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# COMPARISON OF LEAF ANATOMICAL CHARACTERISTICS OF *HIBISCUS ROSA-SINENSIS* GROWN IN FAISALABAD REGION

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**Abstract**

The genetic potential of different plant species to different environmental conditions differ in relation to different physiological, biochemical and anatomical characteristics. Of these varying attributes leaf anatomical characteristics play most important role for the establishment of that cultivar in varied environmental conditions. So, the present study was conducted to assess the inter-cultivar genetic potential of Hibiscus in relation to leaf anatomical characteristics. To fulfill the study requirements *Hibiscus rosa-sinensis* and its six cultivars (were well adapted to their specific natural habitat) were collected from different locations of district Faisalabad Pakistan that have great environmental changes round the year. Results showed significant variability among cultivars in relation to analyzed anatomical characteristics. Cultivars Lemon shiffon and Wilder's white emerge more promising among others by possessing more epidermal thickness, increased epidermal cell area, high cortical cell area and incremented stomatal density as compared with other cultivars. On the other hand, cultivars Cooperi alba, Mrs. George Davis and Frank green possessed least cortex cell area, lowest xylem region thickness and minimum phloem region thickness respectively. Overall, it can be concluded that anatomical genetic potential has endorsed cultivars Lemon chiffon and Wilder's white with enormous capability to grow well under variable environments.

## Introduction

The planet earth is under pressure from climatic changes that directly or indirectly affecting the vegetation. In this regard the continent Asia, is particularly facing formidable environmental and socio-economic challenges due to over population and industrialization. Pakistan is located in an area having wide range of temperature range round the year and varied environmental conditions. There are evidences of prominent increases in the intensity and/or frequency of many extreme weather events such as heat waves, tropical cyclones, prolonged dry spells, intense rainfall, tornadoes, snow avalanches, thunderstorms, and severe dust storms in that region during last few years (Cruz *et al.,* 2007). Such climate changes have a profound effect on the future distribution, productivity, and health of the plants (Edwards & Richardson, 2004; Christensen *et al.,* 2007; Hameed *et al*., 2013). Increased risk of extinction for many plant species has become obvious due to the synergistic effects upon life (Christensen *et al.,* 2007; Cruz *et al.,* 2007; Abbas *et al*., 2012; Asghar *et al*., 2013). So, the plants belonging to different plant families have varied genetic potential to adopt wide range of environmental conditions. This adaptation potential might be due to varying physiological, biochemical and anatomical characteristics (Noman *et al*., 2012). Of different leaf anatomical characteristics to adopt varied environmental conditions, the number of stomata and trichomes per unit area with simultaneous reduction in the size of the guard cells appeared to be the most important one, which provide the support to the plant for their survival in such conditions (Azmat *et al.,* 2009). Of other adaptations that take place in plant leaf to changing environmental conditions are included the curtailed water loss, shrinked cell size, high stomatal and trichomatous densities are also important ones (Bonnet *et al.,* 2004).

Unequivocally, among all plant families, the Malvaceae stand sublime morphologically, anatomically and economically and the genus *Hibiscus*, the most important member of this family had adopted the tropical and sub-tropical regions of northern and southern hemispheres (Beers & Howie, 1992). This genus comprised approximately 250 species and a wide range of cultivars are being grown throughout the area. The leaves of *Hibiscus rosa-sinensis* and its cultivars are simple, alternate and petiolate (Nwachukwa *et al.,* 2008). Mucilaginous epidermis along with stomata on both sides has been reported. In addition, complex, stellate, glandular hairs are also present. This genus has great feed and medicinal uses as well. Young leaves of *Hibiscus rosa-sinensis* are taken as alternate to spinach in some parts of Nigeria (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).

Naturally adapted characteristic of stress tolerant plants can be used effectively for the exploration of acclimatization mechanisms in relation to leaf anatomical characteristics. For example water stress may result in increased cell wall thickness up to 20 folds in the outer tangential walls in bundle sheath cells and cellular shape alterations in mesophyll cells (Utrillas & Alegre, 1997; Hameed *et al*., 2013). Similarly in plants growing in saline and polluted areas, the increase in leaf area is sensitive to augmented salinity level along with reduced leaf emergence rate (Jafri & Ahmad, 1995; Laghari & Zaidi 2013).

Since genetically based variation in natural populations of plants has not been much investigated under varied environmental conditions, so, it was felt mandatory to find out species/cultivars that have been endowed by nature with strong anatomical attributes as capacity to tackle climatic changes in the best way. Thorough study of literature regarding leaf anatomical features reveals that *Hibiscus rosa-sinensis* possess vital characteristics that could be attached to other taxonomic information and used in their description. Hence, present study was emphasized on the evaluation of leaf anatomical characteristics with reference to their adaptability in presence of incremented environmental hazards that have been added due to large industrial activities in the subject area. In toto, role of such anatomical aspects can never be denied in exploring anatomical genetic diversity among members of family Malvaceae.

## Materials and Methods

In order to explore the variations in the leaf anatomical studies of *Hibiscus rosa-sinensis*, and its cultivars, Faisalabad region was comprehensively explored for the record and distribution of cultivars. Six cultivars of *Hibiscus* (*Hibiscus rosa-sinensis*; *H. rosa-sinensis* cv. Charles September; *H. rosa-sinensis* cv Cooperi alba; *H. rosa-sinensis* cv Mrs. George Davis; *H. rosa-sinensis* cv Frank Green; *H. rosa-sinensis* cv Lemon Chiffon; *H. rosa-sinensis* wilder's white) were selected for present studies. Geographically, Faisalabad is located at latitude 30°30 N, longitude 73°10 E and altitude 213m. The average climatic conditions of the study area during sampling were calculated as means were day/night RH 33.1/75.1% and day and night temperatures 38.28±4°C and 22.82±3.6°C, respectively. The soil was sandy clay consisting of average 65% clay content, 22% sand and 13% silt. The soil pH was ranging within 7.0-8.1 and soil electrical conductivity (ECe) was in range 1.8-3 dS/m.

For recording anatomical observations, appropriate piece from leaf inclusive midrib of each *Hibiscus* cultivar was taken. The leaf material was preserved in alcoholic formalin acetic acid (FAA) solution (Formalin 5%, acetic acid 10%, ethyl alcohol 50%, and distilled water 35%) for fixation. During preparation of sections, the transversally cut leaf sections (T.S) were passed through a graded series of ethanol onward starting with 10% ethnolic solution and finally treated with absolute ethanolic solution (95%). Double stained standard technique was used for preparing the permanent slides of the T.S of leaves following Ruzin (1999). Individual, finally prepared leaf section was placed on the slide and 1-2 drops of safranin were added. Later on, few drops of 95% ethanol were added to remove the extra safranin. After this, few drops of fast green and 95% of absolute alcohol was added respectively. Finally, one drop of xylene was added on the section. To preserve and permanent fixation canada balsam was applied. Camera photographs were taken with Carl-Ziess camera equipped microscope. Data were subjected to statistical analysis using ANOVA for comparison of means. Standard error was calculated following Steel *et al.*, (1997).

## Results

A significant intra-specific qualitative anatomical variability was observed in various cultivars of *H. rosa-sinensis* examined in present study. Of all examined cultivars of *H. rosa-sinensis* cv. Lemon chiffon was remarkable in having high Adaxial epidermis thickness, abaxial epidermal thickness and also increased adaxial and abaxial epidermal cell area (Figs. 1 & 3) and the cv. Charles September was minimum in this attribute. However, the remaining cultivars were at par in these attributes (Figs. 1 & 3).

Data regarding the leaf stomatal area and density shows that significant inter-cultivar variation was recorded in relation to these attributes. Significantly higher leaf stomtal density and per unit area was recorded in cv. Wilder's white followed by cv. Charles September (Figs. 2 & 4). In other cultivars this inter-cultivar variation in relation to sotamatal density and stomatal area was not significant.

Inter-cultivar variation in relation to leaf cortex area was apparent. Significantly high proportion of leaf cortical cell area was recorded in cv. Lemon chiffon (Figs. 1 & 3) as compared with other Hibiscus cultivars, while this attribute was at its lowest value in *H. rosa-sinensis* cv. Cooperi alba (Figs. 1 & 3). As concerned about midrib thickness, the maximum thickness was recorded in *H. rosa-sinensis* cv. Cooperi alba as compared with other cultivars (Fig. 3) and the minimum thickness was that in cv. Mrs. George Davis. However, the leaf lamina thickness was maximum in cv. Wilder's white (Fig. 4) as compared with other cultivars. But, the cultivar Frank green was minimum in this regard as compared with other Hibiscus cultivars.

Results regarding leaf Palisade cell area presented in Figs. 2 and 3 shows that a substantive increase in this was recorded in cv. Cooperi alba (Figs. 2 & 3) as compared with other Hibiscus cultivars. Among others, cultivars, the cv. lemon chiffon, Mrs. George Davis and Charles September were similar in this regard. However, this feature was witnessing its minimum value in *H. rosa-sinensis* cv. Wilder's white. Furthermore, markedly increased spongy cell area was recorded in cultivar Mrs. George Davis (Figs. 2 & 4), but the cvs. Wilder's white and Lemon chiffon were similar in this regard, while this character was the lowest in cultivar Charles September (Fig. 3.).

Leaf vascular bundle area also varied significantly among cultivars. Cultivar *H. rosa-sinensis* was superior as compared to the rest of cultivars (Figs. 1 & 3). On the other hand, cv. Lemon chiffon possessed significantly reduced vascular bundle area as compared to other cultivars. However, other cultivars showed similar trend with regard to this attribute. Furthermore, incremented xylem region thickness was reported in cultivar Cooperi alba (Figs. 2 & 3) as compared with other cultivars, while cv. Wilders white and Lemon chiffon possessed somehow equally thickened leaf xylem region. However, Cultivar Mrs. George Davis was the lowest in having xylem region thickness.

Data for phloem region thickness showed that cv. Frank green was at the highest in this regard (Figs. 2 & 4) while the cv. Lemon chiffon was at minimum in this regard (Figs. 2 & 3). However, in relation to leaf metaxylem area *H. rosa-sinensis* cv. Wilder's white was ranked the at highest as compared with other cultivars (Figs. 2 & 4). While cv. Charles September and Mrs. George Davis were at par with special reference to this characteristics. But, the least metxylem area was recorded in *H. rosa-sinensis* (Figs. 2 & 3).

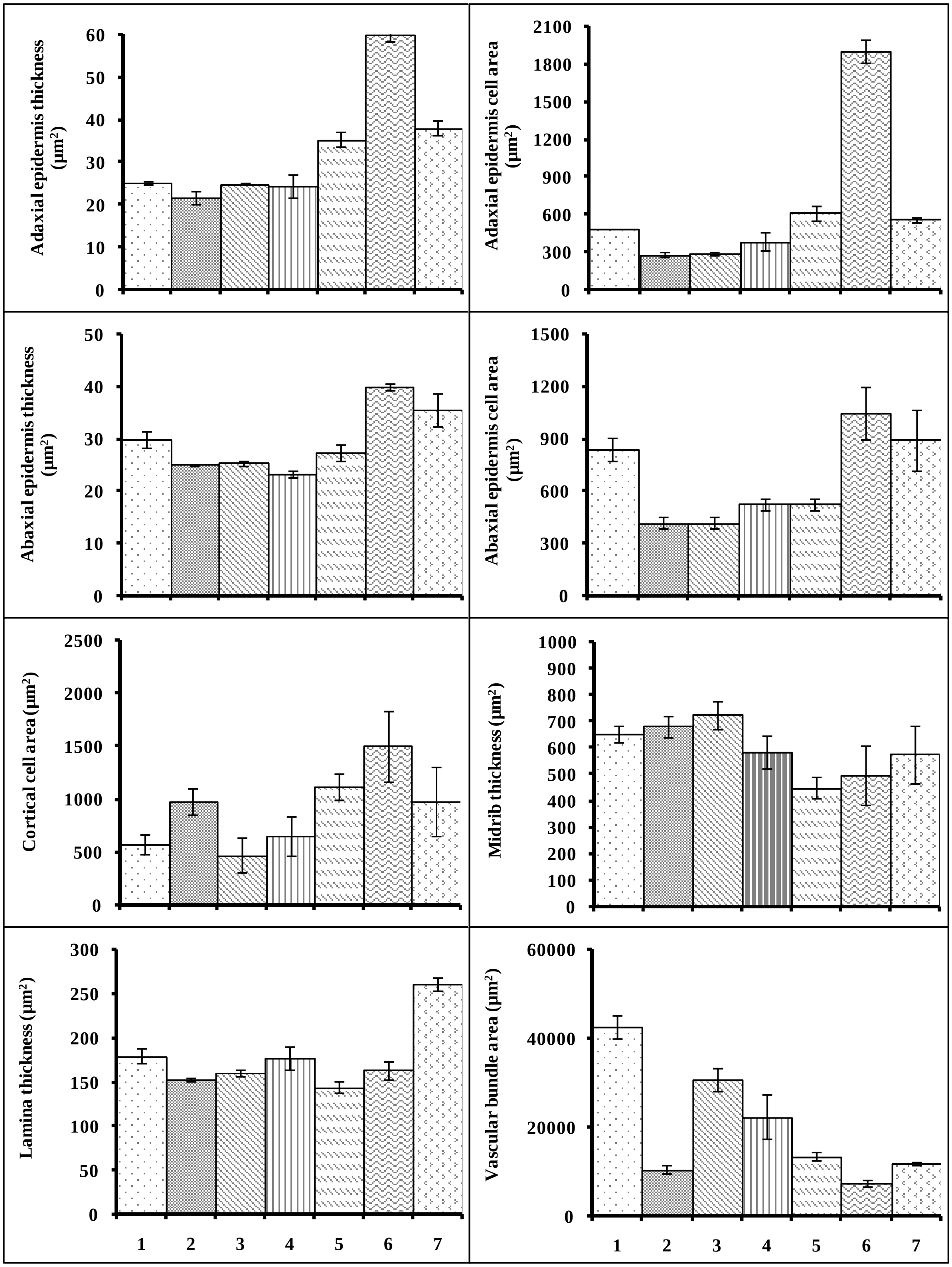



Fig. 1. Leaf anatomical characteristics of different cultivars of China rose (*Hibiscus rosa-sinensis* L.) collected from different areas of Faisalabad region (mean ± S.E.).
1. *H. rosa-sinensis* L.; 2. *H. rosa-sinensis* cv. Charles September; 3. *H. rosa-sinensis* cv. Cooperi alba; 4. *H. rosa-sinensis* cv. Mrs. George Davis; 5. *H. rosa-sinensis* cv. Frank Green; 6. *H. rosa-sinensis* cv. Lemon Chiffon; 7. *H. rosa-sinensis* wilder's white.

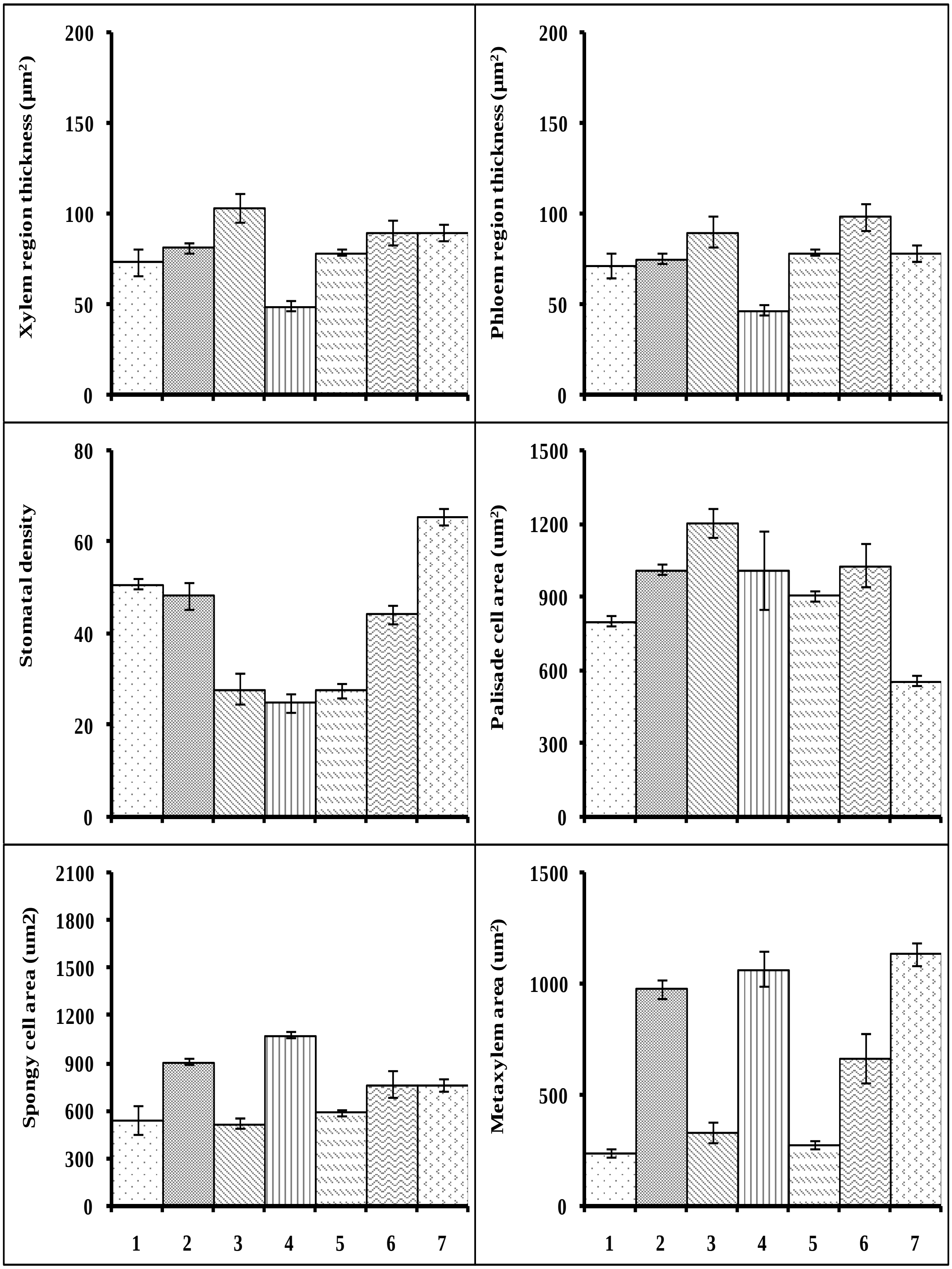
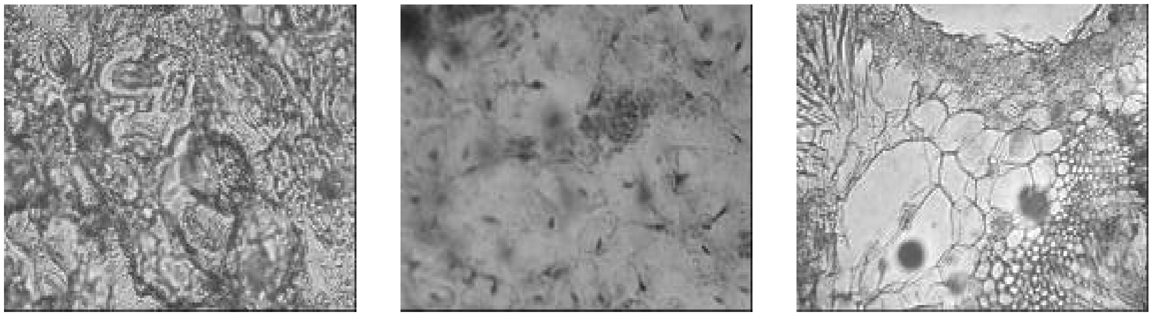
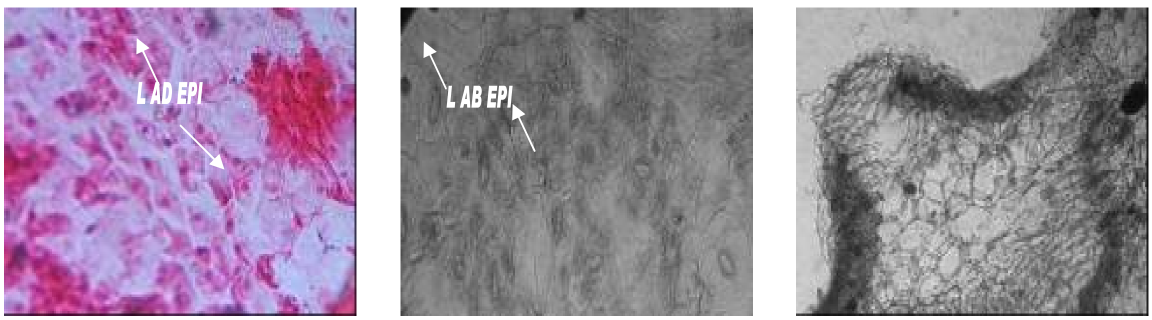


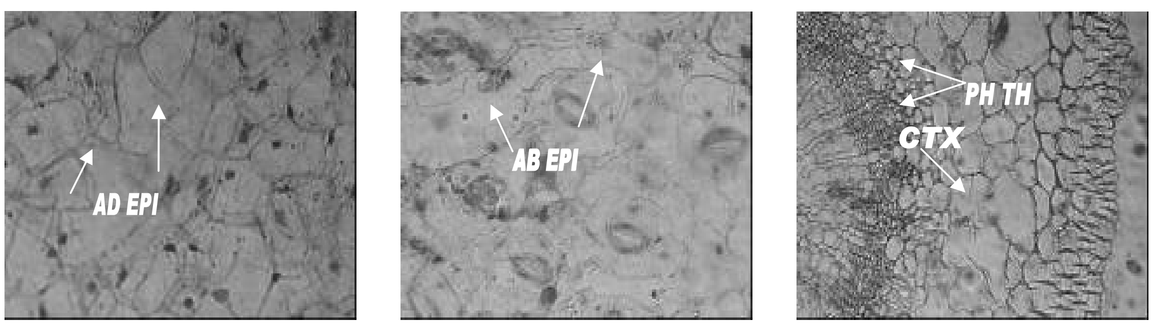

Fig. 2. Leaf anatomical characteristics of different cultivars of China rose (*Hibiscus rosa-sinensis* L.) collected from different areas of Faisalabad region (mean ± S.E.).
1. *H. rosa-sinensis* L.; 2. *H. rosa-sinensis* cv. Charles September; 3. *H. rosa-sinensis* cv. Cooperi alba; 4. *H. rosa-sinensis* cv. Mrs. George Davis; 5. *H. rosa-sinensis* cv. Frank Green; 6. *H. rosa-sinensis* cv. Lemon Chiffon; 7. *H. rosa-sinensis* wilder's white



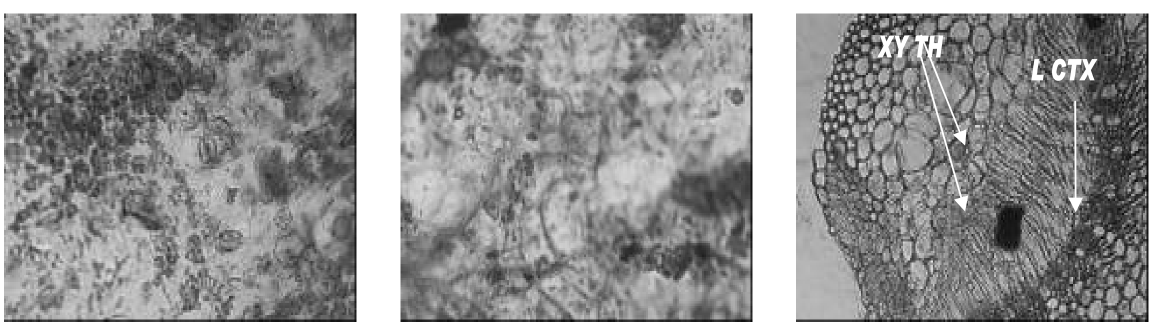
***Hibiscus rosa-sinensis***




***Hibiscus rosa-sinensis* cv. Charles September**



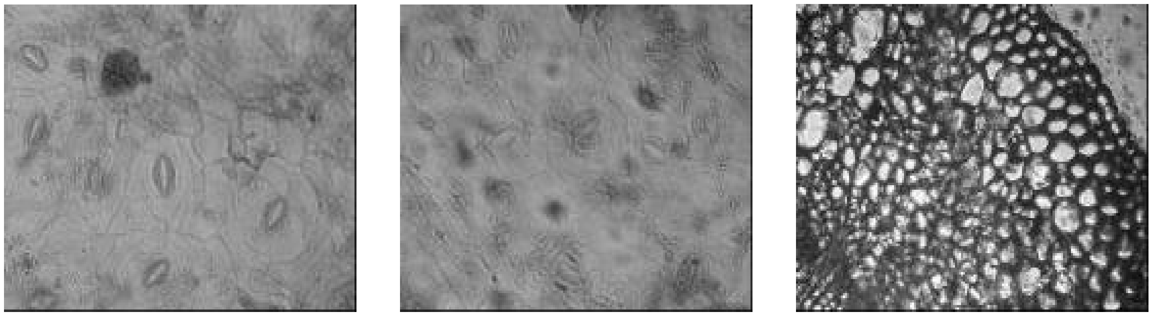

***Hibiscus rosa-sinensis* cv. Lemon Chiffon**



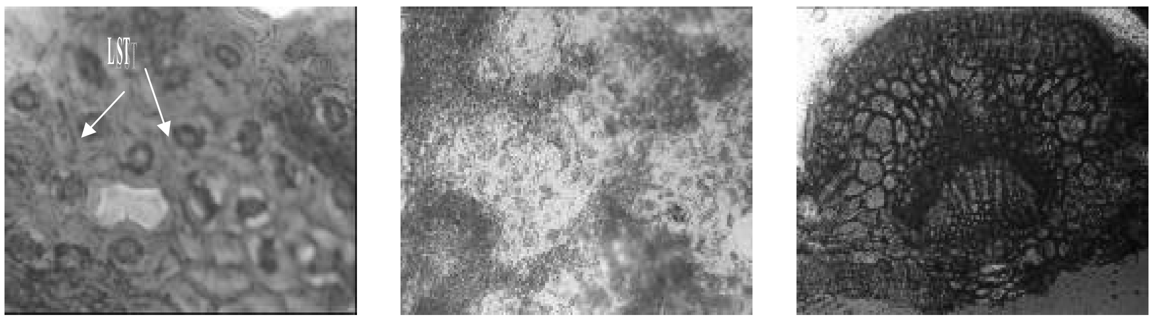

**Adaxial surface** **Abaxial surface** **Midrib**

***Hibiscus rosa-sinensis* cv. Cooperi alba**

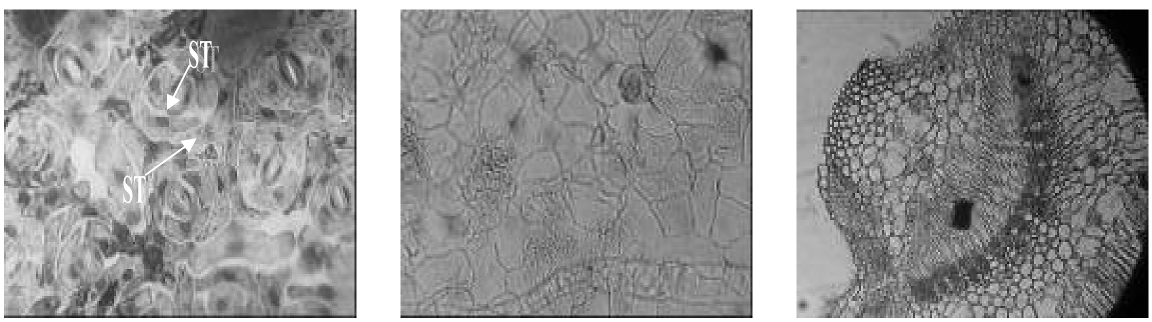
Fig. 3. Leaf anatomical studies of some cultivars of *Hibiscus rosa-sinensis* collected from the Faisalabad region.



***Hibiscus rosa-sinensis* cv. Frank Green**




***Hibiscus rosa-sinensis* cv. Mrs.George Davis**




**Adaxial surface** **Abaxial surface** **Midrib**

***Hibiscus rosa-sinensis* cv. Wilder's White**

Fig. 4. Leaf anatomical studies of some cultivars of *Hibiscus rosa-sinensis* collected from the Faisalabad region.
**AD EPI= A**daxial epidemal cell area and thickness; **AB EPI=** Abaxial epidemal cell area and thickness; **L AD EPI=** Lowest adaxial epidemal cell area and thickness; **L AB EPI=** Lowest abaxial epidemal cell area and thickness; **ST=**High Stomatal density; **L ST** Lowest stomatal density; **CTX=** High cortical cell area; **L. CTX=** Lowest cortical cell area. **PH TH=** Max. Phloem region thickness. **XY TH**= Max. Xylem region thickness.

## Discussion

Being extensively distributed in variety of environmental conditions, plants develop particular physiological, biochemical and anatomical modifications. With special reference to plant species endowed with anatomical alterations, increased chances of survival under multifarious environmental conditions have been recorded from time to time (Cutler *et al.*, 2007; Noman *et al*., 2012). These modified anatomical attributes in different plant body parts are of paramount importance to cope with adverse environments. In the present study different cultivars of *H. rosa-sinensis* appear morphologically similar but, anatomical studies help in their differentiation and identification when correlated with morphological traits.

Plant's internal organization can be significantly influenced by environmental variations (Asghar *et al*., 2013). As self defense system develops in plants under stress 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). It is known that hampered water supply may induce cell wall thickness upto 20%. Plants exposed to longer periods of water scarcity develop folds in their outer tangential walls in bundle sheath cells and cellular shape alterations in mesophyll cells (Utrillas & Alegre, 1997). In addition, increase in leaf area appears sensitive to rising salinity level along with reduced leaf emergence rate (Curtis & Lauchli, 1987; Hameed *et al*., 2013; Leghari and Zaidi 2013).

In present study, different cultivars of *Hibiscus rosa-sinensis* were investigated anatomically. Results indicate significant cultivar variation in relation to various anatomical attributes. These modified anatomical traits in *H. rosa-sinensis* cv. Lemon chiffon including thick adaxial and abaxial epidermis, increased adaxial as well as abaxial cell area and large cortical cell area are indicative of high chances of survival under varying environmental types.. Thick upper and lower dermal layers accompanied with incremented epidermal cell area on both surfaces was at its zenith in this cultivar as compared with others. The presence of such thickened epidermal cell area is consistent with some earlier studies in which they reported a significant inter-specific variation in relation to these leaf anatomical characteristics (Bahaji *et al.,* 2002; Ishida *et al.,* 2008; Nawaz *et al.,* 2011). This thick epidermis along with the thickness of cuticle layers is known to be an adoptive phenomenon for plants grown under water deficit conditions and useful in checking water loss (Rashid *et al.,* 2001; Bahaji *et al.,* 2002). Studies also support that plants with thick leave appear advantageous and can be better adapted under adverse environmental conditions (Brouillette *et al.,* 2006; Donovan *et al.,* 2007; Ristik & Jenks, 2002).

With changing environmental conditions, leaf stomatal conductance among different gas exchange attributes is the first and foremost phenomenon. It directly depends upon the stomatal density. In the present study, high stomatal density was observed in *Hibiscus rosa-sinensis* cv. Wilder's white and stomata were found on both dermises in all cultivars. Such presence of stomata on adaxial as well as abaxial surfaces of leaf can be considered as a reason for ecological success of a species under changing environment (Nawaz *et al.,* 2011). Furthermore, increased stomatal density along with large stomatal area as in the present study is linked to enhanced water use efficiency that directly influences the plant stomatal conductance (Zhang *et al.,* 2007) and it is the indication of the success of a species in a particular area.

As it is reported in the present study that increased palisade and spongy cell area in cv. Cooperi alba and Mrs. George Davis respectively as compared to other cultivars that is known to relates with efficient assimilate synthesis. These findings are in agreement with the findings of Loreto *et al.,* (1992) who described a thick palisade tissue in the leaves and reported that positively correlated with more mesophyll conductance that ultimately increased the photosynthetic activity due to enhanced $CO_2$ diffusion. Similarly, in a number of studies it has been reported that large cortical cells in *Eucalyptus microtheca* and *E. botryoides* are the indication of their wide distribution in a variety of environmental conditions (Zwieniecki & Newton 1995; Baloch *et al.,* 1998; Hamed *et al*., 2013). It is well known that better cortical cell area seems to be related to better storage of moisture that is prerequisite for survival under harsh climates (Ali *et al.,* 2009). Similarly it has also been reported that amplified photosynthetic cells i.e. palisade cells are capable of enhancing photosynthetic capacity (Nawaz *et al.,* 2011). So, in the present study the cultivars with high palisad cell area indicate their genetic potential to more survival under the present conditions. Similarly, leaf succulence in relation with leaf mid rib thickness and enhanced leaf cortical cell area are also the ecologically significant traits to fight against adverse environmental conditions that is also an plant leaf adaptation to servive under varying conditions (Hameed *et al.,* 2009).

Substantial increments of vascular bundle area in *H. rosa-sinensis* appears as one of the most vital feature supporting plant life cycle under environmental variations (Ali *et al.,* 2009). In the present study a substantial increment in vascular bundle area has been recorded in *Habiscus rosa-sinensis* that shows its adaptation under varying environmental conditions. These findings are in line with the results of Ali *et al.,* (2009) stating direct relation of vascular bundle area to alleviated transport of water and nutrients from the soil, and reported that this anatomical adaptation might be of greater importance under reduced moisture availability. Greater vascular bundle size has also been reported by Awasthi and Pathak (1999) in some saline tolerant genotypes of *Ziziphus*. They reported that larger vascular bundles comprising of broad metaxylem vessels and large phloem may prove vital for conduction of water and nutrients as well as translocation of photo assimilates (Stuedle *et al.,* 2000). In the present study approximately equal metaxylem area in cultivars Wilder's white, Charles September and Mrs. George Davis was calculated that appear as an aegis in these plants. Similarly differentially enlarged xylem and phloem area has been recorded in cv. Cooperi alba and Lemon chiffon that shows as an comprehensive indicator for survival of plants under varying environmental conditions. Similarly in an earlier study Hose *et al.,* (2001) reported that presence of greater phloem area in *Hibiscus rosa-sinensis* with enhanced conduction of assimilates shows a good reason of ecological accomplishment of this species under diverse environmental conditions.

From the present study it can be concluded that all the *Hibiscus rosa-sinensis* cultivars showed great diversity in terms of leaf tissue anatomical characteristics. Furthermore, these varied leaf anatomical aspects of the entire Hibiscus cultivars are ample indicators of their better genetic adaptability under varying environmental conditions and, Ipsofacto, prove ecological success of these plants. Presence of specialized feature in relation to leaf anatomy in *Hibiscus rosa-sinensis* cv. Lemon chiffon and Wilder's white appear prudent in determining the fate of these cultivars in multifarious environmental challenges ranging from rising temperature to augmented soil types.

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