

***In vitro* effect of water stress on callus induction and soatic embryogenesis and plantlet regeneration in rice(*oryza sativa* .L)**

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Abstract

Conducted the current study, in laboratory tissue culture of date palm research center ,university of Basra. the spectrum in order to study the effect of water stress of rice plants *Oryza sativa* L. anbar and Jasmin using technology plant tissue culture has been growing mature seeds as for as explant has been the used (MS) medium concentration 4.6 g/l with the full force of some vitamins and amino acids .Secretary has growth regulator used 2,4-D concentration of 8 mg / L scrose concentration of 30 g / L. It has used the sorbitol concentrations (0, 10, 20 and 30) g / L to provide water stress in conditions were clear from the results of the study that the increased concentration of sorbitol in the medea has reduced the weight of callus induced and reduce the number of embryos formed and the number of embryos germinated and the percentage of embryos germinating in the number of plantlets formed as shown by the results outweigh the Anbar cultivar to Jasmine cultivar resistance to aqueous conditions stress.

Key word: tissue culture, Anbar , Jasmine, sor251bitol, stress .

1. Introduction

Rice plant *Oryza sativa* L. of herbal plants which belongs to the grassy family Poaceae and to genus *Oryza* which includes more than 25 species most important of species *O. sativa* and *O. glaberrima* spreads as the first kind in Southeast Asia either Type II Venture in large areas of (Africa Luttet *etal.*, 1998). Is one of the most important food crops in Iraq and has a special place among the Iraqi consumer is ranked second in the forms of food crops after wheat crop. Abound grown in central and southern regions (Obaid, 2015) rice summer crop spends most of the growth period submerged in water therefore requires large amounts of water due to the high temperature and water scarcity and frequent evaporation in the summer and increased salinity seemed grown in contraction and decline (Mohammed 0.2009) so it became necessary to find solutions to this problem, either by providing adequate quantity of water and the outside of the national will because neighboring countries control the water quota entering Iraq and it is linked to the political Baltjazbat so it became necessary to find other solutions, and these solutions to find varieties resistant to water stress. The plant culture technology of the modern methods used to obtain heterozygous plants genetically and that the use of certain chemical compounds in the media center can provide an aqueous tightening conditions on the cell similar to the conditions of the water stress suffered by the plant in the field level (Benn *etal.*, 2013) and this material is sorbitol, which was added to the medium prepared for the induction of callus concentrations (0, 10, 20 and 30) g / L (Hussen *etal.*, 2015).

2. Material and methods

This study was conducted in one of the private-sector laboratories in the district of Shatt al-Arab east of the province of basra. Mature seeds are used for two rice varieties rice (*Oryza sativa* L.) Amber and Jasmin was brought them rice research station in Mashkhab Najaf province. After making a vital test has been de-scaling manually and then underwent surface sterilization by washing the seeds by sterilized water several times to get rid of the impurities and then placed in ethanol (70%) for five minutes with Aharik Pacific and washed afterwards with sterilized water and then placed in clorax (% 50) a encompassed a 5% sodium hypochlorite for 30 minutes, stirring constantly with the addition of one drop per 100 ml substance Tween-20 and then washed the seeds by water sterilized and several times to make sure it is absent from the seeds of the impact of the sterile solution (Carsono and Yoshida,2006). Use MS media (Murashig and Skoog 1968) and added vitamins Gambrog. For the purpose of preparation of one liter of media has been thawed 4.6 of powder MS in 800 ml by distilled sterilized water then put the solution in a volumetric flask capacity Lear one and put the beaker on a double heater mixer magnetic, stirrer hot plate and add 30 g of sucrose as an energy source and then added growth regulators auxin 2-4, D concentration of 8 mg/ l, cytokinine with 0.5 mg/ L and add sorbitol deferent concentrations (0, 10, 20 and 30) g/ l each concentration move to the flask and adjust the pH of the solution at the level of 5.7 using Digital meter device has been tuning and modification by calibration with a solution of hydrochloric acid or sodium hydroxide (0.1M) and then add agar in concentration of 5. g/l for the purpose of hardening of the medium and then complete the volume to one liter and heat the medium until 91 °C for the purpose of dissolving the agar, After that doing the distribution of medium in the test tubes (100 ml) by added 10 ml per each test tube and closed the covers by cotton wool and wrapped by aluminum foil and then sterilized tubes using autoclave sterilization device at a temperature of 121 °C and 1.05 kg/cm³ pressure for 15 minutes and then left to cool the tubs to ready for planting.

Culture seeds and callus induction: seeds sterile planted beforehand, four seeds at a rate of each tube and at 10 replicates for each concentration and for each class of this process have been conducted into the air caste flow table provider by UV sterilized radiation trade by chloride and the ethanol (70%) as sterilized farm tools all of tweezers and saws which used during the culture and then incubated in a growth chamber plantings room lead to growth in the dark and at a temperature of (25 ± 2) °C four weeks after the that initial weight was calculated by callus sensitive balance was directly calculating the percentage callus induced by the equation (1):

$$\text{The percentage of induced callus (\%)} = \frac{\text{Number of seeds Induced}}{\text{Number of seeds planted}} \times 100 \quad (1)$$

Preparation a new medium then done process and the transfer of callus sub culture after one month of the first date the date of the process then prepared new medium as a free of auxin and added cytochinin (BA) with 0.5 mg/l to formation the somatic embryos and germinate then calculating the number of embryos formed, the number of embryos germinated and the percentage of embryos germinated account by the equation (2):

The percentage of embryos germinated % = $\frac{\text{The total number of embryos}}{\text{The number of embryos germinated}} \times 100$

After one month of second moved embryonic callus was transferred to the medium were then calculate the number of plantlets formed in each concentration.

3. Statistical Analysis

Used analysis Completely Randomized Design (CRD) and the experience was factorial experiment two factors are the number of varieties x concentrations of sorbitol and after obtaining the analysis of variance table has a comparison of averages using the least significant difference test average Revised Least Significant Difference Test (RLSD) and the level of 0.01.

4. Results and discussion

4.1: The effect of Caltvar and sorbitol in weight callus mild to plant rice cultivars Anber and Jasmin.

Table 4.1: Effect of cultivars and sorbitol in fresh weight callus to plant rice cultivars Anbar and Jasmin.

Caltvar	Anber	Jasim	Rate
Concentration			
0	1.2	0.97	1.08
10	0.93	0.74	0.83
20	0.81	0.51	0.66
30	0.53	0.34	0.43
Rate	0.86	0.64	

R.L.S.D at the level of 1% of the cultivar 0.036 and medium 0.027 for the central overlap 0.63.

Evidenced by the proven results in Table (1). The addition of sorbitol to the medium prepared for the induction of callus led to lower the weight of the initial callus reaching the highest weight initial callus when free treatment of sorbitol (concentration of 0%), which is 1.08 and then started to decline gradually when you added sorbitol to the medium as it stood at 0.83 g concentration of 10% and 0.66 g when the concentration of 20%, while less than 0.43 g weight stood at 30% concentration. As for the effect of cultivar has also had morally lost outweigh the Anbar cultivar to Jasmin cultivar weight in the first callus reaching 0.86 g product was at the Anbar and 0.64 g when the product was Jasmin this is due to a specific class of genetic factors. As for the interference effect was morally , reaching the highest weight for the initial treatment of callus when the overlap between the ward cultivar and medium

containing sorbitol when the focus 0%, which amounted to 1.2 gm.

4.2 The effect of cultivar and sorbitol in the percentage of callus induction to plant rice cultivars Anbar and Jasmin%.

It is seen from the results shown in Table (2) no significant difference in the percentage of induction of callus reached the highest rate of induction of callus at the Anbar, which stood at 82.5% compared with the rate of induction of callus when the product was Jasmin, which amounted to 69.5% and this is due to genetic differences between cultivars.

Table 4.2: Effect of cultivars and sorbitol in the percentage of callus induction to plant rice cultivars Anbar and Jasmine.

Caltvar	Anber	Jasim	Rate
Concentration			
0	96	87	91.5
10	89	79	84
20	76	67	71
30	69	88	63.5
Rate	82.5	69.5	

R.L.S.D at the level of 1% for the cultivar 1.61 and for the medum 1.82 for the central overlap 2.43.

As for the effect of sorbitol was also was significant in the product of the same table revealed that addition of sorbitol to the medium has led to a decline in the percentage of induction of callus was the highest percentage for the induction of callus rate was at the medium containing sorbitol focus 0% sorbitol, which amounted to 91.5% and then started to decline gradually, reaching 84% at a concentration of 10% sorbitol and 71% at a concentration of 20% sorbitol, while less for callus induction rate was at the focus of 30% sorbitol. As for the treatment of overlap may also was significant as it was the highest percentage for the induction of callus at the treatment of overlap between the variety and sorbitol when the focus 0% in, which amounted to 96 while the lowest percentage was at the treatment of overlap between the product was Jasmine and sorbitol when the concentration of 30%, which amounted to 58%.

4.3: The effect of varaiity and a sorbitol in the number of embryos formed to plant rice cultivars Anbar and Jasmin.

Showing the results shown in Table 2 cultivar surpassed Anbar morally the product was Jasmin in the number of embryos formed as the number of embryos formed when a sperm Item 231.75 a fetus as compared with the number of embryos formed when the product was Jasmin, which amounted to 189.75 fetus and this difference is due to genetic factors each cultivar.

As for the effect of sorbitol it was morally also has shown results at the same table that adding sorbitol to the medium led to a decline in the number of embryos formed has reached the lowest rate of embryos formed at the medium containing the sorbitol a concentration of 30 g./l, which amounted to 145 in the embryo while the highest rate of embryos formed when the medium non-sorbitol (concentration of 0%), which amounted to 263 fetuses.

Table 4.3: Effect of cultivars and sorbitol in the number of embryos formed to plant rice cultivars Anbar and Jasmin.

Cultivar	Anber	Jasim	Rate
Concentration			
0	292	234	263
10	268	213	240
20	211	178	194
30	156	134	145
Rate	231.75	189.75	

R.L.S.D at the level of 1% for the cultivar 6.3 and for the media 6.4 and 12.7for central overlap.

The impact of the overlap between the product and sorbitol it was significant also reaching the highest average number of embryos formed when the treatment of overlap between the ward cultivar and sorbitol concentration of 0%, which amounted to 292 fetuses while the lower rate of embryos formed when the treatment of overlap between the product was Jasmine and sorbitol a concentration of 30%, which totaled 134 embryos).

4.4: Effect of cultivars and sorbitol in the number of embryos germinating plant rice cultivars Anbar and Jasmin.

Table 4.4: Effect of cultivars and sorbitol in the number of embryos germinating plant rice cultivars Anbar and Jasmin.

Cultivar	Anber	Jasim	Rate
Concentration			
0	98	79	88.5
10	82	68	75
20	67	56	66.5
30	59	44	51.5
Rate	76	61.75	

R.L.S.D at the level of 5% of the cultivar 1.36 and for the medium 1.42 for the central overlap 2.78.

Describes the results shown in Table 4 outweigh the Anbar cultiar to Jasmin cultiar morally in the number of embryos germinated has reached the average number of embryos germinated when the Anbar Item 76 fetuses while the number of embryos germinated when the product was Jasmin 61 embryos and this goes back to the respective class genetic traits. Either raise sorbitol indicated results table that adding sorbitol to the medium led to significantly decreased in the number of embryos germinated has reached the lowest rate for the number of embryos germinated at the center containing sorbitol, a concentration of 30 compared to the number of embryos germinated when comparing the treatment of 0% sorbitol reached 88.5 embryo. The effect of overlap between the product and sorbitol have also been morally reached the highest rate of embryos germinated when the treatment of overlap between the ward cultivar and sorbitol concentration of 0%, which amounted to 93 embryos while the fewest amount of embryos germinated when the treatment of overlap between the product was Jasmin and medium teleological containing the sorbitol a concentration of 30 %, which amounted to 44 fetuses. Cultivar and sorbitol in the percentage of embryos germinating plant rice cultivars Anber and jasmin.

4.5: Effect of cultivars and sorbitol in the number of plantlets formed of the plant rice cultivars Anbar and Jasmin.

Table 4.5: Effect of cultivars and sorbitol in the number of plantlets formed of the plant rice cultivars Anbar and Jasmin.

Caltivar	Anber	Jasim	Rate
Concentration			
0	9.6	8.9	9.2
10	8.6	7.3	7.9
20	6.2	5.8	9.0
30	4.4	4.1	4.2
Rate	7.2	6.5	

R.L.S.D at the level of 1% for the cutivar 0.3 and the for the media 0.28 center and for the central overlapping 0.60

As for the effect of overlap between the product was sorbitol was morally also reached the lowest number of plantlets formed when the treatment of overlap between the product was Jasmin and medium containing sorbitol focus 30 g/l amounted to 4.1, while the highest rate was for a number plantlet formation in treatment overlap between the product Anber and medium non sorbitol amounted to 9.6 .

Sacrifice from the results set forth in Tables 6-1 that the addition of sorbitol to the medium has led to a significant decrease in callus mild weight in the percentage of callus induction and the number of embryos formed and germinated in the number of plantlets formed and this is due to the addition of the sorbitol to the medium led to a stress water in the medium and the stress water has several effects, including reducing the amount of water absorbed by the cultured tissue cells due to reduced effort osmoses of the medium as the water moves from the middle voltage high voltage osmoses to the medium of the low osmoses (El-Dawayati *et al.*, 2012) and at the low amount of water in the cellular tissue on, it affects the overall events physiological, biochemical and metabolic inside the cell, as fewer percentage of water in the cellular to increase the concentration of sodium toxic elements, chlorine, led to inhibitor of the activity of certain enzymes to assist in cellular metabolism (Tavkkoli *et al.*,) also reduced the amount of water inside the cell led to decrease of torgor pressure of the cell and thus become shrunken cells decreases in size as the a decrease in the amount of water in the cells leads to a reduction in weight and thus reduce the weight of callus formed (Qadar,1995) as the exposure of plant part to stress water reduces the amount of nutrients absorbed by the cells and thus lowers the metabolic processes and cellular this reduces the weight and callus development (Hussen *et al.*, 2015) as the stress water lead to a decline in building proteins, but increases the decomposition into amino acids in order to reduce water potential within the cellular for their water from the surrounding medium cell (Basu *et al.*, 2002) as the water stress lead to decrease of cell division process and increase the number of cells and thus lead to the growth of vegetation segment growth (Nemeth *et al.*, 2002). Exposure vegetable section to the water tension to increase the effectiveness of Oxygen Free roots may lead (ROS) and these roots play a big role in attacking the cell membranes, proteins and nucleic acids inside the cell which leads to the exposure of the plant part to tensile oxidative which negatively affects the growth of the plant part and development (Rao *et al.*, 2014) as the water affects the tensile be plantlets, development, and that its influence on the photosynthesis process as the water tensile lowers the concentration of chlorophyll in the plant cell as well as its impact on other photosynthetic pigments (Shehab *et al.*, 2010).

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