

# IN THE NAME OF ALLAH THE MOST BENEFICIENT AND MERCIFUL

# ROOT ANATOMICAL CHARACTERISTICS OF SOME DATE PALM (*Phoenix dactylifera* L.) CULTIVARS OF DIVERSE ORIGIN

By

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# My

Siblings

# AND

# My

# **Beloved Aunt**

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## Declaration

I hereby declare that contents of the thesis (Root anatomical characteristics of some date palm (*Phoenix dactylifera* l.) cultivars of diverse origin) are product of my own research and no part has been copied from any published source (except the references, standard mathematical equations/formulas/protocols etc.). I further declare that this work has not been submitted for award of any other diploma/degree. The University may take action if the information provided is found inaccurate at any stage. (In case of any default, the scholar will be proceeded against as per HEC plagiarism policy)

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## Abstract

Worldwide, Pakistan ranks among the five leading producers of date palm (Phoenix dactylifera L.). Date palm is economically the third major fruit crop after citrus and mango in Pakistan. Pakistan appeared on the map of date exporting countries in the beginning of 80s in the last century. In Pakistan, Balochistan is the largest date producing province followed by Sindh, Punjab and Khyber Pakhtunkhwa. Rich soil, abundant sunshine and four distinct seasons make Pakistan an ideal place for cultivating a variety of agriculture crops. The above factors help in creating a very special taste in our farm produce, particularly in fruits: mangoes, apples, and dates. Thirty four date palm (Phoenix dactylifera L.) cultivars were evaluated to compare root anatomy and to examine the ecological significance of root anatomy in identification and ecology of different date palm cultivars. The relative importances of anatomical characters of these cultivars were emphasized and the adaptive component of root anatomy in relation to the habitat ecology was examined. The size of epidermis cells, size and shape of outer cortical region, presence of sclerification in outer cortex, sclerenchyma bundles in cortical region and presence of aerenchyma were quite variable in all the cultivars studied. Similarly endodermal layer thickness, thickness of outer tangential wall of endodermis, shape and size of phloem region, size and arrangement of metaxylem vessels and sclerification in the pith region showed extremely high magnitude of diversity.

## Chapter 1

## Introduction

Thousands of the date palm cultivars exist in different parts of the world. These date palm cultivars are developed through selection by the date palm growers to improve the crop quality and yield. Based on the botanical description, there are 400 cultivars in the Iran, 370 in the Iraq, 250 in the Tunisia, 244 in the Morocco and 400 in the Sudan (Osman, 1984). These cultivars of date palm are identified commonly by wide range of morphological features that described trees and fruits (Nixon, 1950; Zaid and de Wet, 2002; Elhoumaizi *et al.*, 2002; Osman; 2002).

In current palm classification, Palm family contains 183 genera and 2500 species that are divided into five subfamilies: Nypoideae, Calamoideae, Ceroxyloideae, Coryphoideae and Arecoideae (Dransfield *et al.*, 2005). Potentially compensating of architectural liability, extensive diversification of the anatomical structure of leaf within the palms involves many important characters whose alternate state may confer a mechanical or physiological capabilities (Horn *et al.*, 2009).

Of all the genera in Coryphoideae subfamily, genus *Phoenix* contains greatest number of species with biodynamic uses. The Arecoideae subfamily is most diverse and largest of all palm subfamilies. Independent of the species with many uses (*Cocos nucifera*, *Areca catechu*, *Elaeis guineensis* and *Borassus spp.*); genera of this subfamily have medicinal uses with the greatest number of species (Byg and Balslev, 2001; Dransfield and Beentje, 1995).

*Phoenix dactylifera* L. (2n = 36) is cultivated in semi-arid and arid areas of the Asian and North African countries. Being a monocot date palm produces fasciculated and mostly fibrous roots. Primary roots develop from seed and secondary roots develop from primary root. Secondary roots produce on the lateral roots and then tertiary roots. Which are of same type and with the approximately same diameter through their length (Salem *et al.*, 2008).

Architecture of the root is an important aspect in plant productivity. Architecture of the palm root system performs various functions. Roots are morphologically different and have eight types which are distinguished mainly on the base of development pattern and differentiation state: primary vertical and horizontal roots, horizontal and secondary roots, upward growing and secondary vertical roots, downward growing secondary vertical roots and superficial roots, deep tertiary and quaternary roots (Jordan and Rey, 1997).

Palm roots exhibit the determinate growth at adult stage, replaced periodically by the lateral adventitious roots that extend from internodes and usually at base of stem. Cutting of the palm roots do not endanger palm and are done sustainably. Being relatively succulent and pliable, they are commonly mashed or pounded for the creation of decoction (Fisher, 2002).

Roots play critical role in the water absorption, transport and also in water storage in palms. In the rattan palms, from the connections between the adventitious roots and vascular bundles in longitudinal stem water enters the stem base. Water moves along the wide and narrow vascular bundles that are located both in stem center and in the periphery, respectively. All bundles have at least 95% in base of the mature stem and are functional in the transport of the water. There is lack of tuberous roots in Rattans and narrow stems of Rattans have small proportion of the parenchyma that has function in the water storage (Fisher *et al.*, 2002). However, long stems of Rattans with large volume of the water in the wide vessels, represent significant reservoir of water that become available to Rattan if the cavitation of the vessels are occurred during the periods of the extreme stress of water (Holbrook, 1995).

Most of the palm species produce adventitious roots that originate from the root initials present on trunk than relying on the root generation in severed roots. Cabbage palm produces no root tips almost from the cut roots and establishment relies most exclusively on the adventitious roots that are new (Day *et al.*, 2009). Adventitious root system of Palms is composed of the numerous fiber that are first-order roots that grow periodically and independently from root initiation zone, area at base of stem or at the trunk near to the ground level (Tomlinson, 1990).

Most of the palms have root growth that is tended highest during warmer months, from the spring through the fall. *Roystonea regia*, *Washingtonia robusta* and *Phoenix reclinata*, produce a large numbers of the new roots from root initiation zone, while *Syagrus romanzoffiana* and coconut palm (*Cocos nucifera*) grow few (Broschat and Donselman 1984). In latter species (*Syagrus romanzoffiana* and *Cocos nucifera*), however, number of the cut roots, regrowth is surpassed and large number of the new roots are grown from root initiation zone. Offshoots of *Phoenix dactylifera* show similar response, new root of over

two-thirds growth originate from the roots that are severed during the removal from mother palm (Hodel and Pittenger, 2003).

Hodel *et al.* (1998) and Pittenger *et al.* (2000) reported that, root growth of the most palms is tended to be the highest during warmer months which is from the spring through the fall. *Phoenix reclinata, Washingtonia robusta* and *Roystonea regia* grow a large numbers of the new roots from RIZ, while *Syagrus romanzoffiana* and *Cocos nucifera* grow few relatively (Broschat and Donselman, 1984). In latter two species, the number of the cut roots, that regrow is surpassed and large number of the new roots grew from RIZ. Offshoots of *Phoenix dactylifera* responded similarly with the over two-thirds new root growth that originate from the roots severed during the removal from mother palm (Hodel and Pittenger, 2003).

Development of the root structure is important in the survival and growth of the date palm. Water is stored mainly in the Cortex tissue (Ogburn and Edward, 2009). Formation of Aerenchyma in the roots, due to transfer of the cortex, may enhance diffusion of the photosynthetic and atmospheric oxygen from shoot to roots (Naidoo and Naidoo, 1992; Baruch and Merida, 1995). Compactness of exodermal and hypodermal layers in the roots can play role in the preventing collapse of cortex, may also be important structural framework for the aereanchyma formation. Abnormalities in structure of the root vascular tissues can be attributed to the extreme environment in cultivation, such as water potential, air humidity and  $CO_2$  (Seago and Marsh, 1989).

Embryo of oil palm consists of root pole, a single cotyledon and epicotyl shoot apex. Root pole is flattened and blunt. Cotyledon surrounds the Shoot apex. A small slit or an internal cavity, or which is separated from shoot tip zone which is evident from the cotyledon in base of the cotyledon (Kanchanapoom and Domyoas, 1999).

Oihabi (1991) divided the root of date palm into the four zones. Zone I which is also called a respiratory zone. It localized at palm base which surrounds the area with more than 25 cm depth, lateral distribution of maximum of the 0.5m which is away from stripe. Nutritional zone which is called zone II. It is large zone, which contains a highest proportion of the secondary and primary roots. Zone II is developed between the 0.90-1.50m depths and laterally be founded outside of projection of tree's canopy. Zone III, which is absorbing zone. The importance of this zone is dependent on the type of culture and on the depth of

underground water. It is usually found at a depth of 1.5 to 1.8 m, mostly primary roots with a decreasing density from top to bottom are found here. Zone IV, largest portion of zone IV is dependent on the underground water. When underground water is in depth, roots of zone IV could reach greater depth.

In the trees, water conductance of the roots (both axial and radial conductivities) and transpiration rate of the whole plant tend to be more in the fast growing and early successional trees, than in the slow-growing and late successional trees (Meizner *et al.*, 1995; Tyree *et al.*, 1998; Becker *et al.*, 1999). This links to the total absorptive surface area of roots and the finer roots and not to the differences in the anatomy of root xylem (Tyree *et al.*, 1998). Among the cultivars of the citrus rootstock, higher hydraulic conductivity of the root is associated with the components of the radial conductance, not the components of the axial conductance (vessel diameter and number) (Graham and Syvertsen, 1985; Eissenstat and Achor, 1999; Huang and Eissenstat, 2000). Tissues built with the smaller cells and lignified wall materials are associated with the longer persistence of tissue and slower growth (Wahl and Ryser, 2000).

## **Objectives of study are**

- To examine comparative anatomy of roots of date palm grown at Date Palm Research Station, Jhang.
- To study the ecological significance of root anatomy in origin and distribution of date palm.
- 3) To evaluate importance of anatomical characters in cultivar identification.

## Chapter 2

## **Review of Literature**

#### Genetic diversity in date palm

Date palms have astonishingly high genetic diversity. Morocco and Iraq are famous for largest genetic diversity, and many good quality varieties originate from the Tunisia (Deglet Nour) and Morocco (Madjhool). More than 250 of cultivars are characterized in Tunisia (Karim *et al.*, 2010).

In oases of the Morocco, the date palm constitutes main and important income generating activity. Among date palm producing countries, Pakistan comes at the sixth place and in Pakistan, there are more than 325 varieties of the dates that are available. In United Arab Emirates, more than 10 millions of the fruitfull trees of palm are estimated (Jamil *et al.*, 2010). In most of Arabian countries palm is considered very important tree (Mustapha *et al.*, 1983).

Number of the palm varieties distributed in the world is approximately 5000. There are about 450 varieties which are found only in the Saudi Arabia (Bashah, 1996). In the world most of widely grown varieties are characterized morphologically (Ahmed *et al.*, 1979).

Belonging to Angiosperms-Monocotyledones Palmaceae is a family of the 200 genera and1500 species (Dowson, 1982). *Coryphoideae Phoeniceae* is one of the genera which contain dozens of species; all are native to subtropical or tropical regions of the Southern Asia and Africa, including the *Phoenix dactylifera* L. (Munier, 1973).

Elhoumaizi *et al.* (2002) studied the phenotypic diversity of the date palm cultivars (*Phoenix dactylifera* L.) from the Morocco, where 26 cultivars showed wide spectrum of the morphological variations. In some cultivars there are strong relations on basis of the morpho metric characteristics. There are differences between cultivars that were assumed by the characters like the pinnea number, pinnea length, pinnae width, spine width, leaf width and pine length. Length of the pinnaeted part was correlated greatly to the midrib width and the pinnae number. Leaf length was defined by pinnaeted part length. Morphology of the cultivars Azgzao, Aouitobb, Mejhoul and Aguellid were different in relation to each other.

Production of date fruit began in late 1800s in USA on commercial level and is still most important component of the desert agriculture in the Arizona and Southern California (Nixon and Carpenter, 1978; Albert and Hilgeman, 1935; Karp, 2002; Johnson *et al.*, 2002).

Date palm (*Phoenix dactylifera* L.) is most important plant of the desert areas of the Northern Africa, Southern Asia and Middle East. It is called "palm of life". For over 5000 years, it has provided ornament, food, material for the shelter, fuel and fiber in the harsh environment, where only few other plants have ability to grow (Popenoe, 1973; Dowson, 1982; Zaid, 1999).

Areas with the relatively harsh soil and climatic conditions are good for the growth of the date palm tree and where no other crop can be planted. In such areas, date palm tree provide the higher returns to people living. In irrigable desert lands the date palm is irreplaceable tree and it provides the protection to the under crops from the wind, heat, cold weather and plays an important role to stop the desertification and give life to the desert area (Hassan *et al.*, 2006).

Hodel *et al.* (1998) and Pittenget *et al.* (2000) reported that in the Southern California the root growth of the most palm trees is highest during warmer months, spring through fall. Human beings have spread date palm beyond far its historical range. In all regions it showed satisfactory growth (Hodel and Pittenger, 2003).

Extensive diversification of the leaf anatomical structure in the palms involves different characters, whose alternate state can confer the disparate physiological or mechanical capabilities (Horn *et al.*, 2009).

Hammadi *et al.* (2009) performed an experiment on the new approaches for morphological identification of the *Phoenix dactylifera* L. cultivars from the Tunisia. Thirty date palm characters, on the vegetative base were screened. Intra cultivar stability of the some characters was shown by the statistical tests such as maximal width of pinnae, spine length and percentage of the spinned midrib. The characteristics of the leaves showed high diversity among cultivars. Apical divergence angle and pinnae length at top showed the positive correlations. Cultivars of Deglet Nour showed stability in the spine length. Alig cultivar showed stability in the spine length and pinnea length and spined leaf area. Kintichi cultivar had been characterized by the stability in pinnae width and in leaf width. This is phenotypic variability which reflects genetic diversity, when environmental effects were eliminated.

Sack *et al.* (2008) suggest that network of the venation which provides high degree of the vascular redundancy and confers tolerance to the hydraulic disruption. From biomechanical perspective, transverse veins are well-developed in the palmate leaves and function as stringers and it is between ribs (Niklas, 1999).

*Phoenix dactylifera* L. is distinguished from *P. sylvestris* and *P. canariensis* by the several characteristics as production of the offshoots; columnar, tall and thick trunk. Date palm can reach height of more than 20 m (Zaid and de Wet, 2002).

#### **Root anatomy of family Palmaceae**

Mathew and Bhat (1997) conducted an anatomical survey of the 42 species which showed many differences among four genera of the Calamoideae present in India. Vascular bundle in the *Calamus, Korthalsia* and *Daemonorops* is characterized by the two phloem fields and solitary metaxylem vessel, while Plectocomia showed a single phloem and 1-2 metaxylem vessels. Mechanical tissues showed diversity in the *Plectocomia* and *Korthalsia* with the sclereids as yellow cap which is present on outer side of fibrous sheaths of the vascular bundles. The size of different cells, diameter of metaxylem vessel in particular, appeared to relate the species habit, stem size and geography.

Fisher *et al.* (2002) examined the 11 species in the four genera of the rattans (*Calamus, Plectocomia, Korthalsia* and *Daemonorops*) which grow in the native rainforest in Singapore. Above 95% of all the vascular bundles present at base of the mature stem have function to transport water. There are longest protoxylem vessels which ranged from 7.5-62 cm in the length but there was one stem which had exceptional protoxylem vessel which is of 3.0 m. Maximum vessel diameters of metaxylem were correlated positively to the maximum lengths of vessel in these species. *K. rigida* had longest metaxylem vessel which was of 3.96 m in length and 1200 vessel elements constructed it. Widest vessel in the same stem was of 532  $\mu$ m in the diameter. Long and wide vessels had decreased the resistance and had increased the efficiency of water transport. In addition, wide vessels of metaxylem performed an important function which is water storage.

Baker and Zona (2006) investigated the extensive diversification of the root and leaf anatomical structure in the palms. Shape of the epidermal cells may be spindle-shaped, rectangular or hexagonal. Shape of the epidermal cells ranges from the isodiametric, rectangular, hexagonal or elongated with longitudinal and an oblique elongation into the spindle. Shape of the epidermal cells optimizes on each of 100 trees, all changes within the palms. Hexagonal to the spindle-shaped cells are evolved independently in other palms in the tribe Coryphoideae (Caryoteae). Maximum likelihood optimizations of the unambiguously reconstruct state more broadly and assigning it both of the basal nodes of the Arecoideae plus Ceroxyloideae and Ceroxyloideae. Reversions back to the rectangular cells that occurred in the *Pseudo phoenix*, at or near base of the Euterpeae (Arecoideae), at least one time in the Attaleinae (Arecoideae, Cocoseae, clade inclusive *Syagrus* and *Jubaeopsis*).

Slaton and Smith (2002) compared the species with pinnate leaves. Although the leaves of species of *Dypsis*, *Ceroxylon* and *Butia* differ somewhat in external form, degree of difference is not suggested for the remarkable anatomical difference. Major anatomical differences in Lamina of *Dypsis*, *Ceroxylon* and *Butia* have distribution and presence of the fibers. Fibers assumed more load bearing capacity of lamina. Symmetry of lamina histology varies among the three species. *Ceroxylon* and *Dypsis* have dorsiventral arrangement of the tissues; *Butia* exemplifies the isobilateral histology of lamina. *Butia* has abaxial and adaxial leaf surfaces and alike mesophyll. So that disregarding polarity of vascular bundles, they are mirror image equivalents.

Iossi *et al.* (2006) collected the fruit of the *P. roebelenii* from the trees located on campus of Paulista University Brazil. From the sample of the 100 seeds, length, thickness and seed width were determined. Seeds of the *P. roebelenii* are slightly flattened and elliptical, with furrow on ventral face. On dorsal face, operculum can also be observed. They are of albuminous type, contains a hard endosperm that almost completely fill its inner part. Embryo occupies a peripheral and lateral position. It is wedge shaped or cuneiform and funneled end is present towards seed periphery. Plumule emerges towards longitudinal rift which opens at cotyledon of petiole. Seedling completely emerges out through the rift. From anterior portion of primary root, secondary roots started to develop.

Kanchanapoom and Domyoas (1999) excised mature oil palm (*Elaeis guineensis* Jacq.) embryos of variety Tenera and surface sterilized. Embryo of oil palm was white in color from the mature seed, ovate in shape and 3 mm in the length. Embryo of oil palm consists of a single cotyledon, a root pole and epicotyl shoot apex at this stage. Root pole is

flattened and blunt. The cotyledon surrounds the shoot apex. In base of the cotyledon small slit or an internal cavity is evident which separates shoot tip area from cotyledon. Shoot tip contains three leaf primordial and shoot apical meristem. Three cells namely, parenchyma cells, protodermal cells and procambial cells composed the cotyledon. Procambial cells elongate along axis of embryo and are narrow in shape. Procambial strands are individual bundles and are visible.

Avalos and Otarola (2010) conducted the study on the Neotropical palm (*E. precatoria*) at the Quebrada national park and at biological station of La Selva. For analysis of the stilt root structure there were 31 individuals of the Neotropical palm which were selected randomly. The distribution and structure of the stilt roots in the Neotropical palm were determined by the palm size and not by the slope conditions. Stilt root cones reaches at 2 m and roots are usually separated and clustered as in case of *S. exorrhiza*. There was positive and strong scaling between morphological characters that are important in composing palm size and stilt root cone. In the stem, palms developed mechanical properties as present in the conifer and dicotyledonous trees. The timing of expression of the developmental changes in stem is affected by the resource heterogeneity as height vs. diameter relationship observed.

North *et al.* (2008) studied the succulent monocots plants native to the southern California. Anatomically, contractile roots (CRs) of contractile basal zone differed from midroot zone of non-contractile roots in the several ways. Root zone in the *H. whipplei* contained many cellular features, which were characteristic of all species examined and possessed CRs. These features were also examined for the roots of the *H. whipplei* in field. First of all the cells of inner or the middle cortex in the basal zones of the CRs were radially elongated whereas, cortical cells in the midroot which is non contractile zones of same roots were collapsed either as seen in the plants grown in field or nearly isodiametric. In the longitudinal sections the inner cortical cells from basal zone of the CRs were 25–30% shorter and twice wider than the cortical cells in the non contractile zones from same root. Secondly, for the CRs, cortical and endodermis cell walls which is outside the endodermis were less lignified or suberized in the basal than in the midroot zones and metaxylem vessels in basal zone of the CRs were not lignified fully. In contrast for the NCRs, the basal and the midroot zones contained isodiametric cortical cells and adjacent cortex and endodermis cell walls

were more lignified and suberized in basal than in midroot zone. Thirdly, in the longitudinal sections of contracted basal zone the external tissues (outer cortex, epidermis and exodermis) and the vascular tissues (early metaxylem vessels and primarily protoxylem) showed the compression. Outer cortical cells also appeared damaged by the compression whereas exodermis, epidermis, vascular tissues and inner cortical cells remained intact and root hairs were present.

Estrada et al. (2008) examined the root orders in the Vaccinium corymbosum (Ericaceae) which were planted at the research and education center near the State College, USA. Transverse sections of the seven representative orders of root of Vaccinium *Corymbosum* were studied. Ericoid 1<sup>st</sup> order roots had development of minimal cortical layer. Especially in the 1<sup>st</sup> and 2<sup>nd</sup> (lowest order roots) the epidermal cells were absent, squashed or collapsed entirely. Number of the epidermal cells present in the root from the 1<sup>st</sup>-3<sup>rd</sup> order and ranged from the 7-14, 8-12 and 10-18, respectively. Third order of the roots had pent arch xylem pole. Secondary development of xylem occurred in the 4<sup>th</sup> and in the higher orders. Mean diameter of the root varied considerably within the root order. From 1<sup>st</sup>-6<sup>th</sup> order of roots, mean diameters were 40µm, 48µm, 75µm, 120µm, 177µm and 222µm, respectively. Range in the diameter of root for the any order was  $\pm 20\mu m$  for the 1<sup>st</sup> and the  $2^{nd}$  order of the roots, much more for the higher orders of roots. Among the root orders the vascular tissues varied substantially. Diameter of Stele varied with the root orders in the magnitude similar to the root diameter. Mean diameter of stele from the 1<sup>st</sup>-7<sup>th</sup> order of roots was: 17µm, 20µm, 27µm, 38µm, 67µm, 114µm and 239µm, respectively. Mean diameters of stele in the  $1^{st}$  and  $2^{nd}$  roots orders were quite same (P = 0.39). Unlike the stele and root diameter the vessel diameter was conserved over the root order. Mean diameter of vessel from the 1<sup>st</sup>-7<sup>th</sup> order of roots was 2.5µm, 2.9µm, 3.2µm, 3.7µm, 3.7µm, 3.8µm and 4.8µm, respectively. Maximum diameter of vessel from the 1<sup>st</sup>-7<sup>th</sup> order of roots was 6.5µm, 7.2µm, 9.3µm, 10.0µm, 10.0µm, 25.0µm and 31.0µm, respectively. First and 2<sup>nd</sup> roots orders did not differ from the each other in the maximum or mean vessel diameter, however they were smaller (40% and 80%), than those of the higher orders. Vessel numbers varied among the root orders. Mean number of the vessels from the 1<sup>st</sup>-7<sup>th</sup> order was 13, 22, 39, and 812 per root, respectively. Similar to diameters of root number of the vessels, stele and vessel in the 1<sup>st</sup>-2<sup>nd</sup> order of roots was similar. There was considerable increase (about 1150%) in the

number of the vessels between the 5<sup>th</sup> and 6<sup>th</sup> root orders, associated with pronounced growth of radial during the secondary development.

Roots within same category are also same in the function and properties. Also, transport capacity of the roots is linked to the root aging and associated with the radial growth and results in the increase of number of the xylem vessels (Esau, 1965; Steudle and Peterson, 1998; Kumar *et al.*, 2007) and the diameter of largest vessels (Martinez- Vilalta *et al.*, 2002).

Oil palm root system is formed of the primary I, secondary II, tertiary III and quaternary IV roots. Function of I and II types is anchorage basically and transport of the solute and water. Basically, growing points of I and II roots and fine roots are responsible for the nutrient uptake. III and IV (fine roots) reach at the few centimeters in the length can be considered colonizers of substrate but the larger roots which are I and II are pioneers. They precede formation of the absorbing roots and can reach the greater lengths (Ruer, 1967; Jourdan and Rey, 1997). Conditions of soil in particular site can modify characteristics of roots (Albertazzi *et al.*, 2009).

Broschat and Donselman (1984) studied the regrowth of the severed palm roots. Cut root branching responded different among the species of the palms. Percentage of the branched root increases with the increase of the root length in the royal and queen palms. The larger root balls for the branching are necessary and continued the growth of the old roots. Length of the roots had no observable impact on the branching in the cabbage and coconut palms with the half of all the cut roots that branch in the coconuts and none branching in the cabbage palms. New roots initiated from trunks in all of four palms at the rate that is inversely proportional to ability of species that can regenerate the severed root tips. Root pruning in the 2-3 months and prior to the moving palms is necessary for the species such as cabbage and royal palms with the branching of roots minimal and production of new roots extensive. Queen and coconut palms can produce new roots by following the root pruning

Anatomical structures are very important in the family Arecaceae (Palmae). Differentiation of root cortex is a key factor in the transport of compounds inside and outside of the root, subsequently to other parts of the plant body. In roots most internal layer is present and differentiated into endodermis. It is apoplastic barrier which is crucial for selective transport in the root stele through the symplast (Clarkson and Robards, 1975). Like

endodermis the exodermis have the properties of the apoplastic barrier, but it is differentiated in sub epidermal layer of cortex periphery. It enables exodermis to protect the tissue of middle part of cortex. Exodermis is present mostly (90%) in the plant species of the various environments i-e., mesophytes, hygrophytes, hydrophytes and xerophytes. Exodermis formation can be induced by environmental factors (Zimmerman and Steudle, 1998).

### Ecological and taxonomical significance of anatomical characteristics

Dates are traditionally propagated through the offshoot plantation method. An independent plant needs a good root development and is dependent on a good development of the root initiation zone also called RIZ of the offshoot. New roots originate from the existing cut roots and are numerous, less in the diameter from roots originating newly or directly from the offshoots RIZ and also less in the number (Hodel and Pittenger, 2003).

Ali *et al.* (2004) found the differences in the morphology and in the anatomy, with respect to development in the date palm. According to their findings poorly developed date palm has an unorganized development of the week roots. Which lack the taproot differentiation, mesophyll cells and stomata. Leaflets are poorly developed, improper differentiation and have poor development of the parenchymatous cells and vascular bundles.

Anatomy of the palm node has 6 primary strands of vascular bundles, which are present below node. Internode has many vascular traces. These traces are diverged into the nodes. Main shoots have 6 traces and nodes have 2 traces. These traces are diverged into it and are not visible. Anatomically each internode contains 6 traces which are present above the subtending node. Diameter of the vessel members remain unchanged, decrease or increase through the nodes (Ellison *et al.*, 1993).

Distribution and development of the Date palm root depends on the type of culture, depth of underground water, soil characteristics and genotype. Roots of Date palm are present as far 25 m deep. In light soil, 85% of roots are being distributed in zone of the 2 m on the both lateral sides and 2 m deep (Zaid and de Wet, 2002).

Dransfield (1978) summarize that within the *Arecaceae* the stilt roots are present over wide range of the topographic conditions such as soil types and groups. Some of the evidences indicate that the stilt roots can provide a support under steep conditions and waterlogged soils. Stilt root species can survive better the violent tropical storms, along with the buttressed trees than the species lacking of these root modifications (Elmqvist, 1994). Stilt roots have the ability to resprout and favor the palm recovery, when stem has been knocked down by the falling trees or branches (Bodley and Benson, 1980). Early in life cycle development of stilt root cone, it permits rapid elongation without the loss of the stability and allowing the species of stilt root to reach the canopy stature faster than the species lacking the stilt roots (Swaine, 1983). Dransfield (1978) recognized that role of the stilt roots in the most species is not understood completely.

Goto *et al.* (2002) observed the anatomical features of the adventitious and the lateral roots of the sago palm. Two types of the roots are present in the sago palm. Large roots have diameter of 6-11 mm and small roots have the diameter of 4-6 mm. Adventitious roots are large roots. In the stem primodia is present inside of the epidermis, which emerges from the trunk surface and it has down warding growth in soil. Small roots are called lateral roots and have primodia which is present on large roots.

The Allen palm (*Cryosophila guagara*) bears three types of adventitious roots on stems of the young plants: prop roots, crown roots and trunk roots. The crown roots and trunk roots are modified into thorns, but the crown roots are not present in the older plants. Crown roots endogenously arise near apex of stem and can vertically grow downward in stem tissues. Emerge near attachment of the enveloping leaf sheath. Root changes its direction of the growth at site of the leaf attachment and grows upward to top of sheath and give rise ultimately to pendulous or branched thorn. Spines or trunk roots arises as laterals from bases of the crown roots, embedded in tissues of stem. Root apex is converted to thorn through cessation of the meristematic activity. Root caps do not contribute to thorn (Arthur and Steeves, 1969).

Adventitious root system is present in the oil palm. Primary roots are generally 6-10 mm in the diameter, originating from base of trunk and descending at the varying angles and spreading horizontally into soil. Primary roots bear the secondary roots and is of 2-4 mm in the diameter. Tertiary roots are in diameter of about 0.7-1.2 mm and branch out from secondary roots. Tertiary roots bear quaternary roots. Quaternary roots are of about 0.1-0.3 mm in the diameter, unlignified, 1-4 mm in the length and are considered to be main absorbing roots (Corley *et al.*, 1976). In the compacted soil, palms produce less primary roots and secondary roots, but production of the longer tertiary roots and quaternary roots can

compensate this. The compaction affects soil physical properties which in turn can affect distribution and growth of the oil palm roots (Yahya *et al.*, 2010).

Foong-Kheong *et al.* (2010) studied the nutrient absorption of primary roots of oil palm. Roots younger parts are white in colour and absorb the nutrient actively. Root tips are active site of nutrient absorption. Roots of oil palm are made up of the aerenchyma cells. Within them are empty spaces and hold water, nutrient and air. Empty spaces are also within the root structure. These spaces are larger than that found in aerenchyma cells. These are formed after they die and decay of aerenchyma cells. Tunnels are formed by the interconnection of these spaces in the roots. Tunnels extend from roots older parts to younger parts. New active roots are developed into the primary or secondary roots. (Corley and Tinker, 2003) suggest that total root length is effective for the nutrient uptake.

Fibrous root system is produced when Paclobutrazol (PBZ) is given to the oil palm. Low concentration of the paclobutrazol (3-6 mg/l) gave medium size of the fibrous roots of the oil palm and high concentration (12 mg/l) of the PBZ expressed the large diameter of the roots. When the PBZ is given, the medium thickening of fibrous roots of the oil palm is common (Bausher and Yolenosky, 1987; Barnes *et al.*, 1989). The number and thickness of the roots greatly increased at particularly high concentration of the PBZ (Nizam and Te-chato, 2009).

Davoodi *et al.* (2002) carried out the study to investigate the anatomical and morphological aspects of the somatic embryogenesis in the date palm (*Phoenix dactylifera* L) If the proembryos remain on medium supplemented with the 2, 4-D, they can lose potential for the maturation (Tautorus *et al.*, 1991). On other hand if the germinating embryos are provided with the same medium, this may stimulate the callus formation in the tissues and organs. Roots which remain inside medium are more exposed to the callus formation. Callus formation in the embryonic tissues not only includes the root tip but also include the cotyledon and haustorium. Dedifferentiation of root can cause lack of the root formation. Lack of the roots and means of the nutrient transport to growing points of germinating embryo can weeken the aerial parts.

Fisher and Jayachandran (1999) observed the root structure of *Serenoa repens* palm. Roots only have primary growth and has thickness range from 8.00 mm to 0.8-2.9 mm. Thickest root is present at the depth of soil greater than 20 cm, fine root is present at the depth 1-60 cm. All the roots have thick epidermis and has outer wall lignified. Thick-walled, single layered exodermis is present in all the roots, except thinnest. Roots are firstly suberized and then lignified. *Serenoa repens* never have root hairs. Next to exodermis is hypodermis which is composed of many layered lignified cells. Hypodermis formed outer cortex. Outer cortex has thin walled radial series which are slightly lignified. Exodermis has no passage cells. Remaining cortex is also composed of parenchyma which is unlignified air canals and lignosuberized endodermis of old roots. Passage cells are endodermis of some thinnest roots.

Ebanyenle and Oteng-Amoako (2003) investigated the Stem anatomy of the Rattan palms: *Eremospatha hookeri, Calamus deeratus, Laccosperma acutijlorum, Laccosperma secundijlorum* and *Eremospatha macrocarpa* in Ghana. Epidermal cells are rectangular in shape, radial length is about 15-26  $\mu$ m, width is about 6.9-11.16  $\mu$ m. width of the cortex is about 45-113  $\mu$ m, cortical cells are in round to oval shape with the varying sizes and are interconnected. Cortical cells are lignified at basal than at the top of internodes. Vascular bundles are not uniform in the structure, unevenly distributed, first two rows are larger in the size and form ring, smaller inner cells are scattered and diffused, diameter is about 275-825  $\mu$ m. proportion of the conducting cells is 18-43%. Protoxylem consisted of the cluster of the 2-6 vessels. Phloem doubles the stranded fields and lying laterally to metaxylem vessels. Fiber sheath is extensive in the peripheral and basal part of the vascular bundles, than the inner and top sides of the internodes vascular bundles. Fiber length is about 0.60-4.2mm, width is 5.8-34.8  $\mu$ m, lumen is 2.9-29 $\mu$ m, wall thickness is 1.45-20.3  $\mu$ m and proportion is 8-35%. Ground parenchyma is oval to round in shape and sometimes is weakly branched, more lignified at the basal than the top internodes.

All orders of the roots exhibit the irregular and longitudinal pattern of the white rings of the surface eruptions. These patterns are result of the proliferation of the sub epidermal cells, and rupturing of epidermis. Proliferated cells have spherical shape, thin walls, highly suberize and many intercellular spaces. Thick walled exodermal cells and outer cortical cells seem as enlarge and undergo into one or two cell divisions which are internally paradermal. This is lenticel like structure and is called pneumathodes, because of the presumed function in the gas exchange. Pneumathodes are common in the roots near surface and on the erect order II roots (Seubert, 1997).

Hummel et al. (2006) investigated the anatomy and root structure of the 14 herbaceous Mediterranean species. Trifolium angustifolium is annual with the interspecific variations in the xylem. Cross sectional area of xylem is larger than cross sectional area of root and ranged from the 376  $\mu$ m<sup>2</sup> for annual Arenarian serpillyfolia to the 5294  $\mu$ m<sup>2</sup> for perennial B. phoenicoides. Eleven fold variations were in the xylem cross sectional area as proportion of the root cross sectional area. Xylem cross sectional area represented the 1.4% of root cross sectional area in annual dicot (V. persica) and 16% in perennial monocot (B. phoenicoides). Mean cross sectional area of the xylem ranged from the 20  $\mu$ m<sup>2</sup> for the *T*. angustifolium to the 134  $\mu$ m<sup>2</sup> for the Tordylium maximum. Root cross sectional area is correlated with cortex and rhizodermis cross sectional area to lesser extent with xylem cross sectional area. Interspecific variation in the root cross sectional area is not related, to the mean xylem vessel. Interspecific variation is larger in the Apiaceae than in the other species. Absolute xylem cross sectional area and xylem cross sectional area as proportion of the root cross sectional area is higher in the perennials than the species with short life span, whereas root cross sectional area and xylem vessel cross sectional area are not different between the life spans.

Reginato *et al.* (2009) studied the root anatomy of the four species (*Pleiochiton micranthum* Cogn., *Pleiochiton setulosum* Cogn., *C. blepharodes* and *P. ebracteatum*). Diameter of four species ranged from the 5-15 mm. In the transverse section, thick periderm coated the root, which contains 11–13 layers of the rectangular cells. Below periderm, cortex is with the several layers of the large parenchyma cells, relatively large, heterogeneous in the size and shape, ranging from the quadrangular to the polyhedral and rectangular. Oxalate and Sclereids druses are very common throughout cortex. Vascular system contains secondary phloem with few numbers of elements which are larger secondary xylem and a cambium. Adventitious roots from erect stem of the *C. blepharodes* are 1 mm in thickness. These roots have periderm with the 6 layers of the rectangular cells, cortex with five layers which are isodiametric, rectangular or polyhedral in shape and more homogenous than ones from cortex from the regular roots. In these roots rotting endodermis is conspicuous. Vascular cambium contains 5-7 layers, phloem has a few elements with 5-7 layers and a few elements that are located externally to the vascular cambium. Xylem has many layers that fill all central portion of root.

Sawidis *et al.* (2005) studied the anatomy of root, the tuber of the *Asphodelus aestivus*. Multiple layered velamen covered the root tuber, which is epidermal system with 4–6 cells. Velamen epidermis is uniseriate and cells are devoid of the cuticle. Velamen cells are single celled hairs and sometimes thick walled. Epidermis cell contains a myelin like structure. Extra cellular space is present between neighboring and positively reacting cells and is not penetrated. Positively reacting cells do not contain a suberized wall usually. Pericycle is uniseriate and cells are isodiametric and periclinally orientated. Root xylem of vascular cylinder is of 20–28 arches. It contains vessels in the short radial rows which alternate with the broadly elliptical to the variable shaped phloem cell clusters. Vascular tissue is under the sharply differentiated, thick walled and polygonal parenchymatous cells. Sclerenchyma cells are usually present. End walls of vessels are mostly scalariform or simple. Pith is of parenchyma comprising the oval and circular, thin cells with the triangular, rectangular and square intercellular spaces.

Linton and Nobel (1999) examined the xylem cavitation in the roots of the *Opuntia ficus-indica* and *Agave deserti. Opuntia ficus-indica* had a smaller mean diameter of root vessel than the *Agave deserti*. The mean diameter of the vessel on basis of the conductance which was  $71 \pm 2 \mu m$  for the *O. ficus-indica* and  $82 \pm 3 \mu m$  for the *A. deserti. O. ficusindica* had only 10% of vessels which were larger than the 73 µm and they accounted for the 45% of overall conductive capacity. *A. deserti* had 10% of vessels which were larger than the 96 µm and contributed to 21% of overall conductance. Mean diameter of vessels increased by 62% with the increasing diameter of stele for the *A. deserti* and increased 38% for the *O. ficus-indica*.

Adventitious root formation does not require any special treatment to initiate in many plant species, while many other plant species require medium supplied with the different growth regulators, usually of auxin nature (Mitsuhasi-Kato *et al.*, 1978; Haissig *et al.*, 1992; Kevers *et al.*, 1997).

Syros *et al.* (2004) studied the adventitious rooting of the *Ebenus cretica* cuttings. Microscopic observations of the adventitious rooting of the *Ebenus cretica* cuttings showed the anatomical differences between the genotypes. Non-rooting and rooting genotypes display many differences in the anatomy, activity of the soluble peroxidases, lignin content and electrophoretic pattern of the soluble isoforms anionic peroxidase. Adventitious rooting in the cuttings is mainly promoted by the treatment with the auxins particularly with the IBA. Differences between the cuttings of non-rooting and rooting genotype were found mainly in amount and layout of sclerenchymatic fibers, in primary xylem and development of secondary xylem and phloem as well as amount of pith. Transverse sections of root zone revealed that the meristemoids proceeded progressively to individualize and polarization of divisions gave rise typical pointed shape to root primordium. Adventitious roots arose from cambial zone, between secondary phloem and xylem on rooting genotype.

Nwachukwu and Mbagwu (2007) studied the anatomical features of the roots and leaves of the *Abelmoschus esculenta* and *Hibiscus rosa sinensis*, found in the different parts of Nigeria with help of light microscope. Variation in epidermal cells such that small and numerous short chains in the *Hibiscus rosa esculentus*, and big and numerous long chains in the *Abelmoschus*, can be used to differentiate these two taxa. Mesophyll layer is irregular and comprised of the 4–6 layers in the *rosa sinensis*, 3–4 layers are regular in the *Abelmoschus esculenta*. It can further strengthen differences among two taxa. There are parenchyma cells of the root anatomy in the *Hibiscus rosa sinensis* which are of small size and there are bigger size cells in the *Abelmoschus esculenta*. Nwachukwu (2005) had reported that the cells of the parenchyma cells are modified for the secretary and photosynthetic functions and they are metabolically active. Nature wise xylem vessels are numerous, circular in the shape and very big in the *Hibiscus rosa sinensis*, further separated it from few, ovoid in the shape and very small xylem cells in the *Abelmoschus esculenta*. While presence of the metaxylem, protoxylem and angular collenchyma cells in both taxa are typical as of most dicot plants.

Scheres *et al.* (1996) studied the anatomical and genetic analysis of the root development of the lateral and primary roots in the *Arabidopsis*. Primary roots are laid down at basal end of embryo. Secondary roots are post embryonically formed in the different context of the development. For example, lateral roots arise from the pericycle cells that are present within primary roots and that recommence divisions. Nevertheless, cellular organization of the both types of roots is virtually identical. During the embryogenesis, mass of the dividing cells form the root primordium. In the *Arabidopsis*, root primordium of the embryo becomes distinct group of cells at heart stage of the embryogenesis. Scheres *et al.* (1994) reported that the daughters of apical cell formed the part of the primordium at first

zygotic division. Another part is derived from hypophyseal cell that is a daughter of basal cell which is formed in first zygotic division. Formation of the lateral root is initiated from the previously nondividing tissue. Hence, separation of root primordium and onset of the cell division coincide during the lateral root formation.

Bielenberg *et al.* (2001) observed the regulation of the root hair density by the phosphorus availability in the *Arabidopsis thaliana*. Phosphorus availability highly regulated the root hair density and increasing significantly in the roots exposed to the low availability of phosphorus. First week of the radical growth produced the root hair and are of same density regardless of the phosphorus availability, high phosphorus had declined the root hair density and low phosphorus increase the root hair density.

Gales and Toma (2006) examined the histo-anatomical data of some *Euphorbia* species from the Romanian flora. Secondary structure at all the taxons taken into study, resulted from the activity of both secondary meristemes, i.e. the cambium and the phellogen. The suber is thin (2-3 cell layers in *E. taurinensis*) formed by elongated tangentially cells and the external cell layers set exfoliated soon (in *E. platyphyllos*). The phellodermis is thick (5-6 cell layers in *E. taurinensis*) and is represented by a tangentially collenchyma in *E. helioscopia*. From the cambium's activity, a thin secondary phloem ring and a very thick central xylem body, riched in libriformous fibers with weakly lignified and partial gelified walls, result. The xylem body is sweeped by numerous parenchymatous-cellulosic (in *E. helioscopia*) or lignified (in *E. platyphyllos*) medulary rays. The primary structure of the central cylinder is of the triarch type, verified by the 3 principal parenchymatous-cellulosic medullary rays, which sweep the secondary xylem body up to the organ axis (in *E. helioscopia* and *E. platyphyllos*), where there are present 3 groups of xylem elements with thick, but cellulosic walls (in *E. taurinensis*).

Nwachukwu *et al.* (2008) studied the anatomical features of the roots and leaves of *Hibiscus Rosa-sinensis* and *Abelmoschus esculenta*. The root epidermal layer of the two taxa studied shows that the epidermal cells are in form of short chains (kioned) small and numerous in *Hibiscus rosa sinensis* while they are of long chains big and numerous in *Abelmoschus esculenta*. Similarly the cortex tissue show the presence of small sized parenchyma cells in *Hibiscus Rosa sinensis* while in *Abelmoschus esculenta* the parenchyma cells are bigger in size. Both taxa show presence of angular collenchyma. The xylem vessels

are numerous circular in shape and are radially grouped in *Hibiscus Rosa sinensis* while they are few and cuboidal in shape in *Abelmoschus esculenta*. The root anatomy of both taxa studied shows presence of calcium oxalate crystal in the cortex region of the two taxa though the crystal are not stained in *Hibiscus Rosa sinensis* while they are dark stained in *Abelmoschus esculenta*.

Ciamporova *et al.* (2009) compared the root anatomy and growth of three (*A. thaliana*, *A. arenosa* and *A. halleri*), *Arabidopsis* species differing in their heavy metal tolerance. Anatomy of all three species showed a similar tissue pattern and trichoblast location. Cell numbers in both *A. thaliana* genotypes were 17 to 18 in epidermis, 8 in outer cortex, and 8 to 9 in endodermis. Roots of *A. arenosa* had higher number only in epidermis (22 to 26) and roots of *A. halleri* also in endodermis (10 to 12). Some endodermal cells in *A. arenosa* and *A. halleri* underwent tangential divisions. A greater diameter of both central cylinder and whole root were found in the tolerant species, *A. arenosa* and *A. halleri* from each locality.

Naruhashi and Ishizu (1992) conducted comparative anatomical studies among D. indica, Ducbesnea cbrysantha and in their hybrids. Roots of the D. indica present a unistratified epidermis, isodiametric cells formed the cortical parenchyma, endodermis shows the casparian strips and pericycle is unistratified. Cortex remains bounded tightly to juvenile peridermis. In the *F. vesca* exodermis and epidermis are unistratified. Isodiametric cells form the cortical parenchyma. Endodermis has thick walls and 1-2 layered pericycle which may become lignified. Endodermis has an amyliferous sheath present outside.

Udovenko *et al.* (1976) studied the structural and anatomical changes in barley, horse bean and wheat plants under salinity conditions. Salinity clearly reduced diameter of the xylem and increase the wall thickness of the bundle sheath cells. Nutrient transport rate is increased by this.

Konarska (2007) investigated the anatomical structure of the *Sorbus aucuparia* with the help of SEM. Changes in development of cuticular epithelium nectary epidermis and difference in degree of the aperture of the stomata. Increase undulation of the surface of gland was founded during flower development. Stomata were found below the epidermal cells of nectary.

Mbagwu *et al.* (2007) examined the anatomical characteristics of roots of *Solanum nigrum* and *Solanum macrocarpum* to a certain level to the relevance of many characteristics

in the establishment of the interspecific differences and similarities found in two taxa. Roots of the *Solanum nigrum* present a unistratified epidermis, isodiametric cells formed the cortical parenchyma, endodermis shows the Casparian strips and pericycle is the unistratified. Cortex remains bounded tightly to juvenile peridermis. In the *Solanum macrocarpum* exodermis and epidermis are unistratified. Isodiametric cells form the cortical parenchyma.

Silva *et al.* (1999) studied the six cultivars of common bean (*Phaseolus vulgaris*) in the water deficit condition. There was increase in leaf hair per unit area of 35% under the water stress. Cell volume was decreased to about 26% and stomata density increased per  $mm^2$  of about 25-150 on adaxial surface and 167-216 on abaxial surfaces.

## Chapter 3

## **Materials and Methods**

In Date Palm Research Station, Jhang, thirty four cultivars of date palm (*Phoenix dactylifera* L.) have been planted since 1968. The material was collected from all over the country, as well as some exotic cultivars native to Iran, Iraq and Egypt have also been planted (Table 1).

 Table 3.1. List of date palm (Phoenix dactylifera L.) cultivars at Date Palm

 Research Station, Jhang.

Sr. No.	Cultivar	Sr. No.	Cultivar
1	Akhrot	1	Koharba
		8	
2	Angoor	1	Kokna
		9	
3	Aseel	2	Kozanabad
		0	
4	Begum Jhangi	2	Makraan
		1	
5	Berehmi	2	Neelam
		2	
6	Champa Kali	2	Peela Dora
		3	
7	Chohara	2	Peeli Sundar
		4	
8	Daanda	2	Qantar
		5	
9	Dakki	2	Rachna
		6	
1	Deglut Noor	2	Saib
0		7	

1	Halawi-1	2	Shado
1		8	
1	Halawi-2	2	Shamran
2		9	
1	Jaman	3	Shamran-2
3		0	
1	Jansohaar	3	Wahan Wali
4		1	
1	Karbalaen	3	Zaidi
5		2	
1	Khudrawi-1	3	Zardu
6		3	
1	Khudrawi-2	3	Zeerin
7		4	

The research station was surveyed during April 2010 for the collection of material. Adventitious roots were collected from base of the each tree and immediately placed in polythene sample bags. The materials were then brought to the laboratory at the University of Agriculture, Faisalabad and about 2 cm long piece from each root were selected for root anatomical studies.

#### **Preservation of root material**

The material was fixed in FAA (formalin acetic alcohol) solution, which contained v/v 5% formalin, 10% acetic acid, 50% ethanol, and 35% distilled water. For long-term preservation material was subsequently transferred to acetic alcohol solution (v/v acetic acid 25%, ethanol 75%).

### Sectioning of the samples

Free hand sectioning technique was used for the preparation of permanent slides of root transverse sections. Potato tubers were used as a support for root sectioning. A number of sections were cut with the help of razor blade, and some fine sections were carefully picked with the help of needle and put in the wash glass for staining.

### Staining of the sections

The sections were passed through a series of ethanol grades for dehydration. The sections were first placed in 30% alcohol solution in a wash glass for 15 minutes. The material was then transferred to 50% alcohol solution for 15 minutes and then in 70% alcohol solution for 15 minutes. For staining the lignified tissues like xylem vessels and sclerenchyma, the material was transferred to safranin (1 g dissolved in 100 ml of 70% alcohol) for 20 minutes. The sections were dehydrated in 90% alcohol solution for 5 minutes, and thereafter stained with fast green (1 g dissolved in 90% ethanol) for one minute. Fast green was used for the staining of subrinized tissues and parenchymatous tissues. Finally, the materials were three times washed with absolute alcohol and then transferred to xylene for clearing the contrast.

### Mounting of sections

Sections were mounted in Canada balsam by putting a drop of resin on a slide and placing the sections on the slide with the help of forceps and needle, and finally placing the cover slip on the sections. The sections were photographed with the help of compound microscope and digital camera

#### **Measurements of anatomical parameters**

Measurements of anatomical parameters were taken with the help of ocular micromerter under a compound microscope, which was calibrated with the help of stage micrometer (Fig.1). Following anatomical characteristics were studied during the investigation:

### Root cross sectional area (mm<sup>2</sup>)

The maximum length and width of the root sections were measured and area was calculated.

#### Exodermis thickness (µm) and its cell area (µm<sup>2</sup>)

Thickness of exodermis was measured randomly from three different sites. For cell area three cells were selected randomly and length and width were measured for the calculation of cell area.

#### Cortical cell area (µm<sup>2</sup>)

Length and width of three randomly selected cortical cells were measured and area was calculated.

### Sclerenchyma thickness (µm) and its cell area (µm<sup>2</sup>)

Sclerenchyma thickness was measured from the outer cortical region at three randomly selected sites, and length and width of three randomly selected cells were measured for the calculation of cell area.

### Vascular region area (µm<sup>2</sup>)

Total length and width of vascular region were measured and area was calculated. The maximum length and width of pith region were measured and area was calculated. It was subtracted from the area of total vascular region to get actual area of vascular region.

### Metaxylem area (µm<sup>2</sup>)

Length and width of three randomly selected metaxylem vessels were measured and area was calculated.

### Phloem area (µm<sup>2</sup>)

Length and width of three randomly selected phloem regions were measured and area was calculated.






### Any special feature

Nature and size of some special features like sclerenrechyma bundles in cortex, presence of crystals, etc were recorded.

Area of different cells and tissues were calculated by using the following formula (which was modified from the area of a circle,  $\pi r^2$ ):

Maximum length x Maximum width

```
Area = ----- x 22
28
```

The data were subjected to multivariate (cluster) analysis using Minitab statistical software to compare similarities in root anatomical characteristics of different cultivar.

## **Chapter 4**

# **Results**

Analysis of variance (ANOVA) of different date palm (*Phoenix dactylifera* L.) cultivars from Date Palm Research Station Jhang is presented in table 4.1.

#### 4.1: Epidermis thickness

Epidermis thickness varied significantly at p>0.001 in all the cultivars of date palm (*Phoenix dactylifera* L.) studied at date palm research station Jhang (Table 4.1, Fig. 2). Two cultivars (Kozanabad and Halawi-1) surpassed all the other cultivars in relation to this parameter, where the maximum epidermis thickness was recorded in Kozanabad measuring (46.297  $\mu$ m). This was followed by four cultivars Berehmi, Dakki, Koharba and Akhrot. The

minimum of epidermis thickness was recorded in three cultivars namely Champa Kali, Chohara and Karbalaen.

#### 4.2: Epidermis cell area

Variation regarding epidermis cell area in the date palm (*Phoenix dactylifera* L.) cultivars was significantly high at p>0.01 (Table 4.1, Fig. 3). Cultivar Shamran showed the maximum cell area (1241.212  $\mu$ m<sup>2</sup>), whereas cultivars Zaidi and Deglut Noor were the second and third best in relation to epidermal cell area. Four cultivars Zardu, Kozanabad, Makran and Begum Jhangi also showed large epidermis cells than that recorded in other cultivars. Two cultivars (Rachna and Champa Kali) showed greatly reduced epidermal cells. Where as Jaman, Kokna, Shado, Halawi-1, Wahan Wali, Karbalaen and Khudrawi-1 also showed small epidermal cell area.

#### 4.3: Sclerenchyma cell thickness

Date palm (*Phoenix dactylifera* L.) was significantly varied at p>0.001 in Sclerenchyma cell thickness in all 34 cultivars (Table 4.1, Fig. 4). Shado showed high Sclerenchyma cell thickness (209.69  $\mu$ m). Three cultivars Aseel, Khudrawi-1 and Shamran-2 also showed maximum Sclerenchyma cell thickness. Two cultivars Zaidi and Deglut Noor showed minimum Sclerenchyma cell thickness. Minimum of Sclerenchyma cell thickness



Fig.2 Root epidermis thickness of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig.3 Root epidermis cell area of *Phoenix dactylifera* L. cultivars *from* Date Palm Research Station Jhang.



Fig. 4 Root sclerenchyma thickness of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.

was recorded in seven other cultivars Berehmi, Peeli Sundar, Zardu, Daanda, Dakki, Qantar and Begum Jhangi.

## 4.4: Sclerenchyma area

Sclerenchyma cell area varied significantly at p>0.001 in all the cultivars of date palm (*Phoenix dactylifera* L.) (Table 4.1, Fig. 5). Zaidi showed maximum sclerenchyma cell area (785.0133  $\mu$ m<sup>2</sup>). This was followed by five cultivars Peeli Sundar, Saib, Shamran, Champa

kali and Wahan Wali. The minimum of sclerenchyma cell area was recorded in two cultivars namely Kokna and Halawi-1. This was followed by nine cultivars Zardu, Kohzanabad, Jaman, Qantar, Kokna, Shado, Halawi-2, Koharba and Zeerin with thin sclerenchyma cell area.

## 4.5: Sclerenchyma patches area

Sclerenchyma patches were significant at p>0.001 in all cultivars of date palm (*Phoenix dactylifera* L.) except Daanda and Jansohar (Table 4.1, Fig. 6). Area of sclerenchyma patches varied greatly in all cultivars. Maximum of sclerenchyma patches area were observed in two cultivars Zardu and Dakki (18950.33  $\mu$ m<sup>2</sup> and 5524.266  $\mu$ m<sup>2</sup>). It was also maximum in three other cultivars Zaidi, Halawi-1 and Beghum Jhangi. Many cultivars possessed minimum sclerenchyma patches area (Peeli Sundar, Shamra, Rachna, Khudrawi-2 and Shamran-2).

#### 4.6: Cortical cell area

Variation regarding cortical cell area in the date palm (*Phoenix dactylifera* L.) cultivars was significant at p>0.001 (table 2, Fig. 7). Chohara, Shado and Karbalaen showed respectively the maximum cell area (2534.969  $\mu$ m<sup>2</sup>, 2342.892  $\mu$ m<sup>2</sup> and 2220.196  $\mu$ m<sup>2</sup>). Three other cultivars Kozanabad, Jaman and Dakki also showed maximum cell area. One cultivar Saib showed the minimum cortical cell area. Nine other cultivars Danda, Deglut Noor, Jansohar, Qantar, Aseel, Angoor, Halawi-2, Koharba and Wahan Wali also showed thin cell area. More than 70% cultivars showed the minimum cortical cell area.

#### 4.7: Cortical region thickness

Variation among the different cultivars according to the cortical region thickness was significant at p>0.001 (table 2) in all the studied cultivars of date palm (*Phoenix dactylifera* L.). More than half of cultivars showed the cortical region thickness greater than 500  $\mu$ m (Fig. 8). Makran had the maximum cortical region thickness (798.7  $\mu$ m). Other cultivars with



Fig. 5 Root sclerenchyma area of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig. 6 Root sclerenchyma patches area of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig. 7 Root cortical cell area of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig. 8 Root cortical region thickness of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.

maximum cortical region thickness were Zaidi, Deglut Noor, Shamran, Aseel and Khudrawi-1 and Jaman showed the minimum cortical region thickness. It was followed by the Dakki, Daanda, Saib and Karbalaen.

#### 4.8: Endodermis thickness

Endodermis thickness varied significantly at p>0.001 (Table 4.1) in all the studied cultivars of date palm (*Phoenix dactylifera* L.) (Fig.9). One cultivar Saib showed the maximum endodermis thickness (49.02  $\mu$ m). Endodermis thickness was also high in some other cultivars like Zardu, Daanda, Khudrawi-1, Makran and Angoor. Three cultivars Zardu, Daanda and Kozanabad showed the same endodermis thickness (40.85  $\mu$ m). Three cultivars Zaidi, Deglut Noor and Begum Jhangi showed the minimum endodermis thickness.

#### 4.9: Endodermis area

There was significant variation at p>0.001 (Table 4.1) in endodermis area in all the cultivars of (*Phoenix dactylifera* L.) (Fig.10). Four cultivars Kozanabad, Wahan Wali, Koharba and Karbalaen showed respectively the maximum endodermis area (476.119  $\mu$ m<sup>2</sup>, 472.01  $\mu$ m<sup>2</sup> 594.3831  $\mu$ m<sup>2</sup> and 467.119  $\mu$ m<sup>2</sup>). Three cultivars Zardu, Berehmi and Chohara also showed the maximum endodermis area. Two cultivars Champa kali and Akhrot showed the minimum endodermis area. It was followed by the other cultivars like Zaidi, Deglut Noor, Dakki, Qantar, Aseel, Angoor, Pela Dora, Kokna and Shado.

### 4.10: Vascular region thickness

Variation regarding the vascular region thickness in the date palm (*Phoenix dactylifera* L.) cultivars was significant at p>0.001 (Table 4.1, Fig. 11). Two Cultivars Chohara and Zardu showed the maximum vascular region thickness (471.96 µm and 460.24 µm), whereas cultivars Shamran and Khudrawi-2 were the second and third best in relation to the vascular region thickness. Four cultivars Begum Jhangi, Halawi-1, Champa Kali and Berehmi also showed large than that the vascular region thickness recorded in other cultivars. Three cultivars (Daanda, Zeerin and Neelam) showed greatly reduced vascular region thickness. Whereas Peeli Sundar, Kozanabad and Peela Dora also showed small vascular region thickness.



Fig. 9 Root edodermis thickness of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig. 10 Root Edodermis area of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig. 11 Root vascular region thickness of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.

## 4.11: Metaxylem Vessel area

Metaxylem Vessel area varied significantly at p>0.001 (Table4.1) in all the cultivars of date palm (*Phoenix dactylifera* L.) studied (Fig. 12). Two cultivars (Shado and Beghum

Jhanghi) surpassed all the other cultivars in relation to this parameter, where the maximum epidermis thickness was recorded in Begum Jhangi measuring (18897.5059  $\mu$ m<sup>2</sup>). This was followed by five cultivars, Zardu, Daanda, Makran, Halawi-1 and Peela Dora. The minimum of metaxylem vessel area was recorded in nine cultivars namely Peeli Sundar, Kozanabad, Jaman, Rachna, Wahan Wali, Koharba, Champa Kali, Halawi-2 and Karbalaen.

#### 4.12: Phloem area

Date palm (*Phoenix dactylifera* L.) had significant variation at p>0.001 (Table4.1) in phloem area in all 34 cultivars (Fig.13). Dakki showed high phloem area (4867.024  $\mu$ m<sup>2</sup>). Four cultivars Kokna, Khudrawi-2, Halawi-1 and Saib also showed maximum phloem area. Two cultivars Champa kali and Kozanabad showed minimum phloem area. Minimum phloem area was also recorded in three other cultivars Rachna, Chohara and Shado.

#### 4.13: Pith area

There was significant variation at p>0.001 (Table 4.1) in pith area in all the cultivars of (*Phoenix dactylifera* L.) (Fig.14). One cultivar Beghum Jhangi showed maximum endodermis area (42.31  $\mu$ m<sup>2</sup>). Six cultivars Saib, Aseel, Angoor, Shado, Akhrot and Khudrawi-2 also showed the maximum pith area. One cultivar Karbalaen showed the minimum pith area. It was followed by the other cultivars like Peeli Sundar, Zardu, Daanda, Zaidi, Deglut Noor, Kozanabad and Jansohar.

#### 4.14: Multivariate analysis

Dendrogram of multivariate analysis showed relationship of different date palm cultivars on the basis of root anatomical characteristics (Fig.15). Several clusters were identified in the dendrogram. Duglet Noor (Plate 1) and Begum Jhangi (Plate 2) were closely associated, but very different from all other cultivars. Two cultivars, Daanda (Plate 3) and Jansohar (Plate 4) were clustered in close groups. Similarly, two cultivars, Zardu (Plate 5) and Halawi-1 (Plate 6) and four cultivars, Zaidi (Plate 7), Shamran (Plate 8), Jaman (Plate 9) and Rachna (Plate 10) showed close association. Three cultivars, Makran (Plate 11), Neelam (Plate 12), and Peela Dora (Plate 34) were isolated clustered, whereas all the other cultivars



Fig. 12 Root metaxylem vessel area of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig.13 Root phloem area of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.



Fig. 14 Root pith area of *Phoenix dactylifera* L. cultivars from Date Palm Research Station Jhang.

were clustered closely. However, in this large cluster, Dakki (Plate 13), Zeerin (Plate 14), and Shamran-2 (Plate 15), Kozanabad (Plate 18) and Karbalaen (Plate 19), Chohara (Plate 21), Champa kali (Plate 22), Aseel (Plate 23) and Khudrawi-1 (Plate 24), Halawi-2 (Plate

25), Koharba (Plate 26) and Wahan Wali (Plate 27) showed very high similarity index, i.e., well over 90%. Saib (Plate 16), Peeli Sundar (Plate 17), Angoor (Plate 28), Shado (Plate 29), Kokna (Plate 30), Akhrot (Plate 31), Khudrawi-2 (Plate 32) and Berehmi (Plate 33) were different from all other cultivars and showed individual clusters.

Chapter 5

Discussion

Root anatomy of Date Palm (*Phoenix dactylifera* L.) planted at Date Palm research station Jhang showed significant variations. The size of epidermis cells, size and shape of outer cortical region, presence of sclerification in outer cortex, sclerenchyma bundles in cortical region and presence of aerenchyma were quite significantly variable in these cultivars. Similarly endodermal layer thickness, thickness of outer tangential wall of endodermis, shape and size of phloem region, size and arrangement of metaxylem vessels and sclerification in the pith region showed significant diversity.

Cultivar Akhrot showed two distinct portions of cortical region which were separated by the well developed sclerenchyma. The outer cortex had elongated, smaller cells and inner pith larger, hexagonal cells. Epidermis composed of thick walled cells. Distinct sclerenchyma bundles were present in inner cortex. Thick epidermis with intensive sclerification in the cortical region not only prevents the water loss from the roots as Mathew and Nobel (1999) examined the loss of axial hydraulic conductance as result of xylem cavitation for the root of CAM succulents, but also provides mechanical strength to the root and this is extremely important under harsh ecological conditions such as drought as Lowell (1940) reported water relation of grasses to drought resistance. Additionally the increased cortical region with densely packed cells, capable of storing additional water and this is vital for surviving under limited moisture environment. This cultivar also showed thick walled endodermis and this is important for checking radial flow of water in the roots as Michael and Ehwal (2010) examined the radial flow of water in maize roots. Metaxylem vessels were smaller in this cultivar and smaller vessels are generally more efficient as Minoru et al. (2005) examined the protoxylem and metaxylem vessel formation in transporting the water and nutrients along with more resistance to imbibition. Central portion of root was composed of thick walled sclerenchyma and this may enhance the mechanical strength of the root as Wright and Illius (1995) studied the mechanical properties of five grasses.

Cultivar Dakki had distinct sclerenchyma region inside the outer cortex with small sclerenchyma bundles in the inner cortex. However large aerenchymatous spaces were recorded in cortical region. Aerenchyma is critical not only under water logged conditions as Philipson and Coutts (1978) examined the tolerance of tree roots to waterlogging, oxygen transport in lodgepole pine and sitka spruce roots of primary structure, but also under drought and salinity. Nadia *et al.* (2010) reported the variation for salinity tolerance in sweet potato

regenerated plants. This cultivar showed large and few metaxylem vessels and intensive sclerification in pith region. This root structure indicated the tolerance of Dakki cultivar to variety of environmental stresses mainly drought, salinity and water logging. This justifies its wide cultivation in the southern KPK province.

Epidermis in cultivar Aseel was comprised of extremely large cells and very well developed sclerenchyma in outer cortical region. Outer cortex was composed of very much reduced parenchymatous cells, whereas in larger rounded and densely packed cells. Distinctive sclerenchyma bundles were recorded in inner cortex. However well developed aerenchyma were present just outside the endodermis. Intensive sclerification was also recorded in the vascular region. The variation in parenchymatous cells that is a small tightly packed cells in the outer cortex. Larger cells in the inner cortex with distinctive sclerenchyma region, sclerenchyma bundles and aerenchyma may indicate the high tolerance level of this cultivar to variety of environmental condition as Vasellati et al. (2001) studied the effects of flooding and drought on the anatomy of Paspalum dilatatum. Flooding increased the aerenchymatous tissue in the root cortex and the leaf sheaths and decreased the number of root hairs per unit of root length. This may not only resist the cellular collapse under limited moisture environment. Tomar et al. (2006) reported that drought decreased the diameter of root metaxylem vessels, thus lowering the risk of embolisms and increasing water-flow resistance, and increased the number of root hairs, thereby increasing water uptake ability in Paspalum dilatatum, but intensive sclerification may also be involved in preventing water loss through the root in addition efficient translocation of oxygen and solute via aerenchyma as Boris and Michael (1997) examined the plant adaptation to anaerobic stress and repoted that mechanisms of tolerance of plants can include metabolic adaptations and developmentally passive tolerance such as that seen in overwintering rhizomes of many wetland species.

Cultivar Halawi-1 showed poorly developed sclerenchyma in outer cortex and irregularly shaped cells in the inner cortex and distinct aerenchyma outside the endodermis. Moreover distinct phloem inside the endodermis and very few large metaxylem vessels, but intensive sclerification in the vascular region. Large phloem area may be responsible for increased translocation of photosynthate but few and large metaxylem vessels may resist efficient transport of water and solute. Ernst and Peterson 1(998) studied how does water get

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through roots and reported that during periods of varying water supply roots are optimized in their abilities to use water resources in the soil. Overall the root structure in Halawi-1 indicated relative sensitivity of cultivar to environmental stresses. This may be the reason of its limited cultivation in Jhang and Faisalabad region.

Cultivar Qantar showed very prominent modification in root anatomy with extremely large aerenchyma in inner cortex and intensive sclerification in outer cortex and vascular region. High proportion of aerenchyma is vital for a variety of environmental stresses. Gibbs *et al.* (1998) studied the response of oxygen deficiency in primary maize roots. Aerenchyma is known to be a feature of equatic plants, but more recently aerenchyma can be related to high stress tolerance against salinity and drought (Rolando *et al.*, 1992).

Structural modifications in the roots of cultivar Makran were large cells in the outer cortex, well developed sclerification, separating outer and inner cortical region and well developed aerenchyma above the endodermis. Moreover endodermis was extremely thick walled whereas large phloem cells alternating the metaxylem vessels in the vascular region present. Thorsten and Fricke (2010) studied the Root pressure and a solute reflection coefficient close to unity exclude a purely apoplastic pathway of radial water transport in barley (*Hordeum vulgare*). Intensive sclerification was also recorded in pith region. The sclerification in roots can minimize the water loss through root in addition to controlling the radial flow of water and nutrients. Michael and Ehwal (2010) examined the radial flow of water in maize roots. Furthermore larger phloem cells and metaxylem vessels are capable of increasing transport of water, nutrients and reserve food more efficiently.

Distinctive anatomical features in Chohara cultivar were large root area, large vascular region with intensive sclerification in pith region. Well developed sclerenchyma in the outer cortex, large sclerenchyma bundles scattered through out the inner cortical region and well developed aerenchyma in the inner cortex. Thick roots are known to be characteristic feature of drought and salt tolerant plant (Guo and Steudle. 1991) and this may justify the wide cultivation of this cultivar in Punjab, Sindh and KPK as it can tolerate a variety of environmental stresses.

Cultivar Zaidi showed extremely large epidermal cells with enormous sclerification in hypodermal region in addition to large closely packed cells. Structural modifications in cultivar Zaidi can be related to high tolerance against abiotic stress modifications like intensive sclerification as Richard *et al.* (2011) investigated the Identification of tissuespecific, abiotic stress-responsive gene expression patterns in wine grape (*Vitis vinifera* L.) based on curation and mining of large-scale EST data sets. All these parameters can play a critical role in moisture conservation and this is extremely useful under drought conditions. Similar structural modifications were also recorded in cultivar Neelam. However in the latter case vascular region is extremely enlarged. This may be again crucial for water conservation (Beebe *et al.*, 2008).

Cultivar Berehmi showed typical characteristics in the root anatomy with well developed sclerification in cortical region, large proportion of aerenchyma, highly enlarged phloem and large metaxylem vessels. Moreover intensive sclerification was also recorded in the endodermal cell walls. Such modifications can help in transport of solute and reserve food (Beebe *et al.*, 2006) and helpful in controlling radial flow of water as Beebe *et al.* (2000) Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments.

Anatomical features in Daanda, Angoor, Zeerin, Kozanabad, Shamran-2, Karbalaen, Peela Dora, Shamran, Peeli Sundar and Halawi-2 were very similar. All these cultivars showed large epidermal cells and distinct sclerenchyma in the cortical region, large proportion of aerenchyma in inner cortex, thick endodermis and intensive sclerification in vascular region. Epidermis along with intensive sclerification in cortex as well as vascular region is characteristics of drought tolerant plants. Blum (2005) examined the drought resistance, water use efficiency, and yield potential. Therefore all these cultivars can be rated as suitable for arid and semi arid regions (Rao, 2002).

Highly enlarged vascular region with large metaxylem vessel and large aerenchyma were recorded in Jaman, Jansohar, Beghum Jhangi, Shado, Khudrawi-1, Khudrawi-2 and Champa Kali. These cultivars also showed distinctive sclerenchyma in cortical region, prominent sclerenchyma bundles and high proportion of aerenchyma in the cortex. However large metaxylem vessels are more prom to collapse, but at the same time they can involve more and efficient transport of water and nutrients. On these bases it can be concluded that these cultivar can perform better under moderate climate. Rao *et al.* (2007) studied phenotypic evaluation of drought resistance in advanced lines of common bean (*Phaseolus vulgaris* L.).

Distinct modification in the root anatomy of cultivar Koharba and Deglut Noor was observed. Large ovoid metaxylem vessels were the characteristic feature in the transverse section. In addition very prominent sclerification was recorded in epidermal and hypodermal region. Both cultivars were characterized by small aerenchyma and highly sclerified pith region. These anatomical features are typical of xeric nature with the main function of efficient transport of water and prevention of water loss through the roots (Ryan, 1993).

Cultivar Kokna and Saib were characterized by relatively poor development of sclerenchyma both in cortical, vascular region and small, numerous metaxylem vessels on the basis of low proportion of sclerification. These cultivars can tolerate mild environmental stresses. On the whole all the cultivars had very specific anatomical features which indicate their adoption to a variety of environmental conditions and also play vital role in taxonomic identification of cultivars. Taxonomically important features were the nature of sclerification in the cortical region, distinct outer and inner cortex, shape, number and size of the metaxylem vessel and size and shape of phloem. On these basis it can concluded that all these cultivars may have evolved independently from diverse origin during their evolutionary history (Singh, 1995).



Plate: 1 Root transverse section of *Phoenix dactylifera* L. cultivar 'Deglut Noor' from Date Palm Research Station Jhang.



Plate: 2 Root transverse section of *Phoenix dactylifera* L. cultivar 'Beghum Jhangi' from Date Palm Research Station Jhang.



Plate: 3 Root transverse section of *Phoenix dactylifera* L. cultivar 'Daanda' from Date Palm Research Station Jhang.



Plate: 4 Root transverse section of *Phoenix dactylifera* L. cultivar 'Jansohar' from Date Palm Research Station Jhang.



Plate: 5 Root transverse section of *Phoenix dactylifera* L. cultivar 'Zardu' from Date Palm Research Station Jhang.



Plate: 6 Root transverse section of *Phoenix dactylifera* L. cultivar ' Halawi-1' from Date Palm Research Station Jhang.



Plate: 7 Root transverse section of *Phoenix dactylifera* L. cultivar 'Zaidi' from Date Palm Research Station Jhang.



Plate: 8 Root transverse section of *Phoenix dactylifera* L. cultivar 'Shamran' from Date Palm Research Station Jhang.



Plate: 9 Root transverse section of *Phoenix dactylifera* L. cultivar 'Jaman' from Date Palm Research Station Jhang.


Plate: 10 Root transverse section of *Phoenix dactylifera* L. cultivar 'Rachna' from Date Palm Research Station Jhang.



Plate: 11 Root transverse section of *Phoenix dactylifera* L. cultivar 'Makran' from Date Palm Research Station Jhang.



Plate: 12 Root transverse section of *Phoenix dactylifera* L. cultivar 'Neelam' from Date Palm Research Station Jhang.



Plate: 13 Root transverse section of *Phoenix dactylifera* L. cultivar 'Dakki' from Date Palm Research Station Jhang.



Plate: 14 Root transverse section of *Phoenix dactylifera* L. cultivar 'Zeerin' from Date Palm Research Station Jhang.



Plate: 15 Root transverse section of *Phoenix dactylifera* L. cultivar ' Shamran-2' from Date Palm Research Station Jhang.



Plate: 16 Root transverse section of *Phoenix dactylifera* L. cultivars 'Saib' from Date Palm Research Station Jhang.







Plate: 18 Root transverse section of *Phoenix dactylifera* L. cultivar 'Kozanabad' from Date Palm Research Station Jhang.



Plate: 19 Root transverse section of *Phoenix dactylifera* L. cultivar 'Karbalaen' from Date Palm Research Station Jhang.







Plate: 21 Root transverse section of *Phoenix dactylifera* L. cultivar 'Chohara' from Date Palm Research Station Jhang.



Plate: 22 Root transverse section of *Phoenix dactylifera* L. cultivar 'Champa Kali' from Date Palm Research Station Jhang.



Plate: 23 Root transverse section of *Phoenix dactylifera* L. cultivar 'Aseel' from Date Palm Research Station Jhang.



Plate: 24 Root transverse section of *Phoenix dactylifera* L. cultivar 'Khudrawi-1' from Date Palm Research Station Jhang.



Plate: 25 Root transverse section of *Phoenix dactylifera* L. cultivar 'Khudrawi-1' from Date Palm Research Station Jhang.



Plate: 26 Root transverse section of *Phoenix dactylifera* L. cultivar 'Koharba' from Date Palm Research Station Jhang.



Plate: 27 Root transverse section of *Phoenix dactylifera* L. cultivar 'Wahan Wali' from Date Palm Research Station Jhang.



Plate: 28 Root transverse section of *Phoenix dactylifera* L. cultivar 'Angoor' from Date Palm Research Station Jhang.



Plate: 29 Root transverse section of *Phoenix dactylifera* L. cultivar 'Shado' from Date Palm Research Station Jhang.



Plate: 30 Root transverse section of *Phoenix dactylifera* L. cultivar 'Kokna' from Date Palm Research Station Jhang.



Plate: 31 Root transverse section of *Phoenix dactylifera* L. cultivar 'Akhrot' from Date Palm Research Station Jhang.



Plate: 32 Root transverse section of *Phoenix dactylifera* L. cultivar 'Khudrawi-2' from Date Palm Research Station Jhang.



Plate: 33 Root transverse section of *Phoenix dactylifera* L. cultivar 'Berehmi' from Date Palm Research Station Jhang.



Plate: 34 Root transverse section of *Phoenix dactylifera* L. cultivars 'Peela Doora' from Date Palm Research Station Jhang.

## Chapter 6

## Summary

Date palm (*phoenix dactylifera* L.) is distributed widely in the different areas of world. There are three main types of date palm fruits according to the moisture content i.e., dry, semi-dry and soft. Phoenix is most important genus of the palmaceae family. Date fruit is marketed all over the world as high-value confectionery and fruit and remains important subsistence crop in deserts.

Material was collected from Date Palm Research Station Jhang. The material was fixed in FAA (formalin acetic alcohol) solution. Free hand sectioning technique was used for the preparation of permanent slides of root transverse sections. The sections were passed through a series of ethanol grades for dehydration. Sections were mounted in Canada balsam by putting a drop of resin on a slide and placing the sections on the slide with the help of forceps and needle, and finally placing the cover slip on the sections. Measurements of anatomical parameters were taken with the help of ocular micrometer under a compound microscope, which was calibrated with the help of stage micrometer. Many anatomical characteristics were studied during the investigation, which include root cross sectional area ( $\mu$ m<sup>2</sup>), exodermis thickness ( $\mu$ m) and its cell area ( $\mu$ m<sup>2</sup>), cortical cell area ( $\mu$ m<sup>2</sup>), metaxylem area ( $\mu$ m<sup>2</sup>) and phloem area ( $\mu$ m<sup>2</sup>).

Epidermis thickness varied significantly at p>0.001 in all the cultivars of date palm (*Phoenix dactylifera* L.) studied at date palm research station Jhang (Table 4.1, Fig. 2). In all cultivars endodermal layer thickness, thickness of outer tangential wall of endodermis, shape and size of phloem region, size and arrangement of metaxylem vessels and sclerification in the pith region showed great diversity. Cultivar Akhrot showed two distinct portions of cortical region which were separated by the well developed sclerenchyma. The outer cortex had elongated, smaller cells and inner pith larger, hexagonal cells. Cultivar Dakki had distinct sclerenchyma region inside the outer cortex with small sclerenchyma bundles in the inner cortex. However large aerenchymatous spaces were recorded in cortical region. Aerenchyma is critical not only under water logged conditions.

Cultivar Qantar showed very prominent modification in root anatomy with extremely large aerenchyma in inner cortex and intensive sclerification in outer cortex and vascular region. High proportion of aerenchyma is vital for a variety of environmental stresses. Cultivar Halawi-1 showed poorly developed sclerenchyma in outer cortex and irregularly shaped cells in the inner cortex and distinct aerenchyma outside the endodermis. Anatomical features in Chohara cultivar were large root area, large vascular region with intensive sclerification in pith region. Well developed sclerenchyma in the outer cortex, large sclerenchyma bundles scattered through out the inner cortical region and well developed aerenchyma in the inner cortex.

Cultivar Berehmi showed typical characteristics in the root anatomy with well developed sclerification in cortical region, large proportion of aerenchyma, highly enlarged phloem and large metaxylem vessels. All these parameters can play a critical role in moisture conservation and this is extremely useful under drought conditions. Modification in the root anatomy of cultivar Koharba and Deglut Noor was observed. These anatomical features are typical of xeric nature with the main function of efficient transport of water and prevention of water loss through the roots.

These cultivars can tolerate mild environmental stresses. On the whole all the cultivars had very specific anatomical features which indicate their adoption to a variety of environmental conditions and also play vital role in taxonomic identification of cultivars. Taxonomically important features were the nature of sclerification in the cortical region, distinct outer and inner cortex, shape, number and size of the metaxylem vessel and size and shape of phloem. On these basis it can concluded that all these cultivars may have evolved independently from diverse origin during their evolutionary history.

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