J. Agric. Sci. Mansoura Univ., 29 (1): 371 - 389, 2004

A STUDY ON *IN VITRO* PROPAGATION OF PINEAPPLE (*Ananas comosus* L. MERR.)

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ABSTRACT

In several trails for surface sterilization by immersing the explants in Noacl (1 , 5 , 10 , 15 %) + (1.0 %) Hg Cl2 for 10 , 20 , 30 minutes , the least contaminated explants were obtained by immersing the explants in NaOcl (15%) + (1.0%) $HgCl_2$ for 30 minutes.During the establishment stage after 3 weeks the explants were cultured on liquid and solid MS media , full and half strength supplemented with BAP at 0 , 1 , 2 , 3 mg/L and IBA at 0 , 0.5 , 1.0 mg/L for other 6 weeks . The best multiplication media was full strength liquid MS media supplemented with 1 mg/L BAP + 1 mg/L IBA .In rooting stage , shootlet were cultured on media containing 0.5 x strength MS salts , 3 mg/L activated charcoal and combinations of IBA at (0 , 0.2 , 0.5 , 1.0 , 2.0 mg/L) and NAA at (0 , 0.2 , 0.5 , 1.0 mg/L) , the rooted shootlets were obtained by culturing on MS media supplemented with 1 mg/L NAA + 0.5 mg/L IBA . At hardening stage of 3 months duration by using mixture of peatmoos : sand , the mixture of 3 peatmoss : 1 sand gave the highest percentage of survivals and leaf number .

The acclimatization lasted for 6 months then fertilization experiments on 12 months old explants were carried out using a mixture of ammonium sulphate (20%) , super phosphate (15%) and potassium sulphate (48%) in ten treatments as follows : 2:1:2 , 5:1:5 , 6:1:6 , 2:1.5:2 , 5:1.5:5 , 6:1.5:6 , 2:2:2 , 5:2:5 , 7:2:7 and 7:1:7 these mixtures were applied to each pots in four doses (0.5 , 1.0 , 1.5 , 2.0 gm) every week during the six months , the highest values of survival percentage , plant length , stem length , stem thickness , number of leaves and leaves area were obtained at ratio of 5:1.5:5 and 7:2:7 using a dose of 1 gm / plant every week .

INTRODUCTION

Pineapple (Ananas comosus L. Merr cv . queen) is a herbaceous , perennial , self sterile , monocotyledonous and short day plant belonging to fam. *Bromeliaceae*.

It was originated in America , then was transferred to many countries and mainly produced by Thailand , Philippine , Brazil , India , Mexico, Indonesia and United states of America (FAO,1982) . Its dry weight contains bromelain , a proteolytic enzyme in addition to being a good source of vitamins A and B .It is usually propagated by suckers , slips and crowns.The in vitro culture is widely used for propagation of pinapple .Various parts of the plant such as syncarps , lateral , buds , young crowns and slips and stem-tips were used for in vitro propagation (Sita *et al.*,1974 ; Mathews & Rangam , 1979 ; Wakasa , 1989 ; El-sherbini , 1991 and Aydieh *et al.*, 1999).

After production of a fruit from the terminal bud , the crown and slips fall to the ground . the axillary stem buds continue to develop and form new plants *(claude et al. , 1986).*

In Egypt , pineapple can be a promising new crop for growing under plastic greenhouses , particularly , in the newly reclaimed lands *(Flath , 1980).* this study was proposed to provide the best media for propagation and the best method of fertilization of this new crop.

MATERIAL AND METHODS

This study was carried out in the laboratory of plant tissue culture, EL-zahria , Agricultural Research Center , the horticulture service unit Ministry of Agriculture , during two successive periods 2001-2002.The acclimatization stage and the experiments of fertilization were carried out under green house conditions in the farm , Horticulture Research Institute , ARC , Ministry of Agriculture , during the successive period 2002-2003 .

Explants Queen – pineapple cv. were obtained from green house , Horticulture Research Institute , ARC , Ministry of Agriculture , Stem – tips (2-3 cm in length) were used as explants material .

The Stem – tips were transferred to the laboratory and leaves were carefully removed and held in tap water for two hours , followed by a soap solution for 5 min , rinsed again under running tap water for 30 min, then re-cut to 1 -1.5 cm in length .

A- Surface Sterilization

The explants were surface sterilized in the laminar air flow by immersion in sodium hypochlorite 20 % (chlorox, 5.25 active ingredient NaOcl) at (1,5,10 ,15%) for (10, 20,30 minutes , few drops of tween -20) (polyoxethelene sorbitan, a liquid detergent) were added as wetting Further agitation for 10 minutes in 0.1 % mercury chloride (w/v) was carried out. Each disinfection treatment was followed by three rinses with sterile distilled water . Data of sterilization treatment were recorded after two weeks of planting

B-Culture media and experimental conditions :

Murashige and Skoog (1962) based medium (ms) served the current studies . chemicals were used in the constitution of Ms-modified medium as shown in Table (1).

All culture media used in this research were adjusted to ph 5.7 before solidification with 0.7 % agar (BDH) . Media were autoclaved at 121C^{o} under pressure 1.2 kg/cm^{2} for 15 minutes

Table (1) Murashige and Shoog Modified Medium Components

Micro elements	Mg/ L
$CoCl_2 .6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
FeNa EDTA	36.70
H_3BO_3	6.20
KI	0.83
$MnSO_4.H_2O$	16.90
$Na_2MoO_4.2H_2O$	0.25
$ZnSO_4.7H_2O$	8.60
Macro Elements	
$CaCl_2$	332.02
KH_2PO_4	170.00
KNO_3	1900.00
$Mg\ SO_4$	180.54
NH_4NO_3	1650.00
Vitamins	
Glycine	2.00
Myo-Inositol	100.00
Nicotinic acid	0.50
Pyridoxine HCL	0.50
Thiamine HCL	0.10

Cultures were incubated in growth room under the following conditions :-

Incubation Conditions :

Temperature was controlled to 25 C^{o} + - 1, photo period at 16 hours light and 8 hours darkness using florescent lamps (110 cm long) and light intensity :3.5 klux (40 µEcm^{-2} . S^{-1}).

C-Establishment Stage :

The explants of all treatment were cultured in basic MS medium (half strength) (Murashige & Skoog,1962).Medium was Supplemented with 3% sucrose and solidified with 0.7% agar (BDH) in addition to 3gm/L activated charcool , data of sterilization treatment were recorded after three weeks of planting .

After 3 weeks , the explants were transferred to establishment medium using liquid and solid MS basic medium (full and half strength) supplemented with N^6 – benzyl aminopurine (BAP) at 0,1,2,3 mg/L and (IBA) at 0.05,1 mg /L , (Table 3) .

The medium which were filteresterilized to the autoclaved medium in pyrex-glass jars (25ml/jar) .

In this experiment twelve plant growth regulators combinations were regarded . Nine replicates , each containing three explants for all kinds of medium (liquid, solid ,full and half strength) cultures were kept three weeks at the growth room then the following parameters were recorded :-

1) shootled formation (number of axillary shoots per explant)
2) shcotlets length (cm)

in order to obtain a sufficient number of shootlets , shootlets were sub cultured every three weeks .

Rooting stage :

Shootlets were cultured on media containing 0.5 X strength MS salts 30% sucrose ,3gm/L activated charcoal , combinations of IBA at (0,0.2,0.5,1.0,2.0 mg/L) and NAA at (0,0.2,0.5,1.0 mg/L) .

The medium solidified with 0.6%agar (BDH)Cultured were incubated at 25 +- 1,16 h daily exposure to 32 $\mu Em^{-2}S^{-1}$ Gro Lux light .

Fifteen shootlets per treatments were used in all experiments after three weeks , the following parameters were recorded :-

1) The number and length of rootlets per explants.
2) The number and area of axillary shoots per explants.

In Hardening –Off And Acclimatization Stage ;

The plantlets produced from rooting stage were washed with tap water to remove agar remains from the rootlets and transplanted in plastic pots of 6- cm diameter , which were filled with sand ,peat moss and sand (1:1),peat moss and sand (2:1) , peatmoss and sand (3:1) and peatmoss .

The culture pots were irrigated with tap water every day , then kept in laboratory for Three month at 30+_ 2 C^{o}, 2 klux (16 hr/day) and 90% relative humidity (R.H.)

During the first month , pots were covered with transparent polyethylene bages which were then gradually removed During the second and third month the plants were kept without polyethylene bages then the plants transplanted in the plastic pots (20-cm diameter) filled with peat moss and sand (3:1) and allowed to grow for 6 month in a polyethylene greenhouse at 31+-5C^{o}, 6 klux (12hr/day) and 90% R.H after 3month , the following parameter were recorded :

1) survival percentage.
2) shootlets height (cm).
3) shootlets leaves number / plant.

In Fertilization Experiments:

After 12 months pineapple plants were transplanted in plastic pots (40cm diameter) filled with peatmoss and sand (3:1) .Fertilizer had been applied by hand in the basal axils of the older leaves on the plastic pots at the base of the plant .

Fertilizers mixture were ammonium sulphate (20%) , super phosphate (15%) and potassium sulphate (48%) in ten treatments as follows :
(2:1:2),(5:1:5),(6:1:6),(2:1.5:2),(5:1.5:5),(6:1.5:6),(2:2:2),(5:2:5),(7:2:1),and7:1:7).

These mixtures were applied to each pots in four doses (0.5,1.0,1.5,2.0 gm) every week during the six months. Each treatment consisted of three replications . Each replication included ten pots. Estimates were carried out on the plant at every dose . Survival percentage (%) , plant length (cm) , stem length (cm) , stem thickness (mm) ,number of leaves and leaves area $(mm)^2$ were estimated

The dried leaf samples were subjected to determination of (N%) according to *Micro –Kjeldohl method (Jackson ,1976)* Phosphorus was estimated by the method of *Truog & Meyer (1929).* Potassium was determined by using Flame photometer according to the method of *Broun & Lilleland (1946).*

The experiment was designed as complete randomized blocks , with three replicates , factorial experimental of data were subjected to analysis of variance according to procedure reported by *Snedecor and Cocchran (1980)* .

RESULTS AND DISCUSSION

Effect Of Surface Sterilization On Microbial Contamination Of Pineapple Explants:

Table (2) represents the effect of different immersion time in NaOcl concentrations and Hgcl2 on contamination percentage of pineapple explants.It is clear that increasing immersion time and concentration of NaOcl + 1.0 % Hgcl2 reduced the percentage contaminated plants .Best treatment was immersion of explants for 30 min in 15%NaOvl +1.0 Hgcl2 . These results are in agreement with the findings of *Russel et al . (1982) , El sherbini (1991) , prakash & Trevor (1991) , Aydieh etal., (1999) and Abd Rabou (2000)* .They used chemical sterilizing agents , such as sodium hypochlorite and mercuric chloride whereas hypochlorites are powerful antimicrobial a gents (being b actericidal . f ungicidal a nd s poricidal) , iodine chloride bactericidal , fungicidal but not sporicidal.They concluded that the best sterlizatioin treatment includes immersing the explants in NaOcl (15%) +1.0 % iodine chloride for 30 min.

Table (2): Effect of immersion time (min), in sodium hypochlorite (NaOCL) concentrations (1, 5, 10, 15 %) and iodine chloride (1.0%) , on contamination percentage of pineapple explants three weeks after culture

Treatments	Contamination %			Mean
	10 min	20 min	30 min	
1% NaOCL + 1.0 iodine chloride	98.67	96.00	92.00	95.56
5% NaOCL + 1.0 iodine chloride	86.33	85.67	83.67	85.67
10% NaOCL + 1.0 iodine chloride	72.67	56.33	43.33	57.44
15% NaOCL + 1.0 iodine chloride	35.33	30.67	23.33	29.78
Mean	73.250	67.17	60.58	

L.S.D Of treatment = 0.572 L.S.D Of times = 0.4954 L.S.D Of interaction = 0.991

Effect of media and plant growth regulators on the multiplication stage :

In Tables (3,4) show that using full strength of liquid MS media gave better results compared to half strength MS media . It is evident from Tables (3,4) that using BAP alone with increased concentrations 0,1 and 2 mg/Ldecreased both the number of shoots as well as shoot length , On the other hand , using BAP alone with a concentration of 3 mg/L increased the number of shoots but still decreased the shoot length . Similar results were obtained by *Wimber* (1965) on cymbidiums , *Mathewa & Rangan* (1979) on pineapple explants ,*Rodriguez* (1982) on castanea and *Yossef* (1994) on acacia . The latter auther reported an increase in shoot multiplication rate by

increasing the level of BA up to 1.0 mg/L and a decrease at the higher levels . This increase could be ascribed to a stimulatory effect on cell division and enlargement. In this respect , it was found that BA enabled geminating seeds and many excised tissues to regenerate on synthetic media by promoting cell division and enlargement (*Letham et al.* 1978 and *Dowradar et al.* 1996).

Table (3) : Effect of concentrations of plant growth regulators (mg/L) and strength of MS-media on shootlet number and shootlet length (cm) per explants of pineapple explants cultured for 3 weeks liquid MS media .

Treatments BAP,IBA mg/L.	Full strength		Half strength	
	No. of shootlets	Shootlets length	No. of shootlet	Shootlets length
0.0 , 0.0	0.0	0.0	1.50	1.30
1.0 , 0.0	3.93	2.5	1.60	1.37
2.0 , 0.0	3.41	2.16	1.78	1.67
3.0 , 0.0	3.91	2.15	2.15	0.50
0.0 , 0.5	3.62	3 .10	2.14	0.90
1.0 ,0.0	3.76	3.93	2.26	1.50
2.0 , 0.5	4.62	0.85	1.81	0.50
3.0 , 0.5	3.19	1.33	2.17	0.60
0.0 , 1.0	4.63	1.50	2.66	1.33
1.0 , 1.0	4.64	2.10	2.50	0.967
2.0 ,1.0	4.11	2.95	2.24	0.76
3.0 , 1.0	3.94	0.70	2.037	0.93
L.S.D 5%	0.049	0.296	0.053	0.054

Therefore , we concluded that MS- basic medium supplemented with 1.0 mg BA /liter was the best for both the number and length of shoots , while using 3 mg BA/ liter was the best for the number of shoots , Accordingly , shootlets originated from the medium containing 1.0 mg BA/liter were subculture every 3 weeks.In this way, liquid subcultures were made to ensure high multiplication rate and low physiological disorder.

Table (4) : Effect of concentrations of plant growth regulators (mg/L) and strength of MS-media on shootlet number and shootlets length (cm) per explants of pineapple explants cultured for 3 weeks liquid MS media.

Treatments BAP,IBA mg/L.	Full strength		Half strength	
	No. of shootlets	Shootlets length	No of shootlet	Shootlets length
0.0 , 0.0	37.67	3.17	28.33	1.90
1.0 , 0.0	55.67	3.50	21.67	2.17
2.0 , 0.0	47.67	3.17	25.00	1.67
3.0 , 0.0	54.67	3.10	30.100	1.33
0.0 , 0.5	50.67	4.10	29.50	1.83
1.0 , 0.5	52.67	5.23	31.67	2.50
2.0 , 0.5	64.70	1.83	25.37	1.00
3.0 , 0.5	44.73	2.33	38.00	1.50
0.0 , 1.0	64.83	2.50	37.23	2.33
1.0 , 1.0	64.90	3.00	35.00	2.00
2.0 ,1.0	57.67	3.00	31.33	1.67
3.0 , 1.0	55.20	1.67	28.17	1.93
L.S.D at 5%	0.983	0.319	0.657	0.369

On the other hand , using concentrations of BA at 0 and 2 mg / liter combined with a fixed concentration of IBA at 0.5 mg/ liter increased the

number of the shoots , but by using concentrations of BA at 1 and 3 mg/ liter with IBA at 0.5 mg/ liter decreased the number and length of shoots , respectively.

To conclude, these results are in coincidence with these of *Wimber* (1965) on cymbidiums ; *Mathewsd Rangan* (1979) on pineapple explants , *Hosoki & Asahira* (1980) and *Zepeda & Sagawa* (1981) on pineapple explants , *Rodriguez* (1982) on Castaned ; *El-sherbini* (1991) on pineapple , *Youssef* (1994) on Cacia and *Aydieh et al.*(1999). They reported that shootlet number and length increased significantly by increasing the dose of BA until 1.0 mg/L then decreased at higher doses. This increase could be ascribed to a stimulatory effect on cell division and enlargement . Therefore we conclude that MS-basal medium supplemented with 1 mg/L BAP combined with IBA at 1 mg/L was the best for both shootlet number and length .

Tables (5 , 6) show that using full strength solid MS media gave better results than half strength MS media. These results are in conformity with the findings of Turk , *et al.*, (1994) on Rulus .They found that more shoots per regeneration leaf found on MS full strength than MS half strength.

Tables (5, 6) show that on using BAP at concentration 0 , 1 and 2 mg/L with a fixed concentration of 0.5 mg/L IBA caused a significant increase in number of shoots and a decrease in shoot length . while the concentration of 3 mg/l BAP and 0.5 mg/l IBA decrease in shoot length while , the concentration of 3 mg/L BAP+0.5 mg/L IBA increased number of shoots as well as length of shoots.

Table (5): Effect of concentration of plant growth regulators (mg/L) and strength of MS-media on shootlet number and shootlet length (cm) per explants of pineapple explants cultured for 3 weeks on solid MS media

Treatments BAP,IBA mg/L.	Full strength		Half strength	
	No. of shootlets	Shootlets length	No. of shootlet	Shootlets length
0.0 , 0.0	0.0	0.0	0.0	0.0
1.0 , 0.0	4.43	0.70	3.21	1.50
2.0 , 0.0	2.71	1.90	3.35	1.10
3.0 , 0.0	2.43	0.70	2.86	1.10
0.0 , 0.5	2.07	2.00	1.95	3.20
1.0 , 0.5	3.21	1.20	3.60	1.53
2.0 , 0.5	3.41	0.60	2.52	0.50
3.0 , 0.5	3.58	2.20	3.60	1.07
0.0 , 1.0	3.21	4.10	2.55	2.17
1.0 , 1.0	3.58	1.10	2.52	1.14
2.0 ,1.0	3.14	1.20	2.98	0.47
3.0 , 1.0	3.62	1.10	3.21	0.50
L.S.D at 5%	0.063	0.255	0.068	0.071

These results proved the role of BA and IBA in increasing number of adventitious buds formed in vitro culture of pineapple explants. These results are in conformity with the findings of *Hosoki & asahira* (1980) , *Zepeda & Sagawa* (1981) *and El-sherbini* (1992). To conclude , the best results for shootlet number and length for plants culture on solid MS media supplemented with 3 mg/L BAP+0.5 mg/L IBA. Therefore , it is recommended to use MS liquid medium supplemented with 1 mg/L BAP + 1mg/L IBA which was found to be better than the highest results cultured on MS solid medium supplemented with 3 mg/L BAP + 0.5 mg/L IBA regarding number and length of shoots.

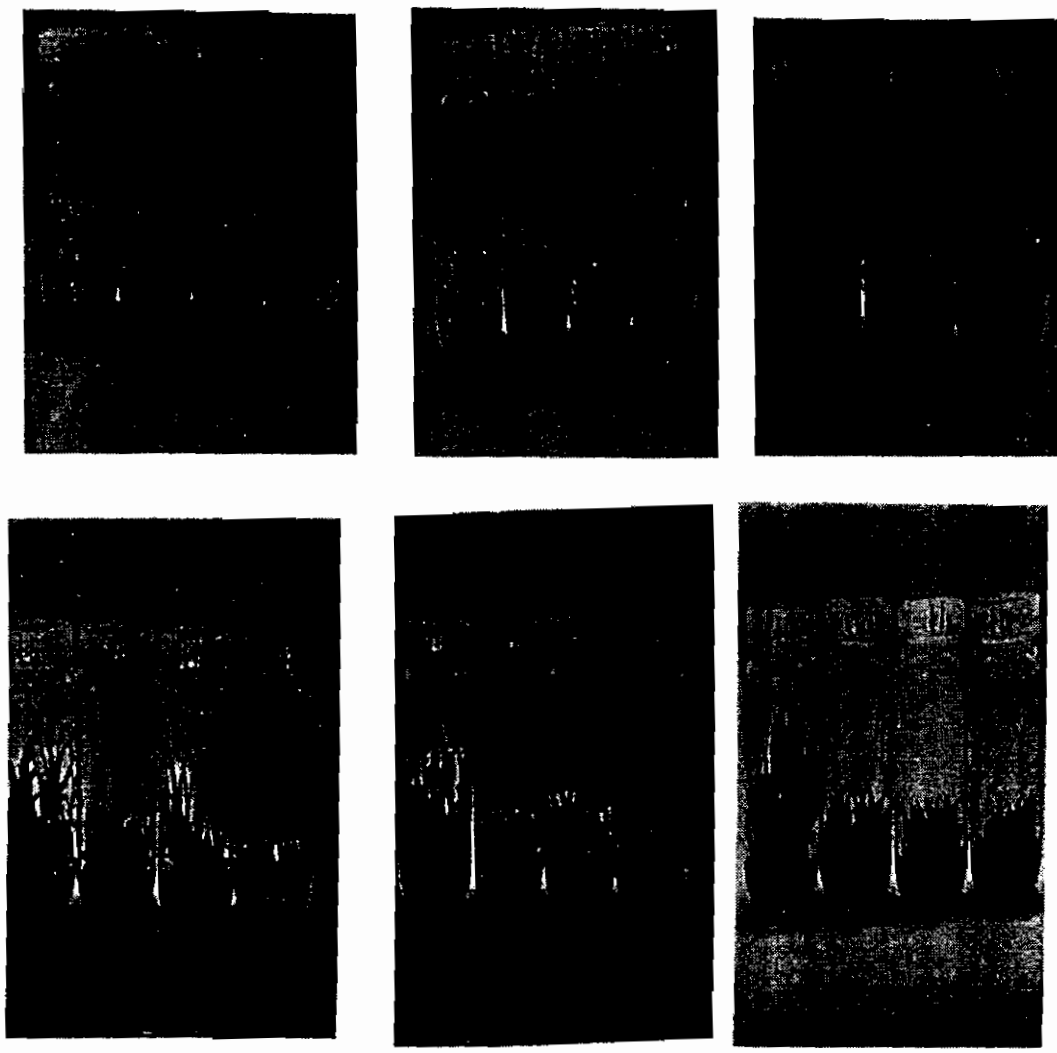

Fig (1): Effect of full strength and half strength liquid MS media on the stage of multiplication of pineapple explants . Notice the effect of variable ratio of plant growth regulators BAP and IBA on number of shootltes / explant and shoot length.

Table (6): Effect of concentration of plant growth regulators (mg/L) and strength of MS-media on shootlet number per explants and shootlet length (cm) of pineapple explants cultured for 3 weeks on solid MS media .

Treatments BAP,IBA mg/L.	Full strength		Half strength	
	No. of shootlets	Shootlets length	No. of shootlet	Shootlets length
0.0 , 0.0	35.00	2.00	45.33	3.03
1.0 , 0.0	48.33	1.7	45.00	2.50
2.0 , 0.0	38.00	2.9	47.00	2.10
3.0 , 0.0	34.00	1.7	40.00	2.10
0.0 , 0.5	29.00	3.00	27.33	4.50
1.0 , 0.5	45.00	2.2	50.33	2.53
2.0 , 0.5	47.67	1.9	35.33	1.33
3.0 , 0.5	50.10	3.2	50.33	2.07
0.0 , 1.0	45.00	5.1	35.67	3.17
1.0 , 1.0	50.17	2.1	35.33	2.17
2.0 ,1.0	44.00	2.2	41.00	1.53
3.0 , 1.0	50.67	2.0	45.00	1.23
L.S.D at 5%	0.532	0.363	0.803	0.302

Effect of plant growth regulators on rooting stage :

Table (7) shows that the control treatment gave the same result for roots number by using high concentration of IBA at 0 ,0.2 , 0.5 , 1 and 2 mg /L combined with a fixed concentration of NAA at 0.2 mg/L . The results were the same concerning the root length except at a concentration of 0.1 mg/L IBA and 0.2 mg/L NAA.

Table (7): Effect of concentrations of NAA(mg/L) and IBA(mg/L) on number of roots , root length (cm) , number of leaves and leaves area (cm^2) of pineapple shootlets cultured for 4 weeks on solid MS media .

Treatments NAA,IBA(mg/L)	No. of roots	Root length (cm)	No of leaves	Leaves area (cm^2)
0.0 , 0.0	4.33	7.00	7.67	13.17
0.2 ,0.0	6.67	9.00	9.33	18.33
0.2 ,0.2	6.00	8.67	6.00	15.67
0.2 ,0.5	6.33	8.33	10.67	13.83
0.2 ,1.0	5.67	5.20	7.33	12.67
0.2 ,2.0	6.33	7.33	8.33	11.97
0.5 , 0.0	8.67	8.33	10.33	19.33
0.5 , 0.2	8.67	12.83	10.33	14.83
0.5 , 0.5	6.33	6.67	9.67	16.33
0.5 , 1.0	6.67	6.00	14.33	17.97
0.5 , 2.0	5.33	7.67	7.67	14.67
1.0 , 0.0	4.67	8.33	7.33	14.67
1.0 , 0.2	6.00	8.67	8.33	15.50
1.0 , 0.5	10.00	8.33	9.33	18.33
1.0 , 1.0	4.67	5.17	9.00	11.67
1.0 , 2.0	7.33	11.00	8.33	18.67
L.S.D at 5%	0.857	0.789	0.905	0.757

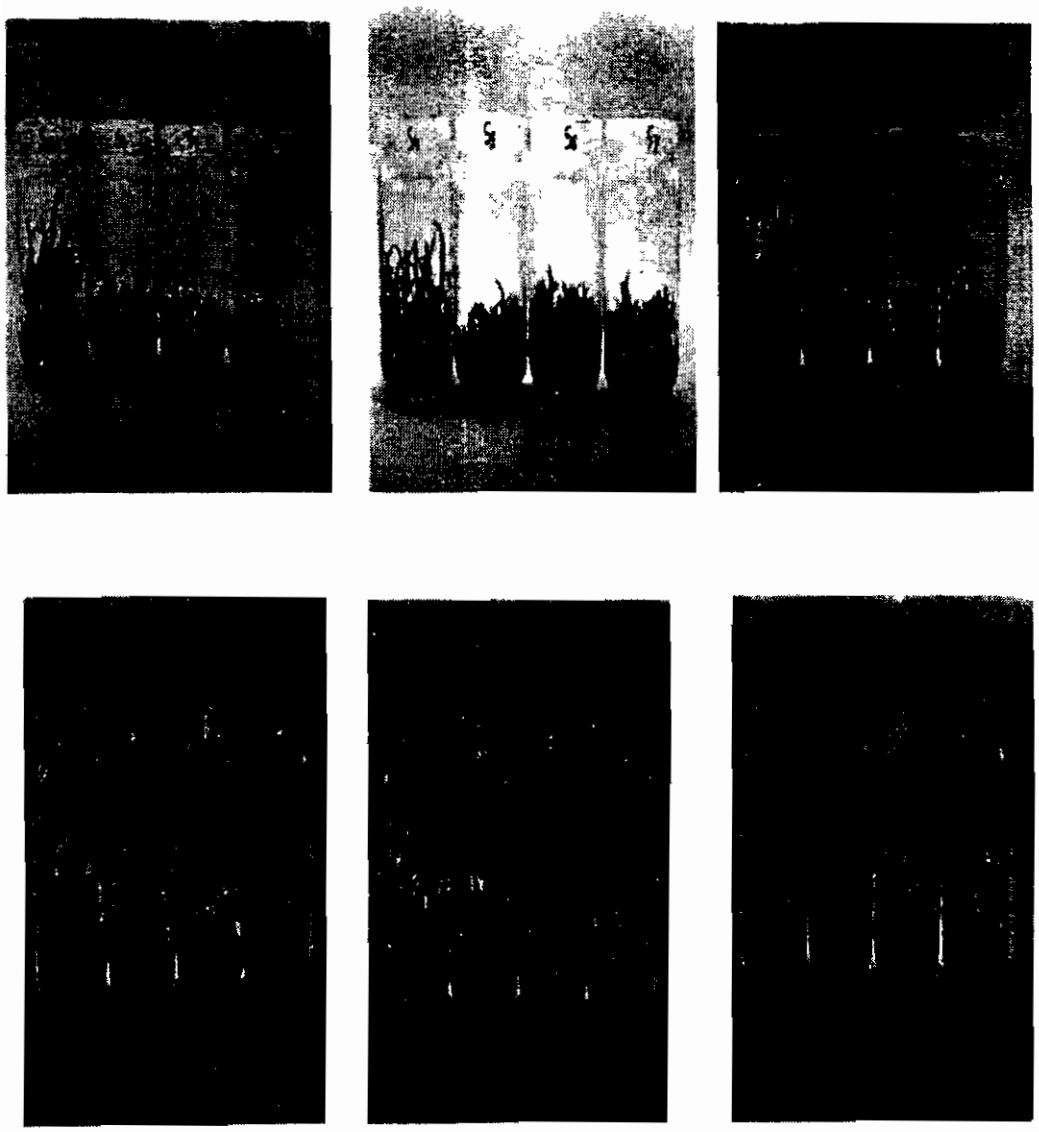

Fig (2): Effect of full strength and half strength solid MS media on the stage of multiplication of pineapple explants . Notice the effect of variable ratio of plant growth regulators BAP And IBA on number of shootlets / explant and shoot length.

As for the number of leaves , the concentrations of 0 , 0.5 , 1 and 2 mg/L IBA and 0.2 mg/L NAA gave nearly the same results but at concentration of 0.2 mg/L IBA and 0.2 mg/L NAA , have the lowest root length. Leaf area was the highest by using 0.2 mg/L NAA and the lowest at 2 mg/L IBA and 0.2 mg/L NAA . By increasing the concentration of NAA to 0.5 mg/L with the same increased concentration of IBA , the number of roots decreased while the lengths of roots were nearly equal except at the concentration of 0.5 mg/L NAA and 0.2 mg/L IBA , it gave the highest score. The number of leaves were nearly equal except at the concentration of 0.5 mg/L NAA and 1 mg/L IBA , it gave the maximum score .

The leaves area was high by using 0.5 mg/L NAA only and by using 0.5 mg/L NAA combined with 1 mg/L IBA. The other concentrations gave lower areas which were nearly the same. By using higher concentration of NAA at 1 mg/L combined with the same increasing concentration of IBA , there were slight differences in number of roots except at 0.5 mg/l IBA and 1 mg/L NAA and a higher number of root length was obtained at 2 mg/L IBA and 1 mg/L NAA while the lowest at 1 mg/L IBA and 1 mg/L NAA.

There were slight differences in number of leaves at these concentrations of 0, 0.2 ,0.5 , 1,2 mg/L IBA and 1 mg/L NAA , while the leaves area was maximum at 0.5 mg/L IBA and 1 mg/L NAA and the lowest at 1 mg/L NAA and 1 mg/L IBA .Therefore increasing the concentration of both IBA and NAA , mostly enhanced the root initiation and elongation , but the magnitude of the response was more pronounced in case of NAA. In this concern , root number and length increasing to maximum (10 and 12.83 cm) at 0.5 mg/L and 0.2 mg/L IBA , respectively and 1 mg/L and 0.5 mg/L NAA , respectively. These results are in coincidence with those of *Mathews & Rang an* (1979) who stated that the best rooting medium , for either IBA or NAA at relatively low concentrations .These findings could be ascribed to the biological activities of IBA and NAA belonging to synthetic axons which promote cell division and elongation *Abu-Grab & Abraham (1998)* reported that both regulators enhanced growth and development of onion by stimulating cell division and enlargement , but NAA had the superior effects.

From these results , we deduced that the best rooting medium for pineapple shootlets in MS – basal medium containing Ms salts at a half strength and supplemented with NAA at a concentration of 1 mg/l + 0.5 mg/L IBA.

Effect of growing media on survival percentage , shootlets height (cm) and number of leaves of pineapple plantlets during hardening off stage.

Table (8) shows that the percentage of survival shoot length and number of leaves were significantly influenced by the growing medium . In case of percentage of survival , means followed by different letters are significantly different at 5 % level Data disclosed that pineapple plantlets grown 2 months (hardening off stage) in peatmos soils were 10% welted ,whereas the other grown in sand were 50% welted . Increasing peatmoss ratio from 0.0 to 75 % gradually and consistently increased both survived plants , Shoot length and number of leaves. In addition ; raising peatmoss ratio , in peatmoss –sand mixture from 1:1 to 3:1 caused an increase in survived plants , shoot length and number of leaves .

Also , it can be noticed that the mix of 3 peatmoss 1 sand achieved the highest values of survivals and leaf number . These results go along with those of *El sherbini(1991)* on pineapple ; *Abou Dahab (1992)* on chlorophytum, Asparagus and *Aydieh et al (1999).*

Table (8): Effect of growing media on survival percentage , shootlets height (cm) and mean number of leaves of pineapple plantlets during hardening off stage

Treatments	Survival %	Shootlets height (cm)	No of leaves	PH
Sand	**47.67**	**10.83**	**8.67**	**7.5**
Peatmoss +sand (1:1)	65.33	16.33	13.00	7.3
Peatmoss +sand (2:1)	75.33	18.00	15.00	7.0
Peatmoss +sand (3:1)	98.67	17.67	16.00	6.5
Peatmoss	92.67	15.67	14.33	3.7
L.S.D at 5%	0.842	0.620	0.769	

Effect of fertilization with different ratios of NPK on plant growth and development

Tables (9 , 10 , 11) show the effect of level of Ammonium sulphate (N) , Super phosphate (P) and Potassuim sulphate (K) fertilization on survival percentage , plant length , stem length , stem thickness number of leaves and leaves area . The control group gave the best percentage of survival (92%) but by increasing the ratio of ammonium sulphate and potassium sulphate , the survival ratio improved and became nearly equivalent to the control . These results agreement with the finding of *Neog – M ; et al (1995) , Velez – Ramos – A ; et al (1995) , Owusu – Bennoah – E ; et al (1997) , Vo – Thi – Gucng ; et al (1997) , and Das – Bc ; et al (1999)* they showed that using N and K application brought an improvement in plant growth. This can be explained by the obvious response of vegetative growth to nitrogen level might be due to the important role of nitrogen in the plant through its presence in the structure of protein molecule . (*Devlin,1972*) explained that the high levels of nitrogen creates a tendency to increase leaf cell number and cell size with overall increase in leaf production . The low nitrogen availability decrease protein synthesis which subsequently cause a decrease in cell size and especially cell division .Whereas potassium is essential as an activator for the enzymes involved in the synthesis of certain peptide bonds . (*Mengel & Kirkby , 1978*) showed that the activation of several kinases by K also enables the synthesis of high molecular weight compounds . Inadequate K supply , therefore, results in an accumulation of low molecular weight sugars and amino acids . More severe K deficiency leads to the synthesis of toxic amines such as purtrescine and agmatine . In addition , K deficiency may lead to more active uptake of Na which has an antagonistic effect on the function of K as an enhancer protein synthesis .

Table (9) shows also that P levels have insignificant effected on vegetative growth . This also confirmed by (*Mengel & Kirkby , 1978*) .The insignificant effect of tested P levels on vegetative growth of mango trees could only be interpretted by the accumulation of this nutrient to an adequate level in the soil through fertilization in years preceding the experiment.

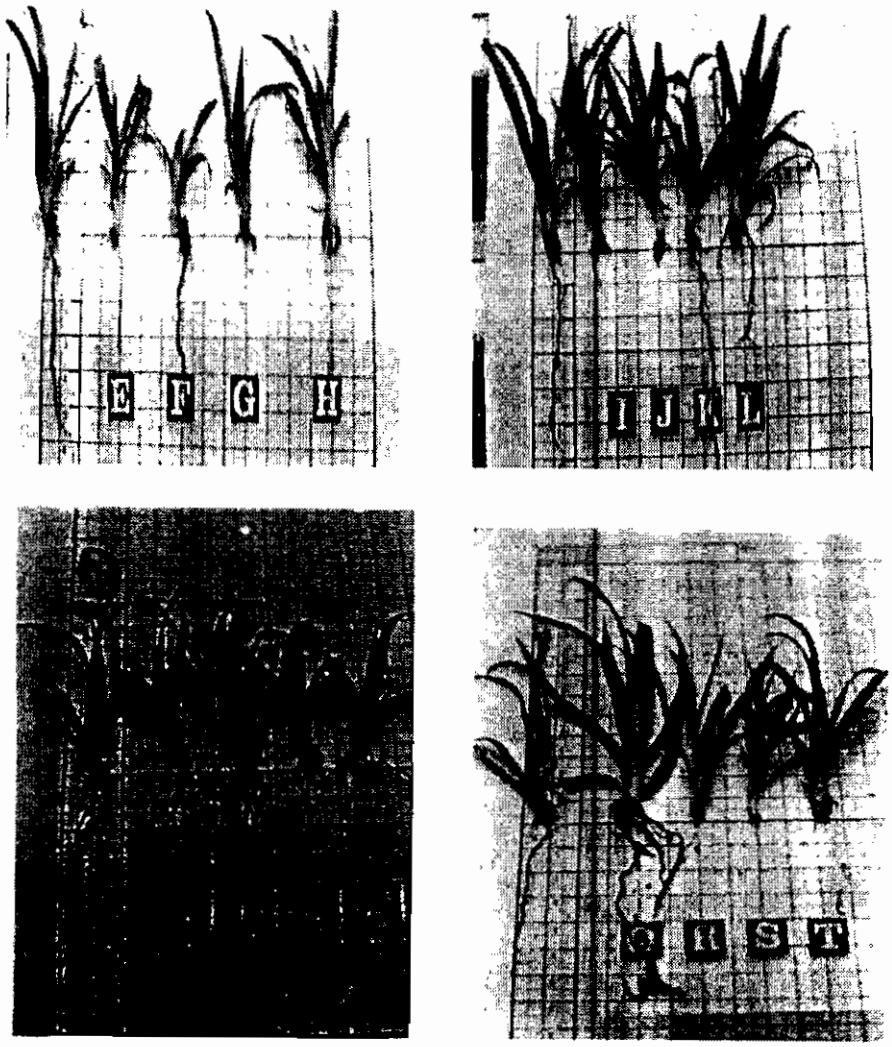

Fig (3): Effect of concentrations of NAA(mg/L) and IBA(mg/L) on number of roots , root length (cm) , number of leaves and leaves area (cm^2) of pineapple shootlet cultured for 4 weeks on solid MS media.

The best dose of the tested treatment was 1 gm per plant that gave the highest percentage of survival . (91.1) . Regarding the plant length , it was evident that giving (N) and (P) at high and equal ratios 5:5 and 7:7 gave the best values of plant length 11.65 cm and 10.42 cm respectively . A dose of 1 gm/plant of the treatment is recommended for high response (8.52 cm) . It was evident from table (10) that using high and equal ratio of (N) and (K) together with low ratio of P as the following N : P : K equal 5 : 1.5 : 5 a significantly high values of length and thickness 10.33 cm , 2.33 mm respectively wherease using the ratio 7:2:7 gave respectively higher values of length and thickness 13.33 cm and 2.33 mm respectively all at a dose of 1 gm/plant .

Table (11) shows also that by using high and equal ratio of N and K with relatively high ratio of P gave the best results for number of leaves and leaves area 9.33 , 25.33 cm^2 with ratio 5:1.5:5 and 8.67 , 26.73 cm^2 with ratio 7:2:7 respectively at a dose of 1 gm/plant . These results are parallel with the findings of *Soerodimedjo , F . w . (1982) and Owusu – Bennoah – E ; et al (1997)* . They explained the important role of using high and equal ratios of N and K in improving plant growth . A dose 2 gm/plant is recommended for the best number of leaves (10.52) and a dose 0.5 gm/plant is recommended for the best leaves area (24.71 cm^2) .Therefore using high and equal ratio of (N) and (K) gave the best results for survival percentage , plant length, stem length, stem thickness , number of leaves and leaves area . (P) has insignificant role in survival percentage , but it is recommended in relatively high ratio e.g: NPK 5:1.5:5 or 7:2:7 , respectively for the best results in plant length (11.63 , 10.42 cm) respectively , stem length (11.36 , 10.42 cm) respectively , stem thickness (2.83 , 3.04 mm) respectively , number of leaves (10.67 , 10.08) respectively and leaves area (28.24 , 25.76 cm^2) . It is better to use a dose of 1 gm/plant of treatment for a good response .

Table (12) shows the chemical analysis of dry leaves of pineapple . It was clear that N and K content increased as N and K increased . It was confirmed that the best ratio of NPK 5:1.5:5 and 7:2:7 . They gave high content of N (0.11 , 0.15) respectively , high content of K (3.85 , 3.9 %) respectively and P content (0.95 , 1.51 %) respectively with dose of 1 gm/plant .

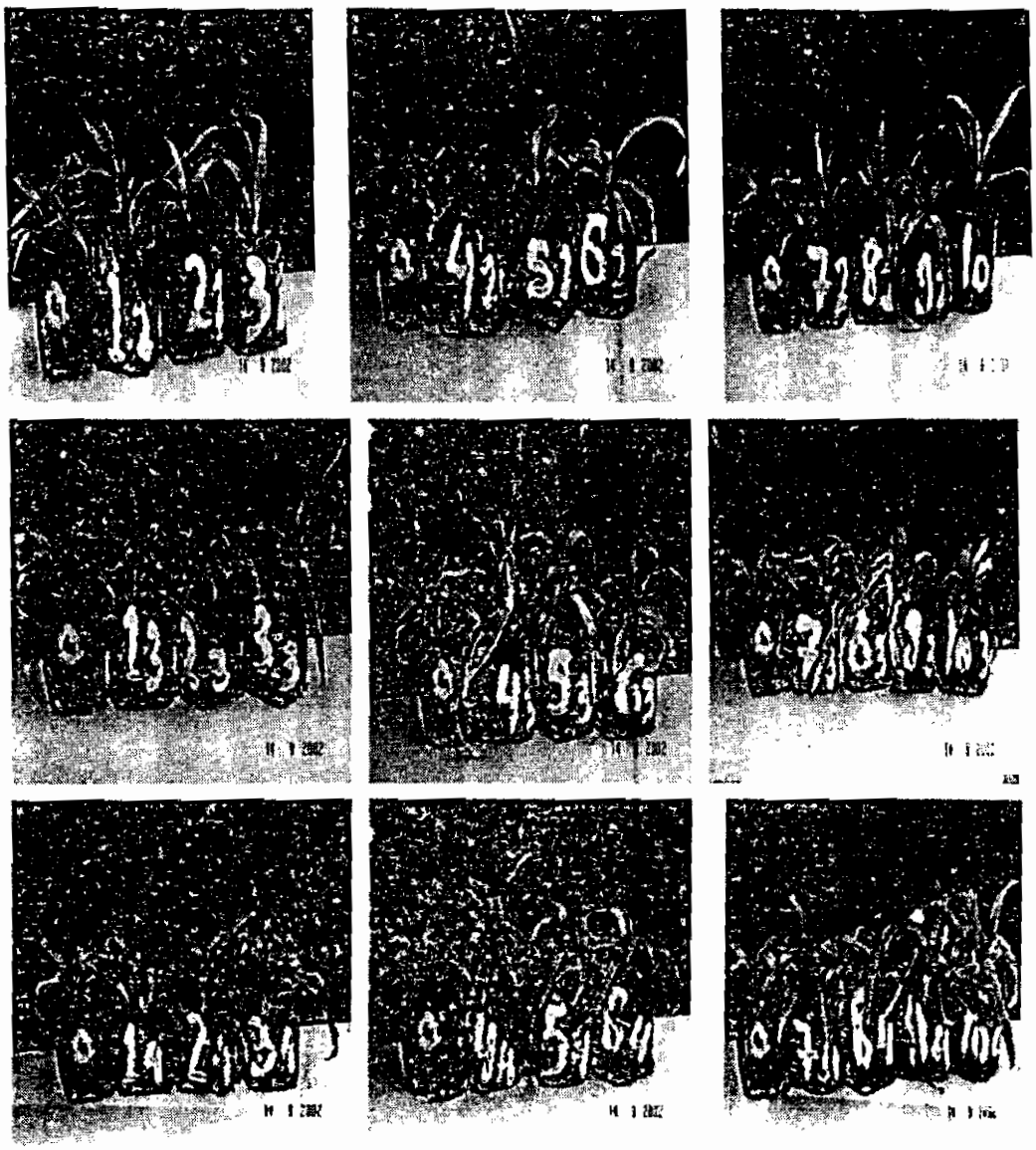

Fig (4): Effect of fertilization with different ratios and doses of NPK on pineapple plants.

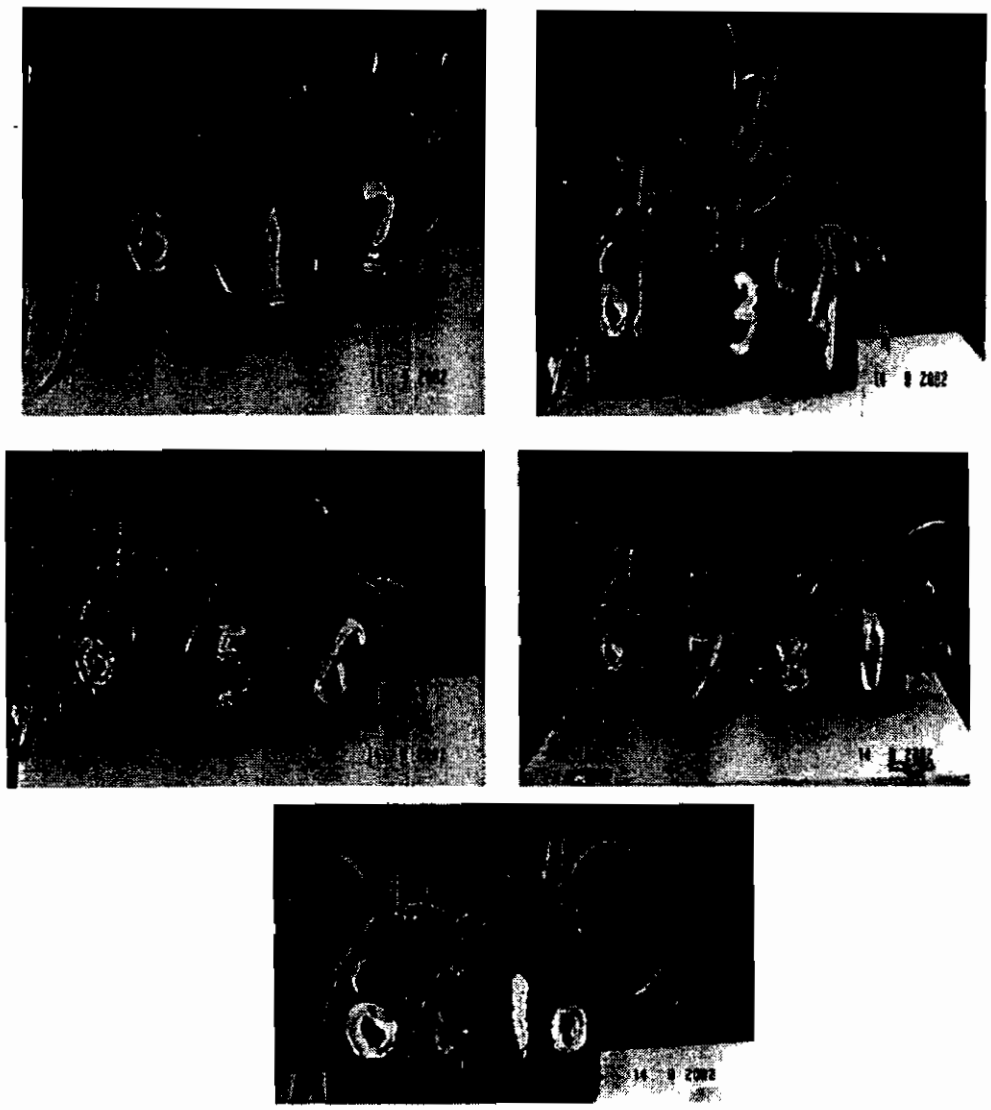

Fig (5): Effect of fertilization with different ratios of NPK on pinapple plants.

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إكثار الأناناس بواسطة زراعة الأنسجة

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في دراسة لإكثار نبات الأناناس صنف كوين استخدمت تقنيات زراعة الأنسجة و ذلك بزراعة القمم النامية للساق. وفى خلال تجارب التعقيم السطحي للأجزاء النباتية المستخدمة تم نقع الأجزاء النباتية في محلول هيبوكلوريد الصوديوم بتركيزات ١، ٥ ، ١٠ ، ١٥% + ١% كلوريد الايودين لمده ١٠ ، ٢٠ ، ٣٠ دقيقة و بعد زراعة الأجزاء النباتية على بيئة موراشيج و سكوج نصف قوة لمدة ٣ أسابيع وجد أن أفضل طريقة للتعقيم والحصول على نباتات خالية من الميكروبات هى نقع الأجزاء النباتية (القمم النامية) في محلول هيبوكلوريد الصوديوم ١٥% + ١% كلوريد الايودين لمدة ٣٠ دقيقة. وفى مرحلة التضاعف تمت زراعة النباتات على بيئات صلبة وسائلة لأملاح المواشيج وسكوج ذات القوة الكاملة والنصف قوة مضافا" اليها تركيزات مختلفة من البنزيل أمينو بيورين بتركيزات صفر ، ١ ، ٢ ، ٣ ملليجرام/ لتر وحمض الأندول بيوتريك بتركيزات صفر,1,0.5 ملليجرام/ لتر لمدة ٦ أسابيع وكانت أفضل البيئات لتضاعف وزيادة النموات الخضرية هى البيئة السائلة ذات القوة الكاملة لأملاح المواشيج وسكوج المضاف اليها ١ ملليجرام/ لتر بنزيل أمينو بيورين + ١ ملليجرام/ لتر حمض الأندول بيوتريك . في مرحلة التجذير زرعت النباتات على بيئة موراشيج وسكوج نصف قوة + ٣ جرام/ لتر فحم نشط مضافا" اليها تركيزات من حمض أندول بيوتريك بتركيزات صفر ، ٠,٢ ، ٠,٥ ، ١ ، ٢ ملليجرام/ لتر و نفثالين حمض الخليك بتركيزات صفر ، ٠,٢ ، ٠,٥ ، ١ ملليجرام/ لتر ، وتم الحصول على أفضل النتائج من البيئة الأساسية لأملاح المواشيج وسكوج بنصف القوة والمحتوية على ١ ملليجرام/ لتر نفثالين حمض الخليك + ٠,٥ ملليجرام/ لتر حمض الأندول بيوتريك . وفى مرحلة التقسية والتى أستغرقت ٣ أشهر أعطت خلطة البيت موس والرمل بنسبة ١:٣ على التوالى أعلى نتائج من حيث عدد النباتات الحية وعدد الأوراق.

في مرحلة الأقلمة قد أستغرقت ٦ أشهر ثم أجريت تجارب التسميد على النباتات البالغة من العمر ١٢ شهر بأستخدام مخلوط من سلفات النشادر ٢٠% و سوبر فوسفات ١٥% و سلفات البوتاسيوم ٤٨% في ١٠ معاملات كالتالى:

٢:١:٢ ، ٥:١:٥ ، ٦:١:٦ ، ٢:١,٥:٢ ، ٥:١,٥:٥ ، ٦:١,٥:٦ ، ٢:٢:٢ ، ٥:٢:٥ ، ٧:٢:٧ ، ٧:١:٧

أعطت بـ ٤ جرعات (٠,٥ ، ١ ، ١,٥ ، ٢ جرام/ نبات) كل أسبوع ، وكانت أفضل النسب من مخلوط النيتروجين – الفوسفور – البوتاسيوم هى ٥:١,٥:٥ ، ٧:٢:٧ على التوالى بجرعة أسبوعيه ١ جرام/ نبات حيث أعطت أعلى نسبة مئوية للنباتات الحية وأعلى طول للنبات والساق وأكثر سمك للساق وأعلى عدد للأوراق وأكبر مساحة للأوراق.