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EFFECTS OF HYDROGEL AND DIFFERENT APPLICATION TECHNIQUES OF INDOLE-3-BUTYRIC ACID (IBA) ON THE STEM CUTTINGS OF SOME FICUS TREES.

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ABSTRACT

This study was carried out during spring of the two successive years of 1998 and 1999 on 20-25 cm length stem cuttings with 1-2 leaves of *F. retusa, F. infectoria* and *F. religiosa* taken from the middle portion of two-years-old branches of trees grown at El- Mansoura University Campus. Hydrogel and different techniques of applying IBA were used in order to improve the rooting of cuttings of the three Ficus species that vary in their rooting ability. These techniques were 1000 mg/L. Indole-3-butyric acid (IBA) in the form of solution, talc powder, and toothpicks loaded with IBA (in the presence or absence of hydrogel in sandy medium), in addition to planting cuttings in sandy medium incorporated with hydrogel-charged with the IBA. The study aimed to investigate the effect of these treatments on rooting and root and shoot characters of the cuttings of the three Ficus species as well as to study the anatomical changes that take place within the cutting as a result of IBA treatment. In addition, in the second season, the relationship between the differences in the internal phytohormones content, the anatomical structure, and the rooting ability and root characters of the three Ficus species were also studied. The results showed that 1000 mg/L. IBA solution quick dip plus hydrogel in the medium produced the highest number of rooted cuttings, the highest survival percentage of the cuttings, and the greatest number of roots. Impregnated toothpicks plus hydrogel in the medium resulted in the fastest rooting, while charged hydrogel incorporated to the rooting medium resulted in the longest and heaviest fresh and dry weight of roots per cutting. As for shoot growth, IBA solution plus hydrogel treatment produced the greatest number of shoots and leaves per cutting, the heaviest fresh and dry weights of leaves, and the largest leaf area. In general, talc powder treatment ranked fourth after the other three application techniques. The results also showed that the presence of hydrogel in the medium improved the effect of any particular treatment. Two weeks after treating *Ficus retusa* cuttings with IBA, the internal anatomy showed great proliferation and extension of the cortex, phloem tissues, and cambial zone, and the formation of meristematic centers which was developed as root primordia. Four weeks after treatment, the roots passed through the cortical tissue and emerged on the cutting and vascular connection tissues were also observed in the growing roots. The untreated cuttings showed similar anatomical changes, but those changes were observed two weeks later.

The comparison among species showed that there was a relationship among the phytohormones contents of the cutting, the anatomical structure of the stem, and the differences in the rooting ability of cuttings of the three species. *Ficus retusa* cuttings had the highest GA_3 and IAA concentrations, but the lowest ABA concentration among the three species, while *F. religiosa* had the lowest GA_3 and IAA and the highest ABA concentration, and *Ficus infectoria* had intermediate concentrations of the three phytohormones. Also, the cuttings of *F. retusa* had the highest percentage of rooting and were the fastest to root, gave the highest percentage of survival after rooting, and the greatest number of new roots, while *F.*

infectoria ranked the second, and *F. religiosa* was the third. On the other hand, *F. retusa* and *F. infectoria* did not significantly differ in either their root lengths or roots dry weight, but both species were significantly higher than *F. religiosa*. The stem structure of both *F. infectoria* and *F. religiosa* had thicker cortex tissue which was composed of more collenchymatious cell layers and had thinner phloem tissue and cambial zone than *F. retusa*. In addition, *F. religiosa* had thicker continuous phloem fiber rings composed of larger number of sclerified fiber cells than *F. infectoria*, while *F. retusa* stem had a discontinuous phloem fiber ring that was composed of smaller number of weak sclerified fiber cells which may explain the differences in rootability among the three species.

INTRODUCTION

Genus Ficus is a member of Family Moraceae, and includes more than 1000 species (Bailey, 1969). Ficus trees are fast grown trees that are tolerant to any soil and light conditions, which make them good street and landscape trees. In Egypt, the use of Ficus trees in landscape is restricted to few species because of poor rooting of most species.

Indole-3-butyric acid (IBA) has been used by most propagators to stimulate rooting of cuttings (Struve *et al*, 1983) because it is nontoxic at relatively high concentrations (Hartmann *et al*, 1990), and is effective over a wide range of species (Couvillon, 1988). The stimulatory effect of IBA on rooting depends on its formulation such as solution and powder, (Bonaminio, 1983), concentration and the duration of treatment (Nunes *et al*, 1982), methods of the application (El-Torkey and El-Sennawy, 1993), type of cuttings (Kwash *et al*, 1989), cultivars within the species, and time of cutting collection (Souidan *et al*, 1995 and Sabbour *et al*, 2001).

Hydrophilic polymers (hydrogels) are high-molecular-weight materials that can absorb high rates of water up to 400 to 500 times their weight under field conditions (Evans *et al*, 1990). Hydrogels reduced water requirements for crops (Blodgett *et al*, 1993), enhanced plant growth (Austin and Bondari, 1992), increased root mass of plants (Kuack, 1987), and improved root development in cuttings (Banko, 1984). They could be also used as carrier for nutrients, insecticides, fungicides, herbicides, and growth regulators (Orzolek, 1991). Hydrogels as carriers of rooting hormones and incorporated into the root medium were used for production of new roots of transplants (Al-Mana and Beattie, 1996).

Many anatomical changes take place in the stem cuttings during the rooting process. In cuttings of *F. pumila* (Davies *et al*, 1982) and *F. retusa* (Sabbour *et al*, 2001) adventitious roots originated from phloem ray parenchyma and cambial zone, while some of the roots developed from callus tissue. Anatomical changes during rooting of *Jasminum sambac* stem cuttings started with proliferation and extension of certain zones of phloem parenchyma and cambial zone (Fouda and Khafagy, 1996).

Poor rooting of stem cuttings in certain species was correlated with an imbalance of plant hormones and presence of growth inhibitors (Biran and Halevy 1973; Davies, 1984; Sagee *et al*, 1992). Stem structure also influenced the initiation of adventitious roots on stem cuttings (Beakbane, 1969 and Fouda, 1995).

In this study, hydrogel and different techniques of applying IBA were used in order to improve rooting of cuttings of three different Ficus species that vary in their ability to root. These techniques were IBA in the form of solution and powder, toothpicks loaded with IBA, and hydrogel-charged with the IBA. The study aimed to investigate the effect of these treatments on rooting and root and shoot characters of the cuttings of *F. retusa, F. infectoria* and *F. religiosa*. and to study the anatomical changes that take place in the cutting as a result of IBA treatment. In addition, the study aimed also to investigate the relationship between differences in the internal phytohormones conetnt, the anatomical structure, and the rooting ability and root characters of the three ficus species.

MATERIALS AND METHODS

Cuttings of *F. retusa, F. infectoria* and *F. religiosa* were taken from two-years-old branches of trees grown at El- Mansoura University Campus on the 1st of March of the two successive years of 1998 and 1999. Cuttings were 20-25 cm in length with 1-2 leaves.

Preparation of Media: the following media were used in this experiment:

A. **Sand medium**.

B. **Non-charged hydrogel medium**: The hydrophylic polymer (SuperSorb C), (a co-polymer acrylamide acrylate manufactured by Aquatrols Corp. of America, Inc. Pennsauken, N.J.), was mixed with sand medium at the rate of 1.2 kg/m^3.

Applcation techniques of IBA:

A. **Charged-Hydrogel**: The charged-hydrogel was prepared according to Al-Mana and Beattie (1996). The charged hydrogel was then mixed with sand medium at the rate of 1.2 kg/m^3. Cuttings were planted in this charged-medium.

B. **IBA solution (1000 ppm)**: 1 g of IBA powder was dissolved in small quantity of ethanol, and then the volume was completed to one liter using distilled water. Cuttings were dipped in this solution for 10 seconds before planting.

C. **Impregnated toothpicks**: round wooden toothpicks were soaked in 1000 ppm solution of IBA for 24 hrs, then completely dried in an oven at 50°C for another 24 hrs. Before planting, two impregnated toothpicks were inserted perpendicularly in 2 mm diameter holes drilled through the cutting at 1cm above the base of the cutting.

D. **IBA powder (1000 ppm)**: the basal 2 cm of the cutting was moistened with water and the base was dipped in a talc powder containing 1000 ppm IBA, and the cutting was shacked gently to remove the excess of the powder before planting.

Treatments:

The cuttings were subjected to the following treatments:

1. Control without hydrogel: cuttings were placed in distilled water and planted in sand medium (A).

2. Non-charged hydrogel: cuttings were placed in distilled water and then planted a non-charged hydrogel medium (B).

3. Charged-hydrogel: cuttings were placed in distilled water and then planted in charged-hydrogel medium.

4. IBA solution: cuttings treated with IBA solution were planted in sand medium (A).

5. IBA solution plus hydrogel: cuttings treated with IBA solution were planted in non-charged hydrogel medium (B).

6. Toothpicks: cuttings treated with impregnated toothpicks were planted in sand medium (A).

7. Toothpicks plus hydrogel: cuttings treated with impregnated toothpicks were planted in a non-charged hydrogel medium (B).

8. IBA powder: cuttings treated with IBA powder were planted in sand medium (A).

9. IBA powder plus hydrogel: cuttings treated with IBA powder were planted in a non-charged hydrogel medium (B).

Planting was done in 20 cm diameter pots filled with the designated medium, and each pot contained 4 cuttings. Each treatment was replicated 3 times, and each replicate contained 5 pots. Additional 4 pots in each treatment were used to check for the appearance of the first visible root on the cutting and for the samples used for the anatomical study. The pots were placed inside a greenhouse at an average temperature of 27 °C ± 2 and 70% relative humidity, and were watered daily throughout the experiment period.

Measurements:

Two weeks after planting, cuttings of the additional 4 sample pots were checked twice a week to determine the rate of rooting expressed as the number of days from planting until the appearance of the first visible root. Three months after planting, number of rooted cuttings was recorded. The survival percentage was measured as the percent of number of survived cuttings divided by the total number of cuttings. The cuttings were removed from the media, gently washed with water to remove the remaining sand off the roots. Number of rooted cuttings was recorded and the roots were separated from the cutting and their number, average length (cm), and fresh and dry weights (g) were measured. Length of the new shoots (cm), number of new shoots and leaves for each successful cutting were recorded. The leaves were separated from the cutting to determine their fresh and dry weights (g) and average leaf area (cm^2).

N.B. Leaf area was measured using a planimeter during the second season only.

Anatomical studies:

In the second season (1999), fresh samples of 4-5 mm long were excised from the basal portion of *F. retusa, F. infectoria* and *F. religiosa* cuttings at the beginning of the experiment for comparison among species. Two and four weeks later, samples of *F. retusa* only were taken from cuttings treated with IBA solution and the control ones to study the effect of IBA on the anatomical changes taken place during rooting. The samples were immediately killed and fixed in FAA solution, dehydrated with ethyl alcohol series, cleared in xylene, and embedded in paraffin wax (55-58°C). Cross and longitudinal sections of 15-30 µm were stained with light green-saffranin combination, cleared in oil cloves and mounted in Canada balsam for microscopic examination and photography (Johansen, 1940).

Determination of endogenous phytohormones:

At the beginning of the experiment of 1999 season, fresh samples from stems of two-year old shoots were taken to determine total indoles, IAA, ABA and GA_3. In this respect, 25 grams collected from fresh stem cuttings were weighed and ground in cold 80% aqueous methanol. The combined acidic ethyl-acetate phase was reduced in volume to be used for GLC determination of the acidic hormones such as IAA, ABA and GA_3. The hormones were determined in the Central Laboratory of Ain-Shams University.

Statistical analysis:

All data were subjected to ANOVA in a split plot design as mentioned by Gomez and Gomez (1984) using SAS computer software (SAS Institute, 1985). Treatment means were compared using the least significant difference test (LSD), (probability 5 %). The experiment consisted of three Ficus species (as main treatments), each of them was subjected to nine hormonal treatments (as sub-treatments). Each of the sub-treatments was replicated 3 times, and each replicate contained 5 pots.

RESULTS AND DISCUSSION

A. Effect of different treatments on cutting characteristics:

A.1. Percentage of rooted cuttings:

Data in Table (1) showed that *Ficus retusa* had the highest percentage of rooted cuttings, followed by *F. infectoria*, and *F. religiosa* was the lowest in both seasons. The highest percentage of rooted cuttings resulted from IBA solution plus hydrogel medium treatment of 75, 45 and 25% for *F. retusa, F. infectoria* and *F. religiosa*, respectively in both seasons. Similarly, Sabbour *et al*, (2001) showed that IBA solution increased rooting percentage of *F. retusa*. In addition, IBA was used in order to enhance rooting of stem cuttings of many *Ficus species* (Kwash *et al*, 1989). The lowest percentage of rooted cuttings was that of the control for *F. retusa* and *F. infectoria*, while the cuttings of *Ficus religiosa* of the control or the non-charged hydrogel treatments did not develop any roots at all. Since *F. religiosa* is a hard to root species, it might need higher IBA concentration. The used IBA concentration in the previous research varied among species and cutting maturity (Davies *et al*, 1982 and Kwash *et al*, 1989). Among treatments without hydrogel, IBA solution gave the highest percentage, followed by toothpicks for *F. retusa*, while the toothpicks gave higher percentage for *F. infectoria* and *F. religiosa*. When treatments included hydrogel, IBA solution plus hydrogel treatment was the highest among all treatments for the three species. Results also showed that any treatment in the presence of hydrogel gave higher percentage of rooted cuttings than when was used without hydrogel in both seasons for all the three species. The hydrogel polymer would contribute to increase rooting percentage of cuttings by its contribution to increase storage of plant available water (Johnson and Veltkamp, 1985).

Table (1): Effect of different IBA treatments on percentage of rooted cuttings of *F. retusa, F. infectoria* and *F. religiosa* during 1998 and 1999 seasons.

Species / Treatments	Rooted cuttings %					
	1998			1999		
	F. retusa	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	26.7	15	0.0	30	16.7	0.0
Non –charged hydrogel	33.3	16.7	0.0	36.7	15	0.0
Charged – hydrogel	65	41.7	20	66.7	43.3	23.3
IBA Solution	58.3	30	10	55	38.3	11.7
IBA Solution + hydrogel	75	45	25	75	45	25
Toothpicks	46.7	38.3	15	45	40	18.3
Toothpicks + hydrogel	56.7	45	18.3	55	46.7	23.3
Powder + hydrogel	45	33.3	18.3	46.7	33.3	16.7
Powder	36.7	31.7	11.7	38.3	33.3	15

A.2. Rooting rate of rooted cuttings (days):

Ficus retusa cuttings rooted faster than *F. infectoria* in most treatments, while the slowest to root was *F. religiosa* cuttings in all cases in both seasons (Table, 2).Among all treatments, the fastest cuttings to root resulted from toothpicks plus hydrogel medium of 21, 30 and 47 days for *F. retusa, F. infectoria* and *F. religiosa*, respectively in the first season, and 20, 32 and 45 days in the second season in the same respective order. The slowest rate was that of the control for *F. retusa* and *F. infectoria*, while the cuttings of *Ficus religiosa* of the control or the non-charged hydrogel treatments did not root at all.

Table (2): Effect of different IBA treatments on rate of rooting of *F. retusa, F. infectoria* and *F. religiosa* during 1998 and 1999 seasons.

Species / Treatments	Rate of rooting (days)					
	1998			1999		
	F. retusa	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	50	50	0.0	50	50	0.0
Non –charged hydrogel	48	50	0.0	50	50	0.0
Charged – hydrogel	34	34	50	32	34	50
IBA Solution	37	40	57	35	40	57
IBA Solution + hydrogel	34	37	53	32	35	52
Toothpicks	21	30	47	20	32	45
Toothpicks + hydrogel	21	30	47	20	32	45
Powder + hydrogel	34	37	50	34	36	50
Powder	34	37	53	34	36	53

Among all treatments, the toothpicks with or without hydrogel resulted in the fastest rooting rate for the three species in both seasons. The promotive effect of the toothpick treatment might be explained that the placement of IBA was in intimate contact with cambial and parenchyma cells involved in adventitious root initiation (Struve and Moser, 1984). In addition, Al-Mana and Beattie (1996) reported that toothpicks impregnated with KIBA (the potassium salt of IBA) at 1000 ppm stimulated root regeneration of both red oak and black gum transplants.

A.3. Survival percentage of the cuttings:

In both seasons, the highest survival percentage of rooted cuttings resulted from IBA solution plus hydrogel medium treatment of 75, 50 and 25% for *F. retusa, F. infectoria* and *F. religiosa*, respectively (table 3).

Table (3): Survival percentage of cuttings of *F. retusa, F. infectoria* and *F. religiosa* during 1998 and 1999 seasons.

Species / Treatments	Survival of cuttings %					
	1998			1999		
	F. retusa	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	23.3	11.7	0.0	20	10	0.0
Non –charged hydrogel	35	18.3	0.0	35	23.3	0.0
Charged – hydrogel	61.7	40	20	66.7	38.3	20
IBA Solution	50	33.3	11.7	50	33.3	11.7
IBA Solution + hydrogel	75	50	25	75	50	25
Toothpicks	38.3	35	10	46.7	35	10
Toothpicks + hydrogel	56.7	45	11.7	60	46.7	11.3
Powder + hydrogel	46.7	33.3	18.3	43.3	30	16.7
Powder	35	25	11.7	38.3	25	10

These results are in accordance with those of Sabbour *et al* (2001) and Sarma (2002), who showed that IBA solution increased survival percentages of different Ficus species. The survival of any rooted cutting shown in this Table was a result of the combination between ability to root (Table, 1) and fast rooting (Table 2). In addition, the growth and development of new roots and shoots depend on root growth and development. Again, it is also clear that any treatment in the presence of hydrogel gave higher survival percentage of rooted cuttings than when was used without hydrogel. It is worthy to note that the charged hydrogel treatment ranked second after IBA solution plus hydrogel. This was a result of IBA stimulation of rooting in addition to the different functions of hydrogel mentioned before. However, all the cuttings of the control or the non-charged hydrogel of *F. religiosa* did not survive until the end of the experiment.

A.4. Number of roots per cutting:

Data in Table (4) showed that *F. retusa* had the highest number of roots per cutting, followed by *F. infectoria*, while *F. religiosa* had the lowest in both seasons. The four methods of applying IBA developed more number of roots especially when combined with hydrogel than the non-charged hydrogel or the control. There were some relative differences among treatments with favor to toothpicks plus hydrogel and IBA solution plus hydrogel treatments.

High number of roots per cutting as a result of IBA solution treatment was reported by Sarma, (2002), while Al-Mana and Beattie (1996) showed that the toothpicks treatment gave the highest number of roots of red oak and black gum transplants. However, many propagators prefer the concentrated solution as a quick dip method to talc application because of the ease of application and the base of cuttings could be dipped in bundles (Bonaminio, 1983).

Table (4): Effect of different IBA treatments on number of roots per cutting of *F. retusa, F. infectoria* and *F. religiosa* during 1998 and 1999 seasons.

Species Treatments	Number of roots/cutting					
	1998			1999		
	F. retusa	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	10.1	2.1	0.0	10.4	2.4	0.0
Non –charged hydrogel	26.7	7.1	0.0	27.6	8.1	0.0
Charged – hydrogel	33.0	9.7	7.1	33.9	10.1	9.9
IBA Solution	34.0	15.6	5.7	34.1	16.1	5.9
IBA Solution + hydrogel	35.7	15.9	13.0	38.6	18.1	13.4
Toothpicks	33.9	16.0	3.4	34.4	16.7	3.6
Toothpicks + hydrogel	35.9	19.6	9.9	35.3	18.4	10.1
Powder + hydrogel	32.9	10.1	7.6	34.0	8.7	8.1
Powder	28.7	8.3	3.1	27.0	8,4	6.9
LSD 5%	7.2			6		

A.5. Effect on average root length (cm):

Data in Table (5) showed that *F. infectoria* had the longest roots, followed by *F. retusa*, while *F. religiosa* roots were the shortest in both seasons. The longest roots resulted from charged hydrogel treatment for *F. retusa* and *F. infectoria*. The hydrogel charged with IBA caused an increasing root length of the rooted cuttings of the three species due to direct contact with the roots in the medium, thus a synergistic effect of both the hormone and the hydrogel was achieved. The hydrogel seemed to be a key for improving root development of the rooted cutting. In this concern, Kuak (1987) reported that root mass of plants was greatly improved with a water-absorbing compound in the medium. The improvement in root growth of the cuttings when polymer was incorporated into the rooting media was due to the fact that polymers contributed to a more uniform distribution of moisture through the rooting medium (Banko, 1984).

Table (5): Effect of different IBA treatments on average root length per cutting of *F. retusa, F. infectoria* and *F. religiosa* during 1998 and 1999 seasons.

Species Treatments	Average root length/cutting (cm)					
	1998			1999		
	F. retusa	*F. infectoria*	*F. Religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	3.5	4.7	0.0	3.6	4.7	0.0
Non –charged hydrogel	4.8	4.1	0.0	4.9	4.8	0.0
Charged – hydrogel	13.9	21.3	1.5	14.0	19.6	2.1
IBA Solution	6.2	12.5	0.9	6.4	14.7	0.9
IBA Solution + hydrogel	10.3	5.5	3.2	10.3	5.6	3.3
Toothpicks	8.5	6.1	0.2	8.6	5.9	0.2
Toothpicks + hydrogel	9.8	6.7	0.7	10.0	6.8	0.7
Powder + hydrogel	11.2	12.0	0.8	15.1	12.1	1.6
Powder	12.2	10.9	1.4	11.5	9.3	1.9
LSD 5%		2.6			2.5	

A.6. Roots fresh and dry weights/cutting (g):

Data in Table (6) showed that *F. infectoria* and *F. retusa* did not significantly differ in their roots fresh and dry weight. The roots weight (fresh and dry) of *F. religiosa* was less than those of either *F. retusa* or *F. infectoria* in most cases. The heaviest roots weight (fresh or dry) resulted from charged-hydrogel treatment in both seasons for *F. infectoria* and *F. retusa*. However, for *F. religiosa*, IBA solution plus hydrogel medium had the heaviest roots fresh weight. It was also clear from the Table that the presence of hydrogel gave higher roots fresh and dry weights than when hydrogel was not applied in both seasons for all the three species. The response of the roots fresh and dry weights were very similar to the response of the root length. These results could be explained on the same bases mentioned before.

A.7. Number of shoots/cutting and shoot length (cm):

Data in Table (7) showed that *F. retusa* had the highest number of shoots per cuttings. The highest number of shoots resulted from IBA solution plus hydrogel medium treatments for the three species in both seasons. The presence of hydrogel in any treatment gave higher number of shoots than when a similar treatment was used without hydrogel for all the three species in both seasons. Data also showed that *F. infectoria* had longer shoots when compared with the other two species in any similar treatments. The rest of the results were identical to those of number of shoots.

A.8. Number of leaves/cutting and leaf area (cm2):

Data in Table (8) showed that *F. retusa* had the highest number of leaves, followed by *F. infectoria* then *F. religiosa* which had the lowest in both seasons. The highest number of leaves resulted from IBA solution plus hydrogel medium treatment for *F. retusa* and *F. infectoria*, while for *F. religiosa* the best treatment was charged-hydrogel in both seasons. Any treatment in the presence of hydrogel gave higher number of leaves than when was used without hydrogel in both seasons for all the three species.

The Table showed also that the effect of different IBA treatments on leaf area of the three *Ficus* species during the second season of the experiment. In general, *F. religiosa* had the largest leaf area, followed by *F. infectoria*, while *F. retusa* had the smallest leaf area. This is a genetically controlled leaf character. However, it might contribute to the results mentioned before, since larger leaf area means more water loss by the vegetative bud. As a consequence, if the cutting did not initiate roots and develop them fast enough to meet with the water requirements of the vegetative bud, it would be expected that these cuttings would not be able to survive. This might be the main cause for the less the survival percentage of *F. religiosa* cuttings.

In case of *F. retusa*, the largest leaf area resulted from powder plus hydrogel medium treatment (8.14 cm^2), while in case of *F. infectoria*, this resulted from charged hydrogel treatment (16.171 cm^2), but for *F. religiosa*, it resulted from IBA solution plus hydrogel medium treatment (13.729 cm^2).

A.9. Fresh and dry weights of leaves/cutting (g):

Data in Table (9) showed that, in similar treatments, *F. retusa* had the heaviest fresh and dry weights, followed by *F. religiosa*, while *F. infectoria* had the lowest in both seasons. The heaviest leaves fresh and dry weights resulted from IBA solution plus hydrogel medium treatment for the three species in both seasons. As for treatment without hydrogel, it was obvious that toothpicks resulted in more fresh and dry weights for *F. retusa* and *F. infectoria*, and that IBA solution was better for *F. religiosa* than the other treatments. It was also clear from the data that any treatment in the presence of hydrogel was better than when was used without hydrogel in both seasons for all the three species.

B. Internal anatomical changes associated with IBA treatment:

The previous results indicated that IBA solution at 1000 mg/L was the most effective treatment to induce fast and better rooting and *F. retusa* had better rooting than other species. Therefore, the anatomical study herein included the anatomical changes accompanying the induction and emergence of adventitious root formation in *F. restusa* cuttings treated with IBA solution in comparison to the untreated ones.

The general structure of *F. retusa* stem (Fig. 1) is as follows: the outer most cell layers constitute the epidermis. The outer most region of the cortex tissue consists of 2-4 cell layers of collenchymatious cells. The middle and inner regions contains more cell layers of paranchymatious cell layers. Primary phloem fibers appear as a discontinuous ring of sclerenchyma.

Table 6: Effect of different IBA treatments on roots fresh and dry weights (g) per cutting of *F. retusa*, *F. infectoria* and *F. religiosa* during 1998 and 1999 seasons.

Species / Treatments	Roots fresh weight (g)						Roots dry weight (g)					
	1998			1999			1998			1999		
	F. retusa	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. Retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	0.28	0.28	0.00	0.51	1.00	0.00	0.02	0.0	0.00	0.21	0.00	0.00
Non –charged hydrogel	0.71	0.58	0.00	0.78	1.61	0.00	0.04	0.11	0.00	0.44	0.15	0.00
Charged – hydrogel	2.46	2.82	1.66	2.58	3.28	2.80	1.02	1.72	0.61	1.16	1.82	0.67
IBA Solution	0.80	1.63	1.01	0.84	1.70	0.92	0.18	0.45	0.22	0.22	0.48	0.23
IBA Solution + hydrogel	2.25	2.76	3.26	2.36	2.72	3.98	0.85	0.79	1.04	0.92	0.95	1.09
Toothpicks	1.03	2.35	0.93	1.44	2.03	1.06	0.36	0.48	0.28	0.45	0.65	0.4
Toothpicks + hydrogel	1.50	2.37	0.54	1.50	3.19	0.53	0.71	0.74	0.19	0.77	0.86	0.23
Powder + hydrogel	2.09	1.88	1.04	2.36	1.84	1.30	0.62	0.62	0.37	0.73	0.53	0.45
Powder	1.00	1.48	0.39	1.01	1.69	0.76	0.33	0.44	0.11	0.40	0.49	0.20
LSD 5%	0.78			0.88			0.25			0.26		

Table 7: Effect of different IBA treatments on number of shoots per cutting and average shoot length of *F. retusa*, *F. infectoria* and *F. religiosa* during 1998 and 1999 seasons.

Species / Treatments	Number of shoots/cutting						Average shoot length (cm)					
	1998			1999			1998			1999		
	F. retusa	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	1.3	0.4	0.0	1.4	0.4	0.0	6.0	2.7	0.0	6.0	2.8	0.0
Non –charged hydrogel	3.1	1.0	0.0	3.3	1.4	0.0	6.9	5.0	0.0	6.2	6.7	0.0
Charged – hydrogel	4.4	2.7	1.0	4.7	2.6	1.3	8.4	13.1	3.5	7.4	10.9	7.1
IBA Solution	5.1	2.3	0.7	5.4	2.6	0.7	11.5	10.6	2.9	11.8	12.5	2.9
IBA Solution + hydrogel	6.6	2.9	1.4	6.7	3.0	1.9	15.7	17.4	5.5	16.2	17.7	4.3
Toothpicks	3.4	2.1	0.4	3.4	2.5	0.4	9.4	10.7	2.0	9.6	11.2	2.2
Toothpicks + hydrogel	4.0	2.4	0.5	3.9	2.6	0.5	10.0	11.7	3.5	10.3	12.1	3.6
Powder + hydrogel	4.1	1.9	0.3	4.3	1.9	0.6	14.7	17.1	3.3	15.1	17.7	5.1
Powder	5.7	2.6	0.4	5.9	2.1	0.7	11.6	14.7	1.5	12.0	17.4	3.1
LSD 5%	1.0			0.9			3.2			3.3		

Table 8: Effect of different IBA treatments on number of leaves per cutting (during 1998 and 1999 seasons) and average leaf area (during 1999 season) of *F. retusa, F. infectoria* and *F. religiosa*.

Species	Number of leaves / cutting						Average leaf area (cm2)		
	1998			1999			1999		
Treatments	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	32.4	2.3	0.0	23.6	2.4	0.00	2.9	1.9	0.0
Non –charged hydrogel	34.0	6.4	0.0	34.6	12.1	0.00	4.3	3.1	0.0
Charged – hydrogel	39.0	11.7	5.0	39.1	12.3	5.43	5.6	16.2	9.6
IBA Solution	40.3	11.3	3.4	39.6	11.3	3.57	5.7	6.0	12.6
IBA Solution + hydrogel	62.1	14.1	4.9	62.6	13.3	3.43	4.2	6.3	13.7
Toothpicks	58.1	11.6	1.0	58.7	11.0	1.00	3.5	3.4	5.7
Toothpicks + hydrogel	35.7	12.9	1.6	38.4	13.1	1.86	6.5	7.1	7.0
Powder + hydrogel	61.9	7.7	1.3	62.0	7.9	2.43	8.4	3.0	8.7
Powder	48.3	13.0	1.6	47.9	12.0	2.43	3.6	2.3	5.4
LSD 5%	4.4			5.1			4.5		

Table 9: Effect of different IBA treatments on leaves fresh and dry weights per cutting of *F. retusa, F. infectoria* and *F. eligiosa* during 1998 and 1999 seasons.

Species	Leaves fresh weight (g)						Leaves dry weight (g)					
	1998			1999			1998			1999		
Treatments	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*	*F. retusa*	*F. infectoria*	*F. religiosa*
Control (sand only)	2.38	0.92	0.00	4.11	3.12	0.00	0.77	0.26	0.00	0.79	0.26	0.00
Non –charged hydrogel	5.71	1.65	0.00	5.66	3.70	0.00	0.88	0.57	0.00	0.96	0.78	0.00
Charged – hydrogel	6.28	2.27	6.90	6.50	2.29	7.59	2.35	2.11	2.76	2.34	3.24	1.39
IBA Solution	4.74	4.59	7.06	4.39	6.24	8.02	1.22	0.76	2.30	1.24	0.91	2.89
IBA Solution + hydrogel	12.32	6.65	10.75	11.03	6.89	12.53	2.85	3.09	3.28	2.87	3.24	2.45
Toothpicks	9.30	5.69	2.29	8.94	6.07	2.92	1.34	2.58	0.93	1.35	2.57	0.94
Toothpicks + hydrogel	7.27	3.38	2.16	8.60	3.60	3.05	0.98	1.52	0.89	0.99	1.55	0.90
Powder + hydrogel	9.84	2.55	3.32	11.70	3.06	4.01	1.62	1.32	0.84	1.65	1.13	1.27
Powder	8.71	2.45	2.43	5.31	3.03	2.91	1.34	0.76	0.36	1.35	0.81	0.74
LSD 5%	3.24			2.67			0.96			0.96		

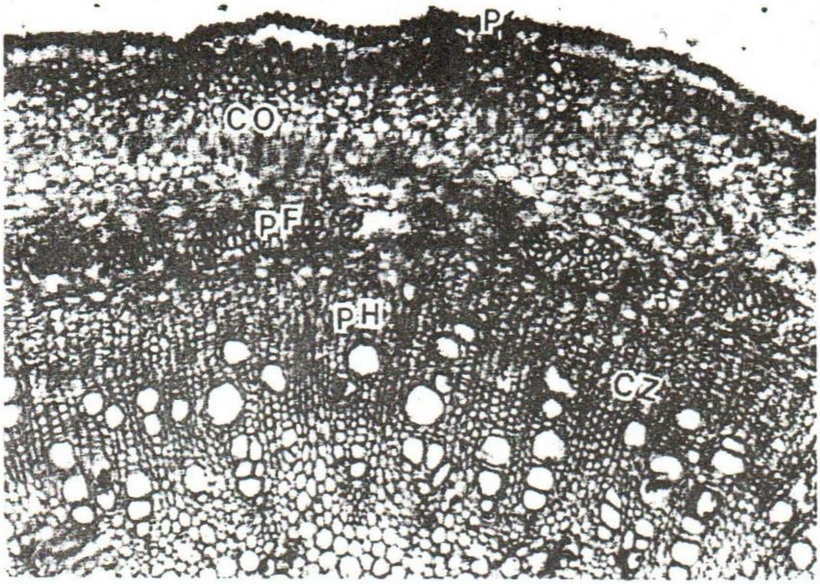

Fig. (1). Cross section of *F. retusa* stem cutting showing the general structure of the stem and discontinuous of sclerenchyma phloem fibers (PF). (P)= periderm, (CO)= cortex, (PH)= phloem, and (CZ)= cambial zone. (Obj – x 10. Oc x 10 x).

Cross section of *F. retusa* stem cutting taken from untreated cuttings two weeks after the start of the experiment (Figure, 2) showed that the most remarkable anatomical feature was extension of the cortex tissues only. Section taken from cuttings treated with IBA solution two weeks after planting showed significant anatomical differences (Figure, 3) compared with those taken from the untreated ones (Figure, 2). The most striking differences observed in IBA-treated cuttings (Figure, 3) were great proliferation and extension of the cortex, phloem tissues, and cambial zone. Along with these modifications, formation of meristematic centers took place, and was developed as root primordia. The cambial region and phloem tissue were the main tissues, which developed root primordia.

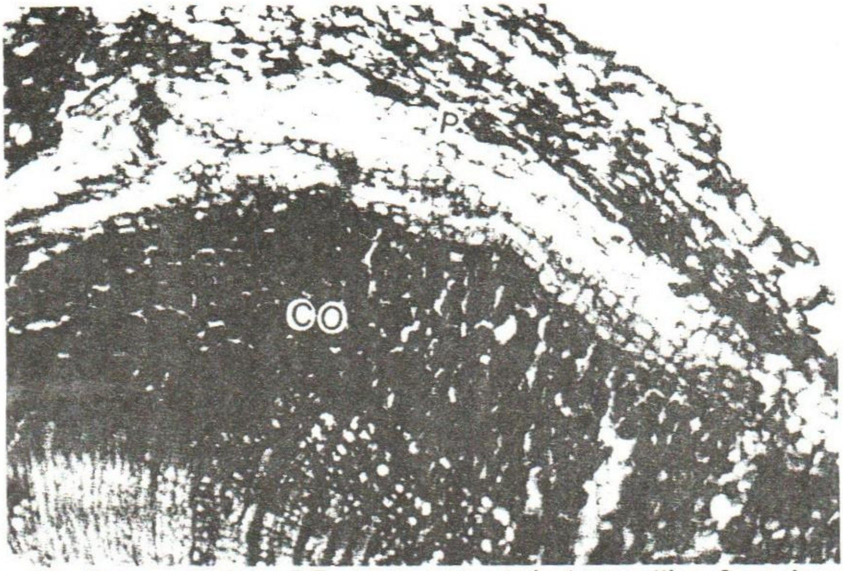

Fig. (2). Cross section of *F. retusa* untreated stem cutting 2 weeks after planting showing formation of periderm tissue (P) and extension in the cortex (CO) tissues. (Obj - x 10. Oc x 10 x).

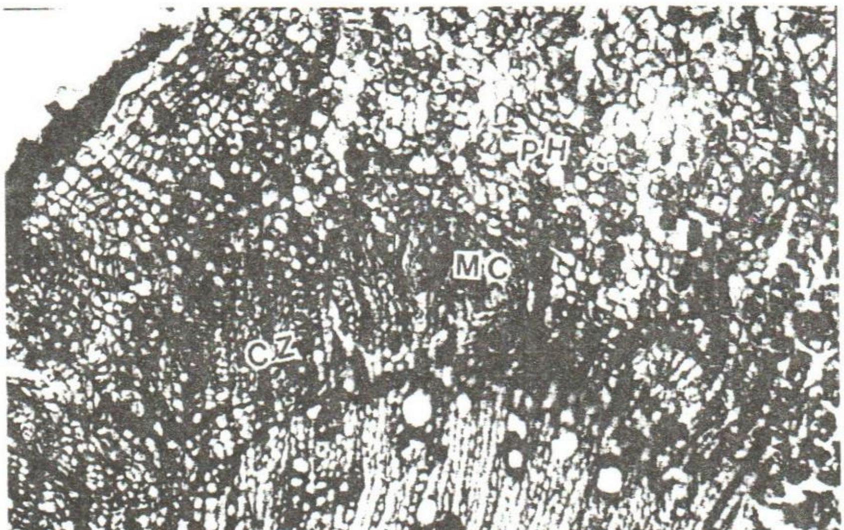

Fig. (3): Cross section through the basal portion of IBA-treated *F. retusa* cuttings 2 weeks after planting. Note proliferation and extension of the cortex, phloem tissues (PH) and cambial zone (CZ) along with formation of meristimatic centers (MC). (Obj - x 10. Oc x 10 x).

Four weeks after planting, the untreated cuttings showed anatomical changes (Figure, 4) similar to those in the IBA-treated cuttings which took place two weeks earlier (Figure, 3), which indicated that IBA hastened these changes in the treated cuttings. On the other hand, in the IBA treated cuttings after four weeks of planting (Figure, 5), the roots passed through the cortical tissue and emerged on the side cutting. Moreover, some of the adventitious roots were developed at the cut surface of cuttings and vascular connection tissues were also observed in the growing roots, which ensure the translocation of nutrients and hormonal factors to growing roots.

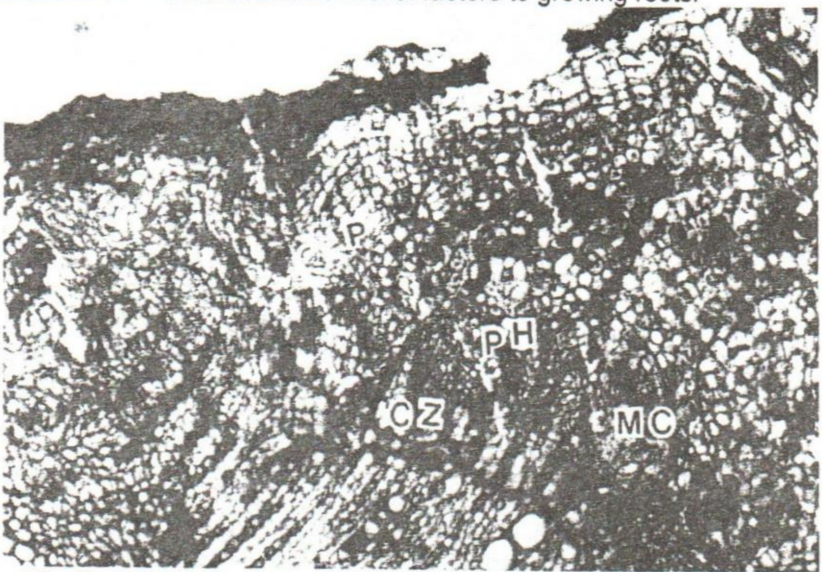

Fig. (4). Cross section of untreated stem cuttings of *F. retusa*, 4 weeks after planting. Note that changes are very similar to those of the IBA-treated cuttings two weeks after planting (Figure, 3). (P) periderm, (PH) phloem, (CZ) cambial zone, (MC) meristimatic center. (Obj - x 10. Oc x 10 x).

The stimulatory effect of IBA on rooting was previously reported on stem cuttings of many Ficus species (Davies *et al*, 1982 and Sabbour *et al*, 2001). The promotive effect of auxins on initiation and development of adventitious roots may be due to auxins can enhance RNA and protein synthesis, which are prerequisites for cell division and DNA synthesis (Webster and Van't- Hof, 1970). Thus, the effect of IBA to enhance adventitious root formation may be attributed to increased cell elongation, swelling of tissue, cell division, and formation of the adventitious roots. In this concern, Rodriguez and Sanchez (1988) reported that auxin was important for both formation of meristematic centers and future development of root primordia. According to Shawky *et al* (1988), the promotive action of IBA on rooting might be partially due to the reduction in ABA (abscisic acid) content especially soon after treatment.

Fig. (5): Cross section of IBA-treated stem cuttings of *F. retusa*, 4 weeks after planting. Note that adventitious roots developed from the cambial zone (CZ) showing the roots developed from phloem tissue (PH), forming vascular connection (VC) and emerging roots. (Obj - x 10. Oc x 10 x).

C. Differences among Ficus species cuttings

C.1. Relationship between the endogenous phytohormone contents and cutting characters:

It is clear from Table (10) that at the beginning of the experiment, the cuttings of *Ficus retusa* had the highest GA_3 and IAA concentrations, but the lowest ABA concentration among the three species. In contrast, *F. religiosa* had the lowest GA_3 and IAA and highest ABA concentrations, while *Ficus infectoria* had intermediate concentrations of the three phytohormones. The Table showed that cuttings of *F. retusa* had the highest percentage of rooted cuttings which was the fastest to root, and the highest percentage of survival after rooting. On the other hand, *F. infectoria* ranked the second, and *F.*

religiosa was the least in all the three previous cutting parameters. The promotive effect of auxins on initiation and development of adventitious roots was discussed earlier.

Table 10: Internal phytohormones (at the time of cutting collection), and percentage of rooted cuttings, rooting rate, and survival percentage of cuttings of the three of Ficus species after three months from planting during (1999) season.

Characters	Phytohormones			Cutting characters		
Ficus species	GA mg/100g	IAA µg/100g	ABA µg/100g	Rooted cuttings (%)	Rooting rate (days)	Survival %
F. retusa	77.45	1451.18	19.76	48.9	34.1	47.2
F. nfectoria	38.74	527.17	95.48	32.8	38.3	31.1
F. religiosa	23.50	312.80	176.50	16.1	52.8	11.1

Table (11) clearly showed that *F. retusa* had significantly the greatest number of new roots, while *F. infectoria* ranked the second, and *F. religiosa* had the lowest number of roots. These significant differences seemed to be related to the differences in IAA concentration among the three species, and thus the ability of the cuttings of each species to form new roots varies. Endogenous auxin is required for initiation of adventitious roots on stems, and the division of the first root initial cells is dependent upon endogenous or applied auxin (Hackett, 1972 and Maldiney *et al*, 1986).

The results also showed that *F. retusa* and *F. infectoria* did not significantly differ in their root lengths or in their roots dry weight, but both species gave significantly higher values than *F. religiosa*. GA_3 promotes cell elongation and thus resulted in longer roots in case of *F. retusa* and *F. infectoria* than in case of *F. religiosa* (which had lowest GA_3 concentration). However, the concentration differences in GA_3 between *F. retusa* and *F. infectoria* did not result in significant differences in either length of their roots or in their roots dry weight in this experiment. However, the formation of roots and the growth characters of roots of the cuttings involve the balance between the three phytohormones, which is a very complicated relationship.
On the other hand, the high level of internal natural ABA in *F. religiosa* resulted in the shortest roots and the least fresh and dry weights of roots which agree with the results of Bascu *et al*, (1970).

Table 11: Root characteristics of the cuttings of the three Ficus species after three months from planting during (1999) season.

Root Characters Species	Root length (cm)	No. of roots/cutting	Roots dW/cutting (g)
F. retusa	9.38	25.6	0.59
F. infectoria	9.28	11.92	0.66
F. religiosa	1.2	6.43	0.36
LSD 5%	1.71	4.05	0.17

C.2. Differences in the anatomical features of the stem cuttings:

The study which deals with the anatomical structure of stem cuttings of three *Ficus* species might give some light on the relationship between the stem anatomy of these species and the ability to form adventitious roots.

The stem anatomy at the time when cuttings were taken from the three *Ficus* species plants was characterized with early formation of both periderm tissues and secondary vascular tissues. The degree of continuity of the ring of phloem fibers varied from one species to another and was greatest in *F. religiosa* cuttings. The stem cuttings of the three *Ficus* species in this study were considered as fair and difficult-to-root species. The poor rooting ability could be related not only to the continuous of phloem fibers ring and amount of phloem fiber groups, but also to the thickness of phloem tissue and cambial zone.

As shown in Figures (6, 7 and 8), the cortex in all *Ficus* species in this study was wider and contained calcium oxalate crystals in case of *F. infectoria* and *F. religiosa* (Figs. 7 and 8); which might act as a mechanical barrier to root emergence.

The stem structure of both *F. infectoria* and *F. religiosa* (Figs. 7&8) had thicker cortex tissue and was composed of more collenchyma cell layers, and had thinner phloem tissue and cambial zone than *F. retusa* (Fig. 6). In addition, continuous phloem fiber rings of these two species was composed of larger number of sclerified fiber cells compared with *F. retusa*. Also, *F. retusa* stem had a discontinuous phloem fiber ring and was composed of smaller number of weakly sclerified fiber cells (Fig. 6).

The formation of fibers and scleroids in the primary phloem acted as a barrier to root formation and the ability of rooting was inversely proportional with the amount of fiber as well as the continuity of the fiberous ring (Edward and Thomas, 1980; and Fouda, 1995). Easy-to-root species had less amount of phloem fiber and large gaps in the sclernchymatic ring

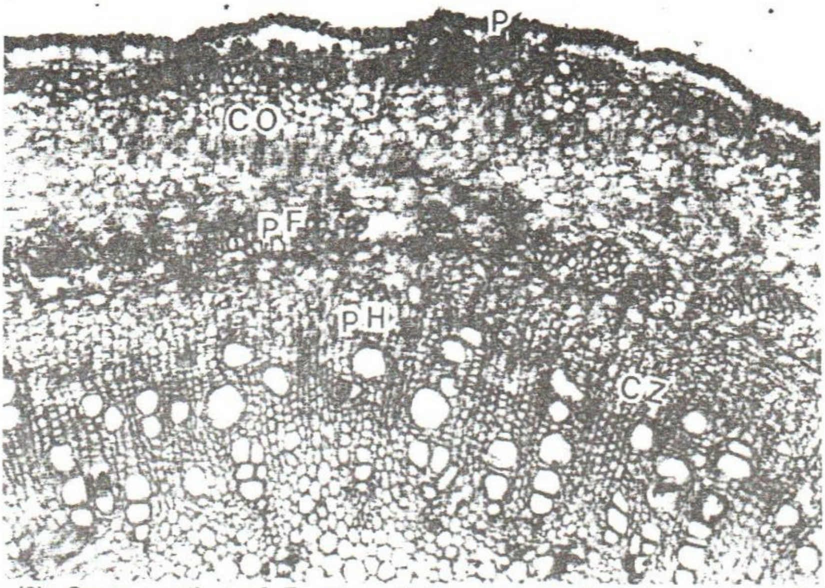

Fig. (6): Cross section of *F. retusa* (fair-to-root species) stem cutting. Note a discontinuous phloem fiber ring and composed of less amount of phloem fiber cells. (P)= periderm, (CO)= cortex, (PF)=phloem fibers, and (CZ)= cambial zone (Obj. x 10 – OC. x 10).

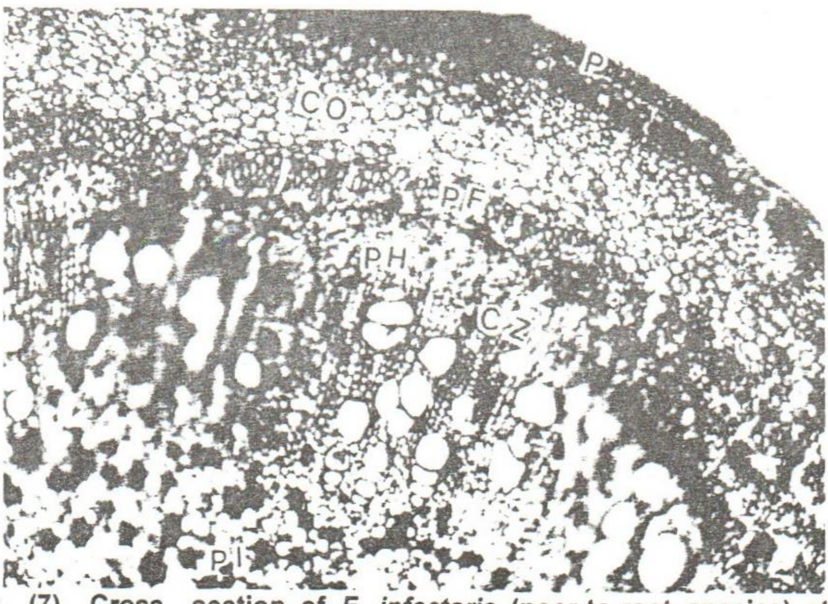

Fig. (7). Cross section of *F. infectoria* (poor-to-root species) stem cutting showing very strong phloem fiber groups. (P)= periderm, (CO)= cortex, (PF)=phloem fibers, and (CZ)= cambial zone (Obj. x 10 – OC. x 10).

(Nelson, 1978), while poor rooting of stem cuttings of certain woody species has been correlated with extensive sclerification in the phloem tissue (Beakbane, 1969 and Kachebay, 1975). In a summary, four anatomical factors seemed to be responsible for the decreased rooting potential in some *Ficus* species; wide cortex tissue and an increase the collenchyma cell layers, continuous phloem fiber ring and large amount of phloem fibers, thinner phloem tissue, and narrow cambial zone.

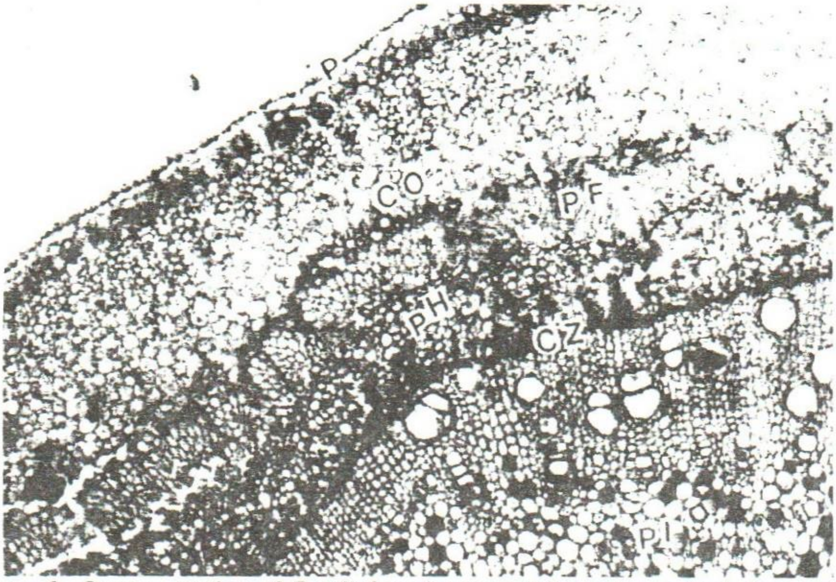

Figure 8. Cross section of *F. religiosa* (very poor-to-root species) stem cutting showing extensive and continuous phloem fiber ring, which composed large amount of phloem fiber cells as well as thinner phloem tissue and cambial zone. (P)= periderm, (CO)= cortex, (PF)=phloem fibers, and (CZ)= cambial zone (Obj. x 10 – OC. x 10).

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تأثير الهيدروجيل و الطرق المختلفة لمعاملة إندول حمض البيوتريك على العقل الساقية لبعض أشجار الفيكس

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تم إجراء هذه الدراسة خلال ربيع العامين المتتاليين 1998 و 1999 على العقل الساقية بطول 20-25 سم و تحمل 1-2 ورقة و المأخوذة من أفرع بعمر سنتين من أشجار الفيكس *F. religiosa F. infectoria F. retusa* النامية فى حرم جامعة المنصورة ، ولقد تم إستخدام الهيدروجل و طرق مختلفة لإستخدام إندول حمض البيوتريك (IBA) بهدف تحسين تجذير عقل الثلاثة أنواع من الفيكس و التى تختلف فى قدرتها على التجذير. و هذه الطرق كانت إستخدام 1000 جم/لتر IBA على صورة محلول أو بودرة التلك أو سلاكات الأسنان الخشبية المحملة بالهرمون و ذلك فى وجود أو عدم وجود الهيدروجيل فى بيئة التجذير الرملية بالإضافة إلى وضع العقل فى بيئة رملية مخلوط معها الهيدروجيل المحمل بالهرمون. ولقد هدفت الدراسة إلى دراسة تأثير هذه المعاملات على التجذير و مواصفات الجذور و المجموع الخضرى لعقل الثلاثة أنواع من الفيكس مع دراسة التغيرات فى التركيب التشريحى للعقلة و التى تحدث كنتيجة لمعاملة ال IBA ، و بالإضافة لذلك فقد تم فى العام الثانى دراسة العلاقة بين الفرق فى كمية الهرمونات النباتية و التركيب التشريحى للساق و القدرة على التجذير و مواصفات الجذوربين الثلاثة أنواع من الفيكس. و لقد أظهرت النتائج أن معاملة العقل ب 1000 جم/لتر من ال IBA على صورة محلول بالغمس مع وجود الهيدروجيل فى بيئة الجذور الرملية قد أنتجت أعلى نسبة تجذير للعقل و أعلى نسبة نجاح للعقل و أكبر عدد من الجذور للعقلة. أما سلاكات الأسنان الخشبية مع وجود الهيدروجيل فى بيئة الجذور الرملية فقد نتج عنها أسرع معدل للتجذير فى حين أن معاملة الهيدروجيل المحمل بالهرمون المخلوط مع البيئة الرملية فقد نتج عنها الجذور الأكثر طولا مع أكبر وزن طازج و جاف للجذور. و بالنسبة للنمو الخضرى فقد أعطت معاملة المحلول بالغمس مع وجود الهيدروجيل فى بيئة الجذور الرملية أكبر عدد من الأفرع و الأوراق للعقلة و أكبر وزن طازج و جاف للأوراق و أكبر مساحة ورقية. و بوجه عام فإن طريقة بودرة التلك كانت الرابعة فى الترتيب بعد الثلاثة طرق الأخرى. و لقد أظهرت النتائج أيضا أن وجود الهيدروجيل فى بيئة التجذير قد أدى إلى تحسين تأثير أى معاملة بالمقارنة مع عدم إستخدام الهيدروجيل.

و أظهرت الدراسة التشريحية أنه بعد أسبوعين من معاملة عقل *F. retusa* بال IBA ظهور إمتداد كبير و زيادة فى أنسجة القشرة و اللحاء و منطقة الكامبيوم مع تكون مراكز مرستيمية تطورت إلى مبادىء للجذور. ولقد كانت منطقة الكامبيوم و أنسجة اللحاء هى الأنسجة الرئيسية التى كونت مبادىء الجذور. و بعد أربعة أسابيع من المعاملة فقد خرجت الجذور من خلال أنسجة القشرة و نبتت من جانب العقلة مع ظهور أنسجة توصيل وعائية فى الجذور النامية. و لقد ظهر فى العقل الغير معاملة تغيرات تشريحية مماثلة للسابق إلا أنها كانت متأخرة عن تلك العقل المعاملة بحوالى أسبوعين.

و لقد أظهرت المقارنة بين الأنواع وجود علاقة بين محتوى الهرمونات النباتية للعقلة و التركيب التشريحى للساق و الإختلافات فى القدرة على التجذير بين الثلاثة أنواع من الفيكس حيث أن عقل *F. retusa* إحتوت على أعلى نسبة من كل من حمض الجبريليك و إندول حمض الخليك و أقل نسبة من حمض الأبسيسيك فى حين أن *F. religiosa* *F.* إحتوت عقله على أقل نسبة من كل من حمض الجبريليك و إندول حمض الخليك و أعلى نسبة من حمض الأبسيسيك و قد كانت عقل *F. infectoria* وسطا بين الإثنين. و أعطت عقل *F. retusa* أعلى نسبة تجذير و التى كانت الأسرع فى التجذير و أعلى نسبة نجاح و أكبر عدد من الجذور فى حين أن *F. infectoria* كان ترتيبها الثانى و كانت عقل *F. religiosa* الأقل. هذا ولم تختلف عقل *F. retusa* و *F. infectoria* معنويا فى طول أو وزن الجذور إلا أنهما كانا أكبر من *F. religiosa*. و لقد ظهر فى التركيب التشريحى لساق كل من *F. infectoria* , *F. religiosa* أن خلايا القشرة ذات سمك أكبر و تتكون من عدة طبقات من الخلايا الكولنشيمية و بها سمك أكبر من خلايا اللحاء و منطقة الكامبيوم عنهما فى حالة *F. retusa* ، هذا بالإضافة إلى أن *F. religiosa* بها حلقة متصلة من ألياف اللحاء الأكثر سمكا و مكونة من أعداد أكبر من الألياف المغلظة عنها فى حالة *F. infectoria* فى حين أن *F. retusa* بها حلقة غير متصلة من ألياف اللحاء و التى تتكون من أعداد أقل من الخلايا القليلة التغليظ و هذا ما قد يفسر إختلاف القدرة على التجذير بين الثلاثة أنواع.