CURRENT UNDERSTANDING OF RESISTANCE TO ALTERNARIA BRASSICAE IN CRUCIFERS

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INTRODUCTION

The blackspot disease caused by Alternaria brassicae is present around the world on many crucifers (Kolte 1985; Tewari 1985). Lesions with necrotic centres and chlorotic margins develop on all aerial plant parts. This causes reduction in the photosynthetic area of the plant and accelerates senescence and defoliation. The pathogen is known to produce abscisic acid (Dahiya et al. 1988) which is a senescence promoting plant hormone. Yield losses in oil-yielding crucifers depend largely on weather conditions that prevail during the silique development and maturation phase. If this period is humid, high yield losses can be expected, as extensive development of blackspot lesions on siliques and inflorescence axes occurs. It is known that siliques themselves produce most of the photosynthates necessary for their growth and that the leaves have little direct effect on the growth of siliques (Allen et al. 1971). In countries such as India, yield losses can range from 10 to 70% with Brassica campestris var. yellow sarson and B. campestris var. brown sarson suffering the maximum losses (Kolte 1985). In Alberta, Canada the disease severity fluctuates considerably from one year to the next. Blackspot was a major disease of canola in 1989 (Conn and Tewari 1990) but a minor one in 1990 (Conn and Tewari 1991). Late summer rains in 1989 caused extensive development of blackspot on canola plants causing direct infection of seeds and increased shattering of siliques. In some fields, the yields were about 40-60% of the expected with high dockage (Conn and Tewari 1990). A monetary value of $23,750,000 was placed on an estimated overall yield loss of 5% in 1989 due to blackspot in canola in Alberta (Tewari 1991a).

SOURCES AND MECHANISM OF RESISTANCE

The reactions of various oil-yielding crucifers to A. brassicae have been summarized by Kolte (1985), Bains and Tewari (1987), Bansal et al. (1990) and Singh and Kolte (1990). Numerous accessions of Brassica spp. were screened by the author and all were found to be susceptible (Tewari, J.P. unpublished data). Similarly, all commercial cultivars of Brassica spp. are susceptible to A. brassicae. However, there are differences in the degrees of susceptibility, for example, cultivars of B. napus ssp. oleifera are less susceptible than those of B. campestris ssp. oleifera (Tewari and Skoropad 1976; Skoropad and Tewari 1977; Conn 1986; Conn and Tewari 1989a). Intraspecific variability in reaction to A. brassicae is present in the species B. campestris and even in the ssp. oleifera (Bains and Tewari 1987; Bansal et al. 1990). In B. campestris ssp. oleifera, the var. yellow sarson is highly susceptible, whereas, the cv. Candle is relatively less susceptible to A. brassicae (Bains and Tewari 1987). There is some variability in susceptibility also present within the var. yellow sarson (Singh and Kolte 1990). Most accessions of B. campestris ssp. rapifera (turnip) are susceptible to A. brassicae. Bains and Tewari (1987) and Conn et al. (1988), however, reported a moderately resistant accession of this subspecies. An accession of Eruca sativa is known to be highly resistant to a Canadian isolate of A. brassicae (Conn and Tewari 1986). The highest resistance to A. brassicae in crucifers is found in two wild types, Camelina sativa (false flax) and Capsella bursa - pastoris (shepherd's purse), where the green leaves do not develop any symptoms when challenged with this pathogen (Conn et al. 1988).

The host-parasite interactions in this disease have been studied in my laboratory for a number of years (Tewari 1991b) with the hope that it would lead to elucidation of the mechanism of pathogenesis and identification of pathogenicity and virulence factors in the pathogen and resistance and susceptibility factors in the host. The interactions/factors so far studied include effects of epicuticular wax, subcuticular growth, calcium sequestration, production of growth regulating substances, strain variation in the pathogen, production and host sensitivity to the phytotoxin, destruxin B, quality, quantity and dynamics of elicitation of phytoalexins and hypersensitive reaction. A number of interactions listed above appear to be related to host resistance/susceptibility and are discussed here.

EPICUTICULAR WAX

The structure of wax in Brassica spp. has been studied by a number of workers (Tewari and Skoropad 1976; Holloway et al. 1977; Conn 1986; Conn and Tewari 1989b). In the canola-type cultivars of rapeseed, the wax is organized into an amorphous layer on which is situated a crystalline layer. The crystalline layer has a layer of plate-like crystals and another layer of erect filamentous and rod-like crystals (Conn and Tewari 1989b). Wax in Brassica spp. is complex both structurally and chemically (Holloway et al. 1977; Conn et al. 1984; Conn 1986). In the canola-type cultivars, the wax has the same nine major constituents (alkanes, esters, ketones, aldehydes, secondary alcohols, ketols, primary alcohols, triterpenols and fatty acids) but in varying proportions (Conn et al. 1984; Conn 1986; Conn and Tewari 1988; Tewari 1991b). In the B. napus ssp. oleifera cv. Altex the major constituents of wax include C_{29} ketone, C_{29} secondary alcohol and C_{40}-C_{48} esters (Conn 1986; Tewari 1991b).

So far as the blackspot disease is concerned, the wax interacts only physically and has no chemical effect (Tewari and Skoropad 1976; Skoropad and Tewari 1977; Conn 1986; Conn and Tewari 1989a). The wax forms a hydrophobic coating and reduces the deposition of water borne inoculum. Free water is required for infection by A. brassicae (see Singh and Kolte 1990) and since the water droplets tend to roll-off from a hydrophobic surface, this aspect also contributes to reduction of infection and disease severity. Wax also reduces the rate of conidium germination and the number of germ tubes formed by each conidium. The crystalline wax layer is fluffy, encloses air pockets and may cause the above two effects by impeding the movement of plant exudates. Plants of B napus ssp. oleifera are very waxy compared to those of B. campestris ssp. oleifera. Also, the former species is less susceptible to A. brassicae than the later. This differential susceptibility is due to varying amounts of wax. Therefore, cultivars of B. campestris ssp. oleifera should benefit from genetic manipulation resulting in increased amounts of wax. Rapeseed in areas of relatively low rainfall should benefit more from this measure as rain erodes the wax layer in Brassica spp. (Baker and Hunt 1986; Tewari, J.P. unpublished data). However, in Brassica spp. wax regenerates after being lost.

SUBCUTICULAR GROWTH

In a number of diseases the pathogens become subcuticular after penetration and wait for some physiological changes to occur in the host tissue before further colonization (Verhoeff 1974). Alternaria brassicae also becomes subcuticular in rapeseed for a brief period of time before further colonization of the leaf tissue (Tewari 1986). The presence of subcuticular growth indicates a degree of resistance to initial colonization of the host and could be a marker for selection for resistance in rapeseed.

CALCIUM SEQUESTRATION

The plant cell walls are rich in calcium which is tightly bound to pectins. Calcium is known to be a factor in disease resistance. The insoluble calcium polypectates are resistant to hydrolysis by pectolytic enzymes produced by the pathogen (Vidhyasekaran 1988). Oxalic acid and possibly other organic acids produced by various pathogens sequester this calcium and thereby strive to overcome this resistance (Punja and Jenkins 1984; Rao and Tewari 1987). Examination of blackspot lesions on rapeseed leaves by scanning electron microscopy in conjunction with energy-dispersive X-ray microanalysis has revealed sequestration of calcium by A. brassicae (Tewari, J.P. and Awasthi, R.P. unpublished data). Therefore, there are possibilities of enhancing resistance to A. brassicae in rapeseed based on normal higher concentration of calcium in the tissues and increase in calcium concentration through soil or foliar application of calcium compounds. Research along most of the aforesaid lines have produced positive results in several disease syndromes (Punja et al. 1986; Rao and Tewari 1988, Vidhyasekaran 1988).

SENSITIVITY TO THE PHYTOTOXIN, DESTRUXIN B

Alternaria brassicae has a multitoxin system and produces at least two phytotoxins. One phytotoxin is the cyclodepsipeptide, destruxin B (molecular formula $C_{30}H_{51}N_5O_7$ molecular weight 593; Ayer and Pena-Rodriguez 1987; Bains and Tewari 1987). Destruxin B is able to recreate symptoms of the blackspot disease, shows activity only against hosts of A. brassicae and satisfies most criteria for a host-specific toxin (Bains and Tewari 1987; Tewari 1991b). The effects of destruxin B parallel with the degrees of susceptibility of various crucifers to A. brassicae. It can even distinguish susceptibility differences among cultivars of B. campestris spp. oleifera (Bains and Tewari 1987). These observations indicate that destruxin B is the primary determinant of the blackspot disease. There are two exceptions where the aforesaid correlation is not seen due to interaction with phytoalexin elicitation. These cases are discussed later. Destruxin B is also produced by Metarhizium anisopliae and some other fungi and has insecticidal properties as well (Gupta et al. 1989). Therefore, destruxin B is a unique toxin that serves both as a phytotoxin and a mycotoxin.

The second phytotoxin, homodestruxin B (molecular formula C_{31}, H_{53} N_5O_7; Ayer and Pena-Rodriguez 1987) affects both the hosts and non-hosts of A. brassicae and acts as a non-host-specific toxin (Bains, P.S., Tewari, J.P. and Ayer, W.A. unpublished data).

PHYTOALEXIN ELICITATION AND HYPERSENSITIVE REACTION

The working definition of phytoalexins is that they are "low-molecular-weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms" (Paxton 1981). So far approximately a dozen different phytoalexins have been reported from curcifers (Takasugi et al. 1986, 1988; Devys et al. 1990; Browne et al. 1991). All phytoalexins elicited by crucifers contain sulphur in their molecules.

Conn et al. (1988) described elicitation of phytoalexins in four cultivars of canola, B. campestris ssp. rapifera (an accession of turnip), C. sativa and C. bursa-pastoris in response to A. brassicae. All canola cultivars and the turnip produced only one phytoalexin with Rf value similar to that of cyclobrassinin described by Takasugi et al. (1986). However, the quantity of cyclobrassinin produced in the turnip was much more than produced in the canola cultivars. This correlated with

their relative responses to A. brassicae. Extracts from C. sativa showed two antimicrobial spots in Cladosporium TLC bioassay plates. Capsella bursa-pastoris extracts also showed two spots, the major one corresponding in Rf value to the upper minor spot from C. sativa and the second minor spot being similar to cyclobrassinin in Rf value. Both these wild crucifers are highly resistant to A. brassicae due to phytoalexin elicitation. Otherwise, they are sensitive to destruxin B (Tewari, J.P., Conn, K.L. and Bains, P.S. unpublished data). It appears from the aforesaid observations that both quality and quantity of phytoalexins are important in responses of crucifers to A. brassicae. Jejelowo et al. (1991) studied the kinetics of phytoalexin elicitation in C. sativa. An accession of E. sativa is reported to show hypersensitive response to A. brassicae (Conn and Tewari 1986), but any possible phytoalexin elicitation has not been studied.

The lower major antimicrobial spot in Cladosporium TLC bioassay plates of C. sativa described above has been separated and described as two new phytoalexins, camalexin and methoxycamalexin (Browne et al. 1991). Both of them are thiazoyl-substituted indole phytoalexins and show strong resemblance with the molecular structure of thiabendazole, a fungicide that has been used for a number of years (Browne et al. 1991). A comparative study of the biological activities of these phytoalexins and thiabendazole is currently in progress in the authors' laboratory.

FUTURE WORK AND CONCLUSIONS

Resistance to A. brassicae in crucifers is layered and multicomponent. Search for souces of high degrees of resistance should be accelerated. Some wild crucifers offer very high degrees of resistance and biotechnological techniques should be employed to make use of this resource. A systematic study of the landraces of rapeseed and mustard in countries, such as India and China, where these crops have been grown for a long time, should prove interesting.

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