

Foliar tissue architectural diversity among three species of genus *Hibiscus* for better adaptability under industrial environment

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ABSTRACT

It is imperative to explore the potential of the species to judge their suitability in any habitat. It depends upon a number of physiological, biochemical and anatomical attributes. In the present study, leaf anatomical attributes of three *Hibiscus* species were evaluated to analyze inter-specific variability under the industrial environment of Faisalabad. Fourteen leaf anatomical traits were investigated through light microscopy. Results indicated significant variations among species depicting their potential to struggle against altering environment. Among all the studied species *Hibiscus tiliaceus* emerge more accomplished among others by possessing maximum epidermal thickness, increased epidermal cell area, high metaxylem area, increased phloem and xylem region thickness and augmented midrib and lamina thickness. On the other hand, *Hibiscus schizopetalous* was behind these two in many attributes e.g. lowest vascular bundle area and minimum lamina thickness respectively. Overall, on the basis of leaf anatomical characteristics, it can be concluded that *Hibiscus tiliaceus* has been endorsed with massive capacity to grow well under polluted environment.

Keywords: *Hibiscus*, environment, leaf anatomy, adaptability

1. Introduction

Being inevitable, environmental conditions in combination with resource availability appear as key factor in determining the distribution and functional characteristics of the species inhabited in a particular region. Industrial polluting agents, gas or solid, are considered permanent aggression factors for air, soil and water quality; this way the plant's way of life is subjected to a generalized stress, which, most often, materializes through an ecological misbalance (Magdalena et al., 2008). Plants usually adapt to high pollutant concentrations and unfavorable environmental conditions which is likely to result in different morphology and anatomy (Wyszkowski and Wyszkowska, 2003). In addition, due to anthropogenic activities e.g. augmented pollution, soil deterioration etc. specific morpho-anatomical and physio-biochemical characteristics are the result of plant's adaptation in contrasting environments (Kovacic and Nikolic, 2005).

Specific anatomical and physiological changes in plants facing stressful environments may enable them to well thrive on such environments. As leaves are organs exposed to the environment, they are expected to reflect in their morphology and structure an optimal adjustment to habitat condition (Margris and Mooney, 1981). For example any specific or predictable alteration in leaf cuticle due to pollution can serve as diagnostic marker of exposure to various pollution types (Baker and Hunt, 1986). Leaves become thin, contain less palisade parenchyma, abridged upper and lower epidermises along with paucity of

chlorophyll “a” and “b” in plants occupying polluted habitats (Stevoic et al., 2010). Under adverse environments, leaf palisade and spongy parenchyma reduce significantly (Bonnet et al., 2004) that lead to disturbance in sclerenchyma tissue formation in leaves of *Poaceae* (Gielwanowska et al., 2005).

Genus *Hibiscus*, native to tropical parts of Asia, is with 250 species approximately, found mainly in tropical and sub-tropical regions of Northern and Southern hemispheres (Beers and Howie, 1992). *Hibiscus* species has many uses including the consumption of their young leaves in diet as alternate to spinach (Nwachukwa et al., 2008). Many bioactive natural products are being yielded by this plant that are of significant value in folk medicinal system especially for curing liver disorders and hypertension (Yasmin, 2010). *Hibiscus tiliaceus* is used as febrifuge, laxative, resolvent etc. This plant is emollient and fruit juice is rubbed on skin to cure weakness. Flowers of *H. tiliaceus* possess antioxidant properties (Kumar et al., 2008).

Faisalabad, known as Manchester of Pakistan, has a good number of textile and dyeing mills, where weaving, dyeing, printing and finishing of cloth is carried out generally. As a result of such operations intensely alkaline liquor high in dissolved materials and suspended soil is produced usually. In the absence of adequate treatment facilities and effective drainage system, bulk of the effluent from these industrial units flow into open land and low lying areas with consequential severe damage to flora and fauna. The offensive smell of stagnant pools of waste water is great source of nuisance to the local people (Javed, 1989).

Present study was conducted with objective to uncover species that have been blessed by nature with strong anatomical attributes of enormous potential to tackle environmental changes in the best way. Detailed study of literature regarding leaf anatomical attributes reveals that different species of *Hibiscus* possess vital characteristics including ecological resilience, physiological adaptation and structural modifications that could be taken as adaptability indicators. Hence, due to limited availability of reports on these species particularly from anatomical point of view, the present study was emphasized on the evaluation of leaf anatomical characteristics with reference to their adaptability in presence of changing environmental conditions of region due to industrial activities.

2. Materials and method

2.1 Collection of Plant material and Effluents

With objective to record various leaf anatomical characteristics that can be crucial for plant adaptability in polluted environments, three species of genus *Hibiscus* namely *Hibiscus rosa-sinensis*, *Hibiscus tiliaceus* and *Hibiscus schizopetalous* were selected. Leaves of naturally growing plants of approximately same age receiving untreated industrial effluents were collected randomly, in triplicate, from different industrial sites of Faisalabad. While control plant samples were taken from plants in Botanic garden, University of Agriculture, Faisalabad. Samples of effluents from different industries irrigating plants were collected and analyzed. Various physio-chemical and Biochemical properties (on average) of industrial discharge are mentioned in table 1.

Featuring an arid climate, the city is located at latitude 30°30 N, longitude 73°10 E and at 213m above sea level. The climate of the district can see extremes, with a summer maximum temperature of 50 °C (122 °F) and a winter temperature of -1 °C (30 °F). The mean minimum and maximum temperature in summer are 27 °C (81 °F) and 39 °C (102 °F)

respectively. In winter, it ranges around 21 °C and 6 °C respectively (<http://en.wikipedia.org/wiki/Faisalabad>). The soil of the all study areas was sandy clay comprising of average 65% clay content, 22% sand and 13% silt.

Table 1: Different Physio-chemical and Biochemical attributes of untreated industrial effluent being supplied to plants under study

Sr #	Physio-chemical/ Biochemical Property	Value
1	pH	9.76
2	Ec (µS/cm)	8.26
3	TDS (mg/L)	3567.40
4	Chloride (mg/L)	1204.00
5	Sulphate (mg/L)	504.30
6	Phenol (mg/L)	0.28
7	BOD (mg/L)	306.30
8	COD (mg/L)	614.80

2.2 Anatomical studies

For recording anatomical observations, one cm piece from leaf inclusive of midrib was taken. The material was fixed in FAA (formalin acetic alcohol) solution, which comprised of Formalin 5%, acetic acid 10%, ethyl alcohol 50%, and distilled water 35%. For long term preservation, the material was then transferred in acetic alcohol (one part acetic acid and three parts ethyl alcohol) solution. The leaf was sectioned transversely by free hand sectioning technique. Double stained standard technique was used for preparing permanent slides of T.S following Ruzin(1999). Camera photographs were taken with Carl-Ziess camera equipped microscope. Following were the attributes studied, leaf epidermis thickness, epidermal cell area, palisade and spongy cell area, cortical cell area, xylem and Phloem region thickness and metaxylem area. Data were subjected to statistical analysis using ANOVA for comparison of means and error of means was calculated following Steel et al. (1997).

3. Results

A significant inter-species qualitative anatomical variability was observed in three species of *Hibiscus* examined in present study. In all species, epidermis was uniseriate on both faces. Of all species of *Hibiscus* under study, the *Hibiscus tiliaceus*, treated with effluents, was remarkable in having high Adaxial epidermis thickness, abaxial epidermal thickness and also in adaxial and abaxial epidermal cell area (Figure 1 and 3) and the *H. schizopetalous* was minimum in this attribute. However, *Hibiscus tiliaceus* and *Hibiscus rosa-sinensis* were at par in possessing adaxial epidermal thickness (Figure 1 and 3).

The study regarding the leaf stomatal area and stomatal density shows that potentially higher leaf stomatal density per unit area was recorded in *Hibiscus rosa-sinensis* followed by *H. schizopetalous* (Figure 1 and 3) as compared to the rest for the same attribute.

Changes in cortex were apparent among these species. Results revealed high proportion of cortical cell area in *H. schizopetalous* (Figure 1 and 3) while this attribute was witnessing its lowest value in *H. rosa-sinensis*. While the most distinguishing factor was presence of black spots in cortex and vascular region of *H. tiliaceus* (Figure 3) as regard rest of the two species no such spots were observed anywhere. As concerned about midrib thickness, maximum was observed in *H. tiliaceus* as compared to other two species (Figure 1).

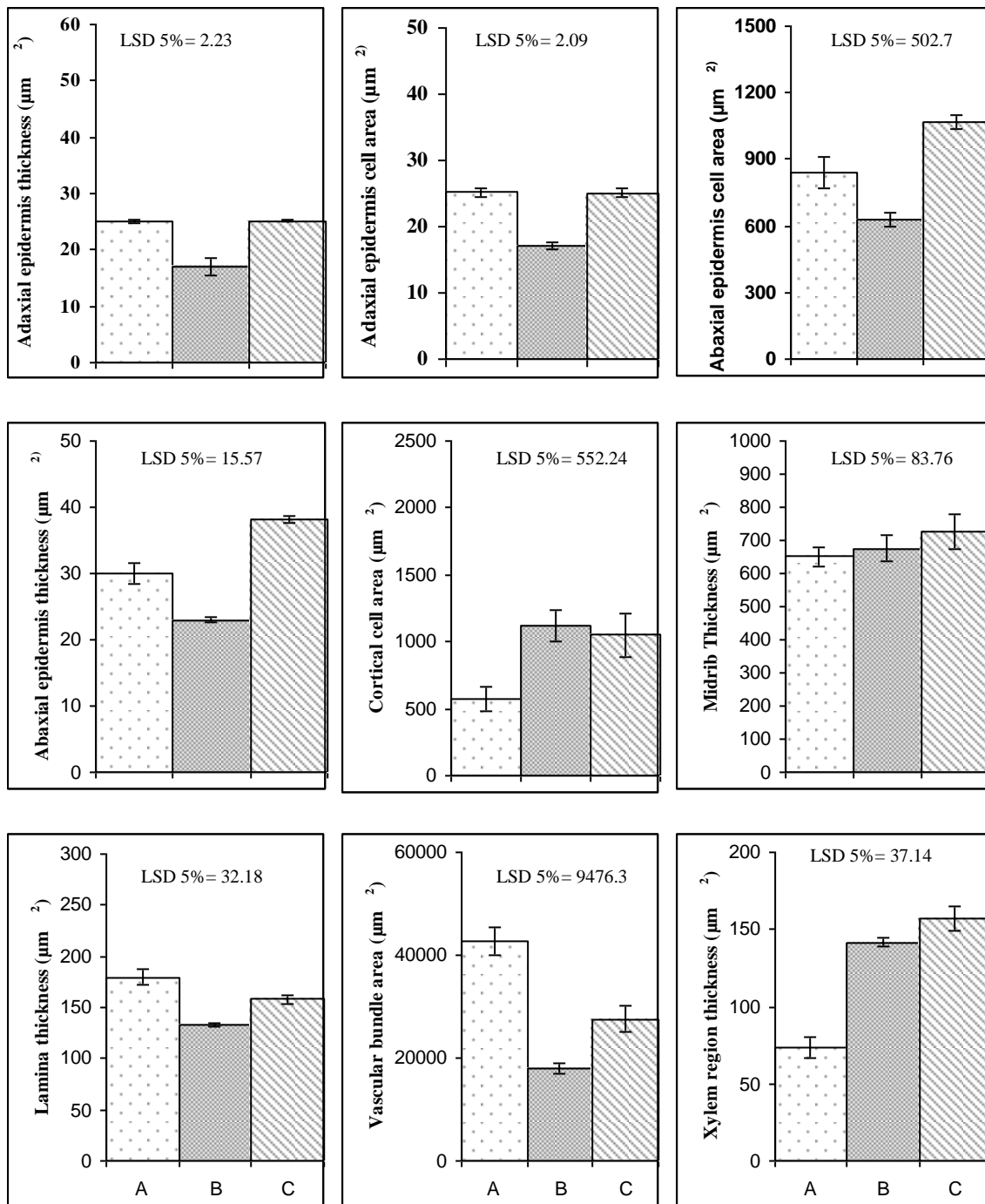


Figure 1: Leaf anatomical variations among three species of *Hibiscus* collected from Faisalabad region (mean±S.E.)

Minor differences were recorded among three species as far as this attribute was concerned. Whereas, in case of lamina thickness the negligible difference was observed between *H. rosa-sinensis* and *H. tiliaceus* (Figure 1). But *H. schizopetalous* was behind these two in this character. Results regarding palisade cell area presented substantive increase in this trait exhibited by *H. tiliaceus* (Figure 2 and 3). Other two were approximately akin for this attribute. Markedly increased spongy cell area was recorded in *H. tiliaceus* (Figure 2), while this feature was at its lowest ebb in *Hibiscus rosa-sinensis* (Figure 3).

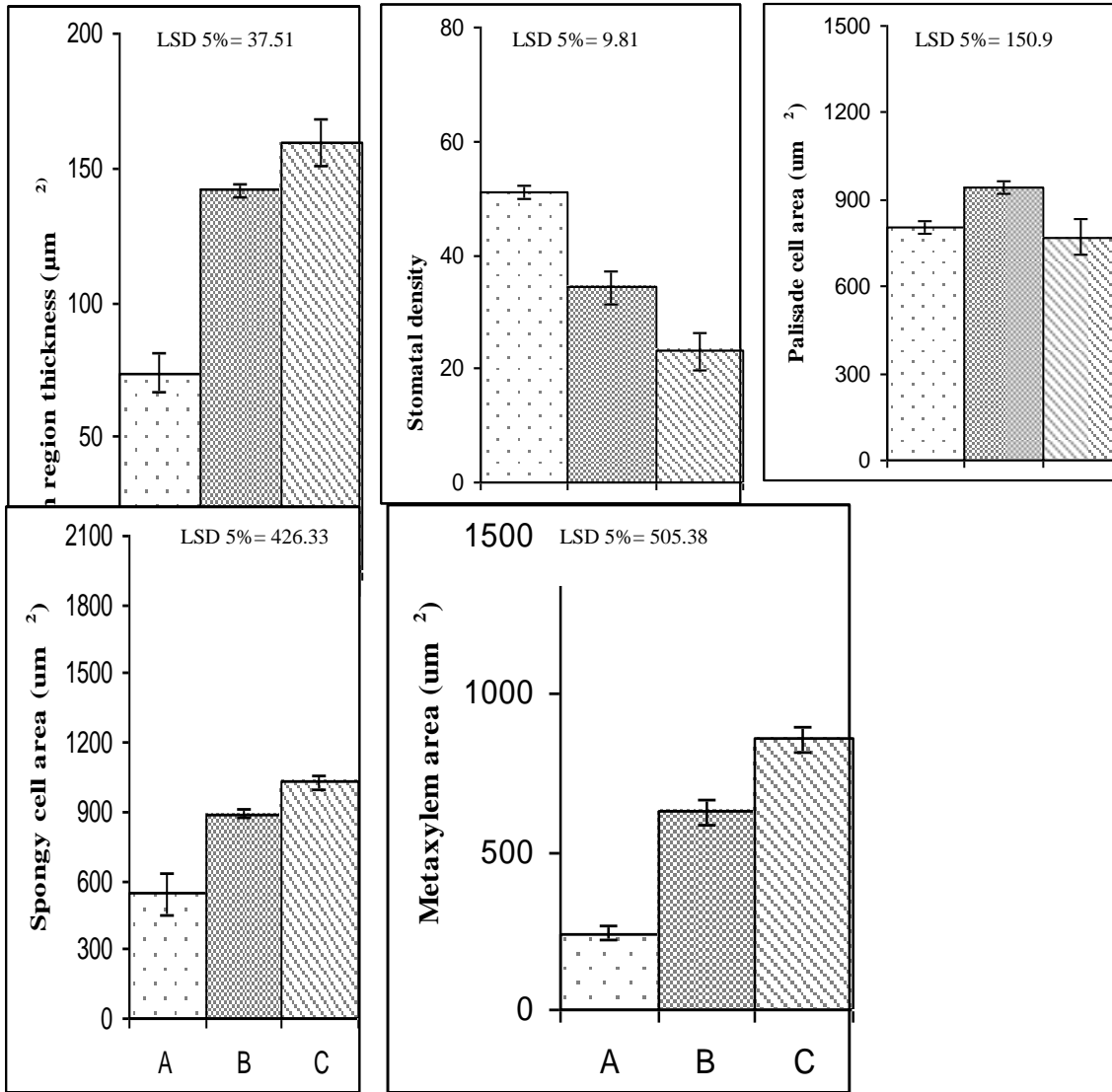
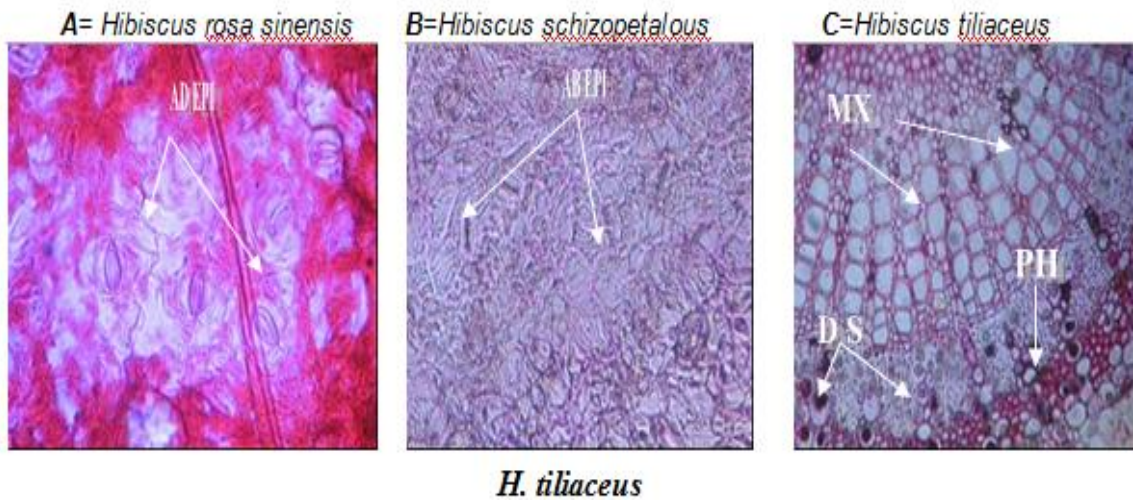


Figure 2: Leaf anatomical characteristics of three species of *Hibiscus* collected from different areas of Faisalabad region (mean±S.E.)



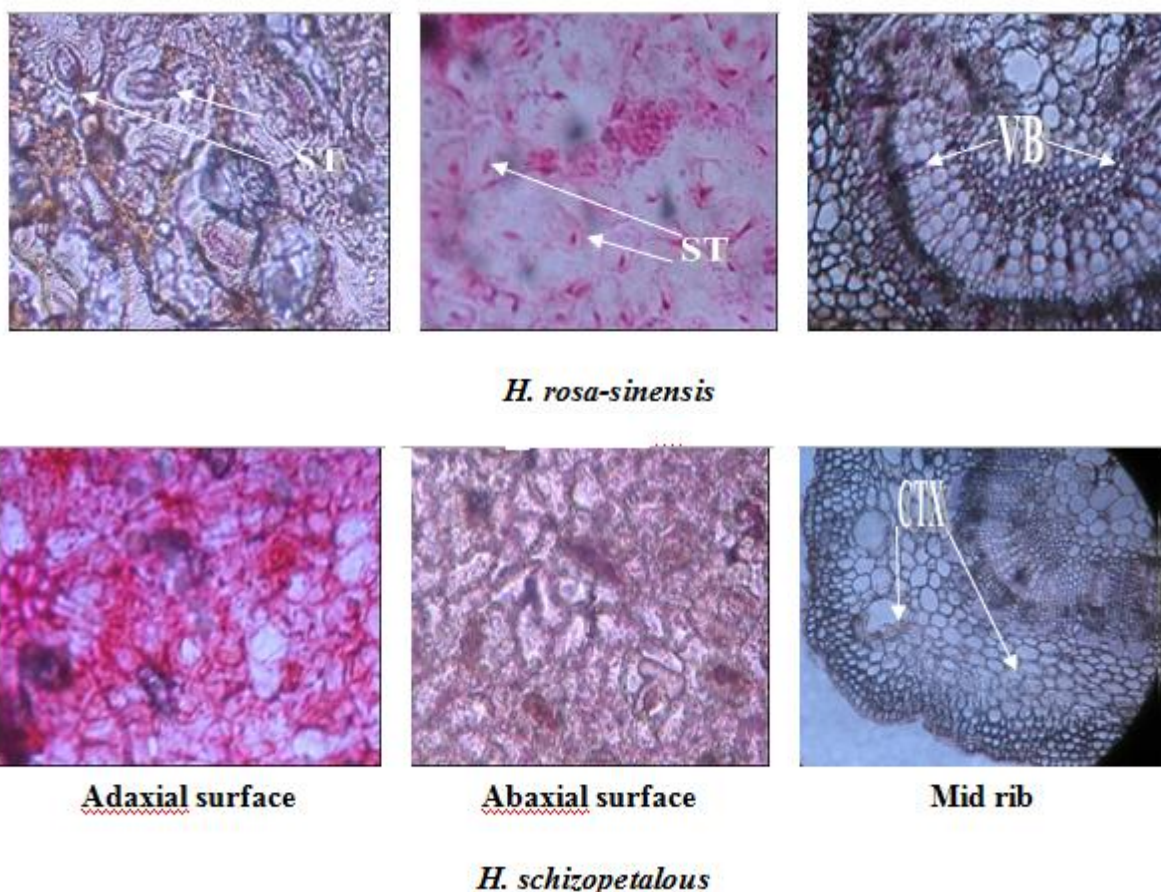


Figure 3: Leaf anatomical characteristics of three species of *Hibiscus* collected from different industrial sites of Faisalabad region.

AD EPI=adaxial epidermal cell area and thickness; **AB EPI**=abaxial epidermal cell area and thickness; **ST**=high stomatal density; **CTX**= high cortical cell area; **PH** =phloem region thickness; **VB**= vascular bundle region thickness; **MX** =metaxylem area; **DS**= dark spots

H. rosa-sinensis was superior to the rest of species as maximum vascular bundle area was recorded in this (Figure 1 and 3). On the other hand, *H. schizopetalous* possessed significantly reduced vascular bundle area as compared to others. Incremented xylem region thickness (Figure 2) was reported in *H. tiliaceus*. Trend was followed by *H. schizopetalous*. *H. rosa-sinensis* was having lowest xylem region thickness. Minimum and maximum phloem region was observed in *H. rosa-sinensis* and *H. tiliaceus* respectively (Figure 2). In comparison among these species, *H. tiliaceus* was prominent in possessing high metaxylem area (Figure 2) followed by *H. schizopetalous* with special reference to this characteristic. However, the least area was recorded in *H. rosa-sinensis* (Figure 2).

4. Discussion

Plants are subjected to multiple environmental types. From time to time, with special reference to plant species endowed with anatomical alterations, increased chances of survival under multifarious environmental conditions have been recorded by different workers. These modified structural attributes in different plant body parts are of supreme importance to cope with adverse circumstances (Cutler et al., 2007). Different species of

Hibiscus appear morphologically similar but, anatomical studies help in their differentiation and identification when correlated with morphological traits.

Responses of plants to the environmental variations are complex involving deleterious or adaptive changes. The anatomical features vary greatly and are of significant value in many plants (Lersten and Curtis, 2001). Studies reveal that the anatomical characters are influenced by the environmental conditions i.e pollution (Ozorgucu et al., 1991). As self defense system develop in plants under contaminated condition, plants experience changes like increase in the number of stomata and trichomes per unit area which prove to be a support to the plant for their survival in contaminated environment (Azmat et al., 2009). In addition, increased abaxial and adaxial epidermal thickness contributes significantly in tackling hazardous effects of heavy metal contamination (Gomes et al., 2011).

In present study, different species of genus *Hibiscus* were investigated anatomically for their survival under industrially polluted conditions. Results indicate significant inter-species variations in relation to different anatomical attributes. These modified anatomical traits in *H. tiliaceus* including thick adaxial and abaxial epidermis, increased adaxial as well as abaxial cell area and large cortical cell area and midrib thickness are indicative of high chances of survival under varying levels of pollution. Presence of thickened epidermises is consistent with studies. Thick upper and lower dermal layers accompanied with incremented epidermal cell area on both surfaces was at its zenith in this species as compared with others. According to Gomes et al. (2011) increased epidermal thickness can be of paramount significance when plants grow in soil toxicated with heavy metals. Plant species capable of survival under water scarcity are generally equipped with thick epidermis (Ristik and Jenks, 2002). Density of main epidermal cells and stomata in the plants from polluted sites tended to increase (Kapitonova, 2002). This could be interpreted as an adaptive response to pollution. In the light of previous records, this increased epidermal thickness along with cuticle deposition appears as an aegis against temperature variations and other environmental imbalances. Therefore, this modified feature can be reckoned as basic support to plants of this species in case of environmental threats inclusive of pollution.

High stomatal density was observed in *Hibiscus rosa-sinensis* while least in *H. tiliaceus* and stomata were found on both dermises in all cultivars. Studies prove that this anatomical characteristic favors survival of plants under harsh environmental types particularly in abridged water availability. Presence of stomata on adaxial and abaxial surfaces of leaf can be considered as a reason for ecological success of Rosa sp. (Nawaz et al., 2011). According to Melo et al. (2007) increased stomatal density coupled with decreased stomatal size would be an alternative to sufficient supply of CO₂ for photosynthesis, without excessive transpiration. This can be considered as an adaptation of plants in response to heavy metal toxicity. Although stomatal density come in front as anatomical attribute yet physiological significance of this trait, supported by different studies and present finding, in terms of its imperative role in adaptability under critical conditions i.e. heavy metal contamination become crystal clear. Increased stomatal density is considered as adaptability indicator to polluted environment (Kapitonova, 2002). High stomatal density in leaves from high polluted sites is the indication of the success of a species in a particular area as reported by Gostin (2009).

Presence of black spots in cortical and vascular region of *H. tiliaceus* is indicative of its response to industrial pollution i.e air and water pollution. These spots are in fact deposited pollutants. Gostin (2009) noted such dark spots in leaves of some members of Fabaceae

subjected to air pollution. Furthermore, spots were described as accumulated phenolics by the same researcher that has also been detected in effluents used in this study. The high accumulation of phenolics and lignin is considered as one of the most frequent reactions of plants to stress (Wild and Schmitt, 1995).

This study highlight increased palisade and spongy cell area in *H. schizopetalous* and *H. tiliaceus* respectively that relates with efficient assimilate synthesis. This finding is in agreement with studies of Loreto et al. (1992) describing thick palisade tissue supporting more mesophyll conductance that in turn leads to increments in photosynthetic activity due to improved CO₂ diffusion. Experiments using olive leaves revealed that thick palisade parenchyma is of superlative importance in plants growing under hampered moisture supply (Guerfel et al., 2009). Zwieniecki and Newton (1995) and Baloch et al. (1998) reported large cortical cells in *Eucalyptus microtheca* and *E. botryoides* as the indication of their wide distribution in a variety of environmental conditions. Enlarged photosynthetic cells i.e. palisade cells are capable of producing high quantity of mandatory metabolites required during ruthless conditions in *Rosa spp.* (Nawaz et al., 2011). Present increased leaf palisade and spongy cell area can be considered as healthier adaptability sign because different researchers (Iqbal, 1985; Gostin, 2009) have prove the injurious effects of industrial effluents on palisade and spongy cell area leading to hampered growth and death of plant also. Leaf succulence in relation with high mid rib thickness and cortical cell area in *H. tiliaceus* and *H. schizopetalous* may be ecologically significant traits to fight abiotic stresses e.g. salinity as leaf succulence is crucial for water storage necessary during period of acute water shortage (Hameed et al., 2009). Enhanced photosynthesis, due to more photosynthetic apparatus, is surely a reason for acclimatization of *Hibiscus tiliaceus* with unique reference to unkind environmental conditions. In addition, worth of this trait can not be denied when distribution of this spp. is taken into account.

Considerable increments in vascular bundle area recorded in *H. rosa-sinensis* appears as one of the most critical feature supporting plant life cycle under ecological variations. Reported attribute is in accordance with findings of Ali et al., (2009) unveiling direct relation of vascular bundle area with alleviated transport of water and nutrients from the soil, and these might be of sublime importance under curtailed moisture supply. Greater vascular bundle size as supportive feature has been reported by Awasthi and Pathak (1999) in saline tolerant genotypes of *Ziziphus* species. Presence of extended phloem is a favour to maintain transport of photosynthates in plants facing deteriorated environmental types (Nawazish et al., 2006). Presence of greater phloem area with better conduction of assimilates can be a good reason of ecological accomplishment of plant species (Hose et al., 2001). Vascular bundles with broad metaxylem vessels and large phloem may prove vital for conduction of water and nutrients along with translocation of photo-assimilates (Stuedle et al., 2000). Potentially enlarged xylem and Phloem in *H. tiliaceus* may appear as a protection in these plants especially under adverse state of affairs. In toto, differentially enlarged xylem and phloem area as recorded in *H. tiliaceus* can be taken as a comprehensive indicator for survival of plants and can be correlated with marked growth particularly in presence of accumulated growth impediments inclusive of various pollution forms and abiotic stresses. Other than this, anatomical capacity of *H. tiliaceus* exhibit potential that can become a base for ecological success under antagonistic conditions.

In conclusion, this study reflects that all the *Hibiscus* species showed immense variety in leaf tissue architecture to fight against polluted environment. Besides, these structural aspects are sufficient signs of eco-physiological plasticity under unstable environmental type and,

Ipsofacto, confirm healthier adaptableness of these plants. Existence of distinctive facets in relation to leaf anatomy in *Hibiscus tiliaceus* along with reported active antioxidant components emerge discreet in determining fortune of this species despite multifarious environmental challenges ranging from rising industrial pollution to augmented soil toxicity etc. Unequivocally, these modifications have established role in fight of plants for survival under contrasting environments and suggesting that species can be further investigated as a potential ingredient in pyhtoremediation.

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