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In Vitro effect of plants hormones on seeds germination and callus induction of four Eggplants cultivars *(Solanum melongena.L)*

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Abstract

An efficient protocol for seeds germination, callus induction and plant regeneration from different seedling explants of four eggplants cultivars (ПОТЕХА,КУЛОН ,ЧЁРНЫЙКРАСАВЕЦ, and ДЛИННЫЙ ПУРПУРНЫЙ) was developed using different combination of plant growth hormones vis. Gibbererllic acid (GA3), 6-Benzyl amino purine(BA), Kinetin (Kint) and Indol acetic acid (IAA).The results reveal different responses among the different explants and cultivars.6mg.l^{-1}of GA3 was found ideal for seed germination percentage and seedling develop into normal shoots. MS or (M2) medium was found best for callus induction identically from shoot tips, hypocotyls, cotyledon leaves and roots of all eggplant cultivars tested. While M1 medium exhibiting different response to callogenesis Further more; the exhibiting variation among different explants could be useful as somaclonal variation for improving eggplant.

Keywords: *Solanum Melongena, In Vitro, callus, GA3, Explants*

Introduction

Solanum melongena.L commonly known as eggplant is an important vegetable crop grown in various tropical and temperate parts of the world (Kashyap *et al* 2002). India is the native of eggplant which is famous in the name of brinjal (Gubu *et al* 2017) and according to Harish and Gubo 2016, India classified as the second largest producer of eggplant in the world with a production of 13.44 m. tons and the productivity stands at 18.6 t/ha (Anon, 2013).Regarding to its medicinal and economic value, eggplant is found to contain low calories and high nutrition value, beside, eggplant has very high water content ,good source of fibers, and vitamins (Collonnier *et al* 2001). In addition, the extract of eggplants has proved its effect in reducing cholesterol rate. Choudhary and Gaur, 2009 reported that, dried shoots of eggplant are used as fuel in rural areas. Numerous tissue culture techniques for seed germination, callus culture have been established by many researchers for eggplants in related in addition to its ability for plant regeneration and somaclonal variation (Sidhu *et al* 2014) as well as improve its efficiency to various abiotic stress(Gobu *et al*, 2017; Siaga *et al*,2016). Available reports regarding to micro propagation from different source of explants, including shoot tips leaves, cotyledon, roots and hypocotyls and induced somatic embryogenesis (Rahman *et al*, 2006; Kantharajah and Golegaonkar, 2004; Zayova *et al*. 2008; Zayova *et al*. 2012; Mir *et al*. 2008)

Nevertheless it is essential to determine a reliable system for micropropagation for further studies, commercial purposes among others. So the present study aim to identify the protocols for optimal culture condition of seed germination, evaluation of four eggplant cultivars for callus induction, direct and indirect plant regeneration from callus by applying various growth regulators

Materials and Methods

The study was carried out in the plant tissue culture Lab. Genetic Engineering Department, Ministry of Science and Technology, during the period 2016-2017. Mature seeds of four eggplants cultivars (ПОТЕХА, КУЛОН, ЧЁРНЫЙ КРАСАВЕЦ, ДЛИННЫЙ ПУРПУРНЫЙ) Belarus originated.

Seeds surface sterilization

Seeds of four eggplant cultivars were surface sterilization using 0.5, 1.0 or 2.0% (v/v) NaOCl (Clorox) for 10, 15 min with vigorous shaking. Seeds were washed three times with sterilized distilled water and transferred to MS medium (Murashige and Skoog, 1962) supplemented with 30 g.l^{-1} sucrose. The pH medium was adjusted to 5.75 before solidified with 7 g.l^{-1} agar and autoclaving at 121˚C and 1.4 Kg/cm^2 for 20 min. After 10 days, contamination percentages have been used as parameter in this stage.

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Seeds germination medium

Seeds of four eggplant cultivars were cultured on MS medium supplemented with different concentrations (0.0, 2.0, 4.0 and 6.0) $mg.l^{-1}$ of GA3 (Gibberellic acid).Two weeks later, seed germination percentage was recorded.

Callus induction medium

Shoot tips, hypocotyls, cotyledon leaves and roots explants of each eggplant cultivar were separated and cultured on two type of media: (M1) medium contain MS+ 4 $mg.l^{-1}$ IAA+4 $mg.l^{-1}$ Kint, (M2) medium contain MS+ 4 $mg.l^{-1}$ IAA+4 $mg.l^{-1}$ BA.All steps have done under aseptic conditions in laminar air flow cabinet, and cultures were incubated at 25 ± 2 ºC, with photoperiod 16/8 hrs (light/dark) and 1000 lux of light intensity condition. Data were recorded after one month later. The experiment were design at completely randomized (C.R.D), five replicates for each parameter and statistical analysis were performed using GenStat software programs ,Means were compared at L.S.D with $P \leq 0.05$ level.

Results and Discussion

Sterilization of seed

According to the results of surface sterilization in table (1A and 1B) higher contamination percentage 78.75 % were found at 0.5 concentration of NaOCl compare to lower 23.33% contamination percentage at 2.0 % of NaOCl concentration (table1A).

Interaction effect between NaOCl and eggplant cultivars was significant and minimum low contamination 48% and 51.00% for ДЛИННЫЙ ПУРПУРНЫЙ and ЧЁРНЫЙ КРАСАВЕЦ cultivars respectively. Also significant interaction between duration time and eggplant cultivars found to be efficient and 15 minimized % contamination to 22.22% and 23.33 in ДЛИННЫЙ ПУРПУРНЫЙ and ЧЁРНЫЙ КРАСАВЕЦ cultivars respectively. In case of the three interactions among (NaOCl concentration, duration time and the eggplant cultivars) no contamination percentage were observed for all seeds of eggplants which were sterilized with 2% NaOCl for 15 min of duration time. The success establishment of in vitro culture free from contamination represents critical stage and challenge to obtain an aseptic explants used (El Kaaby *et al*, 2015; Al-Mohammed *et al* 2014).Sodium hypochlorite is a powerful sterilizing agent used for wide range of in vitro applications, other researcher use antibiotic (Jerico *et al* 2014) or fungicides when routine surface sterilization procedure become insufficient. In our study NaOCl proved its positive role as eliminator of microorganisms due to the hypochlorous acid activity which is formed when the NaOCl reacted with H2O and release Cl (Rodeva *et al* , 2004) .Our results are agree with (Hasnat *et al* 2007; Trigiano and Gray; Gudevand Veselinovska, 2011).

Table 1 A Effect of NaOCl and duration time % seeds contamination of four eggplant cultivars

NaOCl (v/v)	Cultivars				NaOCl
	ПОТЕХА	КУЛОН	ЧЁРНЫЙ КРАСАВЕЦ	ДЛИННЫЙ ПУРПУРНЫЙ	
0.5	79.67	80.33	76.67	78.33	78.75
1.0	65.83	58.33	54.67	50.33	57.29
2.0	26.67	28.33	21.67	16.67	23.33
Mean	57.39	55.67	51.00	48.44	
$L.S.D_{(0.05)}$	Cultivars 4.095		NaOCl×Cultivars 7.093		NaOCl 3.546
Time(min)	ПОТЕХА	КУЛОН	ЧЁРНЫЙ КРАСАВЕЦ	ДЛИННЫЙ ПУРПУРНЫЙ	Mean
10	75.89	80.22	78.67	74.67	77.36
15	38.89	31.11	23.33	22.22	28.89
$L.S.D_{(0.05)}$	Time ×Cultivars 5.791			Time 2.896	

Table 1B Effect of the interaction among NaOCl, duration time and four eggplants cultivars on % contamination

NaOCl	Time (min)	Cultivars				NaOCl × Time
		ПОТЕХА	КУЛОН	ЧЁРНЫЙ КРАСАВЕЦ	ДЛИННЫЙ ПУРПУРНЫЙ	
0.5	10	86.00	90.67	100.00	100.00	94.17
1.0		73.33	70.00	53.33	56.67	91.25
2.0		88.33	93.33	92.67	90.67	46.67
0.5	15	43.33	23.33	16.67	10.00	63.33
1.0		53.33	56.67	43.33	33.33	23.33
2.0		0.00	0.00	0.00	0.00	0.00
$L.S.D_{(0.05)}$	10.031					5.015

Table 2 % seeds germination affect by different concentration of GA3

GA3 mg.l^{-1}	ПОТЕХА	КУЛОН	ЧЁРНЫЙ КРАСАВЕЦ	ДЛИННЫЙ ПУРПУРНЫЙ
0.0	45.12	33.09	50.04	48.23
2.0	48.54	41.18	70.25	77.47
4.0	60.10	68.69	82.15	89.04
6.0	75.30	75.90	87.87	98.11
L.S.D $_{0.05}$	6.71			

Table 3 Effect of different growth hormones on number of shoots, % callogenesis induced from four eggplant cultivars

Media	Explants	Number of shoots/ explant				% Callogenesis			
		ПОТЕХА	КУЛОН	ЧЁРНЫЙ КРАСАВЕЦ	ДЛИННЫЙ ПУРПУРНЫЙ	ПОТЕХА	КУЛОН	ЧЁРНЫЙ КРАСАВЕЦ	ДЛИННЫЙ ПУРПУРНЫЙ
M1	Shoot tips	0.0	0.0	0.0	0.0	+	+	+	+
	hypocotyls	1.15	3.02	7.12	6.22	+++	+++	+++	+++
	cotyledon leaves	0.0	0.0	0.0	0.0	+	++	_	_
	roots	4.5	4.0	3.7	2.8	+++	+++	+++	+++
M2	Shoot tips	2.1	3.4	1.1	2.3	+++	+++	+++	+++
	hypocotyls	5.2	3.1	3	2.3	+++	+++	+++	+++
	cotyledon leaves	2	1.9	3.5	1.4	+++	+++	+++	+++
	roots	0	0	0	0	+++	+++	+++	+++

Seeds germination

According to the results in table 2, % of seeds germination were significantly affected by GA3 concentration comparing to control treatment (MS) media free hormones. At 6.0 mg.l^{-1} of GA3 the highest germination reached 98.11% for ДЛИННЫЙ ПУРПУРНЫЙ as compare to 87.87%, 75.90%, 75.30% in ЧЁРНЫЙ КРАСАВЕЦ, КУЛОН and ПОТЕХА respectively (Fig.1).

Gibberellic acid is a plant growth hormone that plays many roles in plant in plant bioactivity, such as breaking seed dormancy, production of hydrolytic enzymes such as α-amylase by promoting gene expression(Owusu-Mensah *et al*,2011;Rodriguez *et al*,2013; Bezuidenhout, *et al*, 2012) as well as shoots growth and developments (Shivaraj and Rao, 2011). Application of GA3 in our study raised seeds germination percentage of eggplants the results are agree with (El –Kaaby,2016).

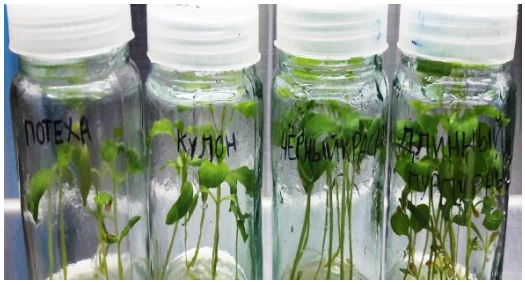

Fig.1 Seeds germination on 6.0 mg.l^{-1} concentration of GA3

Response of different eggplant explants to different combination of plant hormones

Analysis of variance showed significant response among eggplant cultivars and there explants for different traits at various concentrations of Auxins and Cytokinins (Fig 2). In terms of number of shoots and callus induction, the results in table (3) showed significant differences among the cultivars and there explants. In case of M1 medium, maximum multiplication of shoots were obtained indirectly from hypocotyls and roots explants reached (7.12 and 6.22) shoot/explant for ЧЁРНЫЙ КРАСАВЕЦ and ДЛИННЫЙ ПУРПУРНЫЙ respectively as compare to callogenesis only in shoot tips and cotyledon leaves at the same media. Opposite response in M2, all cultivars responded identically in related to callus induction parameter. Also high embryogenic callus were observed in hypocotyls and root explants in the presence of M1 medium whereas no response for shoot formation in cotyledon leaves in media M1 or root explants in media M2 respectively. Cytokinins were reported to overcome apical dominance and promote shoot formation (George,1993) other reported that proper ratio of Cytokinins and Auxins is necessary for morphogenesis (Kim *et al*, 1988). Regeneration potential depends on many factors such as explants and culture media (Kantharajah and Golegaonkar, 2004).Our results are agree with(Sidhu *et al*,2014) in related to the potential of eggplants to produce callogenesis, morphogenesis or both together in the presence of various concentration and combination between auxins and cytokinins.

Fig.2 A hypocotyls on media M1

Fig.2 B. shoot tip on media M1

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