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Full Length Research Paper

Anatomy of the fruit of the halophyte *Crithmum maritimum* L. with emphasis on the endosperm structure and histochemistry

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The halophytes are plants that can survive and reproduce under high salinity. They show high potentiality as new crops plant for biosaline agriculture. *Crithmum maritimum* L. (Apiaceae) is one of the promising halophytes. In this paper, the endosperm structure of the fruit of this oilseeds halophyte was investigated using scanning electrons microscopy (SEM), light microscopy (LM) and fluorescence microscopy (FM). The fruit was composed of a spongy outer coat, a secretory envelope, a thin endocarp reduced to a unicellular layer delimiting the endosperm and an embryo. The endosperm cell appeared limited by thick cell wall and filled with numerous reserve globoids. The histochemical test showed that the cell wall of the endosperm was rich of carbohydrates as revealed by PAS (periodic acid-schiffs). Within the endosperm cells, there were mainly lipid bodies and protein bodies. The starch grains were less abundant. The protein bodies enclose crystal globoids. The x-ray microanalysis revealed that the reserve globoids accumulated mostly Mg, K, Ca, S and P. Taken together, these results highlight the structural features, the biochemical composition and confirm the nutritional quality of *C. maritimum* L. fruit.

Keys words: ***C. maritimum*** L., crystal globoids, the endosperm cells, histochemical test, protein bodies, x-ray microanalysis.

INTRODUCTION

In arid regions, desertification, soil salinization and water shortage constitute the common problems due to a variety of natural and human caused factors (Kinet et al., 1998; Khan and Qaiser, 2006; Koyro et al., 2008). In these areas, the salt-affected soils contain high concentrations of soluble salts that reduce the growth and the productivity of the most conventional crop species (Koyro et al., 2008). Studies conducted by the United Nations Food and Agriculture Organization (FAO, 2005) estimated that an additional 200 million hectares (ha) of agricultural land will be required over the next 30 years. Thus, the research of a new species to be used in salt affected areas to overcome the mentioned problems is needed (Atia et al., 2010). It is well known that in these areas there are specific plants that acquire a specific adaptation which permit them to pass easily the problems related to moisture deficit stress and soil salinization. They were named halophytes. They can tolerate and reproduce viable seeds at concentration not lower than 200 mM NaCl (Flowers and Colmer, 2008). Several species of halophytes show high economical potentiality and have been selected for economical uses. ***Crithmum maritimum*** L. (Apiaceae) is one of the promising halophytes species. This is a perennial species and thrives along rocky coastal ecosystems; this have the capacity to maintain their growth potential up to 300 mM (Ben Amor et al., 2005). ***C. maritimum*** L. is potentially useful for economical and medicinal purposes. Indeed, its leaves display high antioxidant and antimicrobial activities (Meot-Duros et al., 2008; Meot-Duros and Magné, 2009). The fruits of ***C. maritimum*** L. show high accumulation of

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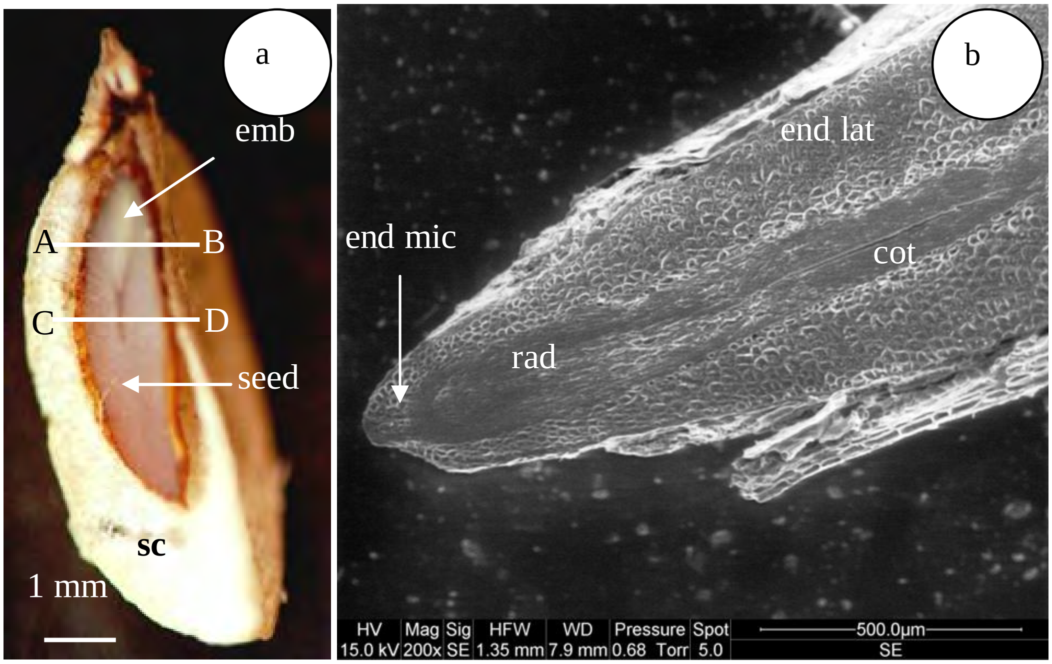

Figure 1. (a) Stereomicroscope views of the longitudinal section on the mericarps showing the spongy coat, the seeds and the embryo; (b) SEM view of longitudinal section at the embryo region showing the endosperm micropylar (end mic), the cotyledon (cot), the endosperm lateral (end lat) and the radicle (rad). Sc, spongy coat; emb, embryo.

essential oils and other biological active compounds (Atia et al., 2009a). They contain up to 44% DW of lipids and show good oils composition that close up to olive oil composition (Atia et al., 2010). Consequently, this species have potential as a saline water-irrigated oilseed crop (Atia et al., 2009b). This species was traditionally consumed by human. In vegetable tissues, the nutriaments that are used in human and/or animal diet is in large part localised in the seed or fruit tissues, in the form of carbohydrates, lipids, proteins, organic phosphates and various inorganic compounds (Bewley and Black, 1983; Coimbra and Salema, 1994). The fruit of ***C. maritimum*** L. is a schizocarp divided into two mericarps; each one contains one endospermic seed. The endosperm tissue of ***C. maritimum*** L. is rich in oil globoids (Atia et al., 2010). The fruit of ***C. maritimum*** L. contains significant amounts of oil, up to 44.4% (w/w), potentially edible due to its fatty acid composition close to olive oil (Atia et al., 2010). Yet, more details about the fruit structure were needed such as the localisation of the proteins, the carbohydrates and the inorganic compounds. Thus, a microscopic study was conducted to more highlight the structure of ***C. maritimum*** L. fruit.

MATERIALS AND METHODS

Mature fruits were collected in December 2008 from plants in the natural population growing in the rocky coast of Tabarka (N 36°57' 12" E 08°45' 18"), located in N-W of Tunisia.

Observations of free-hand sections of seeds were carried out by scanning electron microscope (SEM; type FEI Quanta 200). For light microscopy, fixed materials were used. Fruits were fixed in 1% (w/v) paraformaldehyde solution. Then, they were dehydrated in an ethyl alcohol series, infiltrated and embedded in paraffin block. Embedded tissues were then sectioned with microtome. 0.5 µm thick sections were obtained. The histochemical tests of periodic acid-schiffs (PAS) test were used for polysaccharides (Miller et al., 1999). Light green dye and toluidine bleu (TBO) solutions were used for protein bodies' localisation (Miller et al., 1999). The prepared slides were observed under light microscope (Olympus DX41).

For fluorescence microscopy, free-hand sections were prepared and stained with a 0.001% (w/v) neutral red solution for nucleic acid (DNA) (Dubrovsky et al., 2006), with a 0.1% (w/v) KOH solution for flavonoids (Tzobanoglou et al., 2001) and fuchsin acid for proteins (Fulcher and Wong, 1980).

The x-ray spectra for the compositional analyses were performed for the following elements: K, Mg, Ca, P and S. The scanning electron microscope (SEM) FEI Quanta 200, equipped with x-ray (EDAX) system for microanalysis, was used at 15 kV with a working distance of 10 to 11.4 mm.

RESULTS

The SEM and stereomicroscope observations showed that the fruit of ***C. maritimum*** L. was composed of a spongy outer coat, a secretory envelope, a thin endocarp, which constituted the seed envelopes that delimited the endosperm and an embryo (Figure 1a). Although the latter was very small in size, it was completely differentiated on radicle and cotyledons (Figures 1b and 2a). The SEM view of a transversal section of the fruit showed

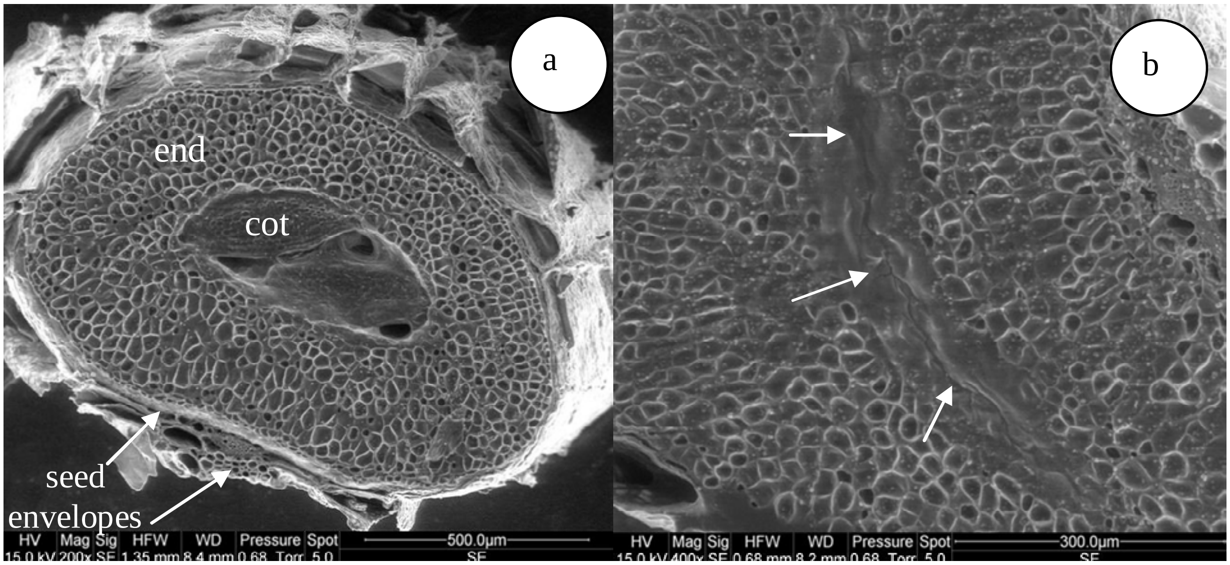

Figure 2. (a) Transversal section at AB level showed in Figure 1 a; this section shows the embryo cotyledons; (b) transversal section at CD level showed in a, showing the endosperm cracks (arrows) that allow the immature embryo elongation.

a crack in the endosperm (Figure 2b). The endosperm cells was limited by a thick cell wall and filled with numerous reserve globoids (Figure 3a). We noted that after distilled water imbibition of the fruit, the cell wall appeared more flexible and the reserve globoids were liberated (Figure 3b).

The histochemical tests revealed that the cell wall of the endosperm was rich with carbohydrates as revealed by periodic acid-schiffs (PAS) coloration (Figure 4a). Some starch grains were also stained by PAS within the cells (Figure 4b). The light microscope observations, after green light dye staining showed that the cell wall was rich in proteins (Figure 4c). The endosperm tissue was filled with the protein bodies which took green coloration and enclosed crystal globoids (Figure 4c). The toluidine bleu stained sections showed that protein matrix of the crystal globoids was stained light (Figure 4b).

The fluorescence microscope observations of the fuchsin acid stained section confirmed the richness of the endosperm cells by the protein bodies (Figure 5a, b). Each endosperm cells contained an intact DNA as revealed by neutral red coloration (Figure 6a, b). The fluorescence microscopic observations revealed the richness of ***C. maritimum*** L. with oil globoids and the richness of the endosperm tissue with flavonoid compounds that appeared as fluorescent corpuscles after KOH staining (Figure 6c, d).

The x-ray microanalysis revealed that globoid reserves accumulated mostly Mg, K, Ca, S and P (Figure 7).

DISCUSSION

Localization of stored reserves inside the ***C. maritimum*** L. fruit was achieved. The fruit was composed of a spongy outer coat, a secretory envelope and a thin endocarp, which constituted the seed envelopes that delimits the endosperm and the embryo which was very small in size. Therefore, the ***C. maritimum*** L. embryo was immature and exhibited a morphological dormancy. Upon imbibetions, the embryo must elongate within the seed through the endosperm crack. This is common in Apiaceae fruit; the embryo is often rudimentary and embedded in the endosperm and exhibits a morphological dormancy (Nikolaeva, 1977; Baskin and Baskin, 2004). Thus, the endosperm is the major part of the seed and constitutes the reserve tissue (Atia et al., 2010). In ***C. maritimum*** L., the endosperm cells appeared limited by thick cell wall and filled with numerous globoid reserves. In angiosperm seeds, the reserve material may be stored in embryo, mainly in the cotyledons or in extra-embryonic tissues mainly in the endosperm or in the perisperm or in both. For instance, in the Leguminosae, and the Cruceferae, the main storage tissue is the cotyledon, in the cereals, Euphorbiaceae and the Apiaceae, the storage tissues is the endosperm (Bewley and Black, 1983) in the Chenopodiacae, the main storage is the perisperm (Prego et al., 1998). In ***C. maritimum*** L., the endosperm accumulates high level of lipids in the oil bodies (Atia et al., 2010). The histochemical tests revealed that the cell wall of the endosperm was rich in carbohydrates as revealed by PAS. Many starch grains were also found within the endosperm cells, however, they were less abundant in comparison with other reserve forms. In ***C. maritimum*** L. endosperm, the carbohydrates were localised in the cell wall. This is common in the Apiaceae. In these species the carbohydrates were mainly localised in the cell wall of the endosperm in form of

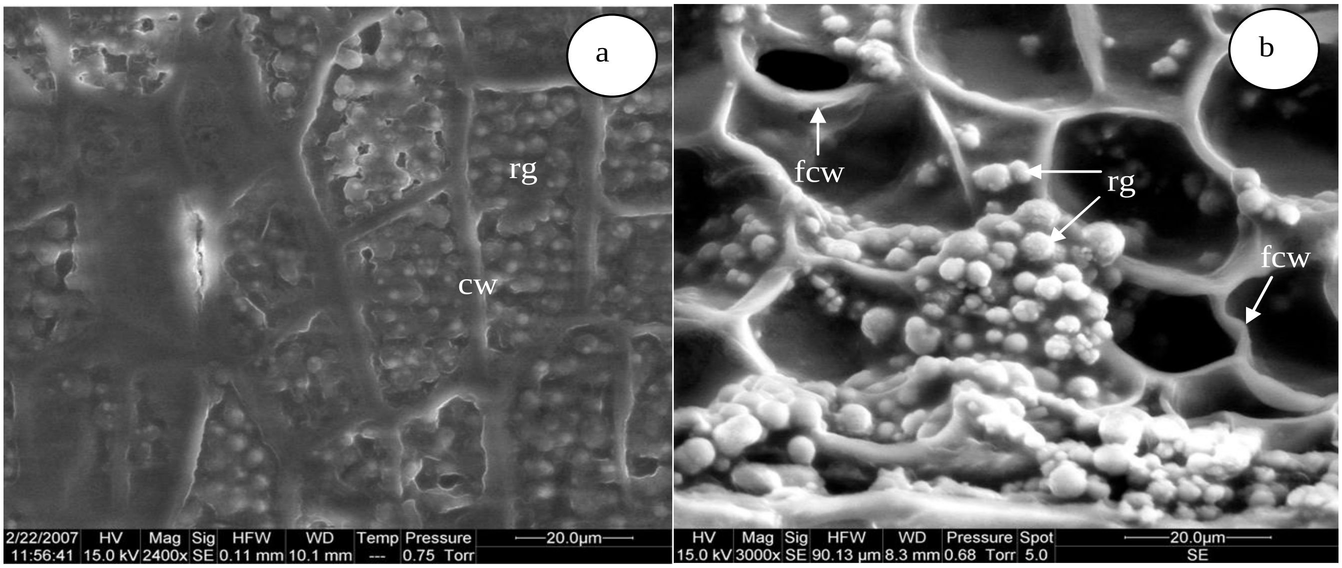

Figure 3. SEM views of the endosperm tissues. (a) SEM view of dry seed section showing a detail view of the endosperm cells which appeared filled with the globoid reserves (rg) and showing a thick and powerful cell wall (cw); (b) SEM view of the endosperm cells of imbibed seed showing the liberation of the globoid reserves (rg) and the flexible cell wall (fcw).

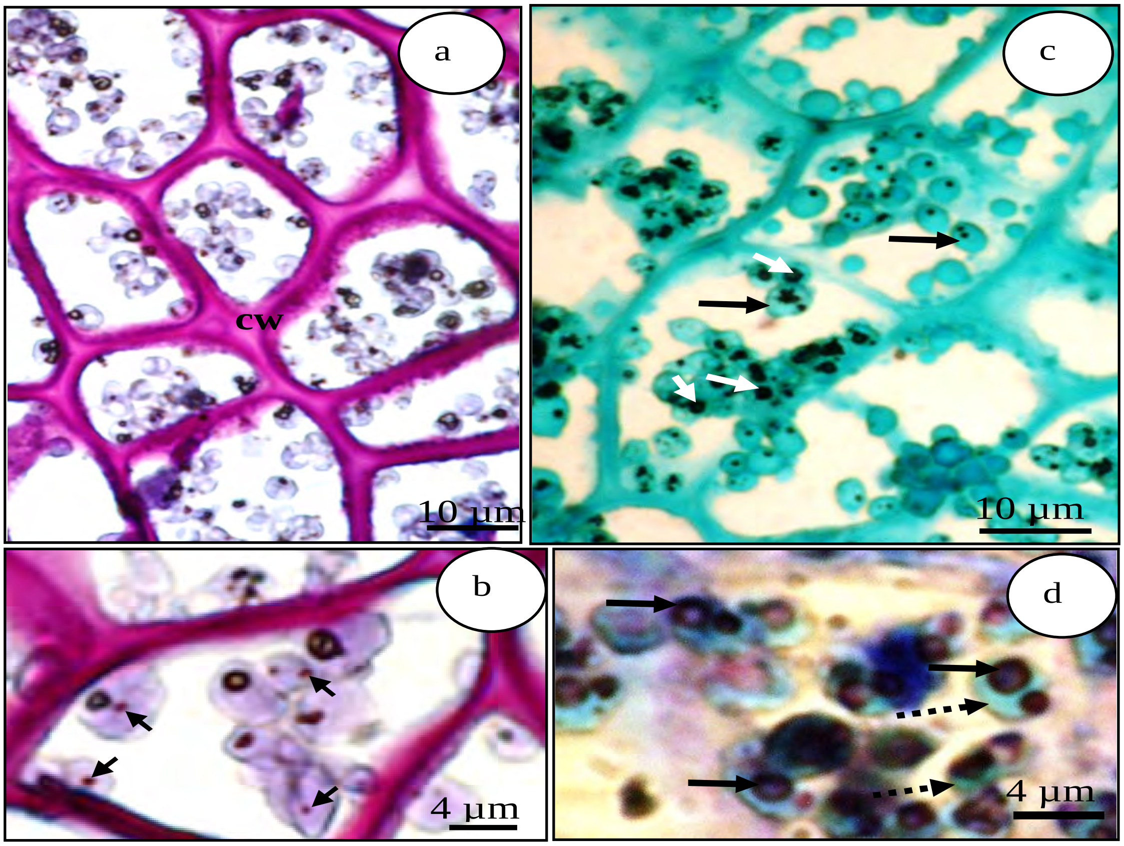

Figure 4. Light microscope observations of the endosperm tissues. (a) Localisation of the cell wall (cw) polysaccharides after coloration with PAS; (b) localisation of starch grains (arrows) after coloration with PAS; (c) localisation of proteins bodies that took green colour after light green coloration (black arrows) and the globoid crystals were dark stained (white arrows). The cell wall (cw) also appeared green; (d) toluidine bleu coloration showing the protein matrix of the crystal globoids that are light bleu stained (discontinuous arrows) and the globoid crystals (continuous arrows).

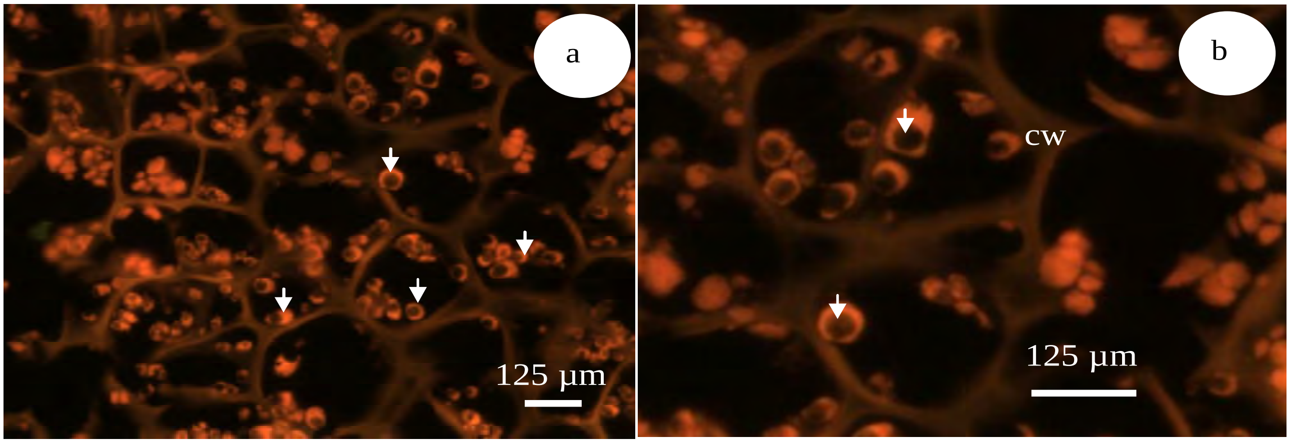

Figure 5. Fluorescence microscopy observations of endosperm tissues. (a) Fuschine acid stained section that reveals the protein bodies (arrows); (b) details view of the crystal globoids (arrows). Note that the crystal globoids which were localised within the protein bodies did not take the coloration.

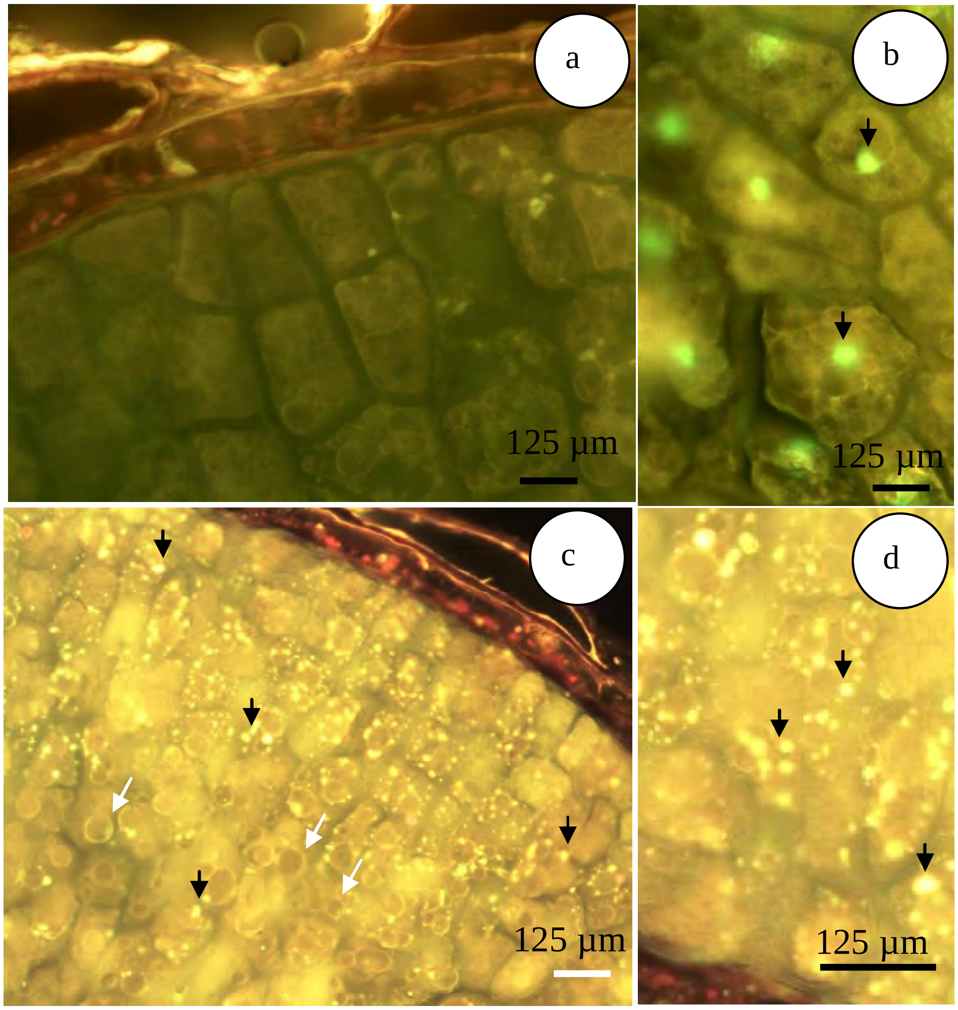

Figure 6. Fluorescence microscopy observations of the endosperm tissues. (a) Autofluorescence of the endosperm cells; (b) observation of the neutral red colored section showing the DNA within endosperm cells; (c) observation of KOH stained section that reveal the flavonoids (Black arrows) and the oil globoids (white arrows); (d) detail view of KOH stained section that reveal the flavonoid corpuscles (arrows).

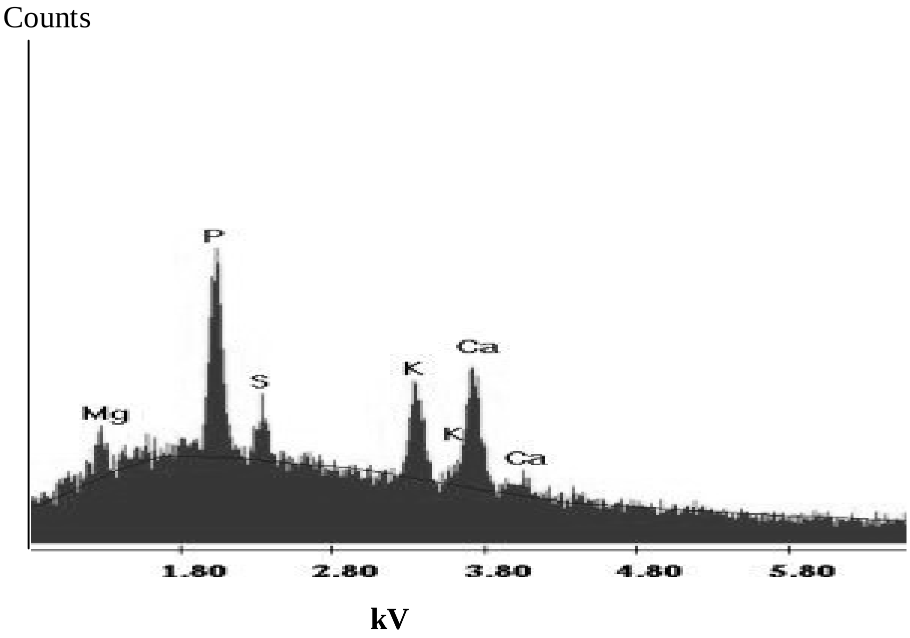

Figure 7. X-ray microanalysis showing the x-ray spectra derived from reserve globoids of the endosperm cells of ***C. maritimum*** L. fruit for K, Mg, Ca, P and S.

of galactomannanes and glucomannanes (Bewley and Black, 1994). The photonic microscopic observations showed that the endosperm tissue was filled with protein bodies that enclose crystal globoids. This confirms the richness of ***C. maritimum*** L. fruit with inorganic compounds. The accumulation of the oil reserves was accompanied by high accumulation of protein bodies in ***C. maritimum*** L. fruit. This is also observed in some oleaginous species such as ***Brassica napus*** (Bewley and Black, 1983). In angiosperms, the proteins are accumulated mainly in reserve globoids (Prego et al., 1998). Storage proteins, which serve as source of carbon, nitrogen and sulfur, reach 90% of the total protein fraction in mature seeds (Kumamaru et al., 2007). The richness of seeds of any species on protein reveals its economic value. For instance, the seed of ***Chenopodium quinoa*** L. was valorised for its high level of proteins (Prego et al., 1998; Konishi et al., 2004). The photonic microscopic observation showed that the protein bodies enclosed crystal globoids. The x-ray microanalysis revealed that the reserve globoids accumulated mostly Mg, K, Ca, S and P. These results highlight the mineral composition of ***C. maritimum*** L. fruit and confirm their richness with essential elements and inorganic compounds. The same composition was also observed for ***Beta*** v***ulgaris*** L., ***Coffea arabica*** L., ***Zostera capricorni*** L. and ***C. quinoa*** L., namely P, K, Mg and Ca (West et al., 1995; Prego et al., 1998). The richness of ***C. maritimum*** L. fruit with sulphur may indicate their richness with sulphured amino acids.

This study showed a significant accumulation of flavonoids in the endosperm. These compounds are known to exhibit some antioxidant and antimicrobial propriety. They inhibit and kill many bacterial strains, and inhibit some viral enzymes, such as reverse transcriptase and protease (Havsteen, 2002). Furthermore, they are none toxic to human cells. Thus, flavonoids are major functional components of many herbal preparations for medical use. In food, the daily intake of flavonoids especially fruits and vegetables are needed for a good human health (Havsteen, 2002). In previous study, we showed that ***C. maritimum*** L. fruit envelope accumulate several forms of biological active compounds (Atia et al., 2009a). The aerial parts of this species were known to accumulate the same molecules (Meot-Duros et al., 2008; Meot-Duros and Magné 2009).

Taken together, these results give new insight about the structural features of the fruit of the oil seed halophyte ***C. maritimum*** L.; the localisation of the protein bodies, the crystal globoids, the polysaccharides and the mineral composition which highlights the biochemical composition and confirms the nutritional quality of the ***C. maritimum*** L. fruit.

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