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Resistance of Common Bean (*Phaseolus vulgaris* L.) Cultivars and Germplasm Lines to the Purple Variant of Bacterial Wilt (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*)

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ABSTRACT

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A growth cabinet study was conducted to determine the resistance reaction of 14 common bean cultivars and lines to the purple variant of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, the causal agent of bacterial wilt of bean. Results of hilum injury/seed inoculation tests revealed significant (P<0.05) differences in disease severity index (DSI) among the cultivars and lines after 14 days of incubation. The great northern cultivar AC Resolute, the pinto cultivar AC Agrinto, the black germplasm line L02F132, and the pink cultivar AC Early Rose, were identified as resistant to the purple variant of the bacterial wilt pathogen, based on the results of least significant difference (LSD) analyses. The study determined that germplasms resistant to the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens* were also generally resistant to the yellow and orange variants of the bacterial wilt pathogen, as shown in a previous study. This study concludes that these bacterial wilt-resistant germplasm lines are an important resource for breeding and developing new bean cultivars for combined resistance to all three variants of *C. flaccumfaciens* pv. *flaccumfaciens*.

Key words: Common bean, *Phaseolus vulgaris*, bacterial wilt, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, purple variant, disease resistance

Bacterial wilt of common bean (*Phaseolus vulgaris* L.) is caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Collins & Jones. The disease is widespread in distribution [(2)]. Symptoms of bacterial wilt of bean include premature seedling death [(9)], wilted leaves on plants due to degeneration of vascular tissues, and discoloration of seeds [(6,7)]. Three cultural variants, yellow, orange, and purple, of *C. flaccumfaciens* pv.

flaccumfaciens have been reported in western Canada. The yellow and orange variants were found in the provinces of Alberta, Saskatchewan, and Manitoba [10,14,17], and the purple variant was found more recently in Alberta [16]. The purple variant of *C. flaccumfaciens* pv. *flaccumfaciens* was previously reported only once, in Nebraska, USA [24].

Recommended measures for control of bacterial wilt of bean include using disease-free seeds for planting, and crop rotation [19]. Chemical control is not an option at present, since no chemicals are registered for control of bacterial wilt of bean [7]. Biological control using seed treatments with *Pantoea agglomerans* (Beijerinck) Gavini *et al.* [8] or *Rhizobium leguminosarum* Jordan [15] appears to have potential as a sustainable method of control for bacterial wilt of bean, but such treatments have not yet been made commercially available. The most promising means of controlling bacterial wilt of bean is breeding for disease resistance.

Inoculation methods for detecting resistance of beans to bacterial wilt include cotyledonary node inoculation [4,18,23], petiole inoculation [21], partial vacuum inoculation of seeds [5], and hilum injury/seed inoculation [11]. Hsieh *et al.* [12] used the hilum injury/seed inoculation technique to test 124 different bean cultivars and breeding lines from 8 different market classes and identified 22 cultivars and lines with resistance to the yellow and orange variants of *C. flaccumfaciens* pv. *flaccumfaciens*. However, no information exists regarding the resistance of bean cultivars and breeding lines to the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens*. The purpose of this study was to determine the status of 14 bean cultivars and germplasm lines for resistance to the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens*.

The common bean entries used in this study included 13 registered commercial cultivars and one germplasm line. The names of the entries and their market classes were: AC Black Diamond, L02F132, and UI906 (black); AC Resolute, AC Polaris and US1140 (great northern); Envoy and AC Morden003 (navy); AC Early Rose and Viva (pink); AC Agrinto (formerly line L02B662) and Othello (pinto); and AC Redbond and NW63 (small red). The cultivars UI906, US1140, Viva, Othello and NW63 are all US-developed cultivars grown commercially in Canada, whereas the cultivars AC Black Diamond, AC Resolute, AC Polaris, AC Morden003, AC Early Rose, AC Agrinto and AC Redbond, and the germplasm line L02F132, were developed by the AAFC bean breeding program in Lethbridge, Alberta and Morden, Manitoba. Envoy was developed in Canada by Gen-Tec Seeds Ltd. (Woodslee, Ontario). The common bean entries were selected on the basis of being either recently developed and commercialized AAFC-bred cultivars, or US standards grown for years in the Canadian prairies; the main navy bean (Envoy) cultivar grown in Manitoba; and the only black germplasm line (L02F132) screened so far showing resistance to the yellow and orange variants of bacterial wilt.

The purple variant of *C. flaccumfaciens* pv. *flaccumfaciens* used for the study was isolate V254 (LRC accession #8478), which was isolated from a purple-discolored seed of great northern bean cultivar US1140 that was found in the cull seeds from a crop grown near Bow Island, Alberta, Canada in 2005 [16]. When grown on modified Burkholder's agar (MBA) [20], isolate V254 produces a blue extracellular pigment [16]. Bacterial inoculum was prepared by growing cultures on MBA agar at room temperature (20±2℃) for 48 hours, washing the resulting colonies in each dish with 10 ml of sterile distilled water to obtain a bacterial suspension, and adjusting the concentration of the suspension to 1×10^8 colony forming units (cfu) per ml. A serial dilution plating procedure was used to confirm the final concentration of the bacterial suspension.

Bean entries were tested for resistance to the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens* using the hilum injury/seed inoculation method [11]. Seeds of each entry were injured by puncturing the hilum with a sterile needle. The seeds were soaked in the suspension of the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens* for 1 h, and were planted in Cornell peat-lite mix [1] in rootrainers™ (Spencer-Lemaire Industries, Edmonton, Alberta, Canada) with a 5×14 cell arrangement, and 1 seed per cell. Rootrainers™ were incubated in a growth cabinet for 14 days at 28/22℃ (16-h day/8-h night) and watered as required. Controls consisted of injured seeds of each entry soaked in sterile water and planted as described above. Treatments (cultivars or lines) were arranged in a completely randomized design with 3 replicates per treatment and 20 seeds per replicate. The experiment was performed twice.

After 14 days of incubation, each seedling was scored for disease severity on a scale of 0 to 5, where 0=no wilt symptoms, 1=wilt on one of the primary leaves, 2=wilt on both primary leaves and no symptoms on first trifoliolate, 3=wilt on first trifoliolate, 4=death of seedling after development of primary leaves, and 5=unemerged seedling or death of seedling before development of primary leaves [11]. For each replicate of each treatment (cultivar or line), a disease severity index (DSI) was calculated, using the formula $DSI=\Gamma(nw)/T$, where n=number of seedlings, w=wilt rating (0 to 5), and T=total number of seedlings. Infection of diseased seedlings was confirmed by recovering seedlings from the soil, surface-sterilizing them in 70% ethanol for 90 s, air-drying, and plating 1-cm-long

segments of lower stem on MBA. Morphology of the resulting bacterial colonies was compared to the original culture of the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens* after growing 3 days at 20℃.

Data of disease severity index (DSI) was analysed by analysis of variance, and Fisher's least significant difference method was used to determine the cultivars and lines belonging to the group with the lowest DSI. This group was designated as 'resistant' to the purple variant of bacterial wilt (DSI<0.47). Analysis was performed separately on data from each run, and on the combined data from both runs. All analyses were completed using Statistical Analysis Software (SAS) version 9.1 [22].

Results of the inoculation tests showed significant (P<0.05) differences in DSI among the 14 common bean genotypes tested for resistance to the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens*. Some bean cultivars had a low DSI, such as the great northern cultivar AC Resolute (DSI=0.10), whereas others had a high DSI, such as the great northern cultivar US1140 (DSI=2.69) and the navy cultivar AC Morden003 (DSI=2.83) (Table 1). Three cultivars, great northern bean AC Resolute, pinto bean AC Agrinto, and pink bean AC Early Rose and one black bean germplasm line L02F132, were identified as resistant to the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens* (DSI<0.47 based on LSD analysis) (Table 1). The four common bean entries that were identified as resistant to the purple variant in this study were also shown to be resistant to the yellow or orange variants of the bacterial wilt pathogen in a previous study [12] (Table 1). For example, the cultivars AC Resolute and AC Agrinto were without symptoms for 14 days after inoculations with the yellow or orange variants of the bacterial wilt pathogen in the previous study, and also had the lowest DSIs (0.10 for AC Resolute; 0.23 for AC Agrinto) after inoculation with the purple variant in the current study. The germplasm line L02F132 had a DSI of 0.06 for both of the yellow and orange variants in the previous study, and a DSI of 0.24 for the purple variant in the current study (Table 1).

This study demonstrates that certain bean cultivars and a germplasm line developed at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada, are resistant to the purple variant of *C. flaccumfaciens* pv. *flaccumfaciens*. The bean cultivars and lines with resistance to the bacterial wilt variants are therefore of importance to the future development of new bean cultivars with improved bacterial wilt resistance. The study further suggests the possibility of finding resistance to the yellow, orange, and purple variants of bacterial wilt from other collections and therefore, an increased screening effort is warranted. In addition to bacterial wilt of bean, other diseases such as anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.], white mold [caused by *Sclerotinia sclerotiorum*

Table 1. Resistance of common bean cultivars and a germplasm line to bacterial wilt caused by the purple variant of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, isolate V254

Cultivar or line	Market Class	Disease Severity Index[1]		
		Purple variant (this study)[2]	Yellow variant (Hsieh *et al*. 2005b)[2]	Orange variant (Hsieh *et al*. 2005b)[2]
AC Resolute	Great northern	**0.10**	**0.00**	**0.00**
AC Agrinto	Pinto	**0.23**	**0.00**	**0.00**
L02F132	Black	**0.24**	**0.06**	**0.06**
AC Early Rose	Pink	**0.36**	**0.25**	**0.20**
Viva	Pink	0.58	1.00	0.90
Othello	Pinto	0.77	0.85	0.80
AC Black Diamond	Black	0.78	1.20	2.20
AC Redbond	Small red	0.80	0.35	0.60
AC Polaris	Great northern	1.12	0.75	0.90
NW63	Small red	1.31	0.70	1.55
UI906	Black	1.87	1.30	2.30
Envoy	Navy	2.55	2.20	2.30
US1140	Great northern	2.69	1.65	1.95
AC Morden003	Navy	2.83	3.50	3.75

[1] Data presented are the mean of two runs, with three replicates per run. Severity of bacterial wilt was assessed on a scale of 0-5, as follows: 0, no wilt symptoms; 1, wilt on one of the primary leaves; 2, wilt on both primary leaves; 3, wilt on first trifoliolate; 4, death of seedling after development of primary leaves; 5, unemerged seedling or death of seedling before development of primary leaves.

[2] Boldfaced number indicates that the cultivar or line was 'resistant' (DSI<0.47 for the purple variant, DSI<0.22 for the yellow variant, DSI<0.23 for the orange variant; Fisher's LSD, P<0.05) to the pathogen.

(Lib.) de Bary] and common bacterial blight (caused by *Xanthomonas axonopodis* Starr & Garces pv. *phaseoli*) are major factors limiting production of common bean in Canada [3,13]. Future bean breeding efforts should focus on combining resistance to bacterial wilt with other desirable traits such as resistance to other bean diseases, improved agronomic characteristics, and superior seed qualities.

Although this study and the study of Hsieh *et al.* [12] have identified common bean entries with resistance to the purple, yellow and orange variants of the bacterial wilt pathogen, there still remain certain market classes such as navy bean, where resistant germplasm has not been reported. A broader screening of bean cultivars and lines may help to identify sources of resistance to bacterial wilt within these market classes. Alternatively, it may be possible to transfer bacterial wilt resistance from other bean market classes into navy bean using traditional breeding. However, the inheritance of bacterial wilt resistance is not well-understood at present. Further studies are in progress to address this issue.

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摘 要

Hung-Chang Huang[1], Hans-Henning Mündel[1], R. Scott Erickson[1, 3], Cheryl D. Chelle[1], Parthiba M. Balasubramanian[2], Ferdinand Kiehn[2], and Robert L. Conner[2]. 2007. 菜豆栽培品系與種原庫品系對菜豆細菌性萎凋病菌紫色變異菌株 (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens* purple variant) 之抗病性調查. 植病會刊 16: 91-95. ([1] 加拿大農業與農業食品部 Lethbridge 研究中心；[2] 加拿大農業與農業食品部 Morden 研究站；[3] 聯絡作者，電子郵件：ericksons@agr.gc.ca；傳眞：+1-403-382-3156)

本研究用種臍穿刺浸泡細菌懸浮液之接種方式 (hilum injury/seed inoculation method)，於植物培養箱中測試 14 種菜豆品系對菜豆細菌性萎凋病菌紫色變異菌株的抗病性，其中以 AC Resolute、AC Agrinto、種原庫品系 L02F132、 及 AC Early Rose 較具抗性。此外，根據已發表的研究報告，自種原庫中找到對菜豆細菌性萎凋病菌黃色與橙色變異菌株有抗性的菜豆品系，在本研究中亦可抗紫色變異菌株，故此抗病品系可用於培育對此 3 種菜豆細菌性萎凋病菌菌株同時具有抗性的新菜豆品系。

關鍵詞：菜豆、細菌性萎凋病、菜豆細菌性萎凋病菌、紫色變異菌株、抗病性