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Salinity effect on wheat *Triticum aestivum* L. callus growth and development

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Abstract

This research was conducted to study the effect of salinity on callus induction and development of two local wheat cultivars Tamoz2 and Iraq. Four different methods were used to sterilize the mature seeds. Sterilizing the seeds before and after soaking them in water with absolute Ethanol for 1 min. followed by 6% NaOCl for 10 min. showed 0% contamination and didn't affect embryos viability. However, there was no significant difference in the percentage of contamination between this method and the method in which 70% Ethanol for 1 min. followed by 4.2% NaOCl for 10 min. before and after soaking them in water. Calli were induced from mature embryos and subjected to sodium chloride of (6, 8, 12, or 14 dSm-1). The results showed that there was significant difference between the cultivars in the calli fresh and dry weights. Tamoz2 showed higher callus induction ability than Iraq. The highest weight was recorded for the control of both cultivars which was reduced significantly as the salinity level increased. However, there was no significant difference between the salinity levels 12 and 14 ds.m-1 in the effect on calli fresh weight. Also Tamoz2 showed significantly higher dry weight than Iraq cultivar. Moreover, significant effect on the dry weight was recorded for the salt levels. The highest dry weight was for 8 ds.m-1 treatment followed by the control. Interaction analysis showed that the highest dry weight was for Tamoz2 grown on 8 dS.m-1 whereas the lowest was for Iraq grown on 12 ds.m1 salinity level. In conclusion Tamoz2 was more salt tolerant than Iraq at the callus level under the condition of this experiment.

Keywords: *Mature embryo, sodium chloride, salt tolerant*

Introduction

Wheat *Triticum aestivum* L. is the most important crop which is widely distributed throughout the world and considered as the major food source for more than third the world population. Wheat growth is affected by salinity which induce sequence of physiological and biochemical changes. Salinity has negative impact on water and nutrient uptake because of osmotic and ionic imbalance. This will produce plants with reduced height, less leaves and tillers as well as reduced yield (O.Yaycili and S. A.likamanoglu, 2012). Since salinity is complicated trait and genetically controlled, plants show different response when they grown under salinity stress according to their genes content (B. Gupta and B. Huang, 2014). Tissue culture provides the best environment to study the effect of salinity on the cell growth as well as the selection of salt tolerant cell lines. A tissue culture response of wheat is influenced by genotypes, explants, the culture medium, and the interactions between them (M. T. Ozgen, et al., 1996, L. Benderradji, et al., 2012). Both mature and immature wheat embryos have been utilized as explants in culture protocols, however mature embryos are available throughout the year thus they have been the preferred choice for callus induction (M. T. Ozgen, et al., 1998). Therefore the objective of this research is to study the effect of salinity on callus induction and development of two local wheat cultivars Tamoz2 and Iraq reflected by the fresh and dry weight of the callus grown under salt stress conditions.

Material and Methods

This study was conducted in the Plant Tissue Culture Laboratories of Genetic Engineering Department of Ministry of Science and Technology. Mature seeds of the two local wheat cultivars (Iraq and Tamoz2) were surface sterilized by four different methods (Table 1).

Mature embryos were excised from the surface sterilized mature seeds and cultured on MS medium (T. Murashige and F. Skoog, **1962)** supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} and 2 mg L g L^{-1} 2,4-D for callus induction. A total of 15 embryos per Petri dish were cultured and 10 replications for each treatment. Contamination percentages were calculated after two weeks. Induced calli were subjected to salt stress in MS medium supplemented with sodium chloride of (6, 8, 12, or 14 dSm^{-1}) as a salt stress factor.

Table 1: Steps of the four surface sterilizing methods

Treatment	Soak in H2O	Ethanol	Wash with H2O	NaOCl	Wash with H2O
Method 1	(1)24 h	(2)Absolute 1min.	(**3**)1 min.	(**4**) 6% for 15 min.	(**5**) 3 times 5 min. each
Method 2	(5)24 h	(1)Absolute 2 min.	(2)1 min.	(3) 6% for 15 min.	(4) 3 times 5 min. each
Method 3	(5)24 h	(1) 70% 1 min. (6) 70% 1 min.	(2)1 min. (7)1 min.	(3) 4.2% for 10 min. (8) 4.2% for 10 min.	(4) 3 times 5 min. each (9) 3 times 5 min. each
Method 4	(5)24 h	(1)Absolute 1 min. (6)Absolute 1 min.	(2)1min. (7)1 min.	(3) 6% for 10 min. (8)6% for 10min.	(4) 3 times 5 min. each (9) 3 times 5 min. each

Table 2: The effect of salinity on callus fresh weight (g) of two wheat cultivars (Iraq and Tamoz2)

	Salinity levels ($ds.m^{-1}$)				
Cultivars	6	8	**12**	**14**	Mean
Iraq	**0.104**	**0.0640**	**0.0372**	**0.052**	**0.064**
Tamoz2	**0.204**	**0.185**	**0.0580**	**0.052**	**0.125**
Mean	**0.154**	**0.124**	**0.047**	**0.052**	**0.094**
LSD 5%	Salinity=**0.025** Interaction=**0.026** Cultivars=**0.018**				

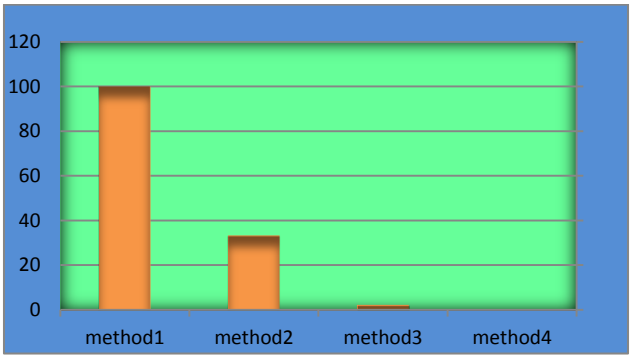

Fig.1 The effect of seeds sterilization methods on the contamination percentage of callus induced from mature embryos of wheat cultivars (Iraq and Tamoz2)

Calli fresh weight were recorded before culture and the effects of salinity on callus growth and development was estimated by measuring fresh and dry weights of the calli which were grown under salt stress *In vitro* for 6 weeks. All cultures were kept in the dark in the culture room at 23 $\pm$ 2 ^{0}. The experiments were arranged in CRD with 10 replications per treatment and the means were compared by LSD at 5%.

Results

The results showed that the sterilization method has great effect on the contamination percentage of the culture (Figure 1). The contamination percentage was 100% when the seeds were soaked before sterilization (method 1). While sterilizing the seeds before and after soaking them with absolute ethanol and 6% NaOCl showed 0% contamination (method 4). However, there was no significant difference between method 4 and method 3 in the percentage of contamination. Methods 3 and 4 were very effective and the embryo viability of the two cultivars was not reduced.

Calli were induced successfully from free mature embryos of both cultivars in the presence of 2,4-D. The results also showed that the induced calli responded differently on the salt stress culture that shown by the significant difference between the cultivars in the calli fresh weights (Table 2). Tamoz2 showed higher callus induction ability (0.125 g) than Iraq (0.064 g) after 4 weeks in culture.

Moreover, there were significant effects for the salinity levels on the average calli fresh weights of both cultivars. The highest was recorded for the control (0.154 g) for both cultivars which was reduced significantly as the salinity level increased. However, there was no significant difference between the salinity levels 12 and 14 $ds.m^{-1}$ in the affect on calli fresh weight.

The interaction analysis showed that there were significant effects for the interaction between the cultivars and the salinity levels on the fresh weight. Tamoz2 at the control level surpassed all other treatments with an average weight of 0.204 g while the lowest was 0.0372 g which was recorded for Iraq at 12 $ds.m^{-1}$.

Table 2: The effect of salinity on callus dry weight (g) of two wheat cultivars (Iraq and Tamoz2)

	Salinity levels ($ds.m^{-1}$)				
Cultivars	6	8	**12**	**14**	Mean
Iraq	**0.0148**	**0.0124**	**0.0063**	**0.0087**	**0.0105**
Tamoz2	**0.0299**	**0.0354**	**0.0081**	**0.0092**	**0.0207**
Mean	**0.0224**	**0.0239**	**0.0072**	**0.0087**	
LSD 5%	Salinity=**0.0084** Interaction= **0.0059** Cultivar = **0.0042**				

Callus dry weight of the two cultivars was also affected by salinity (Table 2). Tamoz2 showed the highest dry weight (0.0207 g) which was significantly different than that of Iraq cultivar (0.0105 g). Moreover, significant effect on the dry weight was recorded for the salt levels. The highest dry weight was (0.0239 g) for 8 $ds.m^{-1}$ treatment followed by the control. The interaction analysis showed that the highest dry weight was (**0.0354** g) for Tamoz2 grown on 8 $ds.m^{-1}$ whereas the lowest was (**0.0063** g) for Iraq grown on 12 $ds.m^{1}$ salinity level.

Discussions

The success of tissue culture is started by the establishment of sterilized culture. Soaking the seeds before sterilization increased the contamination percentage. The contaminants on the surface of the seeds were dispersed inside the seeds during the soaking step. Therefore it is important to sterilize the seeds before and after soaking in water. Sodium hypochlorite is strong sterilizing agent which reacts with H2O and releases [O] in many steps as in the following reactions:

Cl2+H2O=HCl+HOCl
HOCl = HCl + [O]

The released [O] is responsible for the oxidation power of NaOCl (K. G. Ramawat, 2004).

The sterilization had no effect on the embryo's viability and calli were induced from the mature embryos of both cultivars. Wheat mature embryos are available around the year compared with the immature embryos which are season dependant. Mature embryos have been used by many researchers as wheat explants for successful callus induction (S. S. Parmar et al., 2012, H. Turhan and I. Baser, 2004). On the other hand, the induced calli of the two cultivars responded differently under salt stress condition. Salt tolerant is genetically controlled trait. Thus the response of each cultivar depend on it's genetic contain.

The reduction in the calli fresh and dry weights of both cultivars was occurred because of the ions imbalances inside the cells. Accumulations of sodium and chloride ions in the cells will reduce the available water and induce osmotic stress which has negative effects on cell growth (C. Ghoulam, et al., 2002). Water absorption and ions uptake will consume most of the energy available for the cell. Thus cell division and growth of the calli will be reduced as the salinity level increased. This was found by D. W. Rains, *et al*., (1980) when they studied rice callus development under salt stress condition. They attributed the reduction in the fresh weight to the reduction of water availability in the cells. The same result was found when two rice cultivars were subjected to different sodium chloride concentrations (S. H. Wani, *et al*., 2010). Potato callus grown on salt stress medium showed reduction in the fresh and dry weights as the salinity level increased (F. Javed, 2002).

The results of the present experiment are in agreement with the results of other researchers on other crops. V. Nikman *et al*. (2006) found that calli dry and fresh weights of two *Trigonella* species were affected by salinity and they were reduced as the salinity increased. The same results were found when the tobacco leaves calli were subjected to salinity stress (V. Nikman, *et al*., 2004).

Although callus growth was reduced under salinity stress, the cells which survived the high salinity levels are promising cell lines for the regeneration of salt tolerant wheat plants.

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