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Vascular Development And Transition In The Seedling Of Lupinus Albus L.

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Abstract:-Transverse sections made in seedling of lupine (*Lupinus albus* L.) examined by light microscope to study the development and transition of the vascular system. Vascular transition occurred in the root. It started few centimeters below the soil surface, and completed at the basal (superior) end of the root. No rotation was observed during the transition of vascular tissues from exarch radial to endarch collateral arrangement, but the protoxylem elements differentiated gradually on the inner side of metaxylem. Although vascular transition is completed at the base of the root, the vascular system of the hypocotyl axis showed different structure at the different levels of the hypocotyl. Development and course of the leaf traces indicate that there is continuity between the vascular system of the hypocotyl and that of the epicotyl. Regardless the phase of transition; the fascicular cambium was established between the primary phloem and metaxylem. The development and activity of interfascicular cambium varied to some extent according to the level of seedling axis.

Keywords: *Lupinus albus* L., vascular development and transition.

INTRODUCTION

The transition of the exarch radial arrangement of the vascular system of the root into the endarch collateral structure of the stem has been a very interesting topic since the beginning of the 20th century. The arrangements of tissues in the transition region are complex and not easily described, possibly for these reasons there have been few studies of it (Mauseth, 1988). In most seed plants, vascular transition occurs within the system connecting the cotyledons with the root, although the extent of seedling axis that shows the features of transition is variable. Some workers on different plants reported that most stages of vascular transition occurred in the lower portion of the hypocotyl, but transition development is completed in the upper portion of it (*Gossypium*, Spieth (1933) and Hayward (1938); *Cucurbita maxima*, Whiting (1938) and Hayward (1938); *Vigna radiata*, El-Shaarawi et al. (2008) and Soybean, El-Shaarawi et al. (2011). On the other hand many authors found that the transition region restricted to the upper part of the hypocotyl and part of the cotyledons (*Lactuca sigitata*, Lee 1914; *Beta vulgaris*, Archwager 1926; *Raphanus stiva*, Grassley 1932; *Cannabis sativa*, Berkman 1936; and *Arabidopsis thaliana*, Busse and Evert 1999). Moreover, in other plants such as *Pisum sativum* the root-stem transition is not completed in the short hypocotyl, but also involves the first three internodes of the stem (Compton, 1912 and Gourley, 1931). In addition, Rugini et al. (2006) on four dicotyledonous species, mentioned that alternative structure is found only at the lower third of the root; at the middle and superior (basal), the tangential phase is obvious at *Ricinus communis* L. and even overlapped at *Mirabilis jalapa* L. The arrangements of tissues in the transition region are complex and not easily described, possibly for these reasons there have been few studies of it (Mauseth, 1988). The present work was carried out to study the differentiation and transition of vascular system in the seedling of *Lupinus albus* L. Secondary growth in the seedling axis was also discussed.

MATERIALS AND METHODS

The current investigation was carried out in the wire green-house of Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the winter growing season of 2010. Seeds of lupine (*Lupinus albus* L.) cv. Giza1 were obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Seeds of lupine were sown on 1th of November, 2010 in black plastic pots, 30 cm diameter, filled with clay and sand at the ratio of 1:1 by weight.

Specimens were taken at the ages of 7 and 28 days as follows:
1. Different levels of the tap root.
2. Different levels of the hypocotyl.
3. Cotyledonary node and epicotyl.

Samples were killed and fixed for at least 48hrs. in FAA solution, washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series and embedded in paraffin wax (melting point 56-58oC). Transverse sections were cut on a rotary microtome to a thickness of 15-20 microns, double stained with safranin-light green, cleared in xylene and mounted in Canada balsam (Willey, 1971). Sections were microscopically analyzed and photomicrographed.

RESULTS

Germination of lupine is epigeous and takes place after few days from the sowing. Transverse sections were made at different levels of main root, hypocotyl, cotyledonary node and epicotyl at two ages: 1) at the age of 7 days to study the differentiation and transition of vascular system. 2) at the age of 28 days to study the secondary growth along the seedling axis.

Differentiation and transition of vascular system:

Cross sections through the midpart of main root revealed that the primary root of lupine has an exarch, radial and diarch protostele. At the center of the stele, there are 12 to 14 large metaxylem vessels which are sometimes separated by smaller xylem elements. There are numerous of protoxylem elements, 15-17 cells in each protoxylem group. On alternate radii to the protoxylem are two groups of primary phloem that are separated from the central metaxylem elements by parenchyma. The pericycle and endodermis are single layered. The cortex is composed of parenchymatous cells, 9 to 12 layers in thickness, with intercellular spaces. The epidermis is one layer thick (Fig. 1A&B). The main root was oval shaped in the cross section with two dimensions of about 800 and 1250 µ for the whole section and 375 and 437µ for its vascular cylinder

Table 1.Some anatomical measurements (µ) of the transition region in lupine seedling at the age of 7 days.

Level of the seedling axis	Dimensions of the whole cross section	Mean	Dimensions of the vascular cylinder	Mean	Mean cortex thickness
Root:					
Midpart	812&1250	1031	375&437	406	325
4cm.below the soil surface.	1037&1625	1331	475&625	550	406
At the splitting of the protoxylem.	1437&1625	1531	625&662	643	437
At the endarch arrangement.	1937&2375	2156	687&875	781	690
Hypocotyl:					
Basal portion					
At tangential extension of mxand px.	2562&2812	2687	875&1125	1000	795
At arrangement of px. in radial rows.	2687&3937	3312	1000&1187	1093	908
Midpart	3025&4056	3540	1187&1562	1374	1112
Upper portion	3120&4680	3900	1248&1560	1404	1162

Transverse sections of the basal portion of the main root exhibited that transitional development has been started at the level of about 4 cm below the soil surface by the appearance and proliferation of parenchymatous cells between the large central vessels . As a result the vessels separated into two distinct units and a pith differentiated (Fig.1C) . Each unit consists of large number of protoxylem vessels extended centripetally up to the periphery of the developing pith. The metaxylem, instead of differentiating toward the center, diverges laterally from the protoxylem of each unit. The groups of phloem cells are extended tangentially to form two crescent-shaped sections. The whole sectional area as well as vascular cylinder increased in diameter (Table 1).

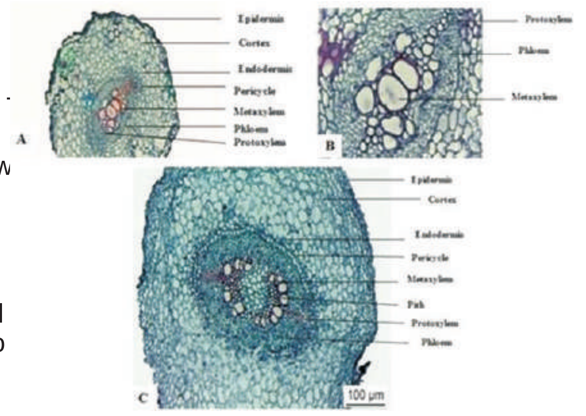

Fig.1: A. Transverse section through the mid-part of the main root of 7-day- old seedling showing its primary structure (x100).

B. Magnified portion of A (x400).

C. Transverse section through the basal portion of the root, about 4cm.below the soil surface, at the beginning of vascular transition (x100).

At higher level, the area of pith increased and each group of the primary xylem divided into two sectors through the splitting of the protoxylem strand (Fig.2A).As a result four groups of primary xylem was formed. Metaxylem of each group arranged tangentially in one or two rows. Part of protoxylem cells of each strand arranged tangentially on the inner side of the metaxylem and the other part appeared in radial row at the primary site of protoxylem strand. At this level of the main root, the vascular cylinder appeared nearly round in shape (Fig.2B).

Transections of the basal portion of the primary root directly below the soil surface show that the vascular cylinder was elliptical in shape. The primary xylem appeared in endarch arrangement due to the concentration of protoxylem vessels on the adaxial side of the metaxylem of each group, and the primary phloem located on the abaxial side of metaxylem. Thus, four endarch collateral vascular bundles were formed. In the cross section the endarch primary xylem of each bundle was nearly triangular in shape, in which the metaxylem constitutes the base of the triangle and protoxylem occupies its inward apex (Fig.2C).

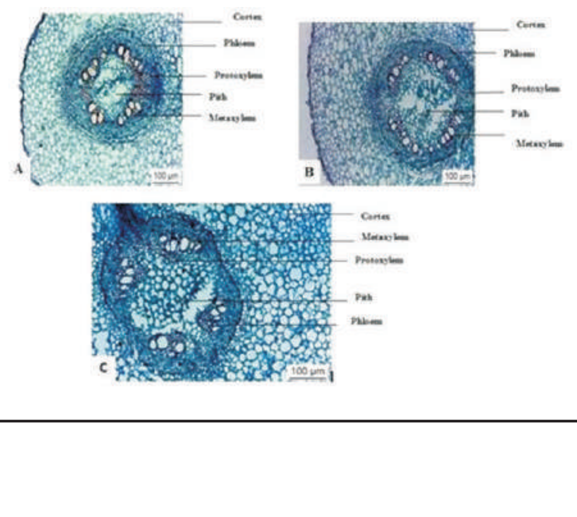

Fig.2: Transverse sections through different levels of the basal portion of the main root of 7-day-old seedling.

A. About 3cm. below the soil surface. Notice the splitting of protoxylem strands.
B. About 2cm. below the soil surface. Notice the increased pith area, arrangement of vascular tissues in four groups and differentiation of protoxylem elements on the inner side of metaxylem.
C. Directly below the soil surface. Notice the endarch collateral arrangement of vascular tissues.

Transverse sections of the basal portion of the hypocotyl, where the epidermis of the transition region is glabrous and cutinized, exhibited that at this level of the seedling axis the diameter of the whole section increased due to the increase in pith area, thickness of cortex and diameter of vascular cylinder (Table 1). At this level each of the endarch collateral vascular bundles extended tangentially that metaxylem as well as protoxylem elements differentiated in one or two tangential rows, developing four vascular plates with higher number of xylem vessels inwardly and primary phloem outwardly (Fig.3A).

At the higher levels of the hypocotyl, tangential dilatation of each vascular plate increased and protoxylem segmented into small groups of vessels and then arranged in radial rows (Fig.3B). Metaxylem as well as primary phloem of each vascular plate is dissected into small groups. As a result ten cauline vascular bundles were differentiated through the mid and upper parts of the hypocotyl (Fig. 3C&D).

Transverse sections through the upper half of the hypocotyl showed that the vascular system arranged in two arcs, the lateral bundles of the arcs constitute the cotyledonary traces, two large bundles for each cotyledon, and the other six bundles established traces of the first two foliage leaves (Fig.4A). Directly below the cotyledonary node, traces of the cotyledons diverged away from the vascular cylinder leaving two wide gaps (Fig. 4B).

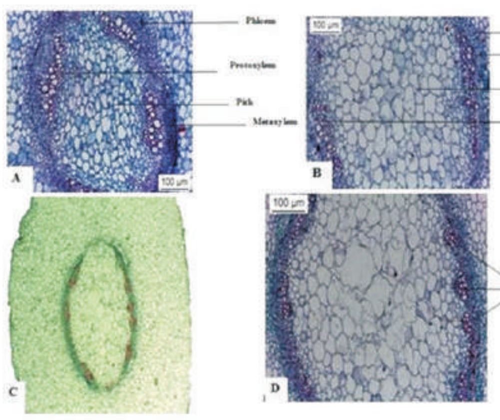

Fig. 3: Transverse sections through the basal half of the hypocotyl of 7-day-old seedling.

A. Directly above the soil surface. Notice the tangential extension of the four vascular groups. (x100)
B. At higher level of the basal portion. Notice the increased tangential dilatation of each vascular plate and differentiation of protoxylem elements in radial rows (x100).
C. At the midpart of the hypocotyl. Notice the development of cauline vascular bundles (x40).
D. Magnified portion of C (x100).

At the cotyledonary node the vascular system of the cotyledonary plate was made up of cotyledonary bundles and the bundles of the foliage leaves. With regard to the cotyledonary bundles, they were two at first and then each bundle divided in cotyledon petiole or midvein into two smaller ones producing four bundles for each cotyledon. With respect to the bundles of the foliage leaves, three bundles were differentiated for each of the first two leaves. Procambial strands of foliar traces of the next two leaves, (third and fourth ones) appeared between the bundles of the first and second leaves except in the region of the two cotyledon gaps (Fig.4C).

Transverse sections through the epicotyl, just above the cotyledonary node, revealed that the traces of the first two leaves showed more differentiation and procambial strands developed in the two cotyledonary gaps (Fig. 5A). At higher level of the epicotyl the traces of the first two leaves diverged away from the vascular cylinder which made up of provascular strands of the next two leaves (Fig. 5B).

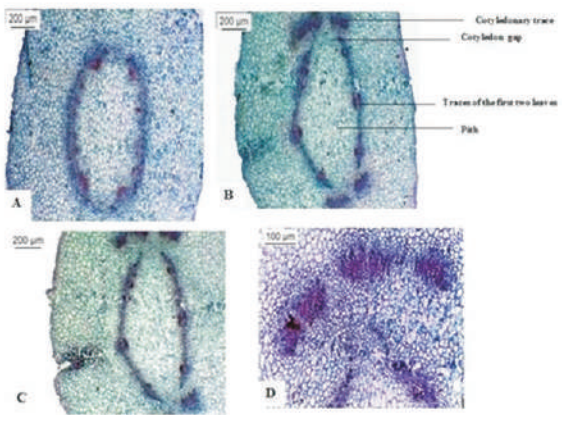

Fig.4:A. Transverse section through the upper portion of the hypocotyl of 7-day-old seedling. Notice the arrangement of vascular bundles in two arcs, and differentiation of the lateral bundles as cotyledonary traces (x40).

B. Transverse section through the upper hypocotyl, just below the cotyledonary node. Notice the divergence of cotyledonary traces, two bundles for each cotyledon, and differentiation of traces of the first two foliage leaves (x40).
C. Transverse section of the cotyledonary node. Notice the formation of four bundles for each cotyledon through the division of each bundle of cotyledonary traces into two smaller ones (x40).
A. Magnified portion of C (x100).

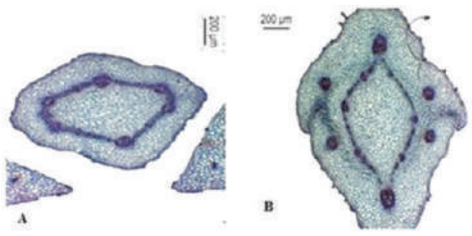

Fig.5: A. Transverse section through the basal portion of the epicotyl of 7-day-old seedling. Notice the formation of provascular tissues incontinuous ring and differentiation of traces of the first two leaves(x40).

B. Transverse section through the upper portion of the epicotyl. Notice divergence of the traces of the first two leaves, three bundles toward each leaf primordium, and development of procambial strands which constitute the traces of the next two leaves (x40).

Secondary growth:

Transverse sections of midpart of the primary root of4-week-old seedling showed that the vascular cambium developed in the fundamental parenchyma lying between the metaxylem and the primary phloem. No active cambium was observed in the pericyclic parenchyma abutting the protoxylem points. Few amounts of secondary phloem were produced, and parenchyma of the primary phloem showed thick walls and extended circumferentially. The vascular cylinder was elliptical in shape with two dimensions of about 561 and 625µ. The mean diameter of the whole section was about 1794µ

At the beginning of vascular transition, the cross sections of main root, few cm. below the soil surface, appeared the vascular tissues in two groups. The establishment of vascular cambium and its activity between the primary phloem and metaxylem were restricted to these two vascular groups. The diameter of the whole section was increased due to the increase in the amount of secondary vascular tissues, specially xylem, and formation of pith. Root diameter was about 1918µ and the two dimensions of the vascular cylinder were about 780 and 936µ (Fig.6A).

Transections of the main root directly below the soil surface exhibited four vascular groups with more amounts of secondary vascular tissues, and interfascicular cambium just initiated in the regions between these four vascular groups. More wall thickening of primary phloem parenchyma was observed (Fig.6B). The diameter of the root increased up to about 3150µ. The vascular cylinder attained two dimensions of about 1248 and 1560µ

Cross sections through the basal portion of the hypocotyl, just above the soil surface, revealed that the diameter of the seedling axis reached its maximum value at this level, being 3588µ. This increase in axis width was due mainly to the increments of the produced secondary tissues and pith area. The two dimensions of the vascular cylinder including the pith were about 1497 and 1778µ. While there was relative increase in the amounts of the thickened walled cells of the primary phloem there was decrease in the degree of thickening of their walls comparing with the corresponding cells in the basal portion of the root. Walls of xylem vessels showed also low lignification (Fig. 6C).

Progressing upward, due to the activity of interfascicular cambium, a continuous ring of secondary xylem was formed, though little amounts of vascular tissues produced by interfascicular cambium. Hollow pith cavity was developed due to the breakdown of cells of the central region of the pith (Fig. 6D & E). At this level both diameter of the hypocotyl and that of its vascular cylinder decreased compared to the corresponding aspects at the lower level of the hypocotyl.

Transections through the upper half of the hypocotyl showed that the vascular tissues arranged in two opposite arcs due to low or even no activity of vascular cambium in two regions, in the cotyledonary plane (Fig.7A). No clear diferences were observed in the diameter of the hypocotyl or that of vascular cylinder between this level of the hypocotyl and the lower one.

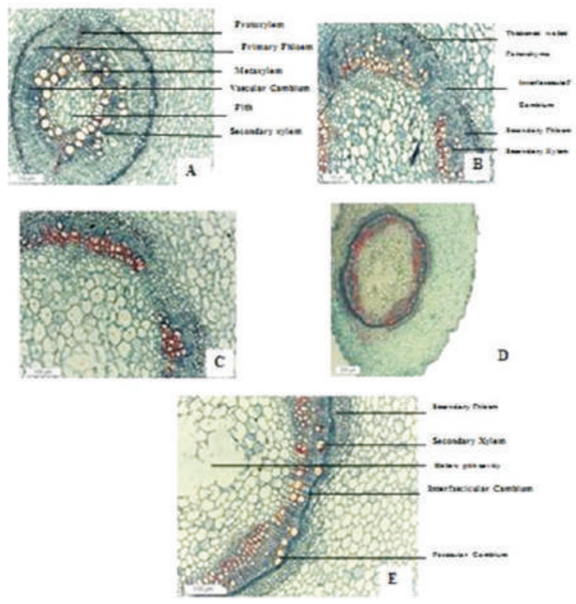

Fig. 6: A. Transverse section through the basal portion of the main root, about 4 cm. below the soil surface. Notice the arrangement of vascular tissues in two groups, and establishment of vascular cambium in these two vascular groups (x100).

B. Transverse section through the basal portion of the main root, directly below the soil surface. Notice the developed four vascular groups, initiation of interfascicular cambium, well lignified of walls vessels and wall thickening of primary phloem parenchyma (x100).

C. Transverse section through the hypocotyl base, just above the soil surface. Notice interfascicular cambium initiation, low lignification of vessels walls and also low wall thickening of primary phloem parenchyma (x100).

D. Transverse section through the basal portion of the hypocotyl, about 1cm. above the soil surface. Notice the continuous ring of both vascular cambium and secondary xylem, well lignified walls of vessels and formation of pith cavity(x40).

E. Magnified portion of D (x100).

Progressing upward, toward the cotyledonary node, the hypocotyl gradually increased in width. This increment was accompanied with increase in diameter and thickness of vascular cylinder. Moreover xylem elements showed more lignification at the upper hypocotyl (Fig.7 B&C).

Directly below the cotyledonary node no vascular cambium developed in cotyledon gaps which were occupied by parenchymatous cells with intercellular spaces. At the cotyledonary node, the vascular cambium initiated in cotyledon gaps and a continuous circle of vascular cambium was developed. In the epicotyl, directly above the cotyledonary node, a continuous ring of vascular tissues was observed.

It is of interest to notice here that in the upper portion of the hypocotyl, the cauline vascular bundles from which the cotyledonary traces diverged, showed normal secondary growth, but the traces which diverged from them were entirely in the primary state (Fig. 7C&D).

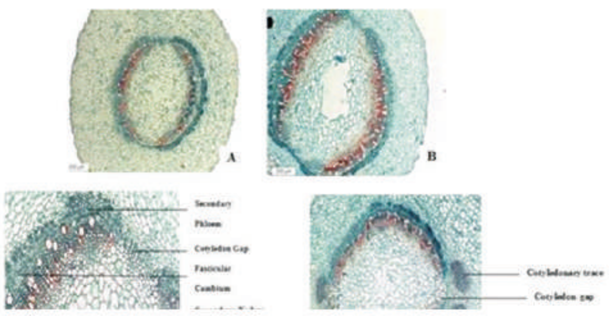

Fig. 7: A. Transverse section through the midpart of the hypocotyl. Notice the arrangement of vascular tissues in two arcs and relatively low amount and maturation of vascular tissues(x40).

B. Transverse section through the upper hypocotyl, near the cotyledonary node. Notice more amount and maturation of vascular tissues, and normal secondary growth in vascular bundles from which the cotyledonary traces will diverge(x40).

C. Magnified portion of B (x100).

D. Transverse section through the upper hypocotyl, just below the cotyledonary node. Notice that the cotyledonary traces are entirely in the primary state (x40).

DISCUSSION

From the foregoing results, it could be concluding that the vascular transition in lupine started at the basal portion of the root, about four centimeters below the soil surface. Moreover, the endarch arrangement of primary xylem and overlapped (collateral) structure took place at the superior end of the root, directly below the soil surface. This result is more or less in accordance with that found in Mirabilis jalapa L. by Rugina et al. (2006). In that regard, Beck (2010) mentioned that transition commonly takes place over a short distance in the hypocotyl of the embryo in provascular tissue, but in some species, e.g., Pisum sativum L., it may extend through several internodes. These changes which would not be conspicuous in the embryo would however, be clearly expressed upon differentiation of primary vascular tissues in the axis of the developing sporophyte. Esau (1965) indicated that in many species the change and orientation of vascular tissue is completed in the cotyledons. A transition of this type has been described in Arabidopsis thaliana by Busse and Evert (1999). In this respect, El-Shaarawi et al. (2011) reported that vascular transition in soybean started at the basal portion of the root and completed in the upper hypocotyl, directly below the cotyledonary node.

The theory of rotation and torsion is assimilated by the majority of the botanists in their studies regarding the histogenesis of the vascular system (Rugina et al., 2006) but, as we have already seen, no rotation was detected during the vascular transition in lupine, instead, the protoxylem elements gradually differentiated on the adaxial side of the metaxylem.

Relatively little is known about the relation between the vascular system of the epicotyl and that of the root-hypocotyl-cotyledon unit. Because the epicotyl generally does not participate in transition, it is often not considered in studies on vascular transition (Busse and Evert 1999).In Linum, the endarch collateral bundles of the epicotyl begin to differentiate basipetally, downward into the hypocotyl.These bundles move toward the metaxylem and metaphloem of the transition region and link to them, forming a continuous conduit between root and stem. The bundles may initially terminate blindly in the hypocotyl parenchyma,but as the formation of metaxylem and metaphloem continuous, linkage occurs (Crooks, 1933 and Mauseth, 1988). In the present work the traces of the first two leaves, as well as cotyledonary traces differentiated along the upper half of the hypocotyl, and well recognized near the cotyledonary node at the cotyledonary node two bundles diverged toward each of the two cotyledons and the traces of the first two leaves, 3 for each leaf, passed upward into the epicotyl and then diverged toward the first two leaf primordia, whereas 6 provascular bundles were differentiated to constitute the traces of the next two leaves . This led to the suggestion that in lupine there is continuity between the vascular system of the epicotyl and that of the hypocotyl through the cotyledonary node. In this connection Grassley (1932) on Raphanus sativus mentioned that change from root structure to stem structure occurs in the upper hypocotyl.The vascular tissue of the hypocotyl at this level form a siphonostele made up of two cotyledonary traces and two leaf traces of the foliage leaves.

Despite the completion of transition of vascular system from exarch radial to the endarch collateral took place at the root base, vasculature of the hypocotyl axis varied according to its level. At the basal portion of the hypocotyl, there were four endarch collateral vascular plates with protoxylem, metaxylem and phloem extended