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MEDIUM CONSTITUENTS AS AFFECTING THE GROWTH OF *Cordyline terminalis* CV. ATOOM *IN VITRO*:
b- EFFECT OF AMINO ACIDS AND VITAMINS OF THE MEDIUM.

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ABSTRACT

In vitro-derived and sterilized shoots of *Cordyline terminalis* were used as explants in this study. In order to investigate the effect of some amino acids and vitamins (as medium constituents) on the growth of cordyline *in vitro*, a recommended medium by Awad et al, (2008a) was used in all experiments. That medium contained 60 g/ L banana pulp, ½ MS, 30 g/ L sucrose and 2 g/ L gelrite. As for the effect of amino acids on shoot number/explant, results indicate that sporadic responses were observed with the used treatments, but the addition of arginine at 25 or 50 mg/ L in the recommended medium replacing glycine significantly increased shoot number/explant in all records (three records) compared to the other treatments. However, Serine at 100 mg/ L significantly showed a similar high number of shoots in all three records. In that concern, glycine (at 2mg/L as in MS) showed lower response in the third record (after 4 months). Some sporadic treatments of amino acids surpassed glycine {as in MS medium (2mg/ L)} in enhancement the colour intensity of cultures. However, that significant increase in colour intensity was only observed during all records (three records) with glutamine at 100 mg/ L and serine at 25 mg/ L. Although, glycine (2mg/ L) observed a significant increase in the growth vigor in the third record only, but some other treatments showed the same high response in all records such as 100 mg/ L glutamine and asparagine at 50 or 100 mg/ L. Concerning the vitamins when examined each alone, medium free of vitamins (control) observed significant increase in shoot number/explant of cordyline compared to the medium contained thiamine at normal level as in MS (0.1mg/ L) or higher level (0.5mg/ L). Using pyridoxine alone at lower level 0.1mg/ L), than that used in MS medium (0.5mg/ L), showed significant high response in shoot formation especially at the third record (after 4 months from culturing). In the final record (3rd record) results show that nicotinic acid at (0.10 mg/ L) showed a significant increase in shoot number when compared to the higher one (0.50 mg/ L as in MS) and the control (free of nicotinic acid). It was clear that the presence of any of those vitamin alone at 0.1 or 0.5 mg/ L was effective in enhancement both colour intensity and growth vigor of cultures compared to control medium.

Combining more than one vitamin in the medium indicated that, although different combinations illustrated positive responses in all measured parameters (shoot number, colour intensity and growth vigor) but using any of those vitamins alone at 0.1mg/ L proved to be sufficient in increasing the shoot number and 0.1 or 0.5mg/ L enhanced both of colour intensity and growth vigor.

INTRODUCTION

Cordyline teminalis, Family: *Liliaceae* is an evergreen tropical perennial shrub with terminal tufts of elongated leaves, mostly grown in tropical southeastern Asia, Australia, Hawaii and Bangladesh. It is known as

Agnishwar in Bangladesh and traditionally used for the treatment of pain and traumatic injury. Leaves are used in inflammation and urinary infection. A thymidine like substances with antiproliferative activity was also isolated from *Cordyline terminalis.* Moreover, *Cordyline terminalis* is considered as one of the most common valuable ornamental plant because it combines the advantages of being adapted to grow in-and out door, and features special combination of colours. Plants synthesis vitamins and amino acids endogenously and these are used as catalysts in various metabolic processes. When plant cells and tissue are grown *in vitro,* some essential vitamins and amino acids are synthesized but only in suboptimal quantitites. Hence it is necessary to supplement the medium with required vitamins and amino acids to achieve the best growth of the tissue (Razdan, 1993). The objective of this study was to investigate the effect of some vitamins (at different concentrations) of the nutrient medium on the growth of cordyline *in vitro*, and examination the using of various concentrations of some different amino acids in the medium to replace glycine.

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory, Genetic Engineering and Biotechnology Research Institute, Minufyia University, Sadat City, during the years of 2003 – T2006.

Source of explants:

In vitro grown shoots of *Cordyline terminalis* were used as explants in this study.

Culture medium

The medium used in all experiments of this study was recommended by Awad et al, (2008a) as a result of the first part of this work. That medium consisted of 60 g/ L banana pulp, ½ MS medium, 1 mg/ L BA, 30 g/ L sucrose and 2g/ L gelrite. This medium was used to study the effect of different amino acids and **vitamins on the growth of cordyline *in vitro.***

This study was designed as following:

(1): Examination of different types of amino acids at different concentrations (in the recommended medium by Awad et al, 2008a) on the growth of *cordyline terminals in vitro.*

Fifteen treatments were carried out, using the chosen medium (60 g/ L banana + ½ MS) as following:

1- Control (medium free of glycine).

2- Adding glycine at normal concentration as in MS (2 mg/ L).

3- The treatments from 3 to 15 contained some other amino acids (to replace glycine), glutamine, asparagine, arginine and serine. These amino acids were added to the media each alone at the concentrations 25, 50, 100 mg/ L, and combinations between them at one concentration of each (25 mg/ L).

Each treatment consists of 10 replicates (Jars). Each Jar contained 30 ml of medium with one explant. Cultures were incubated for one month in growth room. Parameters were taken as, number of shoots, color intensity, growth vigor and number of roots.

These cultures were recultured and incubated for another month under the same conditions. The same parameters were taken again.

After second record the plants were left to grow in Jars without reculutring for two months, and then the previous records were taken.

(2): Examination of different concentrations of some vitamins in the chosen medium (60g/ L banana + $\frac{1}{2}$ MS) on the growth of *Cordyline terminals in vitro.*

This medium was used to carry out different experiments, including different treatments to examine various concentrations (0.0, 0.1, 0.5 mg/ L) of thiamine, pyridoxine and Nicotinic acid each alone and in combinations.

Each treatment consists of 10 replicates (Jars), and each jar contained 30 ml of medium with one explant. Cultures were incubated for one month under the same culture conditions in growth room. Parameters were taken as, number of shoots, colour intensity, growth vigor and number of roots. These cultures were recultured and incubated for another monthin the growth room.The same parameters were taken again.

After second record the plants were left to grow in the Jars without reculturing for two months, and then the previous records were taken.

Incubation conditions: Cultures of all experiments during this study were incubated under the temperature of 24 ^{o}C day and night. Light was provided by fluorescent tubes giving an intensity of 1500 lux at the level of culture jars for 16 hours per day.

All experiments were repeated twice and the represented data were averages. Results of these experiments were analyzed by analysis of variance (ANOVA) according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1- Effect of some amino acid on the growth of *Cordyline terminalis* explants *in vitro.*

a- Number of shoots

Data in Table (1) clearly indicate that sporadic responses were observed , but, the addition of arginine at 25 or 50 mg/ L in the chosen medium replacing glycine significantly increased shoot number/Jar in all records (three records) compared to the other treatments (Fig. 1). However, serine at 100 mg/ L significantly showed a similar high number of shoots in the three records. Interestingly, the free of amino acids (control) significantly observed similar positive result in shoot number as the same of arginine and serine. In that concern, Ghosh and Sen (1994) reported that the development of shoots from callus of *Asparagus plumosus* was on medium containing benzyladenine, L–arginine and level of NAA. Alka (2003) recorded that bulbils of *Dioscorea bulbifera* could be used for direct plantlet differentiation as well as bulbil differentiation on MS + 10.0 micro M IAA + 20.0 micro M Kinetin (Kin) + 30 mg/L Asp. (asparagine) + 30 mg/ L Arg. (arginine) + 30 mg/ L Glu (glutamine) + 10 mg/ L Ad. (adenime) + 10 mg/ L Cys hyd (Cysteine hydrochloride).

Fig, (1): Cordyline plantlets grown in the recommended medium contained 1/2 MS + 60 g/ L banana pulp and arginine at 50 mg/ L.

b- Colour intensity

Data in Table (1) explain that some amino acids surpassed glycine {as in MS medium (2mg/ L)} in enhancement the colour intensity, as glutamine at 100 mg/ L showed the significant increase in colour intensity in the three records. However, asparagine at 50 and 100 mg/ L observed the same significant increase in that concern in the 1st and 3rd records. The significant increase was recorded with the following treatments, serine at 25 mg/ L in the all records and at 50 or 100 mg/ L in the third record only, and arginine at 25 or 50 mg/ L in the third record.

All other treatments including the control (MS without amino acids) showed the lower records.

C- Growth vigor

Data in Table (1) indicate that glutamine (50mg/ L in 1st record and 100mg/ L in all records) and asparagine (50 and 100 mg/ L in all records) significantly observed the highest values of growth vigor. Moreover, arginine at 25 mg/ L significantly showed the value. However, the same significant increase was recorded with glycine treatment in the third record. All other treatments including the control (MS without amino acids) showed the lower records compared to the above mentioned treatments.

These results are in a harmony with that reported by Kamada and Harada (1979) mentioned that the effects of various L–amino acids on *in vitro* organogenesis were also investigated using Kno3 as the principal source of nitrogen in the medium, bud formation was considerably stimulated by alanine and asparagine and slightly by glutamic acid in a medium containing both NAA and BA (in which bud formation of *Torenia fournieri* was easily

induced). Alka (2003) recorded that bulbils of D*ioscorea bulbifera* could be used for direct plantlet differentiation as well as bulbil differentiation on MS + 10.0 micro M IAA + 20.0 micro M Kin + 30 mg/ L aspargine + 30 mg/ L Arginine + 30 mg/ L Glutamine + 10 mg/ L adenine + 10 mg/ L cysteine hydrochloride. Thiruvengadam et al, (2006) recorded that full strength MS medium containing 50 mg/ L PVP and 40 mg/ L glutamine was effective to achieve a high frequency of somatic embryo induction, maturation and further development of an embryogenesis suspension culture of bitter melon.

Table (1): Effect of different amino acids at different concentrations [in the chosen medium (60g/ L banana + 1/2 MS)] on shoot number, colour intensity and growth vigor of *Cordyline terminalis in vitro*.

Characters / Amino acids mg/ L		Shoot number			Colour intensity			Growth vigor		
		Records			Records			Records		
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Control (without Glycine)		abc 4.20	abcde 5.80	efg 9.20	de 2.80	efg 3.00	c 3.20	e 2.20	h 2.40	e 2.40
Glycine as in MS		ab 4.40	abcd 6.20	bc 16.20	bcd 3.20	cde 3.40	bc 3.40	bc 3.40	def 3.40	ab 3.80
Glutamine	25	abcd 4.00	abcde 5.60	def 9.80	de 2.80	bcd 3.60	c 3.20	cde 2.80	defg 3.20	de 2.60
	50	bcde 2.20	De 4.00	fg 8.40	abc 3.40	bcd 3.60	c 3.20	a 4.20	bcd 3.80	cde 2.80
	100	abcde 3.00	cde 4.60	Kg 7.40	a 3.80	a 4.40	ab 3.80	a 4.40	ab 4.40	ab 3.80
Asparagine	25	cde 2.00	de 3.80	fg 8.40	bcd 3.20	cde 3.40	bc 3.40	cde 2.80	fgh 2.80	de 2.60
	50	bcde 2.40	de 3.80	g 5.80	a 3.80	bc 3.80	ab 3.80	a 4.60	abc 4.20	a 4.00
	100	abcde 3.20	bcde 4.80	fg 6.60	ab 3.60	bcd 3.60	ab 3.80	a 4.20	a 4.60	a 4.00
Serine	25	De 1.80	e 3.60	g 5.40	ab 3.60	ab 4.00	a 4.00	cde 3.20	bcd 3.80	ab 3.80
	50	abcde 3.40	abc 7.00	cd 13.20	de 2.80	efg 3.00	a 4.00	bc 3.40	defg 3.20	abc 3.40
	100	abcde 3.80	ab 7.20	a 22.60	cde 3.00	def 3.20	a 4.00	cd 3.20	defg 3.20	ab 3.60
Arginine	25	a 5.00	a 7.80	a 21.00	cde 3.00	bcd 3.60	a 4.00	ab 4.00	cde 3.60	a 4.00
	50	abcde 3.60	a 7.60	ab 19.80	cde 3.00	g 2.60	a 4.00	cd 3.20	efgh 3.00	bcd 3.20
	100	e 1.60	de 4.20	cde 12.60	e 2.60	fg 2.80	c 3.20	cd 3.00	fgh 2.80	de 2.60
Glutamine 25+Asp 25 + Serine 25+ Arginine 25		abcde 3.20	abc 6.80	c 15.40	e 2.60	g 2.60	abc 3.60	de 2.60	gh 2.60	cde 2.80

Asp.= **Asparagine**

2- Effect of different vitamins (thiamine, pyridoxine, and nicotinic acid) at different concentrations in the chosen medium (60g/L banana + 1/2 Ms) on the growth of *Cordyline terminals in vitro.*

A- The effect on shoot number/explant

A1- Effect of single vitamin on number of shoot:

Regarding thiamine, data presented in Table (2) indicate that the higher thiamine concentration (0.5 mg/ L) and the normal level as in MS (0.10 mg/ L) resulted in significant increase in shoot number in the three records compared to the treatment devoid of vitamin in the 1st and 2nd records. However, the 3rd record of that medium free of vitamins showed the same high response as mentioned above. These results agreed with that reported by Le (1980) who stated that adventitious buds were obtained after 8 weeks when *Aeschynanthus hildebrandii* leaf fragments were cultured on Murashige and Skoog basal medium supplemented by 0.1 mg/ L thiamine, 0.5 mg/ L pyridoxin, 0.5 mg/ L nicotinic acid, 100 mg/ L myo-inositol, 0.01 mg/ L alpha NAA, 0.1 mg/ L IBA, 30 g/ L sucrose and 0.8 % agar. Khosh et al, (1984) mentioned that optimum shoot proliferation of myrtle was obtained on medium containing half strength. Murashige and Skoog plus (in mg/ L) nicotinic acid 0.5, pyridoxine HCL, 0.5, thiamine HCL 0.1, glycine 2.0, inosital 100, BA 1.5, and NAA 0.1. Sharma *et al.*, (1984) found that the best results were obtained when explants of date palm were cultured on modified MS medium containing activated Charcoal (0.3%), Na H_2 Po_4 (170 mg/ L), KH_2 Po_4 (200 mg/ L), 2,4-D (100 mg/ L), BA (5 mg/ L) and thiamine (1mg/ L). Kunisaki (1989) found that axillary shoots of Leucospermum hybrid were induced to proliferate in a liquid basal medium containing half strength MS inorganic salts, 0.25 mg/ L each of pyridoxine HCL and nicotinic acid, 0.2 mg/ L thiamine HCL, 2% sucrose, and 0.2 mg/ L BA.

Table (2): Effect of thiamine, pyridoxine and nicotinic acid, each alone, on shoot number/ explant of *Cordyline terminalis in vitro.*

Vitamin Concentration (mg/ L)	Single Vitamin								
	Thiamine			Pyridoxine			Nicotinic Acid		
	Record			Record			Record		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	b 2.53	b 4.87	a 12.24	a 2.73	b 5.00	b 12.67	a 2.84	a 5.64	b 11.93
0.10	a 2.98	a 5.80	a 12.93	a 3.09	a 6.04	a 14.47	a 3.02	a 5.42	a 13.96
0.50	a 3.22	ab 5.47	a 13.07	a 2.91	b 5.09	c 11.16	a 2.87	a 5.07	b 12.36

Concerning pyridoxine, data reveal that, the lower pyridoxine concentration (0.1 mg/ L) mostly showed the significant increase in shoot number in the three records (Fig. 2). Raising the pyridoxine concentration up to 0.50 mg/ L mostly decreased the shoot numbers in the 2nd and 3rd records

As for nicotinic acid, no clear trend can be observed during the 1st and 2nd records with all treatments of nicotinic acid, as they significantly observed similar responses. In the 3rd record data show that nicotinic acid at

(0.10 mg/ L) showed the significant increase in shoot number when compared to the higher one (0.50 mg/ L) and the control (free of nicotinic acid).

These results are in a harmony with that reported by Kunisaki (1989) who found that axillary shoots of Leucospermum hybrid were induced to proliferate in a liquid basal medium containing half strength of MS inorganic salts, 0.25 mg/ L each of pyridoxine HCL and nicotinic acid, 0.2 mg/L thiamine HCL, 2% sucrose, and 0.2 mg/ L BA.

Fig,, (2): Cordyline plantlets grown in the recommended medium contained 1/2 MS + 60 g/ L banana pulp and pyridoxine at 0.1 mg/ L.

A2 - Effect of combination between two vitamins on shoot number.

1-Between thiamine and pyridoxine

Data presented in Table (3) indicate that, Pyridoxine at 0.10 mg/ L alone showed the highest effect in that concern regardless thiamine during all the three records. However, the first record only showed that the presence of thiamine in the medium was important for shoot proliferation.

2- Between thiamine and nicotinic acid

Data in Table (4) indicate that thiamine at the concentration 0.10 mg/ L (in 2nd record only) or 0.50 mg/ L in all records, each alone, proved to be sufficient in increase shoot number. Moreover, when the same levels of thiamine were combined with nicotinic acid at the lower concentration (0.10 mg/ L) showed similar responses in the increase of shoot number.

Table (3): Effect of combination between two vitamins on shoot number of *Cordyline terminalis.*

1- Between thiamine and pyridoxine

Vitamins mg/ L		Record		
Thiamine	**Pyridoxine**	**1st**	**2nd**	**3rd**
0.00	0.00	C 2.20	d 4.13	de 10.67
	0.10	abc 2.87	ab 5.93	a 16.60
	0.50	bc 2.53	cd 4.53	e 9.47
0.10	0.00	ab 3.07	ab 5.73	b 14.20
	0.10	ab 3.07	a 6.53	bc 13.20
	0.50	abc 2.80	bcd 5.13	cde 11.40
0.50	0.00	ab 2.93	bcd 5.13	bc 13.00
	0.10	a 3.33	abc 5.67	bc 13.60
	0.50	a 3.40	abc 5.60	bcd 12.60

Table (4): Effect of combination between two vitamins on shoot number of *Cordyline terminalis.*

2- Between thiamine and nicotinic acid

Vitamins mg/ L		Record		
Thiamine	**Pyridoxine**	**1st**	**2nd**	**3rd**
0.00	0.00	cd 2.60	cd 5.13	d 10.47
	0.10	d 2.33	d 4.27	bcd 12.27
	0.50	bcd 2.67	bcd 5.20	abc 14.00
0.10	0.00	d 2.40	abcd 5.33	d 11.47
	0.10	abc 3.20	ab 6.33	a 15.40
	0.50	Ab 3.33	abc 5.73	cd 11.93
0.50	0.00	a 3.53	a 6.47	abc 13.87
	0.10	a 3.53	abc 5.67	ab 14.20
	0.50	cd 2.60	d 4.27	d 11.13

3- Between pyridoxine and nicotinic acid

Data in Table (5) indicate that, different treatments showed an increase in shoot number, but the only treatment which significantly observed

that increase during the three records was contained pyridoxine at 0.10 mg/ L combined with nicotinic acid at 0.10 mg/ L.

These results are in harmony with that reported by *Kunisaki (1989)* who found that axillary shoots of Leucospermum hybrid were induced to proliferate in a liquid basal medium contained half strength of MS inorganic salts, 0.25 mg/ L each of pyridoxine HCL and nicotinic acid, 0.2 mg/ L thiamine HCL, 2% sucrose, and 0.2 mg/ L BA.

.**Table (5): Effect of combination between two vitamins on shoot number of *Cordyline terminalis.***

3- Between pyridoxine and nicotinic acid.

Vitamins mg/ L		Record		
Thiamine	**Pyridoxine**	**1st**	**2nd**	**3rd**
0.00	0.00	b 2.47	b 4.87	bcd 11.67
	0.10	ab 3.00	b 5.53	bc 13.47
	0.50	ab 2.73	b 4.60	bc 12.73
0.10	0.00	ab 3.07	a 6.80	b 13.73
	0.10	a 3.20	ab 5.73	a 16.67
	0.50	ab 3.00	b 5.60	bc 13.00
0.50	0.00	ab 3.00	b 5.27	d 10.40
	0.10	ab 2.87	b 5.00	bcd 11.73
	0.50	ab 2.87	b 5.00	cd 11.33

A3- Effect of combination between thiamine, pyridoxine and nicotinic acid on shoot number/explant of *Cordyline terminalis*.

Data presented in Table (6) explain that no clear trend could be observed when the three vitamins were added to the medium at various combinations during the three successive records.

B- The effect of vitamins on colour intensity

B1- Effect of single vitamin on colour intensity.

Data presented in Table (7) indicate that.

(A) Mostly, the higher thiamine concentration (0.50 mg/ L) increased colour intensity in the three records, the lower concentration (0.10 mg/ L) significantly observed similar results in the 2nd and 3rd records.

(B) Pyridoxine at both concentrations (0.10 mg/ L and 0.50 mg/ L) increased colour intensity in the three records.

(C) Nicotinic acid at the two concentrations showed similar trend like that recorded with thiamine.

The treatments devoid of vitamins showed lower responses.

Table (6): Effect of the combination between thiamine, pyridoxine and nicotinic acid (mg/ L) on shoot number/explant.

Vitamins mg/ L		Nicotinic acid mg/ L								
Thiamine	Pyridoxine	1st Record			2nd Record			3rd Record		
		0.00	0.10	0.50	0.00	0.10	0.50	0.00	0.10	0.50
0.00	0.00	abc 2.47	abc 3.00	abc 2.70	fg 3.80	efg 4.00	cdef 4.60	hi 9.20	ghi 9.80	cdef 13.00
	0.10	abc 3.07	ab 3.20	abc 3.00	bcde 5.60	abcd 6.00	abcd 6.20	cdefg 13.00	a 18.00	a 18.40
	0.50	abc 3.00	abc 2.87	abc 2.87	abcd 6.00	g 2.80	bcdef 4.80	hij 9.20	ij 8.60	efgh 10.60
0.10	0.00	d 0.25	bc 2.20	abc 2.40	bcdef 5.40	abc 6.60	bcdef 5.20	bcde 13.20	ab 17.00	defgh 12.40
	0.10	abc 2.80	abc 2.80	abc 3.00	ab 6.80	abc 6.40	abc 6.40	bcde 13.80	abc 15.20	efgh 10.60
	0.50	abc 3.00	c 2.00	abc 2.60	fg 3.80	abcde 6.00	bcde 5.60	j 7.40	bcde 14.00	cdef 12.80
0.50	0.00	abc 2.40	a 3.40	a 3.40	bcde 5.40	abcd 6.00	efg 4.00	cdefg 12.60	bcdef 13.60	cdef 12.80
	0.10	abc 2.60	ab 3.20	a 3.40	a 8.00	bcde 4.80	defg 4.20	bcdef 14.40	abc 16.40	fghi 10.00
	0.50	bc 2.20	abc 3.00	ab 3.20	abcd 6.00	abcd 6.20	cde 4.60	abcd 14.60	cdef 12.60	efghi 10.60

Table (7): Effect of thiamine, Pyridoxine and nicotinic acid, each alone, on colour intensity of *Cordyline terminals* explants.

Vitamin Concentration mg/L	Single Vitamin								
	Thiamine (A)			Pyridoxine (B)			Nicotinic Acid (C)		
	Record			Record			Record		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	c 2.91	b 3.33	b 2.96	b 3.00	b 3.18	b 3.07	b 3.00	b 3.38	b 3.18
0.10	b 3.20	a 3.62	a 3.44	a 3.31	a 3.73	a 3.51	b 3.36	a 3.62	a 3.47
0.50	a 3.62	a 3.67	a 3.51	a 3.42	a 3.71	a 3.33	a 3.38	a 3.62	b 3.27

B2- Effect of combination between two vitamins on colour intensity.

1- Between thiamine and pyridoxine

Data presented in Table (8) indicate that, some different treatments were effective in enhancing the colour intensity of *Cordyline terminals* cultures, but the only treatment that showed that effect during the three records contained thiamine with pyridoxine at the higher concentrations of both (0.5 mg/ L).

2- Between thiamine and nicotinic acid

Data in Table (9) explain that, Sporadic responses were observed with some different treatments, but mostly results indicated the combination between thiamine and nicotinic acid was effective in enhancing the colour

intensity, except the treatment of thiamine alone at 0.5 mg/L that significantly showed similar higher effect in that concern.

Table (8): Effect of combination between two vitamins on colour intensity.

1- Between thiamine and pyridoxine

Vitamin Concentration mg/L		Record		
Thiamine	**Pyridoxine**	**1st**	**2nd**	**3rd**
0.00	0.00	f 2.60	e 2.93	c 2.33
	0.10	cd 3.20	ab 3.87	a 3.60
	0.50	de 2.93	de 3.20	b 2.93
0.10	0.00	ef 2.87	de 3.20	a 3.33
	0.10	bc 3.27	abc 3.73	a 3.53
	0.50	bc 3.47	ab 3.93	a 3.47
0.50	0.00	b 3.53	cd 3.40	a 3.53
	0.10	bc 3.47	bc 3.60	a 3.40
	0.50	a 3.87	a 4.00	a 3.60

Table (9): Effect of combination between two vitamins on colour intensity.

2- Between thiamine and nicotinic acid

Vitamin Concentration mg/L		Record		
Thiamine	**Nicotinic acid**	**1st**	**2nd**	**3rd**
0.00	0.00	e 2.60	d 3.20	d 2.87
	0.10	cd 3.20	bcd 3.47	cd 3.00
	0.50	d 2.93	cd 3.33	cd 3.00
0.10	0.00	cd 3.07	bcd 3.47	cd 3.07
	0.10	bc 3.33	ab 3.73	a 3.73
	0.50	cd 3.20	abc 3.67	ab 3.53
0.50	0.00	bc 3.33	bcd 3.47	a 3.60
	0.10	b 3.53	abc 3.67	a 3.66
	0.50	a 4.00	a 3.87	bc 3.27

3- Between pyridoxine and nicotinic acid

Data in Table (10) indicate that Pyridoxine at 0.10 mg/ L when combined with nicotinic acid at 0.10 mg/ L significantly increased colour intensity in the new growth of ***Cordyline terminals*** explants, in the three records.

Table (10): Effect of combination between two vitamins on colour intensity.

3- Between pyridoxine and nicotinic acid.

Vitamin Concentration mg/L		Record		
Pyridoxine	**Nicotinic acid**	**1st**	**2nd**	**3rd**
0.00	0.00	c 2.87	de 3.07	bc 3.13
	0.10	c 2.87	e 3.00	c 2.93
	0.50	b 3.27	bc 3.47	bc 3.13
0.10	0.00	c 2.80	cd 3.40	bc 3.13
	0.10	a 3.80	a 4.20	a 4.13
	0.50	b 3.33	bc 3.60	b 3.27
0.50	0.00	b 3.33	bc 3.67	b 3.27
	0.10	b 3.40	bc 3.67	b 3.33
	0.50	ab 3.53	b 3.80	b 3.40

B_3 - Effect of the combination between thiamine, pyridoxine and nicotinic acid on colour intensity:

Data presented in Table (11) clearly indicate that no clear trend could be observed when the three vitamins were added to the medium at various combinations during the three successive records.

C- The effect on growth vigor.

C1- Effect of single vitamin on growth vigor.

Data in Table (12) indicate that, Thiamine at both 0.10 mg/ L and 0.50 mg/ L mostly enhanced growth vigor when compared to control media, in the three records. The obtained results are in agreement with that reported by Beruto *et al.* (1983) who recorded that buds of *Cordyline terminals* were cultured on Murashige and Skooge medium with 0.5 mg/L thiamine HCL and 0.1 mg/ L BA for 3-4 weeks. Curir *et al.* (1990) mentioned that Embryogenesis occurred on medium containing glycine, calcium panthothenate, thiamine- HCL, myoinositol, zeatin and NAA.

Table (11): Effect of the combination between thiamine, pyridoxine and nicotinic acid on colour intensity

Vitamin Concentration mg/L		Nicotinic acid (mg/ L)								
Thiamine	Pyridoxine	1st Record			2nd Record			3rd Record		
		0.00	0.10	0.50	0.00	0.10	0.50	0.00	0.10	0.50
0.00	0.00	e 2.40	de 2.60	cde 2.80	g 2.60	bg 2.80	def 3.40	i 2.20	i 2.20	hi 2.60
	0.10	de 2.60	a 4.00	cd 3.00	cde 3.60	ab 4.40	cde 3.60	def 3.40	abc 4.00	def 3.40
	0.50	cde 2.80	cd 3.00	cd 3.00	def 3.40	defg 3.20	efg 3.00	fgh 3.00	gh 2.80	fgh 3.00
0.10	0.00	de 2.60	cd 3.00	cd 3.00	efg 3.00	defg 3.20	def 3.40	gh 2.80	cde 3.60	cde 3.60
	0.10	cd 3.00	a 3.80	cd 3.00	defg 3.20	a 4.60	def 3.40	fgh 3.00	ab 4.20	def 3.40
	0.50	ab 3.60	bc 3.20	ab 3.60	abc 4.20	def 3.40	abc 4.20	def 3.40	def 3.40	cde 3.60
0.50	0.00	ab 3.60	cd 3.00	a 4.00	cde 3.60	efg 3.00	cde 3.60	a 4.40	fgh 3.00	efg 3.20
	0.10	cde 2.80	ab 3.60	a 4.00	def 3.40	cde 3.60	bcd 3.80	fgh 3.00	ab 4.20	fgh 3.00
	0.50	ab 3.60	a 4.00	a 4.00	def 3.40	ab 4.40	abc 4.20	def 3.40	bcd 3.80	cde 3.60

Table (12): Effect of thiamine, pyridoxine and nicotinic acid, each alone, on growth vigor during three records

Vitamin Concentration mg/L	Single Vitamin								
	Thiamine			Pyridoxine			Nicotinic Acid		
	Record			Record			Record		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	c 2.53	b 3.00	b 2.84	b 2.89	b 3.16	b 2.71	a 3.02	a 3.47	ab 3.04
0.10	b 3.31	a 3.80	a 3.49	a 3.29	a 3.76	a 3.31	a 3.16	a 3.51	a 3.20
0.50	a 3.44	a 3.80	a 2.89	a 3.16	a 3.69	a 3.20	a 3.16	a 3.62	a 2.98

Pyridoxine at 0.10 and 0.50 mg/ L significantly increased growth vigor in the three records.

Nicotinic acid at 0.10 mg/ L significantly increased growth vigor in three records, followed by the higher (0.5 mg/ L) concentration. These results agreed with that reported by Soczek and Hempel (1988) who stated that shoots of the cultivars clementine, saskia and terravise were multiplied on MS media without or with the addition of thiamin, Pyridoxine, nicotinic acid myoinositol, tyrosine and adenine sulphate, in the original strength or in concentrations reduced to one-half or one-quarter.

C2: Effect of combination between two vitamins on the growth vigor.

1- Between thiamine and pyridoxine

Data in Table (13) indicate that thiamine at 0.10 mg/ L when combined with pyridoxine at 0.10 mg/ L or 0.50 mg/ L significantly enhanced the growth vigor in the three records. Similar result was obtained in the 1st record only when the medium contained the highest concentration (0.5mg/ L) of both vitamins. In that concern, Wang and Ma (1978) found that shoot apical meristem (0.2-0.1mm) of chrysanthemum when cultured on medium supplemented with myoinositol at 100 mg/ L, thiamine at 1 mg/ L, pyridoxine at 5 mg/ L, nicotinic acid at 5 mg/ L, NAA at 0.3 mg/ L, kinetin at 0.1 mg/ L, adenine sulphate at 40 mg/ L and malt extract at 400 mg/ L, first followed callus formation, followed by a single shoot which arose from the centre part of the explant.

Table (13): Effect of combination between two vitamins on the growth vigor. 1- Between thiamine and pyridoxine

Vitamin Concentration mg/L		Record		
Thiamine	**Pyridoxine**	**1st**	**2nd**	**3rd**
0.00	0.00	c 2.27	d 2.33	c 2.20
	0.10	b 3.00	b 3.60	a 3.47
	0.50	c 2.33	c 3.07	b 2.87
0.10	0.00	b 3.00	c 3.20	b 3.07
	0.10	a 3.47	a 4.07	a 3.60
	0.50	a 3.47	a 4.13	a 3.80
0.50	0.00	a 3.40	b 3.93	b 2.87
	0.10	a 3.40	b 3.60	b 2.87
	0.50	a 3.67	b 3.87	b 2.93

2- Between thiamine and nicotinic acid

Data in Table (14) indicate that thiamine at 0.10 mg/ L when combined with nicotinic acid at 0.10 or 0.50 mg/ L significantly increased the growth in the 2nd and 3rd records. Significantly similar result was obtained in the 1st and 2nd records when thiamine at 0.50 mg/ L combined with 0.50 mg/ L nicotinic acid. Lower responses were recorded with the other treatments. The importance if vitamin in the medium was reported by Wang and Ma (1978) who found that shoot apical meristem (0.2-0.1mm) of chrysanthemum when cultured on medium supplemented with myoinositol at 100 mg/L, thiamine at 1 mg/ L, pyridoxine at 5 mg/ L, nicotinic acid at 5 mg/ L, NAA at 0.3 mg/ L, kinetin at 0.1 mg/ L, adenine sulphate at 40 mg/ L and malt extract at 400 mg/ L, first followed callus formation, followed by a single shoot which arose from the centre part of the explant.

Table (14): Effect of combination between two vitamins on the growth vigor 2- Between thiamine and nicotinic acid

Thiamine mg/L	Nicotinic acid mg/L	Record		
		1st	2nd	3rd
0.00	0.00	cd 2.53	c 3.00	de 2.93
	0.10	c 2.73	c 3.07	e 2.80
	0.50	d 2.33	c 2.93	e 2.80
0.10	0.00	b 3.33	b 3.60	bcd 3.20
	0.10	b 3.33	ab 3.73	ab 3.47
	0.50	b 3.27	a 4.07	a 3.80
0.50	0.00	b 3.20	ab 3.80	cde 3.00
	0.10	b 3.40	ab 3.73	be 3.33
	0.50	a 3.87	ab 3.87	f 2.33

3- Between pyridoxine and nicotinic acid

Data in Table (15) indicate that pyridoxine at 0.10 mg/ L when combined with nicotinic acid at 0.10 significantly increased the growth vigor in the three records.

Table (15): Effect of combination between two vitamins on the growth vigor. Between pyridoxine and nicotinic acid.

Pyridoxine mg/L	Nicotinic acid mg/L	Record		
		1st	2nd	3rd
0.00	0.00	d 2.80	d 3.33	de 2.70
	0.10	d 2.80	e 2.93	cde 2.80
	0.50	bcd 3.07	de 3.20	e 2.60
0.10	0.00	cd 2.93	d 3.33	bc 3.13
	0.10	a 3.73	a 4.13	a 3.80
	0.50	bc 3.20	abc 3.80	bcd 3.00
0.50	0.00	b 3.33	bc 3.73	b 3.27
	0.10	cd 2.93	cd 3.47	bcd 3.00
	0.50	bc 3.20	ab 3.87	b 2.33

These results agreed with that reported by Soczek and Hempel (1988) who stated that shoots of the cultivars, clementime. saskia and terravise were multiplied on MS media without or with the addition of thiamin, pyridocine, nicotinic acid, myoinositol, tyrosine and adenine sulphate, in the original strength or in concentrations reduced to one-half or one quarter.

C3 - Effect on the combination between thiamine, pyridoxine and nicotinic acid on the growth vigor.

Data in table (16) explain that: no obvious or clear trend can be observed.

Table (16): Effect of combination between thiamine, pyridoxine and nicotinic acid on growth vigor of *Cordyline terminals.*

Vitamin Concentration mg/L		Nicotinic acid (mg/ L)								
Thiamine	Pyridoxine	1st Record			2nd Record			3rd Record		
		0.00	0.10	0.50	0.00	0.10	0.50	0.00	0.10	0.50
0.00	0.00	h 2.00	gh 2.20	fg 2.60	i 2.00	hi 2.40	ghi 2.60	c 2.00	de 2.20	de 2.40
	0.10	ef 2.80	a 4.00	gh 2.20	ef 3.40	bcd 4.20	efg 3.20	a 3.40	e 4.00	cde 3.00
	0.50	ef 2.80	h 2.00	h 2.20	def 3.60	ghi 2.60	fgh 3.00	b 3.40	e 2.20	e 3.00
0.10	0.00	def 3.00	cde 3.20	ef 2.80	fgh 3.00	efg 3.20	ef 3.40	b 2.60	bcd 3.40	bc 3.20
	0.10	def 3.00	a 4.00	bcd 3.40	efg 3.20	ab 4.60	abc 4.40	a 3.00	ab 3.80	a 4.00
	0.50	a 4.00	ef 2.80	abc 3.60	ab 4.60	ef 3.40	abc 4.40	a 4.00	a 3.20	bcd 4.20
0.50	0.00	bcd 3.40	def 3.00	ab 3.80	a 5.00	efg 3.20	def 3.60	b 3.60	cde 2.80	b 2.20
	0.10	def 3.00	cde 3.20	a 4.00	ef 3.40	def 3.60	cde 3.80	b 3.00	bc 3.60	bcd 2.00
	0.50	cde 3.20	a 4.00	ab 3.80	fgh 3.00	abc 4.40	bcd 4.20	b 2.40	f 3.60	b 2.80

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تأثير مكونات البيئة على نمو نبات الكلورديلين معملياً:
ب- تأثير الأحماض الأمينية والفيتامينات

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استخدمت أفرع نبات الكورديلين المعقمة والناتجة معملياً كأجزاء نباتية لبدء هذه الدراسة، ولدراسة تأثير كل من بعض الأحماض الأمينية والفيتامينات في البيئة على نمو الكورديلين معملياً، فقد أجريت هذه الدراسة باستخدام بيئة تضمنت ½ قوى أملاح موراشيج وسكوج ، ٦٠جم/لترمستخلص موز ، ٣٠ جم/لتر سكروز مع ٢جم/لتر جيلرايت (وهذه البيئة اقترحت لإكثار الكلورديلين معملياً في دراسة سابقة).

بالنسبة للأحماض الأمينية فقد ظهر أن أفضل النتائج من حيث عدد الأفرع كانت مع البيئة المحتوية على ٢٥ أو ٥٠ مجم/لتر أرجنين كبديل للجليسين الذي يستخدم في بيئة موراشيج وسكوج، وقد شجع الحامض الأميني سيرين بتركيز ١٠٠مجم/لتر تكوين الأفرع بدون فروق معنوية عن الأرجينين، أما استخدام الحامض الأميني جليسين بنفس تركيزه في بيئة موراشيج وسكوج (٢مجم/لتر) فقد أظهر نتائج أقل من حيث تشجيع تكوين الأفرع مقارنة بالأرجينين والسيرين، وقد كان جدير بالملاحظة أن بيئة معاملة المقارنة (بدون أحماض أمينية) كان لها تأثيراً معنوياً مشابه لكل من الأرجينين والسيرين على عدد الأفرع الناتجة خلال القراءة الأولى (بعد شهر) والثانية (بعد شهرين) إلا أن هذا التأثير انخفض معنوياً أيضا مقارنة بتأثير نفس الأحماض الأمينية السابقة، وذلك عند القراءة الثالثة (بعد ٤ شهور من الزراعة).

أما بالنسبة لقوة وكثافة اللون بالأفرع الناتجة فبالرغم من أن معاملات مختلفة من الأحماض الأمينية كانت مؤثرة معنوية لزيادة قوة اللون مقارنة بالجليسين المستخدم بنفس تركيزه فى موراشيج وسكوج، لكن التأثير العالي معنوياً في قوة اللون خلال كل الثلاث قراءات كان مع كل من المعاملتين المحتويتين على إما ١٠٠مجم/لتر جلوتامين أو ٢٥مجم/لتر سيرين.

بالنسبة لقوة النمو، على الرغم من الجليسين (٢مجم/لتر) كان مشجعا لزيادة قوة النمو وظهر ذلك خلال القراءة الثالثة (الأخيرة) فقط، ولكن هناك معاملات أظهرت نفس التأثير الإيجابي معنوياً خلال كل الثلاث قراءات مثل المعاملة الخاصة بالجلوتامين (١٠٠مجم/لتر)، اسبراجين بتركيز ٥٠ أو ١٠٠ مجم/لتر.

أما بالنسبة لتأثير الفيتامينات فعند إضافة كل منهما على حدة إلى البيئة أظهرت النتائج أن إضافة الثيامين إلى البيئة بتركيز ١.٠ مجم/لتر (نفس التركيز في بيئة موراشيج وسكوج) أو بتركيز أعلى (٠,٥ مجم/لتر) شجع تكوين الأفرع ولكن بدون فرق معنوي عن البيئة الخالية من الفيتامينات (معاملة المقارنة) ، أما البيريدوكسين فعند أضافته للبيئة منفرداً بتركيز ٠,١ مجم/لتر (كتركيز منخفض عن تركيزه في بيئة موراشيج وسكوج" ٠,٥ مجم/لتر") أظهر تشجيعاً معنوياً لتكوين الأفرع عند القراءة الثالثة (بعد ٤ أشهر من الزراعة بالمعمل).

وأما حمض النيكوتنيل فقد أظهرت النتائج (بعد ٤ شهور من الزراعة) أن أضافته منفرداً بتركيز ٠,١ مجم/لتر اظهر زيادة معنوية في عدد الأفرع الناتجة مقارنة بالتركيز الأعلى (٠,٥ مجم/لتر كما في موراشيج وسكوج) أو الكنترول (بدون فيتامين).

أما بالنسبة لدرجة لون النموات الناتجة وكذلك قوة النمو فقد أظهرت النتائج أن إضافة أي من هذه الفيتامينات منفردا عن تركيز ٠,١ أو ٠,٥ مجم/لتر اظهر تأثيرا إيجابيا في درجة لون النموات وكذلك قوة النمو مقارنة بالكنترول (بدون فيتامينات) والذي اظهر تأثيرا اقل في درجة اللون وقوة النمو.

ورغم أن بعض المعاملات التي تضمنت أكثر من فيتامين (اثنين أو ثلاثة) أظهرت نتائج إيجابية لكل الصفات (عدد الأفرع وقوة اللون وقوة النمو) إلا أن استخدام أي منهما منفرداً بتركيز ١.٠مجم/لتر كان كافيا لتشجيع تكوين الأفرع أو بتركيز ٠,١ أو ٠,٥ مجم/لتر لزيادة قوة اللون والنمو.

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