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GIBBERELLIN A_4 PRODUCED BY SPHACELOMA MANIHOTICOLA, THE CAUSE OF THE SUPERELONGATION DISEASE OF CASSAVA (MANIHOT ESCULENTA)

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Summary: Gibberellin A_4 was identified by combined gas chromatography-mass spectrometry in the culture medium of Sphaceloma manihoticola, a fungus known to cause the "superelongation disease" of cassava (Manihot esculenta). Gibberellin A_4 was synthesized in ageing cultures and reached concentrations as high as 400 µg/l nutrient broth. After Gibberella fujikuroi, Sphaceloma manihoticola is the second phytopathogenic fungus known with certainty to produce an active gibberellin in considerable amounts.

Gibberellins (GAs) are widely distributed as hormones in higher plants. Nevertheless, the richest source for certain GAs, especially GA_3, is the ascomycete Gibberella fujikuroi. This fungus is the cause of the "bakanae disease" of rice, which effects overgrowth symptoms.

Plant pathologists have described various other plant diseases causing an abnormal increase in longitudinal growth, which was suspected to be due to increased GA contents (ref. 1,2). In none of these cases gibberellins were unambiguously identified and it was not shown whether the active principle was produced by the pathogen or by the host plant.

In 1972 J.C. Lozano at the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia, reported a disease of cassava plants, hitherto unknown to scientists (3). It was named the "superelongation disease" because an extensive elongation of the internodes of infected plants occurred. Leaf spots and cancers were further symptoms. Heavy losses due to this disease

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have been observed in various parts of Colombia, where farmers were forced to abandon their cassava crops (4). The fungus Sphaceloma manihoticola was found to be the causal organism (4,5).

Krausz (4) proposed that the superelongation symptom could be caused by a gibberellin produced by the parasitic fungus. However, attempts to isolate the active compound did not give clear results. Because of the compelling nature of the symptoms, we obtained the fungus and searched for gibberellins after cultivation on different media.

MATERIALS AND METHODS

Two strains of Sphaceloma manihoticola were obtained from J.C. Lozano (CIAT, Cali, Colombia). These strains - named No. 5 and No. 43 - had been isolated at different localities in Colombia.

Cultures were maintained on potato dextrose agar. A potato dextrose broth (decoct of 300 g potatoes + 20 g glucose; final volume 1 l) turned out to be a good nutrient liquid. This broth was used in concentrations of 100 % and 10 %. Portions of 100 ml nutrient broth each in a 500 ml flask were inoculated with a small amount of mycelium and incubated on a rotary shaker at room temperature (21 °C).

After removal of the cells on a Büchner funnel the pH of the medium was adjusted to 2.7 with 1 N HCl and the solution was extracted four times with 35 ml each of water-saturated EtOAc. The remaining aqueous layer was further extracted five times with 20 ml each of water-saturated butanol-(1). The combined EtOAc layers were extracted three times with 70 ml each of 0.1 M K_2HPO_4 (saturated with EtOAc), which was then acidified to pH 2.7 with 5 N HCl and extracted four times with 70 ml each of water-saturated EtOAc. The organic phases were combined and washed four times with 14 ml each of water (adjusted to pH 2.7 with HCl) and concentrated to dryness under reduced pressure. The acidic EtOAc extract was purified by TLC on Merck Kieselgel 60 developed with propanol-(2) : 25 % ammonia : water (10 : 1 : 1, v/v). Pure gibberellins had the R_f-values: GA_1 = 0.42; GA_3 = 0.47; GA_4 = 0.54; GA_7 = 0.60; GA_9 = 0.70. Different zones of the chromatogram were scraped off immediately after development, homogenized in a mortar, and eluted with methanol in Pasteur pipets. The recovery rate for GAs over the whole purification procedure may be assumed to be 75 % (6).

Gibberellin activity was measured with the lettuce hypocotyl bioassay (7). Samples for GC analysis were methylated with diazomethane and trimethylsilylated with N-methyl-N-trimethylsilyltrifluoracetamide. GC was carried out using OV 101, OV 11, QF-1, and OV 225 as liquid phases, with 2 % each on Chromosorb W/AW-DMCS (80-100 mesh), in glass columns (183 cm in length; 4 mm i.d.) with nitrogen as the carrier gas. Column temperature

was kept at 180 °C for 3 min and then raised to about 240 °C at a rate of 4 °C/min. For GC-MS analyses, a Varian CH-7 mass spectrometer was coupled with a gas chromatograph over a Biemann-Watson-Separator. Methylated and trimethylsilylated samples were separated on a SE-30 column. The ionisation energy was 70 eV.

RESULTS

Both strains of S. manihoticola grew well in the 100 % potato dextrose medium forming spherical colonies (diameter about 2 mm) of a hard consistency as described in (8). After 14 days of incubation approximately 1.5 g dry weight of mycelium was obtained in 100 ml of medium. In contrast, the mycelium yield in the 10 % medium was only about 0.2 g of dry weight per 100 ml. The color of all cultures was originally yellowish, but strain No. 43 with the 10 % potato dextrose broth became intensively red after approximately 8 days.

Gibberellin-like activity was found by bioassay exclusively in older cultures (cf. Tab. 1). After preparative TLC of the acidic EtOAc extracts, only one zone of activity was present at about R_f 0.55. The acidic butanol extracts did not contain any detectable gibberellin-like activity.

GC analysis of active fractions as Me-, TMS-, and MeTMS-derivatives on the different liquid phases showed a single main

TABLE 1. GROWTH AND GA_4 PRODUCTION OF SPHACELOMA MANIHOTICOLA UNDER DIFFERENT CULTURE CONDITIONS

Strain	Medium Conc. (%)	Age (d)	Color	Dry Weight of Mycelium (g)	GA_4 content (µg/l)
5	100	3	yellow	0.2	---
43	100	3	yellow	0.1	---
5	100	14	yellow	1.5	90
43	10	11	red	0.4	400

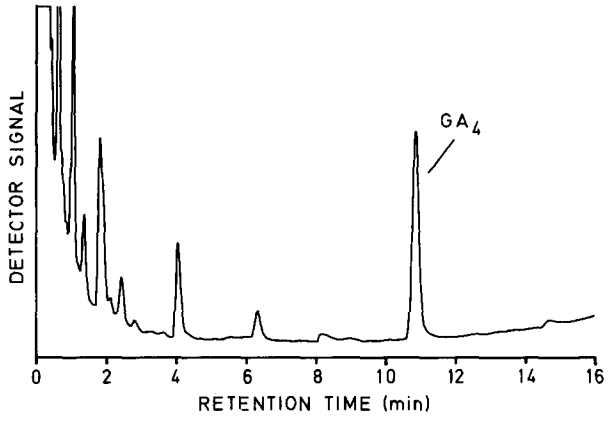

Fig. 1. Gas chromatographic purity of GA_4 obtained from Sphaceloma manihoticola

Strain No. 43 was cultivated for 11 days on a 10 % potato dextrose broth. After TLC of the corresponding acidic EtOAc extract the eluate from R_f 0.5-0.6 was converted to its TMS-derivative and separated on QF-1. (Note that peaks with lower retention times are partly due to impurities in the derivatising agent or by-products of its reaction)

component with a retention time identical to that of GA_4 (Fig. 1). The identity was confirmed by GC-MS, the spectrum of the MeTMS-derivative being identical to the one of GA_4-MeTMS (9). No indications for other gibberellins were found by GC or GC-MS. In spite of the relatively simple purification, samples turned out to be very pure in regard to GA_4 (cf. Fig. 1). The amount of this gibberellin was estimated to be 90 µg/l and 400 µg/l, respectively. In agreement with the results by bioassay, no GAs were detected in the 3 days old samples (Tab. 1).

DISCUSSION

The results show that S. manihoticola produces GA_4 in high amounts. GA_4 is obtained in a very pure state after solvent extraction and single TLC. Besides G. fujikuroi, this is the only other case known in which a phytopathogenic fungus produces an active gibberellin as identified by exact analytical methods.

Although not proved for the situation *in vivo* it is likely that fungal GAs cause the observed overgrowth symptoms in cassava as well as in rice.

At least in strain No. 43 of *S. manihoticola* growing on the diluted potato dextrose medium the production of GA_4 is paralleled by pigmentation, similar to the situation in *G. fujikuroi*. Probably nitrogen is the limiting factor triggering secondary metabolism in *Sphaceloma* as shown for *Gibberella* (e.g. 10).

Under the conditions used here, GA_4 is the only active gibberellin produced by *S. manihoticola*. This indicates that biosynthesis comes to an end at this point of the pathway, whereas in *G. fujikuroi* GA_4 is further converted to GA_7, GA_3, and GA_1 (11). Because of their structural similarity, GA_4 and GA_7 are only obtainable as a mixture in commercial preparations originating from *G. fujikuroi*. By improving culture conditions and selecting suitable strains it might be possible to raise the GA_4-production of *S. manihoticola* thus making another gibberellin easily available in pure form.

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REFERENCES

1. Pegg, G.F. (1976). In: "Encyclopedia of Plant Physiology", Vol. 4 (Heitefuß, R. and Williams, P.H., eds.), pp 592-606, Springer, Berlin-Heidelberg-New York
2. Brian, P.W. (1978). Proc. R. Soc. Lond. B. 200, 231-243
3. In: "CIAT (Centro Internacional de Agricultura Tropical) 1972". CIAT Annual Report 1972. 192 pp. CIAT, Cali, Colombia, as quoted in (4)
4. Krausz, J. (1976). Ph.D. dissertation, Cornell University
5. Krausz, J., Lozano, J.C., and Thurston, H.D. (1976). Proc. Am. Phytopath. Soc. 3, 230

6. Rademacher, W. (1978). Ph.D. dissertation, University of Göttingen

7. Frankland, B. and Wareing, P.F. (1960). Nature 185, 255-256

8. Lozano, J.C. and Booth, R.H. (1974). PANS (Pest Articles and News Summaries) 20, 30-54

9. Binks, R., MacMillan, J., and Pryce, R.J. (1969). Phytochemistry 8, 271-284

10. Bearder, J.R., MacMillan, J., Wels, C.M., Chaffey, M.B., and Phinney, B.O. (1974). Phytochemistry 13, 911-917

11. Graebe, J.E. and Ropers, H.-J. (1978). In: "Plant Hormones and Related Compounds" (Letham, D.S., Goodwin, P.B., and Higgins, T.J.V., eds.), Vol. 1, pp 107-203, Elsevier/North Holland Biomedical Press, Amsterdam-Oxford-New York