Journal of Kerbala University , Vol. 13 No.2 Scientific . 2015

Effect of different concentrations of Turmeric(*Curcuma longa* L.) powder and BA on *in vitro* direct organogenesis from cotyledons of mandarin (*Citrus reticulata* Blanco)

تأثير تراكيز مختلفة من مسحوق الكركم (*Curcuma longa* L.) و BA في التكوين المباشر للأعضاء من فلق نبات اللالنكي (*Citrus reticulata* Blanco) خارج الجسم الحي

Siham Abd Al-Razzaq Salim* * May Abdullah Razzooqee * Abbas Hadi Hashem

e-mail: dr.sihamabdalrazzaq@yahoo.com

***Al – Musaib Technical College**

Abstract

Turmeric(*Curcuma longa* L.) plant is considered one of the important medicinal species belonging to the Zingiberaceae because it is rich with many active compounds. The aim of this research was to study the effect of Turmeric powder and BA on direct organogenesis from mandarin *Citrus reticulata* Blanco. Cotyledons which were used as source of explants as entire cotyledonary segments (EC) and longitudinally cuttings (LC) were cultured on Murashige and Skoog (MS) medium containing 500 mg/L malt extract and supplemented with Turmeric powder (0.0, 200, 400, 600, 800, 1000 and 2000 mg/L) and BA (0.0, 1.0, 2.0 and 3.0 mg/L). The highest frequency of regeneration (100%) was observed in the 2000 and 3.0 mg/L respectively in both EC and LC explants. The highest number of buds/shoots was 8.20 and 7.50 which obtained when EC explants were cultured in MS medium supplemented with 600 mg/L Turmeric + 3.0 mg/L BA and 2000 mg/L Turmeric + 3.0 mg/L BA respectively as compared with other treatments, while the combination: EC + 600 mg/L

Turmeric + 3.0 mg/L BA gave the highest length (6.40 mm) which was significantly higher than other combinations.

Key words: *in vitro, Citrus reticulata, Curcuma longa*, organogenesis.

الملخص

يعد نبات الكركم.*Curcuma longa* L من الأنواع الطبية المهمة العائدة للعائلة Zingiberaceae بسبب غناه بالعديد من المركبات الفعالة. يهدف البحث الحالي إلى دراسة تأثير الكركم و BA في التكوين المباشر للأعضاء من فلق نبات اللالنكي *Citrus reticulata* Blanco . استعملت الفلق كمصدر للأجزاء النباتية كقطع فلق كاملة EC ومقطعة طوليا LC وزرعت على وسط MS المتضمن 500 ملغم/لتر خلاصة المالت والمجهز بمسحوق الكركم (0.0, 200, 400, 600 ملغم/لتر) مع BA (0.0, 1.0, 2.0, 3.0 ملغم/لتر). لوحظ أعلى معدل للإخلاف (100%) في التراكيز العالية من كل من الكركم و BA وخاصة التركيزين 2000 و 3.0 ملغم/لتر على التوالي وفي كلا أجزاء الفلق المستعملة EC و LC . وإن أفضل معدل لعدد البراعم/الأفرع هو 8.20 و 7.50 تم الحصول عليه عند زراعة قطع الفلق الكاملة (EC) على وسط MS مجهزا بالكركم 600 ملغم/لتر + BA 3.0 ملغم/لتر و الكركم 2000 ملغم/لتر + BA 3.0 ملغم/لتر على التوالي مقارنة بالمعاملات الأخرى ,بينما أعطت أجزاء الفلق الكاملة عند زراعتها على التوليفة 600 ملغم/لتر كركم + 3.0 ملغم/لتر BA أعلى معدل لطول البراعم/الأفرع (6.40 ملم) والذي تفوق معنويا على بقية المعاملات.

Introduction

Turmeric (*Curcuma longa* L.) is a perennial herb belonging to the family Zingiberaceae. *Curcuma* rhizome is one of the most common spices for the special taste and color. Many chemical compounds that present in Turmeric like curcumin, feruloyl-metane, camphene, terpines, phenols, stigmasterol, and flavonoids [1]. Turmeric powder has many pharmaceutical actions ; it has been used in treating leucoderma, asthma, tumours and bronchitis [2]. Curcumin prevents flatulence and has a strong anti-inflammatory activity, antioxidant activity. Also it increases bile production [3, 4, 5]. Mandarin is a member of family Rutaceae and a very popular fruit among various citrus species. The fruits are easy peeling for eating, excellent source of vitamin C, fresh consumption, aromatic flavor and low content of saturated fat, cholesterol and sodium [6].

In vitro technique is a useful method to obtain true- to- type regenerated plants [7, 8]. A number of *in vitro* studies have been made on plant regeneration by organogenesis and embryogenesis on various other citrus cultivars [9, 10], but very little information available about procedures for achieving regeneration from *C. reticulata*. Cotyledons have a high potential of regeneration and represent a good source of tissue cultures, also, in last decade, cotyledons and cotyledonary nodal regions were used for transformation mediated by *Agrobacterium* [11, 12].

Because of the important compounds found in Turmeric and not used before in tissue culture, the present research was aimed to study the effect of different concentrations of Turmeric powder on direct organogenesis from cotyledons of *C. reticulata* and to observe any ability of this powder to reduce contamination *in vitro.*

Materials and Methods

Seeds were collected from ripe fruits of *Citrus reticulata* Blanco and transferred to a laminar air flow cabinet where the surface sterilization was achieved with 2% NaOCl + Tween20 for 20 minutes. After removal of two teguments and embryo axis with a scalpel and forceps, entire cotyledons (EC) and longitudinal cut cotyledons (LC) were used as explants. These explants were cultured on MS medium [13] supplemented with other contents (Table-1).

All cultured explants were incubated in a culture room at 25 ± 2ºC under 16 hrs photoperiod. Experiments were performed with 10 replicates and repeated twice. The regeneration frequency (RF) (number of explants producing buds or shoots per total number of explants cultured multiplied by 100), the number of buds/shoots per explants and shoot length (mm) were measured after 90 days of culture. The experiment was designed according to the completely randomized design (CRD) and the data were subjected to analysis of variance (ANOVA). Significant differences were assessed using L.S.D. test ($P \leq 0.05$) [14].

Table(1): Components of culture medium used for the cultures of *C. reticulata* explants

Component	Concentration (mg/L)
MS salts	Full strength
Myo-inositol	100
Thiamine-HCl	0.5
Pyridoxine-HCl	0.5
Nicotinic acid	0.5
Glycine	2.0
Turmeric powder	0.0, 200, 400, 600, 800, 1000, 2000
BA	0.0, 1.0, 2.0, 3.0
Malt extract	500
Sucrose	50000
Agar	7000
pH= 5.7	

Results and Discussion

The frequency of adventitious bud regeneration by direct organogenesis from cotyledonary explants was depending on the type of explant, concentration of Turmeric powder and BA added to the regeneration medium (Table-2). The maximum percentage (49.6 %) was observed in LC explants as compared with EC explants (40.4 %). Cotyledons have the ability of regeneration and represented a good source of tissue cultures, and to obtain true-to-type regenerated plants [7, 8, 11]. The best regeneration percentages reached to 57.5 and 67.5 % at the Turmeric concentrations 600 and 2000 mg/L respectively which were significantly higher than other concentrations, while the control induced poor organogenesis(18.8%). This revealed that Turmeric powder may support the growth of regenerated buds or shoots as it containing many important compounds that are useful for growth as precursors of other physiological compounds or hormones or as co-factors [15]. Results also showed that 3.0 mg/L of BA was the best and significant concentration in the regeneration percentage which gave 75.0% as compared with other treatments. In previous studies showed that the frequency of regeneration increased in the medium supplemented with cytokinins [16]. The requirement of BA for organogenesis and somatic embryogenesis may vary according to the species of plant and explants source [17]. The interaction among explants, Turmeric and BA concentrations showed a significant effect specially in the

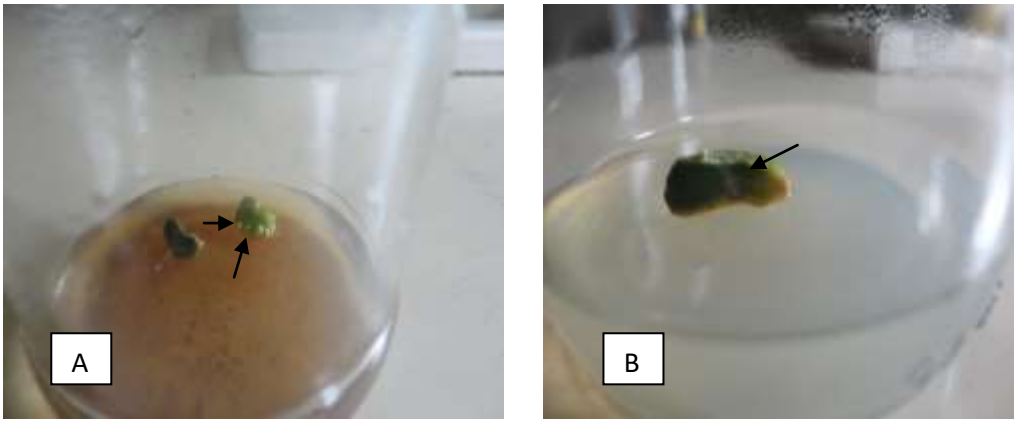
combinations: EC+2000mg/L Turmeric + 3.0 mg/L BA and LC + 2000mg/L Turmeric + 3.0 mg/L BA that gave the maximum response (100%). This revealed that cotyledons have high potential of regeneration [11], as well as the stimulator roles of Turmeric and BA in the medium. No callus was observed in all cultured explants.

The shoots began to emerge directly from the explants as green clusters (Figure-1) that differentiated into buds. The number of regenerated buds/shoots per explant was depending on the type of explant and concentration of Turmeric and BA (Table-3). Results showed that EC explants gave 1.89 bud/shoot per explants which was significant than LC explants (0.93). This showed that entire cotyledons were always more regenerative than segmented cotyledons [18] . previous studies showed that organogenesis from cotyledons was successfully obtained in other plants like: *Capsicum annum* L. [19]; *Pongamina pinnata* L. [20]. Significant differences in the number of buds/shoots per explant with the different concentrations of Turmeric in which the maximum number (2.50 and 2.39) were observed at 600 and 2000 mg/L respectively, whereas the minimum number (0.33) was in the control treatment. It was observed that the contamination was highly reduced in all media that containing Turmeric powder in different concentrations, this revealed the antimicrobial activity of *Curcuma longa* and it contains some minerals and precursors of vitamins [21]. Regeneration was affected by the addition of BA to the MS medium in which the number of buds/shoots per explant increased gradually from 1.09 at 1.0 mg/L BA reaching to the highest number (2.99) in a medium containing 3.0 mg/L BA, while no response was noted when using the hormone-free medium (control). The majority of literatures have been reported that BA as the most active cytokinin in shoot proliferation and multiplication in many plants through inducing cell divisions and preventing apical dominance [22, 23, 24] .

The interaction among type of explants, Turmeric and BA concentrations showed significant differences in the number of buds/shoots per explant. EC explants cultured in MS medium + 600 mg/L Turmeric + 3.0 mg/L BA and MS medium + 2000 mg/L Turmeric + 3.0 mg/L BA gave maximum numbers of buds/shoots per explants(8.20 and 7.50 respectively) as compared with other combinations.

Table(2): Effects of different combinations of Turmeric and BA in MS medium on regeneration frequency(%) from cotyledonary explants of *Citrus reticulata* after 90 days of culture.

Cotyledonary explants	Turmeric Concentration(mg/L)	BA (mg/L)				Mean of Turmeric
		0.0	1.0	2.0	3.0	
EC	0.0	0.0	10.0	30.0	40.0	18.8
	200.0	0.0	30.0	20.0	30.0	22.5
	400.0	0.0	30.0	30.0	80.0	47.5
	600.0	0.0	30.0	50.0	100.0	57.5
	800.0	0.0	60.0	60.0	50.0	46.2
	1000.0	0.0	50.0	60.0	70.0	55.0
	2000.0	0.0	100.0	100.0	100.0	67.5
Mean of EC = 40.4						
LC	0.0	0.0	0.0	20.0	50.0	
	200.0	0.0	20.0	20.0	60.0	
	400.0	0.0	70.0	70.0	100.0	
	600.0	0.0	90.0	90.0	100.0	
	800.0	0.0	50.0	70.0	80.0	
	1000.0	0.0	80.0	90.0	90.0	
	2000.0	0.0	60.0	80.0	100.0	
Mean of LC = 49.6						
Mean of BA		0.0	48.6	56.4	75.0	
L.S.D.(0.05)	Explants = 5.97, Turmeric = 11.17, BA= 8.45 Explants × Turmeric × BA = 31.60					



Figure(1): Direct organogenesis from cotyledonary explants (arrows) of *C. reticulate* cultured on MS medium with different concentrations of turmeric and BA. A) EC explants . B) LC explants .

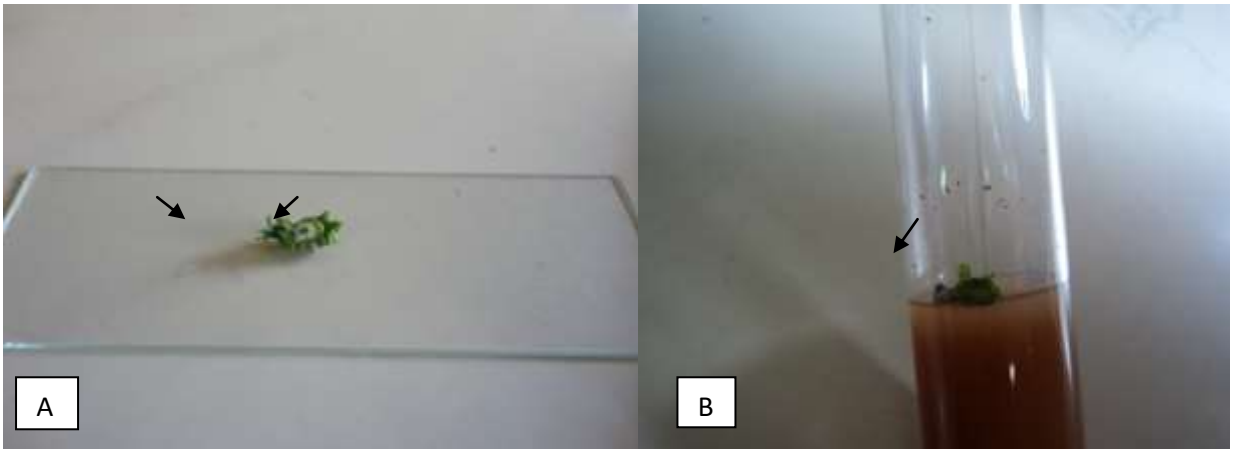
The shoots regenerated from EC explants were significantly much longer (1.66 mm) than those in LC explants (0.61 mm) (Table-4). The shoot length was affected by the Turmeric concentrations in which the concentration 600 mg/L gave the highest length (1.94 mm) which overcame the other treatments, then the length was significantly decreased with the increasing of concentration. Shoot length also was influenced significantly by the BA concentration in which 3.0 mg/L BA gave the best elongation reached 2.09 mm compared with other concentrations. Interaction treatments showed a significant effect on shoot length. EC explants cultured on a medium supplemented with 600 mg/L Turmeric + 3.0 mg/L BA gave the highest length (6.40 mm) as compared with other combinations (Figure-2). It was noticed that explants can be survived for long periods (about 6-8 months) on the same medium that containing Turmeric concentrations specially in the highest concentrations (1000 and 2000 mg/L) (unpublished data). This may reveal the role of Turmeric powder as conservative and antioxidant material. Regeneration from cotyledon explants has been reported in several reports [25, 26, 19] , but it has been recorded in only a few species belonging to *Citrus* genus: adventitious embryos of *Citrus spp.* [7] , indirect shoot regeneration in *C. grandis* (L.) Osbeck (pummelo) [27, 28] and direct organogenesis of *C. clementina* [18] . It was demonstrated that BA is required *in vitro* morphogenesis in *C. reticulata*. It was demonstrated that entire plant can be produced from single cell, tissue or organ depending on the potency of the cells of these explants. Plant growth regulators are the critical components in culture media through determining the developmental pathway of plant cell. So, it has been reported in previous studies the superiority of BA over other cytokinins in proliferation and multiplication of buds or shoots by direct or indirect methods [29, 23, 28] . Also, during cell division and proliferation of buds and shoots, the cell metabolism is active and this causing the high production of free radicals that causing oxidative stress and damage for cells which then prevent the proliferation of buds [30]. So that the addition of Turmeric powder to the MS medium that induced regeneration of buds and shoot may be due to the presence of antioxidant compounds in Turmeric such as phenols, terpens and saponins which have radical scavenging activity to remove toxicity of free radicals and anchorage the formation of buds and shoots without damages [31] .

Table(3): Effects of different combinations of Turmeric and BA in MS medium on number of buds/shoots differentiated per explants by direct organogenesis of *Citrus reticulata* after 90 days of culture.

Cotyledonary explants	Turmeric Concentration(mg/L)	BA (mg/L)				Mean of Turmeric
		0.0	**1.0**	**2.0**	**3.0**	
EC	**0.0**	0.0	0.50	0.50	0.70	0.33
	200.0	0.0	0.60	0.60	2.60	0.65
	400.0	0.0	0.30	0.50	5.10	1.36
	600.0	0.0	2.60	3.70	8.20	2.50
	800.0	0.0	1.00	1.20	5.10	1.45
	1000.0	0.0	1.30	1.90	2.00	1.18
	2000.0	0.0	2.00	4.90	7.50	2.39
Mean of EC = 1.89						
LC	**0.0**	0.0	0.0	0.40	0.50	
	200.0	0.0	0.30	0.50	0.60	
	400.0	0.0	1.40	1.60	2.00	
	600.0	0.0	1.70	1.70	2.10	
	800.0	0.0	1.10	1.40	1.80	
	1000.0	0.0	1.30	1.30	1.60	
	2000.0	0.0	1.20	1.50	2.00	
Mean of LC = 0.93						
Mean of BA		0.0	1.09	1.55	2.99	
L.S.D.(0.05)	Explants = 0.308, Turmeric = 0.576, BA= 0.435 Explants × Turmeric × BA = 1.630					

Table(4): Effects of different combinations of Turmeric and BA in MS medium on length(mm) of shoots differentiated per explants by direct organogenesis of *Citrus reticulata* after 90 days of culture.

Cotyledonary explants	Turmeric Concentration(mg/L)	BA (mg/L)				Mean of Turmeric
		0.0	**1.0**	**2.0**	**3.0**	
EC	**0.0**	0.0	0.20	0.90	1.30	0.43
	200.0	0.0	1.30	0.70	1.50	0.71
	400.0	0.0	0.50	1.70	3.80	1.06
	600.0	0.0	1.60	4.00	6.40	1.94
	800.0	0.0	2.70	2.90	3.00	1.40
	1000.0	0.0	2.10	1.90	3.20	1.25
	2000.0	0.0	1.80	2.30	2.70	1.16
Mean of EC = 1.66						
LC	**0.0**	0.0	0.0	0.30	0.70	
	200.0	0.0	0.40	0.80	1.00	
	400.0	0.0	0.70	0.80	1.00	
	600.0	0.0	0.90	1.10	1.50	
	800.0	0.0	0.70	0.80	1.10	
	1000.0	0.0	0.80	1.00	1.00	
	2000.0	0.0	0.60	0.80	1.10	
Mean of LC = 0.61						
Mean of BA		0.0	1.02	1.43	2.09	
L.S.D.(0.05)	Explants = 0.233, Turmeric = 0.437, BA= 0.330 Explants × Turmeric × BA = 1.237					



Figure(2): Different lengths of buds/shoots that regenerated from different cotyledonary explants cultured on MS medium with turmeric and BA. A) EC explants. B) LC explants.

References

1-Rastogi,R.P. and Mehrotra, B.N.1995. Compendium of Indian Medicinal Plants. CDRI, Lucknow and Publications and Information Directorate, New Delhi.

2-Zaman,K.; Das,S. and Mondal,P. 2013. *Curcuma caesia* Roxb. and it's medicinal uses: A review. Inter.J.Resea.Pharm.Chem., 3: 370-375.

3-Ammon, H.P.T. and Wahl, M.A. 1991. Pharmacology of *Curcuma longa*. Planta Medica, 57: 1-7.

4-Scartezzini,P. and Speroni,E. 2000. Review on some plants of Indian traditional medicine with antioxidant activity. J.Ethnopharmacology, 71: 23-43.

5-Aggarwal, B.B. and Sung, B. 2009. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol.Sci., 30: 85-94.

6-Altaf,N.; Khan, A.R. an Hussain, J. 2008. Fruit variability in kinnow mandarin(*Citrus reticulata*). Pak.J.Bot., 40(2): 599-604.

7-Khawale, R.N. and Singh, S.K. 2005. *In vitro* adventitive embryony in *Citrus* : A technique for *Citrus* germplasm exchange. Current Sci., 88(8): 1309-1311.

8-Mondal, B. and Saha, R. 2014. Identification of zygotic and nucellar seedlings of *Citrus reticulata* and *Citrus aurantifolia* using RAPD. Inter. J. Advan.Biotech.Resea., 15(1): 25-30.

9-Sajeva, M.; Carra, A.; De Pasquale, F. and Carimi, F. 2008. Somatic embryogenesis and plant regeneration from pistil transverse thin layers of lemon (*Citrus limon*). Plant Biosystems, 124(2): 199-203.

10- Siwach,P.; Chanana,S.; Gill,A.R.; Dhanda,P.; Rani,J.; Sharma,K.; Rani,H. and Kumari,D. 2012. Effects of adenine sulphate, glutamine and casein hydrolysate on *in vitro* shoot multiplication and rooting of kinnow mandarin (*Citrus reticulata* Blanco). Afric. J. Biotech.,11(92): 15852-15862.

11- Franklin,G.; Carpenter,L.; Davis,E.; Reddy,C.S.; Al-Abed,D.; Alaiwi,W.A.; Parani,M.; Smith,B.; Goldman,S.L. and Sairam,R.V. 2004. Factors influencing regeneration of soybean from mature and immature cotyledons. Plant Gro.Reg., 43(1): 73-79.

12-Chiancone, B. and Germana, M.A. 2013. Micropropagation of *Citrus spp.* By organogenesis and embryogenesis. Metho.Molec.Bio., 994: 99-118.

13-Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physio.Planta., 15(3): 473-497.

14-Gent SAS. 2007. SAS/Stat User Guide for Personal Computers. SAS Institute Ineae Cary, N.C. USA.

15-Yingming, P.; Ping, L.; Hengshan, W. and Min,L. 2004. An oxidant activities of several Chinese medicinal herbs. Food Chemi., 88: 347-350.

16-Costa, M.G.C.; Alves, V.S.; Lani, E.R.G.; Mosquim, P.R.; Carvalho, C.R. and Otoni, W.C. 2004. Morphogenic gradients of adventitious bud and shoot regeneration in epicotyls explants of *Citrus*. Sci.Hort., 100(1): 63-74.

17-Bhaskaran,S. and Smith, R.H. 1990. Regeneration in cereal tissue culture: A review. Crop Sci., 30: 1328-1336.

18-Lombardo, G.; Alessandro, R.; Scialabba, A.; Sciandra, M. and DePasquale, F. 2011. Direct organogenesis from cotyledons in cultivars of *Citrus clementina* Hor.Ex.tan. Amer.J.Plan.Sci., 2: 237-244.

19-Joshi, A. and Kothari, S.L. 2007. High copper levels in the medium improves shoot bud differentiation and elongation from the cultured cotyledons of *Capsicum annum* L. Plant Cell, Tiss. Org. Cult., 88(2): 127-133.

20-Sujatha, K.; Panda, B.M. and Hazra, S. 2008. De novo organogenesis and plant regeneration in *Pongamia pinnata*, oil producing tree legume. Trees, 22(5): 711-716.

21-Cowan, M.M. 1999. Plant products as an microbial agents. Clin. Microbio.Rev., 12: 564-582.

22-Amoo, S.O.; Finnie, J.F. and Van Staden, J. 2009. *In vitro* propagation of *Huernia hystrix*: an endangered medicinal and ornamental succulent. Plant Cell Tiss. Organ Cult., 96: 273-278.

23-Chavan, J.J.; Nimbalkar, M.S., Adsul, A.A.; Kambal, S.S.; Gaikwad, N.B.; Dixit, G.B.; Bapat, V.A. and Yadav, S.R. 2011. Micropropagation and *in vitro* flowering of endangered plant *Ceropegia attenuate* Hook. J. Plant Biochem. Biotech., 20: 276-282.

24-Gbadamosi, A. E. and Shaibu, B. 2013. Influence of the phytohormones on the *in vitro* regeneration in *Senna alata* (L.) Acad. J. Biotech., 1(3): 41-45.

25-Saafi,H. and Borthakur,D. 2002. *In vitro* plantlet regeneration from cotyledons of the tree-legume *Leucaena leucocephala*. Plant Growth Regu., 38(3): 279-285.

26-Du,N. and Pijut,P.M. 2008. Regeneration of plants from *Fraxinus pennsylvanica* hypocotyls and cotyledons. Scie.Horticul., 118(1): 74-79.

27-Goh, C.J.; Sim, G.E.; Morales, C.L. and Loh, C.S. 1995. Plantlet regeneration through different morphogenic pathways in pummelo tissue culture. Plant Cell,Tiss.Org.Cult., 43(3): 301-303.

28-Begum, F.; Amin, M.N.; Islam, S.; Azad, M.A.K. and Rehman, M.M. 2003. *In vitro* plant regeneration from cotyledon-derived callus of three varieties pummelo(*Citrus grandis* (L.) Osbeck.). J.Bio.Sci., 3(8): 751-759.

29-Chakravarty, B. and Goswami, B.C. 1999. Plantlet regeneration from long-term callus cultures of *Citrus acida* Roxb. and uniformity of regenerated plants. Sci.Horticul., 82(1): 159-169.

30-Gupta, S.D. and Datta, S. 2003. Antioxidant enzyme activities during *in vitro* morphogenesis of gladiolus and the effect of application of antioxidants on plant regeneration. Biol.Plant.,47: 179-183.

31-Diab, D.S. and Mashi, S.K. 2011. Effect of *Curcuma longa* on liver enzymes and the function of the kidney. Wasit J. Sci. Medi., 4(2): 108-115.