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# Correlation between the Nicotine Content of Tobacco Plants and Callus Cultures

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**Abstract.** Callus cultures of two low-alkaloid lines of *Nicotiana tabacum* L. had considerably lower nicotine contents than cultures from the respective high-alkaloid cultivars which were isogenic except for the two loci for alkaloid accumulation. Thus, there was a strong correlation between the nicotine content of callus cultures and the plants from which they were derived.

**Key words:** Callus cultures – *Nicotiana* – Nicotine – Tissue cultures.

The relationship between the alkaloid content of whole plants and of cell cultures derived from these plants has been examined in *Catharanthus roseus* by Zenk et al. (1976) and by Roller (1978). Zenk et al. showed that cultures established from plants having a high content of serpentine and ajmalicine generally had a higher content of these alkaloids than cultures derived from low-yielding plants, but Roller concluded that "serpentine production in the plants must not correlate with serpentine formation in the corresponding tissue culture." In both investigations, some of the cultures from low-yielding plants had a higher alkaloid content than some of those derived from high-yielding plants.

In order to clarify the question of the relationship between the alkaloid content in plants and that in cell cultures derived from these plants, we have investigated the nicotine content of callus cultures established from high- and low-alkaloid tobacco plants (*Nicotiana tabacum* L.) which differ genetically at the two loci coding for production and accumulation of nicotine but which are otherwise isogenic. We find a very high positive correlation between the nicotine contents of the calli and of the plants from which they were derived.

The two cultivars used in this study, and their low-alkaloid derivatives were Burley 21 and low-alkaloid (LA-)Burley 21 (seeds from Dr. Glen Collins, Department of Agronomy, University of Kentucky, Lexington, USA); and NC-95 and LAFC-53 (seeds from Dr. James Chaplin, Oxford Tobacco Research Laboratory, Oxford, N.C., USA). LA-Burley 21 and its relationship to Burley 21 are described in Legg et al. (1970), LAFC-53 and its relationship to NC-95 are described in Chaplin (1975). Seeds were germinated and plants were grown under natural light in a temperature-controlled greenhouse (temperature range, 22–25° C). Explants were obtained from leaves of 2-month-old plants. The leaves were surface-sterilized by immersion in ca. 0.3% NaOCl (6%, v/v, "Clorox", a commercial bleach) for 20 min, followed by two washes with distilled water. Explants were obtained with a 0.6-cm diameter cork borer and were cultured on Murashige-Skoog medium (1962) containing 2.0 mg/l α-naphthaleneacetic acid (NAA) and 0.2 mg/l kinetin (6-furfurylaminopurine), solidified with 0.8% Bacto-agar (Difco Laboratories, Detroit, Mich., USA) in culture tubes, 25 mm diameter, 150 mm long, 10 ml medium/tube, at 25° C in the dark. After callus induction (ca. 3–4 weeks), the calli were transferred onto a medium containing 0.15 mg/l NAA, the optimal auxin concentration for nicotine production according to Ohta et al. (1978). After three weeks, nicotine was extracted from calli using a modification of the procedure described by Tabata and Hiraoka (1976). Callus (2–4 g) was mixed with 10 ml of 5M NaOH and the mixture steam distilled. The steam distillate was collected in 5 ml of 0.5 M HCl to a total volume of 50 ml and nicotine content of the distillate determined spectrophometrically by measuring ultraviolet absorbance at 236, 259 and 282 nm. Nicotine content was expressed as mg/g fresh weight of tissue using the conversion factor given by Willits et al. (1950). The nicotine content of the leaves from which the explants were excised was determined by the same method. The nicotine in distillates from calli and leaves was identified by thin-layer chromatography according to Ohta and Yatayawa (1978); it was the only alkaloid detected in distillates from both sources.

Table 1 shows the average nicotine content of calli from the four tobacco cultivars and of the leaves used to establish the calli. It can be seen that there is a high positive correlation between the nicotine content of callus cultures which have undergone one subculture and the nicotine content of the plants and cultivars from which they are derived. Thus both plants (leaves) and callus of Burley 21 produce about

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**Table 1.** The nicotine content of calli from four tobacco cultivars and of the leaves used to establish the calli

| Cultivar | Nicotine content (mg/g fr. wt.) | |
|---|---|---|
| | Callus culture | Leaves |
| Burley 21 | $0.40 \pm 0.16$[a] (45)[b] | $0.62 \pm 0.28$[a] (8)[c] |
| LA-Burley 21 | $0.02 \pm 0.03$ (44) | $0.03 \pm 0.03$ (8) |
| NC-95 | $0.31 \pm 0.16$ (38) | $0.58 \pm 0.29$ (6) |
| LAFC-53 | 0.0 (33) | $0.07 \pm 0.03$ (6) |

[a] Standard deviation
[b] Number of calli analyzed
[c] Number of different plants from which leaves were taken to obtain explants

20 times more nicotine than plants and callus of LA-Burley 21. We have found no instance where the nicotine content of a callus derived from a low-yielding plant was greater than that of a callus derived from the high-yielding counterpart. The genes that determine nicotine production in the plant clearly also determine the amount of nicotine produced in callus cultures. In addition, however, there is a varietal difference which affects nicotine production in the callus but not in the plant. The nicotine content of LAFC-53 plants was not significantly different from that of LA-Burley 21 plants, but in spite of this we failed to detect even trace amounts of nicotine in any of the 33 calli from LAFC-53, whereas nicotine was readily detectable in calli from LA-Burley 21 (Table 1). Similarly, there was no significant difference in nicotine content between Burley 21 and NC-95 but callus cultures from NC-95 showed a significantly lower yield of nicotine than those from Burley 21 ($t > 95\%$ in Student's t-test). This demonstration of genetic differences which affect alkaloid yield in callus but not in the plant offers a possible explanation for the inconsistencies between the data of Roller (1978) and Zenk et al. (1976). While the plants used in these studies may have been homozygous for alkaloid yield, they may not have been entirely homozygous for other characteristics which may affect yield in cell culture. This could explain the large variation in alkaloid yield seen in callus cultures, and also why the difference in alkaloid yield between high- and low-yielding plants became much smaller in culture.

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