INDUCTION OF GENETIC VARIATION FOR DROUGHT TOLERANCE IN TWO RICE CULTIVARS AMBER 33 AND AMBER BAGHDAD

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ABSTRACT

Seeds of two rice cultivars Amber 33 and Amber Baghdad were presoaked in the chemical mutagen Sodium azide at the concentrations 0.0, 0.5, 1.0 or 2.0 mM for 2, 4 or 6 hrs. The effect of Sodium Azid was examined on seed germination, shoot and root length and promising dose. To increase the genetic variation for drought tolerance, seeds treated with the optimum dose that made 40% in growth reduction in seedling height. Calli were induced from mature embryos on appropriate medium and then transferred to a medium containing 0.0, 0.5, 1.0, 1.5 or 2.0% of Polyethylene Glycol (PEG) (W/ V). Differences were recorded between cultivars and treatments with respect to the studied traits. Results showed that there were no significant differences between cultivars in respect to seeds germination shoot and root length while these parameters decreased with increasing mutagen concentration and soaking time. Results also revealed that there were significant differences between cultivars in % of callus induction and callus fresh weight when callus cultures were transferred to different combinations of 2,4-D and Kin, while there were no significant differences in callus fresh and dry weights between cultivars with increasing concentrations of Sodium Azide and PEG. Callus fresh weight decreased with increasing PEG level in the medium for seeds not exposed to Sodium Azide.

Key words: Drought Tolerance; Cultivars Amber 33; Amber Baghdad.

إستحداث تغايرات وراثية لتحمل الجفاف في صنفي الرز عنبر 33 وعنبر بغداد

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الخلاصة

عرضت بذور صنفي الرز عنبر 33 و عنبر بغداد للتراكيز صفر و 0,5 و 1.0 أو 2.0 ملي مولر من المطفر الكيميائي صوديوم ازايد و لمدة 2 و 4 أو 6 ساعة. درس تأثير الصوديوم ازايد على النسبة المئوية للإنبات و طول المجموع الخضري والجذري و تحديد الجرعة المثلى للتطفير. بعد تحديد الجرعة المثلى للتطفير و التي تسبب انخفاض 40% من طول البادرة، تم تعريض بذور الصنفين لتلك الجرعة فضلاً عن معاملة المقارنة (بدون تطفير). أستحدث الكالس من البذور و زرع الكالس بعد ذلك في وسط يحوي 0,0 و 0,5 و 1.0 و 1,5 أو 2.0% من الاثيلين متعدد الكلايكول. أظهرت النتائج عدم وجود إختلافات معنوية بين الاصناف للصفات نسبة الانبات، طول المجموع الخظري والجذري بينما كانت هناك إختلافات بين التراكيز و مدة التعرض للمطفر صوديوم ازايد. أذ حدث إنخفاض معنوي في النسبة المئوية للإنبات وطول المجموع الخضري والجذري بزيادة التركيز و مدة التعرض للمطفر. كما أظهرت النتائج وجود إختلافات معنوية بين الاصناف في صفتي النسبة المئوية لاستحداث الكالس و الوزن الطري للكالس بإستعمال تراكيز مختلفة من 2,4-D و kin في حين لم يكن هنك فروقات معنوية بين الصنفين في صفتي الوزن الطري و الجاف للكالس بوجود تراكيز مختلفة من ال SA و PEG. كما لوحظ إنخفاض في وزن الكالس بزيادة تراكيز الاثيلين متعدد الكلايكول في البذور غير المعرضة للصوديوم ازايد.

INTRODUCTION

According to the published statistics, the percentage of drought affected land area in the world is more than doubled since the 1970s to the early 2000s (1). Drought is a world - wide problem that seriously has influenced grain production. Increasing human population and global climate change make the situation more serious (2). Rice, as a paddy field crop, world rice production uses about 1,578km^3 of water which represents 30% of the fresh water consumed worldwide (3). Of all the cereals, rice (*Oryza sativa*) is one of the most susceptible crops to damage caused by water deficit (4, 5 and 6). It is estimated that 50% of the world rice production is affected by drought (7).Mutation breeding is a tool used to study the nature and function of gens which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement and economic crops (8). Mutation methodology has been used to produce many cultivars with improved economic value and study of genetic and plant developmental phenomena (9 and10). Induced mutation has great potentials and serves as a complimentary approach in genetic improvement of crops.Various mutagenic agents are used to induce favorable mutation at high frequency that includes ionizing radiation and chemical mutagens (11). Sodium azide (NaN_3) is a chemical mutagen that creates point mutation in the genome of plants by producing metabolites and thus produces protein in a mutant plant has different functions from the normal plant. The mutant plants produced by the treatment of sodium azide are capable to survive under various adverse conditions and have improved yields, increased stress tolerance, longer shelf life and reduced agronomic input in comparison to the normal plants. The selection of plant mutants is based on morphological, biochemical and DNA based markers. In Iraq, mutation breeding program to develop mutants from local rice cultivars has been established. Examination for drought tolerance in the local cultivars is an essential step prior to any mutation program in order to determine the critical doses for this purpose. In this paper, tissue culture techniques in combination with mutagen treatments were applied to increase the probability of selected mutants and to shorten the breeding period and time required for improving the locally grown the above mentioned two rice cultivars.

MATERIALS AND METHODS

Experiment I

Seeds of two rice cultivars Amber 33 and Amber Baghdad were kindly provided by the Agricultural Research Directorate, Ministry of Science and Technology, Baghdad-Iraq. Seeds were treated with 0.0, 0.5, 1.0 or 2.0 mM Sodium Azide for 2, 4 or 6 hrs, washed three times in sterile distilled water, and sown in pots under greenhouse conditions. Percentage of seed germination was recorded 14 days after sowing. Seedlings length was recorded after 34 days of sowing.

Experiment II

Seeds were dehusked and surface sterilized by soaking in 96% ethanol for 2 min and then soaked with 2.5% Sodium hypochlorite (Clorox) for 45 min followed by three rinses in sterile distilled water inside a laminar air flow cabinet.

Sterilized seeds were cultured in test tubes (25 ×150 mm) containing semisolid MS medium (12) supplemented with 1.0, 2.0 or 2.5 mg l^{-1} 2,4-D and 0.0, 0.5, 1.0 or 1.5 mg l^{-1} kinetin + 650 mg l^{-1} proline + 30 g l^{-1} sucrose + 8 g l^{-1} agar. For each treatment, 30 seeds were sown (3 seeds/tube). After four weeks of incubation in dark at 25 ± 2ºC, callus induction frequency (%) was calculated using the following formula (13).

(No. of seeds produced callus/total seeds cultured)*100

For callus growth, the induced calli (initial weight was 150 mg/tube) were sub-cultured, on the same medium.

Experiment III

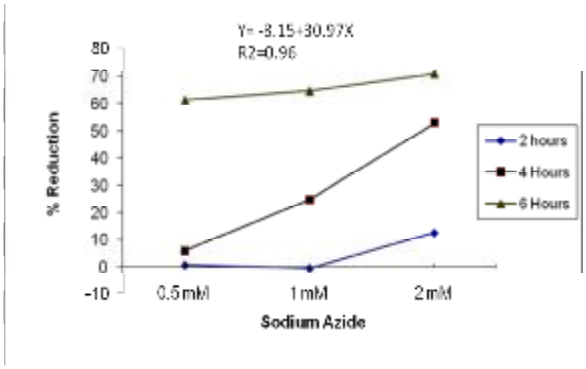
Callus growth was determined after growth on 0.0, 0.5, 1.0, 1.5, or 2.0% of polyethylene glycol 6000 (PEG) which were added to test the effect of PEG on callus fresh and dry weight after 6 weeks incubation in dark. An initial weight of 100mg/tube callus was used with 10 replications for each treatment.

A randomized Complete Block Design was implemented to study the investigated factors. Results were statistically analyzed according to Steel and Torrie (14).

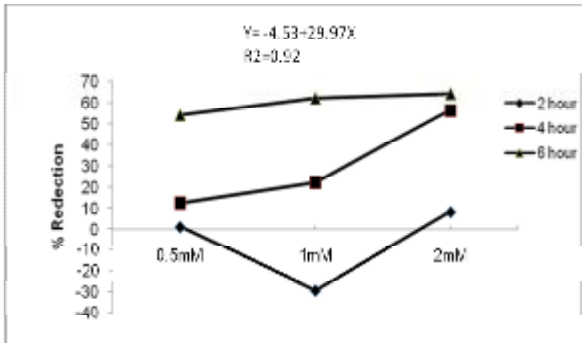
RESULTS AND DISCUSSION

Experiment I

Result showed that there were no significant differences between Amber33 and Amber Baghdad on germination (%), shoot and root length while there were decreases in these characters with increasing the soaking times and Sodium Azide concentrations tables(1,2,3 and 4). The interaction between varieties, soaking time and Sodium Azide concentration revealed that in both varieties there were no significant differences in all studied parameters with increasing the concentrations of Sodium Azide after soaking for 2 hrs. After 6 hours of soaking, shoot and root length significantly decreased with increasing the concentration of Sodium Azide, while after 4 hrs of soaking, there was a significant decrease in mean shoot and root length at 1 and 2mM of Sodium Azide treatment tables(1,2,3). Sodium azide is a strong mutagen and growth of plant parts are strongly inhibited with increasing its concentration and treatment duration. Such reduction in germination, shoot and root length is a common phenomenon; similar observations were made by (15). Percentages of reduction in seedlings length increased with increasing Sodium Azide figures (1 and 2). Generally, for mutagenesis either with physical or chemical mutagens, the dose/concentration that reduces growth of M_1 seedlings to 30-50% of the control is taken as a criterion for promising treatment (16). However, the dose that caused 40% (1.5 mM) reduction was chosen since it gave the highest genetic variation and less sterility for 4 hrs in both Amber33 and Amber Baghdad cultivars.



Figure(1): % reduction in seedlings length for Amber 33.



Figure(2): % reduction in seedlings length for Amber Baghdad.

Table(1):Effect of cultivars, Sodium Azide (SA) concentrations (mM) and soaking time (hrs) on % germination

Cultivars	Soaking time (hrs)	SA (mM)				Mean
		0	0.5	1	2	
Amber33	2	100.000	100.000	100.000	100.000	77.950
	4	100.000	97.033	97.033	63.667	
	6	100.000	36.067	30.533	11.067	
Amber Baghdad	2	100.000	100.000	100.000	100.000	79.147
	4	100.000	94.400	94.400	63.867	
	6	100.000	41.633	36.067	19.400	
Mean		100.00	78.18	76.33	59.66	
LSD 0.05		**Cultivars = N.S ; PEG= 3.37; interaction =8.26**				

Table(2):Effect of cultivars, Sodium Azide (SA) concentrations (mM) and soaking time (hrs) on shoot length (cm)

Cultivars	Soaking time (hrs)	SA (mM)				Mean
		0	0.5	1	2	
Amber33	2	11.475	11.392	11.533	10.039	8.751
	4	11.614	11.039	8.833	5.577	
	6	11.567	4.473	4.086	3.383	
Amber Baghdad	2	10.794	10.608	14.019	9.803	9.070
	4	11.686	10.127	11.587	5.065	
	6	11.478	5.261	4.328	4.078	
Mean		11.43	8.81	9.06	6.32	
LSD 0.05		**Cultivars=N.S;SA= 0.91;Interaction=2.24**				

Table(3):Effect of cultivars , Sodium Azide (SA) concentration (mM) and soaking time (hrs) on root length (cm)

Cultivars	Soaking time (hrs)	SA (mM)				Mean
		0	0.5	1	2	
Amber33	2	6.114	5.928	6.011	5.208	4.592
	4	6.522	5.816	4.577	2.987	
	6	6.053	2.252	1.906	1.733	
Amber Baghdad	2	6.811	5.433	5.506	4.903	4.369
	4	5.858	5.173	4.188	2.628	
	6	5.608	2.225	2.245	1.856	
Mean		6.16	4.47	4.07	3.21	
LSD 0.05		**Cultivars = N.S; SA= 0.32; Interaction= 0.79**				

Table(4):Effect of soaking times (hrs) on % germination, shoot and root length (cm)

Soaking times (hrs)	% Germination	Shoot length (cm)	Root length (cm)
2	100.00	11.20	5.73
4	88.80	9.44	4.71
6	46.84	6.08	2.98
LSD0.05	2.92	0.79	0.27

Experiment II

Results exhibited that % of callus induction was higher in Amber Baghdad compared with Amber33 table (5) while callus fresh weight was higher in Amber33, recording 173.20 and 147.07 mg respectively table(6). Data displayed in table(5) revealed that the concentration of kin at 1.0 and 1.5 mg/l decreased the % of callus induction compared with the 0 and 0.5 mg Kin/l, the percentages of callus induction were 59.20, 62.51, 21.05 and 8.56% at 0. 0.5, 1.0 or 1.5 mg Kin/l respectively.

Adding 2,4-D at 2.0 and 2.5 mg/l significantly increased the callus induction percentage from mature embryo compared with 1.0 mg 2,4-D /l. It is interesting to find that treating seeds with Sodium Azide increased the % of callus induction table(5) while there was no significant differences in 0 and 1.5mM Sodium Azide in callus fresh weight table(6). The interaction between 2,4-D, Kin, varieties and Sodium Azide on % of callus induction table(5) revealed that the highest percentages of callus induction were 90.10 and 93.30 % in non and mutated Amber 33 respectively at 0.5mg kin/l and 2.5 mg 2,4-D /l, while for Amber Baghdad the highest percentage 93.40% for both non mutated and mutated at 0.5mg kin/l and 2.0 mg 2,4-D /l. The different effects of 2,4-D and Kin and Sodium Azide been observed in callus fresh weight table (6), the highest callus fresh weight in non mutated Amber 33 was 616.60 at 0.5mg kin/l and 2.5 mg 2,4-D /l while the highest callus fresh weight in mutated Amber 33, non mutated Amber Baghdad and mutated Amber Baghdad were 799.50, 498.71 and 670.50 mg respectively at 0.5mg kin/l and 2 mg 2,4-D /l . Although, Sodium Azide increased the percentage of callus induction however it decreased the callus fresh weight in all tested media except in the media supplemented with 2.0 mg 2,4-D/l + 0.5mg Kin /l table(6). Callus, mainly comprising masses of undifferentiated cells, is good starting material for *in vitro* manipulation. Moreover, calli induced from the scutellar tissue of mature seeds are an excellent source of cells for *in vitro* regeneration. Differences occurred between media and varieties in callus induction and callus growth, this indicated differential genotypic ability, genotypic. Differences in callus formation were reported to be genetically determined in some cereals including rice (17) and wheat (18). On the other hand, callus growth inhibited with mutagen (callus fresh weight) which is in agreement with the result of Ganesan *et al.* (19).

Table(5):Effect of 2,4-D, Kin, cultivars and Sodium Azide (SA) (mM) on % callus induction

Cultivars	SA (mM)	Kin (mg/l)	2,4-D (mg/l)			
			1.0	2	2.5	Mean
Amber33	0	0	56.70	70.10	43.30	59.20
Amber Baghdad			56.60	76.90	40.00	
Amber33	1.5		50.10	73.50	60.00	
Amber Baghdad			46.70	83.40	53.20	
Amber33	0	0.5	19.90	60.20	90.10	62.51
Amber Baghdad			39.70	93.40	36.80	
Amber33	1.5		33.20	66.80	93.30	
Amber Baghdad			50.00	93.40	73.40	
Amber33	0	1	6.60	16.50	23.30	21.05
Amber Baghdad			23.20	6.60	16.50	
Amber33	1.5		0.00	23.40	16.60	
Amber Baghdad			10.00	46.70	63.20	
Amber33	0	1.5	6.60	3.30	13.20	8.56
Amber Baghdad			0.00	0.00	19.90	
Amber33	1.5		0.00	0.00	19.90	
Amber Baghdad			0.00	10.00	29.90	
Mean			24.95	45.26	43.28	
Mean	**Amber33**		**Amber Baghdad**	**0 SA**	**1.5 SA**	
	35.27		40.39	34.13	41.52	
LSD 0.05	**2,4-D = 5.03; Kin= 5.81; Cultivars = 4.11; SA= 4.11; interaction= 20.14**					

Table(6):Effect of 2,4-D , Kin, cultivars and Sodium Azide (SA) (mM) on callus fresh weight (mg)

Cultivars	SA (mM)	Kin (mg/l)		2,4-D (mg/l)			
			1	1.5	2	2.5	Mean
Amber33	0	0	230.50	202.10	303.60	289.40	219.69
Amber Baghdad			159.60	163.10	186.90	318.70	
Amber33	1.5		178.90	232.86	285.10	208.39	
Amber Baghdad			163.30	158.20	173.10	261.30	
Amber33	0	0.5	135.10	125.40	245.00	616.60	298.56
Amber Baghdad			152.00	144.20	498.71	295.30	
Amber33	1.5		55.26	102.95	799.50	347.30	
Amber Baghdad			148.10	156.20	670.50	284.90	
Amber33	0	1	61.39	104.34	60.24	167.91	77.80
Amber Baghdad			56.82	67.59	73.44	54.92	
Amber33	1.5		39.66	83.42	74.81	161.53	
Amber Baghdad			54.16	61.80	69.65	53.21	
Amber33	0	1.5	82.37	55.04	40.16	68.60	44.50
Amber Baghdad			19.35	44.60	21.48	47.90	
Amber33	1.5		59.69	40.56	28.08	56.75	
Amber Baghdad			25.00	36.30	32.10	54.10	
Mean			101.32	111.16	222.64	205.42	
Mean			**Amber33**	**Amber Baghdad**	**0 SA**	**1.5 SA**	
			173.20	147.07	159.13	161.14	
LSD 0.05	**2,4-D = 21.85; Kin= 21.85; Cultivars = 15.45; SA= non significant; interaction= 87.43**						

Experiment III

The main aim of this experiment was to check the effect of Sodium Azide for induction of genetic variation for drought tolerance in two local Rice cultivars. There was an increase in the mean of callus fresh and dry weight at 1.5 mM Sodium Azide table(7). Results tables(8 and 9) showed that there were no significant differences between varieties in callus fresh and dry weight. Significant reduction in callus fresh

and dry weights started from 0.5 and 1% PEG respectively. Both cultivars showed a similar response under PEG and Sodium Azide (interaction), as the PEG concentration in the medium increased, the callus fresh and dry weight decreased at 0 mM Sodium Azide, while at the treatment 1.5 mM Sodium Azide callus fresh and dry weight in both cultivars increased at 0.5 and 1% compared with the control. The role of PEG in creating chemical drought was observed (20), PEG is a flexible, water-soluble polymer, it can be used to create high osmotic pressures (tens of atmospheres).

It also is unlikely to have specific interactions with biological chemicals. These properties make PEG one of the most useful molecules for applying osmotic pressure in biochemistry experiments, in particular when using the osmotic stress technique (21).

Table(7):Sodium Azide (SA)(mM) on callus fresh and dry weight (mg)

SA (mM)	fresh weight (mg)	dry weight (mg)
0	222.314	23.811
1.5	436.855	38.591
LSD 0.05	41.02	4.53

Table(8):Effect of PEG, cultivars and Sodium Azide (SA) (mM) on callus fresh weight (mg)

Cultivars	SA (mM)	%PEG					Mean
		0	0.5	1	1.5	2	
Ambere33	0	518.700	239.500	148.500	121.260	95.720	336.943
	1.5	634.600	647.900	724.300	157.400	81.550	
Amber Baghdad	0	596.000	200.760	127.100	136.700	38.900	322.226
	1.5	634.700	694.000	674.800	82.800	36.500	
Mean		596.000	445.540	418.675	124.540	63.168	
LSD 0.05	**Cultivars = N.S; PEG= 64.86;interaction= 129.73**						

Table(9):Effect of PEG, cultivars and Sodium Azide (SA) (mM) on callus dry weight (mg)

Table 9. Effect of PEG, cultivars and Sodium Azide (SA) (mM) on callus dry weight (mg)							
Cultivars	SA (mM)	%PEG					Mean
		0	0.5	1	1.5	2	
Ambere33	0	48.900	20.000	14.400	6.000	4.700	29.081
	1.5	46.700	66.500	70.300	4.800	8.510	
Amber Baghdad	0	62.800	41.500	26.400	11.000	2.410	33.321
	1.5	56.200	64.400	56.600	10.200	1.700	
Mean		53.650	48.100	41.925	8.000	4.330	
LSD 0.05	**Cultivars = N.S ; PEG=7.17; interaction= 14.34**						

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