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COMPARATIVE ANATOMY OF ROOT AND STEM OF SOME NATIVE AND EXOTIC *ASPARAGUS* L. SPECIES

TAHIRA NAWAZ[1], MANSOOR HAMEED[1], WAQAR-U-NISA[1], M. SAJID AQEEL AHMAD[1], ADNAN YOUNIS[2] AND HINA KANWAL[1]

[1]*Department of Botany, University of Agriculture, Faisalabad, Pakistan 38040*
[2]*Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan 38040*
[*]*Corresponding author e-mail: hameedmansoor@yahoo.com*

Abstract

Leaf, root, stem and cladode anatomical studies were conducted to evaluate their significance in taxonomy and distribution pattern of *Asparagus* species in the Faisalabad region, Pakistan. *Asparagus adscendens,* an important medicinal plant, was the only native species of the region that is distributed widely in the Punjab plains and sub-mountainous region. It is characterized by relatively thick cuticle and epidermis as well as thick endodermis. *Asparagus densiflorus* and *A. setaceus* were the widely cultivated species in the region and adapted to a variety of environmental conditions. They had thicker roots, larger parenchymatous cells, and well developed vascular tissue than the other species and cultivars. On the whole, a strong relation of descriptive features with plant anatomy was recorded, and therefore, these features can be used as a tool for the taxonomy of the genus *Asparagus*. In addition, by using these anatomical modifications their tolerance to environmental stresses can be easily assessed.

Introduction

Modern taxonomists have placed the genus *Asparagus* in family Asparagaceae of order Asparagales rather than in Liliaceae (Chase *et al*., 2009). The family Asparagaceae contains two other genera, *Myrsiphyllum* and *Protasparagus,* and 370 species (Ali & Khan, 2009). Most of the species are cultivated as ornamentals and as culinary (*A. officinallis*) and are native to Africa. *Asparagus adscendens* distributed in Punjab plains and foothill regions of Pakistan and Kashmir (Chen *et al*., 2000).

Many species of the genus *Asparagus* are medicinally very important and novel chemicals have been isolated form roots, stem and cladodes. Steroidal sapinins (Debella *et al*., 1999) and antiprotozoal compounds (Oketch-Rabah & Dossaji, 1997) have been isolated from the roots of *A. africanus.* Roots of *A. adscendens* contain steroid glycoside (Tandon *et al*., 1990) and steroidal sapinins (Sharma *et al*., 1982), whereas leaf extract contains oligofuro- and spiro-stanosides (Sharma & Sharma, 1984). Chemical constiurents of this species stimulate insulin secretion and inhibits starch digestion (Mathews *et al*., 2006). Capsoneoxanthin (carotenoid) has been isolated from the fruits of *Asparagus falcatus* (Deli *et al*., 2000).

Stem in *Asparagus* species generally consists of 4 major tissues; (i) pith that is free of vascular bundles, (ii) two surrounding layers of parenchyma and fibres containing cortex and vascular bundles, (iii) sclerenchyma sheath, and (iv) epidermis and sub-epidermal (hypodermal) layers (Waldron & Selvendran, 1990a). The dermal tissue is a protective layer. Lignified xylem can also play a role in giving mechanical strength in addition to performing conduction. Among ground tissue, parenchyma is the main storage region, collenchyma gives some support and strength to the plant, and sclerenchyma gives the main mechanical strength (Rodriguez-Arcos *et al*., 2002).

Sclerification, which is mainly due to lignin, is characteristically found in higher plants and makes up 20-30% of the total plant biomass available on the globe (Lewis, 1999). Lignin is the major component of secondary cell walls of fibres, xylem vessels and tracheids, and is mainly responsible for supplying mechanical support to the cells (Monties, 1989) in addition to controlling water movement across the xylem vessels (Northcote, 1989). Lignification in vascular and sclerenchyma tissue is mainly due to xylosyl residues from xylans, cellulose and phenolics (Waldron & Selvendran, 1990b; Holm *et al*., 2003). Nonenzymatic toughening in mechanical tissue and xylem vessels has also been reported in *Asparagus* tissue, which may be related to lignin deposition (Smith *et al*., 1987; Li & Zhang, 2006).

The present study was conducted to study comparative anatomical features of some native and exotic species of the genus *Asparagus,* to evaluate the importance of anatomical parameters in taxonomy, and to relate anatomical modifications to different ecological niches.

Materials and Methods

Faisalabad and its adjoining administrative districts, namely Sheikhupura, Hafizabad, Sargodha, Khushab, Jhang, Toba Tek Singh, Okara, Lahore and Kasur were thoroughly explored for the herbarium record and distribution of native and exotic *Asparagus* L. species/cultivars. Eight species/cultivars were selected for the comparative anatomical studies of stem and root.

For anatomical studies, one cm piece from the base of thickest root from root-shoot junction and for stem anatomy, one cm piece from the central portion of internode were excised. The material was preserved in FAA (formalin acetic alcohol) solution, which contained v/v formalin 5%, acetic acid 10%, ethyl alcohol 50% and distilled water 35%. The material was then subsequently transferred to acetic alcohol (v/v 25% acetic acid and 75% ethyl alcohol) solution for long-term preservation.

The double-stained standard technique was used for the preparation of permanent slides of transverse section following Ruzin (1999). Data were recorded with the help of ocular micrometer under a compound microscope. Camera photographs were taken by Carl-Ziess camera-equipped microscope. Data were subjected to statistical analysis to work out ANOVA for the comparison of means. Standard error was calculated following Steel *et al*., (1997). Cluster analysis was conducted using MiniTab Statistical Software.

Results

Botanical and common names of the species of the genus *Asparagus* L., along with habitat ecology are presented in Table 1.

1. Root anatomy: Root anatomical parameters in *Asparagus* species showed very specific structural modifications. In *A. adscendens,* epidermis was multi-layered composing of large round cells, which was the thickest among all *Asparagus* species (Fig. 1). Sclerified hypodermis was recorded inside the epidermis that composed of a layer of 3-4 cells thick. Large aerenchyma was present in the root with small bundles of sclerenchyma that were scattered throughout the cortical region. This species possessed the maximum of metaxylem and protoxylem vessel area and also the phloem area (Table 2).

Table 1. Habitat ecology of some native and exotic species of *Asparagus* L. from Faisalabad and adjoining districts.

Species	Habitat ecology
Asparagus adscendence Roxb. (Safed musli)	Distributed in Pakistan, Kashmir and India (Mehta & Subramanian, 2005). Tolerant to a variety of environmental stresses, particularly salt and drought stresses (Ali & Khan, 2009).
Asparagus africanus Lam. (Climbing asparagus fern)	Native to S Africa. Moderately tolerant to drought stress (Scott and Batchelor. 2006)
Asparagus densiflorus (Kunth) Jessop (Asparagus fern)	Native of S. Africa; commonly cultivated throughout the world (Ali and Khan, 2009). Good tolerance to salt (Bezona *et al*., 1996) and drought stresses (Rieger & Litvin, 1999).
Asparagus densiflorus (Kunth) Jessop cv. Meyeri (Foxtail fern)	
Asparagus falcatus L. (Sicklethorn)	Native to Africa, distributed in evergreen forest on forest margins and in dense scrub.
Asparagus setaceus (Kunth) Jessop cv. Nanus (Fern Asparagus)	Native to S. Africa., commonly cultivated in most gardens of the Punjab and occasionally becoming naturalized (Chen *et al*., 2000; Straley & Utech, 2002; Ali & Khan, 2009). Tolerant to salt stress (Bezona *et al*., 1996).
Asparagus setaceus (Kunth) Jessop cv. Pyramidalis	
Asparagus umbellatus (Bresler) Kunth. (Ming fern)	Native to Africa. Also distributed in Portugal, Spain, Canary Islands (Jessop, 1966).

Table 2. Anatomical characteristics of root and stem of some native and exotic *Asparagus* L. species from Faisalabad and adjoining districts.

Characteristics	*Asparagus adscendens*	*Asparagus africanus*	*Asparagus densiflorus*	*Asparagus densiflorus* 'Meyeri'	*Asparagus falcatus*	*Asparagus setaceus* 'Nanus'	*Asparagus setaceus* 'Pyramidalis'	*Asparagus umbellatus*
	Root anatomy							
Root area (mm^2)	0.19c	0.15b	0.25d	0.23d	0.12a	0.28d	0.18c	0.19c
Epidermal thickness (µm)	20.52e	11.63bc	7.31a	10.42b	6.58a	15.73d	13.79cd	8.92ab
Cortical thickness (µm)	346.75c	262.14a	482.61f	346.75c	369.02d	389.52e	312.19b	351.59cd
Sclerenchymatous thickness (µm)	578.62f	526.14e	682.54g	686.29g	412.06d	122.93a	297.12b	363.61c
Endodermal thickness (µm)	10.85d	10.02c	9.11b	10.23c	8.29a	12.15g	11.48f	11.09e
Pericycle thickness (µm)	8.02c	5.12a	8.53d	13.93f	6.52b	8.76d	8.79d	9.83e
Vascular region area (μm^2)	29179.83d	2132.27c	5267.19g	3681.11e	4537.81f	1971.89b	1852.64a	5427.82h
Metaxylem area (μm^2)	2559.23h	923.53c	1653.49e	846.69a	2392.73g	1086.53d	897.49b	1876.49f
Protoxylem area (μm^2)	269.19f	152.54c	202.14d	194.47d	246.92e	92.59b	81.92a	315.21g
Phloem area (μm^2)	48.21e	41.72c	44.59d	47.59e	43.95d	31.43b	26.06a	40.63c
Pith area(μm^2)	235.91c	256.84d	492.86g	165.47a	432.56e	469.62f	226.85b	532.82h
	Stem anatomy							
Epidermal thickness (µm)	23.29c	11.57a	29.54de	27.68d	29.82de	19.69b	12.82a	32.51e
Cuticle thickness (µm)	7.83a	13.92cd	15.12d	14.72d	12.64c	14.72d	8.09a	9.73b
Cortical cell area (μm^2)	176.28a	419.73e	493.15f	283.39c	377.31d	369.54d	281.33c	246.48b
Sclerenchymatous thickness (µm)	485.02g	391.46d	406.19e	399.58de	415.89f	382.65c	342.39a	465.32b
Vascular bundle area (μm^2)	3619.08e	2824.57b	3414.82c	3529.65d	4229.64g	2549.14a	2847.54b	3952.12f
Metaxylem area (μm^2)	847.62f	229.71c	231.59c	142.81a	412.62e	213.68b	236.59c	391.09d
Phloem area (μm^2)	6275.02e	6242.52e	6351.11e	3749.62d	525.79a	2951.46b	3019.71b	3125.38c

Means sharing similar letters are statistically non-significant in each row

Asparagus africanus had multi-layered epidermis, which was composed of irregularly-shaped small tightly-packed cells. Cortical region was composed of thin-walled small cells, which were closely packed without intercellular spaces. Endodermis was surrounded by a distinct sclerenchymatous region of 3-4-layers.

The cortical region in *Asparagus densiflorus* showed two distinct regions, the outer consisted of densely-packed, relatively thick-walled elongated cells and inner of irregular-shaped thin-walled cells with large inter-cellular spaces. Sclerenchyma outside the vascular region was highly developed with very thick walls. Pith region also showed intensive sclerification. In contrast, its cultivar 'Meyeri' showed well-developed sclererenchyma outside endodermis. In addition, scattered sclerenchymatous cells (1-3 cells in each group) were distributed throughout the cortical region close to the periphery of the inner cortex. The outer cortex in this case was relatively more prominent and wider as compared to that recorded in *A. densiflorous.* The size of metaxylem vessels was extremely reduced in the cultivar 'Meyeri'. *Asparagus densiflorus* showed the maximum values for root area, cortical thickness, vascular region area and pith area, whereas its cultivar 'Meyeri' showed the maximum values for sclerenchyma thickness, pericycle thickness and phloem area.

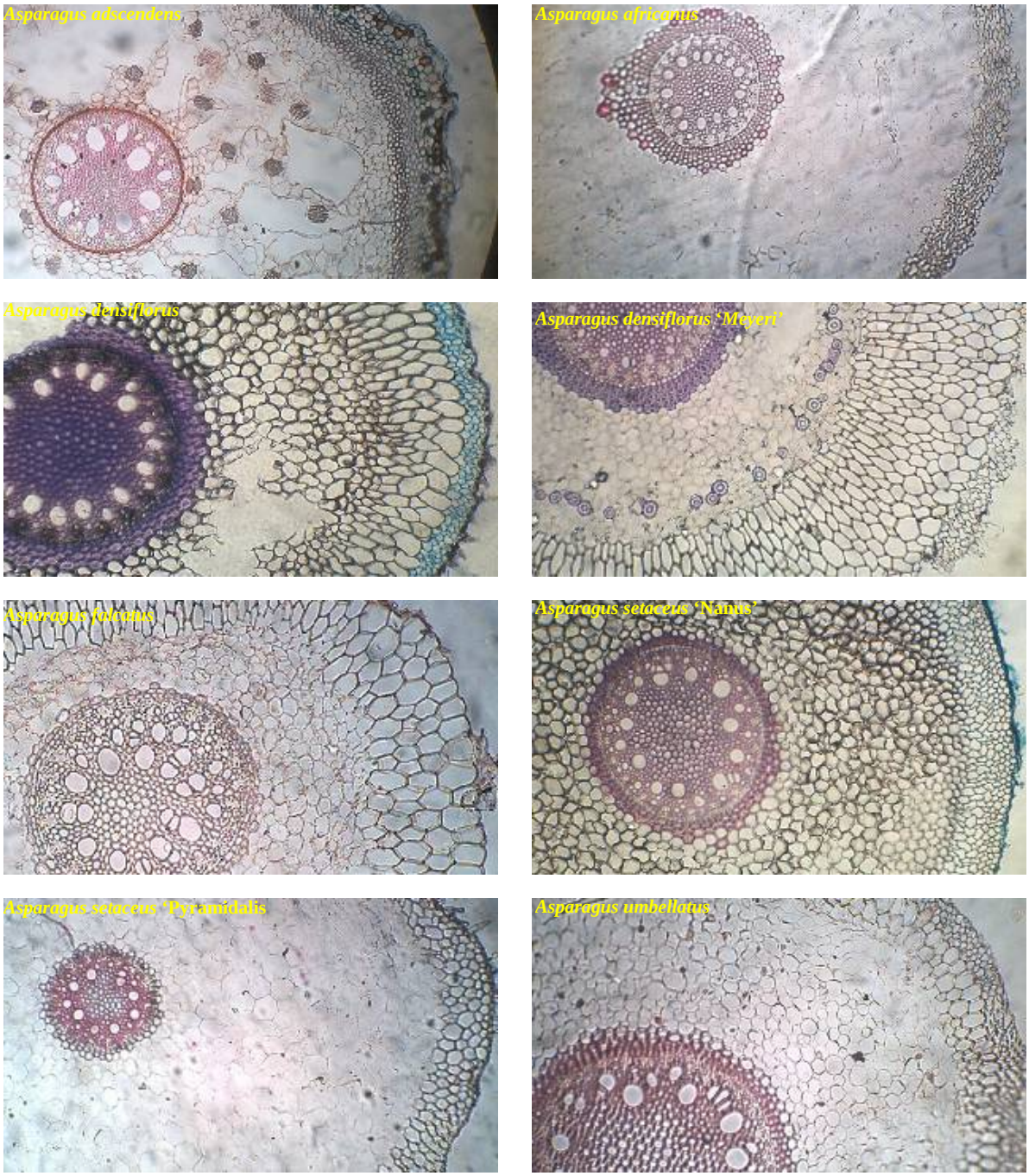

Fig. 1. Transverse sections of root of some *Asparagus* species/cultivars from Faisalabad and adjoining districts.

Asparagus falcatus showed two distinct cortical regions, the outer with large tightly-packed cells and the inner smaller thin-walled cells with large intercellular spaces. Poorly developed sclerenchyma was recorded outside the thin-walled endodermis. The vascular region contained numerous large metaxylem vessels.

Root transverse section of *A. setaceus* 'Pyramidalis' was quite similar to that of *A. africanus*, but in the former case, multi-layered epidermis was comprised of much larger and round cells. Large cortical region was comprised of thin-walled cells with large intercellular spaces. Poorly developed sclerenchyma was recorded outside the endodermis, but the vascular region was intensively sclerified. *A. setaceus* 'Nanus', in contrast, showed cortical region of densely-packed irregularly-shaped cells inside the multi-layered epidermis. Slight sclerification was recorded outside the endodermis and in the vascular region. Root transverse section of *A. umbellatus* showed small-celled multi-layered epidermis, two distinct regions of cortical cells and single-layered sclerenchyma outside the endodermis.

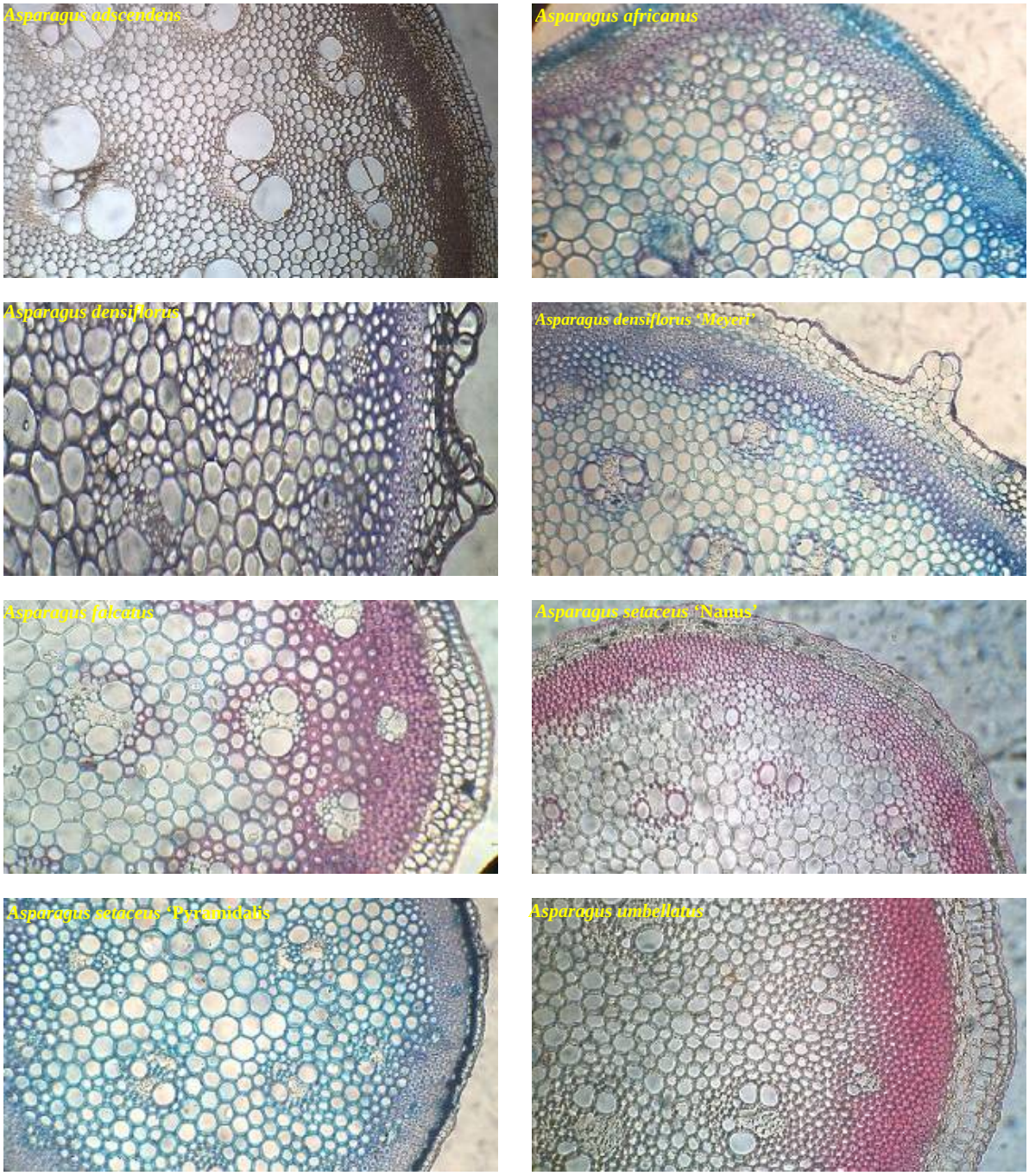

Fig. 2. Transverse sections of stem of some *Asparagus* species/cultivars from Faisalabad and adjoining districts.

2. Stem anatomy: Stem anatomy also showed great diversity in all tissues, e.g., dermal, mechanical, parenchymatous and conducting tissues. *Asparagus adscendens* showed distinctively large metaxylem vessels and multi-layered sclerenchyma comprising of small cells inside the multi-layered parenchymatous hypodermis. Sclerenchyma in *A. africanus* was very much developed with very thick secondary walls (Fig. 2).

Stem transverse section of *A. densiflorus* and its cultivar 'Meyeri' showed thick epidermis, which protruded out by means of greatly enhanced epidermal cell area at distinctive intervals. Both *A. densiflorus* and its cultivar 'Meyeri' showed parenchymatous hypodermis and highly developed sclerenchyma inside the hypodermis.

Asparagus falcatus showed thick epidermal layered with thick cutical surrounding parenchymatous hypodermis. A distinctive highly developed sclerenchyma was recorded below the hypodermis. Vascular bundles were the largest in this species.

Asparagus setaceus 'Pyramidalis' showed highly sclerified epidermis surrounding the sclerified hypodermis comprising large cells. A distinct sclerenchyma with small

cells was recorded inside the hypodermis. However, other cultivar 'Nanus' of this species showed epidermis with thick deposition of cuticle and crushed cells of hypodermis inside the epidermis. A well-developed sclrenchymatous region was recorded inside the hypodermis.

Asparagus umbellatus, on the other hand, showed very explicit structural modification in stem anatomy. A very thick cuticle was recorded outside the epidermis and a single layer of large distinctive parenchyma just below the epidermis. Intensive sclerification was recorded inside the cortical region comprising thick-walled small cells. Vascular bundle area was the maximum in this species.

Discussion

All *Asparagus* species are widely cultivated throughout the world, especially *A. densiflorus* and *A. setaceus.* Both these species are tolerant to osmotic stresses like salinity and drought (Bezona *et al*., 1996). All are native to Africa except *A. adscendens,* which is distributed in arid and semi-arid sub-mountainous regions of Central Asia and Pakistan (Ali & Khan, 2009).

Anatomical parameters relating to root and stem in *Asparagus* species/cultivars were not only species-specific, but the indicators of habitat ecology. Species, which are adapted to osmotic stress conditions, showed some specific anatomical modifications that may play a vital role in the conservation of water, and therefore extremely helpful to cope with environmental hazards.

Asparagus adscendens depicted prominent aerenchyma in roots which is recorded only in this species. This may help not only in gas exchange, but also in the transport of excessive salts, nutrients and water through aerenchyma, as was reported by van der Weele *et al*., (1996) in *Zea mays* and Hameed *et al*., (2009) in *Imperata cylindrica*. Presence of sclerenchyma bands in the cortical region gives strength to the root tissues and may play a critical role in preventing tissue collapse, especially in the presence of large aerenchyma under dry habitats. In addition, thick sclerenchymatous hypodermis and endodermis and large metaxylem vessels can prevent water loss through root surface, (Cholewa & Griffith, 2004), control radial flow of water and efficient conduction of water. All these structural modifications are incredible under extremes of environmental hazards. Vascular tissue with greatly enlarged metaxylem vessels and thickly sclerified hypodermis again plays a vital role in efficient transport of water and nutrients as well as in prevention of undue water loss (Steudle, 2000; Ali *et al*., 2009).

Asparagus densiflorus, however, showed intensive sclerifications, not only outside the endodermis, but also in the pith region of root. In contrast, *A. africanus* and *A. densiflorus* 'Meyeri' showed intensive sclerification outside the endodermis and *A. setaceus* 'Pyramidalis' in the pith region only. Such modification is helpful in providing rigidity to roots and therefore, critical under limited moisture conditions (Read *et al*., 2006).

Intensive sclerification in the hypodermal region of stem was recorded in all *Asparagus* species/cultivars, particularly in *A. falcatus* and *A. umbellatus.* Both these species had a prominent parenchymatous region just inside the epidermis, which comprised enlarged, tightly packed cells. In contrast, *A. densiflorus* and its cultivar 'Meyeri' had very different type of epidermis with protrusion of epidermis consisting of greatly enlarge cells (Ristic & Jenks, 2002). This structure may be of great ecological significance as total surface area increased in addition to forming ridges in the stem surface, which is indeed an important water conservation strategy.

In conclusion, on the basis of several root and stem anatomical characteristics, *A. adscendens*, *A. densiflorus* and *A. setaceus* are better adapted to a variety of environments justifying their wide distribution throughout the world. Anatomical features are species-specific and can be effectively used in taxonomy of the members of family Asparagaceae. Anatomical features are the indicators of habitat ecology, and can be effectively used as markers for stress tolerance in these species.

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