Polarity of Production of Polyphenols and Development of Various Enzyme Activities in Cut-injured Sweet Potato Root Tissue

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ABSTRACT

Investigation of polyphenol production in cut-injured sweet potato (Ipomoea batatas Lam. cv. Kokei 14) roots by histochemical and quantitative methods showed that polyphenols were produced in striking amounts in the proximal side of the tissue pieces (2 cm thick), but only in small amounts in cells of the distal side. In response to cut injury, formation of the enzymes related to polyphenol biosynthesis, phenylalanine ammonia-lyase and trans-cinnamic acid 4-hydroxylase, was also pronounced in the proximal side of the tissue pieces and slight in the distal side. The similar polarity was observed in the development of activities of various enzymes, such as NADPH-cytochrome c oxidoreductase, acid invertase, peroxidase, o-diphenol oxidase, and cytochrome c-O_2 oxidoreductase. Acropetal development of polyphenol contents and of various enzyme activities may be related to the acropetal movement of indoleacetic acid (IAA) in roots of various plants. Treatment of the distal surface of tissue pieces with IAA or 2,4,6-dichlorophenoxacyclic acid caused polyphenol production but treatment with gibberellic acid, abscisic acid, kinetin, or ethylene had little effect. The results suggest that IAA may play a role in the metabolic response to cut injury.

MATERIALS AND METHODS

Plant Material. Roots of sweet potato (I. batatas Lam.) were harvested at Aichi in the summer to autumn and stored until used. Roots of cv. Kokei 14 were used unless stated otherwise. The roots were washed thoroughly with tap water and cut perpendicularly into 2-cm-thick pieces. The pieces were incubated in the dark at 29 ± 1°C under high humidity.

Histochemical Test for Polyphenol Production in Sweet Potato Root Tissues. Polyphenols in the tissue were detected by the histochemical method of Reeve (10) using nitrous acid in the alkaline condition. Isochlorogenic and chlorogenic acids, the principal polyphenols produced in sweet potato root tissue, reacted with the nitrous reagent to form the same red color.

Extraction and Determination of Polyphenols. A cylinder (1.9-cm diameter) was prepared from the tissue piece (2 cm thick) with a cork borer and sliced into 2-mm-thick disks with a razor blade from the proximal side. Polyphenols of each disk were extracted with ethanol and determined as described previously (12).

Preparation of Crude Extract. Two disks (about 1 g) were homogenized with a Potter-Elvehjem glass homogenizer in 2 ml of 50 mM K-phosphate buffer (pH 7.2) containing 0.55 mM d-sorbitol, 1% sodium isoascorbate, 0.5 mM EDTA, and 0.1 g of Polyclar AT. The homogenate was squeezed through nylon cloth and centrifuged at 1,500g for 15 min. The supernatant solution was passed through a column (1.3 × 10 cm) of Sephadex G-25 (coarse) previously equilibrated with 10 mM K-phosphate buffer (pH 7.2) containing 0.55 mM d-sorbitol and 0.5 mM EDTA. The effluent containing protein was used as the crude extract. The membrane fraction (10,000–100,000g) was prepared for the assay of trans-cinnamic acid 4-hydroxylase activity as described previously (12).

Determination of Enzyme Activities and Protein Contents. Activities of phenylalanine ammonia-lyase, trans-cinnamic acid 4-hydroxylase, NADPH-Cyt c oxidoreductase (EC 1.6.2.3) and Cyt c-O_2 oxidoreductase (EC 1.9.3.1) were determined as described previously (12). Activities of acid invertase and peroxidase were determined by the method of Matsushita and Uritani (8). o-Diphenol oxidase activity was assayed by the method of Hyodo and Uritani (1). Protein was precipitated by 10% trichloroacetic acid and determined by the method of Lowry et al. (6) with BSA as a standard.

Treatment of Tissue Piece with Plant Hormones and Other Substances. A sheet of filter paper (5 cm in diameter) moistened with 1 ml of IAA, 2,4-D, GA_3, ABA, kinetin, or dibutyryl cAMP solution was placed on the distal surface of each tissue piece and the pieces were incubated at 29 ± 1°C. In experiments with ethylene, two or three tissue pieces were placed in an air-tight chamber (2.6 liters) and ethylene was injected through a silicone stopper at a final concentration of 10 µl/l. The atmosphere containing ethylene was replaced every 24 hr.

Storage tissues of plants respond to mechanical injury in various ways (3), including synthesis of DNA and RNA (2, 4, 15), an increase in respiration (9), lignification (11), and polyphenol production (12). In sweet potato root tissue, polyphenols, mainly consisting of chlorogenic and isochlorogenic acids, are produced in response to cut injury (5). Phenylalanine ammonia-lyase (EC 4.3.1.5) is synthesized de novo prior to production of the polyphenols (13, 14). Activities of various enzymes related to respiration, carbohydrate metabolism, and other metabolic pathways also develop markedly in response to cut injury (1, 7, 9). However, we observed that in some varieties of sweet potato (Ipomoea batatas Lam.) such as cv. Kokei 14, production of polyphenols in response to cut injury was remarkably concentrated in cells of the proximal side of a 2-cm tissue piece; only small amounts of polyphenols accumulated in cells of the distal side. This polarity of polyphenol production passed unnoticed because many previous experiments on the response to wounding were often performed by using thin disks (1-3 mm thick) and by using the other sweet potato varieties such as Norin 1. The present paper reports the polarity of polyphenol production and development of various enzyme activities in sweet potato root tissue in response to cut injury.

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RESULTS

Histochemical Test for Production of Polyphenols in Response to Cut Injury. Histochemical tests on intact roots showed that red colored nitroso derivatives were localized in the skin area, cambium and vascular bundles but not in parenchymatous cells containing many starch granules (Fig. 1A). In cut-injured tissue pieces, the intense red color reaction occurred in the parenchymatous cells of the proximal side (Fig. 1B). This polarity of polyphenol production was not affected by placing the tissue piece with the proximal surface to the side, up or down. However, such a polarity was not obvious in cut-injured tissue pieces from cultivars of Norin 1, 2, 4 and 10 (data not shown). On the other hand, when the roots (cv. Kokei 14) were cut longitudinally and each half was incubated, the color reaction was restricted to the tissue closely adjacent to the cut surface.

Polarity of the Production of Polyphenols in Response to Cut Injury. Tissue pieces incubated for 5 days were cut into disks 2 mm thick and the polyphenol content of each disk was determined (Fig. 2a). Polyphenols were present in high concentrations to a depth of about 6 mm at the proximal side. On the distal side, they were present in low concentrations and restricted to the surface cells. In cv. Norin 1, such a polarity was not evident and polyphenols were produced in similar amounts at the proximal and distal sides.

Polarity of Development of Various Enzyme Activities in Response to Cut Injury. In response to cut injury, activities of the enzymes related to polyphenol biosynthesis, phenylalanine ammonia-lyase and trans-cinnamic acid 4-hydroxylase, developed markedly in cells of the proximal side but only slightly in cells of the distal side (Fig. 2, b and c). The distribution of enzyme activities was similar to that of polyphenols. A similar polarity was observed in the development of activities of other enzymes such as NADPH-Cyt c oxidoreductase, acid invertase, peroxidase, o-diphenol oxidase, and Cyt c-O_2 oxidoreductase (Fig. 2, d-h). However, constitutive levels of activity of these five enzymes were high to some extent in fresh tissue at the time of cutting.

Time Course of Production of Polyphenols and Development of Various Enzyme Activities in Response to Cut Injury. In 2-mm-thick disks of tissues taken beneath the proximal surface, production of polyphenols in response to cut injury started after a lag period of about 1 day and then continued at an almost constant rate (Fig. 3). In interior tissue layers, polyphenol production occurred at similar rates but only after proportionately longer lag periods. Polyphenol production was low in the top 2-mm tissue layer of the distal side, and almost no polyphenol production was detected in the second layer. Phenylalanine ammonia-lyase activity developed after a lag of about 1 day, reached a maximum after 2.5 days, and remained at a constant level thereafter in the 2-mm tissue layer adjacent to the proximal surface (Fig. 4). In cells of the distal surface, little activity of phenylalanine ammonia-lyase developed. Almost no increase in phenylalanine ammonia-lyase activity was detected in the inner tissue layers below the distal side. Similar distribution was observed in the proximal and the distal sides in the development of activities of the other enzymes such as peroxidase and o-diphenol oxidase (Fig. 5), although the increase in

Fig. 1. Histochemical demonstration of the polarity in polyphenol distribution in fresh (A) tissue and cut-injured (B) tissue (3-day incubation) of sweet potato roots (cv. Kokei 14). The staining of polyphenols on the surface was performed by the method of Reeve (10) using nitrous acid in the alkaline condition. Left side: distal side (for root end); right side: proximal side (for stem).

Fig. 2. Distribution of polyphenols and various enzyme activities in cut-injured pieces of sweet potato root tissue (cv. Kokei 14). Cylinders (1.9-cm diameter) were prepared from individual 2-cm-thick pieces incubated for 3 to 5 days after cutting and sliced into 2-mm-thick disks beginning from the basal side. Polyphenol contents (a) in each disk were determined. A crude extract was prepared from each disk as described under "Materials and Methods" for assay of activities of phenylalanine ammonia-lyase (b), trans-cinnamic acid 4-hydroxylase (c), NADPH-Cyt c oxidoreductase (d), acid invertase (e), peroxidase (f), o-diphenol oxidase (g), and Cyt c-O_2 oxidoreductase (h).
activities of the two enzymes was not as pronounced.

**Effects of Plant Hormones and Dibutyryl cAMP on Production of Polyphenols and Development of Enzyme Activities in the Distal Side.** The distal surfaces of tissue pieces were treated with various plant hormones and dibutyryl cAMP and the pieces were incubated for several days at 29°C. Then production of polyphenols was investigated by the histochemical test and quantitative analysis. Kinetin (10 μM), GA₃ (100 μM), and ABA (10 μM) had almost no or slightly inhibitory effects on polyphenol production (Table 1). Dibutyryl cAMP was inhibitory at a concentration of 10 μM. Tissue pieces incubated with ethylene (10 μl/l) showed no appreciable change in the pattern of polyphenol production in comparison with pieces incubated with air. IAA and 2,4-D stimulated the production of polyphenols when applied to the distal surfaces of tissue pieces. The concentration of 2,4-D for a maximum production of polyphenols was 23 μM (Fig. 6). Activities of phenylalanine ammonia-lyase and acid invertase were also developed by the treatment with 23 μM 2,4-D. When 2,4-D was applied to the proximal surface, no distinctive change in polyphenol production occurred.

**DISCUSSION**

In response to cutting of sweet potato roots (from cv. Kokei 14), production of polyphenols was intense in cells of the proximal side and low in cells of the distal side of cut tissue. The similar polarity was observed in the development of activities of enzymes associated with polyphenol biosynthesis. Therefore, the polarity of production of polyphenols was associated with the enhanced activities of the enzymes involved in their biosynthesis. It is well known that storage tissues respond to cut injury, leading to formation of oxidative enzymes, as well as changes in carbohydrate metabolism. Polarity was not only restricted to the polyphenol production and related enzyme formation, but was also observed in development of activities of various enzymes related to bio-oxidation and carbohydrate metabolism, namely Cyt c-O₂ oxidoreductase. NADPH₂-Cyt c
The results suggest that the propagation of polyphenol production and of the development of certain enzyme activities in the proximal side is similar to the acropetal movement of IAA in roots of various plant (16, 17). The response of the longitudinally cut tissue to cut injury propagated slightly as in the case of IAA movement. Furthermore, treatment of the distal surface of tissue pieces with IAA or 2,4-D caused polyphenol production and enzyme activity development, but treatments with other plant hormones, namely GA3, ABA, kinetin, and ethylene, had little effect. The results suggest that in sweet potato root tissue, IAA may play a part in the metabolic response to cut injury, involving polyphenol production, wound respiration, and other metabolic changes.

The results obtained with roots of cultivars Norin 1, 2, 4, and 10, in which the polarity was not as distinct as in the case of Kokei 14, may be due to considerable basipetal movement of the inducing factor, as in the case of the basipetal movement of IAA in pea roots (16). It will be useful to investigate the polarity shown in roots of cv. Kokei 14 aiming at elucidating not only the regulatory mechanism of the metabolic response to cut injury but also the polarity phenomena in plants. It will be important to determine the quantitative distribution of some hormonal substances such as IAA in tissue pieces after cutting and incubation, using cultivars such as Kokei 14 which show the polarity as well as those as Norin 1 which do not appreciably show the polarity.

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