Micropropagation and Genetic Variations of *Cordyline terminalis* cv. Red Top

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ABSTRACT: Due to the slow, expensive and time - consumed conventional propagation of Cordyline , and the merits of *in vitro* propagation for overcoming on these problems. The present study was undertaken. Simple protocol for *in vitro* regeneration of *Cordyline terminalis* cv. Red Top using shoot tip explants was achieved. For initiation stage , shoot tip explants were cultured on MS medium fortified with both 3% sucrose (w/v) and 0.2% gelrite (w/v). As for multiplication stage, the new formed axillary shoots were cultured on MS fortified with various concentrations of benzyladenine (BA; 0,1, 3, 5 and 7 mg/l) and thidiazuron (TDZ; 0, 0.5 ,1, 1.5 and 2 mg/l) in the presence of 1 mg/l Kinetin (Kin) were used. As for shoot elongation and *in vitro* rooting stage, new formed shoots derived from multiplication stage were culture on MS-PGRs -free medium gelled with 2% (w/v)gelrite . Concerning the acclimatization stage, the rhizogened plantlets were potted in mixture media consists of peat moss and vermiculite (1:1 %). The survival percentage of new formed plantlets after acclimatization was 100 % of the treatments; 1 mg/l BAP +1 mg/l Kin, 7 mg/l BAP +1 mg/l Kin and 1.5 mg/l TDZ +1 mg/l Kin under the light intensity 30 µmol m^{-2} s^{-1}. The procedure outlined has fulfilled the requirements for micropropagation of Cordyline. Randomly amplified polymorphic DNA (RAPD) analysis showed that the polymorphism level was the same at all primers (100%).
Keywords: cordyline terminalis, Micropropagation, regeneration, RAPD, somaclonal variation

INTRODUCTION

Cordyline, Laxmanniaceae, are evergreen perennial shrubs or sub-shrubs; stems clustered, simple or sometime branched with spiral and linear simple evergreen leaves which colored from purple to red or green. Cordyline shrubs are often clustered and palm-like in appearance and originated to Western Pacific Region. The common name of Cordyline is cabbage palm and the generic name is derived from Greek word 'Kordyle' meaning club or cudgel referring to the shape of the roots (Pal, 2006). Cordyline species have creamish white roots that often form root suckers starting from their rhizomes (Ehrlich, 1999; Khan *et al*., 2004 and Kattoor, 2010). It is one of the most economically important ornamental houseplant.

Shoot cuttings and rhizomes are used for the conventional propagation of Cordyline species. However, propagation of Cordyline using rhizome or shoot cuttings is slow, expensive and time consumed plants. Nevertheless, it is known that *in vitro* propagation is very economical method of multiplying a desirable plant species when time, space and laborers are often serious constraints. Also, plant tissue culture technique helps to produce disease- free- uniform plantlets at a required quantity and all the year around. Although, there are several reports on *in vitro* propagation of *Cordyline terminalis* (Kunisaki, 1975; Debergh, 1976; Mee, 1978; Paek *et al*., 1985; Khan *et al*., 2004; Ray *et al*., 2006, 2013 and Kattoor, 2010). However, the micropropagation technique is not the optimal to satisfy the requirements for this plant. Likewise, investigating the genetic variation and fidelity among *in vitro* produced plantlets and mother plant is in

need for further investigation. Genetic fidelity among tissue culture-derived plants of fruit and vegetable crops is very important because of those plants should be true -to- type. However, the variations among plants produced by micropropagation techniques hold ample scope in improvement of ornamental plants. Nevertheless, genetic variation of individual plants within *in vitro* mass propagation is a major advantage of clonal cultivars in commercial production (Krishna and Singh 2013). Larkin and Scowkraft (1981) provided a general term called ‘‘somaclonal variation’’ for plant variants derived from *in vitro* cell or tissue culture. In addition, plant cell and other tissue cultures techniques; could increase genetic variability comparatively faster and without applying a complex technology (Krishna *et al*., 2016). Several instances of genetic variations have been reported in improving ornamental plants (Jain *et al*., 1998).

There are several tools are available for the detection of genetic variations and fidelity which are primarily based on the differences in morphological traits (Nhut *et al.* 2013), cytogenetical analysis (Abreu *et al*., 2014), biochemical (Kar *et al.* 2014) and molecular DNA markers (Bello-Bello *et al*., 2014; and Elmahrouk *et al.,* 2016). Random amplified polymorphic DNA (RAPDs) is a molecular marker that allows the scanning of the genome for genetic changes (Williams *et al.,* 1991). The previous reports have mentioned that RAPD-based detection of genetic polymorphism has been successful in describing somaclonal variability or homogeneity of micropropagated individuals of numerous plant species (Cheruvathur *et al.,* 2013; Haque and Ghosh, 2013 and Kumar *et al.,* 2013). The objective of the present study aims to develop an efficient protocol for *in vitro* propagation of Cordyline terminals cv. Red Top and divulge or detection the genetic variations among *in vitro* -derived -plants using RAPD technique.

MATERIALS AND METHODS

Plant material and culture establishment

Stem with shoot tip explants (4-6 cm) were excised and defoliated from *Cordyline terminalis* Red Top plants, washed under running tap water for 10 min, rinsed three times with sterile distilled water, then surface disinfected for one min. In 70% ethanol followed by immersing for 10 min in a 0.1% (w/v) mercuric chloride solution containing 2-3 drops of Tween 20 (polyoxyethylenesorbitan monolaurate). After three rinses with sterile distilled water, explants were sectioned to 2 cm segments containing two to three nodes and cultured in 375-ml jars, each containing 50 ml of basal MS medium (Murashige and Skoog, 1962) with 3% (w/v) sucrose and 0.2% (w/v) gelrite to induce pre-exitising axillary buds to form new axillary shoots. The pH of all tested media was adjusted to 5.8 before autoclaving at 121°C and 118 kPa for 15 min. All cultures were incubated for 4 weeks at 25 ± 1°C under a 16-h photoperiod provided by cool-white fluorescent light at 30 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD).

Shoot multiplication stage

New formed axillary shoot (1.5 cm long) were cultured on basal MS medium containing 1 mg/l Kinetin (Kin) and supplemented with various concentrations of BAP (benzyladenine ;0,1, 3, 5 and 7 mg/l) or TDZ (thidiazuron;0, 0.5 ,1, 1.5 and 2 mg/l). However, MS basal medium without PGRs served as a control treatment. Both BA and TDZ were added prior to autoclaving the media. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C and 118 kPa for 15 min. Explants were cultured in 250-ml jars containing 25 ml of the tested media. There were three explants per jar and five replicates per treatment. Cultures were incubated at 25 ± 1°C and 16-h photoperiod provided by cool-white fluorescent light under two level of PPFD (30 and 60 µmol m^{-2} s^{-1}). After 4 weeks, number of shoots per explant, shoot length, number of leaves per shoot, number of roots per shoot and fresh weight were recorded.

Shoot elongation and *in vitro* rooting stages

Shoots (2-3 cm long) of *C. terminals* were separated and cultured on MS medium fortified with gelrite (0.2% ;w/v) without plant growth regulators (PGRs) for subsequent growth and root induction. Medium preparation and culture conditions were identical to shoot induction stage.

Acclimatization

Micropropagated plantlets (three to five leaf stage) of *C. terminals* were transplanted into culture pots (plastic coffee cups with three holes at the bottom) filled with sterilized mixture of peat moss and vermiculite mixture (1:1). Before transplanting, all plantlets were treated with 0.1% Rezolix (a fungicide). The plantlets were covered with clear plastic film during the first 15 days of culture in growth chamber to maintain relatively high relative humidity. The environment of the growth chamber was 28 ± 2°C air temperature and 70% relative humidity (RH). Plants were irrigated once a week and a compound fertilizer (20: 20: 20; N: P_2O_5: K_2O) at 1 g L^{-1} was applied twice after 3 and 6 weeks. Survival percentage and growth parameters (i.e. plant height, number of leaves/plant, fresh weight and number of roots) were determined after 8 weeks in the growth chamber.

Photosynthetic parameters

The contents of chlorophyll (Chl) a, Chl b, total Chl and total carotenoids in the fully expanded young leaves of 8-week-old plants were determined using (Spectronic instruments, spectronic 20 D+,115V, 60HZ, 1.0A, lr 45227,Bamko-Surplus company, USA). Chlorophyll contents was extracted from 0.1 g fresh leaf tissue by 10 mL N, N, Dimethyl formamide in a dark at 4 °C for 24 hrs. The absorbance of the extracts was measured at a wave length of 480, 647 and 664 nm. Chlorophyll and carotenoid concentrations were calculated from spectrophotometric data using the formulae of Moran (1982) and Torres *et al*. (2014).

Plant DNA extraction and RAPD polymerase chain reaction conditions

DNA was extracted from fresh leaves (three and four leaves on the plant) of the mother plant and acclimatized plants of all tested treatments by hexadecyltrimethylammonium bromide (CTAB) according to modified protocol of Keb-Llanes *et al* .(2002). Polymerase chain reaction (PCR) was performed and repeated three times using six random decamer primers (Table 1). RAPD-PCR was carried out in presence of 10 µl master mex 2X (i-Taq ^{TM}, Intron Biotechnology),1 µl primer (10 pmol) ,3 µl DNA sample (150 ng) in a total volume of 20 µl. PCR amplification was performed in an automated thermal cycler (Techne, UK) programmed as follows: 95 ºC for 4 min followed by 40 cycles of 1 min for denaturation at 94 ºC, 30 s for annealing at 35 ºC and 2 min for polymerization at 72 ºC, followed by a final extension step at 72 ºC for 7 min. The amplification products were tested by electrophoresis in 1.5% agarose gels in 0.5 X Tris-EDTA Acetic acid (TEA) buffer and 4 µl Ethidium bromid. Finally the gel was visualized with a UV transilluminator at 254nm.

Experimental design and data analysis

Experiments were set up as a factorial experiment outlined in a completely randomized design. Both two factors under the study were as follows: the first factor was 10 treatments of PGRs = Control (PGRs -free- MS medium), with 1 mg/l kin alone, 4 BA concentrations with 1 mg/l kin and 4 TDZ concentrations with 1 mg/l kin; meanwhile, the second factor was two levels of light intensity). The means and ANOVA were calculated using SAS program, version 6(1985) statistical software. The mean separations were carried out using least significant difference tests (LSD) and significance was determined at $p \leq 0.05$, 0.01, and 0.001. Amplification profiles generated by all the different RAPD primers (three replicates) were scored as presence (1) and absence (0) of a band of a particular molecular size to compile a binary matrix which was then subjected to cluster analysis. Both faint and intense bands were scored if shown to be reproducible in separate runs.

Table (1). primers list and their nucleotide sequences

Primer name	Sequence (5' → 3')
OPB-01	GTTTCGCTCC
A09	GGGTAACGCC
P6	AGGGGTCTTG
P5	TGGACCGGTG
G03	GAGCCCTCCA
G10	AGGGCCGTCT

The similarity matrix was estimated using Jaccard's genetic similarity index (Jaccard, 1908). A dendrogram was generated by cluster analysis with the unweighted pair–group method using the arithmetic averages [UPGMA] (Jain and Dubes, 1988). PAST statistics software version 2.17 package was used to analyze the data (Hammer *et al.,* 2003).

RESULTS AND DISCUSSION

Effect of cytokinins and light intensity on *in vitro* shoot multiplication stage:

Shoot multiplication stage of Cordyline was ,significantly, influenced by type and concentration of cytokinins levels, but light intensity had a non-significant effect (Table 2). The highest mean shoot number (4 shoot per explant) was obtained on MS medium (Murashige and Skoog,1962) supplemented with 1.5 mg/l TDZ + 1 mg/l Kin. On the other hand, MS-free, both Kin and BA were less effective than that with TDZ for shoot multiplication of *C. termenalis*. The tallest shoot (7.43 cm) was observed on MS medium augmented with 0.5 mg/l TDZ+ 1 mg/L Kin; whereas, the lowest shoot length were obtained at high TDZ concentrations. However, TDZ-induced morphogenesis, which probably depends on the concentration of hormones and modulates the endogenous auxin level (Huetteman and Preece, 1993; Siddique and Anis, 2007). Hormone TDZ considers the best selection for *in vitro* propagation of many plant species because of its tremendous ability to stimulate shoot proliferation (Ruzic and Vujovic, 2008 and Sharma *et al.*, 2012). In comparison with the most of the other active compounds added to the media, suitable concentrations of TDZ stimulate axillary shoot proliferation of many plant species. The previous reports mentioned that TDZ effect depends on the concentration and the duration of its application (Hvoslef-Eide and Preil, 2005 and Alatar, 2015). Therefore, the morphogenetic response of *C. termenalis* was achieved on MS medium fortified with 1.5 mg/l TDZ + 1 mg/l Kin for shoot proliferation and 0.5 mg/l TDZ + 1 mg/l Kin for shoot length. Similar results ,more or less, were mentioned by Elmahrouk *et al.* (2016) who reported that 1.5 mg/l TDZ; enhanced shoot multiplication of *Aglaonema*. On the contrary, Chen and Yeh (2007) reported that higher concentrations of TDZ decreased shoot elongation. Regarding the main effect of light intensity, the gained results revealed that it did not has significant effect on number of shoot and shoot length. Similarly, Fiola *et al.* (1990) found that light intensity from 0-81 µmol m^{-2} s^{-1} of continuous fluorescent light did not affect the percentage of *Rubus* organogenesis. The highest number of leaves /propagule (7.65) was observed on MS medium plus 0.5 mg/l TDZ+ 1 mg/l Kin. Also, 30 µmol m^{-2} s^{-1} improved the leaves' number as 5.89 leaves/propagule. Singh and Dwivedi (2014) suggested that low concentration of TDZ promoted shoot formation and produced the highest number of leaves, and *vice versa*.

Table (2). Effect of cyokinins and light intensity on *in vitro* shoot multiplication of *Cordyline terminalis* cv. Red Top after 8 weeks in culture

Cytokinins (mg/l)	Number of shoot			Shoot length (cm)			Number of leaves			Fresh weight (g)		
	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average
Control	1	1	1e	6.03	8.52	7.28ab	6.28	6.65	6.47ab	1.15	2.00	1.58ab
1 kin	2.2	2.2	2.2bc	4.89	3.59	4.24cd	4.95	3.63	4.29c	1.72	1.13	1.42ab
1BAP+1Kin	1.6	3.4	2.5bc	6.18	2.53	4.36cd	6.63	2.69	4.66c	1.05	1.36	1.20b
3BAP+1Kin	1	1.2	1.1e	4.56	3.98	4.27cd	7.8	3.3	5.55bc	1.78	0.88	1.33ab
5BAP+1Kin	1.2	2.4	1.8cd	6.39	3.94	5.17bcd	5.2	3.6	4.4c	1.36	1.23	1.29b
7BAP+1Kin	1.2	1.2	1.2ed	5.88	5.83	5.86abc	6.2	5.1	5.65abc	1.57	1.32	1.44ab
0.5TDZ+1Kin	1	1.6	1.3ed	7.02	7.84	7.43a	8.6	6.7	7.65a	1.25	1.85	1.55ab
1TDZ+1Kin	3	2.6	2.8b	4.84	6.39	5.62abc	5.75	4.03	4.89bc	2.19	1.89	2.04a
1.5TDZ+1Kin	4.6	3.4	4a	2.9	3.43	3.16d	3.80	4.42	4.11c	1.65	1.62	1.64ab
2TDZ+1Kin	2.8	2.8	2.8b	3.31	2.94	3.12d	3.68	4.40	4.04c	1.21	0.99	1.10b
Average	1.96a	2.18a		5.2a	4.9a		5.89a	4.45b		1.49a	1.43a	
LSD values at 0.05 level												
Light intensity(L)		[Y]ns			ns			0.18			Ns	
Cytokinins (C)		0.28			2.16			0.39			Ns	
L × C		ns			ns			ns			Ns	

[Y]ns = non-significant

The heaviest shoot (2.04 g) was observed upon culture on MS medium fortified with 1 mg/l TDZ + 1 mg/l Kin, while there was no significant differences between light intensities on shoot fresh weight. Sajid and Aftab (2009) found that the highest fresh and dry weights of potato were observed on MS medium containing low concentration of TDZ. The analyzed and presented results indicated that interactions between the tested levels cytokinin levels and light intensities did not have significant effect on all the given traits. The highest root number (8.5) was observed on control medium in light intensity with 60 µmol m^{-2} s^{-1}. Also, high TDZ concentration, control medium and that fortified with 1 mg/l Kin; enhanced root formation under both light intensities. In contrast, BA had negative effect on root formation. This finding could be attributed to the mode of action of cytokinins in this respect (i.e. antagonistic effect on root formation).

Effect of MS -PGRs-free medium on *in vitro* shoot proliferation, elongation and rooting

Shoot clusters of *C. terminals* were cultured on MS -PGRs-free medium for their subsequent growth and elongation. Shoot multiplication and vegetative growth were increased when responded explants were transferred to a cytokinin - free -MS medium (Table 3 and Figures 1a and 1b). Subculturing process had a significant effect ($p \leq 0.05$) on shoot proliferation of different treatments. Therefore, the highest shoot number (13.4) was observed of explants derived from organogenesis on MS medium fortified with 3 mg/l BA + 1 mg/l Kin. Nevertheless, there was no significant differences regarding tested light intensities on shoot multiplication, but the interaction between the tested light intensities and cytokinin levels; gave a positive response on shoot number with 21 shoot/ explant upon culture on MS medium supplemented with 3 mg/l BA + 1 mg/l Kin at 60 µmol m^{-2} s^{-1}. In contrast, explants responded on MS-free- PGRs

medium gave the lowest shoot number (1.1 shoot/explant) with tallest shoot (12.4 cm). Also, low light intensity (30 µmol m^{-2} s^{-1}) increased shoot length (7.04 cm). The best result of shoot length (13.57 cm) was obtained on MS- free-PGRs medium kept under the highest light intensity 60 µmol m^{-2} s^{-1}. The highest number of leaves (11.08) was recorded on control MS medium. Also, the interaction between 0.5 mg/l TDZ + 1 mg/l Kin and 60 µmol m^{-2} s^{-1}; increased leaves' number (14.36). The heaviest shoots (6.14 and 6.6 g) were obtained on MS medium supplemented with 1 mg/l TDZ +1 mg/l Kin and 1.5 mg/l TDZ +1 mg/l Kin, respectively. In addition, the interaction between the tested cytokinin levels and light intensities, had significant effect ($p \leq 0.05$) on fresh weight. Thus, the treatments of MS medium supplemented with 1 mg/l TDZ +1 mg/l Kin and 1.5 mg/l TDZ +1 mg/l Kin under 30 µmol m^{-2} s^{-1}; led to high fresh weights as 7.61and 8.56 g, respectively. Generally, light intensities did not have significant effects on numbers of leaves and fresh weight. In general, over-supply of cytokinins can be associated with abnormal or unusual organ development (Ramage and Williams, 2004), such as stunting or fasciation of shoots (Toponyanont *et al*. 1999). The abnormal plant growth associated with high TDZ concentrations has been reported by Dewir *et al.* (2006) and Elmahrouk *et al*. (2010) in *Spathiphyllum cannifolium* and *Arbutus unedo*, respectively. Therefore, subculturing of the new formed shoots on MS-PGRs-free medium resulted in reduce endogenous plant hormons which accumulated in the plant tissues. Hence, number of shoots and vegetative growth traits increased after subculture on MS-free- medium. Similar observation was, also, documented in *Rauvolfia serpentine* (Alatar, 2015).

Table (3). Effect of MS -free PGRs- medium on *in vitro* shoot proliferation and elongation of different treatments of *Cordyline terminalis* cv. Red Top after 8 weeks in culture

Cytokinins (mg/l)	Number of shoot			Shoot length (cm)			Number of leaves			Fresh weight (g)		
	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average
Control	1.2	1	1.1e	10.5	13.57	12.04a	11.5	10.67	11.08a	1.77	2.78	2.28ef
1 kin	4.2	2.6	3.4cd	9.48	8.53	9.01bc	7.73	9.81	8.77b	3.4	3.56	3.48cde
1 BAP+1 Kin	1.4	5.2	3.3d	7.05	2.65	4.85e	10.87	7.01	8.94b	1.52	2.1	1.81f
3 BAP +1 Kin	5.8	21	13.4a	2.59	2.16	2.38 f	8.25	8.79	8.52b	1.7	2.37	2.03ef
5 BAP +1 Kin	15.8	3.8	9.8b	1.93	2	1.96f	5.42	5.00	5.21c	3.86	4.01	3.94 bc
7 BAP +1 Kin	4	4.2	4.1c	3.06	4.82	3.94e	7.42	8.52	7.97b	2.22	2.98	2.6def
0.5 TDZ+1Kin	5.2	2.4	3.8cd	6.66	6.11	6.38d	5.08	14.36	9.72b	2.45	2.29	2.37def
1 TDZ +1 Kin	4.6	2.8	3.7cd	8.28	8.18	8.23c	9.04	7.78	8.41b	7.61	4.67	6.14ab
1.5 TDZ+1Kin	4.2	3.8	4c	8.94	10.71	9.82b	8.64	9.08	8.86b	8.56	4.65	6.60a
2 TDZ +1 Kin	3	3.8	3.4cd	11.87	6.41	9.14bc	10.81	7.31	9.06b	5.7	3.69	4.69bc
Average	4.94a	5.06a		7.04a	6.51b		8.48a	8.83a		3.88a	3.31a	
LSD values at 0.05 level												
Light density (L)		[Y]ns			0.51			ns			Ns	
Cytokinins(C)		0.22			1.14			0.31			1.61	
L × C		0.32			1.62			0.44			2.27	

[Y]ns = non-significant

After subculture of the new formed shoots for 8 weeks on MS medium without PGRs for their rooting; all shoots grown on medium containing 1 mg/l

kin and TDZ were rooted under both light intensities (Figure 2B). The highest root number (11 roots/shoot) was obtained on MS medium supplemented with 2 mg/l TDZ +1 mg/l Kin under light intensity of 30 µmol m^{-2} s^{-1}. Although, shoots enhanced on BA gave roots but, the number of roots was few. Regardless of the number of roots/propagule ; all plantlets of all treatments were rooted on MS-free PGRs - medium. Paek *et al*. (1985) mentioned that using 2.0 or 3.0 mg/l IBA was an important factor for increasing rooting percentage in micropropagation of *Cordyline*. However, in the present work, addition of auxins was not required for *Cordyline* rooting. This finding could be account for achieving the appropriate ratio between exogenous cytokinins and endogenous auxines within plant tissues, hence achieved the given rhizogenesis. Similar observation was, also, reported in *Cordyline fruticosa* (Dewir *et al*., 2015). The previous studies indicated that shoot elongation and rooting of palm cultivars were achieved on PGR -free- medium (Mazri 2015; Mazri *et al*., 2016).

Acclimatization stage

new formed plantlets with fully expanded leaves and well-developed roots (Figure 1c) were transferred into pots containing sterile medium (Figure 1d).The obtained data showed that regenerants derived from high TDZ concentrations (1mg/l TDZ +1 mg/l Kin, 1.5 mg/l TDZ +1 mg/l Kin and 2 mg/l TDZ +1 mg/l Kin); gave the tallest

Fig (1). Regeneration steps of *Cordyline terminalis* cv. Red Top, a) shoot multiplication on MS- free PGRs- medium of responded explants on Benzyl adenine (BA); b) shoot elongation on MS-free PGRs-medium; c) plantlets during rooting stage on MS -free PGRs-medium; d) plants tissue culture -derived

plants with 12.3, 15.89 and 15.76 cm, respectively, while these 5 mg/l of BAP +1 mg/l Kin and 0.5 mg/l TDZ +1 mg/l Kin media had no significant effects on plant height (Table 4). The interaction between light intensities and cytokinin concentrations had significant effect on plant height and number of leaves, whereas both characters expressed similar trends. The highest number of leaves (11) was observed on MS medium plus 1.5 mg/l TDZ +1 mg/l Kin, while the lowest one (2.5) was obtained due to growth on MS medium plus 0.5 mg/l TDZ +1 mg/l Kin.

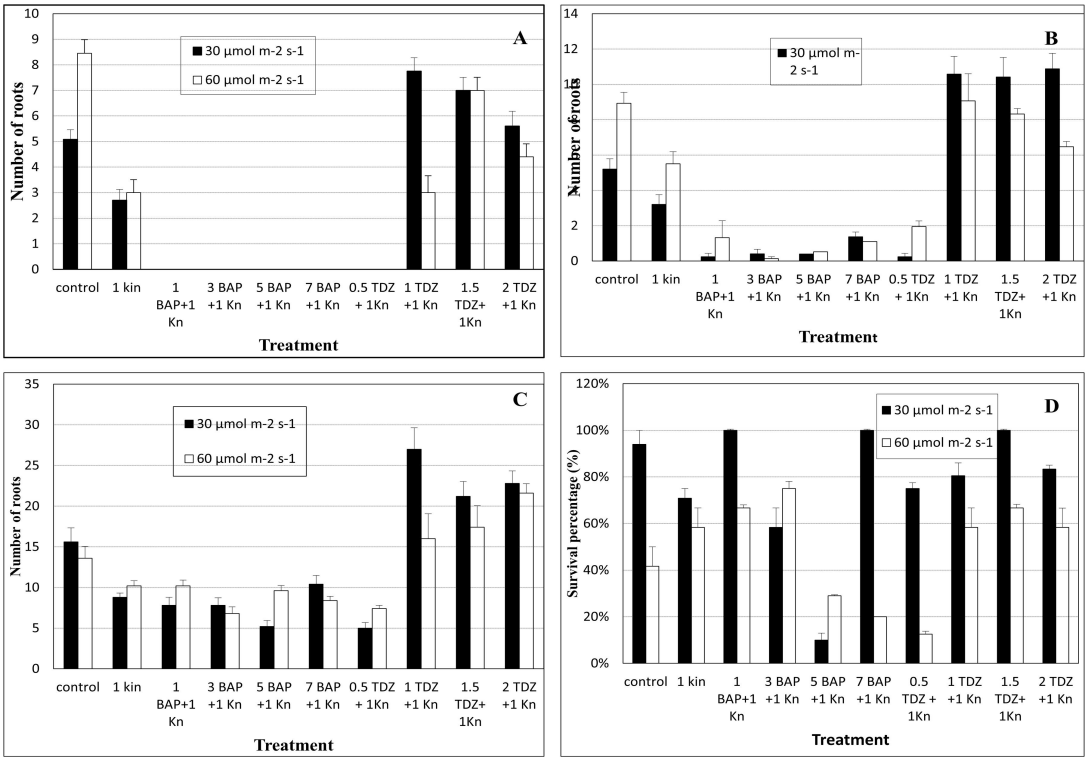

Figure (2). Number of root and survival percentage of *Cordyline terminalis* cv. Red Top, A) root number of shoot during the multiplication stage; B) root number of plantlets on MS -free PGRs- medium during root stage, C) root number of plants during acclimatization stage , D) survival percentage during the acclimatization stage

Notably, all cytokinin treatments; the root length was not significantly different from each other's, except when MS medium was enriched with 0.5 mg/l TDZ +1 mg/l Kin. However ,there was a direct relationship between inclusion of TDZ in culture media containing 1 mg/l Kin and fresh weight. The interaction between light intensity and cytokinin levels had no significant effect on root length and fresh weight. Also, there were no significant differences of light intensity on all studied traits. In spite of plants were well- developed on MS-PGRs- free medium, but they enhanced on media containing cytokinins specially TDZ. However, TDZ is a plant growth regulator widely used in plant tissue culture whereas it promotes cell division and elongation (Murthy *et al*.,

1998). It is usually used in the regeneration and proliferation of cultured explants and in combination with other regulators. Also, TDZ has been successfully used for the propagation of several plants, where the increase in the number and length of cultured shoots were observed (Ahmed and Anis, 2012 and Grabkowska *et al.,* 2014). In the present study, the number of roots of acclimatized plants were increased especially for those grown on MS medium with high TDZ concentration, when compared with other treatments (Figure 2C). Also, the survival percentage which observed after acclimatization was 100 % with the plantlets grown previously, on MS media fortified with the following combinations of PGRs : 1 mg/l BAP +1 mg/l Kin, 7 mg/l BAP +1 mg/l Kin and 1.5 mg/l TDZ +1 mg/l Kin with the light intensity of 30 µmol m^{-2} s^{-1} (Figure 2D). Generally, high survival rate was reported for Cordyline during the acclimatization (Dewier *et al*., 2015).

Table (4). Effect of cyokinins and light intensity on acclimatization stage of *Cordyline terminalis* cv. Red Top after 4 weeks in culture

Cytokinins (mg/l)	plant height (cm)			Number of leaves			Root length (cm)			Fresh weight (g)		
	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average
Control	12.36	10.42	11.39bc	5.8	8	6.9d	9.44	13.36	11.4a	2.41	3.38	2.89abc
1 kin	7.32	12.16	9.74bcd	9	6.8	7.9c	9.84	11	10.42ab	2.11	2.39	2.25bcd
1BAP+1Kin	2.16	10.74	6.45de	2.2	10.8	6.5d	7.18	12.94	10.06ab	0.87	2.31	1.58cde
3BAP+1Kin	5.86	9.78	7.82cd	5.6	9.6		10.3	10.26	10.28ab	0.73	0.77	0.75e
5BAP+1Kin	0.4	5.56	2.98ef	1	7	4f	4.46	13.16	8.81ab	0.41	0.86	0.64e
7BAP+1Kin	10.92	4.7	7.81cd	8.4	2.2	5.3e	11.94	8.6	10.27ab	1.72	1.01	1.36de
0.5TDZ+1Kin	3.72	0.5	2.11f	4.2	1	2.5g	8.18	5.8	7 b	0.94	1.66	1.3de
1 TDZ+1Kin	15.2	9.4	12.3ab	11.8	7.4	9.6b	11.88	10.3	11.09ab	3.14	3.05	3.1ab
1.5TDZ+1Kin	17.52	14.26	15.89a	11.4	10.6	11a	10.44	11	10.72ab	3.45	2.89	3.18ab
2 TDZ+1Kin	16.18	15.34	15.76a	8	11.8	9.9b	10.78	12.52	11.65a	3.73	4.13	3.93a
Average	9.16a	9.29a		6.64a	7.42a		9.44a	10.89a		1.95a	2.24a	
LSD values at 0.05 level												
Light intensity (L)		[Y]ns			ns			Ns			ns	
Cytokinins (C)		4.28			0.94			Ns			1.33	
L × C		6.05			1.33			Ns			ns	

[Y]ns = non-significan.

Photosynthetic parameters

Photosynthetic parameters, viz., Chl a, Chl b ,total chlorophyll and total carotenoids were assessed in regenerated plantlets of *C. terminals* at all treatments after 8 weeks of acclimatization (Table 5). Results showed that cytokinin treatments and the interaction between cytokinin levels and light intensities had significant effect on Chl a, Chl b, total chlorophyll and total carotenoids. On the other hand, light intensity did not have significant effect on the pervious traits. Contents of Chl a (6.33, 7.28 and 5.46 mg/g FW), Chl b (5.09, 5.82 and 4.33 mg/g FW) and total chlorophyll (11.42, 13.10 and 9.78 mg/g FW) showed a increasing of plants derived from explants grown on high TDZ concentrations (1 mg/l TDZ +1 mg/l Kin, 1.5 mg/l TDZ +1 mg/l Kin and 2 mg/l TDZ +1 mg/l Kin). The interaction between the cytokinin levels and light intensities showed that treatments of 1 mg/l TDZ +1 mg/l Kin and 1.5 mg/l TDZ +1 mg/l Kin on 30 µmol m^{-2} s^{-1} ;brought about the highest mean values of Chl a,

Chl b and total chlorophyll. The highest content of total carotenoids (227.21 and 220.83 mg/g FW) was observed on leaves of plantlets grown on 7 mg/l BAP +1 mg/l Kin and 1.5 mg/l TDZ +1 mg/l Kin, respectively; while the lowest one (57.26 mg/g FW) was obtained on 0.5 mg/l TDZ +1 mg/l Kin. The interaction between cytokinin levels and light intensities showed that plant growth on culture medium contained 7 mg/l BAP +1 mg/l Kin when kept under 30 µmol m^{-2} s^{-1} of light intenisty; increased the total carotenoids. This finding could be attributed to the cytokinin mode of action on enhancing chlorophyll biosynthesis. The previous studies mentioned that improvement in the chlorophyll content after *ex vitro* transfer was observed in Oil palm (Rival *et al*., 1997); tobacco (Pospisilova *et al*., 1998) and *Rauvolfia serpentina* (Alatar, 2015) . Dhiman *et al.* (2015) found that spraying cut stems of *Lilium* with 1 mg/l TDZ; increased total chlorophyll content. Also, foliar application of TDZ and BAP; increased the photosynthetic rate and activities of key photosynthetic enzymes in sugar beet, pea, meadow fescue and red fescue (Chernyad'ev, 1994).It is known that TDZ regulates endogenous cytokinin production and metabolism (Mok *et al*., 2000). In addition, TDZ delayed leaf yellowing and senescence in Alstroemeria (Ferrante *et al.,* 2002), Lupinus densiflorus (Sankhla *et al.,* 2003) and cut rose (Rasouli *et al*., 2015). The carotenoid plays an important role in the protection of the chlorophyll pigments under stress conditions that might be generated during acclimatization. Farnese *et al.* (2014) reported that the carotenoid content was increased during subsequent acclimatization stage.

Polymorphism and genetic similarity based on RAPD markers

RAPD markers were used to test genetic variation among mother plant and the acclimatized regenerates . Data showed that polymorphism level was the same in all primers with 100% (Table 6). Eleven out of thirty-six RAPD fragments, were found to be useful as specific markers. The highest number of unique bands (4) was obtained by the primer OPB-01, whereas the lowest one (0) was observed on the primers of P5 and G03.The tested RAPD primers generated random markers for the mother plant and for its *in vitro* regenerants genotypes (Table 7). Both intense and faint bands were produced (Figure.3). According to RAPD results, plantlets derived from the growth on MS medium supplemented with 3 mg/l BAP +1 mg/l Kin treatment; gave similarity of the plantlets on all tested primers. The similarity coefficient based on RAPD patterns ranged from 0.00 to 0.52174 (Table 8). But, genetic similarity between mother plant and its *in vitro* regenerates varied from 0.00% to 33.33%. Also, the most closely related genotypes with mother plant were derived plant from control treatment which had similarity index (0.333). In contrast, the most distant genotypes from the mother genotype were plants derived from culturing on MS medium plus 0.5 mg/l TDZ +1 mg/l Kin and 2 mg/l TDZ +1 mg/l Kin with a low similarity index (0). A dendrogram constructed by UPGMA cluster analysis using RAPD-based genetic distance is presented in (Figure. 4). The overall tree topology suggested a relative convergence between mother plant and the other treatments.

Table (5). Chlorophyll and total carotenoids contents of different treatments of *Cordyline terminalis* cv. Red Top after 8 weeks of acclimatization *ex vitro*

Cytokinins (mg/L)	Chlorophyll a (mg/g FW)			Chlorophyll b (mg/g FW)			Total chlorophyll (mg/g FW)			Total carotenoids (mg/g FW)		
	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average	30 µmol m^{-2} s^{-1}	60 µmol m^{-2} s^{-1}	Average
Control	2.11	3.65	2.88de	1.63	2.53	2.08de	3.74	6.19	4.97d	180.32	89.10	134.71abc
1 kin	2.71	6.23	4.47bcd	2.32	4.45	3.39bcd	5.04	10.68	7.86bcd	220.78	135.77	178.28abc
1 BAP+1 Kin	0.77	3.57	2.18def	0.59	3.35	1.97de	1.37	6.92	4.15de	66.61	170.49	118.56bcd
3 BAP+1 Kin	2.90	4.15	3.53cde	2.49	3.65	3.07cd	5.39	7.81	6.60cd	226.62	201.7	214.16ab
5 BAP+1 Kin	0.5	3.85	2.18ef	0.4	3.95	2.17de	0.9	7.81	4.36ed	100	179.76	139.88abc
7 BAP+1 Kin	4.61	1.81	3.21cde	4.03	1.73	2.88cd	8.63	3.55	6.09cd	369.14	85.26	227.21a
0.5TDZ+1Kin	0.76	0.3	0.53f	0.56	0.4	0.48e	1.32	0.7	1.01e	64.52	50	57.26d
1 TDZ+1 Kin	9.85	2.81	6.33ab	7.31	2.87	5.09ab	17.15	5.69	11.42ab	227.6	131.72	179.68abc
1.5TDZ+1Kin	9.93	4.63	7.28a	6.96	4.68	5.82a	16.89	9.31	13.10a	225.49	216.17	220.83a
2TDZ+1Kin	6.34	4.57	5.46abc	4.44	4.21	4.33abc	10.78	8.78	9.78abc	138.4	235.04	186.72abc
Average	4.04a	3.56a		3.07a	3.18a		7.12a	6.74a		181.96a	149.50a	
LSD values at 0.05 level												
Light density (L)		^{Y}ns			ns			ns			ns	
Cytokinins		2.39			1.88			4.25			100.46	
L × C		3.37			2.64			6.01			141.95	

^{Y}ns = non-significant.

Also, some treatments of BA or TDZ were clustered together. It is notable that most of the plants derived from the same cytokinin treatments were associated, although some bands were shared among the accessions, but there was a distinct pattern of bands amplified from each accession irrespective of its origin. There is overlapping of clusters different treatments regardless of their origin. The dendrogram can be divided into two distinct clusters. The first contained the accessions of mother plant (MP) and plant derived from MS medium that showed more divergence and individuality in their banding outline compared with the other accessions. The second cluster is divided into four subclusters. The first subcluster consisted of accession of these plantlets grown on MS medium plus 0.5 mg/l TDZ + 1 mg/l Kin; the second accessions are MS medium + 1 mg/l Kin and 1 mg/l BA + 1 mg/l Kin; the third accessions are those grown on MS medium supplemented with 1 mg/l TDZ + 1 mg/l Kin, 1.5 mg/l TDZ + 1 mg/l Kin, 5 mg/l BA + 1 mg/l Kin and 7 mg/l BA + 1 mg/l Kin and the fourth accession is 2mg/lTDZ + 1 mg/l Kin.

Table (6). Distribution of RAPD markers among the mother plant and its regenerants on different cytokinins treatments of *Cordyline terminals*

Primer code	Amplicons No.	Polymorphic amplicons	Polymorphism (%)	Unique Bands
OPB-01	5	5	100	4
A09	6	6	100	2
P6	12	12	100	3
P5	5	5	100	0
G03	3	3	100	0
G10	5	5	100	2
Total	**36**	**36**		**11**

The aforementioned subclusters were further divided into groups having a high degree of similarity.The genetic variation during molecular markers has been mentioned in a number of plant species, including ornamental plants (Rout and Mohapatra, 2006 and Kumar *et al.,* 2016). *In vitro* culture medium supplemented with plant growth regulators (PGRs) could increase the occurrence of somaclonal variation (Mujib *et al.,* 2013). Ray *et al.* (2006) observed through rapid multiplication by different PGRs of *Cordyline terminalis* , some genetic variation. Also, foliar applications of the cytokinin (BA) have been used of stimulate shoot production from *Cordyline terminalis* Celeste Queen and a chimera foliage plant (Maene and Debergh, 1982). Several techniques have been used to detect and identify genetic variations (Ruibal-Mendieta and Lints, 1998). However, RAPD is a quick and trusty method for determining genetic variation (Yadav *et al.,* 2012; Elbanna *et al.,* 2013; Elmahrouk *et al.,* 2016 and Elbanna *et al.,* 2017). In addition, RAPD analysis based on PCR in association with short primers of arbitrary sequence has been demonstrated to be sensitive in detecting variation among individuals (Rani *et al.,* 1995). Hence, *C. terminalis* has been high genetic variation rate during micropropagation. Hence, the present study reports a simple protocol for *in vitro* propagation of *C. terminalis* and detection the genetic variation among different treatments using RAPD marker.

Table (7). Survey of the tested six primers of Cordyline terminalis cv. Red Top on mother plant and regenerated plants from different treatment (black boxes=present bands and white boxes= absent bands)

Primers	Ladder	Mother plant	control	MS+ 1 Kin	0.5 TDZ+ 1 Kin	1 TDZ+ 1 Kin	1.5TD+ 1 Kin	2 TD+ 1 Kin	1BAP+1 Kin	3BAP+1 Kin	5BAP+ 1 Kin	7BAP+ 1 Kin
OPB-01	≥10000				■							
	1500						■					
	1000						■				■	
	750						■					
	550-560						■					
A09	2000						■	■			■	
	1900						■					
	1500										■	
	1100						■	■				
	900							■			■	
	725						■	■			■	
P6	2000										■	
	1600										■	
	1500					■					■	
	1000		■	■		■	■					
	900-950					■					■	■
	(750-1000)					■					■	
	800		■	■		■	■				■	■
	750					■	■					
	(520-550)		■			■	■		■		■	■
	500	■	■	■		■	■		■		■	■
	450	■	■			■	■				■	■
	(300-350)					■	■					
	200					■						
P5	1700							■			■	
	1500							■			■	■
	900					■		■			■	■
	600			■				■			■	■
	500		■	■	■	■		■	■		■	■
G03	1500						■	■				
	550					■	■	■	■			
	250					■	■	■	■			
G10	2100										■	
	1500			■		■	■	■	■		■	■
	1000			■		■	■	■	■		■	■
	750			■								
	500			■		■	■	■	■		■	■

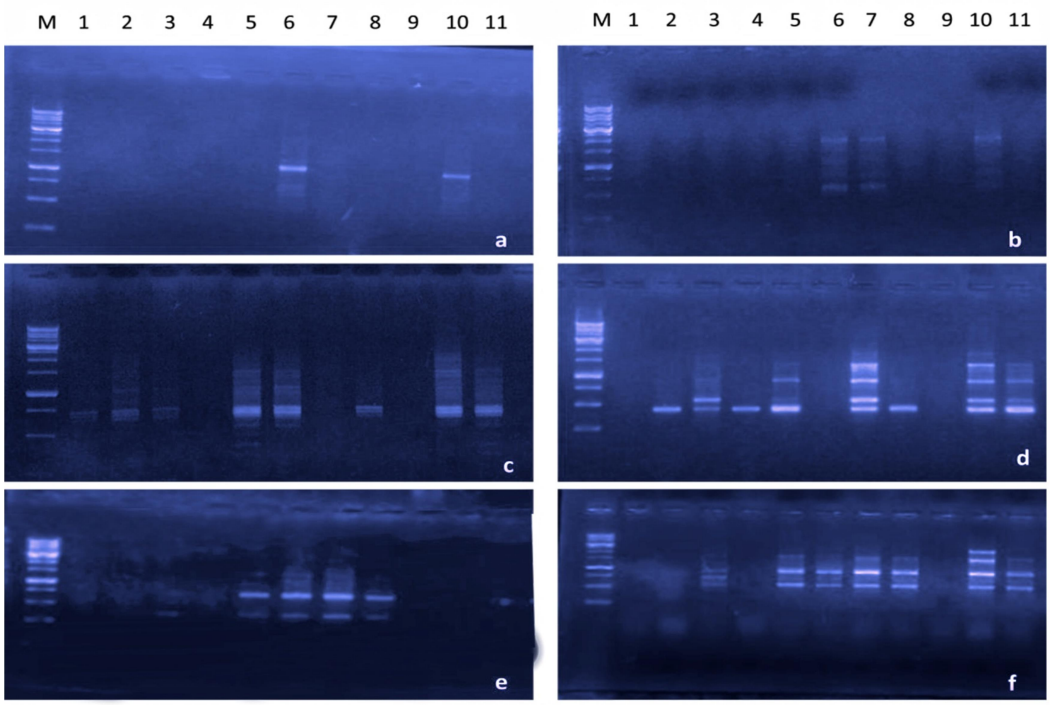

Figure (3). Agarose gel electrophoresis of six primers of RAPD markers in *Cordyline terminalis* cv.Red Top, a) OPB-01 primer; b) A09 primer; c) P6 primer; d) P5 primer; e) G03 primer; f) G10 primer

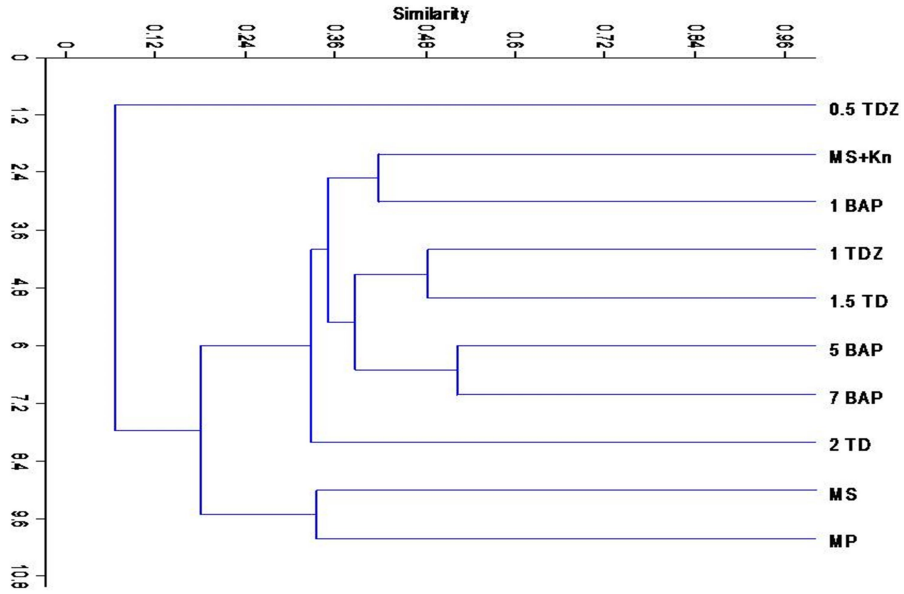

Figure (4). Randomly amplified polymorphic DNA-based dendrogram of mother plant (MP) and 10 accessions constructed using unweighted pair–group method using arithmetic averages and similarity matrices

Table (8). Similarity indices among the mother plant of *Cordyline terminalis* cv. Red Top and its regenerated plants based on RAPD markers

	Mother plant	control	MS+1 Kin	0.5 TDZ+1 Kin	1 TDZ+1 Kin	1.5TD +1 Kin	2 TD+1 Kin	1BAP +1 Kin	5 BAP+ 1 Kin	7BAP +1 Kin
Mother plant	1									
Control	0.333	1								
MS+1 Kin	0.1	0.364	1							
0.5TDZ+1 Kin	0	0.143	0.1	1						
1 TDZ+1 Kin	0.111	0.333	0.35	0.053	1					
1.5TD+1 Kin	0.091	0.217	0.24	0	0.481	1				
2 TD+1 Kin	0	0.05	0.263	0.063	0.269	0.321	1			
1 BAP+1 Kin	0.111	0.273	0.417	0.111	0.444	0.304	0.353	1		
5 BAP+1 Kin	0.087	0.208	0.28	0.042	0.414	0.324	0.407	0.24	1	
7BAP+1 Kin	0.167	0.385	0.5	0.077	0.5	0.308	0.35	0.429	0.522	1

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الملخص العربي

الإكثار المعملي الدقيق والاختلافات الوراثية في نبات الكورداليين

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تم اجراء هذه الدراسة في معمل زراعة الأنسجة بقسم الزهور و نباتات الزينة– كلية الزراعة بالشاطبي – جامعة الاسكندرية خلال الفترة من عام ٢٠١٥ وحتى عام ٢٠١٧ على نبات الكورداليين**'Red Top' cv.** ***Cordyline terminalis*** . وكان الهدف من الدراسة هو ايجاد بروتوكول لإنتاج نبات الكورداليين معمليا ودراسة الاختلافات الوراثية من هذا البروتوكول بين النباتات الناتجة بإستخدام تكنيك RAPD .

ولتحقيق هذا الهدف تم إجراء التجارب كما يلي :

١– إنتاج نبات الكورداليين معمليا بزراعة القمم النامية على بيئة MS المضاف اليها ١ ملجم / لتر كينتين بتركيزات مختلفة من BAP,TDZ واستخدم بيئة MS الخالية من منظمات النمو ككنترول للمقارنة.

٢– استخدام الدلائل الجزيئية Primers بواسطة تكنيك RAPD من أجل دراسة الاختلافات الوراثية بين النباتات الناتجة معمليا.

تأسيس زراعة الأنسجة لنبات الكورداليين:

تم تعقيم القمه النامية بطول (٤– ٦ سم) لنبات الكورداليين وزراعتها على بيئة MS خالية من منظمات النمو.

مرحلة إنتاج المجاميع الخضرية :

تم أخذ القمم النامية بطول (٢– ٣ سم) من نبات . ***Cordyline terminalis*. cv.' Red Top'** ثم زراعتها على بيئة MS مضاف اليها ١ ملجم / لتر كينتين بالإضافة إلى عدة تركيزات من البنزيل أمينو بيورين BAP (٠ , ١ , ٣ , ٥ ,٧ ملجم / لتر) وكذلك تركيزات من مادة TDZ (٠ , ٠.٥ , ١ , ١.٥ , ٢ ملجم / لتر). وتم إستخدام لمبات فلورسنت بنوعين مختلفين من شدة الاضاءة هما (٣٠ – ٦٠ ميكرومول . م $^{-٢}$. ث$^{-١}$) وذلك للحصول على أكبر عدد من المجاميع الخضرية.

أهم النتائج لتأثير السيتوكينين وشدة الإضاءه على إنتاج المجاميع الخضرية معمليا:

تم دراسة الصفات التالية (عدد الفروع , طول الفروع , عدد الأوراق , الوزن الطازج).

وتم الحصول على أعلى عدد فروع (٤ فروع / الجزء النباتي) بإستخدام بيئه موراشيج وسكوج المزودة ب(١.٥ ملجم / لتر TDZ والمضاف اليها ١ ملجم / لتر كينتين). وجد أن تأثير TDZ كان أعلى من تأثير BAP أو Kin أو الكنترول. بينما تم الحصول على أطول فروع من بيئه (٠.٥ملجم / لتر TDZ المضاف اليها ١ مجم / لتر كينتين). بالنسبة للكثافة الضوئية العاليه, لم يكن لها تأثير على عدد الفروع أو طولها . وقد تم الحصول على أعلى عدد أوراق (٧.٦٥) من بيئه (٠.٥ملجم / لتر TDZ المضاف اليها ١ مجم / لتر كينتين).

ومن ناحية شده الاضاءه (٣٠ ميكرومول . م $^{-٢}$. ث $^{-١}$) فقد أعطت أعلى عدد من الأوراق (٥.٨٩ ورقه / فرع). وبالنسبة للوزن الطازج فقد تم الحصول على أعلى وزن من المعاملة (١ ملجم / لتر TDZ المضاف اليه ١ مجم / لتر كينتين).

تأثير بيئة MS الخالية من منظمات النمو النباتية على استطالة وتجذير النباتات الناتجة معمليا:

تم الحصول على أعلى عدد فروع (١٣.٤) من بيئة موراشيج وسكوج المزودة (٣ ملجم / لتر BAP مضاف اليه ١ ملجم / لتر كينتين). ولم يكن هناك تأثيرمعنوي لشدة الاضاءه على إنتاج الفروع الخضرية . وعلى النقيض, تم الحصول على أقل عدد فروع وأطول فروع من النمو على بيئة MS الخاليه من منظمات النمو. كان لإستخدام شده الاضاءه المنخفضة تأثير ايجابي على زيادة طول الفروع (٧.٠٤ سم).

كما تم الحصول على أعلى طول فروع من النمو على بيئه MS الخالية من منظمات النموتحت شدة اضاءه (٦٠ ميكرومول . م $^{-٢}$. ث $^{-١}$) .

كما تم الحصول على أعلى عدد أوراق (١١.٠٨) من بيئه MS الخالية من منظمات النمو(الكنترول).

كما تم الحصول على أعلى وزن طازج من النمو على بيئه موراشيج وسكوج المزودة (١ ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين , ١.٥ملجم / لتر TDZ المضاف اليه ١ ملجم / لتر كينتين) تحت شدة اضاءه (٣٠ ميكرومول . م $^{-٢}$. ث $^{-١}$)حيث أعطت ٧.٦١- ٨.٥٦ جم ,على التوالي .

كما تم الحصول على أعلى عدد جذور (١١ جذر / فرع) من النمو على بيئه موراشيج وسكوج المزودة (٢ ملجم / لتر TDZ المضاف إليه ١ ملجم / لتر كينتين) تحت شده اضاءه (٣٠ ميكرومول . م $^{-٢}$. ث $^{-١}$) .

مرحلة الأقلمة :

تم زراعة الفروع بطول (٢- ٣ سم) على بيئة MS الخاليه من منظمات النمو لإنتاج الجذور. وكان بالنباتات الناتجة بعدد ٣ – ٥ أوراق تم معاملتها بمبيد فطري (ريزولكس) ٠.١ % , ثم زراعتها في أكواب بلاستيك صغيرة محتوية على خليط معقم من (البيتموس : الفيرميكيوليت) بنسبة (١ :١) تم تغطيتها لمدة ١٥ يوم بأكياس بلاستيكية.

الصفات المورفولوجية المسجلة في مرحله الأقلمة على النباتات الناتجة معمليا:

بعد ٨ أسابيع من الأقلمة تم تسجيل الصفات التالية :

طول النبات ,عدد الأوراق / نبات , الوزن الطازج , عدد الجذور/ نبات.

الصفات الكيميائية التى تم قياسها:

تم قياس كلوروفيل أ , ب , والكلوروفيل الكلي , الكاروتينات .

أهم نتائج الصفات المورفولوجية التي تم أخذها في مرحله الأقلمة :

تم الحصول على أعلى طول عند تركيزات (١ ملجم / لتر TDZ مضاف إليه ١ ملجم / لتر كينتين وكان ١٢.٣ سم ,وكذلك عند تركيز ١.٥ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين وكان ١٥.٨٩ سم , وكذلك تركيز ٢ ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين وكان ١٥.٧٦سم .بينما كان لتركيز (٥ ملجم / لتر BAP مضاف اليه ١ ملجم / لتر كينتين , وكذلك تركيز ٠.٥ ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين تأثير سلبي على طول النباتات . فى حين أن أكبر عدد من الأوراق (١١) قد تم الحصول عليه بتركيز ١.٥ ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين. تم الحصول على أقل عدد أوراق (٢.٥)عند النمو على بيئة موراشيج وسكوج المزودة (٠.٥ ملجم / لتر TDZ مضاف إليه ١ ملجم / لتر كينتين).

زادت جميع معاملات السيتوكينينات من طول الجذور عدا عند النمو على بيئة تحتوي على (٥ ملجم / لتر BAP مضاف اليه ١ مجم / لتر كينتين, وكذلك تركيز ٠.٥ ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين). أيضا زاد الوزن الطازج للنبات بصوره منتظمة بإستخدام تركيزات عالية من TDZ . ووجد أن التركيزات العالية من TDZ أعطت أعلى عدد من الجذور للنباتات المتأقلمة بمقارنتها بالمعاملات الأخرى.

أهم نتائج القياسات الكيماوية:

تم الحصول على أعلى تركيزلكلوروفيل أ , ب , الكلوروفيل الكلي عند التركيزات العالية مثل (١ ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين, ١.٥ ملجم / لتر TDZ مضاف إليه ١ ملجم / لتر كينتين , ٢ ملجم / لتر TDZ مضاف اليه ١ مجم / لتر كينتين). تم الحصول على أعلى تركيزللكاروتينات (٢٢٧.٢١ – ٢٢٠.٨٣ مجم / جم وزن طازج) عند تركيزات (٧ مجم / لتر BAP مضاف اليه ١ ملجم / لتر كينتين, ١.٥ ملجم / لتر TDZ مضاف اليه ١ ملجم / لتر كينتين).

استخلاص DNA وقياس الاختلافات الوراثية باستخدام طريقة RAPD :

تم استخدام طريقة CTAB لاستخلاص DNA من الأوراق الطازجة تم استخدام ٦ بريمر لعمل تكنيك RAPD وجد أن تركيز(٣ مجم / لتر BAP مضاف اليه ١ مجم / لتر كينتين) أعطت نتائج متماثلة لكل البريمر المختبرة.

تراوحت نسبة التشابه (٠ –٠.٥٢١٧). بينما كان التشابه الجيني بين النبات الام والنباتات الناتجه معمليا اختلفت بنسبه (٠– ٣٣.٣٣ %) وكان أقرب نبات للنبات الأم هو الكنترول الخالي من منظمات النمو. بينما كانت التركيزات (٠.٥ مجم / لتر TDZ مضاف إليه ١ مجم / لتر كينتين , ٢ مجم / لتر TDZ مضاف إليه ١ مجم / لتر كينتين) أقل نسبة تشابه مع النبات الأم .