Theor Appl Genet (1987) 74:480–482

Herbicide tolerant regenerates of potato

G. Wersuhn, K. Kirsch and R. Gienapp

Sektion Biologie, Humboldt-Universität Berlin, Invalidenstrasse 43, DDR-1040 Berlin, German Democratic Republic

Received December 30, 1986; Accepted March 5, 1987
Communicated by G. Wenzel

Summary. Culture-derived plants and cell cultures of potato (*Solanum tuberosum* L.) respond to the application of the herbicides SYS 67 ME (MCPA) and OMNIDEL (Na-2,2-dichloropropionate) in a comparable fashion. By gradually increasing the herbicide concentration, cell lines were developed which tolerated 50 mg/l of ME or 300 mg/l of OMNIDEL. Any further increase in concentration resulted in the death of all cell cultures. From cell cultures that had been able to grow on media supplemented with 30 mg/l of ME, regenerate plants were obtained that were also tolerant to this concentration. This new trait was retained even after repeated vegetative propagation of the plants.

Key words: In-vitro selection – Herbicide tolerance – Regeneration – Tolerance test – *Solanum tuberosum* L.

Introduction

In countries with advanced agriculture, weeds are mainly controlled by herbicides. However, the selective pressure of herbicides results in the development of tolerant and changed weed populations (Whitehead and Switzer 1963; Gressel and Segel 1982). One way of avoiding these consequences is to use several herbicides. On the other hand, herbicide-tolerant crop cultivars would allow an extension in the application of a herbicide (Faulkner 1982).

Herbicide tolerance may be due to a single dominant or recessive allel, but in most cases it is probably controlled polygenically (e.g. Faulkner 1982). The investigation of progenies obtained from crosses among sorghum lines has indicated that the response to 2,4-D is controlled by a single dominant allel (e.g. Souza Machado 1982).

Although phenoxy-herbicide tolerant cell lines could be selected in several plants except tobacco, no stable tolerant regenerates have as yet been obtained (Chaleff and Parson 1978; Meredith and Carlson 1982). But Harvey and Muzik (1973) have reported that the differential response of intact plants to 2,4-D will be retained in the tissue cultures derived from such plants.

The present paper deals with the in vitro selection of MCPA and Na-2,2-dichloropropionate tolerant cell lines from potato cell cultures, and with experiments to test the tolerance of regenerated plants.

Materials and methods

Plant material and herbicides

Two parthenogenetically developed dihaploid lines (9046, 2278) and two tetraploid cultivars ('Xenia', 'Arkula') of *Solanum tuberosum* were used. Selection was for tolerance to the herbicides SYS 67 ME (2-methyl-4-chlorophenoxy acid, MCPA) and SYS OMNIDEL (sodium-2,2-dichloropropionate), which were both purchased from VEB Synthesewerk Schwarzheide, GDR.

Preparation of cell suspensions

Shoots of aseptically grown and propagated plants were cut into segments, which were incubated in a liquid medium (Opatrny 1979) on a gyratory shaker (180 rpm) at 25 °C in the dark. Suspension cultures available for selection procedures consisted of single cells and cell colonies of different sizes, in which the cell mass doubled every 6 days.

Sensitivity testing and cell-line selection

Here, 2 ml of suspension were plated on each petri dish (9 cm) containing a selective liquid medium, as described by Wersuhn and Fritze (1985). Herbicide solutions were added to the medium in concentrations of 10, 20, 30, 40 or 50 mg/l ME or 90, 120, 180, 240 or 300 mg/l OMNIDEL. Fresh weight was tested on callus cultures developing from pipetted suspensions on selective medium. The entire callus material of each petri

dish was transferred by transmitting the filter to a weighed fresh plate, and then the weight was determined once again. In order to demonstrate the response of in-vitro plants to the application of herbicides, plants were cultivated using a selective medium at 25 °C and 2,000 lux. After every 7 days, the length of the plants was measured and values were given in per cent of control.

To select herbicide tolerant cell lines, suspensions were plated in the manner described above, and after 2 months well-growing callus was transferred to a medium containing higher herbicide concentrations.

Regeneration and tolerance test

Pieces of herbicide tolerant calli were excised and transferred to an agar-solidified medium (Shepard and Totten 1977, modified). Regenerate plants were cloned by rooting of shoot segments, and only well-developed plants were sprayed with the herbicide solution every 3 days throughout the 21 day test time. Thereafter, the height of the plants was measured. In order to verify the results, plants derived from tested clones were propagated once more and included in tolerance tests.

The differences in heights of regenerated and control plants after herbicide application were tested statistically by the *t*-test; when tests were significant, the regenerates were taken as tolerant.

Results

Response of cell cultures and in vitro plants to herbicides

Cell cultures and in vitro plants of four genotypes were exposed to the same range of herbicide concentrations. Since the differences in the results obtained for these genotypes can be neglected, only those for clone 9046 are presented (Figs. 1 and 2). Although there are some quantitative differences in the response of cell cultures and plants, the response is basically identical. This result opens up the possibility of developing herbicide-tolerant plants by selection from cell cultures.

Selection of tolerant cell lines

The results summarized in Figs. 1 and 2 demonstrate that cell cultures for clone 9046 on medium supplemented with 40 mg/l ME or 240 mg/l OMNIDEL cease to grow. This also holds true for other genotypes. Therefore, calli showing normal growth on medium supplemented with 30 mg/l ME were transferred to 40 mg/l and if even under these conditions callus grew well, the herbicide concentration was raised to 50 mg/l. Calli exhibiting a satisfactory increase in cell mass during two subcultures were isolated and developed into cell lines.

The selection procedure for the herbicide OMNIDEL started with a concentration of 180 mg/l. For cv 'Xenia' and clone 2278, a stepwise increase in herbicide level resulted in the development of cell lines growing on 300 mg/l, whereas for clone 9046 no cells surviving on medium supplemented with 240 mg/l could be selected.

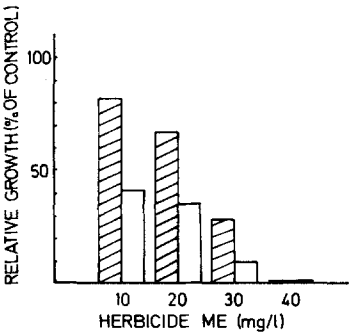

Fig. 1. Growth (% of control) of cell cultures (fresh weight) and plants (height) on selective medium containing herbicide ME after 21 days for clone 9046; □: cell culture; ▨: in-vitro plants

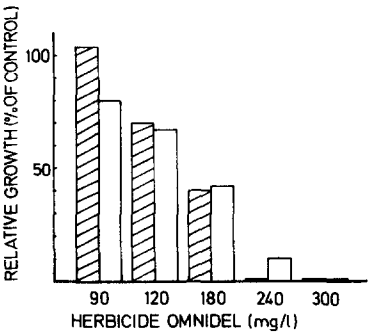

Fig. 2. Growth (% of control) of cell cultures (fresh weight) and plants (height) on selective medium containing the herbicide OMNIDEL after 21 days for clone 9046; □: cell culture; ▨: in-vitro plants

Table 1. Number of MCPA tolerant plant clones of the 9046 and 'Xenia' genotypes; period of herbicide application was 21 days

Genotype	Herbicide concentration (mg/l)	No. of tested clones	No. of tolerant clones	Tolerant clones (%)
9046	30	28	15	54
9046	20	12	0	0
'Xenia'	20	7	0	0

Tolerance of regenerated plants

Callus from cell lines subcultivated under selective conditions (30 mg/l ME) were transferred to regeneration medium. The regenerate clones were tested for tolerance to herbicides using concentrations of the medium from which callus was obtained. Plants of tested clones were again multiplied and tested. The results of these two experiments were about equal (Table 1). Cell lines selected on a medium supplemented with 40 or 50 mg/l ME or 240 or 300 mg/l

OMNIDEL failed to produce any regenerates. One clone of the cv 'Xenia' that had originated from a cell line isolated on medium supplemented with 180 mg/l OMNIDEL showed normal growth and significant differences as against the OMNIDEL-treated control. After application of ME, shoots of tolerant clones exhibited normal growth compared with that of untreated and ME-treated controls, though root development was at first inhibited by callus formation. Root growth returned to normal 10 days after the termination of herbicide application. At present, attempts are being made to transfer tolerant plants to soil culture.

Discussion

There are clearly interrelations between the herbicide concentration applied and the number of tolerant regenerates developed from selected cell lines. Although cell lines growing on medium supplemented with 10 or 20 mg/l ME are characterized by a relatively high regeneration rate, they have not yielded any tolerant regenerates. This is probably due to stringent selection resulting in the isolation of cells with a genetic basis for tolerance. Therefore, the application of high herbicide concentrations is useful for obtaining regenerates in which the new trait is stable. However, according to our observations, regeneration was halted by high concentrations of the selective agent (50 mg/l ME; 300 mg/l OMNIDEL).

The development of tolerant cell cultures following the stepwise increase in ME and OMNIDEL levels need not be due to adaptation processes, but may be attributed to the isolation of single cells from a cell colony, which is promoted by this procedure. Successful selection procedures, including a gradual increase in herbicide concentrations, have also been reported (e.g. Meredith and Carlson 1982).

Although it is very difficult to compare the necessary tolerance levels of crops grown under field conditions with the levels achieved in cell cultures and regenerated plants, a tolerance to 30 mg/l may be assumed.

The relatively high number of tolerant clones (Table 1) might, on the one hand, be explained by a single dominant allel and the propagation of cells during suspension culture. On the other hand, potato suspension cultures and calli developed either from single cells or from small cell colonies will retain a relatively high karyotypic constancy during selection and regeneration (Dathe and Wersuhn, unpublished data).

Acknowledgement. The authors are grateful to Prof. T. Börner for his critical reading of this manuscript and his suggestions.

References

Chaleff R, Parson M (1978) Direct selection in vitro for herbicide-resistant mutants of *Nicotiana tabacum*. Proc Natl Acad Sci USA 75:5104–5107

Faulkner J (1982) Breeding herbicide-tolerant crop cultivars by conventional methods. In: LeBaron J, Gressel J (eds) Herbicide resistance in plants. Wiley, New York Chichester Brisbane Toronto Singapore, pp 235–256

Gressel J, Segel L (1982) The paucity of plants evolving genetic resistance to herbicides: possible reasons and implications. J Theor Biol 75:349–371

Harvey R, Muzik T (1973) Effects of 2,4-D and amino acids on field bindweed in vitro. Weed Sci 21:135–138

Meredith C, Carlson P (1982) Herbicide resistance in plant cell cultures. In: LeBaron J, Gressel J (eds) Herbicide resistance in plants. Wiley, New York Chichester Brisbane Toronto Singapore, pp 275–291

Opatrny Z (1979) Physiological aspects of potato cell and protoplast culture. Ergeb Exp Med 34:107–119

Shepard J, Totten R (1977) Mesophyll cell protoplasts of potato, isolation, proliferation and plant regeneration. Plant Physiol 60:313–316

Souza Machado V (1982) Inheritance and breeding potential of triazine tolerance and resistance in plants. In: LeBaron J, Gressel J (eds) Herbicide resistance in plants. Wiley, New York Chichester Brisbane Toronto Singapore, pp 257–273

Wersuhn G, Fritze G (1985) Ein neues Verfahren für die pflanzliche in vitro-Kultur. Biochem Physiol Pflanz 180: 79–83

Whitehead C, Switzer C (1963) The differential responses of wild carrot to 2,4-D and related herbicides. Can J Plant Sci 43:255–262