Acta Bot. Croat. 48, 35—38, 1989.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 632.23:582.951.4=20

TUMOR TRANSFORMATION IN POTATO TUBER TISSUE

MARIJANA KRSNIK-RASOL and *TATJANA BAKRAN-PETRICIOLI*

(Department of Botany, Faculty of Science University of Zagreb)

Received December 19, 1988

Potato (*Solanum tuberosum* L. cv. Desirée) tuber tissue was infected with *Agrobacterium tumefaciens*, strains: B6S3 (wild type), pGV2255 (rooty mutant), pGV2215 (shooty mutant) and with *Agrobacterium rhizogenes* (8196). Successful tumor induction was achieved by B6S3 (unorganised tumors) and by pGV2255 (rooty tumors). Transformed tuber cells were smaller than normal ones and free from starch. Transformation process caused a significant increase in peroxidase activity which can be taken as a tumorigenetic marker. Electrophoretic protein pattern showed that tumor cells stopped producing a polypeptide of about 40 kd, which was a storage protein dominant in normal tissue.

Introduction

Crown-gall tumor is initiated by a virulent strain of bacterium *Agrobacterium tumefaciens* (Chilton 1983, Nester et al. 1984, Nacmias et al. 1987). A related pathogen *A. rhizogenes* induces »hairy root« disease in susceptible plants (Ooms et al. 1985, Oliver 1986, Hänisch ten Cate 1987). Both diseases are caused by transfer and stable integration of genes from Ti or Ri plasmid into plant chromosomes.

Plant cell transformation offers a powerful tool for studies of differentiation processes in plants (Nakajima et al. 1979, 1981). It also offers a useful way for gene manipulation in plants (Caplan et al. 1983, Chilton 1983).

The present study concerns the analysis of tumorigenesis in potato (*Solanum tuberosum* L. cv. Desirée) tuber tissue which has been undertaken in order to define the conditions for successful tumorigenesis and regeneration of transformed plants.

Materials and Methods

Surface sterilized potato tuber discs (*Solanum tuberosum* L. cv. Desirée) were infected with *Agrobacterium tumefaciens*, strain B6S3 and its shooty (pGV2215) or rooty (pGV2255) derivatives, as well as with *Agrobacterium rhizogenes* (8196). Tuber discs were prepared and inoculated according to Anand and Heberlein (1977). For microscopic analysis of the tissue, thin sections were cut by hand. Soluble proteins were extracted from surface tissue slices of the discs and from developed tumors in 0.1 M Tris/HCl buffer (pH 8.0) with the addition of insoluble polyvinylpyrrolidone, by the method previously described (Krsnik-Rasol et al. 1986). Proteins were separated in 10% polyacrylamide gels (Laemmli 1970), visualized by Coomassie Brilliant Blue R-250, and additionally by silver staining (Blum et al. 1987). The total guayacol peroxidase activity of the extracts was determined photometrically by measuring the increase in absorbance at 470 nm (Mäder et al. 1975). The test solution was prepared after Siegel and Galstone (1967).

Results and Discussion

Tumors appeared on the potato tuber discs infected with *A. tumefaciens*, strans B6S3 and pGV2255 (rooty mutant) within two weeks. In the course of the third week the tumors induced by the strain B6S3 were mainly developing as unorganised, snowy outgrowths; at the same time, numerous roots developed in the tumors induced by mutant strain pGV 2255 (Fig. 1a, b). Tuber tissue used in the present experiment was resistent to the infection with *A. rhizogenes* (8196) as well as to the shooty mutant of *A. tumefaciens* (Fig. 1c, d). Ooms et al. (1985) induced transformed roots on stems of potato (cv. Desirée). Hänisch ten Cate et al. (1987) reported successful induction of hairy roots on the discs of tuber tissue in cv. Bintje. They used bacteria strains different from ours. This indicates that the response of potato tissue to infection by transformation inducing bacteria is not determined exclusively by plasmid, it also depends on the genotype as well as the developmental stage of the host plant, and is therefore not always predictable.

Sections of tumor outgrowths showed relatively large normal cells containing starch grains and small tumor cells free from starch. Unorganised tracheidal elements stretched through older tumors (Fig. 2a, b). The transformation of a normal differentiated cell into a tumor cell initiated by the T-DNA integration into the cell genom is caused by a sequence of callular events, which still remains to be elucidated.

In the course of normal wound healing (suberinisation of the cut tuber disc surface) the total peroxidase activity underwent the typical curve showing an increase in activity during the first two weeks and a decrease during the third week. Tumor transformation induced by B6S3 cause similar changes in peroxidase activity, however at a 2—3 times higher level. Rooting induced by the strain pGV2255 was followed by further increase in enzyme activity in the third week. In spite of the fact that no tumors were induced by the strains A. tumefaciens pGV2215 and A. rhizogenes (8196), changes in peroxidase activity differed from the control sample (Fig. 3). Since a successful tumor induction was reflected in a significant increase in total peroxidase activity, this enzyme

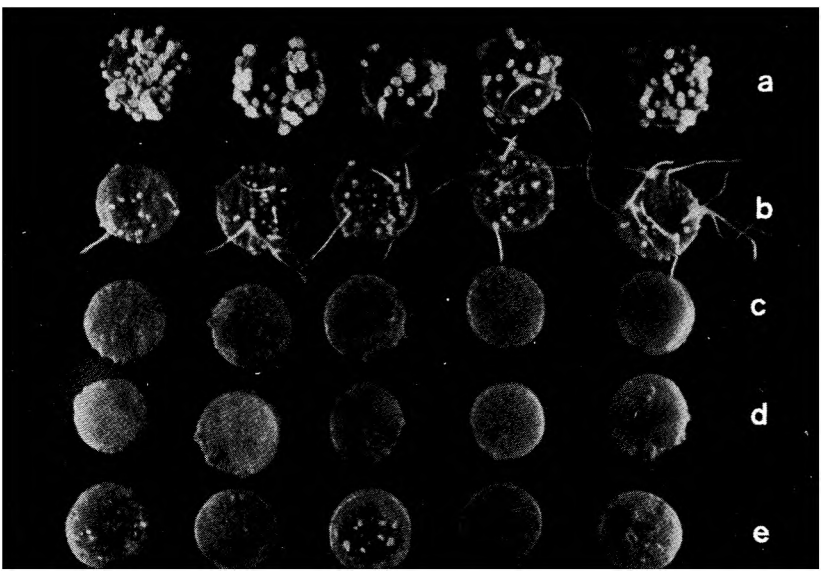

Fig. 1. Potato tuber discs 22 days after infection with: *Agrobacterium tumefaciens*, a — strain B6S3 (wild type), b — pGV2255 (rooty mutant), c — pGV2215 (shooty mutant), d — *Agrobacterium rhizogenes* (8196), e — control (uninfected).

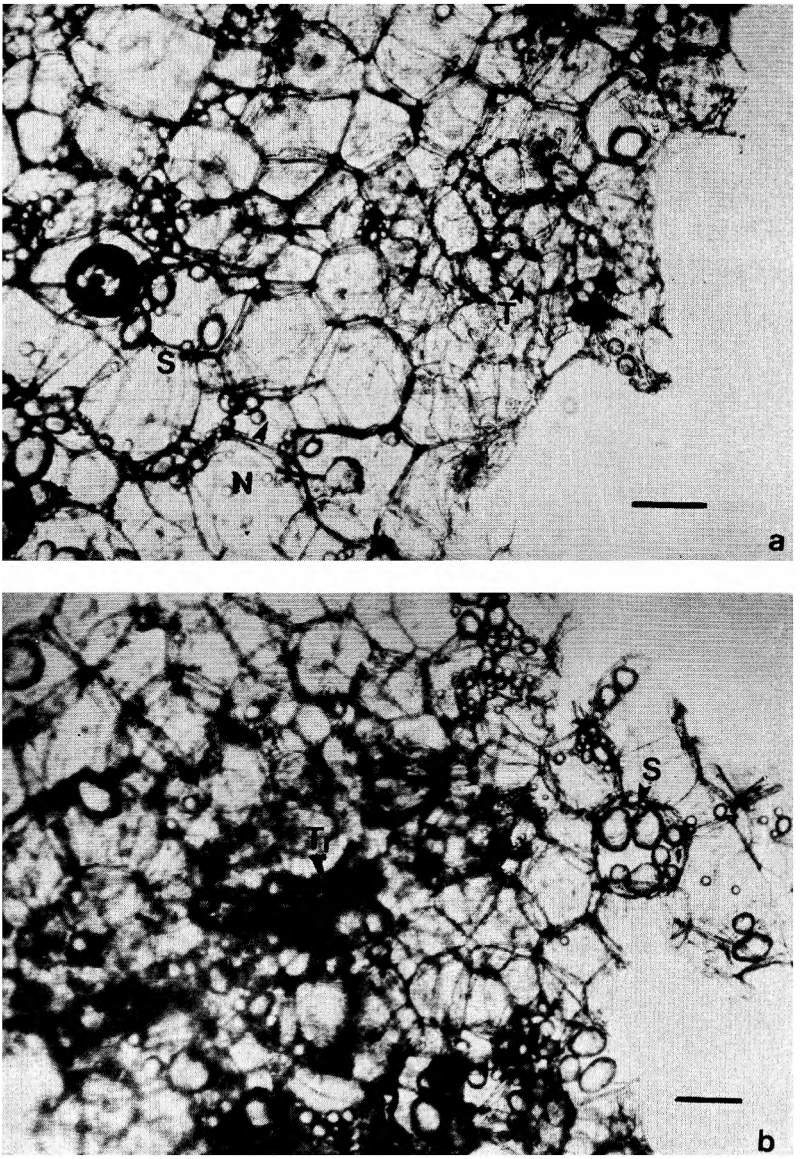

Fig. 2. Section of tumor outgrowths, a — 14, b — 22 days after infection. N — normal cells, T — tumor cells, Tr — tracheidal elements S — starch grains. Bar = 100 μm.

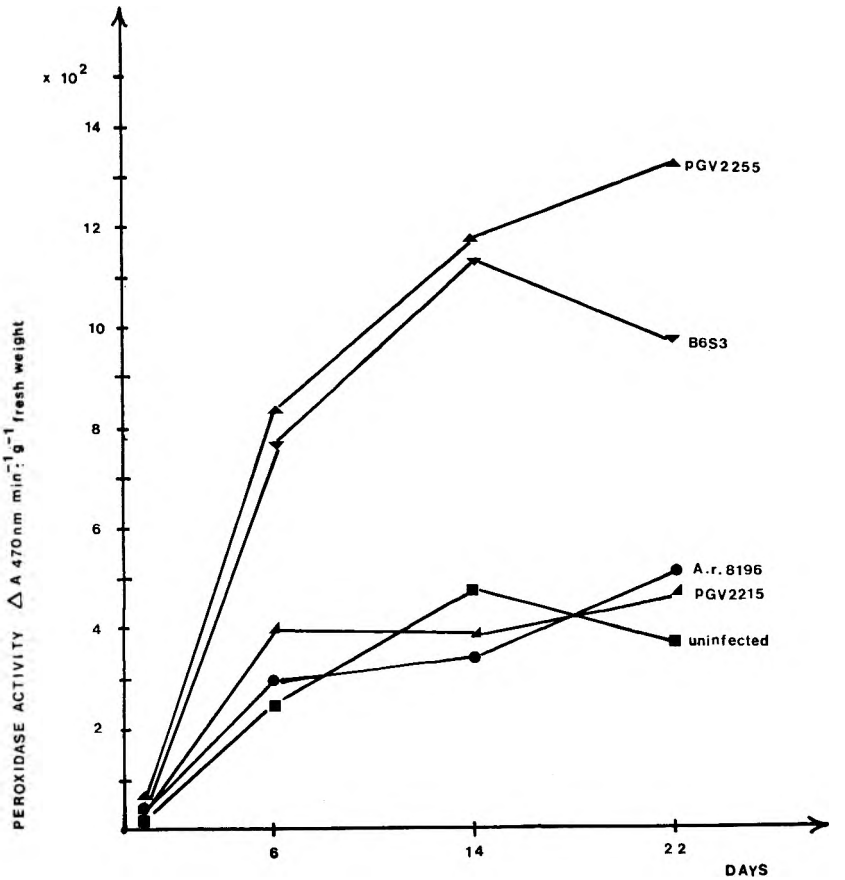

Fig. 3. Variation of peroxidase activity in the tuber tissue during tumor development and normal wound healing.

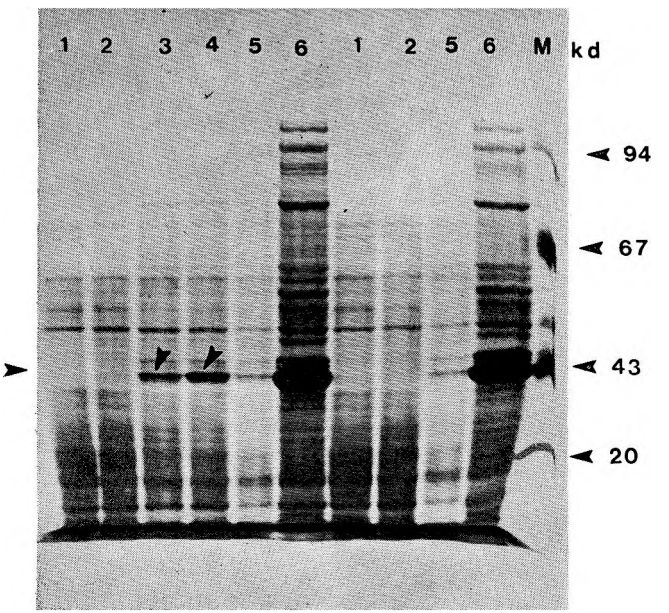

Fig. 4. SDS, 10% polyacrylamide gel fractionated proteins 22 days after infection with: *A. tumefaciens*, 1 — wild strain B6S3, 2 — rooty mutant pGV2255, 3 — shooty mutant, 4 — *A. rhizogenes* 8196, 5 — sterile nutrient broth, 6 — untreated tuber tissue, M — molecular weight markers.

represents a useful marker of tumorigenesis. Studying isozyme gene expression in potato tumors, Oliver (1986) reported that the expression pattern of the monitored isozyme genes was identical, in both, crown galls and non-tumoral callus tissues.

Electrophoretic separation of soluble proteins extracted from normal and tumorous tissue showed that the band of about 40 kd, which was dominant in the normal tissue, disappeared in transformed tissue, both organised (rooty tumor) and unorganised (Fig. 4). The loss of the 40 kd band may be the result of a gene repression for storage protein in actively dividing tumor cells. A comparison with the other protein bands showed little quantitative differences between the normal and the transformed tissue. This seems to be in accordance with the fact that most of the potato isozymes also show a generalized tissue expression pattern (Oliver and Martinez-Zapater 1985, Oliver 1986).

*

This research was supported by the Research Council of SR Croatia (SIZ IV).

The authors are grateful to Prof. Zvonimir Devidé for reading of the manuscript.

References

Anand, V. K., G. T. Heberlein, 1977: Crown gall tumorigenesis in potato tuber tissue. Amer. J. Bot. *64* (2), 153—158.

Blum, H., H. Beier, H. J. Gross, 1987: Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels, Electrophoresis *8*, 93—99.

Caplan, A., L. Herrera-Estrella, D. Inzé, E. Van Hante, M. Van Montagu, J. Schell, P. Zambryski, 1983: Introduction of genetic material into plant cells. Science *222*, 815—821.

Chilton, M. D., 1983: A vector for introducing new genes into plants. Sci. Amer. *6*, 36—45.

Hänish ten Cate, H. C., K. S. Ramulu, P. Dijkhuis, B. de Groot, 1987: Genetic stability of cultured hairy roots induced by *Agrobacterium rhizogenes* on tuber discs of potato cv. Bintje. Plant Science, *49*, 217—222.

Krsnik-Rasol, M., S. Jelaska, D. Šerman, 1986: Peroxidases in pumpkin embryogenic callus lines. Acta Bot. Croat. *45*, 83—89.

Laemmli, U. K., 1970: Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature *227*, 680—685.

Mäder, M., P. Münch, M. Bopp, 1975: Regulation und Bedeutung der Peroxidase-Musteränderungen in sprossdifferenzierenden Kalluskulturen von *Nicotiana tabacum L.* Planta (Berl) *123*, 257—265.

Nacmias, B., S. Ugolini, M. D. Ricci, M. G. Pellegrini, P. Bogani, P. Bettini, D. Inzé, M. Buiatti, 1987: Tumor formation and morphogenesis on different *Nicotiana* sp. and hybrids induced by *Agrobacterium tumefaciens* T-DNA mutants. Developmental Genetics *8*, 61—71.

Nakajima, H., T. Yokota, T. Matsumoto, M. Noguchi, N. Takahashi, 1979: Relationship between hormone content and autonomy in various autonomous tobacco cells cultured suspension. Plant Cell Physiol. *22*, 1489—99.

Nakajima, H., T. Yokota, N. Takahashi, T. Matsumoto, M. Noguchi, 1981: Changes in endogenous ribosyltrans-zeatin and IAA levels in relation to the proliferation of tobacco crown gall cells. Plant Cell Physiol. *29*, 1945—51.

Nester, E. W., M. P. Gordon, R. M. Amasino, M. F. Yanofsky, 1984: Crown gall: A molecular and physiological analysis. Ann. Rev. Plant Physiol. *35*, 387—413.

Oliver, J. L., 1986: Isozyme gene expression in potato tumors incited by Agrobacterium. Theor. Appl. Genet. *72*, 373—376.

Oliver, J. L., J. M. Martinez-Zapater, 1985: A genetic classification of potato cultivars based on allozyme patterns. Theor. Appl. Genet. *69*, 305—311

Ooms, G., J. R. Lenton, 1985: T-DNA genes to study plant development: precocious tuberisation and enhanced cytokinins in *A. tumefaciens* transformed potato. Plant Molecular Biology *5*, 205—212.
Siegel, B. Z., W. Galston, 1967: The peroxidase of *Pisum sativum.* Plant Physiol. *42*, 221—226.

SAŽETAK

TUMORSKA TRANSFORMACIJA U TKIVU GOMOLJA KRUMPIRA

Marijana Krsnik-Rasol i *Tatjana Bakran-Petricioli*

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Tkivo gomolja krumpira inficirale smo bakterijom *Agrobacterium tumefaciens* (sojevi: B6S3, pGV2215, pGV2255) i *A. rhizogenes* (8196). Uspješnu tumorsku transformaciju postigle smo divljim sojem (B6S3) i mutiranim (pGV2255) koji izaziva tumore s korijenom. Svjetlosnim mikroskopom mogle smo razlikovati normalne stanice od tumorskih. Tumorska transformacija odrazila se u porastu peroksidazne aktivnosti te stoga, ovaj enzim može poslužiti kao pokazatelj transformacijskih procesa. Elektroforetskim razdvajanjem topivih proteina u SDS-poliakrilamidnom gelu utvrdile smo da tumorske stanice prestaju sintetizirati proteinsku prugu na položaju koji odgovara molekularnoj težini od oko 40 kd.

Doc. dr. Marijana Krsnik-Rasol
mr. Tatjana Bakran-Petricioli
Botanički zavod IV
Rooseveltov trg 6/III
YU-41001 Zagreb p.p. 933 (Jugoslavija)