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THE EFFECT OF DROUGHT ON PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF SOME RICE GENOTYPES

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ABSTRACT

Seeds of four rice genotypes Amber 33, Amber Baghdad, T_{14} and T_{15} were inoculated into half strength MS medium supplemented with 0.0, 10 or 20% polyethylene glycol 6000 (PEG). The effect of polyethylen glycol % (PEG) was examined on Shoot, root lengths, plant fresh, dry weights, proline and carbohydrate concentrations. The results showed that there were differences between genotypes; genotype T_{15} produced the highest mean plant fresh, dry weight and proline content, with mean values 108.612mg, 11.270mg and 53.387mg/g dry, respectively. While genotype T_{14} exhibited the highest carbohydrate content compared with other genotypes. The PEG caused decreasing in means of all characters studied with increasing PEG concentrations, except the means of proline content. The interaction between genotypes and PEG concentrations had a significant effect on all the measured traits except plant dry weight. Due to the importance of rice especially Amber genotypes in Iraq, its important to improve these drought sensitive genotypes (Amber33 and Amber Baghdad) for drought tolerance using different genetic engineering and tissue culture techniques. Also T_{14} and T_{15} genotypes can be used as stander genotypes for drought tolerance.

Key words: Rice, drought tolerance, PEG

INTRODUCTION

Drought is a world - wide problem that seriously influences grain production. Increasing human population and global climate change made the situation more serious. Rice, as a paddy field crop, is particularly susceptible to water stress Tao *et al.* (16) and Yang *et al.* (19). In fact, drought can cause severe damage at any stage of rice growth and development, which would lead to yield loss. It is estimated that 50% of the world rice production is affected by drought Bouman *et al.* (5). Drought causes delay in the physiological development of rice plant and affects physiological processes like transpiration, photosynthesis, respiration and translocation of assimilates to grain. Plants have many adaptive strategies in response to abiotic environmental stresses; these adaptive mechanisms include changes in physiological and biochemical processes. Adaptation to the stress is associated with metabolic adjustments that lead to the accumulation of several organic solutes that may be classified into two categories: one is nitrogen-containing compounds such as proline and the other is hydroxy compounds, such as sucrose Yancey *et al* (18) and McCue and Hanson (11). Polyethylene glycols (PEG) of high molecular weight has been long used to simulate drought stress in plants as non-penetrating osmotic agents lowering the water potential in a way similar to soil drying Larher *et al*. (10).

Have Many papers been documented that the variation between rice

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genotypes through physiological criteria might be used as a tools in plant breeding.

On the other hand, there is still insufficient data on physiological mechanisms underlying plant responses to drought tolerance for Iraqi genotypes, thus an attempt to investigate the effect of osmotic stress generated by PEG on seedling growth, proline and carbohydrate content in two Iraqi genotypes (Amber33 and Amber Baghdad) and two entries (T_{14} and T_{15}) with the objective to find a criterian for drought tolerance were under taken.

MATERIALS AND METHODS

Seeds of susceptible rice genotypes (Amber 33 and Amber Baghdad) which was kindly supplied by Genetic Engineering Dept., Agricultural Research Directorate, Ministry of Science and Technology, Baghdad, Iraq, and tolerant rice genotypes which was Provided by International Rice Research Institution (IRRI) (T_{14} with code: IRg59007-191-1-b and T_{15} with code: IR60080-AB-A) to water deficit were selected and sterilized in sodium hypochlorite (2.5%) and then washed in distilled water for three times. The seeds were germinated in Petri dishes in an incubator maintained at 25°C, there after the seedlings were transferred to test tubes containing half strength MS media with 0.0, 10 or 20% polyethylene glycol 6000 (PEG). The test tubes were placed in a growth chamber under fluorescent light (14hrs light/10hrs dark) and at an ambient temperature of 25±2°C, the medium was changed every 7days. Shoot, root lengths and plant fresh weights were recorded after six weeks. The dry weight was obtained after drying the plant tissues for 48hrs at 72°C. Proline concentrations were determined according to Bates *et al.* (3). A quantity of 10 mg dry weight of plant seedlings was homogenized with 3% sulfosalicylic acid. The filtrate was mixed with equal volumes of glacial acetic acid and ninhydrin reagent and incubated for 30 min at 100°C. The samples were vigorously mixed with 4ml toluene, light absorption of toluene phase was estimated at 520 nm using a spectrophotometer. Proline concentration was expressed as mg/g dry weight. In this experiment, only the total sugar content (carbohydrates concentrations) was determined without the identification of specific sugar components based on the method of phenol sulfuric acid Herbert *et al*. (7). A quantity 10 mg dry weight of plant seedlings was homogenized with deionized water, extract was filtered, and then treated with 5% phenol and 98% sulfuric acid, the mixture was incubated for 20min at 30°C then absorbance at 485nm was determined by a spectrophotometer. Contents of soluble sugar were expressed as mg/g dry weight.

The experiment was designed as a factorial experiment (two factors, genotypes and PEG treatments) with a Completely Randomized Distribution (CRD) of the treatments with 10 replications (each replication contained 3 seedlings) for shoot, root length, plant fresh and dry weight measurements. Also the values of proline and carbohydrates were determined. Analyses of variance were done by using the SPSS var. 12 software. Differences between means were determined by LSD tests at probability level of 5% Steel and Torrie (5).

RESULTS AND DISCUSSION

Exposure of plants to defiat water environment causes a set of metabolic and physiological changes, which can lead to severe damage and plant death. The present study investigates the effect of PEG-induced water stress on rice. Results showed that there were a significant difference between Amber33 and T_{15} genotypes that caused by PEG treatment on shoot length (Table 1), whereas, there were significant differences among all genotypes in root length (Table 2).

Table 1: Effect of %PEG on the mean plant shoot lengths (cm) of genotypes

Genotype	Mean plant shoot lengths (cm)			Mean
	(%) PEG			
	0.000	10.000	20.000	
Amber33	17.350	16.627	15.570	16.516
Amber Baghdad	17.923	15.047	13.820	15.597
T_{14}	17.010	17.633	12.170	15.604
T_{15}	16.003	14.537	12.003	14.181
Mean	17.072	15.961	13.391	
LSD 0.05	CV =1.51; PEG=1.30; CVxPEG= 2.61			

after 6 weeks of inoculating in half strength MS medium, n=30.

Genotype T_{15} produced the highest mean plant fresh weight, dry weight and proline content reached 108.612mg, 11.270mg and 53.387mg/g□ respectively (Table 3, 4 and 5), while genotype T_{14} gave the highest carbohydrate content compared with other genotypes (Table 6). These results indicate that the drought tolerance which may due to the genetic differences among different genotypes Hamza (6) and Roy *et al.* (13).

Table 2: Effect of %PEG on the mean plant root lengths (cm) of genotypes after 6 weeks of inoculating in half strength MS medium, n=30

Genotype	Mean plant root lengths (cm)			Mean
	%PEG			
	0.000	10.000	20.000	
Amber33	7.300	4.840	4.813	5.651
Amber Baghdad	7.383	4.737	3.753	5.291
T_{14}	4.710	4.677	4.190	4.526
T_{15}	5.340	4.160	5.110	4.870
Mean	6.183	4.603	4.467	
LSD ≤ 0.05	CV =0.33; PEG=0.29; CV x PEG= 0.58			

Table 3: Effect of %PEG on the mean plant fresh weights (mg) of genotypes after 6 weeks of inoculating in half strength MS medium, n=30

Genotypes	Mean plant fresh weights (mg)			Mean
	%PEG			
	0.000	10.000	20.000	
Amber33	106.299	82.499	55.600	81.466
Amber Baghdad	118.799	85.167	56.066	86.677
T_{14}	114.166	102.301	80.934	99.134
T_{15}	124.134	126.600	75.101	108.612
Mean	115.849	99.142	66.925	
LSD ≤ 0.05	CV=12.55; PEG=10.87; CVxPEG= 21.74			

Treatment with PEG showed that there was a markable and significant decreasing in the means of all characters with the increasing of PEG concentrations (Table 1, 2, 3, 4 and 6) except the means of proline content (Table 5) which was elevated linearly with the increase of water deficit (increase PEG concentrations), Values reached 19.687, 40.562 and 44.046mg/g proline dry weight at 0.0, 10 and 20% PEG re 230 **lyethylene glycol is inert, non**

ionic that has frequently been used for inducing water stress and maintains a uniform water potential (Hohl and Schopfer, 1991). Although PEG 6000 exerts osmotic potential, but it does not absorb by plants.

The interaction between genotypes and PEG concentrations exhibited a significant effect on all the measured traits except plant dry weight (Table 1-6) this mean that the response of genotypes different due to PEG. PEG showed no effect on Amber33 shoot length, while the shoot length for Amber Baghdad significantly decreased with increasing of PEG whereas shoot length of genotypes T_{14} and T_{15} significantly decreased at 20% PEG in comparing with 0.0 and 10% PEG (Table1).

Table 4: Effect of %PEG on the mean plant dry weight (mg) of genotypes after 6 weeks of inoculating in half strength MS medium, n=30

Genotype	Mean plant dry weights (mg)			Mean
	%PEG			
	0.000	10.000	20.000	
Amber33	11.023	9.950	9.527	10.167
Amber Baghdad	11.560	9.817	8.900	10.092
T_{14}	11.287	10.823	10.700	10.937
T_{15}	11.513	11.200	11.097	11.270
Mean	11.346	10.448	10.056	
LSD ≤ 0.05	CV =1.02; PEG= 0.88; CV x PEG= n.s			

Table 5: Effect of %PEG on the mean proline concentration (mg/g) of genotypes after 6 weeks of inoculating in half strength MS medium, n=30

Genotype	Mean proline concentrations (mg/g)			Mean
	%PEG			
	0.000	10.000	20.000	
Amber33	6.470	28.638	29.658	21.588
Amber Baghdad	6.214	23.884	29.426	19.842
T_{14}	34.342	46.354	52.035	44.243
T_{15}	31.722	63.374	65.067	53.387
Mean	19.687	40.562	44.046	
LSD ≤ 0.05	CV =6.07; PEG=5.26; CV x PEG= 10.52			

The percentage of the reduction in shoot length at 20% PEG in comparing with control were 11% for Amber33 and 27% for Amber Baghdad, T_{14} and T_{15}. The decrease in plants shoot lengths with the increasing of %PEG may be due to the plant physiological features or it could be another way for drought tolerance as reported by Sadras and Milory (14) mentioned that reduced leaf area is probably the obvious mechanism by which plants and crops restrict their water loss in response to drought.

Table 6: Effect of %PEG on the mean carbohydrat concentrations (mg/g) of genotypes after 6 weeks of inoculating in half strength MS medium, n=30

Genotypes	Mean carbohydrate concentrations (mg/g) % PEG			Mean
	0.000	10.000	20.000	
Amber33	18.245	[illegible]	11.389	14.560
Amber Baghdad	19.405	[illegible]	13.354	15.640
T14	20.973	19.108	16.295	18.792
T15	22.450	21.204	9.100	17.585
Mean	20.268	17.130	12.535	
LSD ≤0.05	CV=2.25; PEG= 1.95; CV x PEG= 3.9			

Evaluation of root length between treatments (Table2) showed significant differences between drought stressed (10 and 20% PEG) and control in both genotypes of Amber33 and Amber Baghdad. Comparison between means showed that the control recorded 7.300 and 7.383cm in Amber33 and Amber Baghdad respectively while the stress treatments reached 4.840 and 4.813cm at 10% and 20% PEG respectively in Amber 33; 4.737 and 3.753cm at 10% and 20% PEG respectively in Amber Baghdad. Concerning genotype T_{14}, there were no significant differences among all PEG concentrations because mean root length reached 4.710, 4.677 and 4.190cm at 0.0, 10 and 20% PEG respectively, and for genotype T_{15} there were no significant differences between the control and 20% PEG (Table 2). Genotype T_{15} recorded the highest root length at 20% PEG reached 5.110cm and the % of the reductions in root length at 20% PEG in comparing with other genotypes, were 51, 76, 11 and 5.5% for Amber33, Amber Baghdad, T_{14} and T_{15} genotypes respectively. It is obvious that % of the reduction in root length in T_{14} and T_{15} genotypes was less than the % of the reduction in root length for Amber33 and Amber Baghdad genotypes in 20% PEG and root lengths provide an important clue to the response of plants to drought stress and can be utilized as screening criteria for stress tolerance, which means that T_{14} and T_{15} are more tolerant to drought compared with Amber33 and Amber Baghdad. The presence of PEG in the medium caused a significant decrease in Amber33 and Amber Baghdad fresh weight compared with the control (0.0% PEG), while a significant decrease occurred in genotypes T_{14} and T_{15} at 20% PEG compared with 0.0 and 10% PEG (Table 3). The % of the reduction in fresh weight at 20% PEG over control were 61, 73, 32 and 39% for Amber33, Amber Baghdad, T_{14} and T_{15} respectively. The addition of PEG to the culture medium decreased the water potential of the medium, thereby induced water stress that adversely affected the turgor maintenance (Munns, (12) and Bajji *et al.* (2), Physiological processes may depend on cell volume particularly in plants sensitive to water deficit and the reduction in plant fresh weight with increasing PEG levels may be due to the osmotic pressure affecting accumulation of soluble elements in cells and then influences other physiological functions in cells including division and growth Binzel *et al*. (14).

Results of this study showed that rice tissues accumulated a significant amount of proline at 10 and 20% PEG compared with the control in all genotypes (Table 5), while a significant decrease occurred in carbohydrate content at 10 and 20% PEG in Amber33 and Amber Baghdad, while in genotypes T_{14} and T_{15} significant differences between control and 20% PEG were recorded for this trait (Table 6). Roy *et al* (15) reported that high proline content is a good index for moisture resistance in genotypes, under water stress conditions since protein degrades and consequently proline content increases. Proline also plays an important role in protection of membrane organelles, proteins and enzymes under stress Ashraf and Foolad (1) and Hoque *et al*. (9). Waldren *et al* (17) found that free proline accumulated significantly when plants severely stressed and wilted and proline accumulation correlates positively to drought. However the degree varies between tolerant and susceptible genotypes, the tolerant one accumulates more proline than the susceptible (Table 5), thus proline accumulation is a sensitive indicator of drought tolerance.

Significant decreases in carbohydrate concentrations were resulted with the increasing of % PEG that may due to that plant cells are using a large amounts of energy including carbohydrates as an energy source in an attempt tolerate stress as reported by Yousif (20).

In conclusion, genotypic variability exists among the investigated rice genotypes for PEG-induced water stress. Water deficit imposed by PEG affected plant growth, proline and carbohydrate concentrations. This study strongly supports the assertion that root length (% of the reduction in root length over control), fresh weight (the % of the reduction in fresh weight) and proline accumulation can be utilized to screen rice genotypes for drought tolerance at early seedling growth stage. Screening procedures are relatively simple, reproducible and less labor-intensive than growing plants to maturity in the field.

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تأثير الجفاف على الصفات الفسلجية والكميوحيوية لبعض اصناف الرز

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الملخص

حضنت بذور اربعة اصناف من الرز (عنبر33، عنبر بغداد، T14 وT15) في وسط MS بنصف القوه ومضافا لها 0.0، 10 أو 20% من الاثيلين متعدد الكلايكول (PEG) كعامل اجهاد و تم الكشف عن تأثير ذلك على معدل طول المجموع الخضري للبادرات، الوزن الطري، الوزن الجاف، تركيز البرولين والكربوهيدرات. أظهرت النتائج وجود فروقات معنوية بين الاصناف; أضهر صنف T15 اعلى معدل وزن طري, وزن جاف, تركيز برولين وصلت الى 108.612ملغم، 11.270ملغم و53.387 ملغم/غم وزن جاف على التوالي. في الوقت الذي اعطى الصنف T14 اضهر اعلى تركيز للكربوهيدرات مقارنة مع باقي الاصناف. أدت اضافة PEG الى تناقص في معدلات الصفات المدروسة مع زيادة تركيز PEG بأستثناء معدل تركيز البرولين والذي ازداد مع زيادة تركيزعامل الاجهاد، كما أظهر التداخل بين الاصناف وتراكيز PEG وجود تأثير معنوي على جميع الصفات المدروسة ماعدا الوزن الجاف للبادرات. و بالنظر الى اهمية الرز و خاصة الصنف عنبر في العراق، هناك حاجة ماسة لتحسين صفة تحمل الجفاف في الاصناف الحساسة (عنبر33، عنبر بغداد) بأستخدام تقنيات الهندسة الوراثية وزراعة الانسجة النباتية. وبالامكان استخدام الصنفين T14 وT15 كمقياس لصفة تحمل الجفاف.

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