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Full Length Research Paper

How to optimize the seed and seed-oil production in the cash crop halophyte *Cakile maritima*?

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Seeds of the medicinal plant *Cakile maritima* contain significant quantities of oil rich in erucic acid. Irrigation with up to 500 mM NaCl had no adverse impact on the plant growth and reproduction of this promising species for domestication. Yet, almost half of the seed yield may be lost at the harvest due to the fall of the fragile ripened siliculas. This is a major practical hurdle that would jeopardize the plant yield in field conditions. Here, we assessed whether the yield and the seed-oil quality were affected by the fruit and seed maturity stage. Results showed that oil content and fatty acid composition did not significantly vary between the immature seeds, whole immature siliculas, and the fully mature ones, indicating that these traits were neither significantly influenced by the maturity stage nor by the simplification of extraction procedure. In the perspective of cultivating *C. maritima*, our results suggest to harvest siliculas ten to fifteen days before their full ripening, despite the lower contents in seed-oil and erucic acid. This should be largely overcome by the large gain in yield traits since siliculas remain attached to the mother plant.

Keys words: *Cakile maritima*, erucic acid, halophyte, maturity stage, oilseed, yield.

INTRODUCTION

Low agricultural productivity in arid and semi-arid regions of the world is to a large extent due to the fact that crops cultivated in these areas are naturally subjected to a multitude of abiotic stresses, among which salinity (Gorham, 1995; Shannon, 1998; Munns, 2002). Today, about 20% of the world's cultivated land and nearly half of all irrigated lands are affected by this environmental constraint (Barrett-Lennard, 2000). The alternative consisting in the irrigation using a poor quality of water (up to 4.5 $g.l^{-1}$ salt concentration) even worsens the situation. Hence, currently approximately one third of the world's irrigated land is salt-affected and 25% of the irrigated perimeters are considered as zones of risk, mainly due to unsustainable irrigation practices.

As salt resistance has already be evolved in naturally-salt tolerant species (halophytes), the cultivation of these plants is emerging as a promising and sustainable management practice for the rehabilitation of marginal saline soil types, when fresh water is not sufficient (Debez et al., 2011). Several researchers in many countries have been investigating the economical potential of halophytes (Aronson, 1989; Ashour, 1993; Ashour and Thalooth, 1993; Abdelly et al., 2006). Some species have been screened for their productivity and/or nutritional potential when irrigated with saline water and even seawater (Glenn and O'Leary, 1985; Debez et al., 2010), while others showed potentials as oleaginous plants, forage plants or food plants. For instance, seeds of *Cakile edentula* (O'Leary et al., 1985) and *Crambe abysinnica* (Mandal et al., 2002) have been reported to contain 50 and 60% oil, respectively. This is also true for

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Cakile maritima, typical of coastal dune vegetation. Tunisian accessions of *C. maritima* contain 25 to 39% seed-oil with erucic acid as major fatty acid (25 to 35% of total fatty acids) (Ghars et al., 2005). This oil is comparable to that of conventional oilseed species such as *Brassica napus* and would be suitable for industrial applications (manufacturing products such like emollients, surfactants, high temperatures lubricants, and plasticizers) (Friedt and Lühs, 1998; Wang et al., 2003). Global demand for erucic acid is steadily increasing throughout the world, rising from 18 million tons in 1990 to 35 million tons in 2010. Alone, the US market need for this product was estimated at about 18 000 metric tons in majority being imported (Bhardwaj and Hamama, 2003), which gives an idea about the extent of the needs in erucic acid.

The actual yield (and the yield potential) of halophytes remains largely unknown since their domestication is still limited despite these plants tolerate extremely harsh conditions. Isolated field studies with *Salicornia europaea* in Mexico, Egypt, and United Arab Emirates reported a production of 20 tons of total biomass with a production of two tons of seeds per ha (Goodin et al., 1990). Glenn et al. (1991) also found similar performances with *Salicornia bigelovii.* Concerning *C. maritima,* data obtained so far were under controlled conditions and indicate that the plant survives and successfully reproduces even at 500 mM NaCl salinity (Debez et al., 2004). Plant growth, harvest index, siliculas number and seeds produced per fruit segment were maximal at 100 mM NaCl (Debez et al., 2008). In addition, seed-oil content was unaffected by salinity, and erucic acid level increased markedly in concomitance with a significant decrease in oleic acid level (Debez et al., 2006). Yet, the fragility of the siliculas at full maturity and their fall from the mother plant is a serious issue to deal with during the harvest of this species, since there is a risk of losing almost the half of the seed yield. To resolve this problem, we thought to harvest siliculas ten to fifteen days before becoming yellow (maturity stage) and to assess whether the yield and the quality of the oil were or not affected by maturity stage.

MATERIALS AND METHODS

Plant species

C. maritima was probably one of the first members of re-invasion floras after successive glaciations (Davy et al., 2006). It grows in sandy habitats along the North Atlantic Ocean, the Mediterranean Sea coasts, the Canary Island and southwest Asia (Clausing et al., 2000). In these regions, it colonizes beach and dune that are frequently disturbed by surf and wind. According to Pottier-Alapetite (1979), in Tunisia, this species is frequent along the coast from north to south. *C. maritima* is an annual diploid species (2n = 18). It has a short life cycle (3 months from seed to seed) and produces a large number of seeds with high germinative capacity even in the presence of salt (Debez et al., 2004). Its annual character is not strict, since some individuals may survive over two or three years. In this case, the number of seeds produced may be almost 20 fold that produced by a single reproductive cycle (Thorne, 1967; Barbour and Rodman, 1970; Boyd, 1988; Boyd, 1993; Gandour et al., 2008). Except for occasional individuals, *C. maritima* is generally self-incompatible (Rodman, 1974; Thrall et al., 2000). In *C. maritima* pollen dispersion is likely to result from wind and various insects (personal observation). The 1.5 to 3 cm long fruit (siliculas) is dimorphic, consisting of two segments (upper and lower) each generally enclosing one seed. The upper segment is deciduous, an adaptive trait for dispersal, while the lower one remains attached to the parent plant (Gandour et al., 2008). The species is considered ecologically beneficial, as its roots bind sand dunes, and its seeds contain appreciable amounts of oil (40% on dry weight basis), rich in erucic acid (Zarrouk et al., 2003), thus potentially appropriate for industrial applications. Its leaves, consumed as salads, constitute an important source of potassium and vitamins B and C.

In the present work, the evolution of fruit ripening was indicated by the change in siliculas color, from green to yellow, the latter corresponding to full ripening. At this stage, siliculas become very fragile and fall easily. Two stages were considered: (1) immature stage (S1), where siliculas are green and completely developed but just before turning to yellow (about two weeks before complete maturity) and (2) mature stage (S2), where siliculas are yellow, dry, and shelled.

C. maritima seeds were harvested from Soliman (20 Km east of Tunis) and sown in pots (40 each containing a seed) filled with sand and irrigated with tap water. The experiment was carried out from January 2009 to April 2009 in the greenhouse of the Centre of Biotechnology of Borj-Cedria. The harvested siliculas were manually shelled and the mature seeds, immature seeds, and immature siluculas were dried (50°C for 48 h).

Oil extraction and fatty acid methylation

Four samples from each stage were finely ground using a bead mill. 10 g of each ground material was extracted in a soxhlet-extractor with 250 ml hexane heated at the nearest boiling temperature (we use here 60°C) (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) for 6 h. The extract was protected from light until use. The solvent and the oil are kept in a balloon of known weight. The solvent was then evaporated by means of a rotary evaporator with 37°C and the mass of oil was determined by double weighting. The oil content or total fat content (TFC), expressed as percent of the dry matter, was calculated using the following formula:

$$TFC\ (\%\ of\ DW) = m \times 100 / M.$$

Where m is the mass of extracted oil, M the mass of dry matter, DW the dry weight, and TFC the total fat content.

The obtained oil samples were stored in tightly cork-wrapped bottles at 4°C until biochemical and GPC analyses were achieved, by which the different constituents and their amounts in the oil were studied.

Preparation of fatty acid methyl esters (FAMEs)

Total fatty acids (TFA) were methylated using sodium methylate according to Cecchi et al. (1985). Methyl heptadecanoate (C17:0) was used as an internal standard. Those fatty acids methyl esters (FAMEs) obtained were subsequently analyzed.

Gas chromatography analysis

The fatty acid methyl esters were analyzed on a HP 6890 gas

Figure 1. Comparison of seed oil content (%DW) between immature and mature seeds of the halophyte *C. maritima*.

chromatograph (Agilent Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The esters were separated on a HP Innowax capillary column (30 m length, 0.25 mm i.d, 0.25 µm film thickness). The oven temperature was kept at 35°C for 1 min, followed by 35 to 205°C at the rate of 3°C/min and finally at 205°C for 10 min. Nitrogen was used as carrier gas at a flow rate of 1.6 ml/min. The injector and detector temperature were maintained at 250 and 300°C respectively. A comparison of the retention times of the FAMEs with those of co-injected authentic standards (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) was made to facilitate identification.

Statistical analysis

All extractions and determinations were conducted in quadruplicate. Statistical analysis was performed by using the SPSS 16 statistical software. Descriptive analyses and one-way ANOVA were used. Differences at a confidence level of 95% were considered significant.

RESULTS AND DISCUSSION

Oil content and seed-oil composition

When expressed on the dry weight basis, seed-oil content was about 35±7% for mature seeds and 30±4% for immature seeds of *C. maritima* (Figure 1). Both values were statistically similar, indicating that the maturity stage had no effect on seed-oil content in *C. maritima*. Theses findings are close to those reported by Ghars et al. (2005) for Tunisian accessions of this species, with seed-oil content ranging from 25 to 39%. Compared to seeds of other halophytes, *C. maritima* seeds appeared to contain more oil. When analyzing six oleaginous halophytes (*Arthrocnemum indicum, Alhaji maurorum, Cressa cretica, Halopyrum mucronatum, Haloxylon stocksii and Suaeda fruticosa*), Weber et al. (2007) found that quantity of oil varied from 22 to 25% but was lower than other *Brassicaceae* such as *Cakile edentula* (O'Leary et al., 1985), or *Crambe abysinnica* (Mandal et al., 2002) (50 and 60%, respectively).

Regardless of the maturity stage seed-oil contained in the decreasing order: triacylglycerol (TAG), polar lipids (PL), free fatty acids (FFA), monoacylglycerol (MAG), and diacylglycerol (DAG) (Figure 2). TAG was by far the major fraction of seed storage lipids as compared to the other components (MAG, DAG, FFA and PL). As reported by Zarrouk et al. (2003), seed-oil of *C. maritima* has similar lipid composition to that of seeds or fruits of conventional oleaginous crops like rapeseed.

Fatty acid composition

GC analysis of FAMEs from mature seed (S2) oils revealed the presence of thirteen (13) fatty acids of which five (5) were saturated and eight (8) were unsaturated fatty acids. The main fatty acids were: erucic acid (C22:1), linolenic acid (C18:3), oleic acid (C18:1), linoleic acid (C18:2), arachidoleic acid (C20:1), palmitic acid (C16:0), stearic acid (C18:0). Percentages of these fatty acids were close to those found by Zarrouk et al. (2003) and Ghars et al. (2005). Palmitic acid (C16:0) was the prominent saturated fatty acid (5.13% of seed-oil) whereas erucic acid (C22:1) was the major unsaturated fatty acid in the oil (26.82%). Quantity of this important fatty acid, from the industrial point of view (Isbell, 2008),

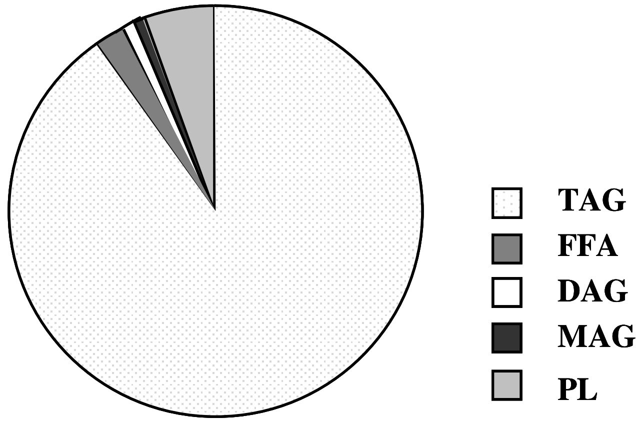

Figure 2. Lipid composition of seed-oil of the halophyte *C. maritima*; triacylglycerol (TAG), polar lipids (PL), free fatty acids (FFA), monoacylglycerol (MAG), and diacylglycerol (DAG).

Table 1. Fatty acid composition of immature seeds compared to that of mature seeds and immature whole siliculas.

	Oil content (% DW)			Significance levels	
	Immature seeds	Mature seeds	Immature whole siliculas	F	F
C14:0	0.0727	0.0715	0.0976	0.016 ns	3.159 ns
C16:0	6.5205	5.1313	9.2290	4.667 ns	3.428 ns
C16:1	0.3838	0.2365	0.4446	4.665 ns	0.579 ns
C18:0	2.5055	1.8550	2.7799	20.011 ns	1.519 ns
C18:1	18.5283	18.5770	16.7225	0.005**	2.250 ns
C18:2	18.2066	17.1486	19.4661	2.668 ns	3.288 ns
C18:3	20.3938	18.3527	23.3205	2.723 ns	2.018 ns
C20:0	1.4817	1.4157	1.3242	0.957 ns	2.471 ns
C20:1	7.8679	8.2928	6.9904	1.131 ns	1.398 ns
C20:2	0.6705	0.7997	0.5560	1.512 ns	8.379 ns
C20:3	0.3276	0.3134	0.5676	0.011 ns	5.236 ns
C22:0	0.5415	0.9768	0.9253	2.029 ns	1.522 ns
C22:1	22.4990	26.8283	17.5755	4.560 ns	3.019 ns

**significant at 0.01, ns: not significant.

was comparable to that found in *Thlaspi arvense*. It was up to 1.5 fold higher than that found by Maršalkienė et al. (2009) in *Erysimum cheiranthoides* and *Cakile baltica* but lesser than that of *Allaria petiolata* (49.3%). Nevertheless, when taking into account both the oil content and the amount of fatty acids, *C. maritima* was found to be more productive in erucic acid than all the cited species. Yet, the high amount of this fatty acid makes the seed-oil of *C. maritima* unsuitable for human consumption, but provides it a high potential for industrial purposes.

Interestingly, the present study revealed other minor fatty acids (less than 1% of total fatty acids) including C20:0, C22:0, C20:2, C20:3, C16:1, and C14:0, that were not detected in previous investigations on the seed-oil of *C. maritima* (Zarrouk et al., 2003; Ghars et al., 2005).

Fatty acid composition of the oil extracted from immature seed (S1) was to a large extent comparable to that of mature seeds (S2) (Table 1). Indeed, except for C18:0, T-test revealed no significant differences among mature and immature seeds for all the fatty acids percentages. This result indicates that fatty acid composition was not significantly influenced by the maturity stage ($P \geq 0.01$) and confirms that the immature stage selected in this study belongs to the end period of fatty acid synthesis. Compared to mature seeds, a slightly lower amount of erucic acid was found in the oil of immature-seeds, but was interestingly still higher than many other oleaginous species from the same family

(Brassicaceae) like *Erysimum cheiranthoides*, *Cakile baltica*, and *Descurainia sophia* (Maršalkienė et al., 2009). Since no significant difference between the two maturity stages was observed with respect to the composition and the quantity of oil, and in the perspective of improving the extraction procedure from *C. maritima* fruit, oil was extracted from siliculas (S1) and compared to that of seeds (Table 1). GC analysis of FAMEs showed largely similar chromatographic profile to that of seed oil, except the amounts of linoleic (C18:2) and linolenic acids (C18:3), which slightly exceeded that of the erucic one (C22:1). Comparing immature seeds to immature whole siluculas for all the fatty acids percentages (T-test) did not show a significant difference, indicating that amount of fatty acids was not affected by simplifying the extraction procedure (Table 1).

Conclusion

C. maritima fruit is characterized by the presence of two segments: an upper one, very fragile and easy-falling at full maturation, and a lower segment that remains attached to the mother plant. As a result of this natural phenomenon, the plant yield at the harvest period in terms of seed production may drastically decrease (up to -50%). As a whole, our findings suggest the possibility of preventing the fall of the upper segment of siliculas by harvesting fruits ten to fifteen days before their maturation. This will result in a two-fold increase of the seed (though immature) production per plant, despite the loss observed in both of seed-oil content and the erucic acid content after direct extraction from fruits (-14 and -30%, respectively). A simple estimation of the erucic acid production when harvesting immature seeds gives potentially a 40% gain as compared to the amount obtained by harvesting mature seeds, due to the augmentation of seed-oil amount (70%) compared to the mature seeds.

More generally, though preliminary, our findings are of practical significance in the context of the biosaline agriculture approach relying on the domestication of halophytes, since they point out the economical potential and the feasibility of cultivating *C. maritima*.

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