Theor. Appl. Genet. 58, 145-148 (1980)

Induced Mutants from Dihaploid Potatoes After Pollen Mother Cell Treatment

T. Przewoźny, O. Schieder and G. Wenzel
Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut), Köln-Vogelsang (Federal Republic of Germany)

Summary. Microspore mother cells of dihaploid *Solanum tuberosum* plants were mutagenically treated during the stage of meiosis. Mutagenesis was performed either by irradiation with x- or γ-rays or by the application of nitrosomethylurethane or methylnitronitrosoguanidine. Then, by use of the anther culture technique, 913 functional plants and 442 untreated control plants were regenerated. From the exposed plants seven distinct mutants could be isolated, predominantly chlorophyll deficient lines, while from the controls no clear-cut mutants arose. One mutant turned out to be photomorphogenetic in addition to having a chlorophyll defect. In addition to the production of mutants the treatments significantly increased the frequency of multicellular structure formation from microspores.

Key words: Anther culture – Mutagenesis – Potato – Haploids

Introduction

One of the major drawbacks in the mutation breeding of higher plants is the formation of chimeras, following the mutagenic treatment of a multicellular organism. Using polyploids, such as the autotetraploid potato with 2n = 4x = 48 chromosomes, the formation of stable mutants is even more restricted. Up until now, only one new mutant variety in *Solanum tuberosum* has been released (see: van Harten 1978). The formation of chimeras may be overcome by producing plants from one mutant cell, e.g. from protoplasts or microspores. Starting with haploid material, i.e. in potato, by taking microspores from dihaploid plants (2n = 2x = 24) with 12 chromosomes, also permits the detection of recessive mutants in the monohaploid offspring. The first experiments using microspores as starting material for mutagenesis were carried out with tobacco (Devreux and Saccardo 1968; Nitsch 1972; Sunderland 1973). While Sunderland could not detect mutants, Nitsch found a white flowered plant after nitrophenylurea treatment. As in potato regeneration of functional plants from microspores in certain dihaploid genotypes is possible with a reasonable frequency (Sopory et al. 1978; Wenzel et al. 1979), we used pollen mother cells and descending microspores to investigate the action of irradiation and chemical mutagens. The aim of this work was to test this system, and to find markers which will be useful for chromosome mapping and for the identification of somatic fusion products and not to produce mutants for direct variety production.

Materials

The microspores were cultured within the anthers and these were harvested from two different interdihaploid potato clones: H^3 3-703 and H^2 258. Further, several clones of the F_1 hybrid family H I, a cross between H^2 236 and *S. phureja* were used as anther donor material. The potato plants were grafted on tomato stocks, to guarantee flowering all through the year, and were grown under a 16 h light regime (illumination if necessary extended by HQI-T 1,000 W Osram lamps, with an intensity of 12.000 lux at the growing point of the plants) at a temperature of 18±2°C. Irradiation was performed by an x-ray machine (Richard Seifert & Co., Hamburg, F.R. Germany), adjusted to 180 kV, 15 mA at a rate of 1,000 R (35 cm distance; time: 10 min), or by γ-rays (^{137}Cs; distance 3 m; with 25 R min $^{-1}$). As the chemical mutagen, N-nitroso-N-methylurethane (NMU) or N-methyl-N-nitro-N-nitrosoguandine (NMG) was used.

Methods

The irradiation was performed at the top of the plants, in the area of the inflorescenses at the developmental stage of the pollen mother cells prior to meiosis. The other parts of the plants were protected by lead shields. γ-ray irradiation was given at doses of 200; 500; 1,000; 2,000 and 3,000 R. For chemical mutagenesis

0040-5752/80/0058/0145/$ 1.00

aqueous solutions of NMU and NMG in concentrations of 0.13; 0.25; 1.0; 1.2 and 2.0 mM and 0.05; 0.1; 0.5; 1.0; and 2.0 mM, respectively, were applied. Inflorescenses with microspore mother cells were dipped into the mutagen solution for 3 h. Control plants were dipped for the same time into tap water. After treatment all exposed parts of the plants were thoroughly washed under running tapwater, and then returned to normal greenhouse conditions. At the time of the first microspore mitosis (normally one week after mutagenic treatment), the buds were harvested, the anthers dissected and handled as described previously (Sopory et al. 1978).

Results

In total, in this experimental series 1355 plants were regenerated from microspores of dihaploid *S. tuberosum* clones. All androgenetic plants doubled up spontaneously during culture and turned out to be dihaploid again when checked for their ploidy level in the functional stage by plastid counts in the stomata. Most probably they can be termed as homozygous since a marker being heterozygous in the anther donor material segregated in a 1:1 manner (Wenzel et al. 1979). Also other characters, like leaf shape, stem colour and tuber skin, showed strong phenotypic variation. From nearly all plants tubers could be harvested, and from these, plants were grown in the field in the next generation. Comparison of the 913 treated and the 442 control plants did not show, however, a striking difference in the frequency of phenotypic variants, but it is probable that additional effects due to the mutagenic treatment appeared amongst the segregants, where already a high amount of variability was present because of pure random meiotic distribution.

Besides those plants with no distinct mutations, seven clear mutants could be isolated in vitro, which means about 1% mutants in relation to the number of regenerated treated plants. Six mutants were derived from the clone H^3 3-703, five of which arose after irradiation either with 1,000 kR or 2,000 kR, and one from the clone H^2 258 after NMU treatment at a concentration of 1.0 mM (Table 1). Three mutants were chlorophyll deficient (Fig. 1A, C), three were viridis and one showed very small filiforme leaves (Fig. 1B). One of the chlorophyll deficient mutants (Fig. 1C) had, in addition, a drastically changed phenotype: it looked like a moss when grown on agar at 500 lux for 16 h (white fluorescent tubes). By stronger illumina-

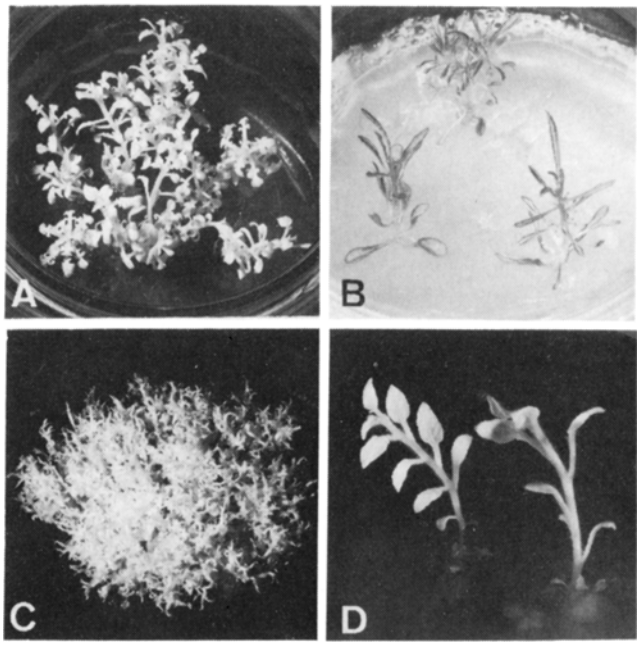

Fig. 1A-D. Mutants obtained after treatment of pollen mother cells of dihaploid *Solanum tuberosum* plants. A Chlorophyll deficient type; B Filiforme mutant; C Moss-like chlorophyll deficient strain under 500 lux and D under 3.000 lux illumination

Table 1. Synopsis of significant results obtained after mutagenic treatments of microspores of dihaploid *Solanum tuberosum* clones

Clone	Mutagen	Dose	Number of plated anthers	macroscopic structures		regenerated plants	distinct mutants	Type
Control	–	–	2.035	1.457	72%	102	0	–
H^3 3-703	NMU	0.05mM	960	825	86%	44	0	–
	NMU	0.1 mM	725	1.388	191%	101	0	–
	NMU	0.5 mM	805	1.310	163%	86	0	–
	NMU	1.0 mM	980	581	59%	14	1	filiforme
	NMU	2.0 mM	920	127	14%	9	0	–
	x-ray	1.000R	1.030	438	43%	62	3	viridis albino albino/photomorph
	x-ray	2.000R	1.120	140	10%	27	2	viridis
H^2 258	NMU	1.0 mM	580	14	2%	3	1	albino

tion (3,000 lux Natura fluorescent tubes) normal morphology could be induced, but it remained albino (Fig. 1d). Most probably this mutant is a photomorphological mutant in addition to having the chlorophyll defect. Furthermore, this mutant showed a strong variability in its anthocyanin content, which was dependent on the phytohormone concentration in the culture medium: It was highest when no cytokinins were added; the plant turned completely white, when, e.g., 1 mg l^{-1} BAP was present. As the albino mutants could not be transferred from in vitro culture into the greenhouse, there exists no data about their mode of inheritance, e.g., via the plastome or the nuclear genome. The chlorophyll deficient mutants are, however, most probably of nuclear origin, as no chimeras were found.

In addition to these findings, the mutagenic treatment acted as an unspecific shock which increased the rate of macroscopic structure formation from microspores developing in the plated anthers. This was true for irradiation, but more enhanced after chemical treatment, particularly after exposure to 0.1 or 0.5 mM NMU. Here, the percentage of macroscopic structure development could be increased from 71% to 191% (Table 1).

Discussion

Up until now, for mutation work in potato, dihaploid material has only occasionally been used, and here predominantly shoot apices served as tissue sources, having the disadvantage of a multicellular nonhaploid-system (van Harten 1978). Starting with microspore mother cells or with microspores from dihaploid potatoes, both the ploidy problem as well as the multi cell nature could be avoided. From such material it was possible to obtain, via mutagenesis, mutant lines which become homozygous and dihaploid via spontaneous doubling, as they most probably arose from monohaploid microspores. From the albino mutants, protoplasts could be isolated and grown into small calluses. Experiments are now in progress, to use such chlorophyll deficient lines as nurse culture for mechanically isolated fusion products amongst mesophyll and callus derived *S. tuberosum* protoplasts (Schieder, Przewoźny and Wenzel, in preparation) according to the system proposed by Menczel et al. (1978).

The comparison of the different ways of mutant induction indicates that irradiation is a more powerful tool: from the 7,515 anthers isolated from irradiated inflorescences 528 plants could be regenerated from clones H^3 3-703 and H^2 258, five of which turned out to be mutants. From the 10,880 anthers which were treated with NMU, 390 plants and 2 mutants arose. In this experimental series, from the 10,775 NMG treated anthers amongst 311 regenerants, no distinct mutant was found. These results indicate, on the one hand, a higher mutagenic capacity of irradiation, as described by Schieder (1976) and Krumbiegel (1979) for chlorophyll deficient mutants obtained from *Datura*. On the other hand, the more severe alteration of mutants derived from irradiation underline that here chromosome mutations with some disadvantages for application (see review Nitzsche and Wenzel 1977) are produced. Besides the mutagenic effect, all treatments increased at certain concentrations the number of regenerated androgenetic macroscopic structures. This is quite probably an unspecific shock similar to those that would be produced by pretreatment or other physical stresses (Nitsch and Norreel 1973; Wenzel et al. 1977). Both irradiation and chemical mutagens, acted similarly; the strongest increase was, however, after NMU treatment. Unfortunately, this enhancement – like many other parameters in microspore culture – was dependent on the genotype. Again, the final result is influenced by genetical, physiological and environmental factors.

Acknowledgement

The expert technical assistance of Miss B. Merkel is gratefully acknowledged. The work was supported partially by a grant of the Deutscher Akademischer Austauschdienst to T.P.

Literature

Devreux, M.; Saccardo F. (1968): Effets des radiations gamma sur les stades successife de dévelopment du gamétophyte mâle de tabac. Rad. Bot. 8, 365-374

Harten, A.M. van (1978): Mutation breeding techniques and behaviour of irradiated shoot apices of potato. Wageningen: Pudoc.

Krumbiegel, G. (1979): Response of haploid and diploid protoplasts from *Datura innoxia* Mill. and *Petunia hybrida* L. to treatment with x-rays and a chemical mutagen. Envir. Exp. Bot. 19, 99-103

Menczel, L.; Lázár, G.; Maliga, P. (1978): Isolation of somatic hybrids by cloning *Nicotiana* heterokaryons in nurse cultures. Planta **143**, 29-32

Nitsch, C.; Norreel, B. (1973): Effet d'un choc thermique sur le pouvoir embryogen du pollen de *Datura innoxia* cultivé dans l'anthère ou isolé de l'anthère. C. R. Acad. Sci. (Paris) **276D** 303-306

Nitsch, J.P. (1972): Haploid plants from pollen. Z.Pflanzenzücht. 67, 3-18

Nitzsche, W.; Wenzel, G. (1977): Haploids in Plant Breeding. Berlin, Hamburg; P. Parey

Schieder, O. (1976): Isolation of mutants with altered pigments after irradiation haploid protoplasts from *Datura innoxia* Mill. with x-rays. Molec. gen. Genet. 149, 251-254

Sopory, S.K.; Jacobsen, E.; Wenzel, G. (1978): Production of monohaploid embryoids and plants in cultured anthers of *Solanum tuberosum*. Plant Sci. Lett. 12, 47-54

Sunderland, N. (1973): Pollen and anther culture. In: Plant Tissue and Cell Culture ed.: (Street, H.E.), pp. 205-239. Oxford, London, Edinburgh, Melbourne: Blackwell Sci. Publ.

Wenzel, G.; Hoffmann, F.; Thomas, E. (1977): Increased induction and chromosome doubling of androgenetic haploid rye. Theor. Appl. Genet. 51, 81-86

Wenzel, G.; Schieder, O.; Przewoźny, T.; Sopory, S.K.; Melchers, G. (1979): Comparison of single cell culture derived *Solanum tuberosum* L. plants and a model for their application in breeding programs. Theor. Appl. Genet. 55, 49-55

Received May 12, 1980
Communicated by H.F. Linskens

Dr. O. Schieder
Dr. G. Wenzel
Max-Planck-Institut für Züchtungsforschung
(Erwin-Baur-Institut)
D-5000 Köln 30 (Federal Republic of Germany)

Dr. T. Przewoźny
Polish Academy of Sciences
Institute of Plant Genetics
Strzeszyńska 30-36
60-479 Poznań (Poland)