Comparative Analysis of Terpenoids in Roots of *Ipomoea* Species Induced by Inoculation of *Ceratocystis fimbriata*

Sir:

Production of various kinds of terpenoids such as ipomeamarone and ipomeanine in sweet potato roots infected by *Ceratocystis fimbriata*, the black rot fungus, is generally thought to be the result of the alteration of the normal metabolism in the host tissue in response to the infection. Further, it has been confirmed that the abnormal enzyme system involved in the production of these terpenoids actively functions through the formation of some enzymes in the system. Thus, we have presented a hypothesis that the abnormal metabolism may be a result of the activation of the genes which are latent under the normal condition. As one of the approaches to confirm this, the root tissues of various strains of sweet potato (*Ipomoea batatas*) and its related species (*Ipomoea* species in section *batatas*) were inoculated with the spore suspension of *C. fimbriata*, and the terpenoids thus produced were investigated by silica-gel plate chromatography. After the incubation at 30°C for 48 hours, 2 g of the infected region of each tissue was homogenized with 20 ml of a mixture of chloroform and methanol (1:1, v/v). The lower layer mainly containing chloroform was evaporated to a syrup by blowing nitrogen gas. The syrup was dissolved in 0.5 or 1.0 ml of chloroform, which was subjected to the chromatography, using two kinds of ethyl acetate-n-hexane mixture (20 or 50 vol. per cent ethyl acetate in n-hexane) as the developing solvents. Silica gel for the chromatography was prepared with 16 g of Merck's Kieselgel G., 1 g of corn starch and 50 ml of H₂O. Ipomeamarone (Ip), ipomeanine (Ipn) and the 16 other components showing positive Ehrlich's reaction were detected on the chromatograms. The 16 components were called component A₁, A₂, A₃, Ip', Ip'', C₁, C₂, C₀₁, C₃, C₄, Ipn', Ipn'', C₀₂, B₁, B₂ and B₃, as indicated in Figs. 1-A and -B. The repeated investigations indicated the chromatograms of various species or strains were constant, as shown in Figs. 1-A and -B. This will give a favorable evidence that alterations of the metabolism in the host tissue under the pathological condition are regularly induced under the genetic control of the host.

Thus, the comparative chromatographic analysis as shown above was applied to confirm the possible phylogenetic relationship among sweet potato and the closely related species. *I. trifida* 6x has been assumed to be the prototype of the modern cultivated sweet potato. Furthermore, a relationship among six other species besides sweet potato and *I. trifida* 6x has been proposed, as shown in Fig. 2-A, from the comparative studies on morphological and physiological characters, chromosome numbers, genome constitutions and isolating mechanisms, the hexaploid species such as *I. trifida* 6x and sweet potato

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FIG. 1. Chromatographic Patterns of the Various Terpenoids from Infected Root Tissues of Twelve Strains of Ipomoea Species.

Developing solvents were 20 vol. per cent (A) and 50 vol. per cent (B) ethyl acetate in n-hexane. Strains used were I. lacunosa (K231), I. triloba (K121), I. leucantha (K221), I. gracilis 2x (K233-1), I. trifida 4x (K222-1 and K222-3), I. gracilis 4x (K134-3), I. tiliacea (K270-1), I. trifida 6x (K123-14 and K123-11) and I. batatas ('Kanto 48' and 'L-4-5'). Numbers in the parentheses indicate the strain numbers. ○: strong coloration, ☐: not strong, but clear coloration.
FIG. 2. Phylogenetic Relationship among Eight Ipomoea Species in Section Batatas.
(A): Proposed from the biological data on leaf characters (herbaceous, He, submembranaceous, SM, and membranaceous, Me), types of reproduction (autogamous, Au, semi-allogamous, SA1, and allogamous, Al), chromosome numbers, genome constitutions (AA, BB, CC, BBB'B', EEFF, and BBB'B'DD) and isolating mechanisms (strong or slightly strong inviability of embryo in F1, I or I I I), strong, slightly strong or weak chromosomal sterility in F1, I V or I V V, or I V V V. B: Proposed from the combined data of the biological and biochemical investigations. Components, Ip', Ip', Ip'', Col and Cot, shown in Fig. 1, have been specially considered as the important biochemical markers.

A > --- B: possible phylogenetic process of an allopolyploid derived from a species or genome, A and another species or genome, B or the relative.

A > --- B: possible phylogenetic process of an allopolyploid derived from A and B, or of an autopolyploid derived from A.

A > --- B: possible phylogenetic process of an allopolyploid derived from A or its relative and another unknown species or genome.
are assumed to have been originated by chromosome doubling of F₁ hybrids between *I. trifida* 4x and *I. gracilis* 2x. The conclusive evidence should be provided by artificial synthesis of the hexaploid that resembles *I. trifida* 6x. Also, *I. trifida* 4x was thought to have been derived from chromosome doubling of *I. leucantha*, or of F₁ hybrids between *I. leucantha* and another species as *I. triloba* or *I. lacunosa*, although the hybridization between *I. leucantha* and the two diploid species has been unsuccessful. The chromatographic study of the terpenoids, especially of three components, Ip⁺⁺, Ip⁺ and Ip⁺⁺⁺, strengthened the possibility of originating *I. trifida* 4x through the latter process, because Ip⁺⁺ and Ip⁺⁺⁺ were detected in *I. leucantha*, Ip⁺ in *I. triloba* or *I. lacunosa*, and Ip⁺⁺, Ip⁺, and Ip⁺⁺⁺, in *I. trifida* 4x, as can be seen in Fig. 2-B. Furthermore, all the other phylogenetic relationships derived from the previous investigations did not contradict to the present data (See Figs. 2-A and -B). Thus, the phylogenetic relationship among *Ipomoea* species in section *batatas* has become more conclusive by the present chromatographic investigation of terpenoids. The outline derived from both biological investigation and chromatography of terpenoids is indicated in Fig. 2-B.

The present results suggest that the comparative biochemistry on abnormal metabolism induced by external factors such as pathogenic infection and other injurious stimuli will be a useful approach to the study of genetic controlling mechanisms and phylogenetic relationship.

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