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Selection and Regeneration of Groundnut Plants Resistant to the Pathotoxic Culture Filtrate of *Cercosporidium personatum* Through Tissue Culture Technology

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ABSTRACT

Callus cultures were established from immature leaf explants of *Arachis hypogaea* on MS medium supplemented with 2.0 mg/L of NAA and 0.5 mg/L of BAP of the susceptible cultivars namely VRI-2 and TMV-7. Three-week-old calli were subjected to mutagenic treatments (gamma rays: 50–250 Gy and EMS: 5–25 m*M*). Mutagen-treated calli were subcultured to fresh medium containing various concentrations (25–100% v/v) of pathotoxic culture filtrates. Calli were challenged in vitro with pathotoxic culture filtrate of the fungal pathogen and were assessed by visible growth ratings expressed as the percent response to the doses/concentrations of mutagen. Selected mutagen-treated calli showed resistance in vitro on media containing *Cercosporidium personatum* pathotoxic culture filtrate. Resistance calli were then transferred to MS regeneration medium supplemented with BAP (2.0 mg/L) and NAA (0.5 mg/L) for shoot bud regeneration. The progeny of the plants produced 13 disease-resistant plants (R_2) in both the cultivars. Among the eight R_2 populations studied, 70.2–82.5% of the plants exhibited enhanced resistance. This study suggested that groundnut plants with resistance to *C. personatum* can be selected

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from mutagen-treated callus of tikka leaf spot-susceptible cultivars using host-specific pathotoxic culture filtrates of *C. personatum* through in vitro technology.

Index Entries: *Arachis hypogaea; Cercosporidium personatum;* in vitro; disease resistance; culture filtrate; plant regeneration.

Abbreviations: MS medium, Murashige and Skoog (1962) medium; NAA, α-naphthaleneacetic acid; IBA, indole butyric acid; BAP, 6-benzylamino purine; KIN, kinetin; GY, gray.

INTRODUCTION

Several techniques have been used to breed crop plants that are disease resistant. One is the introduction of resistance from wild species, although the genetic source is not always available. Another means is the artificial induction of mutations that show resistance (*1*). The in vitro selection and screening of plant cells or tissues resistant to fungal pathogens can be accomplished by using different screening agents, such as purified specific or nonspecific toxins, crude extracts of pathogen cultures, or by co-culture with the fungus itself. Cell culture is a valuable tool for screening the induced variation for direct or indirect utilization in breeding programs for crop improvement. Induction of mutations for resistance to various pathogens at the cellular level and regeneration of plants may facilitate the selection of disease-resistant plants (2). The main advantages of selection in vitro for disease resistance are that (1) experimental procedures can be maintained on defined media and in a rigorously controlled environment that facilitates the measurement of slight quantitative differences in polygenically inherited resistant traits; (2) culture cell lines can be uniformly exposed to selective agents, thus reducing the incidence of escapes; and (3) culture systems can be maintained in a small space, thus replacing large field requirements. However, in order to realize these advantages, it is important that the plant materials have a high regeneration capacity.

Many plant pathogenic fungi produce host-specific and nonspecific pathotoxins that are primary determinants in pathogenesis and induce typical disease symptoms in the absence of the pathogen (3). Toxic culture filtrates and purified toxins have been used for in vitro selection and regeneration of disease resistant plants (4–10).

The fungus *Cercosporidium personatum* is the causal agent of groundnut tikka late leaf spot disease. Ramanujam (*11*) studied the phytotoxic metabolites produced by *C. personatum* in culture filtrate. Two phytotoxic compounds, namely personatin and CP3 pigments (dothistromin and its derivative), which almost certainly play an important role in pathogenesis, have been investigated. The pathogenicity of *C. personatum* probably results, however, in personatin affecting both respiration and permeabil-

ity of leaf cells, but the pigment affects only permeability. For this reason, we preferred to use the pathotoxic culture filtrate of the fungus as the screening agent, rather than the purified toxin, in an attempt to select resistant callus of *Arachis hypogaea* L. (peanut or groundnut).

Because no resistance to *C. personatum* has been found in groundnut by conventional methods (*12*), an alternative approach may be to use pathotoxic culture filtrates to screen tissue cultures for resistance to *C. personatum*. In the present study, we describe the selection and regeneration of groundnut plants from mutagen-treated calli resistant to pathotoxic culture filtrates of *C. personatum* and the evaluation of the regenerated plants for resistance to *C. personatum* in the field.

MATERIALS AND METHODS

Plant Material

The groundnut (*Arachis hypogaea* L.) genotypes used in the present study were VRI-2 and TMV-7 (obtained from Tamil Nadu Agricultural University, Coimbatore, India). Both genotypes are susceptible to *C. personatum*. Seeds were sterilized and germinated as described by Venkatachalam et al. (*13*).

Callus Initiation and Maintenance

Following germination, immature leaf explants were excised and placed on MS medium (*14*) containing B^5 vitamins (*15*), NAA (2.0 mg/L) and BAP (0.5 mg/L), 3% (w/v) sucrose and 0.7% (w/v) agar. The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were kept under artificial lighting at 24 ± 2°C for 16 h (light supplied by cool white fluorescent tubes, irradiance 70–80 $\mu E/m^2s$).

Mutagen Treatment

Gamma Irradiation

Three-week-old calli were subjected to different doses of gamma rays (50, 100, 150, 200, and 250 Gy) from ^{60}Co. These tissues (500 mg) were immediately transferred to MS medium for growth and maintenance.

EMS Treatment

Three-week-old calli were treated with 5, 10, 15, 20, and 25 m*M* of EMS (Ethylmethane sulfonate). The calli were immersed in filter-sterilized EMS solution for 2 h, with continuous shaking (20 rpm) at 25 ± 2°C. Following the EMS treatment, calli were immediately washed four times with sterile liquid MS medium and approx 500 mg of tissue were transferred to MS medium for growth and maintenance.

Pathogen Culture

The fungal isolates of *C. personatum* used were obtained from ICRISAT, Hyderabad, India. Axenic cultures of the fungal isolates were maintained on CDA medium at 20°C in the dark and were routinely transferred to fresh medium every 4 wk.

Preparation of the Pathotoxic Culture Filtrate and Toxic Media

Czapek's dextrose agar (CDA) in petri dishes was inoculated with *C. personatum* isolates and incubated at 20°C in the dark. Two weeks later one piece of CDA with fungal mycelium was transferred to liquid MS medium without growth regulators. The cultures were kept at 24°C in dim light with continuous agitation (80 rpm) and subcultured by transferring 20 mL of the fungal culture to 30 mL of fresh MS medium at 2 wk intervals. Uninoculated fungal culture medium was similarly handled. Any flasks showing abnormal or no mycelial growth were discarded. The liquid cultures were then centrifuged for 15 min at 1500*g* to remove spores and mycelia. Supernatant fractions from the same isolates were pooled. The pH of the filtrate was adjusted to 5.8 and the filtrates were filter-sterilized through a 0.45 μM Millipore filter unit. The phytotoxicity of the toxin was confirmed by inoculation into detached groundnut leaves.

The medium consisted of the MS salts, B^5 vitamins, sucrose 3% (w/v), growth regulators (α-naphthalene acetic acid 2.0 mg/L and 6-benzylaminopurine 0.5 mg/L) and agar 0.7% (w/v). Filter-sterilized volumes of the toxic culture filtrate were added to the MS media after autoclaving, to provide four treatment concentrations (25, 50, 75, and 100%). A control without toxin made up the fifth treatment. The media were poured into 250 mL concial flasks.

Culture Procedure

Callus pieces were used to inoculate MS medium containing various concentrations of fungal culture filtrate and a control flask containing without, toxin to begin the first cycle. The callus was subcultured on fresh media 30 d after the initial culture. After 50, 70, and 90 d, toxin-insensitive (resistant) callus clumps were subcultured to a shooting medium. The viability of the callus clumps was determined by 2, 3, 5-triphenyl tetrazolium chloride (TTC) assay.

Regeneration of Groundnut Plants Resistant to the Pathotoxic Culture Filtrates of *C. personatum*

Surviving callus clumps were transferred to the culture filtrate containing MS medium with 2.0 mg/L of BAP and 0.5 mg/L of NAA. After this step, surviving organogenic calli were continuously transferred to the culture filtrate containing MS medium, with 2.0 mg/L of BAP and 0.5 mg/L of NAA every 2–3 wk for at least six selection cycles, to select

shoots resistant to the pathotoxic culture filtrates. Shoots were also regenerated from the control without culture filtrate. Rooting was induced by transferring the selected shoots to the MS medium containing IBA (2.0 mg/L) and KIN (0.5 mg/L). All cultures were incubated at 24 ± 2°C under 16 h photoperiods (70–80 $\mu E/m^2s$). The selected regenerants were transplanted to soil and grew in the field and set few viable seeds.

Evaluation of Resistance to *C. personatum* in the Progeny of Regenerated Plants

Seeds from the 23 R_1 plants in the immature leaf selection system were planted in the field and 85% of the seeds germinated to produce R_2 plants. The plants in the field were inoculated with *C. personatum* spore suspension. Regenerated plants without toxin treatment were used as controls for evaluation of the resistance to the pathogen. Disease severity was rated using the rating scale of Raguchander et al. (*16*), based on the proportion of diseased leaf tissue. Disease severity and plant growth stage were evaluated at least once a week for 8 wk. Reactions of individual regenerants to *C. personatum* were classified into six groups based on percentage of diseased leaf area: highly resistant (0–10%), resistant (11–20%), moderately resistant (21–40%), moderately susceptible (41–50%), susceptible (51–60%), and highly susceptible (61–100%). Plants that exhibited resistance were harvested individually.

Effects of main factors (mutagen-treated callus, pathotoxic culture filtrate, shooting, and rooting) and their interactions on resistance were examined using analysis of variance (ANOVA). Observations of repeated experiments were subjected to analyses of homogeneity of variance and pooled accordingly. New Duncan's Multiple Range Test was used to determine significance of differences among treatment means.

RESULTS

Selection of Toxin-Resistant Callus

Toxic media were used for selection of calli that were resistant to the culture filtrates of *C. personatum*. Growth on the toxic media is dependent on the size of the callus. The toxins are very likely poorly transported in the callus. In order to get reproducible results, we tried to use callus pieces of nearly identical size (1–5 mm) for selection procedures. We could not make use of smaller callus pieces, because callus pieces of less than 1 mm size also died on nontoxic media. After 4 wk, some of the calli on the toxic media (25%) showed small growing and white areas. Calli that had survived transfer to a toxic medium two or three times could be selected from original mutated calli. After 4 wk of growth on 25% toxic media, putative resistant callus was transferred to 50% toxic media. Surviving calli were again transferred to 75% toxic media at 4 wk intervals. The control calli

(unmutated) do not lose their resistance if they are maintained 6–12 wk on toxic medium. The resistance of the control calli on toxic medium was 6.8 and 5.8% in VRI-2 and TMV-7 cultivars, respectively. The resistance of the callus was gradually increased than the control because of the mutagenic treatment. In VRI-2 cultivar, the percentage of resistant calli ranged from 15.4 to 28.4% and 13.2 to 26.6% in gamma rays and EMS treatments, respectively, and the calli treated with 250 Gy/25m*M* gave the highest frequency (gamma rays: 28.4% and EMS: 26.6%) of resistance (Table 1). In case of TMV-7 cultivar, the percentage of resistant calli ranged from 11.4–25.9% and 11.5–25.2% in gamma rays and EMS treatments, respectively, and the calli treated with 250Gy/25m*M* gave the highest frequency (gamma rays: 25.9% and EMS: 25.2%) of resistance (Table 1). After 12 wk (three subcultures), a stable, resistant callus capable of growth on 75% toxic medium was isolated and used for plant regeneration (Fig. 1).

Regeneration of Groundnut Plants Resistant to Pathotoxic Culture Filtrates

Toxin-resistant calli were isolatd and transferred to shoot regeneration medium. The cultivar VRI-2 exhibited a higher frequency of plant regeneration than TMV-7 cultivar. Among the treated callus cultures in VRI-2, shoot regeneration frequency ranged from 19.4–44.8% and 12.3–35.7% in gamma rays and EMS-treated callus, respectively. In TMV-7, shoot regeneration frequency ranged from 16.9–39.3% and 14.4–32.8% in gamma rays and EMS-treated callus, respectively (Table 2). The shoot regeneration frequency was less in untreated calli in both cultivars (VRI-2, 15.0%; TMV-7, 12.7%). The highest frequency of shoot bud regeneration (44.8%) was observed in 250Gy of gamma ray-treated calli; it was 35.7% in 25m*M* of EMS-treated calli in VRI-2 cultivar. In the case of TMV-7 cultivar, the maximum shoot bud differentiation frequency (39.3%) was observed in gamma ray-treated calli (250Gy); it was 32.8% in EMS-treated calli (25m*M*) (Table 2). Green spots appeared after 3 wk by regeneration of shoots (Figs. 2 and 3) and transferred to rooting medium.

Root induction frequency was also varied because of mutagenic treatments. Well-developed shoots were rooted on rooting medium (Fig. 4) and the rooting frequency of control shoots was 38.4 and 36% in VRI-2 and TMV-7 cultivars, respectively. In VRI-2 cultivar, the root induction frequency ranged from 46.4 to 70.9% and 41.4 to 69.6% in gamma rays and EMS treatment, respectively, and the shoots derived from calli treated with 250Gy/25m*M* gave the highest frequency (gamma rays, 70.9%; EMS, 69.6%) of root induction (Table 3). Among the treatment cultures, rooting frequency of gamma-irradiated, calli-derived shoots ranged from 48.1–61.5% and that of EMS 41.5–63.8% in TMV-7 cultivar (Table 3). The rooting frequency was higher in VRI-2 cultivar than TMV-7 cultivar.

Table 1
Selection of Resistant Callus from Mutagen Treated Calli on NAA (2.0 mg/L) and BAP (0.5 mg/L) of Groundnut

Treatments	No. of calli screened		No. of calli showed resistance		Percent of resistant calli (mean ± SD)	
	VRI-2	TMV-7	VRI-2	TMV-7	VRI-2	TMV-7
Control	220	225	15	13	6.8 ± 2.44d	5.8 ± 1.81bc
Gamma rays (Gy)						
50	240	210	37	24	15.4 ± 4.08b	11.4 ± 2.44b
100	234	215	42	36	17.9 ± 4.81ab	16.7 ± 5.14ab
150	260	250	50	47	19.2 ± 3.68ab	18.8 ± 2.45a
200	245	265	58	54	23.6 ± 2.44a	20.4 ± 2.32a
250	250	262	71	67	28.4 ± 6.20a	25.9 ± 4.08a
EMS (mM)						
5	228	225	30	26	13.2 ± 2.28bc	11.5 ± 1.63b
10	242	230	45	40	18.5 ± 3.67ab	17.4 ± 5.38ab
15	235	244	50	48	20.4 ± 3.75ab	19.6 ± 3.59a
20	255	256	56	55	21.5 ± 4.89ab	21.5 ± 4.49a
25	248	242	66	61	26.6 ± 5.22a	25.2 ± 3.91a

The callus was selected and subsequently evaluated for growth in 75% pathotoxic culture filtrate from the C. *personatum*. Values with the same letter within columns are not significant at the 1% probability level, according to the New Duncan's Multiple Range Test.

Fig. 1. Toxin-resistant callus growing on medium. Fig. 2. Green shoot buds appeared on shooting medium. Fig. 3. Plant regeneration and shoot elongation. Fig. 4. Regenerated shoots were rooted on rooting medium. Fig. 5. Regenerated plantlets were established in plastic cups.

Rooted plants were transferred initially to plastic cups containing red soil and sand in the ratio 1:1 (Fig. 5); they were subsequently transferred to field conditions. Regenerated plants flowered normally and set few viable seeds.

Evaluation of Regenerated Plants Resistant to Pathogen

The progeny of 161 plants were tested with pathogen for their responses to infection. Ninety plants were tested from VRI-2 cultivar and 71 from

Table 2
Plant Regeneration from Selected Toxin Resistant Calli
on MS Medium Containing NAA (0.5 mg/L) and BAP (2.0 mg/L) of Groundnut

Treatments	No. of calli plated for regeneration		No. of calli showing plant regeneration		Percent of plant regeneration (mean ± SD)	
	VRI-2	TMV-7	VRI-2	TMV-7	VRI-2	TMV-7
Control	120	118	18	15	15.0 ± 4.08ef	12.7 ± 1.63de
Gamma rays (Gy)						
50	124	112	24	19	19.4 ± 2.93de	16.9 ± 3.34cd
100	100	120	30	25	25.0 ± 3.67d	20.8 ± 4.08d
150	116	122	38	33	28.4 ± 3.59cd	27.0 ± 3.67bc
200	115	118	42	39	33.9 ± 2.53bc	33.1 ± 2.36b
250	125	117	56	46	44.8 ± 3.26a	39.3 ± 3.51a
EMS (mM)						
5	130	125	21	18	12.3 ± 1.38f	14.4 ± 3.26de
10	118	120	26	22	18.6 ± 2.77e	18.3 ± 3.02d
15	126	113	33	29	23.0 ± 2.44de	25.6 ± 4.40bc
20	112	119	40	36	32.1 ± 1.63c	30.3 ± 4.08b
25	126	128	45	42	35.7 ± 4.32b	32.8 ± 1.79b

Values with the same letter within columns are not significant at the 1% probability level, according to the New Duncan's Multiple Range Test.

Table 3
Root Induction from Regenerated Shoots on MS Medium Containing IBA (2.0 mg/L) and KIN (0.5 mg/L) of Groundnut

Treatments	No. of shoots cultured		No. of shoots showing root induction		Percent of rooting (mean ± SD)	
	VRI-2	TMV-7	VRI-2	TMV-7	VRI-2	TMV-7
Control	52	50	20	18	38.4 ± 6.20c	36.0 ± 4.89cd
Gamma rays (Gy)						
50	56	52	26	25	46.4 ± 4.57bc	48.1 ± 6.45b
100	60	55	30	28	50.0 ± 8.16b	50.9 ± 4.01ab
150	63	57	33	31	52.4 ± 6.04b	54.4 ± 2.93ab
200	66	62	38	36	57.6 ± 6.44b	58.1 ± 8.24a
250	62	65	44	40	70.9 ± 8.16a	61.5 ± 4.22a
EMS (m*M*)						
5	58	53	24	22	41.4 ± 5.22bc	41.5 ± 5.30c
10	60	54	26	25	43.3 ± 2.20bc	46.3 ± 4.65bc
15	68	56	31	30	45.5 ± 4.49bc	53.6 ± 2.59ab
20	60	55	34	32	56.6 ± 5.22b	58.2 ± 4.47a
25	56	58	39	37	69.6 ± 7.34a	63.8 ± 2.91a

Values with the same letter within columns are not significant at the 1% probability level, according to the New Duncan's Multiple Range Test.

TMV-7 cultivar. When screened under artificial conditions, 13 plants exhibited resistance in both the cultivars. Results are shown in Table 4. About 34.4% of the progeny of VRI-2 plants were resistant to tikka disease; none of the control plants showed resistance to this disease. Approximately 21.7% of the progeny of TMV-7 plants were resistant to *Cercosporidium* leaf spot; none of the control plants showed resistance to this disease. Seeds collected from these plants were grown to maturity and subjected to pathogen inoculation and the disease reaction was observed. Of the eight R_2 populations studied, 70.2–82.5% of the plants exhibited enhanced resistance compared to the control. Our results clearly indicate that the resistance shown by these plants was heritable and that it can be transmitted to offspring through sexual reproduction.

DISCUSSION

In this study, we selected and regenerated groundnut plants resistant to the pathotoxic culture filtrate of *C. personatum*. Experimental evidence demonstrates that it is possible to select callus resistant to toxic compounds produced by *C. personatum* from susceptible groundnut callus. Most of the plants regenerated from resistant selections have demonstrated a resistant response when tested against the pathogen. The original plants (VRI-2 and TMV-7) used to initiate the callus were susceptible to the pathogen.

The effects of different doses/concentrations of mutagens on callus cultures are shown in Table 1. The callus induction frequency of the mutagen-treated cultures increased with increase of the mutagen dose/concentration. Resistance of groundnut callus to the culture filtrate of *C. personatum* remained preserved even after regeneration, indicating that resistance in the callus cultures is not caused by a selection of cells or callus with changed gene activity. That changed gene activity of cells or callus can be expected after selection in a callus has been demonstrated by treatment with mutagen (*17,18*). Often the fact that the mutagenic treatment enhances the result of selection procedure is taken as an indication that the mutation has altered the gene activity in selected callus. This is probably the reason why the number of resistant calli could be increased by mutagen treatments.

The present work on groundnut provides some basic information on the effects of gamma rays and EMS on the development of plants from the toxin-resistant callus. The results are summarized in Table 2. An enhancement in plant regeneration was observed in all the doses/concentrations. Generally, mutagenic treatments enhanced the regeneration frequency in groundnut. Similar result was also reported by Pius et al. (*19*) in fingermillet. As in maize (*20*), an enhancement in plantlet development was observed when the embryogenic callus was exposed to gamma rays.

In vitro screening of calluses resistant to T-toxin produced maize plants with increased resistance to *Helminthosporium maydis* (*21,22*). Hartman

Table 4
Number of Plants Showing Different Reactions to Infection with *C. personatum*

	VRI-2 Cultivar							TMV-2 Cultivar					
	No. of plants tested	Disease reaction spectrum						No. of plants tested	Disease reaction spectrum				
Treatment		HR	R	MR	MS	S	HS		HR	R	MR	MS	S	HS
Control	3	–	–	1	–	1	1	2	–	–	1	–	1	–
Gamma rays (Gy)														
50	4	–	1	1	–	1	1	3	–	–	1	1	–	1
100	7	1	2	1	1	1	1	5	–	1	1	1	1	1
150	9	1	3	2	1	1	1	7	1	1	1	1	1	2
200	10	1	3	2	2	1	1	8	1	1	1	1	2	2
250	14	2	4	2	2	2	2	12	1	2	2	2	2	3
EMS (m*M*)														
5	5	–	1	1	1	1	1	4	–	–	1	1	1	1
10	6	–	1	1	2	1	1	4	–	–	1	1	1	1
15	8	1	2	1	1	1	2	6	–	1	1	1	1	2
20	11	1	2	2	1	2	3	9	1	2	1	1	2	2
25	13	1	3	1	3	2	3	11	1	2	1	2	2	3
Total	90	8	22	15	13	14	18	71	5	10	12	12	14	18

HR, Highly resistant; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; HS, highly susceptible.

et al. (*23*) regenerated plants from resistant cell cultures that showed resistance to *Fursarium oxysporum* f. sp. *medicaginis* in alfalfa. Palit and Reddy (*2*) obtained the recovery of as high as 65.6–74.5% of regenerated rice plants from a susceptible cultivar showing resistance to *Pyricularia oryzae*. Recently, Song et al. (*9*) produced soybean plants resistant to the pathogen *Septoria glycines* from embryo cultures selected by the pathotoxic culture filtrates.

All these results and our reported one indicate that the variability in various characters, including disease resistance, found among regenerates, might be present beforehand in the cells from which the plants were derived. It is also possible that cell or callus cultures, or both, might cause genetic alterations by either in vitro or mutagenesis. In fact, variants exist in plants regenerated from callus or single cell origin. In these cases, genetic differences most probably would be generated during cell division in vitro. Nevertheless, toxin-selected mutated calluses do yield a high percentage of disease-resistant plants.

Resistance was found to be controlled by two genes, a dominant gene and an incompletely dominant gene (*23*). This dominant gene mutation was heritable in subsequent generations, when plants segregated into resistant and susceptible plants. Similar result was reported by Palit and Reddy (*2*) in rice.

In the present study, both physical and chemical mutagens were used to induce mutations. Gamma rays were found to be more effective for enhancing the resistant callus and groundnut plants than EMS treatment. Genotypes are also influenced for in vitro studies. The cultivar VRI-2 was found to be more responsive to callus induction and plant regeneration than TMV-7 cultivar.

This approach to generating disease resistance raises a question about whether the trait is caused by a mutation or epigenetic variation (*24,25*). The above results support the conclusion that the disease resistance was inherited. Many plant species have been selected for disease resistance using culture filtrates or purified toxins. Selection of disease-resistant plants through in vitro mutagenesis has been achieved in potato (*5*) and in rice (*2*).

CONCLUSIONS

In the near future we will investigate how culture filtrate resistant plants behave against infection with *C. personatum*. The main aim of this program is to obtain plant from mutagen-treated callus cultures with a general field resistance against the parasite. Byther and Steiner (*26*) have described a case in which resistance to a fungal parasite was correlated with the resistance to its toxin. Recently, Song et al. (*9*) also stated that the selection system using pathotoxic culture filtrates of *Septoria glycines* was correlated with brown spot disease-resistant soybean plants. Self-fertilization of regenerated plants proved resistance to the pathogen and

its toxin produced resistant plants that ranged from 70.2 to 82.5% of the offspring that retained the same degree of resistance to the pathogen. However, the segregation data obtained for the R_1 generation are not significant for determining the mode of inheritance of new traits, because the parental resistance regenerates are suspected to be chimeric. Further, genetic studies are needed to clarify this point and the stability of the selected *C. personatum* resistance.

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