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Crown Gall Teratoma Formation Is Plasmid and Plant Controlled

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Experiments using different species of the plant *Nicotiana* and strains of the bacterium *Agrobacterium tumefaciens* showed that teratoma formation from crown galls was dependent on the combination of bacterial Ti plasmid and host plant used.

Crown gall tumors induced by strains of *Agrobacterium tumefaciens* sometimes spontaneously differentiate abnormal supernumerary organs, which are called teratomas (1, 2, 5). However, only certain combinations of bacteria and plant species give rise to spontaneous teratoma formation (2, 5, 7). Furthermore, tumor formation and general tumor morphology (rough versus smooth tumors) are known to be controlled by bacterial functions (1, 3, 6, 7). In some cases, though, in situ application of phytohormones or tissue culture techniques may induce organogenic development from transformed tissues (4, 8).

This communication reports that spontaneous teratoma development from tumors induced by strains of *A. tumefaciens* is plasmid controlled and that the kind of teratoma formed varies with the host plant used.

Six different *Nicotiana* species and cultivars were inoculated with specially constructed bacterial strains to test the dependence on plasmid type and plant host contribution to the potential of forming a teratoma (Table 1).

The *A. tumefaciens* strains used were: B6S3(pTiB6S3) Sm^r *oct*$^+$; C58(pTiC58) *nop*$^+$; B6S3(pTiC58) Rif^r; C58(pTiB6S3::RP4) Sp^r; T37(pTiT37). The symbols used are: *oct*$^+$, octopine utilization; *nop*$^+$, nopaline utilization; Sm^r, streptomycin resistance; Sp^r, spectinomycin resistance; Rif^r, rifampin resistance.

All plant seeds were obtained from the Queensland Tobacco Research Station (Brisbane). All plants were grown under optimal glass house conditions. The stems of 3- to 5-week-old plants were stabbed at similar positions on different plants by using a needle inoculated with an *Agrobacterium* strain. Control plants stabbed with sterile needles or with needles inoculated with bacterial strains cured of their Ti plasmid were also included in the experiments. Plants were scored after 2, 3, and 5 months of growth for tumor production and teratoma formation. Individual tumors were tested for the presence of the original inoculant bacterium.

Tumorigenesis and teratoma proliferation were observed within 6 to 8 weeks after inoculation. With the stabbing method, almost all wounds produced tumors if inoculated with a virulent *Agrobacterium* strain, whereas the controls failed to generate any tumors and teratomas from wound callus. The virulent strain B6S3 induced tumors in all stabbed wounds without ever giving rise to shoot or root teratomas.

The virulent strain T37 produced tumors on all plants tested, but only *N. tabacum* (three cultivars) and *N. langsdorffii* developed spontaneous teratomas. A similar indication of the importance of the plant host came from studies with strain C58, in which only *N. langsdorffii* and *N. sylvestris* produced teratomas, although all other plants readily developed tumors. However, the *N. sylvestris* teratoma development was characterized by its abundance of roots and lack of shoots. These findings show that teratoma production and morphogenetic development are under both bacterial and plant control.

To verify that the bacterial Ti plasmid governs the teratoma production, bacterial strains were constructed in which their original Ti plasmid was cured and replaced by an alternative Ti plasmid.

All wounds inoculated with these transconjugant strains produced tumors, but those tumors which were derived from strain C58(pTiB6S3::RP4) failed to produce teratomas. This is despite the fact that this strain was constructed in the background of strain C58, except that the Ti plasmid has been replaced with that from strain B6S3. Conversely, tumors induced by strain B6S3(pTiC58) produced teratomas both on *N. langsdorffii* and *N. sylvestris* but not on *N.*

TABLE 1. *Agrobacterium—plant interaction in tumor and teratoma production*

Host plant	Reaction to the following *Agrobacterium* inoculant:[a]											
	Control		B6S3 (pTiB6S3)		C58 (pTiB6S3::RP4)		C58 (pTiC58)		B6S3 (pTiC58)		T37 (pTiT37)	
	onc	tera	onc	tera	onc	tera	onc	tera	onc	tera	onc	tera
N. tabacum												
Sampson	−	−	+	−	+	−	+	−	+	−	+	+
Hicks Q46	−	−	+	−	NT	NT	+	−	+	−	+	+
W360-S	−	−	NT	NT	NT	NT	+	−	+	−	+	+
N. langsdorffii W300	−	−	+	−	+	−	+	+	+	+	+	+
N. sylvestris W314-S	−	−	+	−	+	−	+	+[r]	+	+[r]	+	−
N. glauca	−	−	+	−	+	−	+	−	+	−	+	−

[a] onc, Oncogenic or tumor formation; tera, teratoma formation; +, positive response; −, negative response; r, rhizogenic teratoma; NT, not tested. The table shows pooled data from two separate plantings, each containing four plants for each bacterium-plant combination.

tabacum, thus exhibiting the same properties as strain C58. It is noteworthy that the *N. sylvestris* tumors induced by strain B6S3(pTiC58) were also rhizogenic and not shoot producing. When strain C58 cured of its Ti plasmid was used to inoculate the various plants, no tumors or teratomas were ever observed.

In all cases it was possible to reisolate the original inoculant bacterium out of the tumor, even after 5 months.

The above results show that teratoma formation from crown gall tumors depends on the interaction between the bacterial plasmid and the host plant. In vitro culture methods of plant cells have shown that the plant genotype greatly influences its morphogenetic potential (4, 6). Thus, differing phytohormone regimes are required to achieve organogenesis from tissue cultures of closely related cultivars or species. Crown gall tumors exhibit similar variation of the differentiating tendency toward organized tissue. The organogenic potential of tumors varies even within the same plant, because tumors higher up the stem produce shoot teratomas more easily than ones lower down the stem, perhaps indicating a plant-controlled phytohormone gradient (7). The phenotypic expression of this tendency to form teratomas is governed by the particular bacterial Ti plasmid used. Tumors induced by the Ti plasmid pTiB6S3 did not produce teratomas, whereas those tumors induced by the Ti plasmids pTiC58 and pTiT37 did produce organized growth on the appropriate plant. Similarly, the Ti plasmid from *A. rhizogenes* induces tumors which have profuse root development on a large range of host plants. This teratoma formation could represent an escape from the dedifferentiated oncogenic state or, alternatively, an incomplete transformation to the oncogenic state.

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