Universidade Estadual de Maringá

NUPAGRI

Co-segregation Analysis of Genes Conferring Resistance to Angular Leaf Spot, Anthracnose, and Rust Pathogens in Common Bean

M.C. Gonçalves-Vidigal[1], P.S. Vidigal Filho[1], G. Valentini[1], G. F. Lacanallo[1], L. L. Sousa[1], P. Gepts[2], P.B. Cregan[3], Q. Song[3], M.A. Pastor-Corrales[3]

**Corresponding author: mcgvidigal@uem.br*

COMMON BEAN DISEASES

Several diseases are major limitations to common bean production among them we have Angular leaf spot (ALS), Anthracnose (ANT) and Rust.

ANGULAR LEAF SPOT

The angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola (Sacc.)* Ferraris is one of the most important common bean diseases.

GENETIC RESISTANCE TO ALS

Resistance to ALS in common bean is conferred primarily by the major independent genes identified as symbol *Phg*

RESISTANCE GENES TO ALS

Three resistance major resistance genes to ALS have been mapped to the integrated bean linkage map: *Phg-1*; *Phg-2*, and *Phg-3*.

Phg-1 Gene

The *Phg-1* gene present in Andean cultivar AND 277 is located on linkage group Pv01, and it is linked to the *Co-1*^{4} anthracnose resistance gene.

Phg -2 Gene

The *Phg-2* gene present in Mesoamerican cultivar Mexico 54 is located on linkage group Pv08, and linked to the $OPE04_{700}$ molecular marker.

Phg-3 Gene

The *Phg-3* gene present in Mesoamerican cultivar Ouro Negro is located on LG Pv04, and it is linked with *Co-3*^{4} gene for anthracnose and *Ur-14* for rust resistance.

ANTHRACNOSE

Caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is among the most widespread common bean disease in the tropics.

GENETIC RESISTANCE TO ANTRACNOSE

- **19 anthracnose resistance loci have been identified as *Co symbol*. Multiple alleles exist at the *Co-1, Co-3, Co-4* and *Co-5* loci .**

Relevance of this Study

Phg-1/Co-1^4 locus

The *Phg-1* gene *and Co-1^4 locus* are tightly linked (0.0 cM) to each other on linkage group Pv01 (Gonçalves-Vidigal et al., 2011).

Relevance of this Study

Phg-3/Co-3^4

The *Phg-3* gene and *Co-3^4 locus* in Ouro Negro co-segregated for resistance to Anthracnose and ALS (Gonçalves-Vidigal et al.,2013).

RUST

Rust is a bean fungal disease caused by *Uromyces appendiculatus.*

Resistance Genes to Rust

- **Nine dominant rust resistance genes have been identified and named as *Ur*.**

OBJECTIVE

The purpose of this study was to gather data from co-segregation analysis of the ALS, ANT and Rust resistance genes using F_2 populations and/or two identical sets of $F_{2:3}$ families.

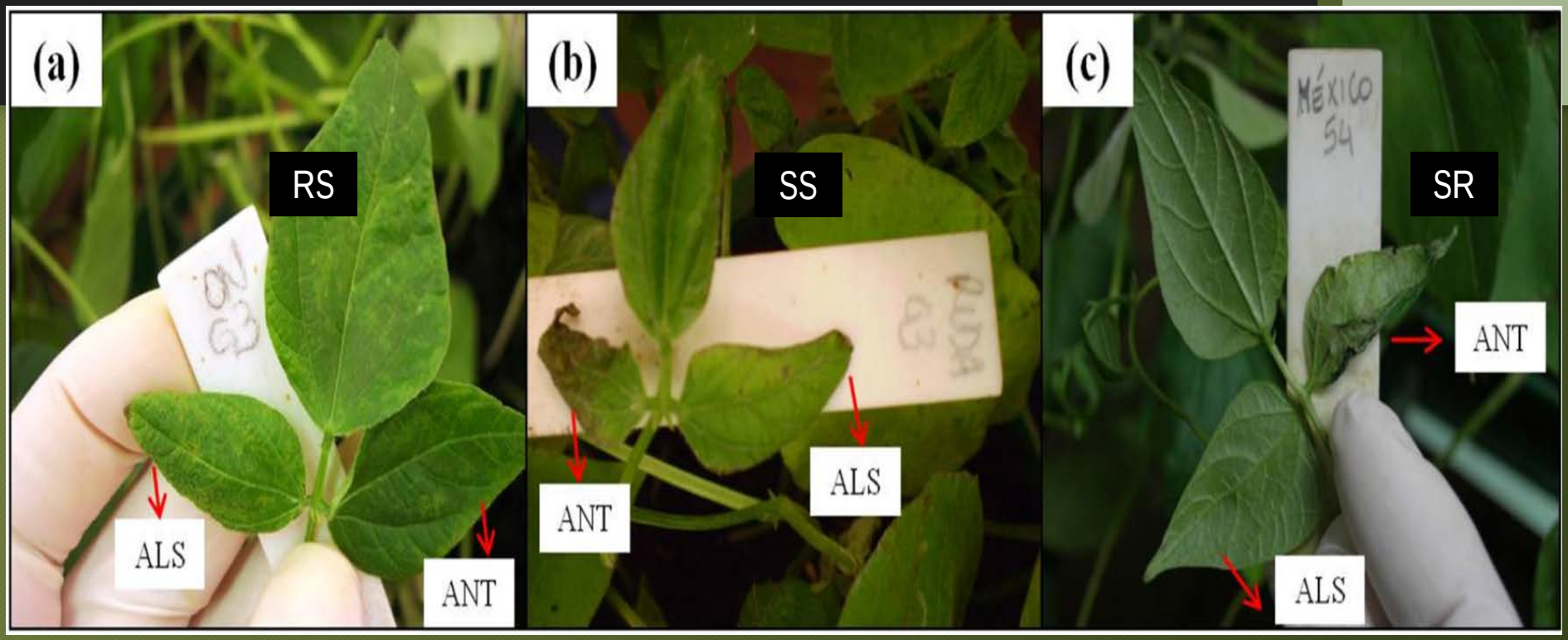
MATERIAL AND METHODS

This work consisted of three studies involving co-segregation analysis.

INOCULATIONS

The simultaneous and separated inoculations were performed according to the methodology proposed by Gonçalves-Vidigal et al. (2011) and Gonçalves-Vidigal et al. (2001), respectively.

INOCULATIONS



Simultaneous inoculation with *C. lindemuthianum* and *P. griseola*: (a) Ouro Negro cultivar resistant to Anthracnose and susceptible to Angular Leaf Spot; (b) Rudá cultivar susceptible to both pathogens; (c) Mexico 54 cultivar susceptible to ANT and resistant to ALS.

Evaluation of Populations

The visual assessments were performed using the severity scales proposed by:
Inglis et al. (1988) for ALS,
Pastor-Corrales et al. (1995) for Anthracnose,
Stavely et al. (1983) for Rust.

FIRST EXPERIMENT

Co-segregation analysis involving *Phg*-1 gene and *Co-1^4 locus* in AND 277.

Cross	Generation	Races inoculated
AND 277 × Rudá	127 F_2 individuals	73 of *C. lindemuthianum* 63-23 *of P. griseola* ***(simultaneously)***
AND 277 × Ouro Negro	129 F_2 individuals	65 of *C. lindemuthianum* 63-23 *of P. griseola* ***(simultaneously)***
AND 277 × Ouro Negro	71 $F_{2:3}$ families	2047 of *C. lindemuthianum* 63-23 *of P. griseola* ***(separately)***

SECOND EXPERIMENT

Co-segregation analysis involving *Phg*-3 gene and *Co-3*^{4} *locus* in Ouro Negro cultivar.

Cross	Generation	Races inoculated
Rudá × Ouro Negro	112 F_2 individuals	73 of *C. lindemuthianum* 63-39 *of P. griseola* ***(simultaneously)***
AND 277 × Ouro Negro	63 $F_{2:3}$ families	7 of *C. lindemuthianum* 63-39 *of P. griseola* ***(simultaneously)***

THIRD EXPERIMENT

Co-segregation analysis involving *Phg*-3/*Co-3*^{4} and *Ur-14 gene* in Ouro Negro cultivar.

Cross	Generation	Races inoculated
Rudá × Ouro Negro	105 $F_{2:3}$ families	73 of *C. lindemuthianum* 41, 47, 53 and 58 *of U. appendiculatus* ***(separately)***

MATERIAL AND METHODS:
Mapping of *Ur-14* and *Co-3*^{4}/Phg-3

The DNA previously isolated from each $F_{2:3}$ families Rudá × Ouro Negro was used for genotyping with 5,399 SNP markers on the BARCBean6K_3 Illumina BeadChip following the Infinium HD Assay Ultra Protocol.

Mapping of *Ur-14* and *Co-3*^{4}/Phg-3

Linkage analyses mapped the SSRs, the *Ur-14* and *Co-3*^{4}*/Phg-3* into linkage group Pv04 using the JoinMap 4.1 software based on the regression mapping method and the Kosambi map function.

RESULTS AND DISCUSSION

FIRST EXPERIMENT

Cross	Generation	Race	Observed ratio	Expected ratio	χ^2	P value
AND 277 × Rudá	F_2	73	95:32	92.25:31.75	0.003	0.96
		63-23	95:32	92.25:31.75	0.003	0.96
AND 277 × Ouro Negro	F_2	65	96:33	96.75:0:0:32.25	0.023	1.0
		63-23	96:33	96.75:0:0:32.25	0.023	1.0
AND 277 × Ouro Negro	$F_{2:3}$	2047	20:33:18	17.75:35.5:17.75	0.46	0.79
		63-23	20:33:18	17.75:35.5:17.75	0.46	0.79

FIRST EXPERIMENT

In the First Experiment the absence of recombinant revealed that *Co-1^4 and Phg-1* are tightly (0.0) on Pv01, conferring resistance to races 65, 73, and 2047 of ANT and to race 63-23 of ALS pathogens.

SECOND EXPERIMENT

Cross	Generation	Race	Observed ratio	Expected ratio	χ^2	P value
Rudá × Ouro Negro	F_2	73	84:28	84:28	0.00	1.00
		63-39	83:29	84:28	0.04	0.83
AND 277 × Ouro Negro	$F_{2:3}$	7	14:34:15	15.75:31.5:15.75	0.43	0.81
		63-39	14:34:15	15.75:31.5:15.75	0.43	0.81

SECOND EXPERIMENT

In the second experiment, co-segregation analysis revealed that *Phg-3* and *Co-3*^{4} were inherited together, conferring resistance to races 7 and 73 of ANT and race 63-39 of ALS.

THIRD EXPERIMENT

Cross	Generation	Disease	Race	Observed ratio	χ^2	P value
Rudá × Ouro Negro	$F_{2:3}$	ANT	73	29:50:26	0.41	0.81
		Rust	41, 47, 53, 58	29:51:25	0.39	0.82

S - Susceptible; R - resistant

(R)

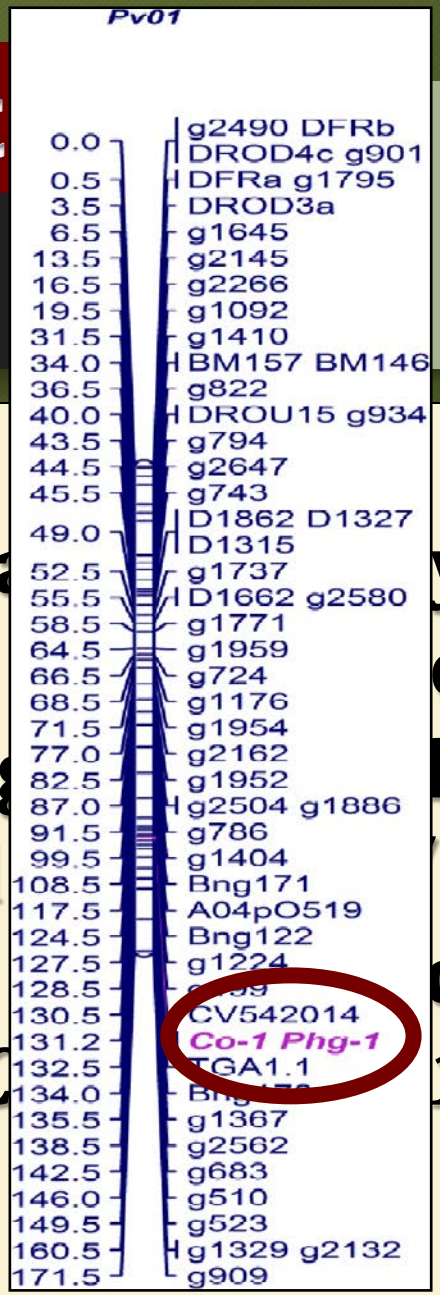
THIRD EXPERIMENT

The results revealed that each pair of $F_{2:3}$ set families showed exactly the same scores when each one was tested independently with two pathogens, except for one family. Indicating that *Phg-3/Co-3*^{4} was tightly linked to the *Ur-14 gene* at 0.5 cM.

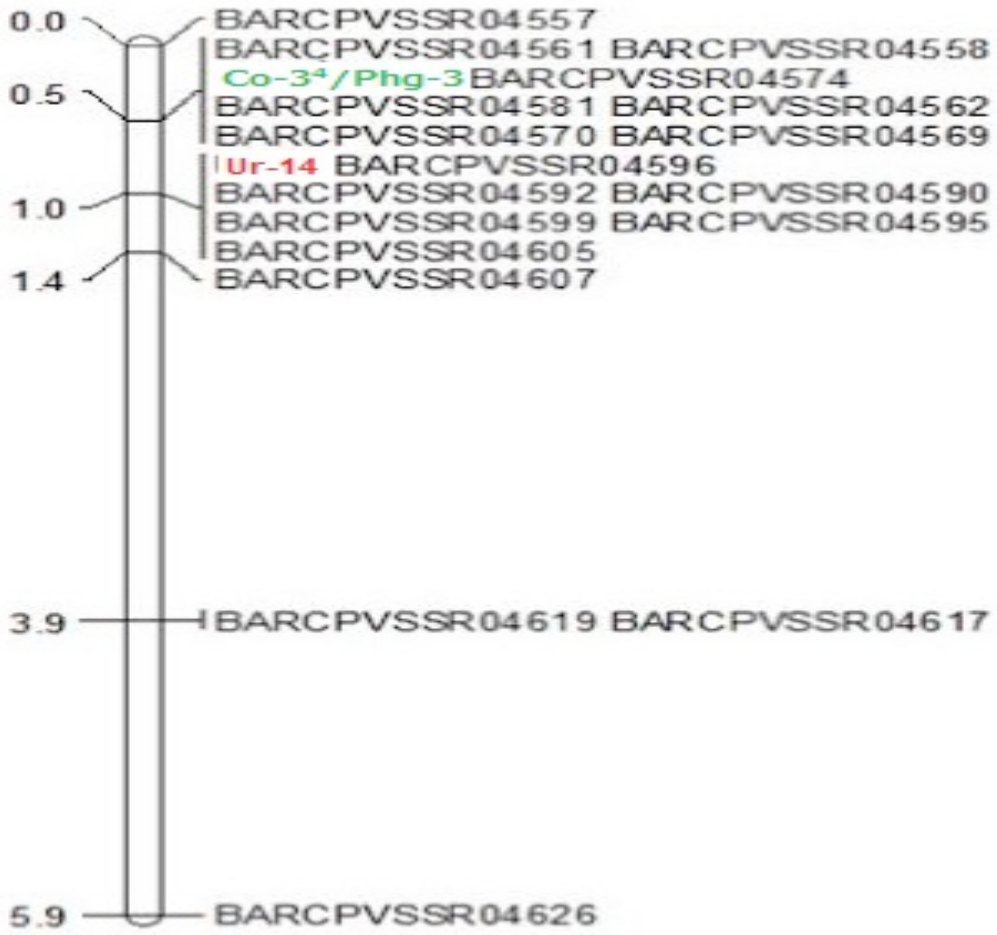
GENES Relevant to this St

Phg-1/Co-1^4 locus

The *Co-1^4* and *Phg-1* locus a y linked (0.0 cM) to each other e group Pv01 (Gonçalves-Vidig l. 2011). The CV542014^{450} and 0 molecular markers are linked d 1.3 cM, respectively from the *C 1* locus in coupling phase.



MAPPING OF THE GENES



Genetic linkage map of the *Ur-14* and *Co-3⁴/Phg-3* region of common bean linkage group Pv04.

CONCLUSIONS

- These results also will reduce the time and cost of pyramiding the *Co-3*^{4}*/Phg-3* and *Ur-14* genes into commercial common bean cultivars.

CONCLUSIONS

- In addition, the many more effective SSR molecular markers were found linked to the above-mentioned gene cluster (*Co-3*^{4}*/Phg-3/Ur-14*) and these should be recommended for use in breeding programs.

Thanks to

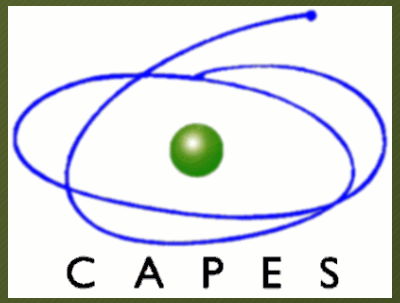
The 20th Anniversary of NUPAGRI, 2014

International Seminars, 2015

ACKNOWLEDGEMENTS

UEM

UEM



WELCOME TO:

Maringá, Paraná state, Brazil

Universidade Estadual de Maringá