Accumulation kinetics of fatty acids in new high-erucic genotypes of winter oilseed rape: EFFECT OF CULTIVAR CHOICE AND TEMPERATURE

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Introduction

Erucic acid is an unsaturated long-chain fatty acid (C 22:1) which confers on oils high oxidation resistance and good viscosity, characteristics in great demand by green chemistry (Nieschlag, 1971). Among the botanical families yielding high quantities of erucic acid, *Brassicaceae* is the one with the highest number of interesting species. *Brassica napus*, in particular, is the species which may guarantee the highest and most stable erucic acid production at world level. This crop has recently undergone massive breeding programs aimed at maximizing the erucic content of its oil and eliminating glucosinolates from its meal. Oilseed rape (OSR) cultivars containing amounts of erucic acid higher than 40-45% are normally called HEAR (High Erucic Acid Rapeseed). New HEAR hybrids have recently been introduced on the market, promising higher and more stable yields in terms of seeds, oil and erucic acid (Seyis et al., 2006). For these newly introduced genotypes, information regarding their fatty acid accumulation kinetics and the possible influences of environmental factors (mainly temperature) are still lacking. With the aim of explaining the relations between fatty acid evolution and temperature during the seed filling-maturity stage in new HEAR genotypes, a two-year field trial was set up at the Experimental farm of the University of Padova.

Materials and methods

The experiment was set up in Legnaro (45°21'N, 11°58'E, NE Italy,) in a silty-loam soil, with a completely randomized block design (n=3). Two commercial genotypes of HEAR were compared during 2007-2009: 'Maplus', a open-pollinated variety, and 'Marcant', a CHH hybrid ('Composite Hybrid Hybrid'), both kindly supplied by NPZ Lembke (Germany). In both years, OSR was sown at the end of September (September 24 2007, September 26 2008) – the optimal time for the environment of NE Italy – with an inter-row distance of 0.45 m and a sowing density of 60 seeds m^{-2}. Immediately after sowing, metazachlor (43%, 2 l ha^{-1}) was sprayed to prevent weed emergence. Fertilizer was applied before sowing (30-90-90 kg NPK ha^{-1}) and before stem elongation (80 kg N ha^{-1}). According to the phenological criteria of the BBCH scale, the end of flowering took place simultaneously for both cultivars (April 27 2008, April 26 2009), as did harvesting (at 54 and 52 days after flowering for 1st and 2nd years, respectively). Fatty acid profile evolution was assessed from the beginning of seed filling until maturity by means of weekly samplings (at 14, 21, 28, 35, 42 and 49 Days After Flowering, DAF) of fully grown pods (three replicates of 130 pods each time). Seeds were manually separated by pod walls and dried at 60°C for 24h. Oil was then extracted by the method of Folch et al. (1956) and fatty acid (FA) contents were determined by gas chromatography. At harvesting, final oil content (Soxhlet-Extraction) was determined.
The effect of temperature on FA accumulation was assessed for both genotypes during the seed filling-maturity period. In this stage, FA evolution is expected to be more stable and their quantities are similar to final ones. In particular, the average values of minimum, mean and maximum temperatures, recorded during the last 30 days before the 35, 42 and 49 DAF samplings, were correlated to the content of oleic (C 18:1), linoleic (C 18:2), linolenic (C 18:3) and erucic (C 22:1) acids determined on the same dates. Daily climatic data (precipitation, minimum, mean and maximum temperature) were collected in both years from the weather station located at the Experimental farm.
FA contents are expressed as percentages (% DM) of total FAs detected in Marcant and Maplus. The differences between cultivars and years were checked for significance by ANOVA ($P \leq 0.05$) with StatGraphics Centurion XV. The significance of correlations was assessed by Excel XLSTAT.

Results and discussion

Effect of genotype: The accumulation kinetics of oil and FAs were found to occur earlier in Marcant than in Maplus (Fig. 1). FA profiles showed high concentrations of oleic and linoleic acids immediately after flowering, after which their amounts decreased, followed by the consequent rapid increase of erucic acid.

Conversely, linolenic acid remained almost stable during the monitored period, reaching higher values in 2007/08 (7.9% DM at 49 DAF) than in 2008/09 (6.9% DM at 49 DAF). The Maplus variety showed constantly higher percentages than the hybrid Marcant (P≤0.05), in both years. Erucic acid content was found to be significantly higher in the hybrid than in the open-pollinated variety (main effect: genotype P≤0.05, in both years) (Fig. 1), the final amount in Marcant being particularly elevated and stable between years, >48% (48.3% and 48.8%, respectively, 1st and 2nd years). Final oleic acid contents were significantly higher in Marcant during the 1st year, and similar between the two genotypes in the 2nd year.

All these differences led to dissimilar concentrations of PUFAs and MUFAs between cultivars; significantly higher MUFAs and lower PUFAs were found in Marcant than in Maplus, in both years (P≤0.05, data not shown).

Oleic acid is a precursor of both erucic and linolenic acid biosynthesis, respectively mediated by the enzymes *elongase* and *desaturase*. Since the percentage of oleic acid was almost the same in the two genotypes, linolenic acid biosynthesis may have been promoted in Maplus and that of erucic acid in Marcant, suggesting that different pathways are followed in the two genotypes.

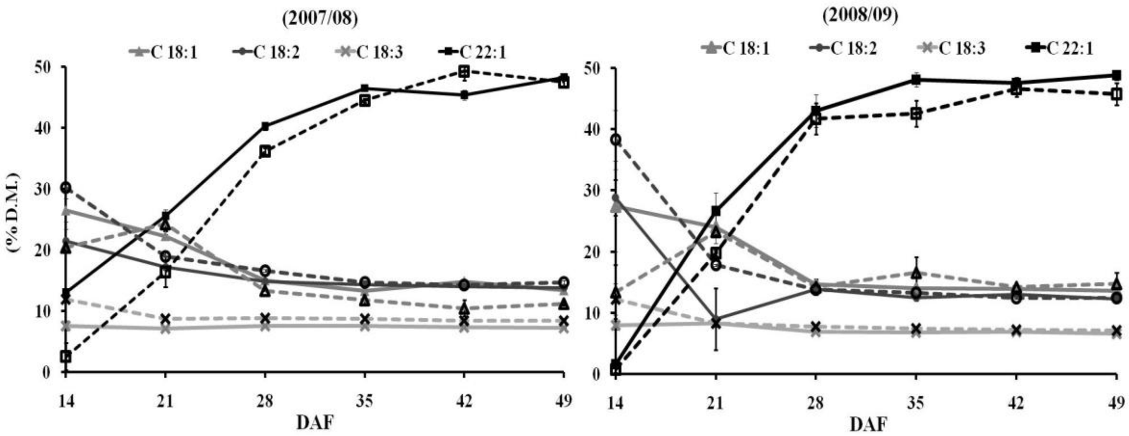

Fig. 1. Contents (% DM) of oleic (C 18:1), linoleic (C 18.2), linolenic (C 18:3) and erucic (C 22:1) acids on total quantity of FAs assessed in Marcant (solid line) and Maplus (dashed line) seed oils from 14 DAF until maturity in 2007/08

Effect of temperature: The two experimental seasons were quite different in terms of temperature and precipitation (Tab.1). During 2007-08 (September-June), the temperature (minimum and maximum) and rainfall were lower than those recorded during 2008-09. Considering the monitored period (April-June), the lst year was characterized by lower minimum and maximum temperatures but higher precipitation than the 2nd year.

As expected, correlation analysis revealed the strong influence of temperature on FA accumulation during the seed filling-maturity stage (Tab. 2). In particular, linoleic and linolenic acids were the most temperature-dependent FAs, especially as regards minimal temperatures (R = -0.85 and -0.66 for C 18:2 and C 18:3, respectively, both cultivars and years, P≤0.05), whereas temperature seemed to have less or no influence on the kinetics of oleic and erucic acids (Tab. 2). This finding matches the assumption that FAD (Fatty Acid Desaturase), regulated by temperature, is only active at low temperature (Baux et al., 2008; Werteker et al., 2010).

As regards the cultivars, Marcant is characterized by a pool of desaturase enzymes less influenced by temperature than Maplus (Tab. 2). Correlation coefficients between linoleic and linolenic acid contents and temperature (minimum and mean) were lower in the hybrid than in the open-pollinated variety. Neither cultivar was affected by temperature as regards percentages of oleic and erucic acids. However, for erucic acid, positive relations were observed in Marcant, whereas the correlation coefficients (for minimum and mean temperature) were negative in Maplus. This result supports the hypothesis based on the different patterns which characterize FA profile evolution in the genotypes tested here.

Tab.1. Monthly temperatures (minimum and maximum) and precipitations recorded during 2007-08 and 2008-09 growing seasons.

Month	2007-08			2008-09		
	T. min (°C)	**T. max (°C)**	**Precipitation (mm)**	**T. min (°C)**	**T. max (°C)**	**Precipitation (mm)**
September	11.9	23.3	104.8	13.3	23.5	60.0
October	8.9	18.5	35.8	10.0	20.5	45.8
November	3.3	12.4	23.4	5.9	12.4	150.8
December	-0.2	7.7	31.6	2.0	7.6	133.2
January	2.0	8.6	67.0	2.0	5.9	57.2
February	0.7	9.8	31.8	1.3	9.7	57.2
March	4.1	12.9	50.6	4.4	13.9	104.6
April	7.7	17.1	107.8	9.8	19.1	126.0
May	12.8	22.7	95.2	14.4	26.1	25.8
June	16,6	26.6	80.0	15.9	27.0	86.6
Mean/sum	6.8	16.0	628.0	7.9	16.6	847.2

Tab. 2. Correlation coefficients (R) of linear relations between minimum, mean and maximum temperatures (°C) recorded during last 30 days before 35, 42 and 49 DAF samplings and corresponding contents (% DM) of oleic (C 18:1), linoleic (C 18.2), linolenic (C 18:3) and erucic (C 22:1) acids assessed in Marcant and Maplus oils (n=15 for each correlation coefficient), values of two years. ***, **, * and ns: P≤0.001, P≤0.01, P≤0.05 and P>0.05.

Fatty Acids	Marcant			Maplus		
	T. min	**T. med**	**T. max**	**T. min**	**T. med**	**T. max**
Oleic (C 18:1)	0.03 ns	0.03 ns	0.02 ns	0.72**	0.63*	0.34ns
Linoleic (C18:2)	-0.81***	-0.80***	-0.64*	-0.91***	-0.89***	-0.62*
Linolenic (C 18:3)	-0.85***	-0.86***	-0.73**	-0.93***	-0.92***	-0.71*
Erucic (C 22:1)	0.37 ns	0.41 ns	0.48 ns	-0.29 ns	-0.16 ns	0.16 ns

Conclusions

The results of our experiments showed that the quality of HEAR oil is affected by both environment and choice of cultivar.

During the seed filling-maturity stage, temperature played a crucial role in defining FA profile evolution and the final percentages of FAs. In particular, low temperatures greatly affect polyunsaturated FA contents (linoleic and linolenic acids) even in a highly monounsaturated oil, like that of HEAR.

In response to temperature, the hybrid Marcant showed more stable behaviour for almost every fatty acid studied. Its final amount of erucic acid was also particularly high, and this, combined with the increased oil content of its seeds and particularly stable FA profile, may make this hybrid more suitable for extensive erucic acid production for green chemistry applications.

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