J. Agric. Sci. Mansoura Univ., 30 (10): 5919 - 5926, 2005

IN VITRO GENETIC ENHANCEMENT THE RESISTANT OF THREE GENOTYPES OF *Zea mays* L. TO *Fusarium oxysporum* Phytotoxicity

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ABSTRACT

The present investigation aimed to increase the tolerance in tissue culture of three hybrids (hybrid 151 , hybrid 158 and hybrid 352) of yellow maize (*Zea maysL.*) against the fungal pathogen (*Fusarium oxysporum*) through *in vitro* mutation induction *via* the chemical mutagen EMS in mature embryos. The induced calli after treated with EMS were selected and tested for their resistant activity against *F. oxysporum*. Then chitinase activity and protein fingerprint of the tested calli were detected. 0.6% EMS showed highly reduction in callus induction of the tested yellow mays (*Zea maize L.*) hybrids than the other EMS doses (0.4%) or the control. Control showed healthy calli ranged from 20 % to 40 % when inoculated with *F. oxysporum*. All treated calli with EMS showed increasing in healthy calli than the controls reached to 250, 150 and 170% of the hybrids; 151, 158 and 352, respectively when treated with 0.4% EMS. Also reached to 250, 167 and 267% of the hybrids; 151, 158 and 352, respectively when treated with 0.6% EMS. The mutagenized maize hybrids with EMS showed increasing in chitinase activity than their control treatments. Whereas the hybrids; 151, 158 and 352 reached to 8.25, 4.82 and 0.32%, respectively when treated by 0.4% EMS, the hybrids; 151, 158 and 352 reached to 30.40, 11.59 and 12.51%, respectively when treated with 0.6% EMS . The induced and inhibited bands from proteins could be used as genetic markers to detect the genetic response of different maize hybrids under EMS effect .

Keywords: *Zea mays L.*, hybrids, tissue culture, mature embryo, mutation, EMS, *F. oxysporum*, chitinase and SDS-PAGE.

INTRODUCTION

Chemical and physical mutagens (irradiation) were used widely for producing mutations and/or increasing genetic variability in target materials. It is well known that mutagens could directly induce physiological changes, point mutations and chromosomal aberrations (Rashed *et al.*, 2000) .

Mutation breeding, using chemical mutagens, e.g., EMS has been found to be a suitable tool and relatively a quicker method for improvement yield as well as other quantitative traits in plants. *In vitro* mutagenesis technique has been proved to be an effective method in many vegetative propagated crops to induce variability in economically important traits at higher pace (Liu *et al.*, 1993).

The biotechnological approach proved to be a powerful tool to complement traditional breeding programs in crops especially bean is now possible. But highly efficient and reproducible protocols for *in vitro* plant callus tissue culture system have been used widely for studying the expression of host resistance in several crops (Moda-Cirino *et al.*, 1995).

Higher plants express a group of proteins, the pathogenesis - related (PR) proteins, upon infection by pathogenic microorganisms, which are reported to have a role in defense against pathogen development. Among these proteins are group of hydrolytic enzymes, the chitinases, which have been extensively studied to determine their roles in plants that are challenged by fungal pathogens (Stintzi *et al.,* 1993 and Graham and Stcklen, 1994).

The present investigation aimed to increase the tolerance in tissue culture of three hybrids (hybrid 151 , hybrid 158 and hybrid 352) of *Zea maize L.* against the fungal pathogen *(Fusarium oxysporum)* through *in vitro* muta ion induction *via* the chemical mutagen EMS in mature embryos.

MATERIALS AND METHODS

Plant material

Three hybrids of maize; Single cross 151 , Single cross 158 and Triple 352 were obtained from the Ministry of agriculture, Gize used in this investigation .

Fungal pathogens and culture media

Stock culture of *Fusarium oxysporum* was obtained from Plant Pathology Department, NRC, Dokki, Cairo, Egypt. Stock culture was prepared using potato dextrose broth (PDB) medium and stored on potato dextrose agar (PDA) slants (Punja and Raharjo, 1996).

EMS treatment

EMS (Ethyl Methane Sulphonate) treatment was a slightly modified method to that developed by Singh *et al.* (2000) where, seeds of tne three hybrids of maize 151 ,158 and 352 were soaked in the mutagen EMS at concentrations of; 0.0, 0.4 or 0.6%. after 24hrs. The seeds soaked in dH2O for 24hrs, then the mature embryos were separated and used to induce callus.

Media and tissue culture conditions

Callus culture for the three hybrids of maize were induced from mature embryos . Following procedures outlined by Ozias-Aktins and Vasil (1983) were used . Mature embryos were rinsed in 70% ethanol for one minute, sterilized for 20 min in a bleaching solution (Commercial Clorox) and washed with sterile distilled water for several times.Then the kernel were soaked in sterile water for 48 hours, after which mature embryos were excised and culture with the scutellum in contact with medium. The culture medium contained the inorganic components of Murashige and Skoog, medium (1962) supplemented with some modifications, 30 g/l sucrose , B5 vitamin Torne *et al* (1984) .

Results of callus initiation results after treated with the mutagen EMS were expressed as frequency as the following :-

Callus initiation frequency = No. of mature embryos callus initiated / total number of mature embryos treated with EMS x 100.

The induced calli after treated with the mutagen EMS were selected as resemble mutants.

Enzyme chitinase activity.

Calli (g) were homogenized in 0.1 mM potassium phosphate buffer, pH 4.7, containing 0,25% sucrose. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was used to determine enzyme chitinase activity.

The substrate (colloidal chitin) was prepared from chitin powder according to the method of Ried and Ogryd-Ziak (1981). Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight calli / 60 minutes (Monreal and Reese, 1969).

Electrophoretic analysis of proteins

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970). Water soluble proteins were extracted from the maize calli and used to study the gene expression of the genotypes after treatmemt with 0.0 , 0.4 ,0.6 % EMS . One ml of water soluble extraction buffer was added, centrifuged for 10 min at 12,000 rpm and the supernatant was collected. Electrophoresis was carried out at 4°C until the bromophenol blue front passed completely through the gel. The gel was stained with silver staining method (Echt *et al.,* 1996). The gel was scanned using Gel Doc 2000 system and analyzed with software supplied by the manufacturer.

RESULTS AND DISCUSSION

Data presented in (Table 1) showed the effect of different concentrations (0-0% as control, 0.4% and 0.6%) of the chemical mutagen, ethyl methane sulfonate (EMS) on callus formation from mature embryos of three yellow mays (*Zea mays L..*) hybrids (151 , 158 and 352 hybrids). The (Table 1), showed, also highly significant reduction in callus induction (reached to 70%) when the seeds of the hybrid 151 treated by 0.6% EMS. Where the other yellow mays (*Zea mays L.*) hybrids showed reduction in callus formation than their control ranged from 10% (hybrid 325 treated by 0.4% EMS) to 40% (hybrid 158 treated by 0.6% EMS). Higher doses of EMS proved to be inhibitory for growth and development of plants, but moderate doses of EMS showed stimulatory effect on growth (Singh *et al.*, 2000). They concoluded that these may be due to the increased in the activity of enzymes involved in biosynthesis of hormones like auxins, cyotokinins etc. in cell at lower doses of mutagen that increases the growth of cell and ultimately whole plant. On the other hand, increase in the dose of chemical mutagens caused damaging effects on biological activities of plants which may be due to inactivation of cells because of mitotic disturbances / chromosomal aberrations at higher doses of EMS (Zargar *et al.*, 1994 and Haggran and Mohamed, 2001).

Table (1):The percentage of EMS effect on callus initiation from mature embryos of three yellow mays (*Zea maize L.*) hybrids.

Treatments	*Zea maize L.* hybrids		
	Hybrid 151	Hybrid 158	Hybrid 352
	% of calli		
Un-treated with EMS	100	100	100
Treated with 0.4 % EMS	60	70	90
Treated with 0.6 % EMS	30	60	70

The mutagenized calli by EMS were tested for their resistant activity against the fungal pathogen *F. oxysporum* (Table 2). Then chitinase activity of the resistant calli were calculated (Table 3).

Concerning enhancement the resistant against fungal disease (Table 2) when un-treated calli with EMS were inoculated with *F. oxysporum*, the hybrid 151 showed the lowest healthy calli (20 %), while the hybrids 158 and 352 showed 40 and 30 % healthy calli, respectively. In general, all treated calli with 0.4% EMS showed increasing in healthy calli than their control. It reached to 250, 150 and 167% of the hybrids; 151, 158 and 352, respectively. Also it reached to 250, 175 and 267% of the hybrids; 151, 158 and 352, respectively when treated with 0.6% EMS. The increase in resistant activity that observed as response to mutagenesis is in agreement with Bravo (1988) who reported that the development of disease resistant lines of chick pea against root rot caused by *Fusarium, Rhizocatonia, Phytophthora, Verticillum* and *Pythium* species were obtained through mutation breeding.

Table (2): The percentage of resistant calli obtained after treated the mature embryos of three yellow maize (*Zea mays L.*) hybrids with EMS, and inoculated them with the spore of *F. oxysporum* and their control .

Treatments		*Zea mays L.* hybrids		
		Hybrid 151	Hybrid 158	Hybrid 352
		% of healthy calli		
Un-inoculation (control)	Un-treated with EMS	100	100	100
	Treated with 0.4 % EMS	100	100	100
	Treated with 0.6 % EMS	100	100	100
Inoculation with *F. oxysporum.*	Un-treated with EMS	20	40	30
	Treated with 0.4 % EMS	50	60	50
	Treated with 0.6 % EMS	50	70	80

Concerning enzyme chitinase activity (Table 3), the results indicated that all mutagenized yellow *maize* (*Zea* mays *L.*) hybrids with EMS showed increasing in chitinase activity than their control treatments. Whereas, in the hybrids; 151, 158 and 352 treated with 0.4% EMS, it reached to 8.25, 4.82 and 0.32% respectively, it reached to 30.40, 11.59 and 12.51%, when they treated with 0.6% EMS, respectively .

Table (3): Chitinase activity of the selected calli, before and after mutagenized with EMS, of three yellow *maize* (*Zea* mays *L.*) hybrids.

Treatments	*Zea mays L.* hybrids		
	Hybrid 151	Hybrid 158	Hybrid 352
	Chitinase activity *		
Un-treated with EMS	1.273	1.493	1.239
Treated with 0.4 % EMS	1.378	1.565	1.243
Treated with 0.6 % EMS	1.660	1.666	1.394

* Chitinase activity measured as m mol N-acetyl glucose amine after 60 minutes^{-1}

Punja and Raharjo (1996) reported that enzyme chitinase overproduction enhanced tolerance to pathogen infection and that plant cultivar may alter the disease response.They also stated that different chitinase genes differ in antifungal activity in plant and pathogenic fungi differ in succeptibility of chitinases *in vivo*.