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Can We "Cultivate" Erucic Acid in Southern Europe?

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Abstract

Over the last fifteen years, considerable progress has been made in the field of "green chemistry", as regards both research aspects and market development. In particular, extraction of erucic acid (C22:1) from plants and its industrial applications have received increasing attention. At present, known species producing oils yielding large quantities of erucic acid belong, with few exceptions, to the *Brassicaceae* family. Among these, the two major sources of erucic acid in the world are HEAR (High Erucic Acid Rapeseed, *Brassica napus* var. *oleifera*) and crambe (*Crambe abyssinica*), both mainly cultivated in the USA. Their cultivation has also recently been considered and extended to southern Europe, supported by specific research projects. The quantity of erucic acid in *Brassicaceae* oils ranges greatly, from 55% in *Crambe abyssinica* to nearly zero in some varieties of *Brassica napus* var. *oleifera*. Even more differentiated and peculiar to each species and variety is adaptability to specific climatic and soil conditions. In this regard, the major limitation to the cultivation of some interesting *Brassicaceae* species, crambe in particular, is their poor tolerance to cold. Among *Brassicaceae* producing erucic acid, the less frequently cultivated species, such as *Brassica juncea* and *B. carinata,* if grown in areas with relatively mild winters, may give yields of seed and oil similar to those of the most productive rapeseed genotypes. Within this framework, in order to achieve high 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 important *Brassicaceae* species for extraction of erucic acid, derived from 15 years of field trials in northern Italy, are discussed in relation to the possibility of autumn or spring sowing.

Key-words: *Brassicaceae*, cold sensitivity, crop management, erucic acid.

1. Introduction

At present the use of oils containing large amounts of erucic acid is receiving increasing attention, according to the general trend of chemical compounds deriving from "green feedstock" the production of which is constantly increasing, so that a real niche market has been progressively created. In a scenario which will allow greater substitution of chemical compounds with "green" ones, the introduction of industrial oil-crops may lead to further expansion of this market.

Alternative uses of crops for non-food purposes may also be an interesting source of profit for farmers, as is happening for high-erucic acid oils. For the latter, the actual chemical demand is still limited: at world level, it is nearly 20,000 tonnes of erucic acid, corresponding to around 57,000 tonnes of oils, used for producing various chemical compounds (Figure 1).

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

Erucic acid is mainly transformed into erucamide, a slip agent for plastic film production. There are also other derived compounds, such as behenic, brassilic and pelargonic acids, obtained through well-known reactions (e.g., hydrogenation, ozonolysis), which find interesting and innovative uses in chemical, lubricant and

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detergent fabrication (Gunstone and Hamilton, 2001). Considering the actual world need of seeds containing erucic acid (around 100,000-120,000 tonnes), it is easy to understand how restricted this market is. Nevertheless, the positive trend observed in the last few years should allow significant extension of such cultivations. This implies that studies on the adaptation of species containing erucic acid in various environments are really essential.

For producing erucic acid, *Brassicaceae* is the most interesting botanical family because of the large number of suitable species and varieties, so that the whole amount of such fatty acid needed in the world derives from plants belonging to this family.

The content of erucic acid ranges greatly among varieties within the same species; as shown in Table 1, variations are very large, even higher than 30% in *Brassica juncea*. This indicates that efficiency in erucic acid production can be greatly improved by adequate screening of genotypes, but also that important genetic resources may be found for breeding purposes. At this regard, an amount of erucic acid corresponding to 66% in oil must be considered, at least in rapeseed, the actual theoretical limit which may be achieved (Renard et al., 1994).

For some species of this family (i.e., *Brassica napus* var. *oleifera*, *Crambe abyssinica*) yield potential and environmental adaptation have been sufficiently studied in southern Europe, whereas there is lack of information on some others, like *Brassica juncea* or *B. carinata.*

This report presents some results on yield potential, obtained from trials carried out in northern Italy for 15 years on various *Brassicaceae* species. In addition, data concerning seed yield, oil content and amount of erucic acid, provided by some genotypes with autumn sowing, are also discussed in relation to their tolerance to cold and winter survival.

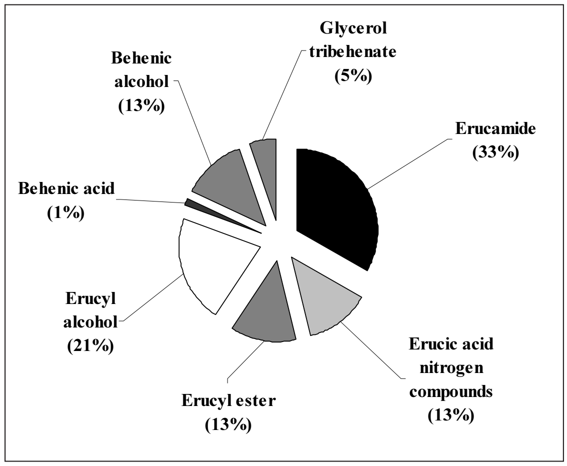

Figure 1. World production of chemical compounds derived from erucic acid, expressed as percentage of total amount (Source: Gunstone and Hamilton, 2001).

2. Species of interest

In this review four different *Brassicaceae* species were considered as the most promising for producing erucic acid, i.e., *Brassica napus* var. *oleifera*, *Crambe abyssinica*, *Brassica juncea* and *B. carinata*.

The results presented here derive from 15 years of field tests, mainly carried out at the experimental farm of the University of Padova (Legnaro; 45° 21' N and 11° 58' E).

Although *Brassica napus* var. *oleifera* and *Crambe abyssinica* are considered the most important crops for producing erucic acid, because of their high, stable yields, the introduction of new species could be an opportunity to satisfy further future needs.

The genotypes of *Brassica napus* var. *olerifera* considered here belong to the HEAR (High Erucic Acid Rapeseed) type, which is – among the sources of erucic acid – the most widely cultivated in the world. This species can provide constant, high yields – around 3.5-4.5 t ha^{-1} with 35-45% of oil – although new hybrids, recently released on the market, may even reach 50% of oil in seeds (De Mastro and Bona, 1998). The most valuable characteristic of HEAR is high tolerance to low temperatures. It

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

Species	Oil content (%)	Erucic acid content (%)
Brassica carinata	37-51	35-48
Brassica juncea	35-45	18-49
Brassica napus var. *oleifera*	35-45	45-54
Crambe abyssinica	30-35	55-60
Eruca sativa	28-30	34-47
Sinapis alba	25-30	33-51

can tolerate down to -13° C (Auld et al., 1984), thus allowing early autumn sowing, long crop cycle and high seed yields in northern Italy.

Although the average yield of *Brassica napus* var. *olerifera* was quite similar around Italy in a 10-year trial (Toniolo et al., 1992), data variability was much higher in the south and in centre with respect to the north of Italy, as evidenced by the range values (Figure 2). The average yield was maximum in central Italy (2.64 t ha^{-1}) and similar between northern and southern parts, being 2.43 and 2.36 t ha^{-1}, respectively.

Crambe abyssinica (crambe) is one of the richest species in erucic acid, reaching around 55% of oil, but its poor resistance to cold allows almost exclusively spring sowing in north Italy. Therefore, this crop may represent an interesting source of erucic acid only for those environments where autumn or early spring sowing is practicable. In locations with mild winters, some inconveniences may also be encountered, a marked reduction in yield being possible in cases of sudden cold spells late in the season.

Crambe has some morpho-physiological peculiarities, such as seeds enclosed in capsules, smaller plants, and lower oil content (average: around 35%) with respect to most of the species considered here.

Brassica juncea (Indian mustard) differs greatly from the other "high erucic" *Brassicaceae* species for several morphological traits, since it has taller plants, smaller seeds and elongated leaves. Its cultivation is widespread, especially in its native geographic area (Far East), because it has an interesting agronomic characteristic, i.e., low incidence of pod shattering, which can significantly reduce yield losses. Despite this, its resistance to cold, which is extremely differentiated among varieties, is generally quite low, although some genotypes tested may tolerate down to -5° C and provide interesting yield results.

Among new *Brassicaceae* suitable for southern Europe, *Brassica carinata* (Ethiopian mustard) may be considered the most promising, because it gives high yield (2.5-3.6 t ha^{-1}) and has two favourable characteristics, lower predation by birds and good tolerance to pests and diseases.

Nevertheless, its poor cold tolerance should be carefully taken into account in order to choose the best sowing time, especially in north Italy.

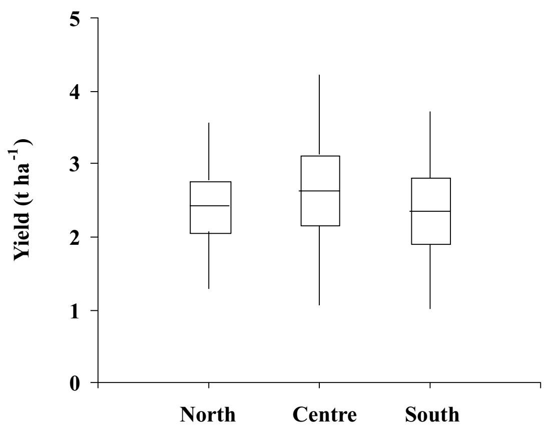

Figure 2. Median yield (10-year period) of *Brassica napus* var. *oleifera* in northern, central and southern Italy. Box and Whiskers plot with 25% and 75% of values (quartiles); vertical bars (whiskers) are defined by least and greatest values.

3. Limitations to crop management

The introduction of *Brassicaceae* species for erucic acid production in southern Europe should be faced with some limitations to their cultivation.

For successful cultivation, as a first step, the choice between spring and winter genotypes must be considered in relation to the specific environment. Nevertheless, for the above-quoted species there are only few varieties available, with the exception of *Brassica napus* var. *oleifera*, which has benefited by intensive breeding programmes. Within the same season, the right time for sowing should also be identified in order to maximize yield; for instance, in north Italy, the best results for HEAR are obtained with early-autumn sowing. For each genotype, it is necessary to know both cold tolerance and the need for chill, to ensure, plant survival on one hand and induction of flowering on the other. In this regard, investigation of intraspecific variability would allow the best genotypes for each environment to be identified.

As cultivation of "00" varieties (i.e., without erucic acid in oil, and absence of glucosinolates in meal) of *Brassicaceae* for food purposes at present prevails over high-erucic varieties, the rate of cross-pollination – even interspecific – among cultivations should be carefully considered, also together with possible effects on oil composition. Most of the literature (e.g., Rieger et al., 2002) has demonstrated that gene flow in

Brassicaceae is extremely variable and depends on many factors – including environmental conditions, plant variety and density, insect behaviour, and genotypic/phenotypic traits (e.g., content of erucic acid, tolerance to herbicides) – but it is greatly limited if the distance between pollen sources is increased (Champolivier et al., 1999).

Furthermore, for the four species considered here, apart from *Brassica napus* var. *oleifera*, there are no hybrids available, because of the high cost of breeding and the little attention paid until today to such species. In any case, because of the great difficulty of restoring male fertility in hybrids and the general low transmissibility of characters, release on the market of true *Brassicaceae* hybrids is still quite limited.

As regards crop management of high-erucic *Brassicaceae*, weed and pest control must be considered with particular attention, especially in southern European countries, where cultivation of *Brassicaceae* is traditionally less important and has led to a limited number of both herbicides and pesticides being registered. For instance, in Italy there are only a few pre-emergence herbicides (active ingredients) with high efficacy against weeds, and they are still very expensive or poorly selective, but they are only allowed for rapeseed (e.g., trifluralin, metazachlor). This is due not only to the current scarce economic interest in these *Brassicaceae* species, but also to the high inter- and intra-specific variability in sensitivity to different active ingredients.

Another essential agricultural practice is nitrogen fertilization. It is known that, although a high amount of N can markedly increase seed yield, at least in rapeseed (Raymer et al., 1990), it can also raise the frequency of lodging, which leads to phytopathological problems, especially when associated with high rainfall. Difficulties in mechanical harvesting are also caused and thus reduce seed and oil yields.

Pod shattering is a common and undesirable characteristic of these *Brassicaceae* species, apart from *Brassica carinata*, and leads to large yield losses, although it can be partially controlled by proper agronomic management. The choice of the optimum harvesting time is essential in order to limit this phenomenon, although new rapeseed cultivars with a lower incidence of pod shattering are now available.

4. Results from field tests

Within the PRISCA and TISEN projects, supported by the Italian Ministry of Agricultural and Forestry Policy (MiPAF), several trials have been carried out at the experimental farm of the University of Padova at Legnaro (NE Italy) over the last 15 years, on the adaptability of high-erucic *Brassicaceae* species. The trials, still in progress, include various accessions belonging to the four above-quoted species.

The genotypes tested here were provided by the North Central Regional Plant Introduction (PI) Station (Ames, Iowa, USA) of the National Plant Germplasm System (NPGS, USDA) and from the Centre for Genetic Resources (CGN, The Netherlands).

The limited amount of seed provided (100 or 300 seeds per accession) was reproduced in conditions of spatial isolation to allow greater scale cultivation (plots), thus making comparisons of accessions more reliable.

Sowing took place on a typical silty-loam soil (fulvi-calcaric-cambisol – USDA classification), with 1.75% organic matter and 0.11% total nitrogen, previously cultivated with winter wheat. After some preliminary trials on tolerance to cold, early-autumn sowing (last days of September) was commonly adopted for HEAR, Indian mustard and Ethiopian mustard, whereas Crambe was sown in early-spring (beginning of April), because of its very high sensitivity to low temperature.

A randomised block design replicated four times was used, with plots 1.4 m wide and 5 m long (4 rows, 0.35 m apart).

Ploughing was done in early September of each year, followed by grubbing and harrowing, so that a fine sowing-bed was obtained at the end of September. A density of 200 seeds per m^2 was commonly used in both autumn and spring sowing.

Weed control was carried out manually throughout the crop cycle. The amounts of fertilisers were the same for all species, being 90, 42 and 80 Kg ha^{-1} of N, P and K, respectively. Phosphorus and K were applied in pre-sowing with small amount of N (30 Kg ha^{-1}); the rest of N was given later when the plants started their fast spring growth.

Harvesting took place between the end of May and 15 June, depending on plant species and accession. Seed yield was evaluated on the two central rows for each plot, by manual harvesting and subsequent mechanical threshing.

Oil was extracted from milled seeds (around 2 g), by means of ether, and its amount was determined after evaporation of the solvent with Soxtec-Tecator equipment (FOSS Analytical, Höganäs, Sweden).

Lastly, the percentage of erucic acid was evaluated on methylated oil (preparation of methyl esters, following the procedure reported by International Standard ISO 5509), through a gas-chromatograph (DANI 8610). Hydrogen was used as carrier gas, and a capillary column (ALLTECH econo-cap carbonwax) 30 m long, 0.01 mm and 0.25 mm, inner and outer diameters respectively, to separate fatty acids. The oven was set to heat the column to an initial temperature of 170° C, maintained for 5 min.; afterwards, 230 °C were reached through progressive increases (+5° C per minute). In the gas chromatograph, a split/splitless (SL/IN) injector with a splitting rate of 100:1 and a Flame Ionization Detector (FID) were installed. Each analysis lasted 35 minutes.

When autumn sowing was adopted, a final plant density (at harvest) of around half the seed density was commonly found, for the three considered species, only after mild winters. This, which also took place in some cases at our experimental site (e.g., growing season 2002-2003, -5° C as lowest value of minimum temperature), allowed all accessions to reach reasonably high yield levels (Table 2). *Brassica napus* var. *oleifera* showed a statistically significant higher yield (3.35 t ha^{-1}) than *Brassica carinata* and *B. juncea*. The latter had the lowest mean value on an hectare base (2.5 t ha^{-1}), but in one accession (AMES 23100) it exceeded 2.9 t ha^{-1}. The intraspecific variability of yield in favourable climatic conditions was almost the same among species, the coefficient of variability (C.V.) being around 16%.

As regard oil contents, almost the same hierarchy was found among species (Table 2), with the highest values again in HEAR (46%). For this trait the C.V. within species was much lower than that found for yield, being 8% in HEAR and 4% in the other two species.

These results suggest that, with autumn sowing, in conditions of relatively mild winters, the best performance for production of erucic acid is that of *Brassica napus* var. *oleifera*, although some accessions of *Brassica juncea* or *Brassica carinata* do allow good yields.

Nevertheless, a different situation was verified in very cold winters. For instance, in crop season 2001-2002, with minimum temperatures ranging between -9 °C and -15 °C for several days (end of December), the more cold-sensi-

Table 2. Yield and oil content (± SE) of high-erucic accessions of three *Brassicaceae* species obtained in Legnaro with autumn sowing and a mild winter (crop cycle 2002-2003). Within each variable (i.e., yield, oil content), different letters indicate statistically significant differences (LSD test, P≤0.05).

Species	Accession	Yield (g m^{-1} DM)	Oil content (%)
Brassica napus	Golden	273 ± 22	45.3 ± 0.5
var *oleifera*	Reston	366 ± 21	46.9 ± 0.5
	PI 469823	361 ± 28	47.1 ± 0.5
	PI 469732 Azuma 156	323 ± 30	45.9 ± 0.2
	PI 469741 Buk Wuk 12	308 ± 8	44.4 ± 0.8
	PI 469778 Dong Mae 23	341 ± 20	45.6 ± 1.1
	PI 469917 Mok Pd 13	370 ± 8	47.8 ± 0.3
Average		335 ± 11 A	46.1 ± 0.3 A
Brassica juncea	AMES 22961	221 ± 13	30.9 ± 1.7
	AMES 23100	292 ± 32	42.4 ± 1.5
	PI 174801	270 ± 18	36.6 ± 1.3
	PI 211000	254 ± 12	39.9 ± 2.0
	PI 426314	212 ± 30	37.4 ± 0.8
Average		250 ± 11 C	37.4 ± 1.1 B
Brassica carinata	PI 274283	274 ± 28	32.8 ± 1.4
	PI 360883	314 ± 16	36.6 ± 0.8
Average		294 ± 17 B	34.7 ± 1.0 C

tive species showed extremely low values of winter survival (Table 3). This caused a marked fall in productivity, especially in *Brassica carinata* and, to a lesser extent, *B. juncea*, with the exception of accession AMES 23100, which was able to ensure 2.36 t ha^{-1} of seeds. As expected, the accessions belonging to HEAR generally showed negligible incidence of winter mortality, but yields were lower than those found in the above-mentioned mild season (2002-2003), because of the high spring rainfall which was responsible for marked lodging and fungal development.

It should be stressed that marked variations in seed yields may be expected from one year to another due to differing climatic conditions rather than plant mortality, at least above a certain minimal level of plant density. It is already known that "00" rapeseed varieties in terms of both aerial biomass and yield are able to compensate low population densities by increasing lateral branching (Barszczak et al., 1993; Szczygielski and Owczarek, 1987).

Oil content was stable in the two years considered in *Brasssica carinata* and *B. juncea*, whereas HEAR was strongly affected by adverse climatic conditions, due to excessive spring rainfall, showing reduced percentages (35.8% vs. 46.1%).

As regards the rate of erucic acid in oil, although the averages were quite similar among species in 2001-2002 (a cold winter), a certain variability was found in accessions. A minimum fraction of 29.4% was found in PI 274283 (*Brassica carinata*) and the maximum (38%) in PI 469778 Dong Mae 23 (*Brassica napus* var. *oleifera*) (Table 3). For the latter, almost 400 kg ha^{-1} of erucic acid may be obtained for industrial uses.

Crambe abyssinica was cultivated only in spring sowing, its sensitivity to cold being its major drawback. The environmental conditions of our experimental site generally allowed only late spring sowing, thus reducing the potential duration of the crop cycle and constraining yields. The average yield of 10 accessions of crambe, in field trials carried out during crop cycle 1994-1995, hardly exceeded 1.0 t ha^{-1}. Although the content of erucic acid in crambe was very high (55%), the small amounts of yield and oil (mean: 25.7%) only yielded a total erucic acid production of around 150 kg ha^{-1}, much lower than in HEAR.

Within the PRISCA project, better yield results were obtained for crambe by experimental stations in central and southern Italy, presumably because earlier spring sowing was possible and the higher temperatures of these re-

Table 3. Plant mortality throughout winter (autumn sowing), seed yield, oil content and amount of erucic acid (± S.E.) in high-erucic accessions of three *Brassicaceae* species, obtained at Legnaro in a cold winter (crop cycle 2001-2002) (n.d. = not determined). Erucic acid was evaluated on one replicate for each accession. Within each variable, different letters indicate statistically significant differences (LSD test, P≤0.05).

Species	Accession	Mortality (%)	Yield (g m^{-2} DM)	Oil content (%)	Erucic acic (%)
Brassica napus	Golden	20	190 ± 32	38.1 ± 4.0	36.5
var *oleifera*	Reston	30	198 ± 25	36.3 ± 2.0	n.d.
	PI 469823	20	208 ± 18	35.2 ± 2.0	34.5
	PI 469732 Azuma 156	30	232 ± 16	34.8 ± 0.5	37.8
	PI 469741 Buk Wuk 12	40	270 ± 24	33.9 ± 1.3	36.7
	PI 469778 Dong Mae 23	20	286 ± 37	35.7 ± 0.3	38.0
	PI 469917 Mok Pd 13	30	263 ± 19	36.6 ± 0.8	37.5
Average		27 C	235 ± 11 A	35.8 ±1.2 A	36.8 ± 0.5 A
Brassica juncea	AMES 22961	80	134 ± 17	37.1 ± 0.9	33.7
	AMES 23100	40	236 ± 33	35.8 ± 0.6	37.6
	PI 174801	90	84 ± 9	34.3 ± 0.5	n.d.
	PI 211000	80	94 ± 6	34.9 ± 1.5	34.7
	PI 426314	90	76 ± 21	38.3 ± 5.0	35.1
Average		76 B	125 ± 16 B	36.1 ±1.1 A	35.2 ± 0.8 B
Brassica carinata	PI 274283	90	49 ± 2.5	35.6 ± 0.9	29.4
	PI 360883	90	37 ± 8.5	38.2 ± 1.5	34.9
Average		90 B	43 ± 0.5 C	36.9 ± 0.8 A	32.2 ± 2.7 B

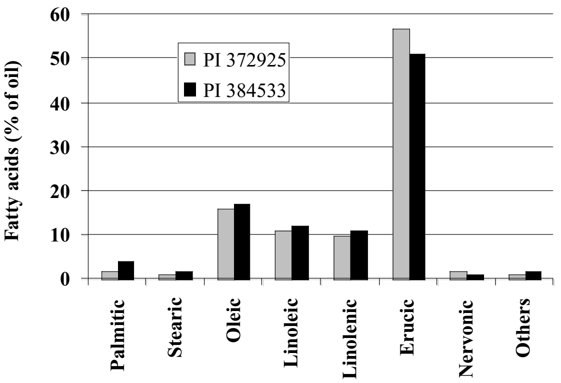

Figure 3. Oil composition of two accessions of *Crambe abyssinica* obtained in Legnaro during crop cycle 1994-1995.

gions were more suitable for the requirements of this species.

Genetic manipulation of the fatty acid composition of *Brassica* oils continues to be an area of active investigation, for both nutritional and industrial needs. For food purposes, the high-erucic varieties of rapeseed have been progressively replaced by zero-erucic ones, to guarantee human health, by substitution of erucic acid with oleic and linoleic acids.

For crambe, we found an amount of erucic acid quite similar to that of the traditional rapeseed variety R-500, for which Shahidi (1990) reports 57%. As shown in Figure 3, the oil composition of two accessions of crambe (i.e., PI 372925, PI 384533) was similar, and differed only slightly from the above-mentioned R-500 rapeseed variety, the amounts of linoleic and linolenic acids both being around 11-12% in all of them.

Because of the large variations in erucic acid content in *Brassicaceae*, considerable variability in oil composition is also to be expected – a fact which should be carefully considered by industry, especially for some applications for correct classification and management of residual by-products after separation of erucic acid.

5. Conclusions

There is the effective possibility of producing high amounts of erucic acid in southern Europe by choosing sowing time and species properly. At a latitude of 45° N or higher, autumn sowing gives good results only with *Brassica napus* var. *oleifera*, although valuable results can also be seen in more cold-tolerant varieties of *Brassica juncea*. The latter, together with *Brassica carinata* and especially *Crambe abyssinica*, are able to grow without any inconvenience in spring sowing, but have lower performances.

The high intraspecific variability found in terms of cold tolerance, yield and amount of erucic acid in oil should allow the best genotypes for each environment to be chosen. However, further investigations among the great number of available accessions and more intense breeding programmes, which allow commercial varieties to be released on the market, are still required.

The farming system adopted in high-erucic *Brassicaceae* is commonly derived from “00” rapeseed, which is widely cultivated, but it should be calibrated for each species or genotype – for instance, through identification of the most suitable herbicides and the most efficient nitrogen dose.

Production of erucic acid from *Brassicaceae* is still a niche market, but it may represent an interesting opportunity for agriculture if, in view of future development of demand, almost all the encountered limitations to *Brassicaceae* cultivation are solved.

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