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TROPANE ALKALOIDS FROM *ATROPA BELLADONNA*, PART II.[1] INTERACTION OF ORIGIN, AGE, AND ENVIRONMENT IN ALKALOID PRODUCTION OF CALLUS CULTURES

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ABSTRACT.—Callus cultures (n = 156) were initiated from one young root and from stems of 12 plants (grown under greenhouse conditions) representing nine European seed samples of *Atropa belladonna*. The calli derived from the same piece of stem showed wide variations in their growth response on modified Wood and Braun's nutrient medium and in their alkaloid production (gc analysis). Levels of alkaloids were not significantly higher in the callus cultures of intact mother plants having a high level of hyoscyamine and scopolamine in seeds, roots, or leaves than in callus cultures originating from plants with low alkaloid production. The maximum hyoscyamine content (ca. 0.2–0.3 g/kg dry wt) was usually found between the 7th and 9th passage in stem callus lines. An exceptionally high alkaloid level (0.8–0.9 g/kg dry wt) was observed at 8th or 9th passage in two stem lines originating from different seed samples. Trace amounts of scopolamine were detected sporadically. After the 9th passage the alkaloid content decreased rapidly, and the repression of synthesis could not be prevented by lower temperature (15° as against 25°) or by lower or higher auxin level of the medium.

Atropa belladonna L. (Solanaceae) plants are one of the most important sources of tropane alkaloids, especially of hyoscyamine. The alkaloids are mainly synthesized in the roots (2,3), but the leaves are also used as a drug. The total alkaloid level varies considerably in the different variants of the species (4). Changes during the growth period and even marked diurnal variation have been observed in the alkaloid composition and concentration (5–7). The hyoscyamine concentration of leaves of *A. belladonna* was found to vary from ca. 2.4 to 3 g/kg dry wt depending on the developmental stage of the plant; scopolamine was at maximum concentration in young leaves (ca. 0.3 g/kg dry wt) and decreased (ca. 0.05 g/kg dry wt) rapidly during the later periods of vegetation (6). Tropane alkaloid production is also affected by climatic and edaphic factors, especially by mineral nutrients (8,9). The application of plant growth regulators may affect the growth and alkaloid levels in intact plants and tissue cultures (10,11).

Before the biotechnological production of biologically active compounds can become economically feasible, plant cell lines demonstrating effective and stable alkaloid formation will have to be developed. Unfortunately, the callus and suspension cultures of species producing tropane alkaloids tend to be labile, and only small amounts of the alkaloids have been reported for *A. belladonna* in some experiments (12–14).

It has been proposed that organogenesis, especially the development of roots, may be a prerequisite for alkaloid biosynthesis in this plant (15). Larger amounts of total alkaloids (0.27 g/kg dry wt), mainly hyoscyamine and scopolamine, have been reported from tetraploid plantlets regenerated from leaf callus than from undifferentiated callus (0.8 mg/kg dry wt) (12). The level of tropane alkaloids obtained from callus cultures of *Duboisia leichhardtii* after 3 months successive culture was rather low (0.002% hyoscyamine and 0.0005% scopolamine) (16). Instability in scopolamine production was

[1]For Part I, see Ylinen *et al.* (1).

also found in suspension cultures of *Hyoscyamus muticus*, and a gradual decrease in scopolamine content to a basic level of 10^{-5} to 10^{-6}% (dry wt) was registered in all cultures (17). Aggregation of suspension cultures of *Hyoscyamus niger* was found to correlate weakly with organ-forming capacity and hyoscyamine synthesis, but there was no clear-cut relationship between organization and alkaloid synthesis (18). Root callus of *A. belladonna* showed higher alkaloid level (0.047–0.053%) (19) than stem callus of *Datura metel* (0.0056%) (20). Alkaloid contents determined according to Worrell and Booth (21) may be too high, however. No alkaloids were detected in stem or leaf callus of *A. belladonna* (19).

The aim of the present work was to find callus lines of *A. belladonna* having high and stable alkaloid production (hyoscyamine and scopolamine) and good growth rates on an inexpensive medium. The following research strategy was applied: (a) a search for a possible relationship between alkaloid production of the mother plant and the respective callus lines, (b) comparison of the callus growths and alkaloid levels of explants of the same plant, and (c) a search for possible influences of callus passages, auxin levels, and incubation temperatures.

TABLE 1. Alkaloid Contents of Seed Samples of *Atropa belladonna*.

Sample[a]	Hyoscyamine (g/kg)	Scopolamine (g/kg)
1	4.1	nd[b]
2	6.9	nd
3	6.5	nd
4	4.3	0.2
5	6.4	0.4
6	—[c]	—
7	4.0	0.3
8	4.9	nd
9	1.8	0.1
10	4.8	0.3
11	5.6	0.5
12	1.2	0.1
13	3.9	nd
14	6.2	0.4
15	5.4	0.2
16	6.9	0.4

[a]Origin of samples: 1. Italy, Reg. Marche (3), Monti Sibillini, Pizzo del Vettore, spont. 2. Italy, Reg. Marche (1), Massa Trabaria, Mt. Maggiore, spont. 3. Italy, Pesaro, M.V.—Monti Sibillini, Urbion, Centro Richerche Floristiche Marche, spont. 4. France, Mieussy, Valère, Jardin Botanique Alpin "La Jaysinia", spont. 5. France, Strasbourg, Jardin Botanique de l'Université Louis Pasteur. 6. France, Côte-d'Or, Essarois, spont. 7. Germany, Niedersachsen, Moringen bei Northeim, spont. 8. France, Nancy, Meurthe-et-Moselle, Laxou, Jardins Botaniques de la Ville et de l'Université de Nancy, spont. 9. Germany, Bad Abbach, Botanischer Garten der Universität Regensburg, spont. 10. Germany, Hessen, Botanischer Garten und Botanisches Museum Berlin-Dahlem, spont. 11. Austria, Alpengarten Villacher Alp, Botanischer Garten Klagenfurt, cult. 12. Germany, Hessen, Caldern, Rimberg, Botanischer Garten Marburg, spont. 13. France, Doubs, Manirolle, spont. 14. Hungary, Budakalász, Pannonicum, Bakonyicum, Visegradense, Institutum Plantarum Medicinalium. 15. Hungary (exact origin unknown; obtained via Leicester, England). 16. Hungary, Budapest, Hortus Botanicus, Universitatis Hungariae, spont./subspont.

[b]nd = not detected.

[c]Small amount of seeds did not permit a reliable analysis in this case.

MATERIAL AND METHODS

CULTIVATION OF MOTHER PLANTS.—Seeds of *A. belladonna*, obtained through exchange with European botanical gardens but mainly collected in nature (Table 1), were sown in a mixture of fertilized peat (Satoturve) and sand (4:1). The material was cultivated in pots (1.5 liter) in a greenhouse, where day and night temperatures varied in the ranges 22–25° and 18–22°, respectively. The plants received supplementary light for 18 h daily from 400 W Osram HQLC lamps. They were watered daily, and a dilute commercial fertilizer (Kemira, Kukkien Y-lannos) was applied once a week when the plants were in stages of effective growth.

COLLECTION OF LEAF AND ROOT SAMPLES.—Full-grown leaves (1.5–4 g fresh wt) were collected from two or three mother plants per strain at the early fruit-bearing stage. Collection was made at about 2 p.m. to avoid the effect of any diurnal changes in the alkaloid level. Leaves were cleaned of aphids, frozen, and lyophilized. The freeze-dried material was stored in a desiccator at 4° until analyzed. The root material was usually collected from plants already having some ripe fruits, in order to avoid damaging the plant before new seed material was available for further experiments. Young lateral roots were dissected at about 1 p.m. and were washed, dried, and stored similarly to the leaf material for alkaloid analyses.

Different mother plants are indicated by Roman numerals I, II, and III, given after the strain numbers (Arabic numerals 6–16), which correspond to the seed samples (Table 1). For example, 13/I = strain 13, plant I, originating from the seed sample 13 (Table 2).

TABLE 2. Tropane Alkaloid (Hyoscyamine and Scopolamine) Content (g/kg dry wt) of Leaves and Roots of Strains 6–16 and Plants I-III of *Atropa belladonna*.

Strain/Plant	Date[a]	Leaves		Date	Roots	
		Hyoscyamine	Scopolamine		Hyoscyamine	Scopolamine
6/I	13 Dec	2.4	0.2	—	—	—
6/II	13 Dec	3.2	nd[b]	—	—	—
7/I	19 Oct	4.0	0.5	10 Jan	2.6	0.7
7/II	10 Oct	2.9	0.5	—	—	—
9/I	13 Dec	4.2	0.2	19 Dec	0.6	0.4
9/II	19 Oct	4.9	0.2	10 Jan	1.8	0.3
10/I	19 Oct	2.0	0.3	19 Dec	1.5	0.4
10/II	13 Dec	2.2	0.1	10 Jan	0.4	0.3
11/I	13 Dec	2.8	0.2	10 Jan	2.3	0.3
12/I	19 Oct	3.4	0.3	10 Jan	3.7	0.3
12/II	13 Dec	1.1	0.3	21 Dec	0.5	0.4
13/I	19 Oct	1.7	0.3	10 Jan	2.2	nd
15/I	13 Dec	4.0	0.3	18 Dec	1.5	0.9
15/II	19 Oct	1.2	0.1	—	—	—
15/III	13 Dec	0.5	0.2	10 Jan	0.7	0.5
16/I	19 Oct	1.0	nd	10 Jan	0.5	nd
16/II	19 Oct	1.3	0.1	—	—	—
16/III	13 Dec	1.3	0.3	10 Jan	1.8	nd

[a]Time period: October 1984–January 1985.
[b]nd = not detected.

INITIATION OF CALLUS CULTURES.—Young, unlignified stem pieces (0.3–0.5 cm in diameter) of the same plants from which the leaf and root material was collected were used as starting material for callus cultures. Pieces about 2 cm long were surface sterilized for 1 min in 70% EtOH and then for 10 min in 3% sodium hypochlorite. The material was rinsed four times in sterile distilled H_2O. The peripheral parts of the stem were removed, and the stem was cut into disks 0.4 cm thick that were used as explants. These were transferred to a nutrient medium favorable to tissue cultures of *A. belladonna* (22). This modified Wood and Braun's medium (23) [macronutrients: $NaNO_3$ (1785 mg/liter), $Ca(NO_3)_2 \cdot 4H_2O$ (235 mg/liter), $(NH_4)_2SO_4$ (790 mg/liter), KCl (910 mg/liter), $MgSO_4 \cdot 7H_2O$ (1740 mg/liter), $NaH_2PO_4 \cdot H_2O$ (250 mg/liter), Na_2SO_4 (190 mg/liter), NaFe(III)EDTA (8.3 mg/liter)] with a rather high nitrogen level (35 mM inorganic nitrogen, NH_4^+/NO_3^-, ca. 1:2) contained micronutrients and vitamins according to White (24), 100 mg/liter myo-inositol, 100 ml/liter coconut milk (Difco), 20 g/liter sucrose, and, as growth regulators, 0.5 mg/liter α-naphthylacetic acid (NAA) and 0.1 mg/liter kinetin. The pH was ad-

justed to 5.2. Solidified (agar 6 g/liter, Difco) medium in portions of about 30 ml was autoclaved in Erlenmeyer flasks (100 ml). Five to seven explants were cultivated in each of the flasks, and five to ten replicates per plant were prepared.

Initiation of root callus from plants cultivated in soil was mainly unsuccessful due to the deleterious effect of surface sterilization (see above) on root tissue. Shorter sterilization time left infection sources. Only one root callus culture was obtained from a plant growing in soil.

CULTIVATION OF CALLUS LINES.—Each tissue explant showing good callus production was cultivated separately and treated as one callus line in order to discover possible somaclonal variation (sensu lato). The callus was transferred to the same medium as in initiation but with the coconut milk omitted. Because the callus growth was not always effective, part of the lines were transferred to a medium containing a higher level of auxin (NAA 2 mg/liter). During the first nine passages the calli were incubated at 25° in the dark. Thereafter, about half of the cultures were transferred to 15° to determine whether the time between passages could be lengthened as a result of the slower growth rate at 15°. The alkaloid production of many callus lines was followed from the 4th to the 13th or 17th passage. They were subcultured every four (25°) or six weeks (15°) and incubated in the dark.

For alkaloid analyses the callus material was rinsed several times with distilled H_2O and lyophilized. Because of the large number of lines, only part of the cultures could be analyzed at different ages and for different environments. The growth of the callus was mainly observed visually and marked from no growth (−) to weak (+), medium (++), and good (+++) growth corresponding to <125, 125–375, 376–600, and >600 mg fresh wt per callus piece, respectively.

TESTS WITH DRAGENDORFF'S REAGENT.—Although the Dragendorff's reagent is unspecific, it was used in rapid preliminary screening when only small amounts of material were available. Small pieces of callus from relatively young cultures were crushed with a glass rod on Whatman no. 1 filter paper to obtain comparable spots of plant material. Reference substances [100 μg of hyoscyamine, scopolamine, and tropanol(= 3α-tropanol)] were applied to the same filter paper, and the paper was stained with Dragendorff's (Munier's) reagent (25).

GAS CHROMATOGRAPHY.—The alkaloids were extracted from freeze-dried plant material (20–200 mg) and purified using disposable extraction columns (Baker-10 SPE™). Capillary gc analysis of hyoscyamine and scopolamine, the main alkaloids present, was carried out as described earlier (1).

RESULTS AND DISCUSSION

ALKALOID CONCENTRATION OF MOTHER PLANTS.—The hyoscyamine concentration was mostly much higher than that of scopolamine in leaf, root, and seed material (Tables 1 and 2). Plants at the same developmental stage and cultivated under the same greenhouse conditions showed considerable variation in hyoscyamine and scopolamine concentration, even when grown from the same seed sample. Relatively large variations were found from plant to plant without clear correlation between the alkaloid levels of roots and leaves (Table 2). The leaves having a high hyoscyamine concentration (4.2 g/kg dry wt) contained scopolamine, the more valuable component, in a rather low concentration (0.2 g/kg dry wt, plant 9/I). However, the plant (7/II) exhibiting the maximum scopolamine level (0.5 g/kg dry wt) in leaves showed only a medium level of hyoscyamine (2.9 g/kg dry wt).

The hyoscyamine concentration in leaf samples of some of the present plants was clearly higher than that reported earlier (6). Some of the earlier results have been given in terms of fresh wt. Only in the case of seeds that have a low H_2O content can the results be compared with some reliability. The previously reported hyoscyamine content of ripe seeds [0.75 g/kg fresh wt (5)] is lower than in our material (Table 1).

Because of the variation during different growth periods, comparable material must be used in evaluating mother plants of different origin. The total alkaloid level and hyoscyamine concentration have been found to be highest (ca. 2 g and ca. 1.6 g/kg fresh wt, respectively) in the shoot tops of flowering *A. belladonna* already bearing some green berries (5). The leaves had maximum alkaloid concentration after flowering and before the fruits were ripe. Young roots and taproots (root stocks) contained more hyoscyamine than older ones.

The wide variation in the alkaloid concentrations of roots may reflect the difficulty

in obtaining comparable samples, for there is much more variation in the morphology and anatomical structure of roots than of leaves. In order to avoid excessive damage to plants, relatively young lateral roots were used for analyses. Strains 7, 9, 12, 15, and 16 showed some morphological characteristics (e.g., large leaves) or alkaloid production capacity that could be considered favorable for further experiments, especially under greenhouse conditions.

Relatively wide variation in the alkaloid concentration was found in the seed samples as well. Some of the seed samples had very similar alkaloid compositions to the corresponding leaves (7/I). One plant (15/III) having a low alkaloid level in leaves produced seeds with exceptionally low alkaloid concentration. This same plant, nevertheless, synthesized a normal level of scopolamine in roots.

Clearly, the quantity of tropane alkaloids in seeds does not predict well the capacity of shoots and roots to produce these compounds. It may be relevant that, while a substantial part of *A. belladonna* seeds are formed by the triploid endosperm, the genes regulating alkaloid biosynthesis may be more dissimilarly regulated in this tissue than in diploid cells. However, the cell structure of the embryo and endosperm is similar in ripe seeds of *A. belladonna* (26).

GROWTH OF CALLUS LINES.—In total, 156 callus lines were initiated from the stems of 12 mother plants and from one root. Owing to the variable number (20–60) of explants and some infections, the ability to form callus could be evaluated only roughly. The number of subculturable, relatively well or excellently growing stem callus lines obtained from a single plant specimen ranged from 3 to 17, with a mean value of 10 (Table 3). The growth of the lines derived from the same stem varied. Poor growth and browning at the beginning of the experiment were observed especially in plants 9/II and 12/II.

Shoots and/or roots were observed in many stem callus lines, mainly when still young (up to 5th passage), independently of the auxin level of the nutrient medium. Although nearly all plants were able to produce one or a few very well growing callus

TABLE 3. Number of Subculturable Callus Lines of *Atropa belladonna*,[a] Highest Hyoscyamine Concentrations Obtained, and the Corresponding Growth.

Strain/Plant	Organ	Number of explants	Number of lines	Passage	NAA[b] (mg/liter)	Hyoscyamine (g/kg dry wt)	Growth[c]
6/I	s	25	9	7	2.0	0.36	++
7/I	s	36	8	7	0.5	0.21	+++
7/II	s	42	6	9	0.5	0.34	++
9/I	s	60	17	4 and 9	0.5	0.30	++
9/II	s	25	3	7	2.0	0.42	++
10/I	s	42	11	4	2.0	0.30	++
10/II	s	25	8	9	0.5	0.88	++
11/I	s	25	14	8	2.0	0.80	++
12/II	s	60	12	4	2.0	0.25	+++
13/I	s	20	7	8	2.0	0.35	++
15/I	s	60	16	8	2.0	0.32	++
16/II	s	60	6	9	2.0	0.33	+++
7/I	r	20	1	4	0.5	0.30	++
Total		500	118				

[a]From stems (s) or roots (r) of 12 mother plants representing 9 strains.
[b]NAA = naphthylacetic acid.
[c]++, 376–600 mg fresh wt per callus piece; +++, >600 mg fresh wt per callus piece.

lines, plants 6/I, 9/I, 11/I, 12/II yielded several of these. Both stem and root callus cultures could be subcultured for 3 years.

THE EFFECT OF ORIGIN AND AGE.—Only a few callus lines could be discarded on the basis of a weak Dragendorff's reaction in spot tests. Nearly all lines gave a very strong positive reaction. However, the reaction was not specific enough to be a good screening method.

The gc analyses showed that both root and stem callus lines of *A. belladonna* were able to synthesize considerable amounts of hyoscyamine, but scopolamine was present in either very low or undetectable concentrations (Table 3). Some earlier results have indicated that the synthesis of tropane alkaloids may be localized in roots and root callus in *A. belladonna* (19). Our results rather support the idea that these alkaloids are synthesized in meristematic cells, not in a specific organ (27), although roots are the main site of synthesis in *A. belladonna*. Secondary products seem to be localized in vacuoles in the tips of roots differentiated from suspension cultures of *A. belladonna* (root origin) (28), and according to some early microchemical observations, alkaloids are present in the shoot meristems as well as in the apices of all buds (29).

The most effective alkaloid production (ca. 0.9 g/kg dry wt) was found in a callus line (9th passage) initiated from plant 10/II and grown on a medium having a low auxin level (NAA 0.5 mg/liter, 25°) and in callus line (8th passage) originating from plant 11/I (ca. 0.8 g/kg dry wt) on a medium supplemented with a higher auxin level (NAA 2 mg/liter, 25°) (Table 3).

Several callus lines having very low or no alkaloid production at the beginning of the cultivation (4th passage) produced 180–350 mg/kg dry wt hyoscyamine in the 7th to 9th passages, independently of the auxin level of the nutrient medium (Figure 1). The alkaloid production declined rapidly after the 9th or 10th passage in nearly all of the lines. In some species it occurs considerably earlier (16,30).

Greening suspension cultures of *A. belladonna* displaying structural differentiation (shoot-like structures and bipolar embryoids) have contained the highest tropane alkaloid levels reported in the literature (up to 1.5 g/kg dry wt cells) (31). The modified Dragendorff's reagent (see above), however, may give a too high total alkaloid level.

The stem callus of *D. metel* contained about 0.155 g/kg (0.0155%) of hyoscyamine and scopolamine (20). Callus cultures initiated from different parts of seedlings of *H. niger* showed no differences in total alkaloid level. One very aggregated suspension culture (8th passage) was reported to synthesize hyoscyamine (18), and the total alkaloid level was rather high (0.75 g/kg dry wt; 0.075%). The spectrophotometric method used in this work [based on the Dragendorff's reaction (32)] may, however, again give too high alkaloid concentrations. Many nitrogenous compounds, for example, several amino acids and many alkaloid precursors, are Dragendorff positive. Arginine is an abundant amino acid in tissue cultures of *Datura stramonium*, where cuscohygrine and pseudotropine (=3β-tropanol) are the principal alkaloids present (33). Hyoscyamine and scopolamine were not found in these cultures.

THE EFFECT OF TEMPERATURE AND AUXIN CONCENTRATION.—Variability in the alkaloid production in callus and suspension cultures of medicinal plants is a significant problem. When rapid screening methods are lacking for alkaloid production at early stages of the callus growth, material must be cultured during several passages until enough is available for chemical analysis and subcultures. The alkaloid production is known to be affected by the culture medium (34).

Production of tropane alkaloids is stimulated in tissue cultures of some solanaceous species (*Datura innoxia*, *D. metel* and *Scopolia japonica*) by a number of growth regulators or their combinations, while other combinations are without any effect or are inhibitory

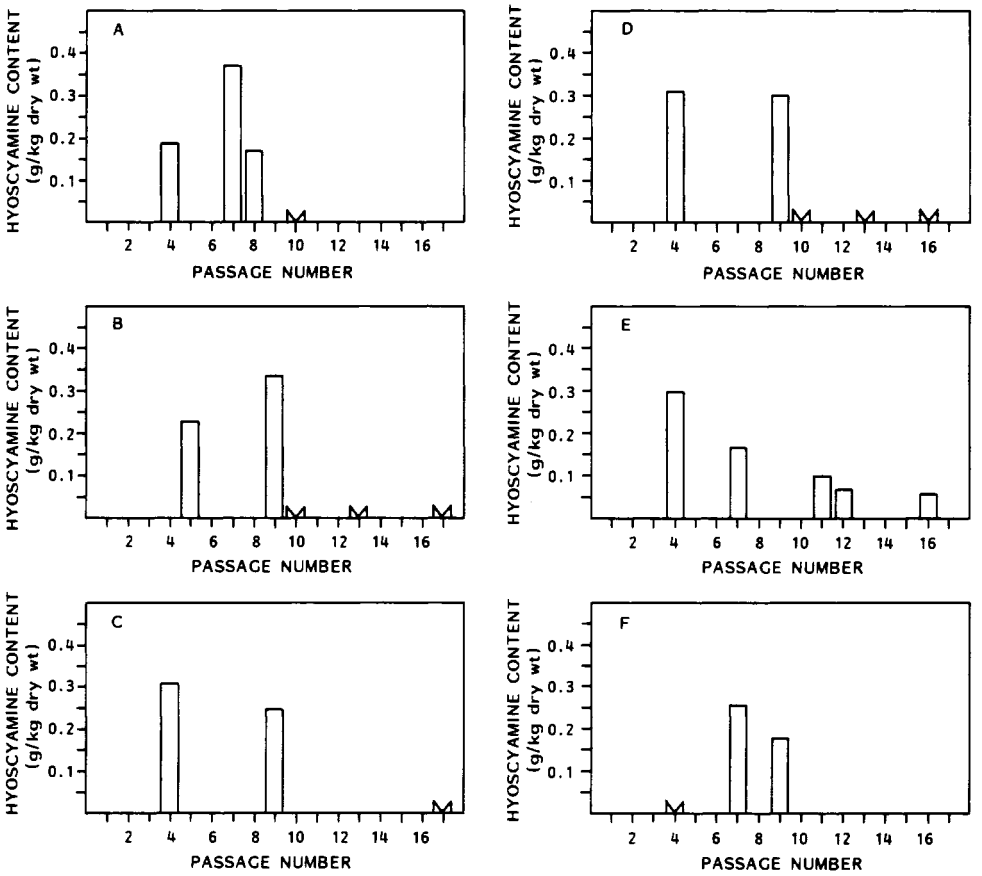

FIGURE 1. Effects of age, origin, and auxin level of the medium on hyoscyamine production in selected *Atropa belladonna* callus lines grown at 25°. Passage interval 4 weeks. M = no detection. A, strain 6/I, line 8, stem origin (NAA = naphthylacetic acid 2 mg/liter); B, strain 7/II, line 4, stem origin (NAA 2 mg/liter); C, strain 7/I, line 1, root origin (NAA 0.5 mg/liter); D, strain 9/I, line 8, stem origin (NAA 0.5 mg/liter); E, strain 10/I, line 6, stem origin (NAA 2 mg/liter); F, strain 11/I, line 5, stem origin (NAA 0.5 mg/liter).

(11, 35–37). In suspension cultures of *A. belladonna*, α-naphthoxyacetic acid (NOA, 2.5 mg/liter) and tropic acid (10 mg/liter), in combination with NAA (0.5 mg/liter), were able to induce root formation, and alkaloid production was stimulated (38). In contrast to our findings in extensive callus line experiments (Tables 3 and 4), in a recent study with suspension cultures (39) tropane alkaloid production was reported only in association with rhizogenesis.

Alkaloid production of many callus lines was followed up to the 13th or 17th passages at 15° and 25° on solid modified Wood and Braun's medium containing 0.5 or 2.0 mg/liter of NAA as growth regulator (Table 4). The callus lines exhibited similar growth on the two media, but they grew more slowly at 15°, so that the time interval between passages was lengthened from 4 weeks (at 25°) to 6 weeks. There was no general trend in the alkaloid production that could be connected with the temperature or nutrient medium. Only a few of the lines were capable of synthesizing hyoscyamine after 13 or 14 passages (age about 1–1.5 years) (Figure 1).

Although the repression of alkaloid synthesis was not followed entirely systematically, it turned out that seven of the lines had a relatively good alkaloid production of 250–340 mg/kg dry wt after nine passages, which is about the same level or somewhat higher than at the beginning of the experiment (4th to 5th passages). The low level of

alkaloids in some lines was not due to rapid catabolism but was a result of repression of biosynthesis because our feeding experiments showed that suspension cultures of *A. belladonna* were able to accumulate exogeneously added hyoscyamine and scopolamine, while their degradation products were not detected in the cells.[2]

TABLE 4. Effects of Age, Temperature, and Auxin Level on the Average Hyoscyamine Content (g/kg dry wt) of Callus Cultures of *Atropa belladonna* during Subculturing. Number of Lines Analyzed in Parentheses.

Passage	NAA[a] (0.5 mg/liter, 25°)	NAA (2 mg/liter, 25°)	NAA (0.5 mg/liter, 15°)	NAA (2 mg/liter, 15°)
4–5	0.14 (14)	0.14 (13)		
7	0.17 (5)	0.24 (4)		
8	0.13 (8)	0.27 (9)		
9	0.14 (14)	0.06 (10)		
10–11	0.04 (12)	0.02 (5)	0.03 (36)	0.03 (17)
12–13	0.02 (10)	0.04 (7)	0.04 (50)	0.03 (8)
14–15	0.04 (3)	0.01 (3)	0 (2)	0 (2)
16–17	0 (6)	0 (7)	0 (1)	0 (1)

[a]NAA = naphthylacetic acid.

The mean content of hyoscyamine in callus lines of *A. belladonna* was smaller than in leaves of intact plants, usually by a magnitude of 10. A much higher difference, e.g., between protoplast-derived cell culture clones and the plant, has been found in *H. muticus* (30).

No negative correlation was observed between the callus growth and alkaloid production in our experiments, and hyoscyamine synthesis was not affected by temperature or the NAA level of the nutrient medium. Earlier results on the effect of auxins and cytokinins on the tropane alkaloid level of tissue cultures were somewhat variable (38–40).

The variation expressed in the alkaloid production of stem callus lines of *A. belladonna* is so great that the alkaloid level of the mother plant can have no significant value as the basis of selection. Substantial somaclonal variation in alkaloid production, changes in the alkaloid composition, and decrease in content upon subculturing have been observed in many medicinal plants (16,18,30,41). The callus cultures of *A. belladonna* were not able to synthesize scopolamine effectively, but some lines showed quite good capacity for hyoscyamine production. Nevertheless, not all lines derived from the same plant organ formed analyzable amounts of this substance. The small number of callus lines in some earlier studies may be the reason for the contrary evidence that callus cultures derived from shoots are unable to synthesize tropane alkaloids and that this ability is restricted to root tissue and calli originating from it (19).

In conclusion, hyoscyamine synthesis was slowly repressed in callus cultures of stem and root origin, but good growth and alkaloid production were obtained in many callus lines grown in our nutrient medium.

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