High level resistance against white rust (Albugo candida) race 2V identified in Brassica juncea genotypes from China and Australia

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Abstract
White rust (*Albugo candida*) is a highly destructive disease of cruciferous oilseed crops such as Indian mustard (*Brassica juncea*) andturnip rape (*B. rapa*). As part of a five year international collaborative project between Australia, India and China, studies were undertaken in Western Australia to develop and apply methodologies to identify resistance to white rust in *B. juncea* germplasm from these three countries.Cotyledon, seedling and flowering stage screening tests all consistently identified the same genotypes with the most resistance, with excellent correlation between the different tests. Across four field trials, both incidence and severity of white rust disease reflected the expression of host resistance equally well in the *B. juncea* germplasm tested. The most resistant genotypes were JM06011 from Australia and CBJ-003 and CBJ-004 from China, with both incidence and severity scored at zero. Other genotypes with high levels of resistance included JM06010, JM06021, JM06004 and JM06013 from Australia and CBJ-001 from China.Australia now has a range of reliable techniques for differentiating levels of resistance to white rust in germplasm under either glasshouse or field tests. This is the first high level resistance (foliage and stagheads) to pathotype 2V available for Australian oilseed *Brassica* breeding programs.

Key words:*Albugo candida, Brassica juncea*, white rust, mustard

White rust, also known as white blister, is caused by the oomycete pathogen *Albugo candida*. It is an important disease oilseed crops and most commercial Indian mustard (*Brassica juncea*) varieties are highly susceptible to this pathogen (Mukherjee et al. 2001; Singh et al. 2010). The pathogen can infect all aboveground parts of *B. juncea* plants, producing characteristic white blisters on cotyledons, leaves, stems and inflorescences and with inflorescence infection also resulting in staghead galls that are the main cause of yield loss in susceptible cultivars (Verma and Petrie 1980). Up to 60% or more yield losses on *B. rapa* and *B. juncea* from white rust have been reported in India (Lakra and Saharan 1989) and losses of up to 20% in Australia (Barbetti 1981). It is essential to identify useful sources of host resistance in *B. juncea* as use of resistant varieties is the most efficient and cost-effective way to control this disease. Canola-quality *B. juncea* is being developed in Australia to extend oilseed *Brassica* production into the lower rainfall areas (Burton et al. 2003; Oram et al. 2005), and the first commercial *B. juncea* cultivar in Australia, cv. Dune, was released in 2006.Rapid identification of new sources of host resistance is a key priority in Australia for its *B. juncea* breeding program. As part of a five year international collaborative project between Australia, India and China, studies were undertaken in Western Australia to develop and apply methodologies to identify resistance to white rust in *B. juncea* germplasm from the three countries.

Materials and Methods

In order to develop disease screening methods, disease development on cotyledons, seedling plant leaves, mature plant leaves and flowers was studied under glasshouse conditions, as was leaf disease incidence and severity and staghead development under field conditions.

Cotyledon test: Seed of genotypes of *B. juncea* were sown into steam treated potting mix in free-draining black plastic pots (70 by 70 by 100 mm) and thinned to four plants per pot after germination. When cotyledons were fully expanded (two weeks after sowing), they were inoculated by a drop-inoculation technique where a single 10 µL drop of the zoosporangial suspension was spotted onto the adaxial surface of each of the two lobes of each cotyledon. Plants were then subjected to 4 days of enhanced humidity (>95% RH) by placing the pots in a high-humidity chamber. Disease reaction was assessed 12 dpi using a 0–9 scoring system according to Singh et al. (1999) where 0=no symptoms or sign of infection; 1=pin-point necrotic flecks at inoculation site, no sporulation;2=larger

necrotic flecks at inoculation site, no sporulation; 3=sparse sporulation, up to 5% of cotyledon surface covered with pustules; 4=6–10%; 5=11–20%; 6=21–30%; 7=31–50%; 8=51–75%; and 9=>75% of cotyledon surface area covered with pustules.

Seedling stage test: Seeds of the test genotypes of *B. juncea* were again sown in the same potting mix, but thinned to only two plants per pot after germination. When genotypes had reached the 4–5 leaf stage (four weeks after sowing when the 5thleaf was emerging), plants were inoculated by spraying a suspension of 10^5 zoosporangia/mL until run-off. Plants were subjected to four days of enhanced humidity by placing each pot into a sealed plastic bag that had been pre-moistened with deionized water. Disease severity was assessed at 14 dpi on the 4thand 5thtrue leaves from the base of each plant according to the percentage of leaf area covered with white rust pustules.

Flowering stage test: The same plants as used for the seedling stage test were used for this flowering stage test. When the majority of the test genotypes had commenced flowering (i.e. where there was at least one open flower on each plant), plants were sprayed with a 10^5/mL zoosporangial suspension until run-off and then subjected to four days of enhanced humidity by covering plants with moistened plastic sheeting. Disease was assessed at 14 dpi by recording the disease incidence (percentage of leaves infected for each plant by recording the number of infected leaves and total leaves on each plant), the disease severity (by estimating the percentage of leaf area covered by white rust pustules), and the mean number of stagheads for each plant (by counting the total numbers of stagheads and the number of plants).

*Field tests (four tests):*The field tests were carried out at the University of Western Australia, Perth, Western Australia. Twenty seeds per genotype were sown in single rows with 1m length and 0.6m spacing in May each year. Plants were not thinned after germination and were irrigated by overhead sprinklers as required during the growing season to supplement any rainfall deficiencies. The source of *A. candida* inoculum utilized was from infected leaves of *B. juncea* plants grown at the same field block in the previous year and then stored at –80^{o}C. These leaves were removed from –80^{o}C and the zoosporangia were scraped from pustules and dispersed in deionised water. The *A. candida* inoculum was propagated and bulked up by applying the zoosporangial suspension on the cotyledons of *B. juncea* seedlings in a growth room at 18/13^{o}C day/night temperature and 16h photoperiod. The newly infected cotyledons and leaves with pustules were then harvested from seedlings two weeks after inoculation and used directly as fresh inoculum. Zoosporangia were dispersed in deionised water and the concentration of the zoosporangial suspension adjusted to 10^5 zoosporangia/mLbefore application to plants in the field. Plants were inoculated twice with the zoosporangial suspension using a hand-held atomiser. The first inoculation was conducted at the seedling stage and thesecond application was two weeks later.Field disease assessment was conducted up to seven times during the growing season. Both disease incidence (percentage of infected leaves) and disease severity (percentage of leaf area covered by white rust pustules) were assessed using a 0–6 scale, modified from Singh et al. (1999) and Mukherjee et al. (2001). For disease incidence, 0 = no symptoms or sign of pustules; 1 = 1–10%; 2 = 11–20%; 3 = 21–30%; 4 = 31–50%; 5=51–75%; 6 = >75% of leaves with white rust pustules.For disease severity, 1 = 1–10%; 2 = 11–20%; 3 = 21–30%; 4=31–50%; 5 = 51–75%; 6 = >75% leaf area covered by pustules. At the end of the growing season, the total number of plants, the total number of plants with staghead, and the total number of stagheads for each row of each genotype were counted. The mean number of stagheads per plant and the percentage of plants with staghead for each genotype were calculated.

Results and Discussion

Cotyledon, seedling and flowering stage screening tests all consistently identified the same genotypes with the most resistance, with excellent correlation between the different tests, clearly demonstrating that that expression of resistance across the different test genotypes was generally independent of type of test conducted (Li et al. 2007). Across the four field tests, both incidence and severity of white rust disease reflected the expression of host resistance equally well in the *B. juncea* germplasm from China, India and Australia (Li et al. 2008). The most resistant genotypes were JM06011 from Australia and CBJ-003 and CBJ-004 from China, with incidence and severity scored zero (Li et al. 2009). Other genotypes with high levels of resistance included JM06010, JM06021, JM06004 and JM06013 from Australia and CBJ-001 from China (Li et al. 2008, 2009).

After glasshouse trials and subsequent field trials over four seasons, Australia now has a range of reliable techniques for differentiating levels of resistance to white rust in germplasm under either glasshouse or field tests. This is the first high level resistance (to foliage and staghead disease) to pathotype 2V available for Australian oilseed *Brassica* breeding programs. There is now an opportunity to introduce resistance to pathotype 2V into all new Australian *B.juncea* cultivars, such that

host resistance can be utilized as the main component of effective management of white rust in Australia.

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