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BIOSYNTHESIS AND DISTRIBUTION OF TOMATINE IN CULTURED EXCISED *LYCOPERSICON ESCULENTUM* ROOTS

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Key Word Index—*Lycopersicon esculentum*, Solanaceae, tomato; cultured roots, biosynthesis, distribution; steroidal glycoalkaloid, tomatine

Abstract—Growth of cultured excised tomato roots in the presence of ^{14}C-mevalonic acid lactone results in labelling of tomatine In the main axis of the root, incorporation was greatest in the apical meristematic region. Tomatine was present in equal concentrations in all parts of the cultured root system

INTRODUCTION

THE STEROIDAL glycoalkaloid α-tomatine is present in all major organs of plants which elaborate it, but the shoot is generally recognized as being the main site of synthesis and accumulation.[1] Tomatine synthesis does occur in the root system[1-3] but almost no information exists regarding its site of synthesis or its distribution in this organ. In a previous communication[4] it was shown that a close quantitative association exists between growth of cultured tomato roots and the level of tomatine, and suggested that this might be indicative of tomatine synthesis occurring principally in actively growing regions of the root. The work reported here provides some information relating to this question and also to the distribution of tomatine in the cultured root system.

RESULTS

Cultured excised tomato roots were grown in standard nutrient medium containing mevalonic acid-[2-^{14}C]-lactone (MVA). After 10 days growth, roots were harvested and extracted and tomatine separated by TLC. Scanning of the TLC plate revealed a single peak of radioactivity corresponding in R_f (0·70) to the tomatine zone. The mean (of four replicates) activity of the extracted tomatine was 31 cpm μg^{-1} corresponding to a 0·09% incorporation of label into the alkaloid. Co-chromatography of "cold" authentic tomatine and labelled MVA and scanning of the TLC plate indicated that the peaks of radioactivity (1 large, 1 small) did not coincide with the tomatine zone. Subsequent counting of this tomatine showed that there was no significant contamination by the labelled precursor.

[1] SANDER, H (1956) *Planta* **47**, 374
[2] RODDICK, J. G and BUTCHER, D. N (1972) *Phytochemistry* **11**, 2019
[3] BRUSKE, H (1966) *Abhandl Deut Akad Wiss Berlin, Kl Chem Geol. Biol* **3**, 105
[4] RODDICK, J G and BUTCHER, D N. (1972) *Phytochemistry* **11**, 2991

In order to determine if the label is incorporated into tomatine at different rates in different parts of the root, experiments involving shorter presentation times were conducted. Roots were grown for 7 days after which labelled MVA was added to the culture medium. After a further 3 days incubation, the roots were harvested, all visible lateral roots removed, and the main axis divided up into three equal lengths (each *ca* 40 mm), designated "apical," "middle" and "basal". Tomatine extracted from the "apical"

TABLE 1 INCORPORATION OF MVA-[2-^{14}C]-LACTONE INTO TOMATINE IN DIFFERENT REGIONS OF THE MAIN AXIS OF CULTURED *Lycopersicon esculentum* ROOTS

Region of main root axis	*ca* Length of region (mm)	Incubation period (days)	Incorporation (cpm μg^{-1} tomatine)
"Apical" $\frac{1}{3}$*	40	3	11 51
"Middle" $\frac{1}{3}$	40	3	4 23
"Basal" $\frac{1}{3}$	40	3	5 63
A_a*	20	2	8 25
A_b*	20	2	1 90
A_1*	10	1	1 75
A_2*	10	1	0 25
A_3*	10	1	0 22
A_4*	10	1	0 21

* Where "Apical" $\frac{1}{3} = A_a (= A_1 + A_2) + A_b (= A_3 + A_4)$

Growth period was 10 days with the label being added in the last 1 2 or 3 days, as shown Data for incorporation are means of three replicates In the 3-day incorporation experiment each replicate extraction was conducted using 20 pieces of the appropriate region, in the 2-day incorporation experiment 30 pieces were used, and in the 1-day incorporation experiment, 100 pieces

region showed a greater incorporation of the label than that from the "middle" or "basal" regions (Table 1). Two further experiments were then set up in which the labelled MVA was added after 8 days growth and 9 days growth in standard medium. Both experiments were terminated 10 days after initiation (so that presentation times were 2 days and 1 day respectively) In each case only the "apical" region was analysed In the 2-day incorporation experiment, this region was divided into two equal lengths (each *ca* 20 mm), designated A_a and A_b (the former being the tip region), while in the 1-day incorporation experiment it was divided into four equal lengths (each *ca* 10 mm), designated A_1, A_2, A_3 and A_4 (A_1 being the tip region) In both experiments, the highest incorporation of label into tomatine occurred in the region which contained the apical meristem, viz A_a and A_1 (Table 1).

To decide if the apical regions of the root are also principal sites of tomatine accumulation, roots were grown for 10 days in standard medium then harvested and divided up into a number of regions, each of which was dried, weighed and extracted. The regions were tip 5 mm of the main root axis; "apical," "middle" and "basal" regions (all of equal length) of the remaining main axis; tip 5 mm of lateral roots (or the whole lateral root if equal to or less than 5 mm), remainder of lateral roots Not only was tomatine present throughout the cultured root, but the amount of alkaloid per unit of dry weight was essentially equal in the various regions tested (Table 2). From these data it was calculated that 2 5% of the total tomatine in a 10-day-old

TABLE 2. TOMATINE CONTENT OF DIFFERENT REGIONS OF THE CULTURED *Lycopersicon esculentum* ROOT

Region	No. of pieces used per replicate	Dry wt (mg)	Total tomatine (μg)	Tomatine concn (μg mg^{-1} dry wt)
Tip 5 mm of main axis	150	0·052	0·22	4·34
"Apical" $\frac{1}{3}$ of main axis	40	0·23	1·00	4·35
"Middle" $\frac{1}{3}$ of main axis	40	0·31	1·18	3·76
"Basal" $\frac{1}{3}$ of main axis	40	0·28	1·23	4·34
Tip 5 mm of lateral roots	From 20 roots	0·53	2·64	4·98
Remainder of lateral roots	From 20 roots	0·58	2·59	4·44

Extractions were carried out on 10-day-old roots Three replicate extractions of each region were conducted Data for dry wt and total tomatine refer to the appropriate region in one cultured root

cultured tomato root is in the tip 5 mm of the main axis, 38·5% in the remainder of the main axis and 59% in the lateral roots. As expected, these values are very similar to those for the relative amount of dry matter in these regions, viz 2·5%, 41·4% and 56·1% respectively.

DISCUSSION

There is no evidence for tomatine synthesis being restricted to one particular part of the root system, although the rate of synthesis does appear (at least in the main axis) to be greatest in the rapidly growing tip region. This finding is of interest in view of the report by Sander[1] that the apical growing points are the principal sites of tomatine biosynthesis in the shoot. Although it is possible that the values for incorporation of the label into tomatine in the "apical" and "basal" regions may have been influenced by the presence of lateral root primordia (too small to excise) and the cut surface respectively, later experiments involving only the "apical" region of the root suggest that any such effects were not of sufficient magnitude to alter significantly the pattern of labelling. On the whole, therefore, these results are compatible with the previously observed[4] association between cultured root growth and tomatine level. A similar association has been reported[5,6] between the level of nicotine and the growth of cultured tobacco roots, but, whereas nicotine biosynthesis was claimed to be restricted to the root tips, tomatine biosynthesis appears to occur only at a greater rate in such regions. When the incorporation of label into tomatine is calculated on a daily basis (i.e. cpm μg^{-1} day^{-1}), it is seen that the rate between days 9 and 10 is much less than that between days 7 and 10 and 8 and 10. The reason for this is not fully understood but it may be the result of the decline in root growth (and biosynthetic activity) which is known to occur around this time.[4]

The finding that the distribution of tomatine in the cultured root closely matches that of dry matter provides further evidence that the biochemical events leading to tomatine synthesis are, in some way, directly and quantitatively connected with the growth processes resulting in dry matter accumulation. It is appreciated that transport systems may be a determining factor in the ultimate distribution of tomatine, but, to date, there is no conclusive evidence that the alkaloid moves in the vascular tissues. In fact, analyses of tomato root culture medium have failed to reveal the presence of tomatine,[1-3] whereas

[5] SOLT, M L (1957) *Plant Physiol* **32**, 480.
[6] SOLT, M L, DAWSON, R F and CHRISTMAN, D R (1960) *Plant Physiol* **35**, 887

nicotine, which is transported from root to shoot via the vascular tissues,[7] accumulates in large amounts in tobacco root culture medium [8]

EXPERIMENTAL

The clone of excised roots was initiated from the radicle of a 7-day-old aseptically-grown tomato seedling (*Lycopersicon esculentum* cv Best of All) and maintained as described by Street and Henshaw [9] The standard nutrient medium was modified White's medium,[9] except that iron was added as Fe–EDTA,[10] and *myo*-inositol was present at 50 ppm The stock clone was maintained by growing roots singly in 50 cm^3 of medium in a 100 cm^3 flask but, for experimental work, 5 roots were grown in 100 cm^3 of medium in a 250 cm^3 flask All cultures were incubated at 25° in the dark

DL-MVA-[2-^{14}C]-lactone (sp act 7·1 mCi $mmol^{-1}$) was dissolved in dist H_2O to give a soln of activity 1μCi cm^{-3} The solution was filter sterilized (Millipore filter, pore size 0·45 μm) and 1 cm^3 aseptically added to 100 cm^3 vol of previously autoclaved medium

Techniques for the extraction and separation of tomatine were as described previously,[2] except that in labelling experiments, (a) the roots or various root segments were washed for 2 hr in running H_2O prior to extraction, and (b) after the second development, the tomatine zone was eluted applied to a second TLC plate and re-developed in the second solvent

The method for assaying tomatine has been reported elsewhere [2] This was done using a known fraction of the tomatine zone from the TLC plate, while the remainder was scraped directly into 11 cm^3 of dioxan-based liquid scintillator and counted for 20 mins Background was 23 cpm and counting efficiency 89%

[7] DAWSON, R F (1942) *Am J Botany* **29**, 66
[8] DAWSON, R F (1942) *Am J Botany* **29**, 813
[9] STREET, H E and HENSHAW, G G (1965) in *Cells and Tissues in Culture* (WILLMER, E N, ed) Vol III p 459, Academic Press, New York
[10] SHEAT, D E G, FLETCHER, B H and STREET H E (1959) *New Phytologist* **58** 128