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EVIDENCE FOR RISHITIN BIOSYNTHESIS IN TOMATO CULTURES

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; cell suspension culture; sesquiterpenoid phytoalexin; elicitation.

Abstract—Rishitin accumulation as well as its biosynthesis from added [2-^{14}C]MVA was observed in tomato cell suspension cultures elicited by yeast extract.

INTRODUCTION

Phytoalexins are plant antibiotics which have been shown to contribute to disease resistance and accumulate rapidly in response to pathogen infection [1]. Studies with intact plants and plant cell cultures have shown that chemical stimuli derived from fungi, so-called elicitors, can trigger this active defence response [2]. Sesquiterpenoid phytoalexins have been detected in potato and tobacco, both in infected tissues and cell suspension cultures [3–8]. In tomato plants, rishitin is the main phytoalexin identified in infected fruits and stem fragments [9–12] but not in leaves [13].

Since yeast extract has been shown previously to contain carbohydrate and glycopeptide elicitor activity [14, 15], we have used it as an elicitor to show that elicited tomato cells synthesize and accumulate rishitin as the main phytoalexin.

RESULTS AND DISCUSSION

TLC (system 1) of the total $CHCl_3$ extract (from the combined cell washings and extracellular medium) of elicitor-treated tomato cultures showed the presence of compound with the same R_f (0.3) as an authentic sample of rishitin. This compound was rechromatographed using system 2 (R_f 0.67). Finally, GC analysis showed only the presence of rishitin (60, 30 and 10%, respectively, in the extracellular medium, and MeOH and $CHCl_3$ washings). Thus, it was observed, for the first time, that the sesquiterpenoid rishitin was produced by tomato cells. The present technique, therefore, offers a new approach to studying the mechanisms of tomato defence responses.

A noticeable browning of the tomato cells treated with yeast extract, a mixture known to have potent elicitation capacities [14], was visible. This was also observed previously in potato cells inoculated with sporangia of *Phytophthora infestans* [7].

The responses of tomato cells treated with different amounts of yeast extract are presented in Fig. 1. Commercial yeast extract had detectable elicitor activity at a concentration of 0.5 mg ml^{-1} and this increased linearly with increasing concentration. At the highest concentration of yeast extract tested, the amount of rishitin produced was 1.8 μg mg^{-1} dry wt which was similar to the amount of capsidiol obtained in tobacco cultures [8, 16]. Nevertheless, traces of rishitin could be observed in untreated cultures.

The incorporation of [2-^{14}C]MVA into rishitin (mean of two independant experiments, expressed as dpm 50 mg^{-1} dry wt) in tomato cells was significantly enhanced in the presence of yeast extract at a concentration of 2.5 mg ml^{-1} (control cells: 1150; treated cells: 7680). The radioactivity present in rishitin was not associated with squalene (R_f 0.95 and 1) and sterols (R_f 0.65 and

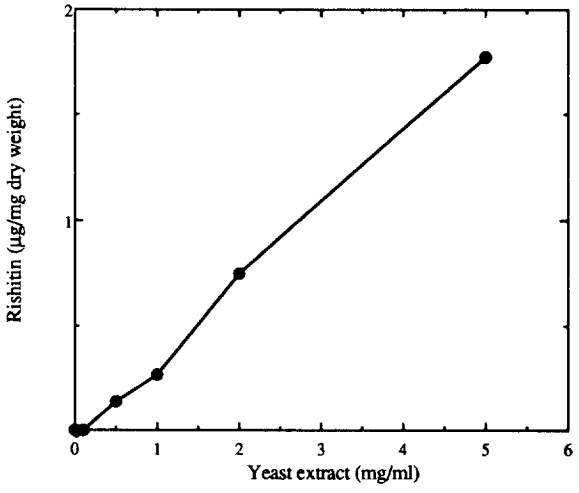

Fig. 1. Relationship between amount of yeast extract and amount of rishitin produced by induced tomato cells.

0.87) as shown by TLC using systems 1 and 2, respectively. Rishitin accumulation and biosynthesis from this precursor has been found in cell suspensions by other workers [7].

EXPERIMENTAL

Cell suspension cultures of *Lycopersicon esculentum* [L.] Mill. line Msk 8 were cultured as described [15]. Four days after subculturing, 10 ml aliquots (40–60 mg dry wt) of the non-chlorophyllous cell suspension were transferred to sterile 125-ml Erlenmeyer flasks and inoculated with yeast extract (Difco, 0–5 mg ml^{-1}). Control and treated cells were analysed for rishitin 16 hr after inoculation.

In the radiochemical experiments, a single dose (18.44 kBq) of [2-^{14}C]MVA (2.18 GBq $mmol^{-1}$; Amersham France S.A.) was administered at the time of elicitation. After 16 hr, the cell suspensions (10 ml) were filtered under vacuum and retained cells rinsed with MeOH (5 ml) and then $CHCl_3$ (2.5 ml). The filtrate (10 ml) and the twice-washed cells (7.5 ml) were bulked, shaken, centrifuged and the resulting $CHCl_3$ layer taken to dryness under vacuum. The $CHCl_3$ extracts were submitted to TLC on silica gel 60 plates (Merck) developed with EtOAc–cyclohexane (1:1) (system 1). The rishitin spots corresponding to an authentic standard revealed by vanillin–H_2SO_4 reagent [17], were scraped off and rechromatographed in $CHCl_3$–MeOH (17:3) (system 2).

The areas corresponding to rishitin were eluted with Me_2CO and analysed by FID-GC (Hewlett Packard 5890) equipped with non-polar capillary column (BP1, SGE), using N_2 gas as a carrier (30 ml min^{-1}) and a temp. gradient of 100 to 300° at 10° min^{-1} (injector temp. 300°; detector temp. 320°). Identification and quantification were carried out using a rishitin standard calibration curve.

The incorporated radioactivity was measured in a liquid scintillation spectrometer (Beckman LC 6000 IC).

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