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ANATOMY AND ULTRASTRUCTURE OF *SALVADORA PERSICA* STEM: ADAPTIVE TO ARID CONDITIONS AND BENEFICIAL FOR PRACTICAL USE

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Stem structure strongly influences the drought response across a diverse group of temperate and tropical tree species. The stem of *Salvadora persica* (miswak), used as a chewing stick in the Islamic world, has a number of distinctive xeromorphic characteristics adapting it to arid or semi-arid conditions. The thick periderm is interrupted at points around the stem by transversely oriented lenticels to moderate exchange of vital gases. On the stem surface are 3-dimensional epicuticular crystals of various shape and size, present to protect against UV exposure, insects and pathogens. The secondary xylem contains groups of xylem fibers which consist of thick-walled narrow cells. Vessels are axially oriented without branching for interconnection. The xylem is also composed of parenchyma cells, which are characterized as ray parenchyma and wood parenchyma. The wood-parenchyma become crushed in the middle, forming a chamber which is later filled with amorphous inclusions or rhombohedral crystals. SEM-EDX analysis revealed sulphur in wood parenchyma cells, likely a defense against pathogenic microorganisms. Apart from its adaptive value, the sophisticated stem anatomy of *Salvadora persica*, in combination with its chemistry, makes it an effective tool for oral hygiene.

Key words: Miswak, chewing stick, *Salvadora persica*, xylem fibers, dental care.

INTRODUCTION

Miswak is a traditional chewing stick prepared from the stems of *Salvadora persica* L. (Salvadoraceae), a small evergreen tree found on rocky slopes and in sandy areas in the Middle East, India and Africa (Eid et al., 1990). This plant is widely used for oral hygiene in traditional Arab medicine (Almas and Al-Zeid, 2004; Al-Otaibi et al., 2004). The use of the chewing stick for cleaning the teeth is deeply rooted in many cultures. Miswaks were used by the Babylonians some 7000 years ago and later throughout the Greek, Roman and Islamic empires. Today, miswaks are used in Africa, South America, Asia and the Middle East (Lewis and Lewis, 1977; Hattab, 1997). For religious and cultural reasons, miswak use is firmly established and widespread in Saudi Arabia and most other Muslim countries (Rispler-Chaim, 1992; Bos, 1993; Al-Sadhan and Almas, 1999; Marwat et al. 2009).

The anti-microbial and cleansing effects of miswak have been attributed to various chemical substances in its extracts (Eid et al., 1990; Hattab, 1997). The biological properties of miswak extracts include significant antibacterial activity, antifungal effects, amelioration of gum inflammation and inhibition of plaque formation (Al-Bagieh et al., 1994; Al-Lafi and Ababneh, 1995; Al-Bagieh and Almas, 1997; Almas et al., 1997; Almas and Al-Bagieh, 1999; Almas and Stakiw, 2000; Darout et al., 2000; Darmani et al., 2003; Al-Otaibi, 2004). Miswak extracts inhibit the growth of several microorganisms *in vitro* (Homer et al., 1991; Almas et al., 1997; Awadh et al., 2001), suggesting that miswak sticks may affect the pathogenesis of periodontal diseases by reducing the virulence of periodontal pathogenic bacteria.

Studies of the miswak have focused on its uses in dental hygiene and the mode of action of its bioactive compounds. Much less attention has been paid to its stem anatomy and ultrastructure. This paper is intended to fill that gap. As will be seen, the anatomy and ultrastructure of miswak adapt it to harsh arid conditions and at the same time make it a good natural toothbrush.

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MATERIALS AND METHODS

Salvadora persica samples obtained in the spring from trees cultivated in Jeddha, Saudi Arabia, were prepared for light and electron microscopy. Stem fragments containing bark attached to wood were taken from apical (1-year-old) and mature (2–3-year-old) regions of a mature living tree. Stem segments were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer (pH 7.2) for 2 h. After rinsing in buffer the tissue was postfixed in 1% osmium tetroxide in the same buffer, dehydrated in an ethanol series and propylene oxide and finally embedded in Spurr's epoxy resin. Semithin sections 0.5–1.0 μm thick from resin-embedded tissue were heat-fixed to glass slides and stained with 0.5% toluidine blue in 5% borax for preliminary LM observations. For histochemical detection of polysaccharides, hand-cut sections or semithin sections of fixed material were treated with the periodic acid-Schiff's (PAS) reaction and examined by LM. To soften the stem for separation of tissue elements, fixed stem segments were left in a high concentration (1 M) of HNO_3 overnight before hand-cutting. Semithin sections were examined under a Zeiss Axioplan light microscope, and ultrathin sections were examined with a Zeiss 9 S-2 transmission electron microscope.

Scanning electron microscopy was done with a 20 kVolt JEOL JMS-840A scanning electron microscope equipped with an energy-dispersive X-ray (EDS) Oxford ISIS 300 microanalytical system and the necessary software for point microanalysis, linear microanalysis and chemical mapping of the surface under examination. Operating conditions were as follows: accelerating voltage 20 kV, probe current 45 nA and counting time 60 s, with ZAF correction provided on-line. The samples were coated with carbon at 200 Å average thickness using a Jeol JEE-4X vacuum evaporator.

RESULTS

STEM CORTEX

The shining white stem of *S. persica* is finely striate on the outer surface. The thick periderm is interrupted at points around the stem by transversely oriented lenticels, which enlarge as the shoot grows due to stretching of the outer surface (Fig. 1a). These macroscopic openings embedded in the corky tissue have loosely connected cells surrounded by an intercellular air space. In young stems the outermost layer of cells forms the epidermis, which is composed of ordinary epidermal cells with a thick, prominent cuticular layer without any trichomes (Fig. 1b). As stems age, this extracellular cuticular layer becomes remarkably thick, develops cuticular pegs, and becomes heterogeneous in morphology.

On the stem surface are epicuticular crystals of various shape and size. The majority of them are spherocrystals, globular in shape and of smooth surface outline (Fig. 1c). A small number of more or less rhomboidal crystals protruding from the amorphous wax layer occur sporadically over the cuticle surface (Fig. 1d). The compositional variation of the surface was examined by SEM with associated energy-dispersive spectroscopy (EDS). EDS detects the % presence on the surface examined, not within the crystal as a whole. EDS analyses of epicuticular spherocrystals showed mean C to be 76.6 wt% and mean O to be 22.9 wt%. For rhomboidal epicuticular crystals the mean percentages are as follows: C 65.6 wt%, O 14.3 wt%, Ca 11.1 wt% and S 9.1 wt%. Typical spectra from the spherocrystals and rhomboidal crystals in Figures 1c and 1d are given in Figures 2a and 2b respectively.

In the bark of young stems, fibers organized in groups occupy the majority of a cross section, allowing small spaces between them for other tissues such as chlorenchyma. These fibers occur in tangential bands alternating with bands containing the rest of the bark tissues (Fig. 3a). The fiber groups in the phloem (extraxylary or bast fibers) are heavily sclerified and stain paler red with Schiff's reagent. Morphologically these fibers are part of the primary phloem tissue, oriented vertically (parallel to the surface), and are the only largely elongated cells of the bark.

The cambium consists of a layer of thin-walled cells, rather uniform in size and rectangular in cross section between the xylem and phloem (Fig. 3b). Stem thickening is due to the continued activity of the cambium, producing a continuous ring of xylem vessels and fibers and a smaller amount of phloem. New xylem elements (vessels, fibers, wood parenchyma) arise from the inside of the cambium opposite the phloem strands (Fig. 3c). At the same time, new phloem elements (sieve tubes, bast fibers, parenchyma) are formed inside the old (primary) phloem from the cambial ring ("included" phloem). A number of solitary brachysclereids also arise from the same meristem (Fig. 3d), forming, together with the phloem fibers, a network in the cortex.

XYLEM

The secondary xylem contains groups of xylem fibers, which are abundant and form a large part of the ground mass of the stem (Fig. 3d). Cellulose cell walls gave a weak positive reaction (red) with Schiff's reagent (Fig. 4a) but stained intensely with toluidine blue (Fig. 4b,c). The fibers elongate dramatically in the axial direction but show very moderate radial expansion with relatively thick walls 3–15 μm in diameter. After mechanical treatment (chewing) or

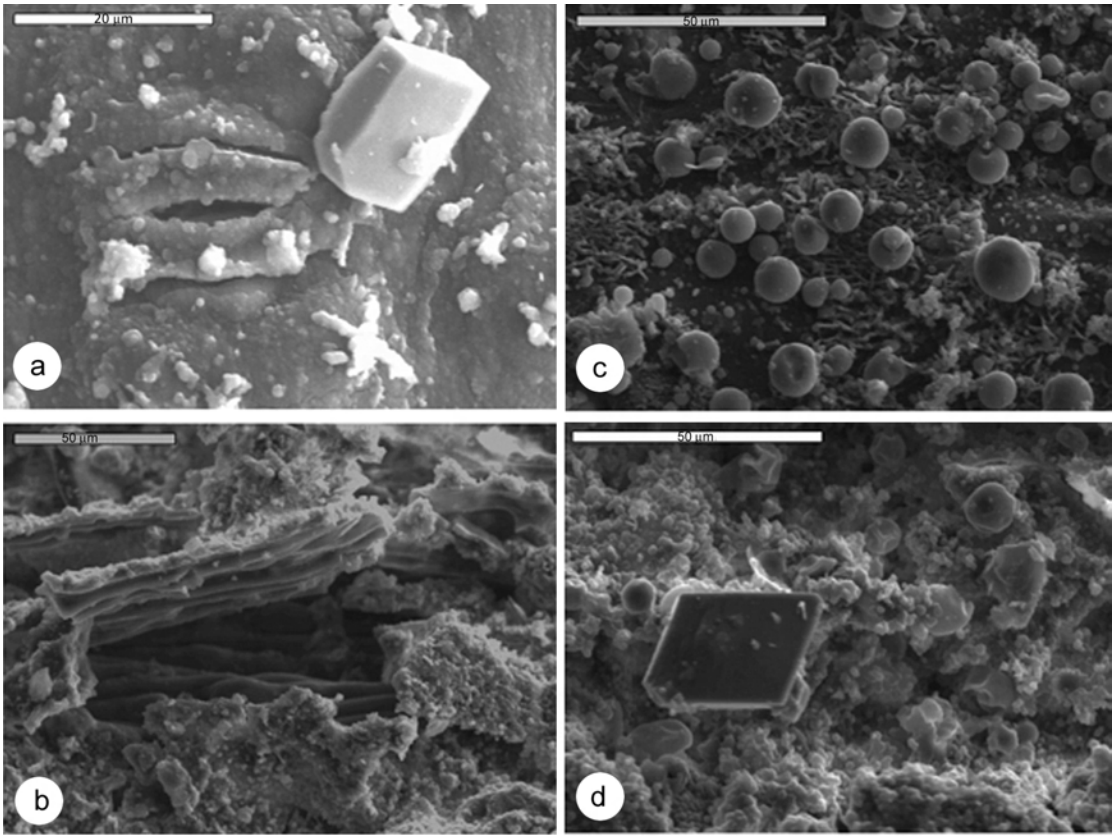

Fig. 1. (**a**) Lenticel in the thick periderm of the outer surface of the stem cortex, (**b**) Detached thick cuticular layer of young stem, showing epidermis cells. Cracks in the wax crust result from shrinking of the stem cortex by air-drying, (**c**) Epicuticular spherocrystals spreading over the stem surface, (**d**) Epicuticular rhomboidal crystals spreading over the stem surface.

chemical treatment with a high concentration of HNO_3 their association loosens (Fig. 4d).

Unlike phloem fibers, which are more compact at maturity without visible cytoplasm (Fig. 5a), xylem fibers are square or polygonal in cross section with slit-like apertures (Fig. 5b). Fiber cells possess lignified cell walls with sparse pits closely associated with vessels. Both types of cells are dead and empty at maturity, but the vessels are easily distinguishable from the fibers owing to their larger diameter (Fig. 5c). These libriform fibers are very long cells (100–1000 µm) with blunt ends (Fig. 5d). They are located between xylem elements and form dense undifferentiated tissue. Like other xylem elements, fibers develop from the same meristematic tissue, the cambium (Fig. 3b,c).

Primary xylem cells are somewhat more irregularly arranged near the outer edge of the pith in a ring of dark-stained cells smaller in diameter than secondary xylem cells. The pith occupies the central area of the stem (Fig. 6a), with numerous parenchyma cells and large intercellular spaces which strongly absorb the stains used in slide preparation. The majority of vacuoles in pith cells react intense purplish red with PAS. As the stem grows in girth, the pith becomes crushed.

The metaxylem consists of large vessels, axially oriented, without branching for interconnection (Fig. 6b). They are surrounded by one or two layers of lignified parenchyma cells which generally are smaller than xylem parenchyma cells. Vessels are roundish, elongated cells 20–40 µm in diameter, supported by xylem fibers smaller in diameter. In most cases they occur singly, occasionally in groups of two or more vessel members. The axial expansion of the vessel elements is more limited than that of fibers. In old stems the vessels become occluded by a balloon-like amorphous substance, obviously impeding water transport (Fig. 6c). SEM-EDS analysis of this amorphous substance gave the following mean percentages: C 25.1 wt%, S 18.8 wt%, O 8.6 wt%, Cl 0.2 wt%, K 1.62 wt%, Ca 44.4 wt%, Zn 0.9 wt% and Cu 0.8 wt%.

PARENCHYMA

Ray parenchyma cells are present in horizontal bands radiating from the pith towards the cortex (Figs. 3d, 6c). Rays are thin sheers varying in length,

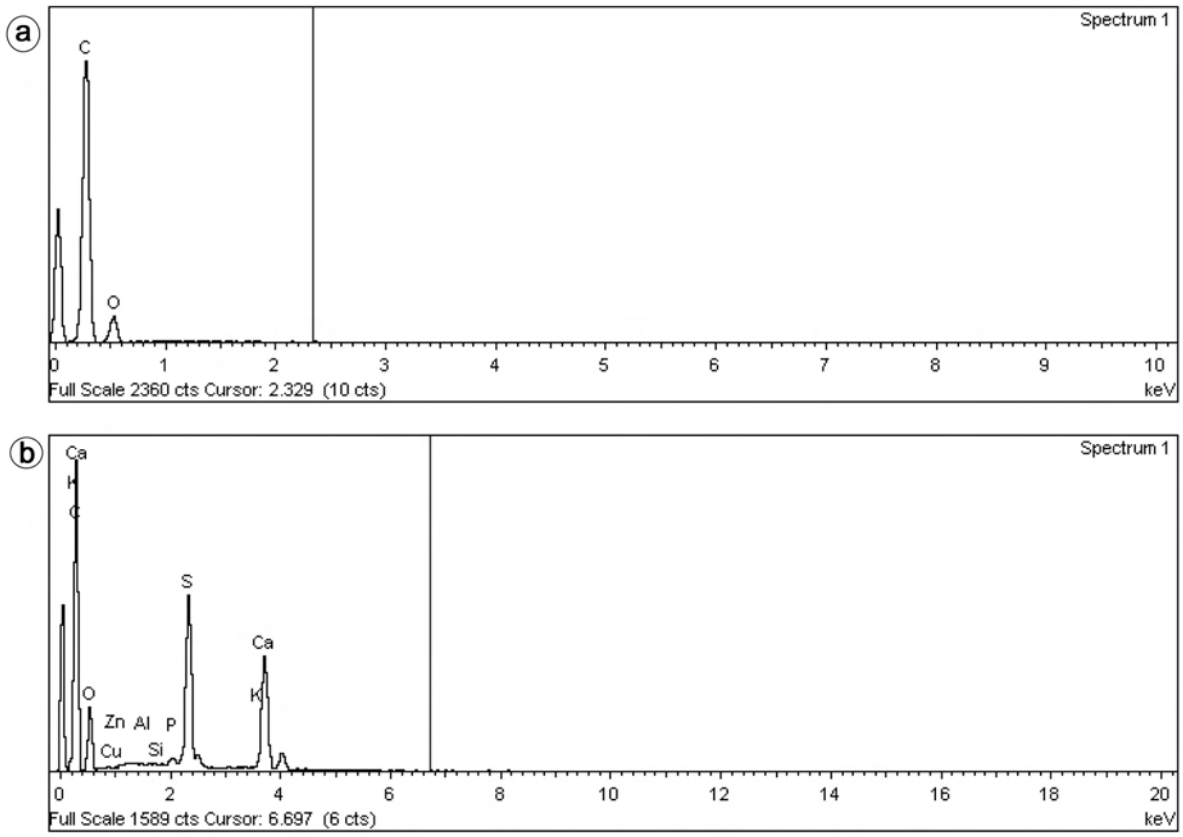

Fig. 2. (**a**) Typical spectrum of epicuticular spherocrystals on the stem surface shown in Figure 1c, (**b**) typical spectrum of epicuticular rhomboidal crystals on the stem surface shown in Figure 1d.

width and depth. They are composed mostly of one or more rectangular cells formed primarily of parenchyma cells possessing a large nucleus, oriented at right angles to the stem's main axis (Fig. 6d). As the stem grows in diameter the cambium adds new parenchymal cells to those rays and also initiates new rays (Fig. 3b).

Another type of parenchyma cell, wood parenchyma, is abundant, regular in shape and relatively thin-walled (Fig. 7a). Those cells are linked spatially with vessels (paratracheal parenchyma) forming groups of variable width. Wood parenchyma consists of small, rectangular, vertically aligned cells with smooth transverse walls. In cross section they are more abundantly distributed within cavitated wood and, unlike xylem fibers or vessels, wood parenchyma cells generally give no reaction (localized neutral coloration) in Schiff staining indicating their thin cell wall (Fig. 3d). As the stem ages the parenchymatous group of cells become crushed in the middle, forming a chamber (Fig. 7b) which later is filled with amorphous inclusions or rhombohedral crystals (Fig. 7c). The newly formed gap among chambered axial parenchyma cells is a suitable place for deposition but amorphous formations are also observed within the intercellular spaces of stem tissues (Fig. 7d).

SEM-EDS surface analyses of the amorphous inclusions shown in Figure 7b gave these mean percentages: Ca 38.8 wt%, C 35.1 wt%, O 23.9 wt% and S 1.6 wt%. Analysis of the rhomboidal crystals shown in Figure 7c gave C 65.6 wt%, O 14.3 wt%, Ca 11.1 wt% and S 9.1 wt%. Analysis of the amorphous material shown in Figure 7d gave Ca 44.4 wt%, C 25.1 wt%, S 18.8 wt%, O 8.6 wt%, K 1.6 wt%, Zn 0.9 wt%, Cu 0.8 wt% and Cl 0.2 wt%. Typical spectra from the inclusions shown in Figures 7b,c,d are given in Figures 8a, 8b and 8c respectively.

The walls connecting vessel elements are perforated, leading to the formation of a tube adapted for water conduction over long distances. Where the vessel elements come in contact with each other tangentially, bordered pits are formed between them (Figs. 9a,b), allowing communication with adjacent vessel elements or parenchyma cells for water and nutrient transport. Pitting through the cell walls of adjacent vessel elements is extensive, and the pits on the vessel walls vary in size. In contrast, the lignified parenchyma cells organized in rays are spatially

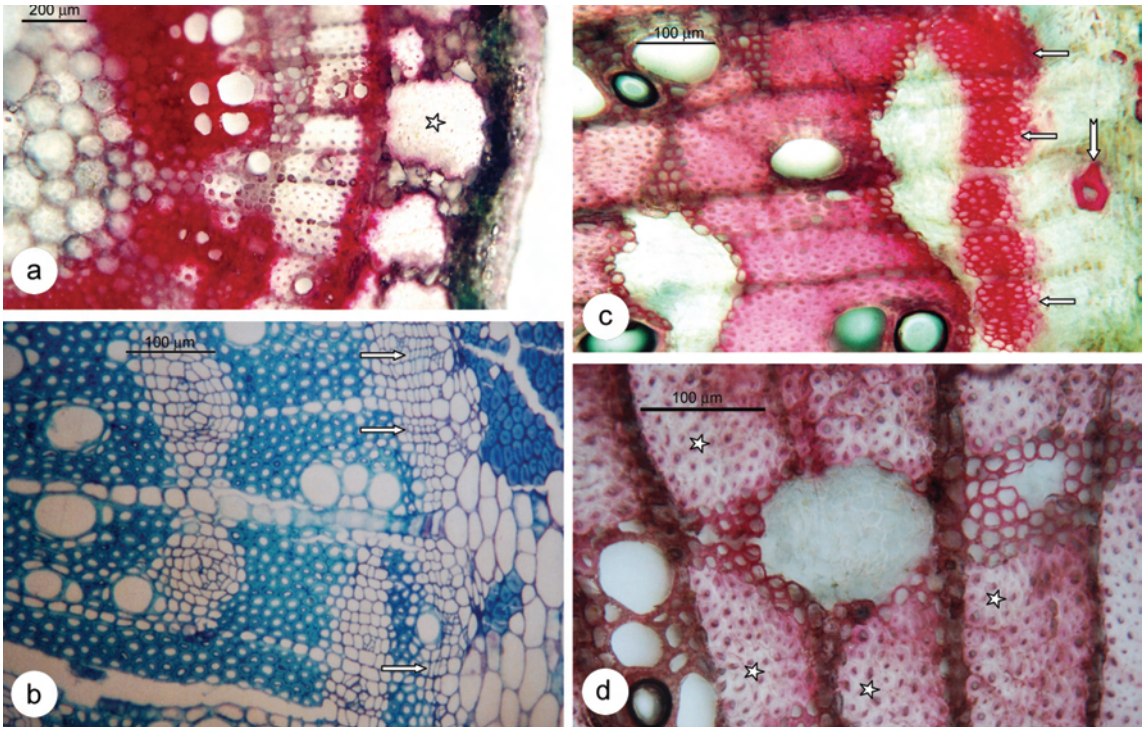

Fig. 3. (**a**) Cross section of 1-year-old stem. Vertically orientated fiber groups (star) alternating with chlorenchyma tissue. Schiff's reaction, (**b**) Cambial cells (arrows) between phloem and xylem tissue, (**c**) Fibers forming from the cambium (inside) as part of the secondary xylem (down arrows). A sclereide differentiated inside the secondary phloem (right arrow), (**d**) Xylem fibers (stars) closely associated with vessels, constituting the majority of the ground mass of the stem.

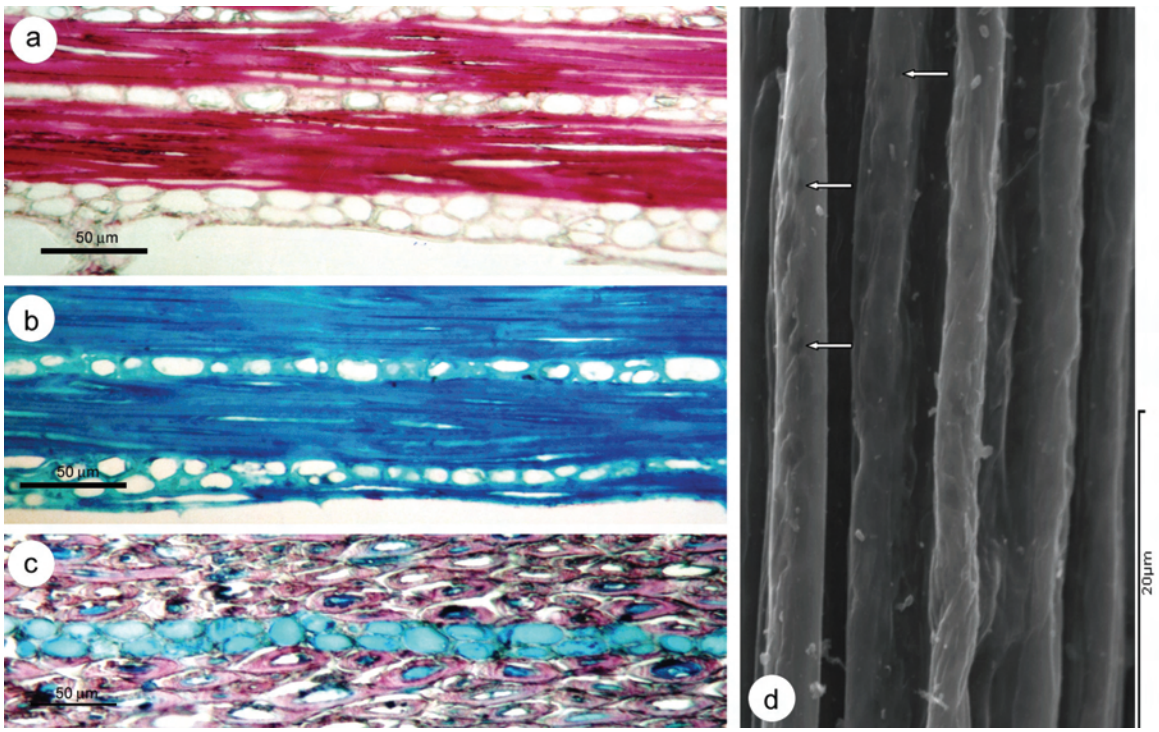

Fig. 4. (**a**) Xylem fibers stained red with Schiff's reagent. Stem tangential section, (**b**) Xylem fibers stained blue with toluidine. Stem tangential section, (**c**) Stem cross section. Staining with toluidine and Schiff's reagent. Ray parenchyma cells between xylem fibers, (**d**) Dense network of very long xylem fibers in longitudinal section. SEM images show the general scarcity of pits in fiber cell walls (arrows).

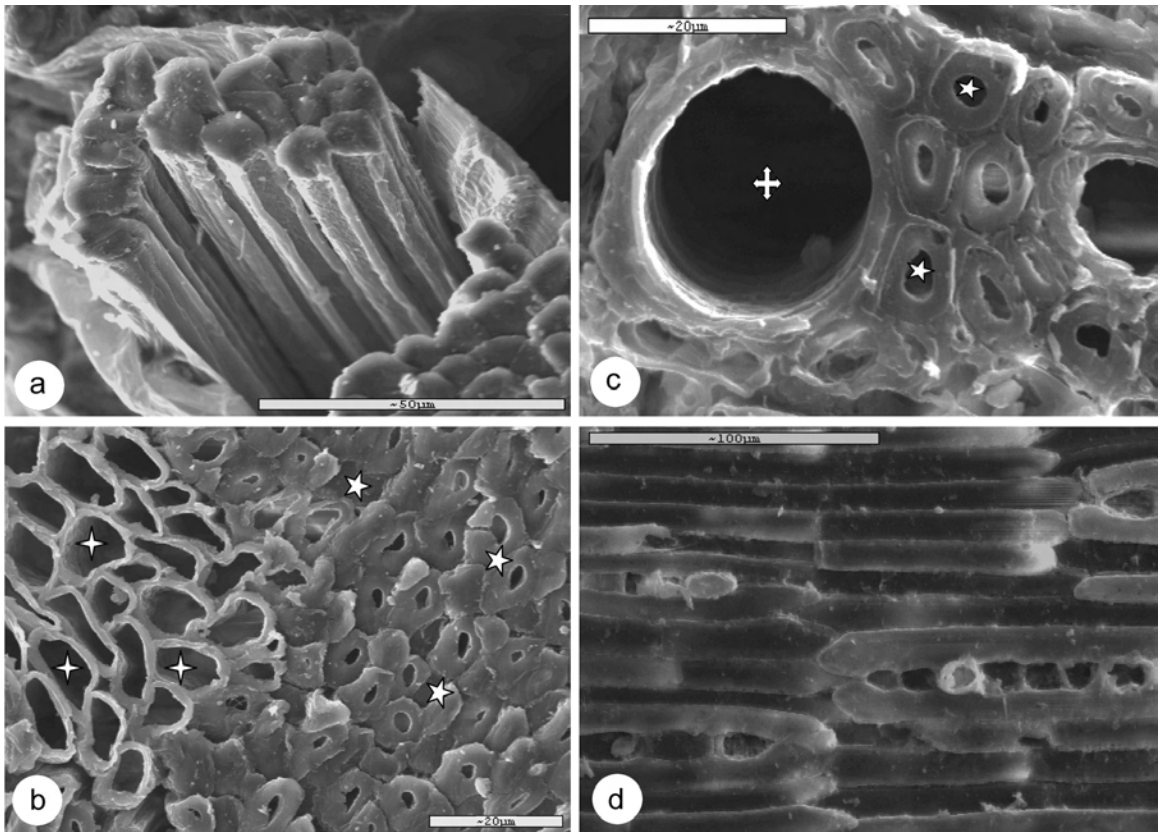

Fig. 5. (**a**) Band of polygonal phloem fibers in cortex with thick lignified secondary cell wall. Fiber cytoplasm is completely absent, (**b**) Flattened fibers (stars) closely associated with parenchyma cells (4-point stars). Fiber cytoplasm is restricted to a small area, (**c**) Xylem fibers (stars) closely associated with metaxylem vessels (quad arrow), (**d**) Xylem fibers in longitudinal section with blunt ends.

connected with the adjoining xylem fibers by a small number of simple or half-bordered pits which contribute to radial transfer of assimilates between the phloem and xylem (Fig. 9c).

DISCUSSION

The stem anatomy of *S. persica*, particularly the amount and distribution of parenchyma, is strongly correlated with tissue capacity during dehydration (Borchert et al., 2002). Parenchyma cells make the plant very efficient in storing water during the long summer drought. They are well protected inside the xylem from shrinkage or mechanical damage, and the stem remains hydrated and turgid. The lignified xylem fibers also have high water storage capacity, which has been referred to as extracellular, capillary or inelastic water storage (Holbrook, 1995). The water storage capacity of stem tissues appears to be the prime determinant of the main strategy observed among tropical tree species adapted to severe seasonal drought (Ludlow, 1989). Species with extensive wood or paratracheal parenchyma are drought avoiders (Borchert and Pockman, 2005).

The presence of a thick cuticle in young stems has important physiological ramifications. The development of a periderm with lenticels embedded in the corky tissue and a thick cuticle would enable more judicious exchange of vital gasses such as oxygen and carbon dioxide than occurs in woody plants in general, giving rise to the notion of carefully regulated cuticular water loss (Buschhaus et al., 2007). Development of a thick cuticular layer restricts the ability of the epidermis to expand as the stem continues to increase. A thick cuticle with 3-dimensional epicuticular structures performs an important protective role against UV exposure, insects and pathogens (Müller, 2006; Mohammadian et al., 2007).

Stem functions related to the distribution and storage of carbohydrates are accomplished in parenchyma cells, which contain protoplasm. Unlike vessels or fibers, parenchyma tissue remains alive for some years after completing development. While the vessels and fibers are involved in water conduction and mechanical support respectively, parenchyma cells function primarily in synthesis,

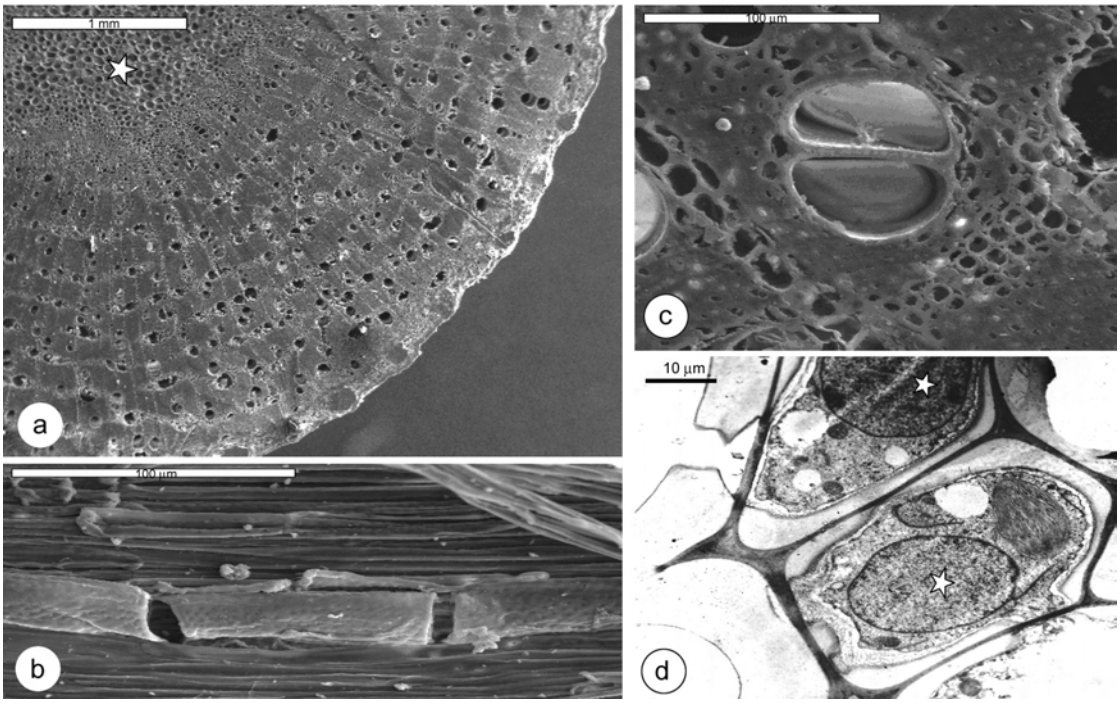

Fig. 6. (**a**) Overview of young stem in cross section. Pith (star) occupies central area of stem, (**b**) Three separated parts of metaxylem vessels and adjoining xylem fibers. Metaxylem vessels filled with balloon-like amorphous material, (**c**) Ray parenchyma cells associated with metaxylem fibers with thick cell walls and simple pits in cross section, (**d**) Wood parenchyma cells, possessing large nuclei (stars), organized in groups.

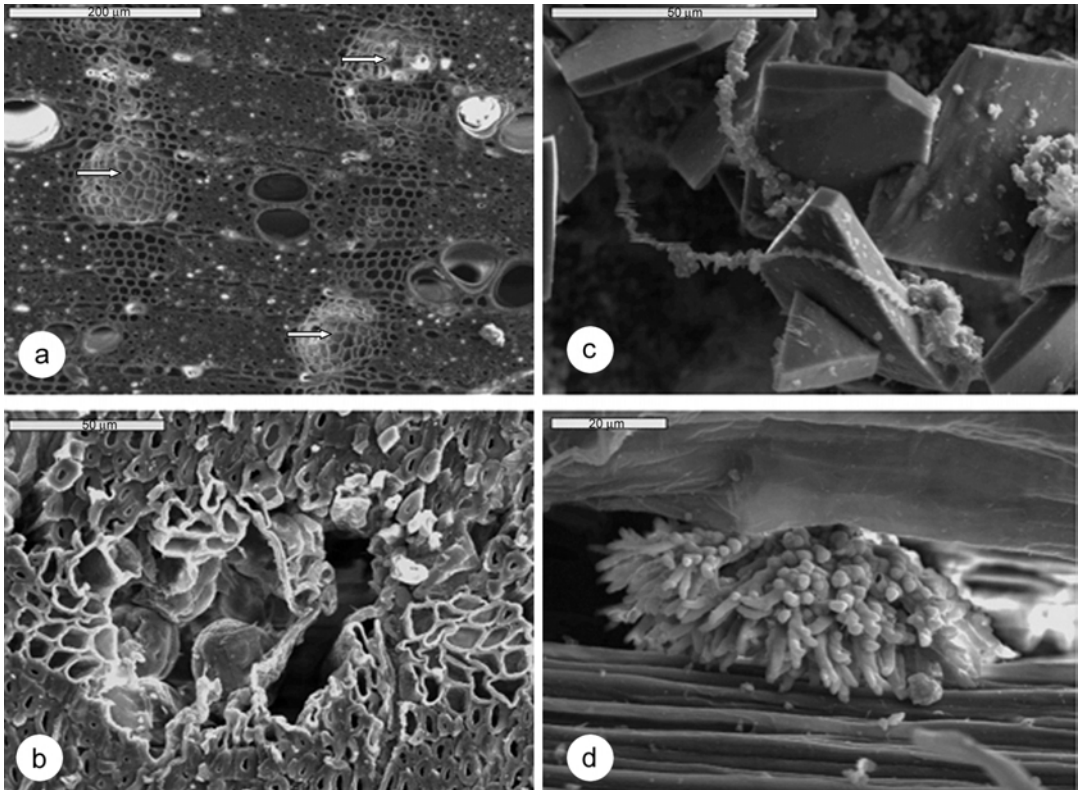

Fig. 7. (**a**) Group of wood parenchyma cells crushed in the middle area (arrows), (**b**) Wood parenchyma cells with amorphous material deposited in the crushed intercellular area, (**c**) Rhomboidal crystals in the crushed intercellular area, (**d**) Amorphous material deposited in the intercellular area between vessels and fibers.

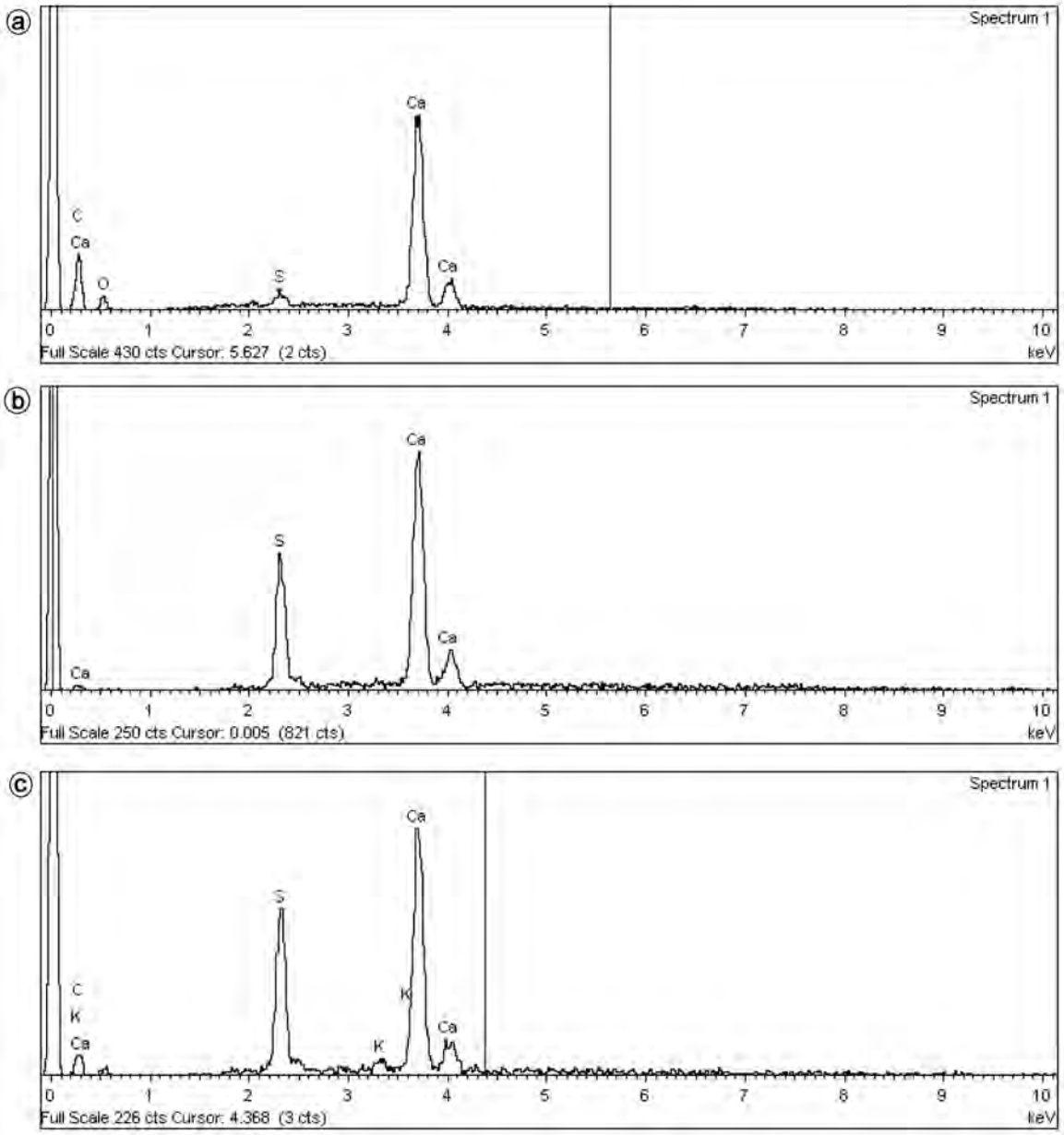

Fig. 8. (**a**) Typical spectrum of amorphous inclusions within the parenchyma cavity (like tyloses), (**b**) Typical spectrum of rhomboidal crystals within the parenchyma cavity, (**c**) Typical spectrum of amorphous inclusions in the extracellular space between xylem fibers and vessels.

temporary storage and lateral transport of assimilates (starch or lipids) and water. As sapwood gradually converts to inactive heartwood, wood parenchyma cells undergo numerous metabolic changes and produce large quantities of heartwood extractives such as phenolic compounds, lignin and aromatic substances which accumulate in the vessels (Magel, 2000).

The content of the wood parenchyma cells of a miswak stem produces a taste described as pleasantly bitter. The antibacterial properties of the stem of this plant can be attributed to its content of sulphur in the form of sulphur oils (Kokwaro, 1993) or other sulphur-containing compounds (Ali et al., 2002). My SEM-EDS analysis revealed sulphur in wood parenchyma cells, a good site for substances to counter pathogenic microorganisms. These substances are located mainly within scattered wood parenchyma cells. SEM-EDS X-ray microanalysis revealed sulphur accumulations at locations suitable for inhibiting a

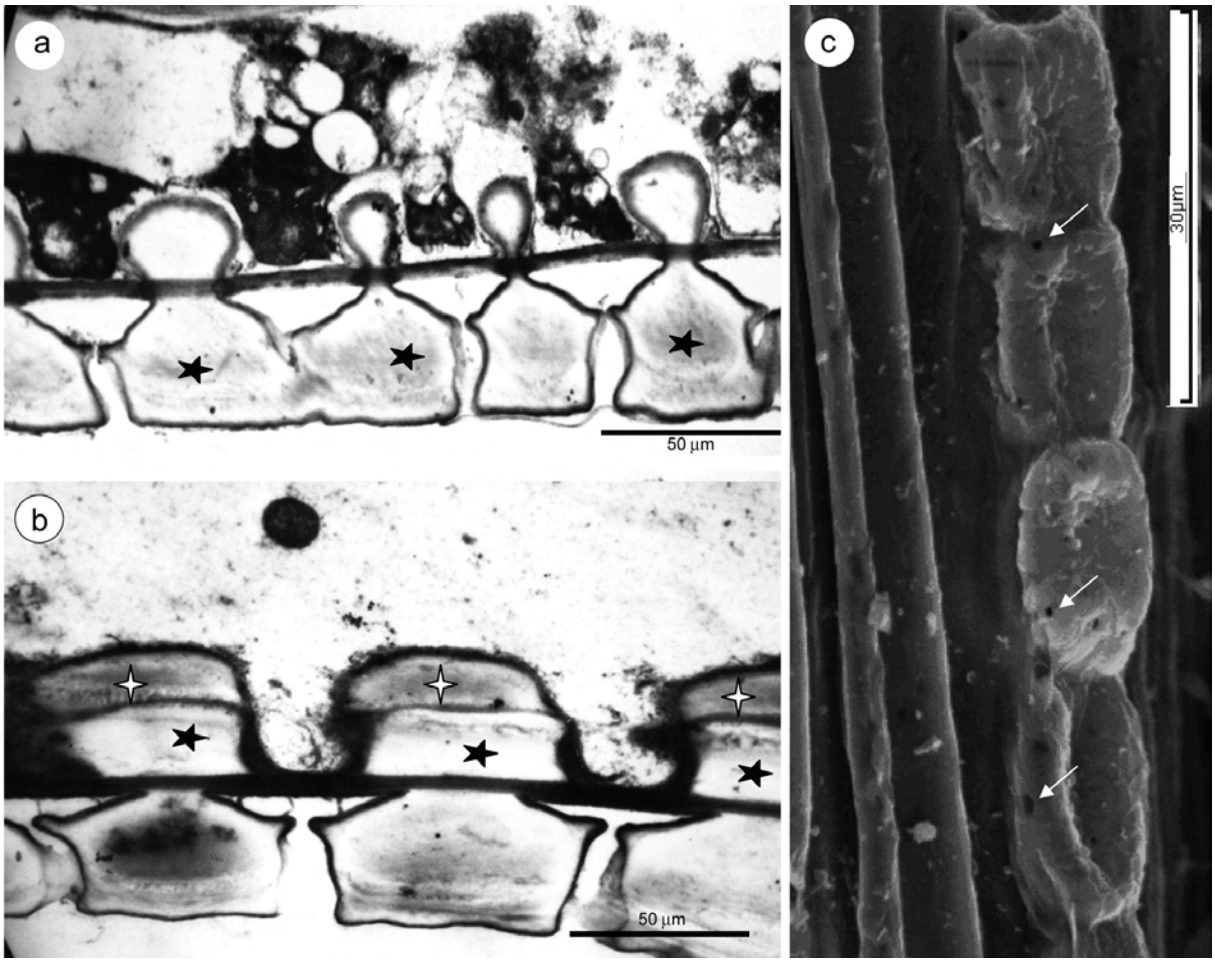

Fig. 9. (**a**) Transmission electron micrographs of longitudinal section or intervessel pits. Remarkably thick pit borders lying at one side of the vessel pair (stars). The structural differentiation apparently arose because the two pitted cell walls were in different stages of pit development, (**b**) Transmission electron micrographs of longitudinal section or intervessel pits. Additional layer (4-point stars) deposited against a previously deposited secondary wall (stars). The two layers are clearly distinguished without additional staining, (**c**) Band of ray parenchyma cells associated with xylem fibers. A few pits (arrows) are visible on cell walls.

xylem-invading microorganism: scattered xylem parenchyma cells or vessel-occluding tyloses.

The latter structures probably are produced as a defense response to occlude vessels and prevent the systemic movement of pathogenic microorganisms (Cooper, 2000; Cooper and Williams, 2004). SEM-EDS revealed sulphur in various places in the *S. persica* stem, but the technique does not distinguish elemental from bound sulphur and may merely reflect the presence of the many sulphur compounds which characterize semi-arid plants (Fahey et al., 2001). Further chemical analysis revealed that the stem contains 19 natural substances that are beneficial for dental health. Its natural antiseptics kill harmful microorganisms in the mouth and its aromatic oils increase salivation, which helps in digestion (Al-Otaibi et al., 2004).

The numerous intervessel pits along adjacent vessel walls enable communication between vessels, playing an important role in water transport (Holbrook and Zwieniecki, 2005). The geometry and structure of the bordered pits is related to the efficiency and safety of sap ascent (Choat et al., 2006). Xylem fibers as well as vessels are produced by cambial activity and undergo processes of secondary cell wall formation, such as lignin incrustation, during differentiation (Groover and Jones, 1999; Fukuda, 2000).

The organic part of *S. persica* sticks consists mainly of lignin as well as cellulose and hemicellulose (Bahabri, 2000). The lignin incrustation of xylem fibers in combination with the interposed parenchyma helps them to separate when the top quarter-inch of bark is pared away and the bristles

are chewed for a minute. This procedure was simulated in this study by subjecting the stem to a high concentration of HNO_3 for several hours. After mechanical (chewing) or chemical separation of the xylem fibers, the stem can be used as a natural toothbrush. The value of chewing sticks is thought to reside in the mechanical cleansing action of the fibrous xylem and the presence of various sulphur substances in the wood parenchyma cells, having antimicrobial activity. Some clinical studies have suggested that the miswak was more effective than toothbrushing for oral hygiene (Hattab, 1997; Al-Otaibi, 2004).

Thus the sophisticated stem anatomy of *S. persica* is of twofold benefit, enabling the plant to thrive in arid conditions and providing an effective and inexpensive way of promoting dental health.

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