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In Vitro Influence of drought on some physiological parameters in callus induced from seeds of four Rice cultivars (*Oryza sativa* L.)

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Abstract

In Vitro seeds of four rice cultivars, Amber 33, Jasmine, IRRI 132 and IR 06L 144 were surface sterilized by using various combinations of ethanol and sodium hypochlorite. For callus induction, mature seeds were cultured on MS medium supplemented with 2,4-D at four concentrations (0.5,1.0,2.0 and 3.0 mg.l^{-1}). Finally callus were exposed to drought stress condition by using Polyethylene glycol (PEG) at four concentrations (0, 20 , 40 or 80 g.l^{-1}). Results revealed that despite treating mature seeds of all rice cultivars with either (90% Ethanol 2 min +3% NaOCl 45min) or (90% Ethanol 2 min +6% NaOCl 45 min) gave no contamination percentage, yet the last treatment yielded no surviving seeds. Furthermore, responses of rice seeds for callus induction were found to be genotype dependent .However, 3.0 mg.l^{-1} concentration of 2,4-D was optimum as compare to 0.5,1.0 or 2.0 mg.l^{-1}. Moreover, a significant reduction in fresh weight when callus of rice exposed to 40 or 80 g.l^{-1} of PEG and significant increase were found in the accumulation of proline and carbohydrate in response to high level of PEG stress.

Keywords: *Rice, In Vitro, PEG, Callus , proline, carbohydrate*

Introduction

Environmental stresses such as drought and salinity are the most important factors limiting crop productivity (Jaleel *et al.* 2009; Piwowarczyk *et al.*,2014).In Iraq, water deficit became the greatest problem which limited the production of many crops. Rice (*Oryza sativa* L.) considered as the most important cereal crop after wheat and maize. According to (Khush, 2005) rice is a primary source of calories and food for human, as it provides one-third of total dietary carbohydrate in Asian countries (Revathi and Pillai,2011). Based on (Puhan and Siddiq, 2013) rice production supports more than 50% of the world population and 50% increment of rice production will be by 2025 (Khush and Virk 2000). Among all cereals, rice is the most susceptible crop to water deficit (Lafitte and Bennet, 2003).

Many biotechnological approaches have been used to improve rice productivity under stress conditions for example Zinnah et al, 2013 evaluated the regeneration capacity of some rice genotypes in NaCl treated media condition. (Karthikeyan *et al*, 2009) study callus induction in regeneration process from mature embryos of rice dry seeds while (Rai *et al* 2011) focused on progress made towards the development of Stress-tolerant lines through tissue culture based in vitro selection.

To study plant physiology through *in vitro* selection most of the researchers tend to supply the culture media with some compound that have influence osmotic stress on the plant such as mannitol (Pant *et al*, 2014.; Hadi *et al*, 2014) polyethylene glycol (El- kaaby, 2016), sorbitol and sucrose (Bidabadi *et.al* ,2012) .Polyethylene glycol (PEG) widely used as supplementary material in the culture media to stimulate drought stress in plants due to its nature as non-penetrating and inert characteristic (Rai *et al.* 2011) therefore, its lowering water potential in a way similar to soil drying (Larher *et al*, 1993.; Abdul-Qadir and Al-Ka'aby,2011.; Matheka *et al*, 2008).

Hence, the main aim of this study was to determine the potent of four rice cultivars Amber33, Jasmine , IRRI 132 and IR 06L 144 to drought stress by supplying media with PEG 6000.

Materials and Methods

The study was conducted at The Ministry of Science and Technology/ Directorate of Agriculture /Genetic engineering labrotary/ Baghdad, Iraq. Seeds of four Rice cultivars namely Amber 33 and Jasmine supplied by Ministry of Agriculture, Baghdad, Iraq , IRRI 132 and IR 06L 144 obtained from International Rice Research Institution (IRRI) in Manila/Philippine. Mature seeds were surface sterilized with various combinations of sterilizant

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such as T1(90% Ethanol 2 min),T2 (70%Ethanol 2 min) T3(3% NaOCl 30 min) T4 (90% Ethanol 2 min +3% NaOCl 45 min) and T5 (90% Ethanol 2 min +6% NaOCl 45 min) and washing three times with D.D. Water (sterile distilled deionized water).Seeds were cultured on MS (Murashige and Skoog, 1962) basal medium (free hormone medium) . For callus induction four seeds of each cultivar were cultured in a glass vial (Fig.1) containing MS basal medium supplemented with four concentrations of 2, 4-D (0.5,1.0,2.0 and 3.0 $mg.l^{-1}$) ,30 $g.l^{-1}$ sucrose and solidified with 7 $g.l^{-1}$agar. The pH medium was adjusted to 5.75 before autoclaving and each treatment triplicated.

To induce osmotic stress, Polyethylene glycol PEG (M.W 6000) at concentrations of (0, 20,40 and 80) $g.l^{-1}$ were added to the same previous MS medium solidified with 7 $g.l^{-1}$agar for the first three concentrations, while liquid medium was used for the last concentration 80 $g.l^{-1}$. Both media supplemented with 2, 4-D at concentration of (3.0 $mg.l^{-1}$) as a best concentration for callus induction. A constant weight (100 mg) of callus from each cultivar was transferred to the stressed media and each treatment was triplicated. Six weeks later, proline was estimating in callus according to (Bates *et al.* 1973), Carbohydrate estimated according to (Dubois *et al*, 1956).

Cultures were incubated in growth room under 16 hours/day light and 8 hours/day darkness, 25 ± 2 °C of temperature conditions. The experiments were design in completely randomized. Data analyzed based on GenStat softwere program and means were compared according to Least Significant Differences (L.S.D.) at $P \leq 0.05$ level.

Fig 1: Rice seeds cultured on callus induction media

Results and Discussion

Surface sterilization of seeds

Ten days after seeds surface sterilization, the contamination percentage was evaluated in Table 1.

Table 1: *In Vitro* effect of different sterilizants on seeds contamination percentage of four rice cultivars

Sterilizants	Concentration%	Contamination%				Mean
		Amber 33	Jasminee	IRRI 132	IR 06L 144	
T1 Ethanol 2 min	90%	100	100	100	100	100
T2 Ethanol 2 min	70%	100	100	100	100	100
T3 *NaOCl 30 min*	3%	60.54	40.90	80.13	20.36	50.48
T4 Ethanol 2 min +NaOCl 45min	90%+3%	0	0	0	0	0
T5 Ethanol 2 min +NaOCl 45min	90%+6%	0	0	0	0	0
Mean		52.11	48.18	56.03	44.07	
L.S.D $_{(0.05)}$	cultivars 4.2	interaction 11.08		Sterilizants 5.54		

Five different treatments with different concentration and period were used in this sterilization experiment, T1 (90% Ethanol 2 min) and T2 (70% Ethanol 2 min) were not suitable as it gave 100% of contamination percentage for all rice cultivars. Also the responses of these cultivars were varied, high contamination percentages reached (56.03% and 52.11 %) in IRRI 132 and Amber 33 respectively. while the response of these cultivars were varied at T3 (3% NaOCl 30 min) treatment from lowest contamination percentage 20.36% in IR 06L 144 to mid (40.90 %) in Jasmine to high contamination percentage reached and 60.54% in Amber 33 and 80.13% in IRRI 132 respectively. Moreover, despite T4 (90% Ethanol 2 min + 3%NaOCl 45 min) and T5 (90%Ethanol 2 min+ 6% NaOCl 45 min) treatments were superior to give no contamination percentage, yet T5 treatment yielded no surviving seeds. In vitro sterilization conditions especially sodium hypochlorite have been reported to control the vital physiological contamination process in plant (explants) due to its activity which have been reported by (Rodeva *et al*, 2004). Our results are agree with Ghobeishavi *et al,*2015; Sakthivelu *et al.*2008) in related to the positive role of NaOCl and Ethanol.

Callus induction media

For callus induction media, the experiment involves four concentration of 2, 4-D. According to (table 2) all 2, 4-D concentrations showed positive effect for callus induction in the form of fresh weight, callus color and texture. However, among the four concentrations 3.0 $mg.l^{-1}$ was superior to give higher callus fresh weight reached 179.28 mg as compare to the other concentrations. Concerning to rice cultivars, both IRRI 132 and Amber 33 pretended to have best response while IR 06L 144 seems to be the weakest. For the interactions, no significant differences were observed between Jasmine and IRRI 132 cultivars at 3.0 mg concentration of 2, 4-D. the variation among the four rice cultivars in related to their response for callus induction parameter may be due their genotype efficiency.

Table 2: *In Vitro* effect of 2, 4-D concentrations on callus induction from mature seeds of four rice cultivars

Rice cultivars	Callus fresh weight (mg) 2,4-D mg.l^{-1}				Mean
	0.5	1.0	2.0	3.0	
Amber 33	78.56	125.10	160.26	227.11	147.76
Jasmine	69.82	131.21	155.09	194.17	137.57
IRRI 132	98.60	128.12	169.87	205.51	150.53
IR 06L 144	66.03	72.09	78.18	90.33	76.66
Mean	78.2525	114.13	140.85	179.28	
L.S.D (0.05) 2,4-D concentrations 13.81 interaction 28.65 cultivars 13.81					

Table 3: Callus of four rice cultivar in stressed media containing different concentration of PEG

Rice cultivars	Callus fresh weight (mg) PEG g.l^{-1}				Mean
	0	20	40	80	
Amber33	188.16	214.33	136.17	100.23	159.72
Jasmine	149.09	227.45	124.14	89.15	147.46
IRRI 132	175.12	238.75	146.32	123.05	170.81
IR 06L 144	198.18	259.08	153.09	117.56	181.98
Mean	177.64	234.90	139.93	107.50	
L.S.D (0.05) PEG concentrations 11.30 interaction 25.89 cultivars 11.30					

Table 4: Accumulation of Proline and Carbohydrate in PEG stressed callus of four rice cultivars

ice cultivars	Proline mg.g^{-1} PEG g.l^{-1}				Carbohydrate mg. g^{-1} PEG g.l^{-1}			
	0	20	40	80	0	20	40	80
Amber 33	2.32	5.03	7.20	9.16	3.10	5.32	6.88	10.93
Jasmine	0.95	4.82	6.15	7.23	1.13	2.78	4.64	8.11
IRRI 132	1.67	5.08	6.01	8.93	2.55	6.14	7.54	9.01
IR 06L 144	3.20	5.14	5.87	7.53	1.34	5.93	6.01	8.32
L.S.D (0.05)	0.59				1.63			

Our results agree with (Revathi and Pillai ,2011; Jaseela *et al*.2009) they referred to the positive role of all 2, 4-D concentrations in forming callus in their experiments. Also agree with Zinnah *et al* 2013 who found highest percentage 75% of callus induction from mature seed embryo of the local variety rice (Chini Kanai) when media MS supplemented with 3.0 mg/L or 5.0 mg.l^{-1} 2, 4-D.

Effect of poly ethylene glycol on callus growth

Data in (Table 3) shown that, calli on control medium with no PEG exhibited normal proliferation and growth. In 20 g.l^{-1} of PEG concentration a slight increase in callus fresh weight for all rice cultivars were noticed. On the other hand, a gradual decrease in callus fresh weight was observed in all rice cultivars at 40 g.l^{-1} or 80 g.l^{-1} of PEG concentration.

Concerning to the cultivars response, no significant differences were observed between IR 06L 144 and IRRI 132 ,they recorded higher callus fresh weight reached 181.98mg and 170.81mg . For the interaction among rice cultivars and PEG levels, IR 06L 144 gave higher callus fresh weight reached 259.08 mg as compare to 214.33 mg for Amber33 at the same level of PEG. The presence of PEG in culture media may be reduced water potential which in turn reduced callus growth. Similar results were found on callus growth of Nemat and Dom siah rice cultivars by (Ghobeishavi *et al*, 2015) and (Shabir *et al*,2010; Shehab *et al,* 2010) in rice and (Aki, 2005) in pepper.

Effect of poly ethylene glycol on proline and carbohydrate in callus of four rice cultivars

Following six weeks of exposing callus to stressed media data in table (4) shown a positive correlation between water stress and accumulation of proline or carbohydrate in callus exposed to (80 g.l^{-1}) of PEG. Moreover, different responses were observed among rice cultivars and maximum average accumulation in Amber 33 reached (9.16 mg.g^{-1} , 10.93 mg.g^{-1}) for proline and carbohydrate respectively. Accumulation of proline in plant callus culture under stress condition have agree with (Al-Bahrany,2002) in rice, and (Al-Taha,2013) in sour orange and also agree with (Khashan.K.T.2016) for proline and carbohydrate in tomato.

Conclusion

In conclusion, drought stress affected negatively on callus growth for all rice cultivars. The reduction in callus growth may be due to the presence of PEG in the growth medium which in turn affected on the amount of endogenous growth regulators which response for cell division. On the other side callus under stress condition accumulate some useful compound like proline and carbohydrate, they are known as osmotic regulators which regulate the growth of cell under abnormal conditions. Hence, further studies are required associated with plant regeneration from stressed callus and investigation whether physiological mechanism or accumulation of secondary compound are responses to drought stresses.

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