Universidad de Oviedo

PROGRAMA DE DOCTORADO DE BIOLOGÍA FUNCIONAL Y MOLECULAR
DEPARTAMENTO DE BIOLOGÍA FUNCIONAL
UNIVERSIDAD DE OVIEDO

ANÁLISIS DE LA HERENCIA DE LA RESISTENCIA A TRES PATÓGENOS EN JUDÍA COMÚN (*Phaseolus vulgaris* L.)

TESIS DOCTORAL

NOEMÍ TRABANCO MARTIN
OVIEDO, 2014

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RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

1.- Título de la Tesis	
Español/Otro Idioma: Análisis de la herencia de la resistencia a tres patógenos en judía común (*Phaseolus vulgaris* L.)	Inglés: Study of the inheritance of resistance to three pathogens in common bean (*Phaseolus vulgaris* L.)

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RESUMEN (en español)

Esta Tesis Doctoral comprende cuatro capítulos en los que se ha profundizado en el conocimiento de la resistencia frente a tres patógenos que inciden en los cultivos de judía común (*Phaseolus vulgaris* L.) en el norte de España.

En los dos primeros capítulos el trabajo se centra en el estudio de la resistencia a oídio, causado por el hongo *Erysiphe diffusa*. La incidencia de esta enfermedad, que también afecta a muchos otros cultivos de interés, ha aumentado considerablemente en los cultivos de faba granja asturiana en las últimas campañas y la interacción *P. vulgaris-E. diffusa* era, hasta el momento, muy desconocida. El trabajo realizado en el primer capítulo comenzó por establecer un método de inoculación en condiciones controladas así como una escala de evaluación de síntomas, en la que se diferenciaron cinco respuestas o tipos de infección (TI0 a TI4). También se evaluaron un total de 245 genotipos de judía, entre los cuales sólo se identificaron seis que mostraron resistencia total al patógeno: Amanda, Belneb, Cornell 49242, Negro San Luis, Porrillo Sintético y la accesión local BGE003161. Finalmente se evaluaron diferentes poblaciones F_2 y $F_{2:3}$ derivadas del cruzamiento entre genotipos con distinta respuesta al patógeno, para estudiar el modo de herencia de la resistencia. Los resultados obtenidos mostraron, en todos los casos, segregaciones de tipo mendeliano. A partir de estos análisis se pudo deducir que el cultivar Porrillo Sintético presenta dos genes de resistencia dominantes e independientes, uno de los cuales produce resistencia total (TI0) y otro que otorga resistencia moderada, permitiendo un crecimiento moderado del patógeno sobre la hoja (TI3). Esta respuesta también se observó en la variedad Cornell 49242, aunque no se puede confirmar que se trate de los mismos genes.

El segundo capítulo aborda el estudio de la resistencia a oídio en la variedad Cornell 49242. Para ello se analizó la respuesta en una población de líneas recombinantes (RIL) derivada de cruzamiento entre esta variedad y la variedad de faba granja Xana, que es altamente susceptible al patógeno (TI4). Los resultados obtenidos confirmaron la

naturaleza cualitativa de la respuesta y se identificaron dos genes dominantes e independientes implicados en su control. Tanto los test de contingencia como el análisis de subpoblaciones y la disección genética realizados confirmaron la localización de ambos genes en dos regiones relacionadas con resistencia a antracnosis: el gen *Pm1*, en el cluster Co-2, en el grupo de ligamiento (GL) Pv11; y el gen *Pm2* el cluster Co-3, en el GL Pv04, respectivamente.

En el tercer capítulo se aborda el estudio de la resistencia a la grasa de la judía en el genotipo Cornell 49242. Esta enfermedad, causada por la bacteria *Pseudomonas syringae* pv. *phaseolicola*, es una de las enfermedades más importantes que afectan a la judía común. En este trabajo se evaluó la respuesta frente a dos aislamientos locales (ITA-812 e ITA-684), sobre la población RIL XC. El genotipo Cornell 49242 presenta una resistencia moderada al patógeno, mientras que la variedad Xana es altamente susceptible. Los resultados obtenidos indicaron que, en el caso de Cornell 49242, la respuesta al patógeno tiene un control cuantitativo. Se pudieron identificar cuatro loci cuantitativos (QTL) implicados en el control del carácter: $Psp4^{812XC}$ y $Psp6.1^{812XC}$, implicados en la respuesta al aislamiento ITA-812 y localizados en los GL Pv04 y Pv06 respectivamente; y $Psp6.1^{684XC}$ y $Psp6.2^{684XC}$, implicados en la respuesta al aislamiento ITA-684, localizados ambos en el GL Pv06. Para confirmar estos resultados se llevó a cabo una disección genética a partir de la cual sólo se observaron diferencias significativas entre diferentes líneas en el caso del aislamiento ITA-684, para el cual ambos QTLs han de estar presentes para que se observe una respuesta de resistencia significativamente mayor que en el resto.

Por último, en el cuarto capítulo se estudia la resistencia a antracnosis en la línea SEL1308. La antracnosis, causada por el hongo *Colletotrichum lindemuthianum*, es una enfermedad importante en los cultivos de judía del norte de España, así como a nivel mundial. La línea SEL1308, que deriva del genotipo G2333, presenta un amplio espectro de resistencia, pero sólo el gen de resistencia $Co\text{-}4^2$ ha sido descrito utilizando la raza 73 del patógeno. En este caso se estudió la respuesta en una población $F_{2:3}$ derivada del cruzamiento entre SEL1308 y la variedad MDRK, frente a las razas 3, 6, 7, 38 y 73, para las que SEL1308 es resistente. Este trabajo permitió identificar una nueva región implicada en la respuesta resistente de SEL1308 a las razas 3, 6, 7 y 38, localizada en el extremo del GL Pv03, una región en la que no se han descrito, hasta el momento, otros genes de resistencia a antracnosis.

Todo este trabajo ha permitido obtener información relevante para la mejora genética de la especie.

RESUMEN (en Inglés)

The aim of this Thesis dissertation is to further analyse the resistance response of common bean to three different pathogens, which seriously affect local bean crops in Northern Spain.

The two first chapters are related to resistance to powdery mildew, caused by the fungus *Erysiphe diffusa*. This incidence of this disease, that also affects other important crop species, has considerably increased in local bean crops in the recent years, and interaction *P. vulgaris-E. diffusa* was quite unknown. The first chapter describes the establishment of an infection method under controlled conditions, as well as an evaluation scale, by which five different responses or infection types were detected (IT0 to IT4). A total of 245 bean genotypes were evaluated, within only six of them showed complete resistance to the pathogen infection: Amanda, Belbeb, Cornell 49242, Negro San Luis, Porrillo Sintético and the local accession BGE003161. Finally, in order to study the mode of inheritance of resistance to powdery mildew, different F_2 and $F_{2:3}$ populations, derived from crosses between genotypes with different response to the pathogen, were also evaluated. Segregations fitted to mendelian ratios in all cases, indicating a qualitative mode of inheritance. From the observed results it could be deduced that genotype Porrillo Sintético presents two dominant and independent resistance genes, one conferring total resistance (IT0) and other one conferring moderate resistance (IT3). This response was also observed in genotype Cornell 49242, although it could not be deduced that Porrillo Sintético and Cornell 49242 share both genes.

The second chapter approaches to resistance to powdery mildew in genotype Cornell 49242. For this purpose a recombinant inbred line (RIL) population was evaluated. This RIL population (RIL XC) derives from the cross between Cornell 49242 and the fabada variety Xana, which is highly susceptible to the pathogen (IT4). Results confirmed the qualitative nature of the response and two dominant and independent genes were identified. Contingency tests and both subpopulation analyses and a genetic dissection allowed to locate these genes on two genomic regions involved in the control of resistance to anthracnose: *Pm1* gene, linked to Co-2 cluster, on linkage group (LG) Pv11; and *Pm2* gene, linked to Co-3 cluster, on LG Pv04, respectively.

The third chapter addresses the study of resistance to halo blight in genotype Cornell 49242. This disease is caused by the bacteria *Pseudomonas syringae* pv. *phaseolicola* and it is one of the most important diseases in common bean crops. In this work the response to two different local isolates, ITA-812 and ITA-684, was evaluated on the RIL XC population. Cornell 49242 shows moderate resistance and Xana is highly susceptible. Results indicated that response of Cornell 49242 to the pathogen is quantitative and four different quantitative trait loci (QTL) involved in this response

were identified: Psp4^{812XC} and Psp6.1^{812XC}, for response to isolate ITA-812, located on LG Pv04 and Pv06 respectively; and Psp6.1^{684XC} and Psp6.2^{684XC}, involved in the response to isolate ITA-684, located both on LG Pv06. A genetic dissection was carried out to confirm these results and significant differences were only observed for isolate ITA-684 when both QTLs were present.

Finally, in the fourth chapter, resistance to anthracnose in bean line SEL1308 was investigated. This line derives from genotype G2333 and shows a wide range of resistance, but only *Co-4*^{2} gene has been identified in this line, using race 73 of the pathogen. Response to five different races (3, 6, 7, 38 and 73) was evaluated on a $F_{2:3}$population derived from the cross between SEL1308 and cultivar MDRK. This work allowed the identification of a new genomic region involved in the control of resistance to anthracnose in SEL1308, located at the end of LG Pv03, a region in which no anthracnose resistance genes have been previously described.

All these results provide useful information in order to a better understand of resistance to pathogenic diseases in common bean, as well as resistance sources for future breeding programs.

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Español: Análisis de la herencia de la resistencia a tres patógenos en judía común (*Phaseolus vulgaris* L.)	Inglés: Study of the inheritance of resistance to three pathogens in common bean (*Phaseolus vulgaris* L.)
Rama de conocimiento: Genética Vegetal	

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4.- Informe

La Tesis tiene por objeto profundizar en el conocimiento de la herencia de la resistencia frente a tres patógenos que inciden gravemente en los cultivos de judía (*Phaseolus vulgaris* L.) de cara a disponer de información para el desarrollo de nuevas variedades resistentes:

- *Erysiphe diffusa* (Cooke & Peck) U. Braun& S. Takam, hongo que causa la enfermedad conocida como oidio
- *Pseudomonas syringae pv. phaseolicola* (Psp), bacteria que causa la enfermedad conocida como grasa
- *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib, hongo que causa la enfermedad conocida como antracnosis

A medida que el trabajo se ha ido concluyendo se han publicando los resultados por lo que la Tesis se ha organizado en cuatro Capítulos que se corresponden con cuatro publicaciones científicas, acompañados de una introducción, discusión y conclusiones generales.

En el primer capítulo se aborda el análisis de la interacción entre *P. vulgaris* y un aislamiento local de oídio. Los resultados de este trabajo fueron publicados en la revista Euphytica (2012) 186:875–882 (Índice de impacto 1.643).

En el segundo capítulo se investiga el control de la resistencia frente a un aislamiento local de oídio en la variedad Cornell 49242. Los resultados de este trabajo fueron publicados en la revista Theor Appl Genet (2013) 126:1503–1512 (Índice de impacto 3.658).

En el tercer capítulo se analiza la resistencia a dos razas de *Pseudomonas syringae* pv. *phaseolicola* presentes en los cultivos locales, en la variedad Cornell 49242. Los resultados de este trabajo fueron publicados en la revista Mol Breeding (2014) 33:577–588 (Índice de impacto 3.251).

Finalmente, en el cuarto capítulo se estudia la resistencia a cinco razas de antracnosis en el genotipo SEL 1308, derivado de la variedad diferencial G2333 uno de los genotipos que mayor espectro de resistencia muestran frente a este patógeno. Los resultados de este trabajo fueron enviados a la revista Theor Appl Genet y ha sido aceptado para su publicación con cambios menores (11/05/2014).

En nuestra opinión creemos que la organización en artículos de la Tesis es la más apropiada puesto que refleja más exactamente el trabajo que desarrolló la doctoranda en su parte experimental de obtención de datos, análisis de resultados y publicación de los mismos.

Villaviciosa, 11 Junio de 2014

de Tesis

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Trabanco N, MC Asensio- Manzanera, E Pérez-Vega, A Ibeas, A Campa, JJ Ferreira. 2013. Identification of QTLs involved in the response of common bean to *Pseudomonas syringae* pv *phaseolicola*. Mol Breed 2014, 33: 577-588 ISSN: 1380-3743

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· Pérez-Vega E, Trabanco N, Campa A, Ferreira JJ (2013) Genetic mapping of two genes conferring resistance to powdery mildew in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 126: 1503-1512

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· Trabanco N, Pérez-Vega E, Campa A, Rubiales D, Ferreira JJ (2012) Genetic resistance to powdery mildew in common bean. Euphytica 186: 875-882

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· Trabanco N, Pérez-Vega E, Campa A, Rubiales D, Ferreira JJ (2012) Genetic resistance to powdery mildew in common bean. Euphytica 186: 875-882

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· Trabanco N, Asensio-Manzanera MC, Pérez-Vega E, Ibeas A, Campa A, Ferreira JJ (2013) Identification of QTL involved in the response to *Pseudomonas syringae* pv. *phaseolicola* in common bean. Mol Breed 33: 577-588

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Y elaborada por Dña. Noemí Trabanco Martín

Valladolid, 11 de marzo de 2014

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Tesis como compendio de publicaciones

La presente tesis doctoral, de acuerdo con el informe correspondiente, autorizado por los Directores y el Tutor de Tesis, se presenta como un compendio de cuatro trabajos previamente publicados. Las referencias completas de los artículos que constituyen el cuerpo de la tesis son las siguientes:

- Trabanco N, Pérez-Vega E, Campa A, Rubiales D, Ferreira JJ (2012) Genetic resistance to powdery mildew in common bean. Euphytica 186:875-882

- Pérez-Vega E, Trabanco N, Campa A, Ferreira JJ (2013) Genetic mapping of two genes conferring resistance to powdery mildew in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 126:1503-1512

- Trabanco N, Asensio-Manzanera MC, Pérez-Vega E, Ibeas A, Campa A, Ferreira JJ (2014) Mol Breeding 33:577-588

- Trabanco N, Campa A, Ferreira JJ (2014) Identification of a new chromosomal region involved in the genetic control of resistance to anthracnose in common bean. Theor Appl Genet (Accepted with major changes)

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En el transcurso de su programa de doctorado, Noemí Trabanco ha sido beneficiaria de una beca INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria).

Índice

Introducción general

1. La judía común

La judía común es una especie diploide (2n = 22), de tipo herbáceo, con reproducción esencialmente autógama y ciclo anual. Sus hojas son compuestas, alternas y trifoliadas y sus flores son las típicas papilionáceas con una quilla que envuelve los 11 estambres junto con el pistilo. El tallo es delgado y con un número variable de nudos y entrenudos, cuya longitud varía según la variedad o genotipo. Dentro de esta especie se han descrito cuatro tipos de hábito de crecimiento diferentes, según el tallo termine o no en botón floral, el número y longitud de los entrenudos y la capacidad o no para trepar de la planta. La semilla presenta un gran polimorfismo en formas, tamaños y colores (Debouck e Hidalgo 1985).

Los parientes silvestres de la especie se han localizado en América. La mayoría de los estudios sobre el origen y domesticación de esta especie se basan en unas proteínas de semilla denominadas faseolinas. Estas proteínas son las más abundantes dentro de las semillas de judía y de ellas depende, en gran medida, la cantidad y la calidad nutricional de las proteínas en las semillas de esta especie (Broughton et al. 2003). Se han definido diferentes patrones de faseolina en función del peso molecular de los polipéptidos que la componen, los cuales oscilan en un rango de pesos de 57,4 a 49,6 kilodaltons (Brown et al. 1981; Gepts y Bliss 1988). A partir del estudio de las faseolinas, Kami et al. (1995) sugirieron que el ancestro silvestre se originó en la región entre el sur de Ecuador y el norte de Perú, a partir de la cual de distribuyó hacia otras regiones. Por otro lado, otra hipótesis sugiere un origen mesoamericano (Rossi et al. 2009). Estudios recientes, que incluyen análisis de secuencias, parecen confirmar esta última hipótesis (Bitocchi et al. 2012). La domesticación de la especie, probablemente tuvo lugar en dos regiones diferentes de forma paralela e independiente, dando lugar a dos acervos genéticos: Mesoamericano y Andino. Estos dos grupos difieren, entre otras características, en el perfil de faseolinas. Generalmente, la presencia de la subunidad de mayor peso molecular (57,4 KDa), presente en los patrones T, C, H y A, se ha asociado con materiales del grupo de germoplasma Andino; la ausencia de esta subunidad, patrón tipo S, se ha asociado con grupos de germoplasma Mesoamericano (Gepts et al. 1986; Gepts y Bliss 1988; Gepts 1990). El acervo Mesoamericano se distribuye por las regiones del norte de Méjico al norte de Colombia y Venezuela, mientras el acervo Andino se distribuye por las regiones de Perú, Chile, Bolivia y Argentina. Rossi et al. (2009) sugieren la existencia de un cuello de botella durante el proceso de domesticación del acervo Andino, que provocó una menor diversidad genética dentro de este grupo. La diversificación dentro de ambos centros de origen y la posterior expansión del

cultivo por otras regiones del planeta, así como el trabajo de los mejoradores, han contribuido a la gran diversidad existente dentro de esta especie.

2. Importancia del cultivo de la judía común

La judía común (*Phaseolus vulgaris* L.) es una leguminosa, siendo su fruto una legumbre o vaina. Como leguminosa tiene la capacidad de fijar nitrógeno mediante la simbiosis con rizobios, contribuyendo a mejorar la fertilidad de los suelos así como a reducir la emisión de gases con efecto invernadero asociados con el cambio climático (Underwood et al. 2013). Esta especie representa una fuente de proteínas muy importante en la dieta de millones de personas, especialmente en América Latina y en muchas regiones de África. Además de su importancia como aporte de proteínas, el consumo de judía proporciona otros nutrientes básicos en la alimentación humana, como fibra o folatos, así como numerosos minerales (Broughton et al. 2003). Esta especie es, dentro del género *Phaseolus* spp*,* la más cultivada en todo el mundo. Existen diferentes formas de consumo humano, tanto de la vaina (judía de verdeo) como de la propia semilla, que puede consumirse directamente tostada o cocida, o tras un proceso de rehidratación. A nivel mundial, la mayor producción se centra en la semilla seca. En el año 2012 se produjeron unos 23 millones de toneladas de judía, siendo Asia el mayor productor, con unos 10,8 millones de toneladas producidas (principalmente en India y China). Le sigue el continente americano, con un total de 7,1 millones de toneladas y África, que produjo unos 5 millones de toneladas de judía. Dentro del continente americano, destaca la producción de Brasil (2,8 millones de toneladas), Méjico (1 millón de toneladas) y los Estados Unidos, que produjeron 1,5 millones de toneladas en 2012 (http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E; revisado enero 2014).

En España, la mayor producción de judía se destina para el uso en verdeo (consumo de la vaina), principalmente en las regiones del sur como Andalucía (más de 63.000 toneladas) y, en el norte, en Galicia (más de 30.000 toneladas). Una parte de esta producción se exporta a países de la Unión Europea como Francia, Alemania o Reino Unido (http://www.magrama.gob.es/). En cuanto al aprovechamiento en forma de semilla seca, las principales regiones productoras son Castilla-León (6.000 toneladas) y Galicia (2.500 toneladas), (http://www.magrama.gob.es/; revisado enero 2014). En algunos casos estas producciones están amparadas por marcas de calidad diferenciada como Indicaciones Geográficas Protegidas (véase http://www.magrama.gob.es/). En el caso de Asturias, la producción se basa en la clase comercial 'faba granja asturiana', que cuenta con una Indicación

Geográfica Protegida (www.faba-asturiana.org). Esta clase comercial muestra un hábito de crecimiento trepador y una semilla blanca, oblonga y alargada, de gran tamaño (unos 100g/100 semillas; Ferreira et al. 2005; Figura 1). Las características de esta semilla son únicas dentro de la especie siendo muy apreciada no sólo a nivel regional sino también a nivel nacional.

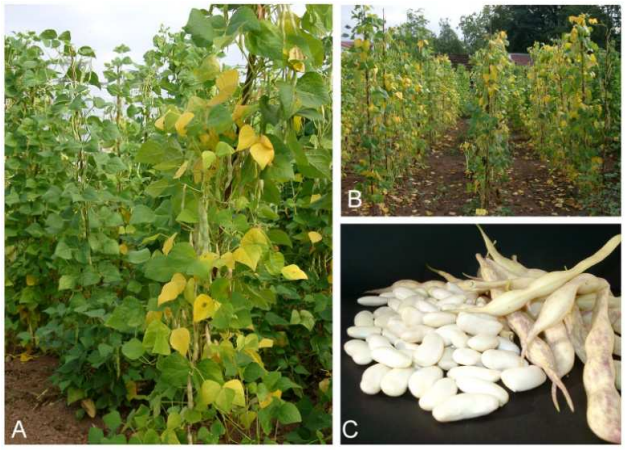

Figura 1. Características de la faba granja asturiana: A) planta con crecimiento trepador; B) vista de un cultivo de faba granja; C) semillas de faba granja asturiana

3. Limitaciones en la producción

El cultivo de esta leguminosa puede verse afectado negativamente por numerosos factores de origen abiótico o biótico. Desde el punto de vista abiótico, ciertas variables relacionadas con las características del suelo (como los niveles de nutrientes, la salinidad o el pH) o la climatología (temperaturas extremas, sequía, niveles de ozono, etc.) son las que más pueden condicionar el desarrollo del cultivo. Los factores abióticos más estudiados en judía son la tolerancia a sequía y a bajos niveles de fósforo en el suelo. Estos factores son condicionantes en importantes regiones productoras de judía, como América Latina o África (Miklas et al. 2006).

En cuanto a los factores bióticos, se puede distinguir entre plagas causadas por insectos, ácaros o nematodos y enfermedades originadas por microorganismos patógenos tales como virus, hongos o bacterias. Dentro de las plagas causadas por insectos, son bastante frecuentes, el pulgón, la araña roja, los trips o la mosca blanca, durante el periodo de cultivo, y el gorgojo durante el almacenamiento de las semillas (Singh y Schwartz 2011). También hay muchas especies de microorganismos y virus capaces atacar y producir daños en esta especie. Dentro de las enfermedades causadas por virus, las más frecuentes son el virus del mosaico común (BCMV), el virus del mosaico amarillo (BGMV) y el virus del mosaico dorado amarillo (BGYMV).

Entre las enfermedades causadas por hongos destacan la antracnosis, la mancha angular, la roya, el moho blanco o las infecciones que provocan pudriciones en la raíz. En el caso de enfermedades de origen bacteriano cabe destacar la bacteriosis común o la bacteriosis de halo, también conocida como 'grasa de la judía' (Schwartz et al. 2005; Singh y Schwartz 2010). En el norte de España, dado que se trata de una región con clima templado y elevada humedad, una de las enfermedades que más afectan al cultivo de judía en Asturias es la antracnosis, pero en los últimos años también se ha visto una incidencia importante de oídio y, en menor medida, de bacteriosis de halo, que resulta muy devastadora en los cultivos de Castilla-León (Rico et al. 2003).

3.1 Oídio

El oídio o mildiu es una enfermedad de origen fúngico. Existen varias especies del patógeno que pueden causar la enfermedad en plantas, la mayoría de ellas pertenecientes al orden Erysiphales. Esta familia de hongos puede atacar a muchas especies vegetales, incluyendo malas hierbas y especies de gran importancia económica como es el caso de la rosa, la vid, la cebada, la soja, el tomate, las habas o el guisante. En el caso de la judía común, se ha descrito que el agente causal de la enfermedad es la especie *Erysiphe polygoni* DC (Schwartz et al. 1981; Ferreira et al. 1999), pero estudios más recientes lo atribuyen a la especie *Erysiphe diffusa* (Cooke & Peck) U. Braun& S. Takam (Almeida et al. 2008). Aunque en judía se la ha clasificado como una enfermedad de importancia secundaria, las últimas campañas del cultivo de faba granja en el norte de España indican una mayor incidencia de la enfermedad, coincidiendo con épocas de baja humedad relativa y temperaturas elevadas (Trabanco et al. 2013).

Este patógeno puede afectar a toda la parte aérea de la planta. La enfermedad se inicia con la aparición de motas polvorientas de color grisáceo sobre hojas y tallo. El micelio del hongo sigue creciendo, llegando a cubrir la planta por completo, lo que produce marchitamiento, caída prematura de las hojas, flores, vainas y, en casos extremos, puede llevar a la muerte de la planta (Figura 2). El mayor problema de esta enfermedad es la facilidad de dispersión del patógeno. Las conidiosporas que se forman sobre el micelio pueden dispersarse fácilmente a través del viento y el agua. De esta misma forma, a través de la ropa o los utensilios de trabajo, el patógeno puede diseminarse por todo el cultivo con mucha rapidez. Además de su fácil dispersión, no existen muchas materias activas capaces de evitar el avance de la enfermedad, sobre todo si el patógeno ya ha alcanzado el cultivo (Trabanco et al. 2013).

La información existente sobre la interacción *P. vulgaris-E. diffusa* es muy escasa. No se ha descrito un método para el manejo del hongo ni para la inoculación y evaluación de síntomas en condiciones controladas. Algunos autores han descrito algunas fuentes de resistencia (Schwartz et al. 1981) y se ha sugerido una respuesta de resistencia de tipo cualitativo (Dundas et al. 1936; Bett y Michaels 1995; Ferreira et al. 1999, 2001). La gran mayoría de las evaluaciones fueron llevadas a cabo en ensayos de campo, probablemente debido la dificultad de manejo del hongo *in vitro* ya que es un parásito obligado que solo crece en tejidos vivos del huésped.

Figura 2. Síntomas de oídio en cultivo de faba granja asturiana: A) primeras motas sobre hoja; B) avance del micelio sobre hoja; C) síntomas en vaina; D) planta seca cubierta de micelio del hongo

3.2 Grasa de la judía

La grasa de la judía, también denominada bacteriosis de halo, es una enfermedad causada por la especie *Pseudomonas syringae* pv. *phaseolicola* (*Psp*). En muchas regiones con cultivo de judía, es considerada como la enfermedad bacteriana más importante, ya que puede provocar la pérdida de alrededor del 50% del cultivo (Singh y Schwartz 2010). Esta enfermedad, extendida por todo el mundo, se produce generalmente en regiones con temperaturas elevadas, como es el caso de los cultivos en Castilla-León y, en menor medida, en la Cornisa Cantábrica. Los síntomas de la enfermedad pueden verse tanto en hoja y tallo como en las vainas. Las más aparentes se producen en las hojas donde, en primer lugar, aparecen unas lesiones redondeadas que van tornando a un color marrón. Alrededor de estas lesiones se

forma un halo clorótico de color verde/amarillo. Los síntomas de clorosis son más pronunciados en temperaturas entre 18 y 23 ºC, ya que es la temperatura idónea para que la bacteria produzca la faseolotoxina que origina la clorosis (Schwartz et al. 2005). La planta comienza a amarillear y debilitarse, pudiendo producirse defoliación prematura e, incluso, la muerte de la planta. Estas plantas infectadas también pueden actuar como foco de infección para las plantas vecinas, por lo que la dispersión de la enfermedad dentro del cultivo puede ser muy rápida y aguda. También las malas hierbas presentes en los cultivos pueden actuar como reservorio del patógeno (Fernández 2013). En las vainas se pueden apreciar lesiones en forma de círculos concéntricos, de aspecto oleoso (Figura 3). Los ataques en vaina provocan la infección de las semillas, que actúan como reservorio y medio de diseminación del patógeno.

Figura 3. Síntomas de bacteriosis: A) inicio de la clorosis en hoja; B) lesiones en vaina; C) avance de los síntomas sobre hoja y vainas. (Fotos cedidas por C. Asensio-Manzaneda, ITACyL)

La respuesta a la interacción *P. vulgaris-Psp* se ha considerado, tradicionalmente, de naturaleza cualitativa y raza-específica. Se han descrito hasta nueve razas diferentes del patógeno, a partir de la respuesta de un juego de variedades diferenciales propuesto por Taylor et al. (1996). De estas nueve, las razas 1, 2, 5, 6 y 7 se distribuyen mundialmente, siendo la raza 6 la más virulenta. Se han identificado varios genes de resistencia con una herencia mendeliana, similar a la descrita en antracnosis (genes *Pse-1* a *Pse-5*). Algunos de estos genes, como *Pse-1*, se localizan en regiones para las que también se han descrito genes de resistencia a antracnosis (Miklas et al. 2006). El gen *Pse-4* está estrechamente ligado al gen *I*, que condiciona la resistencia a potyvirus (virus del mosaico común y necrótico de la judía; BCMNV y BCMV). De hecho aún no se ha esclarecido si *Pse-4* es el propio gen *I* o un gen estrechamente ligado a él (Singh y Schwartz 2010). Para algunos de estos genes se han descrito marcadores ligados que pueden ser utilizados en selección asistida por marcadores (Miklas et al. 2009, 2011). Recientemente, dos nuevos genes de resistencia, *Rpsar-1* y *Rpsar-2*, fueron mapeados en la misma posición donde han sido localizados los *clusters* de resistencia a antracnosis Co-2 y

Co-4 (Chen et al. 2010). Finalmente, resulta conveniente señalar que en algunos genotipos se ha identificado una resistencia de tipo cuantitativo y se han localizado diferentes QTLs (*Quantitative Trait Loci*) implicados en la respuesta (Ariyarathne et al. 1999; Yaish et al. 2006).

3.3 Antracnosis

La antracnosis, causada por el hongo *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib, es una enfermedad que puede causar graves pérdidas sobre el cultivo de judía común, llegando en casos extremos al 100% de la producción (Singh y Schwartz 2010). Se trata de una enfermedad muy extendida, principalmente en regiones con elevada humedad y temperaturas moderadas, como es el caso de la Cornisa Cantábrica.

Este patógeno, que puede dispersarse a través de la semilla, afecta a toda la parte aérea de la planta, produciendo unas heridas denominadas 'chancros'. Las heridas también pueden observarse en las vainas y las semillas (Figura 4). En los casos más severos las lesiones pueden provocar un colapso total de la planta, causando su muerte. El desarrollo de la enfermedad también puede estar influenciado por la temperatura, la humedad o el estado fisiológico tanto de la planta como del propio patógeno (Schwartz et al. 2005; Ferreira et al. 2013).

Figura 4. Síntomas de antracnosis en el tipo faba granja asturiana: A) en tallo; B) en vaina; C) en hojas; D) en semilla

La respuesta a la interacción *P. vulgaris-C. lindemuthianum* presenta generalmente una herencia cualitativa, en la que se pueden diferenciar claramente las reacciones resistentes y susceptibles. La respuesta a esta interacción fue estudiada por primera vez por Barrus (1911), quien también describió la existencia de variantes patogénicas o razas. Hasta el momento han

sido descritas más de 100 razas usando un sistema estandarizado para su caracterización y denominación (Pastor-Corrales 1991; Ferreira et al. 2013). La resistencia genética a esta enfermedad es raza-específica siguiendo, en la mayoría de los casos, el modelo gen a gen descrito por Flor (1955). Los estudios clásicos se basaban en la interpretación de resultados obtenidos a partir de segregaciones F_2. Las poblaciones F_2 obtenidas a partir de un parental resistente (R) y uno susceptible (S) proporcionan información sobre el número y modo de acción de los genes implicados frente a una determinada raza o aislamiento; las poblaciones obtenidas a partir del cruce entre dos parentales resistentes (pruebas de complementación o test de alelismo) se utilizan para la identificación de genes. Hasta el momento, basándose principalmente en estos test de alelismo, se han descrito hasta 20 genes de resistencia diferentes (genes Co-; *Co-1* a *Co-14*, *Co-u*, *Co-v*, *Co-w*, *Co-x*, *Co-y* y *Co-z*). La mayoría de estos genes muestran una dominancia completa, aunque también se han identificado genes de resistencia que actúan de un modo complementario. También se ha propuesto la existencia de genes recesivos, pero nunca se han llegado a demostrar. En el caso de *Co-1*, *Co-3*, *Co-4* y *Co-5*, se ha planteado la existencia de varios alelos para el mismo locus a partir de diferencias en los espectros de resistencias de diferentes variedades (Kelly y Vallejo 2004; Ferreira et al. 2013). Algunos de estos genes de resistencia han sido localizados en el mapa genético de la especie y se han descrito marcadores moleculares estrechamente ligados (Ferreira et al. 2013) lo que resulta de gran interés en la identificación de nuevos genes que protejan frente a este patógeno.

Diferentes evidencias indican que los genes de resistencia a antracnosis se organizan en grupos de genes estrechamente ligados (también denominados *clusters*), en los que pueden localizarse múltiples genes específicos de diferentes patógenos o de diferentes razas de un mismo patógeno. Por esta razón, actualmente se tiende a hablar de *cluster* Co- en lugar de gen *Co-* (Melotto et al. 2004; David et al. 2009, 2010; Ferreira et al. 2013). Considerando la alta especificidad en la interacción *P. vulgaris-C. lindemuthianum*, se ha propuesto un sistema para la nomenclatura de los genes de resistencia a antracnosis basado en la región donde se localizan los genes Co- que tenga en cuenta tanto el genotipo de judía en el que se identifica el gen, como el aislamiento o raza con el que éste se identifica (Ferreira et al. 2013).

4. Estrategias para el control de las enfermedades

Las estrategias para el control de las enfermedades deben evitar la aparición de la enfermedad o, en caso de que esto no sea posible, minimizar sus daños y su propagación. Para ello, pueden

actuar impidiendo o reduciendo la probabilidad de que ocurran alguna o todas las etapas en que se puede dividir el proceso de desarrollo de una enfermedad (véase por ejemplo Agrios 1986): *contaminación*, *penetración e infección, incubación*, *difusión en el huésped, dispersión en el medio* y *supervivencia*. Las estrategias y métodos disponibles son diversos aunque se pueden agrupar en tres grandes categorías: los que intervienen sobre el medio (prácticas culturales adecuadas), los que intervienen sobre el patógeno (tratamientos con organismos vivos o moléculas tóxicas para el control del patógeno) y los que intervienen sobre el huésped (control del material de reproducción y utilización de variedades resistentes).

La producción agrícola actual busca la sostenibilidad, el menor impacto posible en el medio ambiente y una calidad diferenciada (Underwood et al. 2013). En este sentido tienen cada vez más relevancia los modelos de *producción integrada* (Real Decreto 1201/2002) y *orgánica o ecológica* [Reglamento (CE) 834/2007]. En ambos sistemas de producción se usan y combinan diferentes métodos y estrategias para el control de los patógenos sin tener que recurrir necesariamente a tratamientos fitosanitarios convencionales. Además, debe tenerse en cuenta que no siempre existe una materia activa totalmente eficaz contra una determinada enfermedad, y que la política de la Unión Europea tiene una clara tendencia hacia la reducción de las materias activas autorizadas. En cualquier caso, en un modelo agrícola enfocado hacia la sostenibilidad y con el menor impacto posible en el medio ambiente, el genotipo de las plantas cultivadas juega un papel más relevante, si cabe, que en el modelo de una agricultura altamente tecnificada. De esta forma, una de las alternativas más razonables es el uso de genotipos portadores de resistencia genética. Sin embargo, el productor no dispone siempre de variedades resistentes que presenten, además, las características agro-morfológicas y de calidad deseadas, por lo que éstas han de ser desarrolladas mediante programas de mejora genética clásica o mediante estrategias biotecnológicas, como las técnicas de transformación (Schouten et al. 2006). No obstante, los métodos de transformación no son eficaces en todas las especies y el uso de cultivares derivados de transformación (cisgénicos o transgénicos) genera mucha controversia. Además, la respuesta a muchas enfermedades es de naturaleza cuantitativa, por lo que la introducción de genes de resistencia por transformación sería un proceso muy complejo que, en el mejor de los casos, conduciría a incrementar los niveles de resistencia. En todo caso, antes de iniciar un programa de mejora genética resulta recomendable disponer de un detallado conocimiento en los siguientes puntos:

- características del patógeno, como mecanismo de propagación y manejo *in vitro*
- variación en la interacción huésped-patógeno en cuanto a
    i) variantes patogénicas (razas) o agresividad del patógeno local

ii) variación en la respuesta del huésped y, especialmente, fuentes de resistencia disponibles

- herencia de la respuesta frente al patógeno en cuanto a

iii) naturaleza de la resistencia (cualitativa vs. cuantitativa)

iv) herramientas disponibles para la mejora, como genes/QTL descritos o marcadores moleculares disponibles para la selección asistida

En el caso de la judía común, aún se desconocen muchos de los genes implicados en la respuesta a la infección por microorganismos patógenos, así como su mecanismo de acción. El nivel de conocimiento es todavía menor para enfermedades calificadas como secundarias, por su menor incidencia global sobre los cultivos de judía. Avanzar en el conocimiento sobre la resistencia a patógenos, en el descubrimiento de nuevos genes de resistencia, así como de su modo de acción, junto con las nuevas posibilidades para obtener marcadores moleculares que pueden ser utilizados en mejora asistida, permitirá obtener nuevas variedades de interés resistentes a aquellas enfermedades que afectan al cultivo de esta especie.

5. Mecanismos de resistencia en plantas

Las plantas presentan dos tipos diferentes de defensa frente al ataque de patógenos: defensas constitutivas y defensas inducibles. Las defensas constitutivas son las estructuras de una planta que pueden evitar la entrada o desarrollo de los microorganismos. Entre ellas están la propia pared celular o la cutícula de la epidermis. Las defensas inducibles son aquellas que, tras el reconocimiento del microorganismo, activan diferentes mecanismos, como la producción de compuestos tóxicos y enzimas degradantes o como la apoptosis o muerte celular programada (Freeman y Beattie 2008). Estas defensas son mucho más específicas, asemejándose en cierto modo a la respuesta inmune de los animales. En el caso de las plantas se sugieren dos tipos de respuesta inmune: respuestas PTI (PAMP-triggered inmunity) asociadas con el reconocimiento de moléculas asociadas a grupos de patógenos (PAMP) y respuestas ETI (effector-triggered inmunity) inducidas por efectores del patógeno que son reconocidos por proteínas específicas de la planta (proteínas R; Chisholm et al. 2006; Grennan 2006; Dodds y Rathjen 2010). En la respuesta PTI, la planta reconoce proteínas características del patógeno y desencadena una cascada de respuesta. En algunos casos, el patógeno es capaz de superar este mecanismo introduciendo moléculas efectoras que inhiben esta respuesta, causando la enfermedad. Pero, de la misma forma que los microorganismos han sido capaces de desarrollar mecanismos para evitar el reconocimiento por parte de las células vegetales, las

plantas también han desarrollado otras defensas internas, capaces de reconocer estos efectores (respuestas ETI); es lo que se conoce como genes R. Los productos más comunes codificados por estos genes son las proteínas de tipo NBS-LRR, que contienen un dominio de unión a nucleótido (NBS, Nucleotide Binding Site) y un dominio rico en Leucina (LRR, Leucine Rich Repeat). Este grupo de proteínas puede dividirse en dos subgrupos, atendiendo al tipo de dominio que presenten en el extremo amino-terminal: proteínas TIR-NB-LRR, que contienen un dominio TIR (Toll, interleukin-1 receptor) implicado en la señalización intracelular; y proteínas CC-NB-LRR, que contienen un dominio CC (coiled-coil). En el caso de judía común ya se han descrito proteínas de este tipo asociadas a regiones implicadas en resistencia a patógenos, como en el caso del clúster de resistencia a antracnosis Co-3, localizado en el grupo de ligamiento Pv04, en el que se identificaron varias secuencias de tipo CC-NB-LRR en el genotipo de judía BAT93 (Geffroy et al. 2009; David et al. 2009).

El reconocimiento del efector por parte del receptor puede ser directo o indirecto (Dodds y Rathjen 2010; Elmore et al. 2011). En el reconocimiento directo, el efector se une directamente a una parte del receptor. Algunos ejemplos en la bibliografía son el gen *Pi-ta*, de arroz, que produce resistencia frente al hongo *Magnaporthe grisea* (Jia et al. 2000) o la resistencia a la podredumbre del lino, causada por el hongo *Melampsora lini* (Dodds et al. 2006). En estos casos el dominio LRR de las proteínas de resistencia es el que confiere la especificidad, mientras que los dominios TIR y NB promueven la activación y el inicio de la cascada de señalización (Elmore et al. 2011). En el reconocimiento indirecto es necesaria la presencia de una proteína adicional. Existen tres hipótesis diferentes que tratan de explicar esta interacción:

- Hipótesis del guardián o 'guard model'*:* una proteína accesoria, es modificada por la interacción con el efector, y esto es reconocido por el receptor LRR. Un ejemplo de este mecanismo es la proteína RIN4 de *A. thaliana* (Mackey et al. 2002, 2003; Axtell et al. 2003).
- Modelo del señuelo o 'decoy model'*:* una modificación del modelo anterior que sugiere que la proteína accesoria es una "imitadora" del blanco original del efector. Un ejemplo de este modelo de acción es la proteína quinasa Pto del tomate (Mucyn et al. 2006, 2009).
- Modelo del cebo o 'bait model': sería una combinación de los modelos directo e indirecto de reconocimiento. En este caso, la proteína accesoria se une al efector y este complejo interacciona directamente con el receptor LRR. Este modelo fue propuesto por Collier y Moffett (2009).

Por ello, aunque en principio los receptores LRR reconocen a los efectores del patógeno siguiendo el modelo gen a gen, estudios recientes indican que, en algunos casos, pueden actuar en parejas para producir una respuesta inmune completa. En algunos casos es posible que los genes que codifican esas dos proteínas estén estrechamente ligados en un clúster de resistencia (Eitas y Dangl 2010; Elmore et al. 2011).

A la complejidad indicada en la respuesta a los patógenos se puede añadir otro nivel de complejidad, conocido como *alternative splicing*, por el cual a partir de una misma secuencia genómica se pueden obtener diferentes productos (mRNA o proteínas). Este tipo de variación ha sido demostrada en el gen de resistencia RCT1 a *Colletotrichum trifolli* en la leguminosa *Medicago truncatula* (Tang et al. 2013).

En todo caso, un paso previo a investigar en detalle el mecanismo molecular de la resistencia a un patógeno es conocer el control genético de la expresión, así como identificar la región genómica implicada en el control, la secuencia de nucleótidos del gen candidato y su estructura de intrones/exones.

6. Mapas genéticos de ligamiento

Un mapa genético es una representación gráfica de la localización relativa de distintos loci en los cromosomas, obtenido a partir de las frecuencias de recombinación entre ellos. Conocer la distribución de los loci en los cromosomas permite, entre otras cosas, predecir su transmisión en las descendencias y estudiar la herencia de caracteres complejos, como caracteres cuantitativos. A partir de esta información también se pueden iniciar análisis genómicos como aproximación, aislamiento y secuenciación de genes específicos, identificar otros loci que pueden servir como marcadores indirectos o contribuir al ensamblaje de los segmentos en el proceso de secuenciación y re-secuenciación de los genomas (Langridge y Fleury 2011; Morrell et al. 2012). Por todo lo expuesto, los mapas genéticos constituyen una herramienta muy útil en el análisis genético y en la mejora genética actual.

6.1 Poblaciones de mapeo

Los mapas genéticos se elaboran a partir del análisis de la segregación de distintos loci en poblaciones obtenidas a partir de cruzamiento entre genotipos conocidos. Las poblaciones más utilizadas en el caso de especies autógamas, como es la judía común, son las poblaciones F_2 o $F_{2:3}$, las poblaciones de líneas recombinantes consanguíneas (RILs), las poblaciones

obtenidas por retrocruzamiento y, en menor medida, las poblaciones de líneas isogénicas (NILs). Cada una de estas poblaciones presenta ventajas e inconvenientes que deben ser tomados en cuenta a la hora de elegir qué tipo de población usar para el estudio de un determinado carácter. Las poblaciones F_2 son derivadas de la autofecundación de una planta F_1 obtenida del cruzamiento entre dos líneas puras. De las plantas F_2 puede obtenerse el ADN para análisis de marcadores. En cuanto a caracteres de interés, pueden evaluarse directamente las plantas F_2, aunque esta valoración presenta limitaciones, de modo que suele ser habitual autofecundar las plantas F_2 de la población y deducir su fenotipo/genotipo a partir de la evaluación de sus descendientes F_3. La obtención de estas poblaciones no requiere mucho tiempo, por lo que suele ser una opción adecuada para el estudio de uno o pocos caracteres. Para obtener un mapa lo bastante fiable se recomienda una población de, al menos, 100 individuos F_2 (Schneider 2004). Las poblaciones por retrocruzamiento se obtienen de cruzar plantas F_1 obtenidas con uno de los parentales del cruzamiento inicial. Si queremos analizar múltiples caracteres dentro de la misma población, como puede ser la respuesta a diferentes patógenos o a varias razas de un mismo patógeno, quizá este tipo de poblaciones no son la mejor alternativa, ya que no perduran en el tiempo y es fácil que la semilla obtenida se agote. En estos casos suele recurrirse a RILs, obtenidas a partir de varias generaciones de autofecundación de las familias F_2 mediante el método del 'single seed descent' descrito por Fehr (1987). De esta forma, aunque el tiempo y los recursos necesarios para su obtención son mayores, se consigue una población de líneas homocigotas y muy estables, que pueden perdurar en el tiempo y que permiten una mejor estimación de las fracciones de recombinación.

6.2 Marcadores genéticos y mapas en judía común

6.2.1 Marcadores moleculares

Se considera marcador molecular, generalmente, a cualquier variación, polimorfismo o diferencia a nivel molecular, principalmente a nivel de ADN. Los marcadores moleculares se han utilizado en plantas en estudios de diversidad, etiquetado de genes o QTL, en la selección asistida, en el desarrollo de mapas genéticos de ligamiento, en estudios de asociación o en la identificación varietal. Se han descrito diferentes métodos que permiten investigar la variación en secuencia o longitud de fragmentos de ADN, pero actualmente los más usados son aquellos que se basan en la reacción en cadena de la polimerasa (PCR; Polymerase Chain Reaction). Entre ellos cabe citar las amplificaciones aleatorias de ADN (RAPDs: Random Amplified Polymorphism DNA) o los polimorfismos de longitud en fragmentos amplificados (AFLPs:

Amplified Fragment Length Polymorphism). El progreso en las técnicas de secuenciación ha permitido el desarrollo de nuevos marcadores basados en la amplificación de secuencias conocidas. Los más comunes son los marcadores tipo SCAR (Sequenced Characterized Amplified Region) y CAPS (Cleaved Amplified Polymorphic Sequence), desarrollados a partir de RAPDs. Los microsatélites o SSR (Simple Sequence Repeat) han sido también ampliamente utilizados, ya que suelen ser altamente polimórficos, co-dominantes y fácilmente reproducibles. En los últimos años son muy utilizados los marcadores basados en el polimorfismo de nucleótidos o SNPs (Single-Nucleotide Polymorphisms); STS (Sequence-Tagged Site) e InDels, basados en la existencia de inserciones/deleciones. En el caso de la judía las características sobre muchos de estos marcadores (tipo de marcador, secuencia de los cebadores, secuencia amplificada, posición en el mapa genético, etc.) están disponibles en bases de datos abiertas, a partir de las cuales se pueden conocer características de cada uno de ellos (www.css.msu.edu/bic; www.phaseolusgenes.bioinformatics.ucdavis.edu/).

Los marcadores moleculares son más o menos útiles en función de la información que proporcionan: nivel de polimorfismo, secuencia de nucleótidos conocida, posición en el mapa genético, posición física en el genoma, localización en exones, etc. Pero los óptimos son los marcadores funcionales, que son aquellos derivados de regiones codificantes o reguladoras de los genes implicados en la expresión de un determinado carácter de interés.

6.2.2 Mapas genéticos en judía: mapa Xana/Cornell 49242

Los primeros mapas genéticos en judía se elaboraron con marcadores morfológicos (Basset 1991) y bioquímicos (Vallejos y Chase 1991). Eran mapas muy simples, constituidos por pocos loci. Los mapas de Vallejos et al. (1992), Gepts et al. (1993) o Nodari et al. (1993), comenzaron a incluir marcadores basados en la amplificación de ADN. Posteriormente, el primer mapa que integró marcadores de otros mapas genéticos previos realizados en judía fue el mapa de Freyre et al. (1998), utilizando la población RIL BAT93/Jalo EEP558. Éste fue el primer mapa consenso para la especie. Con la aparición de nuevos tipos de marcadores moleculares, como los microsatélites, se han desarrollado varios mapas genéticos con diferentes fines (Blair et al. 2003; Grisi et al. 2007; Rodríguez-Suárez et al. 2007; Hanai et al. 2010; Córdoba et al. 2010a y 2010b). En los últimos trabajos, la mayoría de los mapas genéticos se construyen incluyendo marcadores de tipo SNP (Galeano et al. 2011, 2012), STS (McClean et al. 2010; McConell et al. 2010) e InDels (Moghaddam et al. 2013).

La localización directa o indirecta de los genes en un mapa genético es una parte muy importante para la identificación y caracterización de los mismos. Por ello, resulta conveniente

disponer de un mapa genético de referencia como herramienta para los estudios genéticos que se lleven a cabo dentro de una especie. Con este objetivo, en el año 2002 el grupo de Genética Vegetal del Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA) inicia el desarrollo de una población de mapeo permanente de tipo RIL. Como parentales se seleccionaron dos variedades con genotipos muy extremos, buscando así el máximo polimorfismo. Por un lado, se seleccionó una variedad del tipo comercial fabada, dado que es el tipo varietal que presenta la semilla más grande descrita dentro de la especie y que su cultivo es de gran interés a nivel local. Se seleccionó la variedad Xana, de crecimiento determinado, desarrollada en el SERIDA (Ferreira et al. 2007). Esta variedad presenta características del acervo genético andino. Para el otro parental se seleccionó el genotipo conocido Cornell 49242, perteneciente al acervo mesoamericano. Es una variedad de crecimiento indeterminado, con semilla muy pequeña de color negro. Esta variedad forma parte del set de 12 variedades diferenciales utilizadas en la caracterización de razas de *C. lindemuthianum* (Pastor Corrales 1991).

La población de líneas recombinantes derivada del cruzamiento Xana x Cornell 49242 (RILs XC) se desarrolló por el método 'single seed descent'. Este trabajo se llevó a cabo en invernadero durante el periodo 2002-2005. En total se desarrollaron 6-7 generaciones de autofecundación para un total de 110 líneas. Sobre esta población se construyó un mapa genético constituido, inicialmente, por marcadores de tipo AFLP y algunos marcadores previamente descritos por otros autores de tipo SCAR y SSR, que permitiesen la comparación con otros mapas de judía (Pañeda 2005). Posteriormente se han añadido nuevos marcadores tanto moleculares como morfológicos (Pérez-Vega et al. 2010). Actualmente el mapa consta de 379 loci organizados en 11 grupos de ligamiento. Esta población ha sido también evaluada para los siguientes caracteres:

- caracteres morfo-agronómicos cuantitativos (Pérez-Vega et al. 2010).
- respuesta a *Pythium ultimun* (Campa et al. 2010).
- caracteres de composición de la semilla (Romero del Castillo et al. 2010)
- proteínas de semilla (Campa et al. 2011)
- respuesta a *Sclerotinia sclerotiorum* (Pérez-Vega et al. 2012).
- respuesta a 11 razas de antracnosis (Campa et al. 2014)

Objetivos

Objetivos de la Tesis

El objetivo principal de esta Tesis es profundizar en el conocimiento de la herencia de la resistencia frente a tres patógenos que inciden gravemente en los cultivos de judía. Los objetivos específicos son:

Objetivo 1. Análisis de la interacción entre *P. vulgaris* y un aislamiento local de oídio.

Objetivo 2. Análisis genético de la resistencia frente a un aislamiento local de oídio en la variedad Cornell 49242

Objetivo 3. Análisis genético de la resistencia a dos razas de *Pseudomonas syringae* pv. *phaseolicola* presentes en los cultivos locales, en la variedad Cornell 49242

Objetivo 4. Análisis genético de resistencia a cinco razas de antracnosis en el genotipo SEL1308 derivado de la variedad diferencial G2333

El trabajo se organiza en cuatro Capítulos que se corresponden con cuatro publicaciones. Cada una de ellas desarrolla un objetivo:

Capítulo 1. **Genetic resistance to powdery mildew in common bean**

Capítulo 2. **Genetic mapping of two genes conferring resistance to powdery mildew in common bean (*Phaseolus vulgaris* L.)**

Capítulo 3. **Identification of quantitative trait loci involved in the response of common bean to *Pseudomonas syringae* pv. *phaseolicola***

Capítulo 4. **Identification of a new region involved in the genetic control of resistance to anthracnose in common bean**

Publicaciones

Capítulo 1

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Genetic resistance to powdery mildew in common bean

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Abstract Powdery mildew can cause severe yield losses in bean crops. Limited information about resistance sources, and nature and inheritance of resistance are available to bean breeders and plant pathologist. Sources of resistance were searched in seedling tests under controlled conditions in 44 well-known genotypes and in a Spanish germplasm core collection consisting on 201 accessions. A 0–4 scale was used to describe the infection types (IT) observed. Only six out of the 245 evaluated genotypes showed a complete resistance (IT0) without visible symptoms on the leaves: Amanda, Belneb, Cornell 49242, Negro San Luis, Porrillo Sintetico and the local accession BGE003161. Inheritance of resistance was studied in F_2 and $F_{2:3}$ segregating populations. Observed reactions in the five segregating populations fitted to Mendelian ratios with different modes of inheritance. Results revealed that cultivar Porrillo Sintetico carries two dominant and independent resistance genes: one gene conferring complete resistance (IT0), and another gene conferring IT3, characterized by a moderate mycelial development on the leaves. Both genes show a dominant epistatic relationship. Inheritance of response to powdery mildew in cv. Cornell 49242 was similar to cv. Porrillo Sintetico although the correspondence with the genes described in Porrillo Sintetico was not established. Line X2776 carries one dominant gene conferring IT3, and shares this gene with cv. Porrillo Sintetico. In cv. Amanda, two complementary genes appear to be involved in resistance to this fungus. This information will be relevant for the implementation of breeding programs focused on the development of cultivars carrying genetic resistance to powdery mildew.

Keywords Resistance sources · Plant–fungus interaction · Inheritance of resistance · Plant breeding

Introduction

Powdery mildew is a serious disease for many crops worldwide including common bean (*Phaseolus vulgaris* L.). Common bean powdery mildew causal agent has been frequently ascribed to *Erysiphe poligony* DC. (Ferreira et al. 1999) but recent studies suggest that it is closer to *Erysiphe diffusa* (*Cooke & Peck*) *U. Braun & S. Takam*, formerly *Microsphaera diffusa* Cke. & Pk. (Almeida et al. 2008). Powdery mildews are obligate biotroph fungi that affect all aerial parts of the

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bean plant. The disease is usually first noted as subtle, small, round, greyish or whitish spots on leaves or stems (Schwartz 2005). Progression of the disease leads to enlargement of the spots and coalescinge to a white mass resembling talcum powder on the upper leaf surface, especially in older parts of the plant. On the pods, the fungus can produce greyish cankers. The entire plant can be covered by cottony fungal mycelia and premature senescence of leaves and pods may occur. In the last years the incidence of powdery mildew has significantly increased in bean crops of northern Spain. Under field conditions, fungus colonies appear on bean crops in mid to late July and spread rapidly, colonizing entire leaf surfaces and stems. Conventional methods based on fungicide application have not been efficient in disease control.

The use of resistant bean cultivars can be the most efficient, economical and ecological strategy to provide effective control of this disease. Limited information about sources of resistance to the fungus and the nature and inheritance of resistance are available to bean breeders and plant pathologist. A few sources of resistance to powdery mildew have been described (Schwartz et al. 1981), and a qualitative nature of resistance has been suggested (Bett and Michaels 1995; Ferreira et al. 1999, 2001). The response of common bean to powdery mildew was previously reported to be governed either by a single dominant gene (Dundas 1936); by one dominant and another recessive resistance genes (Bett and Michaels 1995); or by two complementary dominant genes (Ferreira et al. 1999). In general, plant response to powdery mildew has been studied in evaluations carried out in naturally infected fields. However, resistance tests performed under controlled conditions are preferable to ensure repeatable results of the analysis of plant-pathogen interaction (Sillero et al. 2006). In other species, resistance tests developed under controlled conditions and qualitative scales have been used to score the response to powdery mildew (Fondevilla et al. 2006) but this was not applied before in common bean-powdery mildew interaction.

Core collections are usually recommended for the identification of potential sources of resistance against pathogens as well as for the validation of new descriptors, such as the response to plant-pathogen interaction (van Hintum et al. 2000; Pascual et al. 2010). Segregating populations are also a useful tool to investigate the qualitative or quantitative nature of reaction against pathogens as well as to analyze the inheritance. Both sets of materials can also help to test screening methods.

The objectives of this study were to (i) identify resistance sources against powdery mildew in common bean, and (ii) determine the nature of resistance. This information can be of interest for plant breeding programs focused on the introgression of genetic resistance to powdery mildew in bean genotypes.

Materials and methods

Plant material

A total of 245 bean accessions maintained at the Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA, Villaviciosa, Asturias, Spain) germplasm collection were screened for reaction to a local isolate of powdery mildew. The evaluated materials included 201 accessions of the core collection established from the main bean genebank in Spain (Pérez-Vega et al. 2009), and 44 well-known genotypes, cultivars (cvs.) or breeding lines, including: eleven cvs. used for anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib] race characterization: Michelite, Michigan Dark Red Kindey (MDRK), Perry Marrow, Cornell 49242, Mexico 222, Widusa, Kaboon, PI-207262, TO, TU and AB136 (Pastor-Corrales 1991); four lines used as resistant sources to anthracnose: A252, A321, A483, and A493 (Ferreira et al. 2008); seven cvs. used for characterization of the pathogenic variability of *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV): Amanda, Dubbele Witte, Great Northern 31, Imuna, Jubila, Pinto 114, and Topcrop (Drijfhout 1978); four genotypes described as resistant sources to BCMV and BCMNV: Don Timoteo, BRB57, BRB130, and IVT7214; five lines described with moderate levels of resistance to white mold [*Sclerotinia sclerotiorum* (Lib.) de Bary]: A195, G122, Cornell 606, MO-162, and PC 50 (Pascual et al. 2010); the lines BAT93 and Jalo EEP558 used as parents of a major core mapping population (Freyre et al. 1998); four cultivars included in the market class fabada and developed at SERIDA, Andecha, A2806, Xana, and X2776; the cvs. Sanilac and Tendergreen commonly used as reference for the two major gene pools, Middle American and Andean

(Gepts et al. 1986); genotypes A55, Negro San Luis, Belneb, and Chinook described with high levels of resistance to soil pathogens (Román-Avilés and Kelly 2005); and Porrillo Sintético, a genotype described as resistant to powdery mildew (Schwartz et al. 1981).

Five F_2 populations derived from the crosses Xana × Cornell 49242, Xana × Porrillo Sintético, Amanda × Xana, X2776 × G122, and Porrillo Sintético × X2776 were evaluated to investigate the inheritance of the response to powdery mildew. In order to verify the mode of inheritance, 83 $F_{2:3}$ families (obtained by selfing individuals F_2 plants) derived from the cross between the susceptible cv. Xana and the resistant cv. Porrillo Sintético were also evaluated. At least twenty F_3 plants per family were evaluated to characterize the corresponding F_2 plants for resistance to powdery mildew. Number of evaluated F_3 plants was increased in six families to confirm the mode of inheritance.

Inoculation procedure and disease scoring

A local isolate of powdery mildew obtained from a single spot in one infected bean plant at Villaviciosa, Asturias (northern Spain) was used in this study. The pathogen isolate was maintained on plants of susceptible bean cv. Xana in spore-proof chambers. Sequence analysis of ITS region revealed a high similarity of this local isolate with the species *Erysiphe diffusa* (N. Rispail per. com.) which is in agreement with Almeida et al. (2008).

Four seeds of each genotype were planted in a 15 cm plastic pot, 1.5 l volume, containing 80 peat:20 perlite. When the primary leaves were fully developed, seedlings were inoculated by blowing conidia directly from infected leaves onto test plants to give a density of about 5–10 spores/cm^2. Inoculations were performed in sets of 25 pots placed in a box (80 cm × 80 cm × 80 cm). To verify the inoculation procedure, a pot containing susceptible genotype Xana was included in each set as control. Inoculated plants were transferred to another greenhouse and they were maintained at moderate temperature (18–24 °C) and moderate relative humidity (60–70 %). Only inoculated sets in which control Xana showed symptoms were considered. Plant response was recorded as infection type (IT) following a 0–4 scale (Fig. 1) adapted from Mains and Dietz (1930): IT0, seedlings with no visible symptoms; IT1, seedlings with necrotic reaction on leaves with little or no mycelial development; IT2, seedlings with necrotic reaction and moderate mycelial development; IT3, seedlings with moderate mycelial development on leaves without sporulation; IT4, seedlings with abundant mycelial development on leaves and profuse sporulation. Disease assessment was performed 12 days after inoculation when the susceptible control exhibited visible symptoms. Each plant in the pot was rated separately.

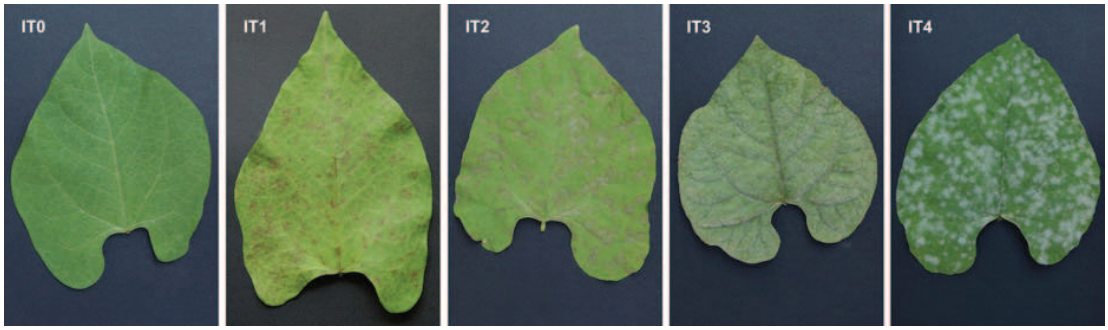

Fig. 1 The five infection types (IT) considered in the classification of response to powdery mildew in common bean: *IT0* primary leaf with no visible symptoms (cv. Porrillo sintético), *IT1* primary leaf with necrotic reaction and no mycelial development (cv. TO), *IT2* primary leaf with necrotic reaction and moderate mycelial development (line A195), *IT3* primary leaf with moderate mycelial development and no sporulation (line X2776), *IT4* primary leaf with abundant mycelial development and profuse sporulation (cv. Xana)

Experimental design and data analysis

Three tests were performed to investigate the reaction of bean materials. In each test, two pots per genotype or accession were arranged in a randomized design. Genotypes or accessions showing different infection types in the same test were considered as mixtures. Genotypes or accessions showing different scores among tests were evaluated in a fourth test and classified according to the most frequent response.

Segregating (F_2 or $F_{2:3}$) populations were evaluated in two different tests, including the corresponding parents. Chi-square was used to test goodness-of-fit of observed to expected ratios.

Results

Response of well-known genotypes

Results presented in Table 1 shows that various types of responses are present in this set of bean genotypes. Five cvs. (Amanda, Belneb, Cornell 49242, Negro San Luis, and Porrillo Sintético) showed complete resistance with no symptoms (IT0). Breeding lines A195, A321, BRB130 and cv. TO displayed high levels of resistance based on macroscopically visible hypersensitive response (IT1 or IT2). Four genotypes (A2806, AB136, Chinook, and X2776) exhibited intermediate resistance based on a diffuse pathogen growth on the leaf (IT3). Cultivar Jubila showed a mixed infection type (IT0/IT4). Thirty remaining genotypes were very susceptible showing symptoms similar to those of the susceptible control Xana (IT4).

Response of the core collection accessions

A total of 201 accessions included in a core collection were screened for their reaction against the local isolate of powdery mildew. Results are shown in Fig. 2. Most of the accessions (146) showed IT4, with clear symptoms and conidia production, like the susceptible control Xana. Ten accessions exhibited IT3 with moderate mycelial development on leaves without profuse sporulation. Clear necrotic reactions on leaves (IT1) were observed in seven accessions, and in 28 accessions the necrotic reaction occurred together with limited pathogen growth (IT2). Infection type 0 (seedlings with no visible symptoms) was only observed in the local accession BGE003161 which has small, oval, and cream colour seeds (30 g/100 seeds)

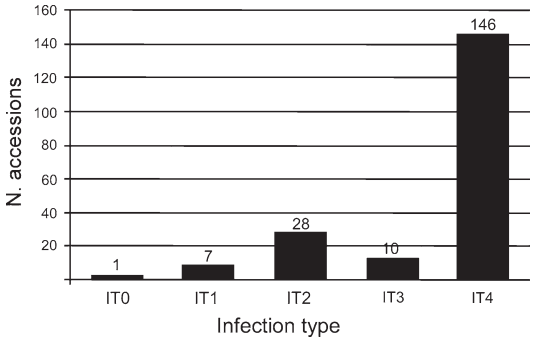

Fig. 2 Histogram showing the distribution for the reaction to a local isolate of powdery mildew for bean accessions included in the core collection described by Pérez-Vega et al. (2009). The nine accessions showing evidences of mixture in the reaction, were not included

Table 1 Reaction of 44 common bean genotypes against a local isolate of powdery mildew observed in three separate tests

Infection type (IT)	Genotypes
0	Amanda, Belneb, Cornell 49242, Negro San Luis, Porrillo Sintetico
1	A195
2	A321, BRB130, TO
3	A2806, AB136, Chinook, X2776
4	A252, A483, A493, A55, Andecha, BAT93, BRB57, Cornell 606, Don Timoteo, Dubbele Witte, G122, Great Northerm 31, Imuna, IVT7214, JALO EEP558, Jubila[a], Kaboon, MDRK, Mexico 222, Michelite, MO162, PC50, Perry Marrow, PI207262, Pinto 114, Sanilac, Tendergreen, Topcrop, TU, Widusa, Xana

Plant response was classified in five classes; *IT0* with no visible symptoms, *IT1* seedlings with necrotic reaction on leaves, *IT2* necrotic reaction together with limited growth of the pathogen on leaves, *IT3* diffuse growth and no sporulation, *IT4* abundant mycelial development on leaves and profuse sporulation

[a] Jubila showed mixture of IT0 and IT4

and an indeterminate prostrate growth habit. A mixture in infection type was observed in nine accessions and one of them, BGE022510, included seedlings with IT0 (8 plants) and IT4 (16 plants).

Inheritance of the response

To investigate the genetic control of resistance and to verify the consistence of the 0–4 scale used, the reaction to powdery mildew was analyzed in five F_2 segregating populations. Reaction of the F_2 seedlings was classified according to the 0–4 scale, suggesting a qualitative nature of resistance. Table 2 shows the observed segregations for the reaction to the local isolate of powdery mildew. Two tests were carried out and the observed segregation of each F_2 population fitted to the same Mendelian ratio in both tests.

Three types of responses were observed in the F_2 plants derived from the crosses Xana (IT4) × Cornell 49242 (IT0) and Xana (IT4) × Porrillo Sintetico (IT0): IT0, IT3, and IT4. IT0 was the most common reaction. Observed segregations showed no significant differences when compared to the expected ratio for two independent dominant genes (12 IT0: 3 IT3: 1 IT4): a gene conferring IT0, and another gene conferring IT3. Both genes showed a dominant epistatic relationship, so that expression of the second gene is masked by the dominant effect of the first gene.

In the two tests, F_2 segregating population derived from the cross Porrillo Sintético (IT0) × X2776 (IT3), F_2 plants showed parental responses, with IT0 as the most common reaction (Table 2). The segregation ratio fitted to the expected ratio for one dominant gene (3 IT0: 1 IT3) suggesting that both parents differ in a dominant gene controlling the reaction IT0.

Two types of responses were observed in F_2 plants derived from the cross X2776 (IT3) × G122 (T4): IT3 and IT4. Infection type 3 was the most common response (Table 2). Observed segregation fitted to the expected ratio for one dominant gene (3 IT3:1 IT4) suggesting that IT3 is controlled by a dominant gene in X2776.

Parental infection types were also observed in the F_2 segregating population derived from the cross Amanda (IT0) × Xana (IT4) and observed segregation fitted to a 9 (IT0): 7 (IT4) ratio, expected in the case of two complementary dominant genes.

Genetic dissection of resistance in Porrillo Sintetico

In order to confirm that two different genes are involved in the response of Porrillo Sintetico, a $F_{2:3}$ population obtained from the cross Porrillo Sintético × Xana was evaluated in detail. The following results were observed: 28 families with all F_3 plants showing IT0; 26 families including F_3 plants with IT0, IT3 and/or IT4; 12 families including F_3 plants with IT0 and IT4; 8 families including F_3 plants with IT3 and IT4; 2 families with all F_3 plants showing IT3; 7 families with all F_3 plants showing IT4. Expected ratio in the case of two dominant and independent genes

Table 2 Observed segregation in five F_2 populations derived from crosses among common bean parents with different types of reaction against a local isolate of powdery mildew

Parent 1 × Parent 2	Response of parents (IT)		Test	Observed segregation			Expected ratio	χ^2	*P*-value
	Parent 1	Parent 2		IT0	IT3	IT4			
Xana × Cornell 49242	4	0	1	55	13	10	12:3:1	5.78	0.06
			2	51	12	4	12:3:1	0.00	0.98
Xana × Porrillo Sintético	4	0	1	76	23	5	12:3:1	1.18	0.55
			2	96	19	2	12:3:1	5.02	0.08
Amanda × Xana	0	4	1	46	–	36	9:7	0.01	0.92
			2	12	–	13	9:7	0.69	0.40
X2776 × G122	3	4	1	–	91	24	3:1	1.29	0.26
			2	–	73	23	3:1	0.00	0.81
Porrillo Sintético × X2776	0	3	1	65	16	–	3:1	1.19	0.27
			2	80	25	–	3:1	0.11	0.73

IT infection type

(A,a and B,b) with a dominant epistatic relationship is, 4 (F_2 genotype, *AA*−; observed infection type in F_3 families, 0): 6 (*AaBB*/*AaBb*; IT0/IT3/IT4): 2 (*Aabb*; IT0/IT4) 2 (*aaBb*; IT3/IT4): 1 (*aaBB*; IT3): 1 (*aabb*; IT4). The observed ratio of these six classes fitted to expected ratio for this hypothesis ($\chi^2 = 6.78$, $P = 0.24$). Number of evaluated F_3 plants were incremented in six F_3 families with monogenic segregation to verify this hypothesis (Table 3): F_3 families 59 and 63 exhibited a segregation for IT0 and IT4; F_3 families 22 and 71 exhibited a segregation for IT0 and IT3; F_3 families 37 and 95 exhibited a segregation for the reaction types 3 and 4. In all cases, observed segregations showed no significant differences when compared to the expected ratio for one dominant gene, confirming that Porrillo Sintetico carries two dominant and independent genes.

Discussion

We adopted a qualitative scale including five phenotypic classes to classify reactions of common bean germplasm to powdery mildew. To our knowledge, this qualitative scale has never been tested before in common bean although a similar qualitative scale was used to record the response against powdery mildew in barley germplasm (Mains and Dietz 1930; Shtaya et al. 2007). Previous studies investigating bean-powdery mildew interactions used a 1-9 quantitative scale based on percentage of the leaf area covered by mycelium to assess the response in field tests (Ferreira et al. 1999). Our results reveal that the responses of bean germplasm, well-known genotypes and segregating populations can be classified in any of the five defined classes. Limited variations in the response among tests were observed in some genotypes. Observed variation was between IT3 and IT4 (e.g. accessions BGE003208 and BGE003246) or IT1 and IT2 (e.g. A321, BRB130 and TO). Environmental factors such as humidity, temperature or light can influence development of the disease, possibly explaining these differences in scores, especially the necrotic reaction. Expression of resistance to powdery mildew associated with high temperatures (25 °C) was described in pea (Fondevilla et al. 2006).

Plant responses of genotypes Cornell 49242, Porrillo Sintético, A195, G122, A2806, AB136, MDRK, Andecha or X2776 observed under controlled conditions were similar to those observed in naturally infected fields at Villaviciosa, Asturias (northern Spain). Genotypes with IT0 such as cvs. Cornell 49242 or Porrillo Sintético did not show symptoms under field conditions, in naturally infected fields. Genotypes with IT1, IT2 or IT3 exhibited limited mildew growth in the field, not affecting the plant development. Genotypes with IT4, such as cvs. Andecha, Xana, G122 or MDRK, showed symptoms in all aerial parts of the plants, as well as leaf drop and premature death when grown in naturally infected fields.

The observed reactions in the F_2 segregating populations analyzed in this study indicate a qualitative nature of resistance to powdery mildew, supporting previous reports (Dundas 1936; Bett and Michaels 1995; Ferreira et al. 1999). A qualitative response in the interaction powdery mildew- common bean is not surprising, since this type of response has already been reported in other legume species such as pea (*Pisum sativum* L.) (Fondevilla et al. 2008; Katoch et al. 2010), mungbean (*Vigna radiata* L. Wilczek; Reddy et al. 1994), or soybean ((*Glycine max* L. (Merr.)) (Lohnes and Bernard 1992; Kang and Rouf Mian 2010).

From observed segregations, it is possible to conclude that several resistance genes controlling

Table 3 Observed segregation in six $F_{2:3}$ families derived from crosses Xana × Porrillo Sintético

F_3 families	Observed segregation			Expected ratio	χ^2	*P*-value
	IT0	IT3	IT4			
F3-59	52	–	15	3:01	0.24	0.62
F3-63	43	–	16	3:01	0.14	0.71
F3-22	42	13	–	3:01	0.00	0.81
F3-71	48	14	–	3:01	0.19	0.66
F3-37	–	43	15	3:01	0.00	0.87
F3-95	–	54	17	3:01	0.00	0.89

different expression of resistance and modes of inheritance, are involved in the response to this fungus. Observed segregation against the local isolate of powdery mildew in the F_2 and $F_{2:3}$ segregating populations derived from the cross Porrillo Sintetico (IT0) × Xana (IT4), can be explained by the presence of two dominant and independent genes in cultivar Porrillo sintético; one gene conferring IT0 (no visible symptoms), and another gene conferring IT3 (limited growth of the pathogen). Both genes show a dominant epistatic relationship. Cultivar Cornell 49242 (IT0) showed the same type of inheritance, although with available data it was not possible to determine if Cornell 49242 and Porrillo Sintético share the same resistance loci. The two segregating populations derived from the cross Porrillo Sintético (IT0) × X2776 (IT3) showed a good fit to the expected ratio for a dominant gene, confirming that cultivar Porrillo Sintético carries a dominant gene which confers reaction type 0. The presence of this dominant gene in Porrillo Sintético was also confirmed in the introgression of complete resistance (IT0) to powdery mildew into line X2776 using backcrossing method. A total of 28 BC_1F_1 plants [derived from X2776 × (X2776 × Porrillo Sintético)] were tested and a 1:1 segregation ratio was observed (12 plants IT0: 14 plants IT3). Segregating populations derived from the cross X2776 (IT3) × G122 (IT4) also showed a good fit to the expected ratio of one dominant gene, indicating that line X2776 carries one dominant gene conferring IT3. From these results, it can also be concluded that a dominant gene controls IT3 in genotype X2776, and this gene is also present in Porrillo Sintético. The 9:7 ratio observed in the population derived from Amanda (IT0) × Xana (IT4) indicates that two complementary genes are involved in the resistance of cultivar Amanda. This type of inheritance has already been described in the interactions powdery mildew—common bean (Ferreira et al. 1999), anthracnose—common bean (Campa et al. 2011), and angular leaf spot—common bean (Mahuku et al. 2011). Additional analysis including allelic tests and mapping will be necessary to characterize the different resistance genes described in this study.

In conclusion, the results of this study (i) show that reactions of common bean genotypes against powdery mildew in controlled conditions can be classified in a 0–4 qualitative scale, (ii) provide clear evidence about a qualitative nature of resistance involving different modes of inheritance, and (iii) identify useful sources of complete and incomplete resistance against the pathogen. This information can be relevant for plant pathologist or the implementation of breeding programs focused on the development of new cultivars carrying genetic resistance to powdery mildew.

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Capítulo 2

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ORIGINAL PAPER

Genetic mapping of two genes conferring resistance to powdery mildew in common bean (*Phaseolus vulgaris* L.)

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Abstract Powdery mildew (PM) is a serious disease in many legume species, including the common bean (*Phaseolus vulgaris* L.). This study investigated the genetic control behind resistance reaction to PM in the bean genotype, Cornell 49242. The results revealed evidence supporting a qualitative mode of inheritance for resistance and the involvement of two independent genes in the resistance reaction. The location of these resistance genes was investigated in a linkage genetic map developed for the XC RIL population. Contingency tests revealed significant associations for 28 loci out of a total of 329 mapped loci. Fifteen were isolated or formed groups with less than two loci. The thirteen remaining loci were located at three regions in linkage groups Pv04, Pv09, and Pv11. The involvement of Pv09 was discarded due to the observed segregation in the subpopulation obtained from the Xana genotype for the loci located in this region. In contrast, the two subpopulations obtained from the Xana genotype for the BM161 locus, linked to the Co-3/9 anthracnose resistance gene (Pv04), and from the Xana genotype for the SCAReoli locus, linked to the Co-2 anthracnose resistance gene (Pv11), exhibited monogenic segregations, suggesting that both regions were involved in the genetic control of resistance. A genetic dissection was carried out to verify the involvement of both regions in the reaction to PM. Two resistant recombinant lines were selected, according to their genotypes, for the block of loci included in the Co-2 and Co-3/9 regions, and they were crossed with the susceptible parent, Xana. Linkage analysis in the respective F_2 populations supported the hypothesis that a dominant gene (*Pm1*) was located in the linkage group Pv11 and another gene (*Pm2*) was located in the linkage group Pv04. This is the first report showing the localization of resistance genes against powdery mildew in *Phaseolus vulgaris* and the results offer the opportunity to increase the efficiency of breeding programs by means of marker-assisted selection.

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E. Pérez-Vega and N. Trabanco contributed equally to this study.

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Introduction

Powdery mildew (PM) can result in a devastating disease in many legume crops, including the common bean (*Phaseolus vulgaris* L.). In Northern Spain, PM is caused by the fungus, *Erysiphe diffusa* (Cooke and Peck) U. Braun and S. Takam (Trabanco et al. 2012) causing significant yield losses in bean crops. The fungus is an obligate biotrophic pathogen which produces small, round, greyish or whitish spots on leaves, stems and pods. In extreme cases, the fungus can cover all aerial parts of the plant resulting in premature defoliation, premature fall of flowers and pods and a reduction in plant development.

Control of the disease is difficult by conventional techniques, because the fungus produces spores that are easily spread by the wind. In addition, the fungus can be spread by infected seeds, plant debris or by some weeds. Use of resistant cultivars provides an effective approach for disease control, eliminates the use of fungicides, minimizes

crop losses and supports sustainable production management. The development of resistant cultivars requires information on the variation in the reaction against this fungus, potential resistance sources and the nature and inheritance of the resistance. In contrast with other legume species, such as mungbean, pea or soybean, only limited information is available to common bean breeders on the genetic control of PM.

With regard to resistance inheritance against this pathogen, several studies have reported a qualitative mode of inheritance controlled by either one dominant gene (Dundas 1936), one dominant and one recessive gene (Bett and Michaels 1995) or by two complementary genes (Ferreira et al. 1999). Recently, Trabanco et al. (2012) investigated the reaction of 245 accessions and five segregating populations using seedling tests under controlled conditions. They found that only 6 out of the 245 evaluated genotypes showed a complete resistance, without visible symptoms on the leaves: genotype Amanda, Belneb, Cornell 49242, Negro San Luis, Porrillo Sintetico, and a local accession, BGE003161. The observed reactions in the five segregating populations fitted Mendelian ratios with two different modes of inheritance. These were either dominant or complementary modes of action. The results also suggested that the resistant reaction in Cornell 49242 could be explained by two dominant and independent resistance genes, where one gene conferred complete resistance, and another gene moderated mycelial development on the leaves. Both genes showed a dominant epistatic relationship. However, the relative position of these resistance genes in the bean linkage map has not been analyzed.

Resistance genes are not distributed randomly in the bean genome. They appear to be clustered on particular chromosomes and specific regions. For example, anthracnose resistance genes are located in specific regions of the genome and they are organized in groups or clusters of loci in which individual gene(s) confer resistance to one isolate or race (Rodríguez-Suárez et al. 2007, 2008; Campa et al. 2009). To date, seven specific regions, which include these resistance specificities, have been identified in linkage groups (LGs) Pv01, Pv02, Pv03, Pv04, Pv07, Pv08 and Pv11 (Ferreira et al. 2012).

Genetic and molecular studies have revealed the presence of many plant disease resistance genes (R genes) in the genomes of monocotyledonous and dicotyledonous plants. R genes can have two functions in the plant–pathogen interaction. They can operate as sensors, directly or indirectly detecting pathogens, or they can activate downstream signaling that, in turn, leads to pathogen resistance (Chisholm et al. 2006; DeYoung and Innes 2006). R genes are often arranged in complex clusters or group of genes (Ameline-Torregrosa et al. 2008b; Meyers et al. 2003; Michelmore and Meyers 1998). Clusters of R genes have also been reported at the end of LGs Pv04 and Pv11 in the bean genome (Chen et al. 2010; David et al. 2009; Geffroy et al. 1998, 2009).

The objective of this study was to investigate the genetic control of PM resistance in the bean genotype Cornell 49242 and to identify the location in the genetic map of the genes conferring resistance against this pathogen. Mapping of these genes can supply markers suitable for marker-assisted selection in bean-breeding programs and provide the basis for the future genomic characterization of these resistance genes.

Materials and methods

Plant materials

A total of 109 F_7 recombinant inbred lines (RILs), derived from the cross Xana × Cornell 49242 by single seed descent (XC RIL population), were used to study the inheritance of the response to PM. Xana is a large white-seeded line bean variety, with determinate, type I growth habit, which belongs to the market-class fabada. It was developed at Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA, Villaviciosa, Spain) from a cross between two Andean genotypes, Andecha and V203. Cornell 49242 is a small-seeded black bean line showing indeterminate prostrate, type III growth habit. It belongs to the Mesoamerican gene pool and is included in the market class black turtle. Cornell 49242 is resistant to PM whereas Xana is susceptible (Trabanco et al. 2012).

Inoculation procedure and disease scoring

An isolate of PM obtained from a single spot in one infected bean plant at SERIDA-Villaviciosa was used in this work. The pathogen was grown and maintained on plants of susceptible bean cv. Xana in spore-proof chambers.

Resistance tests were carried out according Trabanco et al. (2012). Four seeds of each genotype were planted in a 15-cm plastic pot of 1.5 l containing 80 peat: 20 perlite. Plant response was recorded as resistant or susceptible considering the mycelial development on the areal part of the plants. The XC RIL population was evaluated in three separated tests. Two pots per recombinant line were arranged in each test in a randomized design. In each test, the susceptible line Xana and the resistant lines Cornell 49242 and Porrillo Sintético were included as reference of different reaction types.

Molecular marker development and analysis

Genomic DNA was isolated from young leaves using the FastDNA kit (MP Biomedicals, Illkirch, France) following the supplier's instructions. DNA concentrations were quantified photometrically (absorbance measurements at 260 and 280 nm) with Biomate 3 UV–visible spectrophotometer (Thermo Scientific, MA).

Two type of marker loci were added to the genetic map developed in XC RIL population (Pérez-Vega et al. 2010) to saturate specific regions or linkage groups. (1) Markers previously described in the literature with known position in the bean genetic map: the 254G15F SCAR marker developed from the BAT93 BAC clone 254G15 (David et al. 2008), the SQ4 SCAR marker (Awale et al. 2008) and the $OF10^{1100}$RAPD marker located on end of LG Pv04 (Rodríguez-Suárez et al. 2007). Several mapped microsatellite markers (Blair et al. 2003; Gaitán-Solis et al. 2002; Grisi et al. 2007; Hanai et al. 2010) were also analyzed. (2) Microsatellite markers designed from the accessions EU931620.1, FJ817289, FJ817291.1, and FJ817290 deposited in the Genebank database (David et al. 2009) and located in the Co-3/9 region (LG Pv04). WebSat platform (http://wsmartins.net/websat/; Martins et al. 2009) was used to identify microsatellite motifs and the corresponding forward (FW) and reverse (RV) primers. Sequences were screened for all possible dimeric and trimeric repeat motifs. Sequences containing a minimum of five di-nucleotides or four tri-nucleotides motif repeats were considered for primers design. The selected parameters in primers design were: between 18 and 24 bp (optimum 22 bp); annealing temperature between 50 and 68 °C (optimum 60 °C); GC content between 40 and 60 % and predicted size of the amplified fragment between 100 and 300 bp. Annealing temperature was selected using the OligoAnalyzer software (www.uku.fi/∼kuulasma/OligoSoftware).

The polymerase chain reaction (PCR) amplifications were performed in 20 µl solution containing 25 ng of genomic DNA, 100 mM Tris–HCl, 100 mM KCl (pH 8.3), 4 mM MgCl2, 0.2 mM each dNTP (Bioline, London), 0.2 µM each primer, and 1.25 U of Biotaq DNA polymerase (Bioline, London). Amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems, Life Technologies, Foster City, CA) programmed according to the corresponding author or following the recommendations supplied by the OligoAnalyzer software. The SCAR PCR products were resolved on 2 % agarose gels. The microsatellite PCR products were resolved on 8 % polyacrylamide gels. Agarose and polyacrylamide gels were stained with SYBR safe (Invitrogen, Life Technologies, CA, USA) and the amplification products were visualized under UV light. A 100-bp ladder (G.E. Healthcare Life Science, Fairfield, CT, USA) and the software GeneTools V4.01 (Syngene, Cambridge, UK) were used to measure the size of the fragments.

Linkage analyses

A genetic map developed for the XC RIL population was used to locate the genomic regions involved in the control of the response to PM. The linkage map consisted of 294 loci mapped by Pérez-Vega et al. (2010) and 35 added markers that were common to other bean maps (Gaitán-Solis et al. 2002; Grisi et al. 2007; Hanai et al. 2010). MAPMAKER Macintosh version 2.0 software (Lander et al. 1987) was used for map construction. LGs were established with a log of the likelihood ratio (LOD) threshold of 3.0 and a recombination fraction of 0.25. Marker order was estimated based on multipoint compare, order, and ripple analyses. Distances between loci (cM) were calculated using the Kosambi mapping function. The obtained map had 11 linkage groups, which were aligned according to the common bean core linkage map using common molecular markers as anchor points. Linkage groups were named according to Pedrosa-Harand et al. (2008).

Contingency tests were used to investigate the association between the response to PM (classified as resistant or susceptible) and the loci included in the genetic map. The Chi-square test was used to test the goodness-of-fit of the observed-to-expected ratio in the qualitative traits and linkage analysis was performed using MAPMAKER Macintosh version 2.0. Loci showing significant deviations ($p < 0.01$) were not considered in the linkage analysis. Statistical analyses were performed using SPSS V12 software (SPSS Inc., Chicago, IL).

Genetic dissection

To verify the relative position of the resistance genes to PM in specific regions of the genetic map, a genetic dissection was undertaken. Recombinant lines showing resistant responses were selected according to their genotypes for specific loci located in the candidate regions where the resistance loci could be located. Selected recombinant lines were crossed using the susceptible cultivar, Xana, as the female. Crosses between the selected recombinant lines were also performed to study the mode of action of the different genes. F_1 plants were tested for their response to PM and resistant plants were self-crossed. The corresponding F_2 progenies were tested against a local isolate of PM and analyzed for the amplification of the specific markers used in the selection of recombinant lines.

Results

Response of the XC RIL population

Observed responses in the XC RIL population were classified into two groups: resistant and susceptible, like the parent, Xana. A total of 29 recombinant lines showed a susceptible response like the parent, Xana, while the 80 remaining lines were resistant. The observed segregation fitted the 3:1 expected ratio for two independent genes ($\chi^2_{3:1} = 0.15$, $p = 0.70$), which suggested that two independent genes control the resistant reaction in Cornell 49242. The mode of action of the genes (dominant or recessive) could not be deduced from this segregation.

Identification of candidate regions containing resistance genes

To identify the regions associated with PM resistance, contingency tests were undertaken among the loci included in the genetic map and the response to PM. Significant association was found for 28 loci out of a total of 329 mapped loci (Table 1 in ESM). Fifteen of them were isolated loci or formed groups with less than two loci in the genetic map. The thirteen remaining loci were located at three regions: three loci formed a large block in LG Pv04, including SCAR SW12, which is linked to the Co-3/9 anthracnose resistance gene; five loci were located on LG Pv11 and included the SCAR marker SCAreoli, linked to the Co-2 anthracnose resistance gene and; five loci were grouped in a wide region on LG Pv09, including the microsatellite marker BM141 and the AFLP marker McatEag^{131}. The marker locus BM161 (closely linked to SW12 on LG Pv04) and the SCAreoli locus on LG Pv11 showed greater deviations with respect to independent segregation with the response to PM. The deviations were due to an excess of resistant lines containing the Cornell 49242 genotype at these loci. In contrast, the deviation found for the five loci of LG Pv09 was due to an excess of resistant lines showing the Xana alleles (susceptible parent) for these markers, which suggested that this was a random association.

Marker enrichment of LGs Pv04 and Pv11

To precisely locate the genes conferring resistance to PM, twenty loci were added to LGs Pv04 and Pv11, with respect to the genetic map described by Pérez-Vega et al. (2010).

Linkage group Pv04

Fifteen new loci were incorporated into the LG Pv04. Microsatellite motifs were investigated in the accessions: EU931620.1, FJ817289, FJ817291.1 and FJ817290.1. A total of 77 microsatellites were designed: 38 with a dinucleotide repeat motif and 39 with a trinucleotide repeat motif. Twenty-seven microsatellites were tested in cvs. Xana and Cornell 49242 and 24 showed the amplification product (Table 2 in ESM). Seven microsatellites were shown to be polymorphic between both cultivars and they were analyzed in the XC RIL population. Table 1 shows the characteristics and the PCR primer pairs for the seven microsatellite markers that were designed. In all cases, the observed segregation fitted the expected ratio for one gene. Six microsatellites were mapped on LG Pv04, whereas the microsatellite, FZ-E9o, was located at the end of LG Pv11 (Fig. 1). The amplification product of SCAR 254G15F revealed three fragments in 2 % agarose gels, which were closely linked to the SW12 locus (Fig. 1). Twelve SSR markers, previously mapped on LG Pv04, were also analysed for polymorphism and five of them were added to the map: PV-ag004, BMd15, BMd9, BMarc22 and PVBR112. Finally, the RAPD fragment, OF10^{1100}, was added (Fig. 1). The added loci Contig-ah, 254G15E^{550}, 254G15E^{600}, 254G15E^{320}, FZ-E9 h, Contig IIIh, Contig IIIi, and OF10^{1100} were mapped on the Co-3/9 region and they were significantly associated with the response to PM also (Fig. 1; Table 1 in ESM).

Linkage group Pv11

Fourteen microsatellite markers mapped on Pv11 were investigated for polymorphism. Three of them were polymorphic and mapped on LG Pv11; PvBR113, BMd33 and PvM98. Finally, the SCAR marker, SQ4 was also analyzed and added to this LG (Fig. 1). The added loci SQ4 and FZ-E9o were mapped on the Co-2 region and they were significantly associated with the response to PM (Fig. 1; Table 1 in ESM).

Tentative mapping using subpopulations

To verify the involvement of the candidate regions in the response to PM, the resistant/susceptible segregations were investigated in different subpopulations of recombinant lines (Table 2). The subpopulation obtained from the Xana genotype for the McatEag^{131} locus (Pv09) exhibited a 36R:7S segregation for resistance to PM, which fitted the expected ratio for two genes ($\chi^2_{3:1} = 1.74$, $p = 0.19$). This finding suggested that this region on LG Pv09 was not involved in the genetic control of resistance to PM. A change in the segregation type with respect to the observed in the XC RIL population (non-segregation or monogenic segregation) would be expected if this tagged region was involved in the genetic control of the response to PM.

Table 1 Characteristics and PCR primer pairs amplifying seven microsatellite markers designed from the sequence FJ817291.1 and FJ817290.1 deposited in the Genebank data base

Marker name	Genebank entry	Motif		Primer sequence (5′ to 3′)	Tm (°C)	Predicted size
FZ-E9b	FJ817290.1	$(CA)_5$	Forward	ATTGGTAGAAACCGACTTTGGA	55	138
			Reverse	ATCCACAACAGCTTACAGGGTT		
FZ-E9m	FJ817290.1	$(AAG)_5$	Forward	CACTTAACAGGAGAAATCAGCTC	50	250
			Reverse	AGCTTCTGCACTACATCTTGTC		
FZ-E9n	FJ817290.1	$(TA)_{10}$	Forward	CGTTAGCCAAATTACAGAGCAA	50	160
			Reverse	AGACCCTTTCCTTCTCAATGC		
FZ-E9o	FJ817290.1	$(ATA)_4$	Forward	CAATCAAATCATGGAGAGGGTA	50	297
			Reverse	GAATCTGTGAATGGGACGAATA		
Contig-ah	FJ817291.1	$(ATA)_5$	Forward	CTGTTTCACAATCAACGGAT	58	186
			Reverse	TGCAAAAGGGACCTATCTAA		
Contig-III h	FJ817291.1	$(ATT)_4$	Forward	TGTAAGCTCTTTCCTCCCTCTG	58	289
			Reverse	TGACCATTGATTTCAGTAAGCC		
Contig-III i	FJ817291.1	$(AT)_6$	Forward	CACCATGTTCTGCTTCTTTT	50	249
			Reverse	CAGTTCAGAGGTGATTGTTTG		

In contrast, the subpopulation obtained from the Xana genotype for the BM161 locus or from the Xana genotype for the SCAReoli locus exhibited: 21R:24S and 21R:23S segregations, respectively (Table 2). In both cases, the observed segregation fitted the expected ratio for one gene ($\chi^2_{1:1} = 0.2$, $p = 0.35$; $\chi^2_{1:1} = 0.0\,p = 0.76$). These results suggested that one resistance gene to PM was linked to the BM161 locus (Co-3/9 region on LG Pv04) and the other was linked to the SCAReoli locus (Co-2 region on LG Pv11). This hypothesis is consistent with the segregation observed in the two subpopulations established from the Cornell genotypes for the loci BM161 and SCAReoli (Table 2). The segregations for subpopulations obtained from Cornell 49242 genotypes for these loci were: 45R:1S and 43R:1S. Both segregations did not fit the expected ratio for two independent genes or one gene. This finding suggested that a resistance gene was fixed in both subpopulations. The observed susceptible lines may be due to recombination events between the resistance locus and the respective marker loci. Finally, the involvement of both regions in the resistance control was consistent with the non-segregation observed (Table 2) in the subpopulation obtained from the Xana genotype for the BM161 and SCAReoli loci (all the lines were susceptible) or from the Cornell 49242 genotype for the BM161 and SCAReoli loci (all the lines were resistant).

The two subpopulations established from the Xana genotype for the BM161 and SCAReoli loci showed a monogenic segregation for resistance (Table 2) and so they were used in the linkage analysis. Subpopulation established from the Xana genotype for the SCAReoli locus (Pv11) revealed that the BM161 locus was tightly linked to the resistance gene (recombination fraction, RF = 0.02; LOD = 6.84). The SW12 and FZ-E9b loci, located in Co-3/9 region, also showed a close linkage to the resistance gene (LOD > 3.5). Subpopulations established from the Xana genotype for the BM161 locus (Pv04) revealed that the SH13b locus was tightly linked to the resistance gene (recombination fraction, RF = 0.01; LOD = 10.01). Loci SQ4, SCAreoli and PVag001, located in the Co-2 genomic region, also showed a close linkage to the resistance gene (LOD > 7.00). Figure 1 shows the relative position of the two resistance loci inferred from the linkage analysis.

Genetic dissection

A genetic dissection was carried out to confirm the hypothesis that two independent genes located at the end of LGs Pv04 and Pv11 conferred resistance to PM in Cornell 49242. Two resistant recombinant lines were selected according to their genotypes for the block of loci included in the Co-2 and Co-3/9 regions and, they were crossed with the susceptible parent, Xana. In all cases, the susceptible genotype, Xana, was used as the female parent. The resistant recombinant line 22 (XC22) carried Cornell 49242 genotypes for the Co-2 region markers (SCAreoli, SQ4, PVag001 and SH13b) and the Xana genotypes for markers of the Co-3/9 block (SI19, 254-G15F^{550}, 254-G15F^{600}, 254-G15F^{320}, FZ-E9b, SW12, BM161, FZ-E9b and OF10^{1100}; Fig. 1). Resistant recombinant line 217 (XC217) carried Xana genotype for the Co-2 region markers and the Cornell 49242 genotypes for markers of the Co-3/9 region. Seven F_1 plants, derived from the cross Xana × XC22, were tested and all of them were resistant. Eight F_1 plants, derived from

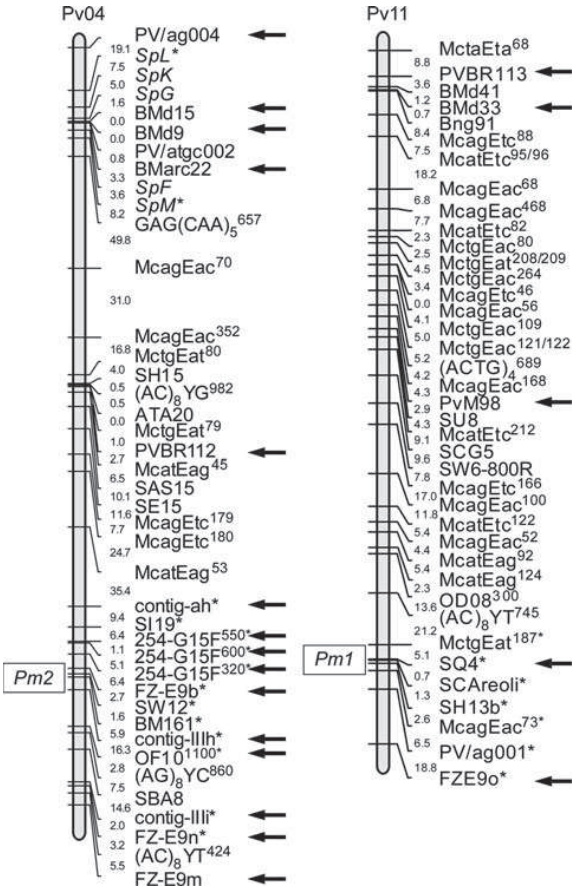

Fig. 1 Linkage map for the linkage groups Pv04 and Pv11 obtained in the RIL population developed from the cross Xana × Cornell 49242. Map distances, on the *left*, are expressed in centimorgans. *Asterisks* near the name of the loci indicate a significant association with the resistance to powdery mildew revealed by contingency tests ($p < 0.05$). *Arrows* indicate the new mapped loci in this study. Markers SCAreoli and SQ4 (Pv11) were described as linked to the genes *Co-2* and *Ur-11* conferring resistance to anthracnoses and rust, respectively. Marker SW12 (Pv04) was described as linked to the anthracnose resistance gene *Co-3* and marker $OF10^{1100}$ was described as linked to an anthracnose resistance gene tentatively named as *Co-10* (Ferreira et al. 2012)

the cross Xana × XC217, were tested and they all were also found to be resistant. Finally, the six F_1 plants obtained from the cross XC217 × XC22 (line XC217 was used as female parent) exhibited a resistant reaction like the resistant parent Cornell 49242.

F_1 plants derived from the crosses Xana × XC22 and Xana × XC217 were self-crossed and the F_2 populations derived from each cross were analysed for their response to PM and for markers located in the Co-2 or Co-3/9 regions. The F_2 population derived from the cross Xana × XC22 (86 plants) showed a 59R:27S segregation, which fitted the expected ratio for one dominant gene ($\chi^2_{3:1} = 1.88$; $p = 0.17$). The result suggested that resistance to PM was controlled by one dominant gene in the XC22 line. Four molecular markers, located in the Co-2 region, were analysed in most of the plants included in this F_2 population (Table 3). Linkage analysis corresponding to the joint segregation for resistance to PM and four molecular markers located in the Co-2 region (Table 3) confirmed that the resistance gene in XC22 line was located in LG Pv11. Resistance locus was closely linked to the SQ4 marker (RF = 3.6 %, LOD = 17.37).

The F_2 population derived from the cross Xana x XC217 (79 plants) showed a 53R:26S segregation. The observed segregation fitted the expected ratio for one dominant gene ($\chi^2_{3:1} = 2.64$; $p = 0.10$), which showed that resistance to PM in the XC217 line was controlled by a dominant gene. Eleven molecular markers, located in the Co-3/9 region, were also analysed in the majority of plants of this F_2 population (Table 4). Linkage analysis corresponding to the joint segregation of resistance to PM with molecular markers mapped in the Co-3/9 region (Table 4) confirmed that the resistance gene in the XC217 line was located in this region. This resistance locus was linked to the SBA8 marker (RF = 24.4 %, LOD = 3.36). In this segregation, seedlings classified as resistant (in contrast to Xana) showed moderate mycelial development on the leaves.

Table 2 Observed segregation on different subpopulations established from Xana or Cornell 49242 genotype for the loci BM161 (Pv04) and/or SCAReoli (Pv11). Adjustment to an expected segregation controlled by two loci or one single locus is indicated

Genotype		Observed Segregation					
BM161 (Pv04)	SCAreoli (Pv11)	R	S	$\chi^2_{1:1}$	p	$\chi^2_{3:1}$	p
Cornell		45	1	32.1	0	12.8	0
	Cornell	43	1	40.1	0	12.1	0
Xana		21	24	0.2	0.35	19.3	0
	Xana	21	23	0	0.76	17.5	0
Xana	Xana	0	19	–	–	–	–
Cornell	Cornell	19	0	–	–	–	–
Xana	Cornell	20	1	17.9	0	5.19	0.02
Cornell	Xana	19	1	15.2	0	4.27	0.03

R, resistant; S, susceptible

Discussion

This study investigated the inheritance of genetic resistance to PM in the bean genotype Cornell 49242, using a RIL population obtained from a cross between Xana and

Table 3 Linkage analysis between the gene conferring resistance to powdery mildew and four molecular markers located in LG Pv11 in a F_2 population derived from the cross Xana × XC22

Marker	Linked gene	Parental genotypes(bp)		F_2 plants										RF	LOD
				Resistant					Susceptible						
		Xana	XC22	XX	X_	XR	R_	RR	XX	X_	XR	R_	RR		
PVag001	*Co-2*	160	150	4		34		18	20		5		0	0.10	10.64
SH13b	*Co-2*	480	500	4		32		20	18		6		0	0.12	9.33
SQ4	*Co-2*	–	1,440	2			54		25			1		0.03	17.37
SCAReoli	*Co-2*	–	1,000	2			52		19			2		0.05	12.80

XX, homozygous for the Xana alleles of the corresponding marker; X_, homozygous for the Xana alleles or heterozygous; XR, heterozygous; R_, homozygous for the XC22 alleles or heterozygous; RR, homozygous for the XC22 alleles

Table 4 Linkage analysis between the gene conferring resistance to powdery mildew and nine molecular markers located in the Co-3/9 region (LG Pv04) in a F_2 population derived from the cross Xana × XC217

Marker	Linked gene	Parental genotypes (bp)		F_2 plants										RF	LOD
				Resistant					Susceptible						
		Xana	XC217	XX	X_	XR	R_	RR	XX	X_	XR	R_	RR		
FZ-E9b	*Co-3/9*	–	160	9			44		14			10		0.25	2.91
FZ-E9n	*Co-3/9*	175	170	4		29		19	8		14		3	0.36	0.94
BM161	*Co-3/9*	185	–		36			16		20			2	0.41	0.24
SBA8	*Co-3/9*	–	530	9			44		14			11		0.25	3.36
SI19	*Co-3/9*	460	–		32			21		19			6	0.41	0.21
SW12	*Co-3/9*	725	–		35			18		20			5	0.41	0.24
254-G15F	*Co-3/9*	–	320	5			46		11			13		0.27	2.23
	Co-3/9	550	–		34			17		20			4	0.37	0.39
	Co-3/9	–	600	3			48		10			14		0.26	2.21
Contig-IIIh	*Co-3/9*	300	–		35			17		20			5	0.41	0.24
Contig-IIIi	*Co-3/9*	250	–		32			18		21			3	0.31	0.93

XX, homozygous for the Xana alleles of the corresponding marker; X_, homozygous for the Xana alleles or heterozygous; XR, heterozygous; R_, homozygous for the XC217 alleles or heterozygous; RR, homozygous for the XC217 alleles

Cornell 49242. Previous studies that have investigated the response in F_2 populations suggested that two independent and dominant loci could be involved in the response against this pathogen (Trabanco et al. 2012). The results of this study revealed that there was a qualitative mode of inheritance for resistance and confirmed the involvement of two independent and dominant genes in the response to PM. The resistance genes were located in the genetic map using three complementary strategies: identifying potential regions associated with the resistance by contingency tests; mapping of resistance using two subpopulations established from the XC RIL population and, linkage analysis in the two F_2 populations obtained from a genetic dissection. All the results supported the hypothesis that a dominant gene (tentatively named as *Pm1*) is located at the end of the linkage group, Pv11 and another gene (tentatively named as *Pm2*) is located at the end of the linkage group, Pv04. Although different relative positions for the *Pm2* gene were estimated on Pv04 in the two linkage analyses (subpopulation of recombinant lines and the F_2 population), a significant linkage (LOD > 3) between the *Pm2* gene and markers for LG04 was found in both cases. Both genes seemed to control a different response to PM; gene *Pm1* conferred complete resistance to PM as the seedlings did not show any visible symptoms, while gene *Pm2* conferred a moderate resistance, in that there was limited mycelial development on the leaves, but there was no sporulation. In contrast, the resistant reaction (no visible symptoms) in the six F_1 plants derived from the cross between XC22 × XC217 suggested that both genes showed a

dominant epistatic relationship. This conclusion agreed with the genetic control reported by Trabanco et al. (2012) for their observed response in F_2 populations.

Resistance genes to PM were mapped at the end of the linkage groups Pv04 and Pv11. These relative positions are in agreement with genetic and genomic evidences. Clusters of resistance genes involved in the response to specific races of anthracnose, caused by the fungus *C. lindemuthianum*, were located in the same relative positions (Ferreira et al. 2012; Rodríguez-Suárez et al. 2007, 2008). Genes controlling resistance to bean rust were also mapped at the end of LG Pv11 and LG Pv04 (Awale et al. 2008; Miklas et al. 2002), as were genes controlling resistance to halo blight, caused by the bacteria, *Pseudomonas syringae* (Chen et al. 2010). Resistance gene analogs (RGAs) were mapped at the end of both linkage groups (López et al. 2003; Mutlu et al. 2006). The vast majority of R genes cloned so far encoded for R proteins containing nucleotide-binding sites (NBS) and the C-terminal leucine-rich repeat motif (LRR) (Dangl and Jones 2001; McDowell and Woffenden 2003). Clusters of sequences encoding NBS-LRR proteins were annotated in the same relative positions (Chen et al. 2010; David et al. 2009; Ferrier-Cana et al. 2003). Therefore, the location of the genes involved in the control of the response to PM in these regions was not unexpected. The involvement of genes encoding LRR proteins in the resistance to PM has been suggested in other legumes. In *Medicago truncatula*, Ameline-Torregrosa et al. (2008a) reported a cluster of genes encoding for LRR protein in the relative position where a QTL associated with the response to PM was mapped. In soybean, the candidate gene Glyma16g34090, encoding for a LRR protein (Jun et al. 2012) has been described at the same chromosome region including a resistance gene to PM. The *Mlo* genes constitute a family of transmembrane proteins, including the leucine rich domain, and many Mlo homologs have been identified in various plants (Singh et al. 2012). The Mlo gene was first identified in barley and its recessive allele leads to broad spectrum resistance against PM (Büschges et al. 1997).

Marker saturation of regions that affect the genetic control of specific traits represents one of the first steps in genomic characterization and can provide useful markers for indirect selection. To pinpoint the location of the gene conferring resistance to PM, a total of 20 new loci were incorporated on the LGs Pv04 and Pv11. The markers were selected according to their relative positions in previously reported genetic maps or were developed from BAC clone sequences obtained for the B4 resistance cluster (David et al. 2009). The resulting LG showed a higher level of saturation than the LGs described by Pérez-Vega et al. (2010). The previous linkage map included a gap in LG Pv04 and only three markers in the Co-3/9 region [SI19 and SW12, reported by Pérez-Vega et al. (2010), and BM161]. The map obtained in this study carried nine new loci in this region and the average genetic distances between loci was 6.1 cM for this region. All the markers were mapped on their expected relative positions, except the marker FZ-E9o, which was mapped on the end of LG Pv11. This microsatellite marker was developed from the FZ-E9 BAC clone sequence, which has been described to be at LG Pv04. A close relationship between the Co-3/9 region (end of LG Pv04) and the Co-2 region (end of LG Pv11) has been described previously (David et al. 2009) and it could explain the relative position of the FZ-E9o marker. The mapping of markers developed from BAC clone sequences confirmed the location of the sequences in chromosome 04.

This study supplies two markers closely linked to the *Pm1* gene (SQ4 and SCAReoli), which conferred complete resistance against the local isolate used in this work. Indirect selection using these markers is being successfully conducted to introgress genetic resistance to PM in common bean cv. Xana. With regard to the *Pm2* gene, the linkage analysis revealed different relative positions on the LG Pv04 in the two analyzed populations. The microsatellite marker BM161 showed the highest linkage to the resistance gene in the subpopulation established from the XC RIL population, whereas in the F_2 population, the highest linkage was with the SBA8 marker. These different relative positions could be explained by the presence of two linked resistance loci in the Co-3/9 genomic region. As previously mentioned, at least ten anthracnose resistance genes have been mapped at this position (Ferreira et al. 2012) and 26 candidate genes, coding for NBS-LRR proteins were annotated in this position with an interval of 30 cM (David et al. 2009). In consequence, the presence of two closely linked loci in the Co-3/9 region conferring to PM in the Cornell 49242 genotype could be possible.

To date, very little information has been available about the inheritance of the genetic reaction to PM in common bean. This has considerably limited the possibility of carrying out breeding programs focused on the introgression of resistance genes against this fungus in common bean genotypes. The results of this work confirmed that resistance to PM in cv. Cornell 49242 was controlled by two dominant and independent genes. One of the genes, located in LG Pv11 (*Pm1*), gave total resistance to this fungus and masked the action of the second gene (*Pm2*), located in LG Pv04. The *Pm1* gene was closely linked to the SQ4 marker, which offers the opportunity to increase the efficiency of breeding programs by means of marker-assisted selection. This is the first report that has investigated the localization of resistance genes against PM in common bean and it will be useful in the future identification of the genomic sequences involved in the genetic control of this resistance.

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Capítulo 3

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Identification of quantitative trait loci involved in the response of common bean to *Pseudomonas syringae* pv. *phaseolicola*

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Abstract Halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkn.) Downs (*Psp*), is an important disease in common bean (*Phaseolus vulgaris* L.). This study investigated the genetic control of the resistance to two local isolates of *Psp* (ITA-812 and ITA-684) in a recombinant inbred line (RIL) population derived from the cross between the bean genotypes Xana and Cornell 49242. The cultivar Cornell 49242 exhibited moderate resistance to these isolates, whereas cultivar Xana was susceptible. The RIL population showed a continuous variation in response to the two isolates. Analysis revealed four significant quantitative trait loci (QTLs): Psp4^{812XC} and Psp6.1^{812XC} located on linkage groups Pv04 and Pv06 (for the response to isolate ITA-812), and Psp6.1^{684XC} and Psp6.2^{684XC} located on Pv06 (for the response to isolate ITA-684). The QTLs Psp6.1^{812XC} and Psp6.1^{684XC} were located in the same genetic region (Psp6.1), close to the Psp6.2 region in which the QTL Psp6.2^{684XC} was mapped. A genetic dissection was undertaken to verify the consistency of these three QTLs located on the end of Pv06. Four sets of RILs were established according to the genotypes (Xana and Cornell 49242) of the underlying markers for the regions Psp6.1 and Psp6.2. Re-evaluation of these sets of lines revealed significant differences relative only to isolate ITA-684. The set of lines with the Cornell genotype in both regions was significantly more resistant than the other three sets of lines. This suggested that both regions were necessary to detect a significant effect in the response to isolate ITA-684. In the physical positions corresponding to these two genetic regions, in silico analysis revealed 16 candidate genes (putative orthologous genes) that carried sequences homologous to the resistance genes *RPM1*, *FLS2*, *RPG1*/*RPG1-B*, and *Pto*—all of which confer resistance to *P. syringae* in different species. The results confirm that, apart from the major genes implicated in resistance to *Psp*, specific bean genotypes exhibit a quantitative mode of inheritance of resistance to *Psp*.

Keywords *Phaseolus vulgaris* L. · Resistance · Inheritance · Quantitative trait loci · Genetic linkage map · In silico analysis · Candidate genes · Plant breeding

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Introduction

Pseudomonas syringae (*Ps*) comprises several related bacterial strains that exhibit different pathogenic abilities and cause plant diseases in a wide variety of agriculturally important crops (Bradbury 1986). *Pseudomonas syringae* pv. *phaseolicola* (Burkn.) Downs (*Psp*), which causes halo blight in common bean (*Phaseolus vulgaris* L.; *Pv*), can attack aerial parts of the plant, particularly leaves and bean pods. Typical symptoms of the disease are water-soaked spots and a yellow–green halo around the point of infection of the leaves. Pod infection appears either as water-soaked spots or as red or brown lesions from where the pathogen enters, causing discoloration and shriveling to the seed. Severe attacks can cause systemic chlorosis, leading to premature leaf drop. Bacteria can produce hypersensitive reaction with local necrosis on the infected tissue in specific *Psp–Pv* interactions (Saettler et al. 2005; Arnold et al. 2011). *Psp* is a seed-borne pathogen, and can be spread by wind-driven rain and insects. Weedy and other cultivated legumes may also harbor the bacteria, and they can also contribute to the spread of the disease. Isolates of *Psp* can also be pathogenic in species as *Glycine max* L. and *Arabidopsis thaliana* (L.) Heynh. (Forsyth et al. 2010; Arnold et al. 2011).

The *Psp–Pv* interaction shows a wide variation in the host response. Both qualitative and quantitative responses to *Psp* have been described in this interaction. Resistance tests often revealed evidence of quantitative variation in reaction to *Psp* isolates, particularly when major genes are not present (Taylor et al. 1996b). Quantitative trait loci (QTLs) associated with the response to two isolates of *Psp* (HB16 and 83-Sc2A) were detected in a recombinant inbred line (RIL) population derived from the cross BelnebRR-1/A55. Three QTLs for leaf reactions to isolate HB16 were found on the linkage groups (LGs) Pv03, Pv05, and Pv10. Four regions on the LGs Pv02, Pv04, Pv05, and Pv09 were significantly associated with leaf reactions to strain 83-Sc2A (Ariyarathne et al. 1999). Two QTLs for resistance to isolate 1375 (classified as race 5) were detected in a linkage map of 103 marker loci developed for a F2 population derived from the cross Jules/Canela (Yaish et al. 2006). Unfortunately, the absence of loci common to the integrated map of the common bean did not allow for the identification of these LGs.

In some cases, *Psp–Pv* interactions appear to be very specific; they essentially follow the gene-for-gene model (Flor 1955). Pathogenic variation was reported that results from a differential response of bean genotypes to different *Psp* isolates. Nine different races (races 1–9) were described using a set of eight differential cultivars (Taylor et al. 1996a, b). Qualitative responses have, in general, been associated with the presence of major resistance genes. Five race-specific genes (*Pse-1* to *pse-5*) were identified among the set of differential cultivars by means of complementary tests. Some of these loci were located in the bean integrated linkage map, and all showed a dominant mode of action, except for the *pse-5* gene. The *Pse-1* gene was first described in differential cultivar Red Mexican UI-3 and it protects against races 1, 5, 7, and 9. The *Pse-1* gene was mapped to linkage group Pv10 (Walker and Patel 1964; Miklas et al. 2009). The *Pse-2* gene, present in the differential cultivar ZAA12, protects against races 2, 3, 4, 5, 7, 8, and 9, and was also mapped in the LG Pv10, although probably in a relative position different from *Pse-1* (Miklas et al. 2011). The location of the *Pse-3* locus was deduced from its genetic linkage with the *I* gene, which confers resistance to the *bean common mosaic virus* (Miklas et al. 2011). The *I* gene was mapped to the end of LG Pv02 (Pérez-Vega et al. 2010). Fourie et al. (2004) also reported evidence for the existence of resistance loci in LG Pv04, which confer resistance to specific races of *Psp*. Finally, two independent *R* genes that confer AvrRpm1-specific resistance were mapped to the end of linkage groups Pv11 (*Rpsar-1*) and Pv08 (*Rpsar-2*) using the RIL population BAT93/JaloEEP558. The genes *Rpsar-1* and *Rpsar-2*, which were mapped in the vicinity of *R* gene, confer resistance to the fungus *Colletotrichum lindemuthianum* (genes *Co-1* and *Co-4*; Chen et al. 2010).

Interactions between *Ps* and a different plant species have been extensively studied because these systems provide good examples of the gene-for-gene model (Flor 1955). Several pathogenicity genes from bacteria have been reported (Arnold et al. 2011). Genetic and genomic studies have also characterized in detail several genes that control resistance to *Ps* in several species, and all this information can be useful in the identification of new resistance genes. The *RPS* and *RPM1* genes from *A. thaliana* encode NB-LRR proteins which carry a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) domain. RPM1

confers resistance to strains of *P. syringae* that express the avirulence genes *AvrRpm1* and/or *AvrB* (Mackey et al. 2002). The bacterial effector protein AvrRpt2 induces resistance in *A. thaliana* genotypes that express the *RPS2* gene. In both cases, RPM1-interacting protein 4 (RIN4) interacts with the type-III effector molecules secreted by *Ps* and is required for RPM1- and RPS2-mediated resistance (Mackey et al. 2002; Axtell and Staskawicz 2003; Day et al. 2006). The *FLS2* gene, which encodes the flagellin receptor, is involved in basal resistance to *Psp* in *A. thaliana* (Forsyth et al. 2010). Similar to the non-orthologous *RPM1* gene from *A. thaliana*, the *Rpg1-B* gene in soybean confers resistance to *P. syringae* pv. *glycinea* strains that express the avirulance gene *AvrB* (Ashfield et al. 1995; Selote and Kachroo 2010). The tomato *Pto* gene encodes a serine/threonine kinase that confers resistance to *P. syringae* pv. *tomato* strains that express the avirulence gene *AvrPto* (Martin et al. 1993; Chandra et al. 1996). The involvement of *R* genes that encode NBS-LRR proteins in resistance against *Psp* was also suggested in common bean (Chen et al. 2010).

A limited number of studies have investigated the quantitative response to *Psp* in common bean. The main aim of this study was to contribute to a better understanding of the genetic control of the resistance response of common bean to infection with *Psp*. In this study, we analyzed the inheritance of the reaction to two different isolates of *Psp* in a RIL population derived from parents that differ significantly in their response to the pathogen, and searched for candidate genes associated with the resistance.

Materials and methods

Plant material

A total of 110 F7 RILs, derived from the cross Xana/Cornell 49242 by single-seed descent (XC RIL population), were used to study the inheritance of the response to *Psp*. Xana is a bean line with very large white seeds, and a determinate growth habit. It belongs to the market class 'fabada'. Cornell 49242 is a cultivar with very small black seeds, and an indeterminate prostrate growth habit. It belongs to the Mesoamerican gene pool and is included in the market class 'black turtle'. Cornell 49242 exhibits moderate resistance to local isolates of *Psp* classified as races 6 and 7, whereas cultivar Xana is susceptible to these isolates.

Two common bean lines, Red Mexican (UI3) and Canadian Wonder, both included in the set of differential cultivars used to characterize pathogenic variation in *Psp* (Taylor et al. 1996a), were used as references to score the response in the XC RIL population. In addition, the local cultivar Cueto was included as a susceptible control.

Inoculation and disease scoring

Evaluations were carried out using two local isolates obtained from naturally infected bean plants collected in northern Spain (isolates ITA-812 and ITA-684). These isolates, which were derived from a single colony, were classified as races 6 (ITA-812) and 7 (ITA-684) using the set of differential cultivars proposed by Taylor et al. (1996a). Bacteria were grown on King's B medium for 48 h at 25 °C.

Resistance tests were performed on primary leaves using the multiple-needle method (Aggour et al. 1989). Each primary leaf was inoculated with one isolate. The inoculated plants were maintained at moderate temperature (from 16 °C at night to 20 °C during the day) in shade and at high relative humidity (80 %). Symptoms were measured 6–7 days after inoculation by using a 1–9 severity scale where 1 = no visible symptoms and 9 = very severe symptoms in the inoculated area (Aggour et al. 1989). The responses of the plants were evaluated in at least two separate tests. There were two replicates of 4–5 plants per test.

Linkage analyses and QTL mapping

A genetic map developed for the XC RIL population was used to identify the genomic regions involved in the control of the response to *Psp*. The linkage map consisted of 349 loci mapped by Pérez-Vega et al. (2010, 2013). MAPMAKER Macintosh version 2.0 software (Lander et al. 1987) was used to construct the linkage map. A new version of the linkage groups was developed, using loci for which data were available for at least 105 lines (95 % of lines). All LGs were established with a log of the likelihood ratio (LOD) threshold of 3.0 and a recombination fraction of 0.25. Marker order was estimated based on multipoint

comparison, order, and ripple analyses. Distances between loci (cM) were calculated using the Kosambi mapping function. The map obtained has 11 linkage groups, which were aligned according to the common bean core linkage map, using common molecular markers as anchor points. Linkage groups were named according to Pedrosa-Harand et al. (2008).

Frequency distributions for the response to *Psp* were tested for normality using the Kolmogorov–Smirnov test, with $p < 0.05$ used to indicate lack of fit. The QGene 4.3.8 software (Joehanes and Nelson 2008) was used to detect QTLs by using composite interval mapping (CIM) analysis with a 10-cM walk speed. A LOD score of 2.5 was used as the threshold to determine the presence of significant QTLs. This value was estimated based on a permutation analysis with 1,000 random data shuffles, as described by Churchill and Doerge (1994), to provide a genome-wide significance level of 0.05. Only QTLs identified in at least one resistance test and in the mean of both tests were considered. Identified QTLs were named according to Miklas and Porch (2010). The name reflects the trait abbreviation (*Psp* = *Psudomonas syringae* pv. *phaseolicola*), linkage group (1–11), order of discovery (1, 2,…), and originating population in superscript (XC = Xana/Cornell 49242 RIL population). The *Psp* isolate was also added in superscript in order to show the specificity in the *Psp–Pv* interaction.

Molecular marker development and analysis

For more precise QTL mapping, new molecular markers were added to specific LGs. Genomic DNA was isolated from young leaves using the FastDNA kit (MP Biomedicals, Illkirch, France) following the supplier's instructions. Concentrations of DNA were quantified photometrically (absorbance measurements between 260 and 280 nm) using a Biomate 3 UV–visible spectrophotometer (Thermo Scientific, MA, USA). Four types of molecular markers were analyzed: (1) markers previously mapped in other populations (Gaitán-Solís et al. 2002; Blair et al. 2003, 2010; Córdoba et al. 2010a, b; Hanai et al. 2010; McConnell et al. 2010; Shi et al. 2012); (2) markers linked to a locus involved in the response to *Psp*, such as the markers SH11, ST8, and SR13 linked to the *Pse-1* gene and the marker SAE15 linked to the *Pse-2* gene (Miklas et al. 2009); (3) insertion/deletion (Indel) markers, which were selected by considering their physical position on the bean genome (http://www.beancap.org/Research.cfm; Moghaddam et al. 2013); and (4) microsatellite (simple sequence repeat; SSR) markers designed from the *P. vulgaris* genomic sequence and deposited in the Phytozome V9.1 database (http://phytozome.net/). Microsatellite markers were designed and analyzed as described by Pérez-Vega et al. (2013).

QTL dissection

Genetic dissection was undertaken in order to verify the consistency and relative positions of identified QTLs associated with the reaction to *Psp*. RILs were selected according to their genotypes (Xana or Cornell 49242) for underlying marker loci located in the regions where the QTLs were mapped. Selected RILs were again tested against the two isolates. Significant differences between means were tested using the least significant difference (LSD, 5 %). Statistical analyses were performed using the SPSS v.12 software (Chicago, IL, USA).

In silico analysis

To investigate the existence in the *P. vulgaris* genome of homologous sequences (putative orthologous genes) to the sequences of the genes *RPM1*, *RPS5*, *RIN4*, *FLS2*, *RPG1*/*RPG1-B*, and *Pto* (all of which contribute to resistance to *Ps*), we took advantage of the *P. vulgaris* genome project. Sequences of the *RPM1*, *RPS5*, *RIN4*, *FLS2*, *RPG1*/*RPG1-B*, and *Pto* genes were obtained from GenBank at NCBI (http://www.ncbi.nlm.nih.gov; April 2013). BLAST searches were performed on the bean genome database deposited in www.phytozome.net V9.1 (Goodstein et al. 2012). Sequences (genes) were considered homolog if their BLAST-based *E* values were $<1 \times 10^{-40}$.

Results

Phenotypic variation

The responses of both parents and three checks to the two local *Psp* isolates were as expected (see ESM Table 1). Cornell 49242 exhibited moderate resistance to both isolates, whereas Xana was susceptible. The differences between the two parents in the responses to

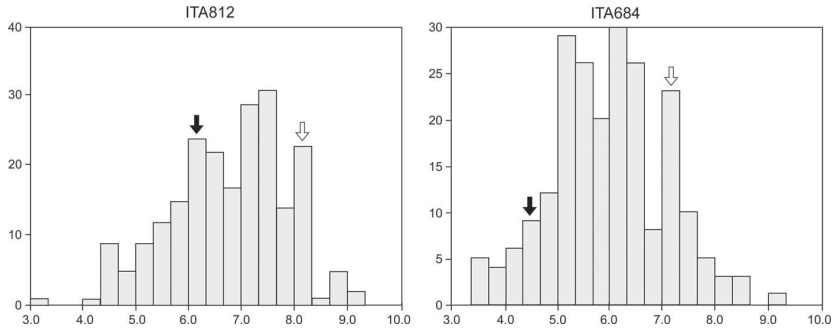

Fig. 1 Distribution (mean obtained from two evaluations) of reaction to two local isolates of *Pseudomonas syringae* pv. *phaseolicola* (ITA-812 and ITA-684) using multiple needle method in the RILs derived from the cross Xana/Cornell 49242. *White and black arrows* indicate the phenotypic values of parents Xana and Cornell 49242, respectively

both isolates were significant. Isolate ITA-812 caused more severe symptoms than isolate ITA-684 for the five bean genotypes tested. The reaction of the differential cultivars Canadian Wonder and Red Mexican was as expected: Canadian Wonder exhibited symptoms against both isolates, whereas Red Mexican only showed symptoms when it was inoculated with isolate ITA-812 (race 6).

Figure 1 shows the distributions of the response against the two *Psp* isolates evaluated in the XC RIL population. No significant deviations from the corresponding normal distributions were observed in either case. Transgressive segregations (phenotypes that are extreme relative to those of either parental lines) were observed. The Pearson correlation coefficient for the response to the *Psp* isolates was significant ($r = 0.58$; $p < 0.001$).

QTL analysis

Four significant QTLs were detected (LOD > 2.5) by CIM analysis (Table 1). In all cases, the alleles from the parent Xana increased the value (increased the susceptibility). Two significant QTLs (Psp4^{812XC} and Psp6.1^{812XC}) were located which confer response to isolates ITA-812 on LGs Pv04 and Pv06. Two other significant QTLs (Psp6.1^{684XC} and Psp6.2^{684XC}) were detected in LG Pv06 for response to isolate ITA-684 (Fig. 2). The QTLs Psp6.1^{812XC} and Psp6.1^{684XC} appear to be located on the same region of LG Pv06. The QTLs Psp4^{812XC}, Psp6.1^{812XC}, and Psp6.2^{684XC} were detected in one of the tests and in the mean of both tests. The QTL Psp6.2^{684XC} was detected in both resistance tests and in the mean of both tests, and it showed the highest value for LOD, for percent variance, and for additive effects (Table 1).

Significant association between the response to the two *Psp* isolates and the loci mapped on the relative position of the major resistance genes *Pse* and *Rpsar* (LGs Pv02, Pv04, Pv08, Pv10, and Pv11) was not detected, although the molecular marker SR13, which is linked to the *Pse-1* gene (Miklas et al. 2009), was included in the XC linkage map. The SR13 marker was mapped on LG Pv10, and was closely linked to the microsatellite marker BM157, which Blair et al. (2003) mapped to LG Pv08. Markers SH11 and ST8, linked to the *Pse-1* gene, and marker SAE15, linked to the *Pse-2* gene, were not polymorphic in this population.

QTL validation and dissection

QTL analysis revealed the presence of three significant QTLs that are located in two adjacent regions at the end of LG Pv06. In order to clarify the relative position of these three QTLs, new molecular markers were added. Polymorphism was analyzed for a total of 73 molecular markers (ESM Table 2) and 22 markers were added on this relative position of LG Pv06. Of these, nine markers had been mapped previously; four were Indel markers, and eight were microsatellite markers developed from bean genomic sequences (ESM Table 2). The resulting map included 32 loci in this relative position, with an average genetic distance of 3.09 cM between loci [interval between the loci BM170 and MvtaEta(57)] (see Fig. 2).

A genetic dissection was undertaken in order to verify the reproducibility of these three significant

Table 1 QTLs identified in XC RIL population by composite interval mapping analysis for reaction to two local *Psp* isolates in two tests and the mean of the two tests

Psp isolate	QTL name	Linkage group	Closest marker[a]	Test 1			Test 2			Mean		
				LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]
ITA-812	$Psp4^{812XC}$	Pv04	*SpL*	4	16	0.60	–	–	–	2.7	11	0.36
	$Psp6.1^{812XC}$	Pv06	BMb1105	3.3	12	0.35	–	–	–	3.3	12	0.30
ITA-684	$Psp6.1^{684XC}$	Pv06	Pv61500b	6.0	22	0.40	–	–	–	3.8	16	0.38
	$Psp6.2^{684XC}$	Pv06	IND691128	6.2	25	0.50	4.2	17	0.42	7	27	0.50

[a] Closest marker is the marker nearest to the peak LOD score

[b] Percent variance: the amount of phenotypic variation explained by each QTL

[c] Additive effect: value of the female parent allele (Xana)

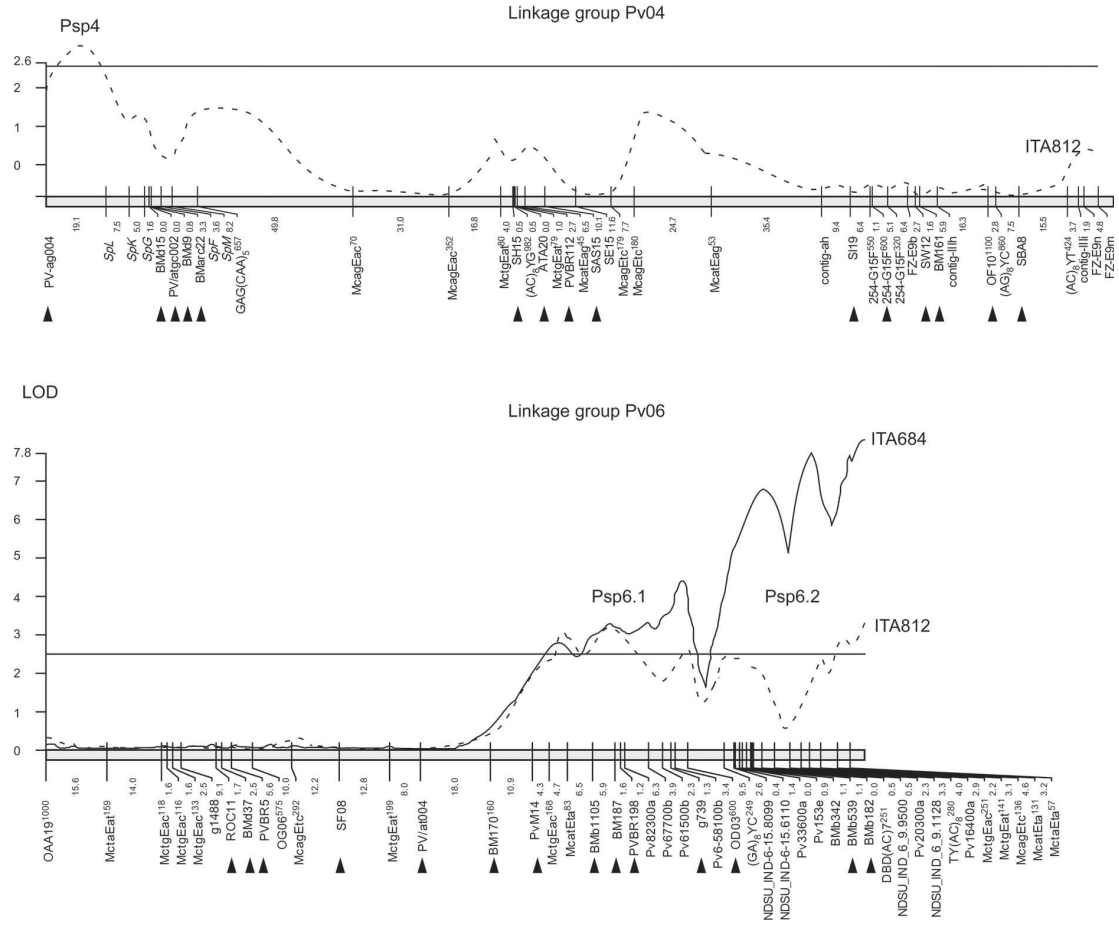

Fig. 2 Localization of the significant QTLs associated with response to two local isolates of *Pseudomonas syringae* pv. *phaseolicola* (ITA-812 and ITA-684) on the linkage groups Pv04 and Pv06. *Black triangles* near the loci designate loci common to the bean genetic map reported

Table 2 Response of four types of RILs against two *Psp* isolates (ITA-812 and ITA-684) using the multiple needle method. Lines within each type were selected according to their genotypes for the loci included between the flanking markers BMb1105 and Pv82300a for the region Psp6.1, and the markers IND_6_15.8099 and Pv16400a for the region Psp6.2

Type	Genotype between the flanking markers				*Psp* isolates	
	BMb1105	Pv82300a	NDSU_IND_6_15.8099	Pv16400a	ITA-812	ITA-684
I	B	B	A	A	6.89	6.01
II	A	A	B	B	7.18	5.38
III	A	A	A	A	7.26	6.09
IV	B	B	B	B	6.69	4.59
LSD ($p < 0.05$)					ns	0.77

A genotype Xana; *B* genotype Cornell 49242; *ns* not significant

QTLs at the end of LG Pv06. Marker loci BMb1105, BM187, PVBR198, and Pv82300a were used for tagging the region 6.1, containing the QTLs Psp6.1^{812XC} and Psp6.1^{684XC} in a segment of length 8.7 cM (ESM Table 3). The thirteen loci included among the NDSU_IND_6_15.8099 and Pv16400a markers were used to tag the region 6.2, which contains QTL Psp6.2^{684XC} within a 15.5 cM segment (ESM Table 3). Four sets of five or six lines each were selected according to their genotypes (Xana or Cornell 49242) for the underlying loci. Type I was established from five lines with the Cornell genotype for region 6.1 and the Xana genotype for region 6.2 (lines 102, 119, 120, 214, and 217). Type II included five lines with the Xana genotype for region 6.1 and the Cornell genotype for region 6.2 (lines 15, 25, 42, 81 and 90). Type III was established from six lines with the Xana genotype for both regions (lines 17, 31, 66, 110, 128 and 200). Type IV included five lines with the Cornell genotype for both regions (lines 6, 41, 71, 79, and 88). The selected lines did not show recombination within the tagged block (ESM Table 3). The four sets of lines were evaluated against the two isolates in three different tests. Significant differences among the four sets were not detected for isolate ITA-812 (Table 2), the most virulent isolate. In contrast, for isolate ITA-684, the set of lines that carried both regions with the Cornell genotype (type IV) showed average scores for the response to the pathogen that were significantly lower than the three sets of lines without the Cornell genotype in both of these regions (lines of types I, II, and III; Table 2). This result confirms the effect of the end of LG Pv06 in the reaction to *Psp*, and suggests that, at least in this set of lines, both regions were necessary to detect a significant effect in the response to isolate ITA-684.

In silico analysis

The physical positions in the bean genome of the regions that included the identified QTLs were estimated from the alignment between the sequences of the flanking markers and the bean genome sequence available from the Phytozome database (ESM Table 3). For the Indel and SSR markers developed, the physical position was known. Markers Pvag004 and BMd15, which flank the QTL Psp4^{812XC}, were aligned with sequences of chromosome 4, at about 44 Mbp (millions of base pairs). Markers Pv16400a and NDSU_IND_6.158099, which flank the QTL Psp6.2^{684XC}, were located at the physical positions 7.9 and 15.8 Mbp, respectively. Markers Pv658100b and BM187 (the sequence of BMb1105 was not available for this estimation), which flank the QTLs Psp6.1^{812XC} and Psp6.1^{684XC}, were located at physical positions 17.0 and 20.2 Mbp, respectively.

Analysis that involved the sequences of *RPM1*, *RPG1-B*, *FLA2*, and *Pto*, four well-characterized genes that confer resistance to *Ps* in different species, identified 16 candidate genes in the physical positions in which the QTLs Psp6.1^{812XC}, Psp6.1^{684XC}, and Psp6.2^{684XC} were mapped (Table 3). Whereas nine of these candidate genes encode serine/threonine protein kinases, seven encode NB-LRR proteins. Interestingly, analysis that involved the sequence of the *RPM1* gene from *A. thaliana* also identified a gene that encodes a domain involved in a cleavage site for the pathogenic type-III effector avirulence factor Avr

Table 3 Candidate genes identified on the position of the identified QTLs that show a high alignment (E value $<1 \times 10^{-40}$) with genes *RPM1*, *FLS2*, *RPG1/RPG1-B*, and *Pto*, all involved in the resistance to *Pseudomonas syringae*. GenBank accessions used in BLAST analysis are indicated. Annotated function and physical location of the genes on the bean genome is also showed

Resistance genes to *Psp*	Species of origin	Genbank accession no.	Identified candidate gene	Annotated function in *P. vulgaris* genome	Identity %	*E* value	Physical location	LG	QTL mapped
FLS2	*A. thaliana*	NP_199445.1[a]	Phvul.004G136500.1	LRR receptor serine/threonine protein kinase	31.1	$5.4e^{-102}$	Chr04: 41556561–41560351	4	$Psp4^{812XC}$
Pto	*G. max*	NP_001241285.1[a]	Phvul.004G164000.1	Serine/threonine protein kinase	45.9	$8.5e^{-65}$	Chr04: 44601151–44605163	4	$Psp4^{812XC}$
FLS2	*A. thaliana*	NP_199445.1[a]	Phvul.004G175700.1	LRR receptor Serine/threonine protein kinase	30.0	$3.4e^{-103}$	Chr04: 45622442–45626397	4	$Psp4^{812XC}$
FLS2	*A. thaliana*	NP_199445.1[a]	Phvul.004G175800.1	LRR receptor Serine/threonine protein kinase	30.8	$9.8e^{-99}$	Chr04: 45628716–45632774	4	$Psp4^{812XC}$
FLS2	*A. thaliana*	NP_199445.1[a]	Phvul.004G175900.1	LRR receptor Serine/threonine protein kinase	30.5	$3.6e^{-96}$	Chr04: 45637544–45641336	4	$Psp4^{812XC}$
Pto	*G. max*	NP_001241285.1[a]	Phvul.006G020700.1	Serine/threonine protein kinase	45.7	$6.4e^{-61}$	Chr06: 09829717–09832983	6	$Psp6^{812XC}$
Pto	*G. max*	NP_001241285.1[a]	Phvul.006G032300.1	Serine/threonine protein kinase	43.9	$5.6e^{-61}$	Chr06: 13444265–13448996	6	$Psp6^{812XC}$
Pto	*G. max*	NP_001241285.1[a]	Phvul.006G039500.1	Serine/threonine protein kinase	42.1	$3.5e^{-67}$	Chr06: 14941940–14945392	6	$Psp6^{812XC}$
RPM1	*A. thaliana*	NP_187360.1[a]	Phvul.006G051500.1	LRR-containing protein ADP binding	28.8	$1.1e^{-100}$	Chr06: 16620560–16623631	6	$Psp6^{812XC}$
RPM1	*A. thaliana*	NP_187360.1[a]	Phvul.006G052300.1	LRR-containing protein ADP binding	29.0	$3.2e^{-93}$	Chr06: 16682590–16685934	6	$Psp6^{812XC}$
RPM1	*A. thaliana*	NP_187360.1[a]	Phvul.006G052400.1	LRR-containing protein ADP binding	28.0	$4.3e^{-83}$	Chr06: 16692492–16696742	6	$Psp6^{812XC}$
RPM1	*A. thaliana*	NP_187360.1[a]	Phvul.006G052500.1	LRR-containing protein ADP binding	31.0	$3.9e^{-82}$	Chr06: 16711093–16713765	6	$Psp6^{812XC}$
RPM1	*A. thaliana*	NP_187360.1[a]	Phvul.006G052600.1	LRR-containing protein ADP binding	30.3	$5.4e^{-86}$	Chr06: 16729788–16738643	6	$Psp6^{812XC}$
RPM1	*A. thaliana*	NP_187360.1[a]	Phvul.006G056500.1	LRR-containing protein ADP binding	30.2	$1.1e^{-75}$	Chr06: 17298018–17300130	6	$Psp6^{812XC}$
RPG1-B	*G. max*	AAR19097.1	Phvul.006G066800.1	LRR-containing protein ADP binding	57.7	0	Chr06: 18542599–18546221	6	$Psp6^{812XC}$
Pto	*P. vulgaris*	AAK52036.1	Phvul.006G080500.1	Serine/threonine protein kinase	46.3	$4.7e^{-50}$	Chr06: 19953718–19967156	6	$Psp6^{812XC}$

[a] Sequences obtained from RefSeq database

from *Ps* (Phvul.006G058100; position 17.5 Mbp) in this physical position (*E* value = 1.9×10^{-8}; identity = 40.6 %). From this gene, marker Pv658100b was developed and polymorphism was detected after digestion with the *Hin*dIII restriction enzyme. It was mapped on the Psp6.1 region (Fig. 2).

Discussion

In this study, we investigated the response to two local isolates of *Psp* (ITA-812 and ITA-684) in a RIL population derived from the cross Xana/Cornell 49242. Isolates were classified as races 6 and 7, which are distributed worldwide and are both especially prevalent in northern Spain (Taylor et al. 1996a, b; Rico et al. 2003). Qualitative variation in the reaction to *Psp* has been reported in common bean. Major genes that confer resistance to specific races or pathogenic variants have been identified from the analysis of segregating populations in which resistant and susceptible reactions were clearly identified (Miklas et al. 2009, 2011). However, our results revealed a continuous variation of the response in the XC RIL population to the two isolates of *Psp*. The lack of identification of clear phenotypic classes such as resistant or susceptible suggests that major resistance genes are not involved in the reaction. Evidence for the involvement of some major resistance genes previously described was not found. Significant association between the response to the two *Psp* isolates and the loci mapped on the relative position of the major resistance genes *Pse* and *Rpsar* (LGs Pv02, Pv04, Pv08, Pv10, and Pv11) was not detected, suggesting that these regions are not involved in the observed response. Quantitative variation in the reaction of bean genotypes to isolates of *Psp* have also been reported. Taylor et al. (1996b) tested 1,048 bean accessions against six races of *Psp*. This screening found 21 accessions that showed a quantitative response to the infection, with no specific reaction, regardless of the race used. Moreover, frequent observation of variation in the reactions of bean genotypes classified as resistant or susceptible suggests that genes with minor effects on disease resistance might also be involved. Obviously, a quantitative variation in the reaction to *Psp* is best observed in the absence of a major resistance gene; for this reason, the XC RIL population is suited for investigation of the genetic background.

QTL analysis was performed using a saturated genetic map that included 349 markers distributed across eleven LGs. Results did not reveal significant QTLs on LGs in which QTLs associated with the response to *Psp* had previously been reported (LGs Pv02, Pv03, Pv05, Pv09, and Pv10; Ariyarathne et al. 1999). In contrast, QTL analysis showed four significant QTLs (LOD > 2.5) on regions of LGs Pv04 and Pv06. Intriguingly, the QTL Psp4^{812XC} was mapped to the end of LG Pv04, in the relative position of the APA locus, which confers resistance to bruchids (Blair et al. 2010). Three significant QTLs were identified on two regions on the end of LG Pv06 (see Fig. 2). Results from the genetic dissection showed that the two regions carrying the QTLs Psp6.1^{684XC} and Psp6.2^{684XC} were necessary to detect a significant effect in the response to isolate ITA-684. Genetic dissection did not confirm the involvement of these regions in the response to isolate ITA-812 (race 6), which exhibited a higher level of virulence to the parents than the isolate ITA-684. The QTLs mapped on the end of LG Pv06 overlapped with a QTL conferring resistance to *Pythium ultimum*, and mapped in this same RIL population (Campa et al. 2010). Using single-factor ANOVA analysis, a significant association was also identified between reaction to strain 83-Sc2A and the RAPD (Random Amplified Polymorphic DNA) marker W13.350, which mapped to LG Pv06 (Ariyarathne et al. 1999). Marker W13.350 was included in the core linkage map between markers P2062 and D1806 (Freyre et al. 1998), which were mapped by Grisi et al. (2007) together with the SSR markers PvBr05 and PvBr198 on LG Pv06. Both markers were included in the XC genetic map, and PvBr198 was significantly associated with the response to the two isolates of *Psp*. This finding suggests that the QTL located in the position of marker W13.350 in the BelnebRR-1/A55 population might be the same as the QTL identified in the present analysis on LGs Pv06.

The difference in the reactions of bean genotypes and the RIL population against the two isolates is in agreement with a high level of specificity in the *Pps–Pv* interaction. Genotype Cornell 49242 exhibited a moderate resistance to isolate ITA-684, but a lower level of resistance to isolate ITA-812 (classified as race 6). It is possible that the reactions of Cornell genotype reflect tolerances rather than resistance. QTL analysis also revealed a specificity in the *Pps–Pv*

interaction. Consistent with the findings of Ariyarathne et al. (1999), two QTLs identified were specific to particular isolates, Psp4^{812XC} and Psp6.2^{684XC}.

Pseudomonas syringae can cause serious damage in a wide range of plant species. Genetic resistance against this pathogen has been studied in many species. Using the sequences of four well-known genes that confer resistance to *Ps* (the majority available in the ReqSeq data base), we were able to identify 16 candidate genes within the QTLs identified in the present study. All of these genes contained typical protein domains involved in resistance responses, such as leucine-rich repeat (LRR) domains. Genes that encode typical R proteins co-localized with the major resistance genes *Pse-3* and *Pse-1* (on the end of LGs Pv02 and Pv10). For example, the genes Phvul.010G082800 and Phvul.010G088600, which encode proteins that contain a LRR domain, were mapped close to the position of the SCAR (sequence characterized amplified region) marker SR13, and thus linked to the *Pse-1* gene (LG Pv10; 32 Mbp). The *Rpsar* genes were also mapped to regions that include genes that encode CC-NB-LRR proteins (Chen et al. 2010). The likely involvement of *R* genes that encode CC-NB-LRR proteins and participate in the response of different species to *Ps*, as well as the location of underlying genes that encode this type of protein in the QTLs identified, suggest that typical *R* genes may be involved in the observed quantitative reaction of *Pv* to *Ps*. However, the possibility that other types of genes might mediate this resistance reaction cannot be dismissed. Changes in the concentrations of enzymes such as isoflavonoid, acetyl-CoA carboxylase, chinases, or lipoxygenases have been associated with the *Psp–Pv* interaction (Voisey and Slusarenko 1989; Croft et al. 1990; García-Ponce and Rocha-Sosa 2000; Bozkurt and Soylu 2011) and any of these might be responsible for the observed response. The ultimate confirmation of the involvement of candidate gene(s) responsible for the identified QTLs might include developing functional markers for each candidate gene, analysis of new segregating populations, construction of near-isogenic lines for specific genomic regions, or gene expression analysis (such as by the identification of expressed QTLs; Collard et al. 2005; Bernardo 2008). However, the limited effect of the QTLs identified on the resistance expression compared with the effects of major genes makes it difficult to verify their contribution to resistance and to use the genes in plant breeding.

To date, little information has been available about the quantitative inheritance of the reaction to *Psp* in common bean. This study confirms that a quantitative mode of inheritance to *Psp* can be present in specific bean genotypes, and shows that QTLs might also contribute to the variation in the reaction of bean genotypes. Moreover, our results also revealed a differential reaction against isolates of *Psp*, and confirmed that genes at the end of LG Pv06 contribute to resistance to this pathogen. Pyramiding of major resistance genes and QTLs associated with resistance, such as the QTLs described in the present study, may contribute to more durable resistance against this important pathogen.

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Capítulo 4

Identification of a new chromosomal region involved in the genetic control of resistance to anthracnose in common bean

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Abstract

Anthracnose caused by *Colletotrichum lindemuthianum* is a major disease affecting common bean crops worldwide. Response to five *C. lindemuthianum* isolates, classified as races 3, 6, 7, 38 and 73, were analyzed in 159 $F_{2:3}$ families derived from the cross between line SEL1308 and cultivar MDRK. SEL1308 was resistant to all five races while MDRK was susceptible to all except race 73. Segregation ratio for response to races 3 and 7 indicated that single dominant genes were responsible for the resistance reaction to each race: *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$. Recombination between both genes was observed and no linkage was found with any of the previously described molecular markers tagging Co- genes/clusters. Linkage analyses allowed location of both genes at the beginning of linkage group (LG) Pv03. *In silico* analysis revealed the presence of seven genes codifying typical R proteins with a NB-ARC domain. Segregation ratio for response to races 6 and 38 indicated that two dominant and independent genes conferred resistance to these races. Contingency tests and subpopulation analyses showed the implication of one region on LG Pv08, corresponding to the Co-4 cluster (genes *Co-4*$^{3\text{-}SEL}$ and *Co-4*$^{38\text{-}SEL}$), and the new region identified on LG Pv03 (genes *Co-17*$^{6\text{-}SEL}$ and *Co-17*$^{38\text{-}SEL}$). For reaction to race 73, the most likely scenario was that two dominant and independent genes conferred resistance: *Co-1* in MDRK and *Co-4* in SEL1308. Results indicated that, in addition to *Co-4*^{2}, SEL1308 carried resistance genes located at the beginning of LG Pv03, in which no anthracnose resistance genes have been previously mapped.

Keywords: *Phaseolus vulgaris* L., anthracnose, genetic resistance, mapping, resistance genes analog

Introduction

Diseases are major limitations to common bean (*Phaseolus vulgaris* L.) production (Singh and Schwartz 2010). Anthracnose, caused by the ascomycete fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.- Scrib., can result in a devastating disease in common bean, particularly in temperate regions. The pathogen can attack all aerial parts of bean plants and cause damage leading to significant yield losses. Common management practices to prevent the disease include crop rotation or use of specific fungicides and host plant resistance.

The response of bean plants to the pathogen exhibits a qualitative mode of inheritance. It is a very specific interaction, so that one resistance gene protects against specific isolates of the fungus (Ferreira et al. 2013). Many pathogenic variants or races have been described using the same set of twelve differential cultivars, a standardized resistance test, and a standardized nomenclature system to name the races (Balardin et al. 1997; Balardin and Kelly 1998; Mahuku and Riascos 2004; Ferreira et al. 2008). Different anthracnose resistance loci conferring resistance to specific races (designated as Co- followed by a number or a letter) have been described in common bean. In general, anthracnose resistance genes show complete dominance, where the dominant allele conditions the resistance reaction. Dominant epistatic inheritance has usually been observed in the presence of several resistance genes, although a complementary mode of action between two resistance genes was described in some cases (Campa et al. 2011, 2014; Muhalet et al. 1981). Most of the anthracnose resistance loci reported have also been located in the genetic map, and markers tagging these genes are available: genes *Co-1*, *Co-x* and *Co-w* were mapped on linkage group (LG) Pv01 (Rodríguez-Suárez et al. 2007; Geffroy et al. 2008); *Co-u* on Pv02 (Geffroy et al. 2008); *Co-13* on Pv03 (Gonçalves-Vidigal et al. 2009); *Co-3*, *Co-9*, *Co-y*, Co-z *Co-10* and *Co-15* on the same position of Pv04 (Geffroy et al. 1999; Alzate-Marin et al. 2003; Méndez-Vigo et al. 2005; Rodríguez-Suárez et al. 2007, 2008; Sousa et al. 2013); *Co-5*, *Co-6* and *Co-v* on Pv07 (Geffroy et al. 1997; Campa et al. 2009); *Co-4* on Pv08 (Melotto et al. 2004; Rodríguez-Suárez et al. 2007); *CoPv09* on Pv09 (Campa et al. 2014,); and *Co-2* on Pv11 (Adam-Blondon et al. 1994). Allelic variants of *Co-1*, *Co-3* and *Co-4* have been proposed from the differences observed in the resistance spectrum between different bean genotypes. Genetic mapping of genes conferring resistance to specific races indicated that some of these Co- genes are not distributed randomly in the genome, but are organized in clusters of race-specific resistance genes (Ferreira et al. 2013).

Breeding line SEL1308, derived from the differential cultivar G2333 (Young and Kelly 1996), is one of the bean genotypes in which resistance to anthracnose has been most studied. In this line, a dominant gene conferring resistance to races 73 and 1545 was described (Young et al. 1998). Results from complementary tests suggested that SEL1308 shared this resistance gene with differential cultivar TO (Young et al. 1998). However, the fact that race 2047 was virulent to cultivar TO but avirulent to line SEL1308 was used to deduce that the resistance gene in SEL1308 was a different allele (named *Co-4*^{2}) at the *Co-4* locus (Young and Kelly 1996). Nevertheless, co-segregation for resistance to different races, including races 73 and 1545, was not investigated in the same segregating population, so that the possibility of two independent or closely linked genes involved in the response against these two races cannot be discounted. Locus *Co-4* was closely linked to marker SAS13 and was physically mapped to bean chromosome 3, which corresponds to LG Pv08 (Melotto et al. 2004). Molecular analysis of a genomic region of SEL1308, including *Co-4* and marker SAS13, revealed the presence of a cluster of genes encoding for proteins with serine–threonine kinase domains (Melotto and Kelly 2001). This type of protein has been associated with plant resistance reaction to pathogens (Dodds and Rathjen 2010). Changes in spatial and temporal gene expression were also investigated in the reaction of line SEL1308 to race 73, and several genes involved in hormone biosynthesis and signaling processes seem to modulate the bean response (Borges et al. 2012; Rodrigues-Oblessuc et al. 2012).

Line SEL1308 exhibits resistance to a wide spectrum of *C. lindemuthianum* races (Balardin and Kelly 1998) but only the genetic control of resistance to races 73 and 1545 has been analyzed in detail in this genotype. In other bean genotypes with a wide resistance spectrum, several independent genes or clustered genes have been described as involved in the response to this pathogen. In the present study, we investigated inheritance of resistance to five *C. lindemuthianum* races, including race 73, in a $F_{2:3}$ segregating population derived from the cross SEL1308 × cv. Michigan Dark Red Kidney (MDRK). Results indicated that, in addition to the gene mapped on the Co-4 region, SEL1308 had resistance genes in LG Pv03, a chromosomal region in which no anthracnose resistance genes have been previously mapped.

Materials and methods

Plant material

A total of 159 $F_{2:3}$ families, derived from the cross between line SEL1308 and the anthracnose differential cultivar MDRK, were used. Line SEL1308, provided by Dr. J. Kelly (Michigan State University), derived from a backcross of cultivar G2333 (Talamanca* 2/G2333; Young and Kelly

1996). Cultivar G2333 is photoperiod sensitive, which makes it difficult to work with in temperate regions, and exhibits resistance against multiple anthracnose races (96% of races tested by Balardin et al. 1997; Balardin and Kelly 1998; Mahuku and Riascos 2004; Ferreira et al 2008). Line SEL1308 exhibited a resistant reaction to 97% of the 34 races tested by Balardin and Kelly (1998). SEL1308 is a middle black seeded line with indeterminate prostrate growth habit. Cultivar MDRK (Michigan Dark Red Kidney) is a large red-seeded line with determinate growth habit. Cultivars G2333 and MDRK are included in the set of 12 common bean anthracnose differential cultivars used to identify *C. lindemuthianum* races (Pastor-Corrales et al. 1991). The remaining 10 differential cultivars were used as a control to confirm the identity of the *C. lindemuthianum* races used in this study.

Inoculation procedure and disease scoring

Five isolates of *C. lindemuthianum* classified in different races according to Pastor-Corrales (1991) were used in this work: races 7 and 73 from the collection of the Crop and Soil Sciences Department (Michigan State University, USA); and races 3, 6, and 38 from the Servicio Regional de Investigación y Desarrollo Agroalimentario collection, (SERIDA Asturias, Spain). All isolates were obtained from monosporic cultures maintained in fungus-colonized filter paper at –20ºC for long-term storage. To obtain abundant sporulation, the isolates were grown at 21ºC in darkness for 10 days in potato dextrose agar (DIFCO, Becton Dickinson and Company, Sparks, MD, USA). Spore suspensions were prepared by flooding the plates with 5 ml of 0.01% Tween 20 (Sigma-Aldrich, St. Louis, MO, USA) in sterile distilled water and scraping the surface of the culture with a spatula. Inoculations were carried out by spraying 10 days-old seedlings per family with a spore suspension containing 1.2×10^6 spores/ml. The seedlings were maintained in a climate chamber at 20–22ºC, 95–100% humidity and 12 hours photoperiod. Responses of the plants were evaluated after 7–9 d using a 1–9 scale (van Schoohoven and Pastor-Corrales 1987). Seedlings with no visible symptoms (severity value 1) or showing limited necrotic lesions (severity values 2–3) were considered resistant (R^x = resistant reaction against race X). Seedlings with large sporulating lesions (severity values 4–8) or dead (severity value 9) were considered susceptible (S^x = susceptible reaction against race X). The response to a specific race in the $F_{2:3}$ population was evaluated by inoculating 16-20 F_3 seedlings per each F_2 family grown in the same tray. Families were individually randomized over the whole climate room. The parents SEL1308 and MDRK and the remaining 11 common bean anthracnose differential cultivars were also included as references in each test.

Resistance genes were named following the nomenclature proposed by the Bean Improvement Cooperative genetics committee (http://bic.css.msu.edu/Genetics.cfm). In order to differentiate the race specific genes located into Co- regions, we included the name of the isolate or race (in superscript) followed by the bean genotype in which the resistance gene was identified. For example, a gene conferring resistance to race N in bean genotype X, located in the Co-2 cluster was named as $Co\text{-}2^{N\text{-}X}$.

Molecular marker analysis

Genomic DNA from F_2 plants was isolated from young leaves using the FastDNA kit (MP Biomedicals, Illkirch, France) following the supplier's instructions. Concentrations of DNA were quantified photometrically (absorbance measurements between 260 and 280 nm) using a Biomate 3 UV-visible spectrophotometer (Thermo Scientific, MA).

In order to identify the gene(s) involved in the resistance to a specific race, different molecular markers were analyzed (for more detail see Table 1 ESM): i) markers tagging those regions in which anthracnose resistance loci were previously located (revised in Ferreira et al. 2013). The InDel markers NDSU_IND_9_29.1822 and NDSU_IND_9_29.1976, linked to complementary genes mapped on LG Pv09 (Campa et al. 2014), were also included in this analysis. When these markers were not polymorphic in the SEL1308/MDRK population, additional markers were analyzed for each region, considering their relative position in genetic maps; ii) markers tagging resistance genes analog (RGA) regions (Hanai et al. 2010); iii) InDel markers, which were selected considering their physical position on the bean genome (http://www.beancap.org/Research.cfm; Moghaddam et al. 2013); iv) microsatellite markers designed from the *P. vulgaris* genomic sequence, deposited in the Phytozome V9.1 database (http://phytozome.net/) or the National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/). New microsatellite markers were designed as described by Pérez-Vega et al. (2013). The polymerase chain reaction (PCR) amplifications were performed in 20 µl solution containing 25 ng of genomic DNA, 100 mM Tris–HCl, 100 mM KCl (pH 8.3), 4 mM MgCl2, 0.2 mM of each dNTP (Bioline, London), 0.2 µM of each primer, and 1.25 U of Biotaq DNA polymerase (Bioline, London). Amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems, Life Technologies, Foster City, CA) programmed according to the corresponding author (see Table 1 ESM) or following the recommendations supplied by the OligoAnalyzer software (www.uku.fi/*kuulasma/OligoSoftware). RAPD and SCAR PCR products were resolved on 2% agarose gels. The microsatellite PCR products were resolved on 8%

polyacrylamide gels. Agarose and polyacrylamide gels were stained with SYBR Safe (Invitrogen, Life Technologies, CA, USA) and visualized under UV light.

Genetic analysis

Goodness-of-fit of observed to expected ratios was tested by chi-square test. To identify the gene(s) involved in the resistance response to a specific isolate, a genetic analysis was performed as follows. In those cases in which the observed segregation ratio suggested the presence of one resistance gene, linkage analyses were performed. In those cases in which the observed segregation ratio suggested the presence of more than one resistance gene, contingency chi-square tests were carried out for the joint segregation of each resistance score and the markers. Significance thresholds were determinate using Bonferroni correction considering $\alpha = 0.05$ (Bonferroni 1936). A significant deviation from random segregation would suggest the involvement of that specific chromosome region tagged with the marker in the resistance response. The putative position of a resistance gene was also investigated through subpopulation analyses. Two subpopulations from the total F_2 population were established per candidate region, considering the parental genotypes for molecular markers linked to each region. If a resistance gene was located in that particular region, changes in the segregation ratio R:H:S were expected in the two established subpopulations, compared to that of the total $F_{2:3}$ population. In the subpopulation established with the resistant parent genotype for the marker tagging the investigated region, the resistance gene should be fixed and a resistant reaction should be observed. In the subpopulation with the susceptible parent genotype, the resistance gene should not be present, so the segregation for resistance should change with respect to the segregation observed in the total F_2 population.

Linkage analyses were carried out with MAPMAKER Macintosh version 2.0 software (Lander et al. 1987) using a log of the likelihood ratio (LOD) threshold of 3.0 and a recombination fraction (RF) of 0.25. Loci order was estimated based on multipoint compare, order and ripple analyses. Distances between ordered loci (in cM) were calculated using the Kosambi mapping function. The resulting LGs were aligned according to the common bean core linkage map using common molecular markers as anchor points and considering the physical position of markers loci. LGs were named according to Pedrosa-Harand et al (2008).

***In silico* analysis**

In order to identify putative candidate genes, possibly involved in control of the resistance reaction to specific anthracnose races used in this work, we took advantage of the *P. vulgaris*

genome project deposited in www.phytozome.net V9.1 (Goodstein et al. 2012). We investigated the annotated function of the predicted genes within the genomic region included between the nucleotide sequences corresponding to the molecular markers closely mapped to the resistance genes identified in Pv03.

Results

Segregation for *C. lindemuthianum* races

SEL1308 was resistant to all races, whereas cv. MDRK was susceptible to all races except race 73. For each race, the $F_{2:3}$ families were classified as resistant (R; when all F_3 individuals were resistant), heterozygous (H; when F_3 individuals were resistant and susceptible) or susceptible (S; when all F_3 individuals were susceptible). Resistant and susceptible phenotypes were clearly differentiated. Table 1 shows the segregations observed in the $F_{2:3}$ families for response to races 3, 6, 7, 38 and 73. Due to the availability of F_3 seeds, it was not possible to evaluate all $F_{2:3}$ families against the five races. Segregation for races 3 and 7 showed a good fit to a 1R:2H:1S ratio, expected for one dominant gene. Segregation for races 6 and 38 showed a good fit to a 7R:8H:1S ratio, expected for two dominant and independent genes. In the segregation for race 73, an excess of families classified as homozygous resistant was observed, with respect to a 7R:8H:1S ratio. The preponderance of R genotypes to race 73 may be an anomaly of the intergene pool cross being studied.

Genetic analyses of the response to races 3 and 7

Segregation for resistance to races 3 and 7 [SEL1308 (resistant or R) × MDRK (susceptible or S)] exhibited a close co-segregation (15 R^3R^7; 55 H^3H^7; 23 S^3S^7; 6 H^3R^7; 2 H^3S^7; 3 R^3H^7; 4S^3H^7). In order to identify the localization of both resistance loci, molecular markers linked to the anthracnose genes/clusters (*Co-1*, *Co-u*, *Co-2*, *Co-3*, *Co-4*, *Co-5*, *Co-13* and *CoPv09*) were analyzed in the $F_{2:3}$ population. At least two marker loci per Co- cluster or gene were analyzed, except in the case of the Co-2 cluster, for which only marker PV-ag001 was polymorphic, and for *Co-13*, to which only marker $OPV20_{680}$ has been described as linked. Genetic linkage between the resistance loci to both races and any of the molecular markers was not found (Table 2). Linkage between the resistance loci and molecular markers closely mapped to resistance gene analogs (RGAs) was then investigated. Twelve microsatellite markers tagging a total of 15 RGAs mapped by Hanai et al. (2010) were analyzed in this population (see Table 1 ESM). These analyses revealed a significant linkage between marker PvM148 and the resistance loci to races 7 (RF=0.23 and LOD = 6.93; Table 2) and 3 (RF = 0.25 and LOD = 5). These results indicated that the location of these resistance loci was probably at the end of LG

Pv03. These genes were named as *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$ considering the symbols *Co-1 to Co-16* have been previously used (Coelho et al. 2013; Ferreira et al. 2013; Gonçalves et al. 2010).

Fine mapping of LG Pv03

For a precise location of the two resistance loci *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$, a set of molecular markers was analyzed, including several markers previously located in this position by other authors and new markers developed from the sequence of genotype G19833. Polymorphism was investigated for a total of 36 molecular markers, and 20 were polymorphic and were added to LG Pv03 (Table 1 ESM): six microsatellite markers (PVBR255, BMd36, BM159, BMb1203 and BMb339) and 13 InDel markers. In all cases the segregations fit a monogenic mode of inheritance. The resulting LG included 20 marker loci (considering the RAPD marker $OPV20_{680}$), ten of them located at one end of the LG (physical position within 0–3,000,000 bp). Resistance loci were mapped at the beginning of LG Pv03 (Figure 1). In a preliminary analysis, the nearest marker to the resistance genes was the InDel NDSU_IND_3_0.0441 at 15 cM from *Co-17*$^{7\text{-}SEL}$ (RF = 0.15; LOD = 9.14). Interestingly, marker $OPV20_{680}$, described as linked to *Co-13*, was mapped in a position distant from the resistance loci (Figure 1). For a more accurate approach to the resistance genes, 10 microsatellites (Table 2 in ESM) were developed from the G19833 sequence (within 0–318,714 bp). Three of them were polymorphic and analyzed in the population (B5, B6 and Pv3_3300a). Marker B5 was mapped 12.9 cM from *Co-17*$^{7\text{-}SEL}$ (RF = 0.12 LOD = 18.94). The co-dominant microsatellite marker B5 was developed from the nucleotide sequence of the gene Phvul.003G000700, coding for a serine-threonine protein phosphatase.

The *in silico* study around the physical position of marker NDSU_IND_3_0.0441 (44,114 bp) in the sequence reported for the bean genotype G19833 (from a total of 1,500,000 bp explored; www.phytozome.net) revealed the presence of a gene cluster between the physical positions 193,409 and 325,742 bp, including seven genes encoding typical R proteins with a NB-ARC domain (Phvul.003G002300, Phvul.003G002400, Phvul.003G002500, Phvul.003G002600, Phvul.003G002700, Phvul.003G002800 and Phvul.003G003000; see Table 3 ESM) and two peroxidases (Phvul.003G003100 and Phvul.003G003200), which have also been related to defense processes, signaling and response to stress. The microsatellite marker Pv3_3300a was developed from the sequence of predicted gene Phvul.003G003000.

Genetic analyses of the response to races 6 and 38

Resistance to races 6 and 38 [cross SEL1308 (R) × MDRK (S)] showed a good fit to a 7R:8H:1S ratio, expected in the case of two independent genes (Table 1). The contingency chi-square

values (Table 3) deviated significantly from the random segregation expected when compared with markers SAS13 and SH18, tagging the Co-4 resistance cluster (Table 3) and the resistance loci *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$ (or linked markers according to Figure 1). These results indicated that resistance to both races might be mediated by one resistance gene located at the Co-4 cluster (*Co-4*$^{6\text{-}SEL}$ and *Co-4*$^{38\text{-}SEL}$) and another gene at the end of LG Pv03 (*Co-17*$^{6\text{-}SEL}$ and *Co-17*$^{38\text{-}SEL}$) in SEL1308. To confirm this scenario, several molecular markers, previously mapped by other authors, were added to LG Pv08. The resulting map was formed by 26 loci, distributed across the whole chromosome: 14 InDel markers, six SCAR markers and four microsatellite markers, one of them developed from the AY341443.1 sequence deposited in GenBank (microsatellite 78L17c; Table 2 ESM). Markers SAS13 and SH18, linked to *Co-4*, were flanked by NDSU_IND_8_3.0216 and microsatellite 78L17c (Figure 1).

Segregation for resistance to races 6 and 38 was investigated in four subpopulations established from the total $F_{2:3}$ population, considering the genotype for markers B5 (tagging Co-17 region) and SAS13 (tagging Co-4 region; Table 4). In the M_B5 subpopulation (families with MDRK genotype for the co-dominant marker B5), resistance to race 6 segregated according to a 1R:2H:1S ratio, expected in the case of one dominant gene. A genetic linkage was found between this gene and marker SAS13 (RF = 0.18; LOD = 4.27). In the case of the Se_B5 subpopulation (SEL1308 genotype for the B5 marker), most of the families were resistant to race 6, suggesting that this region was involved in the resistance reaction. In the M_SAS13 subpopulation (families with MDRK genotype for the dominant marker SAS13), resistance to race 6 segregated according to a 1R:2H:1S ratio (Table 4). Linkage analysis using this subpopulation revealed a close linkage between the resistance gene to race 6 and microsatellite marker B5 (RF = 0.09; LOD = 5.61). In contrast, most of the families were resistant to race 6 in the opposite subpopulation (Se_SAS13 subpopulation, families with SEL1308 genotype for marker SAS13). In sum, all evidence confirmed that loci involved in resistance to race 6 were located in these two regions: Co-4 region (Pv08) and the chromosomal region in which loci *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$ were mapped (Figure 1). The same situation deduced for resistance to race 6 fitted the resistance pattern for race 38.

Genetic analyses of the response to race 73

Segregation for resistance to race 73 [cross SEL1308 (R) × MDRK (R)] did not show a good fit either to a 7R:8H:1S or a 37R:26H:1S ratio, expected in the cases of two or three independent genes, respectively (Table 1). The contingency chi-square values (Table 3) deviated significantly from the random segregation expected compared with markers SAS13 and SH18, tagging the

Co-4 resistance cluster (LG Pv08); TGA1.1 and ATA3 tagging the Co-1 cluster (LG Pv01); and markers SW12 and g1315 tagging the Co-3 resistance cluster (LG Pv04). These results suggest that three chromosome regions could be associated with the genetic control of resistance to race 73 in this population. To confirm this hypothesis, segregation for resistance to this race was investigated in different subpopulations established from the total $F_{2:3}$ population. In the Se_SAS13 subpopulation, susceptible families were not found (58R:25H:0S) while monogenic segregation was observed in the opposite subpopulation (M_SAS13: 4R:17H:6S; $\chi^2_{1:2:1} = 2.11$; p = 0.35). Similarly, in the subpopulation formed for the Co-1 region, using the dominant marker TGA1.1, susceptible families were not detected in the M_TGA1.1 subpopulation (49R:25H:0S) while monogenic segregation was observed in the opposite subpopulation, formed by families with SEL1308 genotype (13R:16H:6S; $\chi^2_{1:2:1} = 3.06$; p = 0.22). These observed results were compatible with the presence of a resistance gene in the Co-1 cluster, derived from MDRK, and another resistance gene in the Co-4 cluster, derived from SEL 1308. However, a significant association was also observed with markers tagging the Co-3 cluster (Table 3). Subpopulation formed by families with SEL1308 genotype for SW12 marker exhibited a good fit to a 7R:8H:1S ratio, suggesting the presence of two independent genes (38R:39H:5S; $\chi^2_{7:8:1} = 0.23$; p = 0.89). In contrast, subpopulation formed by families with MDRK genotype for the SW12 dominant marker exhibited a 24R:4H:1S segregation. This result suggests that this region was likely involved in the resistance reaction, since a change in the segregation ratio with respect to the total $F_{2:3}$ population would be expected in that case. Thus, it was not possible to discount the presence of a third resistance gene to race 73 in this region.

Discussion

The use of resistant cultivars is an economical way to prevent yield losses caused by pathogens. The identification of genomic regions involved in resistance responses allows the development of functional molecular markers, which can be used for assisted selection in breeding programs or genetic analysis. These markers can also help to identify candidate genes. The identification or validation of a candidate gene, controlling a particular trait, is usually preceded by a first step in which the locus/loci are located in the genetic map of the species through genetic analysis. In this study we investigated the inheritance of resistance to five *C. lindemuthianum* races in the well-known bean genotype SEL1308. Results revealed the involvement of two regions in the reaction against races 3, 6, 7, 38 and 73 in SEL1308. One region, located on LG Pv08, corresponding with the genomic region in which the Co-4 cluster was previously mapped, contained three genes for resistance to races 6, 38 and 73 ($Co\text{-}4^{6\text{-}SEL}$, $Co\text{-}4^{38\text{-}SEL}$ and $Co\text{-}4^{73\text{-}SEL}$). In another region, located on LG Pv03, two genes for resistance to

races 3 and 7 were directly mapped (*Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$) and another two genes for resistance to races 6 and 38 were indirectly mapped (*Co-17*$^{6\text{-}SEL}$ and *Co-17*$^{38\text{-}SEL}$). All of them were mapped in a position distant from marker $OPV20_{680}$, described as linked to *Co-13*, which confers resistance to race 73 in the bean cultivar Jalo Listas Pretas (Lacanallo et al. 2010). Resistance gene *Co-13* has not been directly mapped and its relative position was inferred from the location of marker $OPV20_{680}$ in the linkage map developed from the recombinant inbred line population BAT93/Jalo (named V20.700; Freyre et al. 1998). The relative positions of marker $OPV20_{680}$ and resistance genes *Co-17*$^{3\text{-}SEL}$*and Co-17*$^{7\text{-}SEL}$ on the obtained LG Pv03 (Figure 1) suggested that *Co-13* was not involved in resistance to races 3 and 7 in SEL1308.

Apart from *Co-13*, recessive resistance genes to bean common mosaic virus (BCMV) have been identified at the end of LG Pv03 (corresponding to physical position 0). Genes *bc-1*^{2} and *bc-u* were first described by Strausbaugh et al. (1999). Then, the $SBD5_{1300}$ SCAR marker was described as linked to *bc-1*^{2} and mapped at the end of LG Pv03 by Miklas et al. (2000). Blair et al. (2007) identified a recessive resistance gene for bean golden yellow mosaic virus (BGYMV), linked to SCAR marker SR2. This molecular marker was closely linked to the $SBD5_{1300}$ SCAR marker in the DOR364 × G19833 RIL population. It is not surprising that a certain chromosomal region can comprise a cluster of genes related to response against different pathogens, as it has also been described for other chromosomal regions in common bean. Pérez-Vega et al. (2013) reported the localization of two resistance genes to powdery mildew, located on the position of anthracnose resistance clusters Co-2 and Co-3. SCAR marker SQ4 was also described as linked to anthracnose resistance cluster Co-2 and gene *Ur-11*, which confers resistance to rust (Awale et al. 2008).

Previous studies indicated that a single dominant gene on LG Pv08, allelic to *Co-4* from the differential cultivar TO and named *Co-4*^{2}, was responsible for the genetic control of resistance against races 64, 73, 521, and 1545 in SEL1308 (Young et al. 1998). This conclusion was established from the observed segregation in different F_2 populations. Monogenic segregations were observed in the reaction of the F_2 populations Catrachita × SEL1308 and Black Magic × SEL1308 (S × R) to races 73 and 1545, respectively. In both populations, this resistance gene was linked to the RAPD marker $OAS13_{950}$, suggesting that the same chromosome region was involved in the control of the resistance reaction. The presence of one resistance gene was also suggested from complementary tests using the bean genotypes MDRK (carrying gene *Co-1* to race 73), Blackhawk (carrying *Co-2* to race 64), Mexico 222 (carrying *Co-3* to race 521), To (carrying *Co-4* to race 73) and Tu (carrying *Co-5* to race 73).

However, genetic analysis has not so far provided evidence confirming that the same resistance gene in SEL1308 confers resistance to all races included in the complementary tests (73, 1545, 64 and 521; Young et al. 1998). Co-segregation analysis of resistance against all these races would be necessary to clarify this question. Thus, the observed results in the present study were not contradictory with previous reports. In fact, we found monogenic segregations in resistance to races 3 and 7 and they were independent of the *Co-4* locus.

The majority of the genetic analyses carried out by other authors using differential cv. MDRK have considered that resistance is conferred by *Co-1* located on LG Pv01. Inheritance of resistance to race 73 in MDRK was investigated in two F_2 segregating populations derived from R × R crosses (Alzate-Marin et al. 2003; Young et al. 1998). The authors interpreted the observed results considering the presence of two resistances genes, one from each parent. However, both observed segregations also showed a good fit to a 63R:1S ratio, expected in the case of three independent resistance genes. In this analysis, contingency tests suggested that a second gene, located in the Co-3 region, can also play a role in reaction to race 73 in MDRK. However, the results obtained from the subpopulation analyses were not conclusive, either due to the population size or randomness. Monogenic segregation was not expected in the established subpopulations (M_SAS13 and Se_TGA1.1 subpopulations; in which Co-4 and Co-1 regions are not respectively present) in the case of two regions being involved in the response of MDRK (Co-1 and Co-3) and one in resistance of SEL1308 (*Co-4*^{2}). The ultimate confirmation of the participation of the Co-3 region should be based on a genetic dissection in which the two putative resistance genes to race 73 from MDRK will be separated in different segregating populations derived from directed crosses. In any case, evidence found in the present study suggests the presence of two putative independent resistance genes to race 73: one located in the Co-1 region, derived from MDRK and another in the Co-4 region, derived from SEL1308.

Results showed the usefulness of previously mapped RGAs in the identification of new resistance genes. Genes *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$ were located close to the position of RGA RNF240. The marker PvM148, tagging RGA RNF240, was previously mapped on LG Pv03 in the BAT93/Jalo RIL population (Hanai et al. 2010) and, showed a closely linkage to genes conferring resistance to races 3 an 7. In plants, many characterized resistances genes are organized in clusters of loci, which encode proteins containing conserved domains such as serine–threonine kinases, nucleotide-banding site (NB) or leucine rich repeats regions (LRR) (Dodds and Rathjen 2010). The NB domain is also found in other proteins, such as de APAF-1 (apoptotic protease-activating factor-1) or de CED-4 (*Caenorhabditis elegans* death-4 protein),

and so is also known as the NB-ARC domain (van der Biezen and Jones 1998; Tamelin and Takken 2008). This domain has been associated with pathogen recognition and subsequent activation of resistance responses (van Ooijen et al. 2008). Molecular markers based on polymerase chain reaction using degenerate primers have been designed from these conserved sequences and located in the genetic map in different species. In common bean, several studies have located regions including RGAs and, in many cases, their relative positions co-located with resistance genes or previously mapped QTL. RGA markers were mapped on regions in which were located anthracnose resistance genes as *Co-2, Co-3* or *Co-4* (Mutlu et al 2006). In addition, genomic analysis reported by other authors have shown that genes encoding proteins with NB-LRR domains were organized in tandem on chromosome regions corresponding to the Co-3 and Co-2 clusters on LGs Pv04 and Pv11, respectively (Creusot et al. 1999; David et al. 2009; Geffroy et al. 2009), so that it was not surprising to find a new anthracnose resistance gene in a region in which RGAs were previously mapped. All these findings suggest that a typical R protein can be involved in the control of resistance to races 3 and 7 in SEL1308.

In silico exploration in the sequence of bean genotype G19833 revealed the presence of a typical cluster of R genes between the physical positions 193,409 and 325,742 bp of chromosome 3. This cluster included seven predicted genes codifying for proteins with a NB-ARC domain. The microsatellite marker Pv3_3300a was developed from genes included in this cluster. Although the mapping software positioned both resistance genes at the end of a LG, we cannot discard the involvement of this cluster in the reaction to races 3 and 7. It is noteworthy that establishing the relationship between the genetic and the physical maps is complicated. First of all, variations among bean genotypes such as structural changes in the chromosome (e.g. insertion, inversions or translocations) or in the nucleotide sequence are possible. The *in silico* analysis, in this case, was performed using the sequence of the bean genotype G19833, which is available online. Second, many variables can affect the obtaining of a genome sequence, including coverage of the sequencing process and the algorithms and databases used for gene prediction and annotation (Yandell and Ence 2012). Third, genetic linkage maps are based on recombination fraction among loci and many factors can affect their estimation (e.g. accuracy in the phenotyping and genotyping and size of population). Considering the clusters included several R genes, it cannot be discounted that several closely linked epistatic genes were involved in the resistance reaction to a specific race. In this situation, recombinant events between resistance loci would not be detected, linkage could be overestimated and the relative position of resistance loci on the map distorted.

This study contributed to increasing understanding of the genetic control of resistance to anthracnose in common bean. Results from this work revealed a new chromosomal region involved in resistance to anthracnose, located at the beginning of LG Pv03, where no anthracnose resistance genes have been previously identified. Results also showed that, although monogenic segregation can be observed in the response of a bean genotype against different races, it may be controlled by independent race-specific resistance genes: e.g. gene *Co-4*^{2} to race 73 (Young et al. 1998) and *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$ to races 3 and 7 in the bean genotype SEL1308. All of these findings demonstrate again that response of bean plants in the *P. vulgaris–C. lindemuthianum* interaction is complex, highly specific and that different chromosome regions can take part. Depending on the bean genotype, specific resistance genes can be involved in the reaction to a certain pathogenic variant of the fungus. Thus, the description of an anthracnose resistance gene should be based on the bean genotype in which it has been identified and the *C. lindemuthianum* race or isolate used to identify that gene.

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Conflict of Interest

The authors declare that they have no conflict of interest

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Table 1. Observed segregation for resistance to five monosporic isolates of *Colletotrichum lindemuthianum* classified as races 3, 6, 7, and 38, in the $F_{2:3}$ population SEL1308/MDRK.

	Parental phenotype[a]			Observed segregation in the F_2 families[b]				
Race	SEL1308	MDRK	Expected ratio	R	H	S	χ2	*p*-value
3	R	S	1:2:1	18	66	28	5.36	0.07
7	R	S	1:2:1	21	66	26	3.64	0.16
6	R	S	7:8:1	62	80	14	2.49	0.29
38	R	S	7:8:1	50	51	11	2.75	0.25
73	R	R	7:8:1	62	43	6	6.66	0.04

[a]*R* Resistant, *S* susceptible

[b]*R* families with all individuals resistant; *H* families with resistant and susceptible individuals; *S* families with all individuals susceptible

Table 2. Linkage analysis between the gene conferring resistance to race 7, and each one of the markers tagging the clusters Co-1, Co-2, Co-3, Co-4, Co-5 and Co-u as well as the anthracnose resistance genes *Co-13* and *CoPv09*. Microsatellite PvM148 linked to RGA RNF240 was also included

			$F_{2:3}$ SEL1308 x MDRK families[a]															RF	LOD
			R					H					S						
Marker	LG	Co-locus	S/S	S/-	S/M	M/-	M/M	S/S	S/-	S/M	M/-	M/M	S/S	S/-	S/M	M/-	M/M		
ATA03	Pv01	*Co-1*	10		7		4	22		28		16	6		12		8	0.43	0.48
TGA 1.1	Pv01	*Co-1*	8	-	-	10	-	17	-	-	46	-	9	-	-	19	-	0.47	0.03
NDSU_IND_2_40.3966	Pv02	*Co-u*	2	-	12	-	4	19	-	33	-	13	8	-	16	-	3	0.50	0.00
NDSU_IND_2_40.4411	Pv02	*Co-u*	2	-	13	-	3	15	-	35	-	16	8	-	16	-	3	0.50	0.00
$OPV20_{680}$	Pv03	*Co-13*	3	-	-	15	-	16	-	-	50	-	8	-	-	19	-	0.50	0.00
SW12	Pv04	*Co-3*	-	14	-	-	4	-	47	-	-	19	-	21	-	-	7	0.50	0.00
g1375	Pv04	*Co-3*	7	-	7	-	4	21	-	31	-	13	7	-	16	-	5	0.50	0.00
SZ04	Pv07	*Co-5*	2	-	6	-	3	13	-	22	-	10	3	-	10	-	4	0.48	0.01
BM185	Pv07	*Co-5*	3	-	6	-	2	11	-	25	-	9	3	-	10	-	4	0.45	0.07
SAS13	Pv08	*Co-4*	-	17	-	-	1	-	51	-	-	15	-	16	-	-	11	0.36	1.13
SH18	Pv08	*Co-4*	-	17	-	-	1	-	49	-	-	17	-	15	-	-	13	0.34	1.63
NDSU_IND_9_29.1822	Pv09	*CoPv09*	5	-	6	-	7	14	-	37	-	15	6	-	12	-	9	0.45	0.17
NDSU_IND_9_29.1976	Pv09	*CoPv09*	4	-	6	-	8	14	-	37	-	15	6	-	12	-	9	0.47	0.04
PV-ag001	Pv11	*Co-2*	3	-	9	-	3	20	-	28	-	15	2	-	15	-	10	0.43	0.27
PvM148	PV03	-	13	-	7	-	1	10	-	44	-	10	0	-	15	-	11	0.23	6.93

[a] For resistance to race 7: *R* families with all individuals resistant; *H* families with resistant and susceptible individuals; *S* families with all individuals susceptible. For molecular markers genotype: *S/S*, homozygous for SEL1308 alleles; *S/-*, homozygous for the SEL1308 alleles or heterozygous; *S/M*, heterozygous; *M/-*, homozygous for the MDRK alleles or heterozygous; *M/M*, homozygous for the MDRK alleles. RF, recombination frequency

Table 3. Contingency χ^2 tests corresponding to the joint segregations for each one of the resistances to isolates classified as races 6, 38 and 73 and 13 loci tagging the main anthracnose resistance clusters identified in common bean. Loci conferring resistance to race 3 and 7 (*Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$) mapped on LG Pv03 were also included in these tests.

Name	LG	Co- locus	Race 6 χ^2-*p*	Race 38 χ^2-*p*	Race 73 χ^2-*p*
ATA03	Pv01	*Co-1*	2.69 ns	3.35 ns	25.64 *
TGA 1.1	Pv01	*Co-1*	0.49 ns	0.63 ns	17.12 *
NDSU_IND_2_40.3966	Pv02	*Co-u*	6.01 ns	4.05 ns	3.31 ns
NDSU_IND_2_40.4411	Pv02	*Co-u*	7.11 ns	7.41 ns	2.45 ns
Co-17$^{3\text{-}SEL}$	Pv03	*Co-17*$^{3\text{-}SEL}$	43 *	25.9 *	ns
Co-17$^{7\text{-}SEL}$	Pv03	*Co-17*$^{7\text{-}SEL}$	48.9 *	34 *	ns
SW12	Pv04	*Co-3*	1.75 ns	0.32 ns	11.67 *
g1375	Pv04	*Co-3*	1.16 ns	3.06 ns	17.92 *
BM185	Pv07	*Co-5*	2.89 ns	3.9 ns	3.71 ns
SZ04	Pv07	*Co-5*	1.9 ns	2.83 ns	5.88 ns
SH18	Pv08	*Co-4*	45 *	39.2 *	31.39 *
SAS13	Pv08	*Co-4*	38.6 *	30.6 *	35.16 *
NDSU_IND_9_29.1822	Pv09	*Pv09Co*	1 ns	4.13 ns	4.22 ns
NDSU_IND_9_29.1976	Pv09	*Pv09Co*	1.13 ns	3.22 ns	5.22 ns
PV-ag001	Pv11	*Co-2*	1.65 ns	2.48 ns	3.88 ns

ns= not significant, *= significant after applying Bonferroni correction

Table 4. Observed segregation for resistance to races 6 and 38 in four subpopulations formed from the $F_{2:3}$ population for two chromosome regions: Co-4 and Co-17 (tagged using the microsatellite marker B5). Results of the linkage analyses performed in the subpopulation showing a monogenic segregation are also indicated.

	Segregation in Subpopulation within the $F_{2:3}$ population[a]										Segregation in Subpopulation within the $F_{2:3}$ population[b]									
	Se_B5					M_B5					Se_SAS13					M_SAS13				
	Ob. frequency					Ob. frequency					Ob. frequency					Ob. frequency				
Race	RR	RS	SS	$\chi^2_{(1:2:1)}$	*p*	RR	RS	SS	$\chi^2_{(1:2:1)}$	p	RR	RS	SS	$\chi^2_{(1:2:1)}$	p	RR	RS	SS	$\chi^2_{(1:2:1)}$	p
6	27	10	0	47.2	0	9	25	11	0.73	0.7	56	58	1	50.3	0	6	22	12	2.2	0.3
38	21	5	0	43.8	0	5	16	8	0.93	0.6	46	36	1	47.8	0	4	15	9	1.93	0.4

[a] Co-17 region. Subpopulations were established using the codominant marker B5; Se_B5 and M_B5, formed by families showing, SEL1308 and MDRK alleles respectively. [b] Co-4 region. Two subpopulations were established using the dominant marker SAS13; Se_SAS13 and M_SAS13, formed by families showing, SEL1308 and MDRK alleles respectively

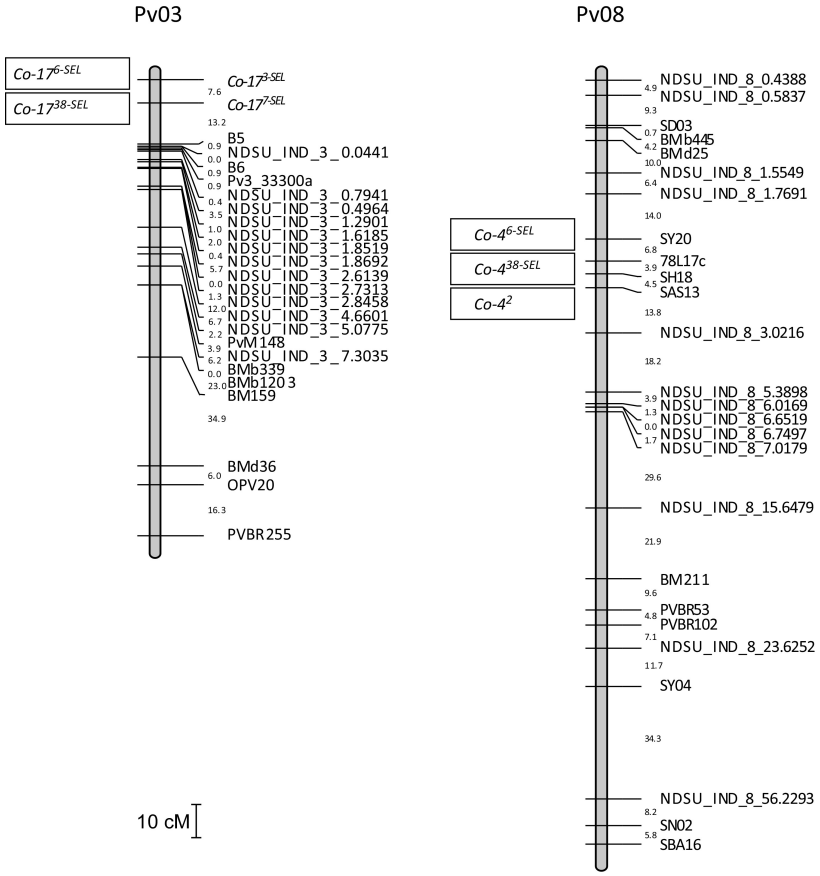

Figure 1. Linkage groups Pv03 and Pv08 developed in the $F_{2:3}$ segregating population derived from the cross SEL1308 x MDRK. Distances between loci (cM) are showed on the left of the locus names. The relative position of two race-specific resistance genes directly mapped on LG Pv03 (name as *Co-17*$^{3\text{-}SEL}$ and *Co-17*$^{7\text{-}SEL}$) and five resistance genes, whose position was indirectly deduced (*Co-4*$^{6\text{-}SEL}$, *Co-4*$^{38\text{-}SEL}$, *Co-4*$^{73\text{-}SEL}$, *Co-17*$^{6\text{-}SEL}$, and *Co-17*$^{38\text{-}SEL}$), is indicated.

Discusión general

1. Uso de resistencia genética en plantas

El uso de variedades resistentes es una de las alternativas más eficaces para reducir las pérdidas causadas por los patógenos en los cultivos, y su empleo presenta significativas ventajas. Entre ellas cabría citar que su uso permite una reducción en la utilización de productos fitosanitarios y, en consecuencia, una reducción del coste económico asociado a la producción, así como un menor impacto del cultivo en el medio ambiente, incluyendo la producción de gases con efecto invernadero que genera la síntesis y aplicación de tales materias activas. Por otra parte, limitar la utilización de productos fitosanitarios permite desarrollar un cultivo más saludable desde la perspectiva de la salud humana y seguridad agroalimentaria. El uso de variedades resistentes puede representar, a corto plazo, un papel más importante del que hasta ahora jugaba, dada la tendencia por parte de las administraciones a limitar el número de materias activas permitidas para el control de patógenos (www.magrama.gob.es/). Además, es una de las pocas soluciones en aquellos casos en los que no existen materias activas realmente eficaces para evitar el desarrollo de una determinada enfermedad.

Pero el uso de variedades resistentes también presenta algunas dificultades. En primer lugar se debe considerar que el desarrollo, obtención, registro y comercialización de variedades resistentes puede requerir una fuerte inversión en recursos económicos, humanos, técnicos y de tiempo. Asimismo, en muchos casos, la especificidad de las resistencias genéticas se limita a uno o unos pocos agentes patógenos específicos o, incluso, a una o pocas variantes patogénicas de éstos. Por otro lado, la co-evolución entre huésped y patógeno puede conducir a la superación por parte de estos últimos, de las resistencias genéticas disponibles en el huésped. Por último, es importante tener en cuenta que no siempre existen fuentes de resistencia genética descritas en un determinado huésped frente a patógenos específicos o variantes patogénicas. En todo caso, antes de iniciar un programa de mejora genética para el desarrollo de nuevas variedades resistentes (vía mejora genética clásica o vía transformación) es importante un conocimiento previo sobre la interacción planta-patógeno, es decir, del control genético de la resistencia. Para ello resulta recomendable contar con las herramientas necesarias, como la disponibilidad de aislamientos del patógeno y su manejo; disponibilidad de test de resistencia confiables y repetibles, así como de una escala de valoración de síntomas o daños; y la existencia de fuentes de resistencia en la especie de interés. Esta Tesis se centra en profundizar en el conocimiento de la herencia de la resistencia frente a tres patógenos que

inciden gravemente en los cultivos de judía común en el norte de España: oídio, bacteriosis común y antracnosis. El nivel de conocimiento del que se partía era distinto para cada uno de los patógenos, por lo que en cada uno de los casos se abordaron etapas diferentes.

2. Resistencia genética a oídio

En el caso del oídio la información de partida era muy escasa, ya que no existen muchos trabajos en la bibliografía que hayan estudiado la interacción entre este patógeno y la judía común. Al iniciar los trabajos con este patógeno no se conocía exactamente la especie responsable de la sintomatología. Basándose en la secuenciación de fragmentos de espaciadores transcritos internos (ITS, Internal Transcribed Spacers) y del posterior alineamiento de estas secuencias con las base del datos del NCBI, se pudo determinar que se trataba de la especie *Erysiphe diffusa* (N. Rispail com. per.), lo que coincide con lo descrito por Almeida et al. (2008) y con un reciente análisis realizado (A. Campa com. per). Por otro lado, los pocos estudios llevados a cabo se habían realizado en condiciones de infección natural en campo (Schwartz et al. 1981), no bajo condiciones controladas. Por esta razón, el trabajo desarrollado en esta Tesis comenzó por establecer un método para el manejo y multiplicación del patógeno, así como un método de inoculación y evaluación de síntomas que permitiese el estudio de la respuesta del material vegetal frente al patógeno en condiciones controladas en invernadero o cámaras de inoculación. Así mismo, se buscó una escala fenotípica para describir las reacciones observadas en plántula, adoptándose una escala previamente descrita en cebada (Mains y Dietz 1930). Llegados a este punto se buscaron fuentes de resistencia frente al aislamiento local conservado. En cuanto al control genético de la resistencia al patógeno, algunos autores han sugerido una herencia de tipo cualitativo. Dundas (1936) describe un gen dominante implicado en la respuesta de resistencia. Bett y Michaels (1995) sugieren la presencia de un gen dominante y otro recesivo, tras la evaluación de varias poblaciones F_2 derivadas de cruzamientos entre diferentes genotipos, mientras Ferreira et al. (1999) describen dos genes complementarios. En ningún caso estos genes fueron localizados en el mapa genético de la especie ni se describieron marcadores moleculares ligados que pudiesen ser utilizados en selección asistida por marcadores.

Los resultados obtenidos en este trabajo indicaron que la reacción frente al aislamiento de oídio tiene una naturaleza cualitativa, coincidiendo con los estudios realizados previamente por otros autores a partir de evaluaciones en campo (Bett y Michaels 1995; Dundas 1936; Ferreira et al. 1999). En este trabajo se ha puesto a punto el método de inoculación y de

manejo del patógeno y se ha aplicado una escala de evaluación sencilla. En los estudios realizados previamente con este patógeno se habían utilizado escalas basadas en el porcentaje de área de hoja afectada, bien escalas 0-4 (Dundas 1936, Bett y Michaels 1995) o escalas 0-9 (Ferreira et al. 1999). En las evaluaciones realizadas en este trabajo fue posible clasificar la reacción de las plántulas en cinco clases claramente diferenciables o tipos de infección (TI): TI0, sin crecimiento visible del hongo en la superficie de la hoja; TI1, reacción de hipersensibilidad sobre las hojas sin apenas crecimiento del patógeno; TI2, reacción de hipersensibilidad y cierto desarrollo del patógeno; TI3, crecimiento moderado del hongo sobre las hojas; TI4, crecimiento intenso del hongo, cubriendo totalmente la hoja. La respuesta TI0 se considera resistencia completa mientras que las respuestas TI1, TI2 y TI3 se consideran resistencia moderada, frente a la respuesta claramente susceptible (TI4). La escala propuesta en este trabajo simplifica considerablemente la evaluación de síntomas en planta.

En cuanto a la herencia de la resistencia, los resultados obtenidos en esta Tesis sugieren que está controlada por un limitado número de genes mayores de naturaleza cualitativa, que pueden ser epistáticos o actuar de un modo complementario. Este tipo de respuesta cualitativa a oídio también ha sido descrita en otras especies de leguminosas de importancia como el guisante (Fondevilla et al. 2008; Katoch et al. 2010) o la soja (Lohnes y Bernard 1992; Kang y RoufMian 2010), así como en leguminosas modelo como *Medicago truncatula* (Ameline-Torregrosa et al. 2010). A partir de las diferentes descendencias F_2 o $F_{2:3}$ evaluadas durante este trabajo, se dedujo la implicación de uno o dos genes en la respuesta de resistencia, dependiendo del cruzamiento analizado, así como distintos modos de herencia. En la población derivada del cruzamiento entre Porrillo Sintético (TI0) y Xana (TI4), se identificaron dos genes dominantes e independientes, uno de los cuales confiere resistencia total (TI0) y otro resistencia parcial (TI3), ya que en la evaluación de la población F_2 se observaron plántulas con tres tipos de respuesta: TI0, TI4 y TI3. Esta observación fue confirmada mediante una disección genética a partir del análisis de varias familias F_3, evaluadas frente al mismo aislamiento del patógeno. Por otra parte, en la población Porrillo Sintético (TI0) x X2776 (TI3), la segregación observada se ajustó a lo esperado para un gen dominante que confiere resistencia total, confirmándose que ambas variedades comparten el gen que produce una resistencia intermedia, TI3. La variedad X2776 (TI3) se cruzó con la variedad G122 (TI4) y la segregación se ajustó también a lo esperado para un gen dominante, que controla la respuesta TI3 de X2776. La variedad Xana (TI4) también fue cruzada por la variedad Cornell 49242 (TI0) y la evaluación de la descendencia F_2 dio como resultado una segregación que se ajustó de nuevo a lo esperado para dos genes dominantes e

independientes. Finalmente, la evaluación de las plantas F_2 derivadas del cruzamiento entre las variedades Amanda (TI0) y Xana (TI4) dio como resultado una segregación que se ajustó a lo esperado para dos genes complementarios, que controlarían en este caso la respuesta resistente en Amanda. Este tipo de interacción génica fue también descrita en judía por Ferreira et al. (1999) y ha sido descrita en la respuesta a otras enfermedades, como puede ser la antracnosis (Ferreira et al. 2013; Campa et al. 2014), por lo que no es sorprendente que una complementación entre genes de resistencia también pueda ocurrir en la respuesta a oídio.

La herencia de la resistencia a oídio en Cornell 49242, uno de los genotipos que mostraron resistencia total al patógeno (TI0), se evaluó con detalle en una población de líneas recombinantes derivadas del cruzamiento entre Cornell 49242 y la variedad susceptible Xana (RIL XC) y para la que se disponía previamente de un mapa genético (Pérez-Vega et al. 2010). La evaluación de esta población RIL XC dio como resultado una segregación que se ajustó a lo esperado para dos genes dominantes e independientes, lo que confirmó lo observado en la evaluación de las poblaciones F_2 derivadas del mismo cruzamiento. Para investigar la localización de estos dos genes se realizaron análisis de contingencia y análisis de ligamiento a partir de subpoblaciones. Los análisis de contingencia indicaron una asociación significativa de la resistencia con marcadores de dos regiones cromosómicas; la región del *cluster* de resistencia a antracnosis Co-2, en el GL Pv11 y la región del *cluster* Co-3, en el GL Pv04. Para confirmar la implicación de dos genes en la respuesta al patógeno, su modo de acción y su localización en estas regiones, también se llevó a cabo una disección genética, mediante el cruzamiento entre la variedad Xana y dos líneas recombinantes, cada una de las cuales sólo presentaba uno de los genes de resistencia a oídio. Tanto las segregaciones obtenidas en las correspondientes poblaciones F_2, como los análisis de ligamiento confirmaron la localización de los genes de resistencia. Un gen dominante, que produce resistencia total al patógeno, en el GL Pv11 (denominado gen *Pm1*) y otro gen, localizado en Pv04 (denominado gen *Pm2*), que produce una resistencia parcial, con moderado crecimiento del patógeno sobre la planta (TI3). Puesto que cuando se cruzaron las dos líneas elegidas entre sí, todas las plantas presentaron resistencia total (TI0), se puede deducir que existe relación de epistasia dominante, de modo que la acción del gen *Pm1* enmascara la de *Pm2*.

El hecho de que ambos genes hayan sido localizados en regiones cromosómicas donde previamente se habían localizado *clusters* de resistencia a antracnosis, puede sugerir un mismo mecanismo de resistencia. Esta co-localización no es sorprendente si tenemos en cuenta que la mayoría de genes R descritos hasta el momento son aquellos que codifican para proteínas de

tipo NBS-LRR y que estos genes suelen encontrarse agrupados formando *clusters* de múltiples genes. En la mayoría de los casos estos genes son secuencias muy parecidas pero, a veces, también pueden contener secuencias filogenéticamente distantes (Marone et al. 2013) que pueden responder a diferentes procesos.

3. Resistencia genética a grasa en el genotipo Cornell 49242

En el caso de la grasa de la judía, la experiencia previa en la interacción *P. vulgaris-Psp* era mayor dado que se trata de una enfermedad muy extendida en el cultivo de esta especie (Singh and Schwartz 2010). Se disponía de un método de inoculación y de un método de identificación de variantes patogénicas, mediante el uso de un juego de variedades estandarizado (Taylor et al. 1996). También se han descrito fuentes de resistencia y genes mayores que protegen frente a razas específicas del patógeno (Chen et al. 2010; Fourie et al. 2004; Miklas et al. 2009, 2011; Taylor et al. 1996) así como respuestas de tipo cuantitativo (Ariyarathne et al. 1999; Yaish et al. 2006), por lo que parece que la resistencia a este patógeno no está controlada necesariamente por genes mayores. En el caso del genotipo Cornell 49242, las pruebas realizadas en colaboración con el ITACyL (Instituto Tecnológico Agrario de Castilla y León) mostraron que este genotipo presenta una resistencia moderada frente a varias razas del patógeno y que esta respuesta era significativamente diferente de la respuesta de la variedad Xana, que es altamente susceptible. Por esta razón, se utilizó la población de líneas recombinantes derivada del cruzamiento entre ambos genotipos para estudiar la herencia de la resistencia a *Psp* en Cornell 49242.

La mayoría de los trabajos describen una resistencia de naturaleza cualitativa a la grasa de la judía y se han llegado a describir 5 genes de resistencia (Fourie et al. 2004; Miklas et al. 2009, 2011). Pero también algunos trabajos han descrito una respuesta de tipo cuantitativo, en aquellos casos en los que los genes mayores no están presentes (Ariyarathne et al. 1999; Yaish et al. 2006). En esta Tesis se investigó la respuesta del genotipo Cornell 49242, mediante la evaluación de la población de RIL XC frente a dos aislamientos del patógeno (ITA-812 e ITA-684), clasificados como razas 6 y 7, respectivamente. Para ambas razas, se observaron diferencias significativas entre parentales, pero la respuesta en las líneas evaluadas no mostró una clara diferenciación entre respuestas susceptibles y resistentes, sino una variación continua que parecía indicar un control genético de tipo cuantitativo. Los análisis permitieron identificar 4 QTLs significativos (LOD > 2.5) en los grupos de ligamiento Pv04 y Pv06. El QTL identificado en el GL Pv04, $Psp4^{812XC}$, fue mapeado en una región cromosómica que

corresponde con la misma en la que se ha localizado el locus APA, que confiere resistencia a gorgojo (Blair et al. 2010). En el grupo de ligamiento Pv06 se detectaron 3 QTLs diferentes, dos para el aislamiento ITA-684 (Psp6.1^{684XC} y Psp6.2^{684XC}) y uno para el aislamiento ITA-812 (Psp6.1^{812XC}). Para confirmar la implicación de cada una de estas regiones en la respuesta al patógeno, se diseñó una disección genética, re-evaluando varias líneas con genotipos de ambos parentales para los marcadores moleculares localizados en cada una de las regiones. Se observó que, en el caso del aislamiento ITA-812 (raza 6), la respuesta de las líneas con genotipo del parental más resistente para estas regiones cromosómicas no variaba significativamente respecto a la respuesta de las líneas con genotipo del parental susceptible. Hay que tener en cuenta que en el caso de la variedad Cornell 49242 este aislamiento resultó ser más virulento que ITA-684. En el caso del aislamiento ITA-684, se observó que para que haya una diferencia de respuesta significativa, es necesaria la presencia de los dos QTLs localizados en el grupo de ligamiento Pv06.

4. Resistencia genética a antracnosis en el genotipo SEL1308

En el caso de la resistencia a antracnosis la experiencia de partida era mayor, ya que se trata de una enfermedad ampliamente estudiada en judía común. De hecho, las primeras referencias datan de principios del siglo XX. Al iniciar este trabajo se disponía de aislamientos del patógeno, método de inoculación, escala de valoración de síntomas y caracterización de variantes patogénicas. Asimismo, en judía se han descrito genes mayores que condicionan resistencia a razas específicas, muchos de los cuales han sido localizados en el mapa genético de la especie (Ferreira et al. 2013). En este caso, el trabajo se centró en el estudio de la resistencia a antracnosis en la línea SEL1308. Esta línea deriva de la variedad diferencial G2333, utilizada en la caracterización de razas del patógeno, y presenta un alto espectro de resistencia [fue resistente al 97% de las 34 razas testadas por Balardin and Kelly (1998)]. Sin embargo, en el genotipo SEL1308 solamente se ha descrito el gen de resistencia *Co-4*^{2}, localizado en el grupo de ligamiento Pv08, que confiere resistencia frente a las razas 73 y 1545 del patógeno (Young y Kelly 1996; Melotto et al. 2004).

Para investigar la herencia de la resistencia a antracnosis en esta línea, se analizó la respuesta a cinco razas en una población $F_{2:3}$ derivada del cruzamiento entre SEL1380 y la variedad diferencial MDRK (Michigan Dark Red Kidney). En los test de resistencia los fenotipos resistente y susceptible fueron claramente diferenciables, de modo que se puede descartar un modo de herencia cuantitativo. Las evaluaciones frente a las razas 3 y 7 indicaron la presencia

de un solo gen dominante implicado en la respuesta. Aunque se observó recombinación entre ambos genes, los dos loci fueron mapeados en una misma región del extremo de Pv03 (genes *Co17*$^{3\text{-}SEL}$*y Co17*$^{7\text{-}SEL}$). El origen de estos nuevos genes de resistencia no puede ser deducido ya que no se conoce la respuesta frente a ambas razas de los genotipos Talamanca y G2333, a partir de los cuales, se originó la línea SEL1308. En el caso de las razas 6 y 38, la segregación se ajustó a lo esperado para dos genes dominantes e independientes. A través de los test de contingencia y los análisis de subpoblaciones, se pudo deducir que para cada una de las razas la respuesta de resistencia vendría dada por un gen localizado en el GL Pv08, coincidiendo con la región del *cluster* de resistencia Co-4 (genes *Co-4*$^{6\text{-}SEL}$ *y Co-4*$^{38\text{-}SEL}$), y por otro gen localizado también en el extremo de Pv03.

En el GL Pv03 se describió previamente la existencia del gen *Co-13* en el genotipo Jalo Listas Pretas, ligado al marcador RAPD $OPV20_{680}$ (Lacanallo et al. 2010), el cual había sido incluido previamente en el mapa genético consenso (Freyre et al. 1998). Los resultados obtenidos indican que no se trataría del mismo gen. Los análisis de contingencia y los análisis de ligamiento tanto directos, como a partir de subpoblaciones, no revelaron una relación estrecha entre los genes de resistencia y este marcador, lo que indica que, al menos para las razas analizadas, *Co-13* no estaría implicado en la respuesta. No obstante, en este extremo cromosómico donde se localizaron los genes *Co17*$^{3\text{-}SEL}$ *y Co17*$^{7\text{-}SEL}$ también se han descrito otros genes de resistencia a otros patógenos, como el virus del mosaico común (Miklas et al. 2000) o el virus dorado del mosaico amarillo (Blair et al. 2007). Para la raza 73, los resultados obtenidos en este trabajo coinciden con lo descrito hasta el momento, es decir, que el genotipo SEL1308 presenta un gen de resistencia localizado en el *cluster* Co-4 (*Co-4*^{2}). En suma los análisis de la respuesta a antracnosis en esta población han permitido identificar una nueva región cromosómica implicada en la resistencia a antracnosis en judía común, localizada en el extremo del GL Pv03.

5. Genes candidatos

En los últimos cinco años han ocurrido dos hechos relevantes para los análisis genéticos en plantas. El primero de ellos ha sido la aparición de las nuevas técnicas y equipos para la secuenciación del ADN (Illumina, Ion Torrent, Pyrosequencing, SOLiD), lo que es conocido como 'next-generation sequencing' (NGS). Esta tecnología permite reducir los costes de la secuenciación y el tiempo de análisis significativamente, aunque supone una gran complejidad en el análisis bioinformático (Feuillet et al. 2011; Langrdge y Fleury 2011; Michael y Jackson,

2013; Morrell et al. 2012). Una primera consecuencia de la disponibilidad de tecnologías de secuenciación es un crecimiento enorme de las bases de datos de secuencias, que ponen a disposición del investigador nuevas herramientas para el análisis genético, como pueden ser:

- el desarrollo de marcadores específicos para regiones genómicas concretas (marcadores a la carta), bien para saturar una región o bien para desarrollar marcadores funcionales asociados a la expresión de un carácter.

- la búsqueda de genes con secuencias homólogas (genes ortólogos o parálogos), en lo que se conoce como genética inversa, es decir, a partir de la secuencia de un gen con una función descrita, identificar genes con secuencias similares en otra especie.

La tecnología NGS también ofrece la posibilidad de re-secuenciación de diferentes genotipos (o regiones genómicas) dentro de una especie con relativa facilidad y de este modo analizar la variación dentro de una especie a nivel de genes.

Otro hecho relevante ha sido la publicación de la secuencia del genoma de judía incluyendo la predicción automática de genes, su posible función, los niveles de expresión en mRNA y las secuencias de proteínas (www.phytozome.net). El genotipo secuenciado fue G19833, con unas características morfológicas y moleculares que lo incluyen en el grupo de germoplasma andino. Aunque con ciertas limitaciones, la disponibilidad de este genoma permite ir más allá de los análisis genéticos clásicos, esto es, de la identificación de la región donde se localizan los genes implicados en la expresión de un carácter. Una vez localizado un gen o QTL en el mapa genético es posible relacionarlo con la posición en el mapa físico (secuencia de nucleótidos de cada cromosoma) mediante el alineamiento de las secuencias de los marcadores flanqueantes de la región de interés. Acotada la región física en la que se encuentra un gen mayor o QTL, es posible conocer los genes predichos en la región, sus funciones anotadas y sugerir los genes que tentativamente pudieran estar implicados en la expresión del carácter. Este tipo de análisis (conocido como análisis *in silico*) ha sido utilizado para proponer genes candidatos en los QTL asociados a la respuesta a *Psp* en Cornell 49242. Dado que la región acotada era muy extensa, se buscaron secuencias homólogas de genes conocidos implicados en la respuesta a *Psp* en otras especies. Se utilizaron secuencias de genes conocidos como *RPM1*, *RPG1-B*, *FLA2* y *Pto*, de diferentes especies y se realizó un BLAST entre las secuencias de estos genes y la secuencia genómica del genotipo G19833. Dentro de las regiones que se corresponden con los QTLs identificados en este trabajo se encontraron un total de 16 genes candidatos, que presentaron alta homología con las secuencias utilizadas en el BLAST (valor E < $1x10^{-40}$). De todos ellos, 9 codifican proteínas con motivos LRR, relacionados estrechamente con procesos

de resistencia a patógenos. Los otros 7 codifican proteínas de tipo serina-treonina quinasa, 4 de las cuales también incorporan receptores de tipo LRR. Aunque conocer todos los genes implicados y su acción es más difícil en el caso de respuestas cuantitativas, es importante investigar el funcionamiento de estos QTLs en respuestas de resistencia en ausencia de genes mayores. También es preciso delimitar de una manera más eficaz, las regiones cromosómicas implicadas en estas respuestas, para que mediante mejora asistida por marcadores, sea más fácil introgresar estas regiones en genotipos de interés y obtener nuevas variedades resistentes al patógeno.

En el caso de la resistencia genética a las razas 3 y 7 de antracnosis, presente en el genotipo SEL1308 y mapeada en el extremo del GL Pv03, se pudo identificar en el entorno un *cluster* de genes candidatos en la posición física entre las 193.409 y las 325.742 pb. En este *cluster* han sido anotados 3 genes que codifican para proteínas relacionadas con resistencia a enfermedades y otros 4 genes que contienen también dominios NBS-ARC, relacionados con resistencia a patógenos. Además, en este mismo *cluster* se han anotado dos genes que codifican para proteínas de tipo peroxidasa, también relacionadas con procesos de respuesta frente a patógenos, mediante la formación de especies reactivas del oxígeno o ROS (Liu et al. 2010; O'Brien et al. 2012). En este punto conviene señalar que, aunque se desarrolló un marcador de este *cluster* (Pv3_3300a, a partir de la secuencia del gen Phvul.003G003300) y el extremo del GL Pv03 estaba saturado de marcadores, los genes de resistencia a las razas 3 y 7 no se ligaron estrechamente a ningún marcador, por lo que no es posible asegurar que este *cluster* esté implicado en la resistencia a las razas evaluadas. Este hecho pone de manifiesto la dificultad en relacionar el mapa físico con el mapa genético debido a factores como:

i) Variación entre genotipos, como cambios estructurales en cromosomas (inserciones, deleciones, duplicaciones, inversiones o traslocaciones) o cambios en secuencia de nucleótidos. Hay que tener en cuenta que el análisis *in silico* se realizó sobre el genoma de G19833, genotipo diferente al genotipo de interés, ya que es el único cuya secuencia se encuentra disponible.

ii) Hay muchas variables y parámetros que pueden influir en la obtención de una secuencia de nucleótidos y su anotación (Yandell y Ence 2012).

iii) Los mapas genéticos están basados en la frecuencia de recombinación entre los loci y muchos factores pueden afectar a su estimación, como puede ser la precisión en el 'fenotipado' y 'genotipado', tamaño de la población, azar...

En todo caso, el mapeo genético constituye un primer paso en la localización de la región genómica implicada en el control genético de un carácter por una aproximación directa y más, como en este caso, cuando se trata de nuevas regiones en las que previamente no se habían descrito genes de resistencia para ese patógeno y para las que no existen marcadores ligados.

6. Implicaciones en la mejora genética de judía

Este trabajo ha permitido aportar información relevante para la mejora genética frente a oídio en judía. El trabajo describe un método para evaluar la respuesta a oídio en plántulas bajo condiciones controladas, así como una escala de valoración de síntomas confiable y reproducible. Asimismo, este trabajo permitió identificar 6 fuentes de resistencia y describir dos genes de resistencia, *Pm1* y *Pm*, en el genotipo Cornell 49242. El gen *Pm1*, localizado en el GL Pv11, protege totalmente frente al ataque de este patógeno y sería un candidato a ser introgresado en el tipo faba granja asturiana, muy susceptible frente a este patógeno. Su localización en el mapa genético pone a disposición un abanico de marcadores moleculares para la selección asistida.

Los genes de resistencia a las raza 6 y 38 de antracnosis, localizados en la posición del *cluster* Co-2, fueron introgresados a partir de la variedad SanilacBc6Are (derivada por retrocruzamientos de Cornell 49242) en la variedad de faba granja Andecha (Ferreira et al 2012) para obtener las líneas A1878 y A2826. Ambas líneas presentaron una respuesta TI3 a oídio. Este resultado es coherente con la respuesta de SanilacBc6Are frente al aislamiento de oídio (TI3) y sugiere que en esta posición también hay un locus que condiciona este tipo de respuesta, además del gen *Pm1* derivado de Cornell 49242. Probablemente un evento de recombinación entre los genes de resistencia Co- y el gen *Pm1* ha dado lugar a la pérdida de esta resistencia. Considerando estos resultados, se está desarrollando un programa de retrocruzamientos para introgresar la resistencia a oídio en el tipo faba granja asturiana, usando como fuente de resistencia Porrillo Sintético y como parental recurrente la variedad de faba granja asturiana X2776 portadora de genes de resistencia antracnosis en el *cluster* Co-2 (Trabanco et al. 2013).

En lo que respecta a la resistencia a la grasa de la judía, los resultados no permitieron identificar genes mayores que aporten una alta protección frente a este patógeno, aunque esto no fue sorprendente, dados los niveles moderados de resistencia observados en Cornell 49242. Siempre es deseable disponer de genes mayores ya que son más fácilmente manejables en los programas de mejora genética que los QTL. No obstante, los resultados

pusieron de manifiesto que la respuesta a grasa también puede tener una expresión cuantitativa que no debe ser despreciada en los programas de mejora genética.

Finalmente, este trabajo describe una nueva región genómica implicada en el control genético de la respuesta frente, al menos, cuatro razas de antracnosis en judía común. No podemos confirmar si esta región genómica procede de la variedad diferencial G2333, dado que la línea SEL1308 deriva del cruzamiento Talamanca*2/G2333 y desconocemos la respuesta del parental Talamanca a las razas 3 y 7. En todo caso, esta región puede ser utilizada para el desarrollo de nuevas variedades resistentes o el agrupamiento de genes de resistencia en un determinado genotipo de interés que reúna un amplio espectro de resistencia frente a este patógeno. No obstante, en la mejora frente a antracnosis se debe tener en cuenta la alta especificidad en la interacción hongo-planta (Ferreira et al. 2013), de modo que en el caso de razas diferentes a las usadas en este trabajo, debería analizarse específicamente el control genético y las regiones genómicas implicadas en la resistencia a esas razas.

7. Desafíos en el futuro

Esta Tesis ha permitido identificar seis fuentes de resistencia a oídio, aunque sólo se ha caracterizado en detalle la resistencia en una de ellas, Cornell 49242. Por tanto, resulta recomendable abordar la caracterización de la resistencia a este patógeno en las otras cinco variedades (Porrillo Sintético, Belneb, Amanda, Negro San Luis y BGE03161) de modo que podamos conocer si existen genes de resistencia diferentes a los descritos en Cornell 49242. La caracterización de estas fuentes de resistencia permitirá que también puedan ser utilizadas por los mejoradores.

Por otro lado, uno de los retos actuales en el estudio de la resistencia a patógenos, así como de otros caracteres de interés, es la validación de genes candidatos para confirmar su implicación en el control de un determinado carácter. Es decir, “traducir” los datos genéticos en datos genómicos. El hecho de que el genoma de judía haya sido secuenciado y esté disponible para los investigadores ha facilitado enormemente la obtención de marcadores moleculares “a la carta” para saturar determinadas regiones cromosómicas de interés y aproximase a los genes candidatos. Una de las nuevas alternativas para acotar eficazmente regiones de interés y/o mapeo fino es el genotipado masivo por secuenciación o 'Genotyping by Sequencing' (Elshire et al. 2011). Esencialmente esta técnica combina la digestión del ADN, el acoplamiento de unos adaptadores, la amplificación de los fragmentos mediante PCR y la secuenciación de fragmentos de entre 50 y 150 pb. La variación en la secuencia de estos

fragmentos (Single Nucleotide Polymorphism o SNPs) puede ser detectada y los fragmentos pueden ser posicionados frente a un genoma de referencia (Elshire et al. 2011). Este genotipado ha sido usado en la construcción de mapas genéticos, análisis de paneles de asociación, o en trabajos de selección genómica (Poland y Rife 2012).

En este trabajo de aproximación al gen de interés, la situación deseable es encontrar un ligamiento absoluto entre un gen predicho, etiquetado a través de un marcador, y la expresión de un carácter. Sin embargo, esto no siempre es posible o la población de mapeo no es lo suficientemente amplia como para confiar en el ligamiento estimado. No obstante, el disponer de una región acotada que incluya el gen de interés permite identificar genes candidatos anotados en esa área. Una vez identificados los genes candidatos, el último paso sería validar su función; es decir, verificar su implicación en el control del carácter de interés. Para ello se pueden llevar a cabo diferentes estrategias. En ciertos casos se han usado ensayos de PCR cuantitativa o de actividad enzimática, que muestran la expresión o actividad diferencial de genes concretos en respuesta a diferentes condiciones como puede ser, por ejemplo, entre plantas control y plantas inoculadas con un determinado patógeno. Una prueba alternativa o complementaria a este tipo de ensayos, es la validación mediante silenciamiento génico, ya que en algunos casos, los ensayos de expresión o de actividad enzimática pueden no ser totalmente confirmatorios (Pflieger et al. 2001). En especies cuya capacidad para ser genéticamente transformadas es muy baja o no se pueden obtener genotipos mutantes del gen de interés, como es el caso de *Phaseolus vulgaris*, existen otras alternativas como el silenciamiento génico mediado por virus [virus-induced gene silencing (VIGS); Díaz-Camino et al. 2011; Pflieger et al. 2013]. En este caso, un virus que es capaz de infectar a la planta, produce un silenciamiento post-transcripcional, degradando una parte concreta del ARNm a través del ARN viral, de tipo bicatenario (dsARN). Si en el vector viral se añade una copia del gen que se pretende silenciar, lograremos que el mecanismo del virus suprima la expresión del gen en la planta. En el caso de leguminosas han sido varios los virus utilizados para este tipo de ensayos. En concreto en judía común el virus del moteado del frijol (Bean Pod Mottle Virus o BPMV) ha demostrado ser efectivo para silenciamiento génico (Zhang et al. 2010; Díaz-Camino et al. 2011). Existen varias formas de insertar el vector en la planta. Una de las formas más sencillas es inocular directamente sobre las plantas el virus con el vector. En caso de que nuestro genotipo de interés no sea sensible al virus, pueden utilizarse otros mecanismos, como la transformación transitoria mediante la bacteria *Agrobacterium tumefaciens*, a la que previamente se ha insertado el vector (Pflieger et al. 2013).

Una vez relacionada una secuencia de nucleótidos con la expresión de un carácter, el camino está abierto para iniciar el desarrollo de marcadores funcionales que ayuden en la mejora genética; para profundizar en los mecanismos que llevan a la expresión del carácter; o usar estas secuencias en el desarrollo de nuevos cultivares mediante transgénesis o cisgénesis (Schouten et al. 2005; Jacobsen y Schouten 2009). Las posibilidades son muchas pero en el inicio están un buen fenotipado del material vegetal y la genética clásica o el conocimiento del control genético de la expresión de los caracteres fenotípicos.

Conclusiones

Conclusiones

1. Dentro de la especie *Phaseolus vulgaris* L. existe variación en la respuesta a la interacción con el hongo responsable de los síntomas de oído. Se han identificado seis fuentes de resistencia en las que no se produce desarrollo del patógeno.

2. La respuesta a la interacción judía-oídio siguió un modelo cualitativo, pudiendo clasificarse las respuestas observadas en cinco clases fenotípicas.

3. La resistencia genética frente a oídio en judía es de naturaleza cualitativa. Se han identificado genes de resistencia con un modo de acción dominante y, en cruzamientos particulares, con un modo de acción complementario.

4. El genotipo Cornell 49242, que presenta resistencia total al patógeno, dispone de dos genes de resistencia frente a oídio: *Pm1,* que otorga una protección total frente al patógeno; y *Pm2,* que produce una resistencia parcial, permitiendo un limitado crecimiento del patógeno. Entre ambos genes se ha encontrado una relación de epistasia dominante (*Pm1>Pm2).*

5. La localización de ambos genes en el mapa genético permitió identificar marcadores moleculares ligados, que pueden ser utilizados en selección asistida por marcadores en futuros programas de mejora.

6. La resistencia genética a oídio está siendo introgresada en la clase comercial fabada mediante un programa de retrocruzamientos, utilizando como parental donante el genotipo resistente Porrillo Sintético identificado en este trabajo, y como parental recurrente la línea de faba X2776 que dispone de resistencia a antracnosis. El avance de este programa confirma la naturaleza cualitativa de la resistencia.

7. La resistencia genética a grasa en el genotipo Cornell 49242 tiene una naturaleza cuantitativa.

8. Los resultados obtenidos han permitido identificar QTLs con efecto significativo en la respuesta al patógeno en el GL Pv06. La resistencia que ofrecen estos QTLs es moderada y sólo se han confirmado frente a un aislamiento clasificado como raza 7.

9. El genotipo SEL1308 dispone, además del *cluster* de Co-4, de genes de resistencia para las razas 3, 6, 7 y 38 de antracnosis, localizados en el extremo del grupo de ligamiento Pv03, región en la que no se habían localizado genes de resistencia a antracnosis previamente.

10. La disponibilidad de la secuencia de un genoma anotado automáticamente de *P. vulgaris* desde principios de 2012 ha permitido avanzar hacia la identificación de las regiones genómicas implicadas en el control de la resistencia a grasa, en el GL Pv06, y a antracnosis, en el GL Pv03. Se han identificado varios genes candidatos para la respuesta a la raza 7 de grasa, en Cornell 49242 y para la respuesta a las razas 3 y 7 de antracnosis, en SEL1308. Su implicación debería ser confirmada en posteriores trabajos.

11. Los mapas genéticos y, en general, los análisis de ligamiento genético, son una herramienta muy útil para identificar la región genómica en la que se localizan los genes que controlan un determinado carácter. No obstante, el establecer la relación entre el mapa físico y el mapa genético puede resultar complejo, dada la cantidad de variables que afectan a la obtención de ambos tipos de mapas.

Conclusions

1. In this study, variation in the response of common bean species (*Phaseolus vulgaris* L.) to the interaction with a fungal species producing powdery mildew has been found. Six resistance sources have been identified, in which no symptoms in response to the pathogen were observed.

2. Response to common bean-powdery mildew interaction was qualitative and it was able to be classified into five different phenotypic classes.

3. Genetic resistance to powdery mildew showed a qualitative mode of inheritance. Both dominant and complementary modes of action of genes have been identified within different crosses evaluated.

4. Genotype Cornell 49242 shows complete resistance to the pathogen, controlled by two dominant and independent genes: *Pm1*, conferring total resistance with no development of the fungus; and *Pm2*, conferring moderate resistance, with moderate growth of the pathogen on the leaves. Both genes show an epistatic relationship in which *Pm1*>*Pm2*.

5. Localization of these genes in the genetic map allowed to identify several molecular markers linked to both genes, which can be used in marker-assisted selection in future breeding programs.

6. Genetic resistance to powdery mildew is being introgessed in fabada market class by backcrossing, using Porrillo Sintético as the donor parent, and fabada line X2776 as the recurrent parent. Progress in this breeding program confirms the qualitative nature of resistance to powdery mildew in common bean.

7. Genetic resistance to halo blight in genotype Cornell 49242 shows a quantitative mode of inheritance.

8. Results obtained allowed the identification of significant QTLs with a significant effect in the response to the pathogen. These QTLs confer moderate resistance and their action has only been confirmed against isolate classified as race 7.

9. In addition to Co-4 cluster, resistance genes to anthracnose races 3, 6, 7 and 38 have been identified in line SEL1308 and localized at the end of linkage group (LG) Pv03, a region in which no anthracnose resistance genes have been previously described.

10. The availability of an automatically annotated genome sequence of *P. vulgaris* since early 2012 has allowed to make progress towards the identification of genomic regions involved in the control of resistance to halo blight, on LG PV06; and to anthracnose, on LG PV03. We have identified several candidate genes for response to *Psp* race 7 in genotype Cornell 49242; and for response to anthracnose races 3 and 7, in line SEL1308. Further studies should confirm their involvement on the control of resistance to these pathogens.

11. Genetic maps and genetic linkage analyses are a useful tool for the identification of the genomic regions involved in the genetic control of a particular trait of interest. However, the relationship between genetic and physical maps is still difficult to establish due to the number of variables affecting the development of both types of maps.

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