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Snap beans for organic farming and evaluation of resistance to the common bacterial blight

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ABSTRACT

Snap beans have been widely used in organic farming as a good income source and an alternative to diversify production, with increasing use in crop rotation. This work reports the evaluation of 25 bush-type snap beans accessions for their suitability to integrate a breeding program for organic farming, as well as for their resistance to the common bacterial blight (CBB). Agronomic performance was assessed in two field experiments (September-December, 2013; April-June, 2014), in complete blocks at random, while resistance to CBB was assessed in greenhouse, in a completely randomized trial. Plants were challenged with two isolates, one from *Xanthomonas axonopodis* pv. *phaseoli* and another from *X. fuscans* subsp. *fuscans*. Accessions UEL 402, UEL 405, UEL 407, UEL 408, UEL 412, UEL 417 and UEL 420 were highly productive in both seasons (averages of 10.3, 8.7, 9.5, 9.2, 8.9, 9.3 and 9.2 t/ha, respectively), and are promising for use both as cultivars by organic farmers in the region of Londrina and also as germplasm in breeding programs for developing cultivars adapted to the region. Although all accessions were moderately susceptible to CBB, accessions UEL 407, UEL 409, UEL 411, UEL 412, UEL 424 and UEL 431 presented the lowest values for the area under CBB progress curve for both isolates.

Keywords: *Phaseolus vulgaris*, *Xanthomonas axonopodis*, *Xanthomonas fuscans*, smallholders, agroecology.

RESUMO

Feijão de vagem para cultivo orgânico e avaliação da resistência ao cretamento bacteriano comum

O feijão de vagem vem sendo amplamente utilizado na agricultura orgânica por ser uma boa fonte de renda e uma alternativa para diversificar a produção, com uso crescente em rotação de culturas. Os objetivos deste trabalho foram avaliar 25 acessos de feijão de vagem de hábito determinado para uso em um programa de melhoramento para cultivo orgânico e verificar o nível de resistência destes acessos ao cretamento bacteriano comum (CBC). O desempenho agrônomico foi avaliado em dois experimentos de campo (setembro-dezembro, 2013; abril-junho, 2014), em blocos ao acaso; e sua resistência ao CBC foi avaliada em um experimento em casa de vegetação, inteiramente ao acaso. Foram utilizados dois isolados, um de *Xanthomonas axonopodis* pv. *phaseoli* e outro *X. fuscans* subsp. *fuscans*. Os acessos UEL 402, UEL 405, UEL 407, UEL 408, UEL 412, UEL 417 e UEL 420 apresentaram elevada produtividade para ambas as épocas (médias de 10,3, 8,7, 9,5, 9,2, 8,9, 9,3 e 9,2 t/ha, respectivamente), sendo considerados promissores para uso tanto como cultivares pelos produtores orgânicos da região de Londrina, quanto como germoplasma em programas de melhoramento visando o desenvolvimento de cultivares adaptadas à região. Embora todos os acessos tenham sido moderadamente suscetíveis ao CBC, os acessos UEL 407, UEL 409, UEL 411, UEL 412, UEL 424 e UEL 431 apresentaram valores menores para área abaixo da curva do progresso do cretamento bacteriano para ambos os isolados.

Palavras-chave: *Phaseolus vulgaris*, *Xanthomonas axonopodis*, *Xanthomonas fuscans*, agricultura familiar, agroecologia.

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Snap beans (*Phaseolus vulgaris*) are considered one of the world's main vegetables, reaching an annual estimated production around 21 million tons (FAO, 2015). In Brazil, production figures and information on the importance of snap beans market are scarce. Snap beans are widely grown by Brazilian smallholders, representing a good source of income and as an alternative to diversify production, either through

crop rotation, an increasingly popular practice, or as option during off-season of other vegetables.

Snap beans cultivars are classified according to growth habit and pod type and color. Pole-type cultivars are preferred in Brazil, since usually they outyield bush types. However, pole-type snap beans are more labor intense than bush-types: pole-types need tutoring and have higher incidence of pests

and diseases due to their larger cycles (Moreira *et al.*, 2009). There are two main types of pods: butter and flat; and four basic color groups: dark green, light green, yellow and purple (Myers & Baggett, 1999).

Despite the economic and social importance of snap beans, breeding programs for organic farming are scarce. Organic farming, considered a sustainable cropping system, has been

expanding steadily in last decades in several countries (Willer & Kilcher, 2012). In Brazil, 10,505 farmers currently meet official organic farming requirements, a 56% increase when compared to 2013, when not more than 6.7 thousand farmers were accredited as organic producers (MAPA, 2015).

Vegetable production is pioneer in organic farming. Even though, it still faces difficulties as, for instance, lack of enough organic seeds to meet the certification process throughout the production chain. In addition, cultivars meant for organic farming should be developed according to agroecological principles, using germplasm adapted to local conditions. Very often, in comparative experiments between conventional and organic systems, cultivars with good performance in the first do not necessarily perform well in the second (Kirk *et al.*, 2012, Spagnuolo *et al.*, 2016). In organic farming, the ideal cultivar is expected to be rustic, resistant to pests and diseases and reasonably productive in the absence of soluble fertilizers (Vidal *et al.*, 2007).

Among snap beans diseases, the common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) or *X. fuscans* subsp. *fuscans* (*Xff*) (EFSA Panel on Plant Health, 2014), is considered the main bacterial disease affecting beans in Brazil (Trindade *et al.*, 2012; Silva *et al.*, 2013). *Xap* and *Xff* are present in all environments where *P. vulgaris* is produced, causing damage mainly under high temperature and humidity (Miklas *et al.*, 2003). CBB symptoms are observed throughout plant aboveground part: leaves, stems, pods and seeds (Rava & Sartorato, 1994). At first, leaves show water-soaked lesions that evolve into necrotic spots, usually surrounded by a yellow halo. Adjacent lesions coalesce and, in severe infections, CBB causes leaf death and, consequently, plant defoliation. In pods, lesions are initially circular and soaked, becoming necrotic and reddish. Seeds present discoloration in the thread, spots on the tegument and wrinkling, although in some cases seed infection is symptomless (Bianchini *et al.*, 2005).

CBB must be controlled by

employing several management measures simultaneously, since bactericides alone are not efficient. Among recommended measures, the use on non-infested areas, healthy seeds, crop rotation, adequate irrigation systems and resistant cultivars are highlighted (Trindade *et al.*, 2015). Nevertheless, the development of snap beans cultivars with resistance to CBB is a challenge to breeders. Breeding for CBB resistance in snap beans still waits for the identification of resistance sources in either snap beans or common beans, once high levels of resistance to *Xanthomonas* spp. have not been found in *P. vulgaris* yet.

The present work describes the screening of accessions of bush-type snap beans from the Germplasm Bank of the Universidade Estadual de Londrina (UEL) for their performance in organic farming and resistance to CBB.

MATERIAL AND METHODS

Twenty-five snap beans accessions from the germplasm bank of the Universidade Estadual de Londrina (UEL) were evaluated. Agronomic traits were assessed in two experiments carried out at UEL School Farm (23°20'23"S, 51°12'32"W, altitude 535 m), in an area kept under organic farming for five years. Experiment I was carried out from September to December 2013 and, experiment II, from April to June 2014.

The farm is located under mesothermal humid subtropical climate Cfa (Köppen classification), with hot summers, winters with frequent and severe frost, and no marked dry season. The average annual temperature is 20°C. The soil, a Rhodic Ferralsol, had the following characteristics: $pH_{H_2O} = 5.5$; $P = 3.41 \text{ cmolc/dm}^3$; $K^+ = 0.74 \text{ cmolc/dm}^3$; $Ca^{+2} = 6.8 \text{ cmolc/dm}^3$; $Mg^{+2} = 1.2 \text{ cmolc/dm}^3$; $Al^{+3} = 0.05$; $H+Al = 4.28 \text{ cmolc/dm}^3$; $CEC = 13.02 \text{ cmolc/dm}^3$; $V = 67.13\%$ and Organic Matter = 1.87%.

Experiments were set in complete blocks at random, with three replications, 20-plant plots, and 0.2x0.45 m spacing between plants and rows, respectively. In both experiments, fertilization consisted of 10 t/ha of organic compost with the

following chemical composition: $pH_{H_2O} = 7.2$; $P = 359.30 \text{ mg/dm}^3$; $K^+ = 5.78 \text{ cmolc/dm}^3$; $Ca^{+2} = 9.5 \text{ cmolc/dm}^3$; $Mg^{+2} = 6.9 \text{ cmolc/dm}^3$; $Al^{+3} = 0.0$; $H+Al = 2.19 \text{ cmolc/dm}^3$; $CEC = 24.37 \text{ cmolc/dm}^3$; $V = 91.01\%$ and Organic Matter = 5.5%. We used sprinkling irrigation to water the plants, straw between plants and lines to inhibit weeds, and the Bordeaux mixture (0.5%) before flowering to control diseases. Agronomic performance was measured by means of number of commercial (NCP) and non-commercial pods (NnCP) per plant, mass of commercial pods (MCP), in grams, and commercial yield (Yield), in t/ha, considering pods longer than 10 cm, with no mechanical or disease damages, as commercial.

CBB resistance was evaluated in greenhouse, at the Department of Agronomy (UEL), from December 2013 to February 2014. We used a completely randomized design and with ten replications. Accessions were grown in five-liter pots, filled with a 50%-soil/30%-manure/20%-sand substrate. We planted four seeds per pot, thinned after emergence to two plants per pot.

Inoculation was carried out 35 days after sowing with two bacterial isolates, *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), and *X. fuscans* subsp. *fuscans* (*Xff*), received from the Biological Institute of São Paulo and from the Agronomic Institute of Paraná, respectively. Isolates were cultured in DYGS medium (Rodrigues Neto *et al.*, 1986) in Petri dishes (streaks made with smear loop) and kept in growth chamber, 28°C for 36 hours. After, bacterial colonies were suspended in 100 mL distilled water and concentration adjusted to 10^7 CFU/mL, using a spectrophotometer at 640 nm absorbance. For inoculation, we took a three-blade leaf per plant and inoculated each bacterial isolate in a separate leaflet. Inoculation consisted of two 2-cm cuts per leaflet made with scissors previously immersed in the two bacterial suspensions.

CBB severity was evaluated daily for 30 days, using the diagrammatic scale of Pastor-Corrales *et al.* (1981), where: 1= no symptoms; 2= 1 to 5% necrosis; 3= 6 to 25% necrosis; 4= 26 to 50%

necrosis; 5= over 50% necrosis. Data were used to calculate the area under the disease progress curve (AUDPC). Data from field and greenhouse experiments were submitted to analysis of variance individually and, subsequently, once homogeneity of residual variances was detected, field experiments were submitted to joint analysis of variance. Means were grouped using the Scott-Knott test, at 5% significance, whenever significant differences were found. Data were analyzed with the aid of the R program (<http://www.r-project.org>) using the Agricolae and Scott-Knott packages.

RESULTS AND DISCUSSION

The analysis of variance carried out

independently for each field experiment indicated the occurrence of significant differences among snap beans genotypes for number of commercial and non-commercial pods per plant and productivity in season I; and for mass of commercial pods and productivity in season II, pointing to a wide variability among snap beans accessions. Accuracy estimates had moderate ($\geq 50 < 70$), high ($\geq 70 < 90$) or very high (≥ 90) magnitude in all cases, confirming experimental precision (Cargnelutti Filho *et al.*, 2009). Accuracy is a very robust indicator of experimental precision, since the experimental mean does not influence it (Resende & Duarte, 2007).

Cargnelutti Filho *et al.* (2009) consider selective accuracy more

adequate to assess experimental precision in trials with beans than the coefficient of variation or the honest significant difference used on the Tukey test, as percentage of the mean. The F test for the joint analysis of variance carried out considering the two field experiments indicated significant differences for all traits, for both sources of variation, e.g., genotypes and environments (seasons). Genotype x environment interaction (GE) was significant for all traits as well, indicating a differential response from genotypes to the environmental variation.

Agronomic traits averages were higher for the experiment carried out from April to June 2014 (Season II)

Table 1. Number of commercial and non-commercial pods per plant, mass and yield of commercial pods of 25 bush-type snap beans. Londrina, UEL, 2013.

Genotypes	Number of commercial pods/plant		Number of non-commercial pods/plant		Mass of commercial pods (g)		Yield of commercial pods (t/ha)	
	Exp I	Exp II	Exp I	Exp II	Exp I	Exp II	Exp I	Exp II
UEL 402	9.7 Aa	10.6 Aa	52.3 Aa	35.0 Aa	9.0 Aa	9.3 Ab	9.7 Aa	10.9 Aa
UEL 403	5.8 Bb	9.0 Aa	34.7 Ab	24.7 Aa	10.3 Aa	10.5 Aa	6.7 Bb	10.6 Aa
UEL 404	6.3 Bb	9.6 Aa	29.3 Ab	34.0 Aa	8.9 Aa	9.9 Aa	6.2 Bb	10.5 Aa
UEL 405	7.3 Bb	9.6 Aa	39.3 Ab	37.0 Aa	9.8 Aa	9.1 Ab	8.0 Aa	9.7 Aa
UEL 407	9.6 Aa	9.6 Aa	41.0 Ab	30.3 Aa	8.8 Aa	8.9 Ab	9.4 Aa	9.5 Aa
UEL 408	9.0 Aa	10.4 Aa	35.0 Ab	35.0 Aa	9.0 Aa	8.5 Ab	9.0 Aa	9.3 Aa
UEL 409	8.0 Ba	10.5 Aa	34.3 Ab	34.0 Aa	8.5 Aa	8.2 Ab	7.6 Ab	9.3 Aa
UEL 411	12.0 Aa	8.3 Ba	35.3 Ab	32.0 Aa	7.2 Aa	8.3 Ab	9.5 Aa	7.0 Bb
UEL 412	8.5 Aa	9.0 Aa	38.7 Ab	32.0 Aa	8.4 Aa	10.2 Aa	7.8 Ba	10.1 Aa
UEL 415	7.1 Bb	10.1 Aa	32.0 Ab	31.3 Aa	9.4 Aa	9.8 Aa	7.4 Bb	11.0 Aa
UEL 416	6.1 Ab	7.4 Aa	54.0 Aa	57.3 Aa	8.7 Ba	11.0 Aa	5.9 Bb	8.9 Aa
UEL 417	7.9 Aa	8.4 Aa	43.7 Ab	29.0 Aa	9.8 Aa	11.0 Aa	8.6 Aa	10.0 Aa
UEL 420	9.5 Aa	8.2 Aa	34.3 Ab	34.3 Aa	8.7 Aa	10.1 Aa	9.2 Aa	9.2 Aa
UEL 424	5.6 Bb	8.7 Aa	39.7 Ab	20.3 Aa	8.1 Ba	11.0 Aa	5.1 Bb	10.6 Aa
UEL 427	5.1 Bb	8.1 Aa	37.0 Ab	28.7 Aa	9.3 Aa	10.0 Aa	5.2 Bb	9.0 Ab
UEL 429	9.9 Aa	7.8 Aa	19.3 Bb	39.7 Aa	7.5 Ba	9.9 Aa	8.3 Aa	8.5 Ab
UEL 431	8.3 Aa	9.8 Aa	62.7 Aa	37.3 Ba	7.2 Aa	8.8 Ab	6.6 Bb	9.6 Aa
UEL 436	6.2 Bb	10.8 Aa	65.7 Aa	40.0 Ba	10.0 Aa	9.2 Ab	7.0 Bb	11.1 Aa
UEL 441	5.0 Bb	8.8 Aa	41.7 Ab	48.7 Aa	9.4 Aa	7.8 Ab	5.2 Bb	7.5 Ab
UEL 445	7.0 Bb	10.2 Aa	44.0 Ab	30.0 Aa	8.9 Aa	7.5 Ab	6.9 Ab	8.4 Ab
UEL 446	6.4 Ab	7.1 Aa	41.3 Ab	32.7 Aa	9.8 Aa	11.1 Aa	6.9 Ab	8.7 Ab
UEL 447	7.2 Ab	9.2 Aa	67.3 Aa	25.0 Ba	9.2 Aa	6.3 Bb	7.4 Ab	6.5 Ab
UEL 1	7.9 Aa	8.8 Aa	60.0 Aa	36.3 Ba	9.8 Aa	7.8 Bb	8.5 Aa	7.6 Ab
UEL 2	8.2 Aa	9.6 Aa	57.0 Aa	43.7 Aa	7.8 Aa	8.3 Ab	6.9 Ab	8.7 Ab
Alessa	9.1 Aa	9.1 Aa	52.0 Aa	58.3 Aa	7.3 Aa	9.0 Ab	7.4 Ab	9.1 Aa

Pods longer than 10 cm with no mechanical or disease damages were considered commercial; Means followed by same capital letters in the line and small letters in the column do not differ significantly from each other by Scott-Knott test, 5%.

[number of commercial pods per plant (NCP): 9.15; pod mass (PM): 9.26 g; and commercial productivity (PROD): 9.25 t/ha] than for experiment carried out between September and December, 2013 (Season I) (NCP: 7.71; PM: 8.84 g; and PROD: 7.46 t/ha) (Table 1). The highest pest and disease incidence in season I is likely the most relevant reason associated to differences between environments.

NCP ranged from 5.0 to 12.0 and from 7.1 to 10.8 in seasons I and II, respectively. In season II, accessions did not differ significantly from each other. In season I, best accessions were UEL 402, UEL 408, UEL 409, UEL 411, UEL 412, UEL 417, UEL 420, UEL 429, UEL 431, UEL 1, UEL 2 and cultivar Alessa (Table 1). Moreira *et al.* (2009), evaluating 14 genotypes of bush-type snap beans in conventional farming, observed NCP varying between 2.2 and 7.1, genotypes HAB 415, HAB 441, HAB 448 and UEL 2 having the highest values. Genotypes HAB 415, HAB 441 and UEL 2 were evaluated also in the present study and accessions HAB 415 and HAB 441 had significantly lower NCPs than accessions clustered in the top group. Comparative trials between conventional and organic farming have already shown that cultivars with good performance in conventional systems do not necessarily perform well in organic farming (Kirk *et al.*, 2012; Luby *et al.*, 2013). Mason & Spaner (2006) stated that the increase of plant stress in organic farming can affect cultivar performance strongly enough to justify the development of breeding programs specific for organic farming.

No significant differences among accessions were found for NnCP by the Scott-Knott clustering test in season II. Nevertheless, in season I accessions UEL 402, UEL 416, UEL 431, UEL 436, UEL 447, UEL 1, UEL 2 and cultivar Alessa were grouped as having significantly highest NnCP, respectively 52.3, 54.0, 62.7, 65.7, 67.3, 60.0, 57.0 and 52.0 (Table 1). Differences may be related to the differential anthracnose incidence among accessions, especially in season I.

MCP ranged from 7.2 to 10.3 g in season I and from 6.3 to 11.1 g in season

Table 2. Area under the disease progress curve (AUDPC) for the Common Bacterial Blight in 25 bush-type snap beans accessions inoculated with *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* subsp. *fuscans* (*Xff*). Londrina, UEL, 2013.

Genotypes	<i>Xap</i>	<i>Xff</i>
UEL 402	98.50 A	101.83 A
UEL 403	86.25 A	104.67 A
UEL 404	74.07 B	100.29 A
UEL 405	93.19 A	100.81 A
UEL 407	64.79 B	87.29 B
UEL 408	75.50 B	100.63 A
UEL 409	68.00 B	90.50 B
UEL 411	74.83 B	97.00 B
UEL 412	70.31 B	95.83 B
UEL 415	99.00 A	85.17 B
UEL 416	89.79 A	103.93 A
UEL 417	79.83 B	104.67 A
UEL 420	81.55 A	102.11 A
UEL 424	79.55 B	94.20 B
UEL 427	87.94 A	103.78 A
UEL 429	88.56 A	106.89 A
UEL 431	74.19 B	96.75 B
UEL 436	99.35 A	112.05 A
UEL 441	90.86 A	105.14 A
UEL 445	83.00 A	105.70 A
UEL 446	84.50 A	102.25 A
UEL 447	73.67 B	103.33 A
UEL 1	84.00 A	98.67 B
UEL 2	74.20 B	117.20 A
Alessa	104.25 A	114.67 A

Means followed by same capital letters in the column do not differ significantly from each other by Scott-Knott test, 5%.

II, and no significant differences among accessions were detected by the Scott-Knott test in season I. In season II, MCP differed significantly among accessions: UEL 403, UEL 404, UEL 412, UEL 415, UEL 416, UEL 417, UEL 420, UEL 424, UEL 427, UEL 429 and UEL 446 having the heaviest pods.

Yield varied between 5.14 and 9.72 and between 7.02 and 11.07 t/ha in seasons I and II, respectively. The Scott-Knott test grouped ten and 15 accessions in the top classes in seasons I and II, respectively, with accessions UEL 402, UEL 405, UEL 407, UEL 408, UEL 412, UEL 417 and UEL 420 appearing in both seasons (Table 1). Yield observed here was higher than that reported by Moreira *et al.* (2009). Pinto *et al.* (2001), evaluating the best

harvest age for snap beans, cultivar Novirex, under conventional farming, reported the highest commercial pod yields between 8.6 and 10.1 t/ha.

There are no breeding programs of bush-type snap beans specific for organic farming. In the present work, accessions UEL 402, UEL 405, UEL 407, UEL 408, UEL 412, UEL 417 and UEL 420 have shown to be promising both as cultivars for organic farming in the region and as advanced germplasm in breeding.

The analysis of variance of the area under the disease progress curve (AUDPC) revealed significant differences and wide variability among accessions when inoculated with the two *Xanthomonas* isolates (Table 2). Average AUDPC values were 82.09

(64.79-104.25) and 101.17 (85.17-117.2) for *X. axonopodis* pv. *phaseoli* (*Xap*) and *X. fuscans* subsp. *fuscans* (*Xff*), respectively, indicating that *Xff* was the most pathogenic. This is consistent with Mutlu *et al.* (2008), who also verified higher virulence of *Xff* when compared to *Xap* in beans.

All accessions were moderately susceptible to the common bacterial blight (CBB) within 30 days after we started assessing the disease. Nevertheless, there were isolate-specific reactions. Accessions UEL 404, UEL 408, UEL 409, UEL 411, UEL 412, UEL 417, UEL 424, UEL 431, UEL 436 and UEL 2 had the lowest AUDPC values for CBB-*Xap*; while accessions UEL 407, UEL 409, UEL 411, UEL 412, UEL 415, UEL 424, UEL 431 and UEL 1 had the lowest scores for CBB-*Xff* (Table 2). CBB resistance in *P. vulgaris* has complex nature, oligo or polygenic inheritance, and is strongly affected by the environment (Ferreira *et al.*, 2003). More than 20 QTLs related to CBB resistance, with variable genetic effects, are distributed across all eleven beans chromosomes (Kelly *et al.*, 2003; Santos *et al.*, 2003). Trindade *et al.* (2012), evaluating the resistance of 14 snap beans accessions to two *X. axonopodis* pv. *phaseoli* isolates, found that only accession UENF 1482 showed good performance, since it did not differ significantly from resistant genotypes PI 207262 and BAC 6. The presence of CBB resistance genes in common beans genetic background is an important tool for strategies that intend to breed snap beans for resistance via crosses with selected parents. Among accessions evaluated in the current work, only accessions UEL 407 and UEL 412 combined low AUDPC and high yield. Even then, the introgression of resistance genes, as for example those available in accessions PO 207262 and BAC 6 (common bean), into these accessions are still necessary.

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