Somaclonal variation for resistance to *Verticillium dahliae* in potato (*Solanum tuberosum* L.) plants regenerated from callus

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**Summary**

Plant tissue culture is recognized as an important tool to generate useful genetic variability for crop improvement. Regenerated plants from callus induced from stem explants of *Solanum tuberosum* cv Désirée were assessed by *in vitro* selection, for resistance to *Verticillium dahliae*. This fungus is the causal agent of *Verticillium* wilt, a serious vascular wilt disease both in crops and wild species.

The rate of *in vitro* multiplication by single node cuttings was used as a parameter of screening in two selection cycles with different concentrations of *V. dahliae* filtrate. One resistant clone was selected and then evaluated by inoculation in the growth chamber. Induced damage, and morphological traits (dry weight, leaf area and tuber production) were estimated. The selected clone was comparable to the resistant control, cv Kondor.

The results suggest that genetic variation induced in tissue culture could be utilized to generate disease resistance.

**Introduction**

*Verticillium* wilt is caused by two soilborne fungi, *Verticillium dahliae* Kleb and *V. albo-atrum* Renke and Berthold (Rowe et al., 1987). This disease is a limiting factor for production of all the commonly grown varieties of potato especially in dry climates (Susnoschi et al., 1976). It affects the quality of potato tubers, causing browning of cortical and parenchymal cells and cellular collapse around the xylem. *V. dahliae* invades the vascular elements of host plant mainly through wounds in roots, induces collapse of vessels and fills them with mycelia and gums leading to plant death (Isaac & Harrison, 1968). Although it has no specific hosts, *V. dahliae* infects a wide range of crops (tomato, alfalfa, strawberry, eggplant, watermelon), different genera (*Chenopodium, Taraxacum, Papaver, Capsella*), and even many species of trees (Rich, 1983).

The genetics of resistance is unclear but polygenic control in eggplant, potato, alfalfa and *Capsicum* is reported (Alconero et al., 1988; Goth & Webb, 1981; Latunde-Dada & Lucas, 1983; Palloix et al., 1990; Martin et al., 1993), while in tomato, resistance is controlled by a single dominant gene (Schaible et al., 1951).

The genus *Solanum* is characterized by broad genetic variability and resistance to *V. dahliae* exists within the wild species *S. chacoense*, *S. sparsipil- lium*, *S. microdontum* and *S. torvum*. That resistance could be transferred to *S. tuberosum* but for the necessity to preserve allelic and non allelic genetic interaction in cultivated varieties and the occurrence of sexual incompatibility that hinders the interspecific hybridization.

New genetic variability induced by *in vitro* tissue culture has been reported for many crops (for review see, e.g., Scowcroft et al., 1987; Karp, 1991). A wide range of plant characteristics can be altered as a result of regeneration from cell and tissue culture, including agronomically important traits such as disease resistance (Van den Bulk, 1991). Particularly in potato, the utilization of somaclonal variation made it possi-
ble to obtain resistance to several pathogens such as *Phytophthora infestans* (Matern et al., 1978; Behnke, 1979, 1980a), *Alternaria solani* (Shepard et al., 1980), *Fusarium oxysporum* (Behnke, 1980b), *Streptomyces*, PLRV, PVY (Thomson et al., 1986) and PVX (Wenzel et al., 1987).

Resistance to *Verticillium* wilt induced by *in vitro* tissue culture has been attained in *Medicago sativa* (Latunde Dada & Lucas, 1983, 1987, 1988; Frame et al., 1991), while in potato breeding, *in vitro* techniques have been exploited to transfer this trait from *Solanum torvum* S.W. into *Solanum tuberosum* by protoplast electrofusion (Jadari et al., 1992).

The purpose of the present work was to screen regenerated plants from callus culture of potato stem explants of cv Désirée, for resistance to *V. dahliae*. Disease symptoms and morphological data after inoculation of a selected resistant clone were determined.

**Materials and methods**

**Plant materials**

Potato tubers (*Solanum tuberosum* L.) cv Désirée (tolerant to *V. dahliae*; Susnoschi et al., 1976) and the control cultivar Kondor (resistant to *V. dahliae*; Buchner et al., 1989), supplied by Department of Agricultural Plant Biology, Horticultural and Floriculture Section, University of Pisa, were maintained with dark condition at 25 ± 1 °C in growth chamber until sprouting.

**In vitro multiplication**

Shoots (5 cm long) were cut from tubers and the wounds covered with liquid paraffin. Successively they were surface sterilized for 10 min in 10% ACE (sodium hypochlorite) and then washed three times for 10 min with sterilized distilled water. After sterilization they were aseptically placed on 20 ml solidified (0.8% Bactoagar) medium in 150 ml Erlenmeyer flasks and incubated in growth chamber at 25 ± 1 °C under a 16/8 h day/night regime (60 µE/m²/sec fluorescent light). Cultural medium was composed of MS basal medium (Murashige & Skoog, 1962) containing 0.5 mg/l of α-naphthaleneacetic acid (NAA) and 0.5 mg/l Zeatin riboside (ZR) (modified from Hu & Wang, 1983). Rooted shoots were then multiplied by single node cuttings on MS medium without growth regulators (Hussey & Stacey, 1981).

**Callus cultures and plant regeneration**

Stem explants of cv Désirée (0.5 cm long) from multiplied plantlets were placed in Petri dishes (100 × 15 mm) on callus induction medium composed of MS basal medium supplemented with 2.25 mg/l 6-benzylamino purine (BAP) and 0.186 mg/l NAA (Webb et al., 1983). Cultures were incubated in growth chamber in the same conditions previously described. After 30 days the induced calli were transferred to the regeneration medium (MS basal medium plus 10 mg/l gibberelic acid (GA₃) and 2.25 mg/l BAP) (Webb et al., 1983) and subcultured on fresh medium every three or four weeks. The developed shoots (2 cm long) were excised and placed on solidified (0.8% Bactoagar) MS basal medium without growth regulators for rooting.

**Verticillium dahliae filtrate**

Conidial suspensions of *V. dahliae*, strain PA1 (supplied by Department of Plant Pathology, University of Bologna) were added to 250 ml of liquid medium according to Nachmias et al. (1985) in 1,000 ml Erlenmeyer flasks. These were placed on a rotary shaker (80 rpm) at 25 ± 1 °C in the dark for three weeks. Mycelia and conidia were removed by filtration through Whatman N° 1 filter paper and following centrifugation for 10 min at 3,000 rpm (Durrands & Cooper, 1988). Fungal filtrate pH were adjusted to 5.7 with 1 M KOH before filter sterilization through Millipore membrane (0.22 µm).

**In vitro selection**

Nodes from cvs Désirée and Kondor were grown for two weeks on 20 ml MS liquid medium ammended with three concentrations (12.5%, 25% and 50%) of *V. dahliae* filtrate, in the same conditions described in 'In vitro multiplication'. Each treatment was replicated 50 times and all experiments were repeated at least three times. Vitality was calculated as percentage of well grown shoots obtained from the total nodal explants cultured. Discrimination between resistant (Kondor) and tolerant (Désirée) cultivars was possible at 12.5% and 25% fungal filtrate concentration (Table 1).

Nodal explants from regenerated shoots of cv Désirée were selected (first selection cycle) in 25% fungal filtrate concentration. Developed, non deformed shoots were propagated through the system described in 'In vitro multiplication' and successively submitted.