J. Agric. Sci. Mansoura Univ., 33 (7): 5005 - 5016, 2008

ANATOMY OF THE TRANSITION REGION OF *Vigna radiata* (L.) Wilczek. (MUNG BEAN).

El-Shaarawi, A. I.; Awatif A. Megahed and Rania M. A. Nassar
Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

Seedlings of *Vigna radiata* (L.) Wilczek (Mung bean) were examined by light microscopy to study vascular development through the transition region. The beginning of vascular transition took place about one centimeter below the soil surface by the appearance of a pith between the large central vessels. At the higher level, pith parenchyma increased and the vessels are separated into four groups. The protoxylem maintains its peripheral position, but the metaxylem instead of differentiating toward the center, diverges laterally from the protoxylem. At successively higher levels, the hypocotyl axis widens and the distances between the protoxylem poles as well as the divergence of each xylem arms increased. The reorientation of primary xylem took place gradually upward. The length of the transition region from a point where the axis is exarch and protostele to a point where the xylem form approximately endarch condition was about two centimeter. Thus, most stages of vascular transition in mung bean seedling occur in the lower portion of the hypocotyl, and the reorientation of vascular tissue from exarch radial to the endarch collateral arrangement may be completed in the upper portion of the hypocotyl.

The behavior of vascular cambium activity and the difference in maturation degree at the different levels of the hypocotyl indicating that the upper portion of mung bean hypocotyl is older than the lower one. Adventitious root primordia were recorded at the basal portion of hypocotyl and their development was associated with the activity of vascular cambium.

Keywords: Mung bean, *Vigna radiata* (L.) Wilczek, transition region.

INTRODUCTION

The interface between the root and the shoot is a problem, because the primary vascular tissues of the two organs are arranged differently. In the root, there is an exarch protostele of xylem with independent phloem strands. This must be made to merge smoothly with the endarch collateral bundles of the shoot. The area where this happens is called the transition region, and is usually encompasses all or part of the hypocotyl.

The arrangement 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 the seedling axis that shows the features of transition is variable. At one extreme, the transition region is short and restricted to the upper part of the hypocotyl and part of the cotyledons (*Lactuca sagitata,* Lee 1914; *Beta vulgaris*, Artschwager 1926; *Raphanus sativus*, Grassley 1932; *Melilotus alba,* McMurry and Fisk 1936; *Descurainia pinnata,* Dittmer and Spensley 1947; *Arabidopsis thaliana,* Busse and Evert 1999). At the other extreme the transition region is very long, extending from the root into one or more internodes above the cotyledons (e.g., *Pisum sativum*,Gourley 1931; other dicotyledons with hypogeous cotyledons, Compton 1912, and Muller 1937). The extent of the region is determined partly by the manner in which the hypocotyl elongate (Millor and Wetmore 1945; Bisalputra 1961; Kang and Soh 1991).

The present work was carried out to throw some light on the structure and vascular development of the transition region of *Vigna radiata* (L.) Wilczek. (Mung bean) seedlings. The vascular system showing transitional characteristics is entirely primary; therefore, for an understanding of its development and structure, it is essential to examine the young seedling before the primary tissues are obscured by secondary growth.

MATERIALS AND METHODS

The current investigation was carried out at the green house of Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the season of 2007 to study the structure of the transition region of mung bean plant.

Seeds of mung bean (*Vigna radiata*) cv. Kawmy 1 were obtained from Field Crops Research Institute, National Research Centre, Dokki, Giza, Egypt. Seeds of mung bean were sown on 3rd June, 2007 in black plastic pots, 30 cm diameter, filled with clay and sand at the ratio of 1 : 1 by weight.

Anatomical studies were carried out on specimens as follows:

1- Tap root at its basal and median portions (at the age of 7 days).
2- Hypocotyl at its basal and middle portions (at the age of 7 and 10 days).
3- Epicotyl (at the age of 7 days).
4- Hypocotyl at its basal and upper portions (at the age of 28 days).

Specimens were killed and fixed for at least 48 hrs. in FAA solution, washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series and embedded in paraffin wax of melting point 56° c, sectioned to a thick of 20 microns, double stained with crystal violet – erythrosine, cleared in xylene and mounted in Canada balsam (Willey, 1971). Slides were examined microscopically and photomicrographed.

RESULTS

Transverse sections were made in main root, hypocotyl at different levels, and epicotyl of 7 – day – old seedlings. Structure of the hypocotyl was also studied at the age of 10 and 28 days.

Cross sections through the midpart of main root of 7–day–old seedling revealed that the primary root has an exarch, radial and tetrarch protostele (Fig. 1. A). At the center of the stele, there are four to six large metaxylem vessels which are sometimes separated by smaller xylem elements. There are three to six elements in each protoxylem groups. On alternate radii to the protoxylem are four groups of primary phloem that are separated from the central metaxylem elements by parenchyma. The pericycle and endodermis are single layered. The cortical cells are isodimetric with intercellular spaces, except for compact hypodermal layer in which the cells resemble the epidermal cells. The thickness of cortical parenchyma ranged between 243 and 300 μ . The diameter of the whole sectional area was about 900 μ , while that of vascular cylinder was about 300 μ .

The first transitional development is the increase in the number of parenchymatous cells between the large central vessels. As a result, the vessels are separated into four groups. The protoxylem maintains its peripheral position, but the metaxylem, instead of differentiating toward the center, diverges laterally from the protoxylem. Such order of differentiation leaves the center of the axis unoccupied by vascular elements. In other words, a pith differentiates in this part of the seedling (Fig. 1.B).

At the higher level, the metaxylem differentiates with higher number of large vessels in a ring surrounding cenrally located parenchymatous cells (Fig.1.C).

It is of interest to notice that this change in vascular plan occurred in a segment of the axis, which is about a centimeter in length, consider from the external view as a root, because it developed lateral roots and root hairs (Fig.1.B). The diameter of axis at this level was about 1500 μ , while that of the vascular cylinder was about 480 μ . Cortex thickness ranged between 450 and 600 μ .

At successively higher levels where the epidermis of the transition region is smooth and cutinized, the hypocotyl axis widens and exhibited its maximum diameter, about 2200 μ . Pith parenchyma collapsed, except few peripheral layers, and pith cavity appeared. Diameter of pith area, including pith cavity was about 960 μ , and thickness of cortical parenchyma ranged between 480 – 600 μ .

At this level, the distance between the protoxylem poles increased. Concomitantly the plates of metaxylem associated with each protoxylem pole do not join. Thus instead of one xylem plate as in the root, there are, higher up, four distinct xylem complexes. Each protoxylem strand devided into two sectors owing to the fact that the cells of the central region remain parenchymatous instead of differentiating into metaxylem (Fig. 2.A). In other words, four V- shaped groups of primary xylem in which the protoxylem occupies the outward apex of the V and the metaxylem the extended arms inward are formed.

The divergence of each two xylem arms increased gradually at the higher levels of the hypocotyl and protoxylem and metaxylem become in a tangential plane (Fig. 2.B). In the sequent stages of transition, the two xylem arms of each group separated and the protoxylem pole of each arm occupies a deeper position in the axis than the metaxylem. This orientation signifies that the xylem approaches the endarch condition (Fig. 2.D). Though differentiation of some metaxylem vessels being laid down in tangential direction.

It is worthy to notice that parenchymatous cells, might be of pericyclic in origin, outside the xylem arms exhibited active cell divisions and radial elongation (Fig. 2.D).

Fig. (1): A: Cross section through the main root in primary state.
B and C: Cross sections through the axis at the beginning of vascular transition.
Details: en = endodermis, lr = lateral root, Pe = pericycle, Pi = pith, Px = protoxylem, mx = metaxylem,
Notice the appearance of pith in B and C and the beginning of metaxylem divergence in C.

Fig. (2): Transverse sections through the basal portion of the hypocotyl of 7-day-old seedlings showing the divergence and gradual reorientation of primary xylem from exarch (A) to approximately endarch condition (D).

Details: co = cortex, pf = proliferated and radially elongated parenchyma, pi = pith, px = protoxylem, mx = metaxylem.

The length of the transition region from a point where the axis is exarch and protostele to a point where the xylem form nearly endarch condition was about two centimeter.

At the halfway of the hypocotyl, the axis decreased in width. Diamter of the whole cross sectional area was about 1400 μ, and thickness of the cortical parenchyma ranged between 150 and 180 μ. Diameter of pith area was about 780 μ. At this level of the seedling axis, the centrifugal differentiation of primary xylem attained and endarch condition may be completed (Fig. 3.B).

Transections of the epicotyl just above the cotyledons revealed that the vascular tissues developed as endarch collateral bundles (Fig. 3.D).

The differences in the orientation of the phloem at various levels of the seedling are less pronounced than those of the xylem. Instead of the four phloem strands appearing in the root, there eight in the hypocotyl. Considering the structure from the base upward, one could say that the phloem branches, each phloem strand of the root giving two branches in the hypocotyl. Each of the eight hypocotylary phloem strands is associated with one metaxylem plate. In the part of the hypocotyl where the xylem is endarch, the phloem differentiates on the abaxial side of the bundle. This bundle is, therefore, collateral.

Secondary growth:

Transverse sections of 7–day–old seedlings revealed that at the beginning of vascular transition the diameter of axis increased gradually upward and reached its maximum value at the basal portion of the hypocotyl where the divergence of metaxylem arms took place. The increment of axis width was primary and due mainly to the increase in pith area. At this level of the hypocotyls, the vascular cambium was just initiated between the phloem strands and primary xylem.

Progressing upward, the hypocotyl gradually decreased in width and at the middle portion of the hypocotyl of 7–day–old seedling was about 1500 μ. At this level, the formation of the vascular cambium was more progressive and some mature xylem elements were observed (Fig. 3.A and B).

When the seedlings were 10 days old, the vascular cambium was estaplished in the middle part of the hypocotyl as a continuous ring between the produced secondary xylem and secondary phloem (Fig. 3.C). While at the lower hypocotyl, it was discontinous and only immature secondary vascular elements were observed.

At the age of 4 weeks, the lower portion of the hypocotyl still wider than the upper one, though the later have higher thickness of secondary xylem. The diameters of the axis were about 1950 and 1760 μ for the lower and upper portions, respectively. The thickness of secondary xylem in the cross section was about 290 μ in the upper portion against 250 μ in the lower one. (Fig. 4.C and D).

Fig. (3): and C: Transverse sections through the midpart of the hypocotyl.

B: Magnified portion of A.

A and B: 7-day-old seedlings showing the endarch condition and separation of the protoxylem poles of primary xylem and the beginning of secondary growth.

C:10-day-old seedling showing the formation of vascular cambium in a continuous ring, and considerable amount of secondary tissues was produced.

D: Cross section through the epicotyl of 7-day-old seedling showing the endarch collateral bundles.

Details: co = cortex, mx = metaxylem, pi = pith, pph= primary phloem, px = protoxylem, sph = secondary phloem, sx = secondary xylem, vc = vascular cambium.

Fig. (4): A and B: Cross sections through the basal portion of the hypocotyl of 10- day-old seedling at the beginning of secondary growth showing formation of adventitious root primordium.
C and D: Cross sections through the hypocotyl at the age of 28 days.
C: At the basal portion.
D: At the upper portion.
Details: rp = root primordium, prx = primary xylem, sph = secondary phloem, sx = secondary xylem.
Notice the differences in maturation and amount of secondary tissues between the two levels of the hypocotyl.

It is worth mentioning that adventitious root primordia were recorded at the lower hypocotyl and their development was associated with the vascular cambium activity. No adventitious root primordia were observed when the lower hypocotyl was in the primary state, 7-day-old seedling. It originated only when secondary growth took place at the age of 10 days specially opposite the primary xylem poles (Fig. 4.A and B). This observation led to the suggestion that the adventitious roots might be initiated by divisions in the cambial zone.

DISCUSSION

The vascular transition in mung bean started one centimeter below the soil surface in portion of the axis developed lateral roots and root hairs. In that respect, Whiting (1938) mentioned that the lower face of the transition region in *Cucurbita maxima* may bear root hairs. Busse and Evert 1999, found that in *Arabidopsis thaliana* numerous hairs arise from the epidermis at the base of the hypocotyl. Most stages of vascular transition occurred in the lower portion of the hypocotyl. In that regard, the transition region of mung bean resembles that of *Gossypium* (Spieth 1933 and Hayward 1938) and *Cucurbita maxima* (Whiting 1938 and Hayward 1938). On the other hand many authors, on different plants, found that the transition region restricted to the upper part of the hypocotyl and part of the cotyledons *(Lactuca sagitata*, Lee 1914; *Beta vulgaris*, Artschwager 1926; *Raphanus sativa*, Grassley 1932; *Cannabis sativa,* Berkman 1936; *Arabidopsis thaliana* Busse and Evert 1999).

The results indicate that just above the cotyledons, the vascular tissues of the epicotyl have developed as endarch collateral bundles. In other words, the transition region in mung bean may be restricted to the hypocotyl. In other plants of the same family 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 so that the stele is an endarch dictyostele until the fourth internode is reached (Compton 1912 and Gourley 1931). Unlike the pea, the transition in *Medicago sativa* is entirely hypocotyledonary, and the reorientation of the vascular from exarch radial to the endarch collateral

arrangement is completed in the veins of the cotyledons (Compton 1912 and Winter 1932). Thus, it could be concluded that the extent of the transition region is determined partly by the manner in which the hypocotyl elongate (Bisalputra 1961 and Kang and Soh 1991).

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 continues, linkage occurs (Crooks 1933 and Mauseth 1988).

With respect to the secondary growth in the transition region, it is found that the activity of vascular cambium varied according to the level of hypocotyl axis and seedling age. At the basal portion of the hypocotyl of 7–day–old seedling, the vascular cambium was just initiated. At the higher levels, the secondary growth was evident although largely confuned to the regions between the phloem strand and primary xylem plate in the hypocotyl. At the age of 10 days, the vascular cambium appeared as a continuous ring in the upper hypocotyl and produced considerable amounts of secondary xylem in continuous ring and secondary phloem rich in fibers.

With continued secondary growth and the interpolation of secondary vascular tissues between the primary phloem and primary xylem, the primary vasculature was further disrupted as exemplified by the 28-day-old seedlings. These results are more or less in accordance with that found by Busse and Evert (1999) on *Arabidopsis thaliana*. However they found that opposite the protoxylem poles, the pericycle derived vascular cambium gave rise largely to relative wide parenchymatous rays.

The behavior of vascular cambium activity at the different levels of the hypocotyl indicating that the upper portion of mung bean hypocotyl is older than the lower one. In addition the immature condition of the lower part of the hypocotyl as compared with the middle and upper ones is due to the fact that the former is a region of continued axial elongation resulting from activity of an intercalary meristem. On the contrary, the region of intercalary meristem responsible for axial elongation is found in the upper hypocotyl of *Gossypium* (Spieth 1933).

As mentioned by many authors the parenchymatous cells involved in the process of transition through the formation of pith at the beginning of transition and divergence of metaxylem and increasing the distances between the transitional bundles (Spieth 1933; Whiting 1938; Esau 1965 and Busse and Evert 1999).

In the present study an additional role of parenchyma was recorded. It might be involved in the reorientation of primary xylem by pushing the protoxylem poles inward through its proliferation and radially elongation outside the tangentially oriented primary xylem poles. This parenchymatous cells seem to be pericyclic in origin. In this respect Spieth (1933) found that in *Gossypium*, except over the protoxylem points, where the tissue remains parenchymatous, thick–walled pericyclic cells abut the endodermis, which becomes discontinuous, retaining its identity only over the transition bundle. Busse and Evert (1999) reported that in the hypocotyl of *Arabidopsis thaliana* seedling the pericycle cells external to the phloem had proliferated.

REFERENCES

Artschwager, E. (1926). Anatomy of the vegetative organs of the sugar beet. J.Agric. Res., 33 : 143 – 176.

Berkman, A.H. (1936). Seedling anatomy of *Cannabis sativa* L. Ph.D. Thesis, Univ. of Chikago.

Bisalputra, T. (1961). Anatomical and morphological studies in the chenopodiaceae. II. Vascularization of the seedling. Aust. J. Bot., 91 : 1 - 19.

Busse, J.S. and R.F. Evert (1999). Vasuler differentiation and transition in the seedling of *Arabidopsis thaliana* (Brassicaceae) Int. J. Plant Sci., 160 (2): 24 – 251.

Compton, R.H. (1912). An investigation of the seedling structure in Leguminosae. J. Linn. Soc. Lond. Bot., 41 : 1 – 122.

Crooks, D.M. (1933). Histological and regenerative studies on the flax seedling. Bot. Gaz., 95: 209 – 239.

Dittmer, H.J. and R.D. Spensley (1947). The developmental anatomy of *Descurainia pinnata obroleuca* (woot) Detling. Univ. NM. Publ. Bio., 13 : 1 – 47.

Esau, k. (1965). Plant anatomy. Wiley, New Yourk.

Gourley, J.H. (1931). Anatomy of the transition region of *Pisum sativum*. Bot. Gaz., 32 : 367 – 383.

Grassley, F.E. (1932). The anatomy of the primary body of *Raphanus sativus* L. M.Sc. Thesis, University of Chicago.

Hayward,H.E. (1938). The structure of economic plants. Macmillan, New York.

Kang, K.D. and W.Y. Soh (1991). Vascular transition and hypocotyl eloangation in soybean *(Glycine max)* seedlings. Korean J. Bot., 34 : 275 – 281.

Lee, E. (1914). Observations on the seedling anatomy of certain sympetalae. II. Compositae. Ann. Bot., 28: 203 – 329

Mauseth, J.D. (1988). Plant anatomy. The Benjamin / Cammings, California.

McMurry, E.B. and E.L. Fisk (1936). Vascular anatomy of the seedling of *Melilotus alba*. Bot. Gaz., 98 : 121 – 135.

Millor, H.A. and R.H. Wetmore (1945). Studies in the developmental anatomy of *Phlox drummondii* Hook. II. The seedling.Am. J. Bot., 32 : 628 – 634.

Muller, C. (1937). La tige feuillée et les cotylédons des viciées a germination hypogée. Cellule 46 : 195 – 354.

Spieth, Alda M. (1933). Anatomy of the transition region in *Gossypium*. Bot. Gaz., 95 : 338 – 347.

Whiting, A. Geraldine (1938). Development and anatomy of Primary structures in the seedling of *Cucurbita maxima* Duchesne. Bot. Gaz., 99 : 497 – 528.

Willey, R.L. (1971). Microtechniques: A Laboratory Guide. Macmillan Publishing Co., Inc., New Yourk. 99 PP.

Winter, Clara W. (1932). Vascular system of young Plants of *Medicago sativa*. Bot. Gaz., 94 : 152 – 167.

التركيب التشريحى لمنطقة التحول فى فول المانج

عبد الفتاح ابراهيم الشعراوى ، عواطف عزيز الدين مجاهد و رانيا محمد عبد العزيز نصار
قسم النبات الزراعى - كلية الزراعة – جامعة القاهرة – جيزة – مصر.

تم فحص بادرات فول المانج بواسطة الميكروسكوب الضوئى لدراسة التطور الوعائى خلال منطقة التحول. ولوحظ بداية التحول الوعائى أسفل سطح التربة بحوالى ١ سم و ذلك بظهور النخاع بين أوعية الخشب التالى المركزية. و فى مستوى أعلى حدثت زيادة فى بارنشيمة النخاع و انفصال الأوعية فى أربعة مجاميع. و يظل الخشب الأول فى وضع خارجى بينما ينفرج الخشب التالى الى جانب الخشب الأول بدلاً من تكشفه جهة المركز. و فى مستويات أعلى متتالية حدث اتساع فى محور السويقة الجنينية السفلى و زادت المسافة بين أقطاب الخشب الأول و كذلك درجة انفراج أذرع الخشب. و يتم اعادة ترتيب الخشب الابتدائى تدريجياً كلما اتجهنا الى أعلى. و يبلغ طول منطقة التحول من النقطة التى يكون فيها المحور خارجى الخشب الأول و العمود الوعائى أولى الى النقطة التى يكون فيها الخشب الأول داخلى تقريبا ٢ سم. و بذلك تتم معظم مراحل التحول الوعائى فى بادرات فول المانج فى الجزء القاعدى من السويقة الجنينية السفلى و لكن يكتمل اعادة ترتيب الأنسجة الوعائية من الترتيب القطري خارجي الخشب الأول الى الجانبى داخلى الخشب الأول بالجزء العلوى منها.

يشير سلوك نشاط الكامبيوم الوعائي و الاختلاف في درجة النضج في المستويات المختلفة من السويقة الجنينية السفلى إلى أن الجزء العلوي منها أقدم من الجزء السفلى. وقد تم ملاحظة تكون بداءات الجذور العرضية في الجزء القاعدي من السويقة الجنينية السفلى و قد ارتبط ذلك بنشاط الكامبيوم الوعائى.