Implicit Role of Hydrogen Peroxide on Phytoalexin Production in Higher Plants

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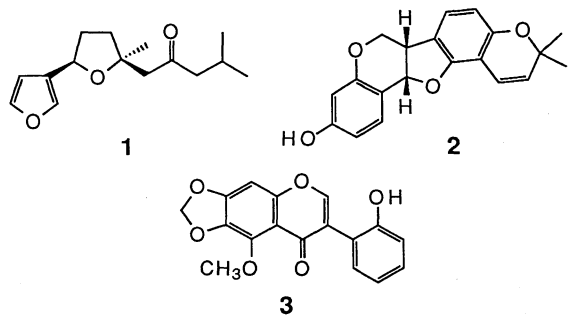
Sweet potato, kidney bean, and sugar beet, when infected with *Fusarium roseum*, *Fusarium solani*, and *Rhizoctonia solani*, respectively, generated H_2O_2 which then elicited accumulation of the phytoalexins in the respective cases. The three intact plant tissues, only when treated with H_2O_2, induced phytoalexin production, respectively.

Very recently, we have reported that H_2O_2 is a dynamic substance for triggering the phytoalexin production in potato plant as one of the representative species in Solanaceae family.[1] Our proposal has been led to the first isolation of the endogenous elicitor, which increased from the potato tuber tissues on treatment with H_2O_2.[2] We have extended our proposal to other plants. In this paper, we describe application of the role of H_2O_2 concerning the accumulation of H_2O_2 and elicitation of phytoalexins to sweet potato, kidney bean, and sugar beet. As the representative phytoalexins produced from these plants have been known ipomeamarone (**1**, from sweet potato),[3] phaseollin (**2**, from kidney bean),[4] and betavulgarin (**3**, from sugar beet),[5] respectively.



The amount of H_2O_2, generated from the surface of the plants, was measured by a direct method using *N,N*-dimethyl-1,4-phenylenediamine and horseradish peroxidase (HRP).[1,6] Sweet potato roots (cultivar Beni-Koukei, harvested in Ibaragi prefecture) were cut into slices (5 mm in thickness, 20 mm in diameter), washed, and aged at 23 °C for 14 h. The slices were inoculated at 23 °C with *Fusarium roseum* Lk. The H_2O_2 generation reached a maximum (217×10^{-3} μmol/10 slices) after 9 h, followed by an initial accumulation of **1** (Figure 1). Next, after incubation with *Fusarium roseum* Lk. at 23 °C for 6, 9, 12, 24, and 48 h, the respective slices were blended with MeOH and crushed ice, and worked up as usual to yield a residue, which was purified by column chromatography over SiO_2 [hexane-EtOAc (30:1)] and HPLC [μ-porasil, hexane-CH_2Cl_2-CH_3CN (46:2:1)] to afford pure samples of **1**[7] (Figure 1). The blank test with deionized water did not indicate formation of **1**. On the other hand, aged slices of intact sweet potato roots (2.5 kg), when treated exogenously with a 3% aqueous H_2O_2 solution (200 ml) at 23 °C for 48 h, produced a pure sample of **1** (38.8 mg)[7] after separation of the extracted mixture.

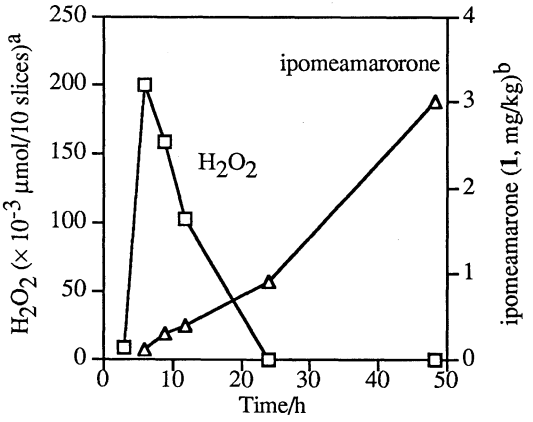


Figure 1. The amounts of H_2O_2 and **1** accumulated in sweet potato slices inoculated with *F. roseum* Lk. Each value represents the average of three experiments. a) These values denote the concentration of H_2O_2 generated per 10 slices of sweet potatoes. b) These values show the weight of **1** per kg of sweet potato slices.

Next, we attempted to check Leguminoceae plant (Figure 2). The pods of kidney bean (cultivar Hiramame, harvested in Kouchi prefecture) were washed, aged at 23 °C for 2.5 h, and opened into two parts in order to remove the seeds. The inner parts were inoculated at 23 °C with *Fusarium solani* f. sp. *phaseoli*. The pods generated H_2O_2 with a maximum (200×10^{-3} μmol/5 pods) after 6 h. After incubation of 6, 9, 12, 24, and 48 h, the aforementioned pods were worked up as usual to afford a residue, which was purified by column chromatography over SiO_2 [PhH-EtOAc (5:1)] and Sephadex LH-20 (EtOH) to provide samples of **2**.[8,9] The blank experiment using deionized water did not show production of **2** even in any trace amount. The compound was also furnished from the pods treated exogenously with 1% H_2O_2 (3.5 mg/kg fresh weight after treatment at 23 °C for 3 d).

Similarly, we tried Convolvulaceae plant (Figure 3). Sugar beet roots (cultivar Monohiru, harvested in Hokkaido prefecture) were cut in slices (5 mm in thickness, 20 mm in diameter), washed, and aged at 23 °C for 1 h. Both surfaces of the slices were inoculated at 23 °C with *Rhizoctonia solani* R. 101. The slices generated a maximum amount of H_2O_2 (192×10^{-3} μmol/10 slices) after 9 h, just when **3**[10] started to be detected (Figure 3). Treatment of the intact tissue with 3% H_2O_2 (23 °C, 3 d) gave rise to the compound (3.5 mg/kg fresh weight), while no trace amount of **3** was detected in the blank test.

Thus, in combination with the previous results on potato,[1] we emphasize herein the generality in the role of H_2O_2 on phytoalexin

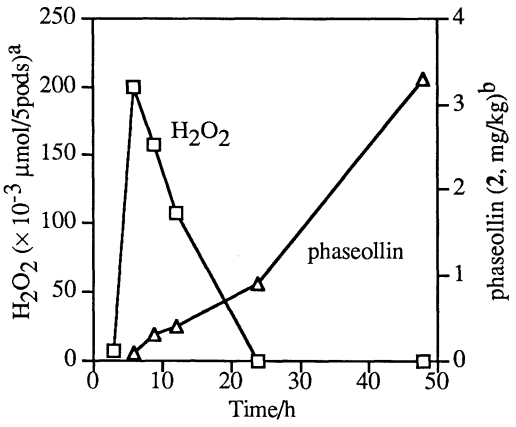


Figure 2. The amount of H_2O_2 and **2** accumulated in the pods of kidney beans inoculated with *F. solani* f. sp. *phaseoli*. Each value represents the average of three experiments. a) These values denote the concentration of H_2O_2 generated per 5 pods of kidney beans. b) These values show the weight of **2** per kg of the pods of kidney beans.

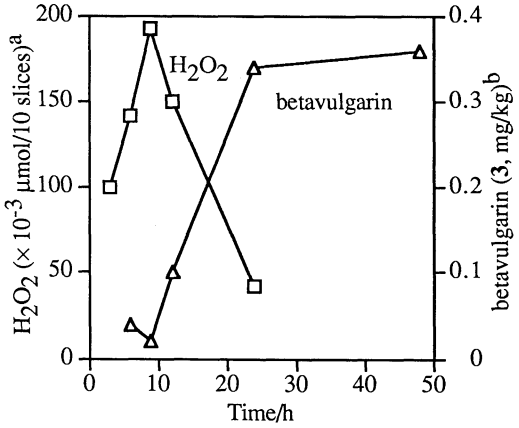
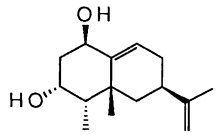


Figure 3. The amount of H_2O_2 and **3** accumulated in the slices of sugar beet roots inoculated with *Rhizoctonia solani* R. 101. Each value represents the average of three experiments. a) These values denote the concentration of H_2O_2 generated per 10 slices of sugar beet roots. b) These values show the weight of **3** per kg of the slices of sugar beet roots.

production in a wide range of plants having no mutually biological relationships. For example, intact tissues of sweet pepper and eggplant produced capsidiol (**4**, 1.2 mg/kg fresh weight)[11] and aubergenone (**5**, 0.4 mg/kg fresh weight)[12] as the respective phytoalexins on treatment with H_2O_2 (23 °C, 2 d). Chemical studies how H_2O_2 would play an implicit role for phytoalexin production are in progress in our laboratory.



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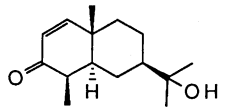

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References and Notes

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7 **1**: a colorless oil; $[\alpha]_D^{20}$ +31.6° (c=1.5, EtOH); IR (neat), 1715 and 880 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz), δ7.38 (1H, s), 7.37 (1H, s), 6.36 (1H, s), 4.92 (1H, m, W_H=15 Hz), 2.68 (2H, ABq, J=14.9 Hz), 2.33 (2H, dd, J=1.95, 6.84 Hz), 1.33 (3H, s), 0.90 (3H, d, J=6.4 Hz), and 0.88 (6.8 Hz); EI-MS, m/z 250 (M^+) and 43 (base).

8 The amount of **2** in each fraction was measured by PTLC (SiO_2, PhH:EtOAc=5:1, Rf 0.43).

9 **2**: mp 182~183 °C; $[\alpha]_D^{20}$ -145.8° (c=1.1, EtOH); ^{1}H-NMR ($CDCl_3$, 400 Mz), δ7.42 (1H, d, J=8.3 Hz), 6.95 (1H, d, J=8.3 Hz), 6.56 (1H, dd, J=2.6, 8.3 Hz), 6.51 (1H, d, J=10.0 Hz), 6.42 (1H, d, J=2.6 Hz), 6.34 (1H, d, J=8.3 Hz), 5.57 (1H, d, J=10.0 Hz), 4.84 (1H, s, -OH), and 1.43 and 1.39 (each 1H, s); EI-MS, m/z, 322 (M^+) and 307 (base).

10 **3**: mp 167~168 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz), δ9.02 (1H, s, -OH), 7.90 (1H, s), 7.32 (1H, dt, J=1.5, 7.8 Hz), 7.08 (2H, dt, J=1.5, 7.8 Hz), 6.93 (1H, dt, J=1.5, 7.8 Hz), 6.70 (1H, s), 6.11 (2H, s), and 4.12 (3H, s); EI-MS, m/z 312 (M^+) and 194 (base).

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