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Fiber cables in leaf blades of *Typha domingensis* and their absence in *Typha elephantina*: a diagnostic character for phylogenetic affinity

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Vertical fiber cables anchored in horizontal diaphragms traverse the air-filled lacunae of the tall, upright, spiraling leaf blades of *Typha domingensis*, *T. angustifolia*, *T. latifolia* and *T.* × *glauca*. The fiber cables may make a mechanical contribution to leaf blade stiffness while allowing flexibility under windy conditions. We examined the very tall, upright, spiraling leaf blades of *T. elephantina*, which can be over 4 m long, for fiber cables. In the tall species of *Typha*, there are two alternative architectures for upright leaf blades. *T. domingensis* utilizes fiber cables to enhance stiffness in the tall, upright concavo-convex leaf blades, whereas *T. elephantina* may maintain their tall stature in the absence of fiber cables by having a different cross-sectional geometry. These alternative architectures can be used as a diagnostic character along with other morphological characters to assess phylogenetic affinity in *Typha*. The very tall *T. elephantina* which lacks fiber cables may be more closely related to the much shorter *T. minima*, which also has no cables, than to the tall *T. domingensis* and *T. angustifolia*, both of which have prominent fiber cables. *T. elephantina* and *T. minima* share other morphological characters as well.

Keywords: cattail; fiber cables; phylogenetic relationship; Typha domingensis; Typha elephantina

Introduction

The long, linear, upright, air-filled leaves of Typha (cattails, reed mace) achieve impressive heights relative to their width, thickness and cross-sectional area. This is especially noteworthy because they are often subject to high winds. In Typha domingensis Pers., leaves may be longer than 4 m, each leaf composed of a basal sheathing portion and a free-standing blade that may be 3 m long and only 1 cm wide. This impressive height-to-width ratio requires rigidity which is provided by outer stiff tissue and an internal hierarchical series of frameworks which prevent the inward collapse of the stiff outer abaxial and adaxial tissues. The light-weight construction of the leaf blade is a grid work of tissue enclosing air-filled lacunae or compartments that facilitate gas exchange for photosynthesis and respiration (Constable & Longstreth 1994). Each compartment is delimited by the abaxial and adaxial leaf surface tissue, longitudinal leaf partitions running the length of the leaf which keep the leaf surfaces apart, and horizontal diaphragms connected at right angles to the partitions (Teale 1949; Marsh 1955; Kaul 1974; Rowlatt & Morshead 1992; McManus et al. 2002; Witztum & Wayne 2014).

In *Typha domingensis*, fiber cables composed of long fiber cells are anchored in the diaphragms of the leaf blades and traverse the air-filled lacunae for most of the

length of the leaf blade, but are absent in the distal part. We have suggested that the fiber cables contribute to the leaf blade stiffness while allowing flexibility under windy conditions (Witztum & Wayne 2014). Similarly, fiber cables are also present in *Typha latifolia* L., *Typha angustifolia* L., and *Typha* \times glauca Godr., although they are absent in the last-formed leaves of flowering plants of *T. latifolia* (Witztum & Wayne 2014, 2015). In this paper, we compare the architecture of the leaf blades of *T. domingensis* to those of *T. elephantina* Roxb. and characterize the fiber cables of *T. domingensis*.

Materials and methods

Leaves of *Typha elephantina* were collected on November 10, 2014 from plants growing on marshy soil covered by sand on the Mediterranean coast near Michmoret, Israel. Leaves of *T. domingensis* were collected on November 20, 2014 from plants growing in a wet wadi in Beer Sheva, Israel. Cross-sections of the leaf blades of both species were cut 20 cm above the highest connection of the leaf sheath and then at \sim 40 cm intervals toward the tip of the leaf blade. Cross-sections and longitudinal sections were photographed with a dissecting microscope (Leica M 125 equipped with a Leica DFC 295 camera;

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Leica Microsystems, Buffalo Grove, IL, USA). Some sections were treated with phloroglucinol–HCl (McLean & Ivimey Cook 1941). Isolated cables were pulled free from diaphragms and photographed with a compound microscope (Leica DM 2500 equipped with a Leica DFC 295 camera) using polarizing optics and a full-wave plate compensator (Wayne 2014).

Fiber cables were macerated in Jeffrey's macerating fluid (McLean & Ivimey Cook 1941) and fiber cell lengths and widest diameters were measured with a compound microscope (Olympus BH-2; SPLAN10 for lengths and SPLAN40 for diameters Olympus, Waltham, MA, USA); AmScope MU300 camera and ToupView (3.7) image capturing software; AmScope, Irvine, CA, USA) using NIH Image J, which is available free from the National Institutes of Health (http://imagej.nih.gov/ij/).

Small pieces of leaf blades of *T. domingensis* were cut to expose leaf partitions, diaphragms and fiber cables. These were prepared for observation with a scanning electron microscope by fixing in 1.5% paraformaldehyde and 4% glutaraldehyde (Karnovsky 1965) in 0.05M sodium phosphate buffer, pH 6.8, for 24 h at room temperature and then rinsing in buffer. The leaf pieces were dehydrated with an ethanol series and then critical-point dried. Pieces were coated with gold (100–200 Å) and photographed with a Jeol SEM (JSM-35CF, acceleration voltage 25 KV; Jeol, Tokyo, Japan).

Breaking loads for 6-cm segments of fiber cables pulled free from leaves of *T. domingensis* were determined on an Instron 5564 (Instron, Norwood, MA, USA; Witztum & Wayne 2014) using a 10 N load cell with a cross-head speed of 5 mm/min. The diameter of fiber cables was measured with a compound microscope and Young's modulus was determined from the Instron data.

Observations

Tall plants of both *Typha elephantina* and *T. domingensis* may exceed 4 or 5 m in height. In both species leaf blades are spirally twisted (left-handed spirals) and a 4-m long blade of *T. elephantina* spiraled through 1.5 turns or 540°. Similarly a 2.38-m leaf blade of *T. domingensis* spiraled through 540°. Other leaves spiraled through 180°, 225°, 360°, and 450° (Table 1).

Leaves are composed of a basal leaf sheath and a freestanding leaf blade which itself can be 4 m long or more in some leaves. Cross-sections of leaf blades from the thick basal (proximal) part of the blade to the thin ribbondistal portion differ in their shape (Figure 1). Cross-sections of leaf blades of *T. domingensis* cut 20-30 cm above the highest attachment of the tapering leaf sheath are convex on the lower (abaxial or dorsal) side and concave on their upper (adaxial or ventral) surface (Figures 2 and 3). This concavo-convex geometry persists along the length of the leaf blade until the leaf blade becomes thin

Table 1. Leaf twist in spiral leaves of *T. elephantina* and *T. domingensis*.

Leaf number	Leaf blade length (m)	Number of turns	Twist in degrees
T. elephantina			
1 (oldest)	4.0	1.50	540°
2	3.52	1.25	450°
3	3.77	1.25	450°
4	3.73	1.25	450°
5	3.59	1.25	450°
6	3.23	1.25	450°
7 (youngest)	2.10	0.63	225°
T. domingensis			
1 (oldest)	2.38	1.5	540°
2	2.23	1.0	360°
3	2.03	1.0	360°
4	1.89	0.5	180°
5	2.27	1.0	360°
6 (youngest)	2.10	1.0	360°

and ribbon-like toward the tip of the leaf (Figure 1). In *T. elephantina* the cross-section at the base of the leaf blade 20 cm above the highest attachment of the sheath is V-shaped (Figures 4 and 5), then becomes trigonal and



Figure 1. Cross-sections of *Typha elephantina* (left) and *Typha domingensis* (right). Upper sections are from the base of the leaf blade (20 cm above the highest attachment of the leaf sheath to the leaf blade). Other sections were progressively cut through the length of the leaf blade (approximately every 30-40 cm). The smallest unit of scale is in millimeters.



Figure 2. *Typha domingensis.* Cross-section of proximal part of leaf blade. The convex abaxial or dorsal surface (d) and concave adaxial or ventral surface (v) are separated by partitions (P) and diaphragm tissue (D). The scale is in millimeters.

finally, in the distal part of the leaf blade, is thin with a median keel on the abaxial surface (Figure 1). In both species longitudinal partitions prevent the stiff adaxial and abaxial tissues from collapsing inwards and are widest at the thick base of the leaf blade where bending moment due to lateral wind load is greatest. In both species, diaphragms composed of aerenchyma cells are connected perpendicularly to the partitions and in *T. elephantina* are connected to the midportions of the partitions.

The dorsal and ventral surface tissues and the partitions in the leaf blades of *T. domingensis* (Figure 6) and *T. elephantina* (Figure 7) contain lignified vascular bundles and become red when reacted with phloroglucinol-HCl, which reacts with lignin.

In longitudinal paradermal sections of leaf blades of *T. domingensis*, cables composed of fiber cells are



Figure 4. *Typha elephantina.* V-shaped cross-section of proximal part of leaf blade. Note wide partitions (P) that separate ventral (v) and dorsal (d) leaf surfaces. Diaphragms (D) are connected to mid-portions of the partitions. The scale is in millimeters.

anchored in the diaphragms and run vertically through the air-filled lacunae of the blade (Figure 8). No fiber cables are present in leaves of *T. elephantina* (Figure 9). In longitudinal sections of *T. domingensis* treated with phloroglucinol–HCl, only lignified cell walls of vascular bundles in the surface tissues or partitions were red. By contrast, the fiber cables in the lacunae did not react with the phloroglucinol–HCl and were colorless (Figure 10). The fiber cables of *T. domingensis* have an outer sheath of parenchyma cells that contain a single prismatic crystal of calcium oxalate in each cell (Figure 11). The cables anchored in the aerenchyma of the diaphragms (Figure 12) are composed of thick-walled fiber cells (Figure 14) which



Figure 3. *Typha domingensis*. Cross-section of proximal part of leaf blade. Diaphragm tissue (D) attached to partitions (P). Fiber cables (FC) are perpendicular to diaphragms. The scale is in millimeters.



Figure 5. *Typha elephantina*. V-shaped cross-section at base of leaf blade. Diaphragms (D) of aerenchyma cells are perpendicular to partitions (P). The scale is in millimeters.



Figure 6. *Typha domingensis.* Cross-section of convexo-concave part of leaf blade treated with phloroglucinal–HCl. Lignified vascular bundles in abaxial and adaxial tissues and partitions are red.



Figure 7. *Typha elephantina*. Cross-section of V-shaped proximal part of leaf blade reacted with phloroglucinol-HCl. Lignified bundles are red.



Figure 8. *Typha domingensis*. Paradermal longitudinal section at base of leaf blade. Fiber cables anchored in diaphragms run vertically through the air lacunae of the leaf blade. Diaphragms are attached perpendicularly to the partitions. The scale is in millimeters.



Figure 9. *Typha elephantina*. Paradermal longitudinal section at base of leaf blade. No fiber cables are present. The scale is in millimeters.



Figure 10. *Typha domingensis*. Longitudinal paradermal section of base of leaf blade reacted with phloroglucinol–HCl. Fiber cables anchored in diaphragms are non-lignified and unstained. Lignified vascular tissue in partitions reacted with phloroglucinol–HCl and are red.



Figure 11. *Typha domingensis*. Fiber cable observed with polarizing light microscope equipped with crossed polars and a fullwave plate compensator. Sheath cells contain prismatic crystals.



Figure 12. *Typha domingensis*. SEM micrograph of cross-section of leaf blade. Fiber cables (FC) traverse aerenchyma of diaphragm (D) which is attached to partitions (P) and to dorsal (d) and ventral (v) leaf surfaces.



Figure 13. *Typha domingensis*. SEM micrograph of cross-section of fiber cable. The fiber cell lumens are black. Thin-walled sheath cells surround the fiber cable.



Figure 14. *Typha domingensis.* SEM micrograph of fiber cables anchored in diaphragm between two air-filled lacunae.



Figure 15. *Typha domingensis*. SEM micrograph of fiber cables anchored in a diaphragm that is two cells thick.

may be two (Figure 15) or three (Figure 16) cell layers thick.

In *T. domingensis*, fiber cables are most numerous at the base of the leaf, are fewer in number in the upper part of the blade, and entirely absent from the very distal part of the blade. In the tall *T. elephantina*, no fiber cables are present at all, but the V-shaped, trigonal and keeled geometry of the leaf cross-sections is different than that of *T. domingensis*.

In material treated with acid, for example phloroglucinal-HCl, the crystals in the sheath of the fiber cables dissolve, but in fresh material or non-acid prepared material for the SEM the crystals persist (Figure 17). The lengths and diameters of the long, narrow and tapering fiber cells from fiber cables of *T. domingensis* macerated in Jeffrey's solution were measured. The lengths of individual fiber cells ranged from 380 to 1630 μ m with an average length of 986.5 ± 260.0 μ m ($\bar{x} \pm$ SD, n = 100). The widest diameter of individual fiber cells, which taper at each



Figure 16. *Typha domingensis.* SEM micrograph of fiber cables anchored in a diaphragm that is two and three cells thick.

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Figure 17. *Typha domingensis.* SEM micrograph of a fiber cable, with sheath cells containing prismatic crystals.

end, ranged from 3 to 10 μ m with an average diameter of 6.43 ± 1.59 μ m ($\bar{x} \pm$ SD, n = 100).

Cables were pulled free from the diaphragm tissue and 6-cm segments were tested on an Instron for load vs. extension (Figure 18). The diameter of cables was determined with a compound microscope and the cross-sectional area of the cable was used to determine Young's modulus (Figure 19). The fiber cables are non-lignified and light-weight. One meter of dry cable weighs less than 1 mg (Witztum & Wayne 2014). The cables have a break load of about 0.1 N and a Young's modulus of about 21,000 MPa.



Figure 18. *Typha domingensis*. Instron data for a 6-cm segment of dry fiber cable. Load in newtons (N) versus extension (mm).



Figure 19. *Typha domingensis.* Young's modulus (stiffness, in MPa) of the cable in Figure 18 after the cross-sectional area of cable was determined from microscopic measurement of cable diameter.

Discussion

Schwendener (1874) realized that the anatomy of plants could be understood in terms of mechanical principles. The two very tall species of *T. elephantina* and *T. domingensis* share leaf anatomy that includes partitions that keep the stiff adaxial and abaxial surfaces apart and diaphragms that are attached perpendicularly to the partitions. In both species, the leaf blade spirals. In barley, leaf spiralization results in a steeper inclination of the leaf blade (Tsunoda 1997) and in *Typha*, a twisted leaf can achieve greater height before bending over due to its own weight (Schulgasser & Witztum 2004).

In *T. domingensis*, light-weight, stiff fiber cables are last in a hierarchical series that prevent the collapse of diaphragms and help redistribute lateral wind loads so that leaves bend but do not break. In *T. domingensis*, the fiber cables that are anchored in the diaphragms traverse the air-filled lacunae formed by the leaf surfaces, the partitions and the diaphragms. The fiber cables prevent collapse of the diaphragms and the diaphragms stabilize the partitions. The fiber cables may be part of multiple load paths (Rowlatt & Morsehead 1992) through which stresses are redistributed so that no particular structure is overstressed to buckling point (Vincent 2012). In *Typha elephantina*, fiber cables are not present.

In T. elephantina, the V-shaped and trigonal cross-section of the leaf blade contributes structural stiffness. The farther away stiff material is from the neutral axis, the more it adds to rigidity (Niklas 1992). The stiff trigonal and keeled parts of the upright leaf allow leaf blades to reach lengths of 4 m or more. The V-shaped proximal part of the leaf blade, as in other folded monocot leaves (Moulia & Fournier 1997 King et al. 1996), increases its structural stiffness. In a comparison of T. elephantina and T. angustata (a synonym of T. domingensis), Sharma and Gopal (1980) noted differences in the leaf shape but did not include observations on fiber cables. The trigonal cross-sectional architecture was used by Frank Lloyd Wright (1957) in his proposal to build a mile-high skyscraper, which was to be known as the Illinois Tower, and has been used to build the tallest towers and buildings in the world.

In studies of other species of *Typha* (Witztum & Wayne 2014, 2015), the presence or absence of fiber cables was noted. The other outstanding species that has no cables in any leaves is *Typha minima* (Witztum & Wayne 2014). The very robust *T. elephantina* and the delicate *T. minima* have been placed in separate "taxonomic groups" by S. Galen Smith (1987). His Group 5 (page 131) is exclusively represented by *T. minima*, which has pistillate bracteoles and pollen in tetrads. His Group 6 is exclusively represented by *T. elephantina*, also with pistillate bracteoles and pollen in tetrads.

The molecular data of Kim and Choi (2011), however, suggested to them that *T. elephantina* is sister to *T. angustifolia* with moderate support and this is the basis

of *T. elephantina* being placed in their Clade I with *T. angustifolia* and *T. domingensis*. From their Figure 4, which is based on four morphological characters, *T. elephantina* differs in one of them. It, unlike the others, has pollen in tetrads. If an additional column were added for a fifth morphological character, the presence or absence of fiber cables, it differs as well in the absence of fiber cables; all the other species in Clade I have cables (Table 2).

All five morphological characters are the same for *T*. *elephantina* and *T. minima*.

- (A) They both have pollen tetrads.
- (B) They both have filiform stigmas.
- (C) They both have bracteoles in the female inflorescence.
- (D) They both usually have a gap between the male and female inflorescences.
- (E) They both lack fiber cables.

There are no fiber cables in the lacunae of the closely related genus *Sparganium* (Kaul 1973). The absence of fiber cables in leaf blades of both *T. minima* Funck ex Hoppe (synonym *Rohrbachia minima* [Funck ex Hoppe] Mavrodiev) and *T. elephantina*, together with other morphological characters that they share, including pollen tetrads, filiform stigmas, the presence of bracteoles in female inflorescences and a gap between the staminate and pistillate inflorescences, suggest a closer affinity than has previously been considered.

While the goal of systematics is to determine true species phylogenies, a determination of the true phylogeny is challenging because the proposed phylogenies based on molecular data from the nuclear genome and the chloroplast genome often differ from each other and may also differ from proposed phylogenies

Table 2. The relationship between species of *Typha* using morphological features (A–D from Kim and Choi 2011) and E (presence or absence of fiber cables).

Species	Characters						
	Pollen unit (A)	Stigma shape (B)	Bracteoles (C)	Gap between inflorescences (D)	Fiber cables (E)		
T. domingensis	0	0	1	0	1		
T. angustifolia	0	0	1	0	1		
T. latifolia	1	1	0	1	1		
T. shuttleworthii	1	1	0	1	1		
T. elephantina	1	0	1	0	0		
T. minima	1	0	1	0	0		
Sparganium sp.	0	0	0	0	0		

Character states: (A) pollen unit: 0, monads; 1, tetrads. (B) Stigma shape: 0, filiform; 1, spathulate. (C) Presence or absence of bracteoles in female flowers: 0, absent; 1, present. (D) Gap between the staminate and pistillate inflorescences: 0, present; 1, absent. (E) Presence or absence of fiber cables: 0, absent; 1, present. based on morphological characters. Indeed, a knowledge of the genetic basis of morphological characters would be invaluable in relating the two methodologies. While the molecular and morphological approaches each have their values as well as their limitations as a result of introgression and stochastic lineage sorting (Doyle 1992, 1997), the addition of a new clearly defined diagnostic character for *Typha* is not only valuable in terms of understanding the diversity of the genus, but also emphasizes the diverse ways that *Typha* species can overcome the challenges of reaching great heights.

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