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EFFECT OF GA_3 AND 2,4-D SPRAY APPLICATION ON THE MORPHOLOGY AND ANATOMY OF DATE PALM ROOT SYSTEM

ABDULLAH R. DOAIGEY AND M.H. AL-WHAIBI

Department of Botany and Microbiology,
College of Science, King Saud University,
P.O.Box 2455, Riyadh 11451, Saudi Arabia.

Abstract

GA_3 and 2,4-D @ 10^{-5} and 10^{-3}M used as foliar spray on date palm (*Phoenix dactylifera* L.) cv. Khedri seedlings affected the length of root and the anatomical characteristics of thick root. GA_3 at low concentration increased the differentiation of fibre, xylem strands and number of vessels in each xylem strand while at high concentration the number, size of lacunae and the number of vessels in each xylem strand decreased. 2,4-D at low concentration increased lacunae, fibre and xylem strands but decreased the number of vessels in each xylem strand, which at high concentration decreased the number of fibre, xylem strands and number of lacunae but increased the xylem vessels in each xylem strand.

Introduction

Plant growth regulators have been used in flowers and fruits as parthenocarpic, thinning and elongating agents (Salisbury & Ross, 1985) besides their use in plant tissue culture for propagaton and crop improvement (Ammar & Ben Badeis, 1983; Reuveni, 1979; De Fossard, 1976; Schroeder, 1970). Of the growth regulators gibberellins which stimulate cell division have been found to enhance stem length in higher plants due to cell elongation in the internodes (Stowe & Yamaki, 1959). Cleland (1969) reported that GA_3 can modify plant growth through an increase in volume of individual cells. GA_3 which increased xylem differentiation in vascular cambium, stem and leaf length decreased the amount of leaves produced by the apical meristem (Amai & Hadad, 1982). GA_3 decreased xylem vessels and xylem parenchyma and also increased xylem fibres. 2,4-D however, increased the differentiation of both xylem vessels and xylem fibres in plant seedling.

Plant growth regulators have been used in the tissue culture of date palm (Tisserat, 1979; Schroeder, 1970; Reuveni, 1979; Tesserat & De Mason, 1980). Ragan (1976) reported the effect of 2,4-D on tissue specialization and its differentiation to organs. GA_3 showed a decrease in root length and increase in shoot length of date palm seedlings (Al-Watban, 1989). Plant growth regulators have also been used to study fruit growth and development of the date palm (Al-Whaibi & Al-Ackhal, 1985).

The effects of 2,4-D and GA_3 application on morphological and anatomical characters of the root and shoot systems of date palm (*Phoenix dactylifera* L.) seedling with special reference to cell and tissue differentiation is presented.

Materials and Methods

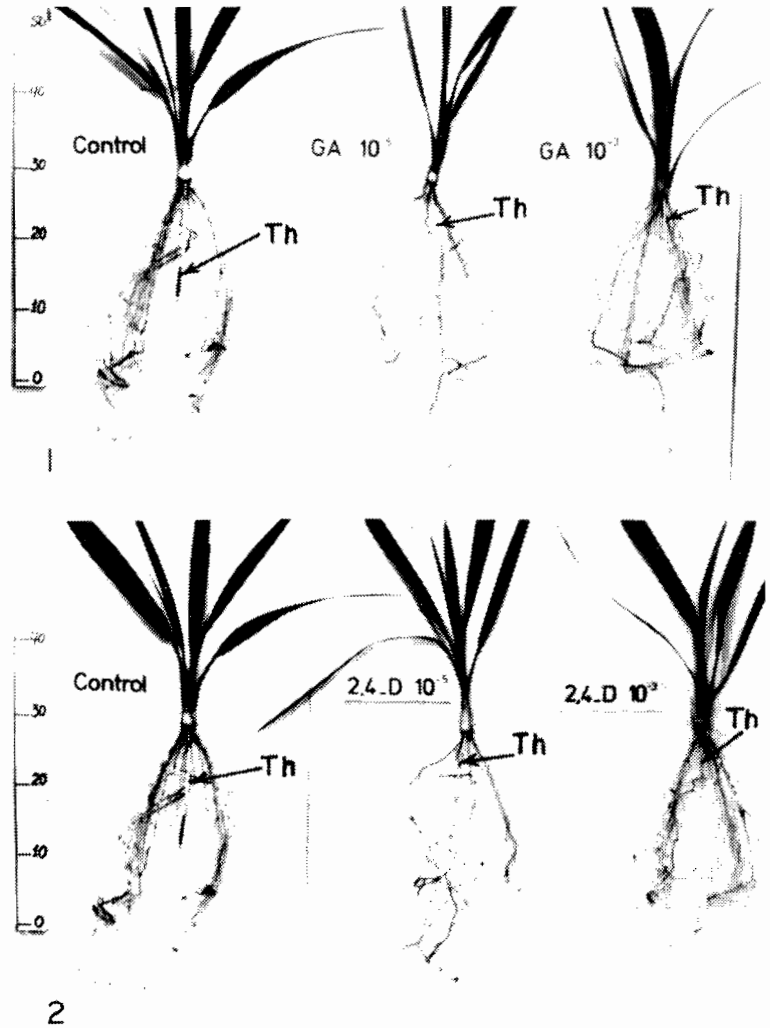
Seeds of date palm (*Phoenix dactylifera* L.) cv. Khedri of 1985 crop were washed and soaked in one litre tap water with aeration for 4 days. Three seeds were planted in each pot containing field soil-peat moss mixture (1:1) and plants were left in the green house at 30°C and 60% R.H. Three month old seedlings were transferred into big pots. The seedlings were irrigated with tap water twice a week and with IX nutrient solution at fortnightly interval. The composition of IX solution is given by Al-Whaibi & Al-Ackhal (1985). A group of 6 seedlings were sprayed with IX solution and with IX solution mixed with 10^{-5}M, 10^{-3}M 2,4-D, 10^{-5}M and 10^{-3}M GA_3 with four drops of Tween 20 to reduce surface tension. The plants were sprayed at 7 day interval upto 4 leaf stage. Seedlings were then harvested and divided into shoots and roots. The first leaf emerging after the begining of spray of each treatment was selected and cut into 2 cm pieces of leaf blade from 3 cm of the base and 3 cm from the blade apex. Similarly from old and young roots, 2 cm segments were taken from 10 cm of the root tips. Each leaf and root segment was divided into 5 mm pieces, fixed in Formalin-Acetic acid-Alcohol, dehydrated in ethanol and embedded in paraffin. A 10-20 μm thick sections were cut using a rotary microtome, stained with safranin and light green and mounted in Canada balsam. Sections were examined and photographed using Zeiss photomicroscope III. Cell wall thickness was determined according to Doaigey *et al.*, (1989) where thick walls were designated at 6-12 μm, slightly thick at 2.5-6 μm and thin at $\leq$2.5 μm.

Results

Two types of roots were observed: Old (thin) commonly long, branched, 3-5 roots in each plant and 10-95 cm long in the control (Figs.1,2; Table 1). GA_3 and 2,4-D treatments reduced the root length where 2,4-D was more effective than GA_3 (Fig.1) with young root thick, short, unbranched, none or one root in each plant and 14-21 cm long in the control. Root numbers, length and diameter of both old and young roots are given in Table 1. No significant trend of the effect could be statistically established.

Table 1. Effect of GA_3 and 2,4-D treatment of date palm seedling on the length, diameter and number of root system.

Treatment	Old root (thin)			Young root (think)		
	Number in plant	Length (cm)	Diameter (mm)	Number in plant	Length (cm)	Diameter (mm)
Control	3-5	10-95	1.75-2	None/1	14-21	3-4
$GA_3 10^{-3}$M	4-6	15-80	1.5-2	1/2	1-24	3.5-4
$GA_3 10^{-5}$M	4-6	12-75	1-1.5	None-3	2-21	5-5.5
2,4-D 10^{-3}M	3/4	15-70	1.5-1.75	None-3	18-27	3-3.75
2,4-D 10^{-5}M	3-8	20-70	1-1.25	None-3	3-24	4-4.25



Figs.1-2. (1) Control and GA_3 treatments showing root system. (2) Control and 2,4-D treatments showing root system.

The internal characteristics of old (thin) and young (thick) roots are given in Tables 3 & 4. The details are as follows:

Epidermis: one layer, cells commonly palisade-shaped with thin suberized walls becoming slightly tubular to irregular shaped after 2,4-D treatment (Figs.3,8,11,12; Tables 3,4).

Exodermis: exodermal cells, 6-10 layers with thick lignified walls. The outer 2 or 3 layers of exodermal cells in old root with cells containing tannin contents either in the control or in low and high concentrations of 2,4-D treatment (Figs.3,6,7; Table 3). The cells lack tannin contents in GA_3 treatments either in low or high concentrations

Table 3. Effect of GA_3 and 2,4-D treatments on the anatomical characters of old (thin) root of date palm seedling.

Characters	Control	GA_3 10^{-3}	GA_3 10^{-5}	2,4-D 10^{-3}	2,4-D 10^{-5}
Epidermis	One layer; cells tabular to palisade-shaped, large with thin walls.	As in control	As in control	One layer, cells tabular to irregular with thin walls.	As in 2,4-D 10^{-3}
Exodermis	Comspicuous; several layers; outer, 2 or 3 layers, cells contain tannins, with thin walls. Other cells with thick lignified walls.	As in control, but cells with tannins not observed.	As in control, but cells with tannin not observed	As in control.	As in control.
Cortex	Lacunae large, many, 17-20, Partitions, one to several cells wide. Fibre strands, 4 or 5 groups; cells with thick, lignified walls. Some cortical cells contain raphides or tannins.	As in control, but lacunae 17-23; partitions one to three cells wide, Fibre strands not observed.	As in control, but partitions one to three cells wide. Fibre strands not observed.	As in control, but lacunae 17-18; partitions one to three cells wide. Fibre strands not observed	As in control, but lacunae 24-27. Fibre strands not observed.
Endodermis	Cells with narrow lumina and U-shaped secondary thickening, passage cells not observed.	As in control.	As in control.	As in control.	As in control.

Table 3 (Cont'd)

Characters	Control	GA_3 10^{-3}	GA_3 10^{-5}	2,4-D 10^{-3}	2,4-D 10^{-5}
Pericycle	One layer; cells with thick lignified walls.	As in control.	As in control.	As in control.	As in control.
Xylem	Xylem, 10 strands; Protoxylem elements with narrow lumen and lignified walls; Metexylem with wide vessels in clusters, 28 vessels in each root.	As in control, but 30 vessels in each root.	As in control. but 24 vessels in each root	As in control, but 25 vessels in each root.	As in control, 13 vessels in each root.
Phloem	Phloem strands alternate with xylem strands; sieve tube elements in two to several rows.	As in control	As in control.	As in control	As in control.
Pith	Narrow, with polygonal parenchymatous cells with thick lignified cell walls.	As in control.	As in control.	As in control.	As in control.

Table 2. Effect of GA_3 and 2,4-D treatment of date palm seedling on the internal structure of root system.

Treatment	Old root				Young root			
	LA	FS	XS	MV	LA	FS	XS	MV
Control	17-20	4/5	10	28	39-43	60-62	30	62
GA_3 10^{-3}M	17-23	2/3	10	30	24-27	60-65	30	45
GA_3 10^{-5}M	17-20	---	10	24	39-43	100-	40	95
2,4-D 10^{-3}M	16-18	---	09	25	31-33	40-42	23	55
2,4-D 10^{-5}M	16-22	---	08	13	60-65	90-95	35	45

LA = lacunae; FS = fibre strand; XS = xylem strands; MV = Metaxylem vessels.

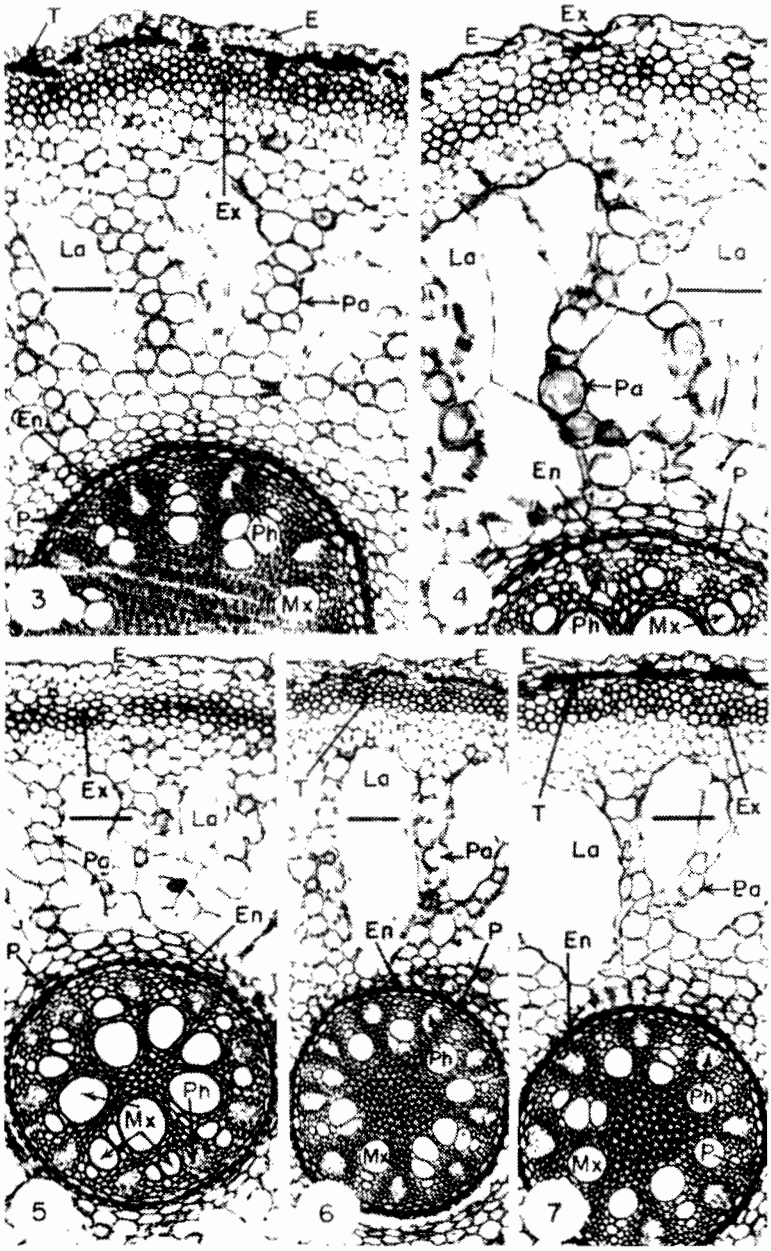
(Figs.4,5; Table 3). On the other hand, the exodermal cells are devoid of tannin contents in young root either in control or in both GA_3 and 2,4-D treatments of low and high concentrations (Figs.8-12; Table 4).

Cortex: small intercellular spaces occur between most cortical cells, in addition large radially elongated intercellular spaces (lacunae) were present either in young or old roots. Lacunae range in number from 17-20 in old root to 40-43 in young root of the control (Fig.3-12; Tables 2,4). On the other hand, lacunae increased in number in 10^{-5}M 2,4-D treatment either in old or young roots (Figs.7,11; Tables 3,4) whereas, they decreased in number in 10^{-3}M GA_3 and 10^{-3}M 2,4-D treatments in young root (Figs.10,12; Table 4) but they were not affected by 10^{-5}M GA_3 treatment in both old and young roots (Figs.5,9; Table 3,4). Partitions comonly several cells wide of parenchymatous cells and inner cortical cells were small and commonly arranged in radial rows (Figs.3-12; Tables 3,4).

Fibre strands: either few, 4 or 5 groups (if present in old root, cells with thick lignified walls (Table 3) or many upto 60 groups in control of young root having cells with unlignified walls, the outer cells of these fibre strands contain silica bodies (Figs.3-12; Tables 3,4). Fibre strands increased in number upto 90 groups in treatments of low concentrations of both GA_3 and 2,4-D (Figs.9,12; Tables 3,4) whereas they decreased in number, (24-34 groups) in treatments of high concentration of both GA_3 and 2,4-D (Figs.10,11; Tables 3,4).

Endodermis: the endodermis very conspicuous, cells with narrow lumina and either U-shaped secondary thickening walls in transverse sections of the old root (Figs.3-7; Table 3) or thickening on all walls in transverse section of young root (Figs.8-12; Table 4). Passage cells were observed in the endodermis of young roots (Table 4). Endodermal cells were not affected by the treatments except in 10^{-3}M 2,4-D treatment where secondary thickening occur on the inner tangential and radial cell walls of young root (Fig.11; Table 4).

Pericycle: one layer thick, cells with either thin cellulosic walls in young root or thick lignified walls in old root (Figs.3,8; Tables 3,4). Pericycle cells were not affected also by both GA_3 and 2,4-D treatments (Figs.3-12; Tables 3,4).



Figs.3-7. TS. of thin (old) root. (3) Control. (4) $GA_3 10^{-3}$ treatment. (5) $GA_3 10^{-5}$ treatment. (6) 2,4-D 10^{-3} treatment. (7) 2,4-D 10^{-5} treatment. Bar = 100 μm

Table 4. Effect of GA_3 and 2,4-D treatments on the anatomical characters of young (thick) root of date palm seedling.

Characters	Control	GA_3 10^{-3}	GA_3 10^{-5}	2,4-D 10^{-3}	2,4-D 10^{-5}
Epidermis	One layer; cells palisade-shaped with thin walls.	As in control.	As in control.	One layer, cells tabular to irregular with thin walls.	As in control.
Exodermis	Several layers; outer, 3-5 layers; cells with thick lignified walls; inner 3-5 layers, cells with thick unlignified walls.	As in control.	As in control.	As in control.	As in control.
Cortex	Lacunae, large 39-43, Partitions several cells wide. Fibre strands, 60-62 groups; cells with thick unlignified walls; outer cells contain silica bodies. Some corical cells contain raphides. Inner cortical cells small, commonly in radial raws.	Lacunae, narrower than that of the control 24-27. partitions several cells wide. Fibre strands 60-66 groups similar to those of the control. Other cortical cells similar to those of the control.	Lacunae and partitions closely similar to those of control. Fibre strands numerous, more than 100 groups similar to those of the control. Other cortical cells similar to those of the control	Lacunae, large 31-33; partitions several cells wide. Fibre strands many, 40-42 groups similar to those of the control. Other cortical cells similar to those of the control	Lacunae, large 60-64. Partitions several cells wide. Fibre strands, 90-95 groups, similar to those of the control. Other cortical cells similar to those of the control.
Endodermis	One layer, cells with thickened, superized	As in control.	As in control.	Incomplete U-shaped secondary thickening	As in control.

Table 4 (Cont'd)

Characters	Control	GA_3 10^{-3}	GA_3 10^{-5}	2,4-D 10^{-3}	2,4-D 10^{-5}
	walls; passage cells with wide lumina and thin walls.			of inner tangenial and radial walls of each cell.	
Pericycle	One layer, cells with thin cellulosic walls.	As in control.	As in control.	As in control.	As in control.
Xylem	Xylem strands, 30 in one ring. Protoxylem elements narrow with lignified walls. Metaxylem vessels with wide lumina unlignified cell walls 62 vessels in each plant.	Xylem strands similar to that of the control. Protoxylem elements being less lignified. Metaxylem vessels similar to those of the control; 45 vessels in each. plant.	Xylem strands 40 in one ring. Protoxylem elements similar to those of the control. Metaxylem vessels similar to those of the control; 95 vessels in each plant.	Xylem strands, 35 in one ring. Protoxylem elements similar to those of the control. Metaxylem vessels similar to those of the control 55 vessels in each plant	Xylem strands, 35 in one ring. Protoxylem elements similar to those of the control. Metaxylem vessels similar to those of the control, 45 vessels in each plant.
Phloem	Conspicuous, alternating between xylem strands, 2 or 3 radial rows of wide sieve-tube elements.	As in control.	As in control.	As in control.	As in control.
Pith	Pith parenchyma wide, cells polygonal with thin walls; central cells polygonal with thickened walls.	Pith parenchyma, narrower than that of the control, central cells similar to those of the control	Pith parenchyma wider than that of the control with more thickened cell walls.	Pith parenchyma narrower than that of the control, cells with thickened walls.	Pith parenchyma wider than that of the control with more thickened cell walls.

Phloem: the phloem strands commonly conspicuous and alternate with xylem strands; sieve tube elements, two to several rows with wide lumina in both young and old roots (Figs.3-12; Tables 3,4). It seems that phloem elements were not affected by GA_3 and 2,4-D treatments in both young and old roots.

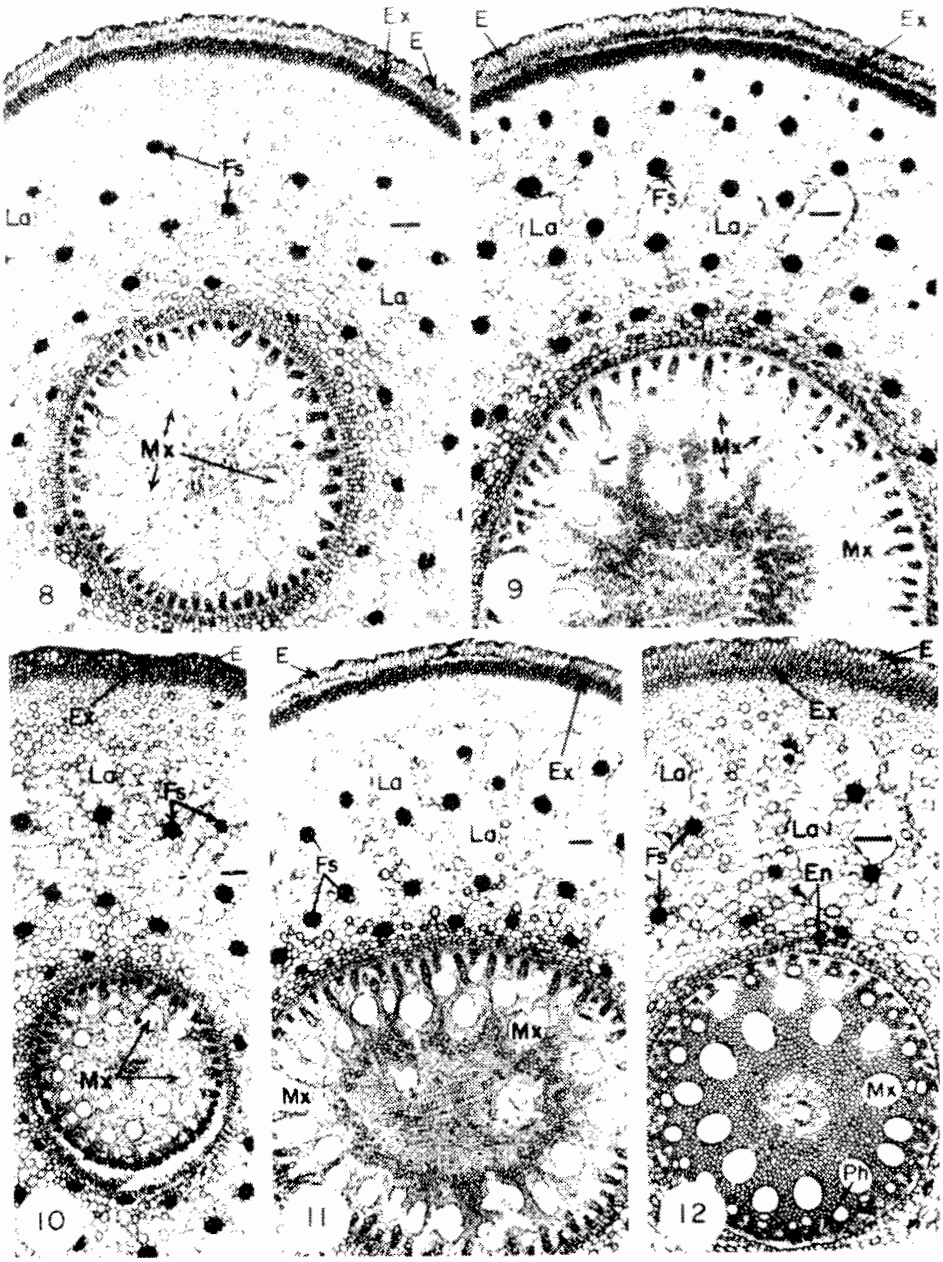
Xylem: the xylem elements either 10 strands in old root or 30 strands in young root alternating with phloem strands. Protoxylem in both roots had narrow vessels with thickened lignified walls (Fig.3; Tables 3,4). Metaxylem of old root contain either small or large vessels with thick lignified walls, occurring frequently in clusters, forming a single ring (Fig.3 Table 2). On the other hand, metaxylem of young root showed large vessels with thick unlignified walls, being in a single ring with several vessels wide (Fig.8; Table 2).

Xylem strands of old root were not affected by low or high concentration of GA_3 and 2,4-D treatments. On the other hand, low concentrations of GA_3 and 2,4-D increased the xylem strands and the size of the stele of young root (Fig.9; Tables 2,4). In contrast, high concentrations of GA_3 and 2,4-D decreased the xylem strands and the size of the stele of young root (Figs.10,11; Tables 2,4). Metaxylem vessels of old root were not much affected by either low or high concentrations of GA_3 and 2,4-D treatments. On the other hand, metaxylem of young root were affected by high concentration of GA_3 and 2,4-D treatments which decreased the differentiation of metaxylem vessels (Figs.10,11,12; Tables 2,4). In contrast, low concentration of GA_3 increased the differentiation of metaxylem vessels (Fig.9; Table 2,4).

Pith: the ground tissue of the stele in young root showed polygonal parenchymatous cells with thin cellulosic walls and conspicuous intercellular spaces, cells with thickened walls were observed in the central part of he stele (Fig.8; Table 4). On the other hand, ground tissue of the stele in old root consists of polygonal parenchymatous cells with thick, lignified walls (Fig.3; Table 3). Ground tissue of the stele in old root was not affected by both GA_3 and 2,4-D treatments either in high or low concentrations. In contrast, high concentration of 2,4-D treatment increased the formation of thickened cell walls in the pith of young root (Fig.11; Table 4).

Discussion

The anatomical characteristics of old root of the untreated control are in general agreement with those reported by Tomlinson (1961) and with the first mature seedling root described by Al-Salih *et al.*, (1985). The length of the old roots was slightly affected by GA_3 and 2,4-D treatments either in low or high concenrations (Table 1) which may be due to the decrease in cell division rate or inhibition of cell elongation. On the other hand, 10^{-5}M of 2,4-D treatment increased the formation of main old roots in each plant (Table 1). GA_3 and 2,4-D growth regulators did not affect most of the anatomical characteristics of the old root, however, the number and size of lacunae (large intercellular spaces) increased at low and high concentrations of growth regulators, whereas GA_3 prevented the accumulation of tannin substances in the outer part of the exodermal cells of old root cortex (Figs.4,5; Table 3). Other anatomical characteristics of the old root which were not affected by GA_3 and 2,4-D treatments may be due to either a complete cell differentiation before spraying by growth



Figs.8-12. TS. of thick (young) root. (8) Control. (9) GA_3 10^{-5} treatment. (10) GA_3 10^{-3} treatment. (11) 2,4-D 10^{-5} treatment. (12) 2,4-D 10^{-3} treatment. Bar = 100 μm.

Figure abbreviations: E, epidermis; En, endodermis; Ex, exodermis; Fs, fibre strand; La, lacunae; Mx, metaxylem; P, pericyle; Pa, partition; Ph, phloem; T, tannins; Th, Thick root.

regulators or that the main old roots ceased to develop because of the formation of another new of young roots.

The results indicate that the length of young thick roots was affected by both GA_3 and 2,4-D treatments, however, low and high concentrations of GA_3 decreased the length of roots. whereas, high concentration of 2,4-D increased its length (Table 1). In addition, 10^{-5}M of GA_3 increased the number of young roots in each plant as well as increased their diameters (Table 1). On the other band, low concentration of GA_3 and 2,4-D stimulated the formation of young roots in each plant but they did not affect their length (Table 1).

Although, the anatomical characteristics of young root were affected by GA_3 and 2,4-D treatments as compared to control, however, 10^{-3}M of GA_3 treatment was found to decrease the number of vessels in xylem strands (Fig.10; Table 4) which confirms the finding of Amar & Hadad (1982). The number and size of lacunae in the root cortex decreased. On the other hand, low concentration of GA_3 was observed to increase xylem strand in the young root and the number of vessel elements in each xylem strand. It also increased the differentiation of fibre strands in the root cortex (Fig.9; Table 4).

Amar & Hadad (1982) reported that 2,4-D increased the differentiation of both xylem vessels and xylem fibres. However, the present study indicated that 10^{-3}M of 2,4-D increased the xylem vessels in each xylem strand, decreased the number of xylem strands of each root and the number of fibre strands and lacunae in the root cortex (Fig.11; Table 4). On the other hand, low concentration of 10^{-5}M 2,4-D was found to increase the differentiation of xylem strands of each root but decreased the number of vessels in each strand. It also stimulated the formation of lacunae and the differentiation of fibre strands in the root cortex (Fig.12; Table 4).

The present report would suggest that spraying the shoot system of date palm seedling with GA_3 or 2,4-D growth regulators may affect the length and anatomical features of the root system, especially those roots which are formed during spraying process.

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