BRIEF COMMUNICATION

Comparison of Hairy Root and Crown Gall Tumors of
Arabidopsis thaliana*

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Abstract. The phenotype appearance of Arabidopsis thaliana hairy roots and crown galls
their teratomas and regenerated plants were compared. Several differences were found, which
converge with T-DNA differences between Ti and Ri plasmids.

The induction of crown galls in Arabidopsis thaliana by Agrobacterium tumefaciens was already demonstrated (AERTS et al. 1979, ONDŘEJ et al. 1984a). A. thaliana has shown an unusual response to crown gall induction; we
demonstrated frequent spontaneous differentiation of opine synthesizing
plants and the opine synthesizing activity was maintained in succeeding
generations (PAVINGEROVÁ et al. 1983). Like in tobacco crown galls (BEZDĚK et al. 1977), A. thaliana crown galls also showed higher resistance to 5-bromodeoxyuridine than untransformed calli (ONDŘEJ 1983). BEZDĚK and VYSKOT
(1981) explain their results in tobacco by modulation of thymidylate kinase
activity due to the increased level of cytokinins. Thymidylate synthase
causes debromination of 5-bromodeoxyuridine in situ in crown galls.

The peculiarity of A. thaliana crown galls is that they are capable of in vitro growth without growth regulators in the light only (ONDŘEJ et al. 1984a). Untransformed A. thaliana calli are also capable of in vitro growth without growth regulators in the medium but the growth is slow and transient (ONDŘEJ et al. 1984b).

Agrobacterium rhizogenes brings its T-DNA of the Ri plasmid into plant
cell nuclei similarly as A. tumefaciens (CHILTON et al. 1982, WHITE et al.
1982) but most of T-DNA genes on Ri plasmid of A. rhizogenes are different
from those on Ti plasmid of A. tumefaciens (WILLMITZER et al. 1982a).
Genes for T-DNA transcripts 1 and 2 (WILLMITZER et al. 1982b) which were
shown to code for enzymes for biosynthetic pathway of IAA (INZÉ et al.
1984) are common in T-DNA of both Ti and Ri plasmids (WILLMITZER et al.
1982a).

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Hairy root tumors differentiate into plants more readily than crown gall tumors (Byrne and Chilton 1983). On the other hand, De Cleene and De Ley (1981) concluded that “the disease symptoms caused by A. tumefaciens (= crown gall) and A. rhizogenes (= hairy root) are similar”. In the study presented here we tried to test the validity of their assumption.

The aim of this study was to describe the habitus, growth pattern and the response to external factors in Arabidopsis thaliana hairy root tumors and their regenerants and to compare the situation with that in crown galls induced in the identical Arabidopsis thaliana cultivars under similar experimental conditions.

A. rhizogenes strains 8196, 15834, TR7 and TR101 were obtained by courtesy of Prof. J. A. Lippincott, Northwestern University, Evanston, Illinois. A. tumefaciens strains C58C1 carrying Ri plasmid A4b (strain 24) and A4 a, b, c (strain 10) were obtained by courtesy of Dr. A. Petit, Orsay, France. The derivation of these strains was described by Petit et al. (1983).

Plants cultivated in vitro to the rosette stage were inoculated by puncturing with Pasteur pipette rinsed in overnight bacterial suspension into the middle of the rosette. Two weeks after inoculation, small tumors appeared and later they became surrounded with roots. Four weeks after inoculation, tumors were detached from plants, put on PG0 agar medium of Negrutiu et al. (1975) containing antibiotics for the elimination of bacteria, but no growth substances. Large proportion of tumors developed shoots or teratomas. Detached roots were unable to proliferate on PG0 medium. On PG1 (medium for callus induction) they produced calli which showed very slow growth on all PG media and which never differentiated, as did untransformed calli on PG3 medium.

There were considerable differences in the regeneration capacity of hairy root tumors induced by different strains. A. rhizogenes 15834 and A. tumefaciens 24 were most efficient in inducing tumors which gave rise to well-growing teratomas or fully differentiated, phenotypically slightly modified fertile plants. In the course of four subcultivations (four weeks each) teratomas became gradually converted to flowering plants.

The behaviour of hairy root tumors, teratomas and plants induced by A. rhizogenes 15834 and A. tumefaciens 24 was studied and compared with the already known behaviour of A. thaliana crown galls.

The first prominent difference was that while crown gall teratomas and regenerants never form roots, hairy root teratomas and differentiated plants showed root formation.

A. thaliana crown galls often grow permanently as undifferentiated tumors which are of hard, compact appearance and green or creamy colour, but hairy root tumors grew only scarcely as undifferentiated tissue in succeeding subcultivation. A. thaliana crown galls and teratomas can be subcultivated without growth regulators only under illumination; hairy root teratomas can be grown on PG0 medium in complete darkness. Another interesting feature, observed only in hairy root tumors but not crown galls of A. thaliana, was disturbance of tropisms. Roots grew mostly horizontally, on the surface of the agar layer, or even upwards. Also leaves and shoots rarely showed positive geotropism, i.e. they showed the tendency to grow downwards, through the agar layer.

Surprisingly, however, hairy root teratomas in our experiments showed
the same level of increased resistance to 5-bromodeoxyuridine as crown gall teratomas, when compared with untransformed calli and plants. Both hairy roots and crown galls tolerate $2 \times 10^{-4}$ M 5-bromodeoxyuridine (the highest concentration tested), which is lethal to untransformed calli and causes bleaching and growth disturbances and retardation in plants. As no increase of cytokinin level in hairy roots is expected, this effect awaits elucidation.

Several phenotypic differences between \textit{A. thaliana} crown galls and hairy roots were found and therefore the assumption of DE CLE~E~N~E and DE LEY (1981) of phenotypic similarity of crown galls and hairy roots cannot be confirmed.

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