EFFECT OF GROWTH REGULATORS ON ANATOMICAL FEATURES OF PALM PLANTLETS INDUCED *IN VITRO*

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Abstract

The current investigation was carried out at the tissue culture Laboratory, Technological Institution for Genetic and Molicular Engineering, Sadat branch, Monofia Univ., during 2004-2005 seasons, aiming to explore the anatomical features of root and leaflets of 24 weeks old plantlets belong to three palm regulators cultivars namely, Gondila, Sewi and Zaghloul. The main obtained results were, all used growth regulators affected the internal structure of the studied anatomical traits to various extents. Average root diameter was relatively differed according to the type of growth regulators. Ansymidol treatments seemed to be the most effective one. Such treatments scored the highest average of root diameter followed by Paclobuetrazol, IBA and (NAA+BA) treatments. These increments in root diameter mainly referred to the appreciably increased in all tissues shared in root structure, specially cortex thickness and diameter of the vascular bundles. Cortex was relatively the thickest with Ansymidol treatment as compared with the other three growth regulators. While, no remarkable differences were found between IBA and NAA treatments.

The three investigated cultivars showed the same response towards different growth regulators treatments with different extents. However, Gondila. showed the highest microscopic measurements and counts as compared with the other two cultivars. Leaf mesophyll thickness was greatly affected by growth regulators. Gondila leaflets always showed thicker sheath of vascular bundle as well as a considerable numbers of both large and small vascular bundles. Growth regulators in general affected the mean values of all anatomical features. These growth regulators could be ranked due to their reduction effects on the anatomical features of the three used date palm cultivars as, Ansymidol foilowed by Paclobutrazol and finally 2,4-D treatments.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is considered one of the most important fruit trees cultivated in Egypt and other Middle East and Arab Countries (Mohammad, 1983, Moursy and Saker, 1996). The cultivated grown cultivars are used for food, fiber, fuel and shelter (Al-Bakr, 1972, Zohary and Spiegel – Roy, 1975). Recently in Egypt, more attention is directed to date palm as being a promising crop for the cultivation in new reclamised soils at the regions of high temperature (Bouchreib and Clarck, 1997). Some cultivars were

imported to Egypt from the Gulf area for their super fruit qualities. The conventional method of vegetative propagation using offshoots is slow and expensive (Pareek, 1984). Therefore the need for a propagation protocol which insures the mass production of true to type plants became very necessary. The use of tissue culture techniques in the clonal propagation of date palm has been investigated by many workers since the pioneer work of Tisserat (1979). Two micopropagation protocols were adopted: a) by somatic embryogenesis via callus as reported by Tisserat, 1982, Sharma *et al.* 1986, Daguin and Letouze, 1988, Zaid and Hughes, 1995 and Diwaker *et al.* 1988) by axillary bud multiplication as reported by Poulain *et al.* 1979, Beauchesne, 1982, Gabr and Tisserat , 1985, Nasir *et al.* 1994 and Al-Karyi and Al-Maarri, 1997.

It has been reported that organogenesis and bud multiplication are less efficient when compared with somatic embryogenesis due to the low number of explants that respond in vitro, the long time required from the initiation phase, the low multiplication rate and the strong influence of the cultivar. (Poulain *et al.* (1979) and Beauchesne (1982).

Although the use of tissue culture techniques for the mass propagation of date palm is already adopted in many countries, yet the adoption level of these techniques is still low. The reasons for that are, the absence of leaflet need among farmers for the incompatibility of this technology, uncertainity about its results and impacts and the wide spread of the seedless fruits phenomenon in certain varieties when propagated by tissue culture (Al Sakran, 2001).

The present study aimed to elucidate some aspects of evaluation of the anatomical variation of leaves and roots of three cultivars regenerated plantlets of date palm under different regulator levels.

Materials and Methods:

The current investigation was performed at the tissue culture laboratory, Technological Institution for Genetic and Molicular Engineering, Sadat branch, Menofia University and at Central Laboratory of Date Palm Research and Development, Agriculture Research Center, Giza, Egypt.

Plant material

Offshoots of the three cultivars, namely, Gondila, Sewi and Zaghloul were secured from different farms, leaves of offshoots were completely removed with bases to expose for sterilization the shoot tip of each offshoot for sterilization.

Shoot tips (5-7 x 3-4 cm) washed with running tap water for 30 minutes and were sterilized in 95% ethanol for 5 min. Thereafter, they were dipped in 20% (v/v) commercial bleach (2.25% sodium hypochlorite) for 30 min and finally in 0.1% mercuric chloride (MC) for 5 min. The explants were rinsed 3 times with sterile distilled

water. Under asptic conditions the outer 3-4 leaf primordia (LP), about 3 cm in length, were dissected from the apical shoot tips (ST). and were longitudinally cut into 3-4 pices. The explants were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 0.5 nicotinic acid, 0.5 pyridoxine – Hcl, 1.0 thiamin Hcl, 100 myoinositol, 2.0 glycine, 3000 agar, 3000 activated charcool and 30000 sucrose (all in mg/L). The pH media was adjusted to 5.7 before autoclaving at 121°C. For callus initiation and proliferation, the medium was supplemented with 2,4-D, 2-ip, Ansymidol and Paclobutrazol. Such media proved the most suitable for callus growth. The cultures were incubated at 25-27°C in darkness, at 6 weeks intervals.

The formed embryos on all media were systematically isolated and cultured on free hormone medium and incubated at 16 hrs. The shoots were rooted on MS Media supplemented with 0.1 Ansymidol, 0.1 Paclobutrazol, 2 mg/L IBA and 0.1 mg/L NAA + 0.05 BA light photoperiod regime with 3000 Lux light intensity to stimulate embryos germination into normal plantlets. The normal plantlets were transferred and planted in container filled with peatmoss and sand at a ratio of 3 : 1, irregated every 2 days for about 1.5 months under green house conditions, which was 27°C of temperature with 60% humidity and 4000 – 6000 Lux light intensity After 6 months leaves and roots from each cultivar were chosen to study the anatomical structure. (Specimens 1 cm long of root, and 1 cm^2 of leaves (from the middle portion of both)., of *in vitro* regenerated plantlets were taken at the stage of two simple foliage leaves (24 weeks old). Specimens were killed and fixed in F. A. A., washed in 50% alcohol, dehydrated in normal butyl and embedded in paraffin wax (55 C mp), (Sass, 1958). Cross sections, 20μ thick were cut, and stained with crystal violet/ erythrosine combination and mounted in Canada balsam (Jackson, 1926). Slides were micoscopically examined. Measurements of different tissues were taken, and averages of 10 readings from 5 slides were calculated.

RESULTS AND DISCUSSION

1- Effect of growth regulators on palm root structure

Regarding the anatomical features of the fibrous roots that initiated in vitro plantlets of the three studied palm date cultivars, Zaghloul, Sewi and Gondila treated by 0.1mg/L Ansymidol, 0.1mg/L Paclobutrazol, 2.0 mg/IBA and 0.1 mg/NAA +0.05 BA. It is evident that, the three cultivars under investigation showed relatively same trend in their response to growth regulators treatments. The effects of such treatments on Zaghloul cultivar is only discussed which will represent too the other cvs. with different measurements .

Data pertaining certain microscopical counts and measurements of fibrous root structure as affected by the four different types of growth regulators, Ansymidol,

Paclobutrazol, IBA and (NAA+ BA) with different concentrations are presented in Table (1) and Figures (1-4). It is clear that, all used growth regulators affected the internal structure of the studied anatomical traits to various extents. Average root diameter was relatively differed according to the type of growth regulator. Ansymidol treatments seemed to be the most effective one. Since, counts and measurements of that treatment scored the highest average of root diameter followed by Paclobuetrazol, IBA and (NAA+BA) treatments. This increments in root diameter mainly referred to the appreciably increased width of all tissues shared in root structure, specially cortex thickness and diameter of the vascular cylinder. Cortex was relatively the widest with Ansymidol treatment as compared with the other three hormonal treatments. The relative increased percentages in cortex thickness due to that treatments were, 16.0%, 53.4%, and 76.7% for Paclobutrazol, IBA and (NAA+BA) treatments, respectively. It is worthy to mention that, this increase was accompanied by an extended increase in both root exodermis (15.7, 65.6 and 67.9 %) and mesodermis. (13.2, 28.6 and 53.6 %) for the same treatments in the same order. It is also evident that there is no significant differences found between average size of cortex cell. As, the number and dimensions of air lacunas that found within root mesodermis were greatly varied. Where, Ansymidol treatment produced high number of air lacunas as compared with the other treatments. The average number of air lacunas due to all growth regulators treatments were, 57,40,31 and 31 for Ansymidol, Paclobutrazol, IBA and (NAA+ BA), respectively. The other factor which may affect the thickness of cortex mesodermis is the changes occurred in Lacunas dimensions due to treatments. Where, Ansymidol treatment caused a remarkable increase in length and width of air lacunas. While, no remarkable differences were observed between the effect of IBA and NAA treatments.

Data presented in Table (1) and Figures (1) revealed that, the thickness of the vascular cylinder was noticeably affected by growth regulators treatments. It is realized that, Ansymidol treatment produced the thickest vascular cylinder as compared with the other three growth regulators. The average thickness of vascular cylinder recorded with ansymidol was 1032 µ as compared with 970,912 and 852 µ for the other treatments, Paclobutrazol, IBA and (NAA+ BA), respectively. This increased in thickness was mainly due to the increasing the number and diameter of xylem vessels. Where, both length and width of vessels increased with Ansymidol treatment more than with the other three growth regulators treatments. It is also realized that, all growth regulators treatments do not show any distinctive differences in phloem thickness. Pith diameter showed a sever reduction as all microscopic measurements recorded for Paclobutrazol, IBA and NAA+BA treatments and proved to be reduced by more than half.

The abovementioned results are in harmony with those reported by Al-Salih *et al.* (1986), Ibrahim *et al.* (1999) and Chin (1982).

Table 1. Measurements and counts of certain anatomical features in trasverse sections of Zaghloul cv. fibrous root as treated by Four different growth regulators .

Treatment / Characters (µ)	Ansymidol	Paclobutrazol	IBA	NAA + BA
Average Epidermis thickness (µ)	44	42	36	34
Average Exodermis thickness (µ)	425	358	146	140
Average thickness of Exodernis.cell (µ)	70.8	44.7	24.3	28.1
Average thickness of Mesodrmis(µ)	1137	1004	884	740
Average thickness of Endodermis(µ)	56	48	38	35
Average Cortex thickness (µ)	1580	1362	1030	894
Average No. of lacunas (µ)	57	40	31	31
Average Lacunae length (µ)	136	100	156	154
Average Lacunae width (µ)	56	54	52	51
Average Vascular bundle cylinder thickness (µ)	1032	970	912	852
Average thickness xylem arm (µ)	758	441	334	297
Average Phloem thickness (µ)	50	50	40	40
Average No. of xylem bundles (µ)	12	12	12	12
Average diameter of Pith (µ)	452	224	168	165
Average diameter of Root (µ)	4288	3794	3022	2610

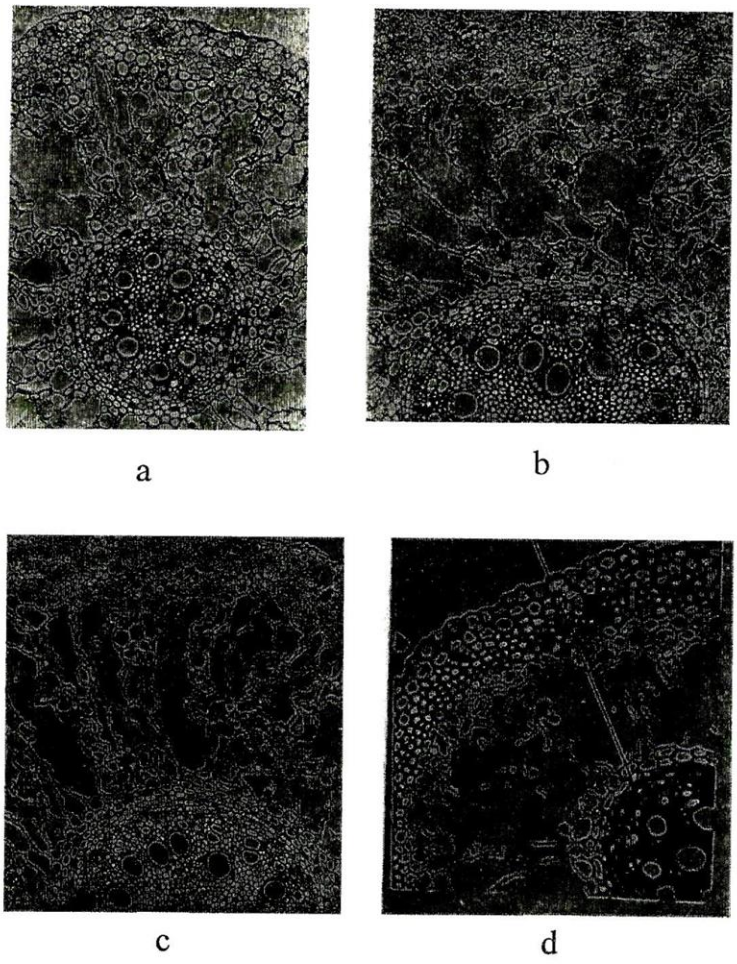


Fig 1. Transections in the root of the date palm plantlets rooted on
a) Ansymidol b) Paclobutrazol
c) IBA d) NAA + BA
X=160

2- leaflet structure

Microscopic measurements as detected in leaflet transverse sections of the three palm cultivars treated by the three types of growth regulators, Ansymidol, PP_{333}, 2,4-D are presented in Table (2) and Figure (2). With regard to the cultivar response to growth regulators treatments,, it is realized that the three investigated cultivars showed the same response towards different growth regulators treatments at different extents. Since, Gondila . showed the highest microscopic measurements and counts as compared with the other two cultivars., the treated leaflets by Ansymidol resulted in the thickest upper and lower epidermis compared with the other two palm cultivars. Relative to Gondila, the average decreased percentages in thickness of the upper epidermis were -27.1 and -18.6% for Sewi and Zaghloul, respectively. The corresponding increased percentages in thickness for the lower epidermis were, 22.2 and 13.8% for the same cultivars, respectively. Leaf mesophyll thickness was greatly affected by growth regulators. However, Gondila leaflets showed the thickest mesophyll tissue as compared with the other two cultivars. Plants treated by Ansymidol showed the thickest mesophyll tissue as compared with the other two palm cultivars which received the same dose of growth regulators. Relative to Gondila, the average decreased percentages in mesophyll thickness were -28.2 and -31.9% for Sewi and Zaghloul, respectively. Same trend was obtained regarding to the average thickness of sclerncyhma sheath of the vascular bundle. As the vascular bundle of Gondila showed a remarkable increase in thickness of both upper and lower vascular sheaths. This increased in thickness of vascular sheath was accompanied by an increase in number of sclenchymatous layers which almost found in both upper and lower portions of the vascular bundle. Gondila leaflets always represented the thickest vascular bundle sheath as well as a considerable number of both large and small vascular bundles compared to other cultivars. The average number of large vascular bundles were 11 bundles in Gondila compared with 6 and 12 bundles in Sewi and Zaghloul, respectively. Whereas, the average number of small vascular bundles were 39 bundle in Gondila compared with 18 and 20 bundles in Sewi and Zaghloul, respectively. Same trend was obtained with regard to xylem and phloem length and width. As, Gondila showed the highest microscopic measurements and counts of leaflets as compared to the other two cultivars.

Regarding the differences in leaflet structure due to growth regulators treatments, the measurements of certain anatomical characters of leaflet lamina transverse sections are presented in Table (2). It was observed that, in all palm cultivars, Ansymidol treatments resulted in a prominent increase in thickness of lamina. All tissues shared in leaflet structure were remarkably affected by the used growth

regulators. It is evident that, regarding the average thickness of both upper and lower epidermis, thickness of mesophyll tissue, average thickness of bundle sheath, average number of large and small bundles as well as the length and width of xylem and phloem. Paclobutrazol, treatments caused a steady decreases.Another steady decrements in the same anatomical measurements and counts were found in leaflets of the three studied palm cultivars due to 2,4-D treatments. As, all tissues shared in leaflet structure were appreciably decreased as compared with the other two growth regulators treatments. Relative to Ansymidol treatments in the three studied cultivars, the average decreased percentages in leaflet mesophyll thickness due to Paclobuetrazol and 2,4-D treatments were, -14.8 and -7.4%, -8.4 and -9.0% as well -2.8 and -4.9% for the three studied cultivars, Gondila, Sewi and Zaghloul, respectively. The corresponding decreased percentages due to the same growth regulators treatments that affected average vascular bundle length were, -14.3 and -10.5, -9.7 and -6.1 as well -21.8 and -14.3 for the three studied cultivars, Gondila, Sewi and Zaghloul, respectively.

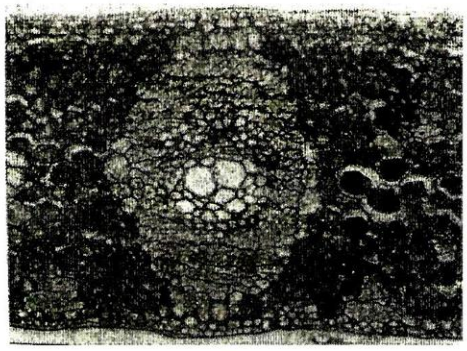
To conclude the abovementioned results, it is worthy to mention that the three investigated palm cultivars showed the same trend in response to growth regulators treatments. However Gondila palm cultivar showed the highest microscopical measurements and counts compared with the other two cultivars. at the mean time, growth regulators in general affected the mean values of all anatomical features. The three used growth regulators obviously affected all tissues sharing in leaflets structure. The three used growth regulators could be ranked due to their reduction effects on the anatomical features of the three used palm cultivars are, Ansymidol followed by Paclobuetrazol and finally 2,4-D.

As far as we aware no detailed study dealing with anatomical structure of any organs of the three palm cultivars was carried out.

Table 2. Measurements of certain microscopical features transverse sections of the leaflets of in vitro plantlets of date palm c.v Gondeila, Sewi and Zaghloul as treated by the growth regulators. (Averages of 10 readings) .

Cultivars	Growth Regulator	Upper epidermis thickness(µ)	lower epidermis thickness(µ)	A.V.Thickness mesophyl(µ)	No. of mesophyl cells	Dimension of vascular bundle				No. of large bundles	No. of small bundles	Large bundle length	Large bundle width	Xylem length(µ)	Xylem width(µ)	Phloem width(µ)	Phloem length (µ)
						Upper cortex(µ)	Lower cortex(µ)	No. of upper layers	No. of lower layers								
Gondila	Ansymidol	59	36	2160	24	560	357	9	6	11	39	1470	810	410	215	89	92
	Paclobutrazol	51	36	2460	24	496	290	4	4	11	39	1260	718	395	200	77	95
	2,4-D 2ip NOA	55	37	2320	18	521	338	4	4	11	39	1315	612	380	178	69	83
Sewi	Ansymidol	43	28	1550	37	150	136	4	5	6	18	920	620	367	244	100	88
	Paclobutrazol	36	23	1680	41	131	106	4	5	6	17	830	667	340	217	63	83
	2,4-D 2ip NOA	41	27	1410	45	142	127	7	5	4	16	894	601	330	198	52	71
Zaglou	Ansymidol	46	31	1470	18	288	96	5	6	12	20	1296	864	312	210	84	81
	Paclobutrazol	42	31	1512	20	246	77	5	5	12	17	1013	812	198	186	66	72
	2,4-D 2ip NOA	46	29	1398	18	265	85	4	4	11	16	1110	800	288	192	55	66

a



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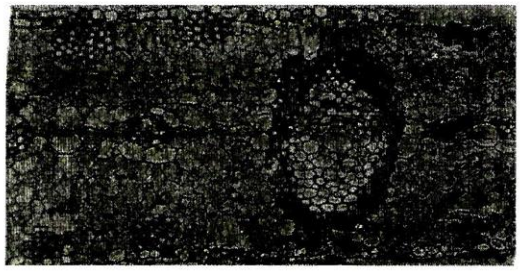


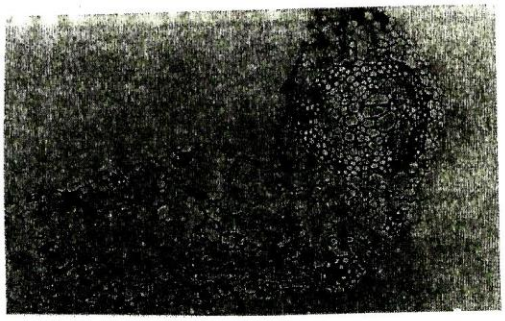
c

Fig 2. Transections in leaflets of Gondila cultivars shoots grown in media containing:

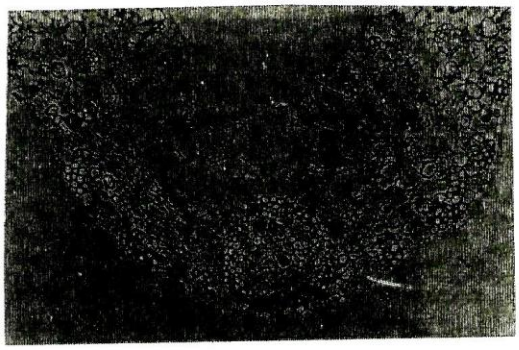
a) Ansymidol
b) Paclobutrazol
c) 2,4-D, 2ip and NOA

X=160

d



e



f

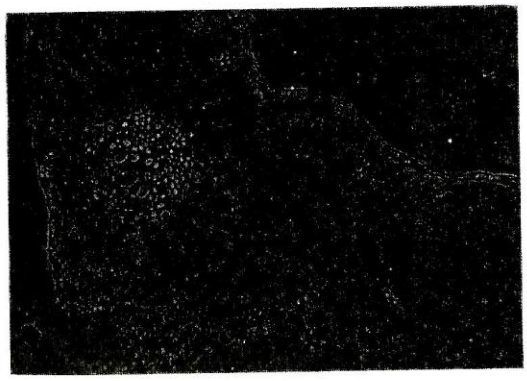
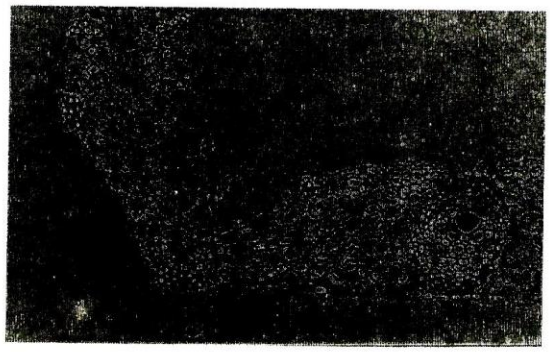


Fig 2. Transections in leaflets of Sewi cultivars shoots grown in media containing:
d) Ansymidol
e) Paclobutrazol
f) 2,4-D, 2ip and NOA
X=160

g



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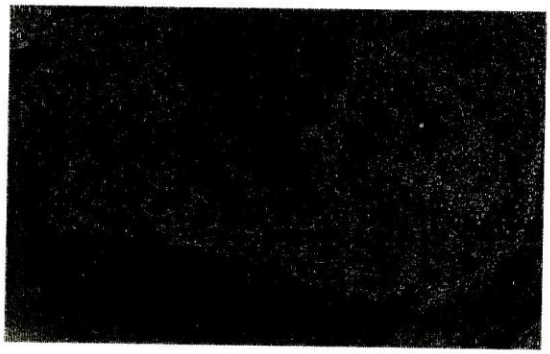

i

Fig 2. Transections in leaflets of Zaghloul cultivars shoots grown in media containing:
g) Ansymidol
h) Paclobutrazol
i) 2,4-D, 2ip and NOA
X=160

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تأثير منظمات النمو على الصفات التشريحية لنبيتات نخيل البلح المستحدثة معمليا

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١. *معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية بمدينة السادات جامعة المنوفية .*

٢. *المعمل المركزي للأبحاث وتطوير نخيل البلح ، مركز البحوث الزراعية .*

تمت هذه الدراسة في معمل زراعة الأنسجة بمعهد الهندسة الوراثية جامعة المنوفية مدينة السادات خلال عام ٢٠٠٤/٢٠٠٥ بالتعاون مع المعمل المركزي للأبحاث وتطوير نخيل البلح – مركز البحوث الزراعية – الجيزة . ويهدف هذا البحث معرفة التراكيب التشريحية لكل من الجذر والوريقة لنبيتات عمر ٢٤ اسبوع تنتمي إلى ثلاث أصناف لنخيل البلح وهي الجندبلة ، السيوي ، الزغلول .

وكانت أهم النتائج المتحصل عليها هي :-

أثرت كل منظمات النمو المستخدمة في التراكيب الداخلية لجميع الصفات التشريحية تحت الدراسة بدرجات متفاوته .

أختلف نسبيا متوسط قطر الجذر تبعا لنوع منظم النمو المستخدم وقد لوحظ أن المعاملة بالأنسميدول أعطت أكثر التأثيرات حيث سجلت المعاملة بهذا المركب أعلى متوسط لقطر الجذر يتبعها المعاملة بالبكلوبترازول ، النفثالين استك اسيد ، الاندول بيوتريك اسيد . وترجع هذه الزيادات في قطر الجذر إلى الزيادة الملحوظة في كل الأنسجة التى يتركب منها الجذر : بصفة خاصة سمك القشرة وقطر الأسطوانة الوعائية وكان سمك القشرة هو الأعلى عند المعاملة بالأنسميدول ومقارنة ذلك مع ثلاث منظمات أخرى . بينما لم يلاحظ أي اختلافات واضحه بين المعاملات سواء بالإندول بيوتريك اسيد أو النفثالين استك اسيد .

وقد لوحظ ان الثلاث أصناف لنخيل البلح لها نفس الاستجابة بالمعاملات بمنظمات النمو على مدى واسع . حيث أظهر الصنف جنديلة أعلى قياسات وأعداد ميكروسكوبية بالمقارنه بالصنفين الأخرين السيوي والزرغلول .

وقد تأثر بصورة كبيرة سمك النسيج الوسطي للورقة بمنظمات النمو . حيث أعطت أوراق صنف جنديله أسمك غلاف للحزمة الورقية وأعلى عدد لكل من الحزم الوعائية الكبيرة والصغيرة . وبصفة عامة أثرت منظمات النمو على متوسط قيم كل الصفات التشريحية تحت الدراسة .

ويمكن ترتيب منظمات النمو المستخدمة حسب تاثيرها على الصفات التشريحية للأصناف الثلاثة المستخدمة لنخيل البلح إلى انسميدول ثم الباكلوبترازول وأخيرا 2,4-D .