BIOLOGIA PLANTARUM (PRAHA)

31 (4) : 312–314, 1989

BRIEF COMMUNICATION

# Potato Transformation by *Agrobacterium rhizogenes* Ri plasmid

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**Abstract.** Potato root cultures transformed by pRiA4 T–DNA do not regenerate plants *in vitro* spontaneously, but regeneration can be induced by a sequence of callogenesis and regeneration media. Both $T_L$ and $T_R$–DNA were found by Southern blotting. Regenerated transformants showed characteristic morphological deviations in the *in vitro* cultures. Plants in the soil showed differences in the inflorescence and tuber development.

The integration of T-DNA of the Ri plasmid of *Agrobacterium rhizogenes* brings T-DNA genes into the plant genome and their expression leads to the hairy root formation. Transformed roots often spontaneously differentiate plants (PETIT *et al.* 1983). We have already shown the ability of spontaneous plant differentiation in hairy roots of *Atropa belladonna* (ONDŘEJ and PROTIVA 1987) and in other objects (ONDŘEJ and BÍSKOVÁ 1985). Here we give a more detailed description of potato pRiA4b transformed regenerants. WHITE *et al.* (1985) have shown that the T-DNA of pRiA4b, which is divided into the left segment $T_L$ and the right segment $T_R$ brings into the plant genome genes for auxin synthesis 1 and 2 together with genes for agropine synthesis localized on $T_R$. The $T_L$ might bring to the plant genome a weak gene for cytokinin synthesis (WHITE *et al.* 1985) and genes which sensibilize plant cells to auxins (CARDARELLI *et al.* 1987). No information on phytohormones in the transformed and regenerated plants is available.

Potato cv. Zvíkov was transformed by *A. tumefaciens* C58Cl (pRiA4b) (pGA472) which brings into the plant genome also the kanamycin resistance gene which resides on the T-DNA of pGA472 vector plasmid (AN *et al.* 1985). Transformation was performed by a modification of the leaf disc method as described by ROGERS *et al.* (1986), but shoot internode segments were used instead

*Received August 5, 1988; accepted January 27, 1989*

of leaves, as described in ONDŘEJ and VLASÁK (1989). After treatment, internodes were placed on the MURASHIGE and SKOOG (1962) medium containing Ticarcilin (500 mg $l^{-1}$) (Beecham Laboratories, England) and kanamycin sulphate (200 mg $l^{-1}$) (Medexport, SSSR). Hairy roots appeared within three weeks on some of the stem segments. After further subcultivation on the same medium, they gave rise to kanamycin resistant roots which grew permanently *in vitro* without growth regulators. Forty root cultures were subcultivated on the same medium at 4 week intervals for one year. Resistance to higher concentrations of kanamycin was also tested and it was found that roots are able to grow, for several subcultivations, in 1 g kanamycin sulphate per litre of medium. During this long term cultivation, no buds and stems appeared and the growth pattern of root cultures did not change. Roots were put on callogenesis medium and after 20 days to regeneration medium (WEBB *et al.* 1983). After two months, three independent shoots were recovered. These were transferred on MURASHIGE and SKOOG (1962) medium without growth regulators. Transformed shoots formed strong root system. Most of the roots, with abundant hairy roots, grew plagiotropically on the surface of the agar and upwards. Roots appeared also on the upper parts of the stems. Stems of the transformed regenerants were stronger than those of controls and leaves were sometimes divided, which is not usual in transformed shoots *in vitro*. The overall picture described here is in agreement with that of OOMS *et al.* (1985), who used another type of Ri plasmid and another potato cultivar. Opines were detected in leaves and roots of the regenerated plants and both agropine and mannopine were found. In later subcultivations, the opine synthesizing activity disappeared, without disappearance of the transformation markers (Figs. 1 and 2). The presence of both $T_L$ and $T_R$-DNA of pRiA4b in the genome of transformed regenerants was detected by Southern blotting (Fig. 3). Because of the high degree of kanamycin resistance of the transformed tissues, we can also suppose the presence of T-DNA of pGA472.

Plants have been transferred into the soil and "second clone generation" plants and tubers were obtained. The plants did not show any symptoms of transformation on stems and leaves, but the inflorescences were condensated and showed many more flowers than controls (Figs. 4 and 5). Tubers of transformed plants were large, prolonged and they had many (20–26) eyes, which were distributed very regularily along the tubers (Figs. 6 and 7). The starch content (17–19% of dry matter) in tubers of transformed plants was equal to that of controls.

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*Figures at the end of the issue.*

## BOOK REVIEW

HAMEL, G.: ORCHIDEEN. Bildtafeln mitteleuropäischen Arten, Formen und Bastarde. Teil 2. Nach Aquarellen von Hermann Walther. – VEB Gustav Fischer Verlag, Jena 1986. M 20,–, Ausland DM 28,–.

Two years after the edition of the first volume has appeared the second part of 32 full-page orchid plates of aquarelles painted by the same artist, H. Walther. The quality of the reproductions is rather better than in the first volume but some species have not, once again, their natural colour nuance, such as *e.g.* the yellow colour of the white *Cephalanthera damasonium* (syn. *C. alba*), the green colour of the pure white flowers of *Goodyera repens* and *Platanthera chlorantha*, or the white form of *Dactylorhiza incarnata*. More bluish nuance is missing in *Limodorum abortivum*.

The second part presents the more rare species in Central Europe and 7 plates of hybrids, some of which are illustrated for the first time. The same authors as in the first volume accompany the illustrations by good descriptions, focussed mainly on biotopes and areas of location in the whole Europe. With respect to endangered types in the GDR, the hybrids are described in the areas of the parental species. Unfortunately, the numbers of the described species refer to the free filed plates, which, however, are not numbered. The aquarelles on the plates are named only in German, which would be confusing for not German-speaking readers. These shortcomings may be removed by careful editing, especially because the two volumes with representative and valuable illustrations are suitable for the amateurs and nature protection.

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