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Selection of Potato Callus for Resistance to Culture Filtrates of *Phytophthora infestans* and Regeneration of Resistant Plants

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Summary. Dihaploid calli from *Solanum tuberosum* were selected, which were resistant to the culture filtrate of *Phytophthora infestans*. Each of the resistant calli was resistant to all four pathotypes of *Phytophthora* used in these experiments. The resistance was not lost through regeneration and the induction of new callus.

Key words: *Phytophthora infestans* – Potato callus – Resistance – Selection

Introduction

Since Binding et al. (1970) demonstrated that it is possible to select callus cultures, many attempts have been made to apply such selection techniques. Many authors investigated only the resistance of the callus itself, whereas others (Maliga et al. 1973; Márton and Maliga 1975) also took into consideration the resistance of plants regenerated from selected callus cultures. The first application of such selective techniques for breeding purposes was reported by Carlson (1973), who was able to produce tobacco plants from callus cultures which were resistant to the toxin of its congenial parasite. Gengenbach et al. (1978) selected callus which was resistant to the toxin of *Helminthosporium maydis*. In this paper we report the selection of a potato callus which is resistant to the culture filtrate of *Phytophthora infestans*. The regeneration of plants resistant to the culture filtrate is also described.

Materials and Methods

Callus was obtained from six dihaploid clones of *Solanum tuberosum*. Such clones were chosen whose callus has a good regeneration capacity (Behnke 1975). The callus was initiated from young leaves on the medium of Linsmaier and Skoog (1965) modified by omitting the cytokinins and adding 5 mg NAA (α-naphthyl acetic acid) per liter. The regeneration medium (a modified Linsmaier and Skoog medium) contained 50 ml coconut milk, 0,1 mg NAA, and 5 mg BAP (benzyl-aminopurine) or 1 mg zeatin-riboside per liter. Young regenerated sprouts were transferred to a B5 – medium of Gamborg et al. (1968) (0,5 mg BAP, no auxins), where they regenerated strong leaves and many roots.

Dr. Erjefält (Svalöv) kindly supplied us the pathotype 1.2.3.4.5.7 and Dr. Schöber (Biologische Bundesanstalt, Braunschweig) the pathotypes 1.2.3.4. and 4. These three pathotypes and a fourth type 'Cologne' isolated by ourselves, were cultivated at 20 °C in 30 ml aliquots of Henniger's synthetic medium (1963). The same pathotypes were also maintained on potato tubers (Grata and Agora). In order to preclude a loss of virulence of the fungus, we inoculated regularly from the tubers to agar solidified Henniger's medium. These agar cultures were checked for infections by microscopical observation and afterwards cultures in fluid Henniger's medium could be initiated from them. The culture fluid was filtrated (pore width 1,5μm; Sartorius) after 3 weeks to remove the sporangia. Pathotype 4 was cultured four weeks because it was growing slower. The undiluted culture filtrates were used for preparing the toxic media. The toxic media contained the culture fluid of one pathotype and the normal ingredients of a Gamborg – B5 medium.

Lots of 20 pieces of callus (size about 1 mm) were placed in petri dishes onto the toxic media. Some of the cultures were irradiated with X-rays (0,516 Coulomb/kg = 2000R) in order to induce the production of mutations. After one week on B5 – medium they were transferred to the toxic media. During selection and regeneration procedures the cultures were illuminated 14 hours a day by white light of about 3000 lux. The callus initiation was obtained in dark. All cultures were held at 28°C.

The resistance of the plantlets to culture filtrates of *Phytophthora infestans* was tested by placing some leaves from sterile cultures on the same toxic media. Callus was again initiated in some leaflets of regenerated plants. This callus was also tested for resistance to the culture filtrate of *Phytophthora*.

Results

Most of the calli showed no further growth on the toxic media becoming brownish and dying after about three weeks. It was not possible to revive them on a non-toxic

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medium. These toxic media were used for selection of calli which were resistant to the culture filtrates of *Phytophthora infestans*. Growth on the toxic media is dependant 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 mm) for selection procedures. We couldn't make use of smaller callus pieces since callus pieces of less than 1 mm size also died on non-toxic media.

After four or six weeks some of the calli on the toxic media showed small growing and white areas (Fig. 1). From 41040 original calli, 153 were growing on the toxic media. After transfer to an identical medium only 36 calli survived. Calli which had survived transfer to a toxic medium three or four times could be selected from all 6 original clones. Spontaneous mutation could have occurred before the selection procedure. Therefore, it would be possible that some of the calli are selected whose resistance originates from the same mutation event. Perhaps the 36 selected calli come from less than 36 mutation events.

The mutation frequency of the irradiated calli was not much higher than that of the non-treated 41040 calli. Twenty out of 1400 irradiated calli were selected, 7 of them survived 2 subsequent transfers on the toxic media. In a second selection cycle 28 growing calli could be found. These were transferred for the second time to the toxic media.

The calli don't lose their resistance if they are maintained four or six weeks on non-toxic medium. They grow on all toxic media regardless of the pathotype of *Phytophthora infestans* used for preparing the toxic medium. With the culture filtrate of type 1.2.3.4.5.7 it is possible to select cells which are resistant to type 4, the reverse is also true.

A part of the selected calli regenerated stems after some weeks. After these stems were allowed to grow to a certain size, 6 or 8 leaflets were removed and tested for resistance to the culture filtrate of *Phytophthora infestans*. Assuming that these calli are a mixture of resistant and sensitive sells we grew a number of stems (up to 30) from each selected callus for these tests. As controls we used plants which had been regenerated from the same original calli without being selected. Leaves of the control plants died and bleached on the toxic media after 3 to 6 weeks. Nearly half of them showed many necrotic flecks. Leaves of the control plants differed from leaves of regenerated plants of selected calli by chloroses and necroses and also by induction of callus on toxic media. After 6 weeks 91 leaves from 224 produced callus clumps on the toxic media while only 6 out of 111 leaves of the control plants grew small brown callus pieces. The callus initiation was best on the culture filtrate of the type Cologne or the type 1.2.3.4.5.7. Probably these types synthesize less toxins than the pathotype 1.2.3.4. A number of the leaves from selected calli also died on the culture filtrate of type 1.2.3.4. after three weeks but leaves of the control plants always showed a stronger toxic effect than leaves of plants from selected calli. This was true even if we took into account the number of chloroses and necroses or the initiation of callus (Fig. 2).

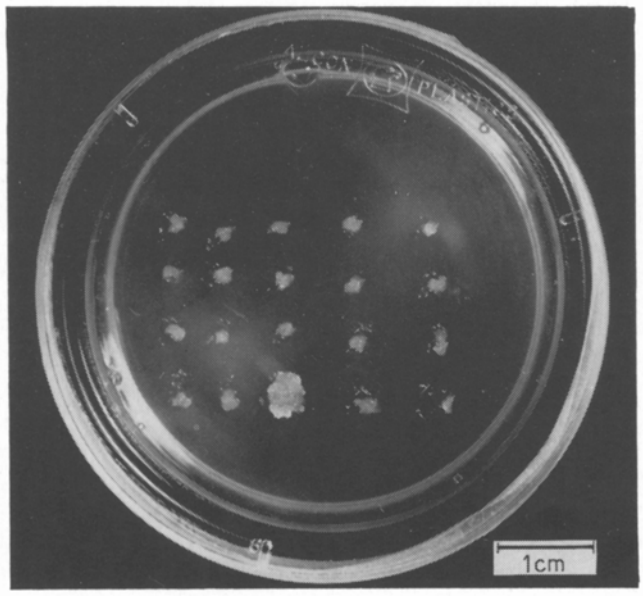

Fig. 1. Potato callus on medium containing culture filtrate of *Phytophthora infestans*. Only one callus piece is growing

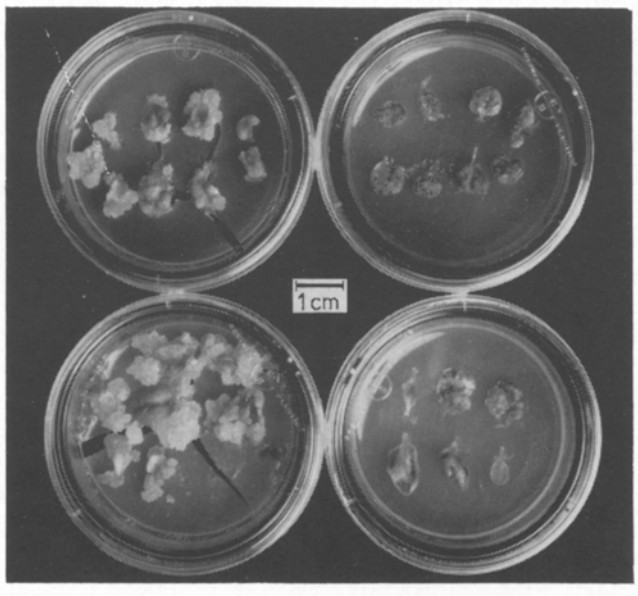

Fig. 2. Leaves of regenerated plants on medium containing culture filtrate of *Phytophthora infestans*. Left = leaves of plants regenerated from resistant calli, right = leaves of control plants

Leaves of all plants from selected calli reacted in the same manner to the same culture filtrate. The same reaction from leaves of stems regenerated from the same callus was observed. Leaves of the control plants, however, responded in a different manner to the toxic media. Leaves of clone HH 140 died very quickly, bleaching totally. It was not possible to revive them on a non-toxic medium. They showed only rarely necrotic spots. Leaves of clone HH 578 often retained green parts after three weeks but they were covered with necrotic spots. Meantimes we stimulated callus initiation in some leaves of regenerated plants. About half of these new calli grew on the toxic media. The selected resistance was not lost through regeneration.

Discussion

Resistance of potato callus to a culture filtrate of *Phytophthora infestans* remained preserved even after regeneration and a second callus induction, indicating that resistance in the callus culture is not caused by a selection of cells with changed gene activity. That changed gene activity of cells can be expected after selection in a callus has been demonstrated by treatment with cycloheximid (Maliga et al. 1976). Often the fact that mutagenic treatment enhances the result of selection procedure is taken as an indication that mutation and not altered gene activity is selected for. The potato is propagated vegetatively, therefore spontaneous mutations will accumulate during the cultivation time. This is probably the reason why the number of resistant calli could not be increased by irradiation with x-rays. The selected calli could still be a mixture of resistant and sensitive cells. As long as it is unknown whether stem regeneration in calli begins from one cell or from numerous cells, it has to be assumed that the toxin resistant plants are chimars consisting of a mixture of resistant and sensitive cells. Some evidence that the regenerated plants are not such chimars is the observation that their leaves react uniformly to the toxic media.

In the near future we will investigate how culture filtrate resistant plants behave against an infection with *Phytophthora infestans*. The aim of this program is to obtain plants with a general field resistance against the parasite. Wheeler and Luke (1955) and Byther and Steiner (1972) have already described cases where resistance to a fungal parasite was correlated with the resistance to its toxins. Also, Matern, Strobel and Shepard (1978) showed that the reaction of potato plants to the toxins of *Alternaria solani* is correlated with the reaction to the parasite itself if they measure the size of the lesions but not if they count the number of lesions. Gengenbach et al. (1978) selected callus which is resistant to Helminthosporosid, the toxin of *Helminthosporium maydis*. From this callus they grew plants which were resistant to the parasite itself. Therefore we can expect a general resistance of the culture filtrate resistant plants to *Phytophthora infestans*. But until now it had not been proven whether *Phytophthora infestans* produces the identical toxins in vivo as it does in Henniger's medium.

We have used the same method in selecting resistance of potato clones to a fungal parasite with the culture filtrate of *Fusarium oxysporum*. We will describe this in subsequent articles.

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