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# Comparative Wood Anatomy of *Atraphaxis* taxa in Turkey

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The wood anatomy of four Atraphaxis taxa that have natural distribution in Turkey—*Atraphaxis billardieri* Jaub. & Spach, *Atraphaxis billardieri* subsp. *tournefortii* (Jaup. & Spach) Lovelius, *Atraphaxis spinosa* L., and endemic *Atraphaxis grandiflora* (Willd.)—were compared in this study. The wood samples were sectioned according to standard techniques. Samples were macerated with Schultze's method. Tangential and radial vessel diameters, intervessel pit diameters, vessel wall thickness, vessel elements length, dimensions of libriform fibres (lengths, widths, cell wall thickness and, lumen diameter), and uniseriate and biseriate ray heights were measured, and the number of vessels per mm$^2$, number of rays per mm, and number of vessels per group were counted. The qualitative features such as growth rings, vessel grouping, presence of helical thickening and storied structure, vestured pits, type of perforation plate, and arrangement of axial parenchyma were determined. These four species of *Atraphaxis* shrubs differ in some wood characteristics such as growth rings, vessel grouping, vestured pits, height and density of rays, number of vessels per mm$^2$, and the dimensions of the vessel.

## INTRODUCTION

The genus *Atraphaxis* L. (Polygonaceae) comprises 36 woody species across Southern Europe, Northern Africa, and predominately Asia (Siberia, China, and Mongolia) (Schuster *et al.* 2011; Yurtseva *et al.* 2014; 2016; Tavakkoli *et al.* 2015). There have been many taxonomic studies (morphologic, palynology, and phylogenetic) on the genus (Pavlov 1970; Haraldson 1978; Ronse Decraene and Akeroyd 1988; Ronse Decraene *et al.* 2000; Sanchez *et al.* 2011; Schuster *et al.* 2011; Sun and Zhang 2012; Tavakkoli *et al.* 2015; Yurtseva *et al.* 2016). However, few studies have been reported on the wood anatomy of *Atraphaxis* genus (Jansen *et al.* 2001; Carlquist 2003; 2010). Jansen *et al.* (2001) has mentioned *A. spinosa* (from Turkey) briefly in their study that vestured pits were investigated, and their taxonomic and evolutionary significance was evaluated. Similarly, species of *A. pungens* (M. Bieb.) Jaub. & Spach and *A. frutescens* (L.) Ewersm. were partially mentioned in studies where wood diversity of Polygonaceae (Carlquist 2003) was examined and Caryophyllales (Carlquist 2010) was evaluated in terms of ontogenetic, phylogenetic, and ecologic factors.

According to floral characteristics (the numbers of tepal, stamen, style, and achene type), the genus *Atraphaxis* is divided into two subgenera *Euatraphaxis* and *Tragopyrum* (Pavlov 1970) or two sections *Atraphaxis* and *Tragopyrum* (Rechinger and Schiman-Czeika 1968). The genus *Atraphaxis* is represented by 5 species in Turkey, one of which

is endemic (*A. grandiflora* Willd.). Four taxa (*A. grandiflora* Willd., *A. angustifolia* Jaub. & Spach, *A. billardieri* Jaub. & Spach, and *A. billardieri* Jaub. & Spach subsp. *tournefortii* (Jaub. & Spach) Lovelius) are indicated in the subgen. *Tragopyrum*, while one species (*A. spinosa* L.) is categorized in the subgen. *Atraphaxis* (Cullen 1967). Although the only Turkish record is the type specimen, the presence of *Atraphaxis angustifolia* in Turkey is doubtful (Cullen 1967).

The goal of the present study is to describe and compare the anatomical characteristics of wood in *Atraphaxis billardieri*, *A. billardieri* subsp. *tournefortii*, *A. grandiflora*, and *A. spinosa.*

## EXPERIMENTAL

### Materials

All studied *Atraphaxis* taxa spread in arid areas and especially dry hillsides and rocky slopes. *A. spinosa* is an erect shrub up to 1 m, *A. grandiflora* (< 30 cm) and taxa of *A. billardieri* (≤ 60 cm) are low shrubs. All of them are deciduous. Plant samples were collected from natural habitats in Turkey (Table 1).

**Table 1.** The Collection Data of Studied Samples

| Taxon | Locality | Voucher Number |
|---|---|---|
| *Atraphaxis billardieri* | Antalya, Elmalı, Avlan Gölü, 1055m 36°34′03′′N, 29°56′57′′ E | Erşen Bak 300 |
| *Atraphaxis billardieri* subsp. *tournefortii* | Artvin, 400 m 41°12′33′′N, 42°02′03′′ E | Erşen Bak 301 |
| *Atraphaxis grandiflora* | Erzincan, Refahiye, 1750 m 39°54′01′′N, 38°45′32′′ E | Erşen Bak 302 |
| *Atraphaxis spinosa* | Iğdır, Aralık, 823 m 39°52′15′′N, 44°30′19′′ E | Erşen Bak & Cesur 9 |

### Methods

Wood samples were obtained from the stem section and were boiled, and stored in equal amounts of ethanol, glycerin, and distilled water (Merev 1998). Wood materials were sectioned with a sliding microtome and cryostat. The sections were stained with safranine O and alcian blue (Ruzin 1999; Ives 2001). Macerations were prepared using Schultze's method (Normand 1972). On sections, tangential and radial vessel diameters, intervessel pit diameters, vessel wall thickness and, uniseriate and biseriate ray heights were measured, and the number of vessels per mm$^2$, the number of rays per mm, and the number of vessels per group were counted. The vessel elements length, libriform fibre dimensions (lengths and widths, the thickness of cell walls, lumen diameter) were measured on macerated specimens. The qualitative features such as growth rings, vessel grouping, presence of helical thickening and storied structure, vestured pits, type of perforation plate, and the arrangement of axial parenchyma were determined. Also, vulnerability ratio (vessel diameter/ numbers of vessels per mm$^2$) and mesomorphy value (vulnerability ratio x vessel element length) were calculated by using vessel member characteristics (Carlquist 1977, 1988). The average value for each feature was based on 30 measurements and/or counts. Vessel diameter was measured on the basis of the lumen and was taken at the widest point. Vessel element length, including its tails, was obtained from macerations (Baas *et al.* 1983;

Carlquist 1988). Measurements and microphotographs were taken with an Olympus BX 53 microscope with DP73 (Tokyo, Japan). Helical thickening and vestured pits were examined in detail using a Zeiss/Evo LS10 scanning electron microscope (Cambridge, UK). All wood terms used conform to the usage of the International Association of Wood Anatomists Committee on Nomenclature (IAWA Committee 1989).

## RESULTS AND DISCUSSION

The wood anatomical properties of the *Atraphaxis* species are listed below. The mean values of selected quantitative data are listed in Table 2.

### *Atraphaxis billardieri*

Wood ring porous, growth ring distinct (marked by thick-walled, radially flattened latewood fibres and vascular tracheids) (Fig. 1a). Vessels *ca.* 146 to 156/mm$^2$, mostly solitary (63%) and in usually tangential multiples of 2 to 3 (4 to 7), or in small radial groups or in small clusters (Fig. 1a), mean number of vessels per group 1.65 (2 to 7); rounded in cross-section, earlywood vessels tangential diameter 30 to 80 µm and radial diameter 30 to 83 µm, latewood vessels tangential diameter 10 to 26 µm and radial diameter 10 to 36 µm, walls 2.28 to 5.54 µm in thickness. Narrow vessels intergrading with vascular tracheids in latewood. Vessel member length 125 to 202 µm. Perforations simple in horizontal to oblique end walls. Intervessel pits vestured (Fig. 2c), alternate, rounded or oval, 3.68 to 5.98 µm in diameter, with slit-like or coalescent apertures. Earlywood (±) and latewood (+) vessels with helical thickenings, vessel members storied. Axial parenchyma scanty paratracheal or vasicentric. Libriform fibres 314 to 491 µm long, 10.31 to 21.36 µm wide, thin- to thick-walls (2.52 to 5.29 µm), simple pits on radial and tangential walls. Rays 1 to 6 / mm, mostly uniseriate (45 to 230 µm, 2 to 9 cells high) and biseriate (80 to 195 µm), very rarely multiseriate (3 cells); heterocellular. Silica bodies were observed and dark-staining deposits in ray and axial parenchyma cells.

### *Atraphaxis billardieri* subsp. *tournefortii*

Wood ring porous, growth ring distinct (marked by thick-walled, radially flattened latewood fibres and vascular tracheids) (Figs. 1b, 1e). Vessels *ca.* 108 to 168 / mm$^2$, solitary (44%) and in usually tangential (predominantly at the beginning of growth ring) to radial multiples of 2 to 4 (8 to 10), or in small clusters (Fig. 1e), mean number of vessels per group 2.5 (2 to 10); rounded in cross-section, earlywood vessels tangential diameter 25 to 80 µm and radial diameter 35 to 85 µm, latewood vessels tangential diameter 15 to 35 µm and radial diameter 10 to 35 µm, walls 3.26 to 9.01 µm in thickness. Narrow vessels intergrading with vascular tracheids in latewood. Vessel member length 115 to 195 µm. Perforations simple in horizontal to oblique end walls. Intervessel pits vestured (Fig. 2b), alternate, rounded or oval, 4.8 to 7.96 µm in diameter, with slit-like or coalescent apertures. Earlywood (±) and latewood (+) vessels with helical thickenings, vessel members storied. Axial parenchyma scanty paratracheal or vasicentric. Libriform fibres 210 to 430 µm long, 11.62 to 23.24 µm wide, thin- to thick-walls (2.49 to 4.15 µm), simple pits on radial and tangential walls. Rays 1 to 5 / mm, mostly uniseriate (45 to 325 µm, 2 to 11 cells high) and biseriate (90 to 525 µm), very rarely multiseriate (3 cells); heterocellular. Silica bodies (Fig. 2j) and dark-staining deposits were observed in ray and axial parenchyma cells.

### Atraphaxis grandiflora

Growth rings distinct. Wood diffuse to semi-ring porous (Figs. 1c, 1f). Vessels *ca.* 140 to 181 / mm$^2$, in tangential and radial group of 2 to 4, or in clusters (5 to 9), or solitary (48%) (Fig. 1f), mean number of vessels per group 2.1 (2 to 9); rounded to angular in cross-section, earlywood vessels tangential diameter 28 to 70 µm and radial diameter 29 to 70 µm, latewood vessels tangential diameter 10 to 23 µm and radial diameter 8 to 29 µm, walls 2.28 to 4.96 µm in thickness. Vasicentric vascular tracheids present adjacent to in vessel grouping. Vessel member length 105 to 220 µm. Perforations simple in horizontal to oblique end walls. Intervessel pits vestured (Fig. 2a), alternate, rounded or oval, 3.84 to 7.03 µm in diameter, with slit-like or coalescent apertures (Fig. 2f). Wide (rare and very slightly) and narrow (+) vessels with helical thickenings (Fig. 2f), vessel members storied (Fig. 2h). Axial parenchyma scanty paratracheal or vasicentric. Libriform fibres 275 to 550 µm long, 11.18 to 45.32 µm wide, thin- to thick-walls (2.12 to 8.65 µm), simple pits on radial and tangential walls. Rays 1 to 5 / mm, uniseriate (40 to 325 µm, 2 to 10 cells high) and biseriate (130 to 430 µm), rarely multiseriate; heterocellular (Fig. 2i).

**Table 2.** Some Wood Anatomical Characteristics of *Atraphaxis taxa*

| Characteristics | A. billardieri | A. billardieri subsp. tournefortii | A. grandiflora | A. spinosa |
|---|---|---|---|---|
| **ETD** | 52.28 | 45.83 | 41.49 | 53.23 |
| **ERD** | 56.38 | 58.83 | 49.74 | 74.09 |
| **LTD** | 18.34 | 20.65 | 15.44 | 19.50 |
| **LRD** | 20.84 | 23.55 | 16.32 | 23.95 |
| **VF** | 150 | 128 | 155 | 185 |
| **VG** | 1.65 | 2.5 | 2.1 | 2.5 |
| **VEL** | 155.93 | 159.50 | 156.50 | 109.08 |
| **LFL** | 400.68 | 348.50 | 396.50 | 322.33 |
| **LFW** | 15.61 | 17.18 | 22.10 | 14.82 |
| **UH** | 115.31 / 4 | 118.95 / 4 | 130.11 / 4 | 127.92 / 6 |
| **BH** | 129.25 | 178.67 | 191.47 | 282.01 |
| **U** | 4 | 4 | 3 | 8 |
| **V** | 0.23 | 0.26 | 0.18 | 0.20 |
| **ME** | 36.71 | 41.42 | 28.74 | 21.44 |

ETD= earlywood vessels tangential diameter, µm; ERD= earlywood vessels radial diameter, µm; LTD= latewood vessels tangential diameter, µm; LRD= latewood vessels radial diameter, µm; VF= number of vessels per mm$^2$;VG= mean number of vessels per group; VEL= vessel element length, µm; LFL= libriform fibre length, µm; LFW= libriform fibre width, µm; UH= uniseriate ray height, µm / cell; BH= biseriate ray height, µm; U= number of rays / mm; V= vulnerability ratio (mean tangential vessel diameter / number of vessels per mm$^2$); ME= mesomophy value (vulnerability ratio x vessel element length).

### Atraphaxis spinosa

Growth rings distinct. Wood diffuse to semi-ring porous (Figs 1d, 1g). Vessels *ca.* 165 to 229 / mm$^2$, in radial group of 2 to 4 (7 to 9), or in clusters, or solitary (42%) (Fig. 1g), mean number of vessels per group 2.5 (2 to 9); rounded to angular in cross-section, vessels of two distinct diameter classes (Fig. 2g), earlywood vessels tangential diameter 32 to 87 µm and radial diameter 42 to 115 µm, latewood vessels tangential diameter 10 to 37 µm and radial diameter 10 to 40 µm, walls 1.96 to 3.99 µm thick. Vasicentric vascular tracheids present adjacent to in vessel grouping. Vessel member length 75 to 137 µm.
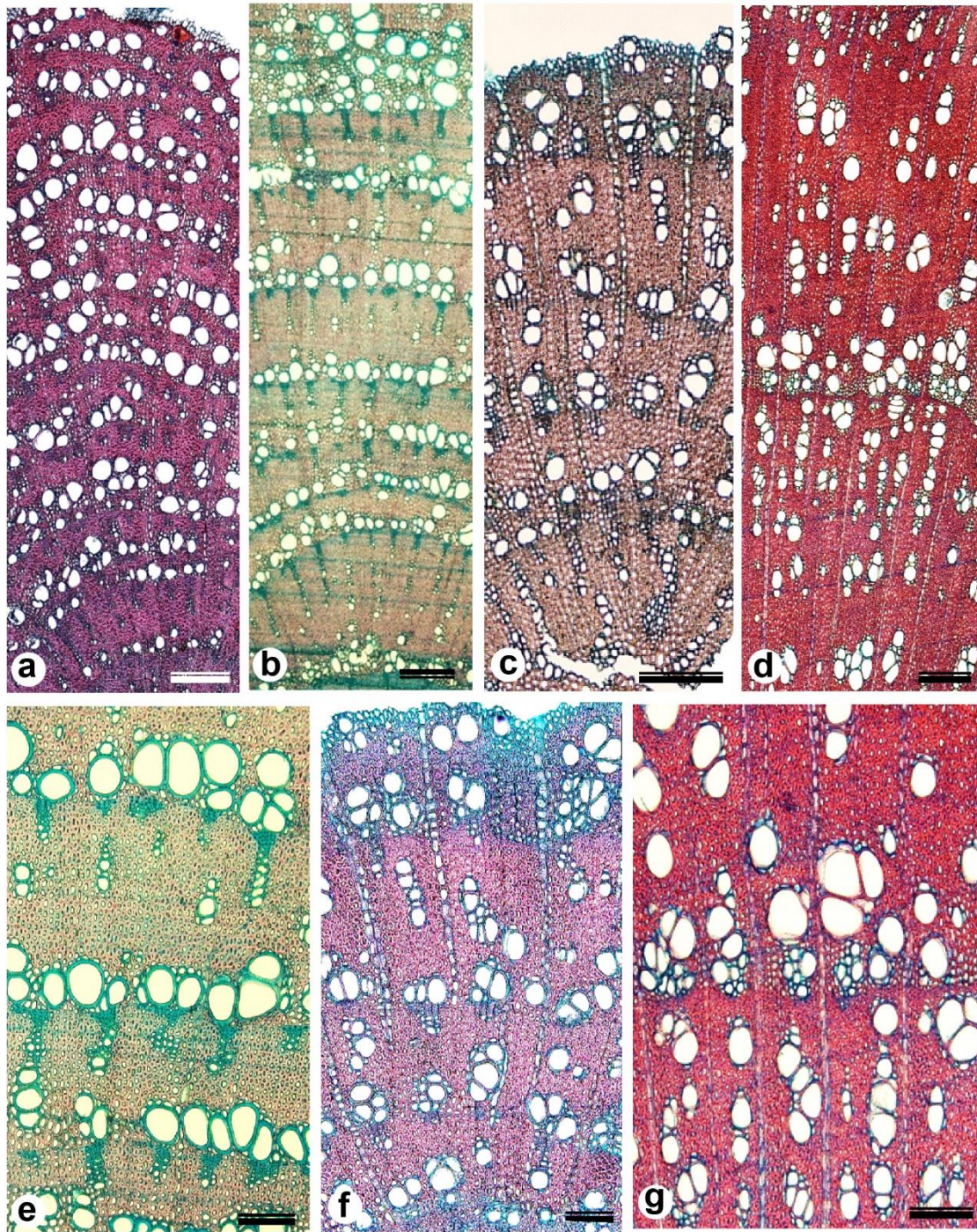
**Fig. 1.** Transverse sections. a: *Atraphaxis billardieri,* b, e: *Atraphaxis billardieri* subsp. *tournefortii*, c, f: *Atraphaxis grandiflora*, d, g: *Atraphaxis spinosa.* a, b: Wood ring porous and growth ring boundaries distinct, c, d: Wood diffuse to semi ring porous and growth ring boundaries distinct, e: Vessels in tangential to radial multiples or small clusters, scanty paratracheal or vasicentric axial parenchyma, f: Vessels in tangential and radial group, or in clusters, g: Vessels in radial multiples of 2 to 4 or in clusters, axial parenchyma scanty paratracheal or vasicentric. Scale bars a-c= 200 µm, e-g = 100 µm.
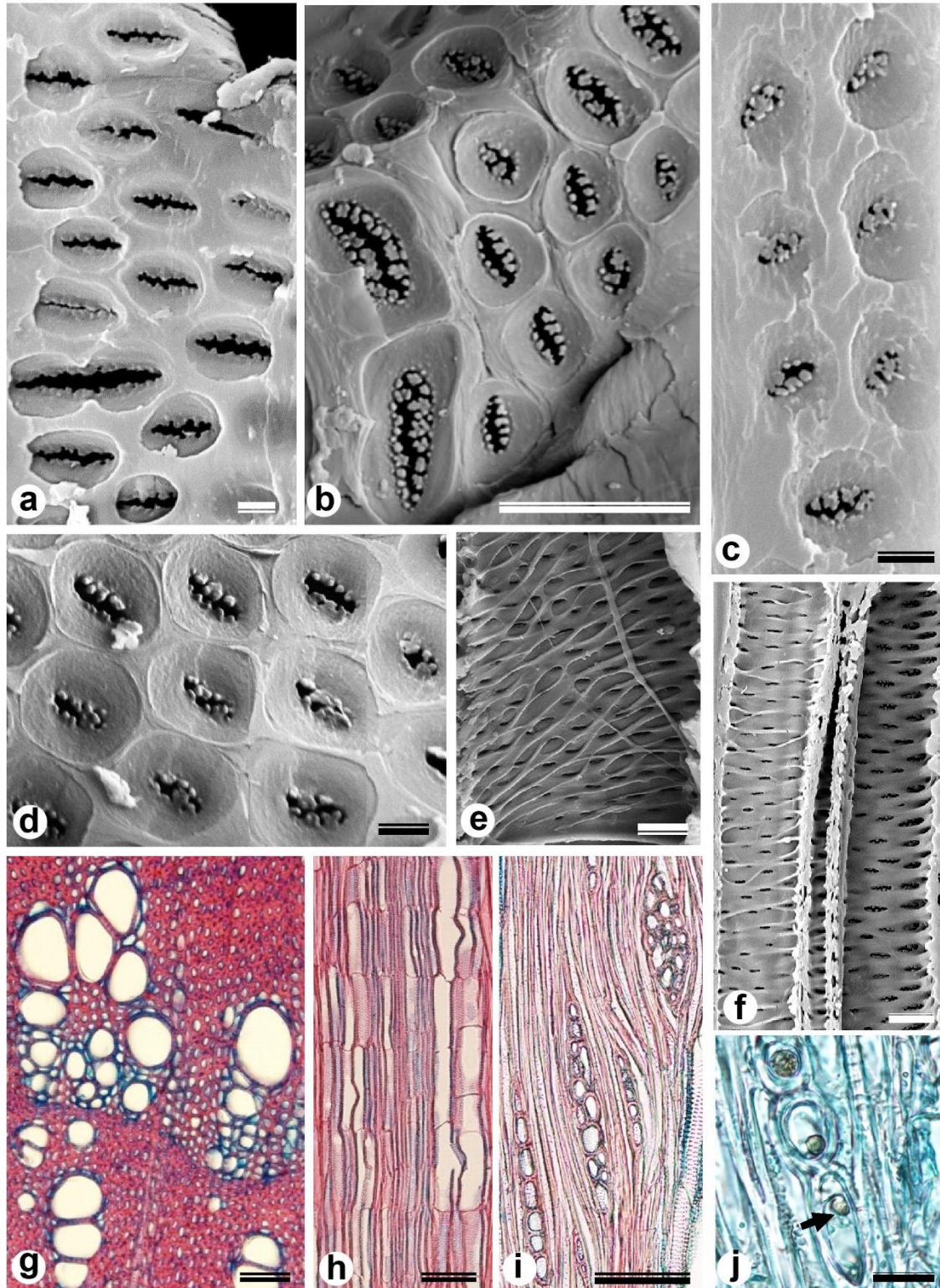
**Fig. 2.** Some qualitative characteristics. a, f, h: *Atraphaxis grandiflora,* b, ı, j: *Atraphaxis billardieri* subsp. *tournefortii,* c: *Atraphaxis billardieri,* d, e, g: *Atraphaxis spinosa.* a-d: Vestured pits on vessel walls, e: Helical thickening in earlywood vessel elements, f: Helical thickening in vessel elements (left) and coalescent pit apertures (right), g: Two distinct vessel diameter classes in ring boundaries, h: Vessel elements storied, i: Heterocellular uniseriate, biseriate and multiseriate rays; j: silica bodies (arrow) in ray cells. Scale bars a, c, d=2 µm; b, e, f= 10 µm; g=50 µm; h, i= 100 µm.

Perforations simple in horizontal to oblique end walls. Intervessel pits vestured (Fig. 2d), alternate, rounded or oval, 4.99 to 6.23 µm in diameter, with slit-like or coalescent apertures. Vessels with helical thickenings (Fig. 2e), vessel members storied. Axial parenchyma scanty paratracheal or vasicentric, and scanty marginal (1 to 2 seriate, initial). Libriform fibres 175 to 400 µm long, 8.3 to 21.58 µm wide, thin- to thick-walls (2.49 to 5.81 µm), simple pits on radial and tangential walls. Rays 5 to 11 / mm, uniseriate (58 to 225 µm, 3 to 13 cells high) and biseriate (110 to 470 µm), rarely multiseriate; heterocellular.

Although growth ring boundaries were distinct in all taxa, it was hard to distinguish latewood vessels in *Atraphaxis spinosa*. Narrow vessels and wide vessels were observed mixed in growth rings, especially in the growth ring initial. Vessel dimorphism, in which vessel diameter tends to be bimodal distribution (nearly), occurs in the *Atraphaxis spinosa* (Figs. 1d, 1g, 2g). Vines and xerophytes often have vessels of two distinct diameter classes (Baas and Schweingruber 1987; Carlquist 1988).

The mean numbers of vessels per group for these species are difficult to obtain because earlywood vessels can be solitary or in small groups, while very narrow latewood vessel groups (include vascular tracheids) can arrange wider patches. It is very difficult to distinguish between very narrow vessels and vascular tracheids in cross-section. Vascular tracheids occur at the end of the growth rings in all studied *Atraphaxis* taxa, and intergrading vessels (Figs. 1e, 1f). Carlquist (1986; 1988) reported vascular tracheids are formed at the end of the growth rings and do not tend to occur intermixed with vessels. In addition, Baas and Zhang (1986) and IAWA Committee (1989) reported they also formed around latewood vessel groups (in a vasisentric position). Because vascular tracheids that lack perforation plate are resistant to embolism, these cells protect the cambium during the dry season, in regions with water stress conditions at the end of the growing season. Vascular tracheids that provide safety to the conductive system are tend to be seen in drought-deciduous species.

*A. spinosa* has the shortest and the narrowest fibers among the studied taxa. There were shorter vessel elements and more numerous vessels in *A. spinosa* than the other *Atraphaxis* taxa (Table 2). According to Baas (1973), Baas *et al.* (1983), Baas and Carlquist (1985), and Wheeler and Baas (1991), vessel elements length and vessel diameter decrease from mesic vegetation towards xeric vegetation, and numbers of vessels in the unit area and vessels grouping rate increase. The calculated mesomorphy values are low for *A. spinosa* (21.44), *A. grandiflora* (28.74), *A. billardieri* (36.71), and *A. billardieri* subsp *tournefortii* (41.42). A mesomorphy value of less than 75 indicates xeromorphy (Carlquist 1977; 1988). *A. spinosa* has more xeromorphic characteristics compared to the others (Table 2). This species is spread in more arid fields. This value was calculated as 4.99 for *A. pungens* depending on vessel member features given in Carlquist (2003). Vessel dimorphism, vessel grouping, more numerous vessel, short vessel elements, and the presence of vascular tracheids are for safety against air embolism and provide increased safety rather than efficiency in the conductive system in drought taxa (Baas *et al.* 1983; Carlquist 1988)

While the diagnostic value of vestured pits is limited (Nair 1998; Jansen *et al.* 1998), they can be employed in wood identification (Bailey 1933; Ohtani and Ishida 1976; Merev 2003). The presence of vestured pits has been reported in Polygonaceae for some genera and species (Jansen *et al.* 1998, 2001; Carlquist 2003). Jansen *et al.* (2001) and Carlquist (2003; 2010) detected vestured pits in *A. spinosa* (from Turkey), and *A. pungens* and *A. frutescens*, respectively. In the present study, vestured pits were photographed with

SEM for *A. spinosa, A. grandiflora, A. billardieri,* and *A. billardieri* subsp. *tournefortii* (Figs. 2a to 2d). The vestured pits examined in this study not only differ among the studied taxa but also are different from the species examined in Carlquist's studies (2003; 2010). Therefore, these findings emphasize the potential significance of vestured pits in the identification of *Atraphaxis* wood.

Carlquist (2003) stated that the width of multiseriate rays is two cells for most members of Polygonaceae. In this study, multiseriate rays ($\geq 3$) are very rare. While biseriate rays are as common as uniseriate rays in *A. spinosa,* uniseriate rays are more common in *A. billardieri, A. billardieri* subsp. *tournefortii*, and *A. grandiflora.* The ray heights (uniseriate and biseriate) of *A. spinosa* and *A. grandiflora* are greater than subspecies of *A. billardieri* (Table 2). In *A. spinosa* and *A. grandiflora*, the mean height of uniseriate rays is 127.92 and 130.11, respectively. In *A. spinosa* the mean height of biseriate rays was found to be longest among studied taxa. For *Atraphaxis pungens*, the mean height of uniseriate rays was 73, and the mean height of biseriate rays was 259 in Carlquist's study (2003). The number of rays per mm in *A. spinosa* more numerous than the other taxa. Ray frequency is more useful in wood identification than ray height because the latter varies depending on ontogeny (Carlquist 1988).

In earlier studies, storied narrow vessels and associated axial parenchyma strands were recorded for *Calligonum arborescens*, *Antigonon leptopus*, *Dedeckera eurekensis, Eriogonum deserticola*, *E. heermannii*, *Gymnnopodium antigonoides*, *Polygonum lapathifolium* (Carlquist 2003), and *Calligonum polygonoides* (Erşen Bak and Cesur 2020) in Polygonaceae. Carlquist (2003) has not reported a storied structure for *Atraphaxis pungens*, although this feature is relatively common in Polygonaceae. In this study, vessels are storied all studied *Atraphaxis* taxa, as shown for *Atraphaxis grandiflora* (Fig. 2h).

Helical thickenings in earlywood and/or latewood vessels are reported for *Atraphaxis* taxa (Figs. 2e, 2f). The presence of helical thickenings in vessels is reported for *Eriogonum fasciculatum*, *E. giganteum* (Carlquist and Hoekman 1985), *Atraphaxis pungens*, *Bilderdykia multiflora*, *Eriogonum giganteum*, *E. heermannii*, and *Muehlenbeckia astonii* (Carlquist 2003) in Polygonaceae. Grooves interconnecting (coalescent) pit apertures (Fig. 2f), which can be confused with helical thickening, were observed especially in earlywood or wide vessel walls of studied *Atraphaxis* woods. These grooves have been reported in vessels of *Antigonon leptopus*, *Calligonum arborescens*, *Coccoloba laurifolia*, *Eriogonum fasciculatum*, *E. kennedyi*, and *Polygonum lapathifolium* in Polygonaceae (Carlquist 2003).

## CONCLUSIONS

1. These four species of *Atraphaxis* shrubs differ in some wood characteristics such as growth rings, vessel grouping, vestured pits, height and density of rays, number of vessels per mm$^2$, and the dimensions of the vessel.

2. The properties such as vessel dimorphism, vessel grouping, vessel density, short vessel member, presence of helical thickening, and tracheids, which provide safety in the water conduction, are common in drought-deciduous species as *Atraphaxis*.

3. Vestured pits may be an important characteristic in identifying *Atraphaxis* wood.

## ACKNOWLEDGEMENTS

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