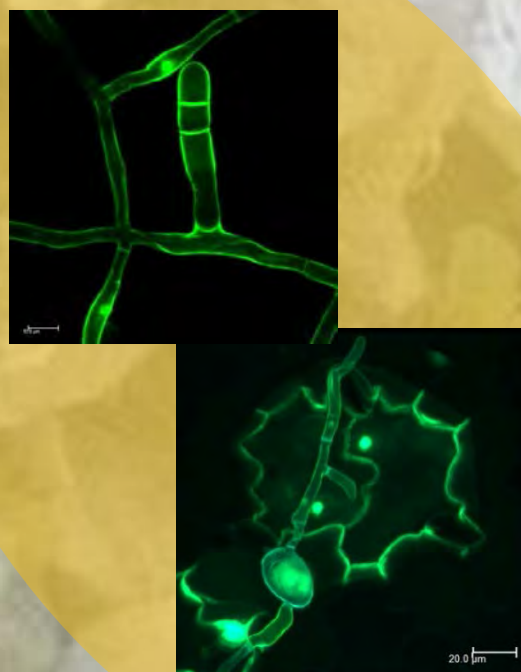
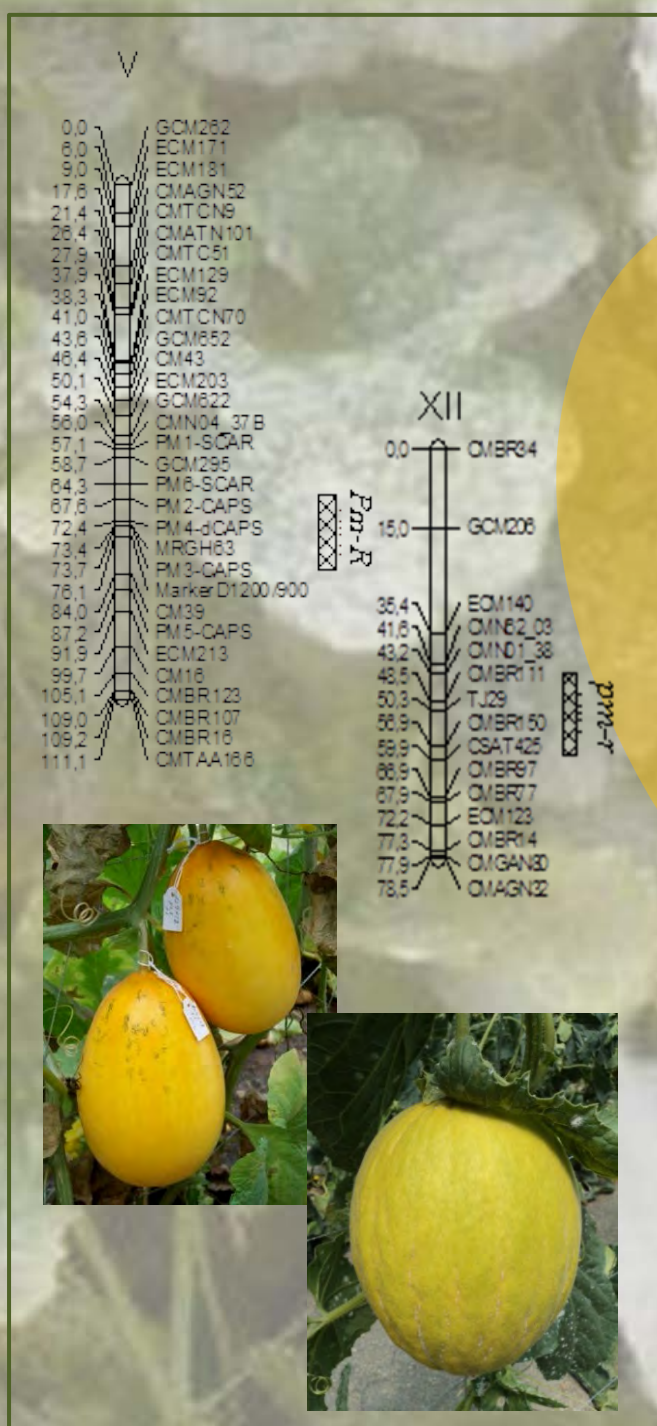


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Memoria presentada por la Licenciada en Ciencias Ambientales Paola Beraldo
Hoischen para optar al grado de doctor por la Universidad de Málaga



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Certifica:

Que la memoria titulada: 'Interacción planta-patógeno e identificación de QTL asociados a la resistencia a *Podosphaera xanthii* y a caracteres agronómicos en una población RIL ('TGR-1551' x 'Bola de Oro') de *Cucumis melo*', presentada por la Licenciada en Ciencias Ambientales Paola Felicitas Beraldo Hoischen para optar al grado de Doctor, ha sido realizada bajo su dirección.

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Ratifica:

La presentación de la citada Tesis por el Departamento de Biología Molecular y Bioquímica de esta Universidad.

Y para que conste a los efectos oportunos, firma el presente en Málaga, a 2 de Marzo de 2015.

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RESUMEN

Resumen

El melón (*Cucumis melo* L.) es una de las especies hortícolas más importantes de nuestro país, cuya producción se ve limitada por la presencia de patógenos y plagas. El oídio, causado por *Podosphaera xanthii*, es una de las enfermedades de naturaleza fúngica que afecta más frecuentemente al cultivo del melón, tanto al aire libre como en invernadero, reduciendo el rendimiento y la calidad de los frutos. La selección de material vegetal resistente, la identificación de los genes o QTL implicados en esta resistencia y la estimación de la colocación con otros QTL vinculados a caracteres de interés agronómico, así como la disponibilidad de un mayor conocimiento de los mecanismos implicados en la interacción planta-patógeno, permite abordar programas de mejora genética encaminados a la obtención de variedades comerciales de melón resistentes a esta enfermedad.

La disponibilidad de una población de líneas recombinantes (RIL) procedentes del cruce entre la entrada multirresistente 'TGR-1551' y el cultivar español 'Bola de Oro' ha permitido estimar la localización de QTL asociados a la resistencia a oídio y a un gran número caracteres morfológicos, así como conocer la respuesta a nivel tisular de varios genotipos de melón al desarrollo del hongo. Para la localización de QTL de resistencia a oídio y de caracteres morfológicos, se utilizó un mapa genético obtenido a partir del genotipado de 114 líneas de la población RIL obtenida a partir del cruce entre 'TGR-1551' y 'Bola de Oro'. Para su construcción se emplearon un total de 216 loci, la mayoría codominantes (138 SSR; 68 EST-SSR; 4 SCAR, 4 CAPS, 1 dCAPS, y el carácter fenotípico de andromonoecia, *a*) que se distribuyeron en 22 grupos de ligamiento, reagrupados en 12, cubriendo una distancia de 992.82 cM, y con una distancia media entre marcadores de 4.6 cM.

En trabajos previos se había descrito que el control genético de las razas 1, 2 y 5 de *Podosphaera xanthii* en el genotipo 'TGR-1551' está conferido por una doble epistasis dominante-recesiva, y, mediante el fenotipado y genotipado de una población F₂, de la que deriva la población RIL disponible actualmente, se localizó un QTL en el grupo de ligamiento V, denominado *Pm-R*, asociado al gen dominante. En este trabajo, mediante tres evaluaciones biológicas (verano y otoño 2010 y primavera 2011) de la población RIL frente a las razas 1, 2 y 5 del *P. xanthii*, y su posterior evaluación molecular, se han identificado QTL en dos regiones genómicas o grupos de ligamiento (GL), para cada una de las evaluaciones realizadas. En el

grupo de ligamiento V se detectó el QTL *Pm-R*, descrito anteriormente, con un porcentaje de la variación fenotípica para la resistencia a las tres razas de oídio testadas (1, 2 y 5) de 51.8-56.6, 16.4-22.4 y 30.8-35.8, en verano 2010, otoño 2010 y primavera 2011 respectivamente. En el GL XII se identificó otro QTL (*pm-r*), posiblemente correspondiente al gen recesivo, con un porcentaje de explicación de la variación fenotípica para las tres razas de 8.1-10.6, 29.8-37.3 y 12.3-13.9% en cada una de las evaluaciones realizadas. Entre estos dos QTL implicados en la resistencia a oídio, *Pm-R* (LG V) y *pm-r* (LG XII), se han detectado interacciones epistáticas observándose un porcentaje de variancia fenotípica explicada por efectos epistáticos aditivos de ambos QTL de entre un 7.8 a un 10.3 %. La expresión del QTL *Pm-R* podría estar influenciada de manera significativa por las condiciones ambientales. Los marcadores moleculares más estrechamente ligados a estos QTL fueron PM3-CAPS, MarkerD y MRGH63 para *Pm-R*, y los microsatélites CMBR111, CMBR150 y TJ29 para *pm-r*. El test de Kruskal-Wallis apoyó los resultados obtenidos con los análisis mediante Interval mapping (IM) y Multiple QTL model (MQM), y también señaló como significativos algunos marcadores del GL II para las tres razas de oídio en los tres ambientes evaluados.

Además de la evaluación de la respuesta a oídio de la población RIL 'TGR-1551' x 'Bola de Oro', esta población se ha caracterizado mediante la evaluación morfológica de más de treinta caracteres agronómicos durante dos años consecutivos (primavera-verano de 2009 y 2010). El posterior análisis de QTL de aquellos caracteres que resultaron más relevantes ha permitido conocer las regiones implicadas en la regulación genética de estos caracteres, y la posible colocalización de los QTL detectados con los de resistencia a oídio antes descritos.

Se detectaron un total de 56 QTL para las dos evaluaciones, la mitad de los cuales fueron consistentes, ya que se localizaron ambos años, bien exactamente en la misma posición dentro del GL (16 QTL) o muy próximos unos de otros (12 QTL). Se observaron correlaciones positivas significativas para los dos años entre aquellos caracteres relacionados con el tamaño de fruto, como son el peso, el diámetro máximo, la longitud, el diámetro de la cavidad central, el grosor de la pulpa. Así mismo se observaron correlaciones positivas entre caracteres relacionados con las formas del ovario y del fruto. Se detectaron correlaciones negativas tanto entre la firmeza de la carne y el contenido de sólidos solubles, como entre los caracteres vinculados al tamaño y el número de frutos por planta. Los grupos de ligamiento con mayor

número QTL fueron el II y el IV, donde la mayoría de ellos estaban relacionados con el tamaño tanto del fruto como del ovario. Veintiún QTL fueron identificados en el GL II, de los cuales doce (seis por evaluación) fueron consistentes entre evaluaciones. En el GL IV se detectaron once QTL. Los QTL de caracteres morfológicos que colocalizaron con los QTL de resistencia a oídio, presentaron efectos aditivos procedentes de alelos de ambos parentales pero en ningún caso afectaron negativamente a la calidad del fruto, por lo que la introgresión de los QTL de resistencia a oídio en líneas comerciales no supondría en principio una merma en la calidad de los frutos resultantes.

Otro de los objetivos perseguidos en este trabajo ha sido profundizar en la interacción entre las razas 1, 2 y 5 de *Podosphaera xanthii* y distintos genotipos de melón, y observar la influencia de factores como la temperatura en esta interacción. Para ello se examinaron varios parámetros relacionados con el crecimiento del hongo y con la respuesta del tejido vegetal de las plantas afectadas. Concretamente se evaluaron en distintos tiempos post-inoculación el número de conidióforos maduros y el número de hifas desarrolladas por el hongo, así como el número de puntos de penetración en las células, el número de células epidérmicas que presentaban deposición de calosa en sus paredes, y el ratio entre ambas variables. En las reacciones incompatibles observadas se pudieron detectar cuatro tipos de comportamientos post haustoriales. Dos de ellos fueron similares a los de tipo I y II previamente caracterizados por Kusuya et al. (2006), y otros dos han sido descritos en este estudio. Uno de ellos, más rápido que el de tipo II, denominado aquí como tipo IIa, y otro más lento, tipo IIb. Este último presenta un fenotipo intermedio entre las interacciones incompatibles con respuesta tipo II y las compatibles. La respuesta tipo I fue observada en 'PMR45' y 'WMR29' inoculadas con la raza 1 a baja temperatura (18-22°C) y 'WMR29' y 'TGR-1551' inoculadas con la raza 2 a alta temperatura (25-30°C). La respuesta tipo II se observó en 'PI 414723' inoculada con las razas 2 y 5, y en las líneas recombinantes ('TGR-1551' x 'Bola de Oro') RIL88 y RIL276, resistentes a oídio posiblemente por portar el gen recesivo de resistencia, inoculadas con las tres razas. La respuesta tipo IIa fue observada en la línea cuasi-isogénica NIL21, resistente a oídio por portar los alelos del gen dominante procedente de 'TGR-1551', para las tres razas, así como en 'WMR29' con la raza 2 a baja temperatura. La interacción de tipo IIb fue detectada en 'TGR-1551' inoculada con las tres razas de oídio a baja temperatura. De acuerdo a estos resultados, la

resistencia de 'TGR-1551' a *P. xanthii* parece estar influenciada en gran medida por la temperatura, pudiéndose éste ser el motivo de la variabilidad observada frente a oídio en algunas líneas RIL de la población examinada.

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Capítulo 1

1 Introducción general

1.1 El melón

1.1.1 Taxonomía

El melón fue descrito por primera vez por Linneo en 1753 en su obra *Species Plantarum*. Es una especie angiosperma dicotiledónea que pertenece a la división Magnoliophyta, clase Dicotyledoneae, subclase Dilleniidae, superorden Violanae, orden Cucurbitales, familia Cucurbitaceae, subfamilia Cucurbitaceae, tribu Melothrieae, subtribu Cucumerinae, género *Cucumis*, subgénero *Melo*, especie *Cucumis melo* (Kirkbride, 1993).

La familia Curcubitaceae está constituida por aproximadamente 118 géneros y 825 especies (Esquinas-Alcázar and Gulick, 1983; Bates et al., 1990) distribuidas en las zonas tropicales y subtropicales de ambos hemisferios del planeta. El género *Cucumis*, que comprende cerca de 30 especies, está dividido en dos subgéneros: el subgénero *Cucumis*, a la que pertenece el pepino (*C. sativus* L), con un número básico de cromosomas $n=7$ y la especie silvestre *C. hystrix* Chakravarty con $n=12$ (Akashi et al., 2002), y el subgénero *Melo*, con $n=12$, que incluye al melón (*C. melo* L.), el kiwano (*C. metuliferus* Mey. Ex Naud), el pepinillo de Indias (*C. anguria* L.) y una treintena de especies silvestres africanas de gran interés para los mejoradores por presentar resistencia o tolerancia a un gran número de plagas y enfermedades.

1.1.2 Origen y clasificación intraespecífica

La localización exacta del origen del melón (*Cucumis melo* L.) es aún un tema de debate. Los taxonomistas del siglo XIX lo situaron en Asia (Naudin, 1859), sin embargo esta hipótesis fue desbancada al encontrar en África gran cantidad de morfotipos de la subespecie *agrestis* viviendo de forma silvestre cerca de regiones de cultivo (Rubatzky and Yamaguchi, 1997; Whitaker and Bemis, 1976). Actualmente, y en base a estudios filogenéticos, se concluye que el antecesor común más reciente de melón y pepino procede de Asia, teniendo ambas especies poblaciones progenitoras en la región del Himalaya y existiendo actualmente una alta diversidad genética de variedades de *C. melo* en India y China (Sebastian et al., 2010).

Los primeros indicios de la domesticación del melón se remontan a miles de años a.C. (Robinson and Decker-Walters, 1997). El centro de diversidad primario, gracias a las

introgresiones desde el continente africano, se sitúa en el Cercano Oriente y Asia central (Jeffrey, 1980). Desde ahí, el melón llegó a los centros de diversificación secundarios (Turquía, China, Afganistán) y en la época Greco-Romana, a Europa (centro de diversificación terciario) (Andrews, 1956; Jeffrey 2001; Szabo et al., 2005) desde donde finalmente, tras la colonización, alcanzó el continente americano (Ware and McCollum, 1980).

La primera clasificación intraespecífica del melón fue la de Naudin (1859), en la que se establecieron diez variedades botánicas: *acidulus*, *agrestis*, *cantalupensis*, *chito*, *conomon*, *dudaim*, *flexuosus*, *inodorus*, *reticulatus* y *saccharinus*. Posteriormente esta clasificación fue revisada y reestructurada por diversos autores (Whitaker and Davis, 1962; Munger and Robinson, 1991; Robinson and Decker-Walters, 1997), obteniéndose una nueva clasificación basada en un total de siete grupos varietales. En esta nueva clasificación *chito* y *dudaim* recibieron el nombre de *dudaim*, y *cantalupensis* y *reticulatus* el de *cantalupensis*. A su vez se eliminó *saccharinus* y se introdujo el grupo *momordica* donde quedaban recogidas aquellas variedades botánicas que no podían ser englobadas en otro grupo. Más recientemente Pitrat et al. (2000) definió 16 grupos y los dividió en dos subgrupos según la pilosidad del ovario, como ya habían propuesto anteriormente Grebenscikov (1953), Jeffrey (1980) y Zohary (1983). Así pues, con ovarios pilosos o lanados se distinguió a la subespecie *melo*, en la se encuentran recogidos un total de 11 grupos: *cantalupensis*, *reticulatus*, *adana*, *ameri*, *chandalak*, *inodorus*, *flexuosus*, *dudaim*, *chito*, *chate*, *tibish*, y con ovarios de pelos cortos a la subespecie *agrestis*, que está representada por cinco grupos: *conomon*, *makuwa*, *chinensis*, *acidulus* y *momordica*. Actualmente diversos estudios basados en el uso de marcadores moleculares confirmaron esta clasificación intraespecífica de *C. melo* en las subespecies *melo* y *agrestis* (Stepansky et al., 1999; Staub et al., 2000; Monforte et al., 2003; Nakata et al., 2005).

1.1.3 Descripción morfológica

El melón es una planta herbácea con crecimiento natural rastrero, con posibilidad de desarrollo en vertical gracias a unas estructuras denominadas zarcillos, y que presenta una gran variedad en cada uno de sus caracteres descriptores. Sus raíces pueden variar desde los 30-40cm hasta alcanzar el metro veinte de profundidad (Zapata et al., 1989) y el tallo principal puede sobrepasar los tres metros de longitud. Presenta ramificaciones primarias, secundarias y terciarias, creciendo, tanto las secundarias como las terciarias, a partir de las axilas de las hojas

(Maroto, 1986). Las hojas son enteras o palmatilobuladas (hasta siete lóbulos). Tanto los tallos, los peciolos, como el haz y el envés de las hojas, suelen presentar pubescencia que varía en densidad y firmeza.

Las plantas de melón pueden ser monoicas (flores femeninas y masculinas, separadas en el mismo tallo), o polígamas. Las polígamas pueden ser de dos tipos: andromonoicas (masculinas y flores hermafroditas) y ginomonoicas (solamente femeninas y hermafroditas) (Zapata et al., 1989). La mayoría de las variedades son andromonoicas. Sus flores presentan un cáliz campanulado o turbinado con cinco lóbulos filiformes y su corola, que puede variar en diámetro entre las distintas variedades, es amarilla con cinco pétalos. Las flores masculinas poseen tres estambres y pueden presentarse tanto de forma solitaria como en racimos de 3 a 5 flores en las axilas de las hojas. Las flores femeninas y hermafroditas aparecen más tarde que las masculinas, generalmente solitarias y en las ramificaciones secundarias o terciarias. Están dotadas de un pedúnculo generalmente corto y grueso. El ovario es ínfero, y está constituido por 3 a 5 carpelos (Zapata et al., 1989) y puede variar en tamaño y en pubescencia. La fecundación del melón es principalmente entomófila.

El fruto recibe la denominación botánica de pepónide y es, sin duda, de todos los caracteres evaluables en la planta del melón, el que presenta una mayor variabilidad. Su peso, puede oscilar, según la clasificación del IPGRI, de menos de cien gramos hasta más de tres mil; su forma, desde esférica hasta fusiforme; el color de la corteza puede ser negro, verde, amarillo, naranja, gris, entre otros, y presentar o no manchas, las cuales pueden ser variables en color y distribución. La piel del fruto puede ser rugosa o lisa, con presencia o no de escriturado, reticulado, y acostillado, y su carne, de distinto grosor, firmeza, textura, contenido en sólidos solubles y color (blanquecina, verdosa, anaranjada). Su cavidad central, de tamaño también variable, puede ser seca, gelatinosa o acuosa en función de su consistencia, y las semillas, que se encuentran insertas sobre el tejido placentario, son fusiformes, aplastadas y de color blanco o amarillento. Otros caracteres donde también se observa alta variabilidad son en el número de melones por planta, la aparición de la primera flor femenina, el porcentaje de flores masculinas y femeninas o hermafroditas, entre otros.

1.1.4 Cultivo y producción

El melón debe cultivarse en climas cálidos y no excesivamente húmedos. La temperatura óptima de germinación se encuentra entre los 28-32°C, la floración ente 20-23°C, y necesita un mínimo de 25°C durante todo el desarrollo del fruto para una adecuada maduración. La planta de melón requiere bastante agua durante la germinación de la semilla y en la maduración con el fin de obtener un fruto de calidad. Sus necesidades de agua y abono dependen del estado fenológico. El fósforo es necesario desde que comienzan a desarrollarse las raíces hasta la floración. Desde la floración hasta el cuajado se ha de controlar el aporte de nitrógeno para evitar el desarrollo vegetativo excesivo, y hasta esta última etapa, los riegos han de ser cortos y frecuentes. Sin embargo, desde el cuajado hasta el desarrollo completo del fruto, tanto la demanda de agua como la de nutrientes aumenta, por lo que se exige un riego más abundante. Finalmente, en la etapa de maduración, el aporte hídrico se reduce y se controlan tanto el potasio, que mejora la calidad del fruto, como el nitrógeno, evitando su exceso.

Actualmente el melón es una de las hortalizas más producida en el mundo después del tomate y la sandía. El máximo productor de melón mundial con diferencia es China. En el año 2011 este país registró una producción de 17.500.000 de toneladas (T) con una superficie cultivada de 600.000 hectáreas (ha) (FAOSTAT, 2012) (Tabla 1.1). España es el principal productor de la Unión Europea y el séptimo a nivel mundial, así como también el mayor exportador mundial. Su producción en 2012 fue de 870.900T con un área dedicada a su cultivo de 27.500ha (FAOSTAT, 2012), y su exportación en el 2011 de 373028T. La tendencia, a partir de los datos observados de los últimos años, es la disminución tanto de la producción como del área de cultivo (MAGRAMA, Anuario de estadística 2012). Las zonas productoras más importantes de España son Andalucía, Murcia y Castilla La Mancha, y las principales provincias exportadoras son Murcia y Almería. La producción española está mayoritariamente destinada al mercado europeo que prefiere los melones de menor calibre. Francia se decanta más por el tipo Charentais, Inglaterra por los tipos Galia y Amarillo, y Alemania por el tipo Cantalupo. El mercado nacional, por el contrario, prefiere variedades de tamaño medio, como Piel de Sapo.

Tabla 1.1. Producción (toneladas), área destinada al cultivo y rendimiento de los mayores productores mundiales de melón en el año 2012.

Región	Producción (T)	Rendimiento (Hg/ha)	Área (ha)
China	17500000	291666	604900
Turquía	1708415	167491	102000
Irán	1450000	176829	82000
Egipto	1007845	250595	40218
India	1000000	223719	44500
Estados Unidos	925060	291541	31730
España	870900	316690	27500
Marruecos	717602	342008	20982
Brasil	575386	252484	22789

1.1.5 Grupos varietales más producidos en España

En España las variedades más comercializadas son del tipo cantalupensis (Cantalupo y Galia) e inodorus (Piel de Sapo, Rochet, Tendral y Amarillo) (Fig. 1.1).

- **Cantalupo:** Las plantas son vigorosas y fuertes. Los frutos precoces (85-95 días), esféricos o ligeramente aplastados. Su peso está comprendido entre 700 y 1200 gramos. Son de piel lisa o reticulada con los meridianos bien definidos de color verde y con casco verde grisáceo. La pulpa, de color naranja, es dulce (11-15°Brix) y con un aroma característico.
- **Galia:** Las plantas son de hojas onduladas. Sus frutos esféricos, con un denso y fino reticulado, con un peso comprendido entre 850-1900 gramos. Su corteza es de color verde que vira a amarillo-anaranjado en la madurez. La pulpa es verdosa, poco consistente y muy dulce (14 a 16 °Brix).
- **Piel de sapo:** Las plantas son vigorosas con frutos elípticos ligeramente asurcados y de peso comprendido entre los 1.5 y 2.5 kg. Su corteza es de color verde con manchas en verde más oscuro, de ahí su nombre, pudiendo presentar escriturado en la madurez. Su pulpa es blanca-amarillenta, compacta, muy dulce (12-15°Brix) y con poco aroma. Su precocidad es media-baja (ciclo de unos 100 días). Se conservan bien (2-3 meses) y son muy aptos para el transporte.

- **Tendral:** Las plantas son vigorosas. Sus frutos son de forma ovalada, con un peso que oscila entre 2-3 kg, de corteza verde oscura, asurcada y rugosa. La pulpa es blanca, muy crujiente y no muy dulce. Gracias al grosor de su corteza presenta gran resistencia al transporte y se conservan excelentemente.
- **Rochet:** Las plantas son vigorosas. Sus frutos son ovalados, con piel lisa o de baja rugosidad. Su corteza es de color verde clara, pudiendo presentar escriturado en la madurez. Su pulpa es blanca, crujiente y muy dulce (14-17 °Brix).
- **Amarillo:** Las plantas son menos vigorosas que los tipos varietales anteriores. Su fruto presenta una corteza de color amarilla, uniforme, de forma redondeada y muy rugosa pero sin escriturado. La pulpa es blanca, de textura crujiente, firme, dulce (12-14°Brix) e inodora. Poseen ciclos tardíos de aproximadamente 120 días.



Figura 1.1. Tipos de melón más frecuentemente cultivados en España.

1.1.6 Enfermedades que afectan al cultivo del melón

El principal factor limitante en la producción del melón es, sin duda, el elevado número de plagas y enfermedades que afectan a este cultivo tanto en invernadero como al aire libre,

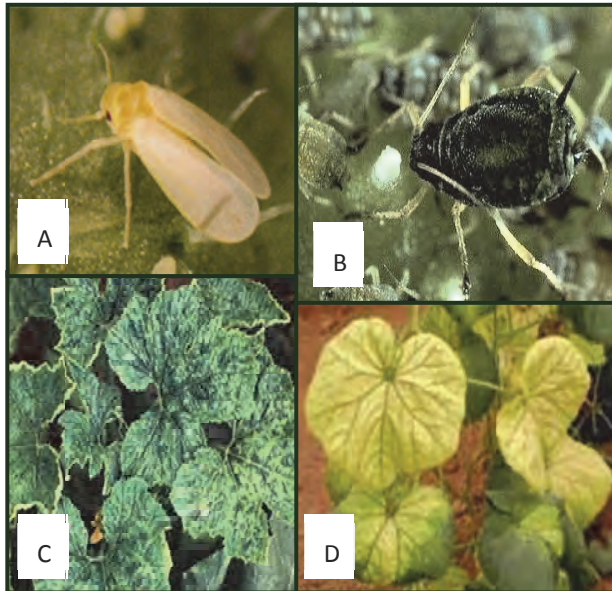


Figura 1.2. Enfermedades y plagas que afectan al melón: mosca blanca (A), pulgón algodonero (B), mildiu (C) y amarillo y enanismo de las cucurbitáceas (D).

ocasionando notables pérdidas (Díaz-Ruiz and García Jiménez, 1994; García-Jiménez, 1997; Jordá, 1997; López-Sesé, 1997; Díaz-Pendón, 2003; Pérez-García et al., 2009) (Tabla 1.2). Según el agente que las origina podemos citar las plagas ocasionadas por insectos como la mosca blanca, el pulgón algodonero (Fig. 1.2) y la araña roja, así como enfermedades ocasionadas por bacterias, por virus transmitidos a través de distintos vectores, como el mosaico del pepino, el amarilleo y enanismo o las manchas necróticas del

melón, enfermedades causadas por nematodos, y las ocasionadas por hongos como la fusariosis vascular, la muerte súbita, el mildiu y el oídio. Tanto los virus como los hongos son los responsables de las enfermedades más importantes detectadas en los cultivos bajo plástico de melón.

Tabla 1.2. Principales plagas y patógenos que afectan al cultivo del melón en España y las enfermedades que ocasionan.

Agente	Especie	Plaga o Enfermedad
<u>Insectos</u>	<i>Tetranychus spp.</i>	Araña roja
	<i>Liriomyza spp.</i>	Minador de hojas
	<i>Trialeurodes vaporariorum</i> ;	
	<i>Bemisia tabaci</i>	Mosca blanca
	<i>Aphis gossypii</i>	Pulgón algodonero
	<i>Frankliniella occidentalis</i>	Trips de las flores
<u>Bacterias</u>	<i>Pseudomonas syringae pv. Lachrymans</i>	Mancha angular
	<i>Xanthomonas campestris pv. Cucurbitae</i>	Mancha bacteriana
	<i>Erwinia caratovora subs. Caratovora</i>	Pobredumbre blanda
	<i>Erwinia tracheiphila</i>	Marchitamiento bacteriano
<u>Hongos</u>	<i>Acremonium cucurbitacearum</i>	Acremoniosis o muerte súbita
	<i>Monosporascus sp.</i>	Puntos negros de las raíces
	<i>Rhizoctonia solani</i>	Pobredumbre del cuello y raíz
	<i>Phytium spp.</i>	
	<i>Phytophthora spp.</i>	
	<i>Fusarium oxysporum f. sp. Melonis</i>	Fusariosis vascular
	<i>Didymela bryoniae</i>	Chancro gomosos del tallo
	<i>Sclerotinia sclerotiorum</i>	Pobredumbre blanca
	<i>Bptrytis cinerea</i>	Pobredumbre gris
	<i>Pseudoperonospora cubensis</i>	Mildeu
	<i>Phodosphaera xhantii</i>	Oídio
<i>Coletotrichum oligochaetum</i>	Antracnosis	
<u>Virus transmitidos por:</u>		
Áfidos	CMV	Mosaico del pepino
	WMV-2	Mosaico de la sandía
	ZYMV	Mosaico amarillo del calabacín
Aleuródidos	CYSDV	Amarilleo y enanismo de las cucurbitáceas
	CVYV	Virus de las venas amarillas del pepino
Coleópteros	SqMV	Mosaico de la calabaza
Hongos	MNSV	Manchas necróticas del melón
<u>Nemátodos</u>	<i>Meloidogyne sp.</i>	Agallas de las raíces

1.2 El oídio

1.2.1 Sintomatología

Oídio es el término que se emplea tanto para denominar a la enfermedad como al agente causal de la misma. La enfermedad, llamada vulgarmente ‘ceniza’, ‘cenicilla’ o ‘mal

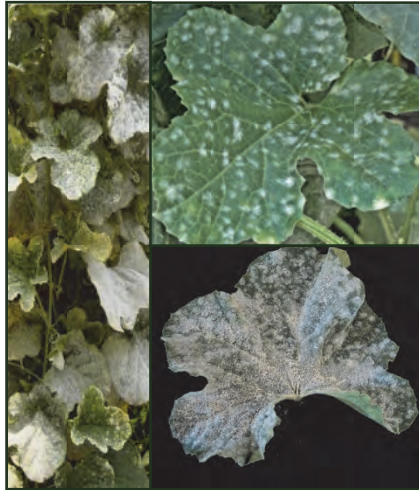


Figura 1.3. Hojas de melón atacadas por oídio.

blanco’, se reconoce por la presencia de manchas redondeadas, pequeñas y blanquecinas con aspecto pulverulento en el haz y en el envés de las hojas (Fig. 1.3). Si la enfermedad prolifera, el oídio puede extenderse a otros órganos de la planta, como a otras hojas, peciolo, tallos, pero raramente a frutos (Sitterly, 1978; Zitter et al., 1996).

El agente patógeno actúa reduciendo la capacidad fotosintética de las hojas dando lugar a tejido necrosado.

La senescencia de estos órganos aéreos deja en ocasiones desprotegidos a los frutos y da lugar a una maduración temprana e incompleta de los mismos. Este hecho afecta directamente a la producción, pudiendo verse reducido el número de melones por planta, afectando también la calidad de éstos (Zitter et al., 1996). El tamaño de los frutos, la firmeza y textura de la carne, así como la concentración de sólidos solubles, son algunos de los caracteres que pueden verse desfavorecidos.

1.2.2 Taxonomía

Los oídios pertenecen a la división Eumycota, subdivisión Ascomycotina, clase Leotiomycetes, orden Erysiphales, familia Erysiphaceae. La clasificación de los distintos géneros y especies se basó, en primer lugar, en la evaluación taxonómica del estadio teleomorfo (Salmon, 1900) y posteriormente, en la del anamorfo. Ferraris (1910) fue el primero que agrupó las especies según el tamaño y forma de las conidias, y Boesewinkel (1977, 1980) creó la primera clave basada en la observación de más de doce características detectables en dicho estadio (anamorfo). Más recientemente, Cook et al. (1997) examinó la superficie de las conidias mediante microscopio electrónico de transmisión (SEM) y separó el género *Oidium* en ocho subgéneros. Actualmente, gracias a nuevos datos de SEM y al estudio de las secuencias espaciadoras transcritas internas del ADN ribosómico (Takamatsu et al., 1998, 1999, 2000; Saenz

and Taylor, 1999; Mori et al., 2000), se ha podido reclasificar a las especies de la familia Erysiphaceae (Braun, 1999; Braun and Takamatsu, 2000).

1.2.3 Estructuras morfológicas

Conidios: Un conidio es una espora asexual inmóvil formada directamente a partir de una hifa o célula conidiógena o esporógena. Su medio de dispersión principal es el aire y cuando es depositado en una planta huésped compatible germina. Son hialinos, unicelulares, uninucleados, con abundantes vacuolas, de pared delgada y contienen en su interior gotas lipídicas y otras inclusiones (Braun, 1987) (Fig. 1.4). El tamaño de los conidios es de 20-60µm de largo y 10-24 µm de ancho y suelen contener o no cuerpos de fibrosina de 2-8µm (Braun, 1987) presentes cuando el conidio está vivo.

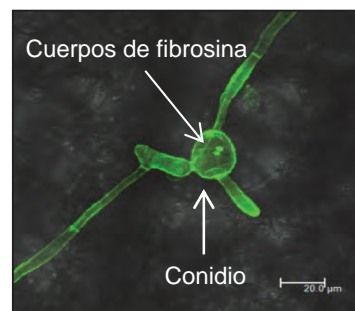


Figura 1.4. Imagen de conidio mostrando cuerpos de fibrosina característicos de *P. xanthii*.

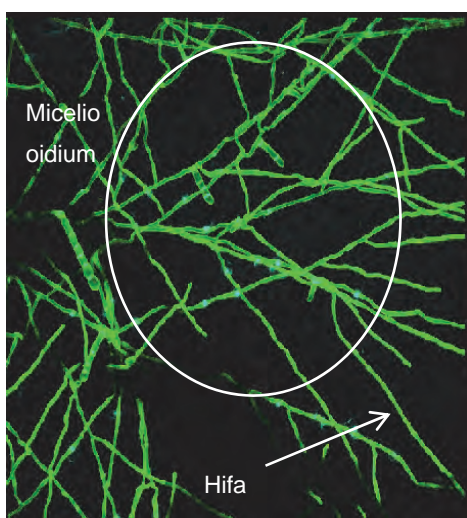


Figura 1.5. Micelio tipo Oidium de *P. xanthii*.

Hifa: Son elementos filamentosos cilíndricos característicos de la mayoría de los hongos que conforman su estructura vegetativa (Fig. 1.5). Están constituidos por una fila de células uninucleadas que miden entre 20 y 150µm de largo y de 2 a 10µm de ancho con numerosas vacuolas. Pueden ser finas y delgadas o anchas robustas como en *P. xanthii* y *G. cichoracearum*. En *P. xanthii* cambian de blancas a marrones pasada la madurez. Las hifas se ramifican en ángulos más o menos rectos y dan lugar al micelio.

Micelio: Se distinguen tres tipos de micelio: el Ovulariopsis (hemiendofítico), el Oídiopsis (endofítico), y el más común entre los oídios, el Oidium (epifítico) (Fig. 1.5). El micelio es hialino, septado y de pared delgada. Las hifas están formadas por células uninucleadas que miden entre 20 y 150µm de largo y de 2 a 10µm de ancho con numerosas vacuolas.

Apresorios: Son ensanchamientos de las hifas que permiten la sujeción del micelio a la planta huésped. El primer apresorio suele ser el ápice del tubo germinativo morfológicamente modificado y engrosado (Emmett and Parbery, 1975) (Fig. 1.6).

Haustorio: Son las únicas estructuras del micelio tipo oídium que penetran en las células epidérmicas del huésped y nutren al hongo (Fig. 1.6).

Conidióforo: Es una hifa fértil simple o ramificada que sostiene o está compuesta por células conidiógenas en las que se producen los conidios. Son hialinos, erectos, de tamaño variable. Están formados por una célula basal (pie) y una cadena de conidios. La conidiogénesis se inicia por la hinchazón y el alargamiento de la célula generatriz (situada inicialmente sobre la célula del pie), que se divide varias veces hasta formar un conidióforo. La célula subterminal retiene la capacidad generativa y la célula terminal dará lugar, cuando madure, al conidio. Según la forma de los conidióforos podemos distinguir dos tipos: el tipo Pseudooidium, con un único conidio terminal y el tipo Euoidium (Fig. 1.7), donde el conidio maduro tarda en desprenderse, lo que permite la formación de una cadena de conidios.

podemos distinguir dos tipos: el tipo Pseudooidium, con un único conidio terminal y el tipo Euoidium (Fig. 1.7), donde el conidio maduro tarda en desprenderse, lo que permite la formación de una cadena de conidios.

Cleistotecio: Es el cuerpo fructífero sexual de los hongos ascomicetos que produce ascas y



Figura 1.8. Imagen de cleistotecio de *P. xanthii* con cuatro ascosporas. http://szolo.blog.hu/2007/11/16/a_zold_reszek_megbetegedesei_lisztharmat.

ascosporas. Es de forma globosa, algo deprimidos en los extremos, y con una pared exterior membranosa y oscura que se rasga cuando las ascosporas llegan a su madurez. Las ascas, que contienen a las ascosporas, pueden oscilar de 1 a 8; son hialinas, ovaladas y pediceladas (Fig. 1.8). Estas estructuras permiten a los oídios sobrevivir en condiciones medioambientales adversas, sin embargo en regiones de clima cálido o subtropical, los oídios suelen sobrevivir en

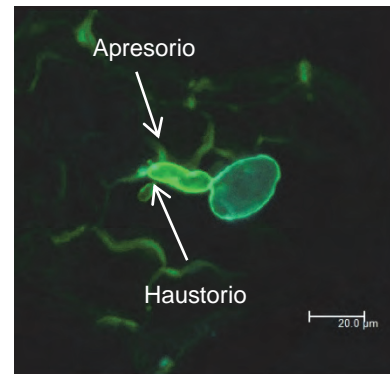


Figura 1.6. Conidio con apresorio y generación de haustorio de *P. xanthii*.

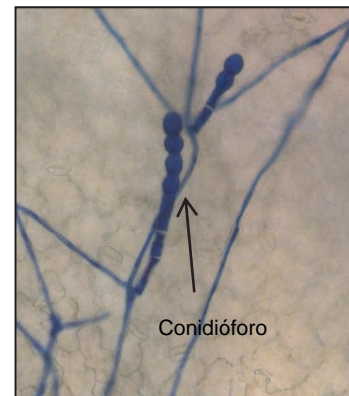


Figura 1.7. Conidióforo de tipo euoidium característico de *P. xanthii*.

forma de conidios (Braun, 1987). En algunas especies esta estructura se denomina casmotecio.

1.2.4 Oídio de las cucurbitáceas

El oídio en las cucurbitáceas está causado fundamentalmente por dos especies, *Podosphaera xanthii* (Castagne) U. Braun and Shishkoff (2000) y *Golovinomyces cichoracearum* (DC.) V. P. Gelyuta (Gc), que si bien inducen síntomas similares, son fácilmente distinguibles bajo el microscopio (Braun et al., 2002). Ambos dan lugar a un micelio superficial y persistente. *Podosphaera xanthii* presenta conidios de forma elíptica, con una relación longitud/anchura de aproximadamente 1.5-1.6, y con cuerpos de fibrosina con forma de bastón o cilindro en el interior. Los tubos germinativos crecen en el lateral de la espora y su germinación puede ser bifurcada. *G. cichoracearum* presenta conidios cilíndricos con razón longitud/anchura de 2, y sin cuerpos de fibrosina en el interior. El tubo germinativo crece desde la parte apical de la espora y no se bifurca. El cuerpo fructífero es una estructura que también puede ser un carácter morfológico discriminatorio de especie. En *P. xanthii* suele contener una sola asca con ocho ascosporas en su interior, mientras que *G. cichoracearum* presenta de 3 a 8 ascas. Esta estructura sexual sólo suele ser visible en climas templados, por lo que su utilidad como carácter diferenciador es limitado. Con respecto a los conidióforos, en ambas especies se desarrollan los conidióforos tipo euoidium.

1.2.5 Ciclo de vida de *P. xanthii*

El ciclo de vida del oídio *P. xanthii* consta de dos fases: una fase asexual y una sexual (Fig. 1.9). El ciclo asexual se inicia cuando una espora o conidio alcanza la superficie de un huésped susceptible. La espora comienza a germinar generando un tubo germinativo que es observable 6 a 8 horas después del primer contacto del patógeno con la planta. Este tubo germinativo termina en un apresorio indiferenciado a partir del cual se forma un haustorio. Tanto desde del tubo germinativo como desde la espora se forma una hifa primaria con su apresorio y haustorio correspondientes (24-48h). De las hifas primarias proliferan hifas secundarias y posteriormente terciarias (72-120h), desarrollándose así el micelio, de color blanquecino, que es el entramado de hifas altamente desarrolladas y ramificadas. A partir de las hifas secundarias se desarrollarán los conidióforos. Este micelio, junto a los conidióforos, constituye el síntoma visible característico de la enfermedad. El ciclo asexual finaliza

aproximadamente en unos diez días (Kuzuya et al., 2006), cuando el conidio está maduro, se desprende y puede llegar a un nuevo huésped. La fase sexual del ciclo vital del hongo suele tener lugar en tejidos altamente infectados en ramas laterales del micelio. Requiere el contacto de hifas de los dos tipos de compatibilidad sexual, y como resultado se genera un cuerpo fructífero redondeado llamado casmotecio (un tipo determinado de cleistotecio), que se caracteriza por contener una única asca con ocho ascoporas (esporas sexuales).

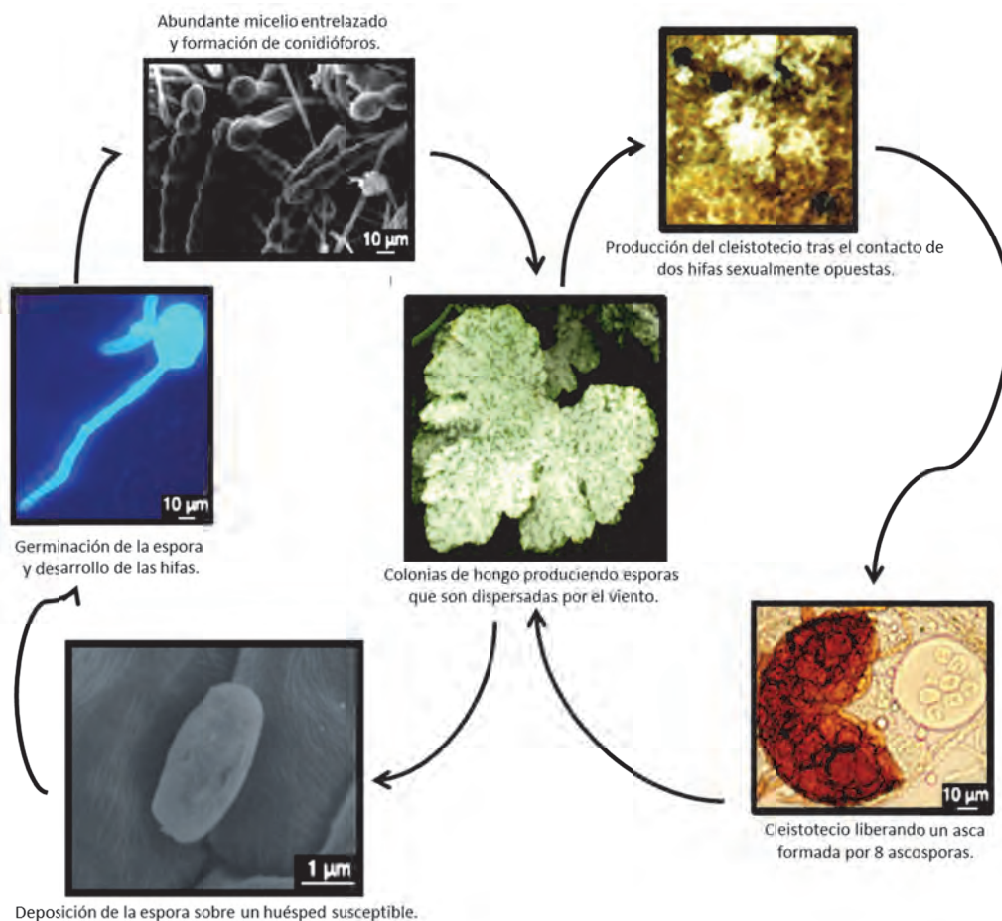


Figura 1.9. Ciclo de vida de *Phodosphaera xanthii* (Pérez-García et al., 2009)

1.2.6 Razas fisiológicas de *P. xanthii*

Phodosphaera xanthii presenta especialización parasitaria, tanto a nivel de especies de cucurbitáceas (patotipos) (Kaur and Jhoo, 1986; Bertrand, 1991; Bardin et al., 1997b), como a nivel de cultivares de una misma especie (razas fisiológicas) (Robinson and Provvidenti, 1975; Thomas, 1978; Sowell, 1982; McCreight et al., 1987; Bertrand, 1991). El patotipo de un aislado determinado del hongo se establece por su capacidad de crecer sobre diferentes especies de cucurbitáceas, mientras que la raza fisiológica se define en función de la capacidad del

aislado para atacar determinados cultivares de una misma especie, y depende de los genes de resistencia presentes en cada cultivar (Caten, 1987).

En melón, se han descrito numerosas razas de *P. xanthii* desde que Jagger et al. (1938) observaron que la línea de melón 'PMR 45', que se creía resistente a oídio, desarrollaba la enfermedad. El empleo de nuevos genotipos diferenciales ha permitido distinguir nuevas razas fisiológicas del hongo hasta llegar incluso a las 28 razas fisiológicas actualmente descritas (McCreight, 2006). El hecho de que la clasificación de las razas se base únicamente en la respuesta a la infección de genotipos diferenciales (indicadores de raza) refleja que el agrupamiento de aislados en razas es completamente dependiente de las plantas huéspedes utilizadas, por lo que la incorporación de un nuevo huésped diferencial suele dar lugar a una nueva raza. Este sistema de clasificación de las razas se establece y utiliza con fines prácticos (Cubero, 2004), ya que hasta el momento no se ha conseguido establecer una asociación entre marcadores moleculares, la virulencia de los aislados y la respuesta de la planta frente al patógeno (Bardin et al., 1997b; Montoro et al., 2004), como tampoco se conocen todos los genes de resistencia implicados en las respuestas en estos genotipos.

Hasta 1990, las únicas razas descritas en los cultivos de melón en España fueron las razas 1 y 2 (Torés et al., 1990), pero hace unos años se identificaron también las razas 4 y 5, en cultivos de las provincias de Málaga y Almería (Olalla, 2001; del Pino et al., 2002).

1.2.7 Métodos de control de la enfermedad

1.2.7.1 Control químico

Es el principal método de control que se utiliza para combatir esta enfermedad. En el caso de los hongos con micelio tipo oídium cuyas hifas tienen un desarrollo epifítico, el fungicida actúa al entrar en contacto con éstas. Una desventaja importante que plantean algunos fungicidas es la aparición de cepas resistentes en las poblaciones del patógeno (Ortuño et al., 2006). Sin embargo esto puede evitarse o reducirse la posibilidad de que ocurra limitando su aplicación, administrándolos en etapas tempranas de la enfermedad, o alternando la aplicación de fungicidas con distintos mecanismos de actuación (Staub, 1991; McGrath et al., 1996; McGrath, 2001).

Existen varios tipos de fungicidas: fungicidas de contacto como el azufre (inorgánico), el dinocap (dinitrofenoles); y fungicidas sistémicos como el prazofos (organofosforados), los QoIs

que inhiben la respiración mitocondrial, y aquellos que están basados en la inhibición de la ruta de biosíntesis del ergosterol (IBE). La actividad antifúngica de los compuestos se basa en la inhibición de la cadena respiratoria, actuando en el sitio de oxidación del ubiquinol (Qo) que se encuentra en el citocromo bc1. La inhibición de la respiración mitocondrial se logra mediante el bloqueo del transporte de electrones entre el citocromo b (CYTB) y citocromo c1 (CYTC1), que en consecuencia conduce a una interrupción del ciclo de energía suspendiendo la producción de ATP. En relación a los fungicidas IBE, el ergosterol es un componente lipídico de la membrana de los oídios, por lo que, cuando su cantidad se reduce, provoca la pérdida y la funcionalidad de las membranas, lo que conlleva a la detención del crecimiento micelial y del desarrollo vegetativo del hongo (Vila et al., 1996). Los IBE pueden diferenciarse en dos tipos según el mecanismo que inhiben. Entre ellos se encuentran los IBE que actúan inhibiendo la dimetilación del C-14, también llamados DMI, como son el imazail (imidazoles), el triadimefón (triazoles), el fenarimol (piridinas), entre otros (Ruíz et al., 2010), y las morfolinás. Así mismo están los IBE que actúan en un paso previo a la dimetilación del C-14, concretamente inhibiendo la isomerización $\Delta 8 \rightarrow \Delta 7$, como por ejemplo el Tridemorf. En cualquier caso la mejor opción parece ser la de alternar fungicidas de contacto con fungicidas IBE para prevenir la aparición de resistencias (de Waard and Fuchs, 1982).

1.2.7.2 Control biológico

La eficacia de los fungicidas no siempre cumple las expectativas deseadas. Además, los límites máximos de residuos permitidos son cada vez más bajos, y cada vez es mayor la demanda del consumo de productos ecológicos. Por ello surge la necesidad de buscar alternativas a los métodos de control químico como son el control biológico y el uso de variedades vegetales con resistencia genética a la enfermedad.

Entre los parásitos más utilizados en el control biológico del oídio se encuentran la levadura *Tilletiopsis*, que reduce los niveles de infección y dificulta la nueva esporulación del hongo, así como los hongos del género *Ampelomyces*, que no logran erradicar al huésped pero sí impiden la reducción de la cosecha (Jarvis and Slingsby, 1997; Sundheim, 1982). El principal inconveniente es que el organismo utilizado para el control biológico necesita un cierto crecimiento del hongo para poder instalarse. Ambos géneros tienen además la desventaja añadida de que requieren humedades relativas muy elevadas, la cual no ha de bajar de 90%.

Otro tipo de parásito, *Sporothrix flocculosa*, un hongo que posee un índice de colonización más rápido que *P. xanthii*, actúa con humedades relativas de 65% (Sundheim and Amundse, 1982) alterando las propiedades de la membrana citoplasmática de *P. xanthii* dando lugar a la pérdida de su contenido (Hajlaou et al., 1994). Las bacterias del género *Bacillus*, mediante la producción de lipopéptidos de las familias iturin y fengicin, han demostrado ser eficientes en el control de distintas enfermedades producidas por hongos (Shoda, 2000; Stein, 2005). Concretamente dos cepas de *B. subtilis* han sido descritas como capaces de detener el crecimiento de *P. xanthii* (Romero et al., 2007) mediante la producción de iturin A o bacillomycin, fengycin y surfactin. La interacción entre estos compuestos antifúngicos y el hongo dan lugar a la inviabilidad de los conidios a través de la inducción de daños ultraestructurales y de modificaciones morfológicas irreversibles (Askary et al., 1997; Wurms et al., 1999; Feio et al., 2002; Wang et al., 2002). Dichas cepas de *B. subtilis*, junto a una tercera y dos productos basados en los micoparásitos *Ampelomyces quisqualis* y *Lecanicillium lecanii*, dan mejores resultados en el control biológico de esta enfermedad a humedades relativas elevadas (90-95%)(Romero et al., 2007).

1.2.7.3 Medidas de control alternativo

Otras medidas de control podrían ser la utilización compuestos que desencadenan la resistencia sistémica adquirida (SAR), mediante la cual se activan los mecanismos de defensa de partes no infectadas de la planta (Ross, 1966; Sequeira, 1979; Heitefuss, 1982) cuando la planta ha entrado en contacto con un patógeno. Existen distintas sustancias que pueden desencadenar esta respuesta sin la presencia del patógeno y proteger así a la planta antes de su interacción con el hongo. Ejemplos de sustancias desencadenantes de SAR podrían ser la aplicación de ácido salicílico, aplicaciones foliares de micronutrientes ($MnCl_2$, $CuSO_4$) y otros “elicitors”(inductores) de naturaleza química (Olalla, 2001).

1.2.7.4 Control genético

El control genético de las enfermedades en los cultivos se basa en la utilización de variedades vegetales que presentan alguna característica que les permita por sí solas reducir o eliminar el ataque de un determinado patógeno. En la mayoría de los casos se basa en el desarrollo de cultivares resistentes mediante la introgresión de genes de resistencia procedentes de distintas fuentes. Esto disminuye o incluso sustituye el uso de otro tipo de control,

aminorando costes y convirtiéndose en una opción más sostenible desde el punto de vista medioambiental (Cuartero, 1993).

1.3 Mejora genética y genes de resistencia

La introducción de genes de resistencia a oídio en líneas comerciales es uno de los fines perseguidos por los mejoradores. Para ello es necesario disponer de variedades con genes de resistencia a oídio y estimar su modo de herencia. El conocimiento de los mecanismos que tienen lugar en la interacción planta-patógeno puede también aportar claves sobre la resistencia genética.

1.3.1 Selección y mejora para la resistencia a enfermedades

Para que el desarrollo de un programa de mejora enfocado a la resistencia a una determinada enfermedad sea posible, hay que disponer de fuentes de resistencia a dicha enfermedad. El tipo de resistencia preferente es aquel que permite un nivel adecuado de producción, sea estable, es decir, difícil de superar por nuevas formas del patógeno, y actúe frente a un espectro lo más amplio posible de éstos.

Lo más idóneo sería que la fuente primaria de genes fuese de la propia especie vegetal y concretamente de aquellos materiales que por su origen hayan estado en contacto prolongado con el patógeno, de lo contrario se ha de optar por especies próximas. Por otro lado, en el caso de que entre variedades no existiese mucha diferencia genética o que hubiese incompatibilidad sexual entre los tipos cultivados y los silvestres que queremos cruzar, se tendría que recurrir a crear variabilidad genética mediante técnicas como la ingeniería genética.

Una vez comprobada la resistencia de las plantas elegidas tanto en campo como en ensayos de inoculación controlada, en los que se simulan las condiciones óptimas de infección y se provoca el contacto planta-patógeno, el segundo paso será estudiar la genética de la resistencia. Este estudio se basa en la realización de cruzamientos dirigidos hacia el parental de interés, y su posterior inoculación y fenotipado. El fenotipado consiste, generalmente, en asignar un valor numérico dentro de una escala a la intensidad de los síntomas observados para cada individuo inoculado (Niks et al., 1993).

El conocimiento de la genética de la resistencia a un determinado patógeno permite establecer el plan de mejora a seguir para el desarrollo de variedades resistentes con valor

comercial. Las resistencias monogénicas u oligogénicas, a pesar de tratarse de genes fácilmente transferibles por simple retrocruzamiento, suelen ser rápidamente superables por nuevas formas más virulentas del patógeno. En el caso del oídio, las poblaciones existentes en los cultivos de melón están constituidas por varias razas, de las que una suele ser predominante a las otras (del Pino et al., 2002). Cuando se desarrolla una variedad resistente frente a la raza dominante, la resistencia es muy eficaz inicialmente, pero, al extenderse su cultivo, su efectividad se ve reducida ya que se favorece indirectamente la selección de razas minoritarias, que llegan a convertirse en la nueva raza dominante (Gómez-Guillamón, 2003). Por ello, el objetivo del mejorador sería la introducción de varios genes de resistencia específicos de raza, o genes con resistencia horizontal, en un mismo cultivar con el fin de obtener una resistencia más duradera. Esto mismo ya fue propuesto por Nelson (1978) bajo el nombre de ‘piramidalización de genes’, que consiste en acumular distintos genes de resistencia contra el mismo patógeno en un mismo genotipo, y por Cubero (2004) con la ‘estrategia de genes duplicados’, que se basa en que una variedad lleve dos genes distintos específicos de raza que son completamente efectivos de manera individual, con lo cual el patógeno tiene que superar una doble barrera para causar la enfermedad.

1.3.2 Genes de resistencia a *P. xanthii* en melón

Es conocido que la localización de la resistencia genética es fundamental en la lucha contra el oídio. Hasta la fecha, a pesar de que han sido descritas en torno a 28 razas fisiológicas de *P. xanthii*, sólo han sido descritos genes de resistencia para las razas 1, 2, 2US, 3, 5, s y pxch1 (Dogimont, 2010-2011).

Frente a la raza 1, se ha descrito que las líneas de melón ‘PMR 45’, ‘WMR 29’, ‘Edisto 47’, ‘PMR 5’, ‘PMR 6’, ‘PI 124111’, ‘PI 124112’, ‘PI 414723’, ‘Negro’, ‘Perlita’ y ‘Bellagarde’ poseen un gen dominante responsable de la resistencia a dicha raza (Jagger et al., 1938; Bohn and Whitaker, 1964; Harwood and Markarian, 1968; McCreight et al., 1987; Pitrat and Lecoq, 1984; Pitrat, 1991; Epinat, 1992; Kenigsbuch and Cohen, 1992; Epinat et al., 1993; Floris and Álvarez, 1995; Anagnostou et al., 2000; Fazza et al., 2013; Ning et al., 2014). Sin embargo, existen algunas discrepancias en el número de genes implicados en la resistencia para algunos de los genotipos mencionados anteriormente. Según Harwood and Markarian, (1968) la resistencia en ‘PMR 5’ a la raza 1 está controlada por un gen dominante mientras que para Epinat et al. (1993)

son dos genes independientes los implicados. Lo mismo ocurre con el 'PI 124112', ya que Epinat et al. (1993) y Kenigsbuch and Cohen (1992) hablan de que un solo gen dominante es el responsable de la resistencia para dicha raza, mientras que Perchepied et al. (2005a) considera que son dos genes independientes los que controlan la resistencia. En el caso de otras líneas de melón como 'PI 235607', 'PI 236366', 'PI 179901' y 'Seminole', la resistencia a la raza 1 es debida a la actuación de dos genes dominantes (Harwood and Markarian, 1968), y para 'Moscatel Grande' (Floris and Álvarez, 1995), la resistencia está conferida por dos genes complementarios. En el cultivar 'PI 313970', ésta es debida a la actuación de un gen recesivo (McCreight, 2003), y en el cultivar 'Amarillo' dicha resistencia está conferida por un gen dominante y un gen modificador (Floris and Álvarez, 1995).

Del mismo modo, han sido varias las líneas de melón en las cuales se ha descrito resistencia frente a la raza 2 de *P. xanthii*. Así, en 'WMR 29', 'Edisto 47', 'PMR 5', 'PI 414723', 'PI 124111', 'PI 124112', 'PMR 6' y 'PI 313970', se ha propuesto que un único gen dominante o con dominancia incompleta es el responsable de la resistencia (Pitrat and Lecoq, 1984; Cohen and Cohen, 1986; McCreight et al, 1987; Epinat, 1992; Kenigsbuch and Cohen, 1992; Epinat et al., 1993; McCreight, 2003). En la mayoría de los casos no queda claro si se trata del mismo gen que controla la respuesta frente a la raza 1 o no. También para la resistencia a la raza 2 los modelos descritos en algunos de las líneas resistentes no son, en muchos casos, coincidentes. Así, Bohn and Whitaker (1964) describieron que la resistencia frente a la raza 2 en 'PMR 5' está controlada por la acción de un gen dominante y dos genes modificadores, mientras que Harwood and Markarian (1968) propusieron que la resistencia en esta línea de melón se debe a la acción de dos genes epistáticos. Por otro lado, Kenigsbuch and Cohen (1992) establecieron que la resistencia presente en 'PMR 6' es controlada por un gen parcialmente dominante y un gen modificador. En 'PI 124112', Perchepied et al. (2005a) sugirieron que la resistencia está controlada por dos genes dominantes, y para el cultivar 'PI 414723', Bardin (1996) determinó que la resistencia se debía a la actuación de dos genes complementarios.

Frente al resto de las razas identificadas del hongo son pocos los estudios realizados encaminados a localizar fuentes de resistencia, así como a describir la naturaleza de la misma. Para la raza 3 de *P. xanthii* se conoce que existe resistencia en 'PI 124111' (Cohen and Eyal, 1983) sin embargo su genética no ha sido investigada, y en 'PI 124112' la resistencia está controlada

por la acción de un único gen dominante (Perchepped et al., 2005a). En 'PI 124112' se ha descrito a su vez resistencia frente a las razas 4 y 5 del hongo, proponiéndose en ambos casos una herencia monogénica dominante (Bardin, 1996; Perchepped et al., 2005a). En el caso de 'PI 414723' frente a estas dos razas, se sugiere la actuación de dos genes dependientes (Bardin, 1996). Fazza et al. (2013) describieron dos genes, uno que confiere resistencia a las razas 1 y 5, y otro ligado al anterior, a la raza 3. El control genético para las tres razas según Fazza et al. (2013) podría ser de carácter dominante conferido por un solo gen o dos genes epistáticos.

Como se observa, existen discrepancias en cuanto al control genético de la resistencia a oídio descrito en un mismo genotipo de melón frente a una determinada raza de *P. xanthii*. Además, a pesar de los numerosos genes de resistencia descritos, la falta de pruebas de alelismo dificulta la comprensión y comprobación de los modelos genéticos que parecen estar involucrados en la resistencia a oídio.

Uno de los principales problemas que surgen a la hora de abordar el estudio de la genética de la resistencia frente a una raza concreta del hongo, es la gran variabilidad observable en las poblaciones del patógeno. Son varios los casos en los que se ha detectado que, al inocular una planta con distintos aislados de una misma raza, se ha obtenido una respuesta distinta en función del aislado utilizado. Así, se comprobó que al inocular los genotipos 'PI 414723' y 'WMR 29' con aislados de la raza 2 de distinta procedencia, éstos daban lugar a una respuesta diferente (McCreight et al., 1987; McCreight and Pitrat., 1993). Según algunos autores, esto podría indicar que los aislados que pertenecen a una misma raza pueden no contener los mismos genes de avirulencia (Cohen et al., 2004) o que la clasificación en razas fisiológicas utilizada debe ser modificada (Álvarez et al., 2000).

Otros factores que pueden influir en la identificación de una raza son, tanto los factores ambientales presentes en el momento de la interacción planta-hongo, como la metodología empleada en la inoculación controlada. Varios son los métodos de inoculación empleados según los autores, como el uso de cotiledones, primeras hojas verdaderas, discos de hoja, inoculación con espátula, con torre de inoculación, con cepillo, sacudiendo una hoja infectada, entre otros (Cohen and Eyal, 1995; Cohen et al., 1996; Leibovitch et al., 1996; Bardin et al., 1999; Nicot et al., 2002; Cohen et al., 2004; Krístková et al., 2004), con, en ocasiones, resultados variables y a menudo poco comparables. Debido a los numerosos factores que pueden estar modificando la

respuesta real de la planta al ataque del hongo, se ha intentado en diversas ocasiones identificar las razas de forma genotípica, buscando marcadores moleculares que pudiesen estar asociados a cada una de las razas (Bardin et al., 1997b; Katzir et al., 2000; Montoro et al., 2004), sin embargo, hasta ahora, no se ha conseguido hallar asociaciones adecuadas entre marcadores y genotipos del hongo que permitan establecer un método fiable de determinación de razas del patógeno; por lo que hasta la fecha la identificación de las distintas razas se basa en la respuesta fenotípica de un conjunto de genotipos indicadores (Bardin et al., 1999).

Todos estos problemas dificultan al mejorador la posibilidad de obtener variedades vegetales resistentes. La detección de genes de resistencia de la planta, su localización en un mapa genético y su estrecha relación con marcadores moleculares, facilitaría y controlaría las introgresiones de dichos genes de interés en variedades comerciales.

1.4 Herramientas para la mejora genética

1.4.1 Marcadores genéticos

Los marcadores genéticos pueden agruparse en marcadores morfológicos y moleculares. Un marcador morfológico es aquel que se detecta a través de variaciones en el fenotipo y que potencialmente se corresponde a la existencia de polimorfismos en los genes. Estas variaciones fenotípicas son visibles a simple vista y pueden deberse a una característica diferencial propia o bien a una mutación. Son marcadores que generalmente se expresan en forma de presencia-ausencia y su número es limitado, siendo altamente influenciados por el ambiente y por la interacción entre distintos genes.

Los marcadores moleculares se identifican a partir de variaciones que tienen lugar a nivel de proteínas (marcadores bioquímicos) y a nivel de ADN (marcadores de ADN). Éstos pueden tener un patrón de herencia dominante (no se distingue el homocigoto del heterocigoto) o codominante (se distingue el homocigoto del heterocigoto), y no están influenciados por el ambiente, así como tampoco es necesario que el individuo analizado esté completo o se encuentre en estado adulto o maduro para que sean informativos.

Como marcadores bioquímicos consideramos tanto a las isoenzimas como también a las proteínas, particularmente de reserva. Las isoenzimas son el conjunto de múltiples formas moleculares de una misma enzima presente en una misma especie, como resultado de la presencia de uno o más genes que codifican para cada una de estas formas. Las más relevantes

como marcadores genéticos son las aloenzimas, que son variantes alélicas del mismo locus y que presentan una migración electroforética diferencial basada en su peso y carga eléctrica. Suelen ser codominantes y son limitados en número, ya que se obtienen de zonas codificantes del genoma.

Los marcadores moleculares basados en el polimorfismo a nivel del ADN se encuentran en zonas codificantes como no codificantes del genoma por lo que son independientes de la expresión génica y su número es prácticamente ilimitado. Un buen marcador de ADN es aquel que es polimórfico, preferiblemente codominante, con un elevado número de copias y cuyas copias se distribuyen de forma aleatoria por el genoma. Se le exige también que sea reproducible entre laboratorios, de fácil utilización y de bajo coste. Actualmente los marcadores de ADN que logran reunir tales características son los SSR (Simple Sequence Repeat) y los SNP (Single Nucleotide Polimorfism), sin embargo, todos los marcadores de ADN pueden ser útiles, según el objetivo que se persiga.

1.4.1.1 Marcadores de ADN

Los marcadores RFLP (Restriction Fragment Length Polimorfism) están basados en la digestión del ADN con endonucleasas de restricción. Los fragmentos son separados mediante electroforesis y posteriormente son trasferidos a una membrana a través de Southern blott y detectados por hibridación con una sonda determinada. El polimorfismo detectado por los RFLP se debe a mutaciones que crean o pierden dianas de restricción, o bien a deleciones o inserciones que, manteniendo las mismas dianas, aumentan o reducen el tamaño de los fragmentos. Sus principales limitaciones son su elevado coste y esfuerzo (Botstein et al., 1980).

Los AFLP (Amplified Fragment Length Polymorphism) se basan en la amplificación por PCR de fragmentos de restricción específicos seleccionados a partir de la digestión del ADN genómico. Esta técnica requiere tres pasos: (1) digestión del ADN con enzimas de restricción y ligación con oligonucleótidos (adaptadores) a los extremos de dichos fragmentos; (2) amplificación de los fragmentos seleccionados y (3) electroforesis del producto de amplificación, generalmente en geles de poliacrilamida por su alta resolución (50-100 fragmentos de restricción visibles por gel) (Vos et al., 1995). Al igual que los RFLP, los AFLP son reproducibles entre laboratorios (Jones et al., 1997) y la mayoría de ellos suelen ser dominantes

(Mueller and Wolfenbarger, 1999), pero, a diferencia de los RFLP, éstos son menos laboriosos, más rápidos y más informativos.

Los marcadores RAPD (Random amplified polymorphic DNA) han sido descritos como un método para detectar polimorfismos basado en la amplificación de segmentos de ADN con un solo primer de secuencia nucleotídica arbitraria (Welsh and McClelland, 1990; Williams et al., 1990). Para que se genere un fragmento RAPD es necesario que las dos hebras del ADN en estudio presenten sitios de hibridación con el oligonucleótido en orientaciones opuestas suficientemente cercanas (menos de 3000bp) como para permitir la amplificación. Estos marcadores suelen ser de carácter dominante, y rápidos y económicos de obtener. La cantidad de ADN necesaria de partida para la amplificación mediante PCR puede ser muy baja, siendo estos marcadores muy útiles para trabajar con genomas de los que no se dispone de información previa (Hadrys et al., 1992). Como limitaciones se destaca que pueden verse afectados por cualquier cambio en las condiciones de la PCR (Williams et al., 1990; Arnold et al., 1991; Carson et al., 1994), pudiendo aparecer bandas inespecíficas debido a la baja temperatura de ligación (35°C) generalmente empleada (Haig et al., 1994), lo que los hace ser unos marcadores poco reproducibles entre laboratorios (Jones et al., 1997).

Los SCAR (Sequence Characterized Amplified Region) son marcadores específicos de PCR que se obtienen a partir de marcadores tipo RAPD, RFLP y AFLP. El fragmento de interés detectado tras la amplificación con cualquiera de los marcadores citados anteriormente puede ser clonado, secuenciado y así utilizado para la construcción de un marcador específico (Paran and Michelmore, 1993). Las ventajas de un marcador tipo SCAR son: la detección de un único locus, una amplificación menos sensible a las condiciones de reacción de PCR y su posible conversión en un marcador de tipo codominante.

Los marcadores denominados ISSR (Inter Simple Sequence Repeats) se obtienen mediante la amplificación por PCR de secuencias que llevan motivos repetidos (2 a 5 nucleótidos) y por lo general algunas secuencias arbitrarias. Los fragmentos amplificados están generalmente situados entre loci microsatélites. Su fundamento es muy parecido a los RAPD, ya que se utiliza un sólo cebador semiarbitrario y como resultado de la amplificación se obtienen fragmentos flanqueados por elementos repetitivos inversamente orientados. Los productos de

amplificación se pueden resolver en geles de agarosa o poliacrilamida, obteniéndose patrones de múltiples bandas (Zietkiewicz et al., 1994).

Los marcadores SSR (Simple Sequence Repeats), también denominados 'short tandem repeats' o microsatélites, se caracterizan por contener unidades repetidas de nucleótidos en tándem, de tamaño entre 1 y 10 pares de bases. Están distribuidos por todo el genoma y son altamente polimórficos, ya que se basan en la variación en número de las unidades repetidas. Según la diferencia de pares de bases existente entre los distintos alelos amplificados, se pueden visualizar en geles de agarosa, de acrilamida o mediante el análisis de fragmentos procedentes de electroforesis capilar en un secuenciador. Son marcadores codominantes, muy informativos, altamente polimórficos y reproducibles entre laboratorios (Jones et al., 1997).

Entre los marcadores SSR destacan los denominados EST-SSR (Expression Sequence Tags-SSR), que son microsatélites asociados a regiones codificantes del genoma. Son marcadores de bajo coste, ya que se pueden desarrollar a partir de las bases de datos EST, y a diferencia de los SSR pueden ser utilizados para especies relacionadas (Gupta et al., 2003).

Los marcadores tipo SNP (Single Nucleotide Polymorphism) están originados por variaciones de un solo nucleótido en la secuencia de ADN producido, bien por un cambio de base, o por pequeñas inserciones o deleciones (Martín, 2002). Se conocen distintas estrategias para generar SNP como son la secuencia directa de un fragmento de ADN a partir de un fragmento aleatorio, de una banda de RAPD o AFLP, de un EST (Expressed Sequences Tag) o de genes conocidos (Rafalski, 2002b). La mayoría de ellos son dialélicos, es decir, sólo se encuentran dos alelos para cada locus estudiado (Weaver, 2000; Gut, 2001). Una de las grandes ventajas de los SNP es el elevado número de ellos que se encuentran distribuidos aleatoriamente por el genoma de cualquier especie, pudiéndose detectar uno cada 0.3-1kb aproximadamente (Edwards and Mogg, 2001; Rafalski, 2002a). Entre las estrategias más utilizadas para la detección de SNP se encuentran la secuenciación de amplicones (fragmentos obtenidos por PCR) y las hibridaciones con microchips de ADN procedentes de varios individuos, cuyas secuencias resultantes posteriormente se alinean para encontrar SNP.

En relación a los marcadores CAPS (Cleaved Amplified Polymorphic Sequences), al igual que en el caso del desarrollo de los SCAR, el producto de amplificación de un RAPD, AFLP o RFLP se secuencia y se observa si en la secuencia existen dianas de enzimas de

restricción (Konieczny and Ausubel, 1993). Posteriormente se elige aquella o aquellas enzimas que tras la digestión puedan dar lugar a polimorfismos y los resultados se visualizan en un gel de agarosa. Estos marcadores de carácter codominante son reproducibles entre laboratorios, aunque son relativamente laboriosos (Jarvis et al., 1994).

Los marcadores denominados dCAPS (derived Cleaved Amplified Polymorphic Sequences) se desarrollan generalmente cuando no es posible crear un CAPS debido a no se obtiene ningún polimorfismo diferencial utilizando endonucleasas de restricción, o no existe ningún sitio de corte en la región amplificada. Lo que se intenta entonces es crear cebadores donde la unión secuencia a amplificar-cebador sí tenga una diana de restricción y genere polimorfismos. Los dCAPS incluyen en los cebadores utilizados en la PCR nuevas mutaciones respecto al ADN molde, que introducen en los productos amplificados las mutaciones nucleotídicas necesarias para generar en uno de los alelos una diana de restricción que es detectada mediante la posterior digestión de los productos de amplificación (Michaels and Amasino, 1998; Neff et al., 1998).

El uso de los marcadores moleculares ha supuesto una increíble mejora a nivel cualitativo y cuantitativo del análisis genético y sus aplicaciones. Algunos de estos avances han sido la caracterización y el análisis de la diversidad genética de poblaciones naturales, líneas de mejora y bancos de germoplasma, la obtención de 'huellas genéticas' (fingerprinting) de individuos, variedades y poblaciones, el establecimiento de relaciones filogenéticas entre diferentes individuos y especies, la selección asistida por marcadores (MAS, Marker Assisted Selection), la construcción de mapas genéticos de alta cobertura genómica para la localización de genes y caracteres de herencia cuantitativa (QTL o Quantitative Trait Loci), la comparación de mapas genéticos, o el clonaje posicional entre otros.

1.4.2 Mapas genéticos

La elaboración de mapas genéticos ha experimentado un gran impulso a partir del desarrollo de marcadores moleculares, lo que a su vez ha renovado el interés por los mismos en diferentes campos de la biología y mejora vegetal. Un mapa genético representa de forma visual la posición lineal relativa de los genes y/o marcadores genéticos obtenidos a partir de inferencias de datos de ligamiento/entrecruzamiento. El orden en el que se sitúan los loci en cada grupo de ligamiento puede corresponder potencialmente al orden físico en los

cromosomas. Las distancias genéticas se basan en la frecuencia de recombinación existente entre dos marcadores contiguos y se miden en centimorgans (cM.).

Para la obtención de un mapa de ligamiento primero se ha de seleccionar la población de mapeo más adecuada según los objetivos perseguidos. Generalmente se utilizan poblaciones procedentes de cruzamientos de líneas puras con valor agronómico y líneas silvestres emparentadas o material exótico, o bien poblaciones segregantes entre líneas de mejora. Las poblaciones más utilizadas suelen ser F2, retrocruces (BC, Backcrosses), dobles haploides (DHL, Double Haploid Lines), líneas recombinantes consanguíneas (RIL, Recombinant Inbred Lines) o líneas casi isogénicas (NIL, Near Isogenic Lines). Una vez seleccionada la población de mapeo se identifican y desarrollan marcadores moleculares que sean polimórficos entre los parentales y se genotipa la población de interés. Posteriormente se calculan las frecuencias de recombinación entre los marcadores; se establecen los grupos de ligamiento, y se estiman las distancias genéticas entre los marcadores y su orden en el mapa (Staub and Serquen, 1996; Jones et al., 1997). En la actualidad existen programas informáticos que facilitan la construcción de los mismos.

1.4.2.1 Mapas genéticos en melón

El melón es una especie diploide con un número básico de cromosomas $n=12$ (Dane, 1983; Ma et al., 1997), para la cual se han descrito aproximadamente unos 160 genes (Dogimont, 2010-2011) que controlan diferentes aspectos de su biología. Su genoma es de pequeño tamaño, de unos 450 millones de pares de bases, y en la reciente secuenciación del mismo se han determinado hasta el momento 27427 genes que codifican proteínas (García-Mas et al., 2012). Entre ellos, más de 410 genes de resistencia a enfermedades, 26 genes relacionados con acumulación de carotenos y 63 genes sobre acumulación de azúcares (García-Mas et al., 2012). Los primeros mapas genéticos en melón se construyeron en base a caracteres morfológicos y genes de resistencia a enfermedades (Pitrat, 1991) mediante el uso de marcadores de tipo isoenzimático (Staub et al., 1997). El primer mapa genético basado en marcadores de ADN fue desarrollado por Baudracco-Arnas and Pitrat (1996) y fue construido a partir de una población F2 obtenida del cruce entre el genotipo coreano 'Songwhang Charmi' ('PI 161375'), y la línea 'Védrantais', de tipo Charentais. Se mapearon un total de 110 marcadores entre los cuales se incluían 68 RAPD, 36 RFLP, una isoenzima, los genes de resistencia a *Fusarium* (*Fom-1* y *Fom-2*),

al pulgón *Aphis gossypii* (Vat), al virus del cribado (*nsv*) y el gen pentamerous (*p*), que determina la presencia de cinco carpelos y cinco estambres en la flor. Los marcadores se distribuyeron en 14 grupos de ligamiento, con una longitud total de 1.390 cM y una distancia media entre loci de 17.7 cM.

Durante algunos años continuó la construcción de mapas poco saturados (Wang et al., 1997; Liou et al., 1998; Brotman et al., 2000, Danin-Poleg et al., 2002; Silberstein et al., 2003), con una distancia media entre marcadores de 7.9 a 11.5 cM y empleando para ellos generalmente poblaciones F2. Sin embargo, como era de esperar, la tendencia ha sido la de desarrollar mapas más densos con poblaciones de tipo RIL y DHL en su mayoría (Oliver et al., 2000; Gonzalo et al., 2005; Zalapa et al., 2007; Cuevas et al., 2008; Fukino et al., 2008), utilizando para ello un mayor número de marcadores tipo microsatélites y SNP (Danin-Poleg et al., 2001; Fazio et al., 2002; Ritschel et al., 2004; Gonzalo et al., 2005; Fukino et al., 2007). El primer mapa genético de melón que estableció un número de grupos de ligamiento igual al número básico de cromosomas de la especie ($n=12$), y estaba compuesto principalmente por marcadores codominantes, fue el desarrollado por Oliver et al. (2001). En este mapa se emplearon 234 RFLP, 94 AFLP, 47 RAPD, 29 SSR, 5 ISSR, 2 isoenzimas y se evaluó el carácter morfológico pentamerous (*p*).

Un año después se comenzó a utilizar la fusión de mapas con el fin de obtener mapas consenso. La fusión de mapas es una técnica muy atractiva ya que permite aumentar la densidad de marcadores sin la necesidad de realizar un genotipado adicional, y a su vez mejora la precisión del alineamiento de los marcadores, aumenta la portabilidad de los mismos y la capacidad de establecer inferencias (Díaz et al., 2011). El primero de ellos fue el desarrollado por Périn et al. (2002a) mediante la combinación de dos mapas obtenidos con dos poblaciones RIL diferentes con un parental en común ('Védrantais'x 'PI 161375' y 'Védrantais'x 'PI 414723') mediante el uso de marcadores dominantes tipo AFLP (346). El segundo mapa consenso, fue desarrollado por Gonzalo et al. (2005), utilizando como puntos de anclaje marcadores codominantes. Este mapa se obtuvo a partir de la fusión del mapa realizado por Oliver et al. (2001) con una población F2 y el establecido por Gonzalo et al. (2005) con una población DHL, procedentes ambos del cruce entre 'PI 161375' y 'Piel de Sapo'. Dicho mapa estaba constituido por 226 RFLP, 97 SSR, 3 SNP y el gen de resistencia al virus del cribado (*nsv*), repartidos en 12 grupos de ligamiento, con una longitud de 1.021 cM y una distancia media entre marcadores de

3.11 cM. Posteriormente se estableció un tercer mapa consenso en el marco de una iniciativa internacional (International Cucurbit Genomics Initiative, ICuGI) que fue considerado como el mapa de referencia de melón. Este mapa se obtuvo con la unión de un primer mapa constituido por 1307 marcadores (110 SNP, 588 SSR, 252 AFLP, 236 RFLP, 89 RAPD, 6 indels, 15 IMA, y 11 caracteres morfológicos) que cubría una distancia total de 1150 cM distribuidos en 12 grupos de ligamiento y con una distancia media entre marcadores de 0.88 cM, con otros 285 marcadores (225 SNP, 52 SSR, 3 RFLP, y 5 indels). resultado de una selección mediante 'bin mapping'. Así el mapa final está basado en datos de ocho mapas independientes de distintas poblaciones y parentales, y consta de 1592 marcadores (640 SSR, 330 SNP, 252 AFLP, 239 RFLP, 89 RAPD, 15 IMA, 16 indels y 11 caracteres morfológicos) y 370 QTL de 62 caracteres diferentes obtenidos de 18 mapas. Cubre una distancia de 1150 cM repartidos en 12 grupos de ligamiento y con una distancia media entre marcadores de 0.72 cM.

El método de Bin mapping o mapeo selectivo propuesto por Vision et al. (2000) es un método que permite situar rápidamente nuevos marcadores en un mapa ya construido anteriormente. La base de este procedimiento consiste en usar solamente algunos individuos de la población de mapeo que, en conjunto, tienen sobrecruzamientos que cubren la totalidad del genoma de una manera prácticamente uniforme. Al tener que examinar solamente una pequeña proporción de las plantas de la población posibilita el estudio de muchos marcadores en un tiempo y coste varias veces inferiores a los requeridos para el análisis de toda la población y con una pérdida relativamente pequeña de resolución. Esta técnica ya fue utilizada previamente por Fernández-Silva et al. (2008) para la construcción de un mapa a partir del mapa de Gonzalo et al. (2005) al que, gracias a la técnica bin mapping, lograron añadirse 121 nuevos SSR. De esta forma, el mapa de bins construido incluye 212 SSR, 80 RFLP, 3 SNP y el locus *nsu*, distribuidos en 122 bins, con una longitud de mapa de 1.244 cM y una distancia media entre loci de 4.2 cM.

1.4.2.2 El genoma del melón

El empleo de marcadores moleculares y el desarrollo de potentes tecnologías de secuenciación de genomas han permitido recientemente generar nuevas aproximaciones para abordar el estudio de la biología de plantas y la mejora vegetal. La información de la secuencia de un genoma facilita el acceso más preciso a regiones concretas (Feuillet et al., 2011), y puede

ser usada para identificar genes candidatos mediante análisis bioinformáticos y comparación de genotipos de referencia con otros de interés (Edwards and Batley, 2010), así como para conocer la estructura de los genes y su expresión (Berkman et al., 2012). Se han abierto también nuevas posibilidades para el análisis de transcriptomas para el estudio de procesos biológicos, permitiendo interpretar los elementos funcionales de un genoma bajo ciertas condiciones de desarrollo o enfermedad (Wang et al., 2009). Los programas de mejora vegetal son los grandes beneficiados, ya que se puede conocer la variación de secuencias génicas y su potencial asociación a la variabilidad fenotípica que se observa en los cultivos, optimizándose los procesos de selección y combinación de genotipos y el desarrollo de cultivares mejorados.

En los últimos años se han secuenciado los genomas de importantes especies hortícolas cultivadas pertenecientes a la familia de las Cucurbitáceas como el pepino (*Cucumis sativus* L.) (Huang et al., 2009), el melón (*Cucumis melo* L.) (García-Mas et al., 2012) y la sandía (*Citrullus lanatus* L.) (Guo et al., 2013). En el caso concreto de *C. melo*, el empleo de la secuenciación masiva ha enriquecido de forma importante el transcriptoma de melón, herramienta clave para la anotación del genoma, situándose también los polimorfismos detectados anteriormente e incrementando, por tanto, su utilidad.

Las nuevas tecnologías de secuenciación han permitido así mismo combinar la generación de secuencias con la identificación de marcadores y el genotipado de los individuos en una misma reacción, lo que se conoce como Genotipado por Secuenciación o GBS (Elshire et al., 2011). De esta forma, en melón, la generación de secuencias procedentes de distintos genotipos ha permitido la identificación *in silico* de un elevado número de SNP y la creación de plataformas de genotipado masivo, de gran utilidad para estudios de diversidad genética, el incremento de la densidad de mapas genéticos y el anclaje del genoma (Deleu et al. 2009; Blanca et al. 2011; 2012; Clepet et al., 2011; Esteras et al., 2013).

La disponibilidad de toda esta información permitirá durante los próximos años identificar genes candidatos implicados en procesos biológicos de interés para la mejora de la calidad de los frutos y la resistencia a plagas y enfermedades.

1.4.3 Análisis de caracteres cuantitativos

Muchas son ya las especies vegetales a partir de las cuales se han desarrollado mapas genéticos. Entre las numerosas utilidades que presentan podemos destacar el mapeo de genes y QTL, la clonación posicional, la disección de epistasis, el análisis del desequilibrio de ligamiento, la comparación de genomas, y la integración de mapas físicos y genéticos (Díaz et al., 2011).

La localización de loci que están vinculados con la expresión de caracteres cuantitativos (QTL) relacionados con resistencias a plagas y enfermedades, así como con caracteres agronómicos de interés, es uno de los principales objetivos en mejora. Un QTL se define como una región del genoma estrechamente vinculada a un marcador cuya variación presenta una correlación significativa con la del carácter cuantitativo al que se asocia (Tanksley, 1993), y su análisis se basa en la búsqueda de asociaciones entre el carácter cuantitativo y los alelos. Para la identificación de QTL consistentes y precisos se recomienda la utilización de mapas genéticos saturados (Tanksley et al., 1992), de poblaciones perpetuas tipo RIL (Périn et al., 2002a), DHL (Gonzalo et al., 2005) y NIL (Eduardo et al., 2007; Eshed and Zamir, 1995) que permite que los caracteres de interés sean evaluados varias veces, por ejemplo en localizaciones diferentes o en la misma localización pero en años o estaciones distintas.

1.4.3.1 QTL asociados a caracteres morfológicos

La mayoría de caracteres morfológicos de importancia agronómica son caracteres cuantitativos que se encuentran bajo un control genético de tipo poligénico. Por ello la mejor forma de conocer las regiones genómicas implicadas en su regulación y lograr su detección es mediante el análisis de loci ligados a caracteres cuantitativos (QTL). Como se ha mencionado anteriormente, este tipo de análisis requiere de mapas genéticos saturados (Tanksley et al., 1992) y de reiteradas evaluaciones de poblaciones perpetuas como son las de tipo RIL, DHL o NIL. Hasta la fecha han sido numerosos los QTL descritos en melón utilizando para ello distintas poblaciones y con diferentes parentales (Périn et al., 2002b; Monforte et al., 2004; Eduardo et al., 2005, 2007; Sinclair et al., 2006; Fernández-Trujillo et al., 2007; Zalapa et al., 2007; Cuevas et al., 2008, 2009; Moreno et al., 2008; Obando et al., 2008; Paris et al., 2008; Obando-Ulloa et al., 2009, 2010; Harel-beja et al., 2010; Díaz et al., 2011.).

Périn et al. (2002b; 2002c), utilizando dos poblaciones RIL del cruce de un parental de forma redondeada 'Védrantais' y dos de forma alargada, 'PI 414723' y 'PI 161375', identificaron numerosos QTL relacionados con la morfología del fruto y el ovario, cinco cosegregantes, y observaron que para ellos la influencia del ambiente era baja.

Monforte et al. (2004), en dos poblaciones obtenidas a partir del cruce entre 'Piel de Sapo' x 'PI 161375' (F2 y DHL), detectaron 35 QTL vinculados con la forma del fruto, el peso, el contenido de sólidos solubles, la precocidad, y los colores de la corteza y de la carne. El análisis de colinearidad entre los mapas de Périn (2002a) y Monforte et al (2004) les permitió detectar además cuatro QTL comunes para la forma de fruto, sugiriendo que existe una alta heredabilidad para este carácter en melón. En relación al color de la piel, los análisis de QTL indicaron que es un carácter muy complejo en cuyo control genético pueden estar implicados más de dos QTL. A partir de esta población F2 se generaron unas 27 NIL con las que también se llevaron a cabo numerosos análisis. Moreno et al. (2008) detectaron cinco QTL para la producción de etileno y cinco para la firmeza de la carne. Eduardo et al. (2007) identificaron 15 QTL para el contenido sólidos solubles, 11 para el peso, 11 para el diámetro, 15 para la forma, cuatro para el color de la carne, cuatro para el color de la piel del futo y cinco para forma del ovario. Utilizando esta población NIL también se detectaron QTL vinculados a desordenes fisiológicos y a la pérdida de sabor (Fernández-Trujillo et al., 2007) y Obando et al. (2008) detectaron 134 QTL: 52 relacionados con caracteres morfológicos, 69 con el color y 23 con el sabor. Obando-Ulloa et al. (2009) localizaron 60 QTL relacionados con el contenido total de azúcares, sacarosa, glucosa y ácidos orgánicos.

Sinclair et al. (2006), mediante dos poblaciones F2 obtenidas a partir del cruce de dos líneas con alto contenido en azúcar y ácido ascórbico ('TAM dulce' y 'Deltex') y una con bajo contenido ('TGR-1551'), identificaron QTL asociados a sólidos solubles totales, sacarosa, fructosa y ácido ascórbico. Así mismo identificaron marcadores tipo RAPD asociados a muchos de los QTL detectados.

Zalapa et al. (2007) con una población de 81 RIL obtenida a partir del cruce entre 'USDA 846-1', una línea altamente ramificada, y el cultivar 'TOP Mark', localizaron un total de 37 QTL vinculados a la producción, entre ellos el número de frutos por planta y peso del fruto. Observaron que 16 de estos QTL eran independientes del ambiente y concluyeron que los genes

de las plantas de melón altamente ramificadas tienen la capacidad de incrementar la cosecha. En esta misma población, Paris et al. (2008) detectó 57 QTL vinculados con caracteres morfológicos de fruto y Cuevas et al. (2008), enfocando su estudio en el valor nutricional del melón, detectó ocho QTL para beta caroteno.

Harel-Beja et al. (2010), mediante 99 RIL procedentes del cruce 'Dulce' x 'PI 414723', hallaron 44 QTL para diez caracteres de fruto como son el porcentaje de reticulado y la firmeza de la carne, y caracteres vinculados al tamaño del fruto, entre otros. Díaz et al. (2011), situaron en un mapa consenso 370 QTL para 62 caracteres obtenidos a partir de 18 mapas, muchos de ellos ya mencionados en este apartado.

1.4.3.2 QTL asociados a la resistencia a oídio

El número de publicaciones relacionadas con QTL implicados en resistencia a plagas y enfermedades en melón es limitado. Los primeros QTL implicados en resistencia a patógenos fueron descritos por Dogimont et al. (2000), que detectaron siete QTL asociados al virus del mosaico del pepino (CMV), y Perchepped et al. (2005b) detectaron nueve QTL implicados en la resistencia a la raza 1.2 de *F. oxysporum* f. sp. *melonis*, así como un QTL relacionado con la resistencia a mildiu causado por *P. cubensis*. Posteriormente, Boissot et al. (2008), usando la misma población que Dogimont et al. (2000), detectaron cuatro QTL con efecto aditivo y dos parejas de QTL epistáticos potencialmente implicados en la resistencia por antixenosis al pulgón *A. gossypii*. Palomares-Rius et al. (2011), con una población RIL ('TGR-1551' x 'Bola de Oro'), detectó un QTL mayor para la resistencia al virus del mosaico de la sandía (WMV).

Con respecto a la resistencia a oídio, son varios los trabajos que recientemente han abordado su estudio desde un punto de vista cuantitativo. Así, en una población RIL del cruce entre 'PI 124112' y 'Védrantais', Perchepped et al. (2005a) identificaron dos QTL para la resistencia a oídio: *PmV.1* para las razas 1, 2 y 3 en el grupo de ligamiento V y *PmXII.1* para las razas 1, 2 y 5 en el grupo de ligamiento XII, así como varios QTL vinculados a la resistencia de la raza 1 de *G. cichoracearum*. Posteriormente, Fukino et al. (2008), con una población RIL del cruce entre 'AR 5' y el cultivar japonés 'Harukei 3', identificaron dos nuevos QTL, ambos relacionados con la resistencia a las razas N1 y 1 de *P. xanthii* en los grupos de ligamiento II y XII. Yuste-Lisbona et al. (2011b), mediante el análisis de una F2 del cruce entre 'TGR 1551' y el cultivar 'Bola de Oro', detectó un QTL, *Pm-R*, para las razas 1, 2 y 5 en el GL V que en un trabajo

posterior tras un mapeo fino de la zona y la búsqueda de genes candidatos para la resistencia a oídio, observaron que se trataba de dos QTL muy próximos: uno para las razas 1 y 2 (*Pm-R1-2*) y otro para la raza 5 (*Pm-R-5*) (Yuste-Lisbona et al., 2011a). Recientemente, Ning et al. (2014), con una población de 631 BC1 obtenidos a partir del cruce entre 'Edisto 47' (resistente a raza 1) y 'Queen' (línea de mejora) inoculados con las cepas PX1A y PX1B de dicha raza, hallaron un QTL para la cepa PX1A (dos genes controlan la resistencia, *Ano2* y *HXC* resistentes) y otro para la cepa PX1B (un gen controla la resistencia, *Ano2* y *HXC* susceptibles) en GL II y V, respectivamente.

1.4.4 Selección asistida por marcadores moleculares

La selección asistida por marcadores tiene como base la identificación y utilización de marcadores moleculares estrechamente ligados a caracteres de interés. Como método plantea numerosas ventajas como son la selección inequívoca en fase temprana de aquellos individuos portadores del carácter deseado (Lavi et al., 1994), el análisis de poblaciones segregantes de gran tamaño, la ausencia de influencia ambiental, y la reducción de gastos en cultivo y personal. Para que un marcador molecular pueda desempeñar esta función, éste ha de cosegregar con el carácter deseado, ha de tener una amplia distribución dentro del genoma, debe existir una tecnología adecuada para analizar grandes poblaciones y ésta debe tener una alta reproducibilidad entre laboratorios, además de ser económica, sencilla y rápida (Mohan et al., 1997; Gupta et al., 1999).

1.4.4.1 Marcadores ligados a la resistencia a oídio en melón

Hasta la fecha han sido mapeados varios genes de resistencia a oídio: *Pm-w*, *Pm-x*, *Pm-y* (Pitrat, 1991; Périn et al., 2002), *Pm-1* (Teixeira et al., 2008), *Pm-8* (Liu et al., 2010), *Pm-AN* (Wang et al., 2011), *Pm-2F* (Zhang et al., 2013) y *Pm-x1,5* y *Pm-x3* (Fazza et al., 2013). También se han localizado en mapas genéticos varios QTL asociados a esta resistencia: *PmV.1* y *PmXII.1* (Perchepied et al., 2005a), dos QTL identificados por Fukino et al. (2008), *Pm-R* (Yuste-Lisbona et al., 2011b) o *Pm-R1-2* y *Pm-R-5* (Yuste-Lisbona et al., 2011a), y *Pm-Edisto47-1* y *Pm-Edisto47-2* (Ning et al., 2014). Aunque todos estos genes y QTL relacionados con resistencia a oídio han sido localizados en mapas genéticos, en pocos casos se han identificado o desarrollado marcadores moleculares muy estrechamente ligados a ellos. Sin embargo sí se han descrito

algunas asociaciones entre genes mapeados y marcadores próximos como son el AFLP M75/H35_155 en GL IX (Teixeira et al., 2008); el SCAR 0305 y el SSR CMBR150 en GL XII (Liu et al., 2010); los marcadores RPW, MRGH63, MRGH5, Vat, SRAP23 en GL V (Wang et al., 2011); CMBR120, CMBR8, SSR02733, SSR02734, CS27, CAPS-Ddel en GL II (Zhang et al., 2013); y H35M75_156 situado entre *Pm-x1,5* y *Pm-x3* (Fazza et al., 2013). Como marcadores asociados a QTL de resistencia a oídio se han identificados los microsatélites: CMBR8 y CMBR120 para el QTL localizado por Fukino et al. (2008) en el GL II, y TJ29, CMBR111 y CMBR150 para el identificado en el GL XII. Yuste-Lisbona et al. (2011a; b) desarrolló los marcadores PM3-CAPS, MRGH5 para *Pm-R1-2* y MRGH63 para el *Pm-R-5* localizados en el GL V. También se han descrito como asociados a QTL de resistencia a oídio los marcadores CMGA36, SSR252089 para *Pm-Edisto47-1* en GL II, y GCM295, DE1469 como aquellos marcadores ligados a *Pm-AN*, en GL V para *Pm-Edisto47-2* (Ning et al., 2014).

1.5 Interacción planta-patógeno

Tres son los distintos tipos de interacción planta-patógeno. La susceptibilidad, donde el patógeno coloniza al huésped ocasionándole graves daños o la muerte; la resistencia, donde el huésped se impone al patógeno dando lugar a la reducción o desaparición del mismo (Eenink, 1977), y por último, la tolerancia, donde aun habiéndose establecido una relación parasítica, los daños producidos por el patógeno se traducen en una mínima reducción de la calidad o cantidad de la cosecha (Robinson, 1969). La tolerancia no suele considerarse en muchos casos un carácter deseable ya que la planta se comporta como reservorio del patógeno (Russell, 1978).

La resistencia es un carácter heredable que puede ser clasificado según varios criterios: la especificidad (específica o no de raza), el grado de expresión (completa o incompleta), el modo de herencia (monogénica o poligénica), la durabilidad (durable o efímera) o su mecanismo de acción (activo o pasivo-constitutivo).

1.5.1 Tipos de resistencia según su mecanismo de acción

En las plantas se han descrito dos mecanismos de defensa: el constitutivo o pasivo y el activo o inducido (Fig. 1.10).

El mecanismo constitutivo o pasivo es aquel en el que la planta hospedante no activa ningún mecanismo de defensa en presencia del patógeno. Éstos a su vez pueden dividirse en

estructurales o químicos. Entre los estructurales podemos citar la presencia de capas gruesas de cutícula, de tricomas y de ceras, y entre los químicos, la acumulación de compuestos tóxicos en las células vegetales.

El mecanismo activo se caracteriza, por el contrario, por una defensa que se activa frente al ataque de un patógeno (Collinge et al., 1994). Este tipo de resistencia provoca cambios claros en el metabolismo del huésped por la expresión diferencial de genes, que es inducida gracias a la presencia de sistemas de reconocimiento específicos por los que la planta detecta al patógeno (Hutcheson, 1998). Los mecanismos que se inducen como consecuencia del reconocimiento del patógeno por parte de la planta pueden ser activados tanto por inductores no específicos como por inductores específicos. Entre los inductores no específicos caben destacar determinados polímeros de azúcares que forman parte de la pared celular, tanto de hongos como de las células vegetales (Darvill and Albersheim, 1984), o la actividad de enzimas hidrolíticas de la planta que pueden liberar ciertos componentes de la pared celular del hongo, que son los que finalmente actúan como inductores. La inducción específica de defensa ha sido observada en las interacciones raza-cultivar. Este tipo de interacción fue descrita por Flor (1955) bajo el nombre de relación 'gen a gen', quien estableció que a cada gen de resistencia (R) en el huésped le corresponde un gen de avirulencia (Avr) en el patógeno (reacción incompatible). La ausencia de alguno de estos dos genes daría lugar al desarrollo de la enfermedad (reacción compatible). A posteriori este tipo de interacción fue denominado como resistencia vertical (Van der Plank, 1963) que junto con la resistencia horizontal son desencadenadas por inductores específicos. Este modelo descrito por Flor (1955) tiene sus excepciones y no se cumple en los siguientes casos (Christ et al., 1987; de Wit, 1992) (Fig. 1.11):

- Los genes de resistencia o avirulencia no son dominantes.
- El número de genes implicados no es el mismo en la planta o huésped y en el hongo o patógeno (epistasia) o que los productos de estos genes estén interactuando, de forma que cada gen puede enmascarar o suprimir la expresión del otro.
- Presencia de genes modificadores o supresores de la avirulencia o de la resistencia.

Los inductores específicos son proteínas codificadas por los genes Avr de avirulencia presentes en el patógeno y son capaces de inducir las respuestas de defensa en cultivares que posean los correspondientes genes R (Parker and Coleman, 1997). Una vez reconocido el

patógeno se activan los mecanismos de defensa, que pueden conllevar varios procesos, como se describe posteriormente: la muerte celular por reacción hipersensible (HR), la acumulación de metabolitos secundarios con actividad microbiana, la acumulación de enzimas hidrolíticas, o la deposición de sustancias de refuerzo que evitan el avance del patógeno (Collinge et al., 1994).

La respuesta hipersensible (HR) es un mecanismo sumamente efectivo que produce una reacción de resistencia casi absoluta basada en una necrosis controlada similar a la apoptosis o muerte celular programada conocida en los tejidos animales (Raff, 1998). Sin embargo, todavía no se sabe con seguridad si la HR es directamente responsable de la resistencia, al privar al patógeno de nutrientes, o si su acción se basa en la acumulación de sustancias tóxicas que matan simultáneamente al patógeno y a las células del hospedador en contacto con el mismo (Heath, 2000).

La acumulación de metabolitos secundarios con actividad microbiana, como las fitoalexinas (Paxton, 1981), es otro mecanismo de defensa. Las fitoalexinas se acumulan tanto en los lugares de penetración del hongo, como en las células y tejidos adyacentes a las células que reaccionan con la HR (Hammerschmidt et al., 1999), y se asocia con la inducción de genes que codifican para ciertas enzimas como la fenilalanina amonio liasa (PAL).

La formación de barreras estructurales es otro mecanismo de defensa que evita el avance del patógeno. Estas barreras se forman mediante la acumulación de lignina y la producción de papilas que son estructuras de resistencia que se producen por modificaciones de las células de la epidermis constituidas principalmente por calosa (Ordeñana, 2002). Estos compuestos son inducidos por la expresión de enzimas como la PAL y la calosa sintasa respectivamente (Kruger et al., 2003; Nishimura et al., 2003).

Las proteínas relacionadas con la patogénesis, denominadas también proteínas PR, son proteínas que se acumula en las plantas durante la infección con patógenos. Algunas PR tienen actividades definidas como las PR-2 (beta-1,3-glucanasa) y PR-3 (quitinasas). Estas proteínas pueden actuar hidrolizando las paredes de las hifas de los hongos ya que están constituidas por quitina y beta 1,3-glucano. La resistencia sistémica adquirida (SAR) es una respuesta de defensa activa, sistémica, de amplio espectro que también está asociada a genes PR (Hammerschmidt, 1999).

1.5.2 Tipos de resistencia según su especificidad

La resistencia activa con inductores específicos se puede clasificar en dos tipos según Van der Planck (1963): la resistencia vertical y la horizontal (Fig. 1.11). La resistencia vertical, también denominada absoluta o específica de raza, está vinculada a aquellos mecanismos de defensa que presentan una herencia de tipo monogénica y su expresión suele ser completa, consiguiendo la eliminación de la enfermedad). Los responsables suelen ser genes de tipo R. La incorporación de este tipo de resistencia a programas de mejora es más sencilla como también lo es la capacidad de los patógenos de superarla. El otro tipo de resistencia activa, según la especificidad es la resistencia horizontal, no específica de raza o poligénica. Esta resistencia actúa indistintamente de la raza del patógeno que esté atacando al hospedador. Es un mecanismo de defensa basado generalmente en más de un gen y/o en genes menores, donde cada gen implicado ofrece una resistencia parcial y su expresión suele ser generalmente incompleta, por lo que, por lo general básicamente ralentiza el crecimiento del patógeno. Este tipo de resistencia es más difícil de vencer por el patógeno, ya que hay más de un gen implicado. Esta característica si bien es muy atractiva para la mejora, obtenerla requiere más tiempo y esfuerzo.

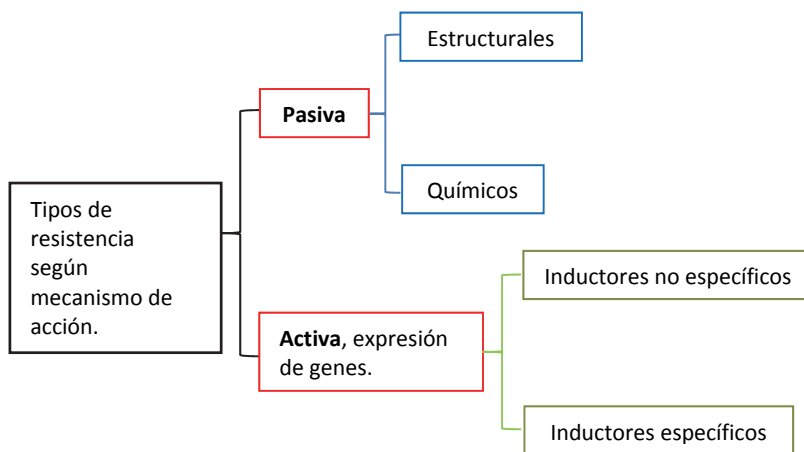


Figura 1. 10. Tipos de resistencia según mecanismo de acción.

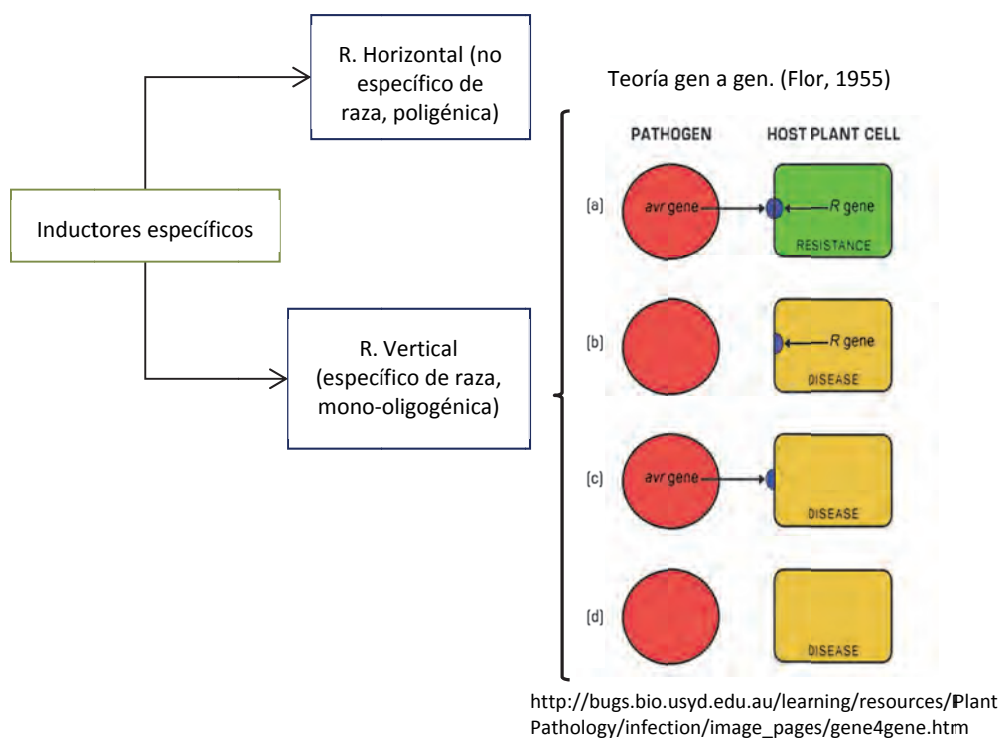


Figura 1.11. Tipos de inductores específicos en la resistencia activa que dan lugar a una respuesta (R) horizontal o vertical (Teoría gen-gen): la resistencia se induce solamente cuando una planta con un gene específico de resistencia (*R gene*) reconoce al patógeno, de una determinada raza, que contiene el correspondiente gen de avirulencia (*avr*) (a). La ausencia del gen de avirulencia (b), del gen de resistencia (c) o de ambos (d) da lugar al desarrollo de la enfermedad.

1.5.3 Mecanismos de defensa específicos en *Podosphaera xanthii*

Se conocen dos mecanismos básicos de resistencia a oídio, la denominada resistencia prehaustorial, asociada a la formación de papilas efectivas, y la resistencia post-haustorial, asociada a la reacción de hipersensibilidad (HR). Sin embargo, en cucurbitáceas sólo se ha identificado la resistencia del tipo post-haustorial.

Kuzuya et al. (2006) describe dos tipos de resistencia post-haustorial que difieren en el tiempo de la inducción de la respuesta hipersensible. La tipo I, en la que el hongo llega a generar el tubo germinativo a partir del conidio, pero éste, no llega a elongarse ni a ramificarse. Sólo se observa ramificación en algunas esporas, y el haustorio que se forma es deforme y no está rodeado por sus lóbulos característicos. Es decir, no se forma adecuadamente la estructura del haustorio (cuerpo haustorial más lóbulos) que se observa en las reacciones compatibles. El tipo de defensa principal en la respuesta tipo I es la HR, que se hace patente mediante la fluorescencia de toda la célula sobre otro mecanismo de defensa, la deposición de calosa alrededor del haustorio y en la pared celular. Esta reacción de hipersensibilidad o HR parece estar asociada con la producción de especies reactivas de oxígeno (ROS) (Kuzuya et al., 2006).

La respuesta tipo I se ha detectado en 'PMR 45', 'PMR 5' inoculados con aislados de la raza 1 de *P. xanthii*, y en 'WMR 29', 'Edisto 47', 'PI 124112' y MR-1' inoculados con aislados de las razas 1 y 2 de este hongo.

En el tipo de respuesta post haustorial tipo II las esporas llegan a formar hifas ramificadas, incluso en ciertos casos se forman conidióforos, y el haustorio y sus lóbulos se desarrollan lentamente. Es decir, en este tipo de resistencia se observa un mayor desarrollo del hongo, incluso llegan a formarse algunos conidióforos. El mecanismo de defensa predominante, a diferencia del tipo I, es la acumulación de calosa alrededor tanto del haustorio como en las paredes celulares, siendo menor el número de células fluorescentes (HR) observadas (Kuzuya et al., 2006). Este tipo de respuesta la presentan 'PI 414723' inoculado con aislados de las razas 1, 2 y 5 de *P. xanthii*, y 'PMR 5' y 'MR-1' con las razas 2 y 5 (Kuzuya et al., 2006). Las diferencias apreciables en el desarrollo del hongo entre estos dos tipos de resistencia parecen deberse al momento en el que comienza a actuar la HR. En el tipo I la HR actúa antes y detiene el desarrollo del hongo en estadios tempranos. En el tipo II actúa más tarde por lo que permite un mayor desarrollo del hongo, lo cual se aprecia en un mayor número de puntos de penetración del hongo en el huésped y por consiguiente de calosa acumulada para un mismo período de tiempo post inoculación. La acumulación de calosa en la resistencia tipo II sigue aumentando tras 120hs después de la inoculación, mientras que en la de tipo I no ocurre así, si no que se mantiene constante a partir de las 48h post inoculación, por lo que la resistencia tipo II parece ser más compleja que la de tipo I (Kusuya et al., 2006). Mecanismos similares a los descritos en melón se han podido observar en otras especies como es el caso de la cebada (*Hordeum vulgare* L.). Así, Hüchelhoven et al. (1999), describieron distintos tipos de respuesta en la interacción cebada-*Blumeria graminis* (Fig. 1.12). Asociaron un fenotipo según el tipo de gen de resistencia a oídio (*Mla12*, *Mlg*, *mlo5*) que portaban una serie de líneas cuasi-isogénicas (NIL) de cebada, así como en una línea de cebada susceptible tratada con el componente inductor de resistencia DCINA (Fig. 1.12). En plantas portadoras del gen *Mla12*, el crecimiento del hongo fue detenido bien por una respuesta hipersensible (HR) desencadenada en las células epidérmicas penetradas desde las 24 a 40hpi o bien por una HR desencadenada en las células del mesófilo debajo de las células epidérmicas atacadas a las 36hpi. En este segundo caso el hongo lograba formar un haustorio y desarrollar hifas secundarias. En la respuesta frente al hongo de plantas

portadoras del gen *Mlg* se observaron papilas efectivas que impidieron la penetración del mismo además de HR en células atacadas pero no penetradas por el hongo desde 18-24hpi. El mismo fenotipo se observó en plantas susceptibles de cebada tratadas con DCINA. En líneas NIL portadoras del gen recesivo *mlo5* se detectó la formación de papilas efectivas que impidieron la penetración por parte del hongo de las células atacadas. En este caso no se desencadenó la HR, por lo que las células atacadas permanecieron vivas. En las interacciones compatibles se observó penetración de la pared celular, formación de haustorio y la presencia de hifas secundarias.

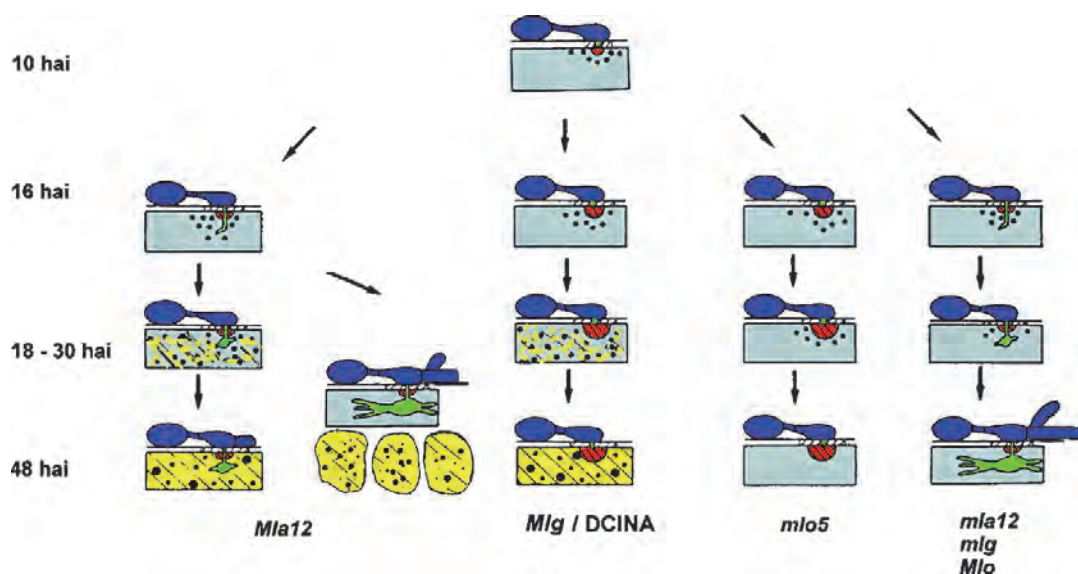


Figura 1.12. Esquema de los fenotipos de interacción predominantes en cebada mediado por los genes de resistencia a oídio: *Mla12*, *Mlg*, y *mlo5*, y por el compuesto inductor de resistencia DCINA tras la inoculación con la cepa BghA6 de la raza 6 de *Blumeria graminis* DC: Fr. f.sp. *hordei* (Hückelhoven et al., 1999).

1.6 Justificación del trabajo de investigación

Dada la evolución incesante de los patógenos, la obtención de variedades de melón resistentes a oídio es un proceso continuo, donde las resistencias monogénicas y específicas, generalmente explotadas a nivel comercial, son superadas rápidamente por nuevas variantes del hongo. El genotipo de melón 'TGR-1551' muestra resistencia a las razas 1, 2 y 5 de *Podosphaera xanthi*, además de a otros patógenos y plagas, como al pulgón *A. gossypii*, al virus del amarillo y enanismo de las cucurbitáceas CYSDV, a su vector la mosca blanca *B. tabaci*, y al virus del mosaico de la sandía WMV-2. Dado el interés de esta línea multirresistente y el fuerte componente ambiental que influye en la expresión de los síntomas de enfermedades como el

oídio, el empleo de marcadores moleculares juega un papel importante en el desarrollo de líneas resistentes, facilitando los programas de mejora mediante la selección de material vegetal resistente e incluso la piramidalización de dichos genes en un mismo genotipo, consiguiendo así una resistencia más duradera a oídio en melón.

La disponibilidad de una población RIL de melón derivada del cruce de esta línea de melón multiresistente con una variedad comercial y la obtención de un mapa genético de la misma, es, por tanto, una herramienta muy útil para la localización de QTL asociados a caracteres de interés, así como para la identificación y desarrollo de marcadores moleculares estrechamente ligados a estos.

Este trabajo se ha enfocado, por este motivo, en la detección de QTL asociados a la resistencia a oídio y a caracteres morfológicos, con la intención de conocer la distribución de los mismos a lo largo de los grupos de ligamiento y la posible relación e interacción entre estos. Además, se ha considerado interesante profundizar en el conocimiento de los mecanismos de resistencia a *Podosphaera xanthii* en distintas entradas de melón, incluyendo genotipos obtenidos del cruce entre 'TGR-1551' y 'Bola de Oro', con el fin de caracterizar los distintos tipos de interacción observados.

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Capítulo 2

2 Objetivos

1. Construcción de un mapa de ligamiento basado en una población RIL (F7:F8) de melón obtenida del cruce entre la entrada procedente de Zimbabwe 'TGR-1551', resistente al oídio causado por *Podosphaera xanthii*, y el cultivar español 'Bola de Oro'. Detección de QTL asociados a la resistencia a las razas 1, 2 y 5 de *P. xanthii*.
2. Caracterización de la población RIL e identificación de QTL asociados a los caracteres morfológicos más importantes.
3. Observación de parámetros relacionados con la interacción planta-patógeno entre las razas 1, 2 y 5 de *Podosphaera xanthii* y distintos genotipos de melón, incluyendo 'TGR-1551' y 'Bola de Oro'.

Capítulo 3

3 Genetic mapping of QTL associated with powdery mildew resistance in a RIL population derived from 'TGR-1551'

3.1 Introduction

Melon (*Cucumis melo* L.; $2n=2x=24$) is an economically important, cross-pollinated, vegetable species of the Cucurbitaceae family. It grows in many temperate, subtropical, and tropical regions of the world, but several pests and diseases limit its production.

Powdery mildew of melon is a fungal disease characterized by a whitish, talcum-like powder that develops on both leaf surfaces, petioles and stems (Sitterly, 1978). It can be caused by either *Golovinomyces cichoracearum* (DC) Gelyuta (Vakalounakis and Klironomou, 2001) or *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff (Shishkoff, 2000). In both cases the symptoms induced are identical, although they can be easily distinguished under light microscope, since *P. xanthii* spores contain fibrosin bodies (Braun et al., 2002). In the coastal zones of Málaga and Almería provinces (Spain), *P. xanthii* has been identified as the sole cause of powdery mildew of cucurbits (Olalla, 2001; Paulitz and Bélanger, 2001), and is responsible for important yield losses in these crops under field and greenhouse conditions (Torés et al., 1990). Although more than 28 putative races of *P. xanthii* have been described worldwide so far (McCreight, 2006), based in the differential response of a set of melon lines, the races 1, 2, and 5 are the most extended races in South Europe (del Pino et al., 2002).

Fungicide applications are the principal management practice (McGrath et al., 1996) to control powdery mildew in crops. However, the development of resistant varieties is considered to be a better strategy to fight against this disease (Hosoya et al., 2000). In many cases, these varieties are obtained by crossing elite melon lines with exotic germplasm, since this exotic germplasm is frequently a valuable source of genes or quantity trait loci (QTL) associated with disease resistance (Tanksley and McCouch, 1997; Zamir, 2001; McCouch, 2004; Fernie et al., 2006)

Several genes and QTL, associated to powdery mildew resistant and previously described in exotic melon accessions, have already been mapped in segregating populations (Pitrat, 1991; Périn et al., 2002b; Perchepped et al., 2005; Fukino et al., 2008; Teixeira et al., 2008,

Yuste-Lisbona et al., 2011a; b; Zhang et al., 2013; Fazza et al., 2013; Ning et al., 2014). Most of them confer resistance to one or two races of *P. xanthii*, and have been localized on linkage groups (LG) II, V and XII. The Zimbabwean melon accession 'TGR-1551', shows resistance to races 1, 2 and 5 of *P. xanthii* (Gómez-Guillamón et al., 1995, 1998). This resistance has been described to be controlled by a double dominant-recessive epistasis, which involves two independent genes, a dominant and a recessive one (Yuste-Lisbona et al., 2010). Until now, by the phenotypic evaluation of an F2 derived from the cross between 'TGR-1551' and the susceptible Spanish cultivar 'Bola de Oro', only a major QTL, *Pm-R*, associated with the dominant gene has been identified and mapped on LG V.

Herein we report the identification of two QTL related to powdery mildew resistance in a recombinant inbred line (RIL) population obtained from the cross 'TGR-1551' x 'Bola de Oro': the QTL *Pm-R* previously described, as well as a new QTL localized on LG XII.

3.2 Materials and methods

3.2.1 Plant material

A RIL population (F7:F8), originated from a cross between the resistant genotype 'TGR-1551' (used as female parent) and the susceptible accession 'Bola de Oro' (used as pollen donor), was obtained using the single-seed-descent method from F2 plants after six to seven selfings.

3.2.2 Inoculation test

The *P. xanthii* isolates used in this experiment were '27' (Race 1), '2204' (Race 2) and 'C8 Cris' (Race 5). Race identification of these fungal isolates was based on the reaction of a set of differential melon lines to each isolate, as described by Bardin et al. (1999). Each isolate conidia was kept in melon cotyledons from the susceptible parent 'Bola de Oro' for race 1, in 'PMR 45' for race 2, and in 'Edisto 47' for race 5, placed on Petri disks with Bertrand medium (Bertrand, 1991) under anoxic conditions. The conidia were subcultured on new cotyledons every two weeks. In order to increase the amount of inocula for the tests, *P. xanthii* isolates were grown on zucchini squash cotyledons (Diamante and Negro Belleza, Semillas Fitó, Barcelona, Spain).

The RIL population, the parental genotypes ('TGR-1551' and 'Bola de Oro'), and the F1 progeny, were evaluated against these three *P. xanthii* races using three plants per genotype in three different time periods (summer 2010, fall 2010 and spring 2011). Plants from the

accessions 'PMR 45', 'Edisto 47', 'PI 414723', 'WMR 29', 'PMR 6', were used as race genotype controls.

All experiments were carried out in glasshouses and under controlled conditions at the Instituto de Hortofruticultura Subtropical y Mediterránea 'La Mayora' (IHSM, CSIC-UMA) in Málaga (Spain). Seventy-four RIL were evaluated in summer 2010 (30-35°C day/20°C night), 77 RIL in fall 2010 (20-25°C day/10-15°C night) and 77 RIL in spring 2011 (22-28°C day/15-20°C night). Seedlings were transplanted in 12cm diameter pots filled with soil-substrate (60% peat, 10% litorite, 30% compost) for inoculation.

Artificial inoculation was performed by placing two small amounts of conidia from each race in the same second true leaf from each plant, in an equidistant position from the central leaf nerve, according to Ferrière and Molot (1988). Twelve days after inoculation, symptoms were scored according to the level of fungal sporulation using a four score scale of 0-3 similar to Yuste-Lisbona et al. (2011b), as follows: class 0= no visible sporulation, class 1= low level of sporulation, class 2= moderate level of sporulation, and class 3= profuse sporulation. Plants scoring 0 and 1 were considered resistant, while plants scoring 2 and 3 were considered susceptible. ANOVA test was employed to estimate significant differences between the response to the three races and environments. All statistical tests were performed using SPSS (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.).

3.2.3 Extraction of genomic DNA and marker analyses

DNA was extracted from young leaf tissue of plants of each genotype using Plant DNAzol kit (Invitrogen, Germany) according to supplier recommendations.

The molecular evaluation of 114 the RIL population derived from the cross between 'TGR-1551' and 'Bola de Oro' genotypes was carried out with a set of different sorts of molecular markers, almost all of them (99.5%) codominants (SSR, EST-SSR, SCAR, CAPS, dCAPS) as well as one phenotypic trait, andromonoecy, conferred by the *andromonoecius* gene, *a*. These markers, previously reported in the literature, were used for map construction, being denominated as follows: CM-, CS- (Danin-Poleg et al., 2001); CSW- (Fazio et al., 2002); CMBR- (Ritschel et al., 2004); CM-N, TJ- (Gonzalo et al., 2005); CMN- (Fukino et al., 2007); CM- (Kong et al., 2007); ECM-, GCM- (Fernández-Silva et al., 2008); MU- (ICuGI). Several markers, described as linked to many resistance genes, were also included: MarkerD SCAR (Dogimont et al., 2004)

linked to the resistance to virus transmission by *Aphid gossipi* (Vat); PM1-SCAR, PM2-CAPS, PM3-CAPS, PM4-dCAPS, PM5-CAPS and PM6-SCAR (Yuste-Lisbona et al., 2011a), linked to powdery mildew resistance; UAL-1 SCAR (Noguera et al., 2005) linked to andromonoecius (*a*), and MRGH63 (melon resistant gen homologue), reported as a NBS (nucleotide binding site) R-gene (García-Mas et al., 2001; Van Leeuwen et al., 2005), and linked to resistance of *P. xanthii* race 5 (Yuste-Lisbona et al., 2011a). PCR amplifications were carried out following the author indications, and fragment visualization were performed using either gel (2-3% agarose gels in SB buffer (10mM sodium boric acid) or capillary electrophoresis using a CEQ 2000XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA) with labelled primers (WellRED fluorescent dyes D2, D3 or D4).

Mapping was carried out using JoinMap® 4.0 software (VanOoijen, 2006). Markers data were assigned to each LG using a minimum LOD score of 4.0 and a recombination frequency value of 0.3. Kosambi mapping function (Kosambi, 1944) was used to translate recombination frequency into genetic distance measured in centimorgans (cM). Segregation ratio distortion at each marker locus was tested against the expected ratio 1:1 for a F7:F8 population using a chi-square (χ^2) test. The molecular markers with *P*-values greater than 0.01, were employed for linkage analysis. Heterozygosis was also checked.

3.2.4 QTL analyses

In order to map powdery mildew resistance in the RIL population, the level of sporulation was evaluated as a quantitative data, using classes 0-3 as numerical values. Thus, QTL analyses were conducted separately for the three RIL evaluations and each powdery mildew race using MapQTL® 5.0 software (VanOoijen, 2004). Interval Mapping model (IM) was first used to detect consistent QTL. Afterwards, the closest markers to the QTL peaks were selected as cofactors and tested with the automatic cofactor selector tool (default *P* value cut off for elimination of a cofactor set of 0.02). Multiple QTL Model (MQM) was then performed with the selected set of cofactors. LOD scores, phenotypic variation percentages and QTL position were then estimated. A permutation test (10.000 cycles) was used to determine the LOD threshold with a confidence interval of 99 %. In addition, the Kruskal-Wallis (KW) method (Lehmann, 1975) of MapQTL, a non-parametric test equivalent to the one-way ANOVA, was

used in order to detect other possible molecular markers with potential significant effect on the expression of powdery mildew resistance.

Since resistance to races 1, 2 and 5 of *P. xanthii* in 'TGR-1551' has been previously described as a double dominant-recessive epistasis (Yuste-Lisbona et al., 2010), QTLNetwork 2.0 software (Yang et al., 2008) was used to identify epistatic interactions between QTL across environments. The mixed-model based on composite interval mapping method (MCIM) was carried out for two-dimensional genome scan to detect epistatic interaction effects. An experimental-wise significance level of 0.05 was designated for the determination of candidate interval selection, putative QTL detection, and QTL x QTL interaction effect. Both, testing and filtration window size, were set at 10 cM, with a walk speed of 1 cM. The critical F value to declare a putative QTL x QTL interaction was determined by a 1000 permutation test at 95% confidence level. The effects of epistatic interactions were estimated by the Markov Chain Monte Carlo method (Wang et al., 1994). The genetic map and the QTL detected were drawn using the MapChart 2.2 software (Voorrips, 2002).

In order to know the influence of each QTL in the powdery mildew resistance, and therefore, to validate the marker-QTL association, the closest markers to each QTL were selected and each RIL score was calculated according to four possible genotype combinations for these markers.

3.3 Results

3.3.1 Evaluation of powdery mildew resistance

The resistant parental line 'TGR-1551' and the F1 line showed resistance to the three races of powdery mildew in the three evaluations. The susceptible parental line 'Bola de Oro' showed clear susceptibility (Table 3.1).

No significant differences were found among RIL for the powdery mildew races evaluated in each environment and among environments ($P < 0.05$) (Table 3.2). Some RIL displayed different levels of fungal sporulation depending on the season when the inoculation was carried out, although each RIL had the same resistant (classes 0 and 1) or susceptible (classes 2 and 3) response to the three races tested.

Table 3.1. Mean values of scores (0-3) for powdery mildew (*Podosphaera xanthii*) resistance to races 1, 2, and 5 of the parental lines, 'TGR-1551' and 'Bola de Oro', F1, and the recombinant inbred line (RIL) population in three evaluations.

Race	Season	Mean values			RIL		
		'TGR-1551'	'Bola de Oro'	F1 line	Mean	R (0-1)	S (2-3)
Race 1	summer 2010	0.0 ± 0.0	2.9 ± 0.2	0.1 ± 0.3	1.11± 1.32	43	31
	fall 2010	0.0 ± 0.0	2.5 ± 0.5	0.9 ± 0.6	1.08± 1.06	50	27
	spring 2011	0.0 ± 0.0	3.0 ± 0.0	0.6 ± 0.6	1.40± 1.20	44	33
Race 2	summer 2010	0.0 ± 0.0	2.8 ± 0.3	0.0 ± 0.0	1.11± 1.3	43	31
	fall 2010	0.0 ± 0.0	2.7 ± 0.5	0.6 ± 0.8	1.04± 1.13	50	27
	spring 2011	0.0 ± 0.0	3.0 ± 0.0	0.6 ± 0.6	1.36± 1.23	44	33
Race 5	summer 2010	0.0 ± 0.0	2.8 ± 0.4	0.2 ± 0.4	1.21± 1.39	43	31
	fall 2010	0.0 ± 0.0	2.6 ± 0.5	0.2± 0.4	1.05± 1.11	50	27
	spring 2011	0.0 ± 0.0	3.0 ± 0.0	0.3 ± 0.5	1.36± 1.21	44	33

R: number of resistant RIL (score 0-1). S: number of susceptible RIL (score 2-3)

The resistant parental line 'TGR-1551' and the F1 line showed resistance to the three races of powdery mildew in the three evaluations, with mean values of 0 for the 'TGR-1551' and lower than 1 for F1 across environments and races evaluated. The susceptible parental line 'Bola de Oro' showed clear susceptibility, with mean values higher than 2.5 (Table 3.1).

The mean values across environments and powdery mildew races evaluated ranged between 1.04 and 1.40 (Table 3.1), being the number of resistant RIL (score 0-1) higher than the number of susceptible RIL (2-3) for the three environments (Table 3.1). Most of the resistant RIL showed score 0 for all the trials, overall in summer. The lower number of susceptible RIL scoring 2 was observed in spring. Some RIL displayed different levels of fungal sporulation depending on the season when the inoculation was carried out, although each RIL had the same resistant (classes 0 and 1) or susceptible (classes 2 and 3) response to the three races tested.

Table 3.2. ANOVA analyses for powdery mildew (*Podosphaera xanthii*) resistance to races 1, 2, and 5 in the recombinant inbred line (RIL) population 'TGR-1551' x 'Bola de Oro' in the three seasons evaluated ($P < 0.05$).

Races 1, 2, 5		Sum of Squares	df	Mean Square	F	Sig.
Summer 2010	Between Groups	,495	2	,247	,138	,871
	Within Groups	393,449	219	1,797		
	Total	393,944	221			
Fall 2010	Between Groups	,091	2	,045	,037	,963
	Within Groups	275,785	228	1,210		
	Total	275,876	230			
Spring 2011	Between Groups	,094	2	,047	,032	,969
	Within Groups	334,654	228	1,468		
	Total	334,747	230			

Seasons		Sum of Squares	df	Mean Square	F	Sig.
Race 1	Between Groups	5,006	2	2,503	1,756	,175
	Within Groups	322,202	226	1,426		
	Total	327,208	228			
Race 2	Between Groups	4,709	2	2,355	1,577	,209
	Within Groups	338,872	227	1,493		
	Total	343,581	229			
Race 5	Between Groups	4,057	2	2,028	1,324	,268
	Within Groups	346,143	226	1,532		
	Total	350,200	228			

3.3.2 Linkage map

The linkage map of the RIL population was carried out with 216 molecular loci (Table 3.3): 138 SSR; 68 EST-SSR; 4 SCAR, 4 CAPS, 1 dCAPS, and one phenotypic trait (*a*). The percentage of skewed markers ($P < 0.01$) was 11%, and the heterozygosity was 2.2%.

Most of the markers (97%) showed less than 7 hybrids. Loci were distributed among 22 linkage groups which were regrouped into 12 LG (Figure 3.1) and designed following the nomenclature proposed by Périn et al. (2002a). The linkage map spanned a total distance of 992.82 cM with an average length between markers of 4.6 cM. Map distances ranged from 48.8 in LG IX to 136.5 cM in LG XI. The most and least saturated LG were LG IV and II, with 3.29 cM and 7.38 cM, respectively. The longest gap among markers, 23.4 cM, was located between ECM133 and ECM197 on LG VI.

Table 3.3. Distribution of molecular markers and one phenotypic (P) trait among 12 linkage groups established on a linkage map using a recombinant inbred line (RIL) population derived from a cross between ‘TGR-1551’ and ‘Bola de Oro’.

Linkage Group	Sub. Groups	Map length (cM)		Nº Markers		Marker density (cM/Marker)		Markers type		
		Total/group	Total/group	Total/group	Total/group	SSRs	EST-SSRs (ECM-CM) MU	SCAR CAPS dCAPS Ptrait.		
I	a	58.5	63.1	15	17	3.90	3.71	7	8	-
	b	4.6		2		2.30		1	1	-
II		110.7	110.7	15	15	7.38	7.38	12	1	2
III	a	55.5	82.0	14	20	3.96	4.1	13	1	-
	b	26.5		6		4.42		2	4	-
IV	a	71.3	88.8	23	27	3.10	3.29	15	8	-
	b	17.5		4		4.38		3	1	-
V		111.1	111.1	31	31	3.58	3.58	14	9	8
VI	a	1.8		2		0.90		2	-	-
	b	65.1	82.3	11	18	5.92	4.57	5	6	-
	c	15		5		3.00		4	1	-
VII	a	6.7		3		2.23		1	2	-
	b	35.1	61.12	12	17	2.93	3.6	10	2	-
	c	19.4		2		9.70		1	1	-
VIII	a	9	67.9	8	16	1.13	4.24	7	1	-
	b	58.9		8		7.36		5	3	-
IX	a	29.6	48.8	3	7	9.87	6.97	2	1	-
	b	19.2		4		4.80		4	-	-
X	a	36.8	62.1	7	12	5.26	5.18	3	4	-
	b	25.3		5		5.06		4	1	-
XI		136.5	136.5	21	21	6.50	6.5	14	7	-
XII		78.5	78.5	15	15	5.23	5.23	13	2	-
TOTAL		992.6		216		4.60		142	64	10

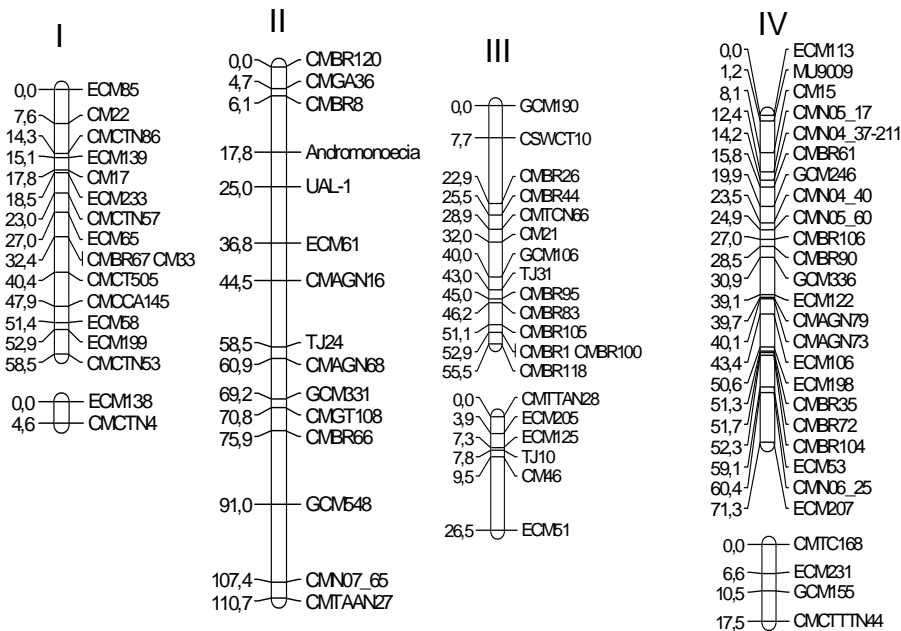
Several molecular markers, described as linked to important resistance genes, were also mapped. Among them, the PCR-based makers associated to powdery mildew resistance described by Yuste-Lisbona et al. (2011a): PM1-SCAR, PM2-CAPS, PM3-CAPS, PM4-dCAPs, PM5-CAPS, and PM6-SCAR, were localized on linkage group V. Marker MRGH63 was located at 0.3 cM from PM3-CAPS, MarkerD, linked to *A. gossypii* resistance, was also set on LG V co-segregating with powdery mildew resistance markers mentioned above (Yuste-Lisbona et al., 2011a), and the marker UAL-1 was situated on LG II at a distance of 7 cM from the *a* locus.

3.3.3 QTL analyses

Two significant QTL were detected in LG V and XII, for each of the three *P. xanthii* races (1, 2 and 5) and each of the three different time periods tested (summer and fall 2010, spring

2011), by all the QTL analyses method used (IM, MQM, and KW) and a confidence interval of 99% (Fig. 3.1).

High LOD scores were also found in LG II with IM and MQM for marker CMAGN16, but they were not significant at the significant level tested (99%). This marker CMAGN16 showed significant values for all races and environments tested according to the KW test. In fall 2010, this was the only significant marker detected in LG II. In summer 2010 and spring 2011, other markers, such as CMBR8 or ECM61, showed significant values for powdery mildew resistance.



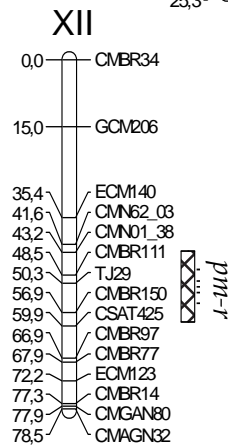
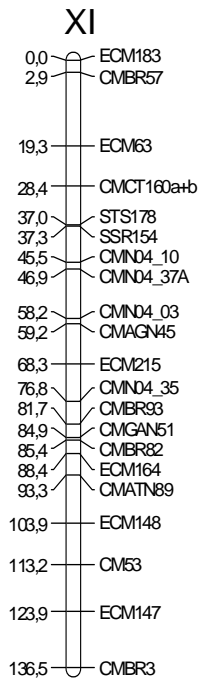
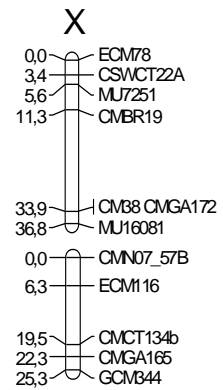
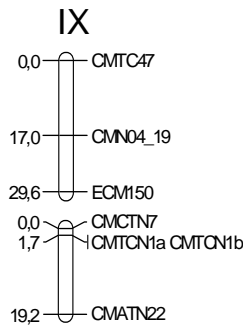
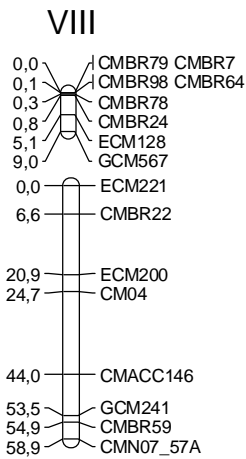
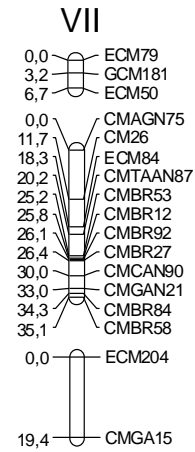
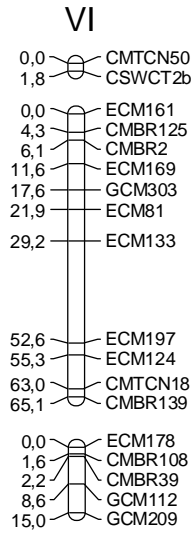
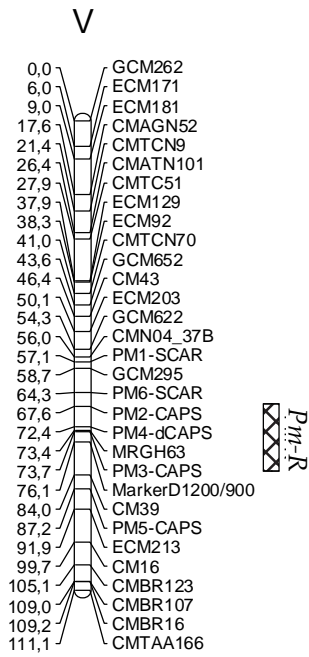


Figure 3.1. Genetic linkage map based on a 114 RIL population (F7:F8) derived from 'TGR-1551' and 'Bola de Oro'. Linkage groups (LG) were named according to Périn et al. (2002a) (I-XII). Markers are shown on the right of the LG and marker positions on the left. Distances are indicated in centimorgans (cM). The putative location of the QTL is represented with a bar on the right side of the LG.

The consistent QTL detected on LG V was supported by LOD scores ranged from 5.75 (R1 fall 2010) to 17.73 (R5 summer 2010) (Table 3.4), and explained between 16.4 (R1 fall 2010) and 56.6 (R5 summer 2010) of the percentage of phenotypic variation (R^2) observed in the RIL population. Maximum values were identified in summer and the minimum in fall. Two markers intervals with significant LOD scores were observed, considering all the time periods tested: one at between 74-83 cM for summer 2010 and spring 2011, and another one between 67-74 cM for fall 2010 (Table 3.4; Fig. 3.2). The markers which presented the strongest linkage with these QTL, independently on the season, were MRGH63, PM3-CAPS and MarkerD, at 73.4; 73.7, and 76.2 cM, respectively. The distance among these markers was lower than 3 cM.

Table 3.4. QTL detected for resistant to races 1, 2 and 5 of *Podosphaera xanthii* in a recombinant inbred lines (RIL) population derived from the cross between 'TGR-1551' and 'Bola de Oro' for a confidence of 99% in three different time periods with the Multiple QTL Model (MQM).

Trait	Season	QTL	Marker interval	LG	cM ^a	LOD ^b	A ^c	R ² (a) ^d
Race 1	summer 2010	<i>Pm-R</i>	MRGH63-CM39	V	74-83	16.49	-0.95	51.8
		<i>pm-r</i>	CMN01_38-TJ29	XII	43-50	5.02	-0.44	10.6
	fall 2010	<i>Pm-R</i>	PM2 CAPS-PM3 CAPS	V	67-74	5.75	-0.42	16.4
		<i>pm-r</i>	CMN01_38-TJ29	XII	43-50	10.92	-0.66	37.3
	spring 2011	<i>Pm-R</i>	MRGH63-CM39	V	74-83	9.6	-0.69	31.5
		<i>pm-r</i>	CMBR111-CSAT425	XII	50-59	4.92	-0.47	13.9
Race 2	summer 2010	<i>Pm-R</i>	MRGH63-CM39	V	74-83	16.51	-0.96	52.6
		<i>pm-r</i>	CMN01_38-TJ29	XII	43-50	4.63	-0.42	9.8
	fall 2010	<i>Pm-R</i>	PM2 CAPS-PM3 CAPS	V	67-74	6.83	-0.50	21.4
		<i>pm-r</i>	CMN01_38-TJ29	XII	43-50	8.84	-0.63	29.8
	spring 2011	<i>Pm-R</i>	MRGH63-CM39	V	74-83	9.17	-0.7	30.8
		<i>pm-r</i>	CMBR111-CSAT425	XII	50-59	4.63	-0.43	13.4
Race 5	summer 2010	<i>Pm-R</i>	MRGH63-CM39	V	74-83	17.73	-1.04	56.6
		<i>pm-r</i>	CMBR111-CSAT425	XII	50-59	4.06	-0.40	8.1
	fall 2010	<i>Pm-R</i>	PM2 CAPS-PM3 CAPS	V	67-74	7.84	-0.53	22.4
		<i>pm-r</i>	CMN01_38-TJ29	XII	43-50	10.56	-0.66	33.3
	spring 2011	<i>Pm-R</i>	MRGH63-CM39	V	74-83	11.18	-0.74	35.8
		<i>pm-r</i>	CMBR111-CSAT425	XII	50-59	4.72	-0.44	12.3

^a Estimated confidence interval of QTL position on the linkage group (LG) in cM (Kosambi).

^b LOD: LOD score detected by Multiple QTL Model (MQM), with a LOD threshold score of 3.8 determined by permutation test for each trait in each environment ($P = 0.01$).

^c Estimated additive effect. Positive values indicate allele arising from 'Bola de Oro' and negative values indicate allele arising from 'TGR-1551'.

^d Percentage of the total phenotypic variation explained by the QTL.

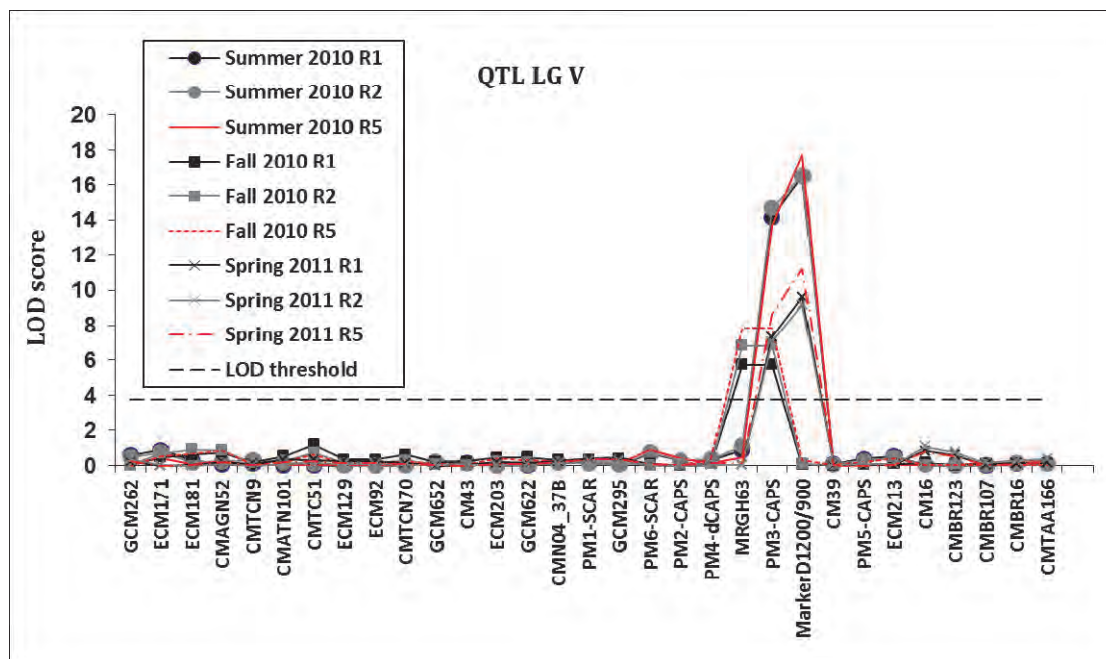


Figure 3.2. Quantitative trait loci detected in linkage group V of melon genome by Multiple QTL Model (MQM) mapping analyses for powdery mildew resistance to races 1, 2 and 5 and in three evaluations (summer and fall 2010, and spring 2011). LOD threshold for a confidence of 99% and 10000 permutations is also represented.

A second QTL for powdery mildew resistance was identified in LG XII. LOD scores obtained for this QTL ranged between 4.06 (R5 summer 2010) and 10.92 (R1 fall 2010), and the R^2 observed in the RIL population ranged from 8.1 (R5 summer 2010) and 37.3 (R1 fall 2010) (Table 3.4). Maximum values were recorded in fall 2010, and minimum ones in summer 2010. Two markers intervals with significant LOD scores were observed considering all the time periods tested: one between 43-50 cM for summer 2010 (races 1 and 2) and fall 2010, and another one between 50-59 cM for summer 2010 (race 5) and spring 2011 (Table 3.4; Fig. 3.3). The markers highly associated with this QTL vary among seasons, and also among races only for the evaluation carried out in summer (Table 3.4). The markers CMBR150 and CMBR111 were strongly linked to this QTL in summer 2010, CMBR111 in fall 2010, and CMBR150 and TJ29 in spring 2011, reporting a maximum distance between markers of 8 cM (Fig. 3.3). The permutation test showed that LOD scores obtained for a confidence of 99% ranged from 3.8 to 3.9 for races 1 and 2, and was 3.8 for race 5 of *P. xanthii*.

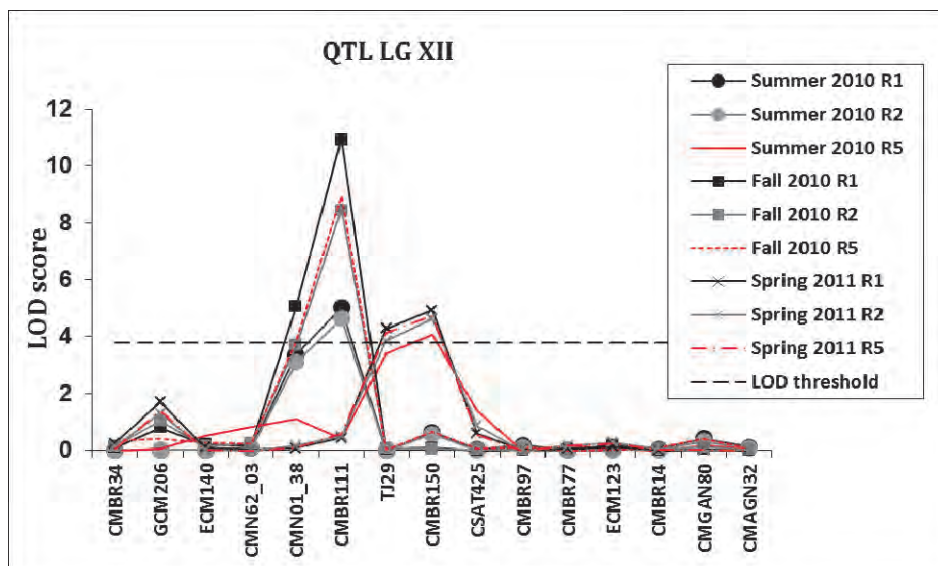


Figure 3.3. Quantitative trait loci detected in linkage group XII of melon genome by Multiple QTL Model (MQM) mapping analyses for powdery mildew resistance to races 1, 2 and 5 and in three evaluations (summer and fall 2010, and spring 2011). LOD threshold for a confidence of 99% and 10000 permutations is also represented.

The QTLNetwork analysis carried out in order to identify epistatic interactions between QTL across environments revealed that there are epistatic interaction between both QTL *Pm-R*, located on LG V, and *pm-r* on LG XII identified for resistance to the three races of *P. xanthii* evaluated (Table 3.5, Figure 3.4). The percentage of phenotypic variance explained by the additive-by-additive epistatic effects ranged from more than 7.8 (race 1) to 10.3 % (race 2).

Table 3.5. QTL × QTL interaction effects detected for powdery mildew (*Podosphaera xanthii*) resistance to races 1, 2, and 5 in a recombinant inbred lines (RIL) population derived from the cross between 'TGR-1551' and 'Bola de Oro' in three different time periods.

E-QTL ⁱ	Marker interval	LG (pos.) ^b	E-QTL ^j	Marker interval	LG (pos.)	F value ^c	AA ^d R ² (aa) ^e
Race 1 - Threshold F value = 5.36							
<i>Pm-R</i>	PM4dCAPS- MRGH63	V (72.4-73.4)	<i>pm-r</i>	CMN01_38- CMBR111	XII (43.2-48.5)	22.57	0.36* 7.8
Race 2 - Threshold F value = 5.40							
<i>Pm-R</i>	PM4dCAPS- MRGH63	V (72.4-73.4)	<i>pm-r</i>	CMN01_38- CMBR111	XII (43.2-48.5)	32.46	0.42* 10.3
Race 5 - Threshold F value = 5.26							
<i>Pm-R</i>	PM4dCAPS- MRGH63	V (72.4-73.4)	<i>pm-r</i>	CMN01_38- CMBR111	XII (43.2-48.5)	24.86	0.39* 8.7

^a E-QTLⁱ and E-QTL^j are the two QTLs involved in epistatic interaction.

^b Linkage group and the estimated confidence interval of QTL position in brackets (in Kosambi cM).

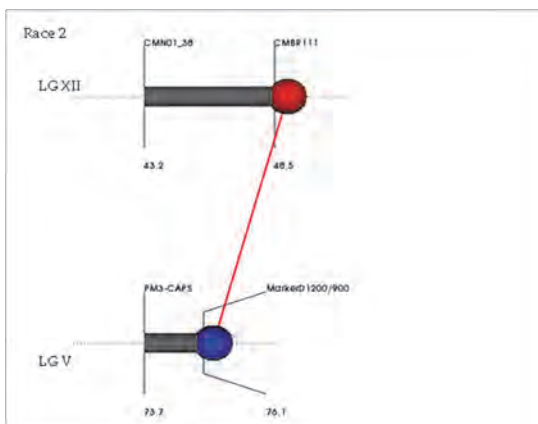
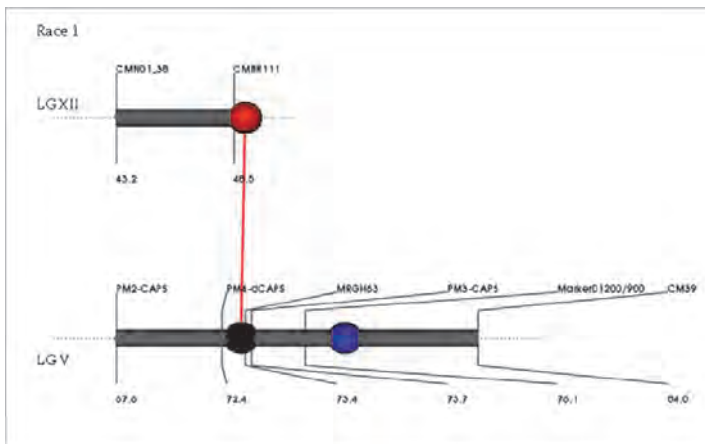
^c F values of significance of each epistatic interaction.

^d Estimated additive-by-additive epistatic effect.

^e Percentage of the phenotypic variation explained by additive-by-additive epistatic effects.

Experiment-wide P value: *P ≤ 0.001.

According to the results from QTLNetwork analyses, the QTL *Pm-R* (LG V) showed environmental effects (Fig. 3.4), so its expression could be environmentally dependent. The positive values of the estimated additive effects (AA) indicated that the alleles coming from ‘TGR-1551’ have positive effects, as expected, on the resistance to the three races of powdery mildew tested (Table 3.5).



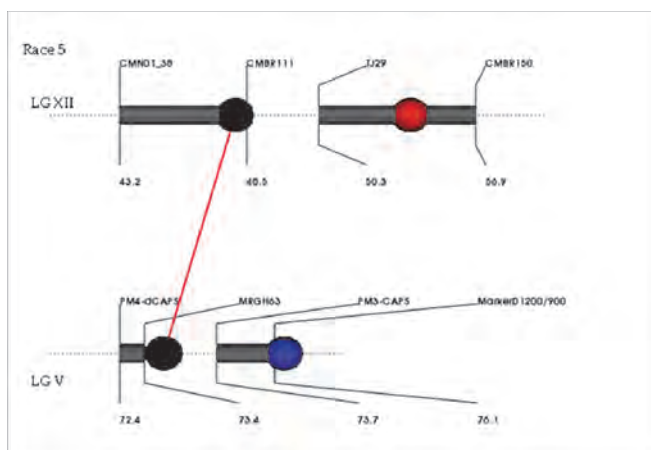


Figure 3.4. Epistatic interaction detected between QTL identified for resistance to races 1, 2, and 5 of *P. xanthii* in the recombinant inbred lines (RIL) population derived from the cross 'TGR-1551' x 'Bola de Oro' across the three environments tested. Genomic regions showing additive effects (A, red circle), both individual additive and environment interaction effects (A+AE, blue circle), and epistatic interactions between QTL (I, red line) are indicated.

3.3.4 Validation of marker-resistance association

In order to know the influence of the QTL detected for powdery mildew resistance, and therefore, to validate the marker-QTL association, two markers, strongly linked with the detected QTL, were selected; PM3-CAPS for the QTL *Pm-R* and CMBR111 for the QTL *pm-r*. Mean powdery mildew symptom scores associated with the allelic combinations of both markers were observed in the RIL population (Table 3.6). Only those RIL carrying these alleles in homozygosis were selected and, although the number of recombinants was really low, they were also discarded (Table 3.6).

In general, mean symptom scores of RIL with 'TGR-1551' alleles in both markers were lower than with 'Bola de Oro' alleles. Homozygous plants for the 'TGR-1551' alleles displayed a mean symptom score between 0 and 0.3, whereas homozygous plants for the 'Bola de Oro' alleles between 2.5 to 2.8. The score reduction produced by 'TGR-1551' allele was slightly higher for *Pm-R*, revealed by the marker PM3-CAPS than for *pm-r* (CMBR111 marker) (Table 3.6).

Table 3.6. Mean symptoms scores of a recombinant inbred lines (RIL) population ('TGR-1551' × 'Bola de Oro') in response to *P. xanthii* races (R) 1, 2 and 5 for three different time periods, associated with the combination of two molecular markers linked to QTL detected in linkage groups (LG) V and XII.

Markers		Symptoms score											
Allelic Combination		Summer 2010				Fall 2010				Spring 2011			
PM3-CAPS(V)	CMBR111(XII)	R1	R2	R5	No. RIL	R1	R2	R5	No. RIL	R1	R2	R5	No. RIL
TGR	TGR	0	0	0	12	0.2	0.2	0.2	13	0.3	0.3	0.2	14
TGR	BO	0.03	0.02	0.02	17	0.5	0.4	0.4	16	0.6	0.3	0.5	16
BO	TGR	0.07	0.07	0.2	10	0.6	0.5	0.7	11	0.3	0.2	0.3	14
BO	BO	2.67	2.65	2.79	24	2.7	2.8	2.7	23	2.5	2.6	2.5	20

No. RIL = number of recombinant inbred lines

TGR: 'TGR-1551' allele; BO: 'Bola de Oro' allele

Scores: 0-1, resistant; 2-3 susceptible.

3.4 Discussion

DNA polymorphism in melon has been reported as moderated to low (Shattuck-Eidens et al., 1990; Neuhausen, 1992). The parental melon lines of the RIL population used herein for mapping were quite distant genetically: 'Bola de Oro' (*C. melo subsp melo*) and the Zimbabwean accession 'TGR-1551', described as *C. melo subsp. agrestis* according to García-Mas et al. (2000). The level of polymorphism detected in this population was elevated and allowed us to achieve a saturated and high informative map constructed with 114 RIL (F7:F8). RIL populations have several advantages for genetic mapping over others: the high homozygosity percentage allows the replication of experiments in different laboratories and environments. Therefore, RIL are very useful for QTL analysis since the environmental component of the total phenotypic variance is reduced, and the precision and accuracy of QTL identification, when more different environments and times are evaluated, are increased (Knapp and Bridges, 1990; Mansur et al., 1996). The residual heterozygosity estimated for our RIL population, 2.2%, was close to the theoretical value. Most of the 216 loci used to genotype our population were codominant, concretely SSR, which are markers of high reproducibility between laboratories and very useful for map comparison and merging (Silberstein et al., 2003; Danin-Poleg et al., 2001; Oliver et al., 2001; Périn et al., 2002a; Gonzalo et al., 2005; Zalapa et al., 2007). The percentage of skewed

markers detected in our map was 11%, slightly lower than those reported previously by Baudracco-Arnas and Pitrat (1996), Périn et al. (2002a) and Wang et al. (1997) (12%, 14%, and 19%, respectively).

Markers were distributed in 22 LG regrouped into 12, number of chromosomes in melon, after comparing the distribution of common markers between our map and those included in other melon maps. This map shares 156 markers with the melon consensus map of Díaz et al. (2011), 97 markers with the F2 map ('TGR-1551' x 'Bola de Oro') of Yuste-Lisbona et al. (2011b), and 52 markers with the RIL map reported by Fukino et al. (2008) which includes QTL for powdery mildew resistance. Collinear order of the common markers was generally observed in the linkage groups. Only a few inversions were detected, mainly on LG IV. Our map spanned a genetic distance of 993 cM with an average inter-marker distance of 4.43 cM between markers. Yuste-Lisbona et al. (2011b), with the F2 of our same population ('TGR-1551' x 'Bola de Oro'), constructed a map with a total distance of 1318.2cM, 111 SSR and 14 specific markers (SCAR, CAPS and dCAPS). Although the saturation of this map was higher (3.1cM between markers), the number of codominant markers was lower. Other melon maps in the literature based on RIL populations and used for QTL analyses covered between 887-1222 cM with a saturation degree from 2.67 to 5.9 cM/markers and employing for its construction from 43 to 386 codominant markers (Zalapa et al., 2007; Cuevas et al., 2008; Fukino et al., 2008; Harel-Beja et al., 2010).

Resistance to races 1, 2 and 5 of *P. xanthii* in 'TGR-1551' has been previously described as a double dominant-recessive epistasis (Yuste-Lisbona et al., 2010), and one major QTL *Pm-R*, linked to the dominant resistant gene, has been described (Yuste-Lisbona et al., 2011b).

Herein, and with a RIL population, we report the identification of a QTL in LG V, as well as another QTL localized in LG XII for the three evaluation and races tested. The QTL detected in LG V shared location with several QTL and genes already mapped, such as *Pm-R* from 'TGR-1551' (Yuste-Lisbona et al., 2011b), *Pm-w* from 'WMR 29' (Pitrat, 1991), *Pm-AN* from Ano2 (Wang et al., 2011), *Pm V.1* from 'PI 124112' (Perchepied et al, 2005) and *Pm-Edisto47-2* from Edisto47 (Ning et al., 2014), which confer resistance, at least, to races 1 and 2. The QTL in LG V observed herein might be *Pm-R*, described by Yuste-Lisbona et al. (2011b) in the F2 derived from the same cross, and the markers strongly linked with this QTL were the same for both populations: PM3-CAPS, a PCR-based marker, and the resistant gene homologue, MRGH63,

which has been described as belonging to a cluster with MRGH6 and MRGH5, all possible candidate genes for *P. xanthii* resistance in 'TGR-1551' (Yuste-Lisbona et al. 2011a). The percentage explained by the QTL obtained with the F2 was 53.6% for *Pm-R1-2* and 65.5% for *Pm-R5*, both higher values than the ones observed with our RIL population. This could be due to the high amount of dominant markers present in the F2 map and that no other QTL was detected in other LG or genomic region. This QTL could be also the same described by Perchepped et al. (2005), *Pm V.1*, which confers resistant to races 1, 2 (two QTL involved, other in LG XII) and 3, with a percentage of the phenotypic variation of 32.9, 38.9 and 88.9% respectively, although no common markers have been observed between both QTL.

Regarding the QTL detected herein in LG XII, possible associated with the recessive gene, it could be related to that one mapped in AR5 (Fukino et al., 2008), since both are located in LG XII and are strongly linked to markers TJ29, CMBR111 and CMBR150. The QTL described by Fukino et al. (2008) is associated to the resistance of two strains of powdery mildew, *P. xanthii* A and *P. xanthii* B, which may be N1 and race 1, respectively. The two QTL related to resistance to *P. xanthii* A explained a 22 % and 28% (LG XII and II, respectively) of the phenotypic variance, and the two involved in resistance to *P. xanthii* B, a 46% (LG XII) and 12 % (LG II). The validation of the marker-resistance association described by this author for both strains in true leaves showed similar values to the ones reported herein. Perchepped et al. (2005) also detected a QTL in LG XII which confers resistance to races 1, 2 and 5 of *P. xanthii*. However, since we did not find any common marker between the LG XII from the two maps, we could not associate both QTL. The QTL (*PmXII.1*) described by Perchepped et al. (2005) explained a 24.8, 28.8 and 93.1 % of the phenotypic variance observed for resistance to races 1, 2 and 5. Other QTL, *PmV.1*, was also involved in the resistant to races 1 and 2. Besides the two QTL reported in this work for 'TGR-1551', other genomic region possibly involved in resistance to races 1, 2 and 5 of *Podosphaera xanthii* was detected in LG II by KW analysis. In the literature, other genomic regions have also been described as involved in powdery mildew resistance in melon. One of these regions is located in LG II, where several genes and QTL were identified: the *Pm-x*; *Pm-x1,5*, *Pm-x3* genes from 'PI 414723' (Pitrat, 1991; Fazza et al., 2013), one QTL detected by Fukino et al. (2008) for races 1, 2 and 3 and linked to markers CMBR120 and CMBR8, as well as one QTL detected by Ning et al. (2014) for strain Px1B, *Pm-Edisto47-1* and located between CMGA36

and SSR252089. Although LOD scores were not high enough to find a putative QTL in LG II in the present report, KW test showed significant values for several markers. So, the marker CMAGN16 was significant for all races and environments examined herein. This marker was located at a distance of 38 cM from the SSR marker CMBR8, described by Fukino et al. (2008) as linked to that QTL involved in powdery mildew resistance in LG II. In the evaluations carried out in summer 2010 and spring 2011 other markers, close to CMAGN16, showed a significant value, including the CMBR8 marker. A fourth genome region has also been described in LG IX as related to powdery mildew resistance in melon according to literature, although only the *Pm-1* gene, resistance to race 1 from 'PI 78374' (Teixeira et al., 2008), has been detected so far.

Data from analyses carried out to identify epistatic interactions between QTL across environments indicated that epistatic interaction exist between *Pm-R* (LG V) and *pm-r* (LG XII), the two QTL detected herein as involved in powdery mildew resistance to races 1, 2 and 5 of *P. xanthii*. Environmental factors seem also to play a role in the expression of *Pm-R* as well as in the interaction between both QTL. Since there are not previous studies concerning QTLxQTL interaction effects involved in powdery mildew resistance, no comparison with the observations mentioned above could be carried out.

More specific studies will be required to confirm the presence of the QTL detected on LG XII and to find candidate genes, as well as to know the influence of environmental factors on each QTL identified and on the interaction among them.

3.5 References

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Capítulo 4

4 Mapping of QTL associated with morphological traits in a RIL melon population

4.1 Introduction

Melon (*Cucumis melo* L.) is an economical important species of the Cucurbitaceae family, whose distribution of wild and cultivated melon types is currently worldwide (Pitrat et al., 2000). Most European and American cultivars are classified within *C. melo* ssp. *melo*, while Africans and East-South Asian cultivars generally belong to *C. melo* ssp. *agrestis*. A high level of molecular and morphological variability in leaf, plant and fruit characters has been described within this species (Kirkibride, 1993; Stepansky et al., 1999; Monforte et al., 2004). In *Cucumis melo* L., most morphological traits can vary enormously among varieties, for example fruit length can range from 4 cm in ssp. *agrestis* to 2m in ssp. *melo* var. 'flexuosus', and fruit weight from 50 g to more than 15 kg (Naudin, 1859). Besides, exotic germplasms are known for being a valuable reservoir of resistance genes or new alleles with favorable effects on fruit quality traits, which can be transferred to elite germplasms as a way to recover part of the genetic variability lost during domestication (Ladizinsky, 1998).

Many genes are involved in the genetic control of a big number of morphological characters. Dogimont (2010-2011) compiled more than 70 genes controlling these traits. Among them, we can find some genes for flowering characters such as andromonoecius (*a*) (Rosa, 1928, Poole and Grimdball, 1939, Wall, 1967), pentamerous number of carpels (*p*) (Rosa, 1928), or male sterility 1 (*ms-1*) (Bohn et al., 1949), some architecture genes such as short internodes 1 (*si-1*) (Denna, 1962) or long main stem internode (*lmi*) (McCreight, 1983), as well as traits related with ethylene production during fruit maturation (Périn et al., 2002c; Fernández-Trujillo et al., 2007). Many of the agronomic characters are, in fact, complex quantitative traits. The identification and mapping of quantitative trait loci (QTL) is nowadays commonly carried out by using populations such us near isogenic lines (NIL; Eduardo et al., 2007; Obando et al., 2008; Obando-Ulloa et al., 2009), recombinant inbred lines (RIL; Périn et al., 2002a; b; c; Zalapa et al., 2007) or double haploid lines (DHL; Gonzalo et al., 2005). These populations are genetically homozygous, so they can be propagated without further segregation, and therefore, they can be

distributed and replicated for experimentation in different laboratories and environments. This is particularly useful for the analysis of quantitative traits, since it allows the reduction of the environmental component of the total phenotypic variance. This fact increases, therefore, the precision and consistency of the detected QTL, as well as the estimation of environment and genetic effects (Knapp and Bridges, 1990; Mansur et al., 1996; Beavis, 1998). Recently, the map positions of many QTL related to fruit quality traits (Périn et al., 2002b; Monforte et al., 2004; Eduardo et al., 2007; Obando et al., 2008; Diaz et al., 2011) and yield (Zalapa et al., 2007) have been determined, many of them localized in several maps constructed with RIL melon populations (Zalapa et al., 2007; Cuevas et al., 2008; Paris et al., 2008; Harel-Beja et al., 2010).

In this work, we present the evaluation and QTL analyses of several morphological traits observed in a RIL population obtained from the cross between the Zimbabwean multi-resistant melon accession 'TGR-1551', and the Spanish cultivar 'Bola de Oro'.

4.2 Materials and methods

A population of 114 RIL (F7:F8) was derived by the single-seed descent method from the Zimbabwean accession 'TGR-1551' and the Spanish cultivar 'Bola de Oro' (Fig. 4.1). The melon line 'TGR-1551' has been described for some authors as belonging to subspecies *agrestis* (Monforte et al., 2003), and its fruits are ovals, with orange skin and eventually green spots, and white, hard and non-sweet flesh. The melon cultivar 'Bola de Oro' (*Cucumis melo ssp. melo*) shows also oval fruits, with yellow skin, and white-greenish, soft and very sweet flesh. This recombinant inbred population (RIL) coming from this cross ('TGR-1551' x 'Bola de Oro') has been used herein to study the genetic control of several morphological traits.

Plants of the parental lines 'TGR-1551' and 'Bola de Oro', the F1, and a variable number of recombinant inbred lines, were grown in a polyethylene greenhouse at the Experimental Station of the IHSM 'La Mayora' (CSIC-UMA) located in Algarrobo Costa (Málaga, Spain) during different periods: 108 RIL in spring-summer 2009, and 93 RIL in spring-summer 2010. Plants were grown under plastic in sandy soil typically used for melon and other vegetable production in Southern Spain, with drip irrigation, and trained for vertical growth on a net, with a planting frame of 1 x 0.8 m² per plant. For each time period (2009 and 2010), two blocks

with around three plants per each RIL, the parental lines and the F1 progeny were distributed in a randomized design. Plants were pruned at five-six leaf stage, leaving a single stem (single stem pruning). During flowering time, one bee hive was placed in the center of the greenhouse to promote pollination.



Figure 4.1. Parental genotypes: 'TGR-1551' and 'Bola de Oro'

Two to three mature fruits per plant were harvested. Mature state was based on several harvest indicators such as fruit growth, period from anthesis (between 32 and 50 days), fruit size, skin waxing appearance, loss of skin trichomes, development of the abscission layer, yellow ring at the pedicel (half to full slip), death of the first leaf beside the peduncle, fruit compactness, ground spot yellowing, netting development, and characteristic skin color (Alarcón et al., 2001; Cantwell and Kasmire, 2002; Fernandez-Trujillo et al., 2007).

In order to characterize our population and detect possible QTL, different plant descriptors were evaluated.

The morphological traits measured in 2009 were categorized as vegetative, phenological and fruit traits. The vegetative traits were: main vein length and number of nodes, both measured two times in the trial (45 and 60 days after transplant), length between 10-11th, 11-12th, 12-13th, 14-15th nodes, width of the main vein on the 10-11th and 14-15th nodes, leaf area, petiole length, number of leaf lobes, lobe depth, corolla diameter and peduncle length of both female and male flowers, and length and maximum diameter of ovary. The phenological traits considered were the date of first female flower in anthesis, fruit setting and harvest. The fruit traits recorded were: fruit weight, length, and maximum diameter, blossom scar size, external and flesh colour, spots color and its distribution pattern, presence of netting, of stripes and sutures, abscission, flesh width, skin width, shape (measured in two ways), seed cavity

diameter, flesh firmness, solids soluble content, and number of complete developed fruits per plant.

A preliminary QTL analysis was carried out for traits measured in 2009. Only characters showing significant QTL were reevaluated in 2010 (Table 4.1). Several vegetative traits were measured as follows: main vein length (MVL) in meters measured 60 days after transplant, leaf area (LA) was measured using an area meter AT MK2 (in cm²) by gathering three leaves per plant from the 10th to 15th node, according to IPGRI. Also one male and one female flower per plant were collected in anthesis and the following parameters were recorded: female and male corolla (C_{fem}, C_{male}) diameter in millimeters, ovary length (OVL) and maximum ovary diameter (OVD) in millimeters, as well as the ovary shape index (OVSI), calculated as the ratio between OVL and OVD. The fruit traits measured were: fruit weight in grams (FW), abscission (ABS) scored according to the strength used to separate the peduncle from the fruit (score between 0= no peduncle-no strength needed, and 4= impossible to separate), blossom scar size (BS), scored between 1 and 4 (5 mm-2 cm Ø aprox.), fruit length (FL) and diameter (FD) in centimeters, fruit shape index (FSI) as the ratio calculated between FL/FD, fruit shape (FS) scored from 1 to 7 based in the IPGRI classification (1=round, 7=elongate), maximum seed cavity diameter (SCD) in centimeters, flesh width (FLW) measured from the skin to the beginning of the seed cavity in centimeters, soluble solid content (SSC), also named as °Brix, estimated by using a hand refractometer (Digital refractometer Atago PAL-1) from homogenized melon flesh, flesh firmness (FIFs), measured with a penetrometer as the breaking force in Newtons using a 4.6-mm-diameter probe (0-100 shore) employed two times in the mid portion of the intact fruit; and flesh (FICol) and skin color (ECol), measured with a hand colorimeter (Minolta CR-200) in tri-chromatic coordinates and recorded in lightness, chroma and hue angle (L*, C*, H* using C illuminant, 0° viewing, and a white plate as reference). Only H* values, known as the 'true color', were considered, though. The number of full developed melon fruits per plant (Nm_{el}) was recorded 90 days after transplant.

Table 4.1. Traits evaluated in a RIL population derived from the cross 'TGR-1551' x 'Bola de Oro' in spring-summer 2009 and 2010.

Traits	Score (unity)	acronym	2009	2010
Vegetative traits				
Main vein length	45 and 60 days (d) after transplant (m)	MVL	x	45 d
No. nodes	45 and 60 days after transplant		x	
Length between nodes	Nodes 10-11 th , 11-12 th , 12-13 th , 14-15 th (cm)		x	
Width of main vein	At the 10-11 th and 14-15 th nodes (mm)		x	
Leaf area	3 leaves, 10-15 th node, area meter AT MK2 (cm ²)	LA	x	x
Petiole length	3 petioles/plant, 10-15 th node (cm)		x	
No. leaf lobes	3 leaves/ plant, 15-18 th node		x	
Lobe depth	Scale (low, medium, high)		x	
Male corolla Ø	One flower/plant in anthesis (mm)	Cmale	x	x
Female corolla Ø	One flower/plant in anthesis (mm)	Cfem	x	x
Peduncle length, female flower	One flower/plant (cm)		x	
Peduncle length, male flower	One flower/plant (cm)		x	
Ovary length	One flower/plant (mm)	OVL	x	x
Ovary maximum Ø	One flower/plant (mm)	OVD	x	x
Ovary shape index	Length/diameter ratio	OVSI	x	x
Phenological traits				
First female flower in anthesis	Days after transplant		x	
Fruit setting	Days after transplant		x	
Fruit harvest	Days after transplant		x	
Fruit traits (3 melons/plant)				
Fruit weight	Measure with a digital weight (g)	FW	x	x
Length	cm	FL	x	x
Maximum Ø	cm	FD	x	x
Fruit shape index	Length/diameter ratio	FSI		x
Fruit shape	Scored from 1 (round) to 7 (elongate), as in IPGRI	FS	x	x
Blossom scare size	Scored between 1 (Ø 5mm) and 4 (Ø 2cm)	BS	x	x
External color	H* values with hand colorimeter	ECol	x	x
Flesh color	Measured as the external fruit color	FICol	x	x
Spots color	Scale from 0.5 (no spots) to 4.75 (brown spots)		x	
Spots distribution pattern	Scale (0.5= no spots, 1= dots, 1.5= no form, 2=band)			
Stripes	Scale from 1 (no stripes) to 4 (many stripes)		x	
Netting	Presence/absence		x	
Sutures	Presence/absence			
Abscission	Scale 0 (no strength needed) to 4 (no separable);	ABS	x	x
Flesh width	From the skin to seed cavity(cm)	FLW	x	x
Skin width	cm		x	
Seed cavity Ø	cm	SCD	x	x
Flesh firmness	Measured twice with a penetrometer (Newtons)	FIFs	x	x
Solid soluble content	Measured with a hand refractometer (°Brix)	SSC	x	x
Fruit harvest date	Days from transplant to mature stage		x	
No. fruits/plant	Recorded 90 days after transplant	Nmel	x	x

Ø: diameter

4.2.1 Statistical analyses

An ANOVA was carried out to detect significant differences among blocks for each time period (2009 and 2010). When no differences among blocks were detected, trait data were grouped. Data were transformed (Ln) after individual examination of their respective normal probability plot. Normality was tested with a Shapiro-Wilk test, P value < 0.05 . Pearson correlation coefficients (r_p) between traits were calculated independently for each evaluation. Correlations were considered significant at P value < 0.05 . Means, standard deviations, trait distribution, pair wise correlation, and ANOVA were conducted with SPSS (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc).

4.2.2 Map construction and QTL analyses

DNA was extracted from young leaf tissue of each genotype using Plant DNAzol kit (Invitrogen, Germany) according to the recommendations of the supplier.

The genotyping of the RIL population was carried out with SSR and EST-SSR markers [Danin-Poleg et al., 2001; Fazio et al., 2002; Ritschel et al., 2004; Gonzalo et al., 2005; Fukino et al., 2007; Fernández-Silva et al., 2008; ICuGI (www.icugi.org)], as well as other markers described before as associated to several resistance genes: MarkerD (SCAR, Dogimont et al., 2008) linked to *Aphid gossipi* (*Vat*), PM1-SCAR, PM2-CAPS, PM3-CAPS, PM4-dCAPS, PM5-CAPS and PM6-SCAR associated to powdery mildew resistance (Yuste-Lisbona et al., 2011a), UAL-1 (SCAR, Noguera et al., 2005) linked to the andromonoecius (*a*) locus, and MRGH63 (melon resistant gen homologue), reported as a NBS (nucleotide binding site) R-gene (García-Mas et al., 2001; Van Leeuwen et al., 2005) as being linked to race 5 of *P. xanthii* (Yuste-Lisbona et al., 2011a). PCR amplifications were carried out following author indications, and data analyses were performed using either gel (2-3% agarose gels in SB buffer (10mM sodium boric acid) or capillary electrophoresis by using a CEQ 2000XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA) with labelled primers (WellRED fluorescent dyes D2, D3 or D4).

Mapping was carried out with JoinMap® 4.0 software (VanOoijen, 2006). Markers data were assigned to linkage groups (LG) using a minimum LOD score of 4.0 and a recombination frequency value of 0.3. Kosambi mapping function (Kosambi, 1944) was used to translate

recombination frequency into centimorgans (cM). Segregation ratio distortion at each marker locus was tested against the expected ratio 1:1 for a F7:F8 population using a χ^2 test. Heterozygosity was also checked. QTL analyses were conducted separately for both RIL evaluations (2009 and 2010) by using MapQTL® 5.0 software (VanOoijen, 2004), MapChart © (Voorrips, 2002). Interval Mapping (IM) was used to detect consistent QTL. Afterwards, the closest markers to the QTL peaks were selected as cofactors and tested with the automatic cofactor selector tool. Multiple QTL mapping (MQM) was then performed with the selected set of cofactors, and LOD scores, phenotypic variation, QTL position and additive effects were then estimated. A permutation test (1000 cycles P value < 0.05) was used to determine the LOD threshold with a confidence interval of 90 or 95%.

4.3 Results

4.3.1 RIL characterization and statistical analyses

Mean, maximum, minimum and mode values of characters from plants of 'TGR-1551', 'Bola de Oro', F1 and the RIL population, evaluated only in 2009, are shown in Table 4.2. Since no significant differences were observed among the length of each of the 10th-11th, 11th-12th, 12th-13th, 13th-14th and 14th-15th internodes of the main vein, as well as among the width of the 10th-11th and 14th-15th internodes, the mean values of those internodes were used for both traits, length and width of internodes. Heterosis and transgressive inheritance were detected for the main vein length measured at 60 days after transplant, the mean of internode length and the number of nodes (both countings). Twenty-five percent of RIL plants had non-lobed leaves, like 'TGR-1551' and F1, whereas most of them (75%) presented leaves with five shallow lobes, like 'Bola de Oro'. Fruits from 'TGR-1551' and the F1 had yellow-brown spots, whereas they were absent in 'Bola de Oro'. Spots in fruit skin were present in some fruits from the RIL population, displayed in orange or green bands, although most of fruits showed no spots (Fig 4.2). Stripes, sutures, and netting, were also observed in fruits from the RIL population. However, since most of the fruits did not show sutures and netting, the presence of stripes was the only trait evaluated in the RIL population. Most of RIL fruits did not show stripes, like 'TGR-1551' and F1, and only around a 21% exhibited a clearly presence of them. 'Bola de Oro' showed few stripes in some fruits.



Figure 4.2. Different melon fruits from the RIL population, 'TGR-1551' x 'Bola de Oro'.

Regarding the traits evaluated in both years in (2009 and 2010), the means and standard deviations from the RIL population, the parental lines and their F1 are shown in Table 4.3. No significant differences were detected among blocks in most of the traits. No transgressive inheritance has been observed, although we did find heterosis for some characters in both years (fruit length, diameter, flesh width, weight, main vein length (first measurement, 45 days after transplant) and leaf area).

Table 4.2. Mean, maximum, minimum and mode values of most of the morphological traits observed in a RIL population, their parental lines ('TGR-1551' and 'Bola de Oro') and the F1 uniquely for the season spring-summer 2009, since no significant QTL were detected for these traits in preliminary QTL analyses.

Traits 2009	Mean value				RIL population		
	'TGR-1551'	'Bola de Oro'	F1	mean	min	max	mode
Main vein length 2nd measurement (m)	3.61	3.23	4.90	4.05	1.85	7.05	3.20-4.20
Nº Nodes 1st measurement	23.00	24.00	27.30	26.20	15.00	45.00	24.00-25.00
Nº Nodes 2nd measurement	42.70	42.30	43.60	45.79	18.00	70.00	40.00
Internode 10-15 length media (cm)	5.38	7.18	8.88	7.37	4.65	11.61	7.1
Internode width 10-11 14-15 media (mm)	15.60	10.80	13.32	12.70	7.09	18.29	11.00
Petiole length (cm)	26.10	22.90	22.50	15.69	8.00	26.20	12.00
Nº leaf lobes	1 lobe	5 lobes	1 lobe	75% 5 lobes	1 lobe	5 lobes	5 lobes
Lobe leaf depth	one lobe	low-medium	low	low	one lobe	high	low
Peduncle fem length (cm)	2.13	1.21	1.82	3.29	0.50	12.50	2.00
Peduncle masc length (cm)	2.10	3.30	2.15	2.20	0.53	8.00	1.50
Skin width (cm)	0.10	0.48	0.10	0.17	0.10	0.70	0.10
Stripes	1.00 (no stripes)	1.28 (no stripes)	1.00 (no stripes)	2.12 (very low)	1.00 (no stripes)	5.00 (high)	1.00 (no stripes)
Spots color	1.83 (yellow-orange)	0.50 (no spots)	1.83 (yellow-orange)	1.55 (yellow)	0.50 (no spots)	4.75 (brown)	0.5 (no spots)
Spots distribution pattern	1.33 (dots)	0.50 (no spots)	1.70 (no pattern)	1.01 (dots)	0.5 (no spots)	3.00 (band)	0.5 (no spots)
Date 1st female flower (days)	56.66	47.66	50.33	45.24	27.00	58.00	48.00
Date 1st fruit setting (days)	61.33	54.67	57.33	54.64	35.00	67.00	56.00
Date 1st fruit harvested (days)	96.00	99.00	102.80	96.53	69.00	115.00	98.00

Table 4.3. Mean, standard deviation, and maximum and minimum values of several morphological traits observed in a RIL population, their parental lines ('TGR-1551' and 'Bola de Oro') and the F1 for two periods (spring-summer 2009 and 2010).

Trait	RIL						'TGR-1551'						'Bola de Oro'						F1									
	Mean		St.dev.		Mean		St.dev.		Mean		St.dev.		Mean		St.dev.		Mean		St.dev.		Mean		St.dev.					
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010				
Main vein length (m) 45days	2.00	1.65	0.50	0.39	1.30	1.17	0.08	0.06	1.82	2.15	0.12	0.28	2.50	2.75	0.42	0.14	4896.13	3563.38	1543.87	1045.94	4497.86	3464.00	854.07	584.40	6802.75	5366.08	1593.43	888.74
Female corolle diameter (mm)	46.56	38.33	10.27	8.30	39.00	37.54	3.00	5.30	63.30	35.90	1.29	2.39	50.60	34.40	2.19	3.53	41.97	34.99	7.76	6.31	53.48	37.09	2.31	3.60	46.10	33.90	4.99	3.88
Male corolle diameter (mm)	18.56	17.50	3.80	3.10	20.08	19.80	1.45	2.06	19.12	13.90	2.42	2.47	22.30	18.00	2.95	0.53	8.98	7.98	1.77	1.40	8.30	8.00	0.37	0.60	7.94	7.86	1.04	1.60
Ovary max diameter (mm)	1015.00	1188.59	493.00	716.38	834.60	864.50	290.14	203.45	1051.30	978.80	154.20	253.94	2498.15	2967.11	303.21	253.57	16.53	17.34	4.61	4.98	14.16	15.54	1.40	1.42	29.10	28.60	1.77	2.09
Fruit length (cm)	10.90	11.33	1.87	2.27	9.80	9.91	0.75	1.58	11.60	10.37	0.60	2.86	13.51	14.32	0.85	0.50	10.90	11.33	1.87	2.27	9.80	9.91	0.75	1.58	13.51	14.32	0.85	0.50
Fruit max diameter (cm)	3.49	3.07	1.02	0.87	3.60	3.20	0.78	0.68	3.30	2.60	0.48	0.30	3.70	3.47	0.98	0.62	3.49	3.07	1.02	0.87	3.60	3.20	0.78	0.68	3.70	3.47	0.98	0.62
Fruit shape index	5.90	6.19	0.97	1.07	6.90	6.72	0.70	0.49	5.28	6.76	0.40	1.05	6.70	6.73	0.33	0.57	5.90	6.19	0.97	1.07	6.90	6.72	0.70	0.49	6.70	6.73	0.33	0.57
Seed cavity diameter (cm)	2.42	2.59	0.69	0.77	1.44	1.59	0.45	0.34	3.20	2.36	0.22	0.544	3.37	3.60	0.39	0.22	2.42	2.59	0.69	0.77	1.44	1.59	0.45	0.34	3.37	3.60	0.39	0.22
Flesh width (cm)	65.52	73.12	14.73	9.12	76.75	66.62	6.00	24.07	15.35	55.20	3.81	18.10	75.09	76.55	8.00	10.25	65.52	73.12	14.73	9.12	76.75	66.62	6.00	24.07	75.09	76.55	8.00	10.25
Solid soluble content	5.40	6.20	1.40	1.98	4.20	4.10	0.81	0.81	7.00	12.90	1.37	2.33	3.80	4.57	0.53	0.61	5.40	6.20	1.40	1.98	4.20	4.10	0.81	0.81	3.80	4.57	0.53	0.61
Blossom scar size	1.30	1.30	0.59	0.66	1.00	1.00	0.00	0.00	1.20	1.46	0.42	0.50	1.00	1.00	0.00	0.00	1.30	1.30	0.59	0.66	1.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Abscission	2.35	2.12	0.96	1.16	1.20	1.95	0.42	0.89	2.70	1.86	0.48	0.79	2.90	2.00	0.30	1.00	2.35	2.12	0.96	1.16	1.20	1.95	0.42	0.89	2.90	2.00	0.30	1.00
External color H*	85.33	85.41	5.82	5.66	78.89	82.90	3.65	3.15	89.93	83.53	2.47	2.93	80.08	80.80	1.31	1.28	85.33	85.41	5.82	5.66	78.89	82.90	3.65	3.15	80.08	80.80	1.31	1.28
Flesh color H*	101.80	102.24	4.90	3.88	100.70	102.20	3.00	2.45	104.30	100.70	4.00	4.10	101.40	102.00	2.20	1.70	101.80	102.24	4.90	3.88	100.70	102.20	3.00	2.45	101.40	102.00	2.20	1.70
Nºmelon/plant	4.92	4.20	2.87	2.40	3.33	4.20	2.16	1.10	6.20	4.30	0.98	0.81	5.20	4.75	0.75	0.50	4.92	4.20	2.87	2.40	3.33	4.20	2.16	1.10	5.20	4.75	0.75	0.50

4.3.2 Phenotypic correlation

Most of the traits were significant and positively correlated and correlation values were consistent for both years (Table 4.4). Only correlation values above 0.4 (absolute value) were considered as being informative.

Traits related with fruit size, such as weight, maximum diameter, length, seed cavity diameter, flesh width, were positive and significant correlated among them, with correlation values (r_p) ranging from 0.6 to 0.8. The number of melons per plant was significant and negatively correlated with these traits ($r_p=-0.4$, only in 2010), though. Most of the traits related with fruit shape (fruit shape index, fruit shape, fruit length, ovary length, ovary shape index) were significant and positively correlated among them in both trials ($r_p= 0.4$ to 0.8). Ovary maximum diameter was negative correlated with the majority of them except for ovary length ($r_p= -0.4$ to -0.5). The solid soluble content was negatively correlated with flesh firmness in both years ($r_p= -0.4$ to -0.5). Both female and male corolla diameters were positively correlated between them ($r_p= 0.5$).

Table 4.4. Pair wise correlations detected for different morphological traits evaluated in two periods (2009 above diagonal and 2010 below it) in a recombinant inbred line population obtained from the cross between 'TGR-1551' and 'Bola de Oro'.

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

	Fruit Weight	Fruit Max. Diameter	Fruit Length	Fruit Shape Index	Seed Cavity Diameter	Flesh Width	Solid Soluble Content	Fruit Shape Index	Fruit Shape	Ov. Max. Diameter	Ov. Length	Ov. Shape Index	Flesh Firmness	Blossom Scar Size	Number Melons	Female Corolla	Male Corolla	External Colour	Flesh Colour	Main Vein Length	Leaf Area	Abscission
Fruit Weight	,879**	,769**	,519**	,839**	,223**	,312**	,112	,153	,181	-,045	,004	-,191*	-,024	,080	,071	-,021	,227*	,343**	,117			
Fruit Max. Diameter	,812**	,513**	,708**	,870**	-,136	-,060	-,048	,171	-,097	-,109	,157	-,183	,086	,069	,094	-,102	,171	,287**	,070			
Fruit Length	,810**	,460**	,203*	,543**	-,286**	,813**	,430**	-,307**	,558**	,154	-,274**	-,193*	-,158	,027	-,025	,167	,191	,328**	,178			
Seed Cavity Diameter	,608**	,730**	,333**	,282**	-,244*	-,227*	-,159	,184	-,101	,084	,172	-,035	,122	,007	,004	-,010	-,132	,138	,071			
Flesh Width	,853**	,870**	,570**	,475**	-,002	,071	,044	,045	-,026	-,215*	,046	-,147	,075	,130	,085	-,075	,297**	,303**	,074			
Solid Soluble Content	-,200	-,012	-,288**	-,094	-,003	-,250**	-,071	,137	-,147	-,209*	-,018	-,008	-,064	-,156	-,095	-,207*	,023	-,291**	-,310**			
Fruit Shape Index	,342**	-,158	,790**	-,105	,061	-,294**	,519**	-,454**	,483**	,744**	-,412**	-,107	-,223*	,012	-,109	,253*	,082	,183	,172			
Fruit Shape	,314**	-,023	,717**	-,054	,095	-,238*	,796**	-,064	,365**	,347**	-,075	-,170	,024	,089	-,150	,019	-,021	,175	-,090			
Ov. Max. Diameter	-,014	,153	-,273*	,157	,072	,090	-,418**	-,415**	,145	-,557**	,350**	,029	,345**	,123	,063	-,104	-,076	-,030	-,139			
Ov. Length	,146	-,102	,403**	-,106	-,058	-,244*	,494**	,212*	,728**	-,262**	-,120	,088	-,029	,029	-,123	,050	-,092	-,059	,003			
Ov. Shape Index	,136	-,192	,538**	-,194	-,098	-,262*	,722**	,719**	,690**	-,439**	-,104	-,180	-,104	-,031	-,164	,131	-,031	-,033	,102			
Flesh Firmness	-,062	-,301**	,031	-,160	-,253*	-,403**	,255*	,132	,285**	-,231*	,126	,285**	-,070	-,052	-,212*	,132	-,046	-,064	,218*			
Blossom Scar Size	-,081	,166	-,323**	,202	,080	,172	-,437**	,342**	-,275**	-,353**	-,053	-,136	,358**	,273**	,198*	-,213*	-,013	,076	-,109			
Number Melons	-,444**	-,439**	-,393**	-,401**	-,422**	-,120	-,186	-,095	-,102	-,018	,160	,067	,159	,076	-,040	,089	,020	,056	,079			
Female Corolla	-,110	-,015	-,150	,035	-,065	,105	-,126	-,066	,091	-,231*	-,201	-,231*	-,021	,248*	,097	,503**	,053	-,007	-,043			
Male Corolla	,072	,073	,012	,140	,012	-,041	-,035	,011	,176	-,032	-,157	,066	,156	-,544**	-,045	-,019	-,078	,227*	-,041			
External Colour	-,243*	-,148	-,250*	-,120	,130	,197	-,157	,162	-,072	-,161	,011	-,020	-,051	,047	-,060	-,063	,070	,020				
Flesh Colour	-,043	-,160	,078	-,169	-,156	-,217*	,169	,228*	-,203	,048	,172	,110	-,280**	-,054	-,040	-,011	-,133	,082	,414**			
Main Vein Length	,181	,117	,209	-,079	,161	-,002	,150	,235*	-,319**	,082	,304**	,000	,124	,211	,071	-,003	,050	,002	,264*	,021		
Leaf Area	,262*	,342**	,196	,185	,296**	,062	,017	,056	-,128	-,048	,057	-,088	,192	,017	,292**	,068	-,026	-,010	,364**	-,056		
Abscission	,236*	,142	,172	,247*	,104	-,297**	,100	,034	,350**	-,152	-,081	-,020	,109	-,261*	,184	-,148	-,079					

4.3.3 Mapping and QTL analyses

Linkage map was obtained with 216 molecular loci: 138 SSR; 68 EST-SSRs; 4 SCARs, 4 CAPSs, 1 dCAPS; and a phenotypic trait (*andromonoecius*, *a*). Markers described as linked to powdery mildew resistance (PM1-SCAR, PM2-CAPS, PM3-CAPS, PM4-dCAPs, PM5-CAPS, PM6-SCAR and MRGH63) were localized on linkage group (LG) V, as well as MarkerD was also set on LG V, co-segregating with them. The molecular marker UAL-1 was situated on LG II at a distance of 7 cM from *a* locus.

The percentage of skewed markers ($P < 0.01$) was 11% and a heterozygosity of 2.2% was observed in the RIL population. Loci were distributed among 22 linkage groups, regrouped into 12, and were designed following the nomenclature proposed by Périn (2002a). The linkage map spanned a total distance of 992.82cM with an average of 4.6cM between markers. Linkage group size ranged from 48.8 cM of LG IX to 136.5 cM of LG XI. The LG IV was the most saturated linkage group, with an average distance of 3.29 cM among markers, and LG II was the least saturated one, with an average distance of 7.38 cM among markers. The longest gap, 23.4 cM, was located between the markers ECM133 and ECM197 in LG VI.

Fifty-six QTL were detected for the 21 traits estimated in both years (Table 4.5, Fig. 4.3): FW=4, FD=6, FL=5, SCD=4, FLW=4, SSC=2, FSI=4, FS=1(only for 2010), OVD=3, OVL=2, OVSI=2, FIFs=2, BS=2, Cfem=2, Cmale=2, MVL=2, ABS=1 (only for 2009), ECol=3, FICol=1 (only for 2009), Nmel=2 and LA=2. These QTL were distributed along 11 linkage groups (LG I=1, LG II=21, LG IV=11, LG V=3, LG VI=4, LG VII=2, LG VIII=2, LG IX=1, LG X=3, LG XI=5, LG XII=3). Sixteen of these QTL were consistently detected, meaning that they were observed in the same map location for both years: two QTL (two for each year) for FL and one (one for in each year) for SCD (LG XI), FSI, OVSI, OVL, BS, and Cfem. For traits such as SDC (LG IV), SSC and FD, the position of the detected QTL for both evaluations varied slightly (QTL overlapped). For Nmel, FIFs and FW, one QTL was identified in the same LG for each year, although they were quite distant from each other.

The proportion of the phenotypic variance explained by the QTL ranged from 10.1 % (*cmale12.1* in 2009 for a confidence interval of 90%) to 37% (*fl2.1* in 2010 for 95%). Major QTL ($R^2 \geq 20\%$) were detected for FW, FD, FL, SSC, FSI, FS, OVSI, BS, Cfem, ranging from 21.1% (*fs2.1* in 2010) to the 37% (*fl2.1* in 2010). The direction of additive effects of the QTL was variable among

traits, except for those with consistent QTL between evaluations. Positive additive effect indicated that 'TGR-1551' alleles improved the trait. The additive effects were provided by 'Bola de Oro' alleles in QTL linked to female and male corolla diameter, leaf area, main vein length, blossom scar size, solid soluble content, flesh color and abscission. On the other hand, 'TGR-1551' alleles increased fruit shape and fruit shape index, both length and shape index of ovary, and flesh firmness. Additive effects for the rest of the characters showed variable directions.

Table 4.5. Quantitative trait loci (QTL) identified for different traits evaluated in two time periods in a RIL population derived from the cross 'TGR-1551' x 'Bola de Oro'. Data of linkage groups (LG), position (in centimorgans), logarithm of odds (LOD) score, LOD threshold (LOD T), percentage of phenotypic variation (%Expl.), and additive effects are indicated.

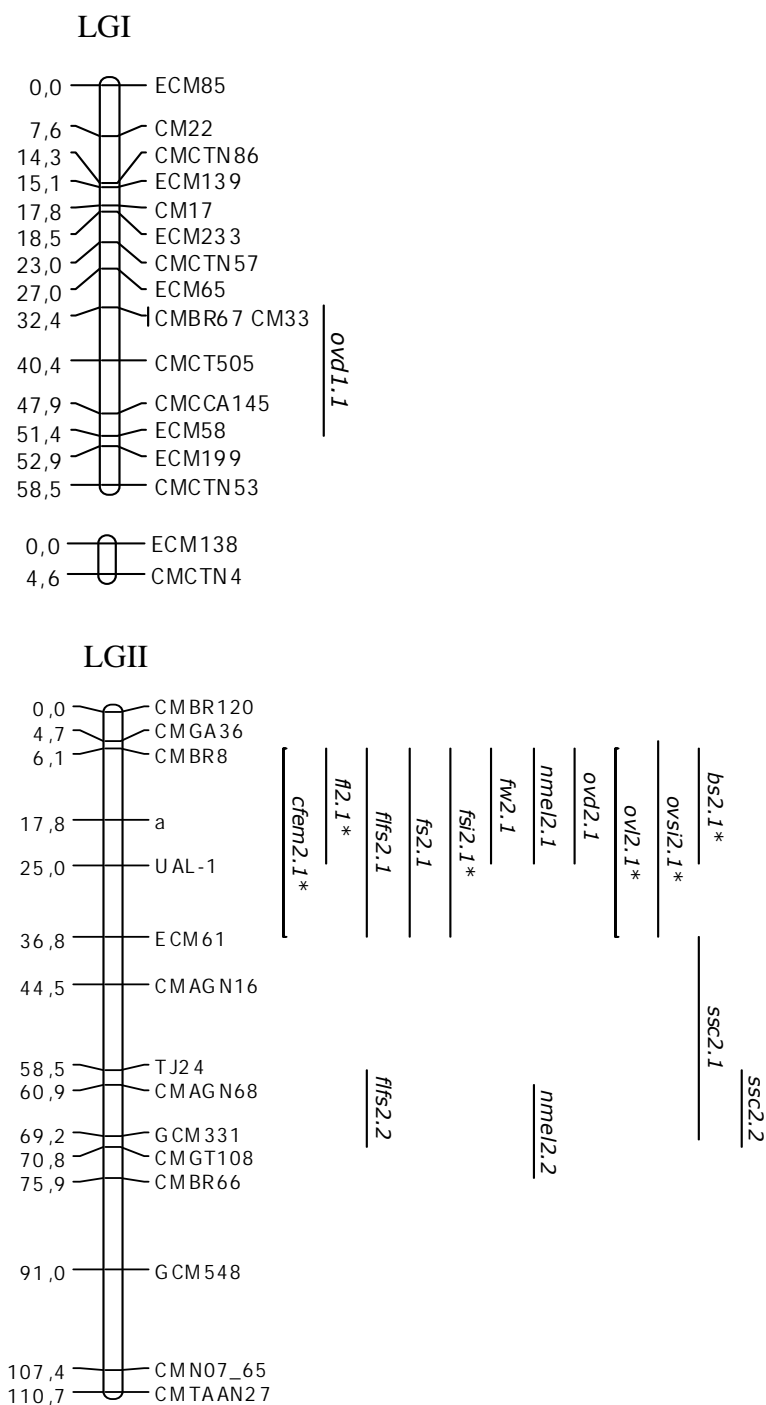
Trait	LG	QTL	Trial	Position (cM)	Flanking markers	Max LOD	Position max LOD	Locus max LOD	% Expl.	Add.Ef.	Sig.	LOD T.	Allele
Abscission	XI	<i>abs11.1</i>	2009	46.80-59.20	CMN04_03-CMAGN45	2.81	58.180	CMN04_03	11.2	-0.30	95	2.8	BO
Blossom Scar Size	II	<i>bs2.1</i>	2009	6.10-24.90	CMBR8-UAL-1	4.86	17.796	Andromonoecius	20.4	-0.27	95	2.7	BO
	II	<i>bs2.1</i>	2010	6.10-24.90	CMBR8-UAL-1	3.76	17.796	Andromonoecius	19.8	-0.25	95	2.9	BO
Female Corolla	II	<i>cfem2.1</i>	2009	6.10-36.80	CMBR8-ECM61	6.91	24.952	UAL-1	25.7	-4.26	95	2.9	BO
	II	<i>cfem2.1</i>	2010	6.10-24.90	CMBR8-UAL-1	3.25	17.796	Andromonoecius	16.0	-2.96	95	2.8	BO
Male Corolla	XII	<i>cmale12.1</i>	2009	59.90-67.80	CSAT425-CMBR77	2.48	66.887	CMBR97	10.1	-2.15	90	2.4	BO
	VIB	<i>cmale6.1</i>	2010	29.20-62.90	ECM133-CMGT108	3.07	55.348	ECM124	15.0	-2.24	95	2.9	BO
External Color	VIIC	<i>ecol7.1</i>	2009	6.00-19.40	ECM204-CMGA15	2.96	19.442	CMGA15	10.4	-1.67	95	2.9	BO
	XB	<i>ecol10.1</i>	2009	19.40-25.30	CMT134b-GCM344	3.50	22.286	CMGA165	12.4	1.78	95	2.9	TGR
	IXA	<i>ecol9.1</i>	2010	17.00-29.00	CMN04_19-ECM150	3.07	29.621	ECM150	14.5	-1.83	95	2.9	BO
Flesh Color	V	<i>fc05.1</i>	2009	99.00-108.00	CM16-CMBR107	2.63	104.790	CMBR123	11.0	-1.38	95	2.5	BO
Fruit Maximum Diameter	IVA	<i>fd4.1</i>	2009	43.30-52.30	ECM106-CMBR104	5.59	51.287	CMBR35	15.2	-0.68	95	2.8	BO
	IVA	<i>fd4.2</i>	2010	52.30-60.30	CMBR104-CMN06_25	3.99	59.122	ECM53	16.0	-0.75	90	2.5	BO
	V	<i>fd5.1</i>	2009	64.30-73.40	PM6-SCAR-PM3-CAPS	3.17	72.244	PM4-dCAPS	7.8	0.48	95	2.8	TGR
	VIC	<i>fd6.1</i>	2009	2.00-5.00	CMBR39-GCM209	4.09	8.612	GCM112	10.7	-0.57	95	2.8	BO
	XI	<i>fd11.1</i>	2009	94.00-123.90	CMATN89-ECM147	6.33	113.183	CM53	17.4	0.82	95	2.8	TGR
	XA	<i>fd10.1</i>	2010	0.00-5.50	ECM78-MU7251	3.82	3.419	CSWCT22A	14.9	-0.73	90	2.5	BO
Fruit Length	II	<i>fl2.1</i>	2009	6.10-24.90	CMBR8-UAL-1	9.69	17.796	Andromonoecius	29.0	2.56	90	2.8	TGR
	II	<i>fl2.1</i>	2010	6.10-24.90	CMBR8-UAL-1	9.93	17.796	Andromonoecius	37.5	2.96	95	2.8	TGR
	IVA	<i>fl4.1</i>	2009	52.30-60.35	CMBR104-ECM207	5.91	60.356	CMN06_25	16.6	-1.95	90	2.8	BO
	IVA	<i>fl4.1</i>	2010	52.30-60.35	CMBR104-ECM207	7.63	60.356	CMN06_25	26.8	-2.45	95	2.8	BO
	VIIIB	<i>fl8.1</i>	2009	44.00-58.00	CMACC146-CMBR59	4.09	53.523	GCM241	10.2	1.50	90	2.8	TGR
Flesh Firmness	II	<i>fff2.1</i>	2009	6.10-36.80	CMBR8-ECM61	4.38	24.952	UAL-1	18.0	5.84	95	2.8	TGR
	II	<i>fff2.2</i>	2010	58.40-70.78	TJ24-CMGT108	2.95	69.190	GCM331	14.0	2.74	95	2.8	TGR
Flesh Width	IVA	<i>flw4.1</i>	2010	52.30-60.35	CMBR104-ECM207	3.40	60.356	CMN06_25	14.9	-0.26	90	2.5	BO
	IVA	<i>flw4.2</i>	2010	12.39-24.90	CMN05_17-CMN05-60	3.19	15.787	CMBR61	11.6	0.24	90	2.5	TGR
	VIB	<i>flw6.1</i>	2009	17.60-29.20	GCM303-ECM133	2.66	21.936	ECM81	11.7	-0.20	90	2.6	BO
	VIIIB	<i>flw7.1</i>	2010	20.20-26.40	CMTAN87-CMBR27	2.57	25.833	CMBR12	10.9	-0.23	90	2.5	BO
Fruit Shape	II	<i>fs2.1</i>	2010	6.10-36.80	CMBR8-ECM61	4.63	24.952	UAL-1	21.1	0.33	95	2.9	TGR
Fruit Shape Index	II	<i>fsi2.1</i>	2009	6.10-36.80	CMBR8-ECM61	8.26	24.952	UAL-1	22.4	0.17	95	2.9	TGR
	II	<i>fsi2.1</i>	2010	6.10-36.80	CMBR8-ECM61	6.63	24.952	UAL-1	28.8	0.20	95	2.9	TGR
	VIIIB	<i>fsi8.1</i>	2009	44.00-54.80	CMACC146-CMBR59	3.91	53.523	GCM241	9.6	0.11	95	2.9	TGR
	XA	<i>fsi10.1</i>	2009	0.00-5.50	ECM78-MU7251	3.10	0.000	ECM78	7.5	0.10	95	2.9	TGR
Fruit Weight	IVA	<i>fw4.1</i>	2009	28.47-38.00	CMBR90-ECM122	3.48	30.862	GCM336	13.9	-1.07	95	2.9	BO
	IVA	<i>fw4.2</i>	2010	52.30-60.35	CMBR104-ECM207	5.55	60.356	CMN06_25	21.3	-3.01	90	2.6	BO
	XI	<i>fw11.1</i>	2010	103.90-123.90	ECM148-ECM147	3.60	113.183	CM53	13.3	2.54	90	2.6	TGR
	II	<i>fw2.1</i>	2010	6.10-24.90	CMBR8-UAL-1	4.00	17.796	Andromonoecius	15.1	2.57	90	2.6	TGR
Leaf Area	XII	<i>la12.1</i>	2009	43.20-50.30	CMN01_38-TJ29	3.10	48.522	CMBR111	13.8	-5.49	95	2.7	BO
	IVA	<i>la4.1</i>	2010	52.30-60.30	CMBR104-CMN06_25	4.00	59.122	ECM53	18.7	-4.28	95	2.7	BO
Main Vein Length (45 days)	VIB	<i>mv6.1</i>	2009	0.00-4.34	ECM161-CMBR125	2.67	0.000	ECM161	11.0	-0.15	90	2.5	BO
	XII	<i>mv112.1</i>	2010	43.20-56.90	CMN01_38-CMBR150	2.58	50.298	TJ29	13.3	-0.12	90	2.7	BO
N°Melons/plant	II	<i>nmel2.1</i>	2009	6.10-24.90	CMBR8-UAL-1	3.91	17.796	Andromonoecius	16.5	-0.98	95	2.9	BO
	II	<i>nmel2.2</i>	2010	60.90-75.90	CMAGN68-CMBR66	2.51	70.784	CMGT108	12.1	0.77	90	2.5	TGR
Ovary Maximum Diameter	II	<i>ovd2.1</i>	2009	6.10-24.90	CMBR8-UAL-1	3.68	17.796	Andromonoecius	16.0	-0.50	95	2.9	BO
	V	<i>ovd5.1</i>	2010	58.60-67.50	GCM295-PM2-CAPS	3.35	64.277	PM6-SCAR	14.6	0.43	90	2.5	TGR
	IA	<i>ovd1.1</i>	2010	32.30-51.36	CMBR67-ECM58	2.53	47.858	CMCCA145	10.7	0.37	90	2.5	TGR
Ovary Length	II	<i>ovl2.1</i>	2009	6.10-24.90	CMBR8-UAL-1	2.58	17.796	Andromonoecius	11.2	1.01	90	2.9	TGR
	II	<i>ovl2.1</i>	2010	6.10-36.80	CMBR8-ECM61	3.61	24.952	UAL-1	17.1	1.17	95	2.9	TGR
Ovary Shape Index	II	<i>ovsi2.1</i>	2009	4.70-24.90	CMGA36-UAL-1	8.43	17.796	Andromonoecius	32.8	0.24	95	3.0	TGR
	II	<i>ovsi2.1</i>	2010	6.10-36.80	CMBR8-ECM61	6.72	24.952	UAL-1	29.4	0.24	95	3.0	TGR
Seed Cavity Diameter	IVA	<i>sca4.1</i>	2009	43.40-51.28	ECM106-CMBR35	3.94	43.397	ECM106	14.4	-0.33	95	2.8	BO
	IVA	<i>sca4.2</i>	2010	52.30-60.35	CMBR104-ECM207	3.07	51.287	CMBR35	13.0	-0.34	90	2.9	BO
	XI	<i>sca11.1</i>	2009	103.90-123.90	ECM148-ECM147	3.48	113.183	CM53	12.6	0.36	95	2.8	TGR
	XI	<i>sca11.1</i>	2010	103.90-123.90	ECM148-ECM147	3.43	113.183	CM53	14.7	0.40	90	2.6	TGR
Soluble Solid Content	II	<i>ssc2.1</i>	2009	36.80-60.90	ECM61-CMAGN68	5.96	58.459	TJ24	23.2	-0.65	95	3.0	BO
	II	<i>ssc2.2</i>	2010	58.50-70.78	TJ24-CMGT108	5.03	69.190	GCM331	22.9	-0.83	95	3.0	BO

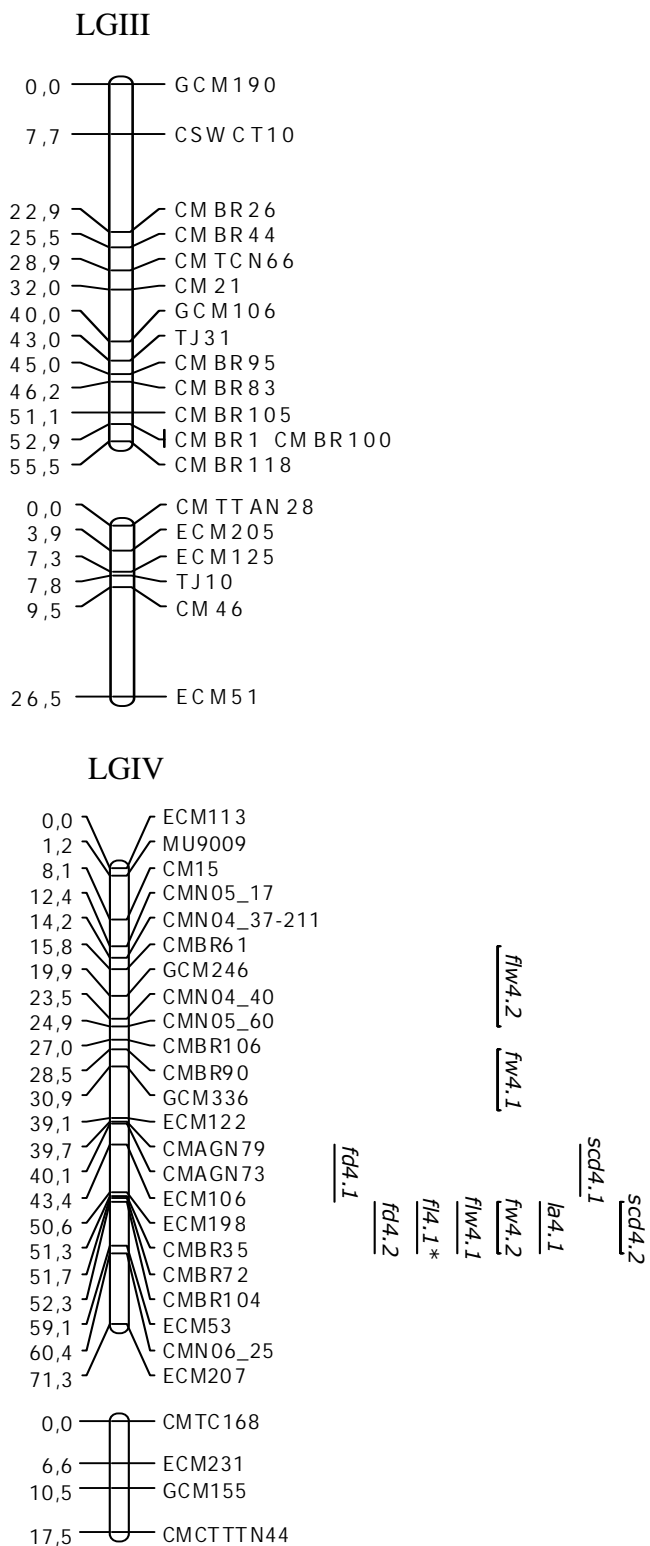
QTL (quantitative trait loci): designated by abbreviated trait name, linkage group number, and QTL number.

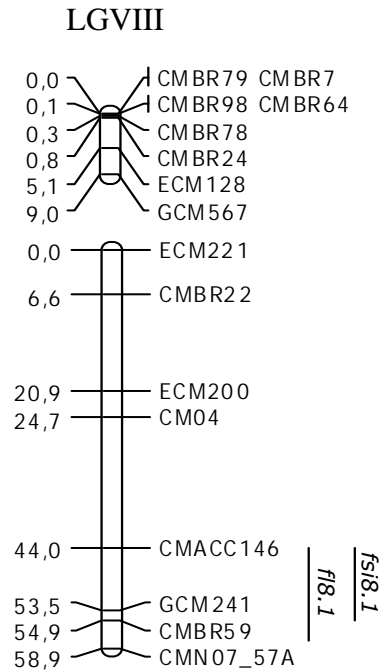
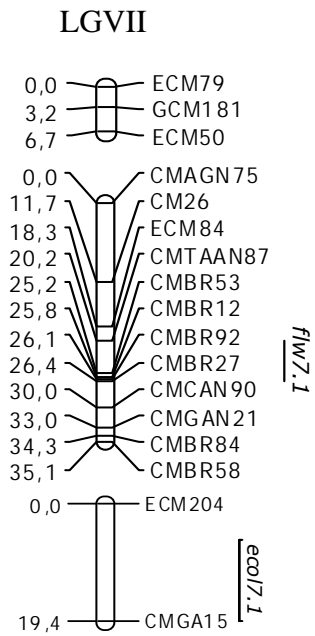
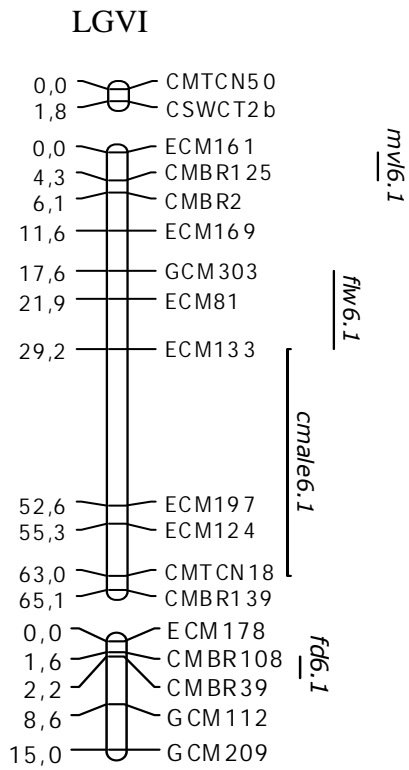
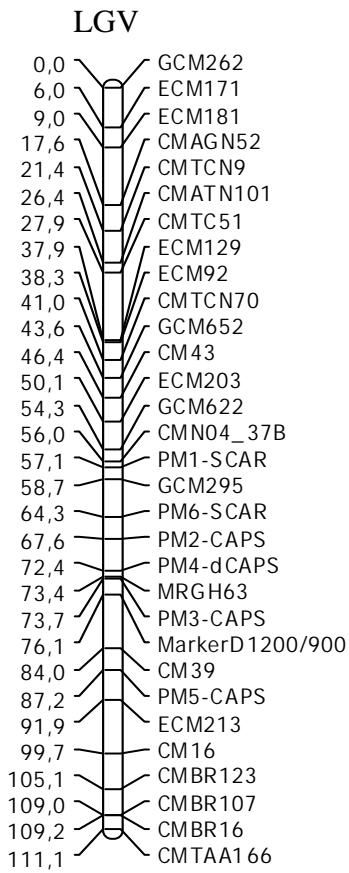
Sig.: QTL significant level.

Add. Ef (additive effects) = positive values mean that the alleles from the parental 'TGR-1551' increases the value for that trait, and the negative values mean that is the parental 'Bola de Oro' who increases the score.

Color code for QTL detected both years for a particular trait: QTL sharing the same precise location are indicated in bold; QTL just overlapping are emphasized in red, and QTL located in different regions of the same LG are shown in grey.







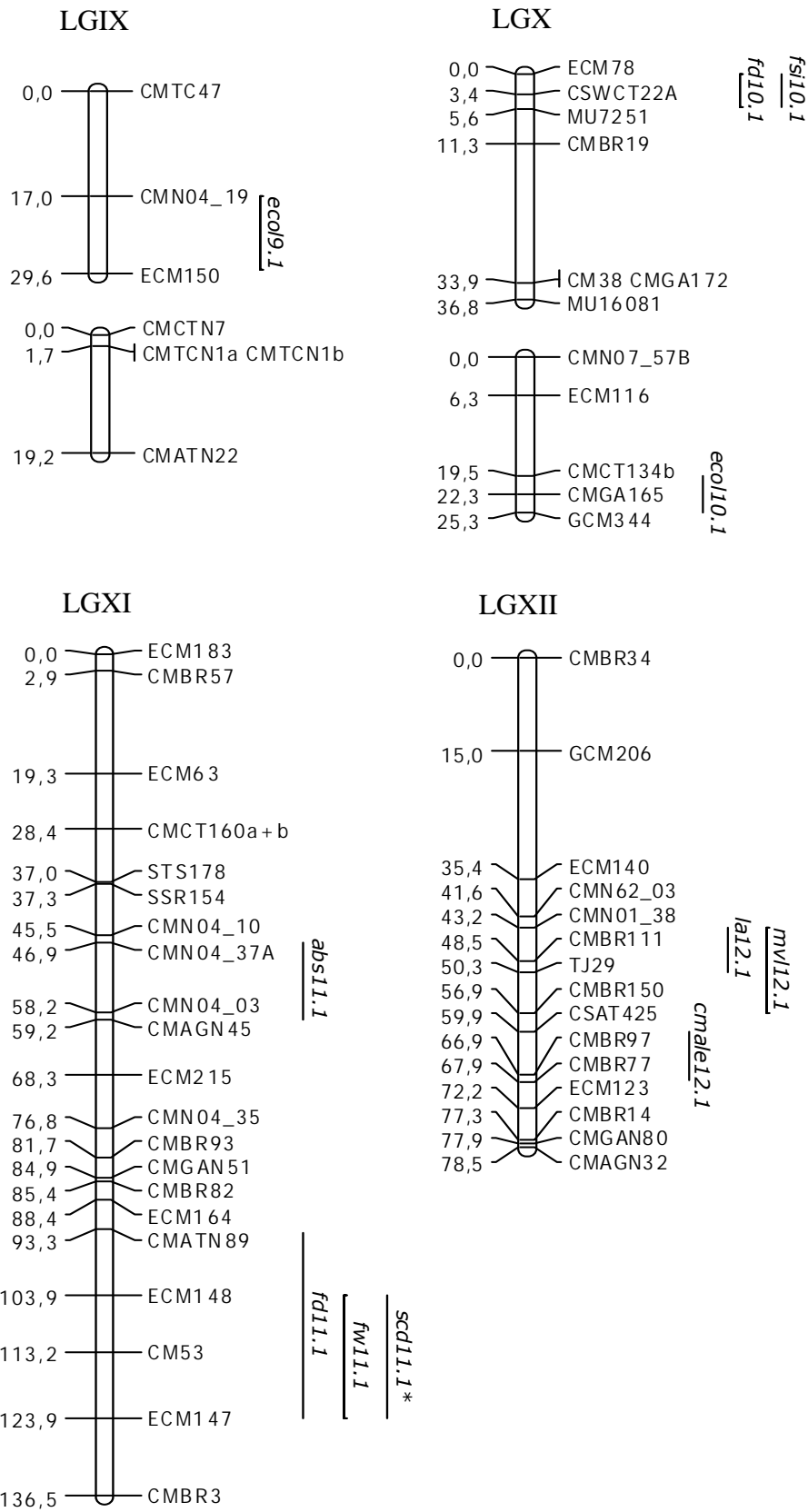


Figure 4.3 .Genetic linkage map based on a 114 RIL (F7:F8) population derived from the cross ‘TGR-1551’ x ‘Bola de Oro’. Linkage groups (I-XII) were named according to Périn et al. (2002a). Markers are shown on the right of each linkage group, while marker positions are on the left. Distances are indicated in centimorgans (cM). The putative location of the detected QTL is represented with a bar on the right side of each linkage group next to marker names.

LG II and IV (Fig. 4.3, 4.4, and 4.5) were the groups presenting the major number of QTL. Regarding QTL distribution, two different regions could be observed in LG II (Fig. 4.4): one region (II-1) between markers CMGA36 (4.7 cM) and ECM61 (36.8 cM), containing *a* locus (17 cM) and the SCAR marker UAL-1 (Noguera et al., 2005), and a second region (II-2) established between markers CMAGN16 and CMAGN66 (44-95 cM). In region II-1, we could find some consistent QTL, detected in both years in the same position, such as *fl2.1*, *fsi2.1*, *ovl2.1*, *ovsi2.1*, *bs2.1*, *cfem2.1*, as well as some which were only present in one trial: *fs2.1*, *fw2.1* in 2010, and *ovd2.1* in 2009. On the other hand, in region II-2 two QTL, almost consistent, for solid soluble content (*ssc2.1-ssc2.2*) were identified. Several QTL for Nmel and FIFs were detected for both years, although in different regions of that linkage group: *flf2.1*, *flfs2.2*, *nmel2.1* and *nmel2.2*. The QTL for solid soluble content (SSC) and flesh firmness (FIFs), important traits for breeding purposes, were estimated as negative correlated between them (Table 4.4), what means that the higher the SSC, the lower the flesh firmness. Another important trait, with two QTL in LG II, is the number of melons per plant (Nmel), which is negatively correlated with fruit size characters (Table 4.4), indicating that the more number of melon in a plant, the smaller size they have. The markers CMAGN16, CMBR8 and ECM61 were significant according to the Kruskal Wallis test carried out for resistance to powdery mildew, as described in our pervious chapter. CMAGN16, located between regions II-1 and II-2, was the only significant for races 1, 2 and 5 of *Podosphaera xanthii*, in the three different environments tested. No putative QTL were detected in this LG though.

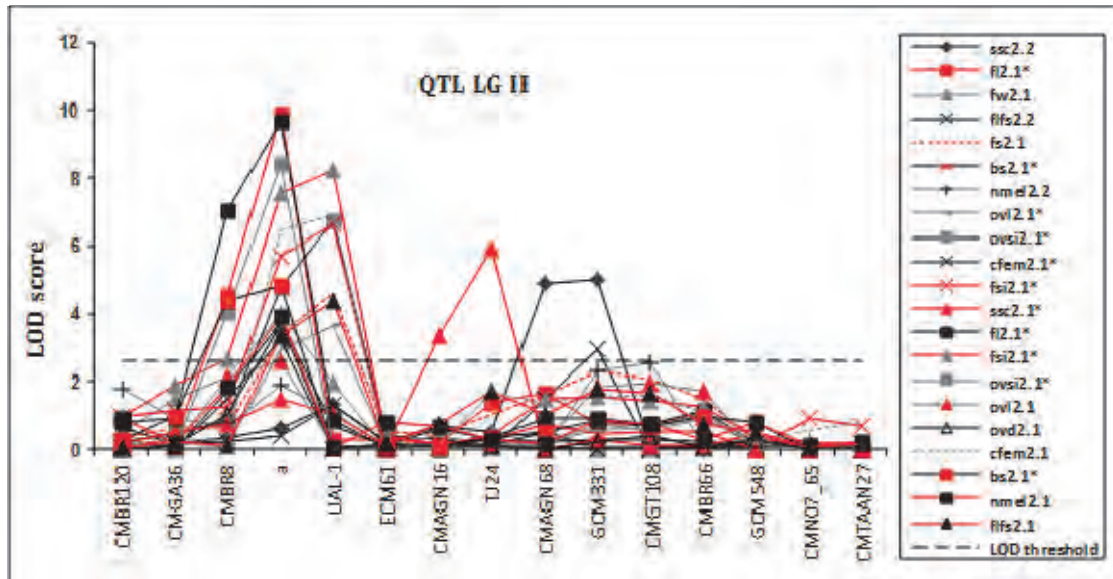


Figure 4.4. Quantitative trait loci detected in linkage group II of the genetic map of a RIL (F7:F8) population, derived from the cross 'TGR-1551' x 'Bola de Oro', by Multiple QTL Model (MQM) mapping analyses for different morphological traits (spring-summer 2009 and 2010). LOD threshold for a confidence of 99% and 1000 permutations is also represented. *=QTL detected for both years in the same position.

In LG IV (Fig. 4.5), we found consistent QTL for fruit length (*fl4.1*) and almost consistent QTL for fruit diameter (*fd4.1-fd4.2*) and seed cavity diameter (*scd4.1, scd4.2*). The QTL for FW (*fw4.1, fw4.2*) were located also in the same LG, although their positions among trials (2009 and 2010) were different. For FLW, two QTL were identified (*flw4.1, flw4.2*) for 2010. No significant QTL was found for this trait in 2009 in LG IV, though.

Overlapping QTL, as well as positive correlations among fruit and ovary sizes, were detected in LG II and V, as expected since both traits are linked, according to literature (Périn et al., 2002c). Flowers with round ovaries will develop generally into round fruits, and elongated ovaries into oval fruits. Two overlapping QTL, one for the fruit diameter and another for ovary diameter (*fd5.1* and *ovd5.1*), were also identified in LG V. They were located next to the PCR allele markers associated to the QTL *Pm-R*, linked to *Podosphaera xanthii* resistance 'in TGR-1551' (Yuste-Lisbona et al, 2011 a; b). The QTL *Ovd5.1* was flanked by the markers GCM295 and PM2-CAPS, this interval containing the marker PM6-SCAR, and being close to PM4-dCAPS. The QTL *fd5.1* was flanked by PM6-SCAR and PM3-CAPS, containing PM2-CAPS, PM4-dCAPS and the homologous resistant gene MRGH63. For these traits, maximum diameter of both fruit and ovary, the additive effect of the QTL were directed to 'TGR-1551' what, in this case, did not

affect the fruit quality regarding these traits, since similar values have been scored for both parental lines. Besides the LG II and V, where QTL for powdery mildew were localized, other QTL related with fruit and ovary sizes were detected in other linkage groups, such as ovary diameter in LG I, flesh width in LG VI and VII, fruit diameter in LG VI, X and XI, fruit length in LG VIII, fruit shape in LG VIII and X, and fruit weight and seed cavity diameter in LG XI. Also in this LG XI we detected the only QTL associated with abscission.

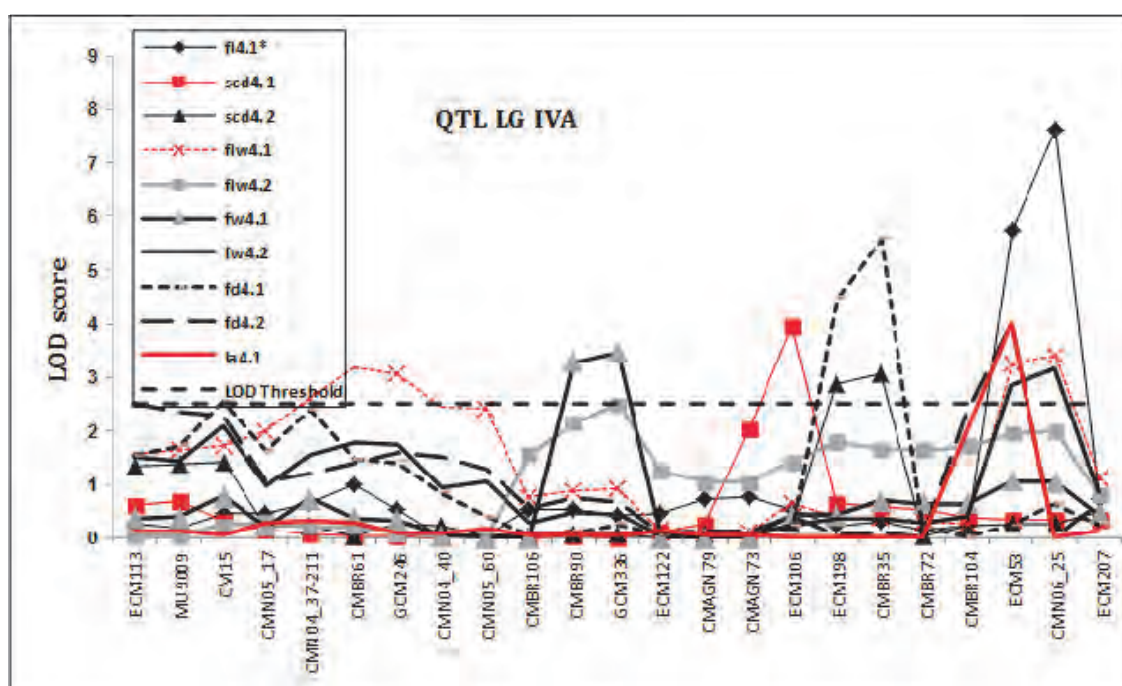


Figure 4.5. Quantitative trait loci detected in linkage group IV of the genetic map of a RIL (F7:F8) population, derived from the cross ‘TGR-1551’ x ‘Bola de Oro’, by Multiple QTL model mapping analyses for different morphological traits (spring-summer 2009 and 2010). LOD threshold (LT) for a confidence of 99% and 1000 permutations is also represented. *=QTL detected for both years in the same position.

Regarding other fruit quality traits, some QTL associated with flesh and external fruit colors were detected in LG V, VII, IX and X. Only one QTL (*fc05.1*) could be identified for flesh color, and three QTL for external color, two QTL in 2009 (*ecol7.1*, *ecol10.1*) and one in 2010 (*ecol9.1*).

Several QTL related to the main vegetative and phenological traits evaluated were observed in LG IV for leaf area, in LG VI for corolla male diameter and main vein length. Other QTL associated also to these traits were identified in LG XII: *mol12.1* for main vein length

(position 43.2-56.9 cM), and *la12.1* for leaf area (43.2-50. cM), sharing one flanking marker (CMN01_38), as well as for male corolla diameter (*cmale12.1*), located next to them, between 59.9 and 67.8 cM. The direction of additive effect from these QTL is to 'Bola de Oro'. No significant correlations ($r_p \geq 0.4$ absolute value) have been observed for these traits, and only it was detected between *Cmale* and *Cfem* ($r_p=0.4$), whose QTL were situated on LG II. The QTL for powdery mildew resistance identified in LG XII (Beraldo-Hoischen et al., 2012) does share localization with the QTL related with leaf area, main vein length, and male corolla diameter, mentioned above. These traits do not play a significant role in fruit quality from breeding purposes, though.

4.4 Discussion

Two hundred and sixteen loci, most of them codominant SSR, were used to genotype our population. The SSR markers are one of the best matches among markers because of their low cost, high reproducibility between laboratories and usefulness for map comparison and map merging (Danin-Poleg et al., 2000; Oliver et al., 2001; Périn et al., 2002b; Silberstein et al., 2003; Gonzalo et al., 2005; Zalapa et al., 2007). The percentage of skewed markers detected in our map was 11%, being of 12%, 14%, 19% previously reported by Baudracco-Arnas and Pitrat (1996) and Périn et al. (2002b), Wang et al. (1997), respectively. The markers were distributed in 22 linkage groups, which were regrouped into 12, number of chromosomes in melon, after comparing the distribution of common markers between our map and some other melon maps (Fukino et al., 2008; Díaz et al., 2011; Yuste-Lisbona et al., 2011 a; b). The map reported herein shares 97 markers with the 'TGR-1551' F2 map of Yuste-Lisbona et al. (2011b), 156 with the consensus map (Diaz et al., 2011), and 52 markers with the RIL map reported by Fukino et al. (2008). Linkage associations between maps have been found and the collinear order of the common markers was generally observed. Only a few inversions were detected, mainly on LG IV. Our map spanned a genetic distance of 993cM with an average of 4.43cM among markers. Other maps found in the literature covered 1150 cM with 0.72 cM/marker (Díaz et al., 2011); 1318.2 cM and 3.1 cM/marker (Yuste-Lisbona et al., 2011b), 877 cM and 5.3 cM/marker (Fukino et al., 2008). Thus, as previous melon maps in literature, the RIL map reported herein is adequate to be used for QTL identification.

LG II and IV were the groups presenting the major number of QTL in our map, concretely in QTL related to fruit size such as weight, length, diameter, seed cavity diameter and flesh width, as well as the shape of fruits. These traits showed positive correlations among them, which might indicate that a tight bond among them may exist. LG II was classified into regions II-1 and II-2. The region II-1 includes the *a* locus and several QTL such as *fsi2.1*, *ovl2.1*, *ovsi2.1*, *bs.2.1*, *cfem2.1*, *fs2.1*, *fl2.1*, *fw2.1*, *ovd2.1*, *flf2.1*, *nmel2.1*. Other previous authors also identified QTL related to these traits in LG II, some of them being also linked to *a* locus. Several genes can interact to influence the expression of yield and quality traits in cucurbit species (Serquen et al., 1997; Fazio et al., 2002), and Périn et al. (2002c) indicated that *a* locus has dramatic effects on fruit shape. Besides, several QTL have been previously described as linked or closely mapped to the *a* locus: Harel-Beja et al. (2010) found three QTL related with fruit size (*fl2.1*, *fsh2.1*, *fw2.1*), Paris et al. (2008) detected two QTL (*fd2.3* and *fs2.3*); other two QTL were identified by Zalapa et al. (2007), one for weight (*fw8.12*) and another for mean fruit weight, *awf* 8.8. [LG 8 corresponds to LG II in our map]. Périn et al. (2002c) also identified four QTL for fruit size (*fw2.1*, *fs2.1*, *fs2.2*, *fl2.1*) and five for ovary size (*ovw2.1*, *ovl2.1*, *ovs2.1*, *ovs2.2*, *ovl2.2*). Zalapa et al. (2007) and Harel-Beja et al. (2010) also found other QTL related to fruit size (*fw8.11* and *fw2.2* respectively) in LG II, although next to marker TJ24, in a region comprised between 33 and 47cM (region II-2 in our map). In this LG II-2 the QTL *ssc2.1*, *ssc2.2*, *nmel2.2* and *flfs2.2* were detected. Zalapa et al. (2007) also identified other two QTL for fruit number per plant (*fn8.7*, *fn8.8*), one linked to *a* locus, and the other next to *fw.8.11*, respectively. Harel-Beja et al. (2010), Paris et al. (2008) and Monforte et al. (2004) found three QTL (*suc2.2*, *suc2.3*, and *tss2.1*), one (*ssc2.2*) and another one (*ssc8.1*) QTL respectively, related with sugar content, also next to TJ24, located in this region II-2. Cuevas et al. (2008) detected three QTL associated with the content beta carotene in LG II: two (*b-car 2.2*, *b-car 2.3*) in the region corresponding to II-2 of our map and one (*b-car2.1*) in region II-1.

The linkage group IV, as mentioned before, contains a high number of QTL, such as *fl4.1*, *fd4.1*, *fd4.2*, *scd4.1*, *scd4.2*, *fw4.1*, *fw4.2*, *flw4.1*, *flw4.2* and *la4.1*. Other authors like Périn et al. (2002c) detected three QTL along LG IV also related with fruit weight and length of fruits and ovary (*ovl4.1*, *fl4.1* and *fw4.1*). No common markers among that map and that one reported herein were observed in order to establish comparison of collocation, though. Other QTL

related to fruit size have also been detected in our map, in LG VII (*flw7.1*), in LG VIII (*fl8.1*, *fsi8.1*), in LG X (*fd10.1*, *fsi10.1*), and in LG XI (*fw11.1*, *fd11.1*, *scd11.1*). Obando et al. (2008) found one QTL for flesh proportion in the same zone of LG VII, a trait related somehow with flesh width evaluated herein. Périn et al. (2002c), Monforte et al. (2004), Zalapa et al. (2007), Paris et al. (2008), and Harel-Beja et al. (2010), also detected QTL related with shape, length, weight and diameter of fruits in the same genomic region of LG VIII. Périn et al. (2002c), Monforte et al. (2004), and Paris et al. (2008) localized QTL for fruit diameter and shape in the same LG XI area, and Périn et al. (2002c), and Monforte et al. (2004) identified a QTL for fruit shape in LG XI.

External fruit color and flesh color, two important fruit quality traits, were also evaluated in the RIL population 'TGR-1551' x 'Bola de Oro'. This first trait was variable among RIL plants. Flesh color was quite homogeneous, though. Color, considered as being under a complex genetic control, has been measured in different ways such as color scale or colorimeter among others, as it is recorded in the literature. The QTL detected herein for external and flesh color by using a colorimeter ('H*' value), showed a similar location to those identified by Obando et al. (2008) using the same method for color evaluation: one for Ecol in LG X and another for Fcol in LG V. Monforte et al. (2004) also identified QTL for external color in LG VII, IX and X; although in that case ECol was recorded as '1' for yellow and '0' for green color.

As observed, many of the QTL reported herein share location with QTL described in the literature. Most of them are related with fruit (Périn et al., 2002c; Monforte et al., 2004; Zalapa et al., 2007; Paris et al., 2008; Harel-Beja et al., 2010) and ovary size (Périn et al., 2002c). Others QTL, such as those related to flesh firmness (LG II), solid soluble content (LG II), number of melon per plant (LG II), external fruit color (LG VII, IX, X), and flesh color (LG V), were also detected by Monforte et al. (2004); Obando et al. (2008); Paris et al. (2008); and Harel-Beja et al., (2010). The QTL identified herein for flesh width was detected in the same genomic region of linkage group II that the one described by Obando et al. (2008), despite the trait was evaluated differently in both cases, and those ones detected for characters such as seed cavity diameter and leaf area seem not to be located in the same LG (Paris et al., 2008; Díaz et al. 2011). The QTL identified herein for some traits such as main vein length (45 days after transplant), female and

male corolla diameters, blossom scar size and abscission have not been reported before in melon in the literature.

Regarding the collocation of QTL associated to agronomical traits and those related to pest and disease resistance coming from 'TGR-1551', some of the QTL already mentioned share linkage group location with QTL such that one linked to WMV resistance (*wmv*) in LG XI (Palomares-Rius et al., 2011) and the QTL for powdery mildew resistance *Pm-R* in LG V (Yuste-Lisbona et al., 2011a; b) and *pm-r* in LG XII (Beraldo-Hoischen et al., 2012). Although QTL for abscission, seed cavity diameter, weight and diameter of fruit were detected in the same LG than *wmv*, none of these colocalized with it. The QTL detected next to *Pm-R*, were next to those associated to the diameter of the ovary and the fruit, and their additive effects did not affect the trait. Those QTL detected close to *pm-r* in LG XII were not related with fruit quality traits. The marker CMAGN16, was located on LG II in the interval of the QTL associated with solid soluble content (*ssc2.1**), which additive effects were directed to the commercial variety 'Bola de Oro'. However, although this marker was significant according to KW test for powdery mildew resistance, no QTL were detected in this LG. All these aspects are positive in the acquirement of high quality lines containing these resistances, since these QTL for *P. xanthii* resistance appear not to be directly related to any non-desirable morphological trait and therefore, the introgression of this resistance from 'TGR-1551' would not carry fruit quality problems. Besides, the data reported herein allow us to extend our knowledge about this RIL population for future breeding programs.

4.5 References

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Capítulo 5

5 Fungal development and callose deposition in compatible and incompatible interactions in melon genotypes infected with powdery mildew

5.1 Introduction

The fungal disease known as powdery mildew is a limiting factor for the production of melon throughout the world (Sitterly, 1978). *Golovinomyces cichoracearum* (Vakalounakis and Klironomou, 2001) and *Podosphaera xanthii* (Shishkoff, 2000), are the two main causal agents of this disease. *Podosphaera xanthii* occurs more frequently in subtropical and tropical areas (Lebeda et al., 2011) and, in coastal zones of the provinces of Málaga and Almería, this species has been identified as the sole cause of powdery mildew of cucurbits (Torés et al., 1990; Olalla, 2001; del Pino et al., 2002). Many races of *P. xanthii* have been identified so far based on the differential response of resistance/susceptibility of a set of melon genotypes (Pitrat et al., 1998; Bardin et al., 1999). Disease resistance is an important objective of melon breeding programs, which implies the knowledge of the inheritance of the resistance and of the mechanisms involved in it.

Plants have evolved sophisticated and efficient mechanisms to prevent the invasion of their tissues by pathogens, and disease rarely occurs. They defend themselves against fungi by mechanisms expressed either constitutively, before pathogen attack, or induced, after fungus recognition, what provokes the activation of a vast array of chemical and physiological changes (Nishimura et al., 2003). Quantitative cytological recordings of incompatible interactions have revealed putative host-cell defence resistances conferring arrest of fungal development at distinct stages (Kita et al., 1981; Koga et al., 1988; 1990). Two of these host-cell resistances can be detected: cell wall reinforcement at sites of attempted penetration (effective papilla), and an active, rapid death of attacked epidermal cells, named hypersensitive response (HR), which can be visualized by whole-cell autofluorescence under UV excitation (Aist and Israel, 1986; Koga et al., 1988; Görg et al., 1993). HR is associated with several processes such as rapid generation of reactive oxygen species (ROS), direct ion flux through the plasma membrane, synthesis of antimicrobial compounds (phytoalexins), strengthening of plant cell via cross linking of cell

wall polymers (callose and lignine) (Brisson et al., 1994; Lin et al., 2005), and transcription of defence-related genes (Pathogenic Related Proteins, PR).

Regarding powdery mildew diseases in general, various mechanisms of resistance have been described so far, and can be roughly classified as pre- and post-haustorial resistances (White and Baker, 1954; Huang et al., 1998; Kuzuya et al., 2006). Pre-haustorial resistance is characterized for the presence of effective papillae under the fungus penetration attempt, what blocks fungal development. This resistance has been observed in barley genotypes containing the *mlo* resistant gene against *Blumeria graminis* (Lyngkjær et al., 2000). It is a race non-specific resistance and it does not damage the cell. Pre-haustorial resistance has not been observed in melon so far. Post-haustorial resistance mechanisms have been described in this species, though. Concretely, regarding powdery mildew in melon, Kuzuya et al. (2006) distinguish two different post-haustorial resistance behaviours, type I and type II, which stop fungal development at different stages depending on the timing of HR in the epidermal cells. In type I, fungal growth is arrested at one or two primary hyphae stage, around 48 hours post-inoculation (hpi), showing few cells with callose accumulation in the penetration points and in their cell walls. In type II, HR in the epidermal cells occurs later in time and a larger fungal development can be observed in a higher number of cells, showing callose accumulation around penetration points and in cell walls. Some conidiophores can also be seen at 120hpi. In a compatible reaction, conidia germinate within 12h post inoculation, an haustorium is formed within 24 h, and germ tube branching and hyphae elongation began within 48 h. New conidiophores were initiated within 120 h, which matured within 240 h (Kuzuya et al., 2006).

The principal aim of this report is the observation, at microscopic level, of the host-pathogen compatible and incompatible interactions between races 1, 2 and 5 of *Podosphaera xanthii* and several melon accessions carrying different resistance genes, including two recombinant inbred lines (RIL) obtained from the cross between 'TGR-1551' (resistant to *P. xanthii* races 1, 2 and 5) and 'Bola de Oro' (susceptible to these three races). The possible association between differential resistance to pathogen and the presence or absence of putative resistance genes are discussed.

5.2 Material and methods

Three to nine plants from the following melon accessions were used in most of the experiments: 'TGR-1551', described as resistant to races 1, 2 and 5 of powdery mildew. This resistance is conferred by one dominant and one recessive gene and it is described as a double dominant recessive epistasis (Yuste Lisbona et al., 2010); 'Bola de Oro', an Spanish cultivar susceptible to these three powdery mildew races; RIL88 and RIL276, two recombinant inbred lines obtained after seven selfings from the cross 'TGR-1551' x 'Bola de Oro', both also resistant to races 1, 2 and 5, due possibly to the presence of a recessive gene from 'TGR-1551' (Beraldo-Hoischen et al., 2012); NIL21, a near-isogenic line obtained after five backcrossing from the same cross 'TGR-1551' x 'Bola de Oro' and resistant to races 1, 2 and 5 possibly due to the dominant gene related to the QTL *Pm-R* (Yuste Lisbona et al., 2011). Also, several melon accessions with differential response to powdery mildew races were used: 'PMR 45', resistant to race 1 and carrying the dominant gene *Pm-1* or *Pm-A* (Jagger et al., 1938; Epinat et al., 1993), 'WMR 29', resistant to races 1, 2 and 3 and carrying one dominant gene *Pm-w* or *Pm-B* (Pitrat, 1991; Epinat et al., 1993), and 'PI 414723', resistant to races 1, 2, 3 and 5 and conferred by different genes according to various authors (Pitrat, 1991; Anagnostou et al., 2000; Périn et al., 2002; Fazza et al., 2013). The experiments with all these melon lines were carried out in two locations and plants were grown in two different environments: at 22°C, 70% RH, 16/8 hours light/dark in a growth chamber in the department of Phytopathology at the Technische Universität München (TUM), in Germany, and at 18-22°C and 25-30°C in a acclimatized chamber at the Experimental Station of the IHSM 'La Mayora' (CSIC-UMA) in Málaga, Spain.

The inoculations were carried out on the second true leaf of each plant using an inoculation tower (Fig. 5.1) with a variable spore density. The *P. xanthii* isolates employed for the experiments were '27' (Race 1), '2204' (Race 2) and 'C8 Cris' (Race 5). Conidia were obtained from monosporic culture which were kept in cotyledons from the susceptible melon accession 'Bola de Oro', and from zucchini squash placed on Petri disks with Bertrand medium (Bertrand, 1991) under axenic conditions. The conidia were subcultured on new cotyledons every two weeks approximately.

Several parameters have been taken into account based on Kuzuya et al. (2006). Thus, the number of conidiophores (new conidia initiation stage) at 120 hours post-inoculation (hpi),

penetration points and cell walls with callose accumulation, both at 48hpi (when the highest differences detected among susceptible and resistant accessions were observed), and number of primary hyphae were recorded in 'PMR 45', 'WMR 29', 'PI 414723', 'TGR-1551', 'Bola de Oro', NIL21, RIL88 and RIL276 for races 1, 2 and 5 of *P. xanthii*. Inoculated leaves were cut in half and kept in tubes with a dilution ethanol- acetic acid 7:1, and employed for fungal development observation, conidiophore counting and callose accumulation detection.



Figure 5.1. Inoculation tower used in TUM.

5.2.1 Spore germination and number of conidiophores

In order to observe conidia, hyphae and conidiophores, half leaf of each inoculated plant was taken and stained with an ink/acetic acid solution. Conidia, primary hyphae, and conidiophores were then coloured in blue and visualized under light microscope (5x, 10x, 20x). The number of conidiophores was counted at 120hpi in 30 colonies per sample.

5.2.2 Callose accumulation in penetration points and cell walls for HR estimation

Callose accumulation in penetration points and in cell walls was detected with the double aniline-blue (Aniline blue 0.01% in 7mM K_2HPO_4) -calcofluor staining technique. For it, each half leaf was kept in aniline-blue from 12 to 24 hours in dark at room temperature. Then each sample was stained with calcofluor during 30 seconds approximately, mounted on microscope slides with the adaxial surface uppermost, covered with a glass coverslip, and examined using a epifluorescence microscope at 5x, 10x, 20x. Fungal hyphae and conidia could then be observed in fluorescent blue, and both, penetration points and cell walls with callose accumulation, in yellow. The number of penetration points, and cells with callose accumulation

in their cell walls (called 'cells with callose' from now on) were counted in 30 colonies per sample. An estimated ratio between penetrations points and cells with callose has been also considered for analyses (a value of 0.9 was used when no cells with callose were observed).

5.2.3 Statistical analyses

An ANOVA analysis was performed to test significant differences among the melon accessions and among the powdery mildew races examined. When only two races or genotypes were tested, differences were observed with a T-test ($P < 0.05$). The statistical analyses were carried out by using SPSS software (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc).

5.3 Results

5.3.1 Conidiophores counting at 120hpi

The highest number of mature conidiophores, more than four per colony, was detected for 'Bola de Oro' inoculated with races 1, 2 and 5, 'PMR 45' inoculated with races 2 and 5, and 'WMR 29' with race 5. In all these cases, the response to powdery mildew was susceptible (compatible interaction). The lowest values, approximately one or less than one conidiophore per colony, were identified in cases of resistant response (incompatible interaction), concretely in 'PI 414723' for races 2 and 5, NIL21, RIL88 and RIL276 for races 1, 2 and 5, in 'PMR 45' for race 1, and in 'WMR 29' for races 1 and 2. The resistant melon line 'TGR-1551' showed, however, intermediate scores for the three races (Fig. 5.2; Fig 5.3).

According to the results from the ANOVA test for the number of conidiophores counted at 120 hpi for the three races tested, three different groups of accessions could be observed based on their response: one with values around zero (incompatible interaction), a second group with values above three (compatible interaction), and a third one with intermediate scores and corresponding to 'TGR-1551'.

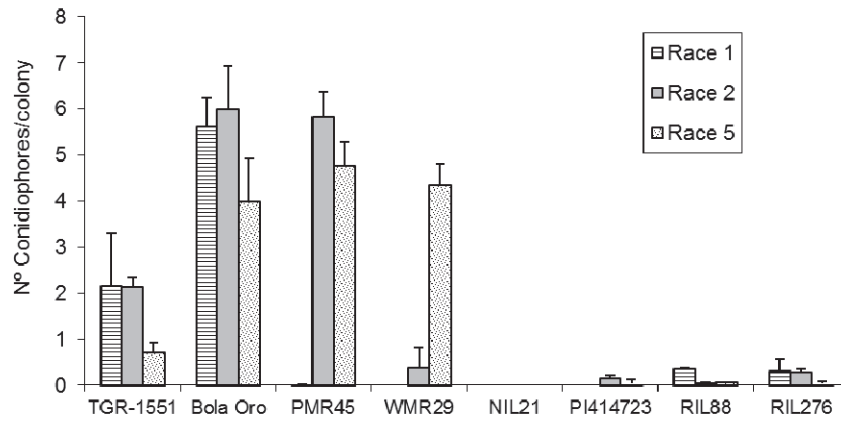


Figure 5.2. Mean number of mature conidiophores per colony (30 colonies/sample counted) in second true leaves of three plants of different melon accessions for three powdery mildew races at 120hpi. No conidiophores were reported for neither the three races in NIL21 nor for race 1 in 'PMR 45' and 'WMR 29'. Race 1 was not evaluated in 'PI 414723'.

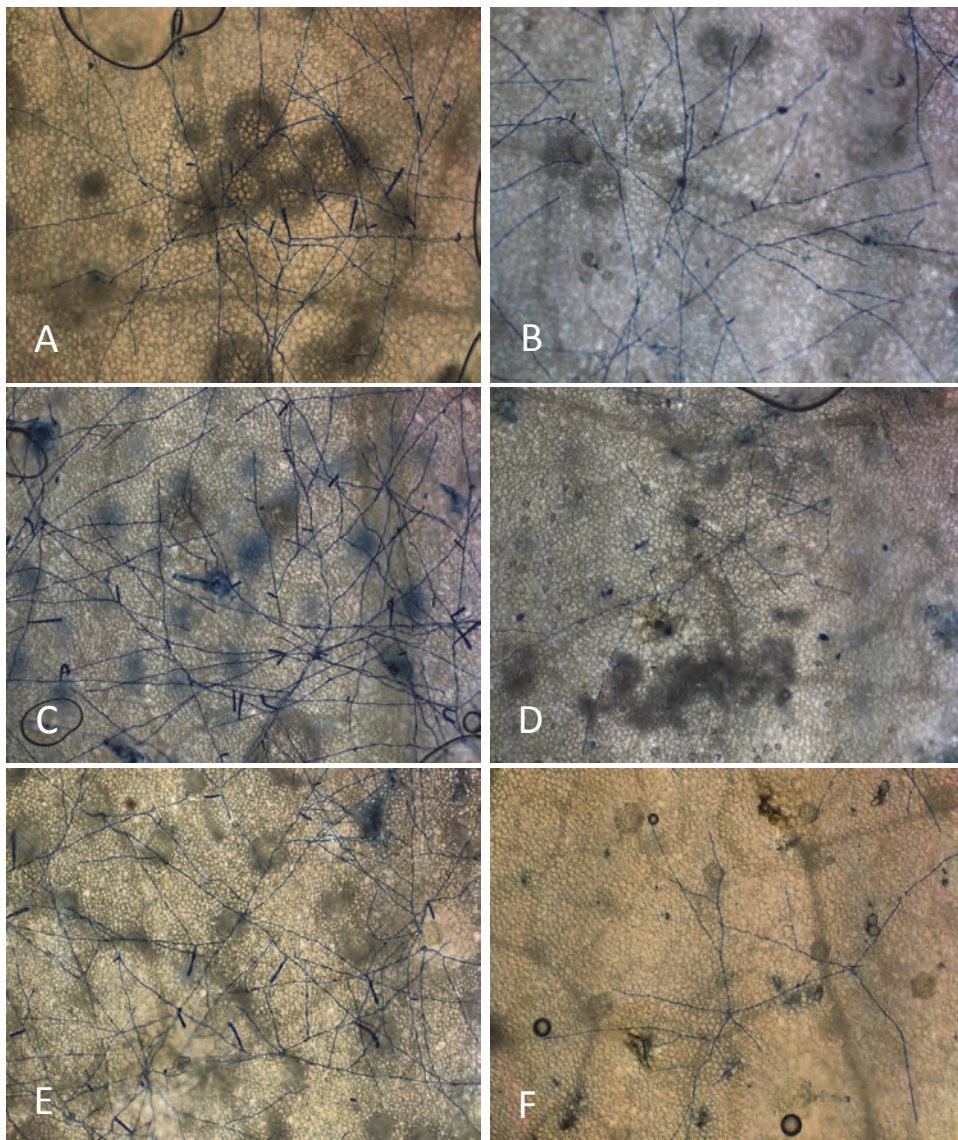


Figure 5.3. Development *Podosphaera xanthii* race 5 observed at 5 days post inoculation in second true leaves from 'Bola de Oro', 'PMR 45', 'WMR 29', 'TGR-1551', NIL21 and 'PI 414723'(A, B, C, D, E and F).

5.3.2 Callose accumulation in penetration points and cell walls at 48hpi

In general terms, no significant differences were detected for the number of penetration points with callose accumulation in 'TGR-1551', 'Bola de Oro', 'PI 414723', NIL21, and RIL276 for the three powdery mildew races tested (Fig. 5.4) with an average value of around 1.7-2 points per colony. These melon lines have been described as showing a similar behaviour to the three races evaluated: all of them are resistant to the three powdery mildew races, except for 'Bola de Oro' which is susceptible. Differences among the resistance to the three races evaluated were, however, observed in 'PMR 45' showing the lowest value for race 1 (around 1) and 'WMR 29' (highest value for race 5, around 4). Both accessions have been described as showing differential response to powdery mildew according to the races, and the values observed for this parameter is in accordance to their, already described, compatible and incompatible interactions. The mean values observed in the recombinant inbred line RIL88, coming from 'TGR-1551', were also different for the three races. The lowest value was detected when inoculated with race 1 (1.43). Regarding powdery mildew race, significant differences were found among lines for each race tested. The response of the different genotypes to each race showed, in general terms, that in the compatible interactions of 'Bola de Oro' (races 1, 2 and 5), 'PMR 45' (races 2 and 5), and 'WMR 29' (race 5), more than two penetration points per colony were observed, being the values below two those one detected for incompatible interactions: 'PMR 45' (race 1), 'WMR 29' (races 1 and 2), and NIL21 (races 1, 2 and 5). This was not observed, however, in 'PI 414723' (races 2 and 5, incompatible interaction) and in 'TGR-1551' (races 1, 2 and 5), which showed more than two penetration points per colony, and were not significant different from each other and from the susceptible line 'Bola de Oro'. The recombinant inbred lines RIL276 and RIL88 displayed above two penetration points per colony in two out of the three races (1 and 5, and 2 and 5, respectively). Based on these results, the counting of penetration points at 48hpi and 22°C seems not to be an adequate parameter to use in order to discriminate clearly between compatible and incompatible interactions, possibly due to that 48hpi is a very early stage in powdery mildew development.

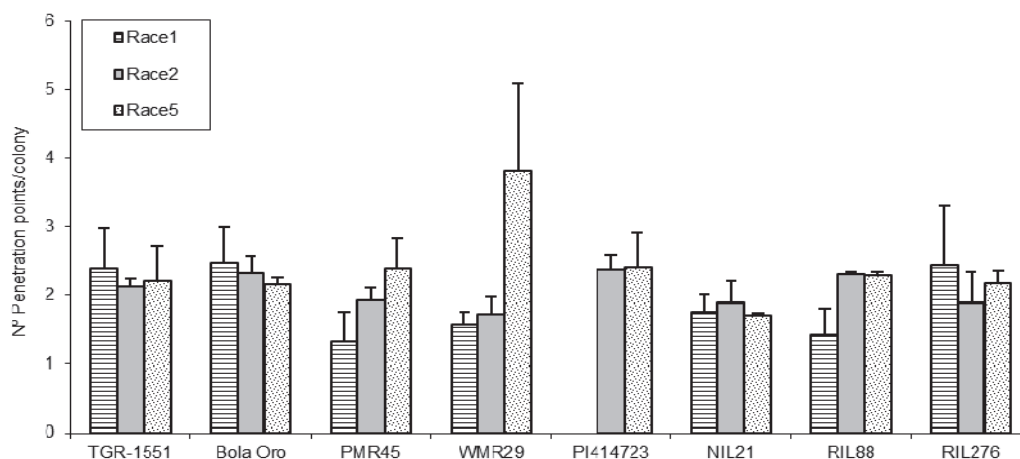


Figure 5.4. Mean number of penetration points with callose accumulation counted per colony (30 colonies/sample counted) in second true leaves of three plans of ‘TGR-1551’, ‘Bola de Oro’, ‘PMR 45’, ‘WMR 29’ (1 plant), NIL21, RIL88 and RIL276 for races 1, 2 and 5; and in ‘PI 414723’ for races 1 and 2 of *Podosphaera xanthii* at 48 hours post inoculation. Race 1 was not evaluated in ‘PI 414723’.

Regarding callose accumulation in cells walls, significant differences were found among races and also among lines for the same race tested. Significant differences among races were observed in ‘Bola de Oro’ (1, 0.44, 0.78), ‘PI 414723’ (1.3 and 1, race 1 was not tested), ‘WMR 29’ (1.3, 1.2, 0.8) (lowest value for race 5), RIL88 (0.5, 1.5, 1.94) and RIL 276 (1.5, 1, 0.7). No differences were noticed for ‘TGR-1551’ (1, 0.7, 1), NIL21 (around 1.3) and ‘PMR 45’ (0.7, 0.91, 0.92) (Fig. 5.5). The lack of differences observed in ‘PMR 45’ could be due possibly to the non-presence or short development of the fungus observed for race 1 (value of 0.79, incompatible interaction) and the absence of a defence mechanism which stopped the fungus development when inoculated with races 2 and 5 at this time after inoculation (compatible interaction, 0.9 approximately).

No differences were observed between ‘Bola de Oro’ and ‘TGR-1551’ for the each of the three races tested. In general, highest values (above 1) were detected in most of the described incompatible interactions, although observations at later time points post inoculation could be needed to discern properly between compatible and incompatible interactions.

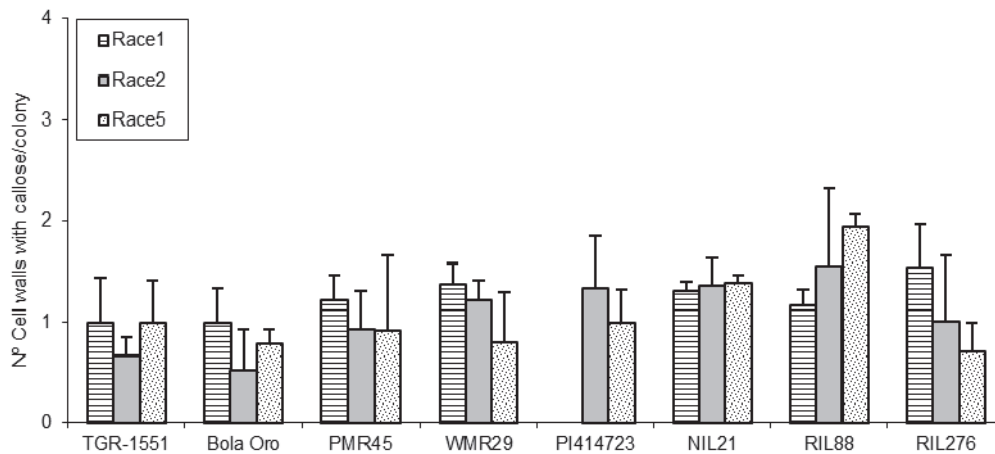


Figure 5.5. Mean number of cells with callose accumulation in their cell walls (n° cell walls with callose), counted per colony (30 colonies/sample counted) in second true leaves of three plants of several melon accessions for races 1, 2 and 5 of *Podospaera xanthii* at 48 hours post inoculation. Race 1 was not evaluated in 'PI 414723'.

According to all these results, none of the parameters evaluated at 48hpi and 22°C (number of penetration points, presence of callose in cell walls, number of primary hyphae, and the ratio penetration points/cells with callose) showed significant differences between the resistant melon line 'TGR-1551' and the susceptible 'Bola de Oro', both parental lines of the NIL and RIL used (Fig. 5.6; Fig. 5.7). Likewise, the number of conidiophores observed at 120hpi in 'TGR-1551' was intermediate between the values observed for the compatible and incompatible interactions. Therefore, it is not clear if the interaction between 'TGR-1551' and *P. xanthii* could be described as a compatible or an incompatible interaction, as estimated by these parameters under the environmental conditions tested.

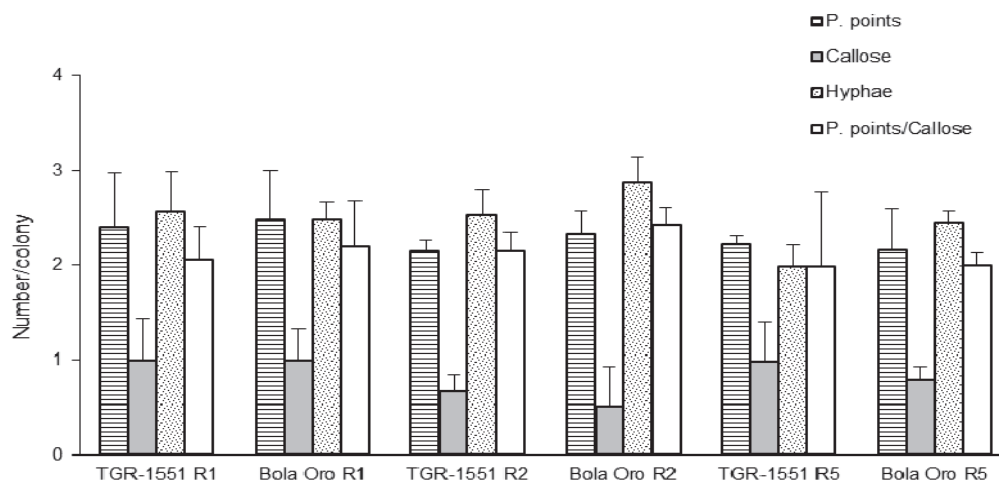


Figure 5.6. Mean number of penetration points with callose accumulation (P. points), cell walls with callose accumulation in their cell walls (Callose), primary hyphae (Hyphae) and the ratio P. points-Callose (P. points/Callose), counted per colony (30 colonies/sample counted) in second true leaves of three plants of 'TGR-1551' and 'Bola de Oro' inoculated with races (R) 1, 2 and 5 from *Podosphaera xanthii* at 48 hours post inoculation.

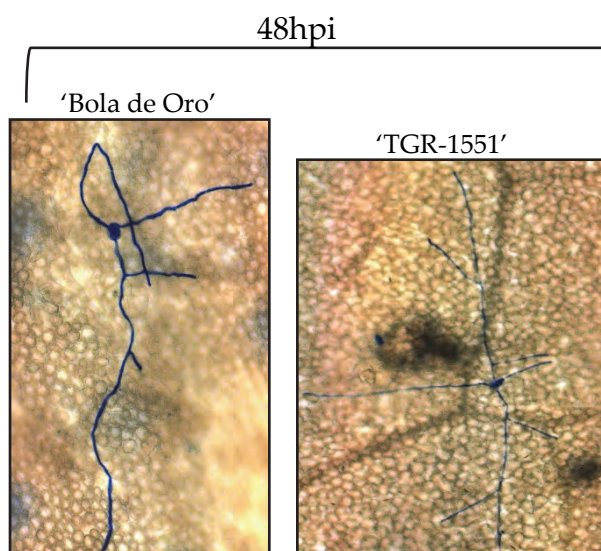


Figure 5.7. Primary and secondary hyphae of *Podosphaera xanthii* developed from conidia in 'Bola de Oro' and 'TGR-1551' at 48hpi (Ink-acetic staining).

In order to clarify and understand the resistance of 'TGR-1551' to powdery mildew, new observations were carried out at different time points, since higher time points could be more informative.

5.3.3 Fungal development at 72hpi and 96hpi and conidiophores observation at 240hpi (conidia mature stage)

Three plants from 'Bola de Oro', 'TGR-1551' and NIL21 (only at 72hpi) (resistant genotype carrying QTL *Pm-R*) were inoculated with races 1, 2 and 5, and samples were taken at 72 and 96hpi in order to estimate fungal development following the methods explained before.

Significant differences were detected among the three accessions tested for the number of cells with callose, number of penetration points, as well as the ratio among these two parameters (Fig. 5.8; Fig. 5.9). The highest divergences were observed only for cells with callose and the ratio penetration points/cells with callose, though. NIL21 showed the lowest number of penetration points among the genotypes tested at 72hpi for the three races.

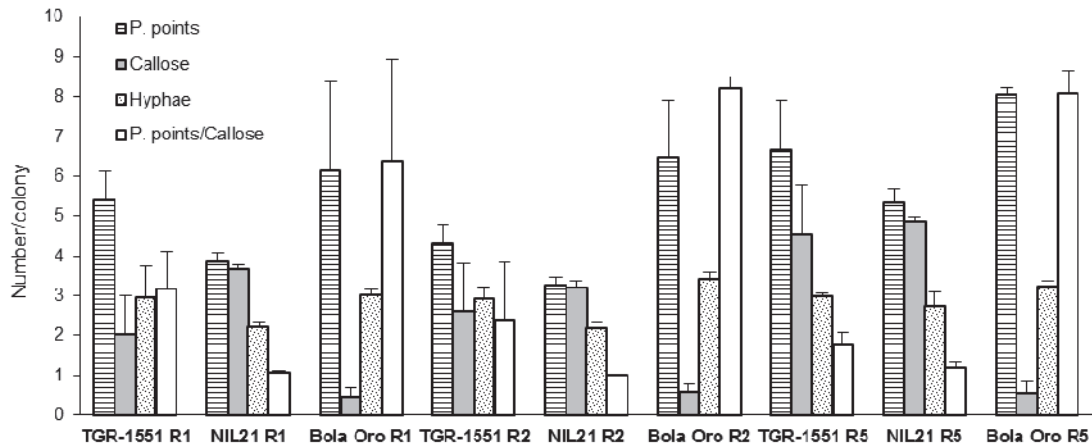


Figure 5.8. Mean number of penetration points with callose accumulation (P. points), cell walls with callose accumulation in their walls (Callose), their ratio (P. points/Callose) and primary hyphae (Hyphae), counted per colony (30 colonies/sample counted) in second true leaves of three plants of 'TGR-1551' and 'Bola de Oro' and NIL21 inoculated with races (R) 1, 2 and 5 of *Podosphaera xanthii* at 72 hours post inoculation.

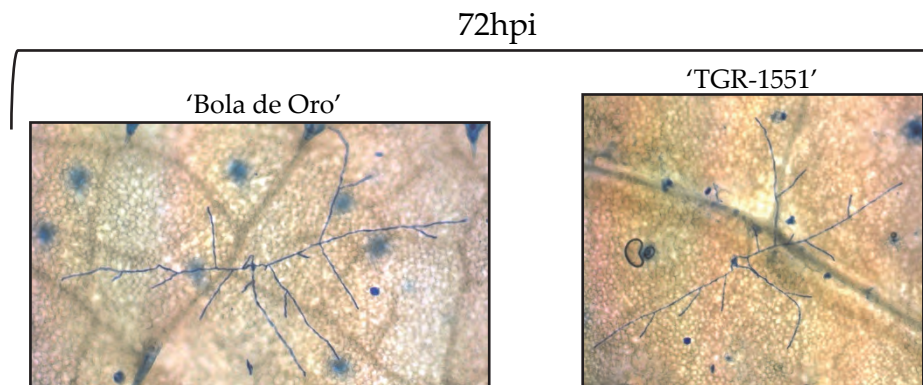


Figure 5.9. Primary, secondary and third hyphae of *Podosphaera xanthii* developed from conidia in 'Bola de Oro' and 'TGR-1551' at 72hpi (Ink-acetic staining).

For the observations at 96hpi, three plants per genotype were inoculated only with race 1, since no differences were observed among races for these parameters. In this case, significant differences were detected between 'TGR-1551' and 'Bola de Oro' for all the variables tested, except for the number of primary hyphae. The highest differences were found in the number of cell with callose and in the ratio penetration points/cells with callose (Fig. 5.10).

We could conclude that 'TGR-1551' is significant different to 'Bola de Oro' at time points above 72hpi for the number of cells with callose, as well as for the ratio established between the number of penetration points and number of cells with callose.

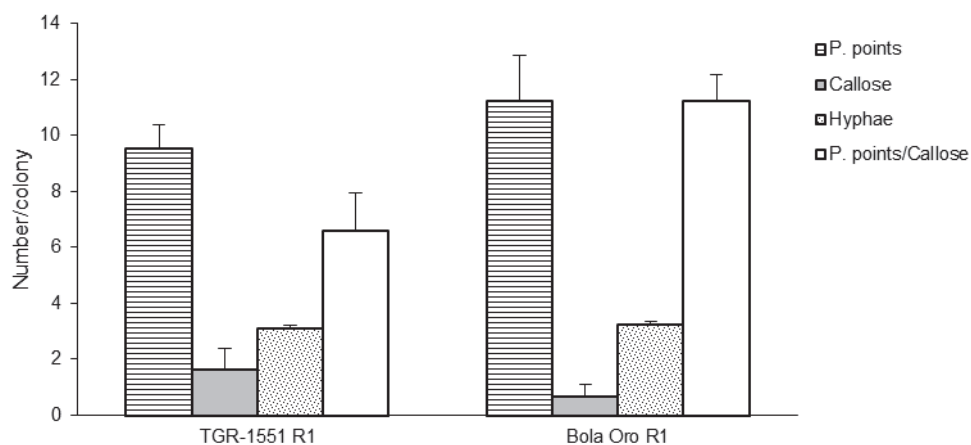


Figure 5.10. Mean number of primary hyphae (Hyphae) penetration points with callose accumulation (P. points), cell walls with callose accumulation in their cell walls (Callose), and their ratio (P. points/Callose), counted per colony (30 colonies/sample counted) in second true leaves of three plants of 'TGR-1551', 'Bola de Oro' and NIL21 for race 1 of *Podosphaera xanthii* at 96 hours post inoculation.

Since conidiophores were already observed and counted at 120hpi in the resistant melon line 'TGR-1551', new observations at 240hpi were considered for mature conidia stage, according to Kuzuya et al. (2006), in plants inoculated with race 1.

Despite we detected some conidiophores in 'TGR-1551' at 240hpi, the number was quite low in comparison to 'Bola de Oro' (Fig. 5.11).

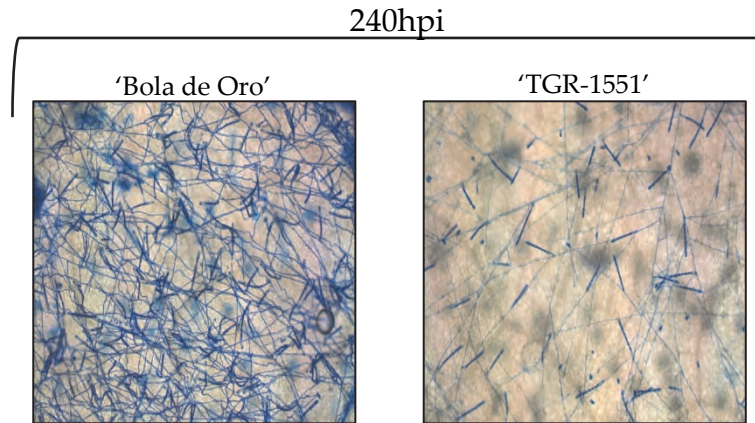


Figure 5.11. Development of *Podosphaera xanthii* in 'Bola de Oro' and 'TGR-1551' at 240hpi (Ink-acetic staining).

In the literature, some melon genotypes have been described as showing different responses to powdery mildew depending on the temperature, such as ANC-57 (Torés et al., 1996), and 'Quincy', ENN2, EMN2, and HN21 (Hosoya et al., 2000), so that the temperature might be playing an important role in fungal development in the accessions examined herein. Since the presence of conidiophores in the resistant melon line 'TGR-1551' could be due to the temperature employed during the experiments (22°C) in TUM (Germany), an additional experiment with different temperatures was carried out in order to observe if this variable could be affecting the fungal development in 'TGR-1551'.

5.3.4 Fungal development at different temperatures

Plants from several melon accessions were used in order to observe fungal development at different temperatures and at different times post inoculation. Concretely, a maximum of nine plants of 'TGR-1551' and 'Bola de Oro' (half leaves taken at 48, 72, 96 and 120hpi) and a maximum of three plants of 'PMR 45', 'WMR 29' and NIL21 (half leaves taken at 48 and at 120hpi), were inoculated with race 2 using an inoculation tower at 70% HR, 16h light/8h dark at 18-22°C and at 25-30°C at the Experimental Station 'La Mayora' (Spain). Callose accumulation in penetration points and cell walls, as well as the number of primary hyphae, were evaluated at 48, 72, 96 and 120hpi. The number of conidiophores was recorded at 120hpi. A T-test was employed to detect significant differences between the two essays carried out at low temperatures (TUM and 'La Mayora') at 72hpi in 'TGR-1551' and 'Bola de Oro' for the number of hyphae, penetration points and cells with callose, as well as the number of mature

conidiophores at 120hpi. Also a T-test or an ANOVA, if the case, were performed to observe significant differences between high and low temperature in each accession and among accessions for the parameters mentioned above for the experiments carried out in 'La Mayora'.

No significant differences were observed in 'TGR-1551' and 'Bola de Oro' at 72hpi for the evaluated parameters between the experiments carried out in both places, TUM (Germany) and 'La Mayora' (Spain), at low temperature. We did find, however, significant differences between both temperatures in the experiments carried out at 'La Mayora' for all the parameters tested for the same genotype and time after inoculation for all the evaluated lines, except for the number of hyphae in 'Bola de Oro' at 48, 96 and 120hpi, and in 'PMR 45' at 120hpi for the number of hyphae and conidiophores. Likewise, we did not find differences for the number of penetration points with callose accumulation in 'TGR-1551' at 48hpi and for the number of cell walls with callose at 72hpi. The number of penetration points with callose increased along the time in all the tested lines inoculated with race 2 for both temperatures, except for 'WMR 29' (resistant to race 2) where the fungus stopped its development in most of the conidia at 48hpi, showing a maximum of one penetration point per colony approximately. The highest values of these parameters for both temperatures were observed, as expected, at 120hpi in the lines susceptible to race 2 ('Bola de Oro', 'PMR 45') and in 'TGR-1551' at low temperature.

Regarding the number of penetration points with callose observed in each line at different temperatures, 'WMR 29' and NIL21 at 48hpi and 120hpi, 'PMR 45' at 48hpi, and 'TGR-1551' at 72, 96 and 120hpi, showed larger scores at low temperature than at high temperature, while 'PMR 45' at 120hpi and 'Bola de Oro' at 48, 72, 96 and 120hpi showed larger scores at high than at low temperature (Fig. 5.12; Fig. 5.13). This parameter, number of penetration points with callose, might be useful to describe the fungal development, since it showed the highest differences between the resistant and susceptible accessions at both temperatures examined.

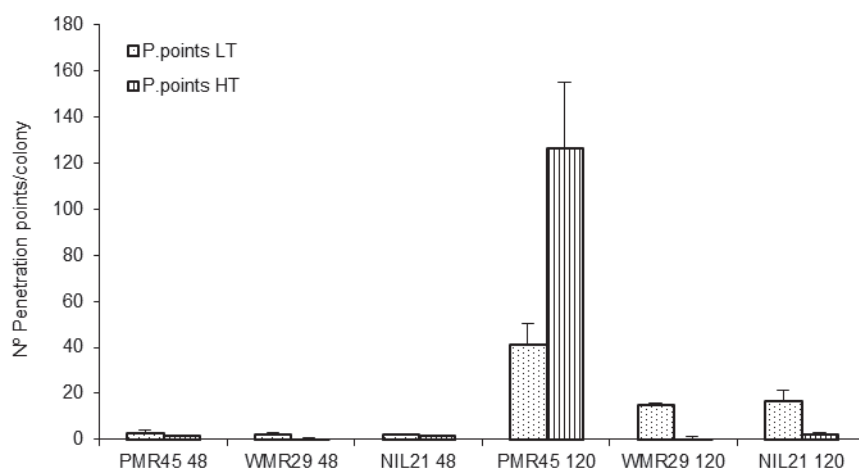


Figure 5.12. Number of penetration points per colony (30 colonies/sample counted) in second true leaves of two to three plants of different melon accessions inoculated with race 2 of *Podosphaera xanthii* at 48 and 120hpi at low temperature (LT, 18-22°C) and high temperature (HT, 25-30°C) (1 plant of 'WMR 29' at LT).

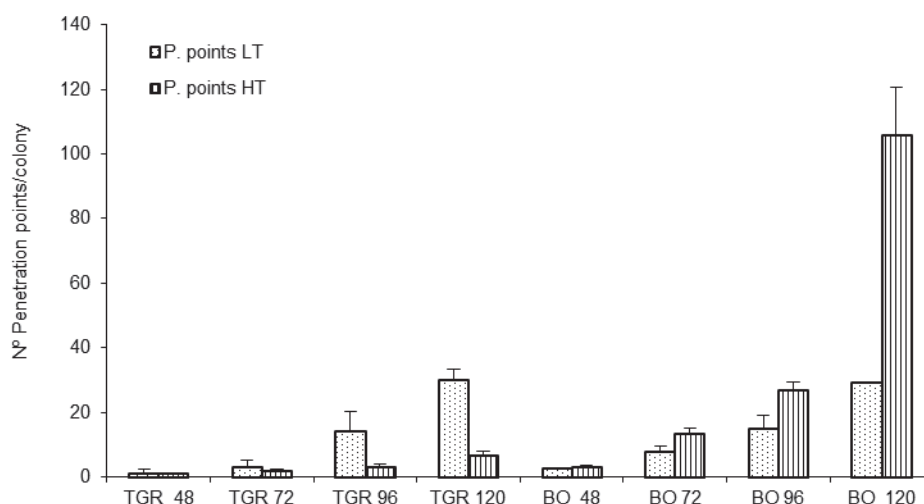


Figure 5.13. Mean number of penetration points per colony (30 colonies/sample counted) in second true leaves of two to three plants of 'TGR-1551' (TGR) and 'Bola de Oro' (BO) in different melon accessions inoculated with race 2 of *Podosphaera xanthii* at 48, 72, 96 and 120hpi at low temperature (LT, 18-22°C) and high temperature (HT, 25-30°C) (1 plant of 'TGR-1551' and 'Bola de Oro' at 72 and 96hpi, LT)

The number of conidiophores counted at 120hpi was significantly different between 'TGR-1551' and 'Bola de Oro' at both temperatures. At high temperature, 'TGR-1551' did not present any conidiophores, and, by contrary, 'Bola de Oro' showed nearly the double of conidiophores per colony (data no shown) than at low temperature.

In summary, differences between 'TGR-1551' and 'Bola de Oro' could be observed at high temperatures at all-time points tested for the number of penetration points (Fig. 5.13, Fig 5.14), number of cells with callose and primary hyphae, and for conidiophores counted at 120hpi. Nevertheless, at low temperatures, as described before, differences among these lines started to be noticed at 72hpi for the number of cells with callose and above 120hpi for the number of conidiophores.

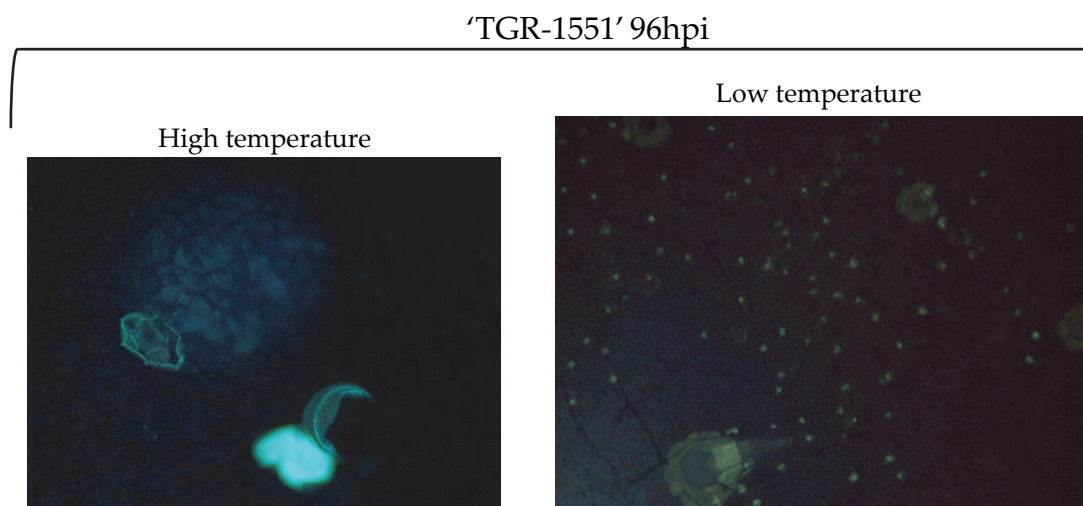


Figure 5.14. Development of *Podosphaera xanthii* in second true leaves from 'TGR-1551' inoculated with race 2 at low and high temperature (18-22; 25-30°C) at 96 hours post inoculation.

5.4 Discussion

The results obtained in this report confirmed the differences between compatible and incompatible interactions observed in plant-powdery mildew system, as described previously for melon and other plant species affected by pathogens causing powdery mildew (Cohen et al., 1990; Thordal-Christensen et al., 1997; Hückelhoven and Kogel, 1998; Kuzuya et al., 2006).

In compatible interactions, fungal development is not stopped by any host defence mechanism of resistance. In incompatible host-pathogen interactions, where arresting of fungal development occurs, several types of behaviours have been described according to the mechanisms involved. Two general types have been described so far in different species (White and Baker, 1954; Koga et al., 1988; Görg et al, 1993; Huang et al., 1998; Hückelhoven et al., 1999; Kuzuya et al., 2006). One is called pre-haustorial, where fungal arrest takes place before the haustorium formation, and the second one is known as post-haustorial, where the haustorium is formed complete or incompletely, allowing fungal development. The pre-haustorial, which

has not been observed in melon yet, has been described to be a non- race-specific response (Carver and Carr 1977, Heath, 1981, Tosa and Shishiyama, 1984), and it is characterized by the presence of an effective papillae under every penetration attempt, which blocks fungal penetration, and fungal development is arrested at germ tube stage (Hückelhoven et al., 1999). On the other hand, in the post-haustorial, which has been described as being a race-specific response, two different powdery mildew-melon resistance behaviours can be distinguished, type I and II (Kuzuya et al., 2006), according to the timing of the presence of HR in the epidermal cells affected. In other species, Hückelhoven et al. (1999) distinguished two different post-haustorial responses: one which takes place in the epidermal cells, similar to that one described by Kuzuya et al. (2006) as type I in melon, and a second one which takes place firstly in the mesophyll cells under the penetrated epidermal cells, affecting finally to those epidermal cells. The facts occurring in the second post-haustorial resistance described by Hückelhoven et al. (1999) might be similar to those which take place in melon for the type II described by Kuzuya et al (2006), where the HR response occurs later. In type I, fungal development stops at germ tube or at one to two hyphae stage. In type II, a higher fungal development is noticed, being observed secondary hyphae, even some conidiophores (White and Baker, 1954; Kuzuya et al., 2006).

Diverse variables have been used to characterize the different responses observed in the melon-powdery mildew interaction. In this work, several main parameters were used: the number of fungus penetration points with callose accumulation, the number of epidermal cells with callose accumulation in their cell walls, and the number of conidiophores developed. The number of primary hyphae and the estimated ratio penetration points/cells with callose were also considered. According to our results, besides the compatible interaction, four different resistance behaviours in the plant-pathogen interaction have been observed: type I and II, as described by Kuzuya et al. (2006), as well as an earlier and a later type II, IIa and IIb respectively.

The number of penetration points, detected as yellow rings by aniline staining, increased along the time in compatible interactions (Romero et al., 2008; Cohen et al., 1990; Kuzuya et al., 2006); haustorium might be well developed since the fungus has not stopped its development (Kuzuya et al., 2006). From our results, we cannot conclude if the fungus was able

to develop an haustorium, although a well-developed haustorium may be needed in order to reach a higher development level such as the one observed in compatible interactions as well as in type II.

In the resistance response type I the number of penetration points was the lowest among the different types of behaviours observed herein and fungal development was arrested at early stages (48hpi). A low number of penetration points and epidermal cells with callose were observed. Normally, under every penetration point, a cell with callose accumulation in their cell wall was detected, showing a ratio penetration points/cells with callose of one, approximately. The number of primary hyphae was around 1 or 2. No conidiophores were observed at 120hpi. We detected this response in 'TGR-1551', 'WMR 29' and 'NIL21' inoculated with race 2 at high temperature, and in 'PMR 45,' and 'WMR 29' inoculated with race 1 at low temperature. Kuzuya et al. (2006) noticed this same resistance response in 'PMR 45' and 'WMR 29' inoculated with race 1, as well as they also observed it in 'WMR 29' inoculated with race 2. According to our results, 'WMR 29' inoculated with race 2 presented a response type I only at high temperatures, since at low temperature, the fungal development was not stopped at first germ tube stage, one or two primary hyphae in some conidia, and even some conidiophores were counted at 120hpi (12 from 90 colonies in one out of three leaves). Such differences have been also described among other authors: 'PI 124112' was considered as showing resistance type I when inoculated with race 2 (Kuzuya et al., 2006), although its development reached advanced development stages in other works (Cohen and Eyal, 1987).

Another resistance response observed in our study, considered as type II, is characterized by a number of penetration points and epidermal cell walls with callose higher than in the resistance considered type I. Also the ratio penetration points/cells with callose is higher than one. The number of penetration points at 48hpi is not significantly different from the observed in the compatible interactions for higher time points (>48hpi). The number of primary hyphae can reach a value of three, like in compatible interactions, and none or only a few conidiophores were detected at 120hpi. This resistance has been clearly observed in our study in 'PI 414723' with *P. xanthii* races 2 and 5, as described by Kuzuya et al. (2006), as well as for RIL88, RIL276 and 'TGR-1551' (at low temperature) inoculated with the three races. A third resistance behaviour, similar to type II but with a faster fungal development, named herein as

type IIa, was observed in 'WMR 29' inoculated with race 2 and in NIL21 with the three races also at low temperature. The fungal development was arrested later than in type I, and nearly for one penetration point a cell with callose has been observed. A fourth resistance behaviour, slower than type II, named IIb, was observed in 'TGR-1551' at low temperature inoculated with the three races, showing an intermediate score between the susceptible and resistant lines for the number of conidiophores. At high temperature 'TGR-1551' presented a clear resistance to powdery mildew. The results obtained in this work showed that temperature could be influencing the fungal development in 'TGR-1551', NIL21 and 'WMR 29'. All these melon lines carry a QTL or a gene in LG V for powdery mildew resistance: 'TGR-1551', NIL21 (resistant to races 1, 2 and 5 due possibly to the dominant gene, *Pm-R*, in LG V) and 'WMR 29' [resistant to races 1, 2 and 3 and carrying one dominant gene *Pm-w* or *Pm-B* (Pitrat, 1991; Epinat et al., 1993)]. According to this information we could suppose that, either the same genes controlling powdery mildew resistance in LG V, or some gene located close to these resistant genes could be significantly affected by temperature.

Other authors have previously described temperature as an environmental factor which could be affecting the resistance of several melon genotypes to powdery mildew (Torés et al., 1996). Other specific temperatures experiments would be necessary in order to verify these results, and identify which mechanisms are involved in these processes. In any case, some associations could be established between the genes involved in the powdery mildew resistance in the different melon genotypes tested, and the phenotype of the different plant-pathogen interactions described herein. The resistance type I was observed in the melon lines 'WMR 29', NIL21 and 'TGR-1551' inoculated with race 2 at high temperature, and in 'WMR 29' inoculated with race 1 at low temperature. These three melon genotypes have at least one gene or QTL controlling powdery mildew resistance which is located in linkage group V (Pitrat, 1991; Périn et al., 2002; Yuste-Lisbona et al., 2011). Kuzuya et al. (2006) also observed this resistance in 'WMR 29' inoculated with races 1 and 2, as well as in other accessions with a dominant gene or QTL in LG V, such as 'Edisto 47' (Ning et al., 2014) inoculated with race 1, and in 'PI 124112' (Perchepped et al., 2005) inoculated with races 1 and 2. The melon line 'PMR 45', inoculated with race 1, showed also this same resistance in our work and in that one of Kuzuya et al. (2006). Its resistance gene, *Pm-1*, was identified in LG IX (Teixeira et al., 2008), though. Other genotypes

with that gene *Pm-1*, such as 'PMR 5' and 'PMR 6' (Bohn and Whitaker, 1964), were also considered to show a resistance type I (Rivera et al., 2002; Kuzuya et al., 2006). It seems that genotypes, carrying a gene or QTL controlling powdery mildew resistance in LG V or IX, show a resistance type I. On the other hand, genotypes with genes or QTL located in LG II or XII seem to have a resistance type II. The melon line 'PI 414723', with a resistance gene located in LG II controlling at least resistance to races 1, 2, 3 and 5 (Pitrat, 1991; Périn et al., 2002; Fazza et al., 2013), showed a resistance type II according to our results, as well as it is described by Kuzuya et al. (2006). This same resistance was noticed in RIL88 and RIL276, both resistant to powdery mildew due to the presence of a possible recessive gene in LG XII (Beraldo-Hoischen et al., 2012). The melon line 'PI 124112', with a QTL in LG XII which confers resistance to race 5 (Perchepped et al., 2005), also showed a resistance type II (Kuzuya et al., 2006).

In further studies and using the information obtained after the sequencing of the melon genome, it would be interesting to saturate the genomic regions likely involved in the resistance to powdery mildew conferred by 'TGR-1551' by using SNP, as well as to determine possible candidate genes associated with this resistance.

5.6 References

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Capítulo 6

6 General discussion

Nowadays the detection of candidate genes and QTL involved in the control of agronomic characters and pest resistances is one of the main objectives pursued by breeders in order to obtain multiresistant varieties. The understanding of the different mechanisms involved in plant-pathogen interaction could also provide, besides basic knowledge, possible alternative options for disease control.

Powdery mildew, caused by *Podosphaera xanthii*, is one of the most important diseases in melon crops due to its negative impact. This biotrophic fungus, which is able to live under a wide temperature range, reduces crop yield and deteriorates fruit quality. The use of sources of resistance instead of chemical treatment is one of the best accepted strategies for the control of this disease. Many studies have been focused on the determination of the genetic control of the resistance to *P. xanthii* in melon. However, the strong environmental influence, the high number of pathogen races already described, and the absence of a consensus standard protocol for the characterization of powdery mildew races (Cohen et al., 2002; 2004; McCreight, 2006), complicate the determination of the genetic control involved in the resistance.

Environmental conditions play a very important role in host-pathogen interaction. Light exposure, temperature and humidity (Schnathorst, 1965; Aust and Hoyningen-Huene, 1986; Torés et al., 1996; Hosoya et al., 2000; Jarvis et al., 2002; Cohen et al., 2002; 2004), as well as the phenological and physiological stages of plants, can interfere enormously in the development of *P. xanthii* (Thomas et al., 1984; Cohen et al., 1995, 2002; 2004; Schuerger and Hammer, 2003; Perchepped et al., 2005; Pitrat and Besombes, 2008). Therefore, these factors should be considered, when different essays are being compared, in order to obtain solid results and conclusions, since, eventually, different responses to *P. xanthii* have been described as depending on the temperature employed during the interaction host-pathogen (Torés et al., 1996; Hosoya et al., 1999; 2000; Pérez-García et al., 2001; Cohen et al., 2004; McCreight, 2006). A standard artificial inoculation method is also required to carry out a good phenotyping and the identification of both, the different pathogen races and the resistant plants.

Twenty-eight different races of *P. xanthii* have been described so far (McCreight, 2006). Nowadays its identification is based on the response of a set of differential melon genotypes to powdery mildew (Bardin et al., 1999), where the fungus strains are grouped according to their responses. Nevertheless, the already mentioned environmental factors can modify this response. Therefore, in order to avoid these problems, the identification of pathogen races based on molecular markers could be a solution. Although this approach has eventually been tried (Bardin et al., 1997; Katzir et al., 2000; Montoro et al., 2004), lacks of good correlations have been established among markers and the different races tested so far.

Regarding the inoculation methods, several types of plant materials have been used in powdery mildew evaluation. The inoculation of leaf disks (Cohen and Eyal, 1987; Bertrand and Pitrat, 1989; Cohen, 1993; Hosoya et al., 1999, 2000; Pérez-García et al., 2001; Cohen et al., 2004; Fukino et al., 2004, 2008; Perchepied et al., 2005; Romero et al., 2008; Pitrat and Besombes, 2008) is the most widely extended technique in powdery mildew evaluation, since environmental conditions are easily controlled and not much room for experimentation is needed. Cotyledons and first true leaves are also frequently used for inoculations according to literature (Aust and Hoyningen-Huene, 1986; Cohen and Eyal, 1995; McCreight et al., 2003; Fukino et al., 2004; Perchepied et al., 2005; Pitrat and Besombes, 2008) in order to obtain fast results. However, no good correlations have been observed in comparison to results obtained in adult plants in field evaluations (Cohen and Cohen, 1986; Floris, 1993; Floris and Álvarez, 1995; Cohen et al., 1995; McCreight, 2006). In the inoculations carried out herein, like in previous works (Cohen and Cohen, 1986; Kenigsbuch and cohen, 1992; Pérez-García et al., 2001; Cohen and Eyal, 1995; Torés et al., 1996; Romero et al., 2008), we have used the second true leaf since, according to literature (Cohen and Eyal, 1995), similar host responses are observed within this material with artificial inoculation and adult plants under natural infection in field screenings, and therefore, the results could be easily extrapolated.

Host responses are classified into compatible and incompatible interactions. A compatible interaction is considered when the fungus is able to grow and produce full developed conidiophores and an interlaced mycelium, and an incompatible interaction when fungal development is stopped and no sporulation is observed. A few full developed conidiophores were eventually detected in some resistant lines, though (Aust and Hoyningen-

Huene, 1986; Cohen and Eyal, 1987; Jahn et al, 2002; McCreight, 2006). Powdery mildew response could be evaluated as a qualitative character, where plants are considered resistant or susceptible (Kenigsbuch and Cohen, 1992; Hosoya et al., 2000), or as a quantitative one, where the level of fungus development and sporulation is considered and a scale with different scores is usually established to estimate fungal growth. Different scales have been used in the literature so far: wide scales from 0 to 9 (Pitrat and Besombes, 2008; Perchepped et al., 2005; Tomason and Gibson, 2006; McCreight, 2003; 2006) or narrow ones, ranging from 0 to 5 (Cohen and Eyal, 1987; Fukino et al., 2005), or from 1 to 4 (Yuste-Lisbona et al., 2010). In our experiments we employed a scale 0-3, where plants showing scores 0 and 1 were considered resistant (incompatible interaction), and scores 2 and 3, susceptible (compatible). Since several phenotypic classes could be frequently observed in plant populations due to environmental and genetic factors; a quantitative scale might be the best option to represent the host response.

The best way to detect the percentage of the genetic contribution in the expression of a quantitative character is throughout Quantitative Trait Loci (QTL) analyses. For it, several phenotypic evaluations are recommended to be carried out under different environmental conditions over the same population which should have been genetically fixed. Some of these populations have been used for different studies in melon, such as populations of double haploid lines (Monforte et al., 2004), near isogenic lines (Eduardo et al., 2007; Obando et al., 2008; Obando-Ulloa et al., 2009), and recombinant inbred lines (Perchepped et al., 2005; Zalapa et al., 2007; Paris et al., 2008; Cuevas et al., 2008; Fukino et al., 2008; Harel-Beja et al., 2010; Boissot et al., 2010; Palomares-Rius et al., 2011). QTL analyses described in this memory have been carried out with an F7:F8 RIL population tested in different time periods.

Regarding powdery mildew in melon, the first QTL detected for resistance were described by Perchepped et al. (2005), Fukino et al. (2008), and Yuste-Lisbona et al. (2011b). Concretely, previous works in our group described that the resistance to races 1, 2 and 5 of *P. xanthii* in the melon genotype 'TGR-1551' was controlled by a double dominant-recessive epistasis, where two genes, one dominant and one recessive, were involved. After genotyping and phenotyping an F2 population obtained from the cross 'TGR-1551' x 'Bola de Oro', only one QTL for the dominant gene, *Pm-R*, was localized (Yuste-Lisbona et al., 2011b). In this work, by genotyping and phenotyping a RIL population obtained from the same cross 'TGR-1551' x 'Bola

de Oro', we were able to detect a second QTL, possible linked to the recessive gene, as well as to confirm the position of the QTL linked to the dominant gene described in the F2 population mentioned above.

Two QTL have been detected for races 1, 2 and 5 of *P. xanthii* in the three evaluations (summer 2010, spring and fall 2011) carried out. One QTL was previously described to be associated to the dominant gene, *Pm-R*, in linkage group (LG) V, and it was strongly linked to PM3-CAPS, a PCR-based marker, and to the resistant gene homolog MRGH63, possible candidate gene for *P. xanthii* resistance in 'TGR-1551' (Yuste-Lisbona et al., 2011a). A second QTL, possible related to the recessive gene and denominated herein as *pm-r*, was located on linkage group XII, and it was linked to CMBR111, CMBR150 and TJ29 markers. Other authors had already detected several genes and QTL associated with *P. xanthii* resistance in this LG XII (Pitrat, 1991; Perchepped et al., 2005; Fukino et al., 2008; Liu et al., 2010), and some of the markers, described herein as linked to *pm-r*, have been previously reported (Fukino et al., 2008). According to our results from Kruskal Wallis (KW) analysis, another genomic region, located in linkage group II, could also be involved in the resistance to races 1, 2 and 5 of *P. xanthii*, since several markers, such as CMAGN16, showed significance for all powdery mildew races and environments tested. The LOD scores estimated were not high enough to find a putative QTL in that LG by MQM analyses, though. According to the literature, LG II, V and XII are the most significant linkage groups related with *P. xanthii* resistance (Pitrat, 1991; Périn et al., 2002a; Perchepped et al., 2005; Fukino et al., 2008; Teixeira et al., 2008).

The resistance of 'TGR-1551' to powdery mildew cannot be considered horizontal since only three races of *P. xanthii* out of more than twenty-eight already described (McCreight, 2006) have been tested so far, and the response of 'TGR-1551' to all those other races is not known. Despite that, the existence of two genes of powdery mildew resistance coming from this melon line could be interesting for breeding programs, since it might be less easy to overcome by the pathogen than a monogenic and/or a race specific resistance. Pyramidalization or introgression of several resistance genes in a same genotype, would allow the development of varieties with a more durable resistance to fight against this disease. The markers described herein as been associated with the putative QTL detected in LG V and XII can be used for MAS, what could allow the accurate selection of those individuals carrying one or both resistance genes in order

to obtain, throughout backcrosses, commercial melon varieties with these genes introgressed. These markers could also be useful for positional cloning techniques throughout genome walking, and also as starting point for the screening of candidate genes from the melon genome annotation.

The morphological characterization of a permanent population, as well as the identification of the QTL associated to the most important agronomical traits, could play a significant role during the introgression of resistances in breeding programs. In the case of the RIL population generated from the multi-resistant melon line 'TGR-1551', the knowledge of the relationships among the QTL associated to key agronomical traits and those involved in the resistance to *P. xanthii*, as well as in other pest and disease resistances, could provide an useful information for breeding strategies. In the characterization presented herein, several traits related with fruits, flowers and vegetative growth were evaluated in two different time periods (spring-summer 2009 and 2010). We reported 14 QTL pairs located at least in the same linkage group for different traits. Linkage groups II and IV were the most saturated groups in QTL related with fruit and ovary sizes observed in our work. Previous authors also found QTL associated with these traits in those linkage groups (Serquen et al., 1997; Périn et al., 2002b; Fazio et al., 2003; Zalapa et al., 2007; Paris et al., 2008; Harel-Beja et al., 2010). In linkage group II we could also find QTL associated with three traits related with fruit quality, as reported also by some other authors: solid soluble content (Monforte et al., 2004; Paris et al., 2008; Cuevas et al., 2008; Harel-Beja et al., 2010), number of full develop melons per plant (Zalapa et al., 2007; Harel-Beja et al., 2010), and flesh firmness.

Regarding the linkage groups V and XII, which include the QTL for powdery mildew resistance, mentioned herein, *Pm-R* and *pm-r*, in LG V, some QTL associated with maximum diameters for fruit and ovary were detected. For these traits, the effect of alleles coming from 'TGR-1551' increased their values, which would not affect fruit quality since similar values for these traits have been scored for both parental lines. In linkage group XII, the QTL for the main vein length, leaf area, and male corolla diameter showed additive effects directed to 'Bola de Oro'. Therefore, the QTL detected in LG V and XII are not directly related to important agronomical trait such as fruit quality or production, and the direction of the estimates additive effects did not affect negatively the evaluated trait. Thus, the introgression of the *P. xanthii*

resistance from 'TGR-1551' would appear not to cause fruit quality problems for future breeding programs, since the genomic regions involved, and related to linkage groups V and XII, did not co-localize with any non-desirable morphological QTL evaluated or detected herein.

Besides the detection of QTL associated with morphological traits and powdery mildew resistance, the following objective was focused on the observation of plant response at microscopic level to estimate the possible mechanisms involved in the *P. xanthii*-melon interaction.

According to literature, post-haustorial resistance behavior is the only one described so far in melon (*C. melo*), and it can be distinguished into type I and type II (Kusuya et al., 2006), depending on the timing of the hypersensitive response (HR). Type I occurs faster: HR takes place in the attacked epidermal cells and stops fungal development at early stages, approximately at 48hpi. In type II, HR takes place firstly in the mesophyll cells under the attacked epidermal cells, what allows a larger fungal development, which stops at approximately 120hpi. According to Kusuya et al. (2006), type I has been observed in 'PMR 45' and 'PMR 5' inoculated with race 1 and in 'WMR 29', 'Edisto 47', 'PI 124112' and 'MR-1' inoculated with races 1 and 2; and type II in 'PI 414723' inoculated with races 1, 2 and 5, 'PMR 5' with races 2 and 5 and 'PI 124112' and 'MR-1' with race 5.

In order to characterize the response to races 1, 2 and 5 of *P. xanthii*, the parental lines of the RIL population used herein ('TGR-1551' and 'Bola de Oro') as well as some genotypes derived from it and carrying different genes for powdery mildew resistance were used. The melon line 'TGR-1551' carrying two genes, one dominant and one recessive (Yuste-Lisbona et al., 2010), RIL88 and RIL276, likely carrying alleles from 'TGR-1551' for the recessive gene, and NIL21, with only the alleles of 'TGR-1551' for the dominant gene. In the resistant genotypes RIL88 and RIL276, a response type II was observed. The melon line NIL21 showed different responses according to the temperature; a faster type II response, named as type IIa, at low temperature, and a type I at high temperature. This response affected by temperature was also observed in the resistant parental line 'TGR-1551': at low temperature showed a slower type II, named herein as type IIb, and a response type I when inoculated at high temperature. With these results we could consider that the resistance of 'TGR-1551' to powdery mildew seems to

be greatly influenced by temperature. This information could help us to understand the different phenotypes observed for a same RIL from the 'TGR-1551' × 'Bola de Oro' population tested in several environments. Some associations could be established between the genes involved in the powdery mildew resistance in the different melon genotypes tested, and the phenotype of the different plant-pathogen interactions described herein. Melon lines containing a gene or QTL in LG V or IX, such as 'TGR-1551', 'WMR29', 'Edisto 47', 'PI 124112', 'PMR 5', 'PMR 45', 'PMR 6' (Pitrat, 1991, Perchepped et al., 2005; Teixeira et al., 2008; Yuste-Lisbona et al., 2011; Ning et al., 2014;) and NIL21, show a resistance type I (Rivera et al., 2002; Kuzuya et al., 2006). Most of these lines with genes or QTL in LG V showed different phenotypes according to the temperature which may mean that, either the same genes controlling powdery mildew resistance in LG V or some genes located close to these resistant genes, could be significantly affected by temperature. On the other hand, those accessions likely with genes or QTL associated to powdery mildew resistance in LG II or XII, such as 'PI 414723', 'PI 124112', RIL88 and RIL276 (Pitrat, 1991; Périn et al., 2002; Perchepped et al., 2005; Beraldo-Hoischen et al., 2012; Fazza et al., 2013), show a resistance response type II (Kuzuya et al., 2006).

Further studies will be needed to confirm the presence and the precise location of the recessive gene for resistance to powdery mildew conferred by 'TGR-1551', to validate the molecular markers linked to the QTL detected, as well as to characterize the mechanisms of defense involved in this resistance and the role of temperature in powdery mildew-melon interaction. Moreover, the current availability of melon genome and several platforms for massive genotyping may facilitate the identification of candidate genes involved in the resistance to powdery mildew, exploring gene expression, as well as estimating the relative role of genes identified in the biological processes occurring during plant-pathogen interaction and the effect of environmental variables in them.

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Capítulo 7

7 Conclusiones

1. Se han identificado dos QTL para la resistencia a las razas 1, 2 y 5 de *Podosphaera xanthii* para cada uno de los tres ambientes evaluados (verano y otoño 2010 y primavera 2011) en el mapa de ligamiento de una población RIL (F7:F8) obtenida a partir del cruce establecido entre 'TGR-1551' y 'Bola de Oro'. Este mapa ha sido construido con 216 loci, la mayoría de ellos codominantes, distribuidos en 22 grupos de ligamiento (GL), reagrupados en 12, abarcando una distancia total de 992.8 cM, con una distancia media entre marcadores de 4.6 cM.
2. El QTL *Pm-R* fue localizado en el GL V y explicó entre un 16.4 y un 56.6 % de la variación fenotípica observada para la resistencia a las tres razas de oídio en los tres ambientes evaluados. Este QTL se corresponde al ya descrito como asociado al gen dominante para la resistencia a oídio en la población F2 de la que deriva la población RIL evaluada. Otro QTL, denominado *pm-r*, y potencialmente asociado al gen recesivo descrito en la literatura, fue identificado en el GL XII y explicó entre un 8.1 y un 37.3 % de la variación fenotípica para la resistencia a las razas 1, 2 y 5 de *Podosphaera xanthii*. Este QTL podría estar potencialmente asociado al gen recesivo descrito en la literatura. Además de estos QTL, otra región genómica localizada en el GL II podría presentar cierta relevancia en la resistencia a oídio, aunque no se detectaron QTL significativos. Un análisis multi-ambiente preliminar parece indicar que existen interacciones epistáticas entre los dos QTL implicados en la resistencia a oídio, *Pm-R* (LG V) y *pm-r* (LG XII). La expresión del primero de ellos podría estar influenciada de manera significativa por las condiciones ambientales.
3. Los marcadores más estrechamente ligados a los QTL detectados fueron MRGH63, PM3-CAPS, y MarkerD para *Pm-R* en el GL V, y CMBR111, TJ29 y CMBR150 para *pm-r* en el GL XII. Las líneas RIL portadoras de los alelos procedentes de 'TGR-1551' para los marcadores PM3-CAPS, asociado al QTL *Pm-R* (LG V), y CMBR111, asociado a *pm-r* (LG XII), mostraron una respuesta claramente resistente. La respuesta de las RIL portadoras de los alelos procedentes de 'Bola de oro' fue de susceptibilidad.

4. Se detectaron un total de 56 QTL asociados a caracteres morfológicos para las dos evaluaciones realizadas. La mayoría de ellos fueron consistentes, observándose ambos años en los mismos GL en una localización genómica similar. Los grupos de ligamiento II y IV presentaron el mayor número de QTL, estando la mayoría de ellos asociados a caracteres relacionados con los tamaños de fruto y ovario. Concretamente se detectaron 21 QTL en el GL II y 11 en el GL IV.

5. Los QTL vinculados a caracteres morfológicos que colocalizaron con los QTL asociados con la resistencia a oídio no presentaron efectos aditivos negativos. Se identificaron dos QTL en el GL V, uno para el diámetro del fruto y otro para el diámetro del ovario. En el GL XII se localizaron tres QTL, uno para el diámetro de la corola masculina, y dos muy próximos entre sí, uno para la longitud del tallo principal y otro para el área foliar.

6. En la interacción planta-patógeno se pudieron observar las reacciones de resistencia post-haustoriales tipo I y II descritas previamente en melón. Se detectaron así mismo otros dos tipos de comportamientos de resistencia denominados tipo IIa (más rápido que el tipo II) y el IIb (más lento que el tipo II). La temperatura parece influir de forma significativa en las interacciones compatibles e incompatibles entre *C. melo* y *P. xanthii*, especialmente en la respuesta a oídio de 'TGR-1551'. La interacción de tipo I ha sido observada en 'TGR-1551' en inoculaciones a alta temperatura. El tipo II fue observado en las líneas resistentes RIL88 y RIL276, ambas RIL portando *pm-r*, potencialmente el gen recesivo de resistencia a oídio. La interacción de tipo IIa ha sido detectada en la línea NIL21, portadora de *Pm-R* (LG V) asociado al gen dominante. El tipo IIb, que presenta un fenotipo intermedio entre el tipo de resistencia II y el descrito en las líneas susceptibles, fue observado en 'TGR-1551' en inoculaciones a baja temperatura.

