

**IDENTIFICATION OF TWO ALLELES AT LOCUS CO-1
IN COMMON BEAN ACCESSIONS USING THREE RACES
OF *Colletotrichum lindemuthianum***

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Abstract

Kiryakov, I., D. Genchev, 2006. Identification of two alleles at locus Co-1 in common bean accessions using three races of *Colletotrichum lindemuthianum*.

In common bean, alleles at locus *Co-1* provides resistance to Middle America (MA) races of *Colletotrichum lindemuthianum*, among them being race 81, which is widespread in Bulgaria. Up to now four alleles have been identified at the locus *Co-1*. Three of them were present in the differential cultivars “MDRK” (*Co-1*), “Perry Marrow” (*Co-1*³) and “Kaboon” (*Co-1*²). Races 6 and 2 of the fungus were virulent to the alleles present in the first two varieties. This study considers the reaction of 190 common bean accessions to races 81, 6 and 2 of *C. lindemuthianum* with the aim to select cultivars and lines possessing alleles *Co-1* and *Co-1*³. The plants were grown in a greenhouse at 20-25° C day temperature and 18-20° C night temperature. Plants were sprayed with spore suspension 10⁶ spores/ml seven days after germination. Spore mass of one isolate from each race of the fungus - 81, 6 and 2, was used as inoculum. Following inoculation, plants were transferred to a moist chamber for 72 hours at 20-22/16-19° C day/night temperature. Disease reaction was read 7-10 days after inoculation according to a 9-degree scale: 1-3 - resistant, 5-9 - susceptible. Twenty-six of the investigated accessions demonstrated resistance to race 81. Accessions “IIRR 7585”, “Trudovets” and “Vranya 1” were susceptible to races 2 and 6; this is an indication that their resistance to race 81 is controlled by allele *Co-1*. Cultivars “Laker”, “Montcolm”, “Mecosta” and “Red Hawk”, and lines “SEQ 1005” and “ICA Linea 64” were susceptible to race 6 and resistant to race 2. These results showed that their resistance to race 81 is controlled by allele *Co-1*³. The above accessions are suitable donors for resistance to the MA races of the fungus widespread in Bulgaria.

Key words: Common bean – Anthracnose – Race - Race-specific gene

Резюме

Киряков, И., Д. Генчев, 2006. Идентифициране на два алела от локус Co-1 в образци обикновен фасул използвайки три раси на *Colletotrichum lindemuthianum*

Ген *Co-1* при обикновения фасул осигурява устойчивост към расите от Middle America (MA) популациите на *Colletotrichum lindemuthianum*, между които е и разпространената в България раса 81. До този момент са идентифицирани четири алела на гена в локус *Co-1*. Три от тях са представени в сортове “MDRK” (*Co-1*), “Perry Marrow” (*Co-1*³) и “Kaboon” (*Co-1*²) включени в диференциация ключ на антракнозата. Раси 6 и 2 на гъбата са вирулентни към алелите представени в първите

два сорта. В настоящото изследване е проучена реакцията на 190 образци обикновен фасул към раси 81, 6 и 2 на *C. lindemuthianum* с цел отбор на сортове и линии притежаващи алели Co-1 и Co-1³. Образците са отгледани в оранжерия при 20-25°C дневна и 18-20°C нощна температура. Растенията са опръскани със спорова суспензия 10⁶ спори/ ml седем дни след поникване. За инокулум е използвана спорова маса от по един изолат на раси 81, 6 и 2 на гъбата. След инокулиране, растенията са поставени във влажна камера за 72 h при 20-22/16-19°C дневна, респективно нощна температура. Болестната реакция е отчетена 7-10 дни след инокулиране по 9 бална скала: 1-3, устойчиви, 5-9, чувствителни. Устойчивост към раса 81 показват 26 от проучваните образци. Образци “ИИРР 7585”, “Трудовец” и “Враня 1” са чувствителни към раси 2 и 6 което показва, че устойчивостта им към раса 81 се контролира от алел Co-1. Сортове “Laker”, “Montcolm”, “Mecosta” и “Red Hawk” и линии “SEQ 100”5 и “ICA Linea 64” са чувствителни към раса 6 и устойчиви към раса 2. Тези резултати предполагат, че устойчивостта им към раса 81 се контролира от алел Co-1³. Посочените образци са подходящи донори за устойчивост към разпространените у нас МА расите на гъбата.

Ключови думи: Обикновен фасул – Антракноза – Раси – Расово-специфични гени

INTRODUCTION

Anthracoze causes serious problems to bean production in the mountainous and semi-mountainous regions of Bulgaria. The disease is of sporadic occurrence in the plain areas but under conditions favorable for its development it can cause considerable damages. The cause agent of the disease, the hemibiotrophic fungus *Colletotrichum lindemuthianum* is characterized by a great virulent diversity. Up to now over 100 physiological races of the fungus have been identified worldwide by using a standard differential set (Rodrigues-Guerra et al., 2003). In Bulgaria seven races have been identified but the main races are 81, 2 and 6 (Kiryakov & Genchev, 2004).

The resistance of common bean to anthracnose is controlled by race-specific genes localized at nine independent loci (Kelly and Vallejo, 2004). Among all loci characterized as conditioning resistance to the fungus, Co-1 is the only locus originating in the Andean gene pool in common bean (Gepts, 1988). Four alleles were identified at the locus Co-1 and three of them are present in the differential cultivars “Michigan Dark Red Kidney” (MDRK), “Perry Marrow” and “Kaboon” (Kelly & Vallejo, 2004). The alleles at the locus Co-1 are efficient against the Middle America (MA) races of the pathogen, race 81 being among them.

The use of molecular markers and physiological races allows identifying of one or more race-specific genes in individual genotypes (Pastor-Corrales & Stavely, 2002). According to Silbernager (1994), the use of 4 strains of BCMV and BCMNV allows to identify race-specific genes in bean accessions, as well as their combination with the inhibitory gene *I*. Pastor-Corrales and Stavely (2002) involved 8 races of *Uromyces appendiculatus* to identify genes *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11* in bean accessions.

This study investigates the reaction of common bean accessions to three races of *C. lindemuthianum* with the aim to select cultivars and lines which possess alleles Co-1 and Co-1³.

MATERIAL AND METHODS

Plant material. The investigation included 190 bean accessions (*Phaseolus vulgaris* L.) from the collection of DAI - General Toshevo. Twelve seeds from each accession were sown in plastic pots of 45 x 30 x 8 cm containing soil-and-sand mixture (3:1), 12 accessions per pot, in two replications. The plants were grown in a greenhouse at 20-25° C day

temperature and 18-20° C night temperature.

Preparation of inoculum. The investigation included a monospore isolate from each of races 81, 6 and 2. The isolates were cultivated on modified Mathur's medium (dextrose, 8 g/l; MgSO₄ 7 H₂O, 2.5 g/l; KH₂PO₄, 2.7 l/l; peptone, 2.4 g/l; yeast agar, 2.0 g/l; agar 16 g/l) (Balardin & Kelly, 1998) at 18±1° C at dark for 10 days. The spore substance was washed with sterile distilled water and after filtration through sterile gauze the suspension was reduced to concentration 10⁶ spores/ml.

Inoculation and reading of reaction. Seven days after germination, plants were pulverized with spore suspension from isolate of race 81 and transferred to moist chamber for 72 h at 20-22/16-19° C day/night temperature. The reaction of the accessions was read 7-10 days after inoculation according to a 9-degree scale (Genchev & Kiryakov, 1994). The accessions with disease reaction 1-3 were considered resistant, and those with reaction 5-9 were susceptible. The accessions resistant to race 81 were inoculated with races 6 and 2 according to the same methodology.

RESULTS

Twenty-six of the investigated accessions showed resistance to race 81 of *C. lindemuthianum* (Table 1).

Table 1. Reaction of 26 bean accessions from the collection of DAI-General Toshevo to races 2, 6 and 81 of *C. lindemuthianum*

Accession	Race 81	Race 6	Race 2	Presence of the locus <i>Co-1</i> / allele
IIRR 7585	R*	S	S	<i>Co-1</i>
Trudovetc	R	S	S	<i>Co-1</i>
Vrany 1	R	S	S	<i>Co-1</i>
SEQ 1005	R	S	R	<i>Co-1</i> ³
ICA Linea 64	R	S	R	<i>Co-1</i> ³
Red Hawk	R	S	R	<i>Co-1</i> ³
Mecosta	R	S	R	<i>Co-1</i> ³
Montcolm	R	S	R	<i>Co-1</i> ³
Laker	R	S	R	<i>Co-1</i> ³
DRK 134	R	R	S	<i>Co-1</i> ; ?
Dunav 1	R	R	S	<i>Co-1</i> ; ?
WA 1921-1-1	R	R	S	<i>Co-1</i> ; ?
Chinook 2000	R	R	R	-
Beluga	R	R	R	-
Astria	R	R	R	-
Contessa	R	R	R	-
Titan	R	R	R	-
Silvert	R	R	R	-
Amanda	R	R	R	-
Imuna	R	R	R	-
A 195	R	R	R	-
A 475	R	R	R	-
Drezden	R	R	R	-
MX 18-34-1	R	R	R	-
NAB 19	R	R	R	-
A 769	R	R	R	-

*R –resistant; S - susceptible

Twelve of these cultivars/lines were susceptible to race 6 and/or 2. Accessions “IIRR 7585”, “Trudovets” and “Vranya 1” were susceptible to both races. The cultivars “Laker”,

“**Montcolm**”, “**Mecosta**” and “**Red Hawk**”, and lines “**SEQ 1005**” and “**ICA Linea 64**” were susceptible to race 6 and resistant to race 2. Cultivar “**Dunav**” and lines “**DRK 134**” and “**WA 1921-1-1**” were susceptible to race 2 and resistant to race 6. The other 14 accessions were resistant to the three races of the fungus.

DISCUSSION

Three of the four alleles identified at the locus *Co-1* are present in the differential cultivars “**MDRK**”, “**Perry Marrow**” and “**Kaboon**” (Table 2) (Kelly & Vallejo, 2004). Allele *Co-1* is present in cultivar “**MDRK**”, which has binary number 2 in the differential set (Melloto & Kelly, 2000). This cultivar is susceptible to races 2 and 6. Allele *Co-1²* is present in cultivar “**Kaboon**” and has binary number 32. This cultivar is resistant to races 2 and 6 and therefore it is not possible to identify allele *Co-1²* through the above races. The third allele at the locus *Co-1* - *Co-1³*, is present in cultivar “**Perry Marrow**” and has binary number 4. “**Perry Marrow**” is susceptible to race 6 and resistant to race 2. Race 81 is avirulent to these cultivars, and to the three alleles in locus *Co-1*, respectively.

Table 2. Reaction of 12 bean varieties included in the differential set of anthracnose to races 81, 6 and 2 of *C. lindemuthianum*

Differential cultivars	Host genes	Binary number	Gene Pool*	Race 81	Race 6	Race 2
				<i>vir/avr genes</i> **	<i>vir/avr genes</i>	<i>vir/avr genes</i>
Michelite	-	1	MA	+	-	-
MDRK	<i>Co-1</i>	2	A	-	+	+
Perry Marrow	<i>Co-1³</i>	4	A	-	+	-
Cornell 49242	<i>Co-2</i>	8	MA	-	-	-
Widusa	-	16	MA	+	-	-
Kaboon	<i>Co-1²</i>	32	A	-	-	-
Mexico 222	<i>Co-3</i>	64	MA	+	-	-
PI 207 262	<i>Co-4³, Co-9</i>	128	MA	-	-	-
TO	<i>Co-4</i>	256	MA	-	-	-
TU	<i>Co-5</i>	512	MA	-	-	-
AB 136	<i>Co-6, co-8</i>	1024	MA	-	-	-
G 2333	<i>Co-4², Co-5 Co-7</i>	2048	MA	-	-	-
G 2333	<i>Co-4², Co-5 Co-7</i>	2048	MA	-	-	-

* A - Andean ; MA – Middle American

** + virulent genes; - avirulent genes

In testing the reaction of 190 bean accessions to race 81 of *C. lindemuthianum*, it was established that 26 lines/cultivars were resistant to the race (Table 1). The susceptible reaction of 9 of these accessions revealed that they possessed locus *Co-1* with one of the two alleles - *Co-1* and *Co-1³*. The susceptibility of “**IIRR 7585**”, “**Trudovets**” and “**Vranya 1**” to race 2 confirmed the presence of allele *Co-1* in them. The resistance of cultivars “**Laker**”, “**Montcolm**”, “**Mecosta**” and “**Red Hawk**” and of lines “**SEQ 1005**” and “**ICA Linea 64**” to race 2 confirmed the presence of allele *Co-1³*. Since race 2 has avirulent genes with regard to allele *Co-1³*, it is possible that locus *Co-1* is represented in two allele conditions of the gene (*Co-1* and *Co-1³*) in some of these accessions.

Contradicting results were observed in cultivar “**Dunav 1**” and lines “**DRK 134**” and “**WA 1921-1-1**” (Table 1). These accessions were resistant to races 81 and 6, but susceptible to race 2. Their susceptibility to race 2 indicated the presence of locus *Co-1*. In this case their reaction to race 6, possessing a virulent gene with respect to this allele, should be susceptible, too. The reason for these contradictory data is the lack of thorough information on the virulent/avirulent genes in the respective races. The virulent phenotype of

the two races is determined by the reaction of the cultivars in the differential set (Table 2). It could be presumed that there are differences between the two races which can not be identified by the differential set of cultivars. These accessions probably possess more than one race-specific gene determining their resistance to anthracnose race 81, and one of them is allele *Co-1*.

The resistance of the other 14 accessions presented in Table 1 to the three races implies presence of other race-specific genes, including alleles from locus *Co-1* (*Co-1*² or *Co-1*⁴) which are to be identified additionally.

A strategy for the management of race-specific resistance to plant disease is to pyramid two or more resistance genes into a single plant genotype (Mundt, 1990). The alleles at the locus *Co-1* of bean provide resistance to the MA races of *C. lindemuthianum*. This makes the accessions possessing this locus a suitable donor of resistance in breeding of beans of MA origin, particularly in those regions where the MA races of the anthracnose fungus predominate (Balardin *et al.*, 1997). The results obtained showed that 12 of the investigated 190 bean accessions have locus *Co-1*. The resistance of accessions “**IIRR 7585**”, “**Trudovets**” and “**Vranya 1**” to race 81 is controlled by allele *Co-1*. Accessions “**Laker**”, “**Montcolm**”, “**Mecosta**”, “**Red Hawk**”, “**SEQ 1005**” and “**ICA Linea 64**” possess allele *Co-1*³. These accessions can be included in the breeding program for resistance to the MA races of the pathogen widespread in Bulgaria. Cultivar “**Dunav 1**” and lines “**DRK 134**” and “**WA 1921-1-1**” have more than one race-specific gene, one of them being *Co-1*. Further researches will be necessary to identify these genes.

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Идентифициране на два алела от локус Co-1 в образци обикновен фасул
използвайки три раси на *Colletotrichum lindemuthianum*
