

ISOLATION OF PHYTOALEXINS FROM INDIGENOUS MALVACEAE SPECIES RESISTANT TO VERTICILLIUM DAHLIAE AND FUSARIUM OXYSPORUM F. SP. VASINFECTUM

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Summary

Fungal diseases cause appreciable cotton yield losses in Uzbekistan and other cotton producing countries. Phytoalexins are one of the active defense mechanism that plants utilize to protect themselves from attack by pathogens, and indigenous plant species may provide compounds that are more potent than those produced in cotton. Cotton (*Gossypium*) belongs to the family Malvaceae, and phytoalexins produced by some Malvaceae are structurally related to those in cotton. The introduction into cotton of genes responsible for the synthesis of more potent Malvaceae phytoalexins could significantly increase resistance to fungal pathogens that attack cotton. Others have shown that plant resistance is correlated with the speed with which the plant recognizes the pathogen and the potency of the phytoalexins produced. Herein, we report our search for more potent phytoalexins from several members of the Malvaceae family that are indigenous to Uzbekistan. The plants investigated were *Hibiscus trionum*, *Hibiscus esculentus*, *Hibiscus manihot*, *Malva sylvestris*, *Malva moschata*, *Malva bucharica*, *Abutilon theophrasti* and *Althea rosa*. The antifungal activities of the phytoalexins from these plants were assayed against the plant pathogens *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum*. Of the plants investigated, the most resistant were *M. sylvestris*, *H. trionum* and *H. esculentus*. In these plants, the phytoalexins achieved maximal concentration within 24 h after inoculation by pathogen spores. Phytoalexins were extracted from the stems of inoculated plants and isolated by chromatography on a silica column. They were further purified by HPLC. IC50 values were determined on partially pure fractions. Structure elucidation of the most active phytoalexins is currently under way.

Introduction

Fungal diseases cause appreciable cotton yield losses in Uzbekistan and other cotton producing countries. Phytoalexins are one of the active defense mechanism that plants utilize to protect themselves from attack by pathogens, and indigenous plant species may provide compounds that are more potent than those produced in cotton. Cotton (*Gossypium*) belongs to the family Malvaceae, and phytoalexins produced by some Malvaceae are structurally related to those in cotton. The introduction into cotton of genes responsible for the synthesis of more potent Malvaceae phytoalexins could significantly increase resistance to fungal pathogens that attack cotton. Others have shown that plant resistance is correlated with the speed with which the plant recognizes the pathogen and the potency of the phytoalexins produced.

Aims of a work

The main aim of a work is searching for more potent phytoalexins from several members of the Malvaceae family that are indigenous to Uzbekistan.

Objects of investigation

The plants investigated were *Hibiscus trionum*, *Hibiscus esculentus*, *Hibiscus manihot*, *Malva sylvestris*, *Malva moschata*, *Malva bucharica*, *Abutilon theophrasti* and *Althaea rosa*. The antifungal activities of the phytoalexins from these plants were assayed against the plant pathogens *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum*.

The main results

We report our search for potent phytoalexins from several members of Malvaceae family that are indigenous to Uzbekistan. The antifungal activities of the phytoalexins from these plants were tested against the plant pathogens *Verticillium dahliae* and *Fusarium oxysporum* (Fig. 1).

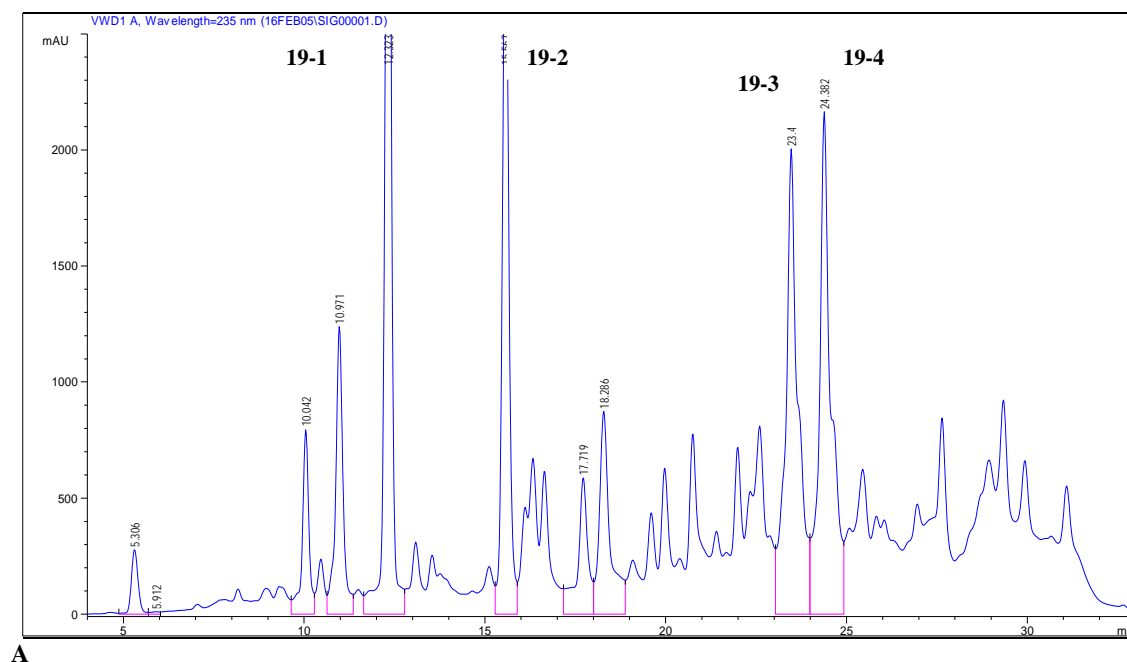


Fig. 1. Investigation of fungitoxic activity of phytoalexins.

Of the plants investigated, the most resistant were *M. sylvestris*, *H. trionum* and *H. esculentus* (Fig.2). In these plants, the phytoalexins achieved maximal concentration within 24 h after inoculation by pathogen spores.

*Malva sylvestris**Hibiscus esculentus**Hibiscus trionum***Fig. 2. Malvaceae plants.**

Phytoalexins were extracted from the stem of inoculated plants, and isolated by chromatography on silica column. They were further purified by HPLC and (Fig.3).



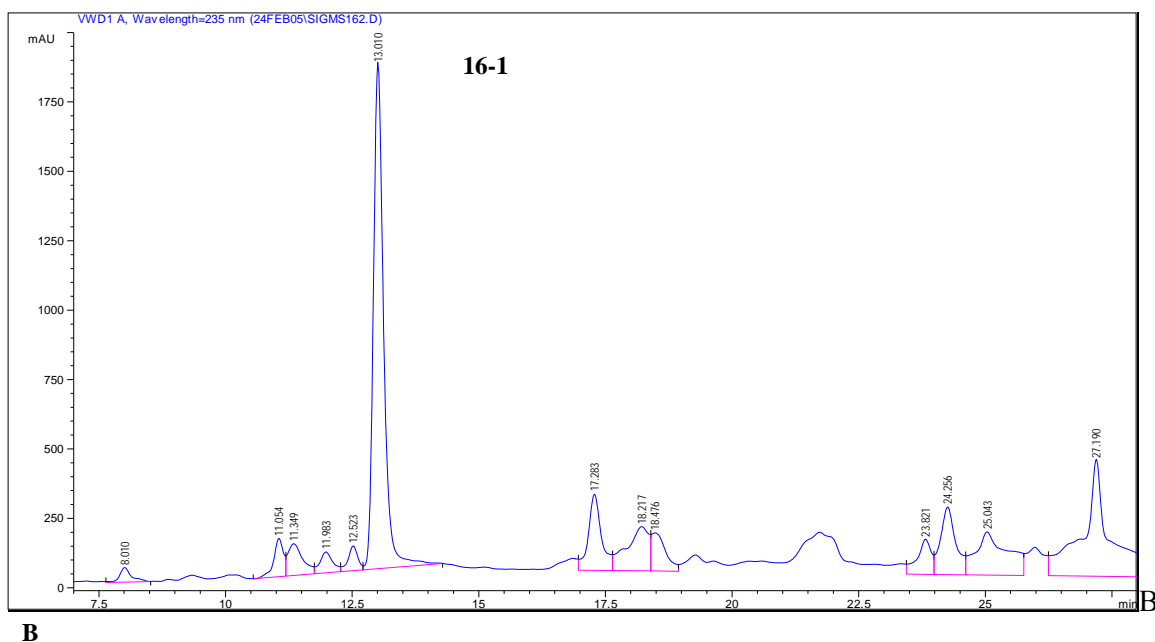
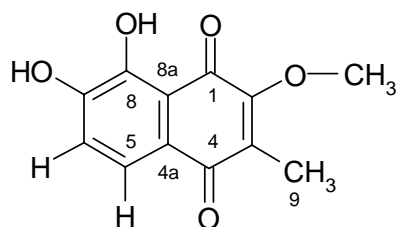
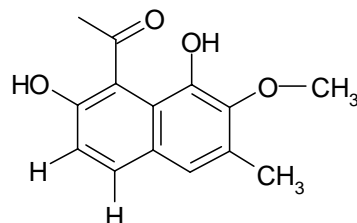


Fig. 3. HPLC analysis of *Malva sylvestris* phytoalexins.

The antifungal activities of individual phytoalexins were tested on fungi and their IC_{50} values were determined. Structure elucidation of the most active phytoalexins from *Hibiscus trionum* and *Hibiscus esculentus* is under way. We have established chemical structure of two phytoalexins from *Malva sylvestris* stems by the methods of NMR- and mass-spectrometry and X-Ray analysis (Fig. 4):



Phytoalexin 19-4 from *Malva sylvestris*
 $C_{12}H_{10}O_5$ Mr 234.21



Phytoalexin 16-1 from *Malva sylvestris*
 $C_{13}H_{12}O_4$ Mr 232.33

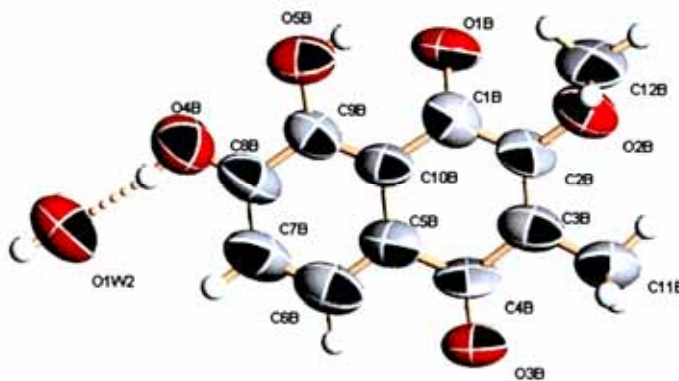


Fig. 4. Model of crystalline structure of phytoalexin 19-4 from *Malva sylvestris*.

Identification of the genes that regulate their biosynthesis would add novel tools to enhance resistance to cotton. This work was supported by USDA ARS, project STCU P-115.