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BRIEF COMMUNICATION

Crown Gall Tumors in *Centaurium*

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Abstract. *Agrobacterium tumefaciens* C58M (a nopaline strain) was used for induction of crown gall tumors in *Centaurium umbellatum* GILIB. The formation of tumors is very rare. After elimination of bacteria the tumor tissues grow in the light on R3B medium without growth regulators. They are positive in nopaline synthase.

Agrobacterium tumefaciens harboring Ti plasmid induces crown gall tumors in many dicotyledonous and gymnospermous plants. The tumor cells contain a part of Ti plasmid (T-DNA) as a stable component of nuclear DNA (CHILTON *et al.* 1977). The transformed cells are autonomous in the synthesis of plant hormones and produce specific substituted aminoacids called opines (HOOYKAAS 1983). According to DE CLEENE and DE LEY (1976) *A. tumefaciens* is virulent in 643 plant species, *i.e.* roughly in 60% of all inoculated species. *A. tumefaciens* ChrIIb does not induce tumors in *Centaurium minus* MOENCH (TAMM 1954). Of other species of *Gentianaceae* *Gentiana asclepiadea* L. is sensitive to *A. tumefaciens* B6; *G. cruciata* L., *G. grombozewski* KUSNETZOW and *G. tibetica* KING are resistant (DE CLEENE and DE LEY 1976). As far as we know there are no other reports on the crown formation in *Gentianaceae*.

We tried to induce crown gall tumors and hormonal autonomy of plant tissues in *Centaurium*. Some species of this genus are important medical plants.

Tissue cultures (marked *C. erythrea* C5, C6 and C7) and seeds (strain C and E) were a gift of ing. M. Kamínek (Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha). Plants regenerated from a tissue culture and cultivated in unsterile soil (strain CK) were kindly supplied by Prof. R. Hončariv (Faculty of Sciences, Šafařík University, Košice).

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TABLE 1

Object	Place of infection	Number of plants	Number of inoculations	Age of cultures or state of plants
Regenerants from calli*	Leaves	59	245	10— 20 days
	Stems	18	90	20— 50 days
Seedlings	Stems	18	90	90—120 days
Wild plants	Stems	10	50	before anthesis

* Strains C5, C6, C7 and CK

Wild plants from a locality near Trocnov (South Bohemia) were generously provided by Mrs. and Mr. J. Fulín. In all cases the regenerated flowering and wild plants were determined as *C. umbellatum* GILIB. subsp. *typicum* (WITTR.) RONN.

Tissue cultures were grown on MS medium (MURASHIGE and SKOOG 1962) with indol-3-ylacetic acid (2 mg l^{-1}) and 6-furfurylaminopurine (0.2 mg l^{-1}). In the light (10 000 lx, 16 h photoperiod) at roughly 28 °C they regenerated roots and leaves, later some of them stems. For axenic cultivation the seeds were surface sterilised with 70% ethanol (1 min) and 6% chloramine (6 min), washed and sown on PG_0 medium (NEGRUTIU *et al.* 1975). Other cultivation conditions were the same as for the cultivation of calli.

The chromosome number was determined in the shoot apical meristem of regenerated plants (strain C5). The diploid number is $2n = 40$ or $2n = 42$. The Feulgen method was used and a more accurate determination was impossible till now. According to BOLCHOVSKICH *et al.* (1969) the diploid chromosome number in *C. minus* Moench (syn. *C. umbellatum* GILIB.) is $2n = 20$, 40 or 42.

A. tumefaciens C58, C58M and T37 (nopaline strains) as well as B6 and B6-806 (octopine strains) were used for infections. The bacterial cultures were incubated for 48 h at 28 °C in the medium of LANGLEY and KADO (1972). A sharp forceps or a needle was used for wounding the plant tissues and for transferring the bacterial cell suspension into the wounds. The kind of plant objects and the number of inoculations are shown in Table 1. The number of inoculations with different bacterial strains was the same in every object.

The inoculation of leaves mostly caused necrosis. There was no necrosis by wounding and inoculating the stems. The infections were evaluated after 6 weeks (axenic regenerants and seedlings) or 12 weeks (wild plants and regenerants CK). The inoculations were performed from March till October 1983.

We found the formation of tumor-like structures in three cases in September only: (1) on the stem of a seedling (strain E) inoculated with the strain T37, (2) on a leaf regenerated from a callus (strain C6) inoculated with the strain C58M (Fig. 1) and (3) on the leaf of a seedling (strain C) inoculated with the strain C58M. Together 6 tumor-like tissues were isolated and transfered onto R3B medium (MEREDITH 1978) without growth factors

and with antibiotics (carbenicilin 250 μg ml^{-1} plus gentamycin 250 μg ml^{-1}). After the elimination of bacteria the tissues were propagated on R3B without antibiotics and growth factors. Three of them (item (2) and (3)) are able to grow after more than 6 months. Using the method of OTTEN and SCHILPEROORT (1978) we found that these tissues are positive in repeated examinations for nopaline synthase. Therefore they are tumors, not normal calli. The tumors cultivated in the light on R3B are light green and do not form teratomas or regenerants (Fig. 2).

C. umbellatum GILIB. is a new species in which it is possible to induce the crown gall tumors. The induction of tumors is a very rare event. We got positive responses in 5 cases from a total of 100 infections with *A. tumefaciens* C58M; the other strains of *A. tumefaciens* were ineffective. An interesting fact is that the tumors were formed only in September. We have no explanation for this result; it is possible to speculate about some environmental and other factors.

It seems that the negative results with crown gall tumor induction in some plant species need not always be definite.

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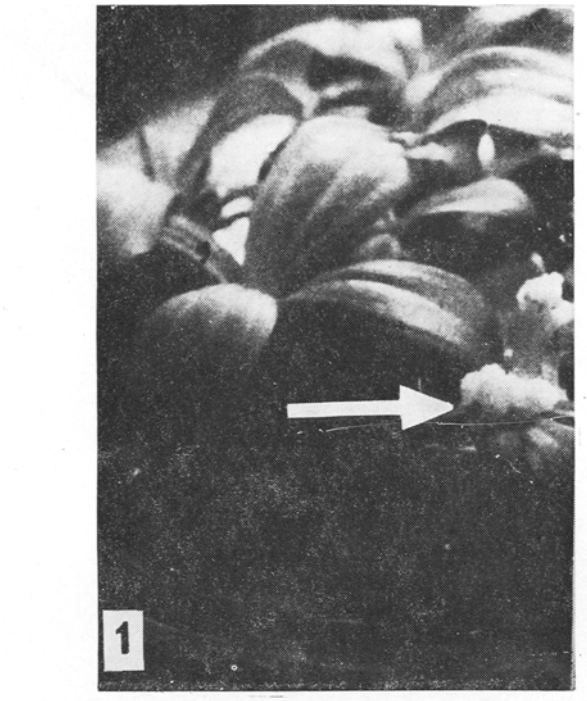
Figures at the end of the issue.



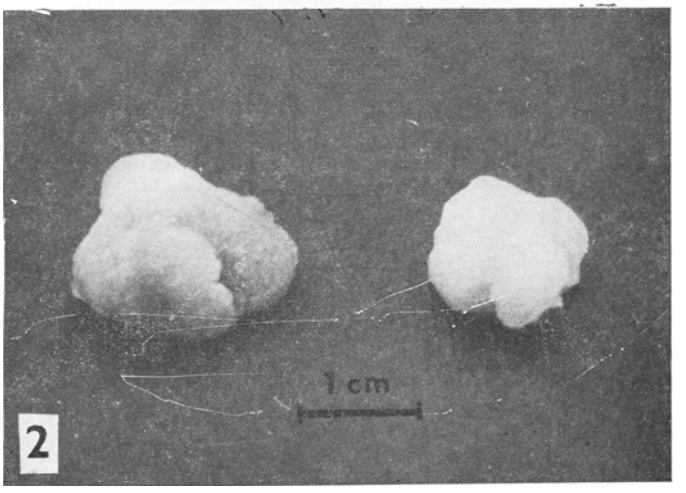


Fig. 1. Tumor-like structure on the leaf of *Centaurium umbellatum* (axenic regenerant of the callus C6).
Fig. 2. Established crown gall tumors of *Centaurium umbellatum* (induced with *A. tumefaciens* C58M).