Morphology and Anatomy of Dwarf Shoots of Some Exotic Species of Pinus Linn. in Kumaon Hills, Western Himalayas

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Abstract

A study was conducted on the Morphology and Anatomy of dwarf shoots of some exotic species of Pinus Linn. In Kumaon Hills, Western Himalayas” and hence morphological and anatomical details of the Pinus growing in above mentioned area. These 5 species of Pinus were collected from different localities of Kumaon Himalayas including Nainital, Ranikhet, Almora, Kaushani and neighbouring areas in Western Himalayas, India. The five species of Pinus considered for the following study included P.canariensis C.Smith (three needles per fascicle), P. densiflora Siebold et Zuccarini (two needles per fascicle), P. engelmannii Carriere (three needles per fascicle), P. echinata Miller (three needles per fascicle) and P. gregii Engelmann (two needles per fascicle). The sections of the dwarf shoots of these species were taken from three different regions and their anatomical details such as shape of epidermal cells, number and position of resin ducts and ratio of cortex to wood were compared. These show interrelationships among different species using morphological, anatomical data. The anatomical details of the Pinus species and their correlation may be incorporated along with other diagnostic features needed for the identification and classification or systematic of these species. This work also attempts to provide a database for the systematics of different species which can be very useful for interspecific comparisons within the same territory and among different territories. The study revealed that anatomical variations in dwarf shoots can be taxonomically very helpful in identification of various species of Pinus.

Introduction

The genus Pinus Linn. belonging to family Pinaceae is widely distributed and about 105 species have been reported worldwide (Mehra, 1988). Pinus is most dominant genus of the family Pinaceae with its wide range of distribution and adaptability to diverse climatic conditions in Northern as well as Southern Hemisphere on the globe.

In an Asiatic continent, Pinus are distributed mainly in Afghanistan, Pakistan, India, Myanmar, Philippines and spread upto Indonesia after crossing the equator. In India, Kalika forest of Uttarakhand has the largest Pinatum of India where several species of Pinus have been introduced which are flourishing and surviving.
Commercially, numbers of Pinus species are the main source of timber, paper, herbal products, scents, etc. and various cottage as well as large scale industrial works on based upon *Pinus* throughout the world. The geographical area of the state is 53,485 sq. kms. The major part of the state of Uttarakhand is hilly. Geographically it is situated in the central Himalayan zone. It extends between 77°34' and 81°02' East longitude and 28°43' to 31°27' North latitude. The state comprises 34 districts. The topography of the Uttarakhand region is characterized by hilly terrain, rugged and rocky mountains, deep valleys, high peaks, sharp streams and rivulets, rapid soil erosion, frequent landslides and widely scattered habitations. The topography of Uttarakhand presents sloppy lands with poor soil depth and fragile ecosystem.

Amongst 105 species of the genus *Pinus* (Mehra, 1988), only six species are indigenous to Indian flora. These species are *Pinus insularis* Endlicher, *Pinus roxburghii* Sargent, *Pinus wallichiana* A.B. Jackson, *Pinus gerardiana* Wall ex Lamb., *Pinus merkusii* Jungh et de Varies and *Pinus armendii* French. However, the Western Himalayan region of India particularly Kumaon Hills are predominantly covered with various numbers of more other *Pinus* species which are introduced and these species have established over there.

The study of the dwarf shoot of the genus *Pinus* dates back since the time of Henry (1837), who described and accurately illustrated and the detail of dwarf shoot organography and certain aspects of its development. Later on, Geylor (1867) work on dwarf shoot was concerned and vascularization of the needles. Subsequently, Schneider (1913) described anatomy of the cataphylls and recognized the spiral arrangement of the needles. The investigation of Borthwick (1899a), Thomson (1914), Prat (1930) were concerned largely with the proliferative capacity of the dwarf shoot. Although the anatomical features of the foliage leaves have been in extensive use in the classification of conifers and other vascular plant groups (Durell, 1916; Harlow, 1931; Fulling, 1934; Marco, 1939; Florin, 1940; Orr, 1944; Buchholz and Gray, 1948; Florin, 1951, 1958, 1963). Sacher (1954) has an intensive study has been made of the structure and seasonal periodicity of the shoot apices of *Pinus lambertiana* and *Pinus ponderosa*. Later on, Sacher (1955), Doak (1935) stated that the ontogenetic fate of dwarf shoot apices probable various considerably from species to species. Konar (1963) investigated the morphology and anatomy of *Pinus roxburghii*. Ghouse and Yunus (1972) was designed and aimed to evolve a simple and workable key for the easy identification of Indian conifers based on their microscopic features of foliage leaves. Debazac (1962), Hanawa (1967), Gabilo and Mogenson (1973) regarded dwarf shoot as microscopic bud and found that the dwarf shoot apex is obliterated during needle development. Owens and Molder (1977) studied on *Pinus longaeva* and revealed that the long shoot terminal bud is strictly monocyclic. Ewers (1982) stated that microscopic measurement and cell counts revealed that with advancing needle age phloem increases radially and transfusion tissue. Later on, Ewers (1983) studied on the dwarf shoot of *Pinus longaeva* and stated that the distal dwarf shoot of each flush have interfoliar buds which often proliferate into long shoots. Ewers and Schmid (1985) stated that the dwarf shoot of *Pinus longaeva* are initiated in the axil of scale leaves in the long shoot terminal bud. Ewers and Aloni (1987) examined that the current year’s needles, 1 year old and 2 year old needles of *Pinus*
strobus and Pinus brutia and more detailed seasonal analysis of vascular differentiation. Lee and Eom (1988) described and compared the qualitative and quantitative anatomical aspects of dwarf shoots of Pinus koraiensis. Chaturvedi (1993) studied the epidermal and anatomical characters in the needle of two species of Pinus viz. P. radiata Don and Pinus yunnanensis Frenchet. Eom and Chung (1994) studied anatomy of tumor wood and normal wood in a branch of Korean red pine (Pinus densiflora Siebold et Zuccarini). Reich et al., (1996) discussed the phenotypic responses in Pinus sylvestris L. and Picea abies L. Lim and Soh (1997) concluded that tracheids in the annual rings of dwarf trees are shorter, narrower and fewer than those of normal trees. Simcha Lev-Yadun (1998) studied relationship between growth ring width and ray size and number. Hafren et al., (2000) studied that developing xylem and mature xylem of Pinus sylvestris is affected by Homogalacturonan. Boratynska and Boratynski (2003) studied abnormal three (or more) needle dwarf shoots of most southern and central European two-needle pine (Pinus) species. They also discussed frequencies of abnormal dwarf shoots in Pinus mugo and Pinus uncinata.

Later on, Donaldson et al., (2004) studied two trees of radiata pine and examined that the presence of compression wood, lignin distribution, microfibril angle, basic density, radial and tangential lumen diameter and cell wall thickness. Esteban et al., (2005) described the anatomical description of the wood as well as an interpretation of the factors related to this resification. Heuret et al., (2006) discussed the morphological features of wood of Pinus pinaster. Subsequently, Zhang et al., (2008) discussed the formation and significance of radial resin canals in Pinus densiflora. Nair et al., (2009) examined that rays and resin canals were the initiation sites of checking. Boratynski et al., (2011) studied the three-needle dwarf shoots of Pinus mugo and these were affected by climatic factors. Antony et al., (2012) studied and described different methods used to define the transition from early wood to late wood in Pinus taeda.

The purpose of the present study was conducted on the morphology and anatomy of the dwarf shoots of some exotic species of Pinus. Their anatomical variations was analysed and inter-relationships among the species.

**Materials and Methods**

The work deals with the “Morphology and Anatomy of dwarf shoots of some exotic species of Pinus Linn. in Kumaon Hills, Western Himalayas” and hence morphological and anatomical details of the Pinus growing in above mentioned area. These 5 species of Pinus were collected from different localities of Kumaon Himalayas including Nainital, Ranikhet, Almora, Kaushani and neighbouring areas in Western Himalayas, India.

During the present study morphological and anatomical aspects of some exotic species of P. have been taken into consideration which constitutes an important aspect of the taxonomical characters of gymnosperms.

The dwarf shoots of some exotic species of Pinus were taken in order to study the anatomical details. They are:

1. Pinus canariensis Smith
2. Pinus densiflora Siebold et Zuccarini
3. Pinus echinata Miller
4. Pinus engelmanii Carriere
5. Pinus greggii Engelmann
Formalin-propionic acid – Ethyl alcohol was used as a killing and fixation fluid followed by dehydration with the tertiary butyl alcohol method and embedded in Tissuemat. Safrannin staining technique was also employed followed by the use of fast green solution (Johansen, 1940).

All the materials were sectioned upto 8 µm in thickness with a razor, then mounted and stained in safrannin and fast green. The microphotographs of transverse sections of dwarf shoots from different regions investigated (taken with help of Nikon Binocular Compound Microscope, FX-35DX).

Results and Discussion

**Pinus canariensis** Smith commonly known as Canary Island Pine is a large tree ranges from 80-100 feet in height and 10 feet or more in girth (Plate 1, Fig. 1). The branches spreading with drooping blanchlets forming a tree of somewhat weeping habit. Bark thick, reddish, slightly fissured, dividing into irregular scales. Young shoots yellow, winter buds large, ovoid with lance-shaped. Leaves (needles) in threes per fascicle, highly glaucous on seedling (Dallimore and Jackson, 1966). The spur length may vary, it is upto 4.2 inch and dwarf shoot about 1 cm in length (Plate 1, Fig. 2).

**Pinus densiflora** Siebold et Zuccarini commonly known as Japanese Red Pine is a large tree ranges from 70-120 feet in height and 6-12 feet in girth (Plate 1, Fig. 6). Bark reddish like that of Scots pine, particularly about the higher parts of tree, peeling off in thin scales. Young shoots green with a glaucous bloom. Winter buds, slender, cylindrical with sharp pointed. Leaves (needles) in pairs per fascicle persisting three years (Dallimore and Jackson, 1966). The spur length may vary, it is upto 3.5 inch and dwarf shoot about 1 cm in length (Plate 1, Fig. 7).

**Pinus echinata** Miller commonly known as short leaf pine is a large tree ranges from 80-100 or occasionally 120 feet in height with a trunk 4-6 feet in girth (Plate 1, Fig. 11). The bark thick, reddish divided into irregular shaped. Young shoots pale green flushed with violet and usually coated with a glaucous bloom changing to brown. Winter buds ovate, sharp pointed. Leaves (needles) usually in pairs but occasionally in threes or fours also have been reported (Dallimore and Jackson, 1966). The spur length may vary, it is upto 3.7 inch and dwarf shoots about 9 mm in length (Plate 1, Fig. 7).

**Pinus engelmanii** Carriere commonly known as Apache pine is a medium sized tree ranges from 20-30 meters in height and a trunk diameter of 35-80 cm (Plate 2, Fig. 1). Bark dark brown, at maturity deeply furrowed. Branches straight to ascending. Winter buds ovoid to conic, resinous. Leaves (needles) usually in threes, often fives per fascicle, persisting two years (Dallimore and Jackson, 1966). The spur length may vary, it is upto 7.8 inch and dwarf shoot about 1 cm in length (Plate 2, Fig. 2).

**Pinus greggii** Engelmann commonly known as Gregg’s pine is a medium size tree ranges from 40-50 feet in height with smooth grey bark except at the base of old trees where it becomes rough and fissured (Plate 2, Fig. 6). Young shoots glaucous at first, greyish-buff the second year. Winter buds slender, cylindrical with sharp-pointed. Leaves (needles) are usually in threes but rarely two in each fascicle, persisting two-three years (Dallimore and Jackson, 1966). The spur length may vary, it is upto 8.2 inch and dwarf shoot about 1 cm in length (Plate 2, Fig. 7).

The sections of the dwarf shoots of above mentioned species were taken from three
different regions and their anatomical details were compared. Many variations were recorded in the anatomical structures of dwarf shoots such as shape of epidermal cells, number and position of resin ducts, ratio of cortex to wood, etc.

The basal region of dwarf shoot was bounded by membranous sheath formed by scale leaves instead of a well defined epidermis. Foster’s (1938) opinion that tunica and corpus represent interdependent growth zones in apical meristem and as such, one cannot exist independently of the other. *P. canariensis* (Plate 1, Fig. 3) has very thick walled cuticle covered with scales leaves which shows similarities with *P. densiflora* (Plate 1, Fig. 8), *P. echinata* (Plate 1, Fig. 13) and *P. greggii* (Plate 2, Fig. 8) while *P. engelmanii* shows thick walled cuticle but some cells constitute the epidermis (Plate 2, Fig. 3).

The cortical region may be differentiated into two zones viz. outer and inner zone. The outer zone consists of parenchymatous cells, six to seven layered in *P. canariensis* (Plate 1, Fig. 3), four to five layered in *P. densiflora* (Plate 1, Fig. 8), five to six layered in *P. echinata* (Plate 1, Fig. 13) and *P. greggii* (Plate 2, Fig. 8) while *P. engelmanii* shows thick walled cuticle but some cells constitute the epidermis (Plate 2, Fig. 3).

The shape and size of outer zone of cortical cells may also vary. The outer cortical cells are oval, elongated, quadrangular, penta to hexagonal in *P. canariensis* (Plate 1, Fig. 3) and elongated, quadrangular, penta to hexagonal in *P. densiflora* (Plate 1, Fig. 8) which shows similarity with *P. echinata* (Plate 1, Fig. 13) where these are oval, quadrangular, penta to hexagonal. *P. engelmanii* (Plate 2, Fig. 3) has oval, elongated, quadrangular to pentagonal while in *P. greggii* (Plate 2, Fig. 8), these are elongated, quadrangular to pentagonal. The size of outer cortical cells may also varied, 22.2-44.4 μm in long and 11.1-33.3 μm in broad in *P. canariensis* (Plate 1, Fig. 3, Table 1), 11.1-44.4 μm in long and 11.1-33.3 μm broad in *P. densiflora* (Plate 1, Fig. 8, Table 1) and *P. engelmanii* (Plate 2, Fig. 3, Table 1), 11.1-33.3 μm in long and 11.1-27.7 μm in broad in *P. echinata* (Plate 1, Fig. 13, Table 1) and 22.2-55.5 μm in long and 11.1-33.3 μm in broad in *P. greggii* (Plate 2, Fig. 8, Table 1).

Resin ducts are absent in this zone in case of *P. canariensis* (Plate 1, Fig. 3), *P. densiflora* (Plate 1, Fig. 8) and *P. greggii* (Plate 2, Fig. 8) while these are present in *P. echinata* (Plate 1, Fig. 13) and *P. engelmanii* (Plate 2, Fig. 3). In *P. echinata* (Plate 1, Fig. 13), the resin ducts distributed irregularly here and there, lined by thin walled, single layered of epithelial cells, placed intermarginally while in *P. engelmanii* (Plate 2, Fig. 3), these are also thin walled but two layered of epithelial cells, placed intermarginally. The cells of inner zone of cortex are relatively smaller, thin walled than the outer zone of cortical cells.

The inner zone also consists of parenchymatous cells, thin walled, small, loosely or compactly arranged. These are five to six layered in *P. canariensis* (Plate 1, Fig. 3), three to four layered in *P. densiflora* (Plate 1, Fig. 8), *P. echinata* (Plate 1, Fig. 13), *P. engelmanii* (Plate 2, Fig. 3) and *P. greggii* (Plate 2, Fig. 8). The shape and size of inner cortical cells may also vary. These are elongated, quadrangular to pentagonal in *P. canariensis* (Plate 1, Fig. 3), *P. densiflora* (Plate 1, Fig. 8) and *P. echinata* (Plate 1, Fig. 13). These shows much affinity with *P. engelmanii* (Plate 2, Fig. 3) and *P. greggii* (Plate 2, Fig. 8) which contain oval, elongated, quadrangular to pentagonal cells.
Table 1: Anatomical details of dwarf shoots at three different regions viz. basal, middle and upper

| SL. NO. | NAME OF SPECIES                | CUTICLE  | EPIDERMIS | HYPODERMIS | OUTER ZONE | CORTICAL ZONE | INNER ZONE | WOOD REGION | PITH REGION |
|---------|--------------------------------|----------|-----------|------------|------------|---------------|------------|-------------|-------------|
|         |                                |          | SHAPE     | SIZE       | SHAPE      | SIZE          | R.D.       | AMOUNT      | SIZE        | R.D.        |
| 1.      | *P. canariensis* Smith         | Scale    | ND        | ND         | ND         | Oval, elongated quadrangular, penta to hexagonal | 22.2-44.4 µm long & 11.1-33.3 µm broad | –           | Elongated, quadrangular to pentagonal | 11.1-22.2 µm long & 11.1-16.6 µm broad | + Less in amount as compared to the amount of cortex | 321.9-333 µm long & 277.5-288.6 µm broad | + Centrally located & less in amount |
|         | *(3 needles per fascicle)*     |          |           |            |            |               |            |             |             |             |
| 2.      | *P. densiflora* Siebold et. Zuccarini | Scale    | ND        | ND         | ND         | Elongated quadrangular, penta to hexagonal | 11.1-44.4 µm long & 11.1-33.3 µm broad | –           | Elongated, quadrangular to pentagonal | 11.1-16.6 µm long & 11.1-22.2 µm broad | + Less in amount as compared to the amount of cortex | 266.4-277.5 µm long & 244.2-255.3 µm broad | – Centrally located & less in amount |
|         | *(2 needles per fascicle)*     |          |           |            |            |               |            |             |             |             |
| 3.      | *P. echinata* Miller           | Scale    | ND        | ND         | ND         | Oval, elongated quadrangular, penta to hexagonal | 11.1-33.3 µm long & 11.1-27.7 µm broad | +           | Quadrangular, elongated to pentagonal | 5.55-11.1 µm long & 5.55-16.6 µm broad | – Less in amount as compared to the amount of cortex | 222-233 µm long & 177-188 µm broad | + Centrally located & less in amount |
|         | *(2 needles per fascicle)*     |          |           |            |            |               |            |             |             |             |
| 4.      | *P. engelmanii* Carriere       | Scale    | ND        | ND         | ND         | Oval, elongated quadrangular to pentagonal | 11.1-44.4 µm long & 11.1-33.3 µm broad | –           | Oval, elongated quadrangular to pentagonal | 11.1-16.6 µm long & 11.1-27.7 µm broad | – Less than the amount of cortex | 321.9-333 µm long & 210.9-222 µm broad | – Less in amount and occupying minimum area |
|         | *(3 needles per fascicle)*     |          |           |            |            |               |            |             |             |             |
| 5.      | *P. greggii* Engelmann         | Scale    | ND        | ND         | ND         | Quadrangular, elongated to pentagonal | 22.2-55.5 µm long & 11.1-33.3 µm broad | –           | Oval, elongated quadrangular to pentagonal | 11.1-16.6 µm long & 11.1-22.2 µm broad | + Less than the amount of cortex | 155.4-166.5 µm long & 166.5-177.6 µm broad | – Least in amount and occupying minimum area |
Table 2

| SL. NO. | NAME OF SPECIES | CUTICLE | EPIDERMIS | HYPODERMIS | MIDDLE REGION | WOOD REGION | PITH REGION |
|---------|-----------------|---------|-----------|------------|---------------|-------------|-------------|
|         |                 |         | SHAPE     | SIZE       | SHAPE | SIZE | R.D. | SHAPE | SIZE | R.D. | SHAPE | SIZE | R.D. | Size | R.D. |
| 1.      | *P. canariensis* Smith | Thick walled | Isodiametric, elongated to rectangular | 11.1 to 16.6 μm long & 11.1 to 16.6 μm broad | ND | ND | Isodiametric quadrangular, elongated to pentagonal | 16.6 to 44.4 μm long & 11.1 to 16.6 μm broad | ND | ND | Quadrangular, isodiametric & elongated | 11.1 to 16.6 μm long & 11.1 to 16.6 μm broad | ND | ND |
|         | (3 needles per fascicle) |         |           |            |       |      |      |       |      |      |       |      |      |      |      |
| 2.      | *P. densiflora* Siebold et. Zuccarini | Thin walled | Oval, elongated quadrangular | 5.55 to 11.1 μm long & 5.55 to 16.6 μm broad | Oval elongated to pentagonal | 5.55 to 11.1 μm long & 5.55 to 16.6 μm broad | ND | ND | ND | Oval, elongated, quadrangular, to pentagonal | 11.1 to 33.3 μm long & 11.1 to 27.7 μm broad | + |
|         | (2 needles per fascicle) |         |           |            |      |      |      |       |      |      |       |      |      |      |      |
| 3.      | *P. echinata* Miller | Thick walled | Oval, isodiametric, quadrangular to elongated | 5.55 to 11.1 μm long & 5.55 to 11.1 μm broad | Oval elongated to quadrangular | 11.1 to 16.6 μm long & 5.55 to 16.6 μm broad | ND | ND | ND | Isodiametric, oval, elongated, quadrangular, to pentagonal | 11.1 to 27.7 μm long & 11.1 to 27.7 μm broad | + |
|         | (2 needles per fascicle) |         |           |            |      |      |      |       |      |      |       |      |      |      |      |
| 4.      | *P. engelmannii* Carriere | Scale leaves | ND | ND | ND | Oval, elongated, isodiametric, quadrantal to hexagonal | 11.1 to 44.4 μm long & 11.1 to 33.3 μm broad | + | ND | ND | ND | ND | ND | ND |
|         | (3 needles per fascicle) |         |           |            |      |      |      |       |      |      |       |      |      |      |      |
| 5.      | *P. greggii* Engelmann | Thin walled | Oval, isodiametric, elongated to rectangular | 11.1-16.6 μm long & 11.1-16.6 μm broad | Oval elongated to pentagonal | 11.1-16.6 μm long & 5.55-11.1 μm broad | Oval, elongated, quadrangular to hexagonal | 11.1-27.7 μm long & 11.1-22.2 μm broad | + | ND | ND | ND | ND |
|         | (3 needles per fascicle) |         |           |            |      |      |      |       |      |      |       |      |      |      |      |

ENDO. = ENDODERMIS; PERI. = PERICYCLE; R.D. = RESIN DUCT; V.B. = VASCULAR BUNDLE; N.D. = NOT DISTINCT; L.D. = LESS DISTINCT; + = PRESENT; – = ABSENT
**Table 3**

| SL. NO. | NAME OF SPECIES | UPPER REGION | LOWER REGION |
|---------|-----------------|---------------|--------------|
|         | CUTICLE          | SHAPE SIZE     | HYPODERMIS SHAPE SIZE | ENDODERMIS VASCULAR ZONE |
|         |                 |               | MESOPHYLLS (ARM PALISADE & SPOGY PARENCHYMA) & R.D. | R.D. |
|         |                 | SHAPE SIZE     | ENDODERMIS SHAPe Size | PERICYCLE | V.B. |
| 1.      | *P. canariensis* Smith (3 needles per fascicle) | Thin walled | Oval, elongated to rectangular | 5.55-11.1 µm long & 5.55-11.1 µm broad | Oval, quadrangular, to elongated | 11.1-16.6 µm long & 11.1-22.2 µm broad | Quadrangular, oval, elongated to pentagonal | 11.1-22.2 µm long & 16.6-27.7 µm broad | + & 2 in no. in each zone | ND | ND | ND | ND | 77.7-88.8 µm long & 133.2-144.3 µm broad | – | – |
| 2.      | *P. densiflora* Siebold et. Zuccarini (2 needles per fascicle) | Thin walled | Oval, elongated to quadrangular | 5.55-11.1 µm long & 5.55-16.6 µm broad | Oval, elongated, quadrangular | 5.55-11.1 µm long & 5.55-16.6 µm broad | Less differentiates, elongated (polygonal) | 11.1-38.8 µm long & 11.1-33.3 µm broad | + & 2 in no. in each zone | ND | ND | ND | ND | 99.9-111 µm long & 155.4-166.5 µm broad | – | – |
| 3.      | *P. gregii* Miller (2 needles per fascicle) | Thin walled | Oval to rectangular | 5.55-11.1 µm long & 5.55-11.1 µm broad | Oval, elongated to rectangular | 5.55-11.1 µm long & 5.55-16.6 µm broad | Oval, elongated quadrangular, circular | 11.1-27.7 µm long & 11.1-27.7 µm broad | + & 2 in no. in each zone | LD | LD | LD | LD | 88.8-99.9 µm long & 188.7-199.8 µm broad | – | – |
| 4.      | *P. engelmannii* Carriere (3 needles per fascicle) | Thick walled | Oval, isodiometric, elongated to rectangular | 5.55-11.1 µm long & 11.15-16.6 µm broad | Oval, elongated to quadrangular to pentagonal | 5.55-16.6 µm long & 11.1-16.6 µm broad | Elongated quadrangular, penta to octagonal | 11.1-22.2 µm long & 16.6-27.7 µm broad | + & 3 in no. in each zone | Elongated to barrel | 11.1-16.6 µm long & 16.6-33.3 µm broad | Elongated, quadrangular, to pentagonal | 11.1-27.7 µm long & 11.1-16.6 µm broad | 66.6-88.8 µm long & 155.4-177.6 µm broad | – | – |
| 5.      | *P. gregii* Engelmann (3 needles per fascicle) | Thick walled | Oval, isodiometric, elongated to rectangular | 5.55-11.1 µm long & 5.55-11.1 µm broad | Oval, elongated to quadrangular to pentagonal | 5.55-11.1 µm long & 5.55-11.1 µm broad | Oval elongated quadrangular, penta to octagonal | 11.1-27.7 µm long & 11.1-33.3 µm broad | + & 2 in no. in each zone | Elongated to barrel | 11.1-16.6 µm long & 16.6-27.7 µm broad | Elongated, quadrangular, to pentagonal | 11.1-27.7 µm long & 11.1-16.6 µm broad | 55.5-66.6 µm long & 99.9-111 µm broad | – | – |

ENDO. = ENDODERMIS; PERI. = PERICYCLE; R.D. = RESIN DUCT; V.B. = VASCULAR BUNDLE; N.D. = NOT DISTINCT; L.D. = LESS DISTINCT; + = PRESENT; – = ABSENT
Plate 1 *Pinus canariensis* fig.(1) Habit, fig.(2) a twig with spur shoot, fig.(3) T.S. of basal region of dwarf shoot X120. fig.(4) T.S. of middle region of dwarf shoot X120. fig(5) T.S. of upper region of dwarf shoot X120. *P. densiflora* fig.(6) Habit, fig.(7) a twig with spur shoot, fig.(8) T.S. of basal region of dwarf shoot X120. fig.(9) T.S. of middle region of dwarf shoot X120. fig(10) T.S. of upper region of dwarf shoot X120. *P. echinata* fig.(11) Habit, fig.(12) a twig with spur shoot, fig.(13) T.S. of basal region of dwarf shoot X120. fig.(14) T.S. of middle region of dwarf shoot X120. fig(15) T.S. of upper region of dwarf shoot X120.
Plate 2 P. engelmannii fig. (1) Habit, fig. (2) a twig with spur shoot, fig. (3) T.S. of basal region of dwarf shoot X120. fig. (4) T.S. of middle region of dwarf shoot X120. fig. (5) T.S. of upper region of dwarf shoot X120. P. greggii fig. (6) Habit, fig. (7) a twig with spur shoot, fig. (8) T.S. of basal region of dwarf shoot X120. fig. (9) T.S. of middle region of dwarf shoot X120. fig. (10) T.S. of upper region of dwarf shoot X120.
The size of inner cortical cells may also vary among the species. 11.1-16.6 µm in long and 11.1-22.2 µm in broad in *P. densiflora* (Plate 1, Fig. 8,Table 1) and *P. greggii* (Plate 2, Fig. 8,Table 1). 11.1-22.2 µm in long and 11.1-16.6 µm in broad in *P. canariensis* (Plate 1, Fig. 3, Table 1). *P. echinata* (Plate 1, Fig. 13, Table 1) has 5.55-16.6 µm in long and 5.55-16.6 µm in broad while *P. engelmannii* (Plate 2, Fig. 3,Table 1) shows 11.1-16.6 µm in long and 11.1-27.7 µm in broad. Resin ducts are absent in this zone in case of *P. echinata* (Plate 1, Fig. 13) and *P. engelmannii* (Plate 2, Fig. 3). In *P. canariensis* (Plate 1, Fig. 3), these are numerous in number, large, distributed here and there, lined by thin walled, single layered of epithelial cells, placed intermarginally while these are few in number, small, two layered of epithelial cells, placed inter marginally in case of *P. densiflora* (Plate 1, Fig. 8) and *P. greggii* (Plate 2, Fig. 8).

Barbaros Yaman (2007) explained that the anatomical properties of the wood of *P. sylvestris* var. *compacta* and compares them with typical *P. sylvestris*. The woods of these taxa have the same qualitative anatomical features but most of the quantitative anatomical characteristics show significant differences.

The vascular cylinders characterized by having well marked secondary growth, due to which large amount of secondary wood is formed. Amount of secondary wood is less than the amount of cortex observed in *P. canariensis* (Plate 1, Fig. 3) which shows affinity with *P. densiflora* (Plate 1, Fig. 8), *P. echinata* (Plate 1, Fig. 13), *P. engelmannii* (Plate 2, Fig. 3) and *P. greggii* (Plate 2, Fig. 8). The size of vascular cylinders may also vary among the species.

Maximum size of vascular cylinders i.e. 321.9-333 µm in long and 277.5-288.6 µm in broad observed in *P. canariensis* (Plate 1, Fig. 3,Table 1) which shows close affinity with *P. engelmannii* (Plate 2, Fig. 3,Table 1) i.e. 321.9-333 µm in long and 210.9-222 µm in broad. Lowest size of vascular cylinders i.e. 155.4-166.5 µm in long and 177.6 µm in broad observed in *P. greggii* (Plate 2, Fig. 8,Table 1) which shows close affinity with *P. echinata* (Plate 1, Fig. 13,Table 1) i.e. 222-233 µm in long and 177-188 µm in broad. 266.4-277.5 µm in long and 244.2-255.3 µm in broad observed in *P. densiflora* (Plate 1, Fig. 8,Table 1). Resin ducts are absent in wood region observed in *P. densiflora* (Plate 1, Fig. 8), *P. engelmannii* (Plate 2, Fig. 3) and *P. greggii* (Plate 2, Fig. 8) while these are present in *P. canariensis* (Plate 1, Fig. 3) and *P. echinata* (Plate 1, Fig. 13). Amount of pith is centrally located but less in amount observed in *P. canariensis* (Plate 1, Fig. 3), *P. densiflora* (Plate 1, Fig. 8), *P. echinata* (Plate 1, Fig. 13) and *P. engelmannii* (Plate 2, Fig. 3) while these are centrally located but least in amount observed in *P. greggii* (Plate 2, Fig. 8).

The middle region of dwarf shoot shows differentiation in structure. The whole structure may be circular or oval in outline but differentiation may occur among the species. The middle region of the dwarf shoots covered with thick walled cuticle observed in *P. canariensis* (Plate 1, Fig. 4), *P. echinata* (Plate 1, Fig. 14) whereas these are thin walled observed in *P. densiflora* (Plate 1, Fig. 9) and *P. greggii* (Plate 2, Fig. 9). *P. engelmannii* (Plate 2, Fig. 4) has cuticle in the form of scale leaves. The shape and size of epidermal cells may also vary in middle region of the dwarf shoots. The cells of epidermis are isodiametric, elongated to rectangular in *P. canariensis* (Plate 1, Fig. 4) whereas in *P. densiflora* (Plate 1, Fig. 9), these are oval, elongated and quadrangular. Oval, isodiametric, quadrangular to
elargèd in *P. echinata* (Plate 1, Fig. 14). In *P. engelmanii* (Plate 2, Fig. 4), the epidermis is not distinct. In *P. greggii* (Plate 2, Fig. 9), these are oval isodiametric, elongated to rectangular in shape. The cells of epidermis are 5.55-11.1 µm in long and 5.55-16.6 µm in broad observed in *P. densiflora* (Plate 1, Fig. 9, Table 2). 11.1-16.6 µm in long and 11.1-16.6 µm in broad in *P. canariensis* (Plate 1, Fig. 4,Table 2) and *P. greggii* (Plate 2, Fig. 9,Table 2). 5.55-11.1 µm in long and 5.55-11.1 µm in broad observed in *P. echinata* (Plate 1, Fig. 14,Table 2).

The very important significant feature of middle region of the dwarf shoots is the distinction of cortex as well as vascular region into two, three, four, five, etc. regions depending upon their needles per fascicle, is already begins by the broadening of medullary rays from pith to cortex and resin ducts are few in number. The wood region is present in the form of vascular cylinders. *P. canariensis* (Plate 1, Fig. 4, Table 2) has 111-122.1 µm in long and 177.6-199.8 µm in broad vascular cylinders. 99.9-111 µm in long and 177.6-199.8 µm in broad in *P. densiflora* (Plate 1, Fig. 9, Table 2), 88.8-99.9 µm in long and 188.7-199.8 µm in broad in *P. echinata* (Plate 1, Fig. 14,Table 2), 111-133.2 µm in long and 188.7-222 µm in broad in *P. engelmanii* (Plate 2, Fig. 4,Table 2) and 66.6-77.7 µm in long and 111-133.2 µm in broad vascular cylinders observed in *P. greggii* (Plate 2, Fig. 9, Table 2).

The feature of secondary wood appears to be an important feature and can be used as a character for identification of species. Another significant feature of the dwarf shoot in the middle region in which fused resin ducts may also present (Plate 1, Fig. 9, Plate 2, Fig. 4).

The very important significant feature of the dwarf shoot at upper region is the distinction of needles into two, three, four, five, etc. zones. Each zone is remains united at innerface while free at the outerface. Each zone has an epidermis, hypodermis, cortex is present in the form of mesophylls, endodermis, pericycle and centrally located vascular cylinders. Here pith is altogether absent (Plate 1, Figs.5, 10, 15, Plate 2, Figs. 5, 10, Table 3). Moreover, shape and size of epidermal cells, amount of cortex, number and position of resin ducts, amount of secondary wood, etc. may vary from species to species of *Pinus*. These important feature can use as a character for identification of species. The study revealed that anatomical variations in different regions of dwarf shoots can be taxonomically very helpful in identification of various species of *Pinus*. 

The cross section of the dwarf shoot at upper region is almost circular in outline with clearly distinct two independent semicircular in *P. densiflora* (Plate 1, Fig. 10) and *P. greggii* (Plate 2, Fig. 10) whereas three independent triangular zones observed in *P. canariensis* (Plate 1, Fig. 5), *P. echinata* (Plate 1, Fig. 15) and *P. engelmanii* (Plate 2, Fig. 5). Each zone is remain united at inner face while free at the outerface. Each zone is rather independent except centrally at inner face and bounded by single layer epidermis.

Annual shoot elongation decreased with increasing tree size. This is described by Moorby and Wareing (1963) for trees of different ages and sizes.

Ewers and Schmid (1981) demonstrated that the variability in leaf longevity in several pine species may involve responses to environmental conditions as well as variability within a genotype. Weideman (1939) provided data for ponderosa pine to support the same conclusion.

The very important significant feature of the dwarf shoot at upper region is the distinction of needles into two, three, four, five, etc. zones. Each zone is remains united at innerface while free at the outerface. Each zone has an epidermis, hypodermis, cortex is present in the form of mesophylls, endodermis, pericycle and centrally located vascular cylinders. Here pith is altogether absent (Plate 1, Figs.5, 10, 15, Plate 2, Figs. 5, 10, Table 3). Moreover, shape and size of epidermal cells, amount of cortex, number and position of resin ducts, amount of secondary wood, etc. may vary from species to species of *Pinus*. These important feature can use as a character for identification of species. The study revealed that anatomical variations in different regions of dwarf shoots can be taxonomically very helpful in identification of various species of *Pinus*.
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How to cite this article:

Dildar Husain. 2016. Morphology and Anatomy of Dwarf Shoots of Some Exotic Species of Pinus Linn. in Kumaon Hills, Western Himalayas. Int.J.Curr.Microbiol.App.Sci. 5(9): 219-233. doi: http://dx.doi.org/10.20546/ijcmas.2016.509.025