Variable Wood Formation and Adaptation to the Alpine Environment of Ephedra pachyclada (Gnetales: Ephedraceae) in the Mustang District, Western Nepal

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INTRODUCTION

The genus Ephedra comprises about 50 species native to arid and semiarid regions of Asia, Europe, northern Africa, western North America and South America (Price, 1996). Its species are mostly erect or sprawling shrubs and grow over a wide altitudinal range from near sea level to 5000 m in the Himalayan and Andean mountains. Ephedra is a member of the Gnetales and is a vessel-bearing gymnosperm with three kinds of tracheary elements, i.e. vessel elements, tracheids and fibre-tracheids. Its wood structure has been studied in relation to vessel evolution in vascular plants (e.g. Thompson, 1912, 1918; Carlquist, 1989, 1992). Carlquist (1996) showed that the wood or stem features of Ephedra differs fundamentally from those of angiosperms and supported the opinion that vessels arose independently in Gnetales and in angiosperms.

In his study of species of Ephedra from all over the world, Carlquist (1988) showed that individuals of Ephedra from high alpine localities of the Andes and the Himalayas become nearly vessel-less, even within a species normally having vessels at lower elevations. He considered this as an adaptation to extremely dry alpine conditions. Although he did not report complete lack of vessels in Ephedra species, this variation in vessel formation seemed to imply that wood formation in Ephedra is quite variable, different from that in dicotyledons. In dicotyledons, a limited number of vessel-less genera never have vessel-bearing species, and all other genera have only vessel-bearing species (Metcalfe and Chalk, 1983; Carlquist, 2001). Moreover, in dicotyledons, wood formation is specific usually to genera or infrageneric taxa such as subgenera, sections, species groups or species, and systematic formation of vessels, axial parenchyma, tracheids, fibres and rays allows identification of taxa at these levels. Besides, in dicotyledons, wood structure is known to vary less at the species level than at the genus level, and adaptive variation in wood structure is usually reported between species within a genus, but not within a species (reviewed in Liu and Noshiro, 2003). In Nepalese species of Rhododendron, for example, wood structure was less variable within species than within the genus (Noshiro and Suzuki, 1995; Noshiro et al., 1995), and alpine species had distinctly small, numerous vessels composed of short vessel elements, probably as an adaptation to frequent freeze–thaw cycles (Noshiro and Suzuki, 2001). Similarly, in the genus Cornus, wood anatomical features had clear latitudinal trends at the genus level, but not at...
the species level (Noshiro and Baas, 2000). Even in Cactaceae with extremely diversified wood structure, each taxon has a specific wood structure, and wood polymorphism in individuals is controlled ontogenetically (Mauseth and Plemons, 1995; Mauseth, 2006). Thus, wood formation and wood structural variation in Ephedra may fundamentally differ from the trends observed in angiosperms.

*Ephedra pachyclada* is a shrubby species distributed in the Himalayan region between Iran and Nepal and, in the Nepal Himalayas, occurs commonly in the montane to alpine zones of dry areas (Lewis, 1978). This species growing between 2600 m and 4800 m a.s.l. in the Mustang district of Nepal seemed to be good material for the study of variation in wood anatomy and wood formation against such non-anatomical factors as altitude and plant size. Occurrence of vessel elements and variation in the lengths of tracheary elements were analysed in this species. Because critical distinction between the three

![Study area and sampling localities (stars) of *Ephedra pachyclada* in western Nepal. White stars show locations where vessel-less specimens were collected.](image-url)
types of tracheary elements was possible only in macerations, the diameter of tracheary elements was not studied for all the specimens.

**MATERIALS AND METHODS**

Thirty-four specimens of *Ephedra pachyclada* Boiss., growing between 2560 m and 4820 m a.s.l. were collected in the Mustang district of western Nepal (Fig. 1; between 28°50’N and 29°10’N in latitude, between 83°45’E and 84°10’E in longitude). The voucher specimens are kept at TI and the Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan. *Ephedra pachyclada* individuals in this district were from 2 mm to 60 mm in stem diameter and from 2 cm to 150 cm in plant height (Table 1). For microscopic observation, two specimens preserved in aqueous 50% ethanol and several air-dried ones were used. In one ethanol-preserved specimen (*Ohba et al. 8340222*), we could distinguish the three types of tracheary elements in cross-section and measured their diameters. For the measurement of tracheary element lengths, wood fragments were obtained from the above-or underground stems of dried specimens, macerated in a 1:1 solution of glacial acetic acid and 30 % hydrogen peroxide for 72–96 h at 60 °C, stained with safranin and gentian-violet or fast-green, dehydrated with an ethanol series, and then observed under a light microscope. For the measurement of tracheary element lengths, at least three preparations for all the specimens and up to five preparations for specimens with few vessels were studied. Ontogenetic trends in tracheary element lengths were studied along a 30-mm radius at 1, 3, 5, 7, 10, 15, 20, 25,
Fig. 2. Wood anatomy of *Ephedra pachyclada*. (A–C) Cross-, tangential and radial sections of a vessel-bearing specimen collected at 2560 m a.s.l. (Ohba et al. 8340222). (A) Growth rings in the mature wood with vessels; (B) multiseriate rays with un lignified ray cells; (C) vessel elements with foraminate perforations plates and spiral thickenings, tracheids with distinctly bordered pits and fibre-tracheids with cell contents. (D) Cross-section of a vessel-less specimen collected at 4450 m a.s.l. (Noshiro et al. 20103263). (E–L) Vessel elements (E, F, G), tracheids (H, I), fibre-tracheids (J, K) and intermediate tracheary elements (L) (Mikage et al. 9465040–9). Scale bars: A, B, E–L = 100 µm; C, D = 50 µm.
29 mm from the pith in the largest specimen (Ohba et al. 8340225). In macerations, the three types of tracheary element were distinguished based on the existence of perforations and pit forms (Esau, 1953; Carlquist, 1989, 1992): (1) vessels have foraminate perforations and fully bordered pits of 10–12 μm in diameter; (2) tracheids lack perforations and, on lateral walls, have circular bordered pits with large borders 8–12 μm in diameter, as well as small bordered pits with small pit cavities and reduced borders 4–6 μm in diameter; (3) fibre-tracheids lack perforations and, on lateral walls, have small bordered pits 3–5 μm in diameter only. For each type of tracheary element, lengths were measured for >30 cells in all specimens using a video micrometer (VM-60 N, Olympus Co., Tokyo, Japan) under a light microscope. Simple correlation between tracheary element lengths and non-anatomical factors were calculated with Microsoft Excel 2004 for Mac (Microsoft Co., Redmond, WA, USA). Multiple regression analysis of tracheary element lengths was carried out using habitat altitude, stem diameter, and plant height as independent variables with SYSTAT 5 (Systat Software Inc., San Jose, CA, USA) on a Macintosh computer.

RESULTS

Wood anatomy of Ephedra pachyclada

Ephedra pachyclada had diffuse-porous wood with distinct to indistinct growth rings (Fig. 2A, 4). Growth rings were defined by differences in vessel size, vessel density, and diameter of tracheids and fibre-tracheids between the outermost latewood and the following earlywood and ranged from 40 μm to 1750 μm wide (Fig. 2A). Most vessels were polygonal in outline, ranging from 13 μm to 41 μm and from 16 μm to 49 μm in tangential and radial diameters, respectively, and the vessel size reduced gradually from earlywood to latewood (Fig. 2A). Vessel elements had foraminate perforations and distinct spiral thickenings...
Tracheids were rectangular or polygonal in outline, ranging from 5 μm to 22 μm and from 3 μm to 29 μm in tangential and radial diameters, respectively. Fibre-tracheids had cell contents and were arranged in short tangential lines (Fig. 2A, C), but were difficult to observe in dried samples. Fibre-tracheids were rectangular or polygonal in outline, ranging from 3 μm to 17 μm and from 3 μm to 18 μm in tangential and radial diameters, respectively. Their walls had secondary walls and were as thick as those of vessels and tracheids. Spiral thickenings were obscure in tracheids and fibre-tracheids. In cross-sections of the latewood, it was difficult to distinguish

| Table 2. Correlation table of non-anatomical data and tracheary element lengths of Ephedra pachyclada |
|---------------------------------------------------|--------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Altitude                                          | Stem diameter                                   | Plant height                     | Vessel element length          | Tracheid length                 | Fibre-tracheid length          |
| 1.000                                             | 1.000                                           | 0.600**                         | 0.894**                        | 1.000                           | 0.652**                        |
| Stem diameter                                     | -0.444*                                         |                                 | -0.600**                       |                                 |                                 |
| Plant height                                      | 0.581**                                         | -0.894**                        | 1.000                           | 0.652**                         | 1.000                           |
| Vessel element length                             | 0.563**                                         |                                 | 0.954**                         |                                 |                                 |
| Tracheid length                                   | -0.737**                                        |                                 |                                 | -0.662**                        |                                 |
| Fibre-tracheid length                             | 0.595**                                         |                                 |                                 | 0.966**                         |                                 |
| Significance level: *, 1 %; **, 0.5%.

Fig. 5. Trends in tracheary element lengths in relation to altitude, stem diameter and plant height of Ephedra pachyclada. Significance level: **, $P < 0.5\%$. 

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vessel elements from tracheids or fibre-tracheids (Fig. 2A). Seven specimens were vessel-less, and wide tracheids formed at the beginning of growth rings had only bordered pits (Fig. 2D). No axial parenchyma was observed. Rays were distinctly heterocellular, mostly multiseriate with sparse sheath cells, rarely uniseriate and often partially lignified (Fig. 2B).

Vessel elements had foraminate perforations, and pits on the lateral walls were circular and fully bordered, 10–12 μm in diameter (Figs. 2E–G). Tracheids had large bordered pits, 8–12 μm in diameter, that usually were concentrated at both ends, and also had small bordered pits with small pit cavities and reduced borders, 4–6 μm in diameter (Fig. 2H, I). Fibre-tracheids had simple or quite small bordered pits, 3–5 μm in diameter, throughout the elements (Fig. 2J, K). The size and shape of bordered pits and the occurrence of perforations varied greatly even within a tracheary element, and tracheary elements intermediate between typical vessels and tracheids or between typical tracheids and fibre-tracheids were often observed (Fig. 2L).

Variation in tracheary element lengths

In the largest specimen, tracheary element lengths increased significantly from the pith to 3 mm distant from the pith and then gently outward from there (Fig. 3). Vessel elements showed a steeper increase in length than tracheids and fibre-tracheids. Vessel elements at 1 mm from the pith were more sporadic in nearly vessel-less tracheids and fibre-tracheids. At other points, lengths of tracheary elements did not differ from each other.

In all the specimens the tracheary element lengths ranged from 293 μm to 733 μm (Table 1). Seven specimens collected above 3700 m a.s.l. completely lacked vessel elements. The lengths of vessel elements, tracheids and fibre tracheids were nearly equal, being significantly different in only six specimens.

Simple correlation between tracheary element lengths and non-anatomical factors were significant at the 0.5% level (Table 2). Between tracheary elements, correlation coefficients were high, all above 0.954. Between non-anatomical factors, plant height and stem diameter were strongly correlated, but altitude had weaker correlation with either of these. Tracheary element lengths decreased linearly as altitude increased, but against stem diameter and plant height, they showed a curvilinear exponential increase (Fig. 5). Multivariate analyses showed that about 60% of variation in tracheary element length was affected by non-anatomical factors, and altitude had a stronger effect on tracheary element lengths than plant height or diameter (Table 3). Coefficients for altitude were significant at 0.5% level, but coefficients for stem diameter and plant height were insignificant.

TABLE 3. Multiple regression analysis of tracheary element lengths of Ephedra pachyclada

<table>
<thead>
<tr>
<th>Character</th>
<th>Partial regression coefficient</th>
<th>Standard regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCC</td>
<td>CD</td>
</tr>
<tr>
<td>Vessel element length</td>
<td>0.769</td>
<td>0.591</td>
</tr>
<tr>
<td>Tracheid length</td>
<td>0.788</td>
<td>0.621</td>
</tr>
<tr>
<td>Fibre-tracheid length</td>
<td>0.790</td>
<td>0.624</td>
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<tr>
<td>Excluding specimens nos.</td>
<td>O-8340222, O-8340222</td>
<td></td>
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<tr>
<td>Vessel element length</td>
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<td>0.448</td>
</tr>
<tr>
<td>Tracheid length</td>
<td>0.749</td>
<td>0.560</td>
</tr>
<tr>
<td>Fibre-tracheid length</td>
<td>0.727</td>
<td>0.529</td>
</tr>
</tbody>
</table>

MCC, multiple correlation coefficient; CD, coefficient of determination.

DISCUSSION

Tracheary element differentiation in Ephedra pachyclada

In genera of Gnetales, tracheids are longer than vessel elements and seem to elongate after differentiation from cambial initials (Carlquist, 1996). In the 84 specimens of Ephedra that Carlquist (1989, 1992) studied, tracheids were longer than vessel elements in 78 specimens, with their ratio averaging 1.109 for New World species and 1.12 for Old World ones. This agrees with trends in woody dicotyledons where imperforate tracheary elements are usually from 1.2 to 3.5 times longer than vessel elements (Bailey and Tupper, 1918; Bailey, 1920; Chattaway, 1936; Metcalf and Chalk, 1983). In Ephedra pachyclada of the Mustang district, however, the three kinds of tracheary element were identical in length within individuals and showed no elongation after differentiation from cambial initials. Besides tracheary element lengths, intermediate tracheary elements between vessel elements, tracheids and fibre-tracheids often occur in Ephedra pachyclada (Fig. 3), as was found in other species of Ephedra by Thompson (1912) and Carlquist (1989, 1992). These seem to show that differentiation between tracheary elements in this Ephedra species is not as clear-cut as in dicotyledons, especially in terms of their length.

Functional studies on conifer tracheids and angiosperm vessels show that tracheids and vessels of the same diameter have similar conduit area conductivities (Sperry et al., 2006). Tracheids of Ephedra have pit membranes similar to conifer species (Bauch et al., 1972), and the similar conductivities for conifer tracheids and angiosperm vessels of the same diameter also seems to apply to the wood of.
Ephedra. Thus, the weak differentiation between tracheary elements in Ephedra pachyclada and the little difference in conductivity between tracheids and vessels of the same diameter may facilitate substitution of vessels with tracheids in this species. In the Mustang district, several individuals growing at high altitudes, between 3780 m and 4800 m, completely lacked vessels (Figs 1 and 2D, Table 1). However, they co-occurred with vessel-bearing individuals around Dhi and between Dhi and Damodar Kunda. All the plants growing in this area were quite small, <10 cm tall and 4 mm in stem diameter, growing on silty gravel, and were identical morphologically. This shows that extreme alpine conditions do not necessarily make Ephedra plants vessel-less, but in such conditions, some individuals or individual groups seem to stop vessel formation completely probably due to their sensitive response to local conditions.

Tracheids and narrow vessels are less prone to embolism caused by freeze–thaw cycles than are wide vessels (Sperry et al., 1994), and loss of vessels in Ephedra pachyclada at high altitudes seems to be an adaptation to such freeze–thaw cycles in temperature. In the Mustang district of Nepal, at altitudes corresponding to the upper temperate to alpine zones on the southern side of the Himalayas, Ephedra plants experience frequent cycles of freeze–thaw. The number of diurnal freeze–thaw cycles are not known for the Mustang district, but freezing temperatures occur, on average, on 73 d from November to April at Marpha (2566 m a.s.l.) and on 191 d from October to May at Lo-Manthang (3705 m a.s.l.) in 1971–1986 (Department of Irrigation, Hydrology and Meteorology, 1977, 1982, 1984, 1986; Department of Hydrology and Meteorology, 1988). In the Khumbu district, eastern Nepal, on the southern side of the Himalayas, diurnal freeze–thaw cycles of air temperature are known to occur on 160–170 d year\(^{-1}\) at 4420 m and about 190 d year\(^{-1}\) at 5000 m, mostly in spring and autumn (Matsuoka, 1984).

For Ephedra pachyclada, however, freeze–thaw cycles may not be that important, considering the diameters of tracheary elements. In an individual from the lowermost habitat, vessels averaged 26 \(\mu\)m and 31 \(\mu\)m in tangential and radial diameters, respectively, and tracheids were all <30 \(\mu\)m. The critical diameter for freezing-induced cavitation is estimated to be 30 \(\mu\)m, and vessels >40 \(\mu\)m in diameter were nearly completely cavitated after a freeze–thaw experiment (Davis et al., 1999). Thus, in the climatic conditions of the Mustang district, half of the vessels and all the tracheids of Ephedra pachyclada would not be cavitated by freeze–thaw cycles. Similar adaptation in vessel diameter was observed in alpine shrub species of Nepalese Rhododendron, and their vessels were mostly below 20 \(\mu\)m and 30 \(\mu\)m in tangential and radial diameters, respectively (Noshiro et al., 1995).

In Nepalese Rhododendron, their inefficiency in water conduction was compensated by high vessel density of over 800 vessels mm\(^{-2}\) (Noshiro and Suzuki, 2001). In Ephedra pachyclada, inefficiency in water conduction is probably compensated by the large number of tracheids forming the ground tissue. Thus, adaptive advantage of vessel-less individuals over vessel-bearing ones is probably not great in terms of defence against frequent freeze–thaw cycles.

Although distribution of vessel-less individuals at high elevations seems to show an adaptation to an alpine environment, as Carlquist (1988) pointed out, vessel loss does not seem to be that important for the survival of Ephedra pachyclada in the alpine environment. Even at comparatively low altitudes, an individual that always formed vessels outward from 2-8 mm from the pith (Fig. 4A) was found to be nearly vessel-less in inner growth rings (Fig. 4B). In this individual, vessel formation fluctuated within growth rings, and vessel formation often resumed after the formation of false growth rings (Fig. 4C). Another individual formed nearly vessel-less growth rings after making vessel-bearing inner growth rings (Fig. 4D). Probably for short Ephedra pachyclada plants that have only scale-like leaves, enough water may be conducted either with vessels or tracheids. Ephedra sinica growing in the Mongolian steppe was found to have a low content of photosynthetic tissues with high photosynthetic activity and low transpiration, and to be highly efficient in water use (Ivanov et al., 2004). Apparently, in Ephedra pachyclada of the Mustang district, cambial derivatives will differentiate into vessel elements only when there can be lateral expansion of tracheary elements, probably from high demand for water for active photosynthesis in twigs and enough precipitation to comply with the demand. However, cambial derivatives remain as tracheids without lateral expansion in late summer to autumn, either from less demand for water for photosynthesis in twigs or from less than enough precipitation to comply with the demand. Probably in Ephedra pachyclada at high altitudes experiencing short cold summers, photosynthetic demand and wood formation are not vigorous enough to allow vessel formation and vessel-less wood results.

**Adaptation to extremely dry alpine conditions**

Within most species of woody dicotyledons, variation in wood structure has a weak or no correlation with non-anatomical factors such as stem diameter, plant height, altitude or latitude (Sastrapradja and Lamoureux, 1969; van der Graaf and Baas, 1974; Noshiro and Suzuki, 1995; Noshiro and Baas, 2000; Liu and Noshiro, 2003). One exception is Alnus nepalensis, a pioneer tree growing between 790 m and 2740 m on the southern side of the Himalayas. Ten features of its wood structure had a significant correlation with non-anatomical factors with coefficients of determination up to 0.417 (Noshiro et al., 1994). In this species, vessel element and fibre lengths had a strong negative correlation with altitude, and their coefficients of determination were 0.315 and 0.415, respectively. These values are, however, lower than those observed in Ephedra pachyclada, and the correlation between tracheary element lengths and altitude in Ephedra pachyclada is exceptionally high. Among dicotyledons, similar altitudinal trends in tracheary element length occur at the genus or family levels (Baas, 1973; van den Oever et al., 1981; Noshiro and Suzuki,
1995; Noshiro et al., 1995), and all the taxa studied show reduction in the length of tracheary elements as altitude increases.

This correlation between tracheary element lengths and altitude in *Ephedra pachyclada* occurs in small individuals <60 cm tall and 15 mm in stem diameter except for two individuals up to 150 cm tall and 60 mm thick. Comparison between gentler ontogenetic trends in tracheary element lengths (Fig. 3) with steeper trends between individuals (Fig. 5) clearly shows that intrinsic differences in lengths of tracheary elements exist between such small individuals. Even if the two largest individuals are excluded, the multiple regression analysis shows that vessel element and tracheid lengths have a correlation only with altitude at the 5% significance level with coefficients of determination between 0.448 and 0.560 (Table 1). Why are shorter tracheary elements in such small shrubs more adaptive at higher altitudes? This is probably an adaptation to extreme droughts occurring every 5 years or so when plants become prone to severe embolism.

Situated in the rain shadow side of the Himalayas, highland steppe prevails in the Mustang district of Nepal. In areas north of Marpha where *Ephedra* plants are common, the summer monsoon provides most of the rainfall. In 1971–1986, annual precipitation ranged from 197 mm to 377 mm, and 65–81% of precipitation, i.e. from 136 mm to 285 mm, fell between April and September of the summer season (Department of Irrigation, Hydrology and Meteorology, 1977, 1982, 1984, 1986; Department of Hydrology and Meteorology, 1988). At Marpha (2566 m a.s.l.), the mean summer precipitation between April and September was 272 mm with the mean monthly temperature ranging from 11.2 °C to 16.8 °C, but the summer precipitation went down to 162 mm in 1973 and 171 mm in 1979. At Lo-Manthang (3705 m a.s.l.), the mean summer precipitation is 136 mm with the mean monthly temperature ranging from 5.7 °C to 13.9 °C, but the summer precipitation recorded 53 mm in 1982 and about 80 mm in 1979 and 1983. In these years, July or August monthly precipitation often went below 30 mm.

In such drought years, cavitation is expected to start from wider vessels and tracheids. Across various plant groups, diameter of tracheary elements is not directly correlated with their vulnerability, but surface morphology of tracheary elements is found to be more important (Tyree et al., 1994). Within the same plant having similar surface morphology of tracheary elements, however, vessels and wider tracheids are known to cavitate more easily than narrow tracheids (Hargrave et al., 1994; Sperry and Saliendra, 1994). In the stem of *Salvia mellefer* from California, USA, Hargrave et al. (1994) showed that conduits over 20–30 μm in diameter tended to embolize and that the probability of embolism increased with increasing conduit diameter. In *Betula occidentalis*, Sperry and Saliendra (1994) showed that roots and trunks with wider vessels tended to cavitate more easily than twigs and petioles and that adult plants with wider vessels tended to cavitate more easily than juvenile plants with narrower vessels. Thus, it is probable that in *Ephedra pachyclada*, vessels and wider tracheids are more prone to cavitation and become hydraulically non-conductive in severe drought years.

In an environment with such extreme drought, pathway redundancy among tracheids would be critical for the survival of plants. Pathway redundancy allows water conductance to be maintained by circumventing embolized tracheary elements (Tyree et al., 1994). Shortening of tracheids in *Ephedra pachyclada* plants at higher altitudes would make circumvention of embolized tracheids easier. Even in vessels, short vessel elements with scalariform perforations tend to inhibit bubble expansion and to contain embolism (Tyree and Zimmermann, 2002). Also, when refilling embolized tracheary elements, shorter tracheary elements are easier to refill than longer ones (Sperry, 2003). Thus, short tracheary elements in *Ephedra pachyclada* at high altitudes must be a mechanism to survive extreme dry summers by minimally retaining water conductance.

**CONCLUSIONS**

In *Ephedra pachyclada* growing in the dry Mustang district of Nepal, lengths of three types of tracheary elements were nearly equal within individuals and decreased among individuals as altitude increased. In several individuals growing at the highest altitudes, reduction in tracheary element lengths was accompanied by the loss of vessels. The differentiation of the three types of tracheary elements in *Ephedra pachyclada* was not so rigid as in dicotyledons. Vessels seemed to be differentiated only when their lateral expansion is allowed, probably induced by high photosynthetic demand for water and enough supply of water from precipitation. Shorter lengths of tracheary elements in individuals at high latitudes seemed to be a mechanism to confine embolism in narrower areas that are easier to circumvent through pathway redundancy. These trends are considered to be an adaptation to extremely dry, unpredictable alpine conditions on the northern side of the Himalayas and differed from the trends observed in dicotyledons.

The complete lack of vessels in several individuals, equal lengths for all tracheary elements, and variation in wood formation observed in *Ephedra pachyclada* seem to imply that this species has a variable system of wood formation different from dicotyledons. In dicotyledons, after differentiation from cambial initials, cell fate seems to be defined before leaving the cambial zone (Catesson et al., 1994; Mellerowicz et al., 2001; Evert, 2006). If this early determination of cell fate applies also to *Ephedra pachyclada*, some growth conditions of the plant seems to negate cell fate to vessels elements for all cambial derivatives, completely suppressing the genes concerned with vessel formation. If, on the contrary, cell fate is determined in the zone of radial cell expansion outside the cambial zone, such growth conditions delays lateral expansion of cambial derivatives in this zone and the successive formation of perforation plates in putative vessel elements. The latter scenario seems to be more plausible judging from weak distinction between vessel elements and tracheids in *Ephedra* including *E. pachyclada* as observed by
Thompson (1912), Carlquist (1989, 1992) and in the present study. The lack of axial elongation in tracheids of Ephedra pachyclada and the similar conductivities for tracheids and vessels of the same diameter (Sperry et al., 2006) seems to facilitate such variable, physiological control of cell fate. In either way, this type of variable wood formation is not known in dicotyledons and supports the opinion of Carlquist (1996) that the wood structure of Gnetales is fundamentally different from that of angiosperms.

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