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MUTATED AMBER 33 AND AMBER BAGHDAD RICE GENOTYPES PERFORMANCE FOR DROUGHT TOLERANCE IN CELL SUSPENSION CULTURES

ПРОЯВЛЕНИЕ ЗАСУХОУСТОЙЧИВОСТИ У РИСА С МУТАНТНЫМИ ГЕНОТИПАМИ 'ЯНТАРНЫЙ 33' И 'ЯНТАРНЫЙ БАГДАДСКИЙ' В КУЛЬТУРАХ КЛЕТОЧНОЙ СУСПЕНЗИИ

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Résumé. Amber 33 and Amber Baghdad rice seeds were presoaked in 1.5mM of Sodium azide (SA) for 4 hrs which is the optimum dose for mutagenesis with SA that caused 40% reduction in seedlings height. This dose gave the highest genetic variation and less sterility in both genotypes. Calli from both SA treated and non-treated genotypes were induced from mature embryos on appropriate medium while cell suspension cultures were initiated by placing about 100 mg callus pieces into 250 ml flasks containing 100 ml of Murashige and Skoog (1962) medium (MS) supplemented with 0.5mg/l kin (Kinetin) and 2.0 mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid), then placed on a rotary shaker at 100 rpm for 30 days. Growth curves of cell suspension cultures were examined during a range of culture periods 0, 5, 10, 15, 20, 25 and 30 days. In all cell lines, the growth rates of cells were initially slow but as the culture proceeded, they increased and accumulated great amounts of biomass over a period of 20 days. The best inoculums for cell plating were determined by plating aliquots of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml of cell cultures into 20ml solidified MS medium poured into Petri dishes. Packed cell volume (PCV) and the number of colonies were recorded after four weeks of cell plating. Inoculums convenient for cell plating were inoculated into MS medium supplemented with different concentrations of PEG6000 (0.0, 0.5, 1.0, 1.5 and 2.0%) for screening and selecting cell lines tolerant to drought. Shoot formation was induced on MS medium supplemented with NAA (Naphthaleneacetic acid) and IAA (Indole 3-acetic acid) at 0.5 mg/l and 4.0 mg/l BA (Benzyle adenine). Proline and carbohydrate concentrations were determined in regenerated shoots of plated cell suspension cultures. Results showed no significant differences between the two genotypes in respect to PCV, mean no. of colonies after screening on different PEG concentrations, mean no. of shoot/cell aggregate. While these parameters significantly increased at 1.5 mM SA treated genotypes compared with untreated. Results also revealed a significant reduction in mean no. of colonies, mean no. of shoot/cell aggregate with the increasing of PEG concentration especially in SA non-treated genotypes.

Аннотация. Семена риса сортов 'Янтарный 33' и 'Янтарный Багдадский' были предварительно замочены в 1.5mM азида натрия (АН) в течение 4 часов, концентрации оптимальной для мутагенеза с SA, вызывающего снижение высоты рассады на 40%. Эта доза дала самую высокую генетическую изменчивость и меньшую стерильность в обоих генотипах. Каллюсы от обоих обработанных и необработанных АН генотипов были выделены из зрелых зародышей на соответствующей среде в то время как суспензионные культуры клеток инициировали, помещая около 100 мг кусочков каллюса в 250 мл колбу, содержащую 100 мл среды Мурашига и Скута (МС) (1962) дополненную 0.5 мг/ л кинетинаи 2.0 мг/л 2,4-D (2,4-дихлорофеноксиацетиловая кислота), а затем помещали на роторный шейкер при скорости 100 оборотов в минуту в течение 30 дней. Кривые роста клеточной суспензии культур были рассмотрены в течение ряда периодов культивирования – 0, 5, 10, 15, 20, 25 и 30 дней. Во всех клеточных линиях, темпы роста клеток были первоначально медленными, но, с продолжением культивирования, они увеличились и в течение 20 дней накапливали большое количество биомассы. Лучшие инокулянты для покровных клеток определяли путем высева определенного количества (1.0, 1.5, 2.0, 2.5, 3.0, 3.5 и 4.0 мл) клеточных культур в 20мл затвердевающейМС среды наливаемой в чашки Петри. Гематокритная величина (ГКВ) и количество колоний регистрировали после четырех недель. Инокулянты соответствующие покровным клеткам были внедрены в МС среду, дополненную различными концентрациями PEG6000 (0.0, 0.5, 1.0, 1.5 и 2.0%) для скрининга и отбора клеточных линий, устойчивых к засухе. Образование отростков индуцировали на МС среде, дополненной нафталинуксусной кислотой и индолом 3-уксусной кислоты в дозах 0.5 мг/л и 4.0 мг/л бензил аденина. Концентрации пролина и углеводов определяли в побегах сформировавшихся из высеянных культур клеточной суспензии. Результаты показали отсутствие существенных различий между этими двумя генотипами по отношению к ГКВ, ни в числе колоний после скрининга на различных концентрациях полиэтиленгликоля (ПЭГ), ни в соотношении наполнитель / клетки побегов. В то время как эти параметры значительно увеличились в 1,5 mM АН обработанных генотипов по сравнению с необработанной. Результаты также показали значительное снижение среднего значения числа колоний, числа всходов на клетку с увеличением концентрации ПЭГ, особенно у генотипов необработанных АН.

Key words: rice genotypes, cell suspension cultures, drought tolerance.

Ключевые слова: генотипические формы риса, суспензии клеточных культур, засухоустойчивость.

Introduction

When callus cultures are introduced into a liquid medium and agitated, the cells disperse throughout the liquid to form a cell suspension culture [Allan, 1996]. As new cells are formed they are dispersed into the liquid medium and become clusters and aggregates. Cells in suspension exhibit much higher rates of cell division than do cells in callus cultures. Thus, cell suspension offers advantages, when rapid cell division or many cell generations are desired, or when a more uniform treatment application is required [Philips et al., 1995]. Plant cell culture techniques facilitate the rapid production of variant cell lines via selection procedures, very similar to those employed in microbial systems. These variant cell lines are useful for research into the genetics and biochemistry of plant cells and also in biotechnology for the production of new plant cultivars and secondary metabolites. Selection techniques have the potential for the production of crop cultivars with new characteristics such as salt tolerance [Freytag et al., 1990], herbicide resistance [Saunders et al., 1992], cold tolerance, disease resistance and metal tolerance [Cresswell, 1995].

Plating technique has made it possible to handle many cells at a time and obtain single cell clones. The effectiveness of Bergmann's technique of plating filtered cell suspensions in agar medium has been confirmed using of tobacco cells [Gibbs, Dovgall, 1963], *Huplopuppus gracilis* and carrot cells [Blakely, Steward, 1964]. Jelodarl et al., 2002] regenerated rice from cell suspension protoplast of two Iranian cultivars. Lee et al., 2004] investigated that rapidly proliferating rice cell suspension culture could be established and characterized by Fluorescence-Activated Cell Sorting Analysis. He-Chun [2011] studied the effect of plant growth regulators on the biomass yield of cell suspension culture in rice.

Mutations have been reported with tissue cultures of barely, wheat, rice, oat, tobacco and corn. Induced mutations have played an important role in improving various plant characters in different crop species. The characters in which improvement has been achieved include yield, disease resistance, drought tolerance, earliness, adaptability and other morphological characters. The mutagen of choice in many cultivated plants is HN3, formed from SA buffered at pH 3. Azide is only marginally mutagenic for humans and animals and Arabidopsis, it is therefore quite safe to use [Ahmad, 2005].

Materials and methods

Seeds of two rice genotypes Amber 33 and Amber Baghdad were surface sterilized with 2.5%sodium hypochlorite and then washed in distilled water for three times. Callus was induced in MS medium supplemented with 0.5 mg/l kin and 2.0 mg/l 2,4-D. Cell suspension cultures were initiated by placing about 100mg callus pieces into 250ml flasks containing 100ml of MS medium supplemented with the same combination of growth regulator as in callus induction medium, and the flasks were placed on a rotary shaker at 100rpm for 30 days. Growth curves of cell suspension cultures were determined during a range of culture periods 0, 5, 10, 15, 20, 25 and 30 days for both genotypes, aliquots of 10ml suspension cultures were transferred to 15cm3graduated test tubes, centrifuged at 1500 rpm for 5 min and then the Packed Cell Volume (PCV) was recorded [Ibrahim, 1990].The best inoculums for cell plating were determined by plating aliquots of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 or 4.0 ml onto 20 ml solidified maintenance medium poured into Petri dishes. Packed cell volumes and number of colonies were recorded. Inoculums convenient for cell plating were inoculated into MS medium supplemented with different concentrations of PEG6000 (0.0, 0.5, 1.0, 1.5 or 2.0%) for screening and selecting cell lines tolerant to drought. Numbers of colonies were recorded with five replicates for each treatment. Shoot formation was induced on an MS medium supplemented with 4.0 mg/l BA and 0.5 mg/l of both NAA and IAA. The effect of genotypes, PEG and SA were examined on the basis of mean no. shoot/cell aggregates.

A Completely Randomized Block Design (CRBD) was implemented to study the investigated factors. Data were statistically analyzed. Means were compared between treated and non-treated samples at $p \leq 0.05$ [Steel and Torrie, 1982].

Results

Determination of cell cultures growth curve.

Figures 1, 2, 3 and 4display the typical growth curve of cell suspension cultures of Amber33 and Amber Baghdad genotypes. PCV increased over time showing the lag phase at the first 5 days then the exponential, followed by linear phase for the period 8-15 days after inoculation. This was followed by clear progressive deceleration phase, and then cells entered the stationary phase at day 20. Finally, cells started deterioration after day 20 and started senescence. While there was a clear delay in the exponential phase, which occurred at day 10 for non-mutated Amber Baghdad genotype, and no clear progressive deceleration phase appeared within mutated Amber33 cell suspension growth curve since cell suspension showed long stationary phase. Also there was a clear delay in the exponential phase of mutated Amber Baghdad growth curves which occurred at day 11 after inoculation.

Determination of inoculums size.

Results displayed in table (1) show that mean no. of colonies significantly increased with the increasing PCV. Values recorded3.4, 5.0, 7.0 and 8.6 mean no. of colonies in 0.25, 0.3, 0.35 and 0.4 PCV respectively for 1.5 *mM* SA non-treated Amber33. The same trend was found in 1.5 *mM* SA non-treated Amber Baghdad. The minimum inoculums (PCV) that gave the highest mean no. colonies was 0.25 PCV for both genotypes at 1.5 *mM* SA non-treated genotypes that values were 3.4 and 2.0 PCV for Amber33 and Amber Baghdad cell cultures respectively.

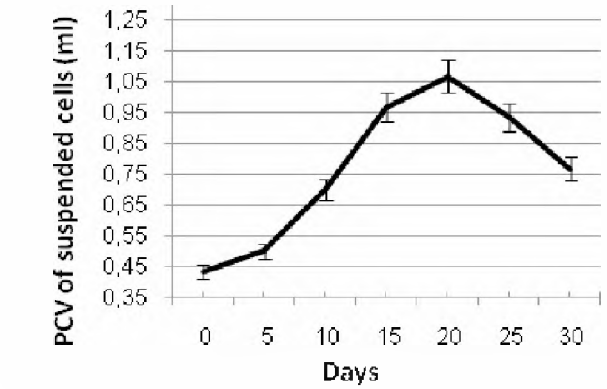

Fig. 1. Cell suspension growth curve of 1.5 *mM* SA non-treated Amber 33, initial weight 100 mg

Рис. 1. Кривая роста клеточной суспензии 'Янтарный 33', не обработанной 1.5 *mM* азида натрия, начальный вес 100 мг

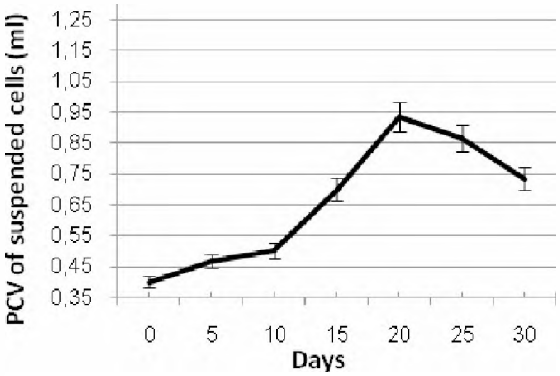

Fig. 2. Cell suspension growth curve of 1.5 *mM* SA non-treated Amber Bagdad, initial weight 100 mg.

Рис. 1. Кривая роста клеточной суспензии 'Янтарный Багдадский', не обработанной 1.5 *mM* азида натрия, начальный вес 100 мг

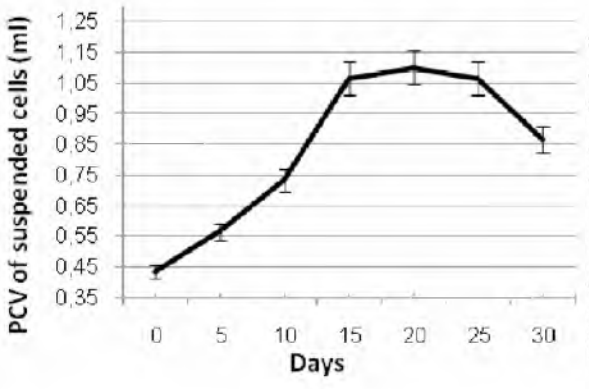

Fig. 3. Cell suspension growth curve of 1.5 *mM* SA treated Amber 33, initial weight 100 mg

Рис. 3. Кривая роста клеточной суспензии 'Янтарный 33', обработанной 1.5 *mM* азида натрия, начальный вес 100 мг

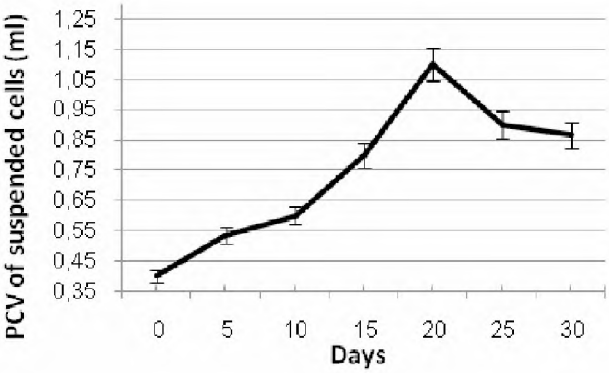

Fig. 4. Cell suspension growth curve of 1.5 *mM* SA treated Amber Baghdad, initial weight 100 mg

Рис. 4. Кривая роста клеточной суспензии 'Янтарный Багдадский', обработанной 1.5 *mM* азида натрия, начальный вес 100 мг

Table 1
Таблица 1

Number of colonies resulted from Amber 33 and Amber Baghdad genotypes cell cultures after and before treatment with 1.5 *mM* SA using different packed cell volumes, *n*=5
Итоговое число колоний генотипов культур клеток 'Янтарный 33' и 'Янтарный Багдадский' до и после обработки азидом натрия 1.5 *mM* с использованием различных гематокритных величин, *n*=5

Genotype	PCV and SA (mM)			
	PCV of non treated	Mean no. colonies in non treated	PCV of treated	Mean no. colonies in treated
'Amber 33'	0.1	0.00	0.15	0.00
	0.15	0.00	0.225	0.00
	0.2	0.00	0.3	4.00
	0.25	3.40	0.375	5.20
	0.3	5.00	0.45	7.00
	0.35	7.00	0.525	7.20
	0.4	8.60	0.6	8.80
'Amber Baghdad'	0.1	0.00	0.13	0.00
	0.15	0.00	0.195	0.00
	0.2	0.00	0.26	0.00
	0.25	2.00	0.325	3.60
	0.3	4.00	0.39	5.20
	0.35	5.80	0.455	7.00
	0.4	7.40	0.52	8.40
LSD 0.05	1.649			

Effect of genotypes and % PEG on the mean no. of cell colonies.

Results showed that 1.5 and 2.0% PEG significantly reduced the mean no. of cell colonies whose values were 2.7 and 0.15 compared with 0.0% PEG, and no significant difference occurred among Amber33 and Amber Baghdad genotype. Results also displayed that the interaction between genotypes and % PEG that 1.5 and 2.0% PEG significantly reduced the mean no. of cell colonies that values were 3.1, 0.2 compared with 0.0% PEG for Amber33 cell culture and 2.3, 0.1 compared with 0.0% PEG for Amber Baghdad cell culture as shown in table 2.

Table 2
Таблица 2

Effect of genotypes and % PEG on the mean no. of colonies of cell cultures after 6 weeks of plating on solid agar MS medium, *n*=5
Влияние генотипов и % ПЭГ на среднее число колоний клеточных культур после 6 недель высева на твердой агаровой МС среде, *n*=5

Genotype	% PEG					
	0.00	0.50	1.00	1.50	2.00	Mean
Amber 33	6.90	6.86	6.65	3.10	0.20	4.80
Amber Baghdad	6.40	6.40	6.30	2.30	0.10	4.34
Mean	6.65	6.65	6.70	2.70	0.15	
LSD 0.05	PEG=1.037; CV=0.656; PEGXCV=1.466					

Effect of SA and % PEG on the mean no. of cell colonies.

Results in table (3) indicate that 1.5 *mM* SA significantly increased the mean no. of cell colonies that value was 5.48 compared with 0.0 mM SA. Results also revealed that mean no. of cell colonies significantly reduced in 0.5, 1.0, 1.5 and 2.0% PEG that values were 5.1, 4.7, 2.0 and 0.0 compared with 0.0% PEG for 0.0 mM SA, But there was a significant increase in the mean no. of cell colonies in 0.5and 1.0% PEG. Values recorded 8.20 and 8.70 compared with 0.0% PEG for 1.5 mM SA, and then significantly reduced in 1.5 and 2.0% PEG that values were 3.4 and 0.3compared with 0.0% PEG for 1.5 *mM* SA.

Table 3
Таблица 3

Effect of SA (*mM*) and % PEG on the mean no. of cell colonies of cell cultures after 6 weeks of plating on solid agar MS medium, *n*=5
Влияние азида натрия (*mM*) и % ПЭГ на среднее количество колоний клеток клеточных культур после 6 недель высева на твердой агаровой МС среде, *n*=5

SA(mM)	% PEG					
	0.00	0.50	1.00	1.50	2.00	Mean
0.00	6.50	5.10	4.70	2.00	0.00	3.66
1.50	6.80	8.20	8.70	3.40	0.30	5.48
LSD 0.05	SA=0.656; PEGxSA=1.466					

Effect of genotypes and SA on the mean no. cell colonies.

Results displayed in table (4) show that 1.5 mM SA significantly increased the mean no. of cell colonies that values were 5.8 compared with 0.0 *mM* SA for Amber33 genotype cell culture and 5.16compared with 0.0 *mM* SA for Amber Baghdad genotype cell culture, also there was no significant difference recorded among genotypes cell cultures in the mean no. of cell colonies.

Table 4
Таблица 4

Effect of genotypes and SA (*mM*) on the mean no. of cell colonies of cell cultures after 6 weeks of plating on solid agar MS medium, *n*=5
Влияние генотипов и азида натрия (*mM*) на среднее количество колоний клеток клеточных культур после 6 недель высева на твердой агаровой МС среде, *n*=5

Genotype	SA mM	
	0.00	1.50
Amber 33	3.80	5.80
Amber Baghdad	3.52	5.16
LSD 0.05	CVXSA=0.927	

Results in table (5) exhibits that there was a significant decrease in the mean no. of cell colonies recorded in 1.0, 1.5 or 2.0% PEG, values recorded 4.8, 2.2 and 0.0 respectively compared with 0.0% PEG for 1.5 mM SA non-treated Amber33 cell culture and 4.6, 1.8 and 0.0 respectively compared with 0.0% PEG for 1.5mM SA non-treated Amber Baghdad genotype cell culture. Results also indicated that 1.0% PEG significantly increased the mean no. cell colonies whose values were 9.0 and 8.4 compared with 0.0% PEG for 1.5 mM SA treated Amber33 and Amber Baghdad genotype cell cultures respectively. Figures 5, 6, 7 and 8 display the effect of genotype, % PEG and SA (*mM*) on the no. colonies/plate of cell culture. Results exhibited that %PEG significantly reduced the mean no. of shoot/cell aggregates whose values were 5.0, 2.58 and 0.0 compared with 0.0% PEG, and no significant difference occurred among genotypes. Results also revealed that the interaction between genotypes and % PEG that the mean no. of soot/cell aggregates significantly reduced in 0.5, 1.0 and 1.5% PEG at which values were 4.33, 1.83 and 0.0 compared with 0.0%PEG for Amber33. The mean no. of shoot /cell aggregates were significantly reduced in 1.0 and 1.5% PEG that values were 3.33 and 0.0 compared with 0.0% PEG for Amber Baghdad as shown in table 6.

Table 5
Таблица 5

Effect of genotypes, SA (*mM*) and (%) PEG on no. colonies of cell cultures after 6 weeks of plating on solid agar MS medium, *n*=5
Влияние генотипов, АН (*mM*) и (%) ПЭГ на число колоний культур клеток после 6 недель высева на твердой агаровой среде МС, *n*=5

Genotype	SA (mM)	(%) PEG				
		0.00	0.50	1.00	1.50	2.00
Ambere 33	0.00	6.80	5.20	4.80	2.20	0.00
	1.50	7.00	8.60	9.00	4.00	0.40
Amber Baghdad	0.00	6.20	5.00	4.60	1.80	0.00
	1.50	6.60	7.80	8.40	2.80	0.20
LSD 0.05	2.074					

Effect of genotypes and % PEG on no. of shoot/cell aggregates.

Table 6
Таблица 6

Effect of genotypes and % PEG on the mean no. of shoot/cell aggregates, after 6 weeks of inoculating in solid agar MS medium, *n*=3
Влияние генотипов и % ПЭГ на среднее число проростков в клеточных агрегатах, через 6 недель инокуляции в агаровую МС среду, *n*=3

Genotype	(%) PEG				
	0.00	0.50	1.00	1.50	Mean
Ambere 33	6.33	4.33	1.83	0.00	3.12
Amber Baghdad	6.83	5.67	3.33	0.00	3.96
Mean	6.58	5.00	2.58	0.00	
LSD 0.05	CV=1.337 ; PEG=1.890 ; PEGx CV=2.673				

Table 7
Таблица 7

Effect of SA and % PEG on the mean no. of shoot/cell aggregates, after 6 weeks of inoculating in solid agar MS medium, *n*=3
Влияние азида натрия и % ПЭГ на среднее число проростков в клеточных агрегатах, через 6 недель инокуляции в агаровую среду МС, *n*=3

SA (mM)	% PEG				
	0.00	0.50	1.00	1.50	Mean
0.00	6.00	4.17	1.33	0.00	2.88
1.50	7.17	5.83	3.83	0.00	4.21
LSD 0.05	SA=1.337; PEGxSA=2.673				

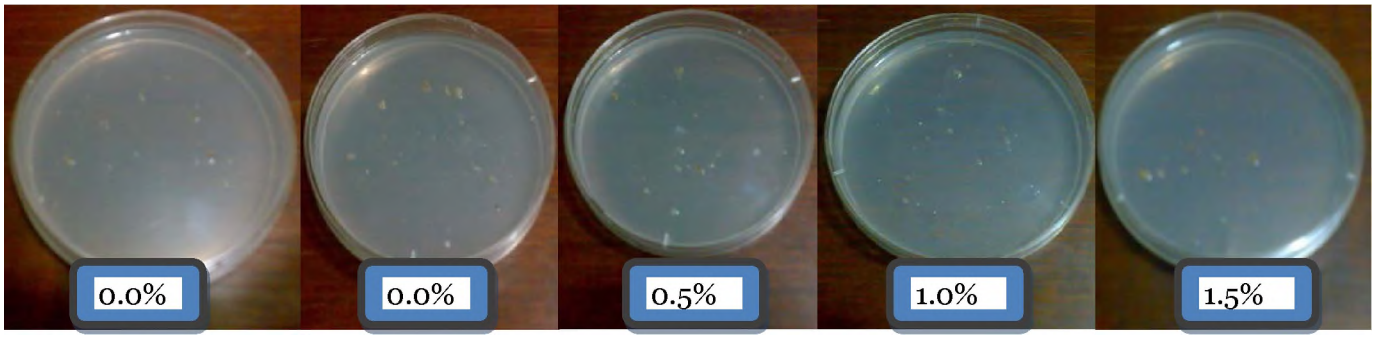

Fig. 5. 1.5 *mM* SA treated Amber Baghdad cell culture exposed to different PEG concentrations showing the effect of PEG on the no. cell colonies, after 6 weeks of plating in solid MS medium *in vitro*
Рис. 5. Культуры клеток Янтарный Багдадский, обработанные 1.5 *мМ* АН, подвергнутые воздействию различных концентраций ПЭГ, показывающие влияние ПЭГ на число колоний клеток, через 6 недель высева в твердой МС среде *in vitro*

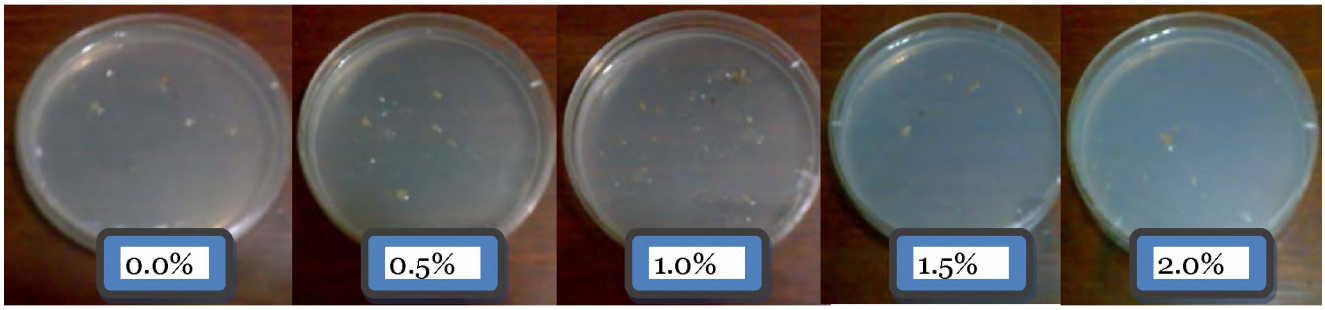

Fig. 6. 1.5 *mM* SA treated Amber 33 cell culture exposed to different PEG concentrations showing the effect of PEG on the no. cell colonies, after 6 weeks of plating in solid MS medium *in vitro*
Рис. 6. Культуры клеток Янтарный 33, обработанные 1,5 *mM* АН, подвергнутые воздействию различных концентраций ПЭГ, показывающие влияние ПЭГ на число колоний клеток, через 6 недель высева в твердой среде МС *in vitro*

Results in table 7 display that 1.5 *mM* SA significantly increase the mean no. of shoot/cell aggregates with a mean value reaching 4.21 compared with 0.0 *mM* SA. Results also indicated that the interaction between SA *mM* and %PEG that 1.0 and 1.5% PEG significantly reduced the mean no. of shoot/cell aggregates whose values were 1.33 and 0.0 respectively compared with 0.0% PEG for 0.0 *mM* SA, but for 1.5 *mM* SA, values were 3.83 and 0.0 respectively compared with 0.0% PEG.

Results displayed in table 8 show that 1.5 *mM* SA significantly increased the mean no. of shoot/cell aggregates with a mean value reached 4.0 compared with 0.0 *mM* SA for Amber33 genotype. Results also indicated that there was no significant difference recorded between 0.0 and 1.5 *mM* SA for Amber Baghdad genotype whose values were 3.50 and 4.42 respectively. Results revealed that 0.0, 1.0 and 1.5% PEG significantly reduced the mean no. of shoot/ cell aggregates whose values were 3.33, 0.0 and 0.0 compared with 0.0% PEG in 0.0 *mM* SA for Amber33 genotype, the mean no. of shoot/cell aggregates significantly reduced in 1.0 and 1.5% PEG reached3.67 and 0.0 compared with 0.0% PEG in 0.0 *mM* SA for Amber Baghdad genotype.

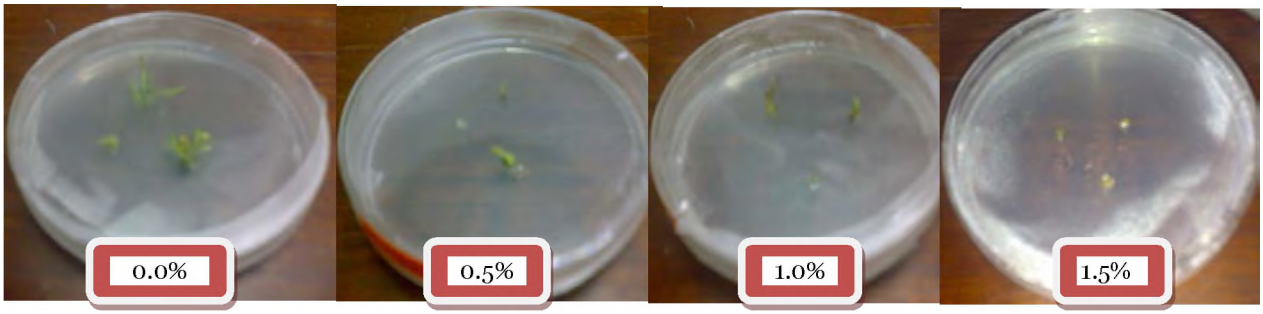

Fig. 7. 1.5 *mM* SA treated Amber Baghdad cell aggregates exposed to different concentrations of PEG showing its effect on the no. shoot/cell aggregates of cell culture, after 6 weeks of inoculating in solid MS medium
Рис. 7. Клеточные агрегаты 'Янтарный Багдадский', обрабртанные 1.5 *mM* АН и подвергнутые воздействию различных концентраций ПЭГ, показывающие его влияние на количественное соотношение проростков в агрегатах культуры клеток, через 6 недель инокуляции в твердую среде МС

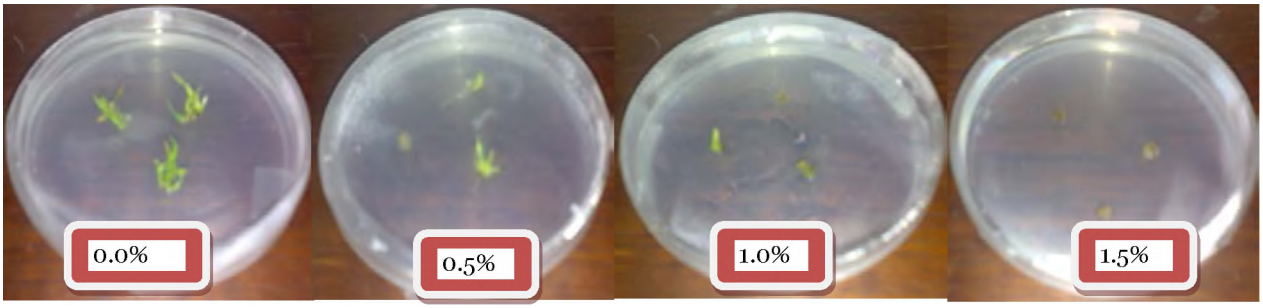

Fig. 8. 1.5 *mM* SA treated Amber 33 cell aggregates exposed to different concentrations of PEG showing its effect on the no. shoot/cell aggregates of cell culture, after 6 weeks of inoculating in solid MS medium
Рис. 8. Клеточные агрегаты 'Янтарный 33', обработанные 1.5 *mM* АН и подвергнутые воздействию различных концентраций ПЭГ, показывающие его влияние на численное соотношение проростков в агрегатах культуры клеток, через 6 недель инокуляции в твердую среде МС

Table 8
Таблица 8

Effect of genotypes and SA (*mM*)on the mean no. of shoot/cell aggregates after 6 weeks of inoculation on solid agar MS medium, *n*=3
Влияние генотипов и азида натрия (*mM*) на среднее число проростков в клеточных агрегатах после 6 недель посева на твердой агаровой МС среде, *n*=3

Genotype	SA mM	
	0.00	1.50
Amber33	2.25	4.00
Amber Baghdad	3.50	4.42
LSD 0.05	CVXSA=1.890	

Results also indicated that there was a significant reduction in the mean no. of shoot/cell aggregates at 1.0 and 1.5% PEG whose values were 3.67 and 0.00 compared with 0.0% PEG for 1.5 *mM* SA treated Amber33 genotype, the same trend was found in 1.5 *mM* SA treated Amber Baghdad genotype as shown in table 9.

Table 9
Таблица 9

Effect of genotypes, SA *mM* and % PEG on the mean no. of shoot/cell aggregates, after 6 weeks of inoculating in solid agar MS medium, *n*=3
Влияние генотипов, азида натрия *mM* и % ПЭГ на среднее число проростков в клеточных агрегатах после 6 недель посева на твердой агаровой МС среде, *n*=3

Genotype	SA (mM)	% PEG			
		0.00	0.50	1.00	1.50
Ambere33	0.00	5.67	3.33	0.00	0.00
	1.50	7.00	5.33	3.67	0.00
Amber Baghdad	0.00	6.33	5.00	2.67	0.00
	1.500	7.33	6.33	4.00	0.00
LSD 0.05	3.780				

Discussion

One prerequisite for the use of plant cell culture in plant improvement is the development of suitable screening and selection methods by which variant can be identified and isolated easily. The widely reported drawbacks in using callus cultures in selection experiments is that cells within callus are not uniformly exposed to the selective agent and a gradient of stress may develop in such cultures, the cells in contact with the medium are the most affected and cells on top are the least affected [Ibrahim, 1990]. Plant cell cultures are normally established and maintained on MS medium containing an auxin and a cytokinin. Removal of either hormone from the medium would normally result in culture death. During the incubation period, the biomass of suspension cultures increased due to cell division and cell enlargement. This continues for a limited period after which the growth stops due to the exhaustion of some factors or the accumulation of certain toxic metabolites in the culture medium [Gurel et al., 2002].

Results shown in figures 1,2, 3 and 4are in agreement with those of Brown and Beevers [1987].The inoculums of cell suspension cultures were determined and the lowest inoculums which produce the highest mean no. cell colonies were used to obtain the best plating efficiency which was 0.25 PCV for both genotypes cell cultures at 0.0 *mM* SA and 0.3 or 0.325 PCV for 1.5 *mM* SA Amber33 and Amber Baghdad genotypes cell cultures respectively, this result is in agreement with those of Ibrahim [1990] who reported that the large inoculums may form a thick layer of cell suspension onto the surface of the agar and again a stress gradient will be established as in callus culture. It was recognized that 1.5 *mM* SA caused an increase in the biomass of suspension culture at which the PCV and the mean no. colonies was higher in 1.5 *mM* SA compared with 0.0 *mM* SA for both genotypes cell cultures as shown in table 1, SA caused an increase in cell growth and division, these results were in agreement with those of Hamza (2007) who reported that the genetic variations that caused as a result of treatment with SA enhance the activation effect for cell division and enlargement. And the differences that occurred during inoculums determination in 1.5 *mM* SA Amber33 genotype, are due either to the sensitivity of Amber33 genotype for SA treatment or to the physiological differences between genotypes. Results in tables 2, 3, 4 and 5 exhibit the effect of SA as a mutagenic agent at which there was a significant increase in the mean no. of cell colonies in 0.5 and 1.0% PEG at 1.5 *mM* SA for both genotypes cell cultures, this may be due to the activity of SA for creation of point mutation as reported by Al-Qurainy and Khan [2009]. While the mean no. cell colonies significantly reduced in 0.0 *mM* SA in all PEG concentrations for both genotypes, this may have occurred because of the cell damage as a result of osmotic pressure (sensitivity to drought), or because of the abnormalities in the nuclear constitution of some cells which make these cells incapable of division or a differentiation of the cells in a way that not all divide under the same conditions.

It is a simple matter to plate some thousand or more cells on a set of Petri dishes and to obtain a large number of clones in a short time that could not be obtained by methods requiring special handling of each cell. The method permits the screening of large numbers of cells for genetic as well as physiologic studies in spite of the relatively small proportion of cells that undergo repeated divisions and establish a tissue. Results in tables 8 and 9were in agreement with those of Kamal et al. [2009] who reported that the mutant genotype produced higher percentages of shoots regeneration than non-mutated genotype. While results in tables 6 and 9 were in disagreement with those of Shah et al. [2012] who established that the regeneration percentage of PEG adapted cell cultures increased to 80% compared with non-adapted cell lines. The induction of high plant regeneration in 1.5 mM SA genotypes compared with 0.0 mM SA genotypes as shown in tables 7, 8 and 9 may be due to the SA ability for chromosomal changes as a result of base pair substitution without chromosomal aberration and this result is in agreement with those of Kleinhofs et al. [1978] who reported that SA creates point mutation in the genome of plants and the mutant plants are capable to survive under various adverse conditions.

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