Plant Physiol. (1977) 60, 190-192

Response to Chilling of Tomato Seedlings and Cells in Suspension Cultures

Received for publication November 15, 1976 and in revised form March 14, 1977

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ABSTRACT

Tomato cell suspensions and seedlings (*Lycopersicon esculentum*) responded comparably when exposed to chilling temperatures (10 C or below). Seedling growth and cellular activities related to cell viability and culture growth (triphenyltetrazolium chloride reduction, fluoroscein diacetate uptake, and hydrolysis) were sharply diminished below 10 C. Arrhenius plots of the respiratory O_2 consumption by both seedlings and cell suspensions had a break at 10 C, as is characteristic for chilling-sensitive species. The acyl chains that were found in the phospholipids of both cell cultures and seedlings were similar. These results indicate the potential usefulness of plant suspension cultures for studies of chilling injury.

Almost all plant species of tropical and subtropical origin are injured or killed by exposure to temperatures of 10 C or below. As reviewed by Lyons (6), evidence arising from many experimental approaches supports the hypothesis that chilling responses are a consequence of the physicochemical properties of the cellular membranes. *In vivo* modification of membrane properties by either biochemical or genetic means offers opportunities to alter the temperature response of chilling-sensitive species, perhaps to practical advantage. Furthermore, the physiological responses of chilling-sensitive plants offer unique opportunities to relate membrane physicochemical properties to membrane function.

In vivo chemical modification of plant membranes has been achieved in tomato seedlings (12) and potato tuber slices (Waring and Laties, personal communication). These modifications have altered the response of tomato seedling tissue to chilling treatment (4). However, modifying treatments and quantitative assessment of their consequences are experimentally difficult with intact seedlings or organs comprised of complex tissues. Suspension cultures of higher plant cells could, perhaps, better serve as systems for membrane modification studies; but little, if anything, is known about their response to chilling treatments.

In order to use plant-cell suspension cultures to study the chilling phenomenon, it is essential to establish that such cultures respond in a manner similar to that of organized plant tissues. If the physicochemical properties of membranes are the critical characteristics of chilling-sensitive species, then these properties must be determined and compared in cell cultures and organized plant tissues. Therefore, we determined the fatty acid composition of the phospholipid fraction, and assessed the temperature dependency of several responses in both tomato seedlings and tomato cell suspensions.

MATERIALS AND METHODS

Tomato cell suspensions (*Lycopersicon esculentum* cv. Marglobe) have been maintained at 25 C through weekly subculture on a Murashige-Skoog mineral-salts medium (8) containing, per liter: 0.5 mg 2,4-D, 5 mg IAA, 0.3 mg kinetin, 100 mg myoinositol, 0.5 mg nicotinic acid, 0.5 mg thiamine-HCl, and 0.5 mg pyridoxine. Cultures are routinely maintained in 200 ml of medium in 2-liter flasks on a gyrorotary shaker.

Suspensions contained several cell types ranging from single cells and small aggregates of isodiametric cells to short filaments several cells in length. Samples of uniform suspensions were transferred, after subculturing in liquid medium, to culture tubes, which were then placed at precisely controlled temperatures for 6 days. Culture temperatures during treatment were maintained on a laboratory-constructed thermal gradient device consisting of 10 (2.5 × 10.2 × 30.5 cm) aluminum blocks, each separated along the 2.5- × 30.5-cm face by a thin layer of insulating material. The complete array of blocks was seated in Styrofoam insulation enclosed by a wooden frame. The space over the 10.2- × 30.5-cm face of each block was compartmented from adjacent blocks by 3.2-mm Plexiglas partitions, and the whole assembly was enclosed by a double-walled, air-insulated Plexiglas cover. The end blocks were thermostated on the warmer end by a thermistor-controlled resistance heater, and on the cooler end by thermistor-controlled pumping of coolant liquid from a −5 C bath. The uniform temperature of each block was measured with permanently fixed thermocouples connected through a multiposition switch to a potentiometer. Temperatures of the blocks were constant within ±0.2 C over the experimental period.

The tubes were slanted in metallo-epoxy casting to maximize thermal contact with the aluminum blocks. Since the tubes were unshaken during treatment, slanting also served to maximize the liquid surface for gas exchange. The suspensions were examined microscopically at the end of the temperature treatment, and portions were then tested for viability by fluorescence microscopy, using fluoroscein diacetate (Nutritional Biochemicals) (13), and by colorimetric measurement of reduced triphenyltetrazolium chloride (Sigma Chemical Co.) (11).

To produce tomato seedlings, lots of approximately 150 tomato seeds were germinated for 2 days in 10.2-cm pots containing vermiculite. The length of 50 randomly selected seedlings was measured and the pots were then transferred to temperature-controlled rooms for exposure for 3 days at each experimental temperature. At the end of this period, 50 seedlings were again measured to obtain the increment in seedling growth.

Oxygen consumption by cell suspensions or seedling tissue samples was measured in thermostated cuvettes with Clark-type O_2 electrodes. The cuvette reaction temperatures were monitored with a low mass thermistor and displayed on a digital thermometer (Paige Instruments, Davis, Calif.). Arrhenius plots were fitted to the experimental data as previously described (1).

Lipids were extracted in boiling isopropyl alcohol (5) followed by chloroform-methanol extraction and NaCl partition (2). The extract was dried with anhydrous Na_2SO_4, flash-evaporated, and the phospholipids separated on Silica Gel H thin layer plates

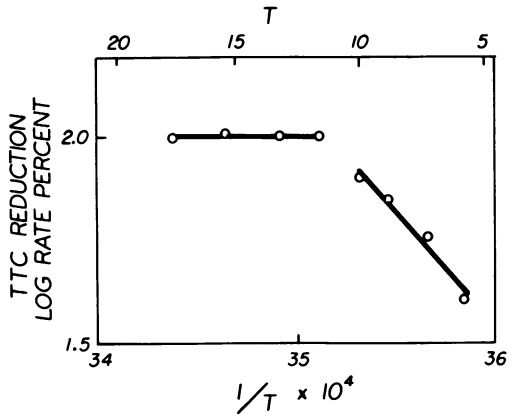

FIG. 1. Arrhenius plot of triphenyltetrazolium chloride (TTC) reduction by tomato cell suspensions that had been exposed for 6 days to precise temperatures. At the end of the period, portions of each of the three cultures treated at a given temperature were incubated for 18 hr at 25 C with TTC.

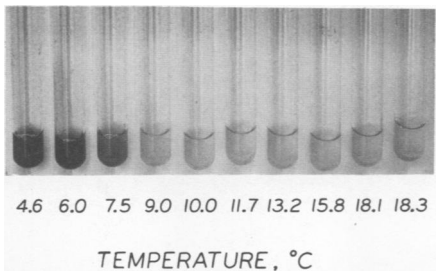

FIG. 2. Appearance of cultures exposed to the indicated temperatures for 6 days. At the end of the exposure, the cultures in each set of three were indistinguishable from one another.

using the solvent system of Gardner (3). The phospholipids were eluted from the silica gel, transesterified with sodium methoxide reagent, and the fatty acid methyl esters separated by GLC, as previously described (12).

RESULTS AND DISCUSSION

Tomato seedling growth was highly responsive to temperature and was sharply reduced below 10 C, with a typical discontinuity in the Arrhenius function. Measurements related to the growth of cell cultures also were drastically reduced below 10 C. Reduction of triphenyltetrazolium chloride (measured at 25 C) was greatly diminished in cultures maintained at temperatures below 10 C, and the Arrhenius function for this parameter was also discontinuous at about this temperature (Fig. 1). Examination with a fluorescence microscope to detect uptake and hydrolysis of fluorescein diacetate revealed a dramatic increase in the number of unstained cells in the cultures held for 6 days below the critical temperature. Furthermore, the visible darkening (presumably caused by oxidation of phenolics leaked from the vacuole into the cytoplasm) of cultures held at chilling temperatures (Fig. 2) paralleled the incidence of necrotic areas in seedlings exposed to chilling temperatures (Fig. 3).

Drastic reduction of respiratory rate below the critical temperature is another characteristic feature of both whole tissues (1, 10) and isolated mitochondria (7), from chilling-sensitive plant species.

The temperature dependency of O_2 uptake by tomato cotyledons was compared with that of tomato cell suspensions. Both Arrhenius plots had a typical discontinuity around 10 C (Fig. 4).

Finally, the fatty acid composition of the phospholipids isolated from tomato cell suspensions was indistinguishable from that of tomato seedling tissues (12), with linoleic acid (62%) and palmitic acid (25%) the major components. Other workers have found that the classes of phospholipids present in cell or tissue cultures from a variety of species were similar to those from organized tissues from the same species (9). Taken together, these data substantiate the hypothesis that cell suspensions of sensitive plant species respond to chilling, and indicate the potential of cell cultures for cell selection systems, chemical membrane modification, and other chilling studies.

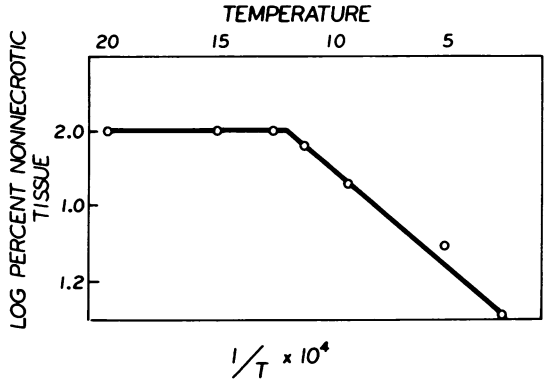

FIG. 3. Arrhenius plot of seedling necrosis (per cent non-necrotic area) estimated for seedlings returned to 25 C for 2 days after exposure to given temperature, as in Figure 1.

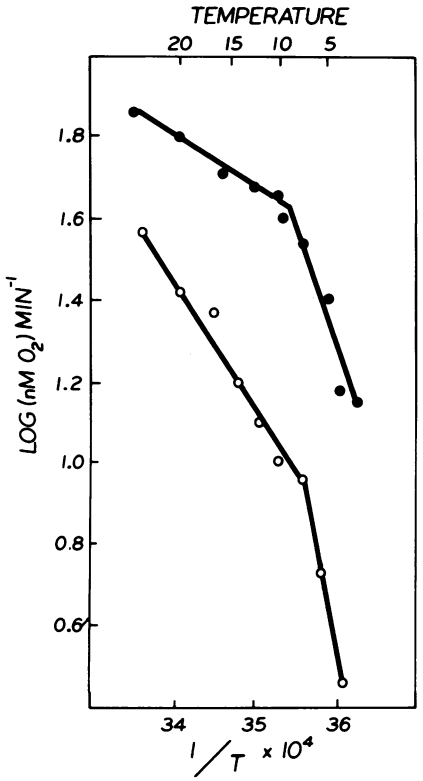

FIG. 4. Comparison of the Arrhenius plot of the respiratory rate of cotyledons of 5-day-old tomato seedlings with that of tomato cell suspensions. O_2 uptake rate for 0.2 g fresh wt of cotyledons was measured in 5 ml of 0.025 M HEPES buffer (pH 7) containing 0.24 M sucrose (●). O_2 uptake rate of cell suspensions was determined for 0.5 ml of packed cells resuspended in 4.5 ml of fresh culture medium (○).

Note Added in Proof. P. J. Dix and H. E. Street (1976 Ann. Bot. 40: 903-910) report selecting stable chilling-resistant cell lines from mutagenized cultures of two chilling-sensitive species, *Nicotiana sylvestris* and *Capsicum annuum*.

Acknowledgments – The authors thank P. Carlson for providing the initial tomato cell culture stocks, and D. F. Paige, who designed and constructed the temperature gradient apparatus. We also thank A. Dalziel, M. Kelly, and K. Yamamoto for maintaining the cultures and providing technical assistance at various times during this work.

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