SHORT COMMUNICATION

COMMON BEAN LINES AS POTENTIAL DIFFERENTIAL CULTIVARS FOR RACE 65 OF *COLLETORICHUM LINDEMUTHIANUM*

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SUMMARY

Colletotrichum lindemuthianum, which is known for its wide diversity in virulence, is the causal agent of anthracnose of common bean (*Phaseolus vulgaris*). Studies of race characterization using the standard set of 12 differential cultivars have shown the predominance of race 65 in Minas Gerais state (Brazil). However, the presence of genetic variability within race 65 has complicated the development of a durable resistant bean cultivar. Therefore, the objective of this study was to identify common bean lines able to differentiate the variation within race 65 to complement the results of the standard set of differential cultivars. A total of 12 common bean lines adapted to Brazilian conditions and 12 fungal isolates classified as race 65 were used. We now propose a new nomenclature to identify the pathogenic variability of race 65 isolates. Using this methodology, five different reaction patterns were observed, allowing the differentiation of regionally important races that could not be discriminated using the standard set of differential cultivars. Furthermore, our approach makes it possible the identification of new genes and sources of resistance to anthracnose.

Key words: anthracnose, common bean breeding, virulence diversity, resistance source, *Phaseolus vulgaris.*

Anthracnose, one of the most significant diseases of common bean (*Phaseolus vulgaris*), has genetic resistance as the main strategy of control. However, *Colletotrichum lindemuthianum*, the causal agent of the disease, is known for its variable virulence, which makes it difficult to obtain effective resistance (Pastor-Corrales and Tu, 1989). Race characterization was done using a standard set of 12 differential cultivars (Pastor-Corrales, 1991) composed of Andean (Michigan Dark, Red Kidney, Perry Marrow, Widusa and Kaboon) and Mesoamerican genotypes (Michelitte, Cornell 49242, México 222, PI 207262, TO, TU, AB 136 and G2333), which possess different resistance genes (Kelly and Vallejo, 2004).

When breeding of common bean for resistance to anthracnose, the various races present in the region of interest should be used for selecting new resistant cultivars. This procedure is effective for selection of the appropriate sources of resistance to be used. The mentioned standard set of 12 differential cultivars has been used by many scientists to characterize the races of the anthracnose pathogen in different parts of the world (Balardin *et al*., 1997; Ansari *et al*., 2004; Mahuku and Riascos, 2004). In the Minas Gerais state (Brazil), the most recent studies have shown a predominance of races 65, 73 and 81 (Silva *et al*., 2007; Ishikawa *et al*., 2008a). However, some fungal isolates classified as race 65 showed differential reaction when inoculated to different cultivars. In principle, this would be an indication that the set of differential cultivars do not distinguish between seemingly different isolates (Davide and Souza, 2009).

If a common bean cultivar is released as resistant to race 65 of *C. lindemuthianum*, it should be so for all fungal strains belonging to this race. If not, this could lead to serious economic and credibility-related consequences for the breeding program. Thus, the objective of this study was to identify new common bean genotypes as potential differential indicators for race 65.

Twelve isolates of *C. lindemuthianum* collected from different cultivars and regions of Minas Gerais state (Table 1) were previously tested on the set of Pastor-Corrales (1991) differential cultivars. These isolates were from the culture collection of Department of Biology, Universidade Federal de Lavras (UFLA). Single-conidium cultures were obtained for each isolate and maintained in M_3S medium (Tu, 1985), were then inoculated in bean pod culture medium and incubated at 22°C for 10-15 days in the darkness to obtain high sporulation. Seedlings with fully expanded primary leaves were sprayed with a suspension of 1.2×10^6 conidia ml^{-1}. One seed of each differential cultivar was sown in a 128 cell polystyrene tray containing Plantmax substrate (Eucatex, Brazil). Two replicate trays were used

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Table 1. Coordinates of the 12 *Colletotrichum lindemuthianum* isolates used in this study.

Isolate	Origin*	Cultivar/ line	Year
LV 111	Ijaci	Ouro Negro	2007
LV 114	Patos de Minas	CVIII-6	2007
LV 115	Patos de Minas	Carioca	2007
LV 117	Lavras	-	2007
LV 120	Lambari	Magno 237	2008
LV 131	Lambari	Magnífico	2009
LV 134	Lambari	Magnífico	2009
LV 136	Lambari	Magnífico	2009
LV 140	Lambari	CNFC 9506	2009
LV 148	Lambari	Pérola	2009
LV 164	Ijaci	Majestoso	2009
LV169	Ijaci	Pérola	2009

* Minas Gerais counties where isolates were collected

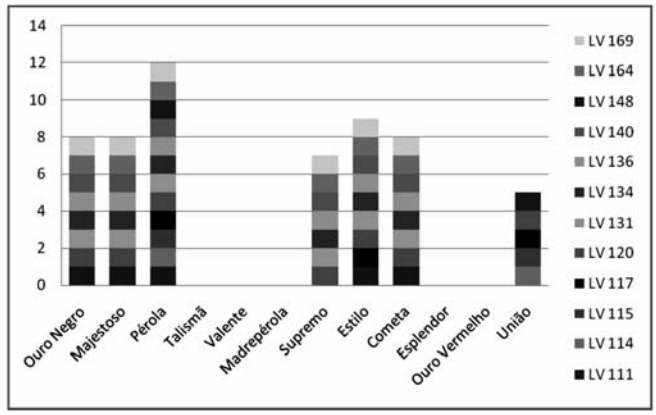

Fig. 1. Number of *Colletotrichum lindemuthianum* isolates compatible with each UFLA common bean cultivar.

per isolate. The inoculated plants remained in a mist chamber at 22°C and photoperiod of 12 h for 3 days.

After 7-10 days from inoculation, plants were evaluated using a scale from 1 to 9 (Schoonhoven and Pastor-Corrales, 1987). Average scores below 3 were considered as resistant, whereas plants scoring more than 3 were susceptible. Identified races were assigned a value based on the binary nomenclature system proposed by Habgood (1970). Fungal isolates were then evaluated on 12 cultivars from the germplasm collection of UFLA (Table 2). The differential cvs Michelitte (2^0) and Mexico 222 (2^6) were used to identify race 65.

The inoculation of the standard set of differentials confirmed that all 12 isolates tested belong to race 65 because of their compatible reaction with cvs Michelitte (2^0) and México 222 (2^6). However, the reaction of the newly tested cultivars to the 12 isolates provided evidence that these isolates differ in their virulence spectra (Table 3) for five different patterns of reaction were observed (Table 3, Fig. 1).

LV 120 was the isolate that infected the highest number of the cultivars (seven), whereas isolates LV 131, LV 134, LV 136, LV 140, LV 164 and LV 169 were compatible with six cultivars (Ouro Negro, Majestoso, Pérola, Supremo, Estilo and Cometa). Isolates LV 131, LV 134, LV 136 and LV 140 were collected from different points of the experimental field in Lambari-MG and probably belong to the same race, which prevailed in that field. Isolates LV 164 and LV 169 were collected more recently in another county (Ijaci, MG) and showed the same reaction. Isolate LV 111 was compatible with five cultivars and LV 117 with only three. Isolates LV 114, LV 115 and LV 148 were compatible with only two cultivars (Pérola and União).

The 12 cultivars (Fig. 1) also differed in their reaction to the 12 fungal isolates used in this study. Estilo was susceptible to 9 of 12 isolates, Ouro Negro, Majestoso and Cometa were susceptible to eight, Supremo was susceptible to seven and União to five isolates. Talismã, Valente, Madrepérola, Esplendor and Ouro Vermelho were resistant to all isolates. Pérola which is susceptible to all isolates, is commonly used as a susceptible control.

C. lindemuthianum shows great virulence diversity, mainly in the regions where Mesoamerican beans are grown, such as Brazil, where the predominant grain types are carioca and black beans (Costa *et al.*, 2010) The existence of variation within races of this fungus has also been ascertained using molecular markers (Mahuku and Riascos, 2004; Silva *et al.*, 2007; Ishikawa *et al.*, 2008b). Unfortunately, this molecular divergence cannot be associated with differences in pathogenicity, as the markers are not linked with virulence genes.

The use of new differential cultivars to mitigate the problem of isolate discrimination was suggested by Davide and Souza (2009). This is, however, difficult to implement because it implies a worldwide expert discus-

Table 2. Common bean cultivars and their corresponding market class from the germplasm collection of the Universidade Federal de Lavras used to differentiate isolates of race 65 of the anthracnose pathogen.

	Cultivar	Grain Type
A	Ouro Negro	Black
B	BRSMG Majestoso	Carioca
C	Pérola	Carioca
D	BRSMG Talismã	Carioca
E	BRS Valente	Black
F	Madrepérola	Carioca
G	BRS Supremo	Black
H	BRS Estilo	Carioca
I	BRS Cometa	Carioca
J	BRS Esplendor	Black
K	Ouro Vermelho	Red
L	BRSMG União	Jalo

Table 3. Average score of the compatible reaction of bean lines inoculated with different isolates of *Colletotrichum lindemuthianum* belongs to race 65. Values below and above 3 were considered as resistant and susceptible, respectively.

Cultivar Isolate	2^{0*}	2^{6}	A	B	C	D	E	F	G	H	I	J	K	L	Race[1]
LV 111	4^{+}	8.1^{+}	8.2^{+}	7.7^{+}	3.9^{+}	1^{-}	1^{-}	1^{-}	1^{-}	7.6^{+}	6.9^{+}	1^{-}	1^{-}	1^{-}	65.3
LV 114	9^{+}	8^{+}	1^{-}	1^{-}	4.8^{+}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	7^{+}	65.8
LV 115	9^{+}	6.2^{+}	1^{-}	1^{-}	3.8^{+}	2^{-}	1^{-}	1^{-}	1.7^{-}	1^{-}	1^{-}	1^{-}	1^{-}	8.5^{+}	65.8
LV 117	9^{+}	5^{+}	1^{-}	1^{-}	3.8^{+}	1^{-}	1^{-}	1^{-}	2.8^{-}	4^{+}	1^{-}	1^{-}	1^{-}	7.7^{+}	65.9
LV 120	9^{+}	9^{+}	7.3^{+}	7.4^{+}	5^{+}	1^{-}	1.7^{-}	1^{-}	6.3^{+}	9^{+}	7.5^{+}	1^{-}	1^{-}	7.5^{+}	65.15
LV 131	6.5^{+}	6.7^{+}	8.7^{+}	8.2^{+}	4.9^{+}	1^{-}	1^{-}	1^{-}	8^{+}	8.1^{+}	6.3^{+}	1^{-}	1^{-}	1^{-}	65.7
LV 134	4^{+}	6^{+}	9^{+}	7.5^{+}	4.2^{+}	1^{-}	1^{-}	1^{-}	3.9^{+}	4.7^{+}	4.6^{+}	1.6^{-}	1^{-}	1^{-}	65.7
LV 136	6.7^{+}	8^{+}	8.1^{+}	6.6^{+}	4.5^{+}	1^{-}	1^{-}	1^{-}	3.3^{+}	7.7^{+}	7.1^{+}	1^{-}	1^{-}	1^{-}	65.7
LV 140	8.4^{+}	8.5^{+}	4.4^{+}	5^{+}	5.9^{+}	1^{-}	1^{-}	1^{-}	5.6^{+}	5.6^{+}	5.4^{+}	1^{-}	1^{-}	1^{-}	65.7
LV 148	7.2^{+}	8^{+}	1^{-}	1^{-}	3.3^{+}	1^{-}	1.3^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	7.4^{+}	65.8
LV 164	7.6^{+}	8.5^{+}	9^{+}	8.5^{+}	6.25^{+}	1^{-}	1^{-}	1^{-}	8.3^{+}	7.5^{+}	8.3^{+}	1^{-}	1^{-}	1^{-}	65.7
LV 169	6.9^{+}	9^{+}	8.8^{+}	8.8^{+}	9^{+}	1^{-}	2.1^{-}	2.2^{-}	8^{+}	8.2^{+}	9^{+}	1.2^{-}	1^{-}	1^{-}	65.7

- Incompatible reaction (resistant); + Compatible reaction (susceptible); 2^{0}* – Michelite; 2^{6} – México 222; A – Ouro Negro; B – Majestoso; C – Pérola; D – Talismã; E – Valente; F – Madrepérola; G – Supremo; H – Estilo; I – Cometa; J – Esplendor; K - Ouro Vermelho; L – União. [1]Race classification using following commercial line and binary number: 2^{0} – Estilo, 2^{1} – Majestoso, 2^{2} - Supremo, 2^{3} - União, 2^{4}- Valente, 2^{5} – OuroVermelho, 2^{6} – Madrepérola, 2^{7} –Talismã.

sions concerning *C. lindemuthianum*, it could take a long time and cause confusion to match past studies with future works.

An alternative and more pragmatic approach could be the inclusion of additional common bean lines to differentiate isolates classified as belonging to race 65, as it allows solving a regional problem. The features of these cultivars may render them good testers in experiments, allowing the rapid assessment and discovery of new races of anthracnose in the field. Figure 1 shows that four cultivars were necessary to differentiate the 12 isolates under investigation: (i) Estilo; (ii) Ouro Negro, Majestoso or Cometa that had the same reaction; (iii) Supremo and (iv) União.

The presence of a gene for resistance to anthracnose (Co-10) had already been identified in cv. Ouro Negro, as it was the susceptibility to some isolates of race 65 (Alzate-Marin *et al*., 2003). Valente, Talismã and Madrepérola, although being resistant to all 12 isolates in this study, were susceptible to some strains used by Davide and Souza (2009), coming from different geographical regions, as stated by the authors. Therefore, these lines could be used to discriminate different isolates from other regions.

Thus, for the identification of the pathogenic variability of isolates within race 65, we suggest the following common bean lines and respective binary numbers: Estilo (2^{0}), Majestoso (2^{1}), Supremo (2^{2}), União (2^{3}), Valente (2^{4}), Ouro Vermelho (2^{5}), Madrepérola (2^{6}) and Talismã (2^{7}). Using this new approach, the isolates of race 65 would be classified according to Table 3. For example, isolate LV 120 would be classified as 65.15 because it was compatible with cvs Estilo, Majestoso, Supremo and União. The adoption of this nomenclature would help the breeder to choose the most virulent isolates classified as race 65 in the search of a more durable resistance. All of these cultivars are well-adapted to the Brazilian conditions and are commercially available, which facilitates their utilization. Future work should anyhow be conducted for the identification of new resistance genes present in these cultivars, as it was done for other common bean lines (Alzate-Marin *et al*., 2003; Gonçalves-Vidigal *et al*., 2009).

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