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Tetraena mongolica Maxim can accumulate large amounts of triacylglycerol in phloem cells and xylem parenchyma of stems

Geliang Wang, Qingqing Lin, Yinong Xu *

Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China

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Abstract

Tetraena mongolica Maxim is a narrowly monotypic genus of Zygophyllaceae found in a very limited area in the western part of Inner Mongolia, China. The plant is called "oil firewood" and its stems and branches are used as fuelwood. As triacylglycerol (TAG) is the main component of the plant oil, the TAG content was analyzed, as were the distribution of oleosomes in different tissues of the stem. This was in order to ascertain whether the term "oil firewood" referred to this storage lipid. Stems of *T. mongolica* indeed contained high levels of TAG (approximately 46 mg/g of dry matter or DM). The concentration of TAG in phloem (90 mg/g of DM) was much higher than that in xylem (20 mg/g of DM), and semi-thin sections stained by Sudan Black B showed that almost all cells in the phloem contained oleosomes whereas in the xylem, oleosomes were found only in parenchymatous cells. These results suggest that *T. mongolica* has a high capacity to accumulate TAG in its stem cells.

Keywords: *Tetraena mongolica* Maxim; Zygophyllaceae; Endemic plants; Phloem; Xylem; Triacylglycerols; Oleosomes

1. Introduction

Tetraena mongolica Maxim, a relic of the Tethys sea-tropical floristic region, is a member of the narrowly monotypic genus of Zygophyllaceae endemic to the western Gobi desert of Inner Mongolia, China (Qian and Chen, 2004). *T. mongolica* appears to be the dominant species in the area, which harbors many ancient plants (Xu et al., 2002). *T. mongolica*, a xerophytic plant with a fully developed root system, is particularly well adapted to its habitat, and can cope with drought and other harsh natural conditions. The plant is a member of an ecologically important group of shrubs in the region, and plays an important role as a windbreak, as well as in stabilizing sand and in conserving soil and water (Zhang et al., 2003). However, because of ovule abortion, *T. mongolica* has virtually lost its ability to set seeds (Wang et al., 2001): the coefficient of seed propagation is as low as 1.26–2.8% (Xu et al., 2003a). The population of *T. mongolica* has declined dramatically over the last few decades because of deterioration of the environment and destruction by people (Zhang et al., 2003) – the species is on the verge of extinction.

T. mongolica, locally referred to as "oil firewood", is used as fuelwood because its branches and stems burn well even while fresh. This suggests that the branches or stems of *T. mongolica* contain some combustible substances, such as lipids. Therefore, it was worth while to investigate whether lipids, such as triacylglycerols (TAG), are involved in the phenomenon.

TAG, a major constituent of oil, serve as storage lipids in many plant species in the form of subcellular particles, the so-called oleosomes (or oil bodies), to be mobilized as needed during periods of active metabolism (Huang, 1996; Tzen et al., 1993); they are synthesized and stored

Abbreviations: TAG, triacylglycerol; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; PrS, primary stems; MeS, medium stems; PeS, perennial stems.

* Corresponding author.
E-mail address: yinongxu@ibcas.ac.cn (Y. Xu).

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at high concentrations mainly in seeds. During seed germination and the growth of seedlings, the stored TAGs are hydrolyzed and serve as a source of carbon and energy (Rawsthorne, 2002; Tzen and Huang, 1992). In higher plants, TAGs are also be found in other tissues and organs, such as the anther, tapetum (Hsieh and Huang, 2004), and phloem in *Brassica napus* (Madey et al., 2002), but only in small amounts. Recently, Piispanen and Saranpaa (2002) reported that sapwood of Scots pine contained significant amounts of TAG (approximately 26 mg/g of DM). These results suggest that stem tissues in some trees accumulate high levels of storage lipids.

In this work, we analyzed the content and distribution of both TAG and oleosomes in different stem tissues of *T. mongolica*. The results showed that cells of phloem and parenchymatous cells of xylem contained high levels of TAG.

2. Results and discussion

2.1. TAG content in whole stems and stem tissues of *T. mongolica*

First, we determined the TAG content in different organs – roots, stems, and leaves – of *T. mongolica* by gas chromatography after extracting and separating the lipids. Only stems contained high amounts of TAG. We then analyzed the TAG content in stems of different ages. As seen in Fig. 1, stems of all ages contained large amounts of TAG (approximately 46 mg/g of dry matter or DM). These results suggest that the content of TAG is independent of the age of the stem – stems of all ages have the capacity to accumulate TAG.

A typical stem of *T. mongolica* consists of cortex, phloem, and xylem, and we analyzed the content of TAG in all the three types of tissues. As can be seen in Fig. 2,

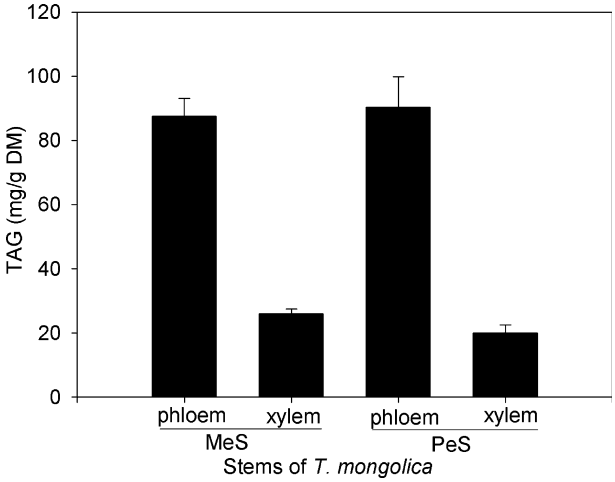

Fig. 1. The content of TAG in stems of *T. mongolica* of different ages: PrS, primary stems taken from current year's growth; MeS, medium stems 2–3 years old; and PeS, perennial stems more than 3 years old. The data are mean ± SD of three independent experiments.

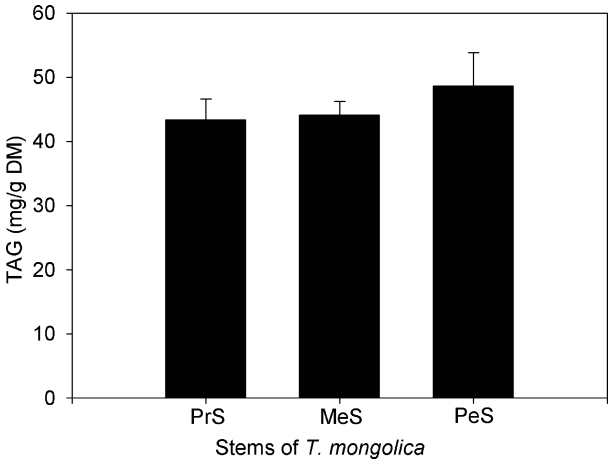

Fig. 2. The content of TAG in stem tissues of *T. mongolica*. Samples were taken from medium (2–3 years old) and perennial (more than 3 years old) stems. The data are mean ± SD of three independent experiments.

TAG was found mainly in the phloem (about 90 mg/g DM) although the xylem also contained a significant amount (20 mg/g DM); only trace amounts were found in the cortex (data not shown), probably due to contamination from the phloem. There was no significant difference in TAG content of corresponding tissues from primary (current season's growth, one year or younger), medium (2–3 years), and perennial (more than 3 years) stems (Fig. 2).

In higher plants, TAG is usually stored in the seed and serves as a source of carbon and energy during seed germination and the growth of seedlings. The lipid is also found in roots (Chinnasamy et al., 2003), leaves (Yatsu et al., 1971), stems (Madey et al., 2002), and other organs (Murphy, 1990) of many plant species, but in small quantities. However, stems of *T. mongolica* contained as much as 46 mg/g of DM, which indicates that stem tissues could also act as major reserves of TAG.

2.2. Fatty acid composition of TAG from stems of *T. mongolica*

The fatty acid composition of TAG from stems of different ages and from different tissues of the same stem was determined by gas chromatography. The results (Table 1)

Table 1
Fatty acid composition of triacylglycerol (TAG) extracted from whole stems, primary stems (PrS, from current year's growth), medium stems (MeS, 2–3 years old), and perennial stems (PeS, more than 3 years old), and from different tissues, namely phloem and xylem

Sample	Fatty acid composition (mol%)				
	C16:0	C18:0	C18:1	C18:2	C18:3
PrS	12.8 ± 0.6	3.4 ± 0.4	12.3 ± 0.7	58.3 ± 2.3	13.1 ± 1.2
MeS	13.1 ± 0.8	2.2 ± 0.2	12.4 ± 1.2	59.9 ± 2.5	12.2 ± 0.7
PeS	12.6 ± 0.5	2.0 ± 0.2	12.1 ± 1.1	62.1 ± 3.9	11.3 ± 0.9
Phloem	12.5 ± 0.5	1.3 ± 0.2	11.7 ± 1.0	62.1 ± 3.7	12.3 ± 1.3
Xylem	12.4 ± 0.8	2.9 ± 0.3	11.2 ± 0.8	61.2 ± 2.9	11.2 ± 0.7

The data are mean ± SD of three independent experiments.

showed that five common fatty acids, namely palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) were the main constituents. The fatty acid composition of TAG from stems of different ages or from different tissues of the same stem were similar. The most abundant fatty acid was 18:2, which accounted for 56.1–67.1% of the total fatty acids in TAG. The polyunsaturated fatty acids (18:2 and 18:3) accounted for about 70% of the total TAG fatty acids. This profile of fatty acid composition is very similar to that of storage TAG in seeds of many other plant species, e.g. soybean, in which 18:1 and/or 18:2 are the dominant fatty acids (Cahoon et al., 2006; Voelker and Kinney, 2001), which suggests that TAG in stems of *T. mongolica* and seeds of some plants is a product of similar processes of biosynthesis. In stems of *T. mongolica*, TAG contained higher levels of 16:0 and 18:3. It has been reported that the level of these two fatty acids is influenced by environmental conditions: generally, low temperatures are associated with higher levels of 18:3 and lower levels of 16:0 in membrane lipids (Xu et al., 2003b). Fatty acid composition of TAG also changes with temperature: Scots pine trees in southern Germany contained higher 16:0 and less 18:3 than those in northern Finland (Fischer and Höll, 1992; Piispanen and Saranpaa, 2002). The concentration of polyunsaturated fatty acids in TAG from Scots pine increased in response to cold stress and during the winter (Fuksman and Komshilov, 1981). Higher levels of both 16:0 and 18:3 in TAG in*T. mongolica* probably resulted from the extreme differences between the day temperature and the night temperature in the region. It seems reasonable to assume that these two fatty acids are involved in the mechanism that enables the plants to adapt to extreme and short-term fluctuations in temperature.

2.3. Distribution of oleosomes in stem tissues of T. mongolica

Plants store TAGs in subcellular particles called oleosomes (or oil bodies) as food reserves in seeds or other organs (Huang, 1996; Murphy, 1990). The oleosomes can be easily detected by staining with lysochromes such as Sudan Black B (Bronner, 1975). To confirm the results of chemical analysis, semi-thin sections of *T. mongolica* stems were examined under a light microscope after staining with Sudan Black B.

It is clear from the photomicrograph of a cross-section of the stem of *T. mongolica* (Fig. 3a) that the stem comprises three distinct parts: cortex, phloem, and xylem. The outermost layer, the cortex, is marked by slender, elongated cells. In these cells, nothing has been stained by Sudan Black B. In contrast, cells in both phloem and xylem show a large number of oleosomes strongly stained by Sudan Black B. At higher magnifications, a cross-section of the phloem shows oleosomes accumulated in almost all cells, although the cells located in the middle layer of the phloem contained a more oleosomes than those adjacent either to the cortex or to the xylem (Fig. 3b and Table 2). Moreover, we also found large amounts of white grains in phloem cells, which were identified as starch grains (see below).

On the basis of these primary observations (Fig. 3a and b), we conducted further studies on the distribution of oleosomes in the phloem and the xylem. Both longitudinal and cross-sections of the stem were prepared and stained with Sudan Black B as well as with periodic acid – Schiff (PAS) (Fig. 3c–f). In cells of the middle layer of the phloem (Fig. 3c), two types of particles are clearly seen: starch granules (the larger particles, stained pink) and oleosomes (the smaller particles, stained black). The starch granules were closer to the plasma membrane whereas the oleosomes were closer to either the plasma membrane or to the starch granules. Oleosomes in these tissues were about 2.0 μm in diameter, very similar in size to those in seeds of most plant species reported previously (Hu and Xu, 1990; Poxleitner et al., 2006). Both starch granules and oleosomes were more densely distributed in the middle layer of the phloem than in the layer adjacent to the xylem (Fig. 3d and Table 2). In the xylem, oleosomes were observed only in parenchymatous cells (Fig. 3e and f). Vertical sections of the xylem showed that the elongated cells in the parenchyma were filled with oleosomes (Fig. 3f), there being 19 oleosomes on average for every square micrometer of cytoplasmic area (Table 2).

These results indicate that the phloem is the main tissue that accumulated oleosomes. Almost all phloem cells contained oleosomes, which suggests that the cells have a great capacity to accumulate TAG. Our results also clearly showed that some cells in the phloem are richer than others in oleosomes (Table 2) and are the main contributors of TAG in stems of *T. mongolica*. It can be seen from Fig. 3a and b that these oleosome-rich cells are distributed in the middle of the phloem. The xylem, on the other hand, was poor in TAG, which accounted for only 2% of the xylem dry matter (Fig. 2). Within the xylem, the oleosomes were observed only in the parenchymatous cells (Fig. 3e and f). Since the parenchyma forms only a small proportion of the xylem, each parenchymatous cell must contain large quantities of oleosomes (Fig. 3f and Table 2).

The large amounts of TAG in stems of *T. mongolica* suggest that this lipid plays an important role in the growth and development of plants. Because *T. mongolica* hardly sets seeds – the ovules abort early – it is likely that the stem has evolved as a sink for carbon storage, the stored TAG being utilized by the new growth of shoots later in the year. This phenomenon is probably one of the adaptive mechanisms that enable *T. mongolica* to grow in a harsh environment, and it is very likely that high levels of TAG in stems are advantageous to survival in such habitats.

3. Concluding remarks

This study shows that stems of *T. mongolica* can accumulate high levels of TAG. Of the cells examined, only

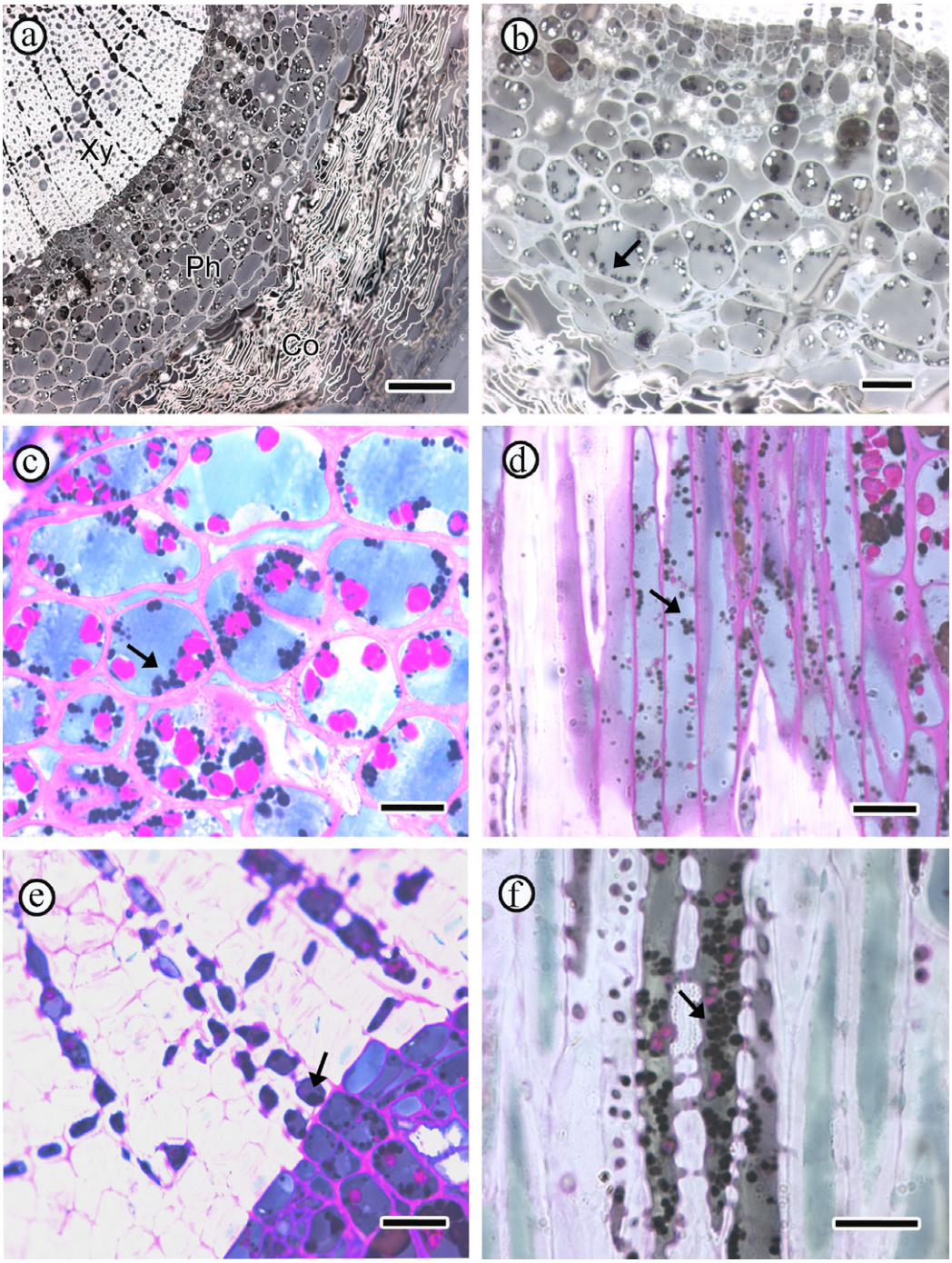

Fig. 3. Photomicrograph of a 1.0-μm semi-thin section of a two-year-old stem of *T. mongolica* showing the distribution of oleosomes (arrows) in different tissues. The oleosomes were stained by Sudan Black B and starch granules and polysaccharides (pink) were stained by PAS reaction: (a) A cross-section of stem showing tissues and cells stained by Sudan Black B. Stems of *T. mongolica* comprise three distinct parts: xylem (Xy), phloem (Ph), and cortex (Co). Oleosomes in both phloem cells and parenchymatous cells of xylem were stained black. The scale bar is 50 μm. (b) Distribution of oleosomes in phloem cells. The number of oleosomes in the phloem parenchyma adjacent to cambium was lower than that in the ray of parenchyma. The scale bar is 20 μm. (c) Photomicrograph at a higher magnification (the scale bar is 10 μm) showing the distribution of oleosomes in phloem cells. Starch grains made visible by the PAS reaction are also present in abundance in all cells. (d) Longitudinal sections showing distribution of oleosomes in the cambium of stems. The scale bar is 10 μm. (e) and (f). Distribution of oleosomes in the transverse (e) and longitudinal (f) sections of xylem parenchyma of stems. Oleosomes are seen mainly in the parenchyma. The scale bar is 10 μm.

the parenchyma of both phloem and xylem contained large numbers of oleosomes, the organelles for storing TAG, indicating that it is these cells that contribute mainly to the high content of TAG. Of all the higher plants reported so far, *T. mongolica* has recorded the highest levels of TAG in stems. Investigating the molecular mechanism of TAG accumulation in stems may have important implications for the plant's exploitation as an energy-rich crop.

Table 2
Distribution of oleosomes in cells from different stem tissues of *T. mongolica*

Tissue	Cells	No. of oleosomes/100 μm^2 of cytoplasmic area
Phloem	Parenchyma	30.7 ± 7.2
	Cambium	16.2 ± 3.2
	Near cortex	9.2 ± 1.3
Xylem	Parenchyma	19.2 ± 1.5
	Sclerenchyma	None present
Cortex		None present

The data are mean ± SD, $n = 15$–28 cells.

4. Experimental

4.1. Plant materials

T. mongolica is a shrub, up to about half a meter tall; a locally dominant but geographically restricted species, it is found mainly over about 2700 km^2 in the western part of Inner Mongolia, China. Being a perennial shrub, *T. mongolica* puts forth new growth from mature stems every spring, which results in the shrub developing a dome-shaped crown over the years. The plant material was collected from several isolated populations in the Wuhai Conservation Zone of *T. mongolica*, Inner Mongolia, China. Vouchers specimens (G.L. Wang 20051029) were deposited in Institute of Botany, Chinese Academy of Sciences Herbarium (PE). Based on age, the stems were classified into primary (generally generated in the current year; diameter $\leqslant$ 0.5 cm; labeled PrS), medium (two- to three-year-old; diameter ~0.5–1.0 cm; labeled MeS), and perennial (more than three-years-old; diameter $\geqslant$ 1.0 cm; labeled PeS).

4.2. Lipid extraction and separation

The tissues of stems were separately ground to a fine powder under liquid nitrogen and represented the following categories: parenchyma of phloem, xylem, cortex (lateral direction), and whole stems. One aliquot of a homogenized sample was used for determining the dry matter content and another for analyzing fatty acids. Lipids were extracted from the samples by the method of Bligh and Dyer (1959) and TAG was separated from the total lipids by thin-layer chromatography (TLC). The plates were developed in hexane:Et_2O:AcOH (70:30:1, v/v). Spots were made visible by spaying the plates with 0.01% primuline in acetone/H_2O (60:40; v/v) and examining the plates under ultraviolet (360 nm) light. Triolein was used as the standard.

4.3. Fatty acid analysis

Fatty acid analysis was carried out by following the method of Xu et al. (2003b). Briefly, the TAGs separated by TLC were transesterified with 5% H_2SO_4 in MeOH at 90 °C for 1 h, and the fatty acid methyl esters (FAME's) were extracted with hexane and separated on a Hewlett–Packard 6890 gas chromatography apparatus supplied with a hydrogen flame ionization detector and a capillary column HP INNOWAX (30 m; 0.25 mm i.d.) with N_2 carrier at 20 mL/min. The oven temperature was maintained at 170 °C for 3 min and then increased in steps to 210 °C, raising the temperature by 5 °C every min. FAME's from TAG were identified by comparing their retention times with known standards (37-component FAME mix, Supelco 47885-U). Heptacanoic acid (17:0, from Sigma) served as the internal standard to quantify the amounts of TAG. Chromatograms of the gas chromatography of TAG extracted from *T. mongolica* stems are provided in the supplementary data.

4.4. Microscopic examination

The different tissues were examined under a light microscope to study the distribution of oleosomes and starch granules, mainly following the method of Bal (1990). The stems of different age groups (described earlier) were cut into thin transverse sections with a clean razor blade, and were fixed immediately in 3% glutaraldehyde in phosphate-buffered saline (PBS) for 4 h at 0 °C. After fixing, the samples were washed three times with the PBS buffer and treated with 1% OsO_4 in the same buffer for 12 h at 4 °C in the dark. After the OsO_4 treatment, the samples were washed and dehydrated in an ethanol series. The samples were treated with 1% *p*-phenylenediamine (*p*PD) in EtOH–H_2O (7:3, v/v) for 45 min during dehydration, after which all samples were embedded in Epon812 and polymerized by heat. For light microscopy, semi-thin sections (1 μm) were made using an LKB-V ultramicrotome (LKB, Bromma, Sweden), stained with or without periodic acid – Schiff (PAS) reaction as described (Hu and Xu, 1990), and then stained in fresh 1% Sudan Black B in EtOH–H_2O (7:3, v/v) for 30 min at 60 °C. The sections were differentiated in EtOH–H_2O (7:3, v/v) for 1 min and then in distilled H_2O. After drying at 40 °C, sections were mounted in glycerin-gelatin. The areas of interest were photographed with a Zeiss photomicroscope and enlarged photomicrographs examined for distribution of oleosomes (Bronner, 1975; Hu and Xu, 1990).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2007.04.040.

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