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ISOLATION OF TOMATINE FROM CULTURED EXCISED ROOTS AND CALLUS TISSUES OF TOMATO

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Abstract—Tomatine was present in cultured excised tomato roots but in lower concentrations than in seedling radicles of the same age. The alkaloid was not detected in 'spent' root medium. Newly-initiated callus cultures of hypocotyl, radicle and cotyledon origin produced roots, and tomatine was isolated from both roots and callus. Roots contained more tomatine than callus, but neither contained as much as the organ explants from which the cultures were initiated. The number of roots produced decreased with time, as did also the tomatine content of the callus tissues. After 447 days, when no organized structures were produced by callus cultures, tomatine was not detected. An established hypocotyl callus contained small amounts of tomatine when grown on certain nutrient media, but a chlorophyllous sub-isolate of this callus did not produce detectable quantities of the alkaloid. Tomatine was not detected in an established root callus isolate or in suspension cultures initiated from established, tomatine-containing hypocotyl callus.

INTRODUCTION

TOMATINE, a glycoside of the steroidal alkaloid tomatidine, has been isolated from a number of species of *Lycopersicum* and *Solanum*.[1] Tomatine synthesis occurs in both the shoot and the root but the former is the main site of accumulation of the alkaloid.[2] Highest levels are found in fully-expanded flowers,[3] and young green fruits are rich in tomatine,[4] but as fruit development proceeds, tomatine degradation occurs.[2,5] Little, however, is known of the sites of, or factors regulating tomatine synthesis in the different organs of the plant. Tomatine has been isolated from cultured excised tomato roots[2] and from crown-gall tumours of tomato,[6] but in neither case was it reported how levels of the alkaloid compared with those in the intact plant. The object of this work, therefore, was to investigate the abilities of cultured excised roots, callus tissues and cell suspensions to accumulate tomatine.

RESULTS

Analysis of Cultured Excised Roots and Seedling Radicles

Growth and tomatine analyses were made of 10-day-old cultured roots and seedling radicles. The excised root clone used had been maintained in culture for 3 yr. Extension growth of the main axis was similar in both cultured and seedling roots but, whereas the former produced large numbers of lateral roots, the latter produced none (Table 1). In appearance, cultured roots were thicker than seedling radicles. These differences were

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reflected in the higher fresh weight (×5) and dry weight (×10) of cultured roots. There was no significant difference in the amounts of tomatine per unit of fresh weight, but, on a dry weight basis, seedling radicles contained almost 3 times as much alkaloid as cultured roots. Tomatine could not be as detected in the 'spent' root culture medium.

TABLE 1. GROWTH AND TOMATINE PRODUCTION IN 10-day-old CULTURED ROOTS AND SEEDLING RADICLES

	Growth analysis					
Callus	Length of main axis (mm)	No. of lateral roots	Total length of all lateral roots (mm)	Fresh wt per root (mg)	Dry wt per root (mg)	Total tomatine per root (μg)
Cultured roots	128 7 ± 3 4	30·4 ± 2 0	385 8 ± 39 7	27 8 ± 2·1	2 7 ± 0 2	11 97 ± 1·32
Seedling radicles	121 6 ± 4 3	0	—	5 9 ± 0 2	0 26 ± 0 01	3 64 ± 0·53

Seedlings were germinated in Petri dishes containing damp filter paper under low light at 25°. For growth analysis, 15 cultured roots and 20 seedling radicles were used. Tomatine data represent the means of 4 replicates. Each replicate of cultured roots and seedling radicles consisted of 5 roots and 20 radicles respectively. Mean values are followed by the standard error (s.e.).

Analysis of Newly-Initiated Callus Cultures

New callus cultures were initiated on chemically-defined medium D (containing 2·0 ppm NAA, 0·1 ppm kinetin, 400 ppm *myo*-inositol and supplementary salts) from the hypocotyl, radicle and cotyledon of a tomato seedling. Analyses of these organs showed tomatine to be present in similar amounts on a dry weight basis (Table 2). The callus tissues which developed from the organ explants gave rise to large numbers of roots. Before extracting callus, the degree of rooting was noted and all visible roots were removed.

TABLE 2. TOMATINE CONTENT OF 7-day-old TOMATO SEEDLING ORGANS USED TO INITIATE NEW CALLUS CULTURES

Callus	No. per replicate	Total fresh wt (mg)	Total dry wt (mg)	Tomatine (μg mg^{-1} fresh wt)
Hypocotyl	40	328·9 ± 50·0	13·6 ± 2·2	0·71 ± 0·07
Radicle	60	278·8 ± 24·7	11·2 ± 0·7	0·68 ± 0·05
Cotyledon	100 pairs	240·4 ± 15·2	68 7 ± 0·2	4·67 ± 0·20

Seedlings were germinated in Petri dishes containing damp filter paper in the dark at 25°. Data represent means of 4 replicates followed by the s.e.

After 38 days growth, only hypocotyl callus yielded sufficient material for analysis, but subsequently, all cultures were examined. Tomatine was present in this callus, although the concentration was only 2·5% of that in the original explant. With increasing age, the tomatine content of all the cultures declined, as did also the number of roots produced (Table 3). With the exception of hypocotyl callus after 176 days, tomatine was detected in callus tissues only when roots were present. Neither roots nor root primordia were produced after 447 days and none of the callus extracts contained tomatine. Growth rates at this time were the highest recorded for these cultures.

Roots which had been removed from callus cultures after 72 days were analysed for