TUMOR TRANSFORMATION IN POTATO TUBER TISSUE

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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


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 guaiacol peroxidase activity of the extracts was determined photometrically by measuring the increase in absorbance at 470 nm (Mader 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, strains 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 pGV2255 (Fig. 1a, b). Tuber tissue used in the present experiment was resistant 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). Hännisch 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 cellular 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 Martínez-Zapater 1985, Oliver 1986).

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References

SAŽETAK

TUMORSKA TRANSFORMACIJA U TKIVU GOMOLJA KRUMPIRA

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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 transformacionih 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.

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