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# EFFECT OF PISATIN INDUCING FUNGI ON PEA POLYAMINES*

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**Key Word Index**—*Pisum sativum*; Leguminosae; pea; polyamine; spermine; spermidine; pisatin.

**Abstract**—The spermine and spermidine content of pea pod tissue is not significantly altered by inoculation with the pisatin-inducing fungi, *Fusarium solani*. Although these polyamines induce pisatin, it appears that they do not accumulate in levels sufficient to serve as internal mediators of pisatin production in infected tissues.

Numerous chemical compounds as well as pathogenic fungi induce the synthesis of the isoflavonoid pisatin in *Pisum sativum* [1–3]. Also, certain mutant lines of peas can produce pisatin constitutively [4]. These diverse means for inducing pisatin have been postulated to share a common mode of action based on the direct effect of the inducer or mutagen on the DNA of the pea cell [1]. However, the possibility that the induction of pisatin is mediated by the enhanced build-up of a low MW compound within the pea tissue cannot be excluded. The most likely candidates for this mediation are the polyamines. The polyamines spermine and spermidine occur naturally in higher plants [5], are pisatin inducers [1, 2], and have been associated with numerous cellular functions [6]. Spermine and spermidine are prominantly involved in nucleotide interactions [5, 7] due to their basic nature. To test the hypothesis that these polyamines play a role in mediating pisatin induction, it was of interest to determine whether any changes in these polyamines could be detected in pea pod tissue inoculated with the pisatin inducting fungi, *Fusarium solani* (Mart.) Appel. and Wr. f.sp. *pisi* (F. R. Jones) Snyd. and Hans. (the natural pea pathogen) and *F. solani* f.sp. *phaseoli* (Burk.) Snyd. and Hans. (the natural bean pathogen).

Table 1 shows that the 24 hr incubation of excised pea pod tissues alone reduces levels of spermine and increases levels of spermidine in all treatments compared to the levels found in fresh pods. However, there was no significant difference in either spermine or spermidine levels between the water treatments which induces no pisatin and the fungal treatment which induce pisatin. The quantities of spermine and spermidine which must be applied externally to induce pisatin range from 100 to 1000 μg/ml. Comparing pea pod polyamine levels found in this study with those found for pea leaves and roots in other studies, pea pods contain comparable levels of spermidine but higher levels of spermine than have been found in pea leaves [8] and much higher levels of both polyamines than have been found in pea roots [9].

Table 1. Spermine and spermidine content of fresh pea pod tissue and pea pod tissue incubated with $H_2O$, *Fusarium solani* f.sp. *pisi* or *F. solani* f.sp. *phaseoli* for 24 hr

| Treatment | μg/g fr. wt | |
|---|---|---|
| | Spermine | Spermidine |
| Fresh | 30 | 41 |
| $H_2O$ | 22 | 53 |
| *pisi* | 19 | 54 |
| *phaseoli* | 17 | 46 |
| LSD(.05) | 10 | 15 |

Each value is the mean of 3 independent trials.

The incorporation of L-arginine-[$U^{14}C$] was evaluated after a 24 hr incubation period to determine if the rate of polyamine synthesis was contributing to the possible regulatory functions of these compounds in infected tissue (Table 2). There was no significant accumulation of label in polyamines in the pisatin inducing fungal treatments compared to the water treatment. Spermidine showed no significant change in label incorporation but spermine showed a statistically significant decline of label incorporation in the fungal treatments compared to that of the water control. This difference in rate of synthesis apparently has little importance since spermine levels were not significantly altered in the fungal treatments (Table 1). A relatively greater percentage of radioactivity

Table 2. Incorporation of $^{14}C$ from L-arginine-[$U$-$^{14}C$] into spermine and spermidine in pea pod tissue incubated with $H_2O$, *Fusarium solani* f.sp. *pisi* or *solani* f.sp. *phaseoli* for 24 hr

| Treatment | Spermine | | Spermidine | |
|---|---|---|---|---|
| | dpm / g fr. wt | % incorp. | dpm / g fr. wt | % incorp. |
| $H_2O$ | 3000 | 0.13 | 16900 | 0.73 |
| *pisi* | 1880 | 0.08 | 18200 | 0.78 |
| *phaseoli* | 2030 | 0.09 | 15900 | 0.68 |
| LSD(.05) | 765 | | 5810 | |

Each value is the mean of 3 independent trials.

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from L-arginine-[U$^{14}$C] was incorporated into spermidine after 24 hr compared to lower levels of incorporation into spermine (Table 2). This result conforms to the present understanding of polyamine synthesis [5, 10] whereby spermidine and spermine are synthesized by successive additions of a propylamine residue to putrescine which is itself synthesized from arginine and/or ornithine.

These results suggest that the induction of pisatin by these fungi is not mediated by altering the internal levels of spermine and spermidine in pea tissue. Thus, the gene activation which occurs in plants following inoculations of pathogenic fungi does not appear to depend upon the formation of spermine or spermidine.

## EXPERIMENTAL

The endocarp of 1-g lots of split immature pea (*Pisum sativum* cv Alaska) pods in Petri plates was inoculated with 1 ml per plate of either *Fusarium solani* f.sp. *pisi* ($10^6$ conidia/ml), *F. solani* f.sp. *phaseoli* ($10^6$ conidia/ml), or $H_2O$ and incubated for 24 hr. Fresh pods (1 g) were harvested and immediately extracted with $HClO_4$ (0.2 N, 4 ml/g) in a mortar and the extract centrifuged at 12000 rpm for 10 min. If dansylation of this supernatant was performed directly, the subsequent TLC separation yielded an intense spot (probably dansylammonia) which smeared the plate. To remove this the supernatant was passed through a phosphorylated cellulose column according to the following modification of the procedure of ref. (11). All of the supernatant (*ca* 5 ml) was added to 20 ml NaPi buffer 10 mM pH 6 and the pH was adjusted to 6.0 ± 0.5 with M NaOH. Each sample was added to a Cellex-P column (3 ml and 5:1 dimensions) and eluted by the following program: 15 ml buffer, 5 ml $H_2O$, 10 ml 0.01 M HCl, 10 ml 0.02 M HCl, 20 ml 0.05 M HCl, and 20 ml 0.5 M HCl. The 0.5 M HCl eluate contained both spermine and spermidine while most of the smaller amines were eluted at lower concns of HCl. Quantitation of the amines was accomplished by the dansylation technique [12, 13]. The amines in the evaporated 0.5 M HCl eluate were re-dissolved in 1 ml $HClO_4$ (0.2 M). An aliquot (0.2 ml) was added to a glass stoppered centrifuge tube and treated with 20 mg $Na_2CO_3 . H_2O$ and 0.4 ml dansyl chloride (30 mg/ml $Me_2CO$) for 16 hr, 0.1 ml proline (100 mg/ml) for 0.5 hr, mixed with 0.5 ml $C_6H_6$, centrifuged, and 25 µl from the benzene layer developed by TLC in EtOAc–cyclohexane (1:1). Plates were desiccated and scanned on a Turner Fluorometer model 111 with TLC scanner and chart recorder (365 nm primary and 525 nm secondary filters). Standard solns of authentic polyamines at 10, 30 and 60 µg/ml were processed in the same way and developed on the same plates as the unknown. The fluorescent intensity of the dansylated standards plotted linearly with concn over the range studied and the unknowns were determined from this standard curve. The identity of the polyamine spots was confirmed by co-migration in 3 solvent systems and co-migration with labelled polyamines. Dansyl-spermine and dansyl-spermidine migrated with $R_f$ of 0.10 and 0.25 resp in EtOAc–cyclohexane (1:1), 0.09 and 0.15 in $Et_2O$–cyclohexane (9:1), and 0.82 and 0.73 in $CHCl_3$–$NEt_3$ (5:1). 2D TLC in EtOAc–cyclohexane (1:1) followed by $Et_2O$–cyclohexane (9:1) showed no spots separating from the spermine or spermidine spots with significant intensity readings on the fluorometer. The same quantity of conidia as was used for pod treatments was analyzed for polyamine content to determine whether the conidia contributed to the polyamine determinations in the pods. Spermine and spermidine were not detectable in these conidial suspensions. Prior to inoculation, L-arginine-[U-$^{14}$C] was evenly distributed over the endocarp of pods (1 µCi/g) and taken up for 10 min under a hood. The pods were then inoculated, incubated for 24 hr and extracted as described above. After $C_6H_6$ extraction of the dansylated amines, the extract was streaked on a TLC plate and developed in EtOAc–cyclohexane (1:1). The spermine and spermidine bands were identified under UV, removed, and counted in 10 ml of Omnifluor scintillation fluid. Counts were corrected by extracting fresh pods in 1 µCi of L-arginine-[U-$^{14}$C] and subtracting the dpm decovered in the polyamine bands during this extraction from the dpm for the 24 hr treatments.

## REFERENCES

1. Hadwiger, L. A., Jafri, A., von Broembsen, S., and Eddy, R. (1974) *Plant Physiol.* **53**, 52.
2. Hadwiger, L. A. and Schwochau, M. E. (1971) *Plant Physiol.* **47**, 346.
3. Hadwiger, L. A., Hess, S. L., and von Broembsen, S. (1970) *Phytopathology* **60**, 332.
4. Hadwiger, L. A., Sander, C., Eddyvea, J., and Ralston, J. (1976) *Phytopathology* **66**, 629.
5. Smith, T. A. (1975) *Phytochemistry* **14**, 865.
6. Tabor, C. W. and Tabor, H. (1976) *Ann. Rev. Biochem.* **45**, 285.
7. Stevens, L. (1970) *Biol. Rev.* **45**, 1.
8. Basso, L. C. and Smith, T. A. (1974) *Phytochemistry* **13**, 875.
9. Smith, T. A. (1970) *Phytochemistry* **9**, 1479.
10. Ramakrishna, S. and Adiga, P. R. (1974) *Phytochemistry* **13**, 2161.
11. Kremzner, L. T. (1966) *Anal. Biochem.* **15**, 270.
12. Seiler, N. (1970) *Methods Biochem. Anal.* **18**, 259.
13. Smith, T. A. (1973) *Phytochemistry* **12**, 2093.