EFFECT OF HEAT SHOCK ON THE EXPRESSION OF DISEASE RESISTANCE RESPONSE GENES IN PEA

Daniels, Catherine H. Department of Plant Pathology
Washington State University
and Lee A. Hadwiger* Pullman WA 99164-6430, USA

The eukaryotic heat shock response is characterized by the increased synthesis of specific proteins concomitant with the suppression of synthesis of most normal proteins. The hypothesis is that this response confers thermotolerance on the organism (6) and that the response entails a major re-direction of the cell's normal regulatory activities. This re-direction affects the ability of pea tissue to actively resist several non-host pathogens (4, 5). Normally, the pea mounts a successful resistance response against the bean fungus Fusarium solani f. sp. phaseoli (3), a response characterized by the increased synthesis and accumulation of specific mRNAs (2) and by the appearance of new proteins as measured by 2-D PAGE methods (9). While the fungal macroconidia do germinate, further growth is arrested.

Previous work has shown that a non-lethal heat shock of 40°C administered to pod tissue for 2 h will: (a) enable this fungus to grow prolifically throughout pea tissue, and (b) affect the protein pattern seen on 2-D polyacrylamide gels (eight new heat shock proteins appear, however, the proteins characteristic of the resistance response disappear). Under several conditions the pea tissue is able to resist the fungus despite a heat shock. One condition is a 9 h recovery period between heat shock and fungal inoculation (4). A second condition involves a 6 h pre-inoculation of the tissue prior to heat shock. Fungal growth is arrested even after a heat shock so long as there is a 6 h pre-inoculation period. Thus, it appears that once resistance is established even a major re-direction of cell activities by heat shock will not break the response. In this report we correlate the relative presence or absence of resistance associated mRNA's after various heat treatment and recovery periods, to the peas resistance or susceptibility to the bean pathogen.

Research was carried out using immature greenhouse grown pea pods (cv Alaska). The heat shock treatment was as previously described (4) except that the time of treatment was shortened from 2 h to 1 h. Pods were held in lighted, moist, 22°C containers except during exposure to heat shock. Pea pods were carefully split along the suture line and the endocarp inoculated with either 4×10^7 Fusarium solani f. sp. phaseoli strain W-8 (American Type Culture Collection 38135) macroconidia/ml at 0.5 ml spore suspension/1.0 g pod tissue, or an equal volume of sterile water.

Total RNA was isolated from pea pods as previously described (9) and electrophoresed on 1.5% agarose gels containing 10 mM Na-phosphate (pH 7.5) and 2.2 M formaldehyde. RNA was loaded as previously described (8) at 25 μg/lane and gels were run at 150 V for 7 h with recirculating buffer. RNA and DNA (markers) were immediately transferred to nitrocellulose using 10X SSC with no pretreatment of the gel. Filters were baked at 80°C under vacuum for 2 h. Replicate blots were run using different lots of total RNA.

*Corresponding author.

Table 1. Effect of heat shock on accumulation of specific mRNAs involved in the disease resistance response to Fusarium solani f. sp. phaseoli

Treatment Number	Description[a]	Disease symptom[b]	Densitometric scores [c]			
			pI 39[d]	pI 49	pI 176	pI 206
1	22°C 1h, H_20 treat for 8h (control)	-	1	1	1	1
2	22°C 1h, fungal inoc for 8h (induced)	R	3.92	2.15 ±0.80	2.50 ±1.46	2.58 ±1.07
3	40°C 1h, fungal inoc for 8h (heat shock)	S	0.69	0.31 ±0.02	0.20 ±0.13	0.58 ±0.66
4	40°C 1h, then H_2O for 8h (heat shock)	-	0.81	0.28 ±0.10	0.42 ±0.10	0.79 ±0.93

[a] Fungal inoculation was 500 µl suspensions (4 x 10^7 spores/ml)/g pod tissue.

[b] Disease reaction: R, no growth of the germ tube in excess of spore length; S, extensive growth, more than 10X spore length; -, control.

[c] Densitometric evaluations of autoradiograms reported here as area under the curve values normalized against water control for comparison purposes.

[d] Data from one experiment only, the remaining values are means of two independent experiments.

The four disease resistance response clones used in this study to measure the accumulation of homologous specific mRNAs were isolated from a cDNA library made from poly (A)+ mRNA extracted from 'Alaska' pea pods inoculated with F. solani f. sp. phaseoli (8). These clones are designated as pI 39, pI 49, pI 176 and pI 206. Plasmid containing cDNA's were ^{32}P-labeled by nick translation (7) and used as probes against Northern blots. All hybridizations and prehybridizations were carried out at 42ºC as previously described (2).

Autoradiograms of each ^{32}P-probed Northern blot were subjected to optical scanning by an LKB Ultroscan XL Laser Densitometer. For comparison purposes, area under the curve values were calculated and data from all treatments were normalized relative to the appropriate water control by dividing the area under the curve numbers by their respective water control values. When duplicate autoradiograms were available, normalized data were averaged to minimize seasonal variation in pod physiology.

As seen in Table 1, mRNA homologous to the four cDNA probes accumulates in pea pods to levels 2-4 times those seen in water control tissue within 8 h of challenge with the fungus. Conversely, when the tissue is subjected to a heat shock immediately prior to fungal inoculation, mRNA levels may fall to as little as 20% of the level of the water control. Although mRNA levels in both induced and heat shocked tissues differ between clones, the abundance of mRNA was increased in induced tissue and decreased in heat shocked tissue.

Molecular studies have indicated that there is an enhanced synthesis of certain proteins which correlates with the expression of disease resistance in peas (1, 9). The most responsive clones from our cDNA library representing pea disease resistance response genes are pI 39, pI 49, pI

176, and pI 206. These clones show an increased accumulation during resistance and decreased levels during susceptibility (1,2). The present data demonstrate that the actual mRNA accumulation is quite low during a treatment which is known to promote susceptibility, i.e., heat shock. This study did not distinguish between increased mRNA degradation of non-heat shock mRNAs and decreased mRNA transcription as explanations for the decrease observed in mRNA accumulation.

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