

THE EFFECTS OF N, P, Ca AND Fe DEFICIENCY IN LINUM USITATISSIMUM L. IN CAMBIAL ACTIVITY AND DIFFERENTIATION

G. ALGAN*, B. TİLEKLIOĞLU**

* Department of Biology, University of Ankara, Faculty of Science Ankara/Turkey.

** Department of Biology, University of 100. Yıl, Faculty of Science Van/Turkey.

ABSTRACT

In the radial growth inhibition of *Linum usitatissimum* L. The most important material deficient was N followed by P and Ca. The least deficient material was found to be Fe. There observed a correlation between the shoot growth and the radial growth when compared with the measured lengths of the plants. The phloem and Xylem differentiation was found to be effected by deficiency of all ions to different extent. The xylem formation was effected most by the deficiency in P while phloem formation was effected most by the deficiency in Ca and N. The deficiency of P effected the fiber formation most.

INTRODUCTION

The importance of macroelements such as N,P, Ca and Fe necessary for plants life upon the plant's growth, development and yield has been known for a long time. If these elements are deficient some of biochemical reactions cannot occur and the physiological activities of the plant go down to a minimum level. This consequently causes the inhibition of growth in the plant and some defects in it. These can easily be seen in the morphological and the anatomical structure of the plant. There are a lot of studies dwelling upon how the deficiency of these minerals effects the normal growth and the development of plants.

Maki (1961), Viro (1965), Terentev et al. (1974) showed that N has a positive effect upon the growth of the plants. Humprey et al. (1977) investigated the protein content in N deficiency, Sugiyama and Sugiyama (1979) proved that N deficiency caused the reduction in the soluble protein and chlorophyll content.

Hobbs (1944), Lyon and Garcia (1944) and Walker et al. (1955) investigated the visible effects caused by P deficiency.

Davis (1949) determined that Ca deficiency caused the reduction of xylem and phloem in *Pinus taeda*. Millikan (1953) claimed that Ca deficiency inhibits the formation of fibers in flax. Walker et al. (1955), Pissarek (1979) and Higaki et al. (1980) examined the effect of Ca deficiency in plants.

Branton and Jacobson (1962) who examined the Fe deficiency, stated that iron is carried to the ends points of the plants. In 1964 Brown et al. obtained similar results. Del Rio et al. (1978) investigated the physiological effects of Fe deficiency in *Pisum sativum*.

The studies carried out up to now are concentrated mainly upon the morphological and physiological effects of various minerals deficiency upon the apical growth. But how the mineral deficiency effects upon the lateral meristem, cambium, which provides the radial growth and xylem and phloem differentiation have not been examined in detail. The aim of this study is to determine the effects of macroelements such as N,P,Ca and Fe deficiency observed in some regions of our country upon the cambial division and therefore upon the cell differentiation and radial growth and establish the necessary criteria for the selection of suitable regions for the plantation of the plants which wood and cortex can be economically utilized.

MATERIALS AND METHODS

In this study *Linum usitatissimum* L. a fibrous plant was employed. The seeds were kept in water for 24 hours and germinated at 27°C for 48 hours in an oven. They were then planted in pots ten by ten, which was filled with silisium sand having the particule diameters of 0.8-1.2 mm. There were 5 pots allocated to each control plants and plants deficient in N, P, Ca and Fe. The nutrition solutions to be given to the plants were prepared according to Witham (1970) method. The plants were watered twice a day with 100 ml. of water. The plant were given 100 ml. of nutrition solution twice a week. The plants were measured and cut approximately 13 weeks later and their sections were prepared using a paraffin method. The increase in cortex (primer and seconder cortex) and xylem were measured in terms of their radii. These measurements were taken from the first internodes.

RESULTS

The average length of the control plants after 13 weeks was measured to be 97 cm. The plants growth which were deficient in N was found to be inhibited and could only reach an average lenght of 23 cm.

The internod lengths significantly decreased and the lamina of the leaves narrowed. The lower leaves died at the end of the experiment. The P deficiency also inhibited the growth of the plants. The average length of the plants was found to be 26 cm at the end of the experiment. The leaves became smaller and There observed anthocyan formation in the lower leaves. The plants which were under Ca deficient conditions reached to an average length of 60 cm at the end of the experiment. The plants which growth was inhibited least were those deficient in iron. They reached to an average length of 66 (Table 1.).

Table 1. The average lengths and thickness of the plants after 13 weeks.

	Average lengths (cm)	Average diameters (mm)
Control	97	2.3
-N	23	0.83
-P	26	0.95
-Ca	60	1.40
-Fe	66	1.52

Cambium- The stems reached to a diameter of 2.3 mm as a result of normal cambial activity in control plants. They had a characteristic cambial zone consisting 4-5 cells. The average radial and tangential widths were found as 8 and 16 micron respectively. The plants deficient in N could only reach to an average diameter of 0.83 mm due to the inhibition of their cambial activity. If one considers that the most of the cells come from the primer structure, it be comes obvious that the cambial cell division occurred in a very small extent. There was no cambial zone differentiated in the stem cross-sections of these plants. The cells were almost completely differentiated. The cells maintaining characteristic cambial cell shape were rarely seen. The radial growth of the stem was very small in the plants which were deficient in phosphorous. The average diameter of the stem was measured to be 0.95 mm. There were two rows of cells extended towards the radial direction, but they were not completely differentiated. There were relatively large radial growth of the stem in the plants under Ca deficient conditions and the diameter reached up to 1.40 mm. The cambial zone formed by 3-5 rows of cambial cells in these plants. The radial widths of these cambial cells was observed to increase. The cambial activity in the plants under iron deficient conditions was the least effected. Their stem diameter reached to 1.52 mm at the end of the experiment. There also was a cambial zone formed by 2-3 rows of cell. The fact that the radial

widths of the cells adjacent to cambial zone reduced and there observed no newly divided cells showed that these were about to differentiate but not completely differentiated (Figure 1).

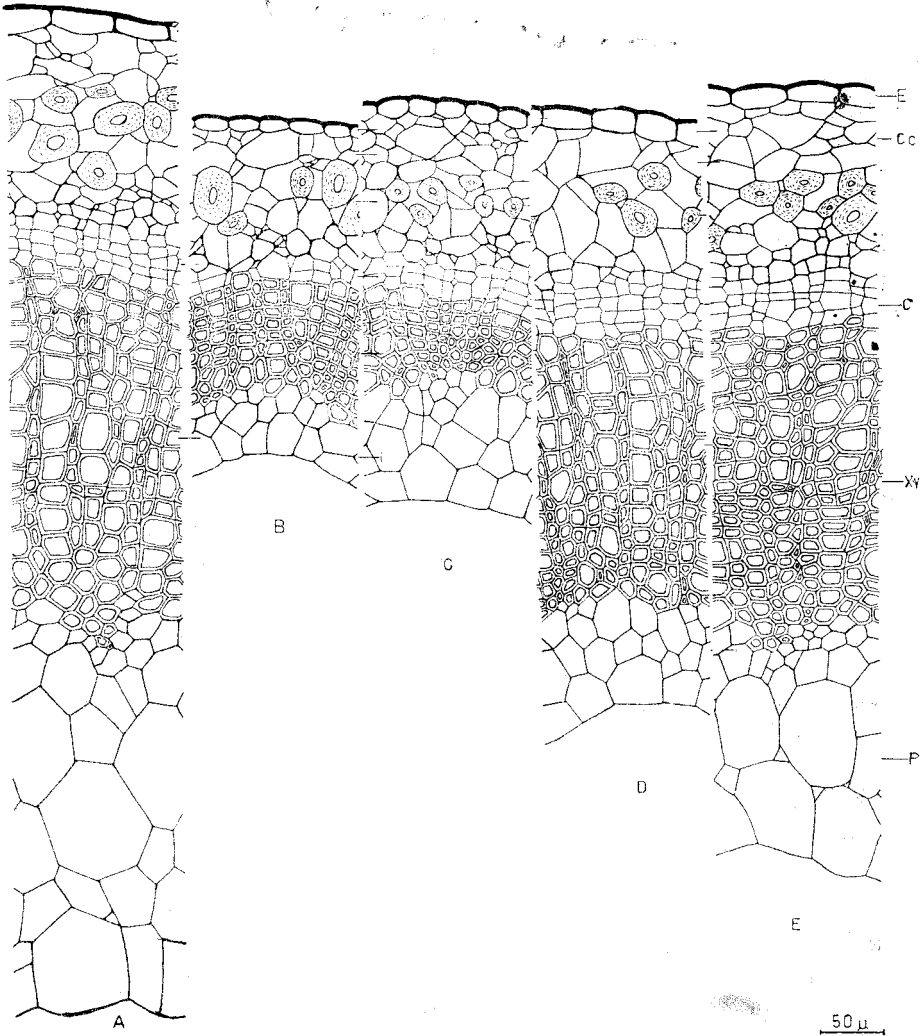


Figure 1. The cross sections taken from the plants at the end of the experiment: A, control; B, The plant deficient in nitrogen; C, The plant deficient in phosphorous; D, The plant deficient in Calcium.

E, The plant deficient in iron. E, epidermis; Co, Cortex; C, Cambium; Xy, Xylem; P, Pith.

Cortex development— The thickness of the cortex layer of the control plants consisted of 10–12 rows of cells, was found to be 193μ (Table 2). The epidermal cells were found to have rectangular shapes from their cross-sections, having the dimensions of $16 \times 48\mu$. The outer layer of the cortex was found to be consisted of parenchymatic cells and fibers. The irregularly oriented phloem cells were seen in inner regions. The cortical cells were seen to have different shapes and lengths (Fig. 1). That was the reason why it was not possible to determine an average length. The fibers are polygonal or oval shaped and thick walled. The average thickness of cell walls and the diameter of fiber cells were 11.5 and 35μ respectively (Table 3). There were small companion cells as well as large size sieve cells in phloem. The shapes and the dimensions of parenchymatic cells were highly different.

Table 2. The thickness of cortex, cambium and xylem amounts of stems which measured in radial direction of their stem cross-sections (μ)

	Cortex	Cambium	Xylem
Control	193	36	345
- N	100	—	89
- P	115	15	80
- Ca	121	25	220
- Fe	124	22	261

Table 3. The average diameter and the wall thickness of fiber cells deficient in different ions.

	Diameter (μ)	Cell-wall thickness (μ)
Control	35	11.5
- N	26	11
- P	20	7
- Ca	25	9
- Fe	35	11

The average thickness of the cortex layer of the plants deficient in N was found to be 100μ . There were 7–10 rows of cell in this layer. Epidermal cells were reduced by a ratio of $2/3$ and their dimensions remained as $10 \times 18\mu$. The average thickness of cell walls and the average diameter of the fiber cells were found to be 11μ and 26μ respectively. The phloem cells were highly heterogenous. The diameter of the cells were relatively narrowed compared with those of control plants.

The cortex of The plants grown under P deficient conditions was found to have a diameter of 115μ and consisted of 7–11 rows of cells.

There was a reduction in the size of the epidermal cells compared with those of controls, and their average dimensions was found to be $12 \times 20\mu$. There was a general reduction of the size of the cortex cells as well. There was a decrease in both the cell wall thickness and the diameters of the fiber cells. The wall thickness and the dimensions of these cells were measured as 7 and 20μ respectively. The maximum inhibition in the fiber development was seen to occur due to P deficiency. (Table 3).

In Ca deficiency, the cortex of the plants was formed by 6-8 lines of cells. The epidermal cells were relatively larger than those of the plants deficient in phosphorous. The tangential and the radial widths of these cells were measured as 22μ and 13μ respectively. The dimension of the cortex cells were found to be similiar even bigger than those of controls. Although the fiber cells were smaller than those of control they are much more developed than those plants deficient in phosphorous. The wall thickness and the diameter of these fibers were found to be 9μ and 25μ respectively.

The cortex of the plants grown under iron deficient condition was formed by 9-12 rows of cells having the total thickness of 124μ . The shapes and the dimensions of the epidermal cells were similar to those of controls. The average dimension of epidermal cells were calculated to be $15 \times 35\mu$. The cortex cells were seen to have different shapes and sizes just as those controls. The fiber cells which has a average thickness of 35μ were similiar to those of controls. The wall average thickness of those cells was 11μ . The plants which showed mininum inhibition were those deficient in iron.

Xylem development- the vessel members forming the xylem in control plants did not have large diameters. They generally showed a radial orientation. There are 15 to 20 cells in these layers. The wall thickness of those cells was homogenous and averaged $3,5\mu$. The diameter of the biggest element is approximately 30μ . The average diameter was around 23μ (Table 4). The thickness of xylem was determined to be 345μ . The piths of the control plants consisted a pith cavity in the middle. The parenchymatic pith region was formed by 5-8 rows of cells having the thickness of 260μ . The average diameter of these cells was around 45μ .

The xylem development of the plants deficient in nitrogen was found to be highly inhibited. The diameters of the water transporting elements were reduced. The diameter of the widest trache was measured to as 16μ and the average diameter was 11μ . The total thickness of

the xylem region was 89μ . The width of the parenchymatic pith up to pith cavity varied between 60 to 65μ it is formed by 2-4 rows of cells radially distributed. The average diameter of the cells was 25μ .

Table 4. The maximum and average diameters of water transporting elements (μ)

	Max. diameter	Average diameter
Control	30	23
- N	16	11
- P	18	16
- Ca	28	20
- Fe	26	17

Maximum inhibition in xylem development occurred under the P deficient conditions. The diameter of the water transporting cells and their numbers were markedly decreased. The diameter of the largest trache was measured as 18μ and the average diameter was found to be 16μ . The thickness of the whole xylem region was only 80μ . The parenchymatic pith thickness around the pith cavity varied between 95 - 100μ . The average cell diameter of this layer formed by 5-6 rows of cells was 28μ .

Although the diameter of the vessel members was reduced, there was not a significant inhibition in plants growth under the Ca deficient conditions. The diameter of the largest vessel member was 28μ while the average diameter was calculated as 20μ . The total xylem thickness was 220μ . This value is approximately $1/3$ of the stem thickness. In some crosssections the cell wall were seen collapsed. The parenchymatic pith region was narrower than that of controls and measured to be 90 - 95μ . The average diameter of the cells was calculated as 30μ .

The minimum inhibition of xylem development was observed in those plants deficient in Fe. The diameter of the largest trache was 26μ . The average diameter of the water transporting elements was calculated as 17μ . The total xylem diameter was 261μ which was the nearest value to that of controls (Table 2). The parenchymatic region thickness of the pith varied between 160 to 180μ . The average diameter was calculated to be 43μ . This was the nearest value to that of controls.

DISCUSSION

The effects of N, P, Ca and Fe deficiencies upon the perpendicular growth and the development of the plants were investigated by various

workers. In our study the maximum inhibition in apical growth was found to be caused by the deficiency of N. This was followed by phosphorous, calcium, and iron. The radial growth was also found to be similarly effected by the deficiency of these ions. Maki (1961) showed that nitrogen caused volumetric and longitudinal growth of the leaves of *Pinus taeda*. Viro (1965) proved that nitrogen containing fertilizers effect shoot growth in *Picea abies* and increased the chlorophyll content of their leaves. Humphrey et al. (1977) found that N deficiency decreased the protein content of *Lemna minor*. Sugiyama and Sugiyama (1979) stated that there observed the reduction in total nitrogen, soluble protein and the chlorophyll content of the lower leaves of *Zea mays*. According to our study the inhibition of lateral growth caused by the deficiency of nitrogen, was due to that it effects the protein synthesis and photosynthesis by entering the structure of chlorophyll. The lack of characteristic cambial zone in the cambial region showed that the cell dividing had stopped long time ago. Terentev et al. (1974) in their study investigating the effects of deficiency of nitrogen in *Linum* reported that the phloem development took place during the first phase of plant growth. The facts that the phloem development was effected much less than that of xylem and increase of the average diameter of the fiber cells in phloem observed in our study could be attributed to this fact.

The fact that radial thickening was most markedly retarded by the deficiency of P can be explained that phosphorous facilitates the synthesis of phospholipids, nucleic acids, nucleo-proteins, enzymes and high energy phosphates. Although the cambial activity was highly retarded there observed two rows of characteristic cambial cells. Lyon and Garcia (1944) claimed that the deficiency of P causes a significant reduction in the amount of phloem in tomato. In our study the amount of xylem reduced. This can be attributed to the role which phosphorus takes in the synthesis of wall compounds. The fact that the maximum inhibition in the phloem fibers was observed in these plants also supports this thesis.

Davis (1949) found the deficiency of Ca cause increases in the diameters of cambial cells of *Pinus taeda*. Higaki et al. (1980) claimed that the deficiency of Ca effects the middle lamella structure of *Anthurium andreaeanum*. The cambial activity was not effected by the deficiency of Ca as much as by the deficiency of N or P at the end of our experiments. The characteristic cambial zone was present, their radial

diameters were larger than those of controls and this supports the aforementioned studies and shows that cambial cells divisions were retarded. The fact that the size of the cortex cells and the diameters of the water transporting elements were similar to those of controls was most probably due to this reason. Millikan (1953) showed that deficiency of Ca inhibited the development of the fiber cells of *Linum*. In our studies the fiber cells were observed narrow and small due to the lack of Ca compared with those of controls. The explanation of why the deficiency of Ca effects phloem formation much more than xylem formation is very difficult.

The cambial division was much less effected by the deficiency of iron than the deficiency of other ions. There a specific cambial zone present. Del Rio et al. (1978) showed that iron effects catalase and peroxidase activities. The slight reduction in the radial thickening compared with controls in our study can be attributed to that breathing was also effected.

LITERATURE

- BRANTON, D. and L. JACOBSON., 1962. Iron transport in pea plants. *Plant Physiol.* 37: 539-545.
- BROWN, A.L., S. YAMAGUCHI. and J. LEAL-DIAZ., 1964. Evidence For translocation of iron in plants. *Plant Physiol.* 40: 35-38.
- DAWIS, D.E., 1949. Some effects of calcium deficiency on the anatomy of *Pinus taeda*. *Am. J. Botany* 36: 276-282.
- DEL RIO, L.A., et al., 1978. Iron deficiency in pea plants. *Plant and Soil* 49: 343-353.
- HIGAKI, T., H.P. RASMUSSE. and W.J. CARPENTER., 1980. Calcium deficiency of *Anthurium andreanum spathe*. *J. Am. Soc. Hortic. Sci.* 105 (3): 438-440.
- HOBBS, C.H., 1944. Studies on mineral deficiency in pine. *Plant Physiol.* 19: 590-602.
- HUMPHREY, T.J., S. SARAWEK. and D.D. DAVIES., 1977. The effect of nitrogen deficiency on the growth and Metabolism of *Lemna minor L.* *Planta* 137 (3): 259-264.
- LYON, C. and C.R. GARCIA., 1944. Anatomical responses of tomato stems to variations in the macronutrient anion supply. *Botan. Gaz.* 105: 394-405.
- MAKI, T.E., 1961. Some effects of fertilizers on loblolly pine. *Trans. 7 th Int. Congr. Soil Sci.* 3: 363-375.
- MILLIKAN, C.R., 1953. Diseases of flax and linseed. *Dept. Agr. Victoria Tech. Bull.* 9.
- PISSAREK, H.P., 1979. Effects of calcium deficiency on the histology of leaves and stems of Maize and sunflowers. *Angew Bot.* 53 (5/6): 215-224.

- SUGIYAMA, N. and T. SUGIYAMA., 1979. Enhanced activity of proteinase in nitrogen-deficient maize and wheat plants. *Soil Sci. Plant Nutr.* 25 (3): 349-356.
- TERENT'EV, V.M., et al., 1974. Fortamion of phloem and Xylem in flax stalk with different doses of nitrogen fertilizers. *Fiziol-Biokhim. Osn. Povysh. Prod. Rast.* 122-132.
- VIRO, P.J., 1965. Estimation of the effect of forest fertilization. *Commun. Inst. Forest. Fenn.* 59.3, 5-42.
- WALKER, R.B., S.P. GESSEL. and P.G. HADDOCK., 1955. Greenhouse studies in mineral requirements of conifers: Western red cedar. *Forest Sci.* 1: 51-60.
- WITHAM, H.F., 1971. *Experiments in Plant Physiology.* Van Nostrand Reinhold Company, New York, 1-3.