Inability of tobacco callus to express resistance against avirulent race of *Phytophthora parasitica* var. *nicotianae* and various non-pathogenic fungi

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Summary : Tobacco calluses derived from stem pith of either the resistant (L-8) or the susceptible (Burley-21) cultivar were equally colonized by race 0 of *Phytophthora parasitica* var. *nicotianae*. Several attempts by changing the cultural conditions of callus such as modifications of the hormonal regime, light, and temperature failed to obtain differential colonization rates by the fungus. A wide variety of the non-pathogenic fungi, which could not normally attack stem of intact tobacco plant, did also colonized on callus. Since the plant stems derived from callus regained the resistance not only to avirulent race of *P. parasitica* var. *nicotianae* but also to the nonpathogenic fungi, it was concluded that the mechanism for loss of resistance in callus is due to other than the deletion of genes governing resistance from genome of callus cells, and presumably being due to repression of the resistance gene expression. Leaves of the resistant and susceptible cultivar were equally attacked by *P. parasitica* var. *nicotianae*, but resisted to certain non-pathogenic fungi. The results, thus, revealed differential degrees of susceptibility among tobacco tissues.

Introduction

Tissue culture systems have been employed for studying host-pathogen interactions in obligate[1,5] and facultative[4,8] parasitisms. Major advantages of the use of such tissue culture systems appear to be that the host material can be grown under defined and repeatable growth conditions, and the study can be made in morphologically and physiologically similar cells, thus avoiding the complexity and physiological difference of whole plants. Futhermore, complication of the interaction by contaminating organisms is eliminated. Tissue culture system could also provide the possibility to study host-pathogen interactions on single cell-single cell basis.

During the course of our approach incorporating tobacco tissue culture system for the study of resistance mechanism, we have found that tobacco callus completely loses resistance not only to avirulent race of the pathogen but to various non-pathogenic fungi which do not normally attack the intact plants. Since tobacco plants recovered from callus regained resist-

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ance, it is likely that the genes for resistance is not deleted from the callus genome but their expression is repressed by unknown mechanisms. We report here the reactions of tobacco callus to various pathogenic and non-pathogenic fungi, comparing with those of stems and leaves of the intact plants.

Materials and methods

The two varieties of tobacco (*Nicotiana tabacum* L.), one resistant (L-8) and the other susceptible (Burley-21) to race 0 of *Phytophthora parasitica* var. *nicotianae* (Breda de Haan)·Tucker, were grown in vermiculite in 50 ml plastic tubes at about 28°C under light for 30 to 45 days. The plants were daily watered with hyponex solution. Callus was induced from pith tissues on Linsmaier and Skoog's medium[7] supplemented with 2 mg indole-3-acetic acid (IAA) and 0.2 mg kinetin per liter of the medium.

Intact plants at 30- to 45-day-old were inoculated by laying a small piece of fungal mycelium on stems or leaves after making a wound by needles. Five-week-old calluses were inoculated as above except wounding and care was taken to avoid direct contact of the inoculum to the medium. The inoculated plants and calluses were incubated at 28°C under 100% relative humidity. Disease development was rated according to the following system: 0=no rot 1=slight rotting 2=considerable rotting 3=severe rotting. At least 10 pieces of calluses and 2 intact plants were used for each individual inoculation experiment.

Results

P. parasitica var. *nicotianae* race 0 successfully invaded stems of intact plants of the sus-

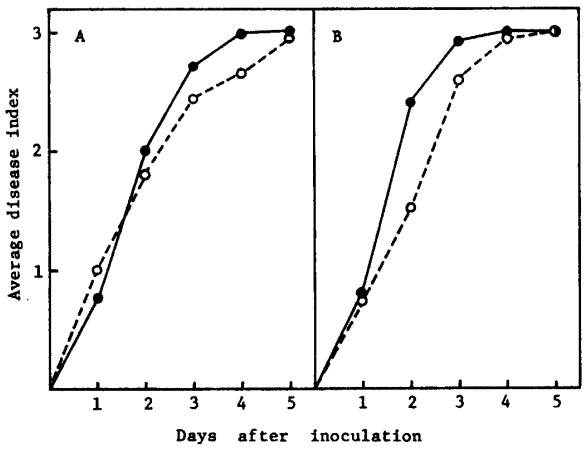

Fig. 1. Time course of disease development on 2-week- (left, A) and 5-week-old (right, B) calluses derived from tobacco stems resistant (L-8, ●—●) and susceptible (Burley-21, ○···○) to *P. parasitica* var. *nicotianae* race 0.

Table 1. Disease development on calluses grown under different cultural conditions following inoculation with *P. parasitica* var. *nicotianae* race 0

Condition for growth of callus	Average disease index[a]	
	Callus derived from resistant tobacco	Callus derived from susceptible tobacco
(Intact plant stem)	(0)	(3)
Standard method[b]	3	3
Kinetin 0.03 mg	2.8	3
2,4-D 0.5 mg, kinetin 0.1 mg	3	3
Sucrose 10g	3	3
Agar 5g	3	3
Agar 15g	2.4	2.7
Dark	2.7	3
20°C	2.8	2.6

a Readings were made at 5 days after inoculation.
b The condition described in Materials and methods in which the medium contained per liter 2 mg IAA, 0.2 mg kinetin, 30 g sucrose, and 8 g agar besides other inorganic ingradients, and callus was grown under light at 28°C.

Table 2. Disease development on various tissues of tobacco after inoculation with different pathogenic and non-pathogenic fungi

Inoculum	Average disease index[a]			
	Intact plant		Callus derived from stem pith	Intact plant derived from callus
	Stem	Leaf		
Phytophthora cactorum (Leb. et Cohn) Schroeter	0	3	3	—[c]
P. capsici Leonian	0	3	3	0
P. castaneae Katsura et Uchida	0	0	3	—
P. cinnamomi Rands	0	2	3	—
P. citricola Sawada	0	3	3	—
P. cryptogea Pethyb. et Laft.	0	2	3	—
P. drechsleri Tucker	0	2	3	—
P. erythroseptica Pethyb.	0	2	3	—
P. melonis Katsura	0	2	2, 3	0
P. palmivora (Butler) Butler	0	3	3	0
P. parasitica var. *nicotianae* (Breda de Haan) Tucker race 0	3(0)[b]	3(3)	3(3)	3(0)
race 1	3(3)	3(3)	3(3)	3(3)
P. parasitca var. *parasitica* (Dastur) Tucker	0	3	3	0
Pythium aphanidermatum (Edson) Fitzp.	0	0	3	0
P. debaryanum Hesse	0	0	3	0
P. irregulare Buisman	0	1	2	—
P. mamillatum Meurs	0	1	3	—
P. manospermum Pringsheim	0	0	3	—
P. nelumbium Takahashi	0	2	3	—
P. ostracodes Drechsler	0	0	3	—
P. spinosum Sawada spud Sawada et Chen	0	0	2	—
P. ultimum Trow	0	2	3	—
P. vexans de Bary	0	2	3	—
P. sp.	—	0	2	—
Alternaria radicina Meier, Dresch. et Eddy	0	0	3	—
Aspergillus niger van Tiegh.	—	0	3	—
Botrytis cinerea Pers. ex Fr.	0	0	3	—
Fusarium sp.	0	0	2	—
Pestalotia sp.	—	0	2	—
Rhizopus nigricans Ehrenb.	0	0	3	—
Sclerotinia sp.	0	0	3	—
Trichoderma sp.	—	0	3	—

a Readings were made at 5 days after inoculation of Burley 21 variety of tobacco which was susceptible to *P. parasitica* var. *nicotianae* race 0.
b The same as above but on resistant tobacco variety (L-8).
c Those which were not tested.

ceptible variety (Burley-21), but not those of the resistant variety (L-8). Calluses derived from either the resistant or the susceptible variety were, however, equally colonized by the fungus, and the colonization was not affected by the ages of calluses (Fig. 1). Thus, calluses apparently lost the resistance to the fungus. Since there is a possibility that the cultural conditions of callus may affect susceptibility, callus was grown under various conditions by mod-

ifications of the hormonal regime, sugar and agar concentrations, temperature, and light, and then followed by the inoculation with fungus. The results indicated that any condition employed did not bring about differential susceptibility in callus (Table 1). Furthermore, callus was extensively colonized by the wide variety of non-pathogenic fungi, including 11 species of *Phytophthora* and *Pythium*, and 8 species of non-pythiaceous fungi (Table 2), none of which could attack the stems of the intact plants. It appears, therefore, that the loss of resistance in callus was not specific to certain species of fungi but non-specific in nature. Since the plant stems derived from callus regained the resistance at least to the several fungi evaluated (Table 2), it is conceivable that genes for resistance were not lost during callus induction. It follows that in callus the resistance genes are not expressed at all or not fully to the extent as in the stems of intact plants. The leaves of intact plants revealed also more susceptibity to fungal invasion compared with the stems, although they resisted to at least several fungal invasion. These results may suggest that qualitatively different resistance mechanisms exist between stems and leaves of intact plants and both types of resistance are deleted from callus.

Discussion

The present study revealed that the resistance of intact tobacco plant to the avirulent race of *P. parasitica* var. *nicotianae* and to the various non-pathogens were completely lost during callus induction. The loss of resistance were possibly due to failure of callus in fully expressing resistance genes, and not due to missing of resistance genes *per se* from callus genome, since the plants derived from callus regained the resistance. This callus system will be a powerful tool for studying resistance mechanism when one can find any condition under which callus becomes to fully express its resistance. Under such conditions, the difference between resistant and susceptible calluses may be only on whether or not the resistance genes are expressed, and the study can be made on morphologically and physiologically similar cells with the same genetic background.

Differential susceptibility of callus to pathogens has been reported in a few host-pathogen systems[2,3,8]. We were, however, unable to obtain such differential susceptibility to either avirulent race of the pathogen or non-pathogens despite our several attempts by changing cultural conditions. It would be possible to find the differential susceptibility in our system also by further manipulations of the cultural conditions since the resistance genes are apparently conserved in callus.

It has been known that treatments such as heat and blasticidin S, a protein synthesis inhibitor, lead intact plants susceptible to the attacks by avirulent races of pathogens and various non-pathogens[9]. The mechanism of the resistance loss in callus would be, however, quite different from that brought by the treatments since it is unlikely in callus cells that protein synthesis is inhibited, as does blasticidin S treatment, or that any biologically important cellular constituent is inactivated, as does heat treatment. In this connection for assessing the mechanism for loss of resistance, it will be of prime interest to examine whether or not callus cells is capable of producing the phytoalexin which has been proposed in pathogen challenged intact tobacco[6].

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摘 要 タバコの羅病性品種（Burley-21）の茎は，その病原菌である *Phytophthora parasitica* var. *nicotianae* によって侵入を受けたが抵抗性品種（L-8）は侵入を受けなかった。しかしながら，両タバコ品種より誘導されたカルス組織は本病原菌により同程度によく侵入を受けた。ホルモン濃度，光，また温度を変化することによりカルスの培養条件を変化させても侵入の程度に差異は認められなかった。また，カルス組織は本来タバコ植物に病原性をもたない多くの非病原菌によってもよく侵入を受けた。これらカルス組織における非特異的な抵抗性消失は，カルス組織より誘導したタバコ植物が再び抵抗性を獲得するので，抵抗性遺伝子がカルス細胞に欠除したためであるとは考え難く，むしろ抵抗性遺伝子の発現が何らかの作用により抑制されていると考えられる。タバコの葉も羅病性ならびに抵抗性品種に関係なく *P. parasitica* var. *nicotianae* によってよく侵入を受けたが，数種の非病原菌に対しては抵抗性を示した。このようにタバコはその組織の違いにより，感受性を著しく異にする。