Histological barriers breached by the race of *Leptosphaeria maculans* that overcomes a single dominant gene-based resistance in *Brassica napus*

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Abstract

Blackleg, caused by *Leptosphaeria maculans,* is a major disease of oilseed rape worldwide. The *B. napus* cultivar Surpass 400 was released Australia-wide in 2000 as the most resistant cultivar to *L. maculans*. This cultivar initially showed excellent resistance to *L. maculans* in the field, with only hypersensitive responses, characterized by small dark brown necrotic local lesions without pycnidia being evident on the cotyledons, leaves and stems. The resistance to *L. maculans* in cv. Surpass 400, which originated from *B. rapa* ssp. *sylvestris*, is considered to be mediated by a single dominant gene. However, in 2003 and 2004, there were reports of breakdown in resistance in Australian commercial cultivars containing this resistance gene in Western Australia and subsequently in other parts of southern Australia. There have been previous studies of the infection processes and histological changes that occur in response to *L. maculans* infection in winter-type and spring-type *B. napus* cultivars and in *B. juncea*. However, nothing was known until now as to how this recent resistance-breaking strain of *L. maculans* actually breaches the histological barriers in cultivars such as Surpass 400, barriers that are effective in restricting damage in such cultivars to a hypersensitive resistant reaction in the presence of non-resistance-breaking strains of *L. maculans*. Studies were undertaken to determine which barriers to the infective processes of *L. maculans* were overcome in cultivars carrying the single dominant resistance derived from *B. rapa* ssp. *sylvestris* when a resistance-breaking strain of *L. maculans* is involved. Our studies to date have shown that the resistance initially displayed by cv. Surpass 400 crops was related to histological barriers that included the hypersensitive response, limited growth of hyphae beyond the restricted lesion, failure of hyphae from cotyledons to cause stem cankers, failure of pycnidia reproduction, high levels of production of phenolics, production of additional cambium in stems, increased deposition of lignin and suberin, and failure of the pathogen to invade pith tissues in the stem. Although histological barriers associated with resistance to *L. maculans* have been reported previously, this is the first series of studies which explains major aspects of the collapse of the single dominant gene-based resistance in cv. Surpass 400 and other cultivars containing this same resistance.

Key words: oilseed rape, blackleg disease, *Phoma*, host resistance

Introduction

Oilseed rape (*Brassica napus*) is an important crop world-wide, including Australia. Blackleg disease, caused by *Leptosphaeria maculans* (anamorph: *Phoma lingam*), is of major economic importance in the oilseed rape growing areas of Australia, North America and Europe (West *et al*., 2001).

The cultivar Surpass 400 was released Australia-wide in 2000 as the most resistant cultivar to L. *maculans*. This cultivar initially showed excellent resistance to L. *maculans* in the field, with only a hypersensitive response, characterised by small dark brown necrotic local lesions without pycnidia being evident on the cotyledons, leaves and/or stems. The resistance to L. *maculans* in cv. Surpass 400, which originated from *B. rapa* ssp. *sylvestris* (Crouch *et al*., 1994), is considered to be mediated by a single dominant gene (Li and Cowling, 2003). However, in 2003 and 2004, there were reports of breakdown in resistance in Australian commercial oilseed rape cultivars containing this single dominant gene-based resistance derived from *B. rapa* ssp. *sylvestris*, initially in Western Australia (Li *et al*., 2003; Hua Li *et al*., 2003) and subsequently in other parts of southern Australia (Anonymous 2004).

Hypersensitive reactions related to *L. maculans* have been reported in winter-type *B. napus* (Somda *et al*., 1999; Roussel, 1999a, 1999b), in *B. juncea* (Chen and Howlett 1996), and more recently in spring-type *B. napus* by Hua Li *et al*. (2003, 2004). However, nothing was known about how this recently occurring resistance-breaking strain of *L. maculans* in Australia actually breaches the anatomical barriers in cultivars such as Surpass 400, barriers that were initially effective in restricting damage in such cultivars to a hypersensitive resistant reaction in the presence of non-resistance-breaking strains of *L. maculans*.

Studies of Hua Li *et al*. (2004, 2006a, 2006b) were undertaken to determine which barriers to the infective processes of *L. maculans* were overcome, in a cultivar such as Surpass 400 that carries the single dominant gene-based resistance derived from *B. rapa* ssp. *sylvestris,* during invasion by a resistance-breaking strain of *L. maculans*. In this paper we discuss the major histological parameters expressed in the response to the single dominant gene-based resistance in cv. Surpass 400 against

representatives of strains of *L. maculans* that were dominant in *B. napus* commercial fields prior to the introduction of this particular type of single dominant gene-based resistance. We also compared these responses to those that occurred in response to challenge by the pathogen strains dominant at and following breakdown of this same single dominant gene-based resistance. These defenses and their breachings were compared in response to their reaction on cotyledons (Hua Li *et al*., 2004, 2006b) and stems (Hua Li *et al*., 2006a). In addition, the responses of *B. napus* cultivars Dunkeld, Outback and Grouse, containing polygenic resistance, and the highly susceptible cv. Westar to the same strains on cotyledons and stems were used as comparisons (Hua Li *et al*., 2006a, 2006b).

MaterialS And Methods

Two L. *maculans* isolates, UWA 192 and UWA P11, were used throughout these studies. UWA 192 is a highly virulent isolate that can overcome the resistance in Australian commercial *B. napus* cultivars containing single dominant gene-based resistance derived from *B. rapa* ssp. *sylvestris* (Hua Li *et al*., 2004). While isolate UWA P11 is known to be highly virulent on cultivars containing polygenic resistance or that are highly susceptible, it is, however, avirulent on cv. Surpass 400 on which it induces a clear hypersensitive reaction to inoculation (Hua Li *et al*., 2003, 2004). The spring-type *B. napus* cv. Surpass 400, which carries the single dominant gene-based resistance to blackleg, cultivars Dunkeld, Grouse and Outback, which carry polygenic resistance, and the highly susceptible cv. Westar were used in these studies. Details of individual methods utilized in these studies are given in the respective papers already published (Hua Li *et al*., 2004) or in press (Hua Li *et al*., 2006a, 2006b).

Discussion

Overall, the studies provided an interesting opportunity to study the evolution and/or development of *L. maculans* strains in relation to blackleg disease in Western Australia (Sivasithamparam *et al*., 2005). The results clearly indicate that strains (such as UWA 192) that became dominant in *B. napus* fields in Western Australia since the commercial release of cultivars with single dominant gene-based resistance from *B. rapa* ssp. *sylvestris*, are clearly different from those (such as UWA P11) which dominated when crops were based on cultivars effectively only carried polygenic resistance and/or that were highly susceptible.

The ability of strain UWA 192 to be highly virulent in cv. Surpass 400 provided an opportunity to study ways by which the resistance of cv. Surpass 400 to previously dominant populations was broken down. Clearly, the major characteristic of the resistance, both on cotyledons and stems, was related to the development of a hypersensitive reaction. This hypersensitive reaction, even at the cotyledon stage, guaranteed prevention of hyphae reaching the stem to cause cankers and associated yield losses. Inoculation with UWA 192 onto cv. Surpass 400 not only resulted in the failure of the hypersensitive reaction to occur, but also led to unlimited hyphal invasion all the way into the stem, and also the production of pycnidia, leading to the initiation of secondary infection cycles. It should be noted that there was no production of pycnidia on cotyledons of cv. Surpass 400 when inoculated with strain UWA P11.

In relation to the response of stems inoculated with UWA P11, there was a dramatic cessation of invasion of stem tissues by the pathogen, resulting from the production of additional cambium and the increased deposition of lignin and suberin in cell walls. It is noteworthy that, in contrast to UWA P11, UWA 192 was able to aggressively invade pith tissues of the stem, which is critical for canker development and stem collapse from loss of tensile tissues. This aspect clearly indicates the basis of histological-based resistance to UWA P11 and how they were breached by UWA 192 in cv. Surpass 400. However, the differences between cultivars relying upon polygenic resistance in comparison with the highly susceptible cv. Westar appear to be more quantitative than qualitative. It is likely that polygenic resistance, where it is expressed, is related, at least in a quantitative sense, to aspects other than or additional to those we determined in cv. Surpass 400.

In contrast to cv. Surpass 400 and cultivars relying upon polygenic resistance, cv. Westar showed high levels of susceptibility to UWA P11 and UWA192 and failed to initiate adequate levels of histological defenses such as additional cambium, lignin, suberin, and/or phenolic compounds. It is also interesting that while the hypersensitive response to UWA P11 took 5-7 days to develop in cv. Surpass 400, it took 12-14 days for lesions to develop both for cv. Westar and for cultivars relying upon polygenic resistance.

While hypersensitive cell death, production of additional cambium, increased production of phenolics and increased deposition of lignin and suberin clearly indicates the barrier posed within cv. Surpass 400 to the population of *L. maculans* that dominated prior to year 2000 and how they were subsequently breached by the strains of *L. maculans* that overcame this single dominant gene-based resistance, defining the nature of polygenic resistance where it has been expressed in the field remains to be determined.

Conclusion

Although histological barriers associated with resistance to *L. maculans* have been reported previously, this is the first series of studies which explains major aspects of the collapse of the single dominant gene-based resistance derived from *B. rapa* ssp. *sylvestris* that occurs both in cv. Surpass 400 and also in other Australian cultivars, including cvs Surpass 501TT and Surpass 603CL that were widely deployed across Western Australia, particularly in the first half of the 2000's.

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