

## MOLECULAR MARKER ASSISTED DIFFERENTIATION OF NEW BEAN BREEDING LINES

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### Introduction

In recent years, various bean breeding lines with similar morphological traits were developed in the North of Spain by SERIDA (Servicio Regional de Investigación y Desarrollo Alimentario). These lines carry different anthracnose resistance genes and exhibit different growth habits. Initially two new breeding lines were developed that carried the anthracnose resistance genes *Co-2* and *Co-9*, originating from the parental donors S34 and A493 respectively. These new breeding lines were phenotypically identical to Andecha, the most important commercial bean in the North of Spain. This is an important advantage in our country where 90% of the cultivated bean have Type IV growth habit type and required a trellis for support or corn to climb. We also developed a new line with determinate growth habit, named Xana that is very similar to Andecha. The breeding programs were combined to develop two additional breeding lines with determinate growth habit that carry the anthracnose resistance genes *Co-2* and *Co-9*.

The combined breeding program described has developed 5 new breeding lines that are phenotypically identical to Andecha, the most commercial bean in the North of Spain. All these materials with different genetic combinations cannot be easily differentiated, therefore molecular markers provide a useful tool for a quick differentiation instead of morphological differentiation. We have chosen molecular markers based in “Polymerase Chain Reaction” as RAPD, SCAR or CAP, because of their easy use (Ortiz et al, 2000). The molecular markers, linked to specific genes, have also the important advantage that most are already mapped. There are already reported quite many molecular markers linked to specific mapped genes that can be used with a wide spectrum of bean materials. The objective of this work was to examine the utility of some molecular markers linked to the known genes *Co-2*, *Co-9* and *Fin*, for the differentiation of 6 materials morphologically identical.

### Results

At the beginning of the work we collected and tested many molecular markers linked to the selected genes (Table 1). Some of the markers were previously developed by our group some years ago, and others come from literature. From a total number of 22 molecular markers analyzed, only 7 were monomorphic in our material. The other 15 displayed polymorphism, and the most useful markers that differentiated our lines are shown on Table 2. Two markers linked to *Co-2* gene and one molecular marker linked to *Co-9*, that worked in our material were chosen.

**Table 1.** Molecular markers analyzed for this study.

Marker	Size (bp)	Type	Gene	Phase	Reference
OQ04	600	RAPD	<i>Co-2</i>	Repulsion	Méndez de Vigo, 2001
OQ04	1440	RAPD	<i>Co-2</i>	Coupling	Young & Kelly, 1996
B355	1000	RAPD	<i>Co-2</i>	Coupling	Young & Kelly, 1996
SCAreoli	500	SCAR	<i>Co-2</i>	Coupling	Méndez de Vigo, 2001
SCAreoli	1000	CAP	<i>Co-2</i>	Coupling	Geffroy et al, 1998
SB12	350	SCAR	<i>Co-9</i>	Coupling	Méndez de Vigo et al 2002
OI19	500	RAPD	<i>Co-9</i>	Coupling	Méndez de Vigo, 2001
OZ10	800	RAPD	<i>Fin</i>	Coupling	Pañeda, 2001
OA04	1100	RAPD	<i>Fin</i>	Repulsion	Pañeda, 2001
OD08	1150	RAPD	<i>Fin</i>	Repulsion	Unpublished results
OI19	375	RAPD	<i>Fin</i>	Repulsion	Pañeda, 2001
OQ03	450	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OF16	1400	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OA17	600	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OA17	950	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OU12	450	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OU19	350	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OU19	450	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
ON12	800	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OV10	250	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OK19	450	RAPD	<i>Fin</i>	Coupling	Park et al, 1999
OT14	800	RAPD	<i>Fin</i>	Coupling	Park et al, 1999

All the molecular markers linked to *Fin* gene (Park et al, 1999) were monomorphic in our material. This is a frequent problem in common bean, because of the big differences between the genetics pools, therefore we initiated a search for new molecular markers linked to this gene. The F2 population proceeded from a cross between Andecha (Fin) and BRB130 (fin), and we characterized all the F2:3 families to detect homozygous and heterozygous individuals. The screening was conducted using BSA method (Michelmore, 1991) with around six hundred random Operon primers and sixteen were found to be significantly associated with the *Fin* gene (Pañeda, 2001). For the present work we have select some RAPD markers that proved useful in our material (Table 2).

**Table 2.** Results for the molecular markers that were polymorphic in our materials

Marker	Gene	Andecha	Xana	S34	A493	A1183	A1220	X1358	X1319
OQ04 <sup>600</sup>	<i>Co-2</i>	+	+	-	+	-	+	-	+
OQ04 <sup>1400</sup>	<i>Co-2</i>	-	-	+	+	+	-	+	-
SCAeroli <sup>500</sup>	<i>Co-2</i>	+	+	-	-	-	+	-	+
SCAeroli <sup>1000</sup>	<i>Co-2</i>	-	-	+	-	+	-	+	-
OI19 <sup>500</sup>	<i>Co-9</i>	+	+	-	-	+	-	+	-
SB12 <sup>350</sup>	<i>Co-9</i>	-	-	-	+	-	+	-	+
OI19 <sup>375</sup>	<i>Fin</i>	-	+	-	-	-	-	+	+
OZ10 <sup>800</sup>	<i>Fin</i>	+	-	+	+	+	+	-	-
OA04 <sup>1100</sup>	<i>Fin</i>	-	+	-	-	-	-	+	-
OD08 <sup>1150</sup>	<i>Fin</i>	-	+	-	-	-	-	+	+

We are also working with another architectural trait because plant architecture is an important trait in breeding programs for common bean. There are some commercial lines (Cimera) in the North of Spain that are morphological similar to Andecha and we need to develop new molecular markers to differentiate between future breeding lines. The main feature that differentiates these two commercial lines is the pod distribution. Cimera is a line with Type IVa growth habit, with the pods uniformly distributed along the plant, whereas Andecha has Type IVb growth habit, with pods mainly in the upper part of the plant (Debouck and Hidalgo, 1985). We studied the genetic of this trait, and we found evidence for a new locus involved in the genetic control of the pod distribution in the indeterminate climbing habits ( $p=0,68$  for the single dominant locus IVa).

The patterns for the polymorphic useful markers are show in Table 2, and from all of them we selected: OQ04<sup>600</sup> and OQ04<sup>1400</sup> linked to the anthracnose resistance gene *Co-2*, OI19<sup>500</sup> linked *Co-9* gene, and OI19<sup>375</sup>, OD08<sup>1150</sup> and OZ10<sup>800</sup> linked to *Fin* gene for further study. The results for the selected markers, confirm the presence of *Co-2* gene in A1183 and X1358 lines and *Co-9* gene in A1220 and X1319 lines. We can conclude with the present results that molecular markers can be used to differentiate without mistake genetically similar breeding lines.

## References

- Debouck D, R Hidalgo. 1985. Morfología de la planta de frijol común. En : Frijol: Investigación y producción. En: Eds: M López, F Fernández, A van Schoonhoven. CIAT, Cali, Colombia, Pg 7-42.
- Méndez de Vigo. 2001. Mapa genético de *Phaseolus vulgaris* L. y resistencia a antracnosis en faba granja asturiana. Tesis Doctoral. Universidad de Oviedo.
- Geffroy V, F Creusot, J Falquet, M Seignac, A-F Adam-Blondom, H Bannerot, P Gepts and M Dron. 1998. A family of LRR sequences in the vicinity of the *Co-2* locus for the anthracnose resistance in *Phaseolus vulgaris* and its potential use in marker-assisted selection. Theor Appl Genet, 96:494-502.
- Méndez de Vigo B, C Rodríguez, A Pañeda, R Giráldez and JJ Ferreira. 2002. Development of a SCAR marker linked to *Co-9* in common bean. BIC 45:116-117.
- Michelmore RW, Paran Y and Kesseli RV 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. USA 88: 9828-9832.
- Ortiz JM, I Aguinagalde, JP Martín. 2000. Identificación varietal. En Los marcadores genéticos en la mejora vegetal. Eds F Nuez y JM Carrillo. Edita SEG, SECH y U. Politécnica de Valencia, 515-559.
- Pañeda A. 2001. Búsqueda de marcadores moleculares ligados al gen *Fin* en *Phaseolus vulgaris*. Seminario de investigación. Universidad de Oviedo.
- Park SO, DP Coyne, JM Bokosi and JR Steadman. 1999. Molecular markers linked to genes for specific rust resistance and indeterminate growth habit in common bean. Euphytica 105:133-141.
- Young RA, and JD Kelly. 1996. RAPD markers flanking the *Are* gene for anthracnose resistance in common bean. Soc. Hort. Sci., 121 (1):37-41