
Effect temperature on the production of non-specific elicitors by *Colletotrichum lindemuthianum* in frenchbean

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The influence of temperature in the production of non-specific elicitors of necrosis by *Colletotrichum lindemuthianum* race γ in leaves of frenchbean (*Phaseolus vulgaris* L.) cultivar Kievitsboon Koekoek was investigated. Inter cellular fluid (IF) from infected leaves incubated at 17°C and from infected leaves transferred to 25°C were injected into seven bean cultivars with differential resistance or susceptibility to *C. lindemuthianum* race γ . Leaves injected with IF from infected leaves transferred to 25°C produced severe necrosis than IF from infected leaves maintained at 17°C. Race-cultivar specificity was not observed although the extent of necrosis varied in different cultivars indicating the production of non-specific elicitors during a compatible host-pathogen interaction.

Key words: Elicitors, *Colletotrichum lindemuthianum*, frenchbean, intercellular fluid

INTRODUCTION

Many plant pathogenic fungi show specificity in causing infection wherein, certain races of the pathogen can infect only certain cultivars of plant species. This phenomenon of race-cultivar specificity was first discovered in the Bean-*Collectotrichum lindemuthianum* interaction which causes anthracnose in bean (Barrus, 1918). Expression of resistance or susceptibility results from the matching of complementary alleles at specific loci in the plant and the pathogen (Deverall, 1977) as explained in the Gene for Gene hypothesis. In resistant cultivars, metabolites of pathogen origin trigger or elicit cell death called 'Hypersensitive reaction'. The infected cell along with a few adjacent cells die thus, restricting further development of fungal hyphae (Heath, 1976). The rapid cell death is attributed to recognition between the host cell and the pathogen immediately after penetration (O'Connell *et al.*, 1985). Phytoalexins and several secondary metabolites are produced subsequent to hypersensitive reaction (Bailey, 1982). According to Bailey *et al.* (1980), resistance to *C. lindemuthianum* is induced in cv.KK bean hypocotyls by incubating inoculated hypocotyls at 16°C for 72 h and then transferring to 25°C. The higher temperature results in premature death of infected cells and inhibition of fungal growth. Such a response is termed 'temperature dependant hypersensitivity'.

The present investigation aims to determine the influence of temperature on the production of elicitor molecules by *C. lindemuthianum* race γ in the frenchbean leaves. The experiment was conducted at the Long Ashton Research Station, Bristol, U. K.

MATERIALS AND METHODS

The fungus *C. lindemuthianum* (Sacc. and Mag.) Briosi and Cav. race γ (LARS Culture No.129) was cultured on Collectotrichum medium (Mathur *et al.*, 1950) at $17 \pm 1^\circ\text{C}$ under 8 W Black fluorescent light (Sylvania F8T5/BLB). The spores were harvested from 9 d old cultures, washed twice by centrifugation at $100 \times g$ for 5 min. The spore concentration was maintained at 5×10^{-6} spores ml^{-1} using a haemocytometer.

The spores were inoculated on the lower surface of fully expanded, primary leaves of 8 d old seedlings of frenchbean cv.KK grown in Fisons 9600 cabinet. The inoculated leaves were incubated at $17 \pm 1^\circ\text{C}$. On the third day after inoculation, some of the leaves were transferred to 25°C . Two days later, the leaves incubated at 17°C and those transferred to 25°C were separately rinsed in running water and vacuum infiltrated with deionised water for 10 min. using a water pump. Intercellular fluid was harvested from the infiltrated leaves according to the method of Crucefix *et al.* (1984). The intercellular fluid was sterilized by passage through a $0.2 \mu\text{m}$ membrane filter.

The presence of elicitor molecules in the intercellular fluid was tested using frenchbean cultivars viz. Kievitsboon KoeKock (KK) (susceptible), Red Mexican (RM), Dark Red Kidney (DRK), Cornell 49-242, Wade, Widusa and Imuna (Resistant) showing differential resistance or susceptibility to *C. lindemuthianum* race γ . 8 d old bean seedlings grown in Fisons 9600 growth cabinet ($25 \pm 1^\circ\text{C}$, 80% RH, $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density and 16 h photoperiod) were injected with the intercellular fluids directly into the veins of fully expanded primary leaves at 5 to 6 different points. The treated seedlings were incubated in Fisons 600 growth cabinet at $17 \pm 1^\circ\text{C}$. The leaves injected with the test fluids were assessed at daily intervals for symptom expression. The intercellular fluid of uninfected leaves of Cv. KK was maintained as control.

Microscopic observation of infected leaf tissues were made using small sections of tissues taken randomly from several leaves. They were cleared in ethanol-chloroform mixture (3 : 1; v/v) containing Trichloroacetic acid (0.15%, w/v) at 70°C for 15 min. The cleared tissues were stained in a solution of aniline blue in lactophenol (0.025%; w/v) for 2 h at room temperature. The tissues were mounted in lactophenol and observed under Nomarski differential interference contrast Microscopy.

RESULTS AND DISCUSSION

The leaves of all cultivars tested showed the first symptoms on the 5th d after injection, which became pronounced by 7th d. The results are summarised in Table 1.

Leaves injected with intercellular fluid of infected leaves incubated at 17°C , showed browning of veins, which was particularly distinct in cvs. RM, Widusa and KK. The intercellular fluid of infected leaves transferred to 25°C produced severe symptoms of large brown spreading lesions in cvs. KK, RM, Widusa and DRK but to a lesser extent in cv.

Table 1 : The production of elicitors of necrosis in the intercellular fluid of *C. lindemuthianum* race γ infected cv. KK leaves, at 17°C and 17°C to 25°C

Cultivars	Treatment			
	Intercellular fluid from leaves maintained at			
	17°C	Control	17° to 25°C	Control
Imuna	+	-	++	-
Widusa	++	-	+++	-
Wade	-	-	-	-
KK	++	-	+++	-
Cornell 49-242	-	-	-	-
RM	+++	-	+++	-
DRK	++	-	+++	-

- = no symptom; + = brown spots at injection site; ++ = browning along the veins; +++ = necrotic lesions.

Imuna However, cvs. Wade and Cornell 49-242 showed negligible or no symptoms in both the treatments. The leaves of cultivars injected with intercellular fluid from healthy leaves did not show any symptoms. This indicated the presence of substance (s) that elicit necrosis in the intercellular fluid from compatible host-pathogen interaction.

Furthermore, the greater response of resistant cultivars to intercellular fluid from infected leaves transferred to 25°C, appeared to be due to the extensive growth of fungus within the host tissue at the higher temperature, thus releasing higher levels of the necrosis inducing elicitor molecules. Microscopic observation of tissues from infected leaves transferred to 25°C, showed, extensive colonisation of the fungus by narrow secondary hyphae compared to those maintained at 17°C. This is contrary to the results on bean hypocotyls where, resistance to the fungus is induced by transferring to 25°C (Bailey *et al.*, 1980). It is not clear why a temperature induced hypersensitive reaction did not occur in bean leaves. It is therefore likely that, in leaves transferred to 25°C, the fungus has entered its necrotrophic phase of infection earlier than in those at 17°C.

These elicitors are non-specific since they cause necrosis in both susceptible (cv.KK) as well as some resistant bean cultivars. However, these molecules may be to a certain extent cultivar-specific as the degree of symptoms varied among the cultivars tested, with no response in cvs. Wade and Cornell 49-242. O'Connell and Bailey (1988) also reported varying levels of resistance or susceptibility of different/bean cultivars to *C. lindemuthianum* races.

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