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Note

Silica gel G interference in the cucumber hypocotyl bioassay

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Thin-layer chromatography was used in an attempt to isolate a plant growth synergist. The present report shows that components of the supposedly inert silica gel G interfere in the isolation of such a material not only by stimulating growth themselves but also by acting synergistically with growth regulators in the assay system.

MATERIALS AND METHODS

Thin-layer chromatographic methods were patterned after MacMillan and Suter[1]. Thin-layer plates of silica gel G (Merck, Darmstadt, F.R.G.) were prepared and activated at 110 to 120°C for 1–2 h. The blank plates were then developed and eluted. The origin was taken as a line 2 cm from one edge of the plate. The plates were removed from the solvent when the front was 3 cm from the opposite edge of the plate for a total migration distance of 15 cm. The plates were developed in one of three solvents: (a) diisopropyl ether–glacial acetic acid (95:5, v/v)[1]; (b) *n*-butanol–ethanol–water (4:1:5, v/v) organic phase[2]; (c) *n*-butanol–acetone–water (5:2:1, v/v)[2].

To elute material from the adsorbent the plate was divided into ten 1.5-cm zones with the first zone near the origin. The absorbent was scraped from each zone and transferred to a centrifuge tube, suspended in 3 ml 95% (v/v) ethanol and centrifuged for 10 min at 600 *g*. The elution was repeated three times after which the 9 ml eluate of each zone was allowed to dry. To test the residue on the bioassay system the residue of each eluted zone was dissolved in 1.0 ml 95% (v/v) ethanol.

The cucumber assay was patterned after the methods of Katsumi *et al.*[3]. Seeds (*Cucumis sativus Linneaus* "National pickling", Burpee Seed Co.) were soaked in water for 2 h and planted in vermiculite. Approximately five days after planting when the hypocotyl length was 2.5 cm an Indian ink mark was placed 2 cm below the cotyledonary node, and the apical bud was removed. The solution to be assayed was placed on the apex of each of seven seedlings chosen at random (0.01 ml per seedling). After three days the distance between the ink mark and the node was measured and recorded. The results from each set of seven seedlings was averaged and a standard error calculated. The growth response of the silica zones in ethanol was considered significant when the response and standard error exceeded the response and standard error of ethanol alone. The growth response of the silica zones in ethanol and growth hormone was considered significant and was described as synergistic when the re-

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sponse and standard error exceeded the response and standard error of the growth hormone in ethanol.

3-Indolylacetic acid (IAA) and gibberellic acid (GA3) was obtained from California Biochemical (Los Angeles, CA, U.S.A.). An amount of 10 μg of IAA or GA3 was dissolved in 0.01 ml ethanol and applied alone or in combination with extracts from the silica plates, and was inoculated at the site of the removed apical bud of the cucumber hypocotyl.

RESULTS AND DISCUSSION

Cucumber seedlings were innoculated with the eluates in ethanol of ten 1.5-cm zones from blank whole plates of silica gel G developed in solvent a (Fig. 1A and B). Plants treated with the eluates from zones 1–3 and 6–10 were significantly longer than those treated only with ethanol. When 10 μg gibberellic acid was added to each of the solutions, plants treated with eluates of zones 4, and 7–10 showed a synergistic growth response. The experiment was repeated with similar results.

Additional silica plates were developed in solvent b and combined so that each test solution contained the equivalent of two and a half plates (Fig. 1C and D). The ethanol solutions were inoculated alone and in combination with 10 μg IAA. Plants treated with silica plate extract in ethanol were not significantly longer than those

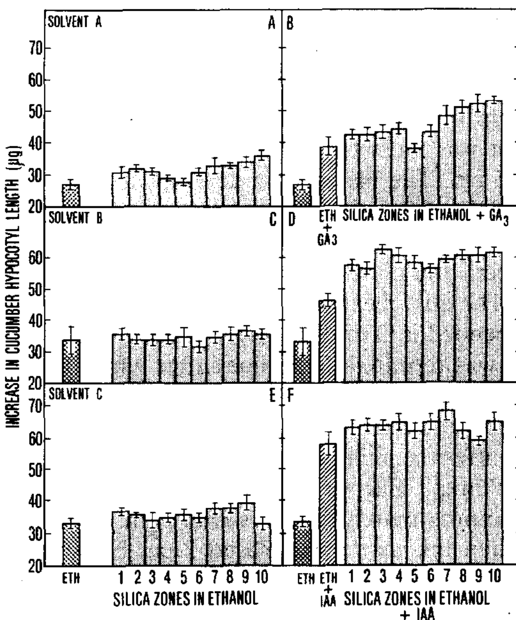

Fig. 1. Effect of silica zone extracts on cucumber hypocotyl growth, alone and in combination with plant growth hormones: (A and B) silica zones extracted with diisopropyl ether–glacial acetic acid (95:5, v/v), (A) silica zones in ethanol, (B) silica zones in ethanol plus GA3; (C and D) silica extracted with *n*-butanol–ethanol–water (4:1:5, v/v), (C) silica zones in ethanol (D) silica zones in ethanol plus IAA; (E and F) silica extracted with *n*-butanol–acetone–water (5:2:1, v/v), (E) silica zones in ethanol, (F) silica zones in ethanol plus IAA. Each column represents the mean value of seven seedlings. The vertical bars represent the standard error. ETH = Ethanol.

treated only with ethanol (Fig. 1C), but a definite synergistic response was obtained when plants were treated with silica extracts of all zones and IAA (Fig. 1D). In solvent c a significant growth increase was obtained with silica zones 7–9 in ethanol and a synergistic response was obtained when plants were treated with IAA and silica extracts of zones 4, 6, 7 and 10 (Fig. 1E and F).

The results indicate that some substance extracted from the silica plates stimulates the growth of the cucumber hypocotyl alone and when this substance is combined with GA3 or with IAA. The ionic mobility of the interfering ion varied according to the solvent system.

The silica gel had a manufacturer's analysis of 0.03% (w/w) maximum iron and 0.02% (w/w) maximum chloride. Although theoretically the silica gel is inert iron in the form of ferrous chloride is soluble in ethanol as is amorphous silica[4]. Dissolved calcium sulfate from silica gel G is reported to contribute to erroneously high values in lipid analysis[5]. Ferrous ions are reported to act as synergists of IAA-induced growth[6–9]. It is possible that the interference of silica gel G in the bioassay is due to the action of dissolved iron, calcium sulfate or even amorphous silica.

We hoped that the silica plates could be used to extract a growth factor from lichens but the growth stimulating property of the ingredients of the silica gel itself interfered in the assay.

While thin-layer chromatography is an excellent and frequently used tool it must be used cautiously when the separated compounds are to be assayed on biological systems.

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