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In Vitro Selection for Resistance against *Fusarium equisetii* in *Robinia pseudoacacia* L.

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ABSTRACT

The objective of this study was to develop resistance *in vitro* against *Fusarium equisetii* in *Robinia pseudoacacia* L. Callus was induced from cotyledon explants of *R. pseudoacacia*, a leguminous landscaping tree, on solid Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of 1-naphthalene acetic acid (NAA; 0.1-0.5 mg/l) and N^6-benzyladenine (BA; 0.1-0.3 mg/l). Among them, 1.0 mg/l NAA and 1.0 mg/l BA was the best combination for callus induction, which was friable and light brown. Callus fresh weight increased and turned pale yellow after subculture. The effect of different concentrations of a toxic culture filtrate of *Fusarium equisetii* (Corda) Sacc. was investigated by plating friable callus on selective medium. Resistant calli were selected at 10% of the culture filtrate on which 15.33% calli survived, although no callus formed at higher concentrations of toxic culture filtrate. Shoots, which regenerated from resistant calli on MS medium supplemented with 0.05 mg/l NAA and 0.6 mg/l BA, could root on half-strength MS medium containing 0.02% activated charcoal. Selected resistant plantlets, which survived on 2.5-10% of the culture filtrate compared to the control, showed reduced wilt incidence (approximately 70%) indicating a resistant response in tissues towards *F. equisetii*.

Keywords: callus, culture filtrate, *Fusarium equisetii*, MS medium

INTRODUCTION

Robinia pseudoacacia Linn. (black locust) is a nitrogen-fixing tree belonging to the family Leguminosae (Papilionaceae). It is a medium-sized, spiny, deciduous tree native of the South Eastern United States (Harlow *et al*. 1979). About 20 species in the genus *Robinia* have been recorded around the world, of which the only multi-purpose and economically important tree recognized globally is *Robinia pseudoacacia* Linn. (Gombu 1972), which has proved to be the species best suited to degraded ecological areas. In addition, it adapts to extreme environmental conditions such as drought, air pollutants, frost and high light intensity (Kanwar *et al*. 2007).

Wilt caused by *Fusarium equisetii* (Corda) Sacc. has become an important disease of black locust. Due to a constantly increasing incidence in areas where it grows around the world (Hangual-Babul 1983), the extent of damage ranged from 12.5 to 52.5% in different nurseries and plantations (Sen 1990). *Fusarium* spp. also induces serious root rot in *Acacia*, pines and other forest nursery plants (Kaushal *et al*. 2003). As the disease progresses, the pathogen produces typical wilt symptoms infecting the crown portion but initially reddish-brown necrotic lesions appear on roots, the finer roots show black steaks which become prominent when bark is removed (Das Gupta and Rai 1947). The roots show rotting at the basal region and bark is easily detectable from the cortex. The cortical regions of the stem and roots also show rotting at the basal region and bark is easily detachable from the cortex. The cortical regions of the stem and root show discoloration and damage. Light brown discoloration is noticed in vascular tissues (Chattopadhyay and Bhattacharjya 1968). Wilted plants later show bark splitting. Leaves turn chlorotic, the stem swells and finally the infection spreads to the vascular tissues leading to distortion and death of the plant (Halász 2002; Chittam and Kulkarni 2008; Gupta *et al.* 2010). The infected plant remains stunted and appears in patches in the field. Many approaches to plant disease management have been reported so far in *R. pseudoacacia* with little success and did not prove very fruitful in the complete eradication of this disease.

Many plant pathogenic fungi produce host-specific pathotoxins that are primary determinants in the pathogenicity and responsible for the induction of typical disease symptoms in the absence of the pathogen (Amusa 2006). *Fusarium* species are well known for inducing phytotoxins). *Fusarium solani* (Mart.) Sacc. f. sp. *piperis* Albuq. and other species of *Fusarium* from the group *Martiella* Wollenweber, to which *F. solani* f. sp. *piperis* belongs, produce several phytotoxic red pigments with a common naphthazarin structure (Duarte and Archer 2003). *F. equisetii* produces various forms of mycotoxins such as nivalenol, diacetoxyscirpenol and estrogenic mycotoxin zearalenone (Bottallico and Perrone 2002; Ezekiel *et al*. 2008; Goswami *et al.* 2008). Toxic cultural filtrates and purified toxins from *Fusarium oxysporum* f.sp. medicaginis were used for *in vitro* selection and regeneration of disease-resistant alfalfa (*Medicago sativa*) plants (Hartman *et al.* 1984; Vidyasekaran *et al.* 1990). During the past few years, interest in using tissue culture for rapid and large-scale propagation of black locust has increased significantly (Kanwar *et al.* 1996, 2000; Kaushal and Kanwar 2003; Kanwar *et al.* 2008, 2009).

In vitro selection involves the selection of calli taking advantage of somaclonal variation in tissue culture (Thakur *et al.* 2002; Kumar *et al.* 2008; Nasir and Riazuddin 2008; Tripathi *et al*. 2008). This variation can be used to develop new variants that retain all the favourable qualities of an existing variety with the additional trait of disease resistance (Evan *et al.* 1984; Purwati and Sudarsono 2007). *In vitro* selection for disease resistance is advantageous to obtain disease-free healthy plants (Kumar *et al.* 2008).

The present study aims to assess the possibility of using *F. equisetii* toxic culture filtrate to select for wilt-resistant plants *in vitro* via callus selection.

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Original Research Paper

MATERIALS AND METHODS

Establishment of callus cultures *in vitro*

1. Explant source

The seeds of *R. pseudoacacia* obtained from the Department of Tree Improvement and Genetic Resources, Dr Y S Parmar University of Horticulture and Forestry, were washed under running tap water for 30 min after adding 4-5 drops 2% (v/v) Teepol (Qualigens, Mumbai, India), a detergent. The seeds were then soaked in distilled water for 1-2 h to soften the seed coat, then surface sterilized with 0.1% mercuric chloride (Merck, Mumbai, India) for 4 min and rinsed with sterilized (autoclaved) distilled water (SDW) three times. The treated seeds were inoculated on sterilized sand to allow them to germinate.

2. Callus induction

Cotyledons from 10-15 days-old *in vitro* germinated seedlings were used as explants. Cotyledon pieces 0.50-0.75 mm in size were cut and inoculated aseptically onto MS medium (Murashige and Skoog 1962) supplemented with different concentrations (0.1-0.5 mg/l) of 1-naphthaleneacetic acid (NAA; Sisco Research Laboratories, Mumbai, India) and 6-benzyladenine (BA; Sisco Research Laboratories) (0.1-0.3 mg/l). The cultures were incubated at 25 ± 1°C under a 16-h photoperiod and 35 $\mu molm^{-2}s^{-1}$ light intensity provided by cool (Philips) fluorescent tubes for 4 weeks. The callus thus obtained was subcultured three times on the same medium at an interval of 4 weeks.

Regeneration of plantlets from calli resistant to *F. equisetii*

1. Isolation of test pathogen (Fusarium equisetii)

The roots of wilt-infected plants (**Fig. 1D**) of *R. pseudoacacia* were procured from the forestry research farm of Dr. YS Parmar University of Horticulture and Forestry, India. The roots were washed with water 2-3 times to remove soil and cut into small pieces (1 cm^2) which were surface sterilized with 0.1% mercuric chloride for 2 min followed by 2-3 washes with SDW under aseptic conditions. The pathogen was isolated on potato dextrose agar (PDA) medium. *F. equisetii* cultures were multiplied and maintained in Petri dishes containing PDA. Thereafter, the Petri dishes were covered and kept at 4°C for further use.

2. Extraction of F. equisetii toxic culture filtrate

A pure culture of the pathogen was cultured in liquid Richard's medium (Goodman 1959). Small bits (1 mm^2) of pathogen mycelium were excised with a sterilized cork borer and inoculated into liquid Richard's medium. After 15 days, the fungal culture was used to prepare the culture filtrate. The fungus was filtered in five distinct phases *viz.* 1) filtration through three layers of muslin cloth, 2) coarse filtration through ordinary filter paper, 3) centrifugation at 8944 × *g* for 30 min in an ultracentrifuge (Remi, Mumbai, India) followed by 4) filtration through Whatman filter paper No. 42. Finally, in step 5, the filtrate was passed through a sintered glass filter (G-5 grade) (Borosil Mumbai, India) which yielded a pure and transparent culture filtrate.

3. Preparation of selective medium and cell plating

In addition to the control medium, medium used to select calli was prepared by mixing the filter-sterilized pathogen culture and molten MS medium to a final concentration of 2.5-20% (v/v) of pure culture filtrate.

4. Plating of calli on selective media

Friable callus obtained on MS medium supplemented with 1.0 mg/l NAA and 1.0 mg/l BA was cut into small pieces (6.0-8.0 mm in size; 20 mg mass) and inoculated onto normal (control) and selective media. The growth of calli was monitored by the ability of cells to divide and form macro colonies following three subcultures at 4-week intervals. Fresh callus from 5 flasks (Borosil, Mumbai, India) of each medium (from of our observations culturing callus in flasks reduced the contamination rate compared to Petri dishes) (control and selective) was weighed prior to each subculture.

5. Selection and isolation of resistant calli

The highest concentration of culture filtrate at which calli from different treatments survived was recorded and surviving calli were subcultured further onto the same medium to compare the susceptibility or resistance to different concentrations of culture filtrate. Thereafter, calli surviving two cycles of selection were multiplied on medium devoid of culture filtrate (Thakur *et al.* 2002).

6. Regeneration of plantlets from resistant calli

The selected calli that were apparently resistant were transferred to conical flasks containing 20 ml of MS medium supplemented with 0.05 mg/l NAA and 0.2-1.0 mg/l BA. These calli began to turn green and produce shoots. Shoots 1.5 cm in size and with 2 nodes and 3-5 leaves were proliferated on MS medium supplemented with 0.2-1.0 mg/l BA. These shoots were subcultured three times at a 4-week interval on the same medium. The final shoots obtained, approximately 2.0-2.5 cm in length, were aseptically removed from culture flasks and dipped in 2.0 ml of 10.00 mg/l indole-3-butyric acid (IBA)(Sisco Research Laboratories, Mumbai, India) for 24 h and cultured in half-strength MS medium supplemented with 0.02% activated charcoal (Sisco Research Laboratories) and incubated for 4 weeks to induce roots. Rooted plantlets were kept at room temperature (30 ± 2°C) under natural light conditions for 7 days. Previously these plantlets had been kept under fluorescent light tubes in the culture room.

In vitro testing of selected plantlets

Plantlets were aseptically removed from culture tubes and inoculated (i.e., treated with) into different concentrations of pure culture filtrate (2.5 and 10.0%) mixed into the medium (half-strength MS + 0.02% activated charcoal).

Statistical analysis of data

The data recorded for different parameters was subjected to statistical analysis. All experiments were arranged in a completely randomized design (CRD) with three replicates per treatment. The software used for statistical analysis was SPSS (SPSS Inc.). The significance of treatment effects on various parameters was determined using analysis of variance (ANOVA). If the treatments were found to be significant, then their comparative performance was tested after obtaining the critical difference ($CD_{0.05}$).

RESULTS

A number of treatments with different concentrations of NAA (0.5-2.0 mg/l) and BA (0.5-2.0 mg/l) were employed to induce callus. The control did not respond at all and most of the treatments induced compact and brown callus. The best combination was 1.0 mg/l NAA and 1.0 mg/l BA which induced friable and light-brown callus. Calli were subcultured every 4 weeks onto the same medium. Calli become loose, pale and friable as the number of subcultures increased, and by the end of the third subculture.

Isolation of fungus

The fungus was isolated and identified as *Fusarium equisetii* on the basis of cultural and morphological characters and its pathogenicity was confirmed by the Department of Mycology and Plant Pathology of Dr YS Parmar University of Horticulture and Forestry, Solan (H.P), India. Thereafter, the culture filtrate was successfully obtained from the isolated pure culture of the pathogen.

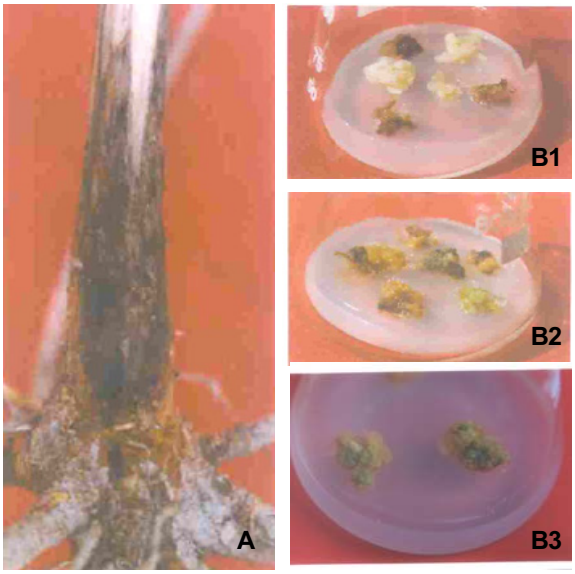

Fig. 1 (**A**) Wilt-infected roots (collar region) of *Robinia pseudoacacia*. (**B**) Callus proliferation on selective medium (1.0 mg/l NAA + 1.0 mg/l BA) supplemented with different concentrations of culture filtrate (CF) after 45 days. (**B1**) 5% CF, (**B2**) 10% CF, (**B3**) control.

Table 1 Callus selection of *Robinia pseudoacacia* against different concentrations of *Fusarium equisetii* culture filtrates after 6 weeks of incubation.

Concentration of culture filtrate (%)	Survival of callus (%)
Control	100.00 (90.00)*
2.5	65.33 (53.94)
5.0	49.33 (44.62)
7.5	35.67 (36.66)
10.00	15.33 (22.98)
12.50	0 (0)
15.00	0 (0)
17.50	0 (0)
20.00	0 (0)
$CD_{0.05}$	4.21 (2.62)

*Values in parentheses are arc-sine transformed values.

Testing of toxicity of culture filtrate

Friable and pale-yellow calli were placed onto selective media (different concentrations of 1.0 ml suspension of pure culture filtrate) and control media (without filtrate). Within a week after inoculation of calli onto selective medium, all calli began to turn brown and after 4 weeks, friable pale-yellow calli became dark brown and necrotic.

Selection and isolation of resistant calli

The highest concentration of culture filtrate at which calli from different treatments survived was recorded and surviving calli were further subcultured onto same medium to compare the susceptibility or resistance to different concentrations of culture filtrate (**Fig. 1A-C**). The control showed 100% survival but survival percentage decreased as culture filtrate concentration increased to 10% (**Table 1**). More than 10% of culture filtrate resulted in 100% mortality while at 5% of culture filtrate 50% mortality occurred. Therefore, callus was selected at 10% culture filtrate which resulted in 15.33% survival. After 4 weeks, this callus was subcultured twice on the same selective medium before placing it on shoot induction medium.

Effect of subculturing on fresh weight of callus

The fresh weight of callus on control and selective media increased continuously over three subcultures. However, the increase was greater in control callus as compared to selected callus. The selected callus was then subcultured on normal and selective medium. The increase in mass of selected callus was greater on normal than on selective medium (data not shown).

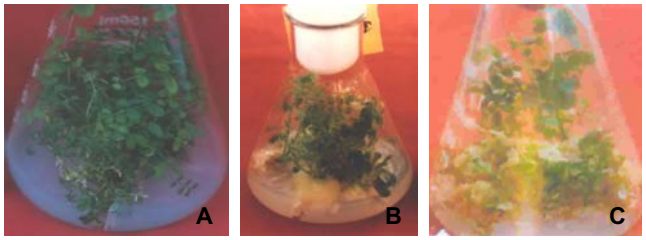

Fig. 2 Induction of small shoots on MS + 0.6 mg/l BA supplemented with different concentrations of culture filtrate (CF) after 30 days. (**A**) Control, (**B**) 5% CF, (**C**) 10 CF.

Regeneration of shoots from callus and shoot proliferation

Normal and *F. equisetii*-resistant calli were transferred to shoot induction medium supplemented with different concentrations of BA (0.2-1.0 mg/l) and NAA (0.05 mg/l) as shown in **Table 2** and **Fig. 2**. BA at 0.6 mg/l + 0.05 mg/l NAA was the best medium to initiate shoot primordia in both callus types (control and selected). Calli turned yellow, then green after 2 weeks and developed small shoot buds/primordia within 3 weeks. The organogenic outcome (callus or shoot formation) was always lower in selective medium than on control medium. Small shoots (2.0 cm) were isolated aseptically and trimmed to 1.0-1.5 cm and transferred to solid MS medium containing various concentrations of BA (0.2-1/0 mg/l) for multiplication. The greatest number of shoots derived from selected calli formed with 0.6 mg/l BA (15.0 and 5.0 shoots/explant for selective and control callus, respectively; Table 2), although shoot growth was always slower than shoots from the control treatment.

In vitro root induction

The shoots obtained after multiplication from control and resistant calli needed to be rooted to obtain plantlets. The shoots (2.0-3.0 cm) formed from *in vitro* grown cultures were aseptically removed from the culture flasks and dipped in 2 ml of 10.0 mg/l IBA for 24 h under dark, aseptic conditions. After dipping, these shoots were transferred to rooting medium (hormone-free, half-strength MS medium containing 0.02% activated charcoal). Roots initiated after 2 weeks and a well developed rooting system was obtained within 6 weeks (**Fig. 3**). Most (60.0%) shoots rooted in the control as compared to 40.0% in selected shoots.

In vitro testing of selected plantlets

Shoots were inoculated onto media with different concentrations of culture filtrate ranging from 2.5 to 10% (w/v). Control plantlets had wilting leaves resulting in death of plantlets whereas selected plantlets survived to varying levels depending on the concentration of culture filtrate (**Fig. 4**).

DISCUSSION

During the present study, cotyledon explants cultured on solid MS medium supplemented with 1.0 mg/l NAA and 1.0 mg/l BA were able to induce callus, which was friable, compact and light brown. By subculturing at regular intervals callus fresh weight increased and changed colour from brown to pale yellow as reported earlier by Kanwar *et al.* (2003) using hypocotyl segments in *R. pseudoacacia*. Resistant calli could be selected from 10% *F. equisetii* culture

Table 2 Effect of different concentrations of BAP along with 0.05 mg/1 NAA on shoot induction from normal (control) and selected callus in *Robinia pseudoacacia*.

BAP (mg/l)	NAA (mg/l)	Shoot induction (%)		Number of shoots per explants	
		Control	Selected	Control	Selected
0.00	0.00	0 (0)*	0 (0)	0	0
0.20	0.05	5.00 (12.88)	0 (0)	0	0
0.40	0.05	10.00 (18.42)	5.00 (12.88)	5.00	2.00
0.60	0.05	40.00 (39.23)	20.00 (26.56)	15.00	5.00
0.80	0.05	15.00 (22.78)	5.00 (12.88)	5.00	3.00
1.00	0.05	5.00 (12.88)	0 (0)	2.00	1.00
		$CD_{0.05}$		$CD_{0.05}$	
		T (treatment) : 0.97 (1.05)		T (treatment) : 1.75	
		I (interaction) : 0.56 (0.60)		I (interaction) : 1.01	
		T × I : 0.97 (1.05)		T × I : 1.75	

*Figures in parenthesis are arc transformed values.

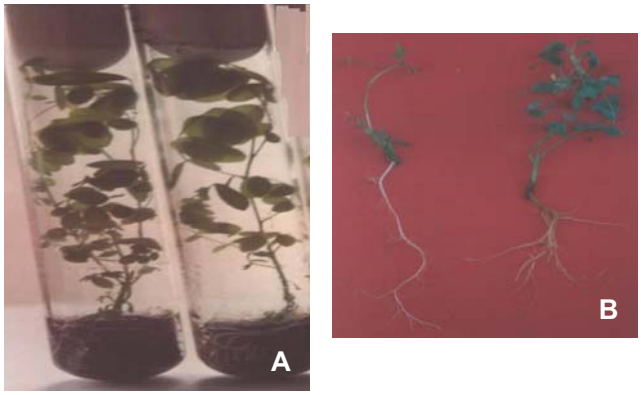

Fig. 3 (**A**) Induction of roots from selected shoots on MS + 0.02% activated charcoal after 28 days. (**B**) Rooted *R. pseudoacacia* plantlets.

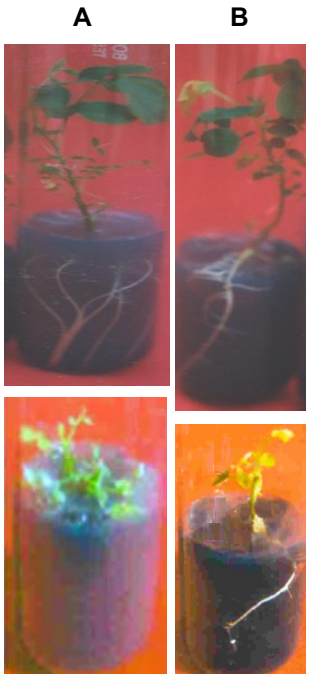

Fig. 4 Selected (left) and control (right) plantlets with 10% (**A**) or 5% (**B**) *Fusarium equisetii* culture filtrate.

filtrate. Although callus at this concentration was brown compared to the control, it was able to regenerate. Similarly, Marunenko *et al*. (1988) used 10% culture filtrate to obtain potato (*Solanum tuberosum* L.) calli resistant against *Ceavibacter michiganesis* sub sp. *sespedonicus* toxin and an enzyme complex of *Erwinia carotovora*. *Fusarium* sp. culture filtrates have already been tested as selective agents in *Medicago sativa* L., *Solanum tuberosum*, *Pisum sativum* L. cv. 'Arkel', respectively (Hartman *et al.* 1984; Botta *et al.* 1994; Kumar *et al.* 1997). In *R. pseudoacacia*, shoots were multiplied on solid MS medium supplemented with 0.6 mg/l BA alone followed by 3 sub-cultures at 4-week intervals. Small shoots could be induced to root by dipping them in 10 mg/l IBA for 24 h and culturing them on solid, hormone-free MS medium supplemented with 0.02% activated charcoal. This follows the advice and methodology of earlier reports (Kanwar *et al.* 1996, 2003, 2007, 2008). An important step in *in vitro* selection for plant improvement is to test the relationship between resistance of the plant culture, culture filtrate and resistance of the plants regenerated from the cultures to the pathogen. This is necessary because resistance to a toxin at the cellular level may or may not be related to whole plant resistance to the pathogen (Ling *et al.* 1985; Jan *et al.* 1993). The results of the findings in this study suggest that toxic metabolites present in culture filtrate of *F. equisetii* may play a role in developing resistance against the pathogen in *R. pseudoacacia* and may be effective screening agents in a cell selection programme. The mycotoxins nivalenol and zearalenone were detected in wheat heads inoculated with *F. equisetii* (Goswami *et al.* 2008). Hammerschlag (1988) and Ritchie *et al*. (1993) obtained resistant plants of geranium and peach against *Xanthomonas campestris* using *in vitro* cell selection. Sacristan (1985) reported higher resistance in plants regenerated from cell lines of *Brassica napus* selected against a culture filtrate of *Phoma lingum* compared to the control. Chawla and Wenzel (1987) obtained calli from immature embryos of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) and screened for resistant cells against a purified culture filtrate of *Helminthosporium*; selection resulted in 6-17% of surviving calli and most regenerated plants were less sensitive to the pathogen. Arcioni *et al*. (1987) and Binarova *et al*. (1990) observed resistance to a culture filtrate of alfalfa (*Medicago sativa* L.) plants to *Fusarium oxysporum* f.sp. *medicagini*s. Resistant calli were obtained on selective media containing 10% culture filtrate. Thakur *et al.* (2002) regenerated carnation (*Dianthus caryophyllus* L.) plants resistant to a culture filtrate of *Fusarium oxysporum* f.sp. *dianthi* which causes vascular wilt of carnation. Resistant lines were selected by culturing calli on growth medium containing various concentrations of the culture filtrate (0, 5, 7.5, 10, 12.5, 15, 17.5 or 20% (v/v)). Resistant calli obtained after two cycles (25 days/cycle) of selection were used for plant regeneration. About 32% of the plants regenerated from the resistant calli had acquired considerable resistance against the pathogen in the field. No phenotypic variation was observed in the selected regenerates. Tripathi *et al.* (2008) obtained disease-tolerant/-resistant onion (*Allium cepa* L. cvs. 'ADR' and 'ALR') cell lines selected against purple blotch disease caused by *Alternaris porri.* Almost 4700 calli obtained from mature embryo and 8300 cell clumps from suspension cultures of two onion cultivars were exposed to the media with LD_{50} (7.8 ml/L) phytotoxin concentration for selection. An *in vitro* pathogenicity test of regenerated plants surviving tolerant/resistant cell lines revealed non-sensitivity against pathogen toxin. Senga *et al.*

(2009) reported *in vitro selection* of sugarcane (*Saccharum officinale* L.) genotypes CoJ88 and CoJ64 against *Collectotrichum facatum*, which causes red rot of sugarcane. The capacity of plantlets to regenerate from callus reduced greatly and one plantlet per callus piece formed. Regenerated plants were evaluated for resistance to culture filtrate and *in vivo* resistance to the pathogen. Three out of eight plants were resistant to the fungus and a high correlation between resistance to culture filtrate and *in vivo* resistance was observed.

Malepszy and Kazaaz (1990) challenged callus culture of two cucumber (*Cucumis sativus* XI) cvs. 'Borszozoagowki' and 'Gy-3' with culture filtrate (1-15%) of *F. oxysporum* f sp. *cucumerinum* races OGI and FOCRI. Resistant callus was isolated with 5% culture filtrate. Botta *et al*. (1994) used culture filtrate of *F. equisetii*, which induces potato wilt and stem end rot, to screen for wilt resistance *in vitro*. Callus responses of the cultivars to the responses of the cultivars to the pathogen in the greenhouse.

REFERENCES

Amusa NA (2006) Microbially produced phytotoxins and plant disease management. *African Journal of Biotechnology* **5 (5)**, 405-414

Arcioni S, Pezzotti M, Damiani F (1987) *In vitro* selection of alfalfa plant resistance to *Fusarium oxysporum* f.sp.*medicagini*s. *Theoretical and Applied Genetics* **74**, 700-705

Binarova P, Nedelnick J, Fellner M, Nedbalkova B (1990) Selection for resistance filtrate of *Fusarium* spp. in embroyogenic cell suspension culture of *Medicago sativ*a L. *Plant Cell, Tissue and Organ Culture* **22**, 191-196

Botta CH, Dimako MP, Melegari L, Huarte MA, Barassi CA (1994) Potential of *Fusarium eumartii* culture filtrate on screening for wilt resistance in potato. *Euphytica* **80**, 63-69

Bottalico A, Perrone G (2002) Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology* **108**, 611-624

Chattopadhyay SB, Bhattacharjya SK (1968) Investigations on the wilt disease of guava (*Psidium guajava* L.) in West Bengal I. *Indian Journal of Agriculture* **38**, 65-72

Chawla HS, Wenzel G (1987) *In vitro* selection for fusaric acid resistant barley plants. *Plant Breeding* **99**, 159-163

Chittem K, Kulkarni S (2008) Effect of media on the growth of *Fusarium oxysporum* f. sp. *gerberae* and *Fusarium oxysporum* f. sp *dianthi. Karnataka Journal of Agricultural Sciences* **21 (2)**, 303-304

Das Gupta SN, Rai JN (1947) Wilt disease of guava (*P. guajava*). *Current Science* **16**, 256-258

Duarte MLR, Archer SA (2003) *In vitro* toxin production by *Fusarium solani* f. sp. *piperis. Fitopatologia Brasileira* **28**, 229-235

Duffy BY, Defago G (1998) *Fusarium* pathogenicity factor blocks antibiotic biosynthesis by antagonistic to *Pseudomonad*s. In: Duffy B, Rosenberger U, Defago G (Eds) *Molecular Approaches in Biological Control*, Switzerland, Delmont, 15-18 Sept, 1997. *IOBC/WPRS Bulletin OILB/SROP* **21**, 145-148

Evan DA, Sharp WR, Medina-Filho HP (1984) Somaclonal and gametoclonal variation. *American Journal of Botany* **71**, 759-774

Ezekiel CN, Odebode AC, Fapohunda SO (2008) Zearalenone production by naturally occurring *Fusarium species* on maize, wheat and soybeans from Nigeria. *Journal of Biological and Environmental Sciences* **2 (6)**, 77-82

Gegenbach BG, Donavan CM (1977) Inheritance of selected pathotoxin resistance in maize plants regenerated from the cell cultures. *Proceedings of the National Academy of Sciences USA* **74**, 5113-5117

Gombu SS (1972) A manual of Indian timbers. An account of growth distribution and uses of tree and shrubs of India and common description of their wood properties. Bishan Singh Mahendra Pal Singh Ltd., Dehradun, India, pp 233-237

Goodman RN (1959) Observations on the production, physiological activity and chemical nature of *Colletotin*, a toxin from *Colletotrichum fuscum.* Laub. *Pflanzen Physiologie* **37**, 187-194

Goswami RS, Dong Y, Punja ZK (2008) Host range and mycotoxin production by *Fusarium equisetti* isolates originating from ginseng fields *Canadian Journal of Plant Pathology* **30**, 255-260

Gupta VK, Mishra AK, Gaur RK, Jain PK, Sharma S (2010) Current status of *Fusarium* wilt disease of guava (*Psidium guajava* L.) in India. *Biotechnology* **9 (2)**, 176-195

Halász G (2002) Canker and wilt of black locust (*Robinia pseudoacacia* L.) caused by *Fusarium* species*. Acta Microbiologica et Immunologica Hungarica* **49 (2-3)**, 249-260

Hammerschlag FA (1988) Selection of peach cells for insensitivity to culture filtrates of *Xanthomonas compestris* pv. *pruni* and regeneration of resistant plants. *Theoretical and Applied Genetics* **76**, 865-869

Hangyal-Balul W (1983) Fungus diseases of *Robinia* seeds and seedlings and possibilities of their control. *Erdeszeti Kutatosok* **74**, 343-349

Harlow WM, Harrar ES, White FM, White FM (1979) *Textbook of Dendrology* (6th Edn), McGraw-Hill, New York, pp 391-399

Hartman CL, McCoy TJ, Knous IR (1984) Selection of alfalfa (*Medicago sativ*a) cell lines and regeneration of plants resistant to the toxin(s) produced by *Fusarium oxysporum* f.sp. *medicagini*s. *Plant Science Letters* **34**, 184-194

Kang Z, Buchennauer H (1999) Immunocytochemical localization of *Fusarium* toxin in infected wheat spikes by *Fusarium culmorum*. *Physiological and Molecular Plant Pathology* **55**, 275-288

Kanwar K, Bhardwaj A, Agarwal S, Sharma DR (2003) Genetic transformation of *Robinia pseudoacacia* by *Agrobacterium tumefacien*s. *Indian Journal of Experimental Biology* **41**, 149-153