	100 N	140 N	<i>P</i> value	Mean	
<b>Yield</b> (t DM ha <sup>-1</sup> )	2.45	2.53	1.78	ns	2.25	1.35 b	1.93 ab	2.71 a	*	1.99	ns
<b>Oil</b> (% DM)	34.7	32.1	35.7	ns	34.2 b	37.5 b	40.0 a	39.2 ab	*	38.9 a	***
<b>Proteins</b> (% DM)	29.7	31.8 a	32.1	ns	31.2 a	24.2 c	28.0 b	30.7 a	***	27.6 b	**
<b>TKW (g)</b>	4.25	4.30	3.70	ns	4.08	4.63 a	2.86 b	3.19 b	*	3.56	ns

Table 7. Seed yield, oil content, protein content, and 1,000 kernel weight (TKW) achieved by *B. carinata* in two experimental sites (2-year means). Letters: statistically significant differences among N rates within same locality ( $P < 0.05$ , Duncan's test). Bold letters: significant differences between localities for each parameter.

ns:  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

content, higher in Legnaro ( $P < 0.05$ ) (Table 7). A similar trend was detected in winter *B. napus*, grown in the same localities and years, with again higher seed yield and protein content in Legnaro and better oil content in Rosolina. The productive performance of *B. napus* was significantly higher than that of *B. carinata*, with ~60% greater seed yield and ~18% greater oil content. As reported in the literature (Getinet et al., 1996), Ethiopian mustard seeds were found richer in protein (+ ~58%, with respect to *B. napus*).

### 3.3 Introduction of newly released genotypes of *Crambe abyssinica* in southern Europe

The growing season of crambe (April-June) of the two experimental years was characterised by different weather conditions. In the 2006 crop cycle, the mean minimum and maximum temperatures were 9.8 and 19.8 °C, respectively, and precipitation was 193 mm. In 2007, mean temperatures were much higher than in 2006 (minimum: 11.8 °C; maximum: 22.8 °C), and were associated with limited rainfall in April (flowering stage) and a surplus (almost double that of the historical average for the location) in May-June (capsule filling-maturation stages) (total 276 mm, during crop cycle).

Yield (capsulated seeds) was much higher in 2006 than in 2007 (mean of varieties: 3.1 vs. 2.3 t DM ha<sup>-1</sup>), probably due to the more favourable climatic conditions, i.e., warmer climate and higher rainfall at flowering. No significant differences emerged among genotypes in terms of yield, although cv. Mario, which is an Italian selection, performed slightly better in both years (Figure 7).

The mean oil content of de-capsulated seeds of crambe was comparable to that of HEAR. For instance, in 2006, Galactica reached a maximum of 47% of oil, a value commonly found in high-erucic oilseed rapes in the same environment (Zanetti et al., 2009). In 2007, the high temperatures during the crop cycle very probably caused a reduction in the final oil

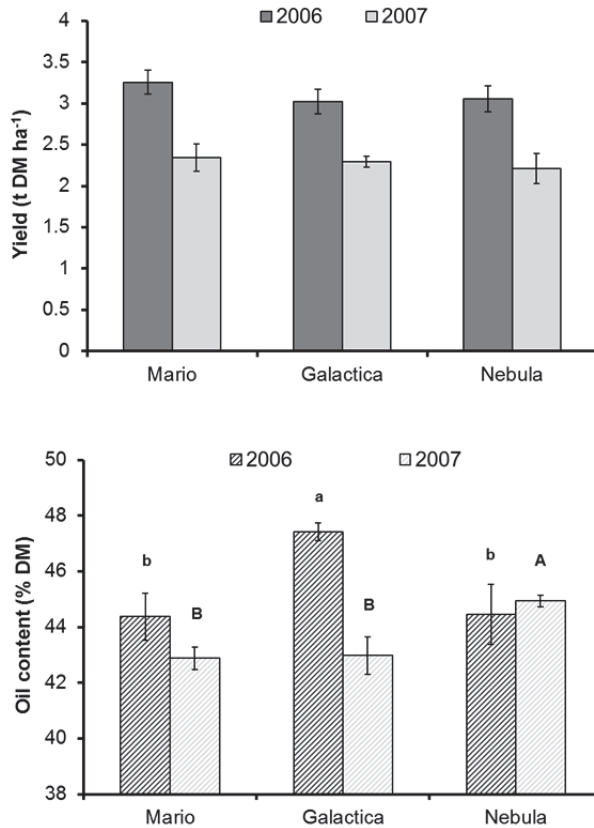


Fig. 7. Capsulated seed yield (above) in varieties of *C. abyssinica* in two-year trial and oil content (below). Letters: significant differences among varieties within same year ( $P \leq 0.05$ , Duncan's test). Vertical bars: standard error.

percentage of Mario (-2%) and Galactica (-5%), but not in Nebula, which showed very stable behaviour across years.

As expected, the content of erucic acid in the oil was very high in all varieties, and in 2006 reached a mean fraction of 58.4%, much higher than that of HEAR (~50%). These results indicate that, in our environment, crambe has lower productivity than HEAR in terms of erucic acid (0.61 vs. 0.89 t ha<sup>-1</sup>), although the higher fraction may facilitate its separation from the other fatty acids.

In view of the short cycle of this crop in spring sowing, the total amounts of both oil and erucic acid produced seems considerable, although significant variations may be expected across years. Greater precocity may allow some genotypes to perform better in terms of oil content but not of seed yield (e.g., Nebula).

The results of the last trial (2008) on different sowing times validated this observation, indicating that the choice of a correct sowing date is essential for final results. A delayed sowing date may cause not only a decrease in final seed yield but also limited oil accumulation. The resulting oil yield may decrease by as much as ~60%, i.e., 1.12 vs. 0.46 t ha<sup>-1</sup> of oil, comparing optimal and delayed sowing dates (Table 8).

Sowing date	Seed yield (t ha <sup>-1</sup> DM)	Oil content (% DM)	Oil yield (t ha <sup>-1</sup> DM)
Optimal (March 20)	2.65	42.4	1.12
Delayed (April 28)	1.16	39.8	0.46

Table 8. Yield performance of *C. abyssinica* cv. Mario, sown on two dates in 2008 at Legnaro experimental farm. Measures in duplicate.

One month's delay in sowing reduced crop cycle length by 18 days, which directly compromised final seed yield (-57%) and oil accumulation (-7%). These results might be even worse in the case of limited rainfall during seed filling, a frequent occurrence in early summer in this environment.

#### 4. Conclusions

A possible increase in the erucic acid market is feasible only if large amounts of oils with high erucic acid content can be stably available on the market at reasonable prices. With this aim, studies on introducing new high-erucic species in promising environments and defining low input management are essential for the progress of this niche market.

From this study, the large-scale cultivation of high-erucic oilseed crops in the plain areas of NE Italy seems feasible with different performance among species. The productive results achieved by all studied species were in any cases encouraging. Although HEAR is the higher-yielding oilseed crop for this environment, *Brassica carinata* and *Crambe abyssinica* showed interesting prospects which should be supported by more intense breeding programmes. Several traits of these new species should be improved, especially yield stability (across years and environments). Currently, the main aspect to be investigated is their optimal sowing date, which seems to be the most important variable affecting yield. *Crambe abyssinica* appeared particularly interesting in view of its short spring cycle which may make it a good alternative to sugarbeet and soybean, two crops extensively cultivated in NE Italy.

All three of these oil crops (*B. napus* HEAR, *B. carinata* and *C. abyssinica*) turned out to be easily adaptable to input reduction, without significant changes in seed yield or quality. In particular, the positive response of these *Brassicaceae* to reduction of N fertilisation means that cultivation costs, which represent an important factor, can be reduced considerably. Other technical aspects, e.g., weed management, must be carefully investigated in future for these new crops, as no herbicides are yet registered on the market. Large inter-row distances and mechanical weeding also makes weed control easier.

Southern Europe seems a promising basin for the cultivation of high-erucic species, in view of the good soil fertility (high OM), mild winter temperatures, and the introduction of new hybrids (HEAR) which are particularly plastic and extremely high-yielding. With this



assumption, stable achievement of 1 ton of erucic acid per hectare would be not only an optimistic dream but also a reliable goal.

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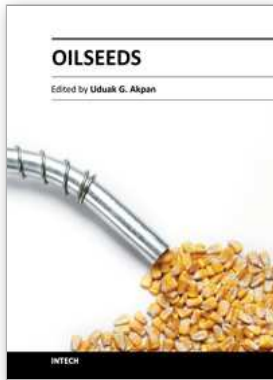
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