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BRIEF COMMUNICATION

Differentiation of *Petunia hybrida* Tissues Transformed by *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens*

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Abstract. *Petunia hybrida* plants were inoculated with different *Agrobacterium rhizogenes* and *A. tumefaciens* strains and developed tumors were further cultivated *in vitro*. Transformed flowering plants differentiated from tumors induced by *A. rhizogenes* strains 8196 and TR101. Transformed but non-rooted plants developed also from tumors incited by *A. tumefaciens* T37. Cultures of roots transformed by *A. rhizogenes* strain 15834 did not show increased incidence of chromosomal aberrations in anaphases in comparison with untransformed control. Permanent growth of isolated untransformed *Petunia* roots was not induced by addition of IAA into the medium.

A. rhizogenes strains carrying large Ri plasmids transform plant cells by a mechanism similar to that of Ti plasmids of *A. tumefaciens* (Chilton *et al.* 1982), *i.e.* by integration of its T-DNA into the plant genome. Several laboratory strains are available, divided into two categories (Petit *et al.* 1983): strains inducing hairy root tumors which synthesize both agropine and mannopine (like 15834 or A4) and strains inducing tumors which synthesize mannopine but not agropine (8196, TR7, TR101). Tissues transformed by *A. rhizogenes*, in contrast to those transformed by *A. tumefaciens*, often differentiate plants (Byrne and Chilton 1983, Tepfer 1983).

In the study presented here we have tested the morphogenetic potential of *Petunia hybrida* tissues transformed by different strains of *A. rhizogenes* and *A. tumefaciens* containing Ri or Ti plasmids.

Four *A. rhizogenes* strains, obtained by courtesy of Dr. J. A. Lippincott, Department of Biological Sciences, Northwestern University, Evanston, Ill., were used: 8196, TR7, TR101, 15834. Two *A. tumefaciens* strains, carrying Ri plasmids, namely C58C1(pRiA4a,b,c) — Nr. 10 and C58C1(pRiA4b) — Nr. 24, obtained by courtesy of Dr. A. Petit, Institut de Microbiologie, Université de Paris-Sud, Orsay, were also included. *A. tumefaciens* strains

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T37 and C58 with appropriate Ti plasmids were used for comparison. The bacteria were cultivated on the complete medium of LANGLEY and KADO (1972) and overnight suspensions were used for inoculation of *Petunia hybrida* cv. Sněženka plants, cultured on MS agar medium. One month old plants were used for inoculation by wounding the stems with Pasteur pipette containing the bacterial suspension. Tumors appeared one week later at almost all sites inoculated. After one month, tumors were transferred to MS medium with antibiotics Ticarcilin (200 $\mu g\ ml^{-1}$), Erythromycin (200 $\mu g\ ml^{-1}$) and Vancomycin (500 $\mu g\ ml^{-1}$). They were transferred to fresh medium at three week intervals. After 5 subcultivations, antibiotics were omitted. Nopaline synthesizing activity of plant tissues was scored by the methods of OTTEN and SCHILPEROORT (1978) and LEEMANS *et al.* (1981). Opines of the agropine type were detected by the methods of LEEMANS *et al.* (1981) and PETIT *et al.* (1983).

A. tumefaciens T37 strain induced crown galls growing mostly as teratomas already on the plants inoculated. One nopaline synthesizing plant without roots also regenerated, but it died before flowering. Crown galls induced by *A. tumefaciens* strain C58 were mostly undifferentiated and rarely grew as teratomas. Crown galls, teratomas and regenerated plants from tumors incited by *A. tumefaciens* never formed roots.

Tumors induced by *A. rhizogenes* and *A. tumefaciens* with Ri plasmids appeared on the plants mostly as undifferentiated tissues. After detachement from the plants and transfer on MS medium with antibiotics, buds and/or roots differentiated, according to the *Agrobacterium* strain used. Mannopine strains induced tumors which formed buds developing to plants. Differentiation was frequent in hairy root tumors induced by the strain 8196, to a lesser extent TR101 and seldom differentiation of leaves, but no plants were observed in TR7 induced tumors. Tissues growing permanently as teratomas were never recovered. Agropine strains induced tumors from which roots proliferated. The root differentiation was very intense in 15834 induced tumors. Tumors induced by *A. tumefaciens* strains 10 and 24 regenerated both roots and leaves, but no plant regeneration was observed. Roots never differentiated to whole plants.

About 50 plants regenerated from hairy root tumors incited by strain 8196 and one clone was propagated for further study. Twelve plants regenerated from tumors induced by the strain TR101 and two clones were chosen for further studies. Chlorophyll defects present in almost half of the originally propagated plants were eliminated during clonal propagation. Clones were propagated by stem segments cultivated on the MS medium. All the segments rooted, grew vigorously and some flowered. Flowers showed no morphological or developmental abnormalities. Currently, plants of all clones are being transferred into soil for hybridization and other studies.

In 16 independently derived tumors induced by *A. rhizogenes* 15834 which differentiated roots, homogeneous root clones were established. Individual root tips of the length 1 cm were used in each of six succeeding subcultivations on MS medium. Most of the root mass grew on the surface of agar end upwards and roots were covered with numerous hairs. The agropine con-

Abbreviations used: BAP = benzylaminopurin; MS = medium of MURASHIGE and SKOOG (1962) without growth substances.

TABLE 1

Cytological evaluation of anaphase abnormalities in three root culture clones, transformed by *A. rhizogenes* strain 15834 and control — untransformed seedling root meristems

Variant of experiment	Anaphases scored	Bridges	Fragments	Laggards	% of abnormal anaphases
Clone 1	309	—	1	2	0.97
Clone 2	204	1	—	1	0.98
Clone 3	248	1	—	2	1.21
Control	244	—	—	2	0.82

tent of roots was the highest observed in all objects studied in our laboratory (*Kalanchoe*, *Arabidopsis*, tobacco, carrot). Cloned roots were transferred to the liquid MS medium. The growth in thin (about 1 cm) layer of the liquid medium was considerably better than that on agar. Roots showed multiple lateral branching. Roots cultivated on the gyratory shaker showed more intense growth and branching than those cultivated in stationary cultures, even in very thin layers of the liquid medium. The velocity of 20—30 revolutions per minute was used.

Addition of 1 μM BAP (Koch-Light) to the agar MS medium resulted in callogenesis of transformed roots; callus growth was initiated in older parts of roots.

An attempt to establish root cultures with untransformed seedling roots was also made. Roots of the 3 cm length were excised from one week old seedlings and put on MS medium with different concentrations of IAA (0, 5, 10, 20, 50 and 100 μM). After one month cultivation in the dark, roots showed swelling and callus formation but never permanent longitudinal growth. The highest IAA concentration used suppressed callogenesis.

Meristems of roots, stained by the Feulgen method were submitted to microscopic analysis for observation of the mitotic activity. Meristematic activity of the individual root tips is blocked progressively with the subcultivation period duration. Subcultivation period was usually 4 weeks but the medium was changed each week. The mitotic activity in individual root tips is an "all or none" process, *i.e.* there is either full mitotic activity or no mitotic activity. In the dividing root meristems, the number of mitoses reached or exceeded that of root tips of the seedlings cultivated in tap water on filter paper in Petri dishes.

Root meristems were submitted to the cytological analysis of anaphase chromosomal aberrations (fragments, bridges) and other chromosomal abnormalities. Meristems of root cultures transformed by *A. rhizogenes* were never cytologicaly studied before, despite there being several reasons why chromosomal aberrations could be involved. These are: 1) the increased auxin synthesis due to the expression of T-DNA auxin genes, 2) the synthesis and accumulation of opines and agrocinopines and, last but not least, 3) long term cultivation of root cultures. Roots used for this cytological analysis were sampled from root cultures kept for almost one year. The results of anaphase analysis are given in Table 1. No increased frequency of chromosomal aberrations and lagging chromosomes and no other ab-

normalities were found. The chromosome number in 30 metaphases scored was invariably 14.

Root cultures of plant cells transformed by *A. rhizogenes* are capable of vigorous, permanent growth and they are chromosomally stable. They can possibly be used in future as an alternative to cell suspension cultures, for instance for the production of alkaloids and other pharmacologically useful secondary plant cell metabolites. The main advantage of these cultures is their chromosomal stability, which was first demonstrated in this study.

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BOOK REVIEW

DE SERRES, F. J. (ed.): CHEMICAL MUTAGENS. PRINCIPLES AND METHODS FOR THEIR DETECTION. VOL. 9. — Plenum Press, New York—London 1984. 306 pp. US $ 42.50.—.

The problems of adequately protecting the human population against mutagenic and carcinogenic hazards are enormous and far exceed national boundaries. The objective of the well acclaimed series "Chemical Mutagens", established in 1971, is to encourage the development and application of testing and monitoring procedures to avert human exposure to environmental genotoxic agents. Volume 9 covers various aspects of testing chemical mutagens. Included are detailed descriptions of seven short-term assays: 1) the grasshopper neuroblast assay, 2) the human lung- and skin-derived fibroblast assay, 3) the L-arabinose resistance assay with *S. typhimurium*, 4) the *Bacillus subtilis* multigene sporulation test, 5) mammalian tissue culture L5178Y/TK gene mutation assay, 6) the bacterophage lambda induction assay, and 7) the rat granuloma pouch assay. A special chapter focuses on the identification of mutagens from the cooking of food, and reviews the formation of the pyrolytic and thermic mutagens and various kinds of genotoxins detected in foods. The concluding article deals with the detection of mutagens in human feces as an approach to the discovery of causes of colon cancer.

The diversity of coverage in this book demonstrates the vitality characterizing the application of new assays for monitoring environmental mutagens. The volume should prove helpful to researchers involved in all aspects of chemical mutagenesis.

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