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GENETIC CONTROL OF NICOTIANA PLANT TUMORS*

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The purpose of this paper is to summarize evidence that: (1) the capacity for spontaneous tumor formation in certain interspecific *Nicotiana* hybrids is genetically controlled; and (2) that genetic change during plant development, i.e. somatic mutation, is not causal to tumor formation.

Genetic Control of Tumor Formation

The arguments for nuclear gene control of spontaneous tumor formation in hybrids between species of *Nicotiana* are as follows:

(1) Of over 300 interspecific *Nicotiana* hybrids reported,^{1,2,3} only 30 produce spontaneous tumors throughout the plant and in entire progeny populations.^{4,5} In 24 additional species combinations genetic tumors have been reported to be produced irregularly in some of the progeny or localized in only certain parts of the plants. Spontaneous tumor formation is restricted, therefore, to a relatively few particular combinations of specific genotypes. According to Lör⁵ these can be placed into at least two groups within which interspecific hybrids are nontumorous and between which the hybrids are tumorous.

(2) No pathogenic causative agent has been isolated from *Nicotiana* hybrid tumors in spite of attempts by workers over a period of years to do so.⁶ Furthermore, tumor induction is not transmitted across the union of

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reciprocal grafts between tumorous and nontumorous types.^{2,4,7,8} Tumors occur only on a stock or scion which is genetically so constituted to form them.

(3) Tumors appear on reciprocal hybrids and, therefore, are not determined by cytoplasmic elements present in only one of the parental species involved.^{2,6} First and second generation progeny of reciprocal crosses between tumorous N. glauca-langedorffii and a nontumorous mutant give equivalent results, thus supporting the evidence for nuclear control.⁸

(4) When all twelve chromosomes of N. glauca (the genome G) are added to diploid N. langedorffii (LL) the resultant triploid plants (LGG) develop tumors, but tumors fail to form in plants with only one or a few glauca chromosomes added to 2n langedorffii.⁴ This result provides evidence that the hereditary capacity for tumor formation is governed by factors residing in the chromosomes.

(5) Tumors form only on crosses between more distantly related species (intersectional hybrids) so that the F_1 's are sterile and amphidiploids breed relatively true. These are unfavorable conditions for genetic analysis. It has been observed^{8,10,11} that N. langedorffii and N. sanderae, two closely related species giving a partially fertile F_1 hybrid, have markedly different effects on tumor formation. The N. langedorffii complement contains genes that enhance tumor expression, whereas N. sanderae germplasm inhibits aerial tumors in appropriate crosses. The F_1 , N. langedorffii x N. sanderae, hybridized in a number of interspecific combinations conducive to tumor formation, showed evidence for segregation of many particulate nuclear genes that enhance or inhibit expression of tumors.⁸

(6) Further indication of genetic control of tumors in segregating populations is afforded by correlations between langsдорffii genes governing small corolla size¹² and tumor enhancement, sanderæ genes for large corolla size and tumor inhibition. These associations are best interpreted as due to genetic linkage.⁸

(7) The above segregants combine three species (N. langsдорffii, N. sanderæ and a third species to provide a tumefacient milieu) and are consequently too sterile for further breeding experiments. In order to establish lines with new recombinations of genes affecting tumor expression, different F_2 segregants of N. langsдорffii x. N. sanderæ were inbred to the F_4 generation. These inbreds were then crossed with an interspecific amphidiploid combination, as N. suaveolens-glaucæ or N. suaveolens-langsдорffii, to produce a tumorous background. The results of such hybridizations with two F_4 's are shown in figure 1. The histograms to the left represent data from a hybrid progeny, the F_4 pollen parent of which contained recombinations of genes giving weak tumor expression. The data arranged in the histograms to the right in figure 1 indicate that the F_4 parent involved contained a recombinant genotype of preponderantly strong tumor expression.

(8) A final point of evidence that genes control tumor formation in Nicotiana hybrids is the induction of gene mutant tumor types. Izard¹³ produced, by X-raying seed, a nontumorous fertile mutant from the characteristically tumorous amphidiploid, N. glaucæ-langsдорffii. This mutant had the same chromosome number as the parent, was dominant to the tumorous condition, and segregated in F_2 and first backcross generations.⁸

Tumor Initiation

One theory for tumor initiation holds that the causal event is somatic mutation. The following lines of evidence, while admittedly inconclusive, tend to support the argument that somatic mutation is not the initial cause of tumor development in *Nicotiana* hybrids

(1) Tumorous types of *Nicotiana* hybrids were synthesized so as to be heterozygous for a single marker gene (P_2) controlling anthocyanin pigmentation in the flower.⁹ These were exposed to chronic gamma irradiation at dosage levels (1.24 to 200 r per day) which gave more than a 100-fold increase in "mutation" rate, as shown by loss of P_2 . Analysis of data on the initiation of tumors in the same plants grown under the same range of radiation exposures showed no significant increase in numbers of tumors with increasing dosages of radiation.¹⁰ There is evidently no simple relation of "somatic mutation" to tumor initiation in these *Nicotiana* types under the conditions imposed in this experiment.

(2) Sterile protuberances extending from teratomatous masses (figure 2) may occasionally elongate into abnormal thickened stems and yield flowers and seed. Sexual progeny produced from these aberrant branches differed in no observable way, including tumor formation, from seed progeny of normal stems. The reverted stem tissue could conceivably be caused by backmutation of previously mutated tumor cells, or could be derived from normal cells within the partly differentiated tumor mass. However, in the writer's opinion, alternative explanations, as noted below, seem more plausible.

(3) Braun¹⁴ has reported that tumor shoots, initiated by crown gall bacterial infection and derived from teratomatous tissue of single-cell origin,

ultimately become normal in every respect when forced into rapid division.

"These findings make somatic mutation at the nuclear gene level appear unlikely as a possible explanation for the nature of cellular alteration in crown gall. They suggest, instead, that some as yet uncharacterized cytoplasmic entity is responsible for the cellular changes that underlie the tumorous state in the crown-gall disease. This does not, of course, preclude the possibility that such cytoplasmic changes may be more or less under control of nuclear genes." An aberrant nuclei acid condition, as for example an accumulative RNA and resultant enzyme imbalance under DNA control, may be causal to the tumorous state in both plants⁸ and animals.¹⁵

(4) Hybrid *Nicotianas* respond differently to irradiation depending on the genotype.⁸ Some show enhanced tumor formation, particularly at levels of chronic gamma irradiation above 200 r per day.^{8,16} The effect could be due to radiation-induced somatic mutation or, alternatively, a response to stress conditions imposed on the cellular metabolism. We are inclined to favor the latter interpretation since similar effects can be initiated by a variety of stress conditions that inhibit growth as, e.g. crowding of seedlings (figure 3) or aging of mature plants.⁸

As a working hypothesis, spontaneous genetically-controlled tumor formation in *Nicotiana* hybrids can be considered in general terms as resulting from:

- (1) a metabolic system which is poorly integrated under the "direction" of the combined activity in hybrid cells of two or more evolutionary divergent genotypes.
- (2) Under conditions of high metabolic rate and rapid growth development is normal, but under less than optimal growth conditions (such

as the stress of heavy irradiation, crowding, aging, wounding or altered chemical environment) abnormal products of DNA-directed-RNA-executed cellular metabolism are synthesized or normal products accumulate to unassimilative levels. (3) By mechanisms presently unknown, phytohormone systems, notably for indoleacetic acid^{4,6} and interactions with kinetin,¹⁷ function to produce more than regulatory amounts of these growth substances¹⁸ in the hybrids and there is a change to autonomous tumor cells. The phenomenon may be viewed within the general framework of a malfunctioning of the normal developmental interaction between cellular environment and genetic or epigenetic controlling elements¹⁹ through failure to repress²⁰ the pluripotency of certain cells in differentiated multicellular organisms.

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FIGURE 1. Differences in tumor expression in progenies produced by crossing two F_4 inbred lines of N. langsdorffii x N. sanderae to N. suaveolens-glauca (SuSxG, above) and N. suaveolens-langsdorffii (SuSuLL, below). The progenies from one F_4 to the left, on both genetic backgrounds, have relatively few tumors, while those on the right from the other F_4 are highly tumorous. Tumor expression is grouped in classes of increasing tumorization from 0 (no tumors) to 4 (highly tumorous).

FIGURE 2. Abnormal branch growing from relatively highly differentiated tumor in Nicotiana suaveolens-langsdorffii.

FIGURE 3. Tumors formed on young seedlings of Nicotiana suaveolens-langsdorffii as a result of stress caused by crowding.

TUMOR EXPRESSION

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