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Effect Of Gelling Agent And Salt Strength Of Nutrient Medium On INVITRO Growth Of MOMORDICA CHARANTIA

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Abstract:-This investigation aimed to study the effect of two types of gelling agent (agar and gelrite) on in vitro growth of Momordicacharantia. Agar was tested at the concentrations (5g/l, 7g/l or 10g/l), while 1.5g/l, 2.5g/l or 3.5g/l gelrite were used. Shoot tip and nodal cutting were used as two types of explant. Nodal cutting explants showed the best performance for in vitro growth. Data indicated that, 7g/l agar gave the highest values of callus formation percentage and both of fresh and dry weight of callus, while gelrite was effective in increasing the shoot and root formation, as 2.5g/l gelrite significantly increased the percentage of shoot and root formation. However, both number of shoots and nods showed to be highest when 3.5g/l gelrite was added to the medium. Shoot length was significantly highest with 1.5g/l gelrite. Data clearly indicated that, highest percentage of survived plantlets which transferred to pots was obtained with 7g/l agar, but 1.5g/l gelrite gave no survival results. Hyperhydricity was significantly increased when 1.5g/l gelrite was used with nodal cutting explants which exposed plantlets grown on medium contained 2 mg/l BA, then sub-cultured on free- growth regulators MS medium. Data were detected after 4 weeks of incubation. For detection the effect of MS nutrient salt strength on in vitro growth using 7g/l agar or 2.5g/l gelrite with nodal cutting explant, data showed that, half strength of MS medium contained 7g/l agar significantly increased callus formation percentage. While full strength MS medium contained 2.5g/l gelrite significantly increased the percentage of shoot and root. Different MS nutrient strengths (0.5, 1.25 or 1.50) gave highest number of shoots without significant difference; however they significantly surpassed full strength of MS in that, concern. Moreover the highest number of nods was observed with 1.5 strength of Ms nutrient medium contained 2.5g/l gelrite. The highest value of shoot length was recorded by using 1.25 strength of MS nutrient medium contained 2.5g/l gelrite.

Keywords: Momordicacharantia, in vitro growth, Agar, Gelrite, Hyperhydricity, MS nutrient salt strength.

INTRODUCTION

Momordicacharantia is one of the most nutritional and medicinal plants belonging to cucurbitaceae family (Tang et al., 2010). Momordica 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). Momordicacharantia 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). Momordicacharantia is considered as minor cucurbitaceous vegetable in spite of having considerable nutritional and medicinal properties. It contains high concentrations of ascorbic acid and iron (Beheraet al., 2008). Animal and human studies suggest 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 (Singhet al., 2011). It has been used as anticancer, showed high antiviral activity and antithelmintic activity, and preventing development of gastric ulcers and duodenal ulcers in rats (Beloinet al., 2004; Alamet al., 2009 and Gunasekaran, 2010). MAP30 is an anti-HIV protein that have been identified and purified from Momordicacharantia (Lee-Huang et al., 1995). Akhtar and Husain (2006) told that highest level of total organic carbon was removed from the model wastewater containing individual phenol or complex mixture of phenols by immobilized Momordicacharantia peroxidase. Moreover it is suggested that Momordicacharantia exhibits a protection mechanism against oxidative damage by maintaining a highly induced antioxidant system (Agarwal and Shaheen, 2007).

Tissue culture technique provides a unique chance for studying many aspects of plant growth and development (Cano et al., 1998; 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; Castillo, 1998).

Gelling agents supplemented in culture medium play role in growth and development of plant cultured in vitro. Generally the concentration of gelling agents has a close relation with water stress. As high concentration of gelling agent causes a high water stress leaded to the difficulty of up taking water and elements from culture medium (Scholten and Pierik, 1998). Many authors reported the influence of gelling agent on development of embryos (Stoltz, 1971), shoot apical meristems (Rombert and Tabor, 1971), somatic embryos (Tremblay and Tremblay 1991), protoplasts (Kodaet al., 1988), and anther cultured in vitro (Kohlenbach and Wernicke, 1978). Both type and quality of gelling agents also produce problems related with hyperhydricity and necrosis of the tissue-e (Chatoet al., 2005).

Momordicacharantiais extremely susceptible to damage by many pathogens, such as fungus, virus and insects, which severely limit the yield (Tanget al., 2003). Furthermore, improvement via genetic transformation pre-requisites the establishment of an efficient, fast and reproducible plant regeneration system. Only few results on Momordicacharantia in vitro studies such as direct shoot regeneration of different explants have been reported (Thiruvengadamet 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 (Thiruvengadamet al., 2006).

Investigations related to tissue culture and in the vitro regeneration system of Momordicacharantiahas 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 to investigate the effect of gelling agent and MS nutrient salt strength on in vitro growth of Momordicacharantia via using shoot tip and nodal cutting as explant.

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 Momordicacharantiawere 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 (Gamborget al., 1968).

Experiment 1: Studying the effect of gelling agent on in vitro growth

shoot tip and nodal cutting were excises from in vitro deriving seedling of Momordicacharantiaand cultured on free- growth regulators MS medium with Gamborg's vitamins using agar at different concentrations (5 g/l, 7 g/l, and 10 g/l) or 1.5 g/l, 2.5 g/l and 3.5g/l gelrite as a gelling agent in order to investigate their effect on in vitro growth. Callus formation estimated as [percentage of explants producing callus and callus fresh and dry weight (g/l)], Shoot formation [percentage of explants producing shoot, number of shoot, number of node, shoot length (cm)], productivity proliferation rate according to Pereira-Torneroet al. (2000), root percentage and in vivo survival percentage of plantlets after one month of culturing in pots contain mixture of peat moss, vermiculite and sand 1:1:2 (v/v) respectively were detected.

Experiment 2: Studying hyperhydricity phenomena

According to the results of the previous experiment nodal cuttings were used to run this experiment. Nodal cuttings excised from plantlets grown on MS media with Gamborg's vitamins free- growth regulators or containing 2mg/l BA (benzyl adenine). Nodal cuttings were cultured on free- growth regulators MS medium with Gamborg's vitamins contains 5g/l, 7g/l or 10g/l agar or 1.5g/l, 2.5g/l or 3.5g/l gelrite as gelling agent in order to study the effect of the previous medium in combination with different types and concentrations of gelling agent on shoot formation [percentage of explants producing shoot, number of shoot, number of node, shoot length], productivity and hyperhydricity

Experiment 3: Studying the effect of MS nutrient salt strength

According to the results of the first experiment 7g/l agar and 2.5g/l gelrite were used with different strengths of MS medium (half, full, one and quarter, and one and half) in order to investigate their effect on in vitro growth of Momordicacharantianodal cutting. In all experiments 3% sucrose was added to the medium as a Carbone source, pH was adjusted and maintained at 5.8. All cultures were kept at a temperature of 27±2^{0}C under 16h 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 at (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: Studying the effect of gelling agent on in vitro growth

The effect of different types and concentrations of gelling agent on different explants (shoot tip and nodal cutting) and their interactions was studied. Data of the main effect of gelling agent on callus percentage and callus fresh and dry weight shown in Table (1), indicate that, 7g/l agar showed the highest significant percentage of callus frequency (38.74) followed by 2.5g/l gelrite (37.41). The

lowest response of callus frequency percentage (17.67) was observed with 1.5g/l gelrite. Concerning the main effect of type of explant, nodal cutting gave highest significant frequency percentage of callus (36.85) compared to shoot tip (20.51). Data of interaction indicated that,the highest percentage of callus formation (51.47) was obtained by culturing nodal cutting on free- growth regulators MS mediumcontained 7g/l agar with Gamborg's vitamins.The lowest response of callus frequency percentage (14.33) was observed with shoot tip cultured on free- growth regulators MS medium contained 1.5g/l gelritewith Gamborg's vitamins. Callus fresh and dry weights weredetected. Data of the main effect of gelling agent indicated that,7g/l agar was the most significant highest percentage for both fresh and dry weight (1.6969 and 0.4824) respectively. The lowest response of callus fresh weight (0.8091) was observed with 1.5g/l gelrite.While the lowest callus dry weight response (0.0800) was observed with 10g/l gelrite. Results showed that, the gradual increase in callus percentage was combined with the gradual increase in both callus fresh and dry weight.Concerning the main effect of type of explant, nodal cutting gave a highest significant value of callus fresh and dry weight (1.5926 and 0.2525) respectively compared to shoot tip (0.7258 and 0.0744) respectively. As for callus fresh weight, data of interaction in Table (1) indicate that, for callus fresh weight nodal cutting on free- growth regulators MS medium contained 7g/l agar with Gamborg's vitamins gave the highest significant value (2.9605). The lowest callus fresh weight response (0.4333) was observed with culturing shoot tip on free- growth regulators MS medium contained 7g/l agar with Gamborg's vitamins. For callus dry weight data of interaction indicated that, nodal cutting on free- growth regulators MS medium contained 7g/l agar with Gamborg's vitamins gave the highest significant value (0.9096).The lowest response of callus dry weight (0.0552) was observed with culturing shoot tip on free- growth regulators MS medium contained 7g/l agar with Gamborg's vitamins.

Shoot formation from shoot tip and nodal cutting was investigated as shown in Table (2).Wherein the base of the shoot tip produced callus or showed swelling without additional growth except the inflated of the leaf(Fig. 1).Data of the main effect of gelling agent showed that, 2.5g/l gelrite significantly gave the highest percentage of shoot frequency (45.17).While the lowest response of shoot frequency percentage(19.07) was observed with 1.5g/l gelrite. Concerning the main effect of type of explant, nodal cutting gave the highest significant percentage of shoot (61.69) compared to shoot tip (0.00). Data of interaction indicated that for shoot formation the highest percentage (90.33) was observed by culturing nodal cutting on free- growth regulators MS medium contained 2.5g/l gelrite. Data of the main effect of gelling agent on number of shoot showed that, 3.5g/l gelrite gave the highest value (2.30) followed by 10g/l agar (2.24) these results may indicate that, harder solid medium induce shoot formation as shown by the results of this experiment.The lowest value of number of shoot (1.00) was observed with both 5g/l agar and 1.5g/l gelrite with no significant difference. Concerning the main effect of type of explant, nodal cutting gave the highest significant value of number of shoot (2.28) compared to shoot tip (1.00). Data of interaction indicated that for number of shoot the highest value (3.60) was observed by culturing nodal cutting on free-growth regulators MS medium contained 3.5g/l gelrite.Data of the main effect of gelling agent on number of nods indicated that, using 7g/l agar gave the highest value (5.37) of number of nodes.While the lowest value of number of nods (2.17) was observed with 5g/l agar.Concerning the main effect of type of explant, nodal cutting gave the highest significant value of number of node (6.54) compared to shoot tip (1.00). Data of interaction indicated that for number of node the highest value (9.73) was observed by culturing nodal cutting on free- growth regulators MS medium contained 7g/l agar.Data of the main effect of gelling agent indicated that, the highest shoot length value (8.49) was observed by using 1.5g/l gelrite. However the lowest value of shoot length (2.02) was observed with 3.5g/l gelrite. Concerning the main effect of type of explant, nodal cutting gave the highest significant value of shoot length (7.25) compared to shoot tip (1.62). Data of interaction indicated that for shoot length the highest value (14.50) was observed by culturing nodal cutting on free- growth regulators MS medium contained 1.5g/l gelrite. Highest productivity (37.71) was observed with 1.5g/l gelriteand that can be explained as very high shoot length value showed when using this type and concentration of gelling agent in comparing with other treatments. Nodal cutting showed to be the highest productivity value (42.22) in compared to shoot tip(1.62) (Fig. 2).

Table 1: Effect of gelling agent on callus formation percentage (%), callus fresh and dry weight (g/explant) of shoot tip and nodal cuttingof Momordicacharantiain vitro deriving seedling grown on MS solid medium with Gamborg'svitamins free growth regulators, incubated for four weeks.

Gelling Agent Type	Callus %			Callus Fresh Weight			Callus Dry Weight		
	Explants Type		Mean	Explants Type		Mean	Explants Type		Mean
	Shoot Tip	Nodal Cutting		Shoot Tip	Nodal Cutting		Shoot Tip	Nodal Cutting	
5g/l Agar	20.50	35.50	28.00	0.7212	1.4101	1.0657	0.0741	0.1427	0.1084
7g/l Agar	26.00	51.47	38.74	0.4333	2.9605	1.6969	0.0552	0.9096	0.4824
10g/l Agar	20.53	43.47	32.00	0.8389	1.8513	1.3451	0.0872	0.1707	0.1290
1.50g/l Gelrite	14.33	21.00	17.67	0.7546	0.8635	0.8091	0.0698	0.1054	0.0876
2.50g/l Gelrite	25.30	49.53	37.42	0.7828	1.4706	1.1267	0.0768	0.1100	0.0934
3.50g/l Gelrite	16.37	20.10	18.24	0.8241	0.9994	0.9118	0.0833	0.0766	0.0800
Mean	20.51	36.85	28.68	0.7258	1.5926	1.1592	0.0744	0.2525	0.1635
LSD at 5%: Gelling Agent				0.7320		0.1853			0.0371
Explants Type				0.4226		0.1070			0.0214
Interaction				1.0350		0.2621			0.5242

Table 2: Effect of gelling agent on shoot formation percentage (%), numberof shoot and node, shoot length (cm) and productivity of shoot tip and nodal cutting ofMomordicacharantiain vitroderiving seedling grown on free- growth regulators MS solid medium with Gamborg's vitamins, incubated for four weeks.

Gelling Agent	Shoot %			No. of Shoot			No. of Node			Shoot Length (cm)			Productivity		
	Explants Type		Mean	Explants Type		Mean	Explants Type		Mean	Explants Type		Mean	Explants Type		Mean
	Shoot Tip	Nodal Cutting		Shoot Tip	Nodal Cutting		Shoot Tip	Nodal Cutting		Shoot Tip	Nodal Cutting		Shoot Tip	Nodal Cutting	
5g/l Agar	0.00	40.47	20.24	1.00	1.00	1.00	1.00	3.33	2.17	2.03	9.27	5.65	2.03	30.87	16.45
7g/l Agar	0.00	57.33	28.67	1.00	2.27	1.64	1.00	9.73	5.37	1.37	5.63	3.50	1.37	47.39	24.38
10g/l Agar	0.00	60.33	30.17	1.00	3.47	2.24	1.00	6.60	3.80	1.23	4.67	2.95	1.23	37.16	19.20
1.5g/l Gelrite	0.00	38.13	19.07	1.00	1.00	1.00	1.00	5.03	3.02	2.47	14.50	8.49	2.47	72.94	37.71
2.5g/l Gelrite	0.00	90.33	45.17	1.00	2.33	1.67	1.00	6.34	3.67	1.50	6.50	4.00	1.50	41.21	21.36
3.5g/l Gelrite	0.00	83.53	41.77	1.00	3.60	2.30	1.00	8.19	4.60	1.13	2.90	2.02	1.13	23.75	12.44
Mean	0.00	61.69	30.85	1.00	2.28	1.64	1.00	6.54	3.77	1.62	7.25	4.44	1.62	42.22	21.92
LSD GellingAgent	0.7254			0.0524			0.1996			0.4210					
Explants Type	0.4188			0.0303			0.1152			0.2430					
Interaction	1.0260			0.0741			0.2823			0.5953					

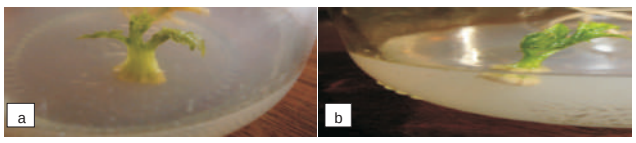

Fig. 1: Shoot tips cultured on MS medium contained different types of gelling agents [7g/l agar (a), 2.5g/l gelrite (b)], for 4 weeks.

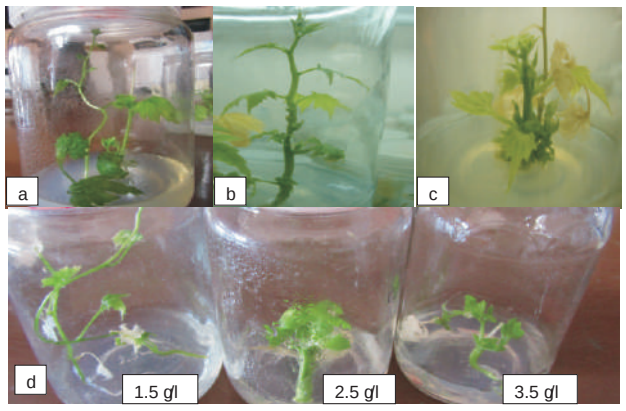

Fig. 2: Nodal cutting cultured on MS medium with different types of gelling agent. Nodal cutting grown on [5g/l agar (a), 7g/l agar(b), 10g/l agar(c) and three cultures on gelrite (1.5g/l, 2.5g/l and 3.5g/l) as labeled above(d)], for 4 weeks.

Data of the main effect of gelling agent on percentage of root and in vivo survival percentage shown in Table (3).As the dataindicate that,1.5g/l gelrite showed the highest significant percentage of root frequency (58.32). The lowest response of root frequency percentage (13.40) was observed with 5g/l agar. Concerning the main effect of type of explant, nodal cutting gave a highest significant frequency percentage of root (61.12)compared to shoot tip (34.98). Data of interaction indicated that, for root formation the highest percentage (81.94) was by culturing nodal cutting on free- growth regulators MS medium contained 2.5g/l gelrite with Gamborg's vitamins. The lowest root frequency percentage response (10.24) was observed with shoot tip cultured on free- growth regulators MS medium contained 5g/l agar with Gamborg's vitamins.In vivo survival percentage was detected after one month of culture on pots containspeat moss, vermiculite and sand 1:1:2 (v/v) respectively.Data of the main effect of gelling agent indicated that,7g/l agar was the most significant highest percentage (22.85) forin vivo survival followed by 2.5g/l gelrite (18.42).The lowest percentage of in vivo response (6.57) was observed with 10g/l agar. While no invivo survival detected by using 1.5g/l gelrite. Concerning the main effect of type of explant, nodal cutting gave a highest significant percentage of in vivo survival(20.08) compared to shoot tip (3.62). Data of interaction indicated that, for in vivo survival nodal cutting on free- growth regulators MS medium contained 7g/l agar with Gamborg's vitamins gave the highest significant percentage (34.33) followed by 2.5 g/l gelrite (29.27).The lowest in vivo survival response (2.77) was observed with culturing shoot tip on free- growth regulators MS medium with Gamborg's vitamins contains 5g/l agar. No detection of in vivo survival by using shoot tip cultured onMS medium contained10g/l agar, 1.5g/l gelrite or 3.5 g/l gelrite or by using nodal cutting culturing on MS medium contained 1.5 g/l gelritewith Gamborg's vitamins (Fig. 3). 1.5g/l gelrite had no survival response plantlets obtained of both shoot tip and nodal cutting, that may be because it showedup-normal growth of the plantlets as shown inTable (2) which the using of 1.5 g/l gelrite gave highest shoot length percentage with low value of number of node, that give a reason why most of plantlets grown on 1.5 g/l gelrite were died after a few days of their transferring to soil mixture (data not shown).This result demonstrated that the prediction of the productivity of Momordicacharantia shoots based on these traits (number of nods and shoot lengths) is not very precise due to the gap between highest shoot length value and the in vivo survival of plantlets.

Table3: Effect of gelling agent of nodal cuttingof Momordicacharantiain vitro deriving seedling grown on free- growth regulators MS solid medium with Gamborg's vitamins, on root formation percentage (%), incubated for four weeks and in vivo survival percentage (%) afterone month of culture on pots contain peat moss, vermiculite and sand 1:1:2 (v/v) respectively

Gelling Agent	Root %			In vivo Survival (%)		
	Explants Type		Mean	Explants Type		Mean
	Shoot Tip	Nodal Cutting		Shoot Tip	Nodal Cutting	
5g/l Agar	10.24	16.56	13.40	2.77	19.00	10.89
7g/l Agar	42.33	54.33	48.33	11.37	34.33	22.85
10g/l Agar	43.68	66.89	55.29	0.00	13.14	6.57
1.5g/l Gelrite	50.35	66.29	58.32	0.00	0.00	0.00
2.5g/l Gelrite	32.58	81.94	57.26	7.57	29.27	18.42
3.5g/l Gelrite	30.72	80.70	55.71	0.00	24.73	12.37
Mean	34.98	61.12	48.05	3.62	20.08	11.85
LSD at 5%: GellingAgent			0.7632			0.6964
Explants Type			0.4406			0.4021
Interaction			1.079			0.9848

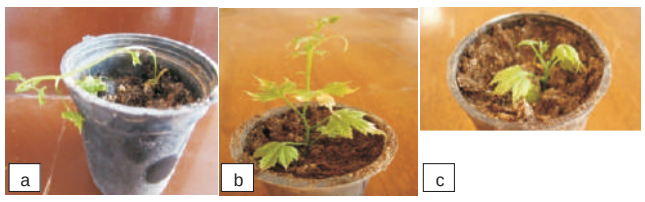

Fig. 3: Plantlets of Momordicacharantia after one month of acclimatization in pots contained mixture of peatmoss, vermiculite and sand 1:1:2 (v/v) respectively. Plantlet obtained from MS medium contained [5g/l agar (a), 7g/l agar (b), 10g/l agar (c), 1.5g/l gelrite (d), 2.5g/l gelrite (e) and 3.5g/l gelrite (f)], for 4 weeks.

Experiment 2: Studying of hyperhydricity phenomena

A physiological phenomenon known as verification (synonymous with glassiness or hyperhydricity transformation) is a serious problem associated with plant micropropagation (Narayanaswamy, 1994). In preliminary experiment constructed to investigate the effect of gelling agent type and concentrations on the hyperhydricity phenomena, data gave a sporadic result (not mentioned in this investigation) that lead to search for the reason causes this muddle information. For Momordicacharantia results shown in Table (4), data of the main effect of gelling agent indicate that, 2.5g/l gelrite gave highest shoot percentage (95.17). Concerning the main effect of type of previous medium, nodal cutting excised from plantlets grown on MS medium contained 2mg/l BA gave the highest percentage of shoot frequency (80.52). Data of interaction indicated that, nodal cutting excised from plantlets grown on MS medium contained 2 mg/l BA and cultured on free- growth regulators MS nutrient medium with Gamborg's vitamins contained 10g/l agar or 2.5g/l gelrite gave the highest percentage of shoot formation (100.00) without significant difference (all explants produced shoots). Moreover both treatments were significantly different than other treatments. The lowest shoot frequency percentage response (38.13) was observed with nodal cutting cultured on free- growth regulators MS medium contained 1.5g/l gelrite with Gamborg's vitamins and was excised from plantlets grown on free- growth regulators MS medium. Data of the main effect of gelling agent indicated that, 3.5g/l gelrite gave the highest value of number of shoot (3.30) followed by 10 g/l agar (3.07). While the lowest response of shoot number (1.63) was observed with nodal cutting cultured on free-growth regulators MS medium contained 5g/l agar with Gamborg's vitamins. Concerning the main effect of type of previous medium nodal cutting excised from plantlets grown on MS medium contained 2 mg/l BA with Gamborg's vitamins gave the highest value of number of shoot (2.47). Data of interaction indicated that, 3.5g/l gelrite gave the highest value of number of shoot (3.60) with nodal cutting excised from plantlets grown on free- growth regulators MS medium with Gamborg's vitamins. The lowest value of number of shoot (1.00) was observed with either 5g/l agar or 1.5g/l gelrite of nodal cutting excised from plantlets grown on free- growth regulators MS medium with Gamborg's vitamins. Data of the main effect of the number of nods indicated that, 7 g/l agar gave the highest value (9.50) followed by 3.5g/l gelrite (8.70). While the lowest response (5.97) of number of node was observed with nodal cutting cultured on free- growth regulators MS medium contained 5g/l agar with Gamborg's vitamins. Concerning the main effect of type of previous medium nodal cutting excised from plantlets grown on MS medium contains 2 mg/l BA with Gamborg's vitamins gave the highest value of number of node (8.81). Data of interaction indicated that, 7g/l agar gave the highest value of number of node (9.73) with nodal cutting excised from plantlets grown on free-growth regulators MS medium with Gamborg's vitamins. The lowest value of number of node (3.33) was observed with 5g/l agar with nodal cutting excised from plantlets grown on free- growth regulators MS medium with Gamborg's vitamins.

Data of the main effect of shoot length indicated that, 1.5g/l gelrite gave the highest response (5.35). While the lowest response of shoot length (2.39) was observed with nodal cutting cultured on free- growth regulators MS mediumcontained 3.5g/l gelrite with Gamborg's vitamins. Concerning the main effect of type of previous medium nodal cutting excised from plantlets grown on free- growth regulators MS medium with Gamborg's vitamins gave the highest value of shoot length (7.28). Data of interaction indicated that, 1.5g/l gelrite gave the highest value of shoot length (14.50) with nodal cutting excised from plantlets grown on free- growth regulators MS medium with Gamborg's vitamins. The lowest value of shoot length (1.87) was observed with 3.5 g/l gelrite of nodal cutting excised from plantlets grown on MS medium contained 2 mg/l BA with Gamborg's vitamins. For the hyperhydricity (Fig. 4), results of the main effect of gelling agent indicated that, 1.5g/l gelrite gave the highest percentage of hyperhydricity formation (34.96). While the lowest percentage of hyperhydricity formation response (20.56) was observed with nodal cutting cultured on free- growth regulators MS medium contained 10g/l agar with Gamborg's vitamins. Concerning the main effect of type of previous medium nodal cutting excised from plantlets grown on MS medium contained 2mg/l BA with Gamborg's vitamins gave the highest percentage of hyperhydricity formation (36.23). Data of interaction indicated that, 3.5g/l gelrite gave the highest percentage of hyperhydricity formation (40.00) with nodal cutting excised from plantlets grown on MS medium contained 2mg/l BA with Gamborg's vitamins. The lowest value of percentage of hyperhydricity formation (5.53) was observed with nodal cutting excised from plantlets grown on free- growth regulators MS medium with Gamborg's vitamins Cultured on medium contained 10g/l agar. Data of the main effect of gelling agent indicated that, highest productivity was shown with using 1.5g/l gelrite (73.00) in combined with using previous medium without growth regulators (42.22) and that, may be because the highly shoot