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EFFECT OF ETHREL, CYCOCEL AND PHOSPHORUS ON THE ANATOMICAL STRUCTURE OF MUNG BEAN PLANTS

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ABSTRACT

This investigation was carried out during the two seasons of 1999 and 2000 in the Agricultural Experiment and Research Station of Faculty of Agriculture, Cairo University, Giza, Egypt to study the response of the anatomical structure of mung bean to different concentrations of ethrel, cycocel (CCC) and phosphorus.

Transections of main stem revealed that treatments with ethrel at 200 and 300ppm or with CCC at 1000 and 1500 ppm enhanced the growth of stem in width by increasing the thickness of cortex, vascular tissues and diameter of pith. Most vessels and ray cells of secondary xylem of ethrel treated stems tended to enlarge in radial direction more than in tangential one. Fertilization of mung bean plants with moderate rate of phosphorus (23.25 kg P_2O_5/ feddan) produced stems with higher amount of conductive tissues and wider xylem vessels than those fertilized with 15.5 or 31.00 kg P_2O_5/ feddan/

Transverse sections of leaflet blades at the median region of main stem showed that ethrel increased sizes of midrib, vascular bundle and cells of ground tissue.

while the rate of 1000 ppm CCC increased the size of midrib and thickness of blade (palisade and spongy tissues) the rate of 1500 ppm decreased them. Plants received 23.25 kg P_2O_5 / feddan produced leaf blades with higher amount of tissues than those fertilized with 15.50 or 31.00 kg P_2O_5 / feddan.

INTRODUCTION

It has been found that treatment with ethrel stimulated the thickness of plant tissues, and modified the cell growth pattern. It stimulated radial cell expansion.

While cell elongation was reduced (Goeschl *et al.*, 1966, Ridge and Osborine, 1969, Apelbaum and Burg, 1971, Poovaiah, 1974, El-Shaarawi and El-Sahhar, 1976, El-Shaarawi and Golban, 1981, Ibrahim *et al.*, 1990 and Sanchez *et al.*, 1992).

Many workers reported that application of CCC to different legume plants caused increase in stem thickness by promoting the thickness of different tissues. On the other hand stem elongation was decreased due to CCC treatment (El-Shaarawi and Megahed, 1976, Soliman, 1984, Khafaga *et al.*, 1986, El-Fouly *et al.*, 1988, Hussein, 1990 and Abdo, 1992.

Reduction in number and area of vascular bundles in the transverse sections of stem has been noticed under phosphorus starvation (Bakr, 1946). Goma'a (1996) reported that phosphorus deficiencies decreased diameter of xylem vessels and amount of vascular tissues.

This investigation aimed to study the effect of two growth regulators (ethrel and CCC) and phosphorous on the internal structure of mungbean plant.

MATERIALS AND METHODS

This investigation was designed for studying the effect of ethrel at 200 and 300 ppm, cycocel at 1000 and 1500 ppm and phosphorus fertilizer at 15.5, 23.25 and 31.00 kg P_2O_5 / feddan on anatomical features of mung bean plant. Spraying with the different concentrations of the two plant growth regulators was carried out 30 days after sowing and the rates of phosphorous were added to the soil before sowing. The experiments were carried out during the two seasons of 1999 and 2000 in the Agricultural Experiment and Research Station of Faculty of Agriculture, Cairo University, Giza, Egypt.

Samples of main stem (basal, median and upper internodes) and leaf (leaflet blades at the median region of main stem) at the two ages of 45 and 60 days were killed and fixed in F. A. A. (10 ml. Formalin, 5 ml. Glacial acetic acid, 85 ml. Ethyl alcohol 70%). Fixed materials were dehydrated by normal butyl alcohol method and embedded in paraffin wax, melting point 54 –58 C°. Sections 15 – 20 μ thick were cut. Crystal violet–erythrosin combination method (Jackson, 1926) was used for staining. Stained sections were mounted in Canada balsam (Willey, 1971).

RESULTS AND DISCUSSION

Stem Structure:

Effect of ethrel:

Transverse sections were made in samples taken from different levels of the main stem, upper, median and basal internodes. At the age of 45 days, two weeks after spraying, transections of the upper portion of the stem (7^{th} internode) showed different modifications in the primary structure of main stem (Table 1 and Fig.1).The diameter of the whole section increased from 2560 μ for untreated stem up to 3472 and 3441 μ by treatments with 200 and 300 ppm of ethrel respectively.This increment was accompanied by increase in number and size of vascular bundles, thickness of cortex and diameter of pith.

Transverse sections of the median part of main stem (Fifth internode) revealed that treatment with ethrel especially at the rate of 200 ppm enhanced the growth of stem in width. The values of whole section diameter were 6751 and 5887 μ for stems treated with 200 and 300 ppm of ethrel, respectively, against 5122 μ for untreated stems. Thickness of vascular cylinder increased from 785 μ up to 1216 and 824 μ by treatment with 200 and 300 ppm of ethrel, respectively. The increase in thickness of vascular cylinder was due mainly to the increase in the thickness of xylem. Thickness of cortex increased by ethrel treatment specially at the rate of 200 ppm, while pith diameter decreased with the application of 300 ppm of ethrel. The diameter of xylem cylinder increased from 4513 μ for control up to 5495 and 4945 μ by treatment with 200 and 300 ppm of ethrel, respectively.

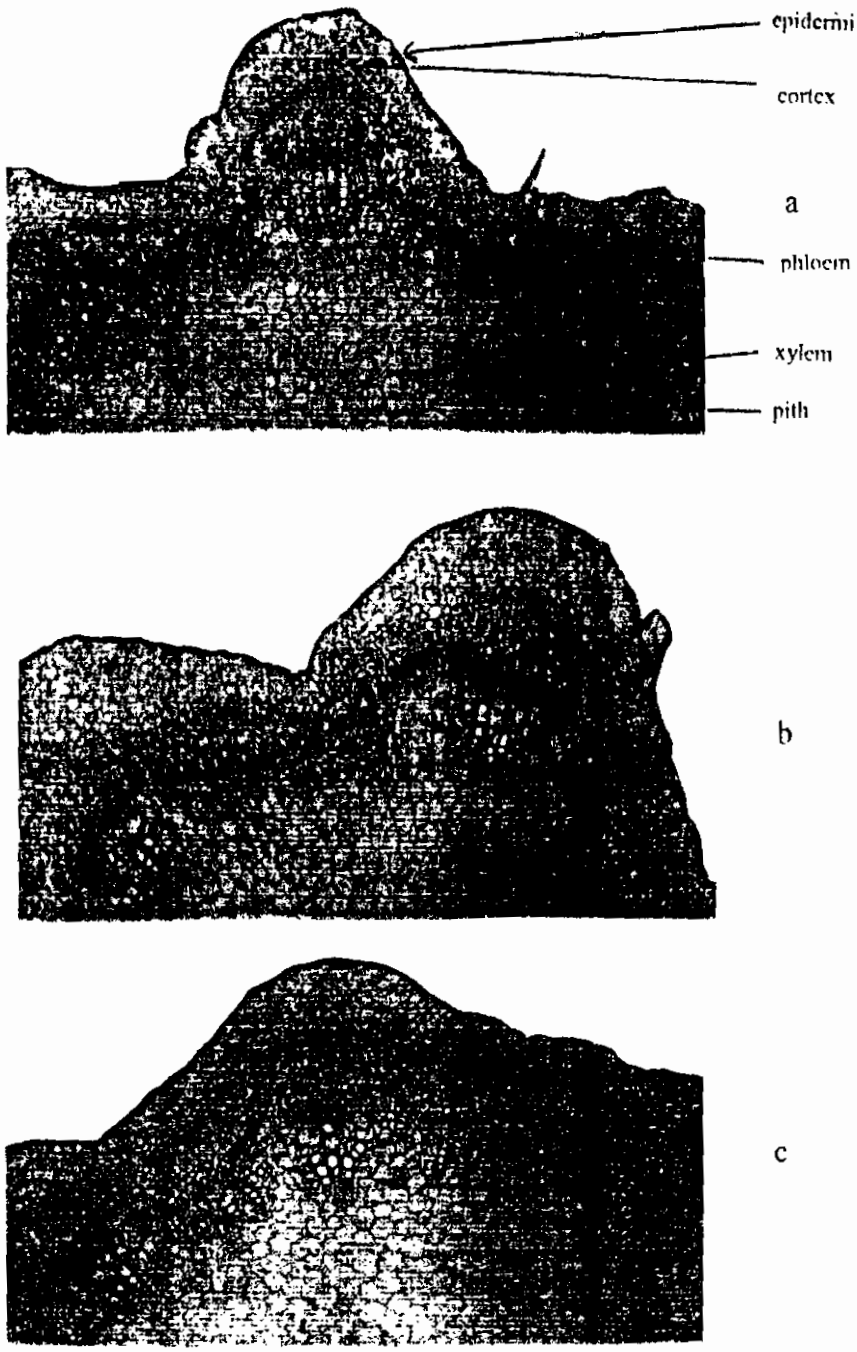

Fig 1: Transverse sectioms of main stem upper internode of mungbean plant at the age of 45 days as affected by different rates of ethrel. (x40).
a: control b: ethrel, 200 ppm c: ethrel, 300 ppm

Table (1): Means of different anatomical parameters of the upper internode of mung bean main stem treated with ethrel and cycocel.

Treatments / Parameters (in micron)	Control	Ethrel (ppm)		CCC (ppm)	
		200	300	1000	1500
Diameter of whole section	2560.6	3472.0	3441.0	3050.4	2480.0
Thickness of cortex	186	217.0	192.2	251.1	124.0
Number of vascular bundles	18	21	20	22	15
Length & width of :					
-large bundle:					
length	496	620	620	520.8	372
width	310	372	558	372	434
-small bundle:					
length	248	372	372	372	272.8
width	124	148.8	248	248	248
Number of vessels / bundle:	17	25	17	22	16
Diameter of pith	1798	2108	1891	1829	1767

Thus it could be concluded that treatment with ethrel especially at the rate of 200 ppm increased the amount of xylem tissue of stem by enhancing both thickness and diameter of xylem cylinder. This might be due to the promoting effect of ethrel on secondary growth (Table 2 and Fig. 2).

Transverse sections of the third basal internode of untreated stems at the age of 45 days revealed higher amount of secondary growth and the effect of ethrel on stem structure was more pronounced at this level of main stem (Table 2 and Fig. 3). Diameter of the whole section of untreated stem was 5848 μ, increased up to 7222 and 8026 μ by treatment with 200 and 300 ppm of ethrel, respectively. The other anatomical characters of this basal internode such as thickness and diameter of xylem cylinder and diameter of pith were enhanced by treatment with 200 or 300 ppm of ethrel. It is of interest to notice that, despite of the cross sectional area appeared higher at the rate of 300 ppm of ethrel when compared with the rate of 200 ppm, the thickness of vascular cylinder was higher at the rate of 200 ppm. On the contrary, pith and cortex of stems treated with 300 ppm of ethrel was wider than those of stems treated with 200 ppm. It could be said that at the age of 45 days, mung bean stems treated with 200 ppm of ethrel had higher amount of vascular tissues at the basal region than those treated with 300 ppm, though the latter appeared wider.

Examination of transverse sections of main stem at the age of 60 days revealed that the effect of ethrel on stem structure showed nearly the same trend as mentioned above at the age of 45 days, though stems of treated and untreated plants showed more amount of different tissues at the age of 60 days.

It could be concluded therefore, that spraying mung bean with ethrel at the age of 30 days, enhanced the growth of main stem in width by increasing the amount of different tissues, specially xylem and phloem. The increment in vascular tissues of the median and basal internodes could be attributed to the simulative effect of ethrel on cambial activity.

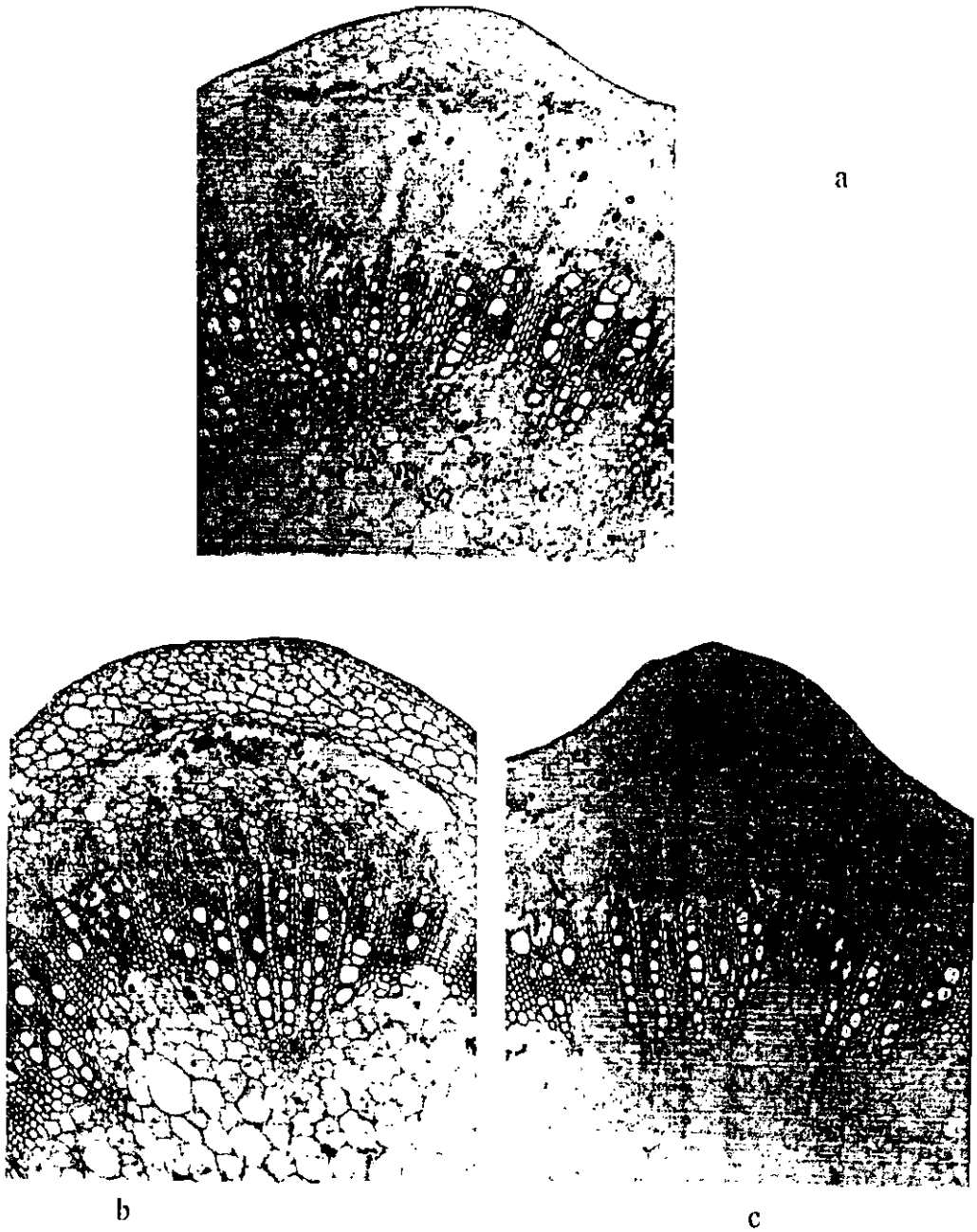

Fig 2: Transverse sectioms of main stem median internode of mungbean plant at the age of 45 days as affected by different rates of ethrel. (x40).
a: control b: ethrel, 200 ppm c: ethrel, 300 ppm

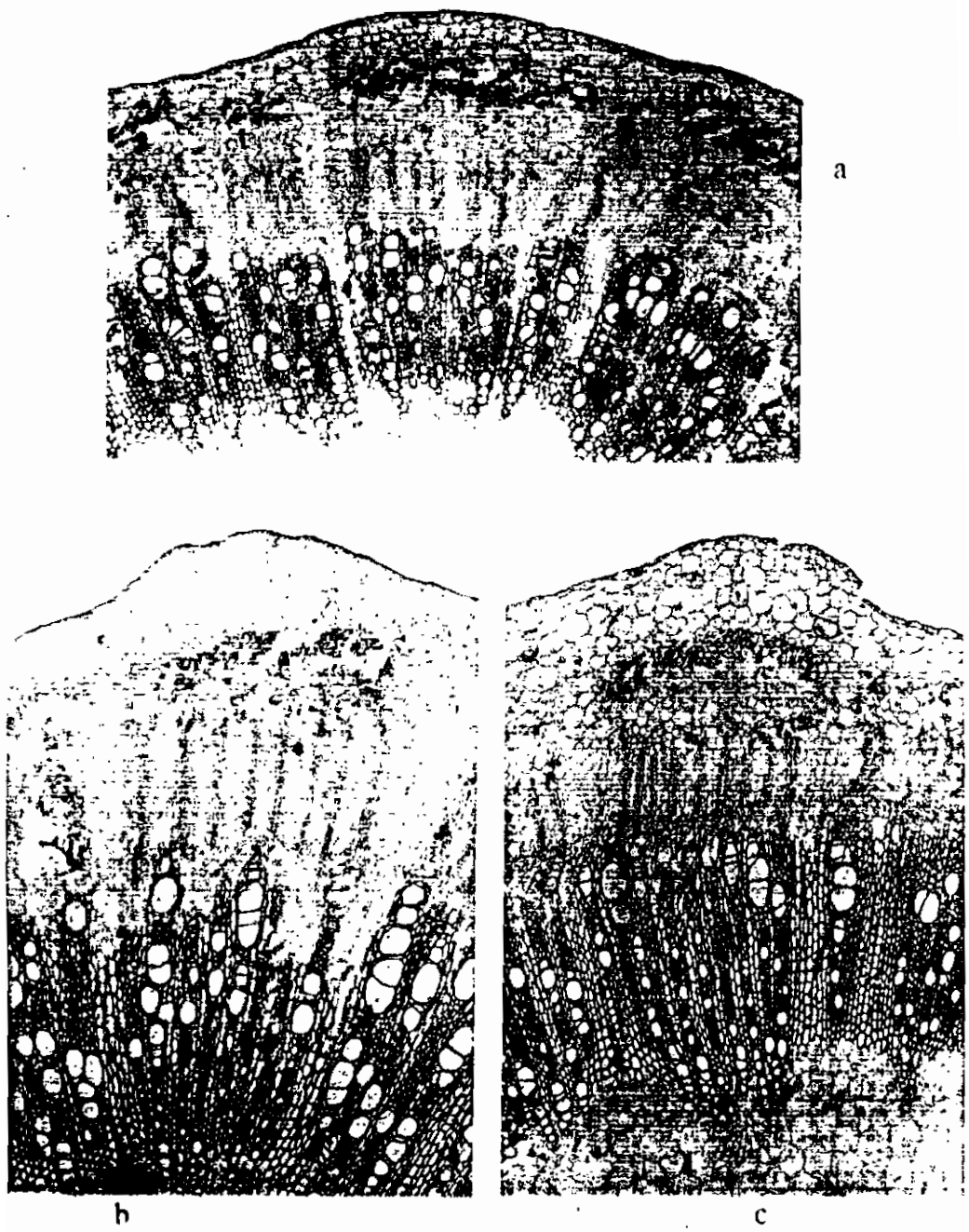

Fig 3: Transverse sectioms of main stem basal internode of mungbean plant at the age of 45 days as affected by different rates of ethrel. (x40).
a: control b: ethrel, 200 ppm c: ethrel, 300 ppm

Effect of CCC:

Transverse sections of the upper region of main stem (seventh internode) at the age of 45 days revealed that the effect of CCC varied according to concentration used. The rate of 1000 ppm of CCC increased diameter of whole section, thickness of cortex, number of vascular bundles, size of vascular bundle, number of vessels per bundle and diameter of pith (Table 1 and Fig. 5). On the other hand, all of these anatomical characters decreased when the rate of 1500 ppm CCC was used, in comparison with control. It could be concluded therefore, that while the rate of 1000 ppm stimulated the growth of upper internode in width the rate of 1500 ppm CCC retarded it.

In addition to promotive effect of ethrel on the amount of stem tissues, it modified the pattern of cell growth. The two rates 200 and 300 ppm of ethrel increased the enlargement of cortical cells and xylem vessels in comparison with those of untreated stems. Most vessels and ray cells of secondary xylem of treated stems tended to enlarge in radial direction more than in tangential one (Fig. 4). This modification in direction of cell growth might be shared in the increased thickness of vascular cylinder.

It was observed also that the density of xylem vessels was deceased in ethrel treated stems. The vessels appeared in control stems in clusters, long radial chains and solitary. In ethrel treated stems the xylem vessels occurred mostly in short chains and solitary (Fig. 3). This might be due to proliferation of ray and axial parenchyma localized between the vessels in treated stems.

The modifications in the structure of mung bean stem recorded in the present work due to treatment with ethrel are more or less in agreement with the findings of some previous investigations on different legume plants. Goeschl *et al.*, (1966) recorded lessened growth in length and radial enlargement of tissues (swelling response) in pea seedlings in response to ethylene. Ridge and Osborine (1969) reported that ethylene was found to alter the direction of cortical cell expansion in pea. Apelbaum and Burge (1971) worked on etiolated pea plants with ethrel and mentioned that the polarity of cell expansion was altered due to changes in the direction of deposition of cellulose micro fibrils at the inner surface at of the wall. Poovaiah (1974) reported that foliar application of ethephon to two week old bean plants induced radial enlargement of stems, and swelling of the stem was occurred about a week after the treatment. He mentioned that swelling was the result of extensive radial enlargement of cells rather than cell division in the vascular region following ethephon treatments. El-Shaarawi and El-Sahhar (1976) mentioned that when *Vicia faba* plant sprayed with ethrel, the rate of 200 ppm increased stem wall thickness, amount of conducting elements and resulted in an expansion of pith and cortical cells in the radial direction. The rate of 400 ppm decreased main stem diameter and amount of vascular tissues.

Transverse sections of the median region (fifth internode) or basal one (third internode) of mung bean main stem showed that treatment with 1000 ppm or 1500 ppm of CCC promoted the radial growth of main stem at both regions.

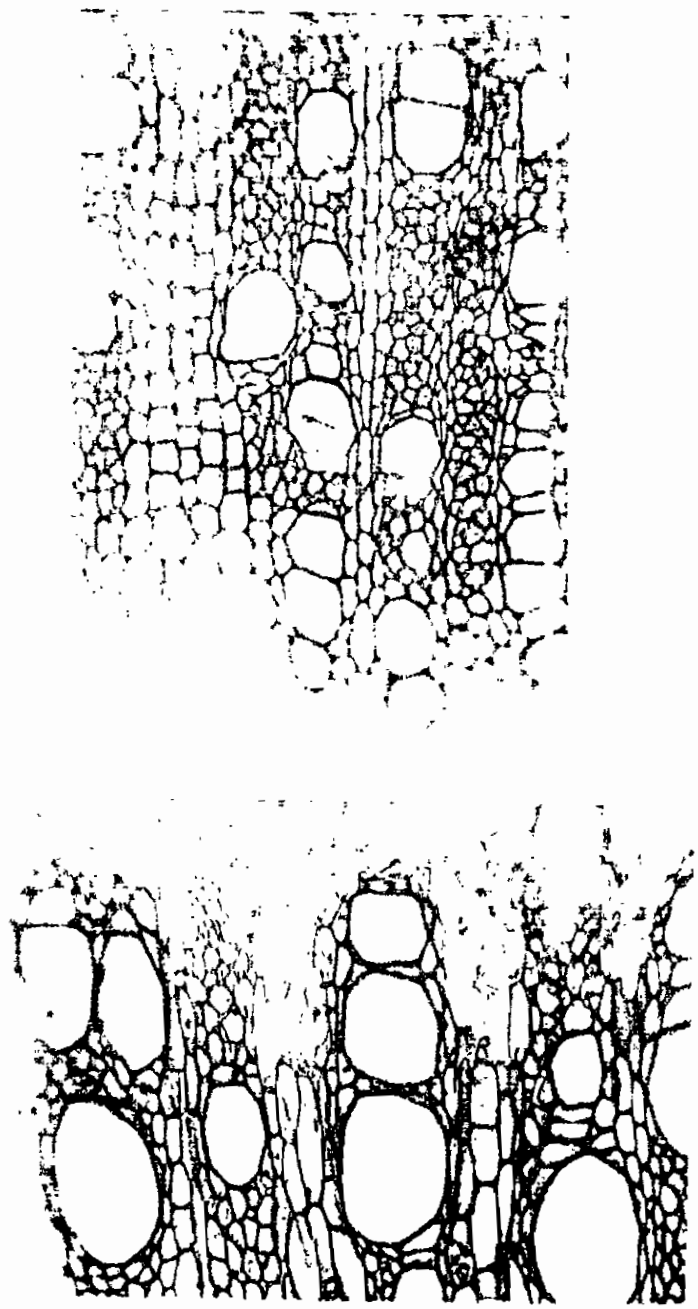

Fig 4: Transverse sectioms in basal internode of mungbean plant at the age of 45 days showing radial enlargement of xylem vessels and ray cells of ethrel treated stem (x 160).
a: control b: ethrel, 200 ppm

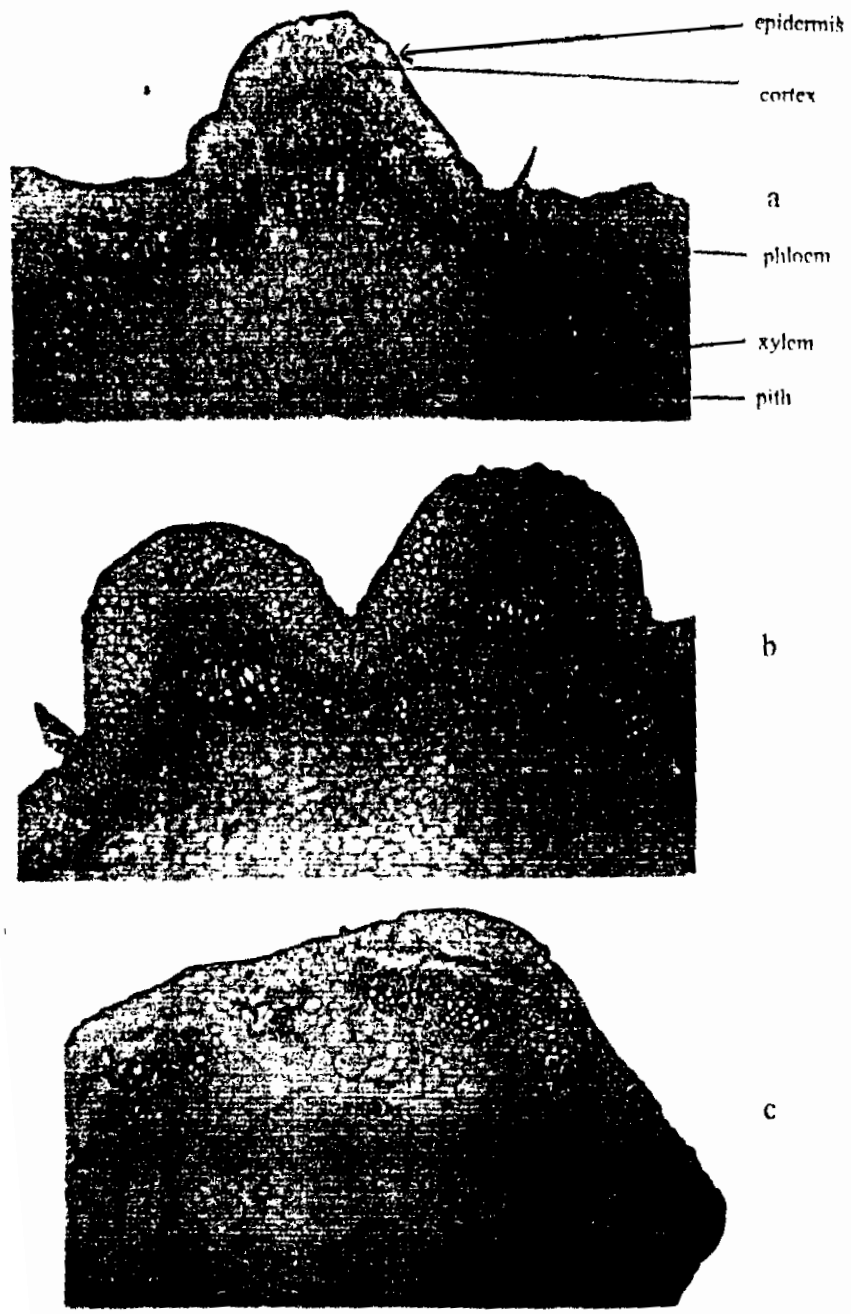

Fig 5: Transverse sectioms of main stem upper internode of mungbean plant at the age of 45 days as affected by different rates of cycocel. (x40).
a: control b: cycocel, 1000 ppm c: cycoce, 1500 ppm

The promotive effect occurred through increasing diameter of xylem cylinder, thickness of cortex, thickness of vascular cylinder, thickness of phloem and diameter of pith. Consequently the diameter of whole section increased due to treatment (Table 2, Fig. 6). Thickness of xylem increased by using the rate of 1000 ppm and slightly decreased at the rate of 1500 ppm of CCC when compared with that of untreated stems. The increase in thickness of cortex could be attributed to the increase in number of cortical layers and size of cells. The suggested stimulative effect of CCC on cambial activity might be responsible for the increased thickness of vascular cylinder of treated stems. It is worthy to notice that, the two rates 1000 and 1500 ppm of CCC enhanced the radial growth of main stem when compared with control and the stems treated with 1000 ppm of CCC surpassed those treated with 1500 ppm in this respect. These results are in accordance with the findings of El-Shaarawi and Megahed (1976) and Sakr (1977) on bean, Soliman (1984) on soybean, Khafaga *et al.*, (1985) on pea, Khafaga *et al.*, (1986) on soybean, Nagdy *et al.* (1989) on sybean, Hussein (1990) on pea, Abdo (1992) on bean, and Hussein (1994) on Lupins.

Table (2): Means of different anatomical parameters of the median and basal internodes of mung bean main stem treated with ethrel and cycocel.

Treatments / Parameters (in micron)	Median internode					Basal internode				
	Control	Ethrel (ppm)		CCC (ppm)		Control	Ethrel (ppm)		CCC (ppm)	
		200	300	1000	1500		200	300	1000	1500
Diameter of whole section	5122.1	6751.0	5887.5	7967.8	6986.5	5848.3	7222.0	8026.6	6281.8	5887.5
Diameter of xylem cylinder	4513.8	5495.0	4945.5	4749.3	4867.0	3768.0	5377.3	6044.5	6201.5	4867.0
Thickness of cortex	235.5	529.9	345.4	628.0	608.4	215.9	196.3	333.6	471.0	176.6
Thickness of vascular cylinder	785.0	1216.8	824.3	1746.6	804.6	1059.8	1609.3	1432.6	1923.3	1138.3
Thickness of xylem	490.6	824.3	522.0	1256.0	471.0	745.8	1079.4	1059.8	1373.8	726.1
Thickness of phloem	293.9	392.5	314.0	490.6	333.6	314.0	529.9	372.9	549.5	412.1
Diameter of pith	3434.4	3218.5	3846.5	4396.0	4082.0	3297.0	3728.8	3925.0	3611.0	3454.0

Effect of Phosphorus:

Transverse sections of the third basal internode of main stem were examined at the two ages of 45 and 60 days to study the differences in stem structure due to application of different levels of phosphorus to mung bean plants. At the age of 45 days, stems of plants received 31.00 kg P_2O_5 / feddan were thinner than those of plants fertilized with 23.25 or 15.50 kg P_2O_5 / feddan. This differences in stem girth was due to the decrease in thickness of different stem tissues at the level of 31.00 kg P_2O_5 / feddan (Table 3 and Fig. 7). Comparing between the two rates of 15.50 and 23.25 kg P_2O_5 / feddan, it is obvious that the rate of 23.25 kg P_2O_5 / feddan showed a slight increase in diameter of whole section and clear increase in thickness of vascular cylinder (both xylem and phloem), a slight decrease in thickness of

cortex and diameter of vascular cylinder, and no difference in pith diameter than those recorded for the rate of 15.50 kg P_2O_5 / feddan.

The thickness of xylem tissue was 612,745 and 431 μ for stems fertilized with 15.50, 23.25 and 31.00 kg P_2O_5 / feddan respectively. The rate of 23.25 kg P_2O_5 / feddan not only increased the thickness of xylem tissue but also increased the enlargement of xylem vessels as compared with the other two rates of phosphorus (Fig. 7).

At the age of 60 days, the difference between the rate of 23.25 kg P_2O_5 / feddan and the other two rates increased with respect to stem structure. The values of whole section diameter were 6280, 7457 and 5495 μ for stems received 15.50, 23.25 and 31.00 kg P_2O_5 / feddan, respectively. The superiority of the medium rate in this respect was due to the increase in thickness of vascular tissues and diameter of xylem cylinder. Diameter of pith Fir2 in stems fertilized with the medium rate was lesser than that of stems received lower or higher rate of phosphorus (Table 3 and Fig. 8).

It could be concluded therefore that fertilization of mung bean plants with moderate rate of phosphorus (23.25 kg P_2O_5 / feddan) produced stems with higher amount of conductive tissues and wider xylem vessels.

In this connection, Bakr (1946) reported that when broad bean was grown under phosphorus starvation, reduction in the number and area of vascular bundles as well as in the size of their water conducting elements were noticed in the transverse sections of stem. Goma'a (1996) found that deficiency of phosphorus in bean plant decreased diameter of xylem vessels and amount of vascular tissues specially the secondary xylem. Wanke *et al.* (1998) found that cortical cells of roots in P-deficient plants had large vacuoles, invagination of the plasmalemma and condensed forms of mitochondria, which, were not seen in P-sufficient roots.Table 3

Table (3): Means of different anatomical parameters of the basal internode of mung bean main stem treated with differen levels of phosphorus.

Plant age (day)	45			60		
Treatments / Parameters (in micron)	Phosphorus (kg P_2O_5 / fed.)			Phosphorus (kg P_2O_5 / ed.)		
	15.50	23.25	31.00	15.50	23.25	31.00
Diameter of whole section	5809.0	5848.3	4631.5	6280.0	7457.5	5495.0
Diameter of xylem cylinder	4199.8	3768.0	3454.0	4867.0	5809.0	4765.0
Thickness of cortex	302 2	215.9	235.5	141.3	157.0	184 5
Thickness of vascular cylinder	883.1	1059 8	686.9	753.6	1923.3	608 4
Thickness of xylem	612.3	745.8	431.8	608.4	1432.6	451.4
Thickness of phloem	270 8	314.0	255.1	157.0	490.6	157 0
Diameter of pith	3297 0	3297.0	2629.8	4105.6	3002 6	3768 0

Leaf structure:

Transverse sections of leaflet blades at the median region of main stem 45 days-old were examined to study the response of leaf structure to treatment with ethrel, CCC and different levels of phosphorus.

It is clear from Table (4) and Fig. (9) that ethrel increased size of midrib, midvascular bundle and cells of ground tissue.

The size of midvein increased due to increment in both length and width of midvascular bundle. The thickness of blade decreased in ethrel treated plants due to decrease in thickness of both palisade and spongy tissues.

These results are in accordance with the findings of El-Shaarawi and El-Sahhar (1976) on *Vicia faba L.*

Response of leaf structure to CCC treatment varied to some extent according to concentration used. While, the rate of 1000 ppm CCC increased the size of midrib, the rate of 1500 decreased it (Table 4 and Fig. 10). The area of midvein in transection decreased due to treatment with 1000 or 1500 ppm of CCC, specially the latter rate as a result of decrease in both xylem and phloem tissues. Thickness of leaflet blade, thickness of palisade and spongy tissues increased with the rate of 1000 ppm of CCC and decreased with the rate of 1500 ppm of CCC. The increased thickness of leaflet blade recorded in the present work due to treatment with CCC, is in accordance with the findings of Sakr (1977), Khafaga *et al.* (1985), Hussein (1990) and Hussein (1994) on different legume plants.

Table (4): Different anatomical parameter of mung bean leaf blade treated with different levels of ethrel and cycocel.

Treatments / Parameters (in micron)	Control	Ethrel ppm		CCC ppm	
		200	300	1000	1500
Thickness of midrib	992.0	1016.8	1178.0	1054 0	781.2
Width of midnb	682.0	868.0	806.0	744 0	620.0
Dimensions of midivein:					
-length	310.0	372.0	372.0	248.0	186.0
-width	310.0	496.0	372.0	310.0	198.4
-xylem thickness	223.2	248.0	223.2	186.0	124.0
-phloem thickness	86.8	124.0	148.8	62 0	62.0
Number of vessels:					
-large bundle	54	41	35	44	30
-small bundle	6	14	12	9	6
Thickness of blade	260.4	226.3	217.0	293.5	186.0
Thickness of palisade tissue	173.6	151.9	136.4	202.5	111.6
Thickness of spongy tissue	86.8	74.4	80 6	90 9	74.4

With regard to the effect of different levels of phosphorus on leaf structure, it is clear from Table (5) and Fig. (11), that plants received 23.25 kg P_2O_5 / feddan produced leaf blades with higher amount of tissues than those fertilized with15.50 or 31.00 kg P_2O_5 / feddan. The medium rate surpassed both lower and higher rates with respect to sizes of midrib and midvein and thickness of blade. The increase in medvein size was due to the enhancement of both xylem and phloem thickness, as well as the increase in blade thickness which reasoned by higher thickness of both palisade and spongy tissues.

Table (5): Different anatomical parameter of mung bean leaf blade treated with different levels of phosphorus fertilization.

Treatments / Parameters (in micron)	Phosphorus (kg P_2O_5/fed)		
	15.50	23.25	31.00
Thickness of midrib	818.4	992.0	868.0
Width of midrib	620.0	682.0	558 0
Dimension of midvein:			
-length	248.0	322.4	248.0
-width	372.0	372.0	310.0
-xylem thickness	161.2	186.0	161.2
-phloem thickness	93.8	136.4	93.8
Number of vesseis			
-large bundle	30	32	24
-small bundle	3	10	3
Thickness of blade	257.3	269.7	257.3
Thickness of palisade tissue	164.3	176.7	167 4
Thickness of spongy tissue	93.0	93.0	89.9

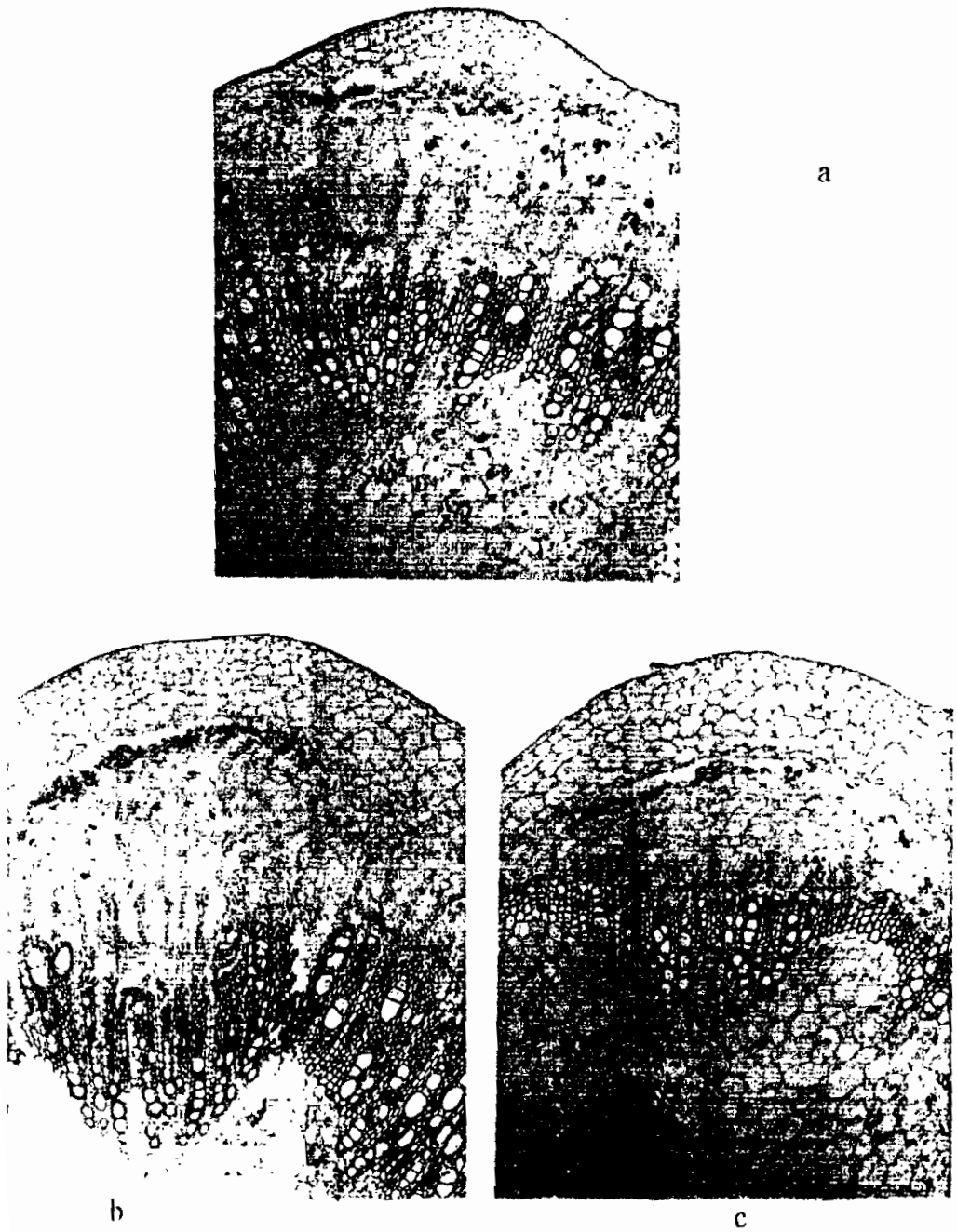

Fig 6: Transverse sectioms of main stem median internode of mungbean plant at the age of 45 days as affected by different rates of cycocel. (x40).
a: control b: cycocel, 1000 ppm c: cycocel, 1500 ppm

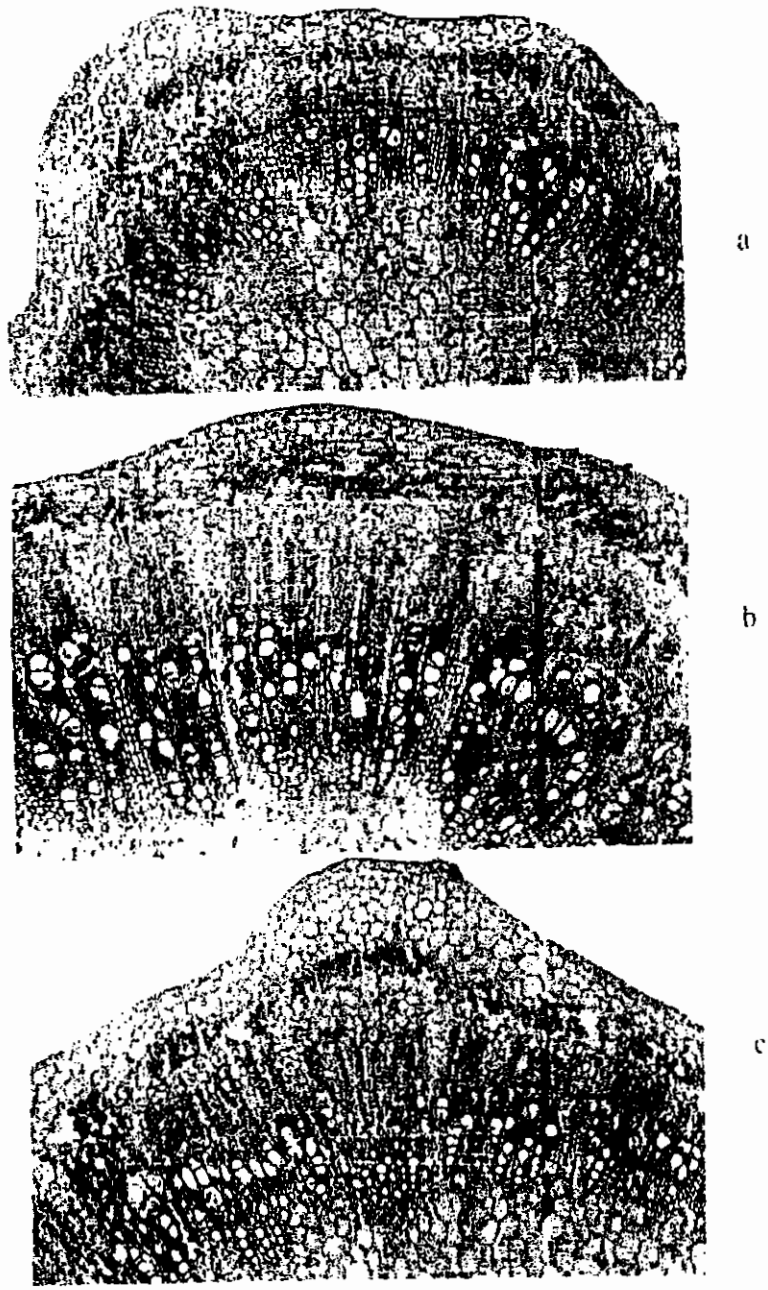

Fig 7: Transverse sectioms of main stem basal internode of mungbean plant at the age of 45 days as affected by different levels of phosphorus (x40).
a: 15.50 kg P_2O_5 / fed b: 23.25 kg P_2O_5 / fed c:31.00 kg P_2O_5 / fed

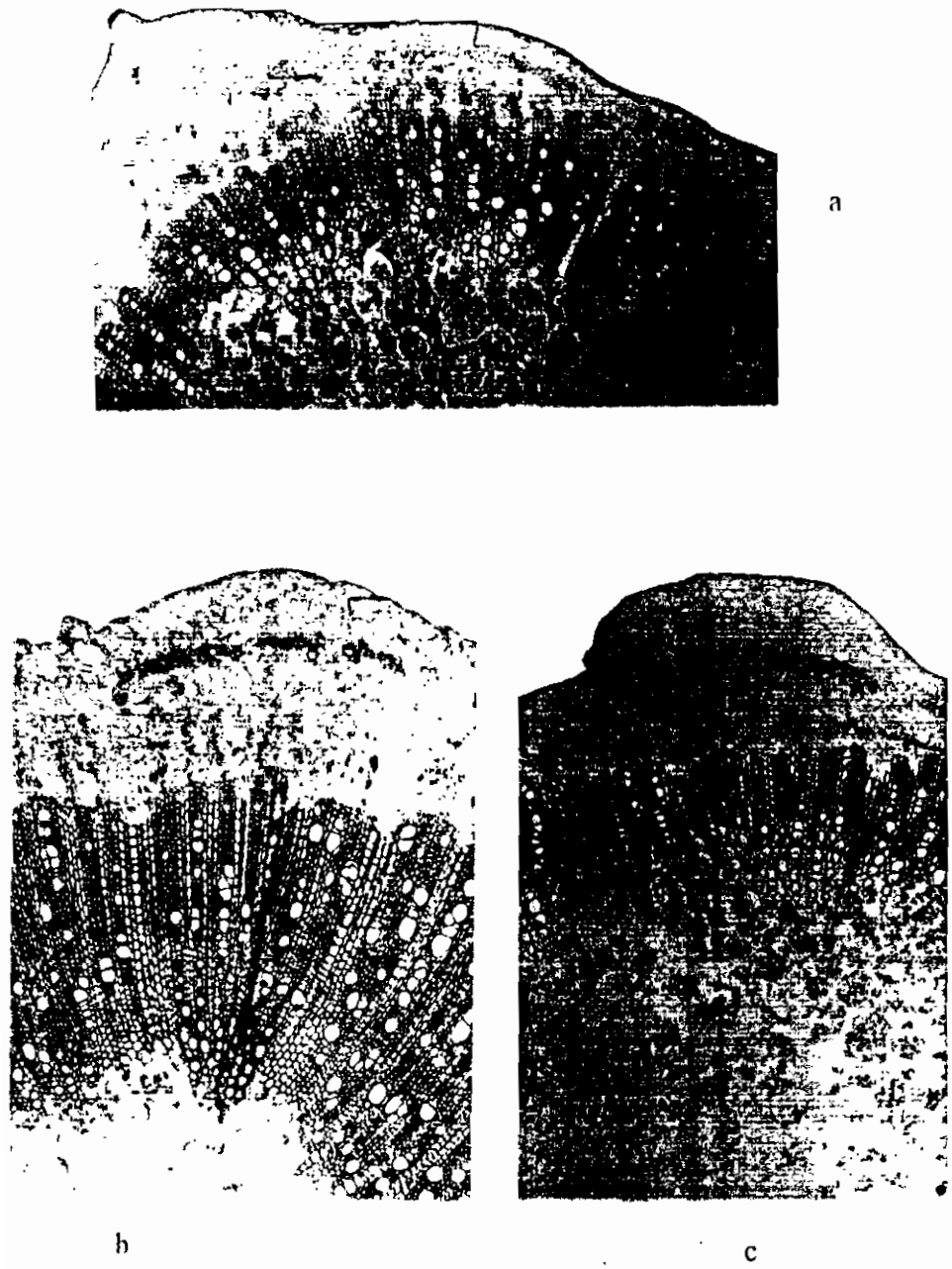

Fig 8: Transverse sectioms of main stem basal internode of mungbean plant at the age of 60 days as affected by different levels of phosphorus (x40).
a: 15.50 kg P2O5 / fed b: 23.25 kg P2O5 / fed c:31.00 kg P2O5 / fed

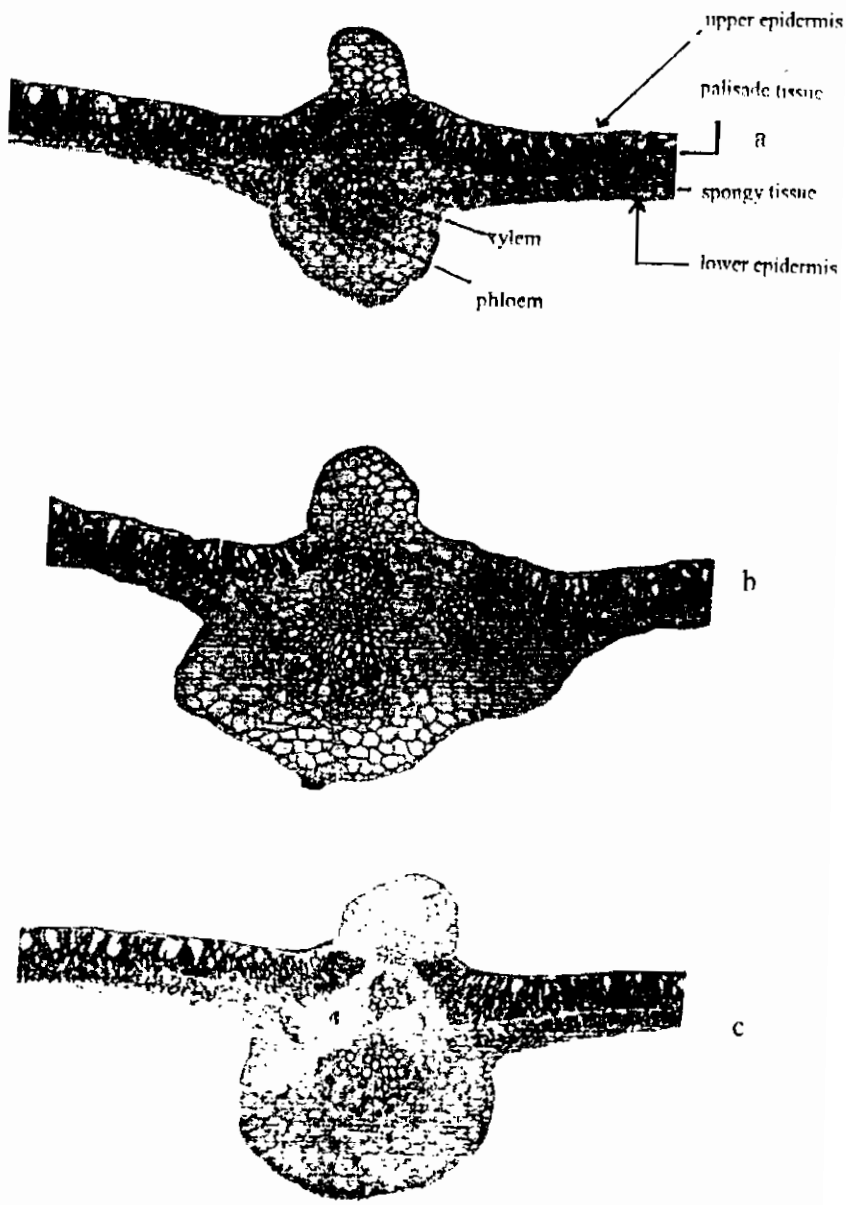

Fig 9: : Transverse sections of leaflet blades , at the age of 45 days, as affected by different rates of ethrel. (x 40)
a: control. b: ethrel, 200 ppm. c: ethrel, 300 ppm.

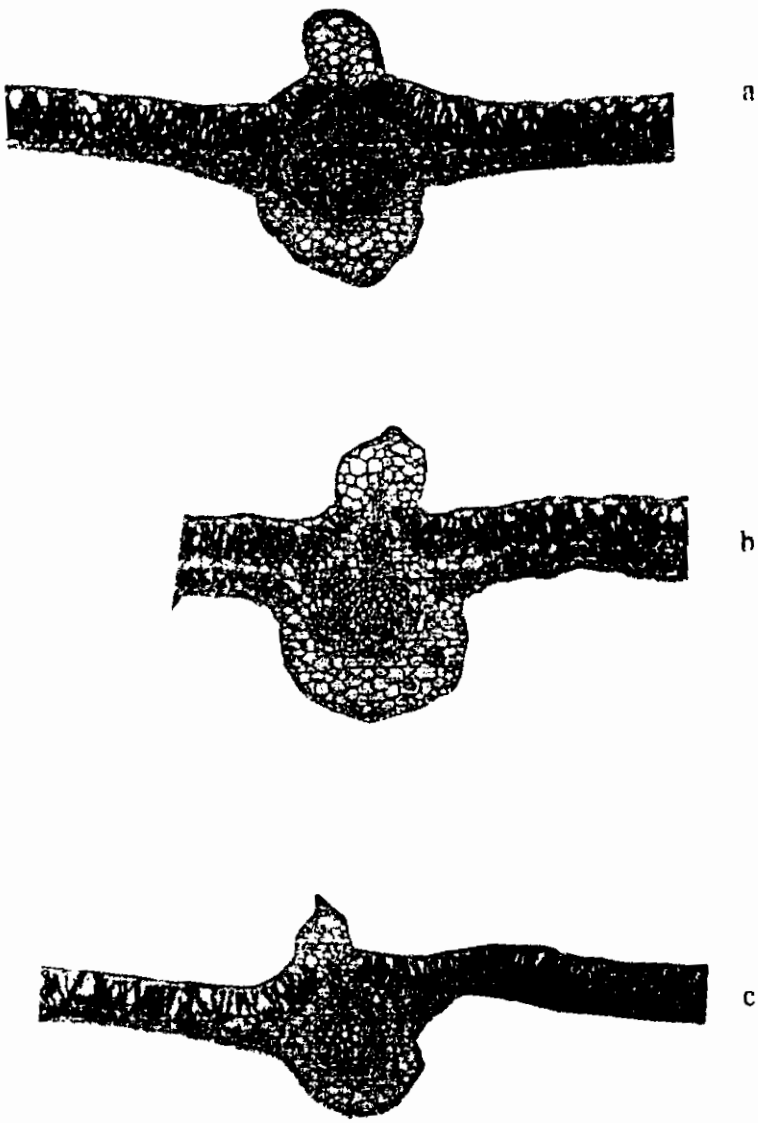

Fig10.: Transverse sections of leaflet blades , at the age of 45 days, as affected by different rates of cycocel. (x 40)
a: control. b: cycocel, 1000 ppm. c: cycocel, 1500 ppm.

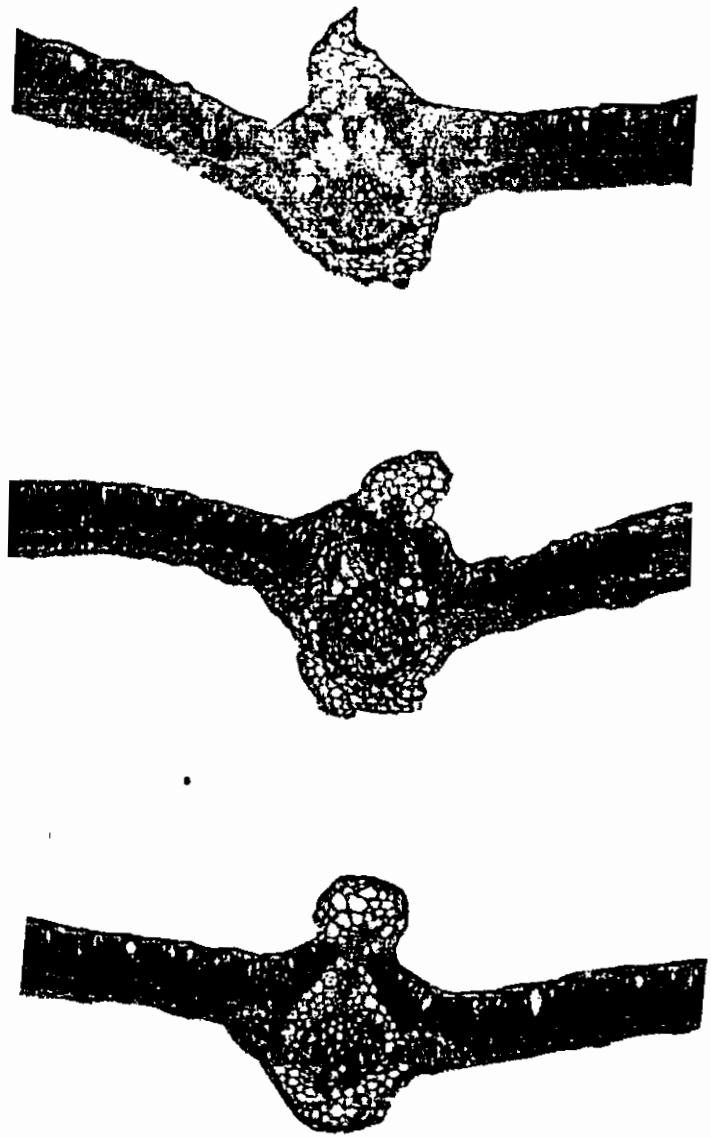

Fig11: Transverse sections of leaflet blades , at the age of 60 days, as affected by different levels of phosphorus. (x 40)
a : 15.50 kg P_2O_5/fed. b: 23.25 kg P_2O_5/fed. c: 31.00 kg P_2O_5/fed.

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تأثير الاثريل والسيكوسيل والفوسفور على التركيب التشريحى لنبات فول المانج

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أجرى هذا البحث خلال موسمي النمو ١٩٩٩، ٢٠٠٠ بمحطة التجارب والبحوث الزراعية بكلية الزراعة – جامعة القاهرة وذلك لدراسة استجابة التركيب التشريحى لنبات فول المانج لتركيزات مختلفة من الاثريل والسيكوسيل والفوسفور.

وأوضحت القطاعات العرضية فى الساق الرئيسى ان المعاملة بتركيزات ٢٠٠ ، ٣٠٠ جزء فى المليون من الاثريل ، ١٠٠٠ ، ١٥٠٠ جزء فىالمليون من السيكوسيل قد أدت إلى تنشيط نمو الساق في السمك نتيجة الزيادة فى سمك القشرة والأنسجة الوعائية وفى قطر النخاع .وأظهرت معظم الأوعية وخلايا الأشعة بالخشب الثانوي للساق المعاملة بالاثريل نموا فى الاتجاه القطري أكثر منه فىالاتجاه المماسى، التسميد بالمعدل المتوسط (٢٣,٢٥ كجم P2O5 للفدان) أدى إلى زيادة فى كمية الأنسجة الوعائية وفى اتساع الأوعية بالساق مقارنة بالتسميد بالمعدل الأقل أو الأعلى .

أوضحت القطاعات العرضية فى أنصال الوريقات المأخوذة من المنطقة الوسطى من الساق أن المعاملة بالاثريل أدت إلى زيادة حجم العرق الوسطى نتيجة الزيادة في حجم الحزمة الوعائية وخلايا النسيج الاساسى .بينما أدت المعاملة بتركيز ١٠٠٠ جزء فى المليون من السيكوسيل إلى زيادة فى حجم العرق الوسطى وكل من النسيجين العمادى والاسفنجى وان زيادة تركيز السيكوسيل الى ١٥٠٠ جزء فىالمليون قد نتج عنه نقص فى تلك الصفات.التسميد بالمعدل المتوسط من الفوسفور (٢٣,٢٥ كجم P2O5 للفدان) أدى إلى زيادة فى كمية الأنسجة بنصل الوريقة عن مثيلتها بأوراق النباتات المسمدة بالتركيز الأعلى أو الأقل.