IV. RESULTS AND DISCUSSION

IV.1. Pineapple:

IV.1.1. Acclimatization:

IV.1.1.a. Effect of agricultural medium:

It is clear from **Table (2)** and **Photo (1)** that the combination treatment 50% vermiculite + 50% peatmoss was significantly superior in improving most of the studied parameters as it increased the percentage of survival percentage (98.00%), shoot length (0.83), number of leaves (3.33), leaf length (4.43), leaf width (0.49), root length (4.64) and number of roots (3.67) followed by the treatment (100% peat moss) then the combination treatments (33.3% vermiculite +33.3% perlite + 33.3% sand) and (50% perlite + 50% peatmoss) as compared with the other used treatments.

Also, the combination treatment (33.3% vermiculite + 33.3% perlite + 33.3% peatmoss) succeeded in increasing shoot thickness to the highest level (0.72) then followed with combination treatment (25% vermiculite + 25% perlite + 25% peatmoss + 25% sand) and (25% vermiculite + 25% peatmoss + 25% sand + 25% loam) as compared with the other used treatments.

The above results showed that using of vermiculite combined with either peatmoss or perlite or sand maximized survival percentage and most of growth parameters. These results may be due to the used combinations of agricultural media encouraged formation of a lot root hairs which enhanced absorption ability and in turn improved most of growth parameters. These results are partially agreed with the findings of **Madhuri and Shankar (1999).** They found that the combination of vermiculite and peatmoss (1:1 v/v) induced the best acclimatization percentage of date palm plantlets.

Table (2): Effect of different agricultural media on survival percentage, growth, and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

No.	Parameters / Agricultural media	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of root
1	100 % Vermiculite	88.67 C	0.43 EF	0.66 CDE	3.00 AB	4.17 AB	0.87 AB	4.30 CD	3.00 ABC
2	100% Perlite	79.00 E	0.37 FG	0.63 DEF	2.67 ABC	3.53 DEF	0.77 BC	3.47 F	2.67 BCD
3	100% Peat moss	94.67 B	0.53 CD	0.69 ABC	3.33 A	4.33 A	0.93 A	4.63 A	3.67 A
4	100% Sand	28.00 Q	0.27 H	0.53 H	1.33 E	1.30 N	0.47 IJKL	1.90 M	1.33 FG
5	100% loam	27.67 Q	0.33 GH	0.59 FG	1.67 DE	2.70 KL	0.63 DEFG	1.57 N	1.00 G
6	50% Vermiculite + 50% Perlite	74.33 FG	0.37 FG	0.63 DEF	2.33 BCD	3.10 HIJ	0.70 CDE	3.43 FG	2.33 CDE
7	50% Vermiculite +50% Peat moss	98.00 A	0.83 A	0.68 ABC	3.33 A	4.43 A	0.97 A	4.67 A	3.67 A
8	50% Vermiculite + 50% Sand	60.00 K	0.33 GH	0.61 EF	2.00 CDE	1.27 N	0.47 IJKL	3.07 I	1.33 FG
9	50% Vermiculite + 50% loam	65.00 IJ	0.37 FG	0.63 DEF	2.33 BCD	2.47 LM	0.37 LM	2.87 J	1.67 EFG
10	50% Perlite + 50% Peat moss	95.00 B	0.63 B	0.67 BCD	3.33 A	3.70 CDE	0.53 GHIJ	4.63 A	3.33 AB
11	50% Perlite + 50% Sand	50.00 M	0.37 FG	0.59 FG	1.67 DE	1.00 NO	0.33 M	2.73 J	1.67 EFG
12	50% Perlite + 50% Loam	30.00 P	0.27 H	0.52 H	1.33 E	0.57 Q	0.33 M	2.17 L	1.33 FG
13	50% Peat moss + 50% Sand	50.00 M	0.33 GH	0.58 FG	1.67 DE	0.93 OP	0.43 JKLM	2.87 J	2.00 DEF
14	50% Peat moss + 50% Loam	60.00 K	0.37 FG	0.63 DEF	2.33 BCD	3.77 CD	0.73 CD	2.80 J	1.67 EFG
15	50% Sand + 50% Loam	57.67 L	0.33 GH	0.61 EF	2.33 BCD	1.20 NO	0.73 CD	2.73 J	1.67 EFG
16	33.3% Vermiculite+33.3% Perlite + 33.3% Peat moss	90.00 C	0.57 BC	0.72 A	3.00 AB	3.67 CDE	0.53 GHIJ	4.43 BC	3.00 ABC
17	33.3% Vermiculite+33.3% Perlite + 33.3% Sand	95.33 B	0.53 CD	0.65 CDE	3.00 AB	3.77 CD	0.60 EFGH	4.60 AB	3.33 AB
18	33.3% Vermiculte+33.3% Perlite + 33.3% Loam	38.00 O	0.33 GH	0.55 GH	1.67 DE	0.60 Q	0.67 CDEF	2.43 K	1.33 FG
19	33.3% Vermiculte+33.3% Peat moss+ 33.3% Sand	44.67 N	0.37 FG	0.56 GH	2.00 CDE	0.67 PQ	0.47 IJKL	2.70 J	1.67 EFG
20	33.3% Vermiculte+33.3% Peat moss+ 33.3% Loam	75.67 F	0.43 EF	0.68 ABC	2.33 BCD	3.27 FGH	0.63 DEFG	3.27 GH	2.33 CDE
21	33.3% Vermiculte+33.3% Sand+ 33.3% Loam	63.67 J	0.37 FG	0.63 DEF	2.00 CDE	2.17 M	0.33 M	3.13 HI	2.00 DEF
22	33.3% Perlite + 33.3% Peat moss + 33.3% Sand	39.67 O	0.27 H	0.55 GH	1.67 DE	0.57 Q	0.37 LM	2.40 K	1.67 EFG
23	33.3% Perlite + 33.3% Peat moss + 33.3% Loam	95.33 B	0.53 CD	0.66 CDE	3.00 AB	3.97 BC	0.67 CDEF	4.43 BC	3.00 ABC
24	33.3% Perlite + 33.3% Sand + 33.3% Loam	68.33 H	0.37 FG	0.63 DEF	2.00 CDE	2.93 IJK	0.47 IJKL	3.13 HI	2.33 CDE
25	33.3% Peat moss + 33.3% Sand + 33.3% Loam	66.00 I	0.37 FG	0.63 DEF	2.33 BCD	2.27 M	0.47 IJKL	3.17 HI	2.00 DEF
26	25% Vermiculite + 25% Perlite + 25% Peat moss + 25% Sand	90.00 C	0.57 BC	0.71 AB	3.00 AB	3.43 EFG	0.57 FGHI	4.37 CD	3.00 ABC
27	25% Vermiculite + 25% Perlite + 25% Peat moss + 25% Loam	75.00 FG	0.37 FG	0.65 CDE	2.33 BCD	3.17 GHI	0.50 HIJK	3.47 F	2.33 CDE
28	25% Vermiculite + 25% Perlite + 25% Sand + 25% Loam	95.33 B	0.43 EF	0.68 ABC	2.67 ABC	2.77 KL	0.37 LM	4.07 E	2.67 BCD
29	25% Vermiculite + 25% Peat moss+ 25% Sand + 25% Loam	68.33 H	0.47 DE	0.71 AB	3.00 AB	2.77 KL	0.47 IJKL	4.23 DE	3.00 ABC
30	25% Perlite + 25% Peat moss + 25% Sand + 25% Loam	66.00 I	0.37 FG	0.66 CDE	2.33 BCD	2.80 JK	0.43 JKLM	3.27 GH	2.33 CDE
31	20% Vermiculite+20% Perlite+20% Peat moss+20% Sand+20% Loam	90.00 C	0.33 GH	0.61 EF	2.00 CDE	2.37 M	0.40 KLM	3.27 GH	2.00 DEF

Means of different agricultural media treatments followed with the same letter within each column are not significantly different from each other at 1% level.

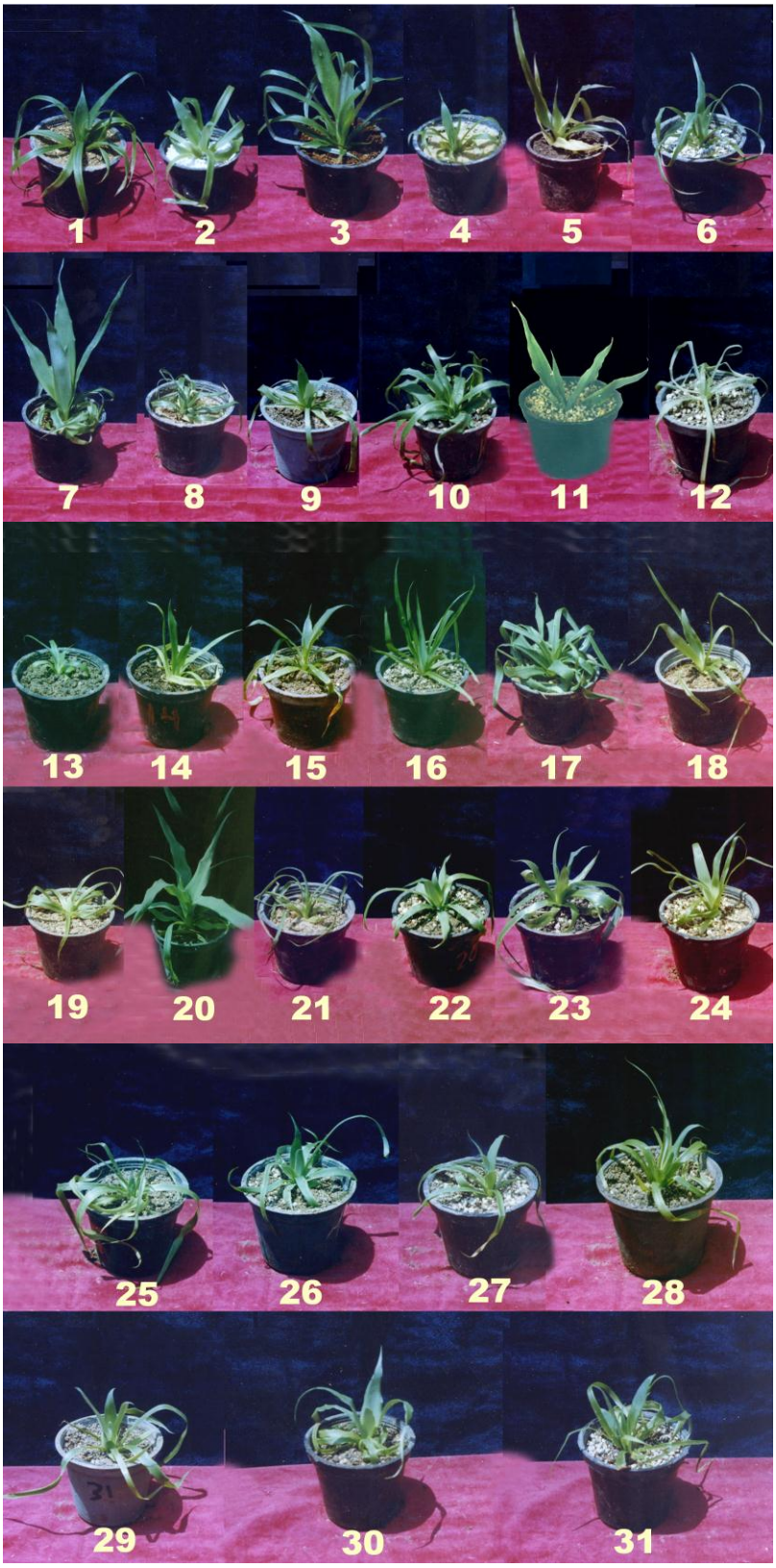


Photo (1): Effect of different agricultural media on growth of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

IV.1.1.b. Effect of microclimate space:

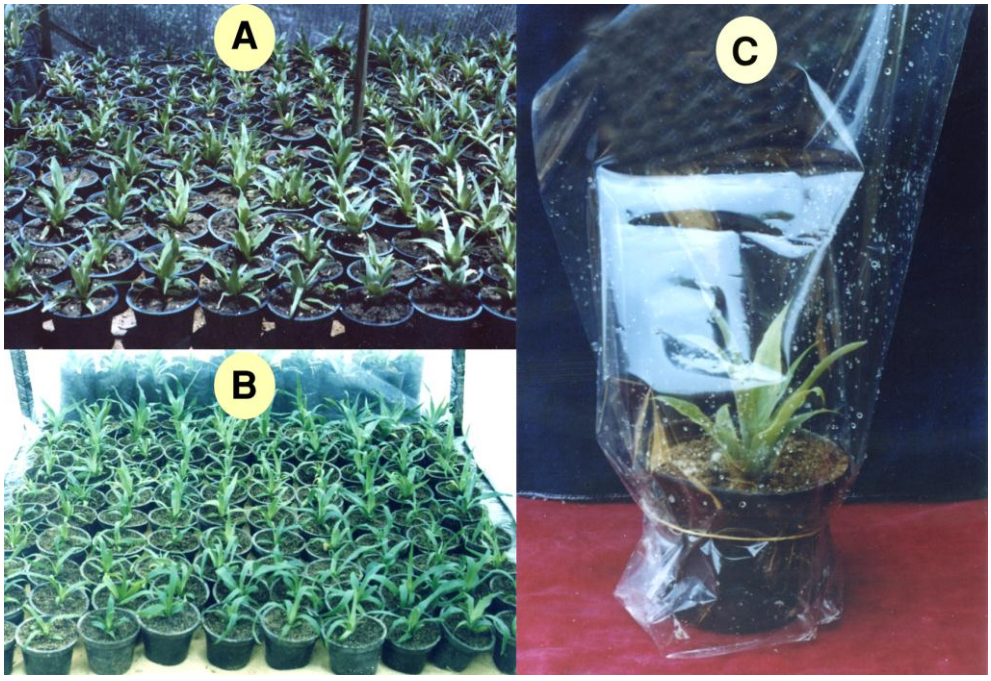
Table (3) and **Photo (2)** show the effect of different microclimatic space on survival percentage and growth parameters of *in vitro* acclimatized pineapple plants. It is quite evident that survival percentage and shoot length were significantly increased when either plastic cage or plastic bag was used in comparison with greenhouse. On the other hand, statistical differences were lacking when shoot thickness, number of leaves, root length and number of roots parameters were considered. However, leaf width was significantly increased when plastic cage was used followed by plastic bag in comparison with greenhouse. Meanwhile, plastic bag was more effective in increasing leaf length as compared with both plastic bags and greenhouse.

The aforementioned results showed the importance of plastic cage or plastic bag in improving most used parameters under study. These may be due to that both plastic cage or bag had smaller climate space compared with greenhouse. Consequently, high relative humidity was accumulated in plastic bags and cage which resulted in maximizing acclimatization parameters. These results go in line with the finding of **Short *et al.* (1987).** They concluded that optimum hardening of cauliflower and chrysanthemum occurred when plantlets were cultured at 80% relative humidity. In addition, **Hazarika (2003)** found that plants under lower relative humidity have fewer transpiration and translocation problems *ex vitro*, and persistent leaves look like normal ones.

Table (3): Effect of different microclimate spaces on survival percentage, growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

Parameters / Microclimatic conditions	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Greenhouse	77.00 B	0.83 B	0.69 A	3.67 A	6.60 B	0.77 C	4.63 A	3.33 A
Plastic cage	93.00 A	1.07 A	0.74 A	4.33 A	6.73 B	1.30 A	4.70 A	4.00 A
Plastic bags	97.00 A	1.23 A	0.71 A	4.67 A	7.17 A	0.93 B	4.77 A	4.00 A

Means of different microclimate spaces treatments followed with the same letter within each column are not significantly different from each other at 1% level.



A: greenhouse B: Plastic cage C: Plastic bags

Photo (2): Effect of different microclimate spaces on survival percentage, growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

IV.1.1.c. Effect of pot size:

Table (4) and **Photo (3)** show the effect of different pot sizes on survival percentage and growth parameters of *in vitro* acclimatized pineapple plants. It is quite evident that using of large pot size (11 cm) succeeded significantly in maximizing the percentage of survival percentage, shoot thickness, leaf length, leaf width and root length in comparison with the other pot sizes. Similarly, pot size 9 cm was significantly increased shoot length, shoot thickness, number of leaves / plant and root length parameters in comparison with either pot size 10 or 6 cm. On the other hand, different pot sizes failed to induce any statistical differences when number of roots parameter was considered.

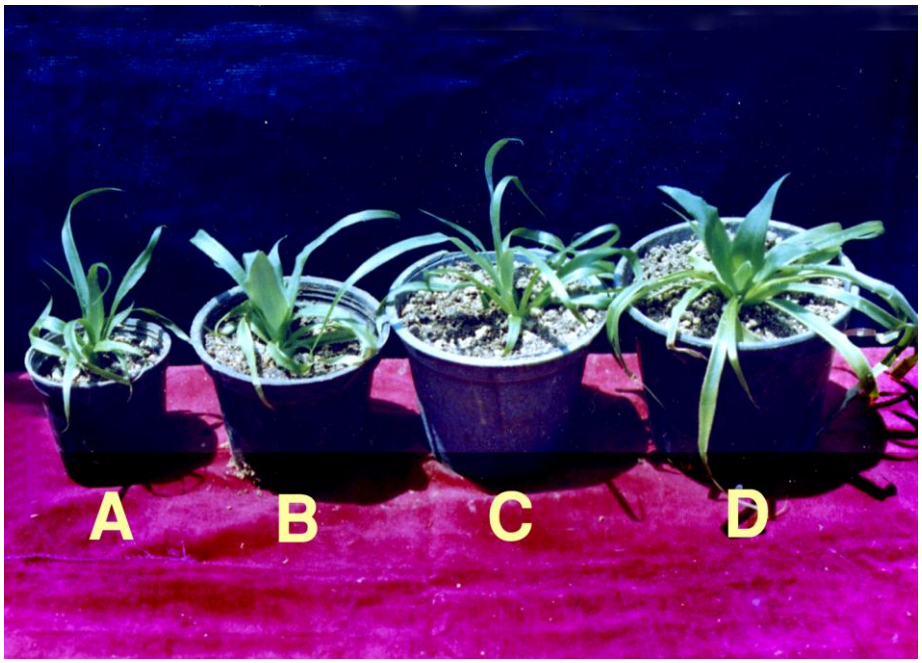
Table (4): Effect of different pot sizes on survival percentage, growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

Parameters / Pot size	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
11 cm	97.00 A	1.50 A	0.84 A	5.67 A	7.43 A	1.43 A	5.07 A	4.33 A
10 cm	83.33 D	0.90 C	0.74 C	4.33 B	6.40 C	0.77 D	4.77 B	4.00 A
9 cm	95.00 B	1.33 A	0.81AB	5.33 A	6.80 B	1.23 B	4.93AB	4.33A
6 cm	89.67 C	1.13 B	0.77BC	4.67 B	6.60BC	1.07 C	4.87 B	4.00 A

Means of different pot sizes followed with the same letter within each column are not significantly different from each other at 1% level.

Generally, the above results can be summarized that the largest pot size (11 cm) induced the highest percentage of survival and growth parameters under study. These results partially agreed with the findings of

Folliot and Marchal (1991). They found that the increase in plants weight of pineapple cv. Cayenne lisse which propagated *in vitro* and grown in pots during acclimatization resulted from using of larger pots which induced good root development.



A= 6cm B=9cm C=10cm D=11cm

Photo (3): Effect of different pot sizes on growth of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

IV.1.1.d. Effect of growth retardants:

Data of **Table (5)** and **Photo (4)** show the effect of different growth retardants with different concentrations on growth parameters of *in vitro* acclimatized pineapple plants. It is clear from **Table (5-A)** that Cycocel (CCC) treatment had significantly increased survival percentage, shoot length, leaf width, root length and number of roots in comparison with paclobutrazol. Similar results was achieved by using of coumarin as it significantly increased shoot length, leaf length, and leaf width in

relation to PP_{333}. However, shoot thickness was significantly increased as PP_{333} was concerned. On the other hand, number of leaves was not statistically affected by different growth retardants. Moreover, **Table (5-B)** show that control and lower concentration of growth retardant (0.125 mg/L) had significantly improved both shoot length and leaf length as compared with higher concentrates (0.25 and 0.50 mg/L). Meanwhile, using of higher concentration (0.50 mg/L) was statistically maximized survival percentage, shoot thickness, leaf width, root length and number of roots in relation to control. However, root length was significantly increased when 0.125 and 0.50 mg/L concentrations were used in comparison with (0.0 and 0.25 mg/L) concentrations. The interaction between growth retardants and concentrations is tabulated in **Table (5-C)** and **Fig. (2)** indicate that 0.50 mg/L of cycocel was significantly effective in increasing survival percentages, leaf width, root length and number of roots in comparison with the other interactions. However, 0.50 mg/L PP_{333} concentration recorded the highest significant values of shoot thickness in relation to the others. Besides, application of either 0.125 or 0.50 mg/L of coumarin or 0.125 cycocel were significantly increased leaf length. On the other hand, different interactions between growth retardants and concentrations failed to induce any statistical differences when shoot length and number of leaves were concerned.

Firstly, pineapple plantlet roots treated under greenhouse conditions with coumarin showed elongation in root tissues increased with concentration increasing, had large cortex, lacked fibers and small xylem and phloem, small pith. Roots of plantlets treated with cycocyl (CCC) showed reduction in elongation increased with concentration increasing, cortex consists of two large layers separated by large ring of sclerenchymatous tissues (fibers), extensive xylem and phloem.

Meanwhile, roots of plantlets treated with paclobutrazol (PP_{333}) showed increasing in diameter with concentration increased, had a two layer cortex with collenchyma, lacked to fibers, had a suitable viscular bundle surrounded with clear healthy pericycle. On the other hand, roots of plantlets previously treated with 0.50 mg/L PP_{333} treated again with 100, 200, 300 and 400 ppm of gibberellins (GA_3) increased root-hairs, cortex size, root diameter, viscular bundle with the concentration increasing, fibers of sclerenchymatous tissues were clear and intensive at 100 and 200 ppm concentrations, but less or no fibers at 300 or 400 ppm concentrations.

The present work was supported with many investigation dealing the effect of plant growth retardants on the anatomical differences in several plant species as follows:

Itoh (1976) determined how roots became thicker, the length and width of cells of the cortex and pith. The number of layers in the cortex and pith, which were about 1 cm from the apical meristem, was determined in maize roots. The number of cell layers forming the cortex and pith was similar in both treated and control roots. Although the light of pith cells was comparable in all tested solutions, there was a difference in the length of the cortical cells, depending on the compound. Cells in maize roots treated with coumarin became shorter and wider compared to the control. In contrast to cells of the cortex, the length of the cells in the pith did not change in response to the coumarins. Since the root given coumarin were much shorter than the controls, cells of the pith must divide with only half the frequency of those in the cortex, suggesting a selective influence of these compounds on different tissues.

Bausher and Yelenosky (1987) observed that progressively higher concentrations of paclobutrazol-markedly induced significant changes in the morphology, growth, and development of roots of Valencia seedlings

and rough lemon (*C. limon* (L.) Burm. F.) leaves. Initial change was readily evident in reduced lateral and fibrous root development at the lower concentrations (10^3 ppm). Higher concentrations (10^5 ppm) resulted in no secondary root formation and progressive basal enlargement terminating in a bulbouslike apex of the primary root.

Kupidlowska *et al.* (1994) found that roots of cucumber and maize treated with coumarin increased markedly in diameter above the apical meristem, whereas those of pea treated with coumarin became slightly thickness. coumarin inhibited the elongation of roots in developing seedlings of cucumber and maize, but only slightly retarded it in pea. stem of etiolated pine seedlings reacted in a similar way when treated with coumarin. Our observations are similar to those on plants treated with antigibberellins- for instance, when cells of lettuce and garden pea were treated with CCC and ancymidol, root growth was inhibited and the diameter of cortex cells increased.

Abenavoli *et al.* (2001) reported the effects of coumarin, an allelopathic compound, on root anatomy and growth, nitrate uptake and translocation to the shoot, as well as respiration in *Triticum durum* (cv. Simeto) seedlings. Coumarin, in the range 25 µM–1 mM, decreased the relative growth rate of roots and increased the area of the root vessels. Within this concentration range, coumarin alone did not significantly affect net nitrate uptake. In seedlings exposed simultaneously to 100 µM coumarin and to 50 µM nitrate, the net nitrate uptake was significantly stimulated. In the presence of nitrate, even the lowest coumarin concentration tested significantly stimulated nitrate translocation from the root to the shoot.

Abenavoli *et al.* (2004) investigated the effects of coumarin on the length, diameter, and branching density of different root types in maize seedlings (*Zea mays* L. cv. Cecilia). Maize seedlings were grown in a

hydroponic culture for 6 days, and then coumarin (at concentrations of 0, 25, 100, and 400 µM) was added to the nutrient solution. Coumarin inhibited root length, but effects differed depending on the root type.

Steffens *et al.* (2006) demonstrated that gibberellin (GA) is ineffective on its own but acts in a synergistic manner together with ethylene to promote the number of penetrating roots and the growth rate of emerged roots of rice. Studies with the GA biosynthesis inhibitor paclobutrazol revealed that root emergence was dependent on GA activity. Abscisic acid (ABA) acted as a competitive inhibitor of GA activity.

Second, pineapple plantlet stems treated under greenhouse conditions with coumarin encouraged the growth and increased elongation cells with the concentration increase, epidermis covered with thickness cuticle, two layer parenchyma cortex, suitable viscular bundle. Stems of plantlets treated with cycocyl (CCC) showed decrease in the cell elongation with the concentration increase, large cortex layer, epidermis with thin cuticle, clear small viscular bundles. Meanwhile, stem of plantlets treated with paclobutrazol (PP_{333}) showed decrease in the cell elongation and xylem size with the concentration increase, medium cortex. On the other hand, stems of plantlets previously treated with 0.50 mg/L PP_{333} treated again with 100, 200, 300 and 400 ppm of gibberellins (GA_3) enhanced the cuticle thickness, increased cortex size, viscular bundle, plastid size, with the concentration increasing.

Anatomical differences between plantlets treated with different doses of plant growth retardants to enhance the plant productivity was reported such as:

Baldev and Lang (1965) stated that the growth retardants CCC inhibited flower formation and stem elongation in *Samolus parviflorus*, a long-day rosette plant, under inductive conditions.

Bostrack and Struckmeyer (1967) reported that gibberellic acid at 500 mg/l caused less elongation of the sub-apical region than at 50 mg/l and 100 mg/l in *S. splendens*. Treated plants had a smaller stem diameter and more xylem parenchyma.

Biddington (1986) found that the uses in the field of the growth promoter gibberellin or the growth retardant chlormequat chloride (CCC) appear to be increased stem diameter, epinasty or a change in sex expression.

Bañón *et al.* (2003) the ability of paclobutrazol (PBZ) and ethephon (ETH) to control the growth and development of the aerial part of this plant was investigated. PBZ (0.2, 0.3, 0.4 and 0.5 mg/pot) and ETH (25, 50, 75 and 100 mg/pot) doses were applied as a single soil drench 25 days after transplantation into 11 cm plastic pots. The most effective treatments were 0.5 mg/plant for PBZ and 100 mg/plant for ETH, which reduced plant height by 73.1% and 50.3%, respectively. PBZ (0.2 mg) and ETH (25 mg) significantly reduced plant width, aerial part dry weight, number of flowering, stems and number of inflorescences per plant. The results suggested that both PBZ and ETH could well be used to control the stem height of *Reichardia tingitana* and improve its ornamental value.

Third, pineapple plantlet leaves treated under greenhouse conditions with coumarin encouraged the growth and increased elongation cells with the concentration increase, epidermis covered with thickness cuticle, Spongy mesophyll depth was greater than palisade mesophyll, had little plastid, increase viscular bundle. Leaves of plantlets treated with cycocyl (CCC) showed increase in the cell elongation with the concentration increase, epidermis covered with thin cuticle, Spongy mesophyll depth greater than palisade mesophyll, had little plastid, clear small viscular bundles. Meanwhile, leaves of plantlets treated with paclobutrazol (PP_{333})

showed decrease in the cell elongation and xylem size with the concentration increase, epidermis covered with clear cuticle layer, Spongy mesophyll depth greater than palisade mesophyll, had intensive plastid. On the other hand, leaves of plantlets previously treated with 0.50 mg/L PP_{333} treated again with 100, 200, 300 and 400 ppm of gibberellins (GA_3) enhanced the cuticle thickness, viscular bundle, plastid size, with the concentration increasing, Spongy mesophyll depth seems the approximately equal with palisade mesophyll, all concentrations induced hypertrophy in the abaxial leaf side only which showed crinkle.

Leaves of *ex vitro* pineapple plantlets treated with aforementioned plant growth retardants was subjected to light microscopic examination and results of many works recorded as follows:

Bostrack and Struckmeyer (1967) reported that development of leaves and stipules which were not significantly affected by foliar applications of a 50 mg/liter aqueous solution of gibberellic acid (GA). Reduction of cell diameter resulted in a reduction in diameter of stems in GA-treated plants. Cell elongation was a major factor in the rapid extension of axillary shoots of treated plants.

Inamdar and Gangadhara (1975) recorded that gibberellic acid, though a growth promoter, acted as an inhibitor at high concentration. Coumarin at 50 ppm reduced the size of guard cells and the stomatal frequency, and commonly induced persistent stomatal initials, while at 100 ppm only the radicle emerged and the cotyledons failed to emerge from the seed-coat.

Mohamed *et al.* (1991) reported that plant growth regulators applied *in vitro* affected strawberry plant performance *ex vitro* for a period of up to 4 months. Benzyl-adenine and gibberellin enhanced juvenile characteristics; in general, more runners and monofoliolate leaves were produced. In contrast, abscisic acid and a gibberellin biosynthesis

inhibitor, paclobutrazol, resulted in a more adult phenotype; specifically, flowering was earlier, net photosynthesis and leaf diffusive resistance rates were equal to those of adult plants, and fewer leaves were monofoliolate.

Burrows *et al.* (1992) treated *Chrysanthemum* cv. Lillian Hoek plants with a paclobutrazol (PBZ) and histologically examined after 3 months. PBZ application resulted in thicker leaves, reduced stem diameter, and roots with an increased diameter. Spongy mesophyll depth was also greater and the individual cells were more rounded and the volume of intercellular space was reduced. The narrower stems had an increased development of secondary xylem, but had a marked reduction in the number of sclerenchyma bundle caps. Increased root diameter was due to an increase in the number of rows and diameter of cortical cells. In PBZ-treated plants, root cortical cell length was 50–70% less than in untreated plants.

Tabuchi *et al.* (2005) anatomical study of leaves with GA_3 and GA_3+BA treatments, palisade and spongy tissue became compact and starch grains and chloroplasts increased as flower development proceeded. As with BA, leaf epidermal cells (both adaxial and abaxial) thicken and breakdown. Tannins or lignin deposition was observed in the palisade and spongy tissues. Our observations suggest an association between the localized accumulation of condensed tannins or lignin and appearance of the dark-stained spots that precede the necrotic lesions in *Gloriosa* leaves following BA treatment.

The above mentioned results could be summarized that CCC at higher concentration (0.50 mg/L) surpassed paclobutrazol and coumarin in improving root length, number of roots and leaf width.

Table (5): Effect of different growth retardants with different concentrations on survival percentage growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

Table (5-A): Effect of growth retardant.

Parameters / Growth retardants	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Paclobutrazol	72 B	1.63 B	1.10 A	7.17 A	5.96 C	1.48 B	8.61 B	11.58 B
Coumarin	65 C	1.82 A	0.94 C	7.33 A	7.80 A	1.54 A	6.86 C	9.67 C
Cycocel	80 A	1.79 A	0.98 B	7.67 A	7.32 B	1.58 A	10.86 A	13.92 A

Means of different growth retardants treatments followed with the same letter within each column are not significantly different from each other at 1% level.

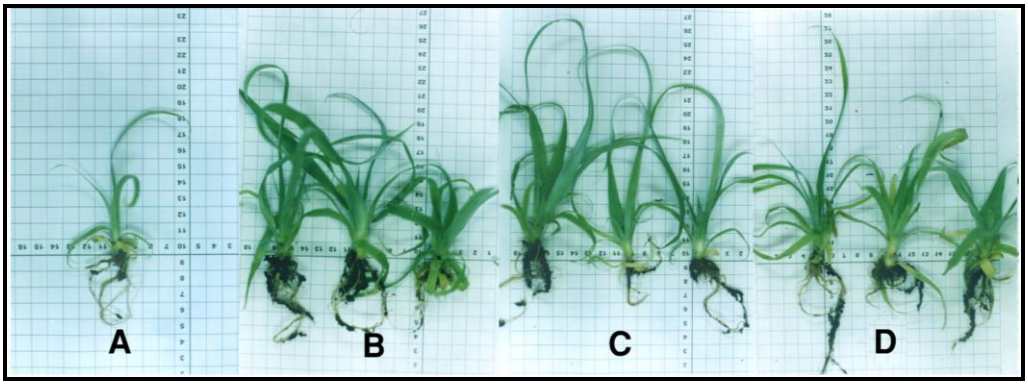
Table (5-B): Effect of concentration.

Parameters / Concentration (mg/L)	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	65 D	1.93 A	0.85 D	8.67 A	7.60 A	1.40 D	5.10 C	4.33 D
0.125	80 B	1.93 A	0.91 C	7.56 B	7.82 A	1.48 C	10.44 A	10.00 C
0.25	72 C	1.60 B	1.06 B	6.89 C	6.34 B	1.58 B	8.61 B	14.11 B
0.50	85 A	1.52 B	1.21 A	6.44 C	6.33 B	1.68 A	10.94 A	18.44 A

Means of different growth retardants treatments followed with the same letter within each column are not significantly different from each other at 1% level.

tab5c

fig2



A = Control

B = 0.125 mg/L (CCC, Coumarin, PP_{333})

C = 0.250 mg/L (CCC, Coumarin, PP_{333})

D = 0.500 mg/L (CCC, Coumarin, PP_{333})

Photo (4): Effect of different growth retardants with different concentrations on survival percentage growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

As known, plant growth retardants are applied in agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering plant productivity. Most growth retardants act by inhibiting gibberellin (GA) biosynthesis (**Rademacher, 2000**). In this study, some plant growth retardants were used to enhancement pineapple growth in *ex vitro* conditions after transfer from *in vitro* conditions. So, comparative anatomical studies *via* light microscopic examinations were made to investigate the effect of coumarin, cycocel (CCC) and paclobutrazol (PP_{333}) with different concentrations (*i.e.* 0.125, 0.25 and 0.50 mg/L) on the anatomical characteristics of pineapple plantlets. In addition, treated plantlets which received 0.50 mg/L PP_{333} (as control) with of 100, 200, 300, and 400 ppm of gibberellins (GA_3) was also histological studied.

These results agreed partially with the findings of **Hazarika *et al.* (2001).** They reported that preconditioning citrus microshoots with

paclobutrazol influence higher *ex vitro* survival by intensifying internode length, thickening of root and reducing leaf dehydration by regulating the stomatal function and increasing epicuticular wax per unit area of leaf, besides more chlorophyll synthesis.

The abovementioned results showed the superiority of coumarin and cycocel at low concentrations (0.125 or 0.50 mg/L) in enhancing most parameters under study. This may be due to that coumarin and cycocel have less adverse effect in retarding growth parameters compared with paclobutrazol. These results are in harmony with the findings of **Dalziel and Lawrence (1984).** They found that paclobutrazol is active as a growth retardant in a broad spectrum of species. Also, **Grosser and Chandler (1986).** They reported that adding 90-150 mM coumarin to the culture medium of Swingle citrumelo citrus plantlets enhanced root formation and increased the number of whole plants recovered per up to 5 fold.

IV.1.2.e. Effect of gibberellic acid (GA_3):

Table (6), Fig. (2) and **Photo (5)** show that higher concentration of GA_3 (400 ppm) had significantly improved most parameters under study since it significantly increased shoot length, shoot thickness, leaf length, leaf width, root length and number of roots as compared with the lower levels (0.0 and 100 ppm). However, number of leaves was significantly increased when either 300 or 400 ppm was used in comparison with lower concentrations i.e. 0.0 and 100 ppm.

Table (6): Effect of different concentrations of GA_3 on growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets treated with paclobutrazol (PP_{333}).

Parameters / Conc. of GA_3 (ppm)	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
0.0	1.37 E	1.44 D	6.33 C	8.67 E	1.90 D	22.83D	16.67CD
100	3.93 D	1.49 CD	7.33 C	13.67 D	2.10 C	16.00 E	15.33 D
200	6.67 C	1.52 C	13.33 B	17.33 C	2.43 B	33.83 C	18.33 C
300	8.77 B	1.58 B	16.00 A	23.50 B	2.60 AB	39.67 B	22.33 B
400	11.50 A	1.65 A	17.33 A	30.67 A	2.77 A	44.67 A	28.33 A

Means of different GA_3 concentrations treatments followed with the same letter within each column are not significantly different from each other at 1% level.

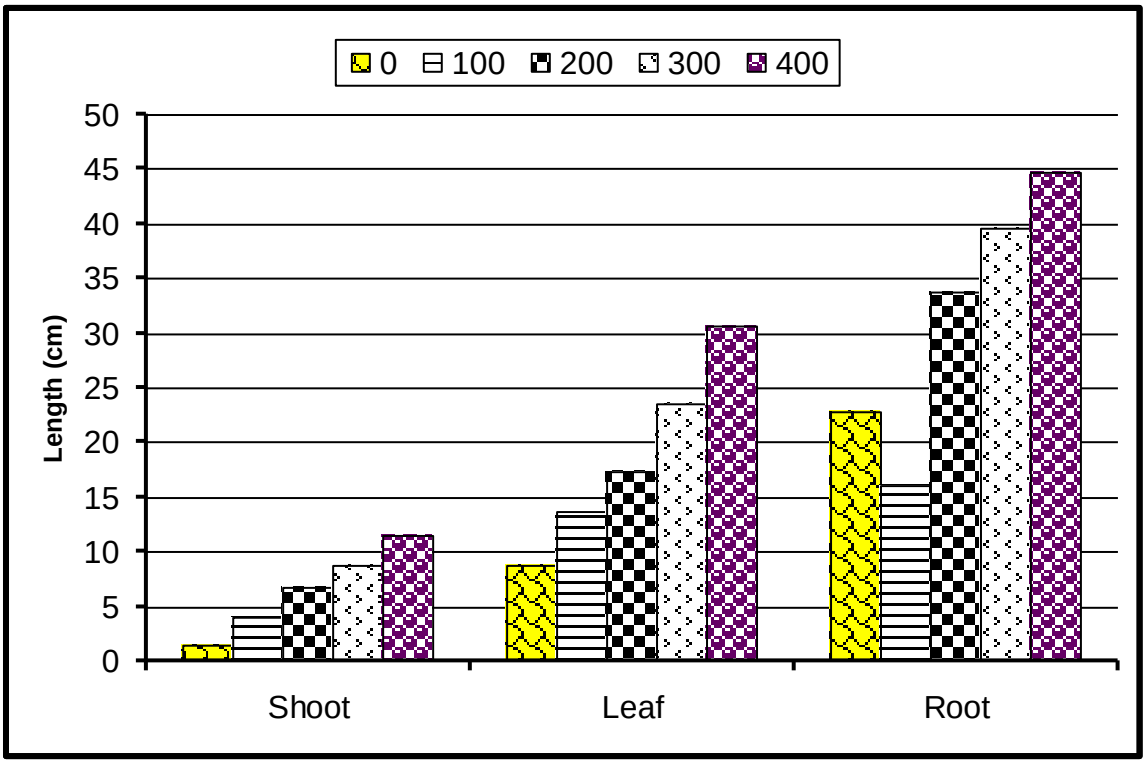


Fig. (3): Effect of different concentrations of GA_3 on growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets treated with paclobutrazol (PP_{333}).

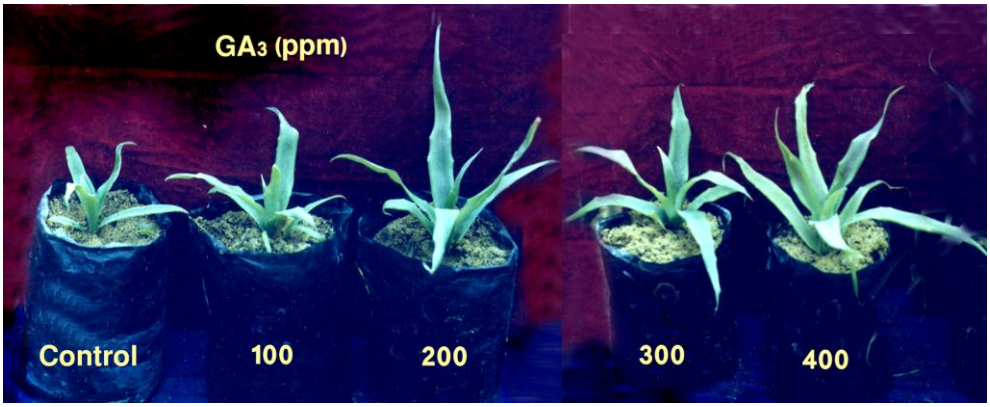


Photo (5): Effect of different concentrations of GA_3 on growth of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets treated with paclobutrazol (PP_{333}).

The aforementioned results concluded that GA_3 at the rate of (400 ppm) is the most suitable concentration for improving all parameters under study. These results are in agreement with the findings of **Yanes-Paz *et al.* (2001).** They found that using of GA_3 at 100 mg/L level twice a month increased the growth of *in vitro* pineapple cv. Smooth cayenne plants during the acclimatization.

IV.1.3. Changes occurred during different acclimatization phases:

It is clear from **Photo (6)** that different changes appeared specifically unacclimatized *in vitro* plantlets (number 1) as compared with those in different phases of acclimatization. The changes were noticed in leaf thickness and length as they increased greatly by acclimatization as well as the roots which changed to a real root with hairs and increased progressively by increasing adaptation phases.

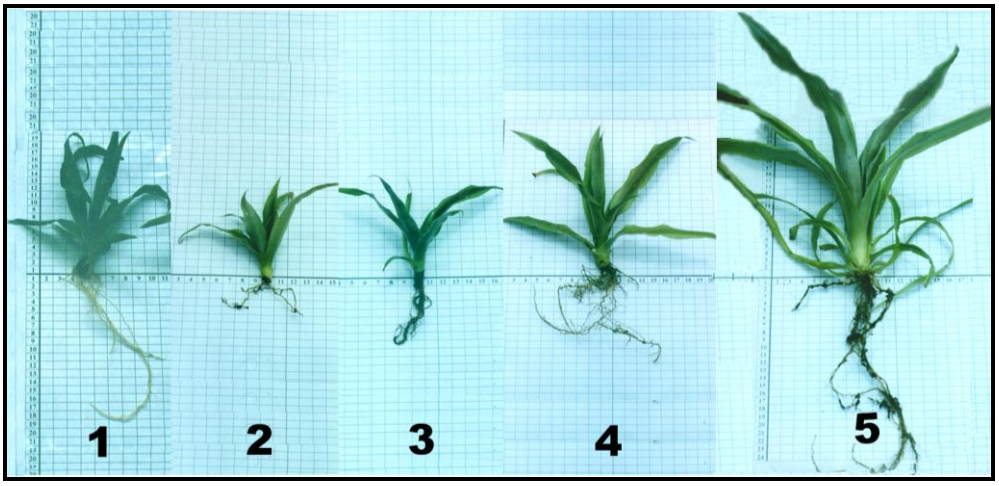


Photo (6): Changes occurred during different acclimatization phases of pineapple plants.

1- *In vitro* unacclimatized plantlet.

2- Laboratory acclimatized plant.

3- Starting phase of greenhouse acclimatization.

4- Developing phase of green house.

5- Fully acclimatized plant.

In the same time, the cytology reflect the changes occurred in anatomy of the plants. Concerning the roots *in vitro* of unacclimatization plantlet roots were smaller, exodermis had intensive root-hairs, cortex consists of two layers of parenchyma without fibers, cortex and collateral vascular bundles forming a ring around the pith, meanwhile, *ex vitro* plantlet roots were larger in size, exodermis had less intensive root-hairs, cortex consists of three layers, sclerenchymatous tissue mediated two layers of parenchymatous tissues, pericycle cells were larger, primary xylem were larger and surrounded by phloem and separated by zone of parenchyma cells, pith appears like star sclerenchymatous tissue **(Photo, 7).**

These findings were in agreement with those obtained by **Ballester *et al.* (1999).**

Pineapple stems of plantlet *in vitro* were smaller than those acclimated *ex vitro* one, epidermis had less cuticle thickness, one layer of parenchyma cortex, smaller viscular bundles emerged in the parenchymatous tissues, no pith, meanwhile, *ex vitro* plantlet stems were larger in diameter, epidermis had very thickened cuticle, cortex consists of large parenchyma cells, clearly large viscular bundles emerged in the parenchymatous tissues, no pith.

These results were harmony with those recorded with **Donnelly *et al.* (1985) and Apóstolo and Llorente (2000)**.

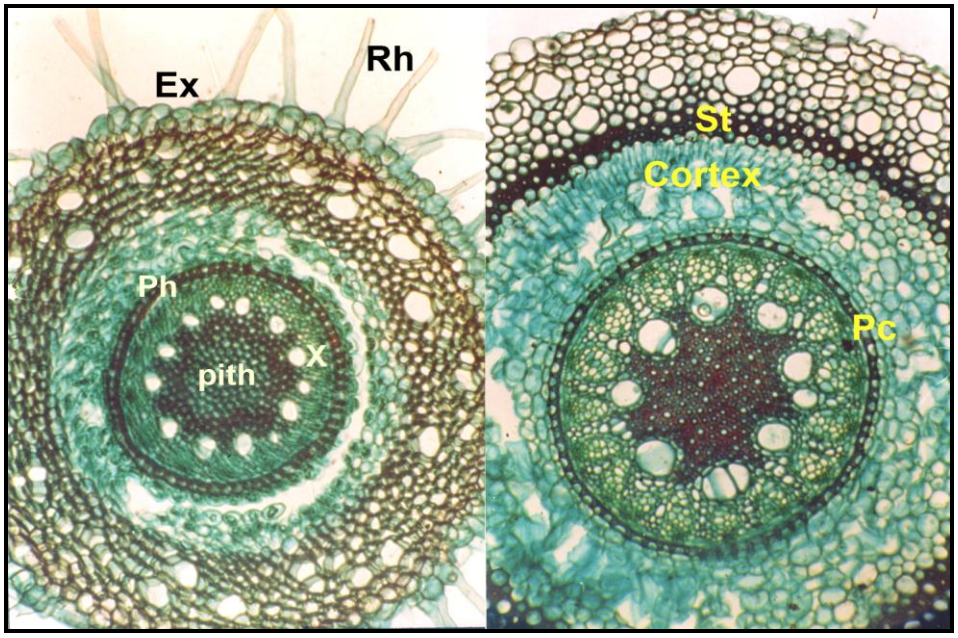


Photo (7): Cross section showed anatomical comparative between *in vitro* (left) and *ex vitro* (right) pineapple plantlet roots.

Ex: exodermis, **Rh:** root-hairs, **St:** sclerenchymatous tissues, **Pc:** pericycle, **X:** xylem, **Ph:** phloem.

Leaves formed *in vitro* were relatively thin and were characterized by poorly-formed palisade cells and large air spaces, meanwhile, those transferred to *ex vitro* (greenhouse) conditions showed distinct palisade and spongy tissues mesophyll and a high cell density. Acclimated *ex vitro*

leaves had a well developed cuticle unlike leaves from culture, developed vascular bundles **(Photo, 8)**.

These observations was confirmed with those obtained by **Hess and Sachs (1972)**, **Wetzstein and Sommer (1982), and Apóstolo *et al.* (2005).**

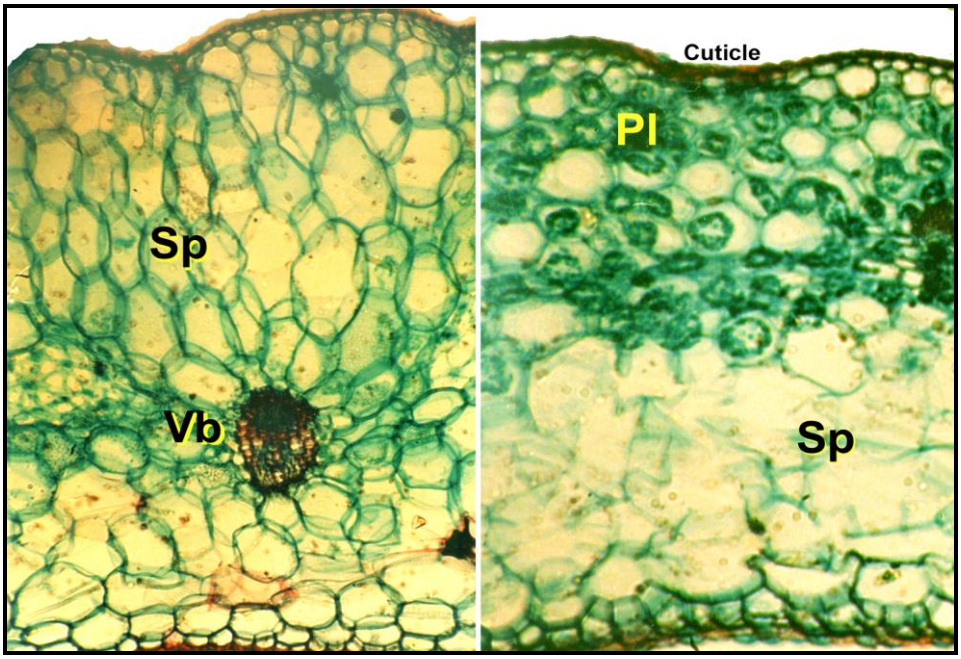


Photo (8): Cross section showed anatomical comparative between *in vitro* (left) and *ex vitro* (right) pineapple plantlet leaves.

Sp: spongy tissue, **Pl:** plaside tissue, **Vb:** viscular bundle

All aforementioned results came in the same line with data recorded by many investigators who compared the anatomical differences between plantlets or seedling cultured *in vitro* and transfer with *ex vitro* conditions for many plant species **(Donnelly *et al.*, 1985; Sutter and Dunston, 1986; Gilly *et al.*, 1997; Louro *et al.*, 1999; Pospíšilová *et al.* 1999; Barry-Etienne *et al.*, 2002 and Rohr *et al.*, 2003).**

IV.1.4. Nutritional studies:

IV.1.4.a. Effect of nutrient medium salts:

It is clear from **Table (7), Fig. (4)** and **Photo (9)** that using combination treatment of MS + B5 salts treatment was significantly succeeded in improving all growth and rooting parameters i.e. shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots. These followed by Gamborg (B_5) salts treatment in comparison with control and using of Murashige and Skoog salts only.

The above results summarized that combination treatment (MS + B5) induced the best improvement in all growth parameters. These results are somewhat partially agreed with the findings of **Garcia-Rodriguez *et al.* (1987).** They mentioned that, doubling the salts concentration of the basal medium increased the number of canary Island banana (*Musa* spp.) shoots but decreased number of roots. Meanwhile, **Vicentini *et al.* (1996)** treated *in vitro* banana (cv. Grand Naine) plantlets grown under greenhouse conditions, with the addition of 5.6, 11.1, 16.7 or 22.2 g mono ammonium phosphate (MAP) / plant at intervals of 8, 12 or 25 days after transplanting. It is appeared that, irrespective of application interval, the lowest rate of 5.6 g / plant increased plant height by 35.2%, dry weights of shoots and rhizomes by average of 158.9%.

Table (7): Effect of different medium salts on growth and rooting parameters of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

Parameters / Medium salts	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	1.53 E	1.17 F	7.33 E	5.27 E	1.83 D	15.50 F	21.00 E
Murashige & Skoog (MS)	1.57 E	1.90 EF	7.67 DE	7.53 D	1.87 D	15.83 EF	21.67 DE
White (Wh)	2.17 BC	1.28 BC	9.33 BC	9.43 C	2.00 BC	16.63BC	23.67ABC
Gamborg (B5)	2.30 B	1.31 AB	9.67 AB	10.50 B	2.10 AB	16.73 B	24.00 AB
MS + white	2.10BCD	1.25 CD	8.67BCD	9.00 C	1.93 CD	16.43BCD	23.67ABC
MS + B5	2.53 A	1.35 A	10.67 A	11.83 A	2.20 A	17.37 A	24.67 A
White + B5	1.90 D	1.22DEF	8.33CDE	7.53 D	1.87 D	16.03DE	22.67CD
MS + white + B5	1.97 CD	1.23CDE	8.67BCD	7.83 D	1.87 D	16.23CDE	23.33BC

Means of different medium salts followed with the same letter within each column are not significantly different from each other at 1% level.

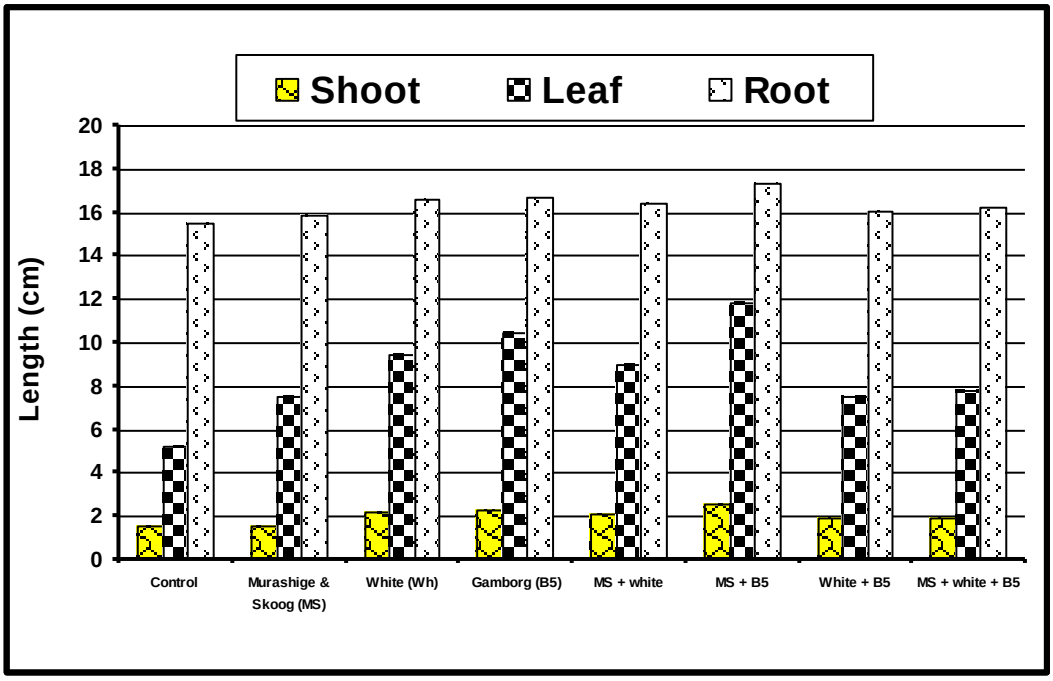
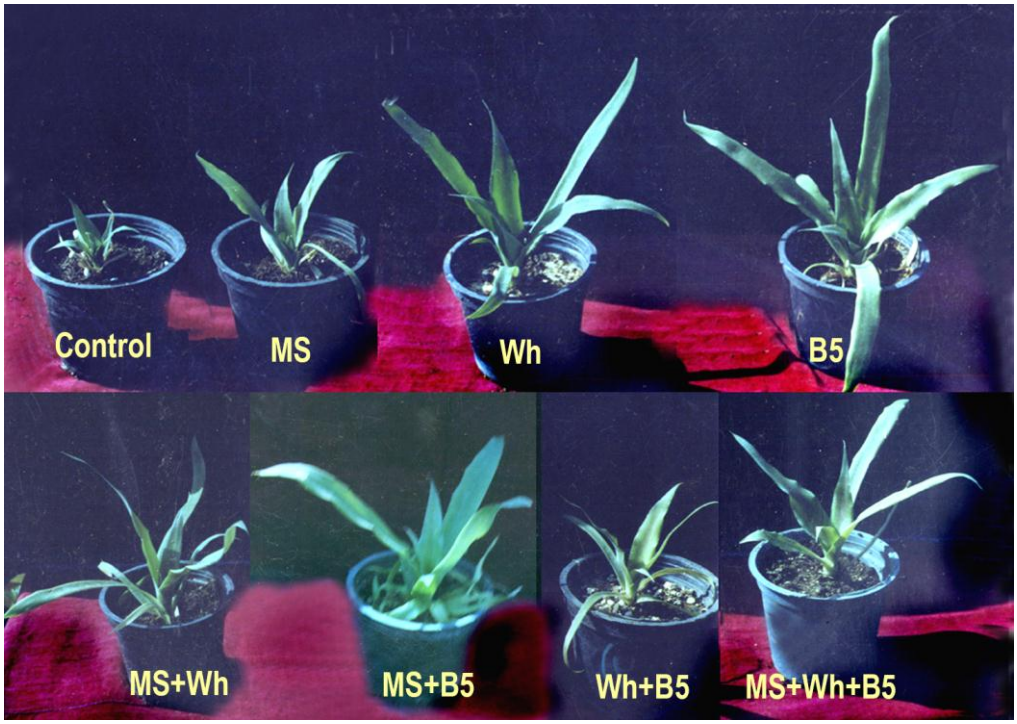


Fig. (4): Effect of different medium salts on growth of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.



MS= Murashige and Scoog Wh= White B5=Gamborg

Photo (9): Effect of different medium salts on growth of *in vitro* acclimatized pineapple cv. Smooth Cayenne plantlets.

IV.1.4.b. Effect of vitamins:

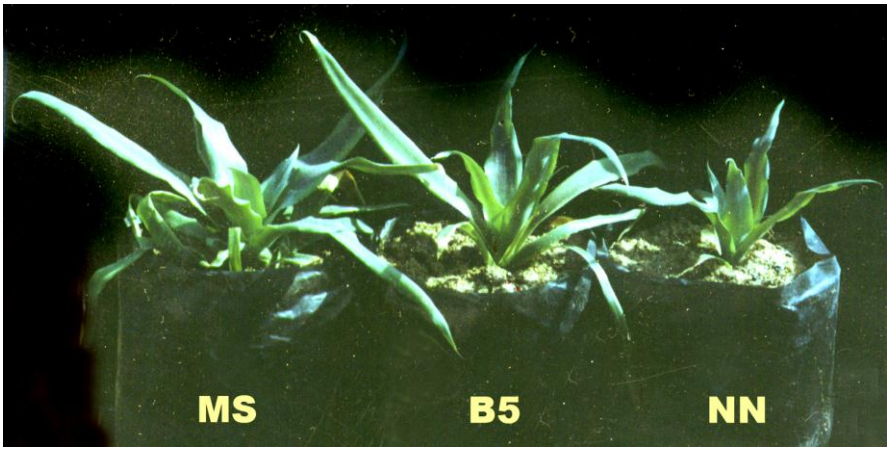
Data in **Table (8)** and **Photo (10)** showed that supplementation of MS medium with MS vitamins had significantly increased all growth and root parameters *i.e.* shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots followed by B5 vitamin then N & N vitamin as compared with the control in a descending order.

From the previous results it could be concluded that MS vitamins is important for growth and root parameters.

Table (8): Effect of different medium vitamins on growth and rooting parameters of acclimatized pineapple cv. Smooth Cayenne plantlets.

Parameters / Medium vitamins	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	2.63 B	1.36 B	11.33 C	12.27 C	2.17 B	17.53 B	24.67 B
Murashige & Skoog (MS)	3.100 A	1.44 A	14.33 A	13.63 A	2.43 A	19.50 A	26.00 A
Gamborg (B5)	2.87 AB	1.41 AB	12.67 B	13.03 B	2.33 AB	18.27 B	25.67 AB
Nitsch & Nitsch (NN)	2.73 B	1.38 B	11.67 BC	12.53 C	2.23 B	17.73 B	25.00 AB

Means of different medium vitamins followed with the same letter within each column are not significantly different from each other at 1% level.



MS= Murashige and Scoog B5=Gamborg NN= Nitsch and Nitsch

Photo (10): Effect of different medium vitamins on growth of acclimatized pineapple cv. Smooth Cayenne plantlets.

IV.1.4.c. Effect of organic additives:

The results of **Table (9), Fig. (5)** and **Photo (11)** explain the effect of different organic additives on growth parameters of acclimatized pineapple plants. It is quite evident that addition of yeast extract at the level of 2.0 g/L induced significant increase in all parameters under study then followed with adenine at rate 0.1 g/L, then yeast extract at 1.0 g/L in a descending order. Most parameters under study in comparison with others. However, coconut milk at different levels were less significantly effective in increasing different parameters under study. On the other hand, control treatments recorded the least significant values compared with different organic additives.

Table (9): Effect of different organic additives on growth and rooting parameters of acclimatized pineapple cv. Smooth Cayenne plants.

Parameters / Organic additives	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	3.07 E	1.51 F	14.33 D	13.77 F	2.43 D	19.77 F	26.33 E
Yeast							
0.5 g/L	3.33BC	1.57CDE	15.33 CD	14.27DE	2.50BCD	20.40CDE	27.33CDE
1.0 g/L	3.40 B	1.61 BC	17.33 AB	14.70 C	2.57AB	21.20 B	28.33ABC
2.0 g/L	3.43 AB	1.67 A	17.67 A	15.47 AB	2.63 A	22.03 A	29.00 A
Adenine							
0.025 g/L	3.37 B	1.56DEF	14.33 D	14.53CD	2.47CD	20.20DEF	27.00 DE
0.05 g/L	3.43 AB	1.60BCD	15.00 D	15.17 B	2.53BC	20.53 CD	27.67BCD
0.1 g/L	3.53 A	1.63 AB	15.33 CD	15.67 A	2.57AB	21.23 B	28.67 AB
Coconut milk							
2 %	3.20 D	1.52 F	14.67 D	14.00EF	2.43 D	20.03 EF	26.67 DE
4 %	3.23 CD	1.54 EF	16.33 BC	14.50CD	2.47 CD	20.40 DE	27.33CDE
8 %	3.33 BC	1.58BCDE	15.33 CD	15.17 B	2.53BC	20.77 BC	27.67BCD

Means of different organic additives followed with the same letter within each column are not significantly different from each other at 1% level.

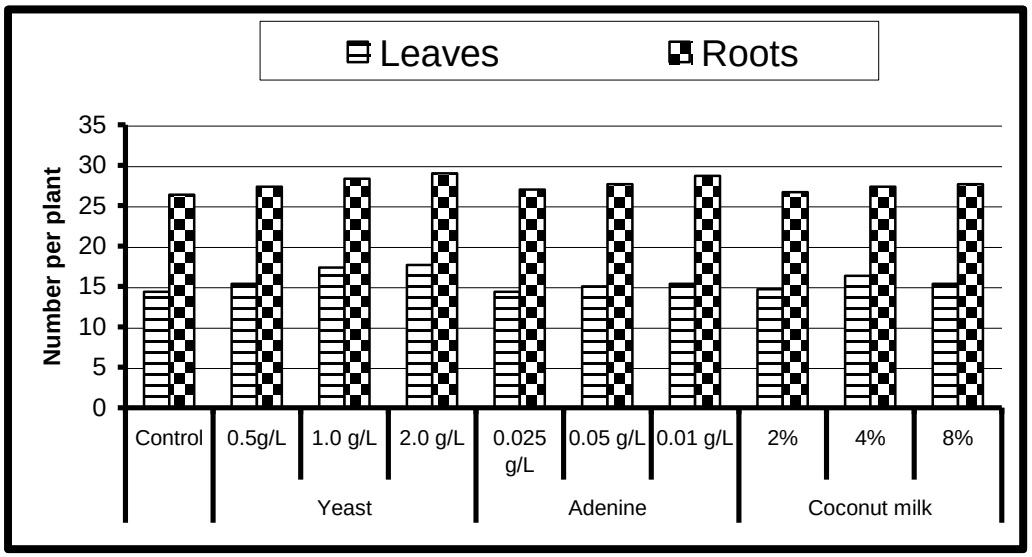


Fig. (5): Effect of different organic additives on number of leaves/ plant and root/plant of acclimatized pineapple cv. Smooth Cayenne plants.

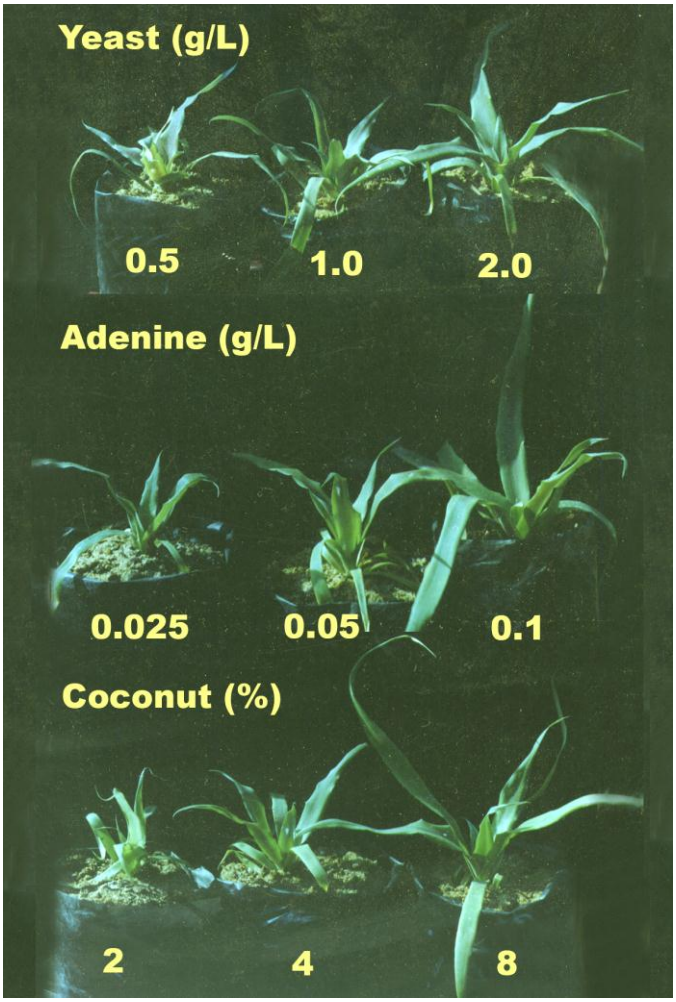


Photo (11): Effect of different organic additives on growth of acclimatized pineapple cv. Smooth Cayenne plants.

IV.1.4.d. Effect of organic additives combinations:

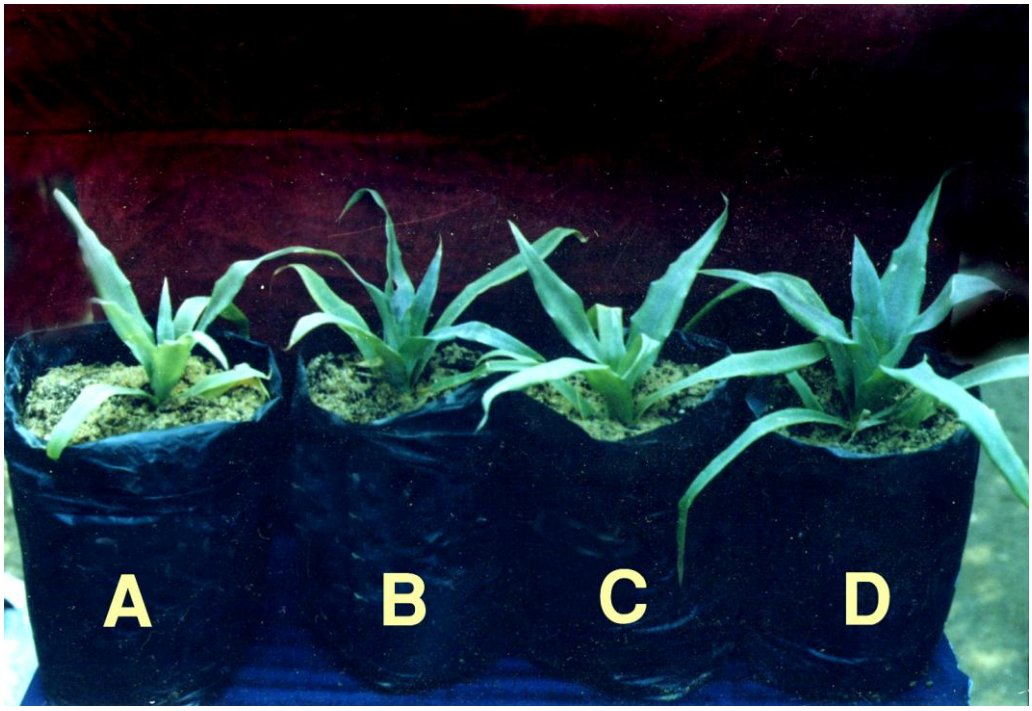
Table (10) and **Photo (12)** showed the effect of the best levels of organic additives combinations on growth parameters of acclimatized pineapple plants. It is appear that the combination of yeast + adenine sulphate + coconut milk (2 g/L+ 0.1 g/L + 8%) had significantly increased all growth and root parameters *i.e.* shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots followed with adenine sulphate + coconut milk (0.1 g/L + 8%) then yeast + coconut milk (2 g/L + 8%) then yeast + adenine sulphate (2 g/L + 0.1 g/L) as compared with the control in a descending order.

Table (10): Effect of the best levels of organic additives combinations on growth and rooting parameters of acclimatized pineapple cv. *Smooth Cayenne* plantlets.

Parameters / Organic additives	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	3.47 C	1.71 B	18.33 C	15.60 D	2.63 B	22.27 C	29.33 C
Yeast + adenine sulphate (2 g/L + 0.1 g/L)	3.60BC	1.73 B	18.67BC	15.77CD	2.63 B	22.77 C	29.67BC
Yeast + coconut milk (2 g/L + 8%)	3.70 B	1.76AB	19.33ABC	15.90 C	2.77AB	23.77 B	31.00 B
Adenine sulphate + coconut milk (0.1 g/L + 8%)	3.77 B	1.74 B	19.67AB	16.17 B	2.67 B	23.50 B	30.67BC
Yeast + adenine sulphate + coconut milk (2 g/L + 0.1 g/L + 8%)	4.00 A	1.81 A	20.33 A	16.37 A	2.87 A	25.03 A	33.67 A

Means of different organic additives combinations followed with the same letter within each column are not significantly different from each other at 1% level.

Thus it could be concluded that combination treatment of yeast + adenine sulphate + coconut milk (2 g/L + 0.1 g/L + 8%) is most superior for growth and root parameters.



A= Yeast + adenine sulphate (2 g/L + 0.1g/L)
B= Adenine sulphate + coconut milk (0.1g/L + 8%)
C= Yeast + Coconut milk (2 g/L + 8%)
D= Yeast + Adenine sulphate + Coconut milk (2 g/L + 0.1 g/L + 8%)

Photo (12): Effect of best levels of organic additives combinations on growth of acclimatized pineapple cv. Smooth Cayenne plantlets.

These results partially in harmony with the findings of **Kalpana *et al.* (2002)**. They found that using medium supplemented with a combination of 3% banana pulp (BP) (w/v) and 10% coconut water and shoot formation, while using 20% CW (v/v) independently promoted root formation along with velamen tissue of dendrobium.

IV.1.5. Flowering and Fruiting:

Table (11) reflect the effect of different flowering treatments on growth and flowering parameters of acclimatized pineapple Smooth Cayenne plants. It is appear from **Table (11-A)** and **Photo (13)** that flower length, flower width and number of old leaves were significantly increased by using Ethephon treatment as compared with the other used treatments. However, combination of Amino Vit and yeast extract induced significant increase in shoot length, shoot thickness and leaf length over the other used treatments. Meanwhile, number of new leaves was significantly increased when either Amino Vit., yeast extract, or combination treatment was used. Regarding the effect of different concentrations on growth and rooting parameters as shown in **Table (11-B).** It is obvious that using higher concentration (1.00 mg/L) profoundly increased shoot length and shoot thickness. However, both concentrations (0.25 and 1.00 mg/L) were significantly increased flower length as compared with the other used concentration (0.50 mg/L). Meanwhile, 0.5 mg/L was significantly increased leaf length and number of new leaves than others. On the other hand, different concentrations failed to induce any statistical differences when number of old leaves and flower width parameters were concerned.

Dealing with the interaction between flowering treatments and concentrations as shown in **Table (11-C)** clarified that 0.25 mg/L Ethophon concentration induced a significant increase in flower length over other combinations. However, 0.50 mg/L of either Amino Vit or yeast extract enhanced significant increase in number of new leaves over the other combinations. Meanwhile, either 0.5 or 1.0 mg/L of combined treatment (Amino Vit + yeast) or 1.00 mg/L Ethephon enhanced significant increase in shoot length over other combinations. On the other hand, different interactions failed to induce any statistical differences when number of old leaves and flower width were concerned.

Table (11): Effect of different flowering treatments on growth and flowering parameters of acclimatized pineapple cv. Smooth Cayenne plants.

Table (11-A): Effect of flowering treatment.

Parameters / Flowering treatments	Shoot length (cm)	Shoot thickness (mm)	Leaf length (cm)	No. of leaves		Flower length (cm)	Flower width (cm)
				New	Old		
Amino Vit.	9.08 B	4.20 C	45.00 C	12.00 A	17.67 B	0.00 B	0.00 B
Yeast	7.78 C	3.87 D	39.39 D	12.33 A	17.67 B	0.00 B	0.00 B
Amino Vit + yeast	12.22 A	5.91 A	55.44 A	12.22 A	19.00 B	0.00 B	0.00 B
Ethephon	9.72 B	5.04 B	49.67 B	7.67 B	24.11 A	5.94 A	3.17 A

Means of different flowering treatments followed with the same letter within each column are not significantly different from each other at 1% level.

Table (11-B): Effect of concentration.

Parameters / Flowering Treatments (ml/L)	Shoot length (cm)	Shoot thickness (mm)	Leaf length (cm)	No. of leaves		Flower length (cm)	Flower width (cm)
				New	Old		
0.25	8.33 C	4.24 C	43.67 B	10.75 B	18.75 A	2.04 A	0.79 A
0.50	9.92 B	4.93 B	51.96 A	12.00 A	19.75 A	0.79 B	0.67 A
1.00	10.85 A	5.09 A	46.50 B	10.42 B	20.33 A	1.63 A	0.92 A

Means of different flowering treatments concentrations followed with the same letter within each column are not significantly different from each other at 1% level.

tab11c

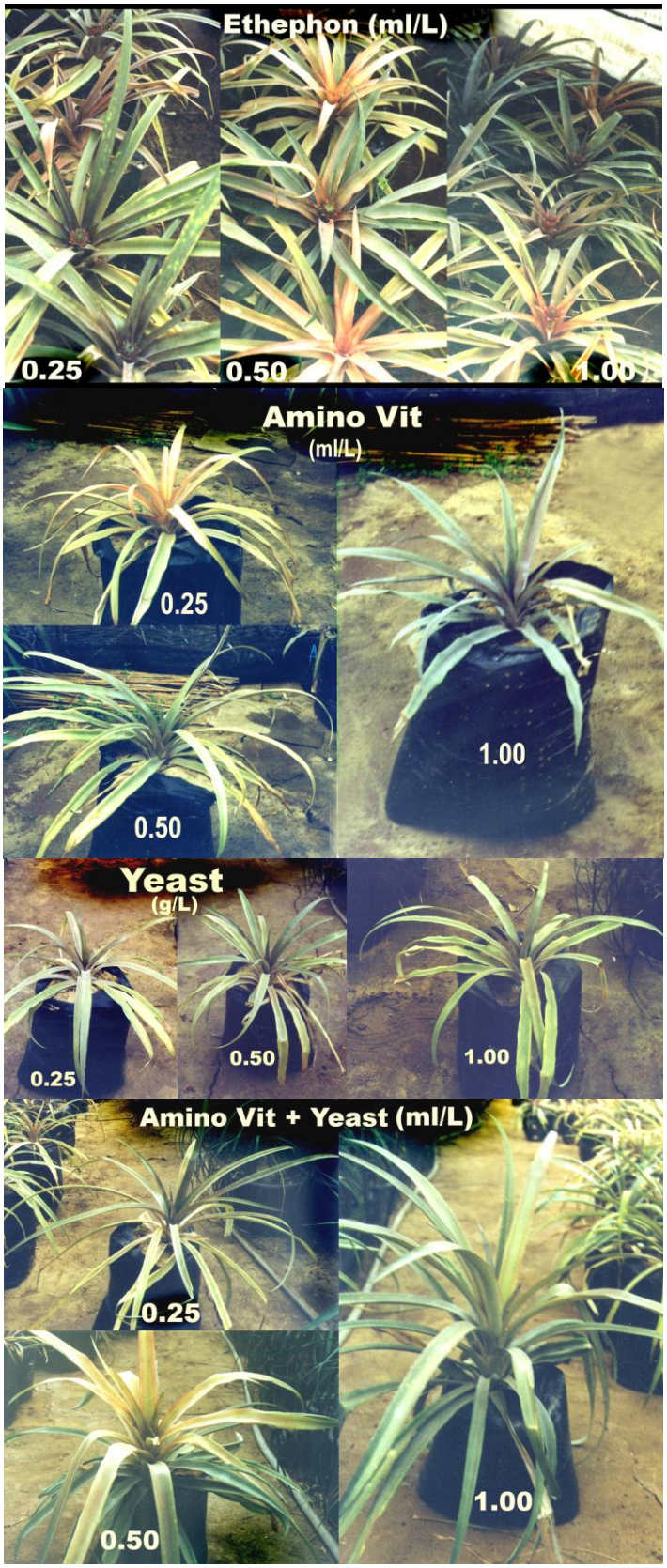


Photo (13): Effect of different flowering treatments on growth flowering parameters of acclimatized pineapple cv. Smooth Cayenne plants.

Generally, it is easy to conclude that Ethophon was more superior than Amino vit, yeast and Amino vit + yeast in flowering parameters. These results go in line with the findings of **Goburdhun (1994).** He found that the highest percentage of flowering was obtained with Ethrel at 750 ppm since it gave 67.5 and 71.5% for the plant and ratoon crops, respectively in 1 year of pineapple. In addition, **Singh *et al.* (1999)** found that pineapple, flowering was the highest in plants treated with 100 ppm ethrel (Ethephon). Treated plants flowered earlier in both seasons.

Dealing with the effect of different ethephon concentrations on fruiting of acclimatized pineapple plants as recorded in **Table (12)** and **Photo (12)**, it seemed that low concentration of ethephon (0.25) was significantly maximized number of fruitlets and improved neck length in relation to the other concentrations tested. However, higher concentration (1.00) was statistically improved crown width, number of crown leaves, and neck thickness as compared with the other concentrations. On the other hand, different concentrations of ethephon failed to show any significant differences when fruit length, fruit width, and crown length parameters were considered.

Table (12): Effect of different ethephon concentrations on fruiting of acclimatized pineapple *Smooth Cayenne* plants.

Parameters / Ethephon (ml/L)	Fruit length (cm)	Fruit width (cm)	No. of fruitlet	Crown length (cm)	Crown width (cm)	No. of crown leaves	Neck length (cm)	Neck thickness (cm)
0.25	5.10 A	5.20 A	28.00A	4.67A	3.17B	41.67B	8.00A	1.07B
0.50	3.90A	4.33A	14.00C	4.00A	2.83B	38.33B	4.00B	1.20B
1.00	4.23A	5.17A	22.67B	4.73A	3.77A	51.33A	4.67B	1.43A

Means of different flowering treatments followed with the same letter within each column are not significantly different from each other at 1% level.

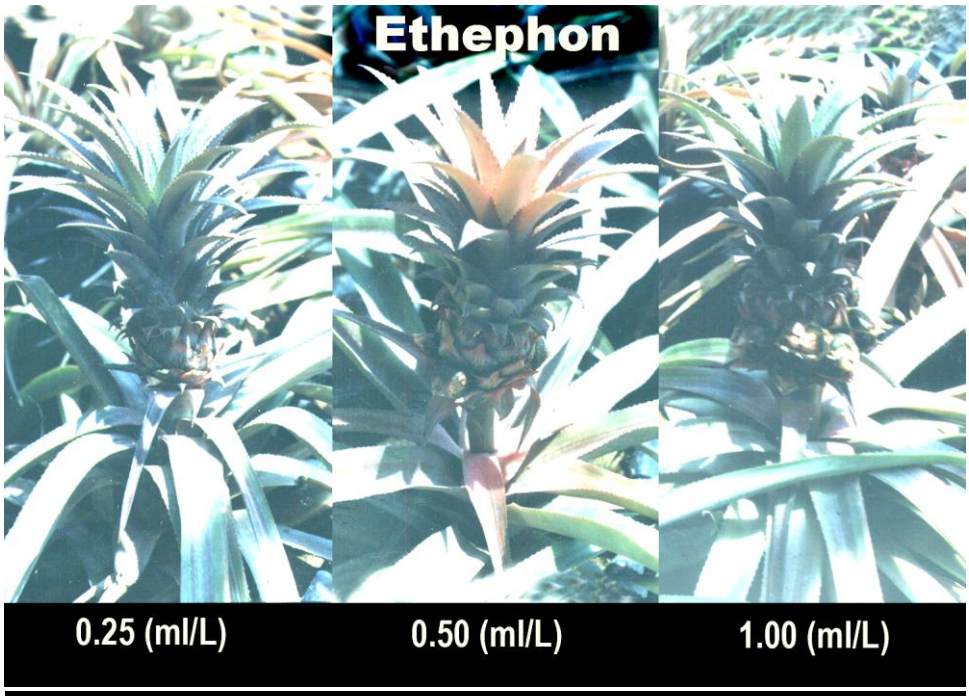


Photo (14): Effect of different ethephon concentrations on fruiting of acclimatized pineapple *Smooth Cayenne* plants.

Photo (15) reflects the developmental stages of pineapple fruit development.

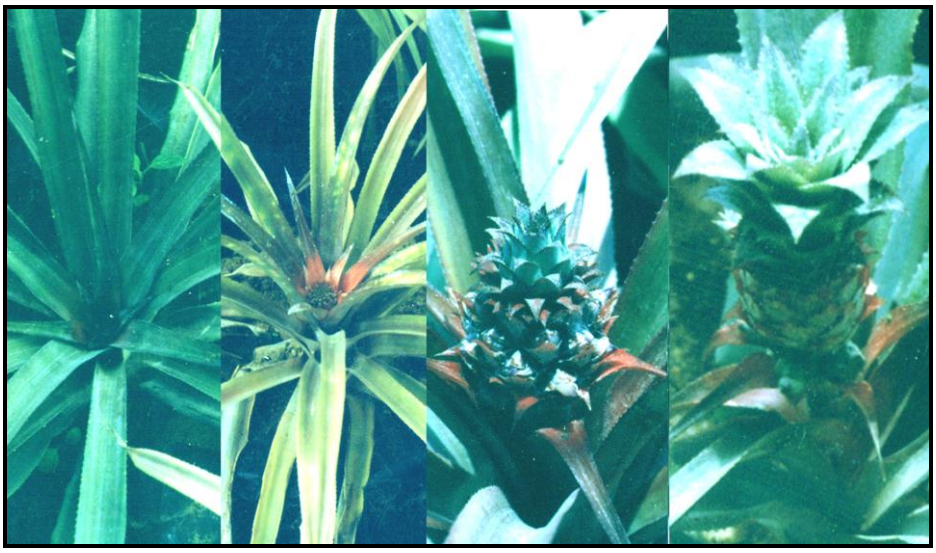


Photo (15): Developmental stages of pineapple fruits.

IV.2. *Grand Naine* banana:

IV.2.1. Acclimatization:

IV.2.1.a. Effect of agricultural media:

Table (13) and **Photo (16)** show the effect of different agricultural media on survival percentage and growth parameters of *in vitro* acclimatized *Grand Naine* banana plants. It is quite evident that the combination treatment (25% vermiculite + 25% peatmoss + 25% sand + 25% loam) succeeded in maximizing the percentage of survival up to the highest level (96.67%) then followed by the combination treatment of (33.3% vermiculite + 33.3% peatmoss + 33.3% sand) induced 95% of survival compared to the other agricultural media. Also, the data clarified that using of any of the agricultural media alone in acclimatizing *Grand Naine* banana plants had an adverse effect on survival percentage. In addition, the combination treatment (25% vermiculite + 25% peatmoss + 25% sand + 25% loam) had a positive effect on most of growth parameters under study since it encouraged significant increase of increased shoot length, shoot thickness, number of leaves, leaf length, root length and root number compared with those alone agricultural media. However, the combination treatment (25% vermiculite + 25% perlite + 25% sand + 25% loam) succeeded in significantly increase of leaf width as compared with the other treatments.

Generally, the above results recommended using the combination treatment (25% vermiculite + 25% peat moss + 25% sand + 25% loam) for inducing the highest percentage of survival, shoot length, shoot thickness, number of leaves, root length and number of roots. These results partially agreed with the findings of **El-Bahr *et al.* (2003).** They stated that the highest survival percentage was observed when combination of washed sand peat moss + vermiculite perlite at the ratio

1:1:1:1 was used for acclimatization of date palm cv. Zaghloul. Also, with the findings of **Aish *et al*. (2004)** when they found that transferring of banana plantlets to pots containing sand and clay (1: 1) and maintained in the green house for acclimatization for 8 weeks induced the highest number of shoots (297).

Table (13): Effect of different agricultural media on survival percentage, rooting and growth parameters of *in vitro* acclimatized *Grand Naine* banana plantlets.

No.	Parameters / Agricultural media	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of root
1	100 % Vermiculite	42.33 N	3.07 QR	0.53 HIJ	1.33 H	2.33 KL	1.67 LM	2.60 I	2.67 DEF
2	100% Perlite	28.33 P	2.60 ST	0.45 JK	1.33 H	2.13 LM	1.23 O	2.17 J	2.00 FG
3	100% Peat moss	60.00 J	3.97 KLM	0.71 EFG	2.33 PG	3.17 HI	2.33 EFGHI	3.67 FG	3.00 CDE
4	100% Sand	27.00 P	2.25 T	0.42 JK	1.00 H	1.83 M	1.33 NO	1.83 K	1.67 G
5	100% loam	58.00 K	3.40 OPQ	0.57 HI	1.67 GH	2.52 JK	2.10 IJ	1.80 K	2.00 FG
6	50% Vermiculite + 50% Perlite	44.33 MN	3.20 PQ	0.56 HI	1.33 H	2.23 KL	1.77 KL	2.63 I	2.33 EFG
7	50% Vermiculite +50% Peat moss	75.33 G	4.27 IJKL	0.71 EFG	2.67 EF	3.40 EFGH	2.60 BCD	3.93 EF	3.33 BCD
8	50% Vermiculite + 50% Sand	45.00 M	3.17 PQ	0.43 JK	1.67 GH	2.37 KL	1.93 JK	2.63 I	2.33 EFG
9	50% Vermiculite + 50% loam	70.67 H	4.33 HIJK	0.64 FGH	3.33 CDE	3.50 DEFG	2.57 BCDE	3.93 EF	3.33 BCD
10	50% Perlite + 50% Peat moss	60.00 JK	3.83 MN	0.62 GHI	2.67 EF	3.07 I	2.23 HI	3.60 G	3.00 CDE
11	50% Perlite + 50% Sand	36.67 O	2.77 RS	0.37 K	1.33 H	2.33 KL	1.53 MN	2.50 I	2.00 FG
12	50% Perlite + 50% Loam	55.00 L	3.50 NOP	0.51 IJ	2.67 EF	2.70 J	2.23 HI	3.23 H	2.33 EFG
13	50% Peat moss + 50% Sand	90.33 D	5.00 BCDE	0.88 BC	3.67 BCD	3.50 DEFG	2.47 CDEFGH	4.73 B	3.67 ABC
14	50% Peat moss + 50% Loam	85.67 E	4.67 EFGH	0.74 EF	3.67 BCD	3.60 CDEF	2.57 BCDE	4.23 D	3.33 BCD
15	50% Sand + 50% Loam	58.67 K	3.80 MN	0.51 IJ	2.33 FG	3.17 HI	2.30 FGHI	3.43 GH	2.67 DEF
16	33.3% Vermiculite + 33.3% Perlite + 33.3% Peat moss	70.67 H	4.30 HIJKL	0.73 EF	3.33 CDE	3.37 EFGHI	2.67 BC	4.37 CD	3.00 CDE
17	33.3% Vermiculite + 33.3% Perlite + 33.3% Sand	58.67 K	3.77 MNO	0.59 HI	3.00 DEF	3.33 EFGHI	2.33 EFGHI	3.27 H	2.67 DEF
18	33.3% Vermiculte + 33.3% Perlite + 33.3% Loam	63.33 I	3.80 MN	0.62 GHI	3.33 CDE	3.30 FGHI	2.37 DEFGH	3.57 G	3.00 CDE
19	33.3% Vermiculte + 33.3% Peat moss + 33.3% Sand	95.00 AB	5.33 B	1.04 A	4.33 AB	4.00 AB	2.73 B	5.23 A	4.33 A
20	33.3% Vermiculte + 33.3% Peat moss + 33.3% Loam	30.33 D	4.93 CDEF	0.74 EF	4.00 ABC	3.63 CDE	2.50 BCDEFG	4.63 BC	3.67 ABC
21	33.3% Vermiculte + 33.3% Sand + 33.3% Loam	76.33 G	4.47 GHIJ	0.71 EFG	3.67 BCD	3.43 DEFGH	2.53 BCDEF	4.17 DE	3.33 BCD
22	33.3% Perlite + 33.3% Peat moss + 33.3% Sand	81.00 F	4.60 FGHI	0.77 DE	3.67 BCD	3.47 DEFG	2.47 CDEF	4.63 BC	3.33 BCD
23	33.3% Perlite + 33.3% Peat moss + 33.3% Loam	64.67 I	4.10 JKLM	0.62 GHI	3.00 DEF	3.66 CDEFG	2.27 GHI	3.63 G	3.00 CDE
24	33.3% Perlite + 33.3% Sand + 33.3% Loam	58.33 K	3.93 LM	0.53 HIJ	2.67 EF	3.27 GHI	2.60 BCD	3.43 GH	2.67 DEF
25	33.3% Peat moss + 33.3% Sand + 33.3% Loam	93.33 BC	5.10 BCD	0.99 AB	3.67 BCD	3.73 BCD	2.53 BCDEF	4.90 B	4.00 AB
26	25% Vermiculite + 25% Perlite + 25% Peat moss + 25% Sand	75.00 G	4.47 GHIJ	0.62 GHI	3.67 BCD	3.53 DEFG	2.57 BCDE	4.33 D	3.33 BCD
27	25% Vermiculite + 25% Perlite + 25% Peat moss + 25% Loam	92.67 C	4.77 DEFG	0.91 BC	4.00 ABC	3.63 CDE	2.27 GHI	4.83 B	2.67 ABC
28	25% Vermiculite + 25% Perlite + 25% Sand + 25% Loam	64.00 I	4.07 KLM	0.59 HI	2.67 EF	3.53 DEFG	3.10 A	3.53 DEFG	3.00 CDE
29	25% Vermiculite + 25% Peat moss + 25% Sand + 25% Loam	96.67 A	6.00 A	1.09 A	4.67 A	4.23 A	2.47 CDEFGH	4.23 A	4.33 A
30	25% Perlite + 25% Peat moss + 25% Sand + 25% Loam	90.33 D	5.00 BCDE	0.74 EF	3.33 CDE	3.60 CDEF	2.60 BCD	3.60 CDEF	3.67 ABC
31	20% Vermiculite + 20% Perlite+ 20% Peat moss + 20% Sand + 20% Loam	93.00 BC	5.23 BC	0.86 CD	3.67 BCD	3.87 BC	2.46 CDEF	3.87 BC	4.00 AB

Means of different agricultural media treatments followed with the same letter within each column are not significantly different from each other at 1% level.

Photo (16): The numbers listed in the photos are the same numbers in the Table (13).

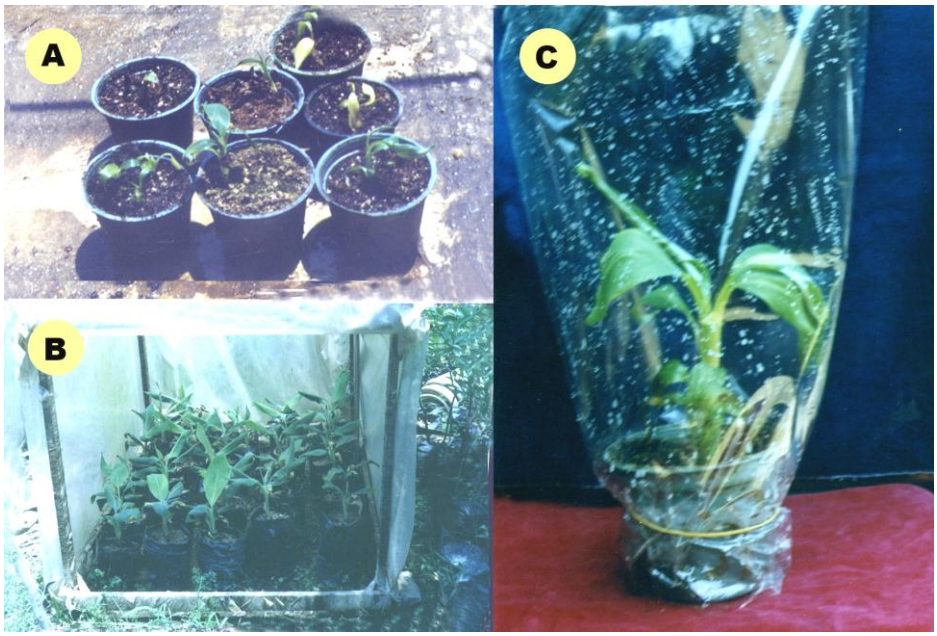
IV.2.1.b. Effect of microclimate space:

Table (14) and **Photo (17)** show the effect of different microclimate space on survival percentage and growth parameters of *in vitro* acclimatized *Grand Naine* banana plants. It is found that plastic bags significantly increased the survival percentage, shoot length, leaf length and number of roots followed by plastic cages and greenhouse in a descending order. On the other hand, different used microclimate spaces failed to induce any statistical differences as both shoot thickness and number of leaves parameters were considered. Meanwhile, plastic cages and bags were more effective in increasing root length as compared with those of greenhouse..

Table (14): Effect of different microclimate spaces on survival percentage, growth and rooting parameters of *in vitro* acclimatized *Grand Naine* banana plantlets.

Parameters / Microclimatic conditions	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Greenhouse	20.32 C	6.13 B	1.02 A	3.33 A	4.10 B	3.03 B	4.03 B	3.00 B
Plastic cages	56.67 B	6.30 B	1.03 A	3.67 A	4.37 B	3.17 AB	5.23 A	3.67 AB
Plastic bags	92.67 A	7.27 A	1.07 A	5.00 A	5.63 A	3.67 A	5.70 A	4.67 A

Means of different microclimate spaces followed with the same letter within each column are not significantly different from each other at 1% level.



A= Greenhouse B= Plastic cage C= Plastic bage

Photo (17): Effect of different microclimate spaces on growth of *in vitro* acclimatized Grand Naine banana plantlets.

In general, the above mentioned results reflect that the highest survival of *Grand Naine* banana plants was obtained when plastic bags were used as it reached 92.67%. These results disagreed with the finding of **Kodyn and Zapata (1998) and Silva *et al.* (1998).** They found that survival percentage of *Grand Naine* banana plants (100%) were reached with greenhouse.

IV.2.1.c. Effect of pot size:

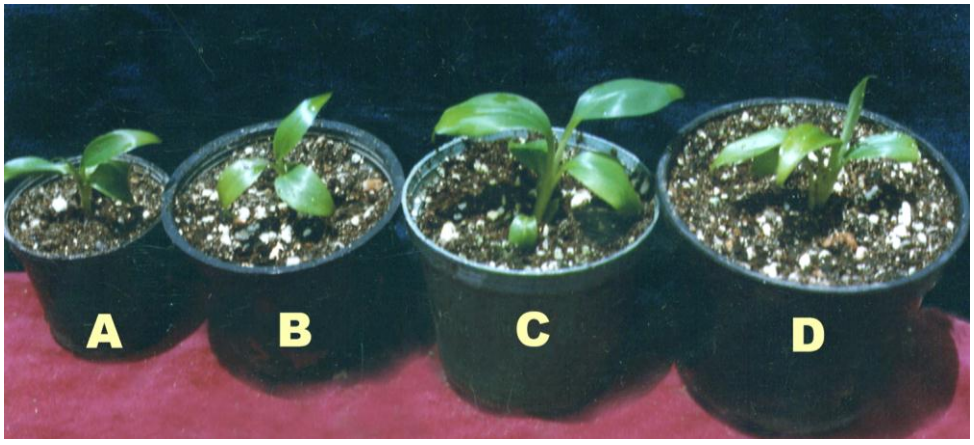
Table (15) and **Photo (18)** clarify that acclimatizing of the Grand Naine in pots size (10 cm) had a positive improvement in all used parameters under study since it significantly increased survival percentage, shoot length, number of leaves, leaf length, leaf width and root length followed by pot size (9 cm) then pot size (6 cm) and pot (11 cm) in a descending order. On the other hand, number of roots was not statistically affected by different used pot sizes.

Table (15): Effect of different pot sizes on survival percentage, growth and rooting parameters of *in vitro* acclimatized *Grand Naine* banana plantlets.

Parameters / Pot size (cm)	Survival %	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
11	77.33 D	7.33 C	1.03 C	3.67 B	4.27 C	3.40 C	6.17 C	3.67 A
10	96.33 A	9.00 A	1.19 A	5.33 A	7.27 A	4.43 A	7.93 A	4.67 A
9	91.33 B	8.13 B	1.12 B	4.33AB	5.37 B	3.93 B	7.07 B	4.33 A
6	84.67 C	7.53 C	1.09BC	3.67 B	4.67 C	3.53 C	6.83 B	4.00 A

Means of different pot sizes followed with the same letter within each column are not significantly different from each other at 1% level.

In general, the above mentioned results reflected that the highest survival percentage of Grand Naine banana plants was obtained in pot size (10 cm) reached to 96.33%. These results agreed with the findings of **Zaied (2001)** who found that lower plant density 1 plant / pot improved all parameters under study as compared with two and three plants of Grand Naine per pot**.**



A= 6cm B= 9cm C= 10cm D= 11cm

Photo (18): Effect of different pot sizes on growth of *in vitro* acclimatized Grand Naine banana plantlets.

IV.2.1.d. Effect of growth retardant:

Table (16) shows the effect of different growth retardants with different concentrations on growth parameters of *in vitro* acclimatized *Grand Naine* banana plants. It is obvious from **Table (16-A)** and **Photo (19)** that paclobutrazol (PP_{333}) treatment induced significant superior effect in increasing shoot thickness, leaf width and number of roots in relation to the other treatments. However, coumarin encouraged a significant increase in number of leaves/plant, leaf length and survival percentage in comparison with the other treatments. However, coumarin and cycocel were significantly more superior than PP_{333} in improving root length.

Furthermore, it is clear from **Table (16-B)** and **Photo (17**) that higher concentration of growth retardants (0.5 mg/L) had significantly increased survival percentage, shoot thickness, leaf width and number of roots as compared with the lower level (0.125 mg/L) and control. However, both leaf length and root length were significantly increased when lower concentration (0.125 mg/L) was used in comparison with higher concentration (0.50 mg). Meanwhile, number of leaves was significantly increased when 0.125 and 0.25 mg/L of growth retardants were used in comparison with lower concentrations (0.0 and 0.50 mg/L).

Table (16): Effect of different growth retardants and different concentrations on survival percentage growth and rooting parameters of *in vitro* acclimatized *Grand Naine* banana plantlets.

Table (16-A): Effect of growth retardant.

Parameters / Growth retardants	Survival	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Paclobutrazol	70 B	10.28 C	1.47 A	6.08 C	8.30 C	5.14 A	7.74 B	10.17 A
Coumarin	75 A	11.02 B	1.28 B	7.42 A	9.32 A	4.32 B	8.99 A	7.92 B
Cycocel	56 C	11.98 A	1.01 C	6.75 B	8.87 B	3.83 C	9.13 A	6.50 C

Means of different growth retardant treatments followed with the same letter within each column are not significantly different from each other at 1% level.

Table (16-B): Effect of concentration.

Parameters / Concentration (ml/L)	Survival	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	58 D	12.33 A	1.22 B	6.33 B	8.77 B	4.40 B	8.60 B	5.33 D
0.125	69 C	11.61 A	1.04 C	7.44 A	9.79 A	4.13 C	8.91 A	8.44 C
0.25	72B	10.61 B	1.29 B	7.00 A	8.78 B	4.41 B	8.57 B	9.11 B
0.50	75A	9.83 C	1.48 A	6.22 B	7.98 C	4.77 A	8.41 B	9.89 A

Means of different growth retardant treatments followed with the same letter within each column are not significantly different from each other at 1% level.

In addition, (0.0 and 0.125 mg/L) levels were significantly effective in increasing shoot length as compared with 0.25 and 0.50 mg/L concentrations.

Dealing with the interaction between different growth retardants and concentrations, it is quite evident from **Table (16-C), Fig. (6)** and **Photo (19)** that 0.5 mg/L PP_{333} recorded a significant increased in shoot thickness, leaf width and number of roots. However, leaf length was significantly increased when 0.125 mg/L coumarin level was added as compared with the other combinations. On the other hand, different interactions failed to induce any statistical differences when number of leaves was concerned.

tab16c

fig6

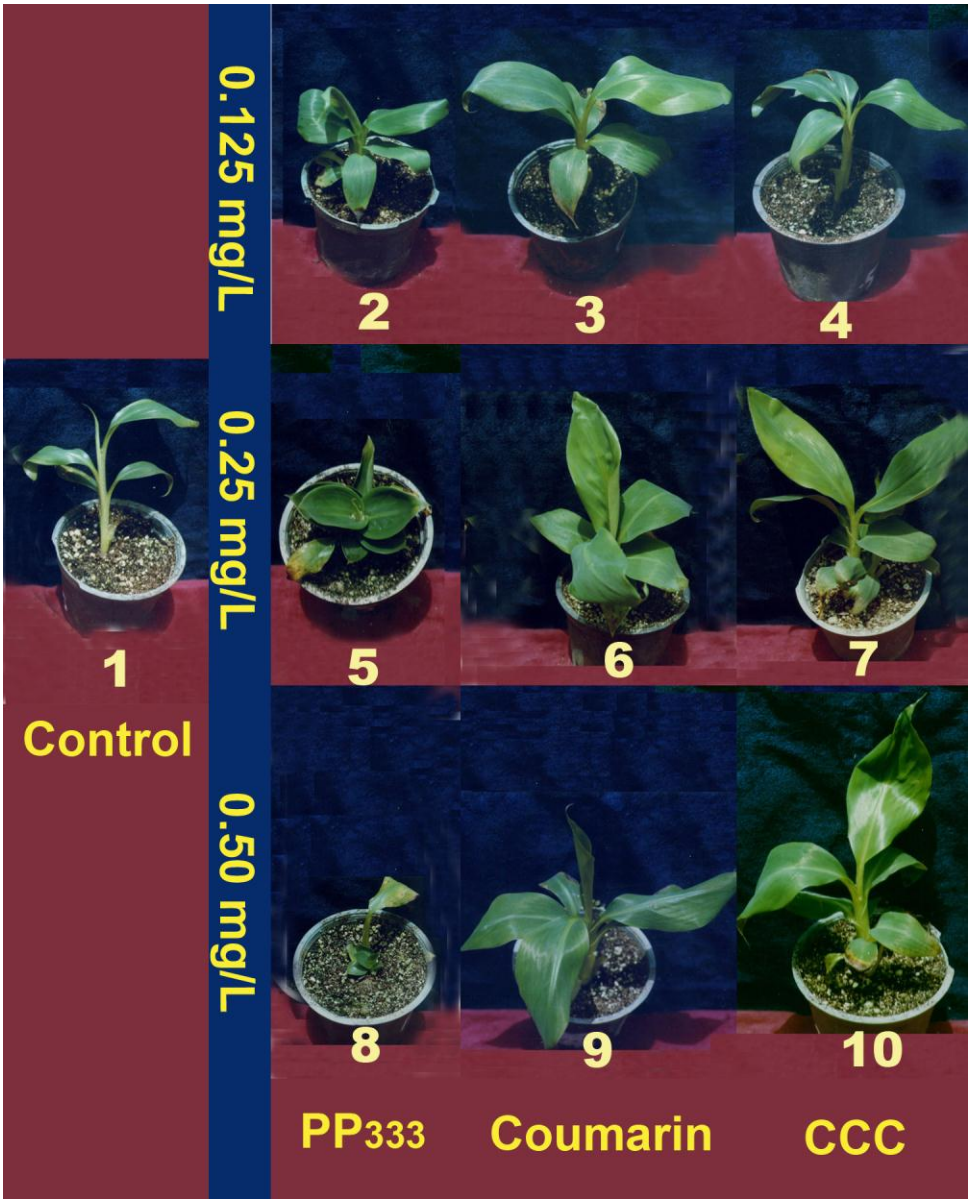


Photo (19): Effect of different growth retardants with different concentrations on survival percentage growth and rooting parameters of *in vitro* acclimatized *Grand Naine* banana plantlets.

The previous results summarized that PP_{333} at high concentration (0.5 mg/L) enhanced shoot thickness, leaf width and number of roots. While coumarin at the rate of (0.125 mg/L) improved leaf length. These results are in harmony with the findings of **Dalziel and Lawrence (1984).** They found that, paclobutrazol is active as a growth retardant in a

broad spectrum of species. In addition, **Graebe (1987)** reported that plant growth retardants generally induce a shortening effect of the internode of higher plants *in vivo* and have some additional effects such as a reduction in leaf size, intensification of green colouration of leaves and thickening of roots. He added that PP_{333} inhibits kaurene oxidase which blocks the oxidative reactions from ent-kaurene to ent-kaurenoic acid in the pathway leading to the gibberellic acids.

IV.2.1.e. Effect of gibberellic acid (GA_3):

Data of **Table (17), Fig. (7)** and **Photo (20)** revealed the effect of different concentrations of GA_3 on *in vitro* acclimatized *Grand Naine* plants treated with PP_{333}. It is clear that spraying of high concentrations of GA_3 (500 ppm) had significantly better effect in improving most parameters under study sine it increased shoot length, shoot thickness, leaf length, leaf width, root length and number of roots as compared with the lower concentration (100 ppm) and the control. However, number of leaves was significantly increased when 400 and 500 ppm treatments were used followed by spraying with 300 ppm then 200 and 100 ppm in a descending order as compared with the control.

At all events, one can conclude that spraying GA_3 at 500 ppm increased shoot length, shoot thickness, leaf length, leaf width, root length and number of roots, while, spraying GA_3 at 400 and 500 ppm levels improved number of leaves. These results are partially in harmony with the findings of **Yanes-Paz *et al.* (2001).** They verified that addition of GA_3 at 100 mg/L twice a month increased the growth of *in vitro* plants during the acclimatization of pineapple plants.

Table (17): Effect of different concentrations of GA_3 on growth and rooting of *in vitro* acclimatized *Grand Naine* banana plantlets treated with PP_{333}.

Parameters / Conc. of GA_3 (ppm)	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
0.0	8.60 E	2.03 C	5.67 C	7.77 E	6.17 F	6.40 D	13.67 C
100	10.00 D	2.03 C	6.67 BC	13.40 D	6.77 E	6.80 D	13.67 C
200	12.83 C	2.07 C	7.00 B	14.33 C	7.33 D	7.77 C	14.33BC
300	13.73 B	2.17 B	7.67 AB	14.83 C	7.90 C	8.50 BC	14.67 B
400	14.03 B	2.20 AB	8.33 A	17.60 B	8.53 B	9.10 B	15.00AB
500	15.23 A	2.27 A	8.33 A	18.43 A	9.70 A	11.53 A	15.67 A

Means of different GA_3 concentrations followed with the same letter within each column are not significantly different from each other at 1% level.

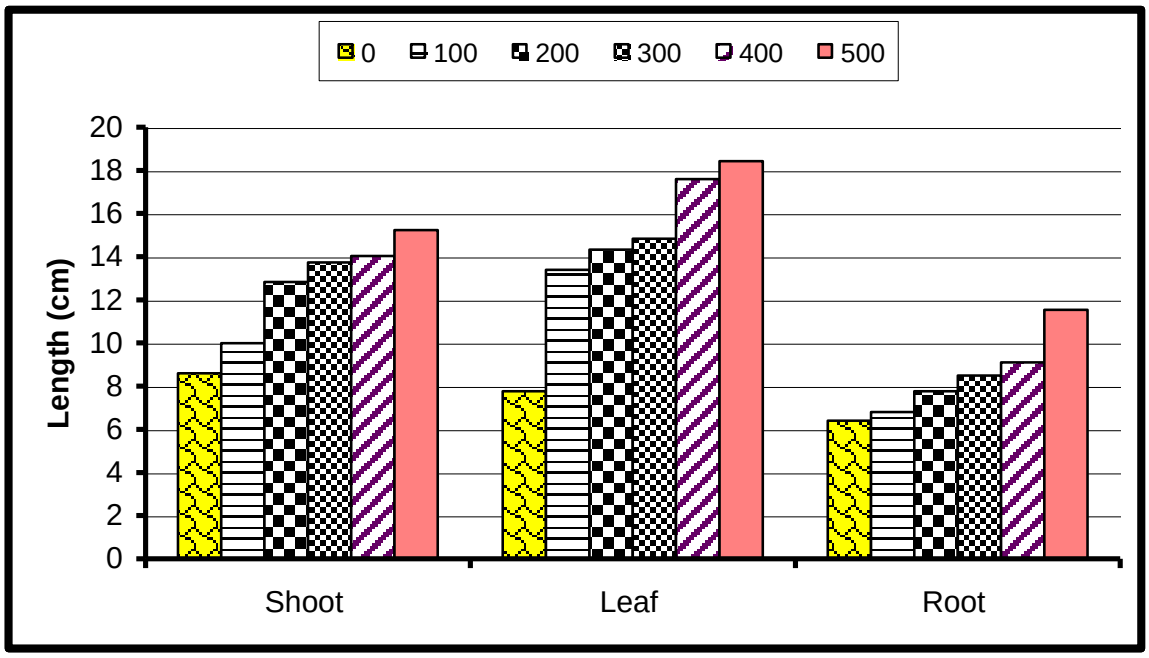


Fig. (7): Effect of different concentrations of GA_3 on plants treated with PP_{333} on shoot length, leaf length and root length of *in vitro* acclimatized *Grand Naine* banana plantlets.

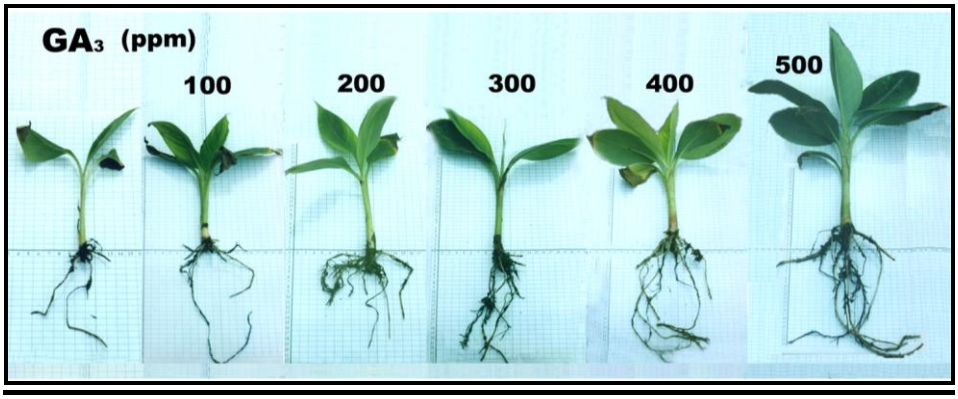


Photo (20): Effect of different concentrations of GA_3 on growth and rooting of *in vitro* acclimatized *Grand Naine* banana plantlets treated with paclobutrazol (PP_{333}).

IV.2.2. Nutritional studies:

IV.2.2.a. Effect of nutrient medium salts:

Table (18), Fig. (8) and **Photo (21)** pointed out the effect of different medium salts on growth parameters of Grand Naine plants. It is appear that the combination of Murashige & Skoog (MS) + white (Wh)+ Gamborg (B5) medium salts had significantly better improved all parameters under study since it increased shoot length, leaf length, leaf width and root length as compared with all other medium salts used. However, all combined medium salts treatments induced the best significant effect on shoot thickness, number of leaves and number of roots as compared with the control. Also, data clarified that using of any medium salts alone for acclimatizing Grand Naine banana plants had an adverse effect on growth as compared with the combination treatments.

Generally, the above results disclosed that the combination medium salts treatments enhanced the highest improvement of all growth parameters under study. These results partially agreed with the findings of

Garcia-Rodriguez *et al.* (1987). They stated that, doubling the salts concentration of the basal medium increased the number of canary Island banana (*Musa* spp.) shoots but decreased number of roots. In addition, **Economou and Read (2003)** found that the percentage of rooted microshoots as well as the number of roots/microshoot were increased by the putrescine treatment reaching up to 93% and 4% respectively of olive.

Table (18): Effect of different medium salts on growth and rooting parameters of *in vitro* acclimatized *Grand Naine* banana plantlets.

Parameters / Medium salts	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	9.83 G	1.57 C	6.33 C	8.27 G	5.100 E	8.50 G	10.67 D
Murashige & Skoog (MS)	10.93 F	1.60 BC	7.33 AB	8.87 F	5.30 DE	12.70 E	12.00BC
White (Wh)	13.70 C	1.53 C	7.00 BC	11.67 C	5.13 E	15.70 C	11.67 C
Gamborg (B5)	12.00 E	1.60 BC	7.67 AB	9.67 E	5.43 CD	13.47 E	12.33ABC
MS + white	14.43 B	1.70 A	8.00 A	12.23 B	6.23 A	18.70 B	12.67 AB
MS + B5	12.57 D	1.67 AB	8.00 A	11.17 D	5.57 BC	14.50 D	12.67 AB
White + B5	13.70 C	1.70 A	8.00 A	11.67 C	5.77 B	15.70 C	12.67 AB
MS + white + B5	16.27 A	1.70 A	8.00 A	14.00 A	6.43 A	21.50 A	13.00 A

Means of different media salts followed with the same letter within each column are not significantly different from each other at 1% level.

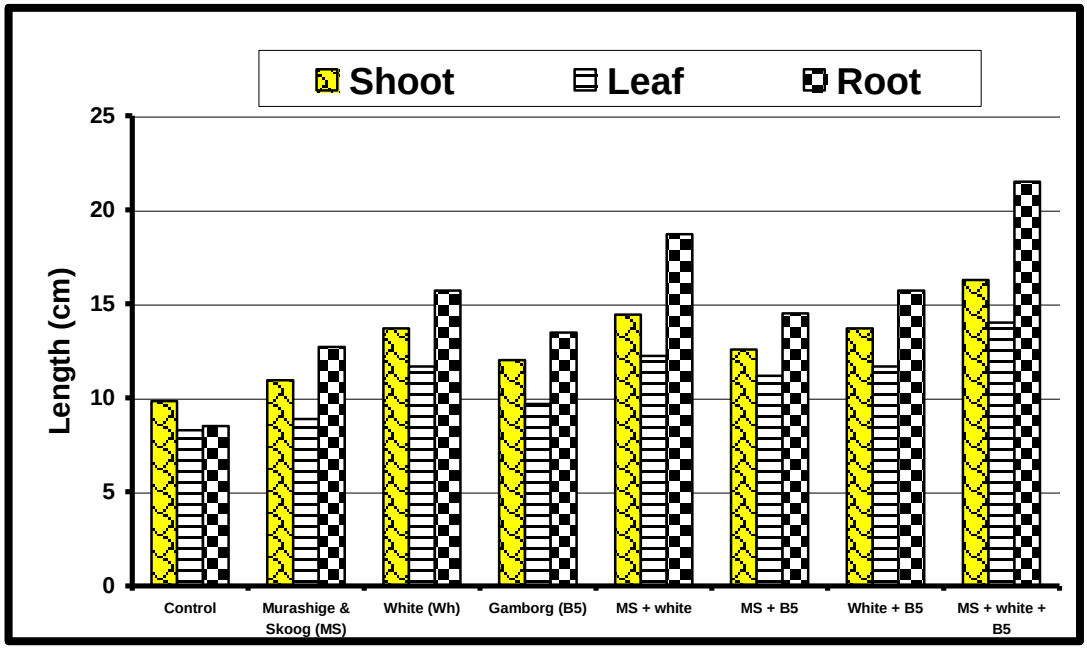
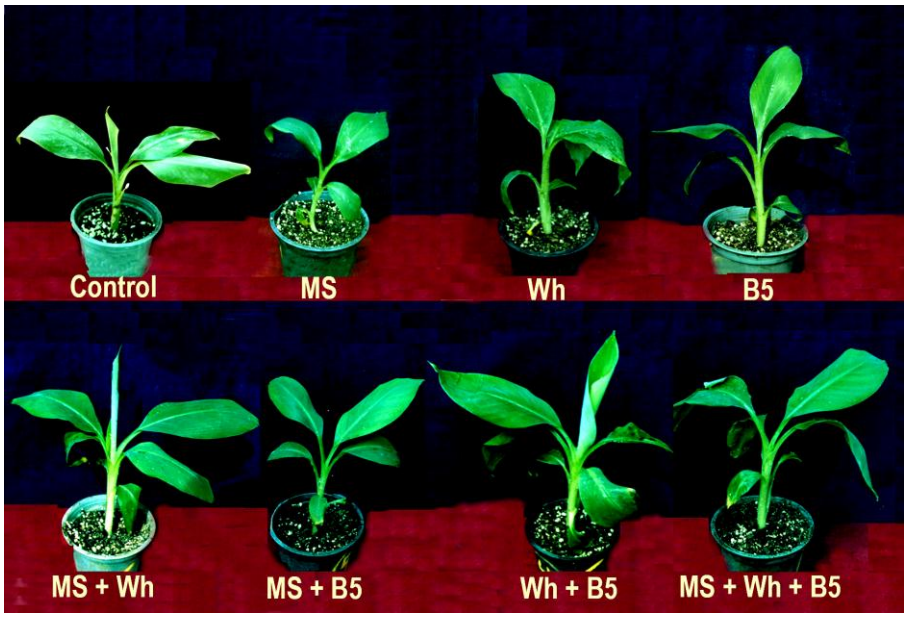


Fig. (8): Effect of different medium salts on growth and rooting parameters of *in vitro* acclimatized *Grand Naine* banana plantlets.



MS= Murashige and Scoog Wh=White B5= Gamborg

Photo (21): Effect of different medium salts on growth of *in vitro* acclimatized Grand Naine banana plantlets.

IV.2.2.b. Effect of vitamins:

Table (19), Fig. (9) and **Photo (22)** showed that using Murashige and Skoog vitamins had significantly increased all growth and rooting parameters i.e. shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots followed by B5 vitamins then N & N vitamins in a descending order as compared with the control.

From the previous results it can be concluded that MS vitamin is more suitable for improving growth and rooting parameters. The results go in line with the findings of **Omar (1988)** who declared that MS salts supplemented with 100 mg/L inositol, 0.4 mg/L thiamin. HCl enhanced acclimatization of date palm plants.

Table (19): Effect of different medium vitamins on growth and rooting parameters of acclimatized *Grand Naine* banana plantlets.

Parameters / Medium vitamins	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	17.33 D	1.70 C	8.00 C	14.37 C	6.60 C	23.87 D	13.00 B
Murashige & Skoog (MS)	21.93 A	1.87 A	9.67 A	16.78 A	7.80 A	26.93 A	14.00 A
Gamborg (B5)	20.23 B	1.83 AB	9.3 AB	14.93 B	7.03 B	26.03 B	13.67 AB
Nitsch & Nitsch (NN)	18.73 C	1.73 BC	8.33 BC	14.63BC	6.73 BC	25.13 C	13.33 AB

Means of different medium vitamins followed with the same letter within each column are not significantly different from each other at 1% level.

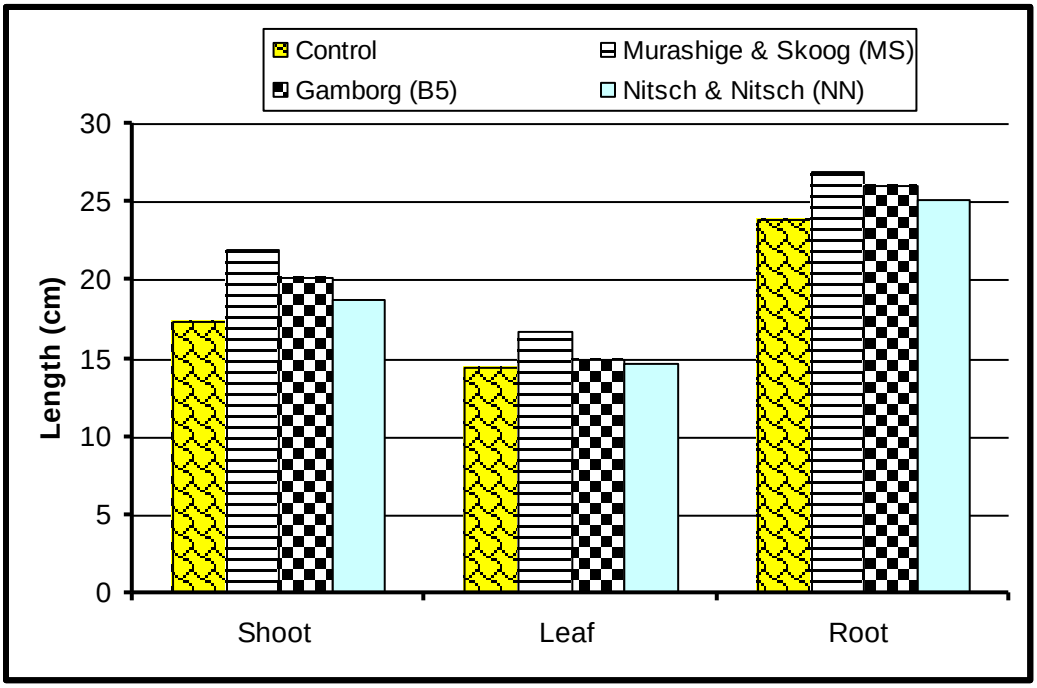
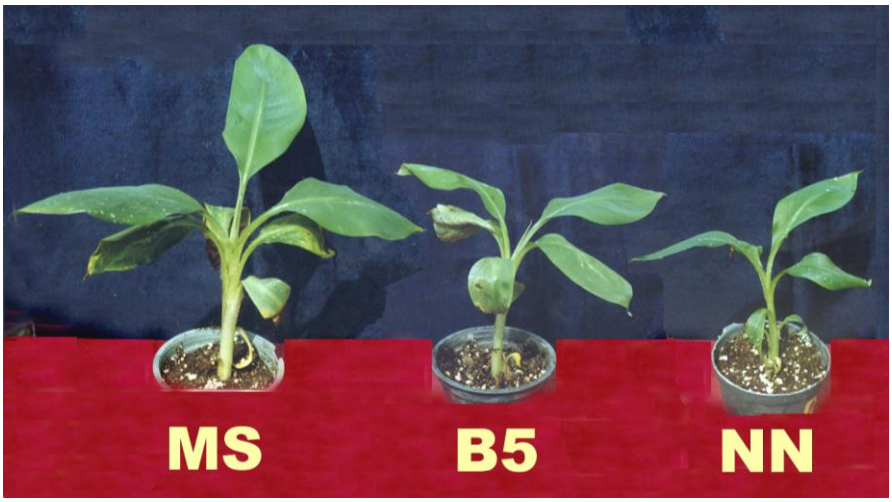


Fig. (9): Effect of different medium vitamins on shoot length, leaf length and root length of acclimatized *Grand Naine* banana plantlets.



MS= Murashige and Scoog **Wh=White** **B5= Gamborg**

Photo (22): Effect of different medium vitamins on growth and rooting parameters of acclimatized *Grand Naine* banana plantlets.

IV.2.2.c. Effect of organic additives:

The data of **Table (20), Fig. (10)** and **Photo (23)** reflect the effect of different organic additives on growth parameters of acclimatized Grand Naine banana plants. It is clear that 2.0 g/L of yeast extract was significantly effective in increasing all parameters used (i.e. shoot length, shoot thickness, leaf length, leaf width, root length and roots number as compared with the other organic additives under study. Moreover, all treatments used were significantly improved all parameters under study in relation to control treatment.

Table (20): Effect of different organic additives on growth and rooting parameters of acclimatized Grand Naine banana plants.

Parameters / Organic additives	Shoot length (cm)	Shoot thickness (mm)	No. of leaves / plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	22.83G	1.87D	9.67B	17.27G	8.07G	27.17I	14.33E
Yeast							
0.5 g/L	25.37B	1.97C	10.67AB	20.07B	9.77B	33.83C	16.67AB
1.0 g/L	25.37B	2.17B	10.67AB	20.07B	9.77B	35.10B	16.67AB
2.0 g/L	26.27A	2.27A	11.00A	24.53A	13.50A	36.00A	17.00A
Adenine							
0.025 g/L	23.83ED	1.90CD	10.00AB	18.13E	8.90E	29.53F	15.33CD
0.05 g/L	23.57F	1.90CD	9.67B	17.73EF	8.67EF	29.03G	15.00DE
0.1 g/L	23.03G	1.87D	9.67B	17.43FG	8.43F	28.07H	15.00DE
Coconut milk							
2 %	24.23DE	1.90CD	10.33AB	18.67D	9.23D	30.00F	15.00DE
4 %	24.83C	1.97C	10.67AB	19.43C	9.60BC	30.53E	16.00BC
8 %	24.33D	1.90CD	10.33AB	18.93D	9.40CD	32.93D	15.33CD

Means of different organ additives followed with the same letter within each column are not significantly different from each other at 1% level.

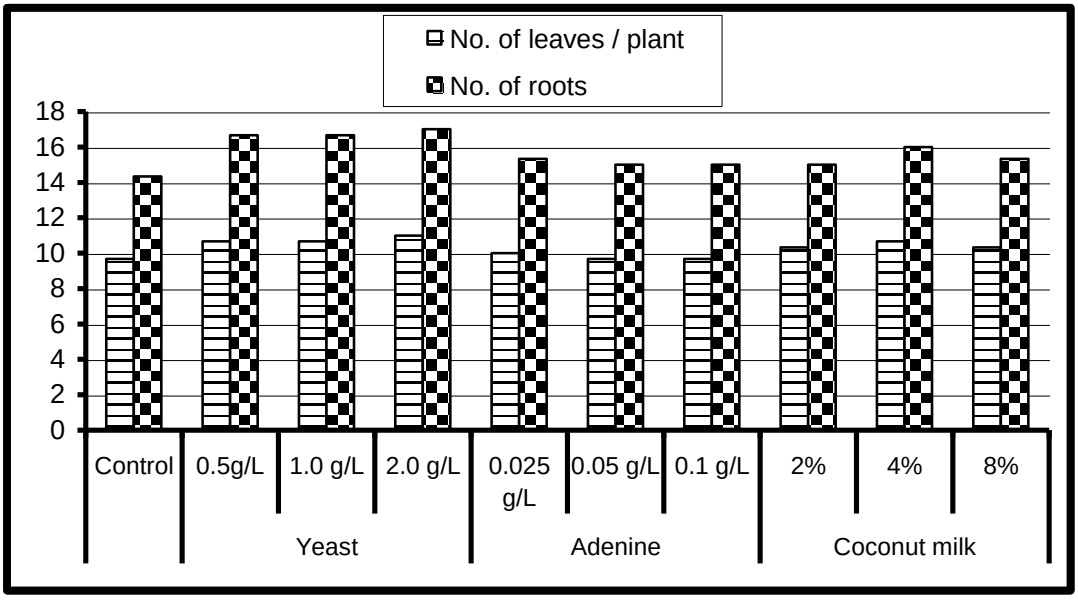


Fig. (10): Effect of different organic additives on No. of leaves/plant and No. of roots of acclimatized Grand Naine banana plants.

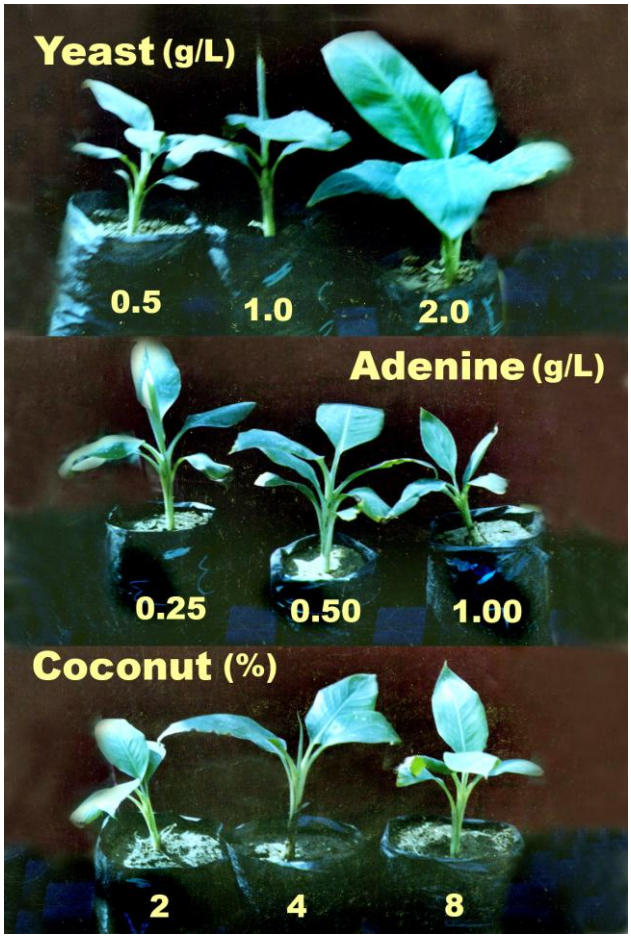


Photo (23): Effect of different organic additives on growth of acclimatized Grand Naine banana plants.

IV.2. 2.d. Effect of organic additives combinations:

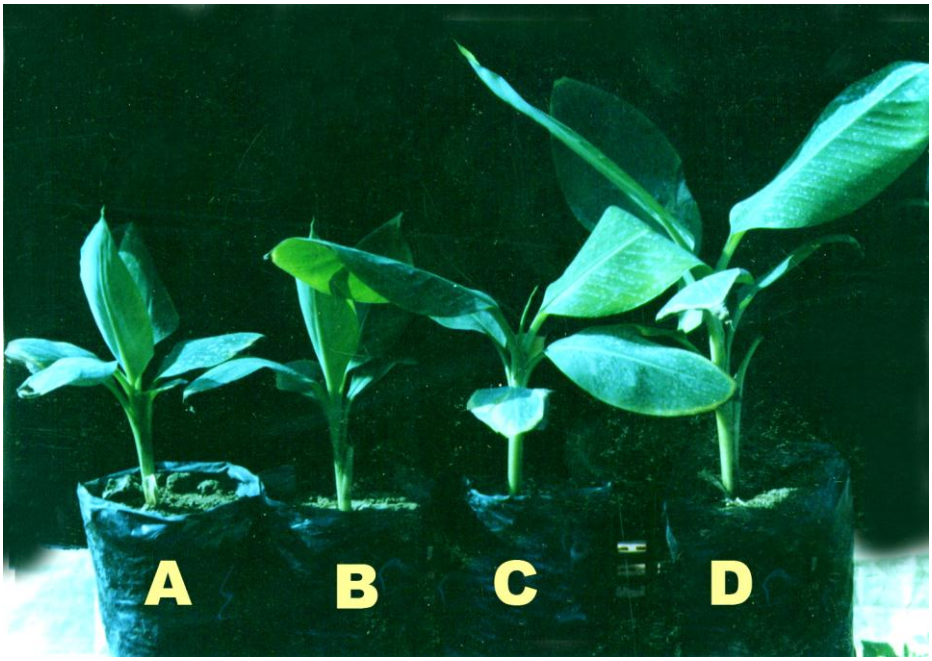
Table (21) and **Photo (24)** showed that the combination of yeast + adenine sulphate + coconut milk (2 g/L+ 0.025 g/L + 2 %) had significantly effect in increasing all growth and root parameters i.e. shoot length, shoot thickness, number of leaves, leaf length, leaf width, root length and number of roots. However, no statistical effect were noticed in shoot thickness between yeast + adenine sulphate (2 g/L + 0.025 g/L), adenine sulphate + coconut milk (0.025 g/L + 2%) and yeast + adenine sulphate + coconut milk (2 g/L + 0.025 g/L + 2%) treatments. Similarly, number of leaves / plant parameter of yeast + adenine sulphate (2 g/L + 0.025 g/L) and yeast + coconut milk (2 g/L + 2%) yeast + adenine sulphate + coconut milk (2 g/L + 0.025 g/L + 2%) treatments were used.

Generally, the above results can be recommended that combination of yeast + adenine sulphate + coconut milk (2 g/L + 0.025 g/L + 2%) are important for growth and root parameters.

Table (21): Effect of best organic additives combinations on growth and rooting parameters of acclimatized Grand Naine banana plantlets.

Parameters / Organic additives (ml/L)	Shoot length (cm)	Shoot thickness (mm)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Root length (cm)	No. of roots
Control	26.77 D	2.27AB	11.00 B	23.77 D	13.20 D	36.60 D	17.33 B
Yeast + adenine (2 g/L + 0.025 g/L)	27.53BC	2.30 A	12.00 A	24.57 C	13.70 C	38.80 C	18.33AB
Yeast + coconut (2 g/L + 2%)	27.70 B	2.23 B	12.00 A	25.13 B	15.03 B	41.00 B	18.33AB
Adenine + coconut (0.01 g/L + 2%)	27.20 C	2.30 A	11.33 B	24.50 C	13.37 D	36.83 D	17.67 B
Yeast+adenine+coconut (2 g/L + 0.025 g/L + 2%)	28.93 A	2.30 A	12.00 A	27.53 A	15.53 A	43.83 A	19.33 A

Means of different organ additives combinations followed with the same letter within each column are not significantly different from each other at 1% level.



A= Yeast + adenine sulphate (2 g/L + 0.025g/L)
B= Adenine sulphate + coconut milk (0.025g/L + 2%)
C= Yeast + Coconut milk (2 g/L + 2%)
D= Yeast + Adenine sulphate + Coconut milk (2 g/L + 0.025 g/L + 2%)

Photo (24): Effect of best organic additives combinations on growth and of acclimatized Grand Naine banana plantlets.