Variability Of Fruit And Seed-Oil Characteristics In Tunisian Accessions Of The Halophyte Cakile Maritima (Brassicaceae)

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Ghars Mohamed Ali
Centre de Biotechnologie, Technopole Borj…


Ahmed Debez
Centre de Biotechnologie, Technopole Borj…


Abderrazak Smaoui
Centre de Biotechnologie, Technopole Borj…


Chedly Abdelly
Centre de Biotechnologie, Technopole Borj…

CHAPTER 4

VARIABILITY OF FRUIT AND SEED-OIL CHARACTERISTICS IN TUNISIAN ACCESSIONS OF THE HALOPHYTE CAKILE MARITIMA (BRASSICACEAE)

MOHAMED ALI GHARS[1], ALMED DEBEZ[1], ABDERRAZZAK SMAOUI[2], MOKTAR ZARROUK[2], CLAUDE GRIGNON[3]AND CHEDLY ABDELLY[1, 3]

[1]INRST, Laboratoire d'Adaptation des Plantes aux Contraintes Abiotiques, BP 95, Hammam-Lif 2050, Tunisia.
[2]INRST, Laboratoire de Caractérisation et de la Qualité de l'Huile d'Olive, BP 95, Hammam-Lif 2050, Tunisia.
[3]B&PMP, Agro-M INRA, 34060 Montpellier CEDEX 1, France.
[3]corresponding author: chedly.abdelly@inrst.rnrt.tn

Abstract. The annual coastal halophyte *Cakile maritima* shows considerable ecological and economic importance. This study describes the variability of the fruit characteristics in twelve accessions of this species, harvested along the Tunisian littoral. Depending on the plant-sampling site, seed-oil contents ranged from 25% to 39% on the seed fresh weight basis. Reserve lipids (Triacylglycerols) represented 80% to 97% of the total lipids, and erucic acid, was the major fatty acid (25% to 35% of the total fatty acids). Fruit morphology and ion status within stems, siliques, and seeds exhibited great fluctuation. *Cakile maritima* seems to protect its reproductive organs from the salt harmful accumulation.
Abbreviations: 16:0: palmitic acid, 16:1: palmitic acid, 17:0: heptadecanoic acid, 18:0: stearic acid, 18:1: oleic acid, 18:2: linoleic acid, 18:3: linolenic, 20:0: arachidic acid, 20:1: gadoleic acid, 22:0: behenic acid, 22:1: erucic acid, DAG: diacylglycerols, DW: dry weight, FFA: free fatty acids, HEA: high-erucic acid, MAG: monoacylglycerols, MAR: Mean annual rainfall, PL: polar lipids, TAG: triacylglycerols, TLC: thin layer chromatography.

1. INTRODUCTION

Coastal areas worldwide are among the most vulnerable ecosystems threatening progressive salinization (Van Zandt et al., 2003). The negative impact of this phenomenon is particularly exacerbated in the irrigated semi-arid and arid regions, characterized by unfavorable environmental-soil features like high water consumption for irrigation, salt drainage, greater evapo-transpiration rates and secondary root-zone salinization (Smedema & Shiati, 2002). In Tunisia, the (semi)

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arid Mediterranean bio-climatic regions (rainfall ranging from 250 to 700 mm) are frequently irrigated with salinized water. Consequently, about 10% of the whole territory and 20% of the cultivated lands are salinized (Hachicha et al., 1994).

Halophytes are exposed to high salt levels in their native habitats, have evolved several mechanisms to deal successfully with these stressful conditions (Zhang, 1996; Donovan et al., 1997). Several among them are recognized as new crop-species (Grieve & Suarez, 1997; Glenn et al., 1998, Khan & Duke, 2001), either like fodder (Khan et al., 2000) or for oil production (Puppala & Fowler, 2002; Bashan et al., 2000; Pita Villamil et al., 2002; Dierig et al., 2003). However, exploiting halophytes requires first the identification of species and their habitats, the ecophysiology and their eventual economical utilization (Khan & Duke, 2001; Kefu et al., 2002).

The present work is a part of a Tunisian project, which aimed at identifying local plant genetic resources of saline ecosystems, presenting potential ecological and/or economical interest. The exploration of the Tunisian coastal areas allowed us to recognize three oilseed halophytes: *Zygophyllum album* (Zygophyllaceae), *Crithmum maritimum* (Umbelliferous), and *Cakile maritima* (Brassicaceae). Oil extraction from *C. maritima* seeds revealed appreciable oil content (ca. 40% on the fresh weight basis). In addition, this oil was rich in erucic acid (Zarrouk et al., 2004), thus potentially appropriate for industrial applications. Alone, the US market demand for this product was estimated at about 18000 metric tons with the majority being imported (Bhardwaj & Hamama, 2003), which gives an indication of the extent of the need for erucic acid.

The purpose of this investigation was to evaluate the variability of seed-oil composition and of fruit and seed mass. Salt distribution among the harvested organs was also determined.

2. MATERIALS AND METHODS

2.1. Description of the species

The mediterranean annual halophyte *C. maritima* achieves its whole life cycle exclusively on coastal sandy dunes and is naturally absent from the inland localities or rocky coasts (Clausing et al., 2000). Nitrogen is required for the development of this succulent plant (Pakeman & Lee, 1991), which shows optimal growth at moderate salt levels (50-100 mM NaCl) and survives even at salinity close to seawater (500 mM NaCl), despite accumulating large amounts of sodium in the leaves (Debez et al., 2004). The fruit consisting of a two-segmented silique (Rodman, 1974), contributes to the plant dispersal when transported away by the wind and waves, owing to its long-term floating ability (Barbour & Rodman, 1970; Maun & Payne, 1989).

2.2. Sampling sites and plant sampling methodology

Though characterized as mediterranean, Tunisian climate displays multiple bioclimatic stages, ranging from the humid (northern) to the saharan (southern). The semi-arid stage prevails in the main part of the country. Twelve harvest sites were selected along the coast, from the north of the country to the sub-saharan region (Figure 1). Here are the different regions and the corresponding bioclimatic stage characterized by the mean annual rainfall (MAR): Tabaraka (humid, MAR > 1500 mm) for, Bizerte (sub-humid, MAR 1000 mm - 1500 mm), Raoued, La Marsa, Hammamet, Enfidha, Sousse, Monastir and Mahdia (semi-arid with temperate winter, MAR 200 mm - 700 mm), Sfax, Jerba and Zarzis

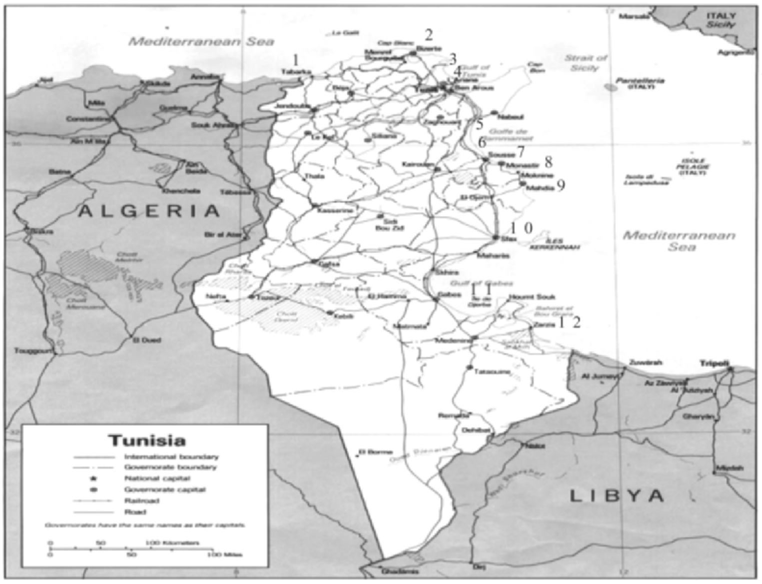

Figure 1. *General map of Tunisia showing the different sampling sites of* C. maritima *seeds. The numbers correspond to the following accessions: 1- Tabarka, 2- Bizerte, 3- Raoued, 4- La Marsa, 5- Hammamet, 6- Enfidha, 7- Sousse, 8- Monastir, 9- Mahdia, 10- Sfax, 11- Jerba, and 12- Zarzis.*

(Arid with temperate winter, MAR 100 mm - 200 mm). Mature siliques and desiccated twigs of C. maritima were harvested between August and October 2001.

2.3. Fruit and seed mass

Samples (n = 300) from each accession were used to estimate fruit and seed mass variability. Masses of each silique and of the corresponding seed manually extracted from the fruit were determined with a Mettler microbalance.

2.4. Ion relationships

Na^+ contents were determined by flame emission photometry on 0.5 % HNO_3 extracts (Corning, UK). Chloride was assayed by coulometry (Büchler chloridometer) on the same extract. Five replicates of the different plant organs (twigs, siliques, and seeds) were used.

2.5. Lipid analysis

Seed total lipids were extracted as described by Allen & Good (1971), with methanol/chloroform/water (1:1:1, vol/vol/vol). According to Mangold (1964), triacylglycerols (TAG) were separated by thin layer chromatography (TLC), using silica gel plates (Merck G 60). Fatty acid methyl esters were prepared (Metcalfe et al., 1966) and analyzed by Flame Ionization Detector-Gas Chromatography using a Hewlett-Packard model 4890 D with a HP innowax (30 m x 0.25 mm) capillary column. The column was maintained isothermally at 210°C . Fatty acid methyl esters were quantified by adding heptadecanoic acid (17:0) as an internal standard. Results are the means of three samples.

2.6. Statistical analysis

Data were statistically analyzed (one-way ANOVA) using the SPSS. Duncan post-hoc test was carried-out if significant differences at $P < 0.05$ occurred among the accessions.

3. RESULTS

3.1. Seed-oil composition

Significant differences among the accessions were found for the seed-oil content, with values ranging from ca. 25% to 39% on the fresh weight basis (Figure 2A), thus confirming the oleaginous potential of this halophyte. Seeds harvested from the humid and sub-humid regions (respectively Tabarka and Bizerte) exhibited the highest seed-oil contents (38.8%), whereas the lowest level was found in La Marsa accession (25.4%). Seed-oil percentage in the remaining provenances was important, ranging from ca. 32% for Raoued and Zarzis to 37% for Monastir.

Saturated, monounsaturated and polyunsaturated fatty acids were found in *C. maritima* seed-oil (Table 1). Regardless of the sampling site, erucic acid (22:1) was the most abundant fatty acid, accounting for 25% to 32% of the total fatty acids. Mahdia (semi-arid region) and Zarzis (arid region) showed the highest erucic acid contents (up to 31.9 %), while the lowest contents (ca. 25 %) were recorded in Hammamet and Raoued (the both from the semi-arid region). Appreciable levels of C_{18} unsaturated fatty acids were found: oleic (18:1), linoleic (18:2), and linolenic (18:3) acid percentages reached up to ca. 25 %, 18 %, and 16 % respectively (Table 2).

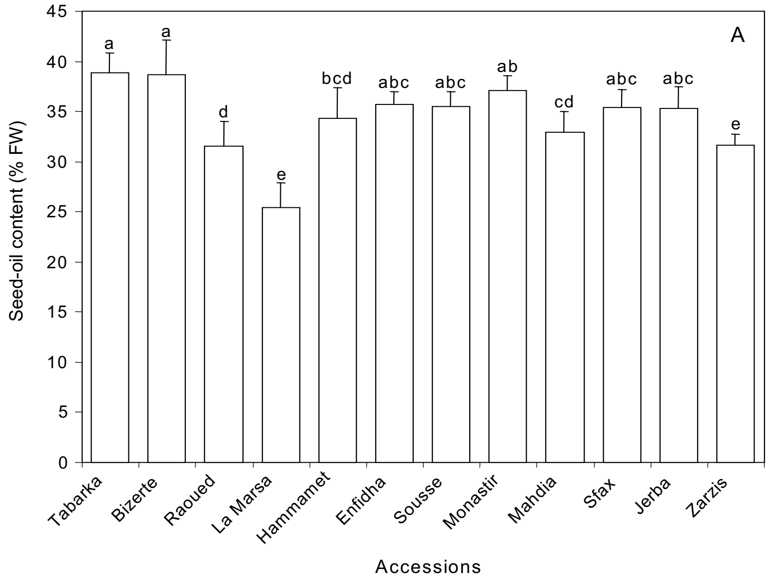

Figure 2a. *Variability of seed-oil content and erucic acid proportion in seed*
Tag. A: Oil content in seeds of C. maritima *accessions.*
Means of 3 replicates ± standard error. Means followed by at least one same letter were not significantly different between the accessions at P < 5 %.

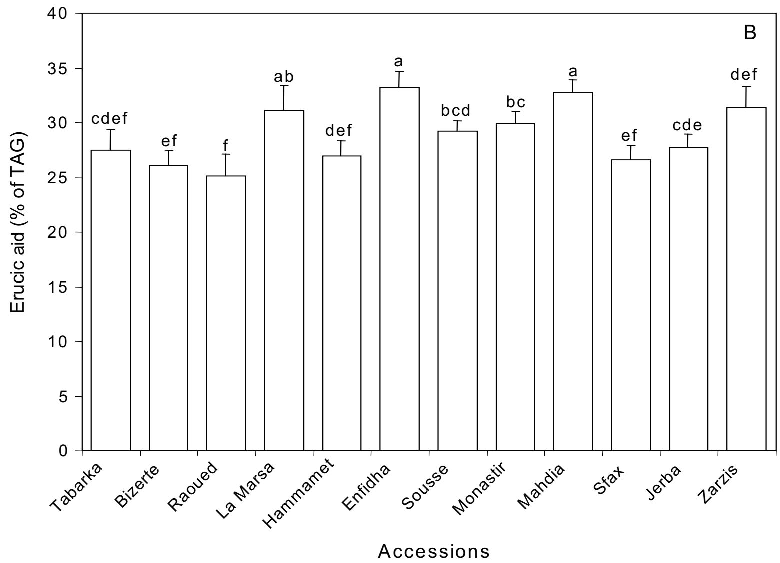

Figure 2b. *Variability of seed-oil content and erucic acid proportion in seed*
Tag. B: Erucic acid percentage in seed of C. maritima accessions. Means of 3 replicates ± standard error. Means followed by at least one same letter were not significantly different between the accessions at P < 5%.

The lipid categories identified were triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG), free fatty acids (FFA), and polar lipids (PL) (Table II). For all the accessions, TAG representing the main form of reserve lipids in oil-seeds, were the major lipid class, contributing for up to 97% of the total lipids. Significant differences were found depending on the sampling site, Jerba being characterized by the highest level (96.9%), unlike Raoued, which showed the lowest one (82.6%). Similarly to what was described for the seed-oil, TAG fatty acid pattern analysis revealed once more the prevalence of erucic acid, which accounted for 25.1% (Raoued) to 33.2% (Enfidha) of this lipid category (Figure 2B).

3.2. Variability of fruit mass and ion relationships

One way-ANOVA statistical analysis revealed significant differences in mean individual masses of fruits, teguments and seeds. The both accessions harvested from the humid and sub-humid bioclimatic regions (Tabarka and Bizerte) were characterized by high seed masses (ca. 7.0 mg), while Sfax accession displayed the heaviest seeds (8.8 mg) (Figure 3). Jerba and Zarzis accessions, belonging to the same bioclimatic stage, but closer to the sub-saharan coast, showed the lowest seed mass (3.5-4.5 mg). This tendency was also true when considering the tegument and silique mass.

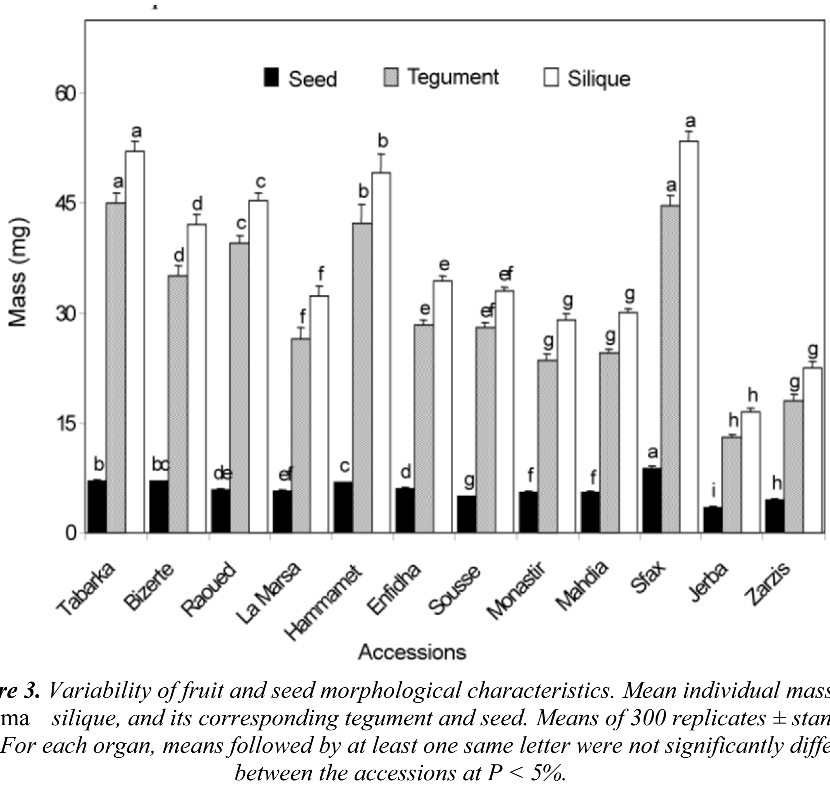

Figure 3. *Variability of fruit and seed morphological characteristics. Mean individual mass of* C. maritima *silique, and its corresponding tegument and seed. Means of 300 replicates ± standard error. For each organ, means followed by at least one same letter were not significantly different between the accessions at P < 5%.*

Table 1. Variability of fatty acid composition in seed-oil among C, maritima accessions. Means of 3 replicates ± standard error. Means followed by at least one same letter were not significantly different between the accessions at P < 5%.

Accession	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
Tabarka	5.71 ± 1.09ab	1.41 ± 0.03a	1.97 ± 0.33ef	19.70 ± 3.83bed	16.70 ± 2.85ab	16.95 ± 2.11de	1.44 ± 0.27de	8.98 ± 1.00ab	1.24 ± 0.17de	25.90 ± 1.89bcd
Bizerte	5.00 ± 0.74abcd	0.72 ± 0.05c	2.34 ± 0.44cde	21.22 ± 3.57b	14.04 ± 2.73cd	17.25 ± 2.75de	1.56 ± 0.47bcde	8.36 ± 1.32abc	1.66 ± 0.27c	27.84 ± 1.19b
Raoued	5.86 ± 0.80a	0.89 ± 0.80b	2.28 ± 0.24cdef	19.95 ± 1.32bcd	16.51 ± 1.11ab	18.05 ± 1.09cd	1.60 ± 0.12abcd	8.88 ± 0.21ab	0.60 ± 0.24g	25.38 ± 1.10cd
La Marsa	4.98 ± 0.34abcd	0.24 ± 0.06fg	2.43 ± 0.23cde	20.19 ± 0.74bc	16.47 ± 0.81ab	15.70 ± 0.58c	1.90 ± 0.41abcd	8.20 ± 1.13bc	2.54 ± 0.22a	27.35 ± 0.53bc
Hammamet	5.92 ± 0.07a	0.40 ± 0.07d	2.21 ± 0.05cdef	15.97 ± 0.13ef	17.60 ± 0.11a	22.29 ± 0.16a	1.41 ± 0.08c	8.32 ± 0.10bc	1.06 ± 0.10ef	24.82 ± 1.86d
Enfidha	4.88 ± 0.16bcde	0.21 ± 0.05fg	2.98 ± 0.08ab	16.00 ± 0.08ef	16.88 ± 0.06ab	21.63 ± `0.17ab	1.62 ± 0.18abcde	8.30 ± 0.18bc	0.74 ± 0.11fg	26.77 ± 1.19bcd
Sousse	5.51 ± 0.35abc	0.26 ± 0.08ef	2.07 ± 0.20def	16.41 ± 0.55ef	17.68 ± 1.2la	19.81 ± 1.31bc	1.48 ± 0.41cde	7.80 ± 0.93bc	1.67 ± 0.46c	27.30 ± 1.49bc
Monastir	5.38 ± 0.65abc	0.27 ± 0.12ef	2.46 ± 0.25cde	18.72 ± 0.38bcde	17.95 ± 0.68a	16.83 ± 1.19de	1.93 ± 0.27abc	8.71 ± 0.34ab	0.75 ± 0.10fg	27.01 ± 1.52bcd
Mahdia	4.71 ± 0.26cde	0.24 ± 0.05fg	1.86 ± 0.21f	15.69 ± 1.34f	14.98 ± 1.13bcd	20.18 ± 1.45ab	1.56 ± 0.18bcde	7.32 ± 0.58c	1.58 ± 0.24cd	31.89 ± 1.23a
Sfax	4.11 ± 0.43de	0.15 ± 0.02g	2.44 ± 0.36cde	17.73 ± 1.05cdef	12.69 ± 1.01cd	21.71 ± 1.46ab	1.49 ± 0.27cde	8.61 ± 0.09abc	0.98 ± 0.10efg	30.10 ± 1.35a
Jerba	3.97 ± 0.45c	0.43 ± 0.04d	3.09 ± 0.39a	24.55 ± 1.94a	16.57 ± 1.63ab	12.80 ± 1.20f	2.05 ± 0.24a	9.68 ± 0.82a	2.22 ± 0.46a	24.64 ± 1.40d
Zarzis	5.23 ± 0.62abc	0.34 ± 0.04de	2.61 ± 0.30bc	17.04 ± 1.13def	14.66 ± 1.27bcd	16.97 ± 0.65de	2.10 ± 0.19ab	8.11 ± 0.69bc	2.17 ± 0.34ab	31.21 ± 1.73a

Table 2. Variability of lipid categories proportion in seed-oil among C, maritima accessions. Means of 3 replicates ± standard error. Means followed by at least one same letter were not significantly different between the accessions at P < 5%.

Accession	TAG	DAG	MAG	FFA	PL	Total
Tabarka	91.63 ± 1.27b	1.73 ± 0.25e	1.41 ± 0.35d	0.55 ± 0.12f	4.67 ± 0.7ed	100
Bizerte	88.90 ± 1.61ed	3.23 ± 0.33c	4.08 ± 0.41a	2.82 ± 0.31d	0.97 ± 0.40f	100
Raoued	82.62 ± 2.72e	2.73 ± 0.58d	3.47 ± 0.20a	4.50 ± 0.71e	6.42 ± 1.70b	100
La Marsa	95.65 ± 1.71a	1.39 ± 0.06ef	0.54 ± 0.06ef	0.91 ± 0.08f	1.51 ± 0.23f	100
Hammamet	91.32 ± 2.09bc	1.45 ± 0.07ef	1.74 ± 0.09cd	1.69 ± 0.13e	3.80 ± 0.11de	100
Enfidha	91.27 ± 1.55bc	2.89 ± 0.17cd	0.86 ± 0.10e	1.92 ± 0.11e	3.07 ± 0.13e	100
Sousse	84.17 ± 1.78e	2.44 ± 0.31d	0.88 ± 0.38e	4.21 ± 0.42c	7.68 ± 0.58a	100
Monastir	92.01 ± 1.55b	1.76 ± 0.31e	0.70 ± 0.11e	1.56 ± 0.23e	3.98 ± 0.19cd	100
Mahdia	88.13 ± 1.19d	4.73 ± 0.51b	2.18 ± 0.33b	1.91 ± 0.20e	3.06 ± 0.32e	100
Sfax	84.30 ± 1.36e	5.22 ± 0.51a	0.91 ± 0.09e	3.41 ± 0.68cd	6.15 ± 0.60b	100
Jerba	96.86 ± 1.64a	0.53 ± 0.07g	0.21 ± 0.05f	0.61 ± 0.09f	1.80 ± 0.11f	100
Zarzis	84.63 ± 1.45e	1.19 ± 0.16f	2.11 ± 0.39bc	7.15 ± 0.54a	4.92 ± 0.51e	100

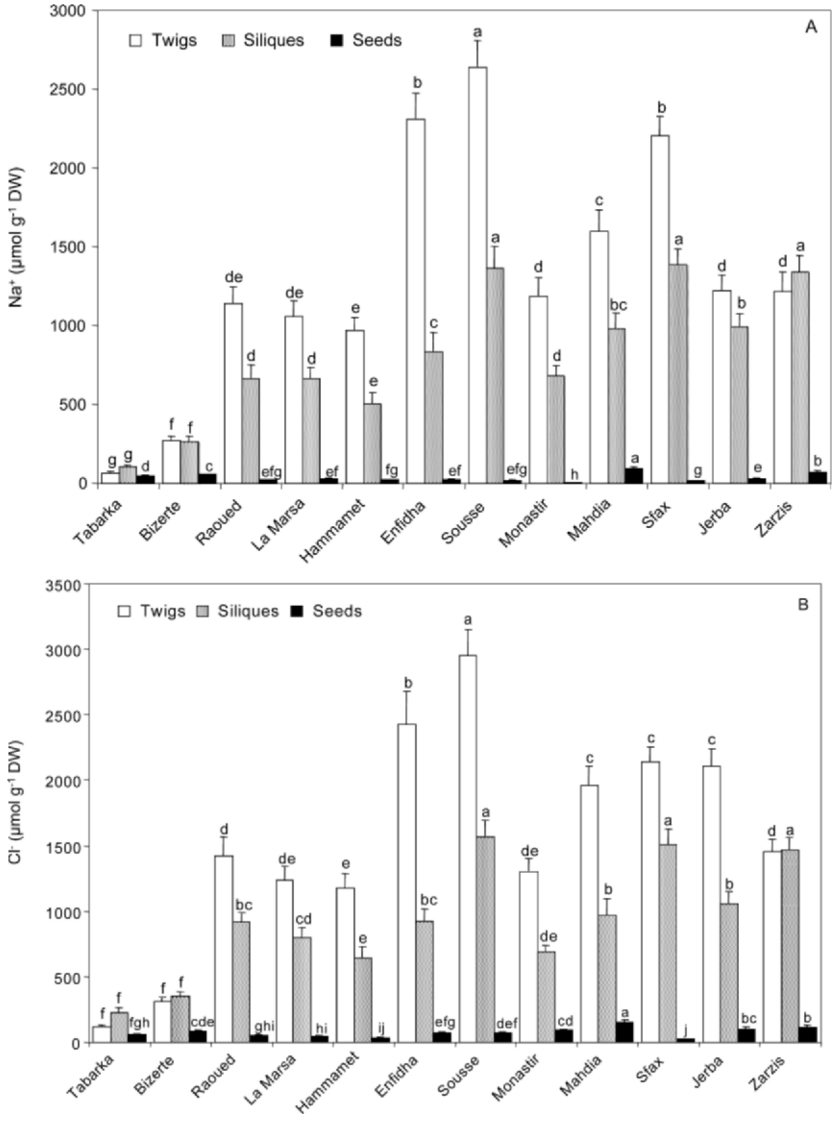

***Figure 4.** Na^+ and Cl^- distribution pattern in C. maritima organs.*
*A**:** Sodium accumulation in desiccated twigs, siliques and seeds of* C. maritima *accessions. Means of 5 replicates ± standard error. For each parameter, means followed by at least one same letter were not significantly different between the accessions at P < 5%.*
*B**:** Chloride accumulation in desiccated twigs, siliques and seeds of C. maritima accessions. Means of 5 replicates ± standard error. For each organ, means followed by at least one same letter were not significantly different between the accessions at P < 5%.*

Significant variation in Na^+ and Cl^- accumulation pattern within C. maritima organs occurred between the twelve accessions (Figures. 4A & 4B). In addition, irrespective of the accession, the lowest sodium or chloride contents were registered in the seeds, in comparison with the levels accumulated in the shoots or in the silique teguments. This constitutes a strong evidence for the ability of *C. maritima* to protect the reproductive organs from the harmful salt accumulation. As shown by figure 4A, Na^+ amounts reached 64.8 and 118.3 µM g^{-1} DW in the desiccated twigs and siliques from Tabarka respectively, unlike the arid and semi-arid area accessions, especially Sousse which exhibited the highest Na^+ amounts in the twigs and siliques (ca. 2645 and 2955 µM g^{-1} DW respectively). Seeds collected from Mahdia were the most salt-charged (93.1 µM g^{-1} DW). Similar trends were found for chloride levels (Figure 4B).

4. DISCUSSIONS AND CONCLUSION

Our data show that *C. maritima* is characterized by great intra-specific heterogeneity, which seems to be accession-dependent. Furthermore, this halophyte could be considered as a promising crop with regard to its seed-oil content. Indeed, its seeds contained up to 39% of oil, a level close to that of several conventional oleaginous species like *Carthamus tintorius*, *Sesamum indicum* (Karleskind, 1996), and some rapeseed genotypes (Bhardwaj & Hamama, 2003). This Brassicaceae showed higher seed-oil contents when compared with other halophytes, among which *Mesembryanthemum crystallinum* (ca. 8%) (Pasternak et al., 1985), Salicornia *bigelovii* Torr. (ca. 30%) (Glenn et al., 1991; Bashan et al., 2000), 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).

Previous studies on *C. maritima* ecotypes from Morocco and Australia showed the existence of such a variability in seed-oil content, with up to 49 % for the former accession (Pasternak et al., 1985) versus ca. 35% for the latter (Hocking, 1982). This intra-specific variability in the amount and the quality of the extracted oil could be related to environmental factors, such as precipitation (Rana & Ahmed, 1981; Pannelli et al., 1994), temperature (Champolivier & Merrien, 1996), and salinity (Zarrouk & Cherif, 1983). Seed-oil of *Cakile maritima* contained high proportions of erucic acid (22:1) (25% to 32%), a compound considered as anti-nutritional for both humans and animals (Rajcan et al., 1999), but widely used in the industry (Domergue et al., 1999;Kaushik. & Agnihotri, 2000).

Seed size was close to that of conventional crops like mustard, winter colza and sunflower (Karleskind, 1996). The smallest fruits and seeds were produced by the arid zone accessions (Jerba and Zarzis), particularly contrasting with those from the humid and sub-humid areas (Tabarka and Bizerte). This corroborates previous studies, which showed such diversity within *C. maritima* populations harvested from central Europe or from Santa Cruz (California) (Barbour, 1970;

Maun & Payne, 1989). It was suggested that seed and fruit variability might improve the ability of halophytes to establish and cope with fluctuating conditions in their native environments (Mandák, 2001). In this way, Maun & Payne (1989) showed the presence of a morphological variability for *Cakile maritima* silique and seed mass. This variability was correlated with the dispersal ability, seedling emergence, and more generally, the plant response. A further dimorphism was reported for seed size in relation with their position on the fruit (Barbour, 1970).

Na^+ and Cl^- ions were not uniformly accumulated between the vegetative organs (twigs), silique teguments and seeds of *C. maritima*, their repartition within the plants organs showing the existence of a decreasing gradient from the twigs to the seeds. Indeed, the twig:silique:seed Na^+ accumulation ratios reached up to 176:99:1 for Monastir accession, while those of Cl^- establishing at 85:60:1 for Sfax accession. Barbour (1970) found a similar pattern of Na^+ and Cl^- repartition among the above cited organs of *C. maritima*, with values reaching 359:74:1 for sodium and 200:30:1 for chloride. Thus, ion relationship investigation confirmed the aptitude of *C. maritima* to control salt repartition within its different tissues, avoiding the harmful salt accumulation in the reproductive organs. This is of high ecological adaptive significance, since it determines the seed viability and thus the species establishment and distribution in saline biotopes. However, the amplitude of the shoot-silique-seed Na^+ or Cl^- accumulation ratios would traduce variability in the efficiency of this high ecological significance feature. This suggests also that the studied populations cope with different salinity levels in their original ecosystems.

The silique salt charge has been reported to impair the germination of this Brassicaceae and could partially explain the salt-induced seed dormancy (Hocking, 1982), which would also require salt leaching from the soil to germinate in its natural habitats (Ungar, 1978). More recently, we showed that seed germination of *C. maritima* was inhibited by salinities higher than 200 mM NaCl. Nevertheless, seeds remained viable and fully recovered their germination capacity once transferred to distilled water (Debez et al., 2004). On the other hand, avoiding salt accumulation in the fruits seems decisive for the use of crop plants in saline conditions. Indeed, Brassica plants overexpressing AtNHX1, a gene enabling salt accumulation in leaves and not in the seeds, were able to grow with unaffected seed yield and seed-oil quality up to 200 mM NaCl (Zhang et al., 2001).

Finally, *C. maritima* is potentially a HEA-crop though high intra-specific variability occurred. The preliminary field yields estimations (ca. 2 tons ha^{-1} for Raoued accession), the salt tolerance capacity of this species, and its ability to limit salt accumulation in the seeds in particular, are encouraging elements for the future exploitation of this halophyte. Several parameters (the financial profitability of the farming systems, the efficiency of salt uptake by the plants, and long-term salt accumulation in the root-zone and the watertable) still need to be taken in account for a reliable evaluation of the sustainability of the introduction of this halophyte as an alternative crop (Barrett-Lennard, 2002; Bañuelos et al., 2003). In this way, large-scale field trials with the most interesting accessions and the impact of the irrigation with salty water on the yield, the seed viability, and the seed-oil quality of *C. maritima* are currently being investigated.

5. ACKNOWLEDGEMENTS

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