

# Examination of pathogenic variation among Australian white rust (*Albugo candida*) isolates from *Brassica juncea* and implications for breeding resistant canola quality *B. juncea*

A. M. Gurung<sup>1</sup>, W. A. Burton<sup>2</sup>, C. Franke<sup>3</sup>, P. A. Salisbury<sup>1,2</sup>

<sup>1</sup>Faculty of Land and Food Resources, The University of Melbourne, Victoria 3010, Australia

<sup>2</sup>Victorian Department of Primary Industries, Horsham, Victoria 3400, Australia

<sup>3</sup>Saskatchewan Wheat Pool Inc., Research and Development, 201-407 Downey Road, Saskatoon, Saskatchewan, S7N 4L8, Canada

Email: a.gurung@unimelb.edu.au

## Abstract

White rust of *Brassica juncea*, caused by the oomycete *Albugo candida* is a potential threat to the emerging canola quality *B. juncea* (juncea canola) industry in Australia. Isolates of *A. candida* that infect *B. juncea* are classified as race 2 and can be subdivided into two pathotype groups, 2A and 2V, based on their virulence to particular Canadian *B. juncea* differential cultivars. To determine which races and pathotypes of *B. juncea*-infecting *A. candida* are present in Australia, isolates were collected from infected juncea canola in 2003 and 2004 from 8 locations in Australia, and tested against a set of Canadian differentials. All isolates were classified as race 2 pathotype 2A, thus, further surveying is underway to determine if pathotype 2V is present in Australia. An Australian 2A isolate was subsequently used to assess the resistance of Australian advanced breeding lines of juncea canola and in addition, the same Australian juncea canola lines were screened in Canada using Canadian pathotypes 2A and 2V. There was a significant correlation between white rust disease ratings for the lines using the Australian and Canadian 2A isolates (Pearson's correlation coefficient = 0.87) and resistance to pathotype 2A was observed in some of the advanced juncea canola breeding lines. However, no Australian breeding lines exhibited resistance to the Canadian 2V pathotype. Although pathotype 2V is yet to be identified in Australia attempts are underway to incorporate resistance to 2V into an Australian canola quality *B. juncea* background with the aim of releasing juncea canola cultivars resistant to 2V in the future.

**Key words:** Indian mustard, white rust, pathogenic variability, resistance

## Introduction

White rust or white blister, caused by the oomycete *Albugo candida* (Pers.) Kuntze, is a disease of many *Brassicaceae* species, including the condiment mustard and oilseed crop *Brassica juncea*. The pathogen produces localised lesions on the leaves and systemic infection which results in distorted, sterile inflorescences (called stagheads). Resistance to the *B. juncea* attacking race 2 was introduced into Canadian *B. juncea* germplasm from brown and oriental *B. juncea* accessions (Petrie, 1988). However, in Canada in 1989 a pathotype of race 2 that attacked the three commonly grown resistant *B. juncea* cultivars was discovered (Petrie, 1994). The avirulent isolate of race 2 was thereafter referred to as 2A and the "new" virulent pathotype was designated 2V (Petrie, 1994; Rimmer *et al.*, 2000). Resistance to pathotype 2V has not been identified in *B. juncea* germplasm, although resistance to 2V in *B. napus* has been introgressed into *B. juncea* (Franke *et al.*, 1999).

*B. juncea* (Indian mustard) is only grown on a small area in Australia (less than 3000 ha), mainly for condiment production (Oram *et al.*, 2005). However, canola quality *B. juncea* (juncea canola) has been developed to extend oilseed *Brassica* production to the lower rainfall areas within the southern Australian wheatbelt, as this crop is better adapted than *B. napus* canola to hotter and drier areas (Burton *et al.*, 1999). Small scale juncea canola production is anticipated in 2007, with significant increases in area expected thereafter. Hence, the threat of white rust to this new crop needs to be assessed. The aims of these experiments are to determine the pathotype(s) of *B. juncea* infecting *A. candida* in Australia, and to assess advanced breeding lines of juncea canola for resistance to Australian pathotypes and the Canadian pathotypes 2A and 2V.

## Materials and Methods

*Pathotypic variation among isolates:* Twenty four single pustule isolates (SPI) of *A. candida* were prepared from samples collected from *B. juncea* at eight sites in south eastern Australia (Victoria and New South Wales) during 2003. No white rust was observed in *B. juncea* trials in South Australia and Western Australia during 2003 and 2004. Pathogenicity of the isolates was examined using a set of cultivars that express differential responses to race 2 and race 7 (i.e. *B. rapa* cvv. Torch and Reward – susceptible to race 7, *B. juncea* cv. Commercial Brown – susceptible to race 2 (pathotypes 2A and 2V) and cvv. Vulcan and Arid – resistant to 2A and susceptible to 2V).

The cotyledons of each cultivar were droplet inoculated using a  $1 \times 10^5$ /ml zoospore suspension (10 $\mu$ l/cotyledon) and plants were maintained at 100% RH for 48 hrs in a randomised block design. Symptoms were assessed 8 to 9 days after inoculation using a visual 0-9 white rust rating scale based on the appearance of white rust pustules on the adaxial and abaxial surfaces of the cotyledons, where 0 = no pustules on either surface (Fox and Williams 1984). The experiment was repeated using four of the isolates, which were selected to represent the range of variation observed on the differential *B. juncea*

cultivars.

*Resistance screening of Australian juncea canola with pathotypes 2A and 2V:* Advanced lines from the Victorian Department of Primary Industries juncea canola breeding program were screened for resistance to *A. candida* in Australia in 2004 and 2006 and in Canada in 2006. Canadian *B. juncea* cultivars resistant or susceptible to pathotypes 2A and 2V, *B. rapa* and *B. napus* cultivars were also included.

Seedlings were spray inoculated with a  $1 \times 10^4$ /ml suspension of zoospores and kept humid for 24 hours. Symptoms were assessed 8 to 9 days after inoculation as described for the pathogenic variation experiments. The experiments were set out in a replicated randomised complete block design.

*Statistical analysis:* Analysis of variance (AVOVA) were performed using Genstat and least significance difference ( $LSD_{0.05}$ ) was calculated, enabling pairwise comparisons of white rust rating means. Pearson's correlation coefficient was calculated to compare the white rust rating of *B. juncea* lines screened in Australia and Canada using pathotype 2A.

## Results and Discussion

*Pathotypic variation among isolates:* There were no significant differences between isolates for white rust rating and no significant interaction between the effect of white rust isolate and *Brassica* differential on white rust rating, indicating that all isolates tested were the same pathotype. All isolates of *A. candida* caused significantly less infection on the *B. rapa* differentials Torch and Reward than the *B. juncea* differentials, which indicated that all 24 isolates were race 2. *B. juncea* differential Commercial Brown developed significantly more severe white rust symptoms than Vulcan and Arid (Fig. 1), which indicated that the isolates were pathotype 2A.

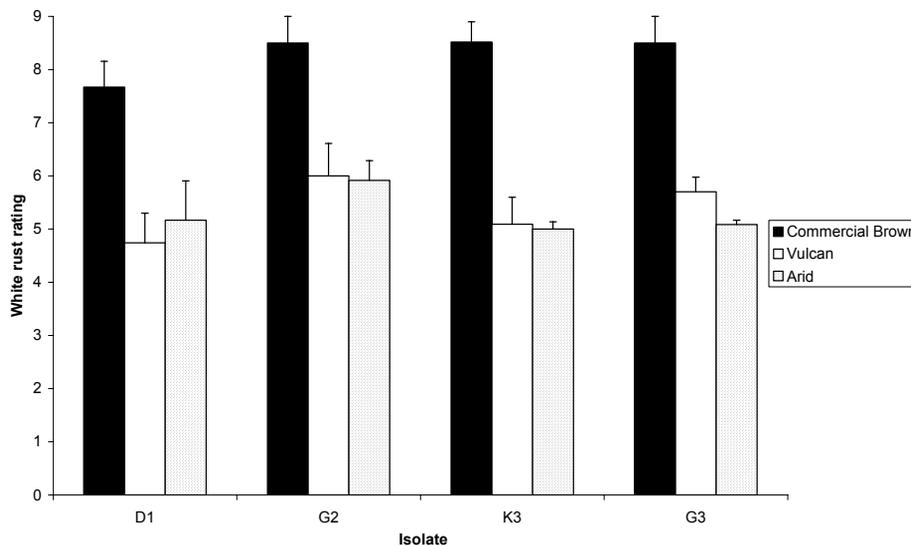


Fig. 1. Average white rust rating for *B. juncea* differentials Commercial Brown, Vulcan and Arid using four *A. candida* SPIs (standard error bars are shown).

*Resistance screening of Australian juncea canola:* There were significant differences in white rust rating between the breeding lines ( $p < 0.05$ ). The Australian *B. juncea* lines ranged from susceptible to resistant to both the Australian and Canadian 2A isolates, whereas, all *B. juncea* lines were susceptible to the Canadian 2V isolate (Fig. 2). There was a highly significant ( $p < 0.01$ ) correlation between the white rust ratings of the lines inoculated with the Canadian 2A and Australian 2A pathotypes (Pearson's correlation coefficient = 0.87), which supports the classification of the Australian isolates as pathotype 2A and suggests that these 2A isolates are the same in both countries.

## Conclusions

All of the *A. candida* isolates collected from eight *B. juncea* trial sites in south eastern Australia were classified as pathotype 2A. Thus, further collection and screening of isolates is underway to determine if pathotype 2V is present in Australia.

Resistance to Australian and Canadian isolates of *A. candida* (pathotype 2A) was observed in well adapted Australian advanced breeding lines from the Victorian juncea canola breeding program, which indicates that selection can be undertaken for resistance to 2A in Australia without access to additional genetic resources. In comparison, all the Australian juncea canola breeding lines were susceptible to pathotype 2V, which represents a significant threat to Australian cultivars should that pathotype be found in Australia, as resistance to pathotype 2V has not been reported in *B. juncea* germplasm.

The current Australian breeding programs have not included the Canadian *B. napus*-derived source of 2V resistance, although attempts are underway to incorporate the *A. candida* 2V resistance from *B. napus*, which was introgressed into *B. juncea* by Franke *et al.* (1999), into an Australian background. Further pre-emptive breeding and selection will be required to ensure resistant cultivars are released in the future.

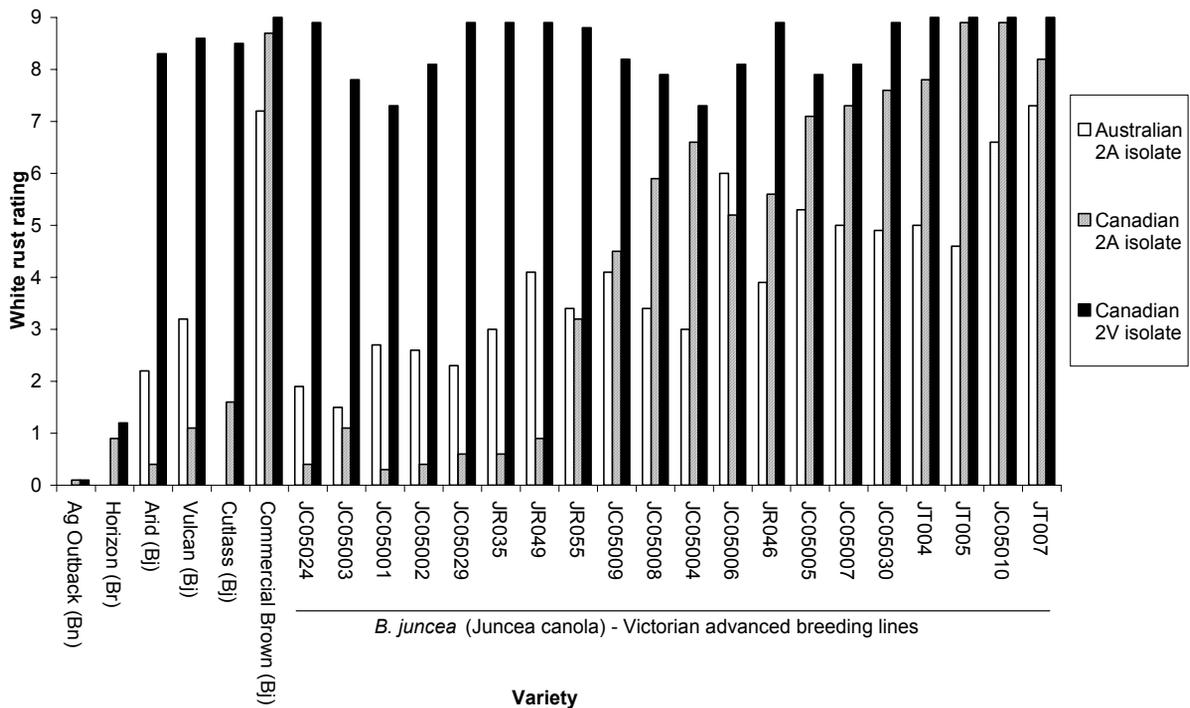


Fig.2. White rust rating for *B. juncea* (Bj), *B. rapa* (Br) and *B. napus* (Bn) cultivars and breeding lines screened during 2006 with Australian and Canadian *A. candida* isolates.

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