Phytopathol. Mediterr. (2010) 49, 89–94

Response of traditional cultivars of Fagioli di Sarconi beans to artificial inoculation with common bacterial blight agents

Pietro LO CANTORE, Giovanni FIGLIUOLO and Nicola Sante IACOBELLIS

Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy

Summary. Cultivars of the Fagioli di Sarconi are a pool of high value common bean (*Phaseolus vulgaris* L.) traditional cultivars selected from various landraces. These cultivars are protected by the European Union (Reg. CEE n° 1263/96) with the mark PGI (Protected Geographical Indication) and are cultivated in Basilicata (southern Italy) for the production of dry seeds. Fagioli di Sarconi cultivars are susceptible to common bacterial blight (CBB), a disease caused by the varieties *fuscans* and non-*fuscans* of *Xanthomonas axonopodis* pv. *phaseoli.* Five Fagioli di Sarconi cultivars (Tondino bianco, Verdolino, Cannellino, Tabacchino and Ciuoto) were artificially inoculated with two virulent strains of this bacterium, representative of 59 recently characterized strains of both the *fuscans* and non-*fuscans* varieties, with the aim to evaluate the susceptibility of these cultivars to the pathogens. Four CBB–resistant breeding–lines were used for comparison. Suspensions of the bacterium were injected into the first trifoliate leaflets of bean plants and produced typical CBB symptoms on the Fagioli di Sarconi cultivars, and hypersensitive necrotic lesions on the CBB–resistant breeding–lines. When cultivars were inoculated with the strain of the variety *fuscans*, Tondino bianco, Verdolino and Cannellino were more susceptible than Tabacchino and Ciuoto, whereas when they were inoculated with the non–*fuscans* strain, Tondino bianco, Tabacchino and Verdolino were more susceptible than Ciuoto and Cannellino. The tolerant cultivars appeared good candidates for the introgression of CBB–resistance characters in a breeding programme. The varying response of the traditional Fagioli di Sarconi bean cultivars to artificial inoculation suggests that several plant and pathogen factors are involved in the interaction between *X. a.* pv. *phaseoli* varieties and the bean cultivars.

Key words: breeding-lines, plant-pathogenic bacteria, plant-resistance evaluation, virulence.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume, widely cultivated (FAO Statistics Division, 2009), due to its commercial value and its high content of quality nutrients (carbohydrates, proteins, minerals, and vitamins). FAO data on bean production in Europe indicate that in 2007 Turkey, Spain and Italy were the main bean producers. In Turkey, bean cultivation covered an area of 172.205 ha (63.000 for string and 109.205 for dry beans), in Spain, 23.900 ha (15.100 for string and 8.800 for dry beans) and in Italy 29.306 ha (20.537 for string and 8.768 for dry beans). In 2007, bean production was 674.211 tons in Turkey (519.968 for string and 154.243 for dry beans), 231.700 tons in Spain (220.100 for string and 11.600 for dry beans) and 202.237 tons in Italy (187.190 for string and 15.047 for dry beans, FAO Statistics Division, 2009). In Italy, besides the commercially grown cultivars there is an abundance of landrace cultivars with unique flavors and nutritional features and for which there is a traditional market (Dinelli *et al*., 2006). This is the case of the Fagioli di Sarconi, a pool of traditional high-value cultivars, selected from various landraces (Masi *et al*., 1999; Piergiovanni *et al*., 2000) and protected by the European Union (Reg. CEE No. 1263/96) with the mark PGI (Protected Geographical Indication). The Fagioli di Sarconi are cultivated in the National Park of the Agri valley in Basilicata (southern Italy) for dry seed production (Brandi *et al*., 1998). The germplasm of this pool should be conserved

Corresponding author: N.S. Iacobellis
Fax: +39 0971 205702
E-mail: iacobellis@unibas.it

and characterized to enable the genetic resources of the bean pool to be exploited by farmers. The germplasm, preserved on farm by the "Consorzio di Tutela dei Fagioli di Sarconi", has been extensively characterized for its biochemical and nutriceutical traits (Piergiovanni *et al*., 2000; Lioi *et al*., 2005; Dinelli *et al*., 2006) but nothing is known about its response to diseases. These traditional cultivars are plagued by Common Bacterial Blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*) and *X. a.* pv. *phaseoli* var. *fuscans* (*Xapf*) (Vauterin *et al*., 1995), and there have been a number of severe outbreaks of this disease in the last decade. Investigations of bean fields in 2001–2002 found that most fields showed the typical symptoms of CBB and that by the end of the production cycle nearly 100% of the plants were infected, with heavy crop loss (Lo Cantore *et al*., 2004). The response of individual cultivars during these outbreaks was not recorded. Different varieties of the pathogen cause similar symptoms on susceptible common beans and they have similar biochemical and physiological characteristics (Van den Mooter and Swings, 1990). Strains of *Xapf* are distinguished from those of *Xap* only because the *Xapf* strains produce a brown pigment when grown on some agar media (Schaad *et al.,* 2001). Nevertheless, there is considerable genetic diversity between *Xap* and *Xapf* strains (Chan and Goodwin, 1999; Mkandawire *et al*., 2004; López *et al*., 2006; Mahuku *et al*., 2006), as recent AFLP analyses have confirmed (Lo Cantore and Iacobellis, 2007; Alavi *et al*., 2008, Lo Cantore *et al*., unpublished data) and this supports the recent proposal to classify the pigment–producing strains as *Xanthomonas fuscans* subsp. *fuscans*, and the non-pigment-producing strains as *X. campestris* pv. *phaseoli* (Schaad *et al*., 2005, 2006).

The CBB bacterium is seed-borne, and therefore pathogen-free seed is a prerequisite for a healthy crop, but apart from exclusion measures, there is as yet no established method to disinfect the seed (Lo Cantore *et al*., 2009). For the control of CBB, the use of bean cultivars with genetic tolerance/resistance to the disease is the most practical method (Coyne *et al*., 1974; Yoshii *et al*., 1978). The highest levels of CBB resistance are found in tepary bean (*P. acutifolius* A. Gray), whereas only low to intermediate resistance occurs in common bean (*P. vulgaris* L.) and scarlet runner bean (*P. coccineus* L.) cultivars (Coyne *et al*., 1963; Valladares-Sanchez *et al*., 1979; Schuster *et al*., 1983; Drijfhout and Blok, 1987; Singh and Muñoz, 1999).

The objective of the present work was to evaluate the response to CBB of five traditional French bean cultivars of the Fagioli di Sarconi pool (Tondino bianco, Verdolino, Cannellino, Tabacchino and Ciuoto), comparing them to four available resistant bean breeding-lines (VAX-4, USDK-CBB-15, ABC-Wiehing and USCR-CBB-20) (Singh *et al*., 2001; Miklas *et al*., 2006; Mutlu *et al*., 2008), using foliar inoculation with representative strains of the above pathogens. Virulent strains of *X. a.* pv. *phaseoli* var. *fuscans* USB749 (ICMP14929) and *X. a.* pv. *phaseoli* USB771 (ICMP14932) were selected from a collection of 59 strains of these two pathogens (31 from *X. a.* pv. *phaseoli* var. *fuscans* and 28 from *X. a.* pv. *phaseoli*. These strains have been recently characterized for their pathogenicity, phenotypic and genetic characters (Lo Cantore and Iacobellis, 2007; Lo Cantore *et al*., unpublished data). Aliquots of a bacterial suspension (10^8 cfu ml^{-1}), prepared from 48-h-old cultures grown on yeast dextrose calcium carbonate agar medium (Schaad *et al*., 2001) at 29°C were injected with a sterile syringe without a needle into the first trifoliate mesophyll (four inoculations per leaflet, twelve inoculations per plant) of

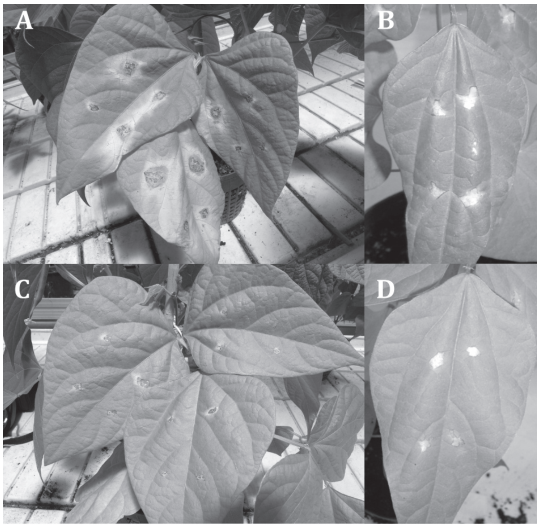

Figure 1. Symptoms on the traditional cultivar Verdolino (A–C) of Fagioli di Sarconi and hypersensitive necrotic lesions on the CBB resistant breeding-line ABC-Wiehing (B–D) 21 days after inoculation with strain USB749 of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (A–B) and strain USB771 of *X. a.* pv. *phaseoli* (C–D).

six bean plants grown for about 20 days in plastic pots containing garden soil (Compo Sana-Compo Agricoltura, Milan, Italy) in a greenhouse at about 22±5°C. Each inoculation covered an area of about 0.5 cm^2. The assays were performed twice.

The typical CBB symptoms, and the necrotic lesions typical of the hypersensitive reaction, appeared on the Fagioli di Sarconi cultivars and the CBB resistant breeding-lines respectively (Figure 1). The traditional cultivars Tondino bianco, Cannellino, Verdolino, Ciuoto and Tabacchino showed at the inoculation sites tiny water soaked lesions about fourteen days after inoculation. These lesions expanded and turned into necrotic spots surrounded by chlorotic halos, as already reported in other studies, on different bean cultivars and strains and using different inoculation techniques (Dursun *et al*., 2002; Marquez *et al*., 2007). The disease response 14, 21 and 28 days after inoculation was assessed with univariate analysis of variance (PROC GLM) using a completely randomized experimental design. Following analysis of variance of the disease

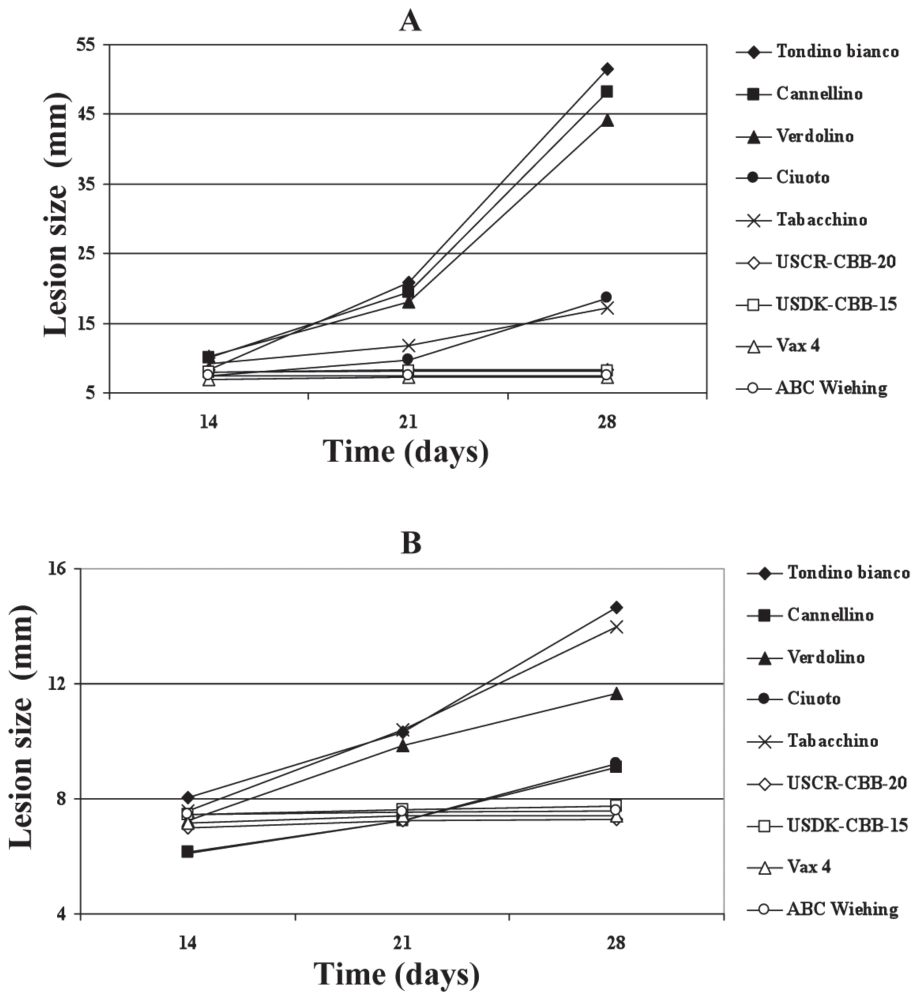

Figure 2. Response given by the traditional bean cultivars of the Fagioli di Sarconi and by CBB resistant breeding-lines to artificial inoculation with strain USB749 of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (A) and strain USB771 (B) of *X. a.* pv. *phaseoli*.

reaction after 28 days, the cultivar means were separated with Fisher's protected least significant difference ($LSD_{.05}$). SAS-STAT software (SAS 9.2, 2002–2003, SAS Institute Inc., Cary, NC, USA) was used for data analysis. The null hypothesis of disease response homogeneity among cultivars was rejected using analysis of variance ($P<0.0001$). The Cultivar × Strain interaction was highly significant ($P<0.001$) (Figure 2). Consequently analysis of variance was also conducted using the disease response at 28 days as a dependent variable. The least significant differences between the means were significant after 28 days ($P<0.05$). At that time the traditional Fagioli di Sarconi cultivars differed in their susceptible response depending on the pathogen used for the inoculation (Figure 2). With strain USB749 of *X. a.* pv. *phaseoli* var. *fuscans*, the lesion sizes on cv. Tondino bianco, Cannellino and Verdolino were significantly larger ($P<0.0001$; $LSD_{.05}=5.14$ mm) than those on cv. Ciuoto and Tabacchino (Figure 2A).

The susceptible response obtained with strain USB771 of *X. a.* pv. *phaseoli* after 28 days differed from that obtained with strain USB749 of *X. a.* pv. *phaseoli* var. *fuscans* (Figure 2). Overall, lesions were smaller with the non-fuscans strain. Specifically lesions on 'Tondino bianco' and 'Tabacchino' were significantly larger ($P<0.001$; $LSD_{.05}=1.82$ mm) than those on 'Verdolino', 'Ciuoto' and 'Cannellino', and on USDK-CBB-15, ABC-Wiehing, VAX-4, and USCR-CBB-20 (Figure 2B). The lesions on 'Ciuoto' and 'Cannellino' did not differ significantly from the lesions on the breeding-lines USDK-CBB-15, ABC-Wiehing and VAX-4 but they differed significantly from those on USCR-CBB-20 (Figure 2B).

In conclusion, the findings that the cultivars Ciuoto and Tabacchino and Ciuoto and Cannellino appear to be less susceptible to CBB caused by *X. a.* pv. *phaseoli* var. fuscans and *X. a.* pv. *phaseoli* strains, respectively, are positive since it is well established that the use of pathogen-tolerant germplasm rather than resistant cultivars should be advisable in order to avoid the selection of the pathogen population, leading to the resistance being overcome. This is particularly true for sustainable farming. The above cultivars, apart from their agronomic, biochemical and nutriceutical traits, appear good candidates for the introgression of CBB resistance characters in a breeding programme.

The differing response of the traditional 'Fagioli di Sarconi' bean cultivars to artificial inoculation suggest that several plant and pathogen factors are involved in the interaction between *X. a.* pv. *phaseoli* varieties and common bean, and that these factors differ between the traditional cultivars of Fagioli di Sarconi and also between the pathogen varieties. However, further experiments with a more representative number of strains of both bacterial varieties, possibly from a different geographic origin, are necessary to determine this. In general the resistance of breeding-lines VAX-4, ABC-Wiehing, USCR-CBB-20, and USDK-CBB-15 was confirmed, although at least in the experimental conditions, there were differences between them. The results further suggest that the inoculation procedure is suitable to evaluate the susceptibility/tolerance and the resistance of bean cultivars and/or breeding–lines. Nevertheless, the response of the traditional bean cultivars to inoculation needs to be confirmed by using different plant parts (i.e. pods), as well as different bacterial strains, possibly of different geographic origin and at different concentrations. It is well known that *Phaseolus* spp. differ in their resistance or susceptibility depending on the plant organ they are tested upon: each organ has its own degree of resistance/susceptibility (Valladares–Sanchez *et al*., 1979; Schuster *et al*., 1983; Aggour *et al*., 1989; Rodrigues *et al*., 1999; Marquez *et al*., 2007).

Acknowledgements

This research was supported by the Ministry of Agricultural Alimentary and Forest Politics with funds released by C.I.P.E. (Resolution 17/2003). Dr. Phillip Miklas, of the Vegetable and Forage Crops Research Laboratory, USDA, WA, USA, and the Consorzio di Tutela dei Fagioli di Sarconi are acknowledged for a generous supply of CBB-resistant breeding-lines and traditional cultivar seed lots.

Literature cited

Aggour A.R., D.P. Coyne and A. Vidaver, 1989. Comparison of leaf and pod disease reaction of beans (*Phaseolus vulgaris* L.) inoculated by different methods with strains of *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. *Euphytica* 43, 143–152.

Alavi S.M., S. Sanjari, F. Durand, C. Brin, C. Manceau and

S. Poussier, 2008. Assessment of the genetic diversity of *Xanthomonas axonopodis* pv. phaseoli and *Xanthomonas fuscans* subsp. *fuscans* as a basis to identify putative pathogenicity genes and a type III secretion system of the SPI-1 family by multiple suppression subtractive hybridizations. *Applied and Environmental Microbiology* 3295–3301.

Brandi M., D. Cerbino, G. Laghetti, A.R. Piergiovanni, G. Olita, R. Rizzi and S. Martelli, 1998. Una carta di identità per il fagiolo di Sarconi e Rotonda. *L'Informatore Agrario* 27, 55–61.

Chan J.W.Y.F. and P.H. Goodwin, 1999. Differentiation of *Xanthomonas campestris* pv. *phaseoli* from *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* by PFGE and RFLP. *European Journal of Plant Pathology* 105, 867–878.

Coyne D.P. and M.L. Schuster, 1974. Breeding and genetic studies of tolerance to several bean (*Phaseolus vulgaris* L.) bacterial pathogens. *Euphytica* 23, 651–656.

Coyne D.P., M.L. Schuster and S. Al–Yasiri, 1963. Reaction studies of bean species and varieties to common blight and bacterial wilt. *Plant Disease Reporter* 47, 534–537.

Dinelli G., A. Bonetti, M. Minelli, I. Marotti, P. Catizone and A. Mazzanti, 2006. Content of flavonols in Italian bean (*Phaseulus vulgaris* L.) ecotypes. *Food Chemistry* 99, 105–114.

Drijfhout E.W. and J. Blok, 1987. Inheritance of resistance to *Xanthomonas campestris* pv. *phaseoli* in tepary bean (*Phaseolus acutifolius*). *Euphytica* 36, 803–808.

Dursun A., M. Figen Dönmez and Ş. Fikrettin, 2002. Identification of resistance to common bacterial blight disease on bean genotypes grown in Turkey. *European Journal of Plant Pathology* 108, 811–813.

FAO Statistics Division, 2009. Food and Agriculture Organization of the United Nations from http://faostat.fao.org/site/567/default.aspx#ancor.

Lioi L., A.R. Piergiovanni, D. Pignone, S. Puglisi, M. Santantonio and G. Sonnante, 2005. Genetic diversity of some surviving *on–farm* italian common bean (*Phaseulus vulgaris* L.) landraces. *Plant Breeding* 124, 576–581.

Lo Cantore P. and N.S. Iacobellis, 2007. Characterization of strains of the varieties *fuscans* and non *fuscans* of *Xanthomonas axonopodis* pv. *phaseoli*. *Journal of Plant Pathology* 89 (Supplement), S44.

Lo Cantore P., C. Nigro, V. Castoro and N.S. Iacobellis, 2004. Presenza e diffusione delle batteriosi in coltivazioni di "Fagiolo di Sarconi" in Basilicata. *Informatore Fitopatologico* 6, 41–47.

Lo Cantore P., V. Shanmungaiah and N.S. Iacobellis, 2009. Antibacterial activity of essential oil components and their potential use in seed disinfection. *Journal of Agricultural and Food Chemistry* 57, 9454–9461.

López R., C. Asensio and R.L. Gilbertson, 2006. Phenotypic and genetic diversity in strains of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) in a secondary center of diversity of the common bean host suggests multiple introduction events. *Phytopathology* 96, 1204–1213.

Mahuku G.S., C. Jara, M.A. Henriquez, G. Castellanos and J. Cuasquer, 2006. Genotypic characterization of the common bean bacterial blight pathogens, *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* by rep-PCR and PCR-RFLP of the ribosomal genes. *Journal of Phytopathology* 154, 35–44.

Marquez M.L., H. Teran and S.P. Singh, 2007. Selecting common bean with genes of different evolutionary origins for resistance to *Xanthomonas campestris* pv. *phaseoli*. *Crop Science* 47, 1367–1374.

Masi P., G. Figliuolo and P.L. Spagnoletti Zeuli, 1999. Landraces of bean (*Phaseolus vulgaris* L.) collected in Basilicata, Italy. *Plant Genetic Resources Newsletter* 119, 51–55.

Miklas P.N., J.R. Smith and S.P. Singh, 2006. Registration of common bacterial blight resistant dark red kidney bean germoplasm line USDK-CBB-15. *Crop Science* 46, 1005–1006.

Mkandawire A.B.C., R.B. Mabagala, P. Guzman, P. Gepts and R.L. Gilbertson, 2004. Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggests pathogen coevolution with the common bean. *Phytopathology* 94, 593–603.

Mutlu N., C.A. Urrea, P.N. Miklas, M.A. Pastor-Corrales, J.R. Steadman, D.T. Lindgren, J. Reiser, A.K. Vidaver and D.P. Coyne, 2008. Registration of common bacterial blight, rust and bean common mosaic resistant great northern common bean germoplasm line ABC-Weihing. *Journal of Plant Registration* 2, 53–55.

Piergiovanni A.R., D. Cerbino and M. Brandi, 2000. The common bean populations from Basilicata (Southern Italy). An evaluation of their variation. *Genetic Resources and Crop Evolution* 47, 489–495.

Rodrigues R., N.R. Leal, M.G. Pereira and A. Lam–Sanchez, 1999. Combining ability of *Phaseolus vulgaris* L. for resistance to common bacterial blight. *Genetics and Molecular Biology* 22, 571–575.

Schaad N.W., J.B. Jones and G.H. Lacy (ed.), 2001. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. Third edition, APS press, St Paul, MN, USA, 373 pp.

Schaad N.W., E. Postnikova, G.H. Lacy, A. Sechler, I. Agarkova, P.E. Stromberg, V.K. Stromberg and A.K. Vidaver, 2005. Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel *et al*., 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv. *malvacearum* (ex Smith 1901) Dye 1978 as *X. smithii* subsp. *smithii* nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and Jones, 1935) Dye 1978 as *X. alfalfae* subsp. *alfalfae* (ex Riker *et al*., 1935) sp. nov. nom. rev.; and "var. fuscans" of *X. campestris* pv. *phaseoli* (ex Smith,

1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. *Systematic and Applied Microbiology* 28, 494–518.

Schaad N.W., E. Postnikova, G. Lacy, A. Sechlera, I. Agarkova, P.E. Stromberg, V.K. Stromberg and A.K. Vidaver, 2006. Emended classification of xanthomonad pathogens on citrus. *Systematic and Applied Microbiology* 29, 690–695.

Schuster M.L., D.P. Coyne, T. Behre and H. Leyna, 1983. Sources of *Phaseolus* species resistance and leaf and pod differential reactions to common blight. *Hort Science* 18, 901–903.

Singh S.P. and C.G. Muñoz, 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Science* 39, 80–89.

Singh S.P., C.G. Muñoz and H. Terán, 2001. Registration of common bacterial blight resistant dry bean germoplasm VAX 1, VAX 3, and VAX 4. *Crop Science* 41, 275–276.

Valladares-Sanchez N.E., D.P. Coyne and M.L. Schuster, 1979. Differential reaction of leaves and pods of *Phaseolus* germplasm to strains of *Xanthomonas phaseoli* and trangressive segregation for tolerance from crosses of susceptible germplasm. *Journal of the American Society for Horticultural Science* 104, 648–654.

Van den Mooter M. and J. Swings, 1990. Numerical analysis of 295 phenotypic features of 266 *Xanthomonas* strains and related strains and an improved taxonomy of the genus. *International Journal of Systematic Bacteriology* 40, 348–369.

Vauterin L., B. Hoste, K. Kersters and J. Swings, 1995. Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology* 45, 472–489.

Yoshii K., G.E. Galvez and G. Alvarez, 1978. Screening bean germplasm for tolerance to common bacterial blight caused by *Xanthomonas phaseoli* and the importance of pathogenic variation to varietal improvement. *Plant Disease Reporter* 62, 343–347.

Accepted for publication: February 8, 2010