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ECOLOGICAL SIGNIFICANCE OF DIVERSITY IN LEAF TISSUE ARCHITECTURE OF SOME SPECIES/ CULTIVARS OF THE GENUS *ROSA* L.

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Abstract

Six species/cultivars were selected for the comparative anatomical studies of leaf in the genus *Rosa* from Faisalabad and adjoining administrative districts. The most widely cultivated *R. damascena* showed some specific anatomical modifications in leaves such as thick leaves (lamina), thick upper epidermis, large palisade cells, wide protoxylem vessels, large phloem area and large and more stomata particularly on adaxial epidermis. This species showed reduced cortical cell area, lower epidermis thickness, spongy cell area, vascular bundle area and metaxylem area. These characteristics indicated ecological success of this species to a variety of environmental types. The second most widely cultivated species, *R. bourboniana* 'Gruss-an-Teplitz' showed thick leaves (lamina), large cortical cell area, large vascular bundle area, large metaxylem vessels and large phloem area. All the *Rosa* species/cultivars showed great diversity in leaf tissue architecture, which are the indicators of distribution and ecological success of the genus *Rosa* in the Punjab plains, particularly Faisalabad environments.

Introduction

Economically Rosaceae is an important family with great morphological diversity. Morphological and chemical assessments (Challice, 1974) has been reported that genetic analysis based sequences strongly support the monophyly of the family Rosaceae (Morgan *et al*., 1994). However, Dickinson *et al.,* (2007) combined morphological and molecular characters from members of the Rosaceae, they recircumscribed Maloideae and Rosoideae, the two largest subfamilies and rejected Amygdaloideae and Spiraeoideae, neither of which proved to be monophyletic. More recently, Potter *et al*., (2007) proposed a new classification of Rosaceae based on molecular phylogenetic analyses, in which they recognized three subfamilies viz., Rosoideae, Dryadoideae and Spiraeoideae. The newly defined Spiraeoideae includes all genera previously assigned to Amygdaloideae and Maloideae.

The family Rosaceae comprises about 125 genera and 3500 species, cosmopolitan in distribution, but abundant in North Temperate Zone (Landrein *et al*., 2009). Members of Rosaceae are well represented in Pakistan with great economic and scientific importance. This family contains a great number of fruit trees of temperate regions. Some plants in the genus *Rosa* containing essential oils or with a high vitamin content are used in industries (Lu *et al*., 2003). Numerous species are used for medical purposes or are cultivated as ornamentals (Yü *et al*., 1986).

About 25 species of wild roses have been reported growing in many parts of the world, mainly in temperate climates including Pakistan. Many of them have contributed to the development of highly-priced modern cultivars. Classification of *Rosa* species is little difficult and that is because of relatively high hybridization potential (Yan *et al*., 2005), and this may be the reason that the wild types of some modern forms are not always known (Wissemann, 2000). A large number of cultivated varieties and hybrids with great diversity in flower shape, size and color have been developed from many of the wild species.

In Rosaceae, the research has been focused on morphological-based or genetic-based variations, which are used for the classification (Jan *et al*., 1999; Hancock *et al*., 2004; Chang *et al*., 2007; Evans *et al*., 2007). However, anatomical-based markers for exploring genetic diversity of *Rosa* species cultivars are expected to be quite high and they can be efficiently used for species identification in addition to structural adaptive features for different environmental conditions (Mohapatra & Rout, 2006; Yan *et al*., 2005). The present study was focused on the evaluation of diversity in leaf tissue architecture and the relation of these adaptive anatomical features with the environmental hazards.

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 record and distribution of native and exotic *Rosa* L., species/cultivars. Six species/cultivars were selected for the comparative anatomical studies of leaf.

For anatomical studies, one cm piece from the leaf centre along with midrib was taken. The material was preserved in FAA (formalin acetic alcohol) solution for fixation, which contained formalin 5%, acetic acid 10%, ethyl alcohol 50% and distilled water 35%. The material was then transferred in acetic alcohol (one part acetic acid and three parts ethyl alcohol) solution for long-term preservation.

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

Results

Among all the *Rosa* species/cultivars studied, the most widely cultivated were *R. damascena* and *R. bourboniana* 'Gruss-an-Taplitz' (Table 1). *Rosa bourboniana* and *R. centifolia* were the less frequently cultivated species, whereas *R. chinensis viridiflora* and *R.* 'Yellow Sunblaze' were less frequently cultivated in the Punjab plains. However, these species and cultivars can be rated as most tolerant and most sensitive to environmental stresses > *R. damascena* > *R. bourboniana* 'Gruss-an-Taplitz' > *R. centifolia* > *R. borboniana* > *R. chinensis viridiflora* > *R.* 'Yellow Sunblaze'. Transverse sections of leaf midrib and lamina are presented in Figs. 1-3.

All the leaf anatomical characteristics varied significantly among *Rosa* species/cultivars (Table 2). Midrib thickness was the maximum in *R. bourboniana* 'Gruss an Teplitz', whereas, in other *Rosa* species/cultivars, there was a little variation in midrib thickness. In contrast, variation in lamina thickness was relatively less as compared to midrib thickness. All *Rosa* species/cultivars varied slightly regarding lamina thickness except *R. chinensis viridiflora,* which showed greatly reduced lamina thickness.

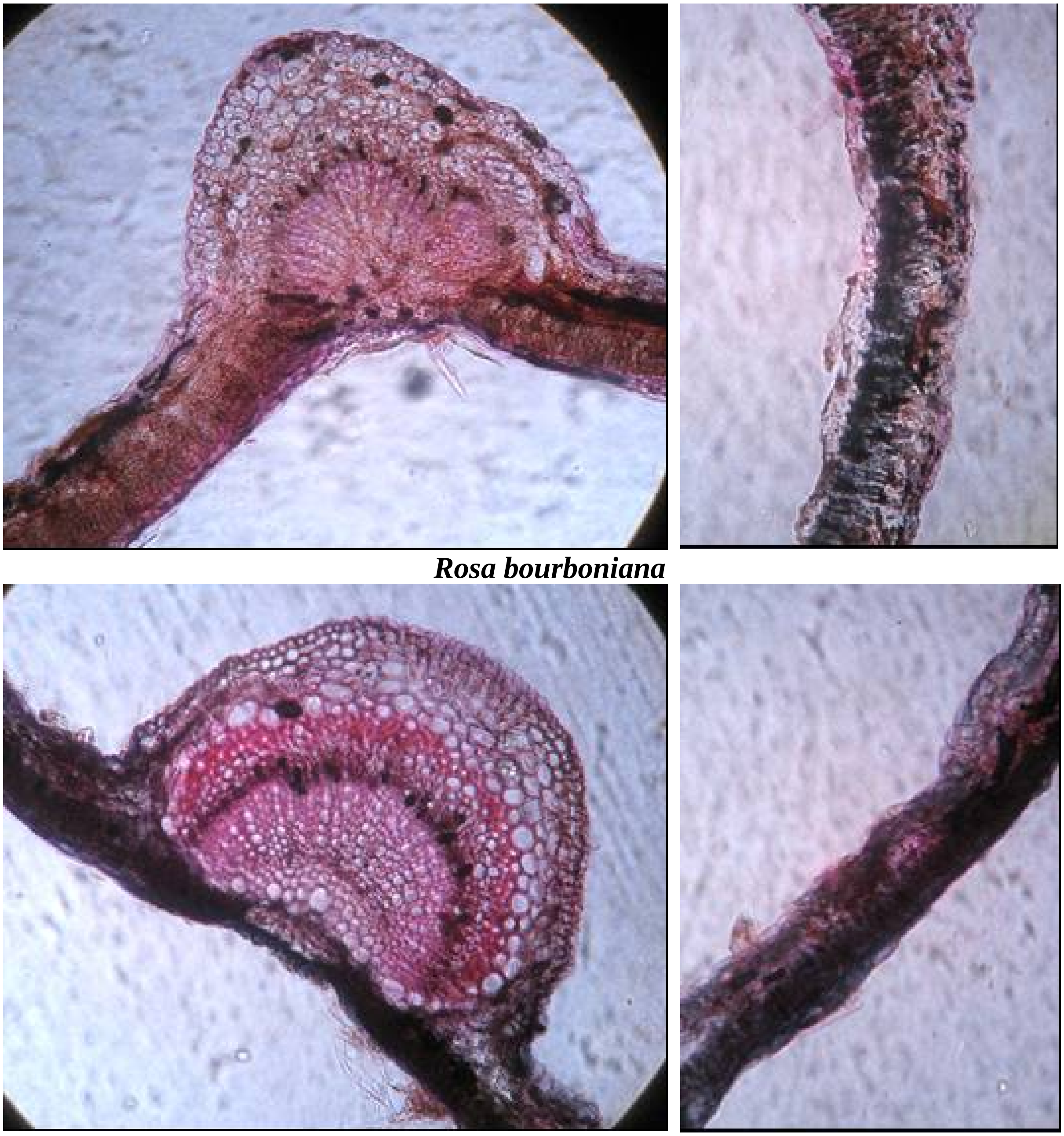
Table 1. Ecology of different *Rosa* species/cultivars.

Species/cultivar	Vernacular name	Ecology
Rosa bourboniana L.	Edward rose, Bourbon rose	Widely cultivated for its scented flowers (Gudin, 2000)
Rosa bourboniana cv. Gruss-an-Taplitz	"Surkha gulab"	Extensively used as a rootstock for modern cultivars and for its fragrance and oil production (Hussain & Khan. 2004)
Rosa centifolia L.	Cabbage rose, Pink rose	Widely cultivated for oil production. Not known in a truly wild situation (Tucker &Maciarello, 1988)
Rosa chinensis var. *viridiflora*	Green rose	Native to China, widely cultivated elsewhere (Lu *et al*., 2003)
Rosa damascena Mill.	'Gulkand gulab', Damask rose	Widely cultivated for its ornamental and medicinal importance (Kashani *et al*., 2010). Widely cultivated for oil production (Kaur *et al*., 2007)
Rosa cv. Yellow Sunblaze	Miniature rose	Miniature roses are frequently cultivated as ornamental (Pati *et al*., 2006)

Table 2. Leaf anatomical characteristics of some *Rosa* species/cultivars (means ± SE).

Characteristics	*Rosa* species/cultivars					
	R. bourboniana	*R. bourboniana* 'Gruss an Teplitz'	*R. centifolia*	*R. chinensis viridiflora*	*R. damascena*	*R.* 'Yellow Sunblaze'
Midrib thickness (µm)	424.84d ± 39.46	547.39e ± 45.27	400.33c ± 41. 54	340.42a ± 38.88	375.82b ± 39.22	427.56d ± 45.61
Lamina thickness (µm)	89.87b ± 8.22	111.65c ± 12.89	136.16d ± 11.95	43.57a ± 8.2	157.95e ± 14.58	114.38c ± 12.64
Epidermal thickness-adaxial (µm)	87.40b ± 8.77	118.00d ± 9.93	297.19f ± 15.99	69.92a ± 8.45	161.71e ± 13.98	103.35c ± 9.44
Epidermal thickness- abaxial (µm)	52.44a ± 5.42	78.67c ± 5.77	174.81f ± 11.89	144.22e ± 10.82	69.93b ± 5.57	80.77d ± 9.80
Cortical cell area (μm^2)	550.68e ± 50.88	961.50f ± 70.97	506.97d ± 48.44	445.78c ± 37.32	201.04a ± 22.28	297.19b ± 25.30
Spongy cell area (μm^2)	113.63a ± 13.32	388.97d± 31.46	157.33b ± 15.40	445.78e ± 41.54	358.38c ± 29.44	524.46f ± 47.89
Palisade cell area (μm^2)	148.59a ± 14.26	287.58c ± 29.34	354.88e ± 37.87	332.15d ± 32.51	415.19f ± 39.92	174.82b ± 19.30
Vascular bundle area (μm^2)	23285.83d ± 2150.76	59438.31f ± 3468.99	28635.28e± 2581.55	7761.94a ± 805.23	18758.03b ± 1554.58	20331.43c ± 1892.85
Metaxylem area (μ^2)	332.15e ± 30.58	209.78d ± 18.32	161.70c ± 14.44	52.38a ± 5.98	78.67b ± 9.27	52.40a ± 5.99
Protoxylem area (μ^2)	384.60c ± 30.55	152.97a ± 30.21	157.33a ± 30.23	201.04b ± 30.34	559.42d ± 30.54	856.61e ± 30.88
Phloem area (μ^2)	113.63b ± 11.45	148.59d ± 12.53	122.37c ± 11.42	87.40a ± 8.33	152.97d ± 11.55	113.33b ± 10.41
Adaxial stomatal area (μ^2)	0.00a ± 0.00	0.00a ± 0.00	0.00a ± 0.00	87.40b ± 11.43	161.71c ± 12.39	0.00a ± 0.00
Abaxial stomatal area (μ^2)	52.73a ± 5.33	144.22d ± 11.88	174.81e ± 21.45	52.66a ± 4.88	69.93b ± 7.76	78.67c ± 7.55
Adaxial stomatal density	0.00a ± 0.00	0.00a ± 0.00	0.00a ± 0.00	21.34b ± 3.54	58.45c ± 6.04	0.00a ± 0.00
Abaxial stomatal density	41.72bc ± 4.56	43.83c ± 3.94	66.54d ± 6.78	37.41b ± 4.21	72.48e ± 7.23	32.29a ± 4.55

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



Rosa bourboniana

***Rosa bourboniana* ‘Gruss-an-Taplitz’**

Leaf midrib **Leaf lamina**

Fig. 1. TS of leaf of *Rosa bourboniana* and its cultivar ‘Gruss-an-Taplitz’.

Epidermal thickness on both abaxial and adaxial leaf surfaces was maximum in *R. centifolia* as compared to those recorded in other *Rosa* species/cultivars. The minimum of this parameter was recorded in *R. chinensis viridiflora* on adaxial surface and *R. bourboniana* on abaxial surface.

Cortical cell area was maximum in *R. bourboniana* ‘Gruss an Teplitz’. The minimum of this characteristic was recorded in *R. damascena* and *R.* ‘Yellow Sunblaze’. However, in other three roses, a little variation was recorded in cortical cell area.

Variation was relatively high in palisade cell area as compared to spongy cell area among mesophyll tissues. The maximum of palisade and spongy was recorded in *R. damascena* and *R.* ‘Yellow Sunblaze’, respectively, whereas, *R. bourboniana* showed the minimum of both palisade and spongy cell area.

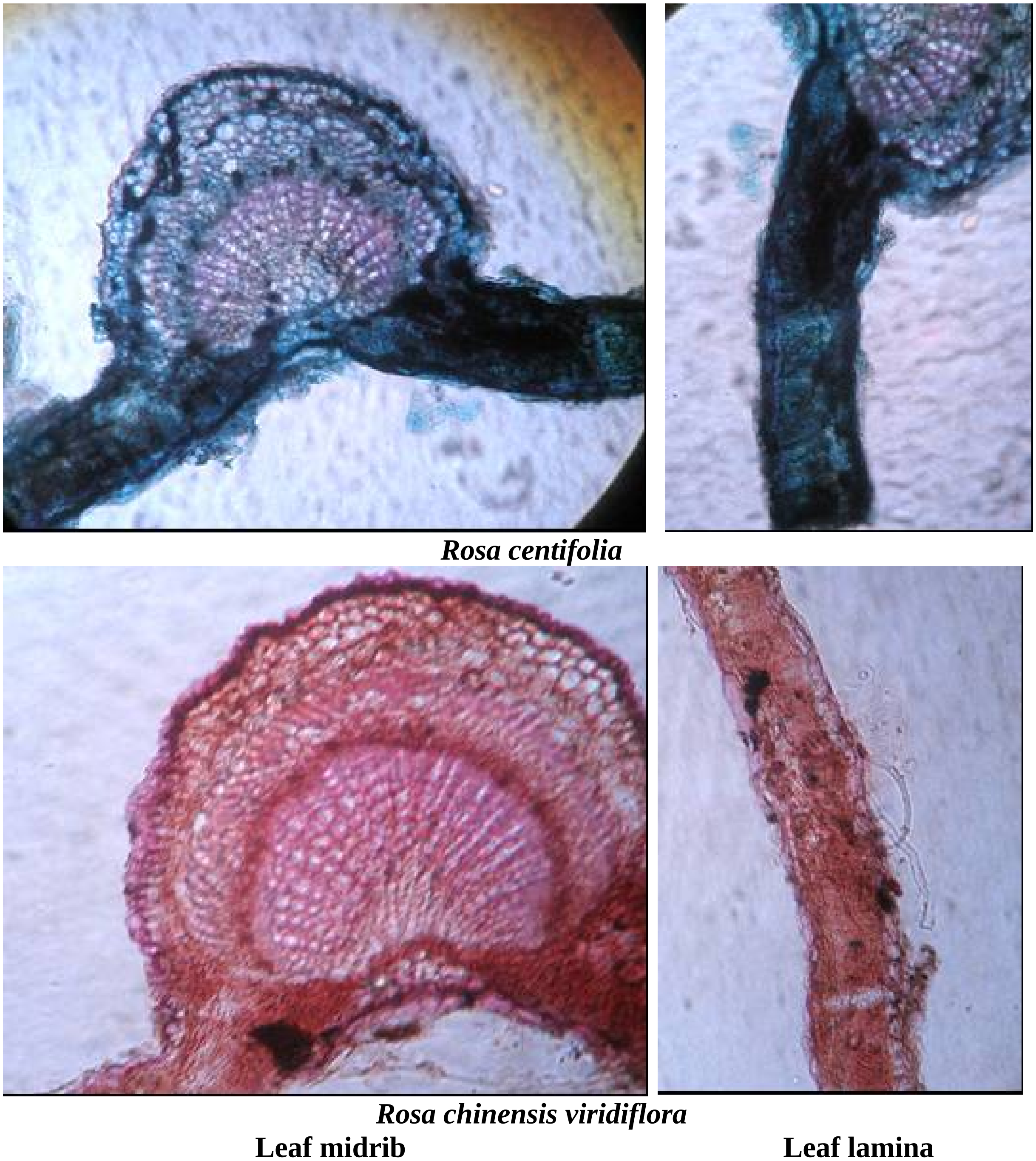


Fig. 2. TS of leaf of *Rosa centifolia* and *R. damascena*.

The maximum vascular bundle area was observed in *R. bourboniana* 'Gruss-an-Teplitz', whereas the minimum of this character was recorded in *R. chinensis viridiflora,* which showed greatly reduced vascular bundle area as compared to those recorded in other species. The maximum of metaxylem area was recorded in *R. bourboniana,* which was followed by the metaxylem recorded in *R. bourboniana* 'Gruss-an-Taplitz'. However, *R. chinensis viridiflora* and *R.* 'Yellow Sunblaze' showed greatly reduced metaxylem vessels. Protoxylem area was the maximum in *R.* 'Yellow Sunblaze' and the minimum in *R. bourboniana* 'Gruss-an-Taplitz'. Phleom area was one of the least variable characteristics in *Rosa* species/cultivars. The maximum phloem area was recorded in *R. damascena* and the minimum in *R. chinensis viridiflora.*

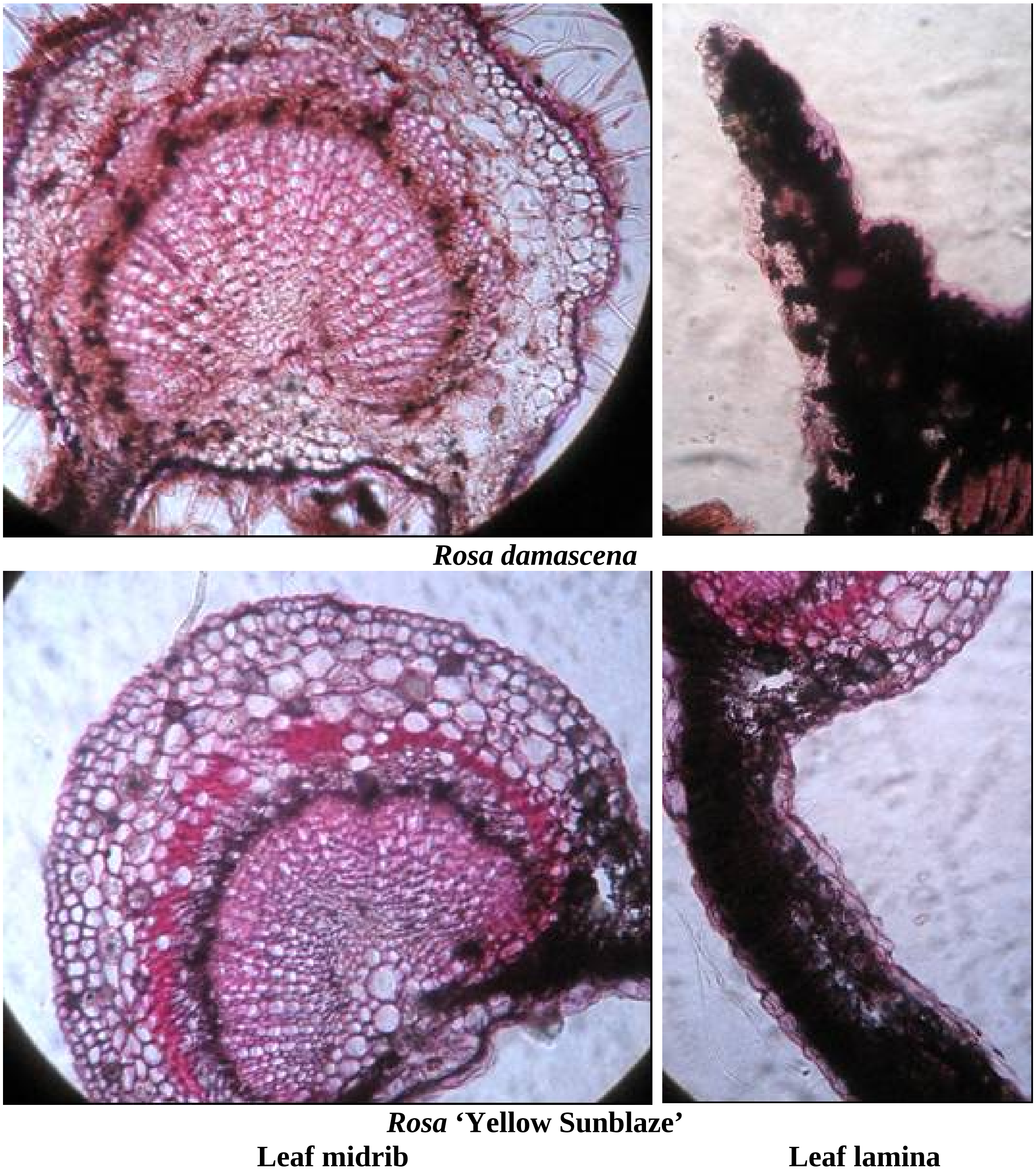


Fig. 3. TS of leaf of *Rosa chinensis viridiflora* and *R.* 'Yellow Sunblaze'.

Four roses, *R. bourboniana*, *R. bourboniana* 'Gruss-an-Taplitz, *R. centifolia* and *R.* 'Yellow Sunblaze' showed stomata only at abaxial leaf surface (Figs. 4-5). Stomatal area was the maximum in *R. damascena* at adaxial leaf surface and in *R. centifolia* at abaxial leaf surfaces, however, the smallest stomata were recorded in *R. chinensis viridiflora* at both leaf surfaces. Stomatal density, in contrast, was the maximum in *Rosa damascena* at both leaf surfaces, whereas its minimum was recorded in *R. chinensis viridiflora* at adaxial surface and in *R.* 'Yellow Sunblaze' at abaxial surface.

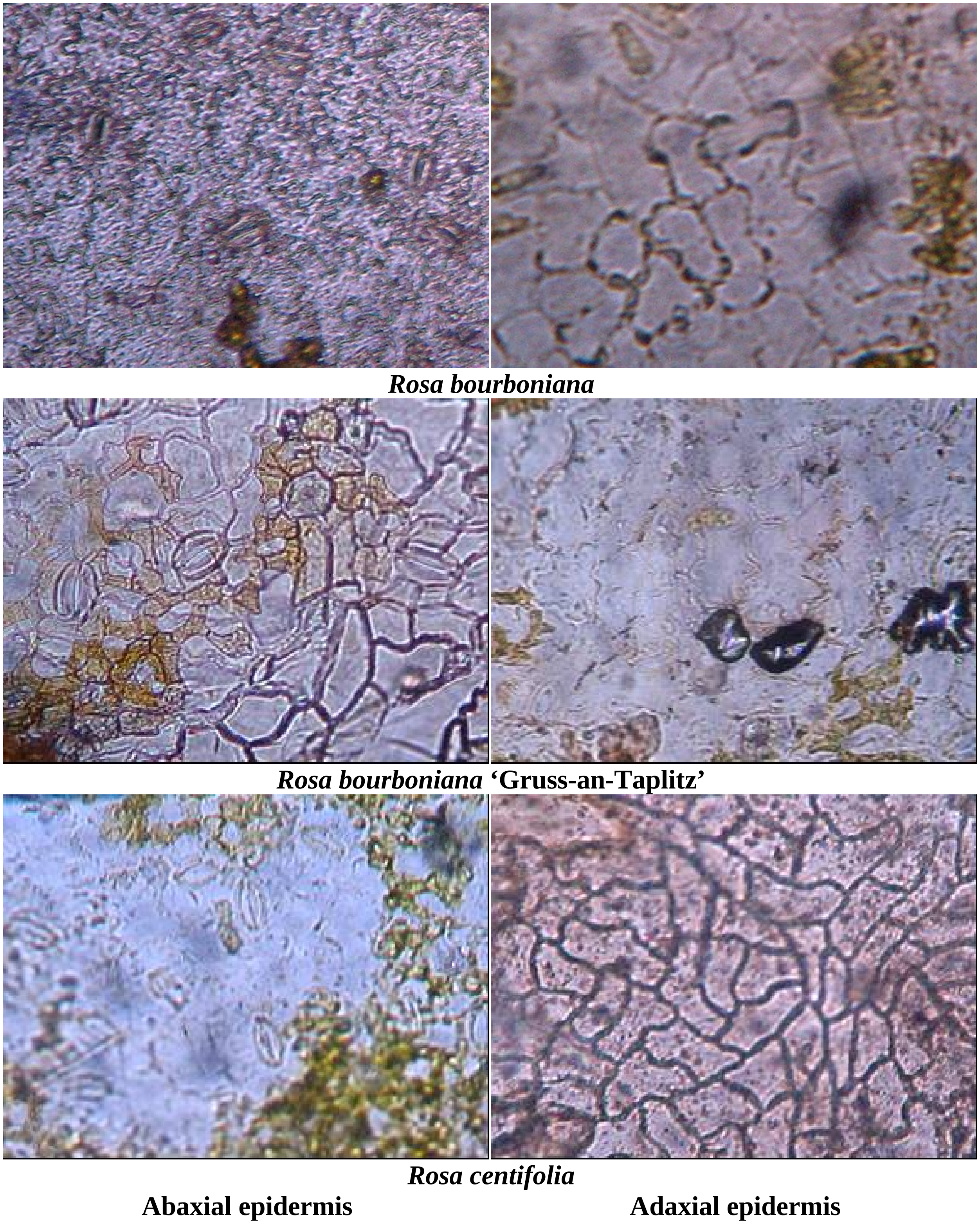


Fig. 4. Surface view of leaf epidermis of *Rosa bourboniana* and *R. centifolia*

Discussion

The most widely cultivated and hardy species, *Rosa damascena* showed some specific anatomical modifications, which may be the reason for its success in a variety of environmental conditions. These modifications include thick leaves (lamina), thick upper epidermis, large palisade cells, wide protoxylem vessels, large phloem area and large and more stomata particularly on adaxial epidermis. However, cortical cell area, lower epidermis thickness, spongy cell area, vascular bundle area and metaxylem area were relatively reduced in this species.

Thick leaves are advantageous, especially under osmotic stress condition, as succulent leaves are capable of storing more water that is vital under adverse conditions (Brouillette *et al*., 2006; Donovan *et al*., 2007). However, Diaz *et al*., (2004) and Ishida *et al*., (2008) rated angiospermic species with thick leaves as tolerant to osmotic stresses.

Thick epidermis, particularly on adaxial leaf surface, is crucial for preventing water loss through leaf surface, which aids in water conservation. This may be the most effective mechanism under osmotic stress conditions against water loss through leaf surface (Jenks & Ashworth, 1999), as tolerant species have been reported to be generally equipped with thick epidermis (Ristic & Jenks, 2002). Large photosynthetic cells e.g., palisade cells are capable of enhancing photosynthetic capacity in this species, which was also reported by Bongi & Loreto (1989) in olive and Brugnoli & Bjorkman (1992) in cotton. Thick palisade helps in more mesophyll conductance and hence enhanced CO_2 diffusion that may increase photosynthetic rate (Loreto *et al*., 1992).

Larger protoxylem vessels can improve water and nutrient conduction (Cholewa & Griffith, 2004), but at the same time smaller metaxylem vessels may prevent embolism (Facette *et al*., 2001), and this again is beneficial under moisture deficit conditions. Large phloem area can enhance the conduction of assimilates (Hose *et al*., 2001), which again indicates the ecological success of this species.

Another reason for the ecological success of *R. damascena* is the presence of stomata on both adaxial and abaxial leaf surfaces. This indicates that this species is capable of maximizing leaf conductance to CO_2, and hence the enhanced photosynthetic efficiency (Mott & Michaelson, 1991). In addition, higher stomatal density along with large stomata are closely linked to water-use efficiency as this influences stomatal conductance (Zhang *et al*., 2007). This may be the reason of the adaptation of this species to a variety of environments, as reported earlier (Spence *et al*., 1986; Martinez *et al*., 2007).

More so, intensive hairiness in leaf can minimize water loss in addition to harmful solar radiations (Naz *et al*., 2009), which is again valuable in relation to the distribution of this species. Presence of epidermal trichomes is a xeromorphic trait (Bezic *et al*., 2003) and therefore, *R. damascena* can withstand osmotic stresses as it is capable of minimizing water loss through leaf surfaces.

The second most widely cultivated species, *R. bourboniana* 'Gruss-an-Teplitz' showed thick leaves (lamina), large cortical cell area, large vascular bundle area, large metaxylem vessels and large phloem area. Leaf succulence in relation to midrib thickness and cortical cell area may provide ecological significance to cope with osmotic stresses like salinity and drought as this can conserve vital water necessary for successful survival under limited water environments (Hameed *et al*., 2009). In addition, large vascular bundles with broad metaxylem vessels and large phloem may provide efficient moisture and nutrient conduction as well as translocation of photosynthates (Steudle, 2000). Stomata were observed only on abaxial leaf surface that indicates that they do not face direct sunlight and expose less transpiration (Esau, 1977).

In conclusion, all the *Rosa* species/cultivars showed great diversity in leaf tissue architecture. Furthermore, leaf structural features are the good indicators of distribution and ecological success of the genus *Rosa*. Most widely cultivated *R. damascena* and *R. bourbonuana* 'Gruss-an-Taplitz' showed specific anatomical modifications, and this may be the reason for their ecological success in a variety of environments.

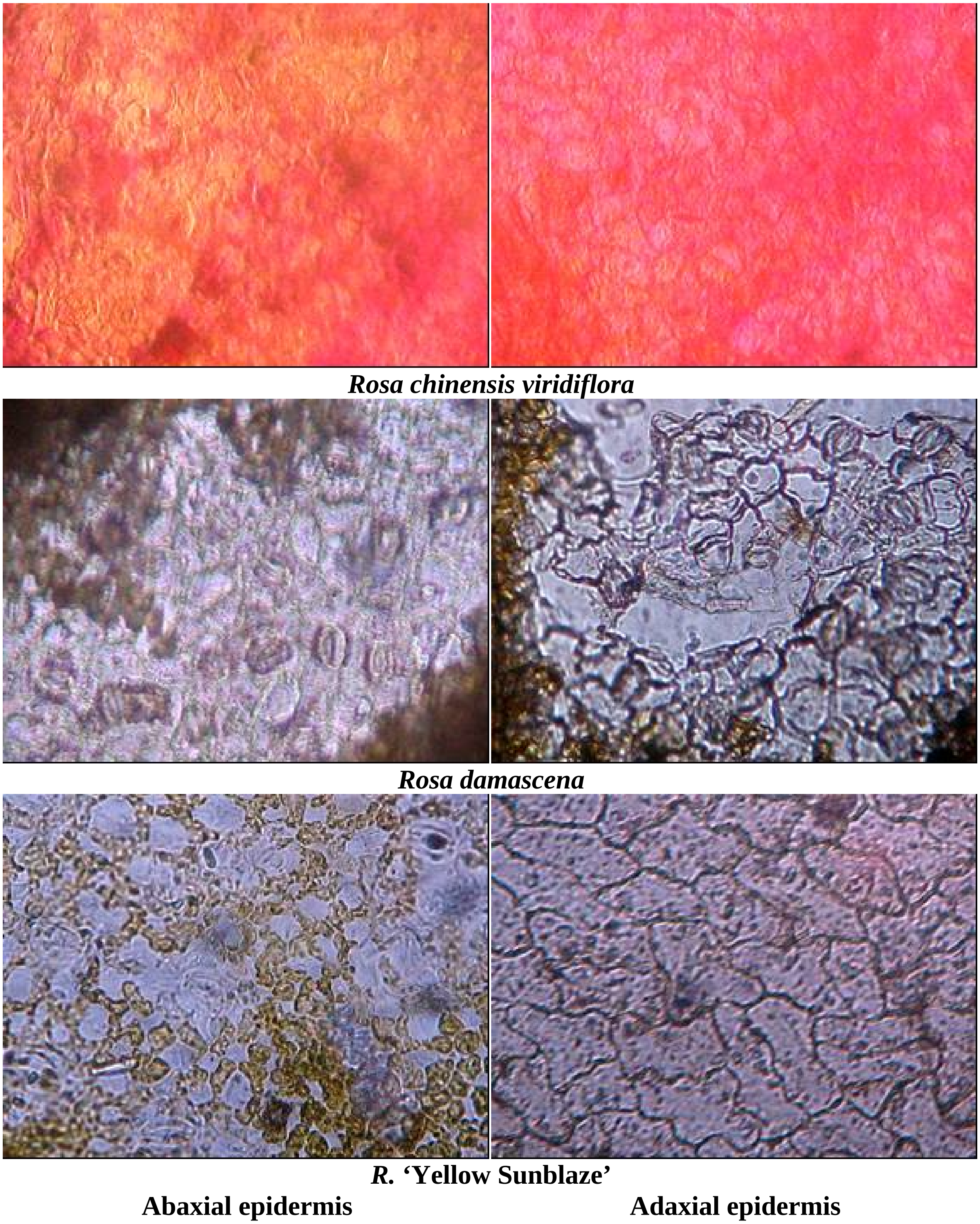


Fig. 5. Surface view of leaf epidermis of *Rosa chinensis, R. damascena* and *R.* 'Yellow Sunblaze'.

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