Pergamon

PII: S0031-9422(96)00796-0

Phytochemistry, Vol. 45, No. 1, pp. 47–49, 1997
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0031-9422/97 $17.00+0.00

A PHYTOALEXIN FROM ROOTS OF *MUSA ACUMINATA* VAR. PISANG SIPULU

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(*Received in revised form* 16 *July* 1996)

Key Word Index—*Musa acuminata*; musaceae; banana; phenylphenalenone-phytoalexins; *Radopholus similis*; resistance.

Abstract—A phenalenone-type phytoalexin has been isolated from infected roots of banana plants (*Musa acuminata* AA 'Pisang Jari Buaya' group cultivar Pisang sipulu). This product was produced *de novo* by the plants upon infection with the nematode species *Radopholus similis*. The structure of this phytoalexin was elucidated using spectroscopic evidence and chemical correlation using reverse phase HPLC and ^{1}HNMR analysis. This compound has previously been described as a natural product, anigorufone from *Anigozanthos rufus* (Haemodoraceae) and has recently been described as a phytoalexin-like compound from *Musa acuminata* (AAA) Grand Nain. This compound is new to diploid species of *Musa* and is the first compound known to be produced in response to nematode invasion in the banana. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The burrowing nematode *Radopholus similis* is the most devastating of all the plant parasitic nematodes that infect the roots and rhizome of *Musa* spp. [1]. Resistance to nematodes is unknown in any of the commonly grown banana or plantain cultivars. Resistance to *R. similis* has been identified in some diploid and triploid material originally collected from Africa and South East Asia [2] (Binks, R. H., unpublished results). Cultivars from the 'Pisang Jari Buaya' (PJB) group of *Musa acuminata* (AA) are the most promising because of their superior agronomic and disease resistance qualities. At present the mechanisms of resistance are unknown, but it is thought that cell wall thickenings along with phenolic acid complexes are possible mechanisms of resistance in this group of cultivars. (Binks, R. H., unpublished results). Resistance, in part, is mediated by a hypersensitive response to invasion from *R. similis* [3]. There is also evidence that this response is associated with the mechanisms of resistance in plants to nematodes [4].

The production of *de novo* defence compounds (phytoalexins) by plants in response to biotic, physical or chemical agents is well documented [5]. Recently several phenylphenalenone phytoalexins have been isolated from the rhizomes of *Musa acuminata* (AAA) Grand Nain elicited with kanamycin, or infected with the fungi *Mycosphaerella fijinsis* [6], the causal agent of Black Sigatoka disease in banana plants, and *Fusarium oxysporum f.* sp. *cubense* Race 4 which causes Panama disease in banana plants [7, 8].

To our knowledge, the role of phytoalexins in resistance to banana and plantain nematodes has not been investigated. Furthermore, no studies have been made concerning the role of phytoalexins in resistance to any burrowing nematode of banana and plantain, although phytoalexin compounds have been found to be produced in the roots of other plant species in response to nematode invasion [9].

We report here on the production, isolation and characterization of the phytoalexin, 2-hydroxy-9-phenylphenalen-1-one (**1**) [7], for *Musa acuminata* AA 'Pisang Jari Buaya' group (cultivar Pisang sipulu) and its association with the hypersensitive reaction to *Radopholus similis*.

RESULTS AND DISCUSSION

Root samples from plants which had been inoculated with nematodes showed a significant increase in phytoalexin production, compared to physically

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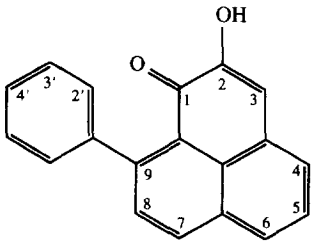

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Fig. 1. 2-Hydroxy-9-phenyl-phenalen-1-one.

damaged and control plants. Phytoalexin production in healthy plants has been suggested to be a natural response to cutting stress [7, 8]. This theory is backed up by results obtained from physically damaged plants. When results from HPLC analysis were compared, extracts taken from roots of the susceptible cultivar *M. acuminata* (AAA) Grand Nain, also showed some signs of phytoalexin production. These observations confirm that the production of this phytoalexin is common to other species from the *Musa acuminata* group of bananas [7].

Data obtained from HPLC and ^{1}HNMR (Bruker AMX400 (400 MHz) analysis have shown that the compound originally extracted from *Musa acuminata* (AAA) Grand Nain is identical to the compound extracted from *Musa acuminata* (AA) 'Pisang Jari Buaya' group (cultivar Pisang sipulu). Results obtained from prep. TLC and HPLC analysis have also shown that the compound extracted from field grow plant material is identical to that identified in glasshouse grown material. These results are in agreement with those obtained by Luis and colleagues [7] and show the compound to be 2-hydroxy-9-phenylphenalen-1-one (**1**) (Fig. 1).

Results confirm that the phytoalexin is being produced by the plant in response to damage and that it is suggested that this compound could be playing a significant role in the plant's defence system (Luis, 1996, pers. comm.). This compound had been previously isolated from *Anigozanthos rufus* (Haemodoraceae), [10] and given the name anigorufone. It has also been found to be produced in response to fungal attack in *Musa acuminata* (AAA) Grand Nain, as shown by Luis and co-workers [7]. It is noteworthy that, although these species of plants produce several phenylphenalenones, this product has never been found before in a diploid species from the *Musa acuminata* group of bananas.

EXPERIMENTAL

Production of phytoalexin

Plant material. Meristems of a burrowing nematode susceptible cultivar *Musa acuminata* (AAA) Grand Nain and resistant variety *Musa acuminata* (AA) 'Pisang Jari Buaya' group (cultivar Pisang sipulu) were supplied by the International Network for the Improvement of Banana and Plantain (INIBAP).

Plants were grown *in vitro* using as established technique [11]. After 8 weeks plants (10/variety) were transferred to modules, containing a peat compost mixt. and hardened off in the greenhouse at 27–30° with a minimum photoperiod of 12 hr for a further 2 weeks. After this time plants were transferred to (1 l) pots. Plants were chosen for homogeneity of size 5–10 cm for use in experimental work. A further 20 plants/variety were grown to provide uninfected control material.

Nematodes. Radopholus similis [12, 13] from Onne, Port Harcourt, Nigeria were supplied by the International Institute of Parasitology, St Albans, U.K. Nematodes were monoxenically cultured on carrot discs [14]. Infective aseptic nematodes were recovered in sterile distilled water.

Inoculation of Musa. Inoculation mimicked natural infection. When plants had reached a size of 5–10 cm, they were placed in a fully randomized block design experiment. Plants were then inoculated either with a sterile 'insect' mounting needle so as to simulate physical damage or with *Radopholus similis* (600 nematodes ml^{-1} H_2O). Control plants were not treated. Each treatment was replicated $\times 3$. Plants were left for 24 hr before being watered so as to allow nematodes to invade the plant roots. Plants were watered every 2 days with tap water.

Collection and extraction of phytoalexin. Four weeks after inoculation, plants were removed from pots, and their root systems were excised from the pseudostem. Roots were washed free of soil and chopped into 1 cm pieces. 80% MeOH extracts were made from the root samples. Each extract was filtered through microfibre (Whatman GF/A). The filtered extract was evaporated to dryness *in vacuo* at 40° and redissolved in 1 ml of 80% MeOH.

Analysis by reverse phase HPLC. A Waters system was used for analysis of root extracts, consisting of a multisolvent delivery pump (Model 600), programmable photodiode array detector (Model 994). Each sample to be analysed was evapd to dryness and the residue dissolved in 1 ml of 80% MeOH. This was filtered through a 0.45 μm pore size Gelman Nylon Acrodisc 13 filter. 40 μl of each sample was injected into a μ-Bondapak C-18 phenyl column (4 (i.d) $\times$ 300 mm). Sepn was accomplished by gradient elution, using a combination of two solvent systems A and B. The composition of solvent A was 2% HOAc in H_2O, and of solvent B MeOH–H_2O–HOAc, (18 : 1 : 1). All solvents used for extraction and HPLC analysis were HPLC grade. The gradient system used started at 75% A and 25% B, and changed linearly to 0% A and 100% B in 20 min, respectively. Detection was at 260 and 350 nm. Each analysis was carried out at a constant temp. (25°), and a flow rate of 1.0 ml min^{-1} was maintained.

Isolation and purification of phenylphenalenone phytoalexin from field grown plant material

Due to a shortage of root material from the inoculation experiment, the phytoalexin was extracted from field grown plant material of the resistant cultivar Pisang sipulu (AA). A crude extract was made, using 25 g of oven dried, ground root material. The extract was filtered and evapd to dryness *in vacuo* at 40 and reconstituted in 5 ml 80% MeOH. This extract was streaked onto Whatman no. 3 paper and run in *n*-butan-1-ol–HOAc–H_2O (BAW), (4 : 1 : 5 upper layer). The paper was air dried at room temp. and the band cut out. The compound was eluted into 80% MeOH. The eluent was evaporated to dryness *in vacuo* at 40 .

Further purification of the extract was required to perform ^{1}HNMR analysis. The mixture was further extracted in 2 ml $CHCl_3$. The extract was filtered through a 0.45 μm pore size Gelman Nylon Acrodisc 13 filter. The compound was purified by prep. TLC (on pre-coated 0.25 mm silica gel plates (G. Schleicher and Schull), using as solvent—$CHCl_3$–Me_2CO (99 : 1). To concentrate and align the compound on the TLC plate the plate was placed in 80% EtOAc. Plate was removed when solvent had reached the compound band. The band was collected in a glass column containing Sephadex LX-20, previously equilibrated with EtOAc. The compound was eluted through the column with EtOAc. Eluent was evaporated to dryness *in vacuo* at 40 and reconstituted in $CDCl_3$.

2-*Hydroxy*-9-*phenylphenalen*-1-*one* (**1**). Yellowish orange compound in visible light; $R_f = 0.31$, $R_t =$ 23.30 min, UV (EtOH) λ_{max}nm: 269, 334, 350, 364. ^{1}HNMR δ: 4.81 (1H, *bs*, -OH), 7.15 (1H, *s*, H-3), 7.39 (2H, *m*, H-2′ and H-6′), 7.48 (3H, *m*, H-3′, H-5′ and H-4′), 7.60 (1H, *t*, $J = 7.8$ Hz, H-5), 7.61 (1H, *d*, $J = 8.3$ Hz, H-8), 7.86 (1H, *dd*, $J_1 = 1.3$ Hz, $J_2 = 8.3$ Hz, H-6), 7.96 (H-4), 8.25 (1H, *d*, $J = 8.3$ Hz, H-7).

Acknowledgements—This work was supported by funding from the Natural Resources Institute as part of a Ph.D. in Nematology based in the Department of Agriculture, at the University of Reading. Thanks are given to Dr P. Rowe at the Fundacion Hondureña de Investigacion Agricola for providing roots from field grown *Musa* material and to Mr El Hassane Lahlou for his help in preparing material for ^{1}HNMR. Thanks are also given to Dr J. Bridge at the International Institute of Parasitology, St. Albans, U. K., for providing original nematode cultures and to Dr D. Jones at the International Network for the Improvement of Bananas and Plantains in Montpellier, France, for arranging supply of *in vitro Musa* germplasm material.

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