Transplantation of Plant Tumors of Genetic Origin

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(Received for publication June 29, 1944)

The occurrence of tumorous growths on hybrids between *Nicotiana langsdorffii* and *N. glauca* has been reported and the behavior of these growths described in considerable detail by Kostoff (1-3), Whitaker (6), Levine (4), and others. Their resemblance to certain types of animal and human teratomas has been pointed out by Kostoff and Levine. Kostoff made some unsuccessful attempts to transplant these tumors.

In 1937 tissue cultures were isolated from growths of this type (7) and were used in studies on control of differentiation in undifferentiated tissue masses (8, 5). These cultures are now (May 10, 1944) in the 144th passage in vitro. Later (1941) similar cultures were isolated from bacteria-free crown-gall tissue of sunflower having an entirely different, nongenetic origin (10). These have likewise been maintained in vitro and are now in the 76th passage.

The successful implantation of tissue of bacteria-free crown-gall cultures into sunflower and artichoke after long cultivation in vitro, the production of new tumors therewith, and the bearing of these grafts on the whole question of homology between plant tumors and animal neoplasms (10, 11) suggested the importance of reexamining the genetic tumors for similar properties. The work on bacteria-free crown-gall tumors also furnished valuable experience in the necessary technic. This paper presents the results of experiments on the transplantation from one host to another of tissue derived from a plant tumor of genetic origin.

MATERIALS AND METHODS

Attempts to graft small pieces of tumor tissue from *Nicotiana langsdorffii* × *N. glauca* hybrids directly into other hybrid plants are obviously doomed to failure,

![Image](cancerres.aacjrnl.org)
since any wound to such a plant regularly results in a massive callus overgrowth that would automatically destroy anything but a fairly broad graft union. Grafts to non-tumefacient species of Nicotiana would avoid this mechanical difficulty but might, as Kostoff has emphasized, encounter physiological barriers or incompatibilities. Grafts attempted on Lycopersicon esculentum, Nicotiana tabacum, and N. glutinosa did, in fact, consistently fail. This physiological difficulty should, however, be minimal when grafts are made back upon either parent of the hybrid. Grafts to Nicotiana langsdorffii gave occasional possible "takes" but these were uncertain, because of the extensive callusing characteristic of all wounds on this species. Grafts to Nicotiana glauca were much more unequivocally successful and will be reported in detail.

For this purpose rapidly growing 5 year old tissue cultures were cut into thin slices, about 0.2 ram. thick and 2 to 3 mm. in diameter. Young stems of Nicotiana glauca were stripped of their leaves 3 to 5 cm. back of the growing point. In each, a pair of parallel incisions about 3 mm. apart and 7 to 8 mm. long were made with a sharp scalpel, the cuts being so placed as to meet at or a little below the cambium level. This formed a flap of cortex which, when the stem was bent toward the cut side, could be made to buckle outward, exposing the cambial region. A slice of tumor culture tissue was inserted in this incision and the stem allowed to return to its normal position, thus pulling the cortical flap tight. The incision was wrapped with "Sterilastic" rubber tape and allowed to heal. The bandages meet at or a little below the cambium level. This would usually record as possible was relegated to the negative class; and 8 of the negatives remained unchanged. Photographs of two series set up on February 25, 1943, and October 4, 1943, are shown in Fig. 1. The percentage of takes (40 per cent in the February 2nd series, 32 per cent in the February 25th series, and 54 per cent in the October 4th series) was excellent. The tumors formed were small in comparison with those produced on Helianthus by grafting bacteria-free tumor tissue cultures, but were essentially similar to these in general appearance.

Sections showed a structure similar to that observed in Helianthus tumor grafts (Fig. 2). In the example shown in transverse section (Fig. 2A) the implant had spread out both to right and left, producing tumors in both incisions, which were externally discrete but internally continuous (No. 2 from the left, bottom row, Fig. 1). A considerable mass of unaltered cortex, including vascular strands, was left at the center. Characteristic disoriented masses of cells occurred at the boundary between tumor and healthy cortex (Fig. 2B) and between tumor and host xylem (Fig. 2C). The rapidly growing outer part of the tumor was even more disorganized. The contrast between size of nuclei in rapidly growing and in degenerating portions of this mass is especially striking (Fig. 2C).

**DISCUSSION**

The spontaneous tumors that regularly arise at traumatized loci on plants of the hybrid, Nicotiana langsdorffii × N. glauca, were long ago recognized as malignant in the sense of regularly destroying plants upon which they appear (1). Levine (4) considered the degree of disorganization somewhat limited since he believed that he could recognize definite bud primordia in the superficial regions of all tumors in situ. In tissue cultures maintained on an agar substratum these bud primordia are no longer recognizable although they can be evoked by reducing the oxygen

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**DESCRIPTION OF FIGURE 2**

A. General picture. At right, host stem with normal pith, wood, and cortex; at left, a tumor (compare Nos. 1, 2, and 10 of bottom row, and Nos. 1, 2, and 3 of top row, Fig. 1). Note fairly wide region of normal epidermis and cortex (left, center) between the two tumors. Mag. X 145.

B. Detail of tumor mass at region of contact between tumor and healthy host cortex. At right, host xylem (wood) with phloem bundle at upper right, flanked above and below with cortical parenchyma. The disorganized tumor mass occupies center and left hand portion of picture. A sharp, dark line of gummy material bounds the tumor mass, and at the bottom is hybrid tumor tissue cultures under bark of Nicotiana glauca. Some evidence of crushing of cortical parenchyma. Scalariform cells, characteristic of tumor tissue (compare White and Braun [10] Fig. 6; White [9] Fig. 67), evident at many points in tumor mass. Mag. X 112.

C. Detail of contact between tumor tissue (left) and host xylem (right). Line of original incision shown by dark, gummy masses at right. Much of tumor mass likewise shows gummy deposits on the cell walls. Note again scalariform cells in tumor tissue. Mag. X 112.

D. Detail of central portion of another younger tumor. Note complete lack of orientation in cells, and scattered areas containing very small, pycnotic nuclei, mostly in regions that show considerable breakdown of the tissue. Mag. X 112.
tension of the medium (8), by reducing the intensity of illumination (5), and probably by other means. The degree of disorganization is thus not definitely fixed. The experiments reported here have demonstrated that these tumors possess the additional property, commonly attributed to neoplasia, of producing new tumors of similar type upon grafting into healthy individuals of a related species (the parent species, Nicotiana glauca), which is not naturally subject to tumefaction and never produces spontaneous tumors. The writer considers the retention of the tumefacient property after 5 years in vitro and its expression in a non-tumefacient host as evidence of the fundamental character of this property, in this case a property of genetic origin.

SUMMARY

Tissues isolated from stems of the hybrid, Nicotiana langsdorffii × N. glauca, which normally produces tumorous overgrowths spontaneously, after being maintained as in vitro tissue cultures for 5 years, were grafted into healthy plants of Nicotiana glauca. Here they produced typical tumors. They thus appear to possess the property of propagating their tumorous nature, in this case of genetic origin, indefinitely. Possible implications of this fact are discussed briefly.

REFERENCES

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*Cancer Res* 1944;4:791-794.

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