

## **GENOTYPIC RESPONSE TO *Fusarium culmorum* TOXIN ON SOME EGYPTIAN WHEAT GENOTYPES DURING *IN VITRO* CULTURE**

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### **ABSTRACT**

This work deals with the effect of *Fusarium culmorum* toxin on the induction of callus, regeneration and root induction during tissue culture of mature embryos of four Egyptian wheat (*Triticum aestivum*) genotypes, i.e. Sids 1, Sids 8, Sids 9 and Giza 167. The two concentrations of *F. culmorum* toxic filtrate (10% and 20%) in addition to the control were applied to treat mature embryos and their calli. All genotypes; turned to be very sensitive to the toxic filtrate, but it was found that the level of sensitivity depends on genotype. The four genotypes differed from one another significantly in their ability to induce root formation, the highest response was observed in Giza 167. But, there were no significant differences due to the toxic medium and toxic medium X genotype interaction. It was concluded that the highest ability of root induction (in the presence of toxic filtrate) and the highest percentage of survival calli were encountered for Giza 167, and the lowest values were obtained for Sids 1. Whereas, the percentage of callus induction (in the presence of toxic filtrate) was determined in the highest values for Sids1.

### **INTRODUCTION**

Plant improvement has been further accelerated by biotechnological tools of gene transfer, to engineer new traits into plants that are very difficult to introduce by traditional breeding. The successful deployment of transgenic approaches to combat insect pests and diseases of important crops like rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and cotton (*Gossypium hirsutum* L.) is a remarkable accomplishment (Jauhar, 2006).

Among biotechnological approaches, tissue culture generates a wide range of genetic variation in plant species which can be incorporated in plant breeding programmes. By *in vitro* selection, mutants with useful agronomic traits, e.g. salt or drought tolerance or disease resistance, can be isolated in a short duration. The successful use of somaclonal variation is very much dependent on its genetic stability in the subsequent generations for which molecular markers such as RAPDs, AFLPs, SSRs and others can be helpful. The potential of somaclonal variation has yet to be fully exploited by breeders, even though a few varieties have been developed in crops such as *Brassica juncea*, rice and others (Jain, 2001).

Plant tissue culture, in recent years, has attracted growing interest from plant pathologists and breeders aiming for disease resistance, since it could meet the requirements for screening a large number of individuals in a small space. Large-scale screening of cell populations using *in vitro* procedures successfully produced resistant plants against pathotoxins (Wenzel, 1985).

Tissue culture systems might also be useful for understanding of the mode of action of toxins, and the resistance responses and physiological

changes occurring in plant cells infected with fungal and bacterial pathogens (Daub, 1986).

Bajaj and Saettler, (1968) initiated studies to understand the effect of pathotoxins on tissue cultures and to select toxin tolerant/resistant cell lines. and significant progress has now been established.

Genotype and callus induction medium played a dominant role in plantlet regeneration. These results will facilitate genetic transformation work with durum wheat (*Triticum turgidum* L.) (Satyavathi *et al.* 2004).

This work was carried out to study the effects of *Fusarium culmorum* toxin filtrate on the induction of callus, regeneration and root induction during tissue culture of mature embryos of four Egyptian wheat (*Triticum aestivum*) genotypes, i.e. Sids 1, Sids 8, Sids 9 and Giza 167.

## **MATERIALS AND METHODS**

### **Wheat varieties :-**

Four varieties of wheat (*Triticum aestivum* L), namely Sids 1, Sids 8, Sids 9 and Giza 167, were used in the present study. The seeds of these varieties were obtained from the Nobaria Research Center, Ministry of Agriculture, Egypt.

### **Fungal strains :-**

One pure culture (isolate) of *Fusarium culmorum* was employed in this study. The isolate was obtained from the Department of Plant Pathology, Faculty of Agriculture, Alexandria University.

### **Establishment of wheat calli :-**

For the initiation of wheat callus cultures, mature embryos of three varieties; Sids 6, Sids 8 and Sids 9 were used as explants in the preliminary study, and the four varieties; Sids 1, Sids 8, Sids 9 and Giza 167 were used along the rest of the experiments. The mature seeds were surface sterilized as suggested by Barakat (1996), as follows: -

- 1- The mature seeds were rinsed in distilled water. Thereafter, all steps were carried out in laminar airflow cabinet (FELL CLEAN AIR (1971) LIMITED).
- 2- Clean seeds were soaked in sterilized distilled water for 24 hours (to facilitate the excision of the embryos from the seeds).
- 3- The seeds were surface sterilized by immersing them for 1 minute in 95% ethanol, followed by immersion in 0.1% mercuric chloride for 15 minutes.
- 4- The seeds were then washed with six changes of sterilized distilled water.
- 5- The seeds were transferred to sterilized filter paper till usage.

Mature embryos were aseptically excised and placed in petri dishes (10 cm in diameters) containing 20ml of culture medium as shown in figure (1). The cultures were incubated at  $25 \pm 2$  C° under 16 hours illumination (2000 lux day light fluorescent tubes).

### **Culture Media:-**

Two media protocols were used for calli induction. For the first protocol (A), the culture medium contained the inorganic salts of Murashige and Skoog (1962), supplemented with 150 mg/L. asparagine, 0.5 mg/L thiamine, 1.0 mg/L 2, 4 – D, 20 g/L sucrose and 7.0 g/L agar (Barakat, 1996). For the second protocol (B), the culture medium contained the

inorganic salts of Murashige and Skoog (1962) supplemented with 100 mg/L inositol, 0.5 mg nicotinic acid, 0.5-mg/L pyridoxine-HCL, and 0.1-mg/L thiamin- HCL, 0.1 mg/L kinetin, 1.0 mg/L 2, 4-D, 30 g/L sucrose and 7 g/L agar (Barakat and Abdel-Latif, 1995). In this preliminary study, the first protocol (A) was applied using four concentrations of 2, 4-D, i.e. 0, 1, 2, 3 and 4 mg/L. The second protocol (B) was applied using the same previous concentrations of 2, 4-D.

After preparing the final media, pH was adjusted up to 5.8. Agar was added then the media was sterilized by autoclaving at 121°C for 20 minutes.

**Calli evaluation:-**

Three traits; callus size, number of induced callus and rhizoid formation were determined after one week of culturing in the preliminary study, for three wheat genotypes (Sids 6, Sids 8, and Sids 9).

Split-plot experiment was conducted, three genotypes, five 2,4-D levels and two types of medium protocols (30 treatments were evaluated). Data were analyzed by split-split-plot design. Four replications were used for each treatment. Data were represented as means and compared by Least Significant Difference "L.S.D." (Snedecor and Cochran, 1967). All data except the callus size were subjected to an angular transformation according to Steel and Torrie (1960).

The traits; number of induced callus, weight callus were determined after five weeks of culturing, the third trait (number of calli with shoot) was determined after four weeks of transfer the calli to regeneration medium. Protocol A supplemented with 1 mg/L 2,4-D four callus induction was used for four wheat genotypes (Sids 1, Sids 8, Sids 9 and Giza 167). Regeneration medium for the previous genotypes was the same as callus induction medium except that 2,4-D was 0.1 mg/L instead of 1mg/L 2,4-D. The percentage of callus induction, shoot formation and means of callus weight for the previous genotypes were recorded.

Data were analyzed by randomized complete block design (4 treatments were evaluated). Six replications were conducted for each treatment. Data were calculated in means and compared by Least Significant Difference "L.S.D." (Snedecor and Cochran, 1967). Shoot formation data were subjected to an angular transformation ( Steel and Torrie, 1960).

**Preparation of *Fusarium Culmorum* Toxic Filtrate :-**

To prepare enough quantities of *F. culmorum* toxin filtrate, the isolate were grown in 250 ml flasks, each contains 100 ml of potato dextrose medium (P.D), according to Shahin and Shepard (1979) method. The flasks were autoclaved, then inoculated with 5mm P.D. discs of *F. culmorum* isolate which was taken from margin of two weeks old cultures. The inoculated flasks were incubated at 25°C for 4 weeks. Next, the upper layer of the fungus was removed. The filtrates were collected through Whatman No .1 filter paper, followed by filtration through sterilized bacterial filter ( Seitz filter) before using.

**Determination of phytotoxicity of the toxic filtrate on callus induction :-**

To determine the phytotoxicity of the toxin filtrate on callus induction, each mature embryo of the four varieties; Sids 1, Sids 9, Sids 8, and Giza 167 were cultured on medium according to protocol A. This medium was

supplemented with two concentrations of sterilized toxin preparations from culture filtrate, i.e. 10% (B) and 20% (C). In addition, the non-toxic control cultures were used without the addition of sterilized toxin filtrate (12 treatments were used, with six replications for each treatment). Cultures were incubated at 25°C under 16 hours illumination and 8 hours darkness. The percentage of mature embryos, which had callused after five weeks on initiation medium, was recorded.

**Determination of phytotoxicity of the filtrate on callus regeneration :-**

To study the phytotoxicity of the toxin filtrate on callus regeneration, calli of the four varieties; Sids 1, Sids 9, Sids 8 and Giza 167 (five weeks in age) were transferred to culture medium which was the same as the culture medium in protocol (A) except that 2, 4-D 0.1 mg/L was used instead of 1.0 mg/L (Barakat, 1996). This medium was supplemented with two concentrations of sterilized toxin preparations from culture filtrate, i.e. 10% (B) and 20% (C) from the total volume of the medium. In addition, the non-toxic control cultures were used without the addition of sterilized toxin filtrate (A) (12 treatments were used, six replications for each treatment). The cultures were incubated at 25°C under 16 hours illumination and 8 hours darkness. The percentage of shoot formation was recorded after four weeks on regeneration medium.

**Determination of phytotoxicity of the filtrate on root induction:-**

The effects of phytotoxicity of the toxin filtrate on root induction were determined for the calli of the four varieties, Sids 1, Sids 9, Sids 8, and Giza 167 which formed shoots during callus initiation. They were transferred to root induction medium contained the inorganic salts of Murashige and Skoog (1962) supplemented with 2 mg/L glycine, 100 mg/L inositol, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine-HCl, 0.1 mg/L thiamine-HCl, 30 g/L sucrose and 8 g/L agar (Barakat and Abdel-Latif, 1995). This medium was supplemented with two concentrations of sterilized toxin preparations, i.e. B and C. In addition, the non-toxic control cultures were used without the addition of sterilized toxin filtrate. Twelve treatments were used, six replications for each treatment. Cultures were incubated at 25°C under 16 hours illumination and 8 hours darkness. The percentage of root induction was recorded after four weeks on root induction medium.

Data were analyzed as factorial arrangement of genotypes and four different concentrations of toxic filtrate (12 treatments used ) in randomized block design with six replications (Snedecor and Cochran, 1967). Data were subjected to an angular transformation (Steel and Torrie, 1960).

**Treatment of callus by toxic filtrate :-**

Mature embryos of the four varieties; Sids 1, Sids 9, Sids 8, and Giza 167 were cultured on medium. This medium was supplemented with two concentrations of sterilized toxin preparations from culture filtrate, i.e. 10% (B) and 20% (C). In addition, the non-toxic control cultures were used without the addition of sterilized toxin filtrate (12 treatments were used, with six replications for each treatment). Cultures were incubated at 25°C under 16 hours illumination and 8 hours darkness. Calli of the four varieties; Sids 1, Sids 9, Sids 8 and Giza 167 (five weeks in age) were, also, transferred to culture medium which was the same as the culture medium supplemented

with the same two concentrations of sterilized toxin preparations from culture filtrate used before for callus initiation stage, i.e. 10% (B) and 20% (C) from the total volume of the medium in addition to control A and were incubated at the same conditions of temperature and light.

## RESULTS AND DISCUSSION

### A. Optimization of 2,4-D concentration and growth media:

Table (1) shows the analysis of variance for callus size, number of induced callus for mature embryo explants, and rhizoid formation for callus of the three wheat genotypes used in the preliminary study. Highly significant differences were obtained for callus size and the number of induced callus from the three wheat genotypes and the variance due to medium protocol used, the concentration of 2,4-D, the interaction between the medium protocol and the concentration of 2,4-D, the interaction between medium protocol and the genotypes, the interaction between the concentration of 2,4-D and the genotypes and the interaction among the three previous factors turned to be also highly significant. On the other hand, highly significant differences were obtained for rhizoid formation for the three genotypes, the variance due to the concentrations of 2,4-D, and the interaction between them. But the variances due to the other factors were not significant. From these results, it was concluded that the genotypes responded differently to the different concentrations and combinations of 2,4-D and medium protocol. There is no general trend of increase or decrease of the three previous characters for the three genotypes in relation to the increase or decrease of 2,4-D. This result was nearly similar to the result which was obtained by Mohammed, (1997) on tomato genotypes through tissue culture techniques. The best combination of the type of medium protocol and the concentration of 2,4-D was found to be protocol A (which depends on using amino acids) and was supplemented with 1 mg/L 2,4-D for all previous genotypes concerning callus size. These results are in agreement with those of Maddock *et al.* (1983), who reported that 1 mg/L of 2,4-D was the optimum concentration for wheat callus growth. However, Ozias-Akins and Vasil (1983 a and b) reported that the optimum concentration of 2,4-D for callus growth was 2 mg/L. Also these data support the results obtained using protocol A in the present study, since it depend on using amino acids, as observed by Ouedraogo *et al.* (1998) who found that the amino acid-supplemented medium was the best for embryogenesis and regeneration and reduced the percentage of albino plants in growth cabinet.

On the other hand, the combination of the concentration of 2,4-D and the medium protocol, that gave the highest value for the number of induced callus and the values of rhizoid formation ability, was not the same for the different genotypes.

Finally, this study showed that the genotype effects were highly significant. This in partial agreement with the results obtained by Maes *et al.* (1996) who reported that the relative importance of genotype effect prevailed over all factors. In this study, not only genotype effects were proved to be significant, but also medium effect and medium X genotype effects were

highly significant. These findings agreed partially with that mentioned by Quimuo and Zapata (1990), and that reported by Barakat and Abdel-Latif (1995) who found that the callus induction and callus weight were highly significantly influenced by differences in genotypes, medium protocols and explants. All the two way interactions and the three way interaction were highly significant. Moreover, Bayliss and Dunn (1979) showed that callus proliferation from mature embryos of different barley genotypes varies with culture medium.

Another experiment was carried out to determine callus induction ability, callus weight and shoot formation ability for four wheat genotypes; Giza 167, Sids 8, Sids 9 and Sids 1. Protocol A with 1 mg/L 2,4-D to induce callus formation, and with 0.1 mg/L 2,4-D to induce shoot formation. The results of callus induction and callus weight were taken after five weeks of culturing (Figure 1).

The above results suggest that, all previous differences of morphogenesis responses which appeared among the four wheat genotypes, calli of four can be attributed to genotype effect. Mathias and Fukui (1986) found that the lines of hexaploid wheat differ in their initial growth rates and their ability to organize shoot primordia and regenerate shoot. The 4 B chromosomes had very significant effect on all these characters. Also, Felsenburg *et al.* (1987) found that there is a correlation between the presence or absence of specific chromosomes and chromosomal arms of the B genome of common wheat and the growth and differentiation capabilities of calli. Kaleikau *et al.* (1989a) indicated that the group 2 chromosomes of wheat (*Triticum aestivum*), in particular chromosome 2 D, posses genetic factor promoting callus growth and regeneration. Kaleikau *et al.* (1989b) suggested that major tissue culture response gene is located on 2 DL and that 2 AL and 2 BS posses minor tissue culture response genes. Ben Amer *et al.* (1995) found that chromosomes 2 B and 6D are critical for tissue culture response of wheat. Whereas, chromosome 1 D affects callus weight only.

**Table (1): Analysis of variance and coefficient of variation for callus size, No. of induced callus and rhizoid formation for the three wheat genotypes**

Source	D.F	Callus size*		No. of induced callus		Rhizoid formation	
		Mean square	F-value	Mean square	F-value	Mean square	F-value
Medium protocol	1	0.328	184.3**	0.589	1291**	0.003	0.8109
Error	3	0.002		0.000		0.003	
Concentrations of 2,4D	3	0.191	501.1**	0.321	768**	0.028	10.8**
Medium X Conc.	3	0.138	360.2**	0.257	614**	0.002	0.67
Error	18	0.000		0.000		0.003	
Variety	2	0.395	444.3**	0.150	181**	0.061	19.2**
Medium X Varsity	2	0.026	29.33**	0.029	35.5**	0.005	1.43
Conc. X Varsity	6	0.010	11.61**	0.010	12.5**	0.010	3.07**
MediumIXConc.XVarsity	6	0.011	12.37**	0.010	12.2**	0.001	0.37
Error	48	0.001		0.0001		0.003	
<b>Coefficient of variation</b>		10.21%		2.58%		7.50%	



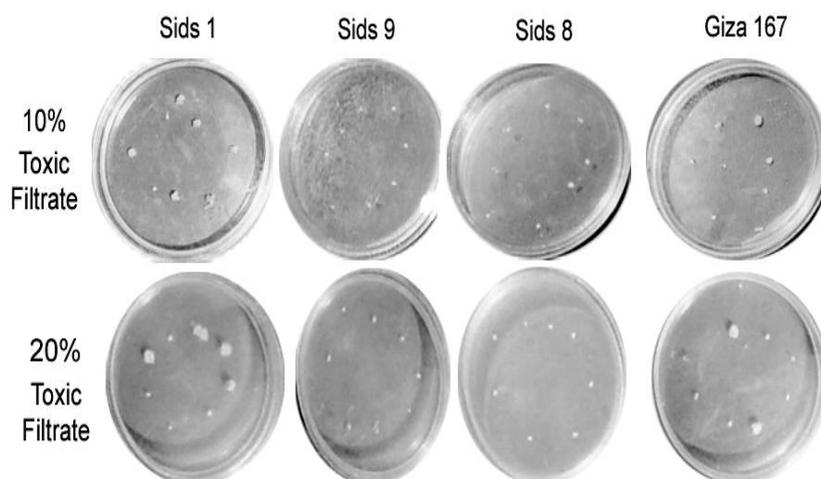
**Figure (1):** Morphogenesis response of mature embryos of four wheat genotypes on callus induction medium after five weeks.

**B- The effect of toxic filtrate of *Fusarium culmorum* on callus induction**

Table (2) shows the effect of two concentrations of toxic filtrate i.e. 10% (B) and 20% (C) on callus induction, after five weeks of culturing the mature embryos on toxic callus induction medium of protocol A with 1 mg/L 2,4-D. From Table (2), there were differences among the four genotypes in their response for callus formation. It was observed that, all tested genotypes turned to be very sensitive to the toxic filtrate, but the level of sensitivity depends on genotype. *Fusarium culmorum* culture filtrate (two concentrations) inhibited callus induction for two genotypes; Sids 8 and Sids 9 (Figures 2). This result was in agreement with that found by Lijuan *et al.* (1991) who observed that *F. graminearum* culture filtrate inhibited the induction of callus.

**Table (2):** The effect of different concentrations of toxic filtrate on the induction of calli from mature embryos of wheat genotypes.

Toxin concentration %	Callus induction (%) of wheat genotypes			
	Giza167	Sids 8	Sids 9	Sids 1
0	100	100	100	100
10	15	-	-	48.75
20	8.7	-	-	33.75



**Figure (2): Morphogenesis differences of callus induction between four wheat genotypes after four weeks of treatment by 10% and 20% *F. culmorum* toxic filtrate.**

On the other hand, it was observed that Giza 167 and Sids I had small size callus, induced on medium which contained toxic filtrate. Whereas, the ability of Sids 1 to induce callus on the toxic medium was higher than that of Giza 167. The percentage of callus induction for Giza 167 and Sids 1 decreased in relation to increased concentration of the toxic filtrate (Table 2 and Figures 2).

#### **C- The effect of toxic filtrate of *Fusarium culmorum* on regeneration**

Table (3) shows the effect of two concentrations of toxic filtrate i.e.10%. (B) and 20% (C) on callus regeneration, after four weeks of transfer calli (5 weeks age) obtained from callus induction culture after five weeks) on toxic regeneration medium.

From Table (3), it seems that the control and the calli of two genotypes, Giza 167 and Sids 8 did not regenerate, completely (Figure 3). Whereas, 33.3% and 9% of calli of Sids 9 and Sids I regenerated respectively (induced shoot formation), as shown in Figures 9 and 10.

It was found (on the toxic regeneration medium) that the minority of calli of Giza 167 turned brown and died, the ratio of brown died calli increased in Sids 8 and Sids 9 (Table 3 and Figure 3), respectively. But, in Sids 1, the majority of calli to be turned brown and then died (Figure 3). It was clear that the calli of Sids 1 was very sensitive to the toxic filtrate in relation to the other genotypes. It was observed that an increase in toxic concentration from 10% to 20% in the medium caused increase in the number of brown and died calli.

The previous results agreed with that indicated by Ahmed *et al.* (1996) who reported that culture filtrate of *F. graminearum* in tissue culture after 4 weeks of contact caused that, the majority of calli turned brown and died, inhibition of growth and decay of calli.

It can be also seen (Table 3), that the culture filtrate of *F. culmorum* in the regeneration medium (10% or 20%) inhibited callus regeneration completely in two genotypes; Sids 9 (Figure 3) and Sids 1 (Figure 3), which formed shoots on the control regeneration medium. The results were in agreement with that indicated by Ahmed *et al.* (1996) who found that culture filtrate of *F. graminearum* in wheat regeneration medium decreased the regeneration ability or was completely lost, some varieties developed roots only on the regeneration medium. Also, Menke-Milczarek and Zimmy (1991) observed an increase in toxic concentration in the medium and this caused a decrease in regeneration ability of the calli.

**Table (3): The influence of different concentrations of toxic filtrate on regeneration of calli derived from four wheat genotypes.**

Toxin concentration %	wheat genotypes							
	Giza167		Sids 8		Sids 9		Sids 1	
	R.C	S.C	R.C	S.C	R.C	S.C	R.C	S.C
0	-	100	-	100	33.3	100	9	100
10	-	100	-	90	-	78.7	-	17.8
20	-	91.7	-	83.4	-	70	-	16.6

R.C.= Percentage of regenerated callus

S.C.= Percentage of surviving callus

#### **D- The effect of toxic filtrate on root induction**

Table (4) shows the effect of two concentrations of toxic filtrate i.e. 10% (B) and 20% (C) on root induction, after four weeks of transfer calli with shoots (which obtained from callus induction culture after five weeks) on the toxic root induction medium. The highest percentage of root induction, on the control root induction medium, was observed in Giza 167, which was 100% (Figure 4). On the other hand, the lowest percentage was observed in Sids 9, which was 30%. Whereas, Sids 8 had a good ability in root induction but it was lower than the ability of Giza 167. Finally, Sids 1 had no ability to form roots on this medium (Figure 4).

There were no differences between the control root induction medium and the toxic root induction medium (10% toxic filtrate from total volume) for two genotypes; Giza 167 and Sids 9 (Figure 4). In Sids 8, on the toxic medium, the percentage of root induction was reduced, in comparison to the control medium (Figure 4). The root induction ability of the genotypes can be arranged according to their percentage of root induction in the following rank; Giza 167, Sids 8 and Sids 9.

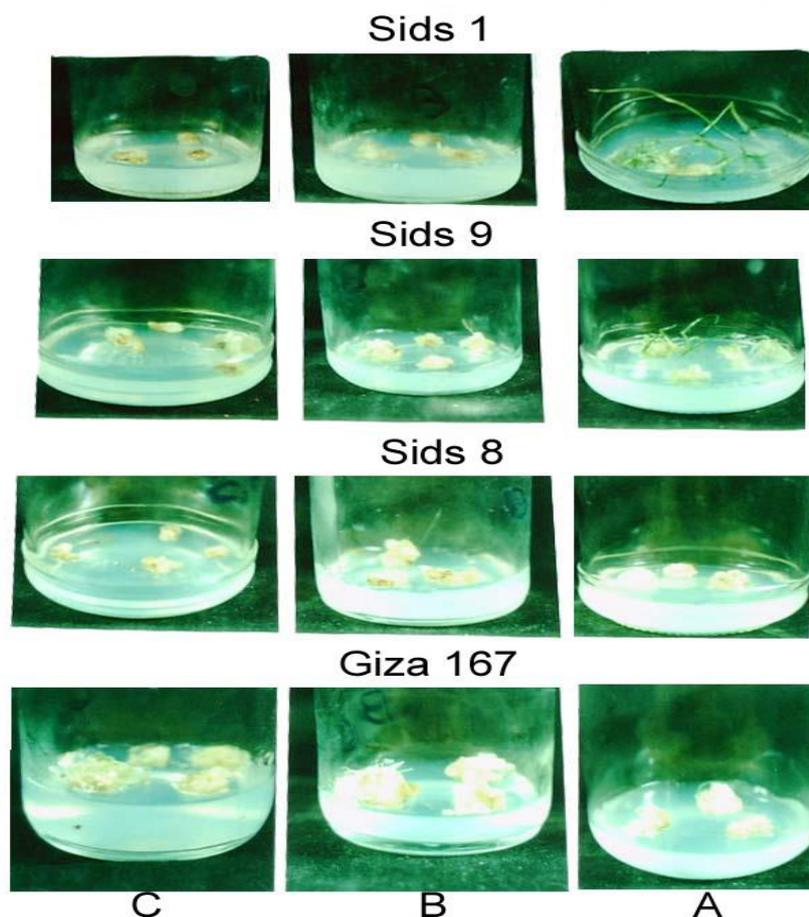


Figure (3): The effect of *F. culmorum* toxic filtrate concentrations (A=control, B=10% and C=20%) on the regeneration of callus of four wheat genotypes after four weeks of calli treatment.

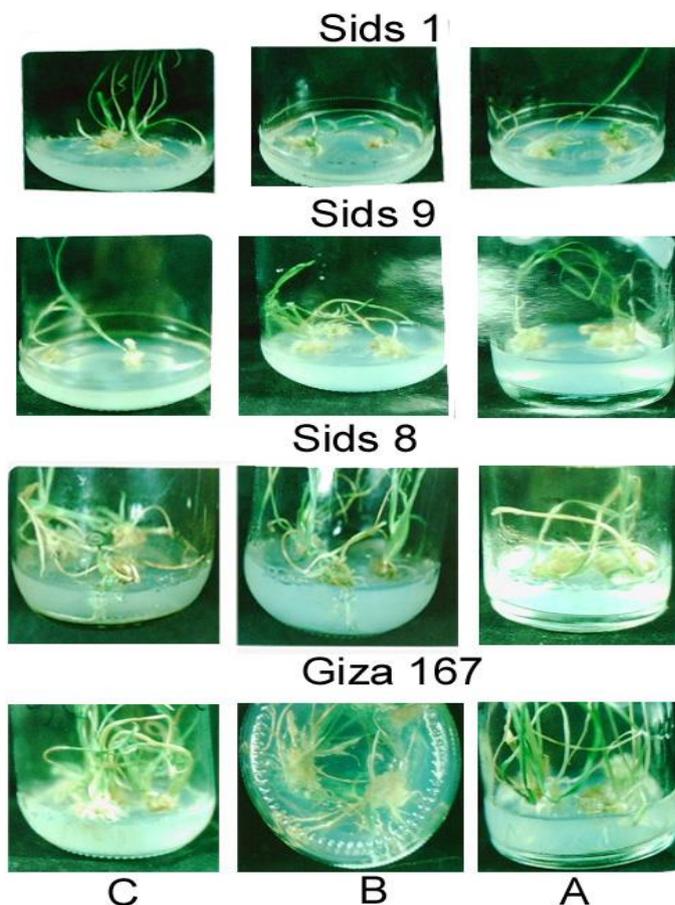
Table (4): The effect of different concentrations of toxic filtrate on the induction of calli from mature embryos of wheat genotypes.

Toxin concentration %	wheat genotypes			
	Giza167 (R.I%)	Sids 8 (R.I%)	Sids 9 (R.I%)	Sids 1 (R.I%)
0	100	80	30	-
10	100	75	30	-
20	93.3	80	-	-

(R.I%)= The Percentage of root induction

On toxic root induction medium which contained 20% toxic filtrate from total volume, it was observed that the percentage of root induction of Giza 167 was reduced, in comparison to the control medium (Figure 4). While, in Sids 8, there were no differences between the control root induction medium and toxic one, and was increased in comparison to 10% toxic

medium (Figure 4). While in Sids 9, root induction ability was lost completely (Figure 4). The root induction ability of the genotypes can be arranged according to their percentage of root induction in the following descending order, Giza 167, Sids 8 and Sids 9.



**Figure (4):** The effect of *F. culmorum* toxic filtrate concentrations (A=control, B=10% and C=20%) on root induction of four wheat genotypes after four weeks of treatment for regenerated calli.

Table (5) represents the values of the analysis of variance for root induction for calli with shoots for the four wheat genotypes, which were transferred on toxic and nontoxic root induction media. Highly significant differences were obtained for root induction for the four wheat genotypes. But, the variance due to the different concentration of the toxic filtrate, and the interaction between the genotypes effect and the toxic filtrate (toxic medium) effect were not significant. These results agreed partially with the results which were obtained by Bruins *et al.* (1993) who observed that the

genotype and the medium (which contains DON) effects were significant on seedlings root growth. But, there were no genotype X medium interactions.

The results from Table (6) show that mean values of root induction for each genotype did not differ significantly, except for the mean value which was obtained from the 20% toxic root induction medium for Sids 9. This value differed significantly from two other values for Sids 9. This may be attributed to the non-toxic effects of the toxic filtrate on root induction, it seems that Sids 9 is more sensitive to the toxic filtrate than the other genotypes. These results were partially similar to those found by Ahmed *et al.* (1996) who reported that *F. graminearum* toxic filtrate decreased the regeneration (Shoots and roots) ability or was completely lost, some varieties developed roots only on the regeneration medium.

**Table (6): Means of root induction (%) of four wheat genotypes.**

Genotype	Toxin concentration %		
	0	10	20
Giza 167	10.02a	10.02a	9.72ab
Sids 8	8.97ab	8.66b	8.97ab
Sids 9	3.91c	3.9c	0.7d
Sids 1	0.7d	0.7d	0.7d

Data were subjected to an angular transformation (Steel and torrie, 1960).

Values within a column followed by the same letter(s) are not significantly different at the  $p=0.05$  level according to the Least Significant Differences (Snedecor and Cochran, 1967)

**Table (5) : Analysis of variance for root induction ability of calli with shoots of four wheat genotypes.**

S.O.V	D.F	Sum of squares	Mean of squares	F-value
Genotype	3	1097.37	365.79	156.32**
Toxic medium	2	11.37	5.68	2.43
GenotypeX Toxic medium	6	30.58	5.1	2.18
Error	55	128.75	2.37	

Data were subjected to an angular transformation (Steel and torrie, 1960).

\*\*Highly significant at  $p<0.01$

Finally, the four wheat genotypes can be arranged according to the percentage of survival callus and the percentage of root induction on the toxic medium in the following rank; Giza 167, Sids 8, Sids 9 and Sids 1. Contrary to the highest percentage of survival callus and root induction was that observed in Giza 167, the highest percentage of callus induction (obtained from toxic medium) was observed in Sids 1.

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### أستجابة التراكيب الوراثية لتأثير سم فطر *Fusarium culmorum* فى بعض أصناف القمح المصرية خلال مراحل زراعة الانسجة م. م. ياقوت وم. أ. السهريجي وم. م. الحداد وه. م. شكيم قسم الوراثة - كلية الزراعة - جامعة الاسكندرية

يدرس هذا البحث أستجابة التراكيب الوراثية لسم المسبب المرضى *Fusarium culmorum* فى أربعة أصناف قمح (*Triticum aestivum*) مصرية هى سيدس ١ وسيدس ٨ وسيدس ٩ وجيزه ١٦٨ باستخدام تقنية زراعة الانسجة. تضمن البحث دراسة تأثير تركيزين من الراشح السام للفطر (١٠% و ٢٠%) على أستحثات تكوين الكالس وإعادة التشكل (تكوين أوراق) وعلى أستحثات تكوين الجذور. أظهرت النتائج أن الأربعة أصناف سيدس ١ وسيدس ٨ وسيدس ٩ وجيزه ١٦٨ ذات حساسيه شديده للراشح الفطرى السام بتركيزيه بالنسبه لإستحثات تكوين الكالس ولكن كان ذلك بدرجات متفاوتة. كما وجد أن الأربعة أصناف السابقة تختلف عن بعضها معنوياً فى قابليتها لتكوين الجذور على بيئه أستحثات تكوين الجذور. وأعلى قابليه لتكوين الجذور تم الحصول عليها فى جيزه ١٦٨. ولكن لم تكن هناك اختلافات معنوية راجعة للبيئه السامة أو للتفاعل الناتج بين البيئه السامة والتراكيب الوراثي. يمكن أنه بالنسبه للقدرة على تكوين الجذور وأيضاً النسبة المنوية للكالس الحى (فى وجود الراشح الفطرى السام) تم الحصول عليها بأعلى قيمة فى جيزه ١٦٨ وبأقل قيمة بينما القدرة على تكوين الكالس فى وجود الراشح الفطرى السام وجدت بأعلى ما يمكن فى سيدس ١.