The Phytoalexin Response of *Lathyrus sativus* (Grass Pea)

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Lathyrus sativus was investigated for the nature of its phytoalexin response with the result that leaflets, immature pods, and seeds were induced to produce 6a-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan (pisatin). Evidence is presented for the metabolism of pisatin, by the wound pathogen *Botrytis cinerea*, to a phenolic congener, 3,6a-hydroxy-8,9-methylenedioxypterocarpan.

Introduction

Phytoalexin accumulation in plant tissues is now the most intensively studied mechanism of plant disease resistance [1]. However, phytoalexin induction in the family Leguminosae is also of taxonomic interest [2]. Consequently 64 species of the tribe Vicieae, including 31 *Lathyrus* species, were screened in a recent survey to determine their phytoalexin response [3]. Because of the economic importance of certain species, notably *Lathyrus odoratus* L. (sweet pea) and *Lathyrus sativus* L. (grass pea), phytoalexin production by these plants was investigated more thoroughly. Phytoalexin production by the popular ornamental, *L. odoratus*, has already been reported in some detail [4]. *L. sativus* in grown in agriculture as a forage crop and also as a pulse, most extensively in India where it has the advantage of being a very dependable cropper under drought conditions. This paper is devoted to the results of a study of phytoalexin induction in this agriculturally important legume.

Results and Discussion

The now standard drop-diffusate technique [5] was applied to leaflets of *L. sativus*, the *Drechslera*

state of *Cochliobolus carbonus* Nelson (*Helminthosporium carbonum* Ullstrup) acting as exogenous elicitor. This resulted in the accumulation of an (induced) isoflavonoid in diffusate from inoculated tissue which was readily detected as a UV-quenching band and which exhibited marked activity in the TLC antifungal assay technique [6]. This phytoalexin was identified (UV, TLC, MS) as the known pterocarpan pisatin (6a-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan) (**1**). In a time-course study of phytoalexin accumulation in diffusates, pisatin was detected (after conversion to its 6a-11a-anhydro derivative [7]) in only trace amounts ($< 3 \mu g/ml$) 24 h after inoculation of detached leaflets with *H. carbonum*. After a further 24 h the concentration had risen sharply to approx. $50 \mu g/ml$. Pisatin was also induced in immature pods by *H. carbonum* and accumulated to a concentration of 55 µg/ml in the diffusate after 48 h.
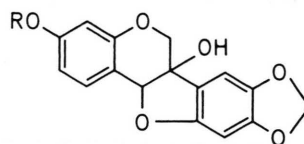
1: R = CH₃
2: R = H

Pisatin was also induced in the leaflets of *L. sativus* by the wound pathogen *Botrytis cinerea* Pers. ex Pers., and abiotically by exposure to short λUV radiation (max emission 254 nm, 30 min exposure). When UV light acted as elicitor *L. sativus* yielded a second compound (LS2), absent from untreated control leaflets, which was resolved from pisatin by TLC in *n*-hexane: Me₂CO, 2 : 1, (R_f 0.15). Its UV spectrum in EtOH (λ_{max} 303 nm) exhibited a bathochromic shift (to λ_{max} 342 and 358 nm) upon treatment with HCl, as has been demonstrated for 6a-hydroxypterocarpans [7, 8] (*cf.* λ_{max} (EtOH) 6a-11a-anhydropisatin 339 and 358 nm). The MS (M⁺ 344) was also typical of a 6a-hydroxypterocarpan with a major ion at M⁺-18 [8] (*m/z* 326(100%)), consistent with dehydration across the 6a-11a bond. The neutral UV spectrum also indicated the presence of a methylenedioxy substituent in the molecule. However, since this compound was present in relatively trace amounts and it showed only weak antifungal activity the elucidation of its structure was not further pursued.

Table 1. Concentration of pisatin in diffusate and leaf tissue of *L. sativus* 48 h after inoculation with *H. carbonum*.

Pisatin concentration	Accession number				
	1[a]	1[b]	2	3	4
Diffusate (µg/ml)	43	57	42	74	61
Leaf tissue (µg/g)	472	422	357	425	439

[a] First experiment.
[b] Second experiment.

Pisatin was induced by *H. carbonum* in the leaflets of a total of four different accessions of *L. sativus*. Concentrations of phytoalexin present in diffusates and in underlying leaf tissue 48 h after inoculation is presented in Table I. These data suggest that pisatin accumulation may be a major factor in preventing pathogenic invasion of *L. sativus* by *H. carbonum* (ED_{50} of pisatin against mycelial growth = approx. 39 µg/ml [4]), as well as by other non-pathogens of *L. sativus*.

Imbibed seeds of *L. sativus* upon treatment with a spore suspension (10^6/ml) of *Botrytis cinerea* yielded pisatin at a concentration of approx. 454 µg/g fresh weight in the top 2 mm of (melanized) tissue. In addition a phenolic compound, tentatively identified (UV, TLC, MS comparison with literature data [9]) as 3,6a-dihydroxy-8,9-methylenedioxypterocarpan (2), was present in *B. cinerea* inoculated tissue (concentration − 90 µg/g). Since 2 was not induced in *L. sativus* by UV irradiation or *H. carbonum* it seems probable that the wound pathogen *B. cinerea* can detoxify pisatin by 3-O-demethylation, a reaction known to be performed by certain other phytopathogenic fungi [10]. This conclusion is further supported by the earlier observation of *B. cinerea* − mediated demethylation of the pterocarpan medicarpin [8].

The phytoalexin response of *L. sativus* is clearly similar to that of *Pisum sativum* in that pisatin, a comparatively uncommon minor phytoalexin within the Papilionoideae [2, 11], is produced as a major phytoalexin by both species. Nevertheless, there is a notable difference in the response of the two plants in that maackiain, which is a minor though significant component of the phytoalexin response of both *P. sativum* and *P. fulvum* [3, 12] was not present in inoculated tissues or diffusate of the grass pea.

Experimental

Lathyrus sativus accessions

Accessions 1 and 2 were from the Institute for Genetics and Plant Breeding, Sofia, Bulgaria and the Botanic Garden of Copenhagen respectively. Accessions 3 and 4 were cv. Favetta and cv. Canberra City respectively, both supplied by the Agrobotanic Institute, Tapioszele.

Induction and isolation of phytoalexin

(a) *Leaflet and pod diffusates.* The drop-diffusate technique was performed as previously described [4]. (b) *Leaf-tissue* see [4]. (c) *Seeds.* Induction and isolation of phytoalexin was carried out as in [13] with the exception that EtOH was used as extracting solvent.

Compound LS2

EtOAc extracts of diffusate from incubated leaflets (48 h) previously exposed to UV irradiation yielded a single quenching band after Si gel TLC in $CHCl_3$:MeOH, 50:1 (R_f 0.50). Elution of this band (EtOH) and TLC of the eluate in *n*-hexane: Me_2CO, 2:1 afforded pisatin (R_f 0.25) plus LS2 (R_f 0.15). For UV data see "Results and Discussion". MS (rel. int.) 344 (M^+, 62%), 329 (5), 326, M^+-H_2O (100), 325 (21), 285 (16), 163 (22), 151 (15).

3,6a-Dihydroxy-8,9-methylenedioxypterocarpan

TLC Si gel ($CHCl_3$:MeOH, 25:1) R_f 0.25. UV and MS data agreed with Lit. values [9], within the limits of experimental error.

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