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Effect Of Pre treatment And Media Composition On IN VITRO Pr opag ation Of MOMORDIA CHARANTIA Using Different Explants.

Hassanien S.H.[1], Ibrahim I.A.[2], Emara H.A.[2], Bekhit M.H.[2], Enass S. Amer[2]

[1]Department of Genetics, Faculty of Agriculture, Ain Shams University, Egypt
[2]Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt

Abs tract:-This investigation was conducted to study the effect of dark pr treatment with different thermal conditions (32±2°C or 22±2°C), explant type and gr owth r egulator concentrations on callus pr oduction and regeneration of Momor dica charantia. Explants cultur ed on MS medium contained diff er ent concentrations of plant growth r egulators with Gamborg's vitamins. All explants that wer e exposed to dark at 32±2°C showed the highest callus per centage compar ed to the cultur es exposed to dark at 22±2°C. MS medium contained 0.5 mg/l TDZ + 1.5 mg/l NAA with Gamborg's vitamins showed to be the effective medium for callus induction. Data indicated that both cotyledons and hypocotyls gave the highest per centage of callus formation after one week of incubation at dark. Moreover cotyledons, hypocotyls and physiological base explant cultur ed on MS medium contained 0.50 mg/l TDZ+ 1.50 mg/l NAA or 1.50 mg/l TDZ+ 0.5 mg/l NAA+ 0.60 mg/l AgNO3 with Gamborg's vitamins and exposed to dark pretreatment for one week at 32±2°C gave the highest callus per centage. Data detected three weeks after transferring all cultur es to 27±2°C under 16h photoperiod. As for r egeneration, physiological base explant cultur ed on MS exposed to dark pr etreatment at 32±2°C gave the best performance for shoot formation compar ed to explants cultur ed at 27±2°C under 16 h photoperiod without dark pr e-treatment. Data clearly indicated that highest per centage of shoot formation was obtained with physiological base explant cultur ed on MS medium contained 1.5 mg/l BA with Gamborg's vitamins. While explant cultur ed on MS medium contained 4mg/l BA + 2 mg/l Kin with Gamborg's vitamins significantly incr eased shoot formation per centage. In that concern, negative r esults wer e detected with leaf, cotyledons or hypocotyl cultur ed on all medium compositions used in this investigation.

Keyw ords: Momor dica charantia, In vitro growth, Pretreatment.

INTRODUCTION

Momor dica charantia is one of the most nutritional and medicinal plants belonging to cucurbitaceae family (Tanget al., 2010). Momor dica means, "to bite" referring to the jagged edges of the leaf, which appear as if bitten. All parts of the plant, including the fruits taste bitter. The fruit is emerald green that turns to orange- yellow when ripe (Grover and Yadav, 2004). Momor dica charantia plant is a slender tendril climbing, annual vine believed to be originated in Asia and in tropical areas of Africa, the Caribbean and south America and is commonly consumed as a vegetable (Thiruvengadamet al., 2006).

Momor dica charantia is considered as minor cucurbitaceous vegetable in spite of having considerable nutritional and medicinal properties. It contains high concentrations of ascorbic acid and iron (Beloinet al., 2008). Animal and human studies suggested that the fruits, seeds and leaf extracts of this plant possess hypoglycemic effects (Danset al., 2007). It is a potent hypoglycemic agent due to alkaloids, insulin-like peptides, and a mixture of steroidal sapogenins known as charantin. It has been used as anticancer, showed high antiviral and antithelmintic activity and preventing development of gastric and duodenal ulcers in rats (Beloinet al., 2004; Alamet al., 2009; Gunasekaran, 2010). MAP30 is an anti-HIV plant protein that have been identified and purified from Momor dica charantia (Lee-Huangel al., 1995). Akhtar and Husain (2006) told that highest level of total or ganic carbon was removed from the model wastewater containing individual phenol or complex mixture of phenols by immo-bilized Momor dica charantia peroxidase. Moreover it is suggested that Momor dica charantia exhibits a protection mechanism against oxidative damage by maintaining a highly induced antioxidant system (Agarwal and Shaheen, 2007).

Pretreatment of tissues at 4 C is routinely used to increase the embryonic potiential of excised anther (Swartzet al., 1990). Tanget al. (2012) stated that brown callus derived from anther limited the application of the anther culture in Momor dica charantia. After pre-treatment at 4°C for 1 day, callus induction rate was the highest and browning rate was the lowest. Pretreatment also used with leaves, cotyledons, nodal segment, hypocotyls and seeds with dif ferent protocols like submer ged in liquied MS with colshicine and TDZ (1-phenyl-3- (1,2,3-Thiadiazol-5-yl) urea for 3 days, or submer ged in water, or explants kept in

a sterile cabin under an air flowfor 30 min then immersed in MS solution containing 1 mg/ l BA (6-benzylaminopurine)and 0.02 mg/l NAA(1-Naphthaleneacetic acid)for 15 min then explants were cultured on MS medium without any growth regulators and in addition explants could be pretreated by exposed to dark exposed to dark (Swartz et al., 1990; Yildiz and Özgen, 2004; Yildiz et al., 2010 and Mendieta et al., 2010).

Tissue culture technique provides a unique chance for studying many aspects of plant growth and development (Cano et al., 1998 and Shatnawi, 2006). Formation of basal nutrient salts significantly influenced gel strength. High nutrient salt concentrations, may possibly contribute to the lower gel strength of MS medium (Baker and Wetzstein, 1994). Many studies have been conducted the effect of MS nutrient salt strength on in vitro germination, shoot formation, and root length (Arnold et al., 1995 and Castillo, 1998).

Momordica charantia is extremely susceptible to damage by many pathogens, such as fungus, virus and insects, which severely limit the yield (Tan et al., 2003). Furthermore, improvement via genetic transformation pre-requisites the establishment of an efficient, fast and reproducible plant regeneration system. Only few results on Momordica charantia in vitro studies such as direct shoot regeneration of different explants have been reported (Thiruvengadam et al., 2007). Production of callus and its subsequent regeneration are the prim steps in plant to be manipulated by biotechnology means (Saharan et al., 2004). There are reports of limited in vitro studies in Momordica tissue cultures (Thiruvengadam et al., 2006).

Investigations related to tissue culture and the in vitro regeneration system of Momordica charantia has not been established yet in Egypt. These were detrimental to the conservation and propagation of such varieties as this plant is not a domestic vegetable in Egypt. Here we reported the establishment of in vitro regeneration system. The aim of this work was study the effect of dark pretreatment with different thermal conditions (32±2°C or 22±2°C), explant type and growth regulator concentrations on callus production and regeneration of Momordica charantia.

MATERIALS AND METHODS

The present study was carried out through 2010 to 2012 at the Laboratory of Tissue Culture Center, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt.

Seeds of Momordica charantia were obtained from 'Horizon Herbs' for strictly medicinal seeds, USA. Seeds without their coat were washed thoroughly under running tap water for 10 to 15 min., then surface sterilized with 0.1 % (m/v) mercuric chloride along with 1- 2 drops of Tween-20 for 15 min followed by rinsing seven times with sterile distilled water to remove traces of $HgCl_2$ under a laminar airflow cabinet. Decoated seeds then inoculated in free-growth regulators MS medium (Murashige and Skoog, 1962) contained 7g/l agar and Gamborg's vitamins (Gamborg et al., 1968).

Experiment 1: Callus formation.

Leaf, cotyledon sections, hypocotyl, physiological base and root were excises from in vitro seedling plantlets, then cultured onto MS medium free of growth regulators or contained either 1.5 mg/l TDZ+ 0.5mg/l NAA+ 0.6 mg/l $AgNO_3$ or 0.5 mg/l TDZ+ 1.5 mg/l NAA. Cultures were kept in the dark at 32±2°C or 22±2°C for one week. Data were taken as percentage of callus. Then jars were transferred and incubated at 27±2°C under 16 h photoperiod for three weeks. The experiment was carried out to study the effect of the dark with different thermal conditions as a pretreatment, explant type and growth regulator concentrations on callus formation, and data were recorded as [percentage of explants producing callus and callus fresh and dry weight (g/l)].

Experiment 2: Regeneration.

All treatments of the previous experiment showed no sign for regeneration. So, callus produced on MS medium free growth regulators were used to run a regeneration experiment in order to avoid the effects of the residual of the previously used growth regulators. That callus which obtained from different explants (Leaf, cotyledon sections, hypocotyl, physiological base and root) were cultured separately on MS medium contained different growth regulators as following: 1.5mg/l 2iP 6-(ã,ã-dimethylallyamino) purine, 1.5mg/l Kin (6-furfurylaminopurine), 5.0mg/l kin., 1.5 mg/l BA (6-benzyl adenine), 1.5 mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid), 1.5mg/l NAA, 0.1mg/l NAA, 1.0mg/l BA+ 0.1mg/l NAA, 1.5mg/l BA+ 0.1mg/l NAA, 0.5mg/l BA+ 2.0mg/l NAA, 0.5mg/l BA+ 2.0mg/l NAA+ 2.0mg/l 2,4-D, 2.0mg/l BA+ 1.0mg/l Kin., 4.0mg/l BA+ 2.0mg/l Kin., 1.5mg/l TDZ+ 0.5mg/l NAA+ 0.6mg/l $AgNO_3$ and 0.5 mg/l TDZ+ 1.5mg/l NAA with Gamborg's vitamins. That lead to 16 treatments for each explant type, each treatment had 50 replicates. Data were recorded after 4 weeks as shoot formation [percentage of explants producing shoot, number of shoot, number of nodes, shoot length] and productivity proliferation rate according to Perez-Torner oet al., (2000).

In all experiments 3% sucrose was added to the medium as a Carbone source, pH was adjusted and maintained at 5.8. Cultures exposed to 27±2°C under 16 h photoperiod at 3000 lux from fluorescent tubular lamps.

STATISTICAL ANALYSIS

The experiment was conducted under controlled conditions and were design in factorial completely design. The comparative LSD multiple range test (P=0.05) was used to determine differences between treatments. Data were compared according to method described by Snedecor and Cochran (1989) with the help of MSTAT software version 2.10.

RESULTS AND DISCUSSION

Experiment 1: Callus formation.

Pretreatment of one week on total darkness.

The effect of dark with different thermal conditions 32±2°C or 22±2°C on callus initiation percentage has been studied. Different Momordica charantia explants were

cultured on different types of MS medium with Gamborg's vitamins.Data detected after one week of culture as shown in Table (1). Data of the main effect of medium compositionindicate that significant difference of callus initiation percentage(81.43) was found withMS medium contained0.50 mg/lTDZ+ 1.50mg/l NAAwith Gamborg's vitamins.While thelowest responseof callus initiation percentage (44.77) was observed with free-growth regulators MS mediumwith Gamborg's vitamins. Concerning the main effect of thermal conditions, the condition of dark32±2 ¡ÛC showed the highest significant callus initiation percentage (88.70) compared to 22±2 ¡ÛC (43.44). Concerning the main effect of explant, cotyledon gave the highest significant frequency of callus initiation percentage (85.24).While the lowest response of callus initiation percentage (50.34) was observed with root.

Data of interaction between medium composition and thermal condition indicated that,both types ofMS medium contained 1.50 TDZ+ 0.50 NAA+ 0.60 AgNO3 or0.50 mg/lTDZ+ 1.50 mg/lNAAwith Gamborg's vitaminsused with total darkness at 32±2 ¡ÛC was significantly highest of callus initiation percentage (100).While the lowest response of callus initiation percentage (23.43) was observed by using MS medium free of growth regulators with Gamborg's vitamins and total darkness at 22±2 ¡ÛC. Data of interaction between Medium composition and explant types indicated that, the highest percentageof callus initiation (96.20) was obtained by culturing cotyledon on MS medium contained 0.50 mg/lTDZ+ 1.50 mg/lNAAwith Gamborg's vitamins.While the lowest response of callus initiation percentage (16.51) was observed by culturing root on MS free growth regulatorswith Gamborg's vitamins. Data of interaction between thermal condition and explant type indicated that, both cotyledon and hypocotyl cultured in total darkness at 32±2 ¡ÛC gave the highest percentage of callus initiation (100).The lowest response of callus initiation percentage (23.01) was observed by using root withtotal darkness at 22±2 ¡ÛC. Data of interaction for the three studied factors indicated that, at the condition of 32±2 ¡ÛC, all used explants gave the similar results of callus initiation percentage (100) with all used media compositions except the treatment of control with leaf, physiological base and root explants.In that concern Summart et al.(2008) studied the effect oflight condition on growth of the rice cell culture and illustrated that, calli grown under dark condition had higher cell mass than that under light condition (16/8 h light/dark cycle).

Table 1: Effect of darkat 32 ± 2¡ÛC or 22 ± 2¡ÛC on percentage of callus initiation fromdifferent Momordicacharantiaseedlingderived explants. Explants werecultured on MS solid medium with Gamborg's vitamins containeddifferent concentrations of growth regulators and incubated forone week.

Conc. mg/l	Total darkness (32 ±2¡ÛC, Dark)						Total darkness (22 ±2¡ÛC, Dark)						Mean of media
	Explant Type					Mean	Explant Type					Mean	
	Leaf	Cotyledons	Hypocotyls	Physiological Base	Root		Leaf	Cotyledons	Hypocotyls	Physiological Base	Root		
Control	60.33	100.00	100.00	37.20	33.01	66.11	38.14	58.66	0.00	20.36	0.00	23.43	44.77
1.50 TDZ+ 0.50 NAA+ 0.60AgNO3	100.0	100.00	100.00	100.00	100.0	100.0	40.70	60.39	39.70	49.72	29.70	44.04	72.02
0.50 TDZ+ 1.50 NAA	100.0	100.0	100.0	100.0	100.0	100.0	76.47	92.39	39.67	66.39	39.33	62.85	81.43
Mean	86.78	100.0	100.00	79.07	77.67	88.70	51.77	70.48	26.46	45.49	23.01	43.44	

Leaf	Cotyledons	Hypocotyls	Physiological Base	Root
69.28	85.24	63.23	62.28	50.34

LSD 5% For

Media Composition	0.1124
Thermal Condition	0.0917
Media Composition XThermal Condition	0.1590
ExplantType	0.1451
Media Composition X ExplantType	0.2513
Thermal Condition X ExplantType	0.2052
Media Composition XThermal Condition X ExplantType	0.3554

Transferring of cultures to light on photoperiod conditions.

After one week of total darkness all jars after grown at 32±2 ¡ÛC or 22±2 ¡ÛC were transferred and incubated at 27±2 ¡ÛC under 16 h photoperiod. Data were detected after three weeks in order to study the effect previous treatments as pretreatment on callus formation [percentage of explants producing callus and callus fresh and dry weight (g/l)].

As shown inTable (2), Data of main effect of medium composition indicate that significant difference of callus production percentage (85.75) was found with MS medium contained 0.50 mg/lTDZ+ 1.50 mg/l NAAwith Gamborg's vitamins.While the lowest response of callus production percentage (62.13) was observed with free-growth regulators MS medium with Gamborg's vitamins. Concerning the main effect of pretreatment dark at 32±2 ¡ÛC showed the highest significant callus production percentage (97.32) compared to 22±2 ¡ÛC (56.01). Concerning the main effect of explant, cotyledon gave the highest significant frequency of callus production percentage (93.07).While the lowest response of callus initiation percentage (60.44) was observed with root.In that concern,Thiruvengadam et al. (2010) conducted that high callus percentage was obtained when mature leaf explants in vivo Momordicacharantia grown on MS medium with Gamborg's vitamins, and 7.7ìM NAA and 2.2ìM thidiazuron (TDZ). Regenerationof adventitious shoots from callus was achieved on MS medium containing 5.5ìMTDZ, 2.2ìM NAA, and 3.3ìM silver nitrate (AgNO3), however no sign for regeneration found in this experiment.The differences between our results with those previously reported are may be due to the difference in Momordicacharantia cultivar genotype, used in this investigation.

Data of interaction between medium composition

and thermal condition indicated that, both types of MS medium contained 1.50 mg/l TDZ+ 0.50 NAA+ 0.60 AgNO3 or 0.50 mg/l TDZ+ 1.50 mg/l NAA with Gamborg's vitamins exposed to total darkness at 32±2 °C as a pretreatment four significant highest of callus production percentage (100). While the lowest response of callus production percentage (32.28) was observed by using MS medium free growth regulators with Gamborg's vitamins that exposed to total darkness at 22±2 °C as a pretreatment. Data of interaction between Medium composition and explant types indicated that, the highest percentage of callus production (100) was obtained by culturing both leaf and cotyledon on MS medium contained 1.50mg/l TDZ+ 0.50 NAA+ 0.60 AgNO3 or 0.50mg/l TDZ+ 1.50mg/l NAA with Gamborg's vitamins. While the lowest response of callus production percentage (45.83) was observed by culturing root on MS free growth regulators. Data of interaction between thermal condition and explant type indicated that, leaf, cotyledon and hypocotyl exposed to total darkness at (32±2 °C) gave the highest percentage of callus production (100). The lowest response of callus production percentage (30.42) was observed by using root that exposed to total darkness at 22±2 °C. Data of interaction for the three studied factors indicated that, at the condition of 32±2 °C, all used explants gave the similar results of callus formation percentage (100) with all used media compositions except the treatment of control with leaf and root explants (Fig. 1).

These results were in a harmony with a study on dark grown Rose leaves formed callus, which results showed that the dark treatment of the leaves is important for callus formation. The highest rate of callus formation and highest number of callus colonies were obtained in the presence of TDZ, none of these calli formed shoot (canli, 2003). Guoet al. (2011) found that TDZ has shown both auxin and cytokinin like effects, although, chemically it is totally different from commonly used auxins and cytokinins.

Table 2: Effect of dark at 32 ± 2 °C and 22 ± 2 °C as a pretreatment for one week on callus formation percentage of different Momordicacharantiaseedling-derived explants. Explants were cultured on MS medium with Gamborg's vitamins contained different growth regulator concentrations and incubated for three weeks at 27 ± 2 °C 16 h photoperiod.

Conc. mg/l	Explants Exposed to Dark Pretreatment at (32±2 °C dark)						Explants Exposed to Dark Pretreatment at (22±2 °C dark)						Total Mean
	Explant Type					Mean	Explant Type					Mean	
	Leaf	Cotyledons	Hypocotyls	Physiological Base	Root		Leaf	Cotyledons	Hypocotyls	Physiological Base	Root		
Control	88.46	100.0	100.0	100.00	71.38	91.97	40.19	58.42	20.29	22.25	20.27	32.28	62.13
1.50 TDZ+ 0.50 NAA+ 0.60 AgNO3	100.0	100.0	100.0	100.00	100.0	100.0	100.0	100.0	40.68	50.11	30.50	64.26	82.13
0.50 TDZ+ 1.50 NAA	100.0	100.0	100.0	100.00	100.0	100.0	100.0	100.0	50.34	66.69	40.49	71.50	85.75
Mean	96.15	100.0	100.0	100.0	90.46	97.32	80.06	86.14	37.10	46.35	30.42	56.01	

Leaf	Cotyledons	Hypocotyls	Physiological Base	Root
88.11	93.07	68.55	73.18	60.44

LSD 5% For

Media Composition	0.0859
Thermal Condition	0.0701
Media Composition X Thermal Condition	0.1214
Explant Type	0.1108
Media Composition X Explant Type	0.1920
Thermal Condition X Explant Type	0.1567
Media Composition X Thermal Condition X Explant Type	0.2715

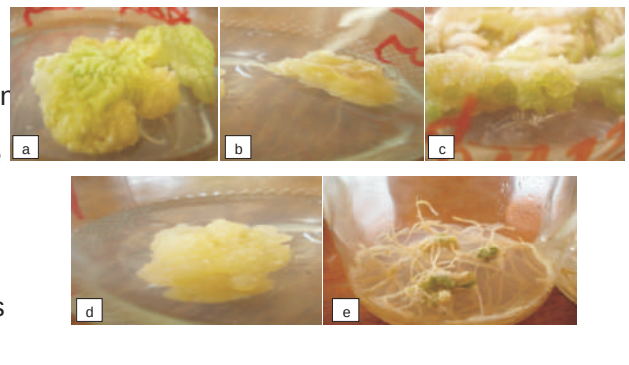

Fig.1: Callus of Momordicacharantiadifferent explants [Leaf (a), Cotyledon (b), Hypocotyl (c) Physiological base (e) And root (f)], for 4 weeks.

Estimation of callus fresh and dry weight.

Callus percentage and callus fresh and dry weight were detected, as showed through following diagrams (Fig. 2, 3, 4 and 5). Data of the main effect of explant types that exposed to dark pretreatment at 32±2 °C on callus fresh weight, indicate that, leaf gave the highest significant fresh weight (2.96) followed by cotyledon and root (2.77 and 2.73, respectively) with no significant difference between both of them. The lowest response of callus fresh weight was observed with hypocotyl and physiological base (2.34 and 2.53, respectively) with no significant difference between both of them. Concerning the main effect of medium composition, nosignificant difference of callus fresh weight between both types of MS medium contained 1.50 mg/l TDZ+ 0.50 NAA+ 0.60 AgNO3 or 0.50mg/l TDZ+ 1.50 mg/l NAA with Gamborg's vitamins, both gave the highest response (3.65 and 3.57, respectively). The lowest response of callus fresh weight (0.77) was observed with MS medium free growth regulators with Gamborg's vitamins. Data of interaction indicated that, the highest percentage of callus fresh weight (4.4234) was obtained by culturing leaf on MS medium contained 1.50mg/l TDZ+ 0.50 NAA+ 0.60 AgNO3 with Gamborg's vitamins. The lowest response (0.0875) of callus fresh weight was observed with hypocotyl cultured on free- growth regulators MS medium with Gamborg's vitamins.

However, in Fig. (3), data show the main effect of explant types that exposed to dark pretreatment at 32±2 °C on callus dry weight. These data indicated that, leaf and root gave the highest significant dry weight (0.1922 and 0.1470, respectively) with no significant difference between both of them. The lowest response of callus dry weight was observed with cotyledon, hypocotyl and physiological base (0.0650, 0.0480 and 0.0892, respectively) with no significant difference between them. Concerning the main effect of medium composition, nosignificant difference of callus dry weight between both types of MS medium contained 1.50 mg/l TDZ+ 0.50 NAA+ 0.60 AgNO3 or 0.50 mg/l TDZ+ 1.50 mg/l NAA with Gamborg's vitamins, both gave the highest response (0.1611 and 0.1186, respectively). The lowest response of callus dry weight (0.0903) was observed with MS medium free growth regulators with Gamborg's vitamins. Data of interaction indicated that, the highest

percentage of callusdry weight was obtained byculturing leaf on MS mediumfree growth regulators or culturing root on MS mediumcontained 0.50 mg/lTDZ+ 1.50 mg/lNAA with Gamborg's vitamins, (0.2890 and 0.2329, respectively) with no significant difference between both of them.The lowest response of callus dry weight (0.0059) was observed with hypocotyl cultured on free- growth regulators MS medium with Gamborg's vitamins.

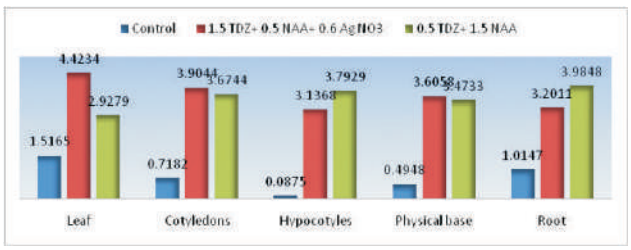

Fig. 2: Fresh weight (mg/explant) of different 32oC condition-derived explants cultured on MS medium with different concentrations of growth regulators (mg/l). Data were detected after three weeks of incubation in photoperiod conditions.

LSD 5 % for:

Explant type	0.1827
Media composition	0.1416
Interaction	0.3165

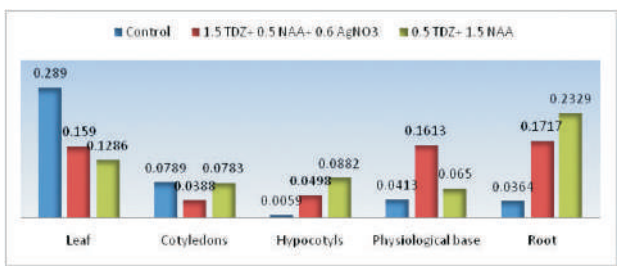

Fig. 3: Dry weight (mg/explant) of different 32 oC condition-derived explants cultured on MS medium with different concentrations of growth regulators (mg/l). Data were detected after three weeks of incubation in photoperiod.

LSD 5 % for:

Explant type	0.0601
Media composition	0.0465
Interaction	0.1041

Data in Fig. (4), show the main effect of explant types that exposed to dark pretreatment at 22±2 C on callus fresh weight.These data indicated that, leaf gave the highest significant fresh weight (3.321). The lowest response of callus fresh weight (1.548) was observed with root. Concerning the main effect of medium composition, significant difference was detected of callus fresh weight by using MS medium contained 1.50mg/l TDZ+ 0.50 NAA+ 0.60AgNO3with Gamborg's vitamins (3.080).The lowest response of callus fresh weight (0.4520) was observed with MS medium free growth regulatorswith Gamborg's vitamins. Data of interaction indicated that,the highest percentage of callus fresh weight was obtained by culturing leaf on MS mediumcontained 1.50mg/l TDZ+ 0.50 NAA+ 0.60 AgNO3 or 0.50 mg/lTDZ+ 1.50mg/lNAAwith Gamborg's vitamins (4.5888 and 4.0123, respectively) with no significant different between both of them.The lowest response of callus fresh weight (0.0385) was observed with hypocotyl cultured on free- growth regulators MS medium with Gamborg's vitamins.

However, in Fig. (5), data show the main effect of explant types that exposed to dark pretreatment at 22±2C on callus dry weight.These data indicated that, leaf gave the highest significant dry weight (0.155). The lowest response of callus dry weight was observed with hypocotyl and physiological base (0.064 and 0.0784, respectively), with no significant different between both of them. Concerning the main effect of medium composition, nosignificantdifference of callus dry weight between both types of MS medium contained 1.50 mg/lTDZ+ 0.50 NAA+ 0.60AgNO3 or 0.50mg/lTDZ+ 1.50mg/lNAAwith Gamborg's vitamins (0.1388 and 0.10932, respectively), both gave the highest response.The lowest response of callus dry weight (0.0401) was observed with MS medium free growth regulatorswith Gamborg's vitamins. Data of interaction indicated that,the highest percentage of callus dry weight was obtained byculturing leaf on MS mediumcontained 1.50mg/l TDZ+ 0.50 NAA+ 0.60 AgNO3 or 0.50mg/lTDZ+ 1.50mg/lNAAwith Gamborg's vitamins (0.1996 and 0.1947, respectively), with no significant difference between both of them.The lowest response of callus dry weight (0.0067) was observed with hypocotyl cultured on free- growth regulators MS medium with Gamborg's vitamins.

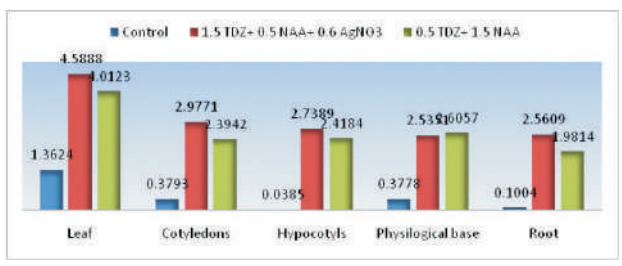

Fig. 4: Fresh weight (mg/explant) of different-22C condition-derived explants cultured on MS medium with different concentrations of growth regulators (mg/l). Data were detected after three weeks of incubation in photoperiod.

LSD 5 % for:

Explant type	0.9501
Media composition	0.0736
Interaction	0.1646

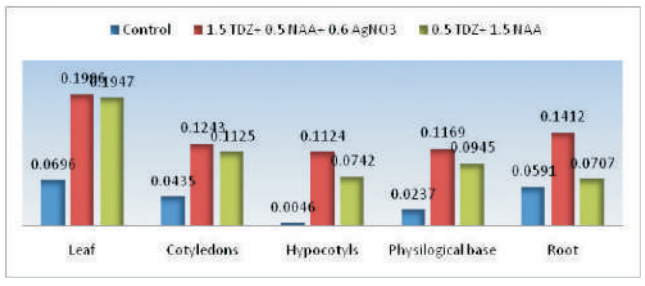

Fig. 5: Dry weight (mg/explant) of different-22 C condition-derived explants cultured on MS medium with different concentrations of growth regulators (mg/l). Data were detected after three weeks of incubation in photoperiod.

LSD 5 % for:

Explant type	0.3439
Media composition	0.2664
Interaction	0.5956

Experiment 2: Regeneration.

According to the previous experiment's results, studying of Momordica charantia callus differentiation has been established. Callus from leaf, cotyledon, hypocotyl, physiological base, and root that exposed to dark at 32±2 C for one week as a pretreatment and were cultured on MS medium free of growth regulators (in order to avoid the effect of residual effect of growth regulators) were compared with those exposed to 27±2 C under 16 h photoperiod without pretreatment. Different types of MS medium with Gamborg's vitamins used with different kind and concentrations of growth regulators as described previously, including media compositions used in the first experiment. Data taken after four weeks of culture.

Negative results were detected with leaf's, cotyledon's and hypocotyl's callus. While physiological base's and root's callus were indicated positive results with only few types of MS medium were used in this investigation(Fig.6).

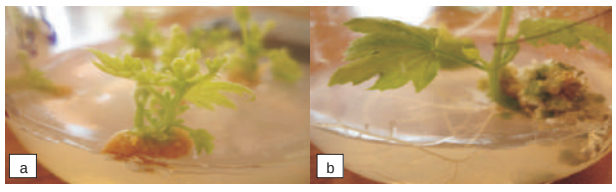

Fig. 6: Callus differentiations and shoot formations of Momordica charantia different explants[Shoots formation from physiological base callus (a). Shoots formation from root callus (b)], for 4 weeks.

Shoot formation from physiological base's callus was investigated as shown Table (3). Data of the main effect of medium composition showed that, MS medium contained 1.50 mg/l BA with Gamborg's vitamins significantly gave the highest percentage of shoot frequency (48.83). While the lowest response of shoot frequency percentage (15.50) was observed with MS medium contained 2.00 mg/l BA+ 1.00 mg/l Kin with Gamborg's vitamins. Concerning the main effect of pretreatment, physiological base exposed to pretreatment dark at 32±2 C for one week gave the highest significant percentage of shoot (36.84) compared to without pretreatment condition (24.58). Data of interaction indicated that for shoot formation the highest percentage (52.33) was observed by culturing physiological base's callus on MS medium contained 1.50 mg/l BA with Gamborg's vitamins and exposed to dark at 32±2 C for one week as a pretreatment.

Data of the main effect of medium composition on number of shoot showed that, MS medium contained 0.50 mg/l BA+ 2.00 mg/l NAA+ 2.00 mg/l 2,4-D or 2.00 mg/l BA+ 1.00 mg/l Kin with Gamborg's vitamins gave the highest value (2.67 and 2.70, respectively) with no significant difference between both of them. The lowest value of number of shoot (0.88) was observed with MS medium contained 1.50 mg/l 2,iP with Gamborg's vitamins. Concerning the main effect of pretreatment, physiological base exposed to dark pretreatment at 32±2 C for one week gave the highest significant number of shoot (2.36) compared to without pretreatment condition (1.47). Data of interaction indicated that for number of shoot the highest value (3.40) was observed by culturing physiological base's callus exposed to dark pretreatment at 32±2C for one week on MS medium contained 2.00 mg/l BA+ 1.00 mg/l Kin with Gamborg's vitamins.

Data of the main effect of medium composition on number of nods indicated that, using MS medium contained 2.00 mg/l BA+ 1.00 mg/l Kin with Gamborg's vitamins gave the highest value (1.50) of number of node. While the lowest value of number of nods (1.38) was observed with MS medium contained 1.50 mg/l 2,iP with Gamborg's vitamins. Concerning the main effect of pretreatment, physiological base's callus exposed to dark pretreatment at 32±2C for one week gave the highest significant value of number of node (5.76) compared to without pretreatment condition (5.16). Data of interaction indicated that for number of node the highest value (1.67) was observed by culturing physiological base's callus exposed to dark pretreatment at 32±2C for one week on MS medium contained 2.00 BA+ 1.00 mg/l Kin with Gamborg's vitamins.

Data of the main effect of medium composition indicated that, the highest shoot length value (3.28) was observed by using MS medium contained 1.50 mg/l BA Gamborg's vitamins. However the lowest value of shoot length (1.49) was observed with MS medium contained 1.50 mg/l 2,iP with Gamborg's vitamins. Concerning the main effect of pretreatment, physiological base exposed to dark pretreatment at 32±2 C for one week gave the highest significant value of shoot length (2.84) compared to without pretreatment (2.05). Data of interaction indicated that for shoot length the highest value (3.33) was observed by culturing physiological base's callus that exposed to dark pretreatment at 32±2 C for one week on MS medium contained 1.50 mg/l BA with Gamborg's vitamins.

Highest productivity (33.36) was observed with MS medium contained 2.00 mg/l BA+ 1.00 mg/l Kin with

Gamborg's vitamins.Physiological base's callus exposed to pretreatmentdark at 32±2 C for one week showed to be the highest productivity value (15.40) in compared to without pretreatment condition (13.11).That was in harmony withChuenboonngarmet al. (2001) who detected that BA was superior to 2iP giving more shoots per explant when same concentrations of the two plant growth regulators werecompared.The effect of BAinduction period on shoot differentiation, with increase inthe duration of culture, there was increase in the numberof shoot buds with a simultaneous decrease in furtherelongation in the elongation medium (Paul et al., 2000).

Shoot formation from root's callus was investigated as shown in Table (4). Data of the mainfect of medium composition showed that, MS medium contained 4.00mg/l BA+ 2.00 Kinwith Gamborg's vitaminssignificantly gave the highestpercentage of shoot frequency (27.84).Whilethe lowest response of shoot frequency percentage (15.00) was observed with MS medium contained 5.00 mg/l Kinwith Gamborg's vitamins. Concerning the main effect of pretreatment, root's callus exposed to dark pretreatmentat 32±2°C for one week gave the highest significant percentage of shoot percentage (27.42) compared to without pretreatment condition (15.33). Data of interaction indicated that for shoot formation the highest percentage (34.67) was observed by culturing root's callus on MS medium contained 4.00mg/l BA+ 2.00 Kinwith Gamborg's vitamins and exposed todark at 32±2°C for one week as a pretreatment.

Data of the main effect of medium composition on number of shoot showed that, MS medium contained 4.00 mg/l BA+ 2.00 mg/l Kin or free growth regulatorswith Gamborg's vitaminsgave the highest value (2.17 and 2.00, respectively) with no significant difference between both of them.The lowest value of number of shoot (1.67) and (1.50) was observed with MS medium contained 1.50mg/l BA and 5.00mg/l Kinwith Gamborg's vitamins, respectively without significant difference between both of them. Concerning the main effect of pretreatment, root exposed to dark pretreatment at 32±2°C for one week gave the highest significant number of shoot (2.08) compared to without pretreatment condition (1.58).Data of interaction indicated that for number of shoot the highest value (2.33) was observed by culturing root's callus exposed to pretreatment dark at 32±2°C for one week on MS medium contained 4.00mg/l BA+ 2.00mg/l Kinand Gamborg's vitamins with no significant difference and with MS medium contained 1.50mg/l BA, 5.00 mg/l Kin and free growth regulatorswith Gamborg's vitamins (2.00) exposed to pretreatment and with MS medium contained 4.00 BA+ 2.00 Kin or free growth regulatorswith Gamborg's vitamins and without pretreatment.

Data of the main effect of medium composition on number of nods indicated that, using MS medium contained 4.00mg/l BA+ 2.00mg/l Kinwith Gamborg's vitamins gave the highest value (7.17) of number of nodswhile the lowest value of number of nods (2.50) was observed with MS medium contained 5.00 mg/l Kin. Concerning the main effect of pretreatment, root's callus exposed to dark pretreatmentat 32±2°C for one week gave the highest significant value of number of node (4.84) compared to without pretreatment condition (4.42). Data of interaction indicated that for number of node the highest value (7.67) was observed by culturingroot's exposed to pretreatmentdark at 32±2°C for one week on MS medium contained 4.00 BA+ 2.00mg/l Kinwith Gamborg's vitamins.

Data of the main effect of medium composition indicated that, the highest shoot length value (3.42) was observed by using MS medium contained 5.00 mg/l Kinwith Gamborg's vitamins. However the lowest value of shoot length (1.57) was observed with MS medium free growth regulatorswith Gamborg's vitamins. Concerning the main effect of pretreatment, root's callus exposed to pretreatment dark at 32±2°C for one week gave the highest significant value of shoot length (2.94) compared to without pretreatment (2.68). Data of interaction indicated that for shoot length the highest value (3.53), (3.27) and (3.37) for MS medium contained 5.00mg/l Kin, 1.50mg/l BA and 4.00mg/l BA+ 2.00 mg/l Kinwith Gamborg's vitamins, respectivelyexposed to dark pretreatmentat 32±2°C for one week and (3.30) and (3.00) for MS medium contained 5.00mg/l Kin and 4.00mg/l BA+ 2.00mg/l Kinwith Gamborg's vitamins respectively without pretreatment, with no significant difference between all of them.

Highest productivity (22.93)was observed with MS medium contained 4.00 mg/l BA+ 2.00mg/l Kinwith Gamborg's vitamins. Root exposed to pretreatmentdark at 32±2°C for one week showed to be the highest productivity value (14.93) in compared to without pretreatment condition (12.18).

In that concern,Al Munsuret al. (2009) detected that explants of nodal and root segments of Momordicacharantiawere cultured on MS supplemented with various concentrations of BA in combination with either 2,4-D or NAA. Nodal segments produced the highest percentage of callus in MS supplemented with 1.0 mg/l 2,4-D and 1.0 mg/l BA whereas, root segments produced the highest callus in 0.6mg/l NAA and 2.5mg/l BA combination. A combination of 1.0 mg/l 2,4-D and 1.0 mg/l BA exhibited 75.00% shoot regeneration from nodal segments. The highest shoot lengthwas recorded with 2.5mg/l BA and 0.2 mg/l IAA from nodal segments. Cytokinin such as 2-iP and kinetin are well known to promote rapid shootmultiplication (Jayanandet al., 2003 andKiranet al., 2005).In another study,shoots were weak with more internodal elongation.Therefore, for subsequent experiments, shoot elongationwas achieved on MS medium supplemented with kinetinin combination with 2-iP(Anwaret al., 2010).

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Table 3: The difference between cultures exposed to dark at (32 ± 2°C) as a pretreatment for one week and cultures without pretreatment of physiological base which obtained from Momordica charantia seedling-derived on shoot formation [percentage of explants producing shoot, number of shoot, number of nodes, shoot length and productivity]. Data detected four weeks after re-culture on MS solid medium contained different types of growth regulators.

Conc. mg/l	Shoot %			No. of Shoots			No. of Nods			Shoot Length (cm)			Productivity		
	(27 ± 2 °C, 16 h photoperiod)		Mean	(27 ± 2 °C, 16 h photoperiod)		Mean	(27 ± 2 °C, 16 h photoperiod)		Mean	(27 ± 2 °C, 16 h photoperiod)		Mean	(27 ± 2 °C, 16 h photoperiod)		Mean
	With Pretreatment	Without Pretreatment		With Pretreatment	Without Pretreatment		With Pretreatment	Without Pretreatment		With Pretreatment	Without Pretreatment		With Pretreatment	Without Pretreatment	
Control	39.87	35.57	37.72	2.00	1.67	1.84	7.40	7.47	7.44	2.13	1.97	2.05	15.76	14.72	15.24
1.50 2,iP	35.38	0.00	17.69	1.75	0.00	0.88	2.75	0.00	1.38	2.97	0.00	1.49	5.72	0.00	2.86
1.50 BA	52.33	45.33	48.83	2.00	1.00	1.50	3.00	2.92	2.96	3.33	3.23	3.28	9.99	9.43	9.71
0.50 BA+ 2.00 NAA+ 2.00 2,4-D	35.97	31.67	33.82	2.67	2.67	2.67	4.00	4.10	4.02	2.83	2.17	2.50	11.32	8.90	10.11
2.00 BA+ 1.00 Kin.	20.67	10.33	15.50	3.40	2.00	2.70	11.67	11.33	11.50	2.93	2.87	2.90	34.19	32.52	33.36
Mean	36.84	24.58	30.71	2.36	1.47	1.92	5.76	5.16	5.46	2.84	2.05	2.45	15.40	13.11	14.26

LSD 5% For				
Media Composition	0.7036	0.4274	0.5307	0.1711
Pretreatment Conditions	0.4450	0.2703	0.3357	0.1082
Media Composition X Pretreatment Conditions	0.9950	0.6044	0.7505	0.2420

Table 4: The difference between cultures exposed to dark at (32 ± 2°C) as a pretreatment for one week and cultures without pretreatment of roots which obtained from Momordica charantia seedling- derived on shoot formation [percentage of explants producing shoot, number of shoot, number of nodes, shoot length and productivity]. Data detected four weeks after re-culture on MS solid medium contained different types of growth regulators.

Conc. mg/l	Shoot %			No. of Shoots			No. of Nods			Shoot Length (cm)			Productivity		
	(27 ± 2°C, 16 h photoperiod)		Mean	(27 ± 2°C, 16 h photoperiod)		Mean	(27 ± 2°C, 16 h photoperiod)		Mean	(27 ± 2°C, 16 h photoperiod)		Mean	(27 ± 2°C, 16 h photoperiod)		Mean
	Pretreatment	Control		Pretreatment	control		Pretreatment	control		Pretreatment	control		Pretreatment	control	
Control	31.00	12.50	21.75	2.00	2.00	2.00	3.00	3.00	3.00	1.60	1.53	1.57	4.80	4.59	4.70
5.00 kin.	19.67	10.33	15.00	2.00	1.00	1.50	2.67	2.33	2.50	3.53	3.30	3.42	9.43	7.69	8.56
1.50 BA	24.32	17.50	20.91	2.00	1.33	1.67	6.00	5.67	5.84	3.27	2.90	3.09	19.62	16.44	18.03

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