CONTINUOUS CULTURES OF TOMATO AND CITRON ROOTS IN VITRO

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SUMMARY

Initial trials with tomato-root cultures disclosed the desirability of employing a gently agitated liquid medium containing iron in the chelated form. For the normal cultivars "Ace" and "Tropic," subcultures were best achieved by utilizing sectors that possessed one or more newly emerged laterals. Continuous cultures of a nonlateral-forming tomato mutant, "Diageotropica," and of citron were accomplished by subculturing tips of the elongating primary roots. The tomato roots were cultured in White's medium with the Fe$_2$(SO$_4$)$_3$ replaced by 0.03 mM NaFeEDTA. Sustained growth of citron-root tips necessitated the use of a medium containing Murashige and Skoog salts, 7.5% sucrose, 100 mg per l each of citric acid and thiamine·HCl, and 5000 mg per l i-inositol. The success with citron-root cultures was extendable to all cultivars of *C. medica* L., but not to other *Citrus* species relatives. Both citron and "Diageotropica" root cultures manifested undiminished elongation through repeated subcultures; but neither produced laterals in response to any cultural treatments.

Key words: root cultures; *Citrus*; tomato; citron; *Lycopersicon*.

INTRODUCTION

Root cultures are intended mainly as tools of investigations that require exclusion of correlative influences by other organs and interference by unwanted microorganisms. They have served advantageously in exploring root morphogenesis (1-4), root nutrition (5-9), and symbiosis with bacteria (10) and fungi (11,12). Inasmuch as synthesis of some secondary metabolites, e.g. alkaloids (13-15), occurs undiminished in excised roots, root cultures may be superior to cell cultures as in vitro sources of some economically significant plant products.

Although White's (16) first continuous culture of tomato roots was achieved more than 40 years ago, and, although the effort has been extensive, comparable successes have remained confined to a handful of other species (17). The experience has resulted in categorization of the culturability of excised roots along taxonomic lines. If the criterion of success is infinite subculturability, root cultures are expected more readily with herbaceous dicots and less probably with grasses and trees. These categories do not always apply; major differences may be encountered among cultivars within a species. Street (18) recognized even intraclonal variations.

This investigation was initiated primarily because root cultures were desired as an aid in exploring certain biological phenomena of *Citrus*. Moreover, the failure of an earlier attempt to culture citrus roots (19) presented an added challenge. The current optimism has been justified by some recent successes with other tree species, e.g. several *Pinus* species (20,21). The continuous culture of excised-citron-root tips is now reportable.

MATERIALS AND METHODS

An initial series of cultures was attempted with tomato (*Lycopersicon esculentum* Mill.) roots to gain the necessary skills and to establish a reference system. The more typical behavior of tomato roots was experienced with cultures of "Ace" and "Tropic" cultivars. The mutant "Diageotropica," lacking in ability to generate lateral roots, was employed in addressing the question of
degree of permanence of cultured root apices and in identifying factors that might underlie lateral root initiation.

Several species of Citrus were first compared with respect to their culturability. None showed sustained growth of subcultures. The citron C. medica L., "Citron of commerce," was chosen for detailed study because its year-round flowering and fruiting habit assured continuous supply of fresh seeds. These citron roots also showed some lateral root formation in culture, a feature absent in the other citrus roots.

Microorganism-free root tips were obtained from aseptically established seedlings. Seeds were disinfested by immersing 10 min in a solution containing 0.5% sodium hypochlorite (commercial bleach diluted 10-fold) and a few drops of Tween 20 emulsifier. It was necessary to remove both seed coats from citrus seeds (but not tomato) to ensure completeness of disinfestation. The disinfested seeds were rinsed 3 times with autoclaved distilled water prior to sowing in germination media. Tomato seeds were germinated in 250-ml Erlenmeyer flasks at the rate of 1 seed per tube. Germination occurred in darkness and at constant 27°C. Excisable tomato-root tips were obtained within 5 days. Citrus seedlings were ready after 14 days.

White's (22) medium with the Fe₂(SO₄)₃ replaced by 0.1 mM NaFeEDTA was used in germinating tomato seeds. The medium contained (in mg per liter): Ca(NO₃)₂·4H₂O (300); KNO₃ (80); KCl (65); KI (0.75); Na₂SO₄ (200); MgSO₄·7H₂O (720); KH₂PO₄ (20); MnSO₄·4H₂O (7); ZnSO₄·7H₂O (1.5); H₃BO₃ (1.5); Na₂EDTA (37.3); FeSO₄·7H₂O (27.8); thiamine·HCl (0.1); pyridoxine·HCl (0.1); nicotinic acid (0.5); glycine (3); and sucrose (20,000). The pH of the solution was set at 4.7 ± 0.1 before sterilization. The solution was dispensed into germination flasks in 50-ml aliquots. To keep from being submerged, the seeds were sown on a supportive platform placed at the bottom of each flask. The platform was made by putting together two circles of 9-cm diameter Whatman No. 42 filter paper and folding a 1-cm strip downward along the circumference. The flasks were closed with polyurethane plugs and autoclaved 15 min at 1.05 kg cm⁻² (121°C). All nutrient solutions employed in this investigation were prepared with distilled water that had been purified further by passage through a mixed-bed column of ion-exchange resins.

The seed germination medium for citrus contained Murashige and Skoog salts (23) and 1% washed Difco Bacto-agar or GIBCO Phytagar. This medium was distributed into test tubes at the rate of 25 ml per tube. The nutrient tubes were capped with Bellco Kaputs and also autoclaved 15 min at 1.05 kg cm⁻².

The freshly excised tomato- and citrus-root tips were 1 cm long and lacked laterals. One such root tip was placed in each culture. Sectors containing a newly emerged lateral were used as inocula when subculturing "Ace" and "Tropic" cultivars of tomato; they were used at the rate of one sector per culture. Subcultures of "Diageotropica" tomato and citrus required continuous use of 1-cm long apical segments, severed from the elongated primary roots; the subcultures employed one root tip per culture, except in the last few experiments in which two tips were employed.

Tomato roots were grown in a nutrient solution, the basal composition of which was the same as that used in seed germination. The basal medium of citrus root cultures contained, in addition to the Murashige and Skoog salts, organic substances of the Murashige and Tucker citrus callus culture medium (24). They were (in mg per liter): sucrose (50,000); L-ornithinol (100); thiamine·HCl (10); nicotinic acid (5); pyridoxine·HCl (10); and glycine (2). The pH of citrus-root culture medium was set initially at 5.7 ± 0.1.

Culture vessels for tomato and citrus roots were 125-ml De Long flasks. Each flask contained 25 ml nutrient solution and was capped with a Morton stainless-steel closure. The nutrient medium for root cultures was sterilized also by autoclaving.

The cultures in initial experiments were maintained in the stationary state whether they involved agar-gelled or liquid medium. As soon as gently agitated media were observed to be superior to the stationary cultures, the standard practice was modified by placing inoculated flasks on a gyratory shaker (New Brunswick Scientific Model G-10) and providing constant agitation at a rate of 80 rpm. The cultures were kept in darkness and at constant 27°C.

Each experimental variable was examined with 10 root tips or sectors. The passage lengths were 7 days for tomato and 14 days for citrus. Growth measurements after each passage included (depending on the applicability) increase in length of root tips, elongation of the original lateral of sectors, number of new laterals, and sum of lengths of all laterals per sector. Standard errors were...