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The *pro* Gene Causes an Enhanced Cell Expansion Response to Fusicoccin in Tomato

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Abstract. The response to fusicoccin (FC) of the elongated tomato mutant procera was tested. Hypocotyl sections from etiolated procera seedlings responded to a range of FC concentrations similar to that of normal sections, but at a given FC concentration the mutant sections exhibited about 40% greater elongation than normal. The initial rates of elongation in FC were similar for normal and procera sections, but elongation continued at a high rate for a longer period in the mutant. Measurements of outer cortical cells demonstrated that the enhanced elongation of the procera hypocotyl sections was due to enhanced cell expansion.

Key Words. Cell expansion—Fusicoccin—Mutant—Tomato

The procera phenotype of tomato (*Lycopersicon esculentum*), caused by the recessive mutant gene *pro*, has longer stems than normal plants. Procera plants are similar to GA_3-treated normal tomato plants in shoot morphology and cellular anatomy (Jones 1987, Jupe et al. 1988) and in stem peroxidase activity (Jupe and Scott 1992). However, the mutant is not a gibberellin overproducer (Jones 1987).

This paper examines the response of procera to another growth-promoting plant growth regulator, the fungal toxin fusicoccin (FC). Recently there have been reports of *Arabidopsis thaliana* mutants exhibiting reduced responses to FC consequent upon reduced binding of the toxin (Holländer-Czytko and Weiler 1994) or decreased response capacity of its target H^+ pump (Gomarasca et al. 1993, Marrè et al. 1995). In contrast, we report here that the procera mutant displays an enhanced cell expansion response to FC.

Abbreviations: GA, gibberellin; FC, fusicoccin.

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Materials and Methods

Dose-Response Experiments

The procera tomato (*L. esculentum* Mill.) line GCR 380, homozygous for the mutant *pro* gene, was used in comparison with the near isogenic cultivar Ailsa Craig as the normal genotype (Maxon Smith and Ritchie 1983). Seedlings were grown in Levingtons compost (Fisons Garden Products, Ipswich, UK) for 7 days in the dark at about 23°C. Hypocotyl sections (11.4 mm) were excised from immediately below the apical hook and incubated ($n = 20$) on a shaker at 25°C in diffuse fluorescent lighting (4.5 μmol m^{-2} s^{-1}) in 20 ml of FC solution containing 2.5 mM potassium phosphate, pH 6.0; 1 mM $Ca(NO_3)_2$; 2.5 mM KCl; 3% sucrose (Kelly and Bradford 1986). All solutions contained 0.1% ethanol, which was used to dissolve FC (Prof. G. S. Muromtsev, Institute of Agricultural Biotechnology, Moscow). After 20 h, hypocotyl lengths were measured at ×10 magnification.

Time Course and Cell Measurement Experiments

Seedlings were grown in dark conditions for 12 days, and 5-mm sections were cut from just below the hypocotyl hook. Sections ($n = 20$) were incubated at about 20°C in 2.5 mM potassium phosphate buffer, pH 6.0, containing 0.1% ethanol ± FC, under video monitoring equipment attached to a low magnification light microscope. Measurements of section lengths were made (to the nearest 0.25 mm) from the video recording. Data were assessed by analysis of variance.

For the cell measurements, sections were fixed for 24 h in 70% ethanol:acetic acid:formalin (90:5:5 by volume) prior to dehydration in a graded ethanol series and embedding in acrylic LR White resin (London Resin Co., Basingstoke, UK). Longitudinal sections (10 μm thick) were stained with periodic acid-Schiff's reagent (O'Brien and McCully 1981), and outer cortical cell lengths were measured at ×100 using an eyepiece graticule. Mean values were estimated from 25–30 cells from each of four to six sections.

Results

Dose-Response Curves

The responsiveness of procera seedling hypocotyls to FC was examined in a typical bioassay procedure (Kelly and Bradford 1986). Mean hypocotyl lengths (±S.E.) of eti-

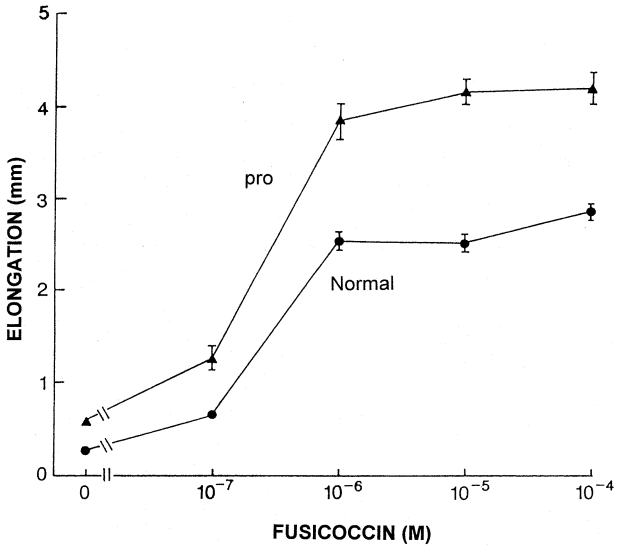

Fig. 1. Effect of FC concentration on elongation of hypocotyl sections (n = 20, initial length 11.4 mm) from normal (*circles*) and procera (*triangles*) tomato seedlings. Data are means ± S.E. of two experiments.

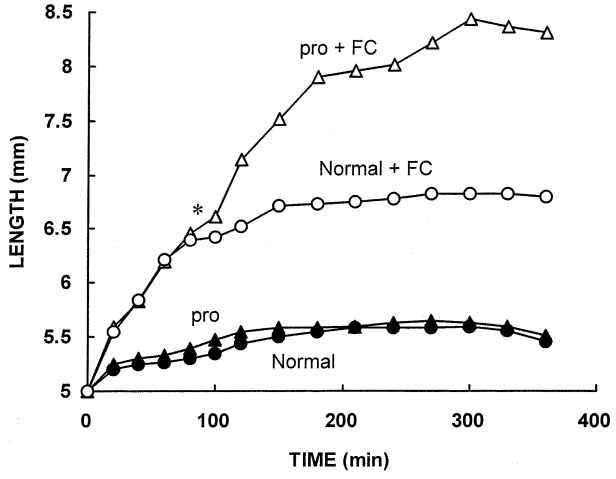

Fig. 2. Mean lengths of hypocotyl sections (n = 20, initial length 5 mm) from normal (*circles*) and procera (*triangles*) tomato seedlings during incubation in control solution (*closed symbols*) or in FC (10^{-5} M) (*open symbols*). *indicates time after which there was a significant difference ($p < 0.05$) between mean lengths of procera and normal sections in FC solution.

olated 7-day-old seedlings were: normal, 80.6 ± 3.0 mm; procera, 94.9 ± 3.4 mm. Sections were excised from immediately below the hypocotyl hook of both genotypes and incubated in a range of FC concentrations. Fusicoccin induced considerable elongation of sections of both genotypes, but the maximum response of procera sections was more than 40% greater than normal (Fig. 1).

Time Course of Response

To investigate whether the enhanced response of procera sections to FC was due to a higher initial growth rate or to a prolonged period of growth, video recordings of incubated sections were analyzed. Fig. 2 shows the time courses of elongation at 10^{-5} M FC. Prior to 80 min of incubation, there was no significant difference between the elongation rates of the normal and procera sections in FC, but thereafter the elongation of the procera sections continued at a high rate for a longer period, to reach a greater final length (Fig. 2).

Cellular Basis of Response

Confirmation that the greater response of procera sections was based on a greater expansion of individual cells was obtained by microscopic examination of sections before and after 6 h of incubation in FC (10^{-5} M), when maximum elongation had been reached. Prior to treatment, outer cortical cells of procera hypocotyls were on average 9.3% longer than equivalent normal cells. The procera cells expanded much more than normal in FC (Fig. 3), and this cell expansion effect was sufficient to account for the greater expansion of the hypocotyl sections. Thus, the mean length of the procera hypocotyl sections after FC treatment was 166% of initial size, whereas the mean cortical cell length was estimated at 164% of initial size; for the controls, mean lengths after treatment were 136 and 135% for sections and cells, respectively.

Discussion

Procera hypocotyl sections showed a much greater than normal capacity for cell expansion in response to FC treatment. The expansion of procera cells in FC was prolonged but not more rapid than normal. The reason for these characteristics remains to be elucidated, and the procera tomato mutant provides an interesting experimental system for study of the control of cell expansion.

The dose-response characteristics of the procera sections (Fig. 1) did not indicate a greater FC sensitivity of procera in the sense of a shift of the dose-response curve to lower concentrations; rather, the response capacity at each concentration was greater in procera. The displacement of the mutant's dose-response curve was comparable (in the opposite direction) to that obtained for the 5-2 *Arabidopsis* mutant by Marrè et al. (1995) and, as noted by these authors, did not suggest an altered receptor affinity.

The prolongation of FC-induced expansion in procera cells could have been due to a greater osmotic concentration or to a greater capacity for acid-induced wall loosening (Van Volkenburgh et al. 1985). Unfortunately, we were not able to demonstrate an acid growth effect in

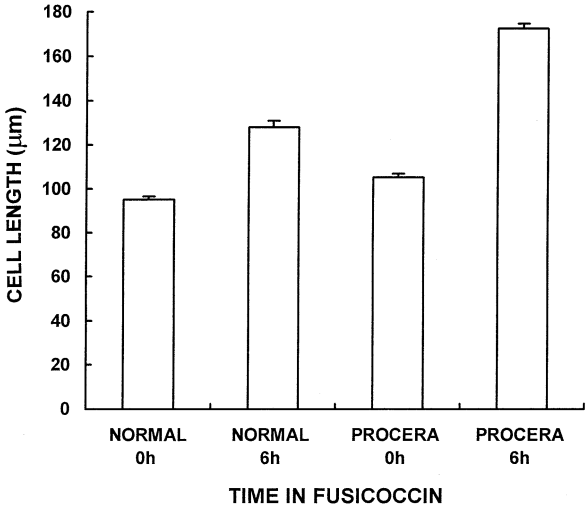

Fig. 3. Mean lengths ± S.E. of outer cortical cells ($n \geq 100$) of normal and procera tomato hypocotyl sections before and after 6-h incubation in FC (10^{-5} M).

this system by adjusting the pH of control buffers in the range 3.0–6.0. Demonstration of acid growth is known to be difficult without removal of the cuticular barrier (Rayle and Cleland 1992), for which tomato hypocotyls are not an ideal material. Studies on shoot extension mutants in other species have indicated differences in cell wall properties (Behringer et al. 1990, Pollock et al. 1990), and the procera tomato could also prove interesting in this respect.

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