TESIS DOCTORAL

GENÉTICA DE LA INTROGRESIÓN DE GENES DEL ALMENDRO (*Prunus dulcis* Mill.) EN EL MELOCOTONERO [*P. persica* (L.) Batsch]: DESARROLLO DE UNA ESTRATEGIA DE SELECCIÓN DE LÍNEAS CASI ISOGÉNICAS (NILs) CON MARCADORES MOLECULARES

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A mi abuelita

por construir(me) un mundo mejor

Vincent van Gogh (1888) Orchard with peach trees in blossom

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RESUMEN

El melocotonero es el frutal de hueso más importante a nivel mundial, y el tercer árbol de fruta dulce cultivado después del manzano y el peral. A nivel genético es una de las especies mejor caracterizadas de la familia *Rosaceae*. El melocotonero presenta un bajo nivel de variabilidad genética pero es sexualmente compatible con otras especies de *Prunus,* como el almendro, que podrían ser una posible fuente de nuevos genes para enriquecer su genoma. Estudiamos un conjunto de caracteres asociados a la flor, fenología, calidad de fruta, morfología de la hoja y la resistencia a enfermedades en dos poblaciones de almendro ('Texas') x melocotonero ('Earlygold'): una F_2 (TxE) y un BC_1 (T1E) del parental 'Earlygold'. Tres mapas genéticos de alta densidad se construyeron utilizando un chip de SNP de 9K y 131 marcadores microsatélite: el mapa F_2 (llamado TxE) y los mapas de los parentales del BC_1 denominados T1E (para el híbrido) y E (para 'Earlygold'). La comparación del mapa intraespecífico de E con los mapas interespecíficos de TxE y T1E mostró una mayor tasa de recombinación en los cruzamientos intraespecíficos que en los cruzamientos interespecíficos. El mapa de E presentó aproximadamente la mitad de su genoma sin marcadores polimórficos, lo que se interpretó por la presencia de regiones idénticas por descendencia debido a su alto nivel de coancestría. La presencia de plantas androestériles en ambas poblaciones y su segregación fue consistente con la existencia de androesterilidad citoplasmática, debido a que los individuos que tienen el citoplasma del almendro requieren la presencia del alelo del almendro en al menos uno de los dos genes restauradores, *Rf1 y Rf2*, para ser fértil. Varios caracteres como el tipo de flor (*Sh*), el tipo de fruta (Ft), la jugosidad (Jui), el color de la antera (*Ag* y *Ag2*), la pulpa antociánica (*Bf2*), el color de la flor (*Fc2*) y la resistencia al oídio (*Vr3*) se mapearon como caracteres morfológicos. El estudio de caracteres cuantitativos permitió identificar 63 QTLs consistentes en 32 caracteres relativos a flor (2), fenología (7), calidad de fruta (15) y hoja (8). Nuevos alelos del almendro en rasgos importantes como el color de la piel, la pulpa antociánica o la resistencia al oídio son útiles para enriquecer el pool genético del melocotonero cultivado. Proponemos la estrategia de Introgresión Asistida por Marcadores Moleculares (IAM) para integrar los fragmentos cromosómicos del almendro en el melocotonero cultivado en un breve período de tiempo (2-3 generaciones). Esta estrategia incluye tres etapas: la primera consiste en la selección de individuos con bajo número de introgresiones (preNILs) en una amplia población BC_1. En esta etapa obtuvimos nueve individuos con tres o menos introgresiones de 882 individuos T1E, lo que demuestra que esta etapa es factible. La segunda etapa implica el mapeo de genes mayores de interés utilizando

una colección de preNILs. En esta etapa seleccionamos 18 preNILs con cuatro o menos introgresiones y demostramos que todos los genes mayores podían ser mapeados en sus posiciones esperadas, aunque esto sólo fue posible para uno de los dos QTLs mayores estudiados. La tercera etapa consistiría en autofecundar o retrocruzar y autofecundar algunas preNILs para obtener NILs (líneas casi isogénicas) homocigotas con una sola introgresión de almendro en el fondo genético del melocotonero. Una colección completa de NILs (cubriendo todo el genoma del almendro) es una poderosa herramienta para el análisis genético de caracteres complejos. Además, las NILs con genes de interés se pueden introducir rápidamente en un programa de mejora genética. Esta tercera etapa está actualmente en progreso y se retrasará una generación debido a la androesterilidad citoplasmática. Proponemos la estrategia de IAM utilizando otras especies de *Prunus* como una forma de incrementar la variabilidad genética del melocotonero incorporando un conjunto de resistencias a plagas y enfermedades y mejoras en la calidad de la fruta necesarias para la mejora del melocotonero en las próximas décadas.

SUMMARY

Peach is the most important stone fruit crop of the world in cultivated surface and the third among temperate fruit after apple and pear. It is one of the best genetically characterized species of the Rosaceae family. Peach present a low level of genetic variability and it is sexually compatible with other *Prunus* species, as almond, that could be a source of new genes for enrichment its genome. We studied the genetics of traits related to flower, phenology, fruit quality, leaf morphology and resistance to diseases in two almond ('Texas') x peach ('Earlygold') progenies: an F_2 (TxE) and a BC_1 (T1E) to the 'Earlygold' parent. High density maps were developed using a 9k peach SNP chip and a collection of 131 SSR markers. Three maps were obtained: the F_2 map (named TxE) and the maps from the two parents of the backcross, T1E (for the hybrid) and E (for 'Earlygold'). Comparison of the intraspecific E map with the interspecific TxE and T1E maps showed that recombination rates were much lower in interspecific than in intraspecific crosses. The E map had approximately half of its genome without polymorphic markers, which we interpreted as being identical by descent in the fixed regions due to its high level of coancestry. Male sterile plants were recovered in the F_2 and BC_1 generations and their segregation was consistent with the existence of a cytoplasmic male sterility, where individuals having the almond cytoplasm required the presence of the almond allele in at least one of two independent restorer genes, *Rf1* and *Rf2*, to be fertile. Several traits as flower type (*Sh*), fruit type (*Ft*), juiciness (*Jui*), anther color (*Ag* and *Ag2*), blood flesh (*Bf2*), flower color (*Fc2*) and powdery mildew resistance (*Vr3*) have been mapped as single genes. The genetics of quantitative traits has been studied and 63 significant QTLs that had a consistent behavior across years have been identified for 32 flower (2), phenology (7), fruit (15) and leaf (8) traits. New alleles from almond on important traits such as red skin color, blood flesh or powdery mildew resistance have been identified that may prove to be useful for the introduction of new variability into the peach commercial gene pool. We propose the strategy of Marker Assisted Introgression (MAI) to integrate chromosomal fragments of almond into the peach background in a short time span (2-3 generations). MAI includes three steps: the first consists of selecting a set of individuals with a low number of introgressions (preNILs) from a large BC1 progeny, and we obtained nine individuals with three or less introgressions from 882 T1E progeny, showing that this step is feasible. The second MAI step involves mapping some of the major genes of interest using a collection of preNILs. We selected 18 peach-almond preNILs with four or less introgressions and showed that all major genes tested could be mapped to their expected positions, although this occurred in only one

of the two major QTLs assayed. The third step consists of selfing or backcrossing and selfing some of the preNILs to extract homozygous NILs (Near Isogenic Lines) having a single almond introgression in the peach background. A complete collection of NILs (covering the complete almond genome) is a powerful tool for genetic analysis of complex characters and NILs containing genes of interest can be readily introduced into peach breeding to obtain cultivars with novel genes. This third step is currently in progress and will be delayed one generation due to the presence of the cytoplasmic male sterility. The extension of the MAI strategy to other donor *Prunu*s species is proposed as a way to incorporate in the peach genome needed genes for pest and disease resistance and fruit quality for the cultivars of next decades.

RESUM

El presseguer es el fruiter d'os més important a nivell mundial en superfície conreada, i el tercer entre els fruiters de clima temperat, després del pomer i el perer. A nivell genètic, és una de les especies millor caracteritzades de la família de les Rosàcies. El presseguer presenta una baixa variabilitat genètica però es compatible sexualment amb altres especies del gènere *Prunus*, com l'ametller, que poden ser una font de nous gens. En aquesta Tesis, s'ha estudiat un conjunt de caràcters associats a la flor, la fenologia, la qualitat de fruita, la fulla i resistència a malalties en dues poblacions interespecífiques entre l'ametller 'Texas' i el presseguer 'Earlygold': una F_2 (TxE) i un BC_1 (T1E) amb el parental "Earlygold'. Els mapes genètics d'alta densitat es van obtenir utilitzant el xip d'SNPs 9K de préssec i una col·lecció de 131 marcadors microsatèl·lits. En total, es van construir tres mapes genètics: el mapa de la població F_2 (TxE) i els mapes dels dos parentals de la població BC_1, T1E (per l'hibrid) i E (per 'Earlygold'). La comparació del mapa intraespecífic E amb els mapes interespecífics TxE i T1E va mostrar que els nivells de recombinació eren molt més baixos en els creuaments interespecífics que en els intraespecífics. La meitat del mapa E no presentava marcadors polimòrfics, el que es va interpretar per la presencia de regions que eren idèntiques per descendència degut a l'alt nivell de coancestralitat. Tant en la població TxE com en T1E es van identificar individus androestèrils, i la seva segregació era consistent amb la existència d'una androesterilitat citoplasmàtica, on els individus amb el citoplasma d'ametller, necessitaven de la presencia de l'al·lel d'ametller, en almenys un dels dos gens restauradors, *Rf1* i *Rf2*, per a recuperar la fertilitat. Varis caràcters com el tipus de flor (*Sh*), el tipus de fruit (*Ft*), la sucositat (*Jui*), el color de la antera (Ag i Ag2), la polpa antociànica (Bf), el color de la flor (Fc2) i la resistència a l'oïdi (Vr3) van poder ser mapejats com a caràcters monogènics. La genètica dels caràcters quantitatius també es va estudiar i es van identificar 63 QTLs amb un comportament consistent per a 32 caràcters relatius a la flor (2), la fenologia (7), el fruit (15) i la fulla (8). Al final s'han pogut identificar nous al·lels d'ametller per a caràcters d'interès com el color de la pell, la polpa antociànica o la resistència a oïdi, que poden ser útils per a introduir nova variabilitat genètica en el pool genètic del presseguer comercial. Finalment es proposa una estratègia d'Introgressió Assistida per Marcadors (IAM) per a integrar fragments cromosòmics de l'ametller en el fons genètic del presseguer en un curt període de temps (2-3 generacions). La IAM inclou tres etapes: la primera consisteix en la selecció d'un conjunt d'individus amb un baix nombre d'introgressions (preNILS) a partir d'una amplia població BC_1. Nosaltres vam obtenir 9 individus amb 3 o menys introgressions a partir de 882 individus BC_1, demostrant que

aquesta primera etapa es factible. La segona etapa de la IAM consistiria en el mapeig de gens majors utilitzant les preNILs. Nosaltres vam seleccionar 18 preNILs amb 4 o menys introgressions i vam demostrar que tots els gens majors es van poder mapejar en les seves posicions esperades. Això nomes va ser possible amb un dels dos QTLs majors analitzats. La tercera etapa consisteix en autofecundar o tornar a retrocreuar i autofecundar les preNILs per obtenir NILs (Línies Quasi Isogèniques) en homozigosis que presentin un únic fragment cromosòmic d'ametller en el fons genètic del presseguer. Una col·lecció de NILs complerta (cobrint tot el genoma de l'ametller) es una molt bona eina genètica per a l'anàlisi de caràcters complexos, i a mes a mes, les NILs amb gens d'interès es poden incorporar ràpidament en els programes de millora genètica per a obtenir varietats amb nous gens. Aquesta tercera etapa esta en procés i es veurà endarrerida un any degut a la presencia de l'androesterilitat citoplasmàtica. Es proposa la extensió d'aquesta estratègia a altres especies de *Prunus*, com una forma d'incorporar nous gens d'interès a nivell de resistències a malalties o de qualitat de la fruita per a obtenir els cultivars comercials de presseguer de les properes dècades.

1. INTRODUCCIÓN GENERAL

1.1 Agricultura y domesticación

La cultura humana ha vivido diferentes transformaciones en el transcurso de su historia. Una de ellas, la revolución neolítica, cambio profundamente la relación del hombre con su entorno. Uno de los aspectos más relevantes de esta transformación es el comienzo de la agricultura; proceso que se remonta hace aproximadamente 11.000 años atrás en el llamado Creciente Fértil, una región montañosa del suroeste de Asia (Figura 1). Al mismo tiempo comienza el proceso de domesticación de las primeras especies vegetales y animales, fenómeno propiciado por el incremento de tamaño de las poblaciones humanas, y a cambios en la forma de explotación de los recursos locales (Xu, 2010). En el curso de los siguientes 5.000 años plantas silvestres como el trigo (*Triticum turgidum*) y la cebada (*Hordeim vulgare*) en el Medio oriente, el maíz (*Zea mayz* L.) y la patata (*Solanum tuberosum*) en América, y el arroz (*Oriza sativa* L.) en Asia sufrieron el proceso de domesticación, siendo hoy la base alimenticia de la mayor parte de la población mundial.

El desarrollo de los primeros centros urbanos asociado a los orígenes de la agricultura sedentaria, coincide con el inicio de la fruticultura, que implicaba un compromiso de largo plazo con una superficie de tierra. Los primeros frutales domesticados: palma datilera, olivo, higuera, vid, granado y almendro, tienen su origen a fines del Neolítico y durante la Edad de Bronce (Janick, 2005). Por su parte, la domesticación del melocotonero se habría comenzado durante 3.300-2.500 años a.C. (Faust y Timon, 1995).

Figura 1. Mapa del Mediterráneo oriental. La domesticación de las primeras plantas ocurrió en el Cercano Oriente (Creciente Fértil) en la zona delimita por el circulo de color verde, actual sureste de Turquía y norte de Siria (Adaptado de Lev-Yadun et al. 2000).

Según Janik (2005) la domesticación de especies frutales involucra cambios genéticos que incluyen ruptura de dioecia, pérdida de autoincompatibilidad, inducción de partenocarpia y apirenia, poliploidía y alopoliploidía, facilidad de propagación vegetativa y pérdida de sustancias tóxicas, espinas, púas, o de la vellosidad. Otros cambios debidos a la selección incluyen aumento del tamaño del fruto, contenido de azúcares y tiempo de almacenamiento; cambios mediados por recombinación intra e interespecífica, poliploidización y selección continua, entre otros.

1.2 El género Prunus: taxonomía y sistemática

El género *Prunus* (Rosaceae) comprende alrededor de 200 especies de arbustos y árboles caducifolios y de hoja perenne, algunas de ellas económicamente importantes por la producción de frutos, incluyendo: melocotonero [*Prunus persica* (L.) Batsch], albaricoquero (*P. armeniaca* L.), almendro (*P. dulcis* D.A. Webb), cerezo (*P. avium* L.), ciruelo japonés (*P. japonica* Thunb.), ciruelo europeo (*P. domestica* L.), y guindo ácido (*P. cerasus* L.) (Hummer y Janik, 2009; Chin et al. 2014), como por su valor ornamental, maderero o médico (Potter, 2007). Otras especies como *P. davidiana*, *P. ferganensis*, *P. kansuensis*, *P. cerasifera* y *P. mira*, que presentan frutos de baja calidad, destacan por su gran capacidad de adaptación frente a diferentes condiciones de estrés, tanto de origen biótico como abiótico, lo que les ha conferido una importancia en el desarrollo de patrones y, más recientemente, como alternativa para la introgresión de genes de interés en especies cultivadas (Scorza y Okie, 1990; Byrne et al. 2012).

Los frutales cultivados del genero *Prunus*, como el melocotonero y almendro, corresponden a un grupo de especies conocidas como 'frutales de hueso' (Potter, 2011). Su clasificación sistemática es la siguiente:

Reino: *Plantae*
División: *Magnoliophyta*
Clase: *Magnoliopsida*
Superorden: *Rosanae*
Orden: *Rosales*
Familia: *Rosaceae*
Tribu: *Amigdaloidea*
Género: *Prunus*

La clasificación infragenérica del genero *Prunus* ha sido estudiada por diferentes taxónomos a lo largo de los últimos siglos (Wen et al. 2008). Actualmente, la clasificación más aceptada es la propuesta por Rehder (1940), quien adoptó una interpretación amplia del género y lo dividió en cinco subgéneros, los que son posteriormente divididos en secciones. Los aportes de Mason (1913) y Kalkman (1965) también han sido ampliamente aceptados. Esta clasificación consiste en cinco subgéneros: *Amygdalus* (melocotones y almendras), *Cerasus* (cerezas), *Prunus* (ciruela), *Laurocerasus* (Laurel cerezo), y *Padus* (cerezo aliso o cerezo pado) (Chin et al. 2014).

1.2.1 *Amygdalus*: diversidad y recursos genéticos

El melocotonero está estrechamente emparentado con otros miembros del subgénero *Amygdalus* (Scorza y Okie, 1990; Badenes y Parfitt, 1995; Bortiri et al. 2001; Chin et al. 2014) (Figura 2). Las especies silvestres de *Amygdalus* emparentadas con el melocotonero y compatibles sexualmente se presentan como una valiosa alternativa para el enriquecimiento genético de la especie cultivada (Byrne et al. 2000; Gradziel, 2003; Foulogne et al. 2003a). A continuación se enumeran las principales especies silvestres de *Amygdalus* que han producido descendencia fértil en cruces con melocotonero:

- *Prunus davidiana* (Carr.) Franch. es nativa del noreste de China, donde se utiliza como patrón, dada su tolerancia a la sequía, a pesar de ser muy sensible a los nematodos. Además, las variedades muestran una disminución del vigor y una temprana entrada en producción (Wang, 1985). El árbol es alto (hasta 10 m), con una corteza de color marrón rojizo; las hojas son largas y glabras, ovadas-lanceoladas, la flor es de color blanco o rosa claro; el hueso es pequeño y separado de la carne (Scorza y Okie 1990). Cruzamientos de esta especies con melocotonero se han llevado a cabo para introducir resistencia a plagas y enfermedades y para generar patrones tolerantes a suelos marginales o con problemas de replante (Foulongne et al. 2003; Moing et al. 2003; Savala et al. 2013).

- *P. ferganensis* (Kost. & Rjab) Kov & Kost. se encuentra en el oeste de China, clasificada por algunos autores como sub-especie de *P. persica*. Se diferencia por sus hojas más alargadas; con venas paralelas y los huesos con ranuras continuas (Scorza y Okie 1990). Presenta frutos de una gran variabilidad y de mejor calidad que otras especies silvestres (Wang, 1985).

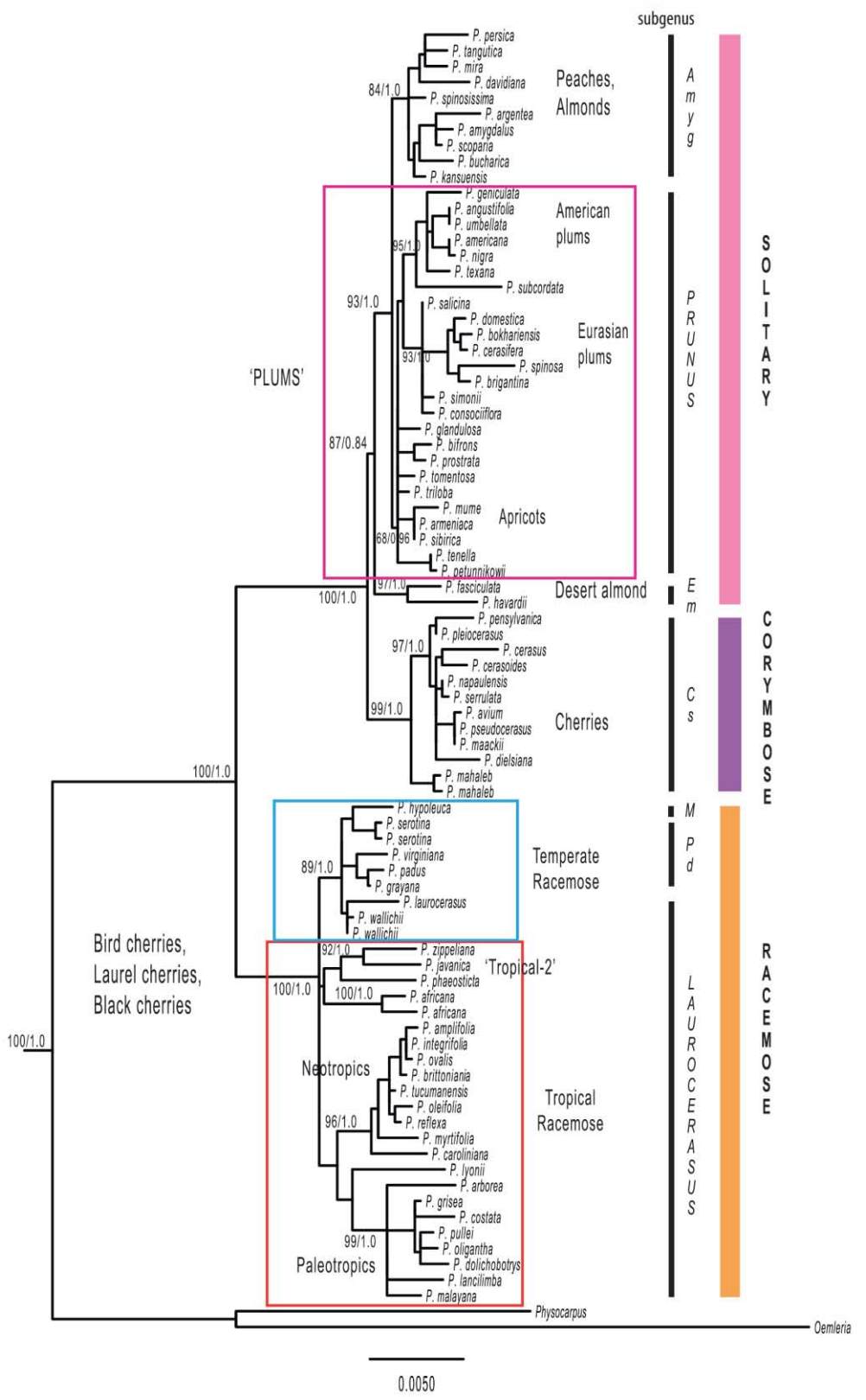


Figura 2. Árbol de máxima verosimilitud de las secuencias de ADN plastidial de cuatro genes concatenados para filogenética de *Prunus*. Los números por encima o por debajo de las ramas indican respaldo 'bootstrap' de la máxima verisimilitud/probabilidad posterior Bayesiana. Las abreviaturas para subgénero: Amyg, Amygdalus; Em, Emplectocladus; Cs, de Cerasus; Lc, Laurocerasus, Pd, Padus; M, 'Maddenia' (Chin et al. 2014).

- *P. kansuensis* Rehd. se encuentra en el noreste de China, donde se utiliza como patrón; se diferencia del melocotonero por sus yemas glabras, pistilos alargados y los surcos paralelos de sus huesos (Wang, 1985). Es un arbusto frondoso, de floración temprana, con flores resistente a las heladas. La calidad de la fruta es muy baja por su elevada astringente (Meader, 1939; Meader y Blake, 1940 citados por Scorza y Okie, 1990).

- *P. mira* Koehne se encuentra en el lejano oeste de China (Tíbet oriental). El árbol es alto, alcanzando alturas superiores a los 20 metros y presenta una gran longevidad, encontrándose individuos de más de 1000 años de edad. Presenta hojas lanceoladas, redondeadas en la base y las flores son de color blanco. El fruto es muy variable en forma, color y tamaño; textura del mesocarpo y adherencia del hueso. El hueso es liso, aunque en algunos tipos se asemeja a *P. persica* (Wang, 1985). Algunas formas se cultivan en el Tíbet y en algunas regiones de India se utiliza como patrón (Scorza y Okie, 1990) (Figura 3).

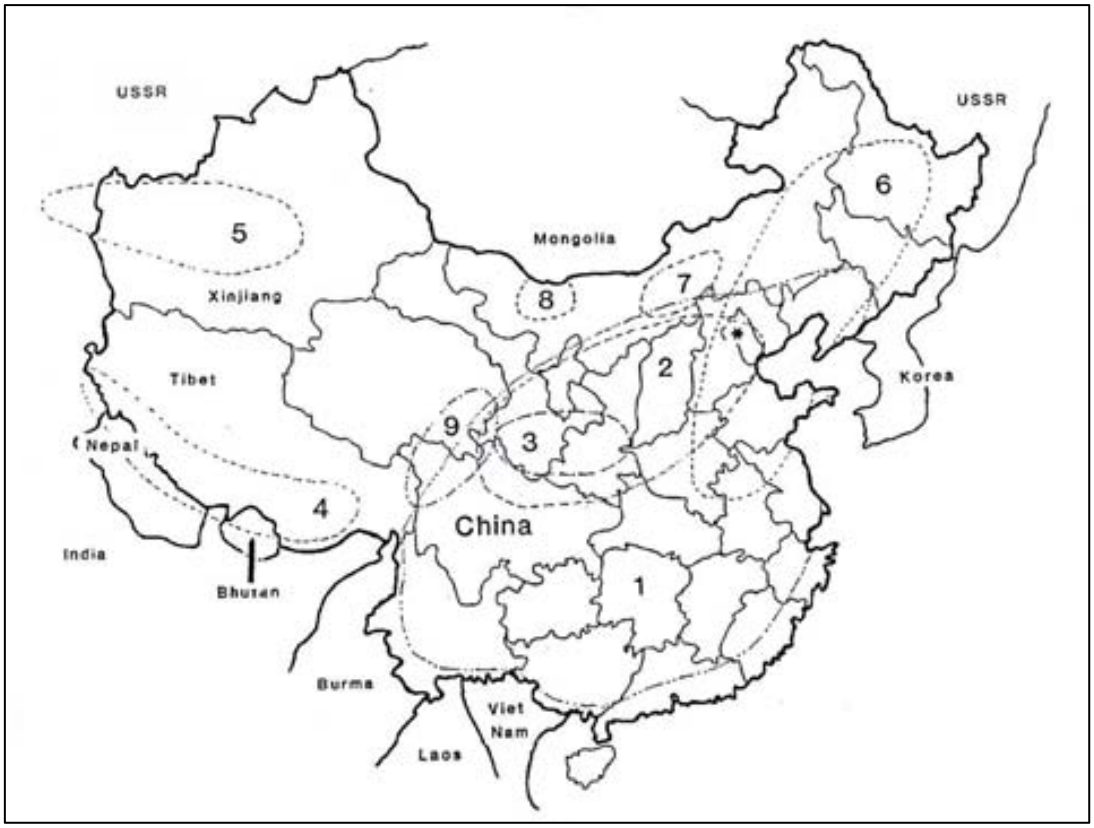


Figura 3. Distribución natural de melocotoneros y almendros en China: *P. persica* (1), *P. davidiana* (2), *P. kansuensis* (3), *P. mira* (4), *P. ferganensis* (5), *P. triloba* (6), *P. mongólica* (7), *P. pedunculata* (8), *P. dehiscens* (9) (Yoshida, 1987 en Skorza y Okie, 1990).

1.3 El melocotonero

1.3.1 Distribución actual

Actualmente el cultivo de esta especie se desarrolla principalmente en regiones temperadas y subtropicales en una franja que se ubica entre los 30º de latitud norte y 45º de latitud sur (Scorza y Sherman, 1996), mostrando una de las mayores adaptabilidades ecológicas entre los frutales, cultivándose con éxito en climas húmedos del sur de China, norte de Egipto y el oeste de EE.UU; en climas semidesérticos de California e Irán; en climas temperados de España y Chile; en climas fríos del norte de China y Canadá y en climas sub-tropicales sin un invierno real como Florida, México e Israel (Faust y Timon, 1995). Sin embargo, las condiciones de alta humedad son un factor limitante para la producción del melocotonero por el aumento de la incidencia de plagas y enfermedades (Byrne et al. 2012).

1.3.2 Botánica

El melocotonero es un árbol caducifolio, robusto y vigoroso, aunque su tamaño está influenciado principalmente por las condiciones edafo-climáticas y el vigor conferido por el patrón (Bassi y Monet, 2008). El sistema radicular es inicialmente profundo, para posteriormente desarrollarse dentro de los primeros 50 a 60 cm de profundidad, dependiendo del tipo de suelo.

El árbol en su etapa adulta presenta diferentes tipos de estructuras vegetativas que difieren en longitud, edad y por su contribución a la producción y calidad de la fruta:

- Brote del año: madera de buen vigor, con yemas florales a lo largo de todo el eje y una yema vegetativa en el ápice; son las más importantes desde el punto de vista productivo debido a que su vigor se correlaciona positivamente con fruta considerada de buena calidad.
- Brindilla: brote de medio vigor (alrededor de 10 a 25 cm). Es la estructura preferida en la industria del melocotón para conserva debido a que produce principalmente frutos de tamaño medio, ideales para su procesamiento.
- Dardo: brote productivo de muy pequeño tamaño (1-5 cm) que eventualmente termina en una yema vegetativa.
- Anticipado: brote lateral que surge de una brotación del mismo año.
- Chupon: brote de excesivo vigor sin yemas florales.

El aspecto general o hábito de crecimiento de esta especie, expresado por la distribución, ángulo y longitud de los entrenudos de ramas fructíferas a través del dosel determinan su aspecto y arquitectura natural; presentando al menos seis tipos característicos (Figura 4) (Bassi, 2003 en Bassi y Monet, 2008).

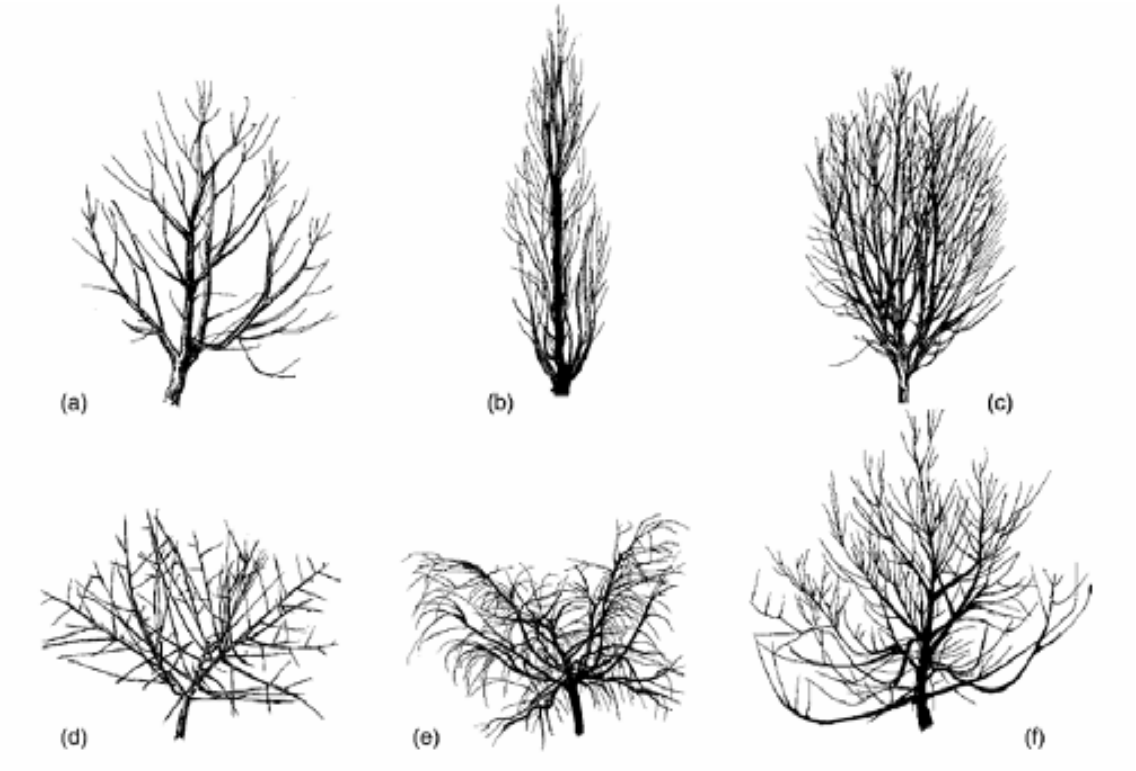


Figura 4. Los principales hábitos de crecimiento definidos en melocotoneros: a) estándar; b) columna; c) vertical; d) compacto; e) colgante; f) abierto. (Bassi, 2003 en Bassi y Monet, 2008).

El desarrollo de los nuevos brotes y las hojas ocurre posterior a la antesis. Las hojas son lanceoladas y acuminadas, pudiendo presentarse de forma plana u ondulada con márgenes o bordes ligeramente aserrados y dos estipulas caducas denticuladas. Las hojas se encuentran espaciadas y colocadas en forma alterna en las ramas (Alvarado y Gonzáez, 1999). En la base del peciolo se presentan glándulas esféricas o reniformes, las que se han utilizado para la caracterización varietal (Castro et al. 1998). En cada nudo normalmente existen tres yemas: dos florales en los extremos y una vegetativa en la parte media. Según el vigor y la distribución de la yemas, el melocotonero puede fructificar en ramas mixtas, ramas de dos años, chifonas o en ramilletes de mayo, originando una flor perígina y hermafrodita, con un solo pistilo, de 20 a 30 estambres. La forma de la corola, compuesta normalmente de cinco pétalos, clasifica las

flores del melocotonero en dos grupos: *Rosacea* o *showy*, cuando los pétalos son largos; y *Campanulacea* o *non-showy*, cuando los pétalos son pequeños, y se observan claramente las anteras entre los pétalos antes del período de antesis (Bassi y Monet, 2008).

Los estados de desarrollo morfológicos de los principales órganos de una especie desde brotación a caída de hojas son conocidos como estados fenológicos (Figura 5). En los frutales caducifolios, como el melocotonero, que presentan mecanismos adaptativos para protegerse de las bajas temperaturas invernales (Faust et al. 1997), la época de floración se ve influenciada por dos mecanismos complementarios: la acumulación del frío durante el invierno es necesario para romper el estado de reposo de las yemas y, posteriormente, la acción de las temperaturas cálidas se requieren para promover el desarrollo vegetativo y floral (Campoy et al. 2011). Sin embargo, los estudios realizados en diferentes especies de Prunus (Ruiz et al. 2007; Alburquerque et al. 2008; Okie y Blackburn, 2008) sugieren que los requerimientos de frío tienen un efecto más importante en la tiempo floración que los requisitos de calor. Por otra parte, las diferentes variedades de melocotonero presentan una variabilidad en la densidad de floración, lo que se conoce como floribundidad. La tendencia de determinadas variedades a producir más yemas florales, les confieren una mayor capacidad de sobrevivir en invierno y a hacer frente a las heladas primaverales (Marini y Reighard, 2008).

La producción de frutos comienza a partir del segundo o tercer año cuando culmina el período de juvenilidad (Bassi y Monet, 2008). El fruto del melocotonero es una drupa, que puede presentar forma redondeada (globoso) o alargada (plano). La pulpa carnosa, o mesocarpo, es de color blanca, amarilla o rojiza, presentándose adherida (pavía) o separado del hueso (prisco), según el tipo de fruto (Alvarado et al. 1999). La curva de crecimiento del fruto sigue una curva doble sigmoidea con tres fases claramente destacadas: i) rápido crecimiento, división celular; ii) estancamiento del crecimiento, endurecimiento del hueso, y iii) segunda fase del crecimiento, elongación celular (Gage y Stutte, 1991). La epidermis del fruto presenta dos tipologías: i) el velloso o melocotón y, ii) el glabro o nectarina, mutación del melocotón estándar probablemente originado por primera vez en el noroeste de China (Faust y Timon, 1995). Según Wen et al. (1995) el *locus* responsable del carácter nectarina estaría ligado o tendría un efecto pleiotrópico con varios otros caracteres como: tamaño, contenido de sólidos solubles y ácidos orgánicos, período de desarrollo del fruto y la exigencia de unidades de frío, lo que lo ha posicionado como una excelente alternativa al melocotonero tradicional, ganando grandes cuotas de mercado en los últimos años.

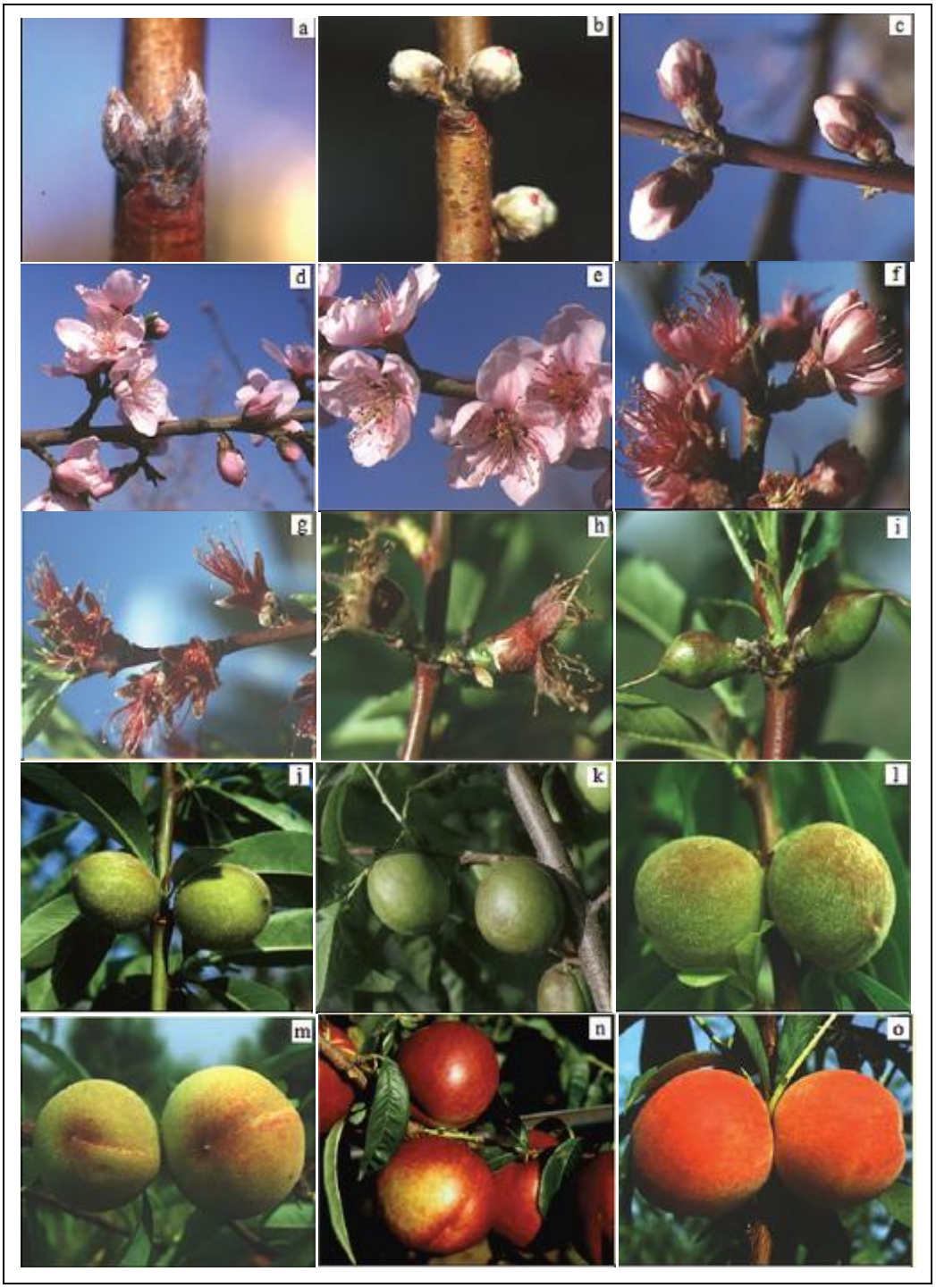


Figura 5. Estados fenológicos del melocotonero. a) yema de invierno, b) inicio de brotación, c) botón rosado, d) inicio de floración, e) plena floración, f) fin de floración, g) caía de pétalos, h) caída de corola, i) fruto cuajado, j) fruto pequeño, k) endurecimiento del hueso, l) fruto en crecimiento, m) cambio de color , n) madurez comercial, o) madurez fisiológica. (Cortesía de E. Bellini, Universidad de Florencia, Italia. Adaptado de Okie et al. 2008)

1.3.3 Importancia económica y social

El melocotonero es uno de los cultivos frutales de mayor importancia a nivel mundial. En los últimos 15 años, la producción mundial del melocotonero prácticamente se ha duplicado como consecuencia del uso de técnicas de cultivo más eficientes, de la introducción de nuevas variedades y de patrones mejor adaptados (Llácer, 2005; Iglesias, 2013) y al rápido incremento de la producción en China (Byrne et al. 2012). Se calcula que en el año 20012 la producción mundial alcanzó la cifra de 21.1 millones de toneladas, siendo la tercera fruta dulce más producida a nivel mundial después del manzano y el peral (Byrne et al. 2012; FAOSTAT, 2014). Los principales productores a nivel mundial son encabezado por China, que concentra el 57% de la producción mundial (Tabla 1) (FAOSTAT, 2014).

Los países de la Unión Europea produjeron 3.4 millones de toneladas de melocotón durante el año 2012, representando el 16% de la producción mundial. El principal productor es Italia (39%), seguido por Grecia (22%), España (21%) y Francia (8%) (FAOSTAT, 2014).

Tabla 1. Principales países productores de melocotón, año 2012 (FAOSTAT, 2014).

País	Producción (t)	Superficie (ha)	Rendimiento (t/ha)
China	12.027.600	770.000	15.6
Italia	1.331.621	71.012	18.8
Estados Unidos de América	1.058.830	56.365	18.8
Grecia	760.200	44.100	17.2
España	747.200	50.000	14.9
Turquía	575.730	28.362	20.3
Irán (Republica Islámica de)	500.000	20.000	25.0
Chile	325.000	20.000	16.3
Argentina	290.000	26.000	11.2
Egipto	285.194	26.611	10.7
Francia	275.521	11.923	23.1
India	250.000	37.500	6.7
Brasil	232.987	19.155	12.2
Argelia	177.986	18.657	9.5
Sudáfrica	175.665	10.200	17.2
Republica de Corea	172.599	14.357	12.0
México	162.866	33.216	4.9
Japón	135.200	9.950	13.6
Túnez	128.000	16.000	8.0
Republica Popular Democrática de Corea	118.500	21.500	5.5

1.4 Mejora genética de plantas

La mejora genética de plantas se inició con la búsqueda y selección de individuos con características superiores en el medio silvestre. Por miles de años este proceso se llevo a cabo de manera empírica e instintiva. Sin embargo, desde el siglo XX en adelante con el redescubrimiento de las leyes de Mendel y el desarrollo de la genética moderna la mejora se ha convertido en un área relevante de las ciencias biológicas; especialmente importante si consideramos que aproximadamente el 50% de los aumentos de rendimiento obtenidos durante la revolución verde, en los cultivos más importantes para la humanidad, son atribuibles a la introducción de nuevas variedades obtenidas mediante mejora (Hayward et al. 1993). Por el contrario, la mejora genética de frutales fue un campo descuidado durante muchos años, debido principalmente a la escasez de mutaciones, su gran tamaño y complejidad biológica (largo período generacional). El trabajo en melocotonero, sin embargo, constó de grandes esfuerzos lo que permitió un mayor conocimiento de su genética (Monet y Bassi, 2008) en comparación con otras especies leñosas.

1.5 Mejora genética del melocotonero

1.5.1 Origen y diseminación

El nombre botánico del melocotonero [*Prunus persica* (L.) Batsch] se refiere a la cuidad putativa de origen, Persia (actualmente Irán), que utilizó Lineó (1758) para nombrarlo por primera vez. Más de 100 años después, gracias al esfuerzo de diferentes botánicos (De candelle, 1883; Hedrik, 1917; Vavilov, 1951 citados por Bassi and Monet, 2008) se conoció y aceptó el verdadero origen de la especie. El melocotonero es nativo de China (Wang, 1985; Huang et al 2008), concretamente de la cuenca del río Tarim (Faust and Timon, 1995), donde aún se encuentra en estado silvestre, presentando la mayor fuente de variabilidad genética de la especie en el mundo (Yoon et al. 2006).

La diseminación del melocotonero desde el oeste de China siguió la ruta comercial a través de Persia, conocida como la 'Ruta de la Seda'. Desde allí se introdujo en Egipto (1.400 a.C.) donde sus frutos eran utilizados como ofrenda al 'Dios de la Tranquilidad' y en Grecia donde los registros de su producción se remontan a 400 y 300 años a.C. Posteriormente, en los primeros siglos de nuestra era, los Romanos expandieron su cultivo por toda la costa Mediterránea (Hancock et al. 2008) y a otros países del continente europeo como Inglaterra, donde es

cultivado desde el año 200 d.C., al mismo tiempo que el melocotonero colonizaba tierras niponas (Yamamoto et al. 2003). Finalmente, durante el siglo XVI los primeros exploradores españoles y portugueses lo llevaron en sus embarcaciones al continente americano, donde se cultivo inicialmente en Florida, México y Sur América, para ser rápidamente adoptado por los nativos americanos y extendido por toda Norte América (Figura 6) (Scorza y Okie, 1990).

La propagación por medio de semillas fue la principal vía para la obtención de cultivares hasta la mitad del siglo XIX en los EE.UU. y Europa, y hasta mediados del siglo XX en América Central y América del Sur. Por lo tanto, existen muchas variedades de melocotón que han sido objeto de varios siglos de selección en cuanto a la adaptación y otras características en toda Europa, América del Norte, América del Sur y Japón (Byrne et al. 2000; Pérez, 1989; Pérez et al. 1993; Yamamoto et al. 2003). Muchas de esas selecciones aún se cultivan localmente (Byrne et al. 2012) y constituyen una valiosa fuente de germoplasma para los programas de mejora actuales (Bouhadida et al. 2007).

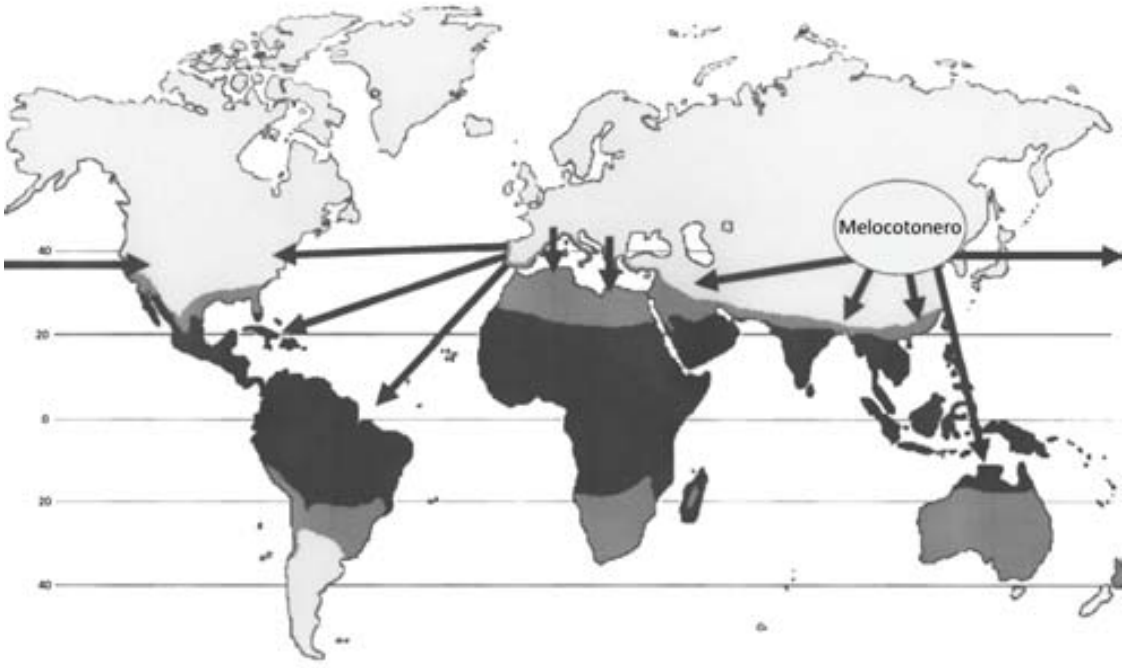


Figura 6. Diseminación temprana del melocotonero (Adaptado de Byrne et al. 2012).

1.5.2 Mejora genética clásica

La mejora genética es un proceso que implica ciclos de cruzamientos entre genotipos con una variación genética útil y la identificación y selección de fenotipos superiores a partir de los que se desarrollan variedades superiores a las precedentes (Torres et al. 2012). Los esfuerzos de mejora en melocotonero, tanto en el sector público como privado, se han dirigido a satisfacer las demandas del mercado y la industria, procurando principalmente el desarrollo de variedades de gran tamaño, alta productividad, completa coloración roja de la piel, ausencia o bajo nivel de pubescencia, forma redondeada, firmeza de la pulpa y frutos de pulpa amarilla o blanca (Byrne, 2005; Okie et al, 2008). Estos programas mostraron un gran dinamismo durante la pasada centuria (Fideghelli et al. 1998; Sansavini et al. 2006) entregando una media de 100 variedades por año en la última decada (Byrne et al. 2012). Sin embargo, la mayoría de ellas se han originado a partir de sólo unos cuantos parentales fundadores que se utilizaron por los primeros programas de mejora en los EE.UU (Faust y Timon, 1995; Scorza et al. 1985). Aranzana et al. (2003; 2010) estudiaron la estructura poblacional de 224 variedades procedentes de programas de mejora de EE.UU., España, Italia, Francia y Canadá, incluyendo 8 de las variedades consideradas fundadoras de los programas de mejora moderna de los EE.UU., para conocer el real impacto genético de los padres fundadores en el fondo genético de las variedades de melocotoneros actuales y encontraron que cinco de ellas ('Elberta', 'Fay Elberta', 'Early Elberta', 'Rio Oso Gem' y 'J.H. Hale') conforman la mayor y directa fuente de la variabilidad genética del melocotonero actual, corroborando los registros y referencias bibliográficas sobre la materia.

A pesar de la escasa base genética, producto de su comportamiento autógamo y a los cuellos de botella surgidos en la historia de la domesticación-mejora de la especie (Aranzana et al. 2010), tres grandes logros durante la mejora genética del melocotonero cultivado son destacables: la expansión de su cultivo a diferentes climas mostrando una gran adaptabilidad climática; la extensión del período de cosecha y la diversificación de su mercado principalmente mediada por el desarrollo de la nectarina (Byrne et al. 2012), y los más actuales melocotones y nectarinas planos.

En la actualidad, la mejora genética del melocotonero enfrenta nuevos desafíos. En primer lugar, el desarrollo de variedades resistentes a plagas y enfermedades son una deseable alternativa para satisfacer la creciente conciencia ambiental entre los consumidores (Byrne, 2002) y para proveer de alternativas sostenibles a los productores, disminuyendo las

aportaciones de productos químicos al ambiente (Pascal et al. 2010). En segundo lugar, la mayoría de las variedades actuales de melocotonero se desarrollaron en EE.UU. y Europa, lugares donde el tiempo transcurrido entre la fecha de cosecha y el consumo de la fruta no sobrepasa los 10-15 días, por lo que el comportamiento poscosecha de las variedades liberadas no fue un objetivo prioritario de los programas. Con el incremento del intercambio comercial y el posicionamiento de algunos países del Hemisferio Sur como potencias exportadoras, el tiempo de almacenamiento en poscosecha de los melocotones ha aumentando considerablemente a una media de 30-45 días, tiempo en el cual los melocotones desarrollan problemas fisiológicos conocidos en su conjunto como 'daño por frió' (del inglés 'chilling injury') que ocasionan una pérdida substancial en la calidad de la fruta, una merma económica a toda la cadena productiva y un detrimento del consumo en contra-estación (Crisosto, 2002). Por tal motivo, numeroso estudios se han conducido para entender las bases genéticas de la problemática (González-Agüero et al. 2008; Ogundiwin et al. 2008; Cantin et al. 2010; Martínez-García et al, 2012; Martínez-García et al, 2013) y diversos programas de mejora tienen como uno de sus objetivos prioritarios el desarrollo de variedades con una mayor vida poscosecha (Infante et al. 1998). Es así como, una nueva gama de variedades son requeridas para ser cosechados en un estado maduro, que soporten un largo almacenaje (Faust y Timon, 2005), e incorporen un conjunto de resistencias a plagas y enfermedades (Byrne, 2005).

Por otra parte, el cambio más dramático que han enfrentado los programas de mejora de melocotonero en las últimas décadas ha sido la reducción de financiamiento público, en contrapartida al incremento de la importancia de los programas privados, los que son responsables actualmente del desarrollo del mayor número de nuevas variedades en EE.UU., Francia, y España. En EE.UU. alrededor de la mitad de los programas públicos de mejora de melocotonero se han clausurado desde 1970. Los que permanecen activos deben utilizar las ganancias generadas por las patentas para cubrir parcial o completamente su financiamiento. Este hecho permite que muchos programas continúen siendo viables, pero tiene el inconveniente que a largo plazo limitará la investigación en genética y recursos fitogenéticos y en el intercambio de germoplasma entre diferentes países y programas (Byrne 2005; Okie et al. 2008).

1.6 El melocotonero: "especie modelo"

El melocotonero ha sido durante mucho tiempo una de las especies mejor caracterizadas genéticamente dentro de la familia Rosaceae (Arús et al. 2012), siendo considerada como especie modelo, junto a *Malus* x *domestica* y *Fragaria vesca* L., para el desarrollo de estudios genómicos y genéticos en virtud de un conjunto de características ventajosas para ello (Abbott et al. 2002; Verde et al. 2005; Bielenberg et al. 2009). Es una especie diploide con ocho cromosomas (2n = 2x = 16) (Jelenkovic y Harrinton, 1972) sin una historia significativa de duplicación dentro de su genoma (Verde et al. 2013); no posee mecanismos de auto-incompatibilidad gametofítica como otras especies cultivadas de su género, lo que permite la creación de poblaciones F_2 para proyectos de mapeo genético y su periodo inter generacional de 2-3 años es relativamente corto comparado con los 5-10 años de otras especies leñosas (Rieger, 2006; Arús et al 2012).

Desde un punto de vista genómico, se puede considerar que tiene un genoma pequeño (≈230 Mb) (Verde et al. 2013) menor a dos veces el tamaño del genoma de *Arabidopsis* y más pequeño que los genomas de *Fragaria vesca* (Shulaev et al. 2011), de *Malus* x *domestica* (Velasco et al. 2010) y el recientemente secuenciado genoma de *Prunus mume* (≈280 Mb) (Zhang et al. 2014).

1.7 Herramientas para la mejora molecular

La comunidad científica actual cuenta con diversas herramientas para el estudio genético y genómico del melocotonero. Las características ventajosas de la especie, descritas en el apartado 6, como el temprano inicio de los estudios genéticos llevados a cabo principalmente en EE.UU. y Europa durante el siglo XX y el esfuerzo de diferentes países y grupos de investigación de EE.UU., España, Italia, Chile y Francia, culminó con la reciente secuenciación y liberación del genoma de la especie (Verde et al. 2013). La integración de toda la información genética y genómica disponible abrirá la era post-genómica, que se caracteriza por la disponibilidad de un genoma completo y las nuevas tecnologías de secuenciación masiva de ADN y ARN. En este sentido, estamos frente a una revolución en el uso de nuevas técnicas de análisis de alto rendimiento, lo que puede significar un cambio de paradigma científico en la identificación de regiones genómicas de interés y su posterior aplicación en la mejora (Salazar et al. 2014).

En las siguientes secciones se describirán los aspectos más importantes relacionados con el desarrollo y utilización de poblaciones genéticas, mapas genéticos y el estudio de QTLs en plantas con un énfasis especial en *Prunus*, así como los aspectos teóricos más relevantes.

1.7.1 Poblaciones genéticas

Diversos tipos de poblaciones pueden ser utilizadas para el desarrollo de estudios genéticos en vegetales: poblaciones F_2, poblaciones F_2 inmortales, poblaciones de retrocruzamiento (BC), doble haploides (DHs), Líneas consanguíneas recombinantes (RILs), y líneas casi isogénicas (NILs) (Tabla 2). En términos generales, las poblaciones F_2 y BC son simples y fáciles de construir, requieren relativamente poco tiempo en su desarrollo, pero son altamente heterocigotas por lo que su reproducción no puede efectuarse por medio de semillas. En cultivos, se utilizan temporalmente para la construcción de mapas genéticos preliminares. Por el contrario, en frutales son las poblaciones más utilizadas. Alternativamente, las poblaciones RILs, DHs, y NILs son completamente homocigotas; pueden ser multiplicadas eternamente mediante semillas, representando un recurso permanente para el desarrollo de estudios genéticos en diferentes ambientes y con la posibilidad de ser transferidas entre diversos grupos de investigación (Boophati, 2013).

Tabla 2. Características de las principales poblaciones usadas en estudios genéticos.

Propiedad	Poblaciones				
	F_2	BC	DH	RILs	NILs
Nº generaciones requeridas	2	2	2	6-8	6-9
Nº de meiosis	1	2	2	múltiples	múltiples
Nº posible de genotipos por locus	3	2	2	2	2
Herencia marcador dominante	3:1	1:0	1:1	1:1	1:1
Herencia marcador codominante	1:2:1	1:1	1:1	1:1	1:1

No existen estudios específicos que determinen el número ideal de individuos que requiere tener una población genética dada (Boophati, 2013). Sin embargo, la precisión de la medición de la distancia genética entre los marcadores, así como la determinación del orden entre ellos, está directamente relacionada con el número de individuos de la población de mapeo (Young et al. 2000). En la práctica se utilizan poblaciones consistentes entre 50-250 individuos, lo que

ha demostrado ser suficiente para construir el esqueleto inicial del mapa (Mohan et al. 1998) y desarrollar estudios genéticos iniciales, aunque para el desarrollo de mapas de alta resolución o para el mapeo fino son necesarios mas de 1.000 individuos (Collard, 2005).

La elección del tipo de población de mapeo para estudios genéticos y utilización en programas de mejora depende de la estrategia reproductiva de la especie. Para especies autógamas, poblaciones tipo F_2 y RILs son ampliamente utilizadas, las que se originan generalmente mediante el cruce de dos padres altamente homocigotos. En especies alogamas o de polinización cruzada, que en su mayoría no toleran la consanguinidad, son mayormente desarrolladas las poblaciones tipo F_1, por medio del cruce entre dos padres heterocigotos. Poblaciones BC y DHs pueden ser utilizadas para ambos tipos de plantas. El método de desarrollo de las principales poblaciones utilizadas en la mejora se presenta en la Figura 7.

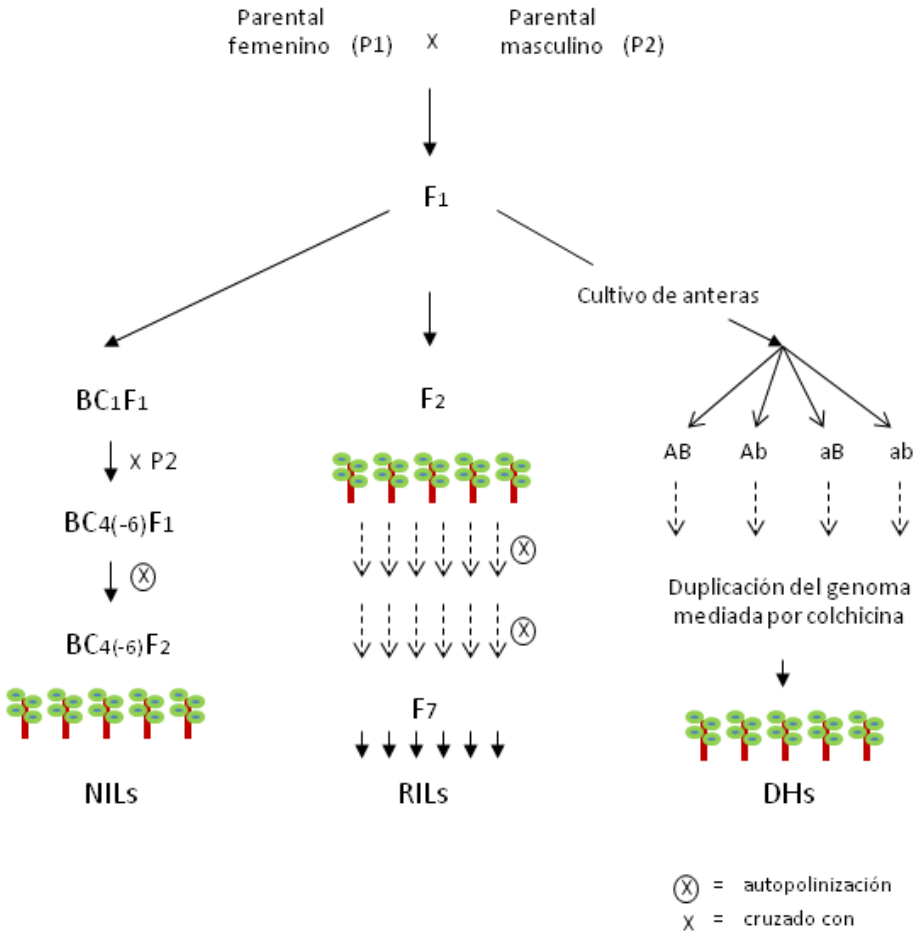


Figura 7. Diagrama de los principales tipo de poblaciones utilizadas para el mapeo genético (Adaptado de Collard et al. 2005 y Boophati, 2013).

Las NILs corresponden a un tipo de población genética diseñadas con la finalidad de superar las limitaciones observadas en el estudio de caracteres cuantitativos observadas en otro tipo de poblaciones y para el aprovechamiento máximo de la variabilidad generada a partir de un cruzamiento. En cada línea casi isogénica se presenta una sola introgresión o fragmento exógeno, procedente de un genoma donante, en el fondo genético de un genoma receptor o recurrente (Eshed y Zamir 1995). Además, la condición que cada línea posee sólo una pequeña fracción del parental donante puede limitar los problemas de fertilidad asociados a los cruzamientos interespecíficos (Eshed y Zamir, 1994).

El desarrollo de este tipo de poblaciones involucra diversas generaciones de retrocruzamiento, asistidas con marcadores moleculares en cada generación para seleccionar las plantas que presentan el menor número de introgresiones o porcentaje del genoma donante, más una o dos rondas de autopolinizaciones para fijar los segmentos exóticos y poder visualizar los rasgos mediados por genes recesivos.

En general, si dos NILs difieren en algún carácter fenotípico se asume que esta variación esta mediada por el efecto de los alelos del fragmento de ADN introgresado en aquella NIL. Esta misma inferencia puede ser realizada contrastando cada NIL contra su parental recurrente, lo que permite la identificación de regiones genómicas de interés para el enriquecimiento genético de especies cultivadas. Por lo tanto, las NILs constituyen poderosas herramientas para el análisis genético y la introgresión de genes de interés. En particular, son extremadamente valiosas para aquellas especies en las que no se cuenta con un protocolo eficiente de transformación donde la incorporación de los genes identificados como de interés mediante la producción de plantas transgénicas sería una alternativa (Boopitha, 2013).

La primera colección reportada de NILs se construyó mediante el cruzamiento de una especie silvestre de tomate (*L. pennellii*) con un cultivar comercial de tomate (*Lycopersicon esculentum)* utilizado en la industria (Eshed y Zamir, 1994). La colección estuvo compuesta de 50 líneas de introgresión y permitió la identificación de 106 QTLs, una cantidad significativamente mayor en comparación con los estudios realizados previamente en poblaciones de plantas (Eshed y Zamir, 1995). En términos generales, el número de QTLs detectados por carácter en una NILs es mucho mayor a los detectados con poblaciones tipo RIL o DH y tiene la gran ventaja que debido a que los QTLs se encuentran fijados en un genotipo concreto, se puede estudiar el grado de dominancia, las interacciones epistáticas entre QTLs,

la heterosis de los QTLs y llevar a cabo el clonaje de gen implicados en un QTL (Torres et al. 2012).

Otra importante ventaja de este tipo de poblaciones es la aplicación directa a la mejora mediante la incorporación de nueva variabilidad genética en los cultivares élite a partir del cruzamiento con una de las líneas de introgresión con regiones genómicas de interés identificadas. En concreto, la variedad de tomate AB2 que se desarrolló a partir del trabajo de Gur y Zamir (2004) introduciendo un QTL de *S. pennellii*, ha sido uno de los cultivares de tomate para industria más cultivados en California en los últimos años (Torres et al. 2012).

1.7.2 Mapas genéticos

El estudio de los genomas mediante el empleo de mapas puede dividirse en dos grandes categorías: (1) mapas genéticos o de ligamiento y (2) mapas físicos (Patterson, 1996). Los mapas físicos identifican la posición de genes y otros elementos del ADN en los cromosomas facilitando el clonaje posicional de genes y el análisis de cromosomas y genomas en detalle. Una alternativa del mapeo físico es el mapeo citomolecular o citológico, en el cual los marcadores de ADN son directamente visualizados en los cromosomas mediante la utilización de un microscopio, pudiendo medir directamente la distancias entre ellos (Patterson, 1996; Boopathi, 2013). En esta revisión, y debido a la naturaleza de nuestro trabajo, se dará énfasis al mapeo genético.

Un mapa genético, de ligamiento o meíotico, es un representación grafica de la localización relativa de diferentes *loci* en los cromosomas (Van Ooijen y Jansen, 2013). Su construcción está basada en el conocimiento de la frecuencia de recombinación de los alelos de diferentes loci en los cromosomas y es el primer paso para la aplicación de los marcadores moleculares en la mejora genética de plantas (Tanksley et al. 1989). El desarrollo de un mapa genético sigue tres pasos principales: (1) la construcción de una población genética; (2) la identificación de polimorfismos y (3) el análisis de ligamiento de los marcadores (Collard et al. 2005). Desde el desarrollo del primer mapa genético en 1913 por Sturtevant la aplicación de esta herramienta, entre otras cosas, ha permitido:

- Realizar análisis genético permitiendo la localización de caracteres cualitativos y cuantitativos en diferentes especies.

- Facilitar la introgresión de genes o QTLs de interés a través de la selección asistida por marcadores moleculares.
- Comparar mapas entre diferentes especies para evaluar similaridad en el orden de los genes.
- Proporcionar un marco para el anclaje con mapas físicos y facilidad el ensamblaje durante el proceso de secuenciación de genomas.
- Iniciar trabajos de clonaje posicional de genes responsables de caracteres de interés agronómico.

En la construcción de un mapa genético dos elementos básicos son requeridos: una población segregante (discutido en el apartado 7.1.) y una batería de marcadores que nos permitan caracterizar genéticamente a la población. Inicialmente se utilizó la apariencia visual (marcadores morfológicos) de los organismos para la construcción de los primeros mapas genéticos, pero su escaso número, y en algunos casos complejo análisis, limitó su utilización en estudios genéticos. Posteriormente el desarrollo de marcadores bioquímicos o isoenzimáticos permitió incrementar las posibilidades y alcances de esta técnica. Sin embargo, fue el desarrollo de los marcadores basados en la variabilidad del ADN los que han ampliado las aplicaciones genéticas de esta herramienta, incluyendo:

- Estudios de diversidad genética, filogenia, domesticación y evolución
- Genética de poblaciones
- Introgresión y flujo génico
- Mapeo de asociación
- Mapeo genético
- Identificación de cultivares y taxonomía
- Certificación de pureza genética de líneas e híbridos
- Detección de la variación somaclonal

En la actualidad, el genoma de las principales especies vegetales de interés económico se encuentra secuenciado, lo que ha propiciado el desarrollo de plataformas de caracterización genética, conocidas como Chip de ADN, lo que permite conocer la variabilidad del ADN en su nivel más profundo, es decir, a nivel de cada nucleótido; con un nivel de automatización nunca antes visto y a precios que decrecen rápidamente. Este tipo de marcadores, conocidos como SNPs están ampliamente distribuidos en el genoma aunque su presencia y distribución varía

entre especies (Boopathi, 2013). El polimorfismo de un solo nucleótido o SNP (Single Nucleotide Polymorphism) es una mutación que afecta a una única base y puede corresponder al cambio de una sola base por otra o mediada por inserciones o deleciones de una base en medio de una secuencia. Este tipo de marcadores presentan una baja tasa de mutación y son estables de generación en generación a través del genoma (Edwards et al 2007). Además, los SNPs tienen varias ventajas para el mapeo genético sobre otros marcadores moleculares: se producen menos errores durante la detección y evaluación que los SSR y es posible mapear loci de rasgos cuantitativos (QTL) con mayor precisión que con marcadores tipo RFLP o SSR (Yu et al. 2011).

1.7.2.1 Mapas genéticos en melocotonero

El primer mapa genético reportado en frutales se desarrolló en una población intraespecífica F_2 de melocotonero utilizando 83 marcadores tipo RAPD, una isoenzima y 4 marcadores morfológicos (Chaparro et al. 1994), marcando un hito muy relevante en el campo de la genética frutal. Sin embargo, debido a la naturaleza de los marcadores utilizados en su elaboración el trabajo no tuvo mayor trascendencia para la construcción de futuros mapas. El desarrollo de marcadores tipo RFLP en el comienzo de la década de 1980 proporcionó una fuente virtualmente ilimitada de marcadores de alta calidad, presentes en todo el genoma, proveyendo de una herramienta para la construcción de mapas en la mayoría de las especies vegetales (Infante et al. 1998). Con este tipo de marcadores se desarrollaron los dos siguientes mapas genéticos en *Prunus* (Rajapakse et al. 1995; Fooland et al. 1995). En los años posteriores los mapas construidos integraron marcadores dominantes tipos RAPD y AFLP con marcadores codominantes como los RFLP y marcadores morfológicos (Dirlewanger et al. 1998) o utilizaron sólo marcadores AFLP (Lu et al. 1998). Sin embargo, estos mapas se consideran incompletos debido a que el número de grupos de ligamiento detectados en ellos era diferente del esperado; tenían una baja densidad de marcadores (4.5-8.5 cM / marcador); incluían grandes intervalos sin marcadores y muchos de ellos no era posible incluirlos en algún grupo de ligamiento (Abott et al. 2008).

El primer mapa de ligamiento saturado en *Prunus* se realizó en una población F_2 interespecífica proveniente del cruce del almendro 'Texas' y el melocotonero 'Earlygold'. Este mapa se construyó exclusivamente con marcadores transferibles (11 isoenzimas y 226 RFLPs), distribuidos en ocho grupos de ligamiento con una distancia total de 491 cM, representando una densidad promedio de 2.0 cM/marcador y ningún intervalo entre marcadores mostró una

distancia superior a 12 cM (1998 Joobeur et al). Posteriormente, Aranzana et al. (2003) y Dirlewanger et al. (2004) aumentaron la densidad del mapa con la incorporación de 176 SSRs y 123 RFLP. Este mapa, conocido como 'T x E', se adoptó por la comunidad científica como el mapa de referencia del genero *Prunus*; permitió el uso de un conjunto de marcadores como referencias para la construcción de otros mapas en diferentes poblaciones, estableció una terminología común para los grupos de ligamiento y el orden de los marcadores dentro de cada grupo (Abbot et al. 1998).

El desarrollo de marcadores tipo microsatélite (SSR) en *Prunus* representó una importante mejora en el área de los marcadores moleculares; facilitando la construcción de mapas genéticos de segunda generación en *Prunus* (Dirlewanger et al. 2006) gracias a su reproducibilidad, herencia codominante, polimorfismo y transferibilidad entre especies (Collard et al. 2005). Diversos mapas genéticos desarrollados en *Prunus* mediante SSR (Salazar et al. 2014) han permitido identificar y mapear la mayoría de los genes mayores (Tabla 3) documentados hasta el momento en el mapa de referencia del genero *Prunus* para melocotonero.

En los últimos años diferentes iniciativas tendientes al desarrollo de chip de SNPs se han llevado a cabo (Ogundiwin et al. (2008); Ahmad et al. (2011) Verde et al. 2012) permitiendo el desarrollo de mapas genéticos de alta densidad (Martínez-Gómez, 2013; Donoso et al. En preparación). Estos trabajos junto a la posibilidad de contar un genoma secuenciado de alta calidad están posibilitando el mapeo fino y la ubicación de genes candidatos, con un mejoramiento en la eficiencia y resolución nunca antes lograda.

1.8 Análisis de caracteres cuantitativos (QTLs)

Posterior al reconocimiento de los descubrimientos realizados por Mendel y el inicial desarrollo de la genética como ciencia, surgió una controversia entre mendelianos y biométricos, debido a que la explicación dada por Mendel sobre la herencia servía para explicar la herencia de los caracteres cualitativos, es decir, para aquellos con segregación discontinua que presentan clases fenotípicas claramente diferenciadas, en contraposición de los caracteres cuantitativos, los que presenta una distribución continua que no permite diferenciar clases fenotípicas claramente. Este tipo de caracteres conocidos como caracteres cuantitativos, también llamados poligénicos, continuos, multifactoriales o complejos, dependen de la acción acumulable de muchos genes. Se ha constatado que la mayoría de los

caracteres de importancia económica en la agricultura están controlados por este tipo de caracteres. A su vez, las regiones específicas dentro de los genomas donde se encuentran los genes asociados a un carácter cuantitativo particular se denominan loci de caracteres cuantitativos (Quantitative Trait Loci) (Collard, 2005).

Tabla 3. Genes mayores documentados que afectan caracteres morfológicos y agronómicos en melocotonero.

Trait	Gene	Linkage group	References
Adaptation			
Siempreverde	*Evg*	1	Rodriguez et al. (1994); Wang et al (2002)
Árbol			
Hábito crecimiento (normal/pilar)	*Br*	2	Lammerts (1949); Scorza et al. (2002)
Altura planta (normal/enana)	*Dw*	6	Lammerts (1945); Yamamoto et al. (2001)
Flores			
Doble flor (única/doble)	*Dl*	2	Chaparro et al. (1994)
Color flor	*B*	1	Jáuregui (1998)
Color flor (rosado/rosado palido)	*Fc*	3	Yamamoto et al. (2001)
Polen (fértil/estéril)	*Ps*	6	Dirlewanger et al. (1999, 2006).
Número de carpelos	*Pcp*	3	Bliss et al. (2002)
Color antera (amarillo/antociánico)	*Ag*	3	Joobeur (1998)
Aborto del fruto	*Af*	6	Dirlewanger et al. (2006)
Forma flor (Campanulacea/Rosacea)	*Sh*	8	Ogundiwin et al. (2009); Fan et al. (2010)
Hojas			
Tamaño hoja (angosto/ancho)	*Nl*	6	Yamamoto et al. (2001)
Color hoja (rojo/amarillo)	*Gr*	6-8[a]	Jáuregui (1998); Yamamoto et al. (2001)
Glandula (reniforme/globosa/eglandular)	*E*	7	Connors (1921); Dettori et al. (2001)
Fruto			
Color mesocarpo (blanco/amarillo)	*Y*	1	Warburton et al. (1996); Bliss et al. (2002)
Color mesocarpo (normal/antociánico)	*Bf*	4	Werner et al. (1998); Bliss et al. (2002)
Color mesocarpo (normal/antociánico)	*DBF*	5	Shen et al. (2013)
Color alrededor del hueso	*Cs*	3	Bliss et al. (2002)
Adhesión del mesocarpo (pavía/prisco)	*F*	4	Etienne et al. (2002)
Fruto no ácido	*D*	5	Dirlewanger et al. (1998, 1999); Etienne et al. (2002)
Vellocidad de la piel (nectarina/melocotón)	*G*	5	Dirlewanger et al. (1998, 1999); Bliss et al. (2002)
Sabor semilla (amargo/dulce)	*Sk*	5	Bliss et al. (2002)
Tamaño fruto (redondo/plano)	*S*	6	Lesley (1939); Dirlewanger et al. (1999); Picañol et al. (2013)
Colo piel[a]	*Sc*	6-8	Yamamoto et al. (2001)
Resistencia pestes o enfermedadaes			
Resistencia nematodo agallador	*Mi*	2	Bliss et al. (2002); Cao et al. (2011)
Resistencia Oídio	*Vr3*	6-8[a]	Pascal et al. (2010)

[a] Genes localizados cerca del punto de translocación entre ambos grupos de ligamiento.

Los caracteres cuantitativos complican enormemente las labores de mejora ya que el fenotipo de un individuo refleja sólo una parte de su potencial genético. Así, al estar controlados por múltiples genes, plantas con el mismo fenotipo pueden llevar alelos diferentes en uno o varios genes. Además, individuos con los mismos QTLs pueden mostrar fenotipos diferentes cuando se desarrollan en ambientes distintos. Finalmente, el efecto de un QTL puede depender de la

constitución alélica de otros QTLs en la misma planta debido a interacciones epistáticas (Torres et al. 2012)

La teoría del mapeo de QTLs fue descrita por primera vez por Sax (1923) y desarrollada posteriormente por Thoday (1961) quien sugirió que la segregación de genes con herencia simple podría ser utilizada para detectar QTLs ligados. La posibilidad de utilizar marcadores moleculares, como los RFLP (Paterson et al. 1988), con éxito en el estudio de QTLs abrió un nuevo interés en el mapeo de QTLs al demostrar que algunos marcadores moleculares explicaban una parte substancial de la variación fenotípica de un carácter cuantitativo. Así, el análisis de QTLs fue el término que se acuñó para estudiar este tipo de variación genética, para localizar los genes responsables y para explorar sus efectos e interacciones (Torres et al. 2012).

En términos generales, la identificación de un gen o de un QTL dentro del genoma de la planta es como encontrar la proverbial aguja en un pajar. Sin embargo, el análisis de QTLs se puede utilizar para dividir el pajar en montones manejables y buscarlos sistemáticamente (Boophati, 2013). En términos simples, el análisis de QTL está basado en el principio de la detección de una asociación entre el fenotipo y el genotipo de los marcadores (Collard et al. 2005). Los marcadores se utilizan para dividir la población de mapeo en diferentes grupos genotípicos basado en la presencia o ausencia de un locus marcador particular, y para determinar si existen diferencias significativas entre los grupos con respecto al carácter cuantitativo que se está midiendo. Por lo tanto, una diferencia estadísticamente significativa entre las medias fenotípicas de los marcadores indica que el locus marcador utilizado para dividir la población de mapeo está vinculado a un QTL asociado al rasgo.

1.8.1 Métodos estadísticos para el mapeo de QTLs

En poblaciones biparentales existen tres principales métodos para la detección de QTLs: (1) análisis de un solo marcador; (2) el mapeo por intervalos simple y (3) el mapeo por intervalos compuesto. En el análisis de marcadores individuales o de un sólo marcador, normalmente se utilizan pruebas estadísticas como la prueba de Student, el análisis de la varianza (ANOVA) y la regresión lineal. La principal ventaja de este método es su simplicidad y que puede ser realizado con programas básicos de estadística. Además, no se requiere el desarrollo de un mapa genético debido a que la correlación se realiza de forma individual para cada marcador con la variación fenotípica (Tanksley, 1993). El método de mapeo por intervalos simple (SIM) es el más popular para el mapeo de QTLs en poblaciones genéticas. El SIM utiliza mapas

genéticos para analizar el intervalo entre pares adyacentes de marcadores ligados a lo largo de los cromosomas, superando las limitaciones del análisis de un sólo marcador (Lander y Botstein, 1989). El mapeo por intervalos compuesto (CIM) es un método que combina el mapeo por intervalos para un único QTL en un intervalo dado con el análisis por regresión múltiple de marcadores asociados con otros QTLs e incluye marcadores genéticos adicionales (cofactores) en el modelo estadístico para controlar el fondo genético. Este tipo de enfoque es más preciso y eficaz que los anteriores (Jansen y Stam, 1994), al eliminar la mayor parte de la varianza genética producida por otros QTLs lo que reduce la varianza residual. Los resultados de las pruebas estadísticas tanto para SIM y CIM se presentan frecuentemente utilizando un valor logarítmico de probabilidades (LOD). Por lo general, el resultado del análisis de QTLs que entregan los programas estadísticos es un grafico con el orden de los marcadores en el eje *x* y la prueba estadística (valor LOD) en el eje de las *y*. (Boophati, 2013).

1.8.2 Posición y efecto de un QTL

Diversos estudios se han realizado con el fin de determinar el umbral crítico que debe superar un QTL para declararlo estadísticamente significativo. Morton (1955) determinó que el umbral crítico para señalar un ligamiento significativo entre dos loci debía ser un LOD superior o igual a 3. Lo que implica una relación de probabilidades de 1.000 a 1 a favor de la presencia de un QTL en una determinada posición del mapa. Posteriormente, otras investigaciones se han llevado a cabo para estudiar el nivel del umbral crítico modulando genomas de diferentes tamaños y densidad de marcadores (Lander y Botstein, 1989; Van Ooinjen 1999). Sin embargo, dado que la detección de QTLs implica múltiples pruebas estadísticas en todo el genoma, el nivel de significancia nominal fijada previamente, dará lugar a un elevado error de tipo I, es decir, a un gran número de falsos positivos.

En términos estrictos se debe considerar valido un QTL cuando el valor del LOD del QTL supera el valor del LOD determinado por un método estadístico más robusto mediante procedimientos de remuestreo, como la prueba de permutación propuesta Churchill y Doerge (1994). En esta prueba los valores fenotípicos de la población se mezclan, mientras que los valores genotípicos de los marcadores se mantienen constantes y el análisis de QTL se realiza miles de veces para evaluar el nivel de falsos positivos en las asociaciones carácter-marcador (por ejemplo 10.000 veces es una razonable aproximación del 1% de confianza) y los niveles de significación pueden determinarse en base al nivel de falsos positivos en las asociaciones carácter-marcador (Torres et al. 2012). Sin embargo, es ampliamente utilizado el valor de LOD

3 como muestra altamente confiable de la validez de un QTL. Además, el LOD 3 supera en la mayoría de los casos el valor entregado por la prueba estadística de permutación.

Dada que la ubicación más probable de un QTL (lugar del mapa en el que se detecta el mayor valor de LOD) representa una aproximación estadística de la verdadera ubicación del locus responsable de la variabilidad genética en el carácter, en la mayoría de los estudios actuales se utiliza la regla de menos uno LOD y menos dos LOD (Van Ooijen, 1992) para calcular los extremos del intervalo de confianza, lo que es fundamental para su empleo en mejora genética y realizar análisis comparativos entre años, localidades y poblaciones.

Una vez localizado el QTL, el siguiente paso es estimar el efecto genético o modo de acción. El efecto genético de un QTL detectado por mapeo por intervalos se calcula a partir de los valores fenotípicos medios de las correspondientes clases genotípicas del QTL en la posición del mapa con máximo valor de LOD. Para poblaciones BC, el efecto genético es una combinación del aditivo y los efectos de dominancia, debido que a falta de una clase homocigótica los efectos de dominancia no pueden ser separados como se realiza en una población tipo F_2. Por el contrario, las poblaciones de líneas homocigóticas (RILs) no permiten estimar los efectos de dominancia, debido a la falta de la clase heterocigótica (Torres et al. 2012).

2. OBJETIVOS

El objetivo general de esta Tesis Doctoral es la evaluación del genoma del almendro como fuente de enriquecimiento del pool genético del melocotonero cultivado. Para ello se han planteado un conjunto de objetivos específicos que se detallan a continuación:

1- Saturar y comparar los mapas genéticos de la población F_2 del almendro 'Texas' y el melocotonero 'Earlygold' (TxE) y del primer retrocruzamiento con 'Earlygold' (T1E) mediante marcadores moleculares tipo SSRs y SNPs.

2- Identificar y mapear los genes mayores/QTLs responsables de la variabilidad fenotípica observada en las poblaciones interespecíficas (TxE y T1E) para un conjunto de caracteres de interés agronómico asociados a rasgos de morfología de flor y hoja, calidad de fruta, fenología y resistencia a enfermedades.

3- Establecer los métodos y estrategias más adecuados para la construcción de una colección de líneas casi isogénicas ("near isogenic lines", NILs) de almendro en el fondo genético del melocotonero cultivado.

3. GENETIC CHARACTERIZATION OF A CYTOPLASMIC MALE STERILITY AND TWO RESTORER GENES IN ALMOND X PEACH PROGENIES USING HIGH-DENSITY SNP MAPS

3.1 INTRODUCTION

Cytoplasmic male sterility (CMS) is driven by rearrangements in the mitochondrial genome that result in plants unable to produce fertile pollen (Pelletier and Budar, 2007). Usually CMS is a binary system where the products of one or more nuclear genes (restorer genes) interfere with the causal mitochondrial proteins and reestablish fertility (Schnable and Wise, 1998; Budar and Pelletier, 2001). CMS is widespread in the plant kingdom and has been described in more than 150 species (Schnable and Wise, 1998) including members of the Poaceae, Leguminosae, Umbelliferae, Brassicaceae, Chenopodiaceae, Solanaceae and Liliaceae. Various cases of male sterility have been reported in the Rosaceae, as in *Fragaria vesca* (Tennessen et al. 2013), peach (Scott and Weimberger, 1938; Werner and Creller 1997), Japanese apricot (Yaegaki et al. 2003), almond (Alonso and Socias, 2003) and pear (Thompson et al. 1976), although CMS has only been documented for peach, Japanese apricot and pear. CMS is an important model for the analysis of the interplay between organelle and nuclear genomes that has implications in the evolution of sex in plants (Caruso et al. 2012) and is a key technological aspect in the development of F1 hybrid seed for many of the most important crops (Kempe end Gils, 2011).

Peach is the only species of the cultivated *Prunus* (including also cherry, almond, plum and apricot) that does not have a functional gametophytic self-incompatibility system and behaves as self-pollinating. This character, along with its important economical value, have made peach a model species for genetic studies of *Prunus* and many major genes have been described (Monet et al. 1992) and mapped on its genome (Dirlewanger et al. 2004; Arús et al. 2012). Peach is also one of the best characterized species of the Rosaceae family and its whole genome sequence has recently been published (Verde et al. 2013).

Self pollination is the major factor that explains the low level of genetic diversity of peach (Aranzana et al. 2010; Aranzana et al. 2012; Verde et al. 2013; Li et al. 2013). In contrast, the almond genome is highly variable as revealed with molecular markers (Byrne 1990; Mnejja et al. 2010), and may be a source of novel alleles for peach that would confer new properties and provide raw materials for characters such as disease resistance, extended fruit shelf life and organoleptic fruit quality. These are some of the major objectives of peach breeding that are difficult to achieve by the scarcity of variability of its gene pool.

One of the key elements of the success of interspecific crosses is the fertility of the F1 and successive generations. F1 hybrids between almond and peach are usually fertile, but we found male sterile plants in their F_2 and BC_1 progenies. In this paper we examine the male sterility found in two crosses between almond and peach: the F_2 between 'Texas' almond and 'Earlygold' peach, a progeny that was used to construct the reference map of *Prunus* (Joobeur et al. 1998; Dirlewanger et al. 2004) and a new population, the backcross one ('Texas' x 'Earlygold') x 'Earlygold', for which we have obtained high density SNP maps that are described here. Our data indicate that the almond cytoplasm confers male sterility to the peach unless the almond allele of at least one of two independent restorer genes (*Rf1* and *Rf2*) is present.

3.2 MATERIALS AND METHODS

3.2.1 Plant material

We used two mapping populations: an F_2 of 80 individuals, obtained by selfing the F1 hybrid plant 'MB 1.37' (almond 'Texas' x peach 'Earlygold'), named TxE. A high density map exists already for this progeny (Dirlewanger et al. 2004) and we have recently added 31 plants, for a final size of N=111, to improve its resolution. The original TxE population is kept at the IRTA Center of Cabrils (Barcelona, Spain) and a copy was planted in the IRTA Experimental Station of Lleida at Gimenells (Spain) both grafted on 'Garnem' rootstocks. A new backcross one (BC_1) population derived from the cross between the hybrid 'MB 1.37' and the peach 'Earlygold' of size N=185 has been created and named T1E. Original trees of T1E were planted on their own roots at Cabrils. Grafted plants of T1E on 'Garnem' rootstocks were also located in the fields of Gimenells.

3.2.2 Phenotyping

The male sterility character was studied in three seasons (2010-2013). Male sterile plants were characterized by the lack of pollen and the presence of empty and white anthers in contrast with the fertile plants that presented pollen and colored anthers ranging from yellow to antocyanic (Figure 8). In addition, the pollen germination capacity in all individuals of T1E was evaluated *in vitro* according the protocol described by Asma (2008) with an additional 15% of sucrose.

Figure 8. a) Male sterile flower with white anthers and absence of pollen, and b) fertile flower with colored anthers and presence of pollen.

3.2.3 Marker detection, linkage map construction and genetic analysis

Genomic DNA was extracted from young leaves using the CTAB method (Doyle and Doyle, 1990) omitting the final RNAse treatment step. Sample DNA quality and concentration were checked and measured with a DNA spectrophotometer (NanoDrop Technologies, Wilmington, USA).

Genetic maps were obtained using a set of 131 selected SSRs with good genome coverage most of them previously located on the TxE map by Aranzana et al. (2003) and Howad et al. (2005) or of known genome physical position based on their sequence (Annex 1). Four of these markers were developed here based on the list of SSRs of the GDR to cover the distal part of G6 (3) and the distal part of the G2 (1). They were named as CPP (C for CRAG, PP for *Prunus persica*) followed by a number that coincides with that of the SSR annotated at the IGA peach genome browser (http://services.appliedgenomics.org/fgb2/iga/prunus_public/gbrowse/prunus_public/). Their main characteristics are provided in Table 4. These markers were studied only in the 31 new individuals of TxE and in all the T1E population. Most of the SSR markers used were common between TxE and T1E (Annex 1). Two additional SSRs (TPScp10 and TPScp10) developed from Japanese plum chloroplast (Ohta et al. 2005) were used to evaluate the almond or peach origin of the cytoplasm in T1E population.

In addition we genotyped 50 individuals of the TxE population and 123 individuals of T1E with the 9k Illumina Infinium SNP chip developed by the Peach International Genome Initiative (Verde et al., 2012). Genotyping was done at the Fondazione Edmund Mach (San Michele

all'Adige, Italy) with DNA (50ng/µl) extracted with the DNAeasy 96 Plant kit of Qiagen as described in Verde et al. (2012). Genotypes were scored with the GenomeStudio Data Analysis software of Illumina Inc. (Gencall threshold 0.15). Markers with GeneTrain score <0.6 were excluded from the dataset used for mapping. SNPs showing skewed segregations ($P < 0.05$) were utilized for mapping and discarded only if unlinked.

Table 4. Characteristics of the SSR markers in linkage group 2 and 6 developed for linkage analysis in the 'Texas' x 'Earlygold' BC_1 population.

Name	Primers	AT[1]	Genome position
CPP8062	Forward: CCTCGCTGAAAACTTGATG	55	2: 10.246.714
	Reverse: GCCAAGGACATTTTGAAAC		10.246.855
CPP20836	Forward: CATTTTTCACCCCAACCATC	55	6: 1.141.321
	Reverse: CATTGTTGGAATTTTGCTTC		1.141.477
CPP21245	Forward:GGGAGATACGGGATTTGGAT	55	6: 3.108.842
	Reverse: AGCCTTTTTGGACCTCCCTA		3.109.025
CPP21413	Forward: ACGTCCTTTGGGAAATCCAT	55	6: 4.150.412
	Reverse: AGCACAACACTCTCGAAATCC		4.150.515

[1] AT= annealing temperature (ºC)

Linkage maps were constructed using MapMaker/exp 3.0 (Lincoln et al. 1992) with the Kosambi mapping function at a minimum logarithm of odds (LOD) grouping threshold ≥3.0. Segregation distortion of individual markers was revealed by a chi-squared test using JoinMap v 4.0 software (Van Ooijen, 2006). Linkage maps were drawn using the MapChart 2.1 software (Voorrips, 2002). For the T1E progeny we elaborated two maps, each one with the data from one of the parents: the 'Texas' x 'Earlygold' F1 hybrid plant used as female parent, that we called the T1E map, and the map from the pollen donor 'Earlygold', called the E map. Linkage group terminology was the usual in *Prunus* (Dirlewanger et al. 2004; Verde et al. 2013).

Given the high number of SNPs identified we mapped only one per genome position or bin, i.e. group of markers with the same genotype for all individuals and separated by at least one recombination event from its neighboring bins. A single SNP was used to label each bin, normally containing more markers. The selected marker was the closest to the origin of the bin based on its physical position that had all or most data for the individuals studied (i.e., excluding SNPs that were scored as dominant or heterozygous for the hybrid individual and 'Earlygold' in the T1E and E maps). Exception to this rule were a few markers (54) of the E map

in which the SNP used to label a specific bin was selected to be one that was in common with TxE and T1E to facilitate the visualization of the map comparison presented in Figure 2. The SSRs were all added as markers in the maps so they could be used as anchor points between the three maps studied and with other *Prunus* published maps.

Results were inconclusive when the male sterility character was mapped as a single gene in the TxE, T1E and E maps. Given these results, we followed a two-step procedure that consisted first of mapping these data as a quantitative character using MapQTL, using values of 2 for fertile and 1 for sterile plants and, second, knowing the approximate positions of the two QTLs detected, we used the ensemble of the genotype data of these two loci to manually find their position in the full dataset of ordered markers of the two linkage groups where these loci were located. A unique and fully consistent position was found for both loci in the positions identified by MapQTL.

3.2.4 Candidate genes analysis

For the PPR genes (Table 5) we used the information of annotated genes in the Prunus genome v1.0 (http://www.rosaceae.org/search/genes; Jung and Main 2014) using 'pentatricopeptide protein' as a keyword. For the non-PPR genes described in Chen et al.(2014) we tried to obtain sequence information from the bibliography and used this information to do a tblastx in the GDR webpage using the default options.

Table 5. PPR genes found in the regions containing *Rf1* and *Rf2* in the *Prunus* genome.

Name	Location	Start (bp)	Stop (bp)
ppa004557m	scaffold_2	1,188,729	1,190,329
ppa003463m	scaffold_2	3,027,943	3,030,377
ppa001444m	scaffold_2	3,894,642	3,897,125
ppa021440m	scaffold_2	4,230,526	4,232,581
ppa023365m	scaffold_6	5,768,164	5,769,950
ppa015993m	scaffold_6	5,799,668	5,803,260
ppa024218m	scaffold_6	5,906,881	5,908,413
ppa025792m	scaffold_6	5,915,202	5,917,143
ppa003571m	scaffold_6	6,024,610	6,028,036
ppa026767m	scaffold_6	6,174,107	6,175,847
ppa023798m	scaffold_6	6,182,493	6,184,532
ppa019799m	scaffold_6	6,204,975	6,206,456

3.3 RESULTS

3.3.1 Map construction and comparison

The three maps obtained, TxE, T1E and E are presented in the Figure 9. The TxE genetic map with 1,948 markers (114 SSRs and 1,834 SNPs) detected the expected 8 linkage groups (G1-G8), covering a total length of 472.1 cM (average density of 0.24 cM/marker). The longest group was G1 with 84.4 cM and the shorter G3 with 43.8 cM and the largest gap was 7.3 cM on G7. The T1E backcross one population gave rise to two genetic maps. The linkage map of the female parent, T1E, consisted of 113 SSRs and 1,919 SNPs and was the most populated with 2,032 markers distributed into eight linkage groups (G1-G8). The total map length was 370.1 cM, with G1 covering the longest genetic distance of 53.6 cM and G5 the shortest with 39.8 cM. The average marker density was 0.18 cM between adjacent markers and the longest gap had 6.7 cM on G8.

The genetic map of E, the peach male parent, had 1,091 segregating markers (1,050 SNPs and 40 SSRs), approximately half of the markers that were polymorphic in the T1E map. Eight linkage groups were identified covering a longer distance than any of the other two maps, 520.4 cM, although the marker density was much lower (0.48cM/marker). In contrast with TxE and T1E where marker distribution was generally uniform, the distribution of markers in the E map was extremely heterogeneous with chromosomal fragments with densities similar to T1E and TxE and others without markers, resulting in large gaps (nine gaps longer than 10 cM, two of which in G2 and G6 longer than 25 cM), vast distal regions of the genome that had no segregating markers with an overall coverage of 155.4 Mb compared to the 207.3 Mb of T1E (75.0%) or the 211.9 Mb of TxE (73.3%), and lower physical coverage between mapped markers of all linkage groups that was very low in certain of them (compared to T1E): a 32.4% of G4, a 36.4 % of G5 or a 70.2% of G8. Considering the gaps >2 Mb, these were rare in TxE and T1E, four in total for each map with a maximum gap of 2.9 Mb, but much more frequent in E where this number was of 23 with a maximum gap of 19.7 Mb in G4 and adding to a total distance of 103.0 Mb, almost half of the distance covered by TxE or T1E.

Ninety-five SSRs were in common between TxE and T1E, all in the same linkage group and with the same order, except for the CPPCT029 and CPPCT053 markers between TxE and T1E. T1E and E shared the 40 SSRs that had in common and of these 31 were also anchor points with TxE. A large proportion of the 1,834 SNPs mapped in TxE were also mapped in T1E (97.5%) and

vice versa (92.9%) for the 1,919 SNPs mapped in T1E. These proportions were much lower in E, where only 478 SNPs (25.9%) were common with TxE and 491 (25.6%) with T1E. The order of the SNPs between TxE and T1E was identical, with the only exception of SNP_IGA_155433 that mapped to a slightly different position in G2 of both maps.

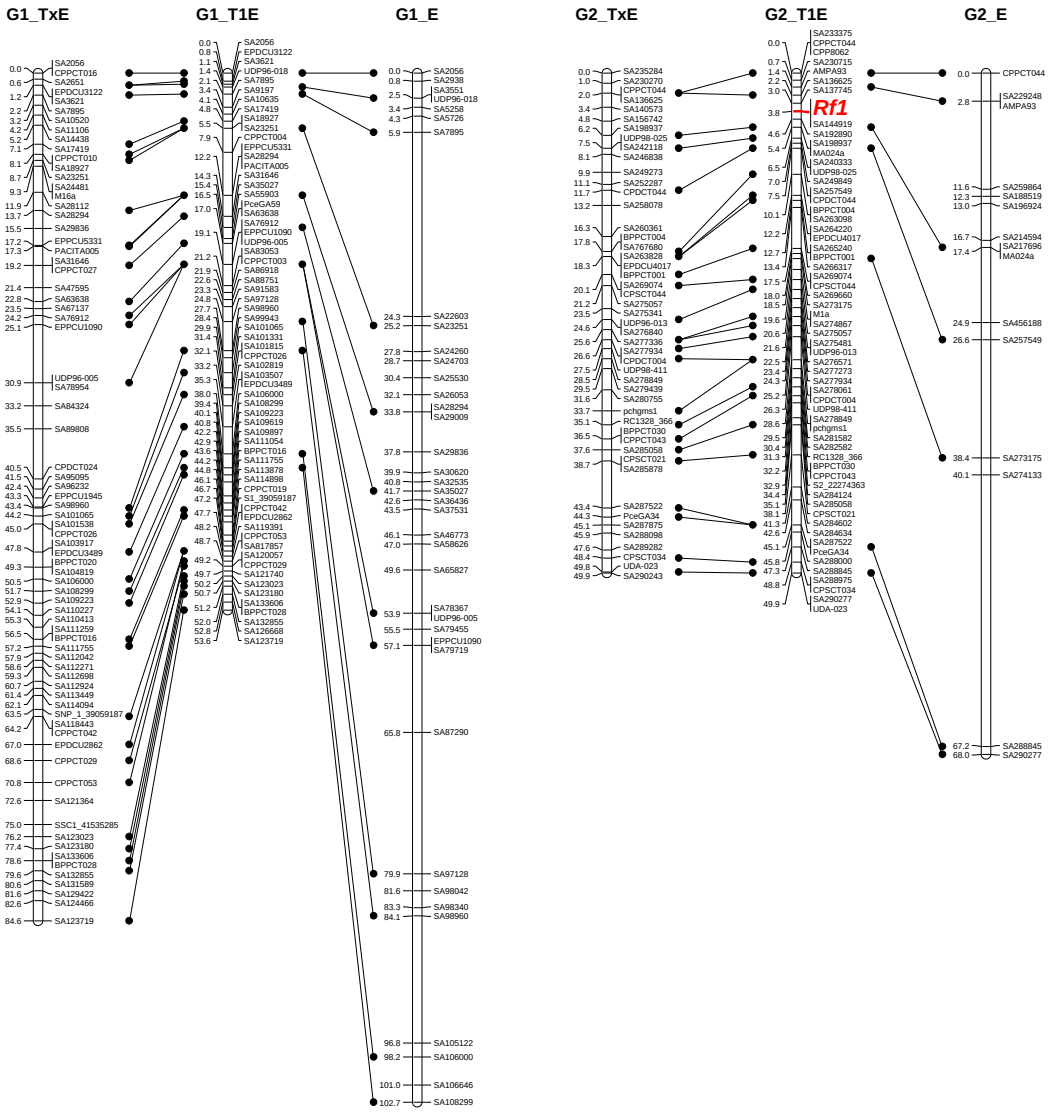
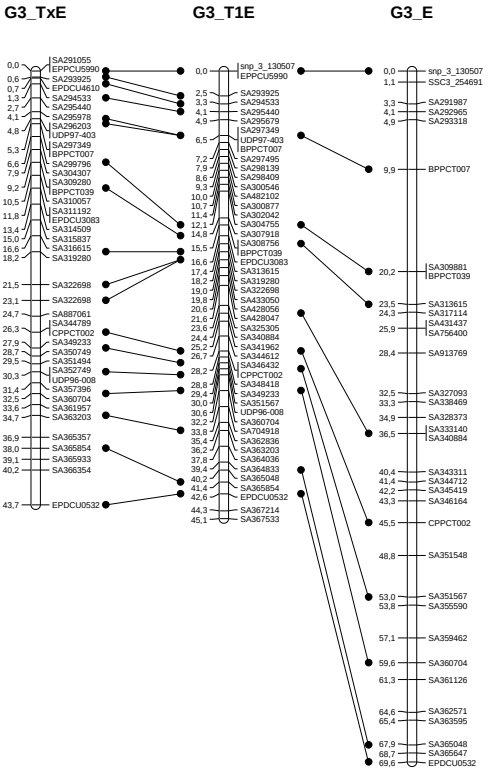
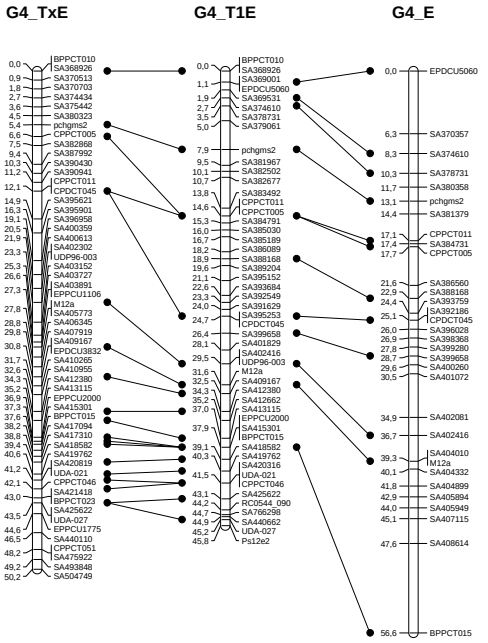
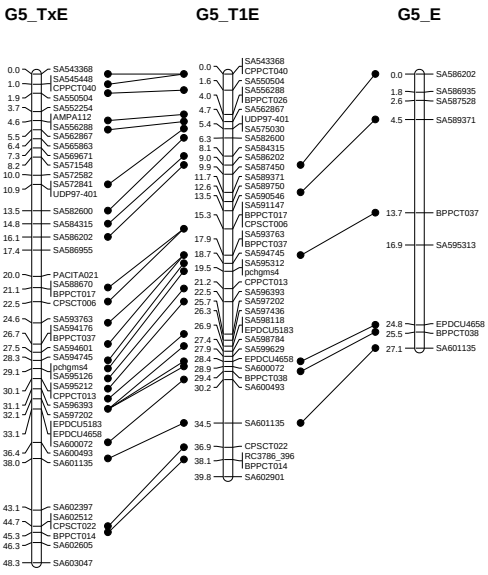
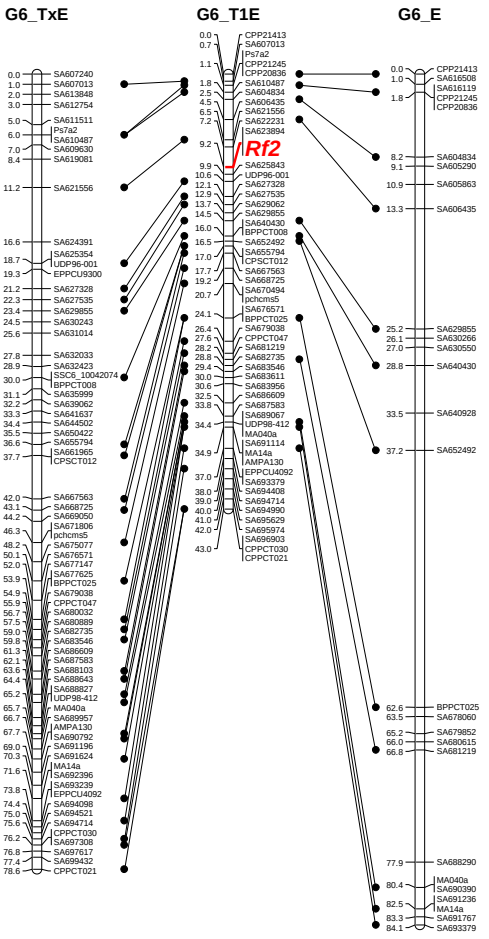


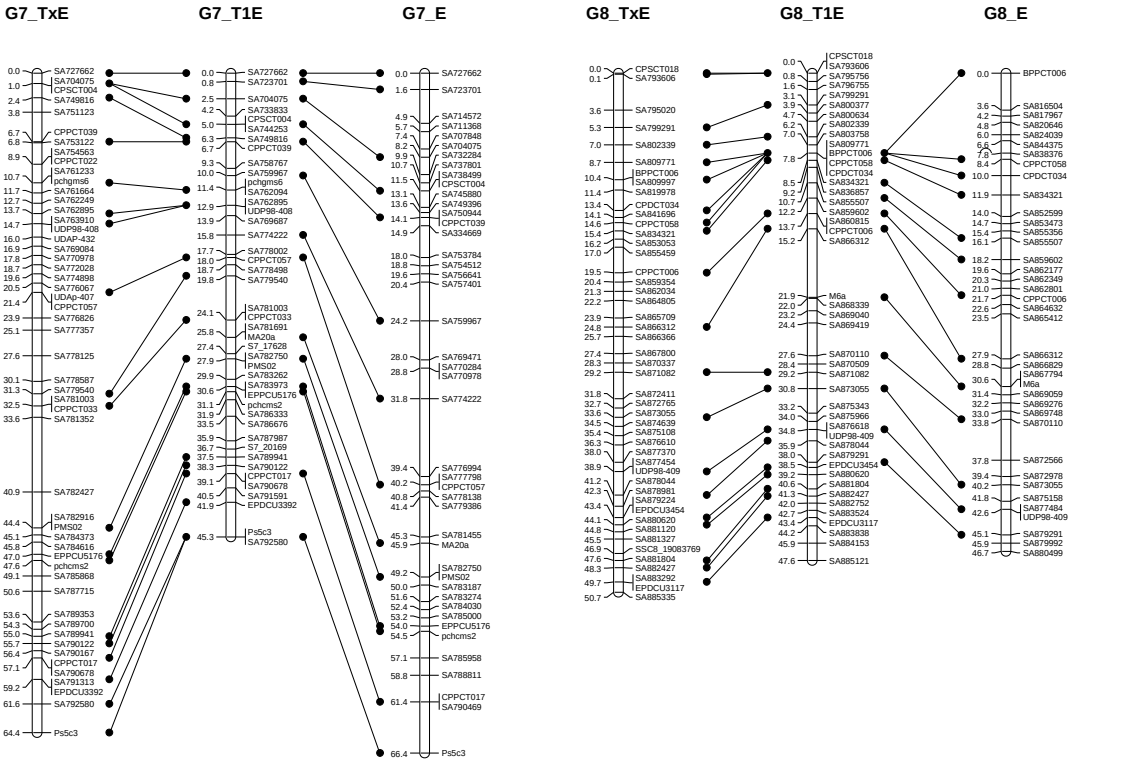
Figure 9. Linkage maps obtained, one with the almond ('Texas') x peach ('Earlygold') F_2 population (TxE), and two more with the backcross one population to peach, one for the hybrid female parent (T1E) and the other for the peach 'Earlygold' male parent (E). Each marker position corresponds to a bin (i.e. a group of markers with the same genotype for all the plants studied that are represented by a single marker). Some of the anchor markers of each map are connected with lines. The selected anchor markers are all the SSRs and some of the SNPs, particularly of the E map to show the completeness of the comparison between T1E and E. The names of the SNP markers are shortened to facilitate the visualization of the maps. The two restorer genes are highlighted in red. (The figure 9 follows in the next pages).


G3_TxE
G3_T1E
G3_E


G4_TxE
G4_T1E
G4_E


G5_TxE
G5_T1E
G5_E


G6_TxE
G6_T1E
G6_E
Rf2



Comparison between linkage and physical maps resulted in the identification of discrepancies, the most relevant of which were two large inversions at the distal end of G1 (>5.1 Mb), and proximal end of G7 (>4.2 Mb), misplacement of a fragment of >1.2 Mb of G2 at the proximal end of this group, a fragment of >2.2 Mb of G4 mapped to the central part of G2 and another fragment of G4 (>0.4 Mb) was located in the central region of G6. Markers of unmapped scaffolds S9, S10, S12 and S17 could be placed at a map position on G3, G3, G2 and G6, respectively. These data are coincident with some of the sequence refinements introduced to the v1.0 version of the peach genome that can be found at the GDR (http://www.rosaceae.org/species/prunus_persica/genome_v1.0_refinements).

3.3.2 Genetic analysis of the male sterility character

Both chloroplast SSRs showed bands of different sizes for 'Texas' and 'Earlygold'. The MB1.37 hybrid and all individuals of the TxE and T1E progenies had the 'Texas' allele, as expected considering that 'Texas' and MB1.37 were used as female parents.

Field observations on male sterility were fully consistent between years and congruent with the pollen viability test *in vitro* in the TxE and T1E populations. In T1E, from a total of 173 plants phenotyped, 121 were fertile and 52 male sterile, and in TxE only one plant was male sterile whereas all the others that could be phenotyped (89) produced fertile pollen. We compared these segregations with those expected considering that this character was determined by two dominant epistatic loci (called *Rf1* and *Rf2*, for restorer of male fertility), where the almond parent carried the two dominant alleles *Rf1* and *Rf2*, the peach parent was homozygous for the recessive alleles, their hybrid MB1.37 was a heterozygous *Rf1rf1/Rf2rf2*, and the individuals of TxE and T1E carrying the double recessive homozygote were sterile and the rest fertile. Data from T1E were in agreement with a 3:1 segregation ratio (χ^2= 2.36; ns), but those of TxE significantly differed from a 15:1 (χ^2= 4.06; P=0.044).

QTL analysis of the male sterility character in the largest T1E population allowed identifying two regions of its map harboring clear QTLs and none in the E map. The two loci were in the proximal parts of G2 and G6 and were detected with LODs 16.7 and 17.4, respectively, explaining 36 and 37% of the phenotypic variability and 67% when considered together. Based in the positions detected by MapQTL, we located manually the two genes in G2 and G6 and found them to have a clear position in these groups. *Rf1* cosegregated with a bin at 3.8 cM from the top of G2 (SNP_IGA_144919 and 119 more SNPs covering a physical distance of 3.4 Mb), flanked by SNP_IGA_144913 (1.118.746 bp) and SNP_IGA_192890 (4.535.916 bp) at 3.0 and 4.6 cM, respectively, corresponding to a total physical distance of 3.4 Mb. *Rf2* co-mapped with two bins containing markers SNP_IGA_623894 and SNP_IGA_625843 (map positions 9.2 and 9.9 cM) and four more SNPs that spanned a distance of 0.7 Mb and was flanked by SNP_IGA_622231 (5.606.752 bp) and SSR UDP96-001 (7.040.897 bp) with cM positions of 7.2 and 10.6 cM, respectively, and covering a total physical distance of 1.4 Mb.

Only the male sterile plant of the TxE progeny had a marker genotype compatible with the positions of *Rf1* and *Rf2* as mapped in T1E and the 89 remaining had not. Nevertheless, the position of these two loci could only be estimated very roughly in TxE, corresponding to the 14.3 cM region between the bins defined by SNP_IGA_230270 and SNP_IGA_260361 in G2 for *Rf1* and the 39.3 cM fragment between markers SNP_IGA_609630 and SSR pchgms5 for *Rf2* in G6.

3.3.3 Search of candidate genes for the Rf

In the peach genome there were a total of 554 genes with PPR motifs annotated, four of which were located in the 3.4 Mb sequence of G2 containing *Rf1* and 16 in the 1.4 Mb of G6 encompassing *Rf2* (Table2S). Any of the non-PPR genes that have been reported as restorer genes (Chen and Liu 2014) and for which sequence information was available has been found in the target regions were *Rf1* and *Rf2* were located. These genes include the *Rf2* from maize (Cui et al. 1996), *Rf2*, *Rf3*, *Rf4* and *Rf17* from rice (Itabashi et al. 2011, Luo et al. 2013, Fuji and Toriyama 2009), and *Rf1* from sugar beet (Matsuhira et al 2012).

3.4 DISCUSSION

High density maps were developed using two interspecific almond x peach populations with the 9k IPGI Illumina Infinium chip and a set of 131 SSRs most of them of known position in the *Prunus* reference linkage map. The three maps constructed, one for the F_2 (TxE) and two for the backcross one progeny (T1E and E) demonstrated the high quality of the data obtained in the following aspects: i) as expected the majority (>92%) of the markers were common to the TxE and T1E maps, as they are derived from the same individual (the hybrid plant MB1.37) ii) anchor markers between maps were syntenic and collinear with almost no exception; iii) the E map had a much higher level of recombination per unit of physical distance than the TxE and T1E maps, suggesting that the recombination rates are higher in intraspecific than in interspecific progenies as observed in other *Prunus* crosses (Arús et al. 2005), iv) the number of markers in the interspecific progenies for the SNP chip were around 2,000 each, whereas only half were segregating in the map of the peach intraspecific parent. Eduardo et al. (2013) used the same 9k IPGI chip in a peach x peach progeny (‘Bolero’ x ‘OroA’) and found also lower numbers of segregating markers (1,450 for ‘Bolero’ and 350 for ‘OroA’). Moreover, the TxE and T1E maps had a homogeneous coverage of the whole genome with only a few gaps >2Mb, none of them longer than 2.9Mb. Independently from the fact that the physical coverage of the maps was usually lower in the intraspecific maps, this results suggest either that ascertainment bias was not an important factor in the almond/peach materials or that it was compensated with a higher level of polymorphism compared to peach alone materials resulting in a similar or higher marker number and coverage. These results indicate also that the IPGI chip can be expanded to almond/peach progenies.

The map constructed with the peach 'Earlygold' was characterized by fragments with high marker density followed by regions without markers, in contrast with the almond x peach maps that had a relatively homogeneous distribution of markers. Similar results have been observed when using high density SNP maps in other peach x peach crosses (Eduardo et al. 2013; Martínez-García et al. 2013). These results indicate that the regions without markers may correspond to genome fragments that are identical by descent, and these fragments may account for a large proportion of the genome, estimated in E as 103.0 Mb (50% of the total physical distance covered by T1E). This hypothesis is supported by the recent history of cultivated peach where most commercial European and North American cultivars come from a bottleneck at the beginning of the US breeding programs leading among other things to a high level of inbreeding (Hesse 1975; Scorza et al. 1985). Moreover, the number of generations between the founders and the current commercial cultivars is probably very low considering the long life, propagation by grafting and breeding schemes (usually cultivars are selected from F1 progenies between two partly heterozygous parents) typical from fruit trees. This would result in the conservation of large chromosomal fragments as supported by the high conservation of linkage disequilibrium of peach (Aranzana et al. 2010; Li et al, 2013). The consequences are that the parents used by breeders are often close relatives with large parts of their genomes and those of the cultivars resulting from their offspring being identical by descent as our results suggest. This has implications for genome analysis and breeding, as only the parts of the genome that are heterozygous will segregate and only the crossovers produced at these heterozygous fragments will result in changes that may produce innovative gene combinations. This means also that only a part of the genome needs to be monitored with markers when using markers for whole genome selection. Then, maps with only partial coverage with markers would be suitable for genetic analysis, provided that the parents are previously tested for a large initial sample of markers with good coverage of the genome, allowing the identification of the segregating regions of each particular parent or cross.

Our results suggest that the male sterility character is determined by the interaction of two restorer genes, *Rf1* and *Rf2*. Presence of the dominant allele (the almond allele) of either gene would result in a fertile plant. This hypothesis is confirmed by the agreement of the observed data on T1E with a 3:1 segregation and by the identification of two genome regions of 3.4 and 1.4 Mb located in G2 and G6, respectively, where these two genes are located. Considering only nuclear inheritance, this hypothesis would conflict with the fertile 'Earlygold' phenotype because the absence of almond alleles at *Rf1* and *Rf2* would have implied its sterility. If one considers the existence of a CMS system where the peach and the almond cytoplasm were

different and individuals with the almond cytoplasm would be fertile only when having one almond allele at *Rf1* or *Rf2*, whereas this will not be a requirement for fertility for individuals with the peach cytoplasm, the data for T1E would completely fit this hypothesis. Other simple hypotheses (involving one or two genes) of only genetic male sterility were discarded by our data, as they imply that the 'Earlygold' parent should be heterozygous for alleles conferring fertility and sterility in at least one locus. This locus should then segregate in T1E and be mapped in the E map, which did not occur.

The situation in TxE fitted well the described two-locus model, although in this case the expected 15:1 segregation was not in agreement with the 89:1 segregation observed. This departure can be explained as the marker segregations had a lower frequency of homozygous peach alleles in both regions, significant in G6 (χ^2=10.45; P=.0012) but not in G2 (χ^2=3.68; P=.055), indicating that they were selected against the peach alleles as it was noticed previously by Joobeur et al. (1998) and we confirm here.

Cytoplasmic male sterility is determined by the interaction between the mitochondrial and the nuclear genomes (Schnable and Wise 1998; Budar and Pelletier, 2001). Our results are compatible with this model, where the products of two almond genes would interact with an unknown mitochondrially-encoded protein of this species and determine the fertile phenotype. In peach these products do not exist, so fertility can only be recovered when the almond alleles are present. The most common nuclear factors involved in fertility restoration are pentatricopeptide repeat (PPR) proteins that are ubiquitous in the plant genomes (Lurin et al. 2004; Liu and Chen, 2014). This also occurs in peach where 554 genes containing PPR motifs were identified in its genome, some of which in the target regions of G2 (4) and G6 (16) where the *Rf1* and *Rf2* genes are. In addition, we identified two other candidates of non-PPR genes with high homology of *Rf* genes described in other species only in the G6 region containing *Rf2*. The numbers of candidates found in G2 were much lower than those of G6 for a much shorter region of the genome (3.4 vs 1.4 Mb). This may be due to the fact that the G2 region that contains *Rf1* was identified as a possible centromeric region by Verde et al. (2013), having low gene density and low recombination, whereas *Rf2* is located at the distal part of chromosome 6 with higher gene density and recombination. In G6, some of the PPR genes are in tandem (ppa026767/ppa023798m and ppa023796m/ppa015333m). Given that *Rf* genes that encode for PPR proteins use to be located in clusters with other non-*Rf* PPR encoding genes (Fujii et al. 2011), these two genes are more likely than others to be the cause of *Rf2*.

Male sterility, cytoplasmic or genetic, has been used as an efficient system for commercial production of F1 hybrid seed in herbaceous species (Kempe and Gils, 2011). This is not a varietal type currently used in peach and most fruit tree crops because grafting is the usual propagation procedure that allows the multiplication of selected vigorous heterozygotes. However, certain male sterile individuals such as cultivars Chinese Cling and JH Hale (Okie, 1998), are among the founders of the first US peach breeding programs, because of their outstanding performance as parents for breeding purposes (Weinberger, 1944; Scorza et al. 1985) and also because all seeds collected from them come from crosses with neighboring plants facilitating the usually tedious pollination procedure. The pollen sterile character was found to be determined by a single gene (*Ps/ps*) by Scott and Weinbeger (1938) that was mapped by Dirlewanger et al. (2006) at position 0.0 cM of G6. The closest marker that can be placed on the physical map, the RFLP FG215 (position 8.8cM on the map and 285,872 bp on the genome sequence) is not compatible with the position of *Rf2* that we have placed in the interval 5,606,752-7,040,897, suggesting that *Ps* and *Rf2* are different loci. The existence of a second male sterility gene *ps2* was reported by Werner and Creller (1997), although its map position has not been determined. A case of male sterility for almond was reported by Alonso and Socias (2003) where a cross between the male sterile cultivar Rof and a fertile peach heterozygous for *Ps* yielded only fertile hybrids, suggesting that if male sterility was of nuclear origin the gene or genes involved were different than *Ps*.

The presence of a cytoplasmic male sterility has been reported in peach (Werner and Creller 1997), coming from accession PI 240928, although no evidence of the existence of restorer genes has been provided. The male sterility of certain accessions of Japanese apricot (*P. mume*) was also shown to be produced by CMS but in this case certain crosses with fertile individuals restored fertility suggesting the presence of *Rf* alleles (Yaegaki et al, 2003). Assuming that the CMS of 'Texas' can be generalized for almond, the consequences of this finding are that the introgression of genes from almond into peach would result in sterile individuals unless one of the *Rf* genes would be also introgressed or if the peach parent is used as mother plant in one of the crosses. This may also occur in progeny between peach and other closely related *Prunus* species and has to be taken into account when planning the crossing schemes to integrate new genes from these species into peach, so that the peach cytoplasm is recovered at some point of the process to avoid undesirable sterility problems.

4. MAPPING MAJOR GENES AND QTLS FOR FLOWER, PHENOLOGY, FRUIT QUALITY, LEAF MORPHOLOGY AND DISEASE RESISTANCE TRAITS IN TWO INTER-SPECIFIC ALMOND X PEACH PROGENIES

4.1 INTRODUCTION

Peach, *Prunus persica* L. (Batsch), is an economically important temperate tree fruit and one of the model species for the *Rosaceae* family (Shulaev et al. 2008). Its genome has been recently sequenced (Verde et al. 2013) and used for comparative studies with strawberry and apple, two other key species of this family (Illa et al. 2011). Since very early, geneticists realized that the genetic basis of USA and European commercial cultivars was very narrow (Scorza et al. 1985), which was later confirmed with molecular markers not only for these materials (Aranzana et al. 2010) but for modern Asian cultivars as well (Li et al.2013). Ancient oriental cultivars and landraces were more variable and could be a source of variability for peach breeding programs (Li et al.2013). Another source of new genetic variability exists in close relatives of peach such as *P. dulcis*, *P. davidiana*, *P. kansuensis* and *P. ferganensis*, where interspecific hybridization is possible and crosses with peach have been used for rootstock development, and for introgression of novel traits (Gradziel 2003; Foulongne et al. 2003a). Linkage drag, enhanced by suppression of recombination, low fertility, and the long time usually required to recover an elite genotype from an interspecific or wide cross, have limited the adoption of this strategy in peach breeding programs.

An almond x peach progeny was developed at IRTA to develop a *Prunus* genetic map that later on became the *Prunus* reference map (Dirlewanger et al. 2004). The high polymorphism of this population facilitated the construction of this map and provided the scientific community with a common terminology and orientation for linkage groups and a large set of transferable markers that were used as anchors for constructing other maps (Arús et al. 2012) and to align the physical map assembled for the construction of the whole genome sequence (Verde et al. 2013).

QTL analysis of biparental populations, mainly F_1 or F_2 populations, has been widely used in *Prunus* and the number of published major genes and QTLs has doubled since 2011 with a total of 760 loci (670 QTLs) for 110 agronomic traits identified (Salazar et al. 2014). In spite of this high number of QTLs none of them has been already cloned, due in part to the size and populations types used in these studies. For this reason the development of experimental populations particularly designed for high quality phenotyping and genetic studies, such as near-isogenic lines (NILs), is needed for QTL dissection and fine mapping (Eshed and Zamir 1995; Eduardo et al. 2005; Xu et al. 2010).

In this work we developed and analyzed two interspecific populations between almond and peach with the objectives of understanding the genetic variability of 42 traits, exploring possible alleles from almond that could be introgressed in peach commercial cultivars, and to generate the basic plant materials to construct a collection of NILs of almond in the peach background.

4.2 MATERIALS AND METHODS

4.2.1 Plant material

In this work we used two interespecific populations. The first one is an F_2 of 111 individuals named TxE, obtained by selfing the F_1 hybrid called 'MB 1.37', a cross between the almond 'Texas' and the peach 'Earlygold'. This progeny includes the 80 individuals used to develop the reference map of *Prunus* (Joobeur et al., 1998) plus 31 additional plants that allow improving its resolution for genetic studies. We obtained data for at least one trait for 88 individuals. The trees of TxE used for this research are kept at the IRTA Center of Cabrils and the Experimental Station of Lleida in Gimenells (Spain) grafted on 'Garnem' rootstocks. A BC_1 population (T1E) of 185 individuals was created from the cross 'MB 1.37' × 'Earlygold'. We obtained data for at least one trait for 178 individuals. Original trees of T1E were planted on their own roots in Cabrils, and grafted plants on 'Garnem' were located in the fields of Gimenells. In Cabrils spacing within and between rows was and 1.0 x 4.0m for T1E and 2.0 x 4.0 for TxE and in Gimenells was of 1.5 x 3.5m for both populations. Standard cultural practices were applied in both locations but trees were not thinned.

4.2.2 Phenotyping

The TxE population was evaluated for 40 traits in one location (Gimenells) during three years (2011, 2012, and 2013). The T1E population was evaluated for the same 40 traits plus the juvenility period and leaf fall date, in two locations (Cabrils and Gimenells) during three years (2011, 2012, and 2013). The exceptions were for leaf fall, juvenility and powdery mildew resistance that were only evaluated in Cabrils, and blooming density and phenology traits that were not evaluated in Gimenells in 2011. The 42 traits have been divided into families of traits and are described below.

Flower traits: Flower type (*Sh*): the typology showy/non-showy was described originally by Connors (1920) as a major gene in peach. Showy flowers are determined by the recessive allele (*sh*) and present large petals. Non-showy flowers show small petals, with anthers emerging from the corolla before full anthesis. Anther color (*Ag* and *Ag2*) was scored as anthocyanic anther or yellow anther in TxE. Given that certain individuals initially scored as yellow had antocyanic spots when observed at the binocular lens, all yellow phenotypes were reexamined with the binocular and re-phenotyped before final scoring of its phenotype. The *Ag* gene was previously described as a single gene in the TxE population and mapped to linkage group 3 (G3) by Joobeur (1998), where the yellow anther was determined by the recessive homozygote *agag*. T1E individuals segregated for orange and red anthers, but none of them had yellow anthers. Flower color (*Fc2*) was classified as pink or pale pink. Pistil length (**PiL**) was scored as very short (1), very short and medium size (2) and normal (3). The blooming density (**BD**) was scored in a scale from 1 to 5 (1: low; 2: low-medium; 3: medium; 4: medium-high and 5: high).

Phenology traits: Beginning of shooting (**BS**) was scored as the number of Julian days at the moment when 5% of shoots were in expansion. Beginning of flowering time (**BFT**) and End of flowering time (**EFT**) were scored as the number of Julian days when a 5% and a 95% of flowers were open, respectively. Flowering duration (**FD**) was obtained by the difference between beginning and end of flowering. Fruit production (**FP**) was scored in a scale from 1 to 4 (1: no fruits, 2: less than 10 fruits, 3: 10 to 50 fruits, 4: more than 50 fruits). Maturity date (**MD**) was scored as the number of Julian days when 50% of the fruits were considered mature based on visual color change and manual evaluation of firmness. Fruit development period (**FDP**) was obtained as the difference between scores of end of flowering and maturity date. Leaf fall (**LF**) was scored as the number of Julian days when 95% of the leaves had dropped. The juvenility period (**Juv**) was scored as the number of years necessary to produce the first fruits after planting.

Fruit traits: Fruit traits were evaluated in five mature fruits of each individual. Fruit type (*Ft*) was scored in the TxE population as peach type or almond type depending on the development of a fleshy mesocarp and a change of external or internal fruit color. In T1E all the fruits were peach type. Juiciness (*Jui*) was scored as the capacity of producing juice (1) or not (2). Blood flesh (*Bf2*) was scored as presence (2) or absence (1) of red flesh color. The intensity of the red skin color (**ISC**) was scored as 1 (light red), 2 (dark red), 3 (dark-violet red) or 4 (violet). Fruit skin color was scored as the percentage of surface covered by anthocyanin coloration (**PSC**) according to the following scale: 1 = 0-25%, 2 = 25-50%, 3 = 50-75%, 4 = 75-

100%. Soluble solid content (**SSC**) was scored as Brix degrees by applying a drop of fruit juice in a digital refractometer PAL-1 (Atago, Tokyo, Japan). For titratable acidity (**TA**) evaluation, two slides from each fruit were cut and stored at -20 ºC until their evaluation. TA was determined as g/l by titrating 10 ml of distilled water and 10 ml of the unfrozen fruit juice or fruit paste with 0.1 N NaOH to pH 8.2. Other traits scored were: fruit weight (**FW**) and stone weight (**SW**) as the average of the fruit and stone weight from the five fruits scored, and flesh weight (**FlW**) as the difference **FW-SW**, all expressed in g. Characters to measure fruit shape were (see Figure 10): fruit polar diameter (**FpD**), fruit cheek diameter (**FcD**), fruit suture diameter (**FsD**), stone polar diameter (**SpD**), stone cheek diameter (**ScD**), stone suture diameter (**SsD**), flesh polar diameter (**FlpD**), flesh cheek diameter (**FlcD**) and flesh suture diameter (**FlsD**), all expressed in cm. Flesh diameters were obtained as the difference between **FpD-SpD**, **FcD-ScD** and **FsD-SsD** respectively.

Leaf traits: each year eight leaves of each tree were collected in July. To minimize leaf variation in each tree, leaves were collected from the middle of sun exposed branches, with medium vigour and at the level of human height. Leaves were scanned and images were saved as jpeg files and imported into Tomato Analyzer 3.0 for automated phenotypic measurements (http://www.oardc.ohio-state.edu/vanderknaap). The analyzed parameters were: leaf perimeter (**LP**), leaf surface (**LS**), leaf blade width (**LBW**), leaf length (**LL**), leaf blade length (**LBL**), and petiole length (**PL**) as the difference **LL-LBL**. The units from these traits were expressed in pixels. Subsequently, the leaves were dried for 3 days at 60 °C and were weighed, to obtain the average leaf dry weight (**LW**) in g. A drawing of the leaf dimensions measured is presented in Figure 1. Chlorophyll content (**CC**) was measured with a SPAD-502 device (Konica Minolta, Osaka, Japan) that emits two wavelengths (650 and 940 nm), and the final measurement of chlorophyll content is the ratio between the two intensities of light after passing through each leaf.

Disease resistance: Powdery mildew resistance (*Vr3*) was characterized as the presence or absence of the disease in both populations by natural exposure. The trees were not treated during the years that this trait was evaluated (2011 to 2013). Trees that were affected at least in one year were considered as susceptible.

4.2.3 Phenotypic data analysis

Statistical analyses were performed using JMP 8.0 software (SAS Institute, Cary, NC, USA). The distribution for each trait was represented in frequency histograms. Correlations between different traits and years were calculated using the Pearson correlation coefficient. The normality of each trait was tested with the Shapiro-Wilk test (Shapiro 1965). The histograms were drawn using R 3.1.0 software (http://cran.r-project.org/bin/windows/base/).

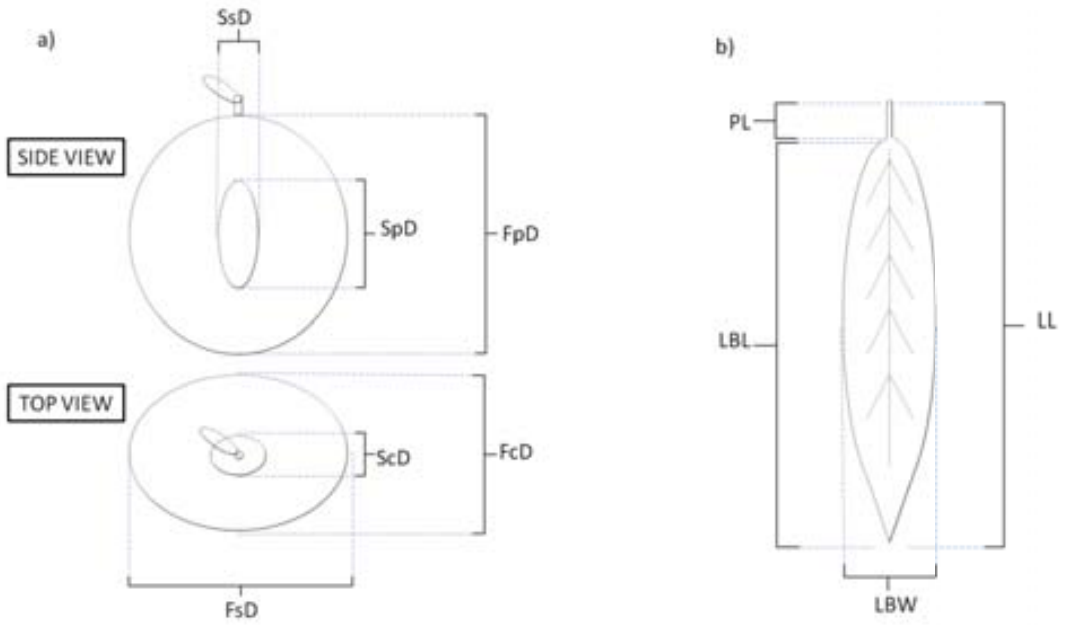


Figure 10. Scheme of a) fruit, stone and b) leaf dimensions.

4.2.4 Genetic linkage maps and QTL analysis

For linkage analysis we used the data from the 9k IPGI SNP Illumina Infinium chip (Verde et al. 2012) in 50 plants of the TxE population and 123 plants of the T1E population. In addition we mapped 131 SSRs covering the whole genome in 88 and 178 plants of TxE and T1E, respectively. Maps were constructed with these markers (see Chapter 3) using MapMaker/Exp 3.0 (Lincoln et al. 1992) and coalesced in the eight expected linkage groups. The TxE map used contained 1,948 markers (114 SSRs and 1,834 SNPs) and covered a total distance of 472.1 cM, and the T1E population was used to construct two maps, one with the markers heterozygous in the female parent, the MB1.37 hybrid plant (the T1E map) that contained 2,031 markers (113 SSRs and 1,919 SNPs) and spanned 370.1 cM and the map obtained with the male 'Earlygold' parent (the E map) with 1,091 markers (40 SSRs and 1,050 SNPs). Most of the

markers (>92%) of T1E and TxE are in common and >43% of markers shared with the other two maps.

Phenotypic characters behaving as simple Mendelian factors were mapped as individual markers using MapMaker v3.0. These were: flower color (*Fc2*), anther color (*Ag2*), juiciness (*Jui*), blood flesh (*Bf2*) and powdery mildew resistance (*Vr3*) were mapped as phenotypic markers in the T1E map, Flower shape (*Sh*) in the E map and Anther color (*Ag*), Fruit type (*Ft*) and Powdery mildew resistance (*Vr3*) in TxE map. Linkage group terminology was the one coined for the TxE *Prunus* reference map (Dirlewanger et al. 2004).

The remaining characters were subject to QTL analysis using the interval mapping method with the mapQTL 4.0 software package (Van Ooijen et al. 2002). QTLs with a LOD score greater or equal to 3.0 were declared significant. When a QTL with a LOD value of 2.5 or higher was consistent with other years in the same trait, with criteria of LOD 3.0 or more, also was considered significant. Maps and QTL positions were drawn using the MapChart 2.1 software (Voorrips, 2002). We considered a QTL as consistent if it was detected all the years in at least one of the locations, having overlapping confidence intervals (those established with two LOD score unit below the maximum for each specific QTL). Major QTLs were those that were consistent and explained at least 20% of the phenotypic variance for any of the years, locations and populations that were studied. In TxE gene action was estimated based on the ratio (*d/a*) between the additive (*a; (*A+B/2)) and dominance (*d;* H-((A+B)/2)) effects and they were classified as underdominant (U; >-1.25), dominant for peach allele (D; -1.25 to -0.75), partial dominant for peach allele (AD; -0.75 to -0.25), additive (A; -0.25 to 0.25), partial dominant for almond allele (D; 0.25 to 0.75), dominant for almond allele (D; 0.75 to 1.25) and overdominant (O; >1.25).

4.3 RESULTS

4.3.1 Trait distributions and correlations

The TxE and T1E populations segregated for many characters as shown in Figure 11. The 42 traits evaluated were classified in five groups: five of flower, nine of phenology, 19 of fruit, eight of leaf and one disease resistance. Eight of these characters could be analyzed as Mendelian genes and the remaining 35 were considered quantitative. Graphs with the phenotype including distributions of the progenies and data from the parents are in Annex 2

and 3 TxE and T1E respectively. Pictures of flowers, fruits, stones and leaves of the parentals and some individuals of their progenies are presented as Annex 4. Variability for most characters was very large, particularly in the TxE population. The hybrid individual MB1.37 presented intermediate values between T and E in certain traits as in blooming density, maturity date, fruit weight and dimensions and petiole length, but had higher values than the two parents as in fruit and stone polar diameter, or similar values than 'Texas' (leaf length) or 'Earlygold' (fruit and stone suture and cheek diameters).

Transgressive segregation was observed in both populations for most traits, except for maturity date, fruit development period and fruit weight. The histograms of all traits evaluated in both populations are available in Annex 5. Fruit dimensions, SSC and leaf morphology (only in TxE) presented a normal distribution. On the other hand, pistil size, blooming density, most phenological traits, TA, intensity and percentage of skin color, and stone weight did not fit a normal distribution. Chlorophyll content in TxE presented a normal distribution but no in T1E. Leaf morphology characters in T1E did not present a clear pattern because it depended on the year and location. For example, leaf perimeter presented a normal distribution in both places during 2013 but not during 2012 and petiole length presented a normal distribution in Gimenells in both years but in Cabrils normality only occurred in 2013.

Correlations between years and locations were significant ($p<0.01$) for all traits except for TA in 2011 in TxE (Annex 6) and for flowering duration and chlorophyll content in 2012 in T1E (Annex 7). Flowering duration also showed the lowest correlations between years in TxE with a value of 0.34. Maturity date showed the highest correlation values between years and locations ranging from 0.79 to 0.93 in T1E and from 0.95 to 0.97 in TxE. Other traits that presented high correlations between years and locations in T1E were intensity of skin color, presenting a range between 0.54 and 0.81, and beginning of flowering time, with a range between 0.53 and 0.78.

Blooming density, beginning of shooting, flowering duration, SSC, TA, chlorophyll content, and juvenility were not correlated with any trait in TxE and T1E. A high correlation was found between pistil size and fruit production in TxE (0.79) and in T1E (0.29). MD and FDP were highly correlated in both populations, but in T1E were not correlated with other traits whereas in TxE were negatively correlated with fruit and flesh weight, fruit dimensions and blood flesh, indicating that earlier fruits were bigger.

In TxE fruit weight was correlated with all the fruit dimension parameters and the same occurred between stone weight and stone dimensions, but fruit and stone parameters were not correlated. This pattern was not as clear in T1E where fruit and stone weight were correlated with other fruit and stone dimension traits in some years and locations but not in others.

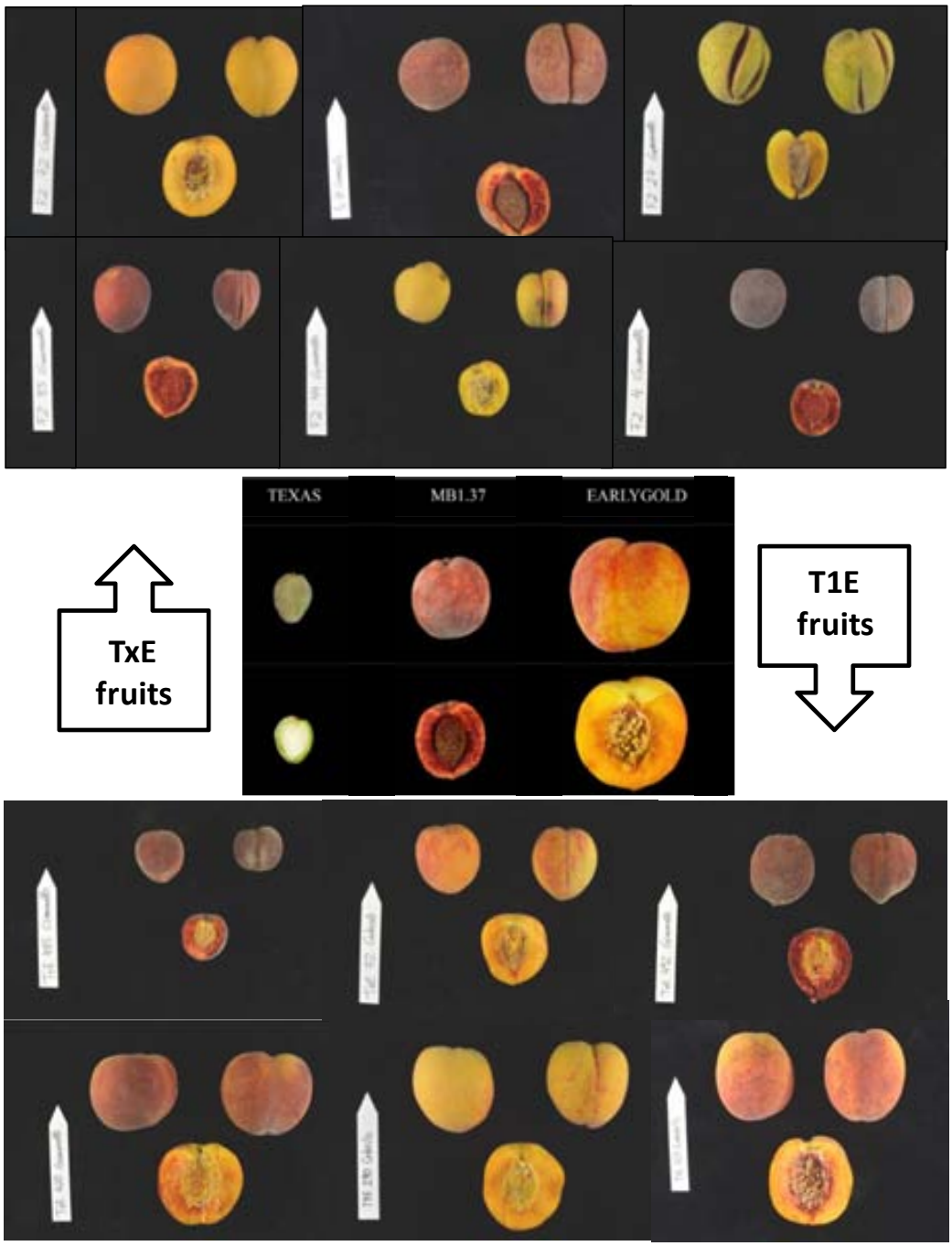


Figure 11. Images of the fruits fromTexas, MB1.37, Earlygold and some individuals from TxE and T1E progenies.

Most leaf traits in T1E (leaf surface, leaf perimeters, leaf length, leaf blade length, leaf blade with and leaf weight) showed high correlations between them in each location, ranging from

0.71 to 0.98 and 0.75 to 0.98 in Cabrils and from 0.60 to 0.97 and 0.66 to 0.98 in Gimenells the years 2012 and 2013, respectively. However, a low level of correlation was observed between locations ranging from to 0.15 to 0.44. This could indicate a location effect for this trait. The same phenomenon was observed in TxE.

4.3.2 Mapping Mendelian traits

Seven of these characters were treated qualitatively and eight Mendelian genes could be mapped in the three maps constructed (Table 6), four (*Fc2, Ag2, Jui*, *Bf2*) in the T1E map, one (*Sh*) in the E map, two (*Ag* and *Ft*) in the TxE map and one (*Vr3*) in both T1E and TxE. One of these characters, anther color, produced information on two genes, one mapped in TxE (*Ag*) and the other in T1E (*Ag2*). All characters had segregation ratios as expected under the single-gene hypothesis except for *Fc2* and *Vr3* (only in TxE) that had an excess of individuals with pink anthers and resistant, respectively (Table 6). Six of these genes are described for the first time: *Fc2*, for flower color, another gene *Fc* was described previously by Yamamoto et al. (2001) but mapped to a different position in G3; *Vr3* is a new gene for powdery mildew resistance mapping at a different position than those described before, one in G6 (*Vr2*; Pascal et al. 2010) and several QTLs in G7 (Verde et al. 2002; Pacheco et al. 2009), G6 and G8 (Foulogne et al. 2003b).

Table 6. Segregation of single genes mapped in this study.

Trait	Symbol	Texas[a]	Earlygold[a]	F1[a]	Population	Expected segregation	Observed segregation		X^{2} [b]
Flower type	*Sh/sh*	showy (*sh/sh*)	non showy (*Sh/sh*)	showy (*sh/sh*)	E	1:1	showy (n=76)	non showy (n=79)	0,06
Flower color 2	*Fc2/fc2*	white (*fc2/fc2*)	reddish (*Fc2/Fc2*)	pale pink (*Fc2/fc2*)	T1E	1:1	pale pink (n=61)	reddish (n=92)	6,28*
Anther color	*Ag/ag*	yellow (*ag/ag*)	red (*Ag/Ag*)	anthocyanic (*Ag/ag*)	TxE	1:3	anthocyanic (n=71)	yellow (n=21)	0,10
Anther color 2	*Ag2/ag2*	yellow (a*g2/ag2*)	red (*Ag2/Ag2*)	anthocyanic (*Ag2/ag2*)	T1E	1:1	red (n=57)	orange (n=48)	0,77
Fruit type	*Ft/ft*	almond (*ft/ft*)	peach (*Ft/Ft*)	peach (*Ft/ft*)	TxE	1:3	almond (n= 11)	peach (n= 29)	0,13
Juiciness	*Jui/jui*	non juicy (*Jui/* -)	juicy (*jui/jui*)	non juicy (*Jui/jui*)	T1E	1:1	juicy (n=65)	non juicy (n=64)	0,08
Blood flesh 2	*Bf2/bf2*	unknown (*Bf2/-*)	yellow (*bf2/bf2*)	red (*Bf2/bf* 2)	T1E	1:1	red (n=73)	yellow (n=57)	1,97
Resistance Powdery mildew	*Vr3/vr3*	resistant (*Vr3/* -)	susceptible (*vr3/vr3*)	resistant (*Vr3/vr3*)	TxE	3:1	resistant (n=75)	susceptible (n=9)	9,06**
					T1E	1:1	resistant (n=75)	susceptible (n=84)	0,51

[a] In parenthesis predicted genotype.
[b] *Significant (P≤ 0.05), **Significant (P≤ 0.01).

In total, six new monogenic traits (*Fc2*, *Ag2*, *Bf2*, *Jui*, *Ft*, *Vr3*) have been positioned in the *Prunu*s genome and other two (*Ag* and *Sh*) that were already mapped have been further defined.

Four of these genes affect flower traits. The flower type (*Sh*) character (showy/non Showy) was located in the E map on G8 co-segregating with the CPPCT006 SSR marker (8: 13659021), between SNP markers SNP_IGA_863252 (8: 13459205) and SNP_IGA_ 864149 (8: 13756987), spanning a region of 1.4 cM (0.30 Mb). *Sh* was previously mapped by Ogundiwin et al. (2009) between markers CPPCT006 (8: 13659021) and Unk5 (8: 17336781) and Eduardo et al. (2011) mapped it cosegregating with the ssrPaCITA15 (8: 13322647). An anther color (*Ag*) gene has been mapped as a morphological marker in TxE on G3, between the markers SNP_IGA_322925 (3: 9071075) and SNP_IGA_344789 (3: 13891040), in a region of 1.7 cM (4.82 Mb). In previous maps of the same population it colocalised with the CC2 (3: 12231238) marker (Joobeur 1998), between markers AG57A (9259448) and CPDCT008 (10023959) in an interval of 1.8 cM (0.76 Mb). Another anther color gene (*Ag2*) has been mapped in G1 of T1E. Its position was between markers SNP_IGA_82861 (1: 23722082) and SNP_IGA_86918 (1:25642323), in a region of 3 cM (1.92 Mb). A flower color gene (*Fc*) was previously mapped to G3 by Yamamoto et al. (2001), whereas the gene that we have found in T1E (*Fc2*) maps on G4 of T1E between the markers pchgms2 (4: 2086474) and SNP_IGA_381287 (4: 2299484), in a region of 3.5 cM (0.21 Mb). While color variability was observed in the TxE population, we were not able to identify a clear color pattern that allowed a qualitative classification.

Three more genes affected fruit traits. The first one is the almond vs. peach-like fruit that we termed as fruit type (*Ft*) that has been located in the TxE map on G4, between the markers SNP_IGA_410955 (4: 10898535) and BPPCT015 (4: 12546880), in a region of 2 cM (1.65 Mb). This trait was not segregating in T1E which was expected given that the allele that confers the almond type (*ft*) is recessive. The other two major genes for fruit traits segregated in T1E and were the Juiciness (*Jui*) gene that mapped between the markers SNP_IGA_107095 (1: 34224252) and SNP_IGA_107417 (1: 34424837) spanning a region of 1.4 cM (0.20 Mb), and the blood flesh (*Bf2*) gene that was mapped on G1, between the markers CPPCT029 (1: 40195426) and SNP_IGA_123023 (1: 41781710) spanning a region of 1.4 cM (1.58 Mb). For *Jui* the dominant allele was that carried by peach that determines juicy fruit, whereas for *Bf2*, red color is dominant and was provided by the almond parent. Other two genes of similar characteristics, *Bf* and *DBF,* were described for blood flesh by Shen et al. (2013). They mapped to G4 and G5 respectively.

Finally, the gene of resistance to Powdery mildew (*Vr3*) was mapped in both populations in the same position in G2 between the markers CPDCT044 (2:14069755) and BPPCT004 (2:18671271) in T1E and between CPDCT044 and SNP_IGA_260361 (2: 14773178) in TxE. Although this trait was evaluated using the natural inoculum in the field data were very consistent between the different years evaluated.

4.3.3 QTL analysis

Considering all years, locations and the three maps analyzed, a total of 336 QTLs were detected in the three maps: 238 in the T1E map, 12 in the E map and 86 in the TxE map (Annex 8). QTLs were identified in the 35 quantitative traits studied, varying between one and four QTLs for each trait. Considering only the consistent QTLs, we found 196 (Figure 12): 145 in the T1E map, 7 in the E map and 44 in the TxE map, representing 58.3% of total QTLs detected. For some traits, as maturity date or petiole length, most of the QTLs detected were consistent, while in others, as SSC, TA, and flowering duration none of the QTLs detected was consistent. Considering consistent QTLs from different years, locations and populations mapping in overlapping map regions as the same QTL, the final number of unique QTLs detected was 64: 43 in the T1E map, 2 in the E map and 19 in the TxE map (Table 7 and Figure 13). We will describe the consistent QTLs in next sections.

4.3.3.1 Flower traits

For pistil length a major QTL explaining a 74.4% of the phenotypic variance was detected in TxE on G6 (qP-PiL6), close to the CPPCT030 marker. In T1E, two minor QTLs were detected, one on G1 (qP-PiL1) and another on the central part of G4 (qP-PiL4). These QTLs explained between 8.4 and 17.8% of the phenotypic variance respectively. For blooming density a major QTL was detected in T1E, at the end of G1, and only in Gimenells (qP-BD1) explaining around 23.8-25.7% of the phenotypic variance. No QTLs were detected for this character in the TxE population.

Table 7. Summary of consistent QTLs identified in this study using the TxE and T1E progenies, including trait name, location, population where it was identified, QTL name (according to the GDR recommendations), year when phenotypic data was obtained, LOD score of the maximum peak, nearest marker, position of the maximum peak, and parameters for genic action estimation (*a, d, d/a*) and gene action.

QTLs Flower

Trait	Location	Population	QTL name	Year	LOD	Nearest marker	Position (cM)	R^2	a^a	*d*	*d / a*	Genic action[b]
Pistil length	Cabrils - Gimenells	T1E	qP-PiL1	2011-2013	3,05	SNP_IGA_107417	39,4	8,4	-0,29			
			qP-PiL4		7,13	CPDCT045	24,7	18,4	0,47			
		TE	qP-PiL6	2011-2013	25,44	CPPCT030	76,2	74,4	0,28	1,21	4,39	O
Blooming density	Gimenells	T1E	qP-BD1	2012	6,70	CPPCT029	49,2	25,7	1,14			
			qP-BD1	2013	7,54	SNP_IGA_109897	42,2	23,8	1,23			

QTLs Phenology

Trait	Location	Population	QTL name	Year	LOD	Nearest marker	Position (cM)	R^2	a^a	*d*	*d / a*	Genic action[b]
Beginning of shooting	Cabrils	T1E	qP-BS8	2011	3,93	CPPCT006	13,7	10,8	3,54			
			qP-BS8	2012	4,38	SNP_IGA_803758	7,0	12,7	2,84			
			qP-BS8	2013	3,75	SNP_IGA_796755	1,6	11,6	4,93			
	Gimenells	T1E	qP-BS2	2012	3,61	SNP_IGA_144919	3,8	12,1	2,38			
			qP-BS2	2013	4,54	CPSCT044	19,0	13,5	1,97			
			qP-BS8	2012	2,55	SNP_IGA_802339	6,2	8,6	2,02			
	Gimenells	TE	qP-BS6	2012	3,14	SNP_IGA_694098	74,4	16,6	-0,73	2,81	-3,85	U
			qP-BS6	2013	2,53	CPPCT030	76,2	13,4	-1,47	3,57	-2,43	U
	Cabrils	E	qP-BS7	2011	4,24	SNP_IGA_779386	41,4	12,1	3,73			
			qP-BS7	2012	7,51	SNP_IGA_779386	41,4	21,8	3,72			
			qP-BS7	2013	8,13	SNP_IGA_779386	41,4	23,5	7,03			
	Gimenells	E	qP-BS7	2012	3,28	SNP_IGA_781455	45,3	11,0	2,30			
Begining of flowering Time	Cabrils	T1E	qP-BFT2	2011	2,77	SNP_IGA_288668	47,3	9,1	-2,94			
			qP-BFT8		3,63	SNP_IGA_802339	6,2	11,5	3,26			
			qP-BFT1	2012	4,27	SNP_IGA_99943	28,4	12,3	-3,06			
			qP-BFT8		5,66	SNP_IGA_803758	7,0	16,0	3,48			
			qP-BFT1	2013	3,95	SNP_IGA_31646	14,3	12,4	-6,52			
			qP-BFT8		4,02	SNP_IGA_796755	1,6	12,7	6,4			
	Gimenells	T1E	qP-BFT1	2012	8,62	SNP_IGA_109897	42,2	26,3	-3,31			
			qP-BFT2		3,14	pchgms1	28,6	10,5	-2,07			
			qP-BFT1	2013	6,39	SNP_IGA_101331	31,4	19,1	-2,82			
			qP-BFT2		2,86	CPPCT044	7,5	9,0	-1,94			
	Gimenells	TE	qP-BFT1	2012	4,47	CPPCT010	80,1	23,8	-2,12	0,10	-0,05	A
			qP-BFT1	2013	3,68	CPPCT010	80,1	19,8	-2,32	0,56	-0,24	A
End of flowering time	Cabrils	T1E	qP-EFT8	2011	3,08	SNP_IGA_796755	1,6	12,1	3,42			
			qP-EFT1	2012	4,53	SNP_IGA_91583	23,3	13,1	-2,95			
			qP-EFT8		4,63	CPSCT018	0,0	13,3	2,96			
			qP-EFT1	2013	6,70	SNP_IGA_96232	24,8	20,6	-6,45			
			qP-EFT8		5,44	SNP_IGA_796755	1,6	17,0	5,86			
	Gimenells	T1E	qP-EFT1	2012	6,79	SNP_IGA_10635	4,1	23,3	-2,42			
			qP-EFT1	2013	6,37	SNP_IGA_91583	23,3	19,8	-3,37			
	Cabrils	E	qP-EFT7	2011	3,62	SNP_IGA_776994	36,8	13,6	3,61			
			qP-EFT7	2012	4,65	SNP_IGA_778138	40,8	14,3	3,07			
			qP-EFT7	2013	2,50	SNP_IGA_776994	36,8	8,9	4,24			
Fruit production	Cabrils - Gimenells	T1E	qP-FP6	2011-2013	13,94	SNP_IGA_683956	30,6	33,3	1,19			
	Gimenells	TE	qP-FP6	2011-2013	11,39	CPPCT030	76,2	45,7	0,36	1,21	3,40	O

QTLs Phenology (continued)

Maturity date	Cabrils	T1E	qP-MD4	2011	8,16	SNP_IGA_413115	36,1	44,8	30,99			
			qP-MD4	2012	12,06	SNP_IGA_409167	31,6	35,9	29,38			
			qP-MD4	2013	4,47	SNP_IGA_412662	34,3	32,9	17,67			
	Gimenells	T1E	qP-MD4	2011	10,91	SNP_IGA_420316	40,6	57,9	40,01			
			qP-MD4	2012	11,81	SNP_IGA_413115	36,1	43,1	32,08			
			qP-MD4	2013	9,71	EPPCU2000	37,0	44,9	33,49			
	Gimenells	TE	qP-MD4	2011	10,05	SNP_IGA_413115	35,2	81,9	45,31	-2,79	-0,06	A
			qP-MD4	2012	11,41	SNP_IGA_412380	34,3	77,7	36,71	1,02	0,03	A
			qP-MD4	2013	9,28	SNP_IGA_412380	34,3	76,1	37,05	4,18	0,11	A
Fruit develepment period	Cabrils	T1E	qP-FDP4	2011	6,96	SNP_IGA_413115	36,1	43,2	29,43			
			qP-FDP4	2012	11,36	SNP_IGA_412380	31,6	34,2	29,07			
			qP-FDP4	2013	3,74	SNP_IGA_412380	33,4	28,3	18,15			
	Gimenells	T1E	qP-FDP4	2012	11,89	SNP_IGA_413115	36,1	48,8	34,63			
			qP-FDP4	2013	9,02	EPPCU2000	37,0	43,8	33,74			
	Gimenells	TE	qP-FDP4	2012	11,52	SNP_IGA_412380	34,3	79,5	37,48	3,88	0,10	A
			qP-FDP4	2013	7,91	SNP_IGA_412380	34,3	75,3	38,11	6,67	0,18	A
Leaf fall	Cabrils	T1E	qP-LF8	2010-2011	3,78	SNP_IGA_796755	1,6	11,1	12,2			
			qP-LF8	2011-2012	4,27	SNP_IGA_796755	1,6	11,3	11,95			
			qP-LF8	2012-2013	3,53	SNP_IGA_869040	23,1	10,7	9,68			
Juvenility period	Cabrils	T1E	qP-Juv6	2006-2012	3,94	BPPCT025	24,1	12,6	-0,61			

QTLs fruit quality

Trait	Location	Population	QTL name	Year	LOD	Nearest marker	Position (cM)	R^2	a^a	*d*	*d / a*	Genic action[b]
Blood Flesh	Gimenells	TE	qP-Bf3	2011-2013	3,95	SNP_IGA_317001	18,0	50,7	-0,50	-0,14	0,29	AR
Juiciness	Gimenells	TE	qP-Jui1	2011-2013	2,76	SNP_IGA_129422	81,6	31,8	0,40	0,21	0,53	AR
Intensity skin color	Cabrils	T1E	qP-ISC1	2012	3,64	CPPCT053	48,7	17,0	0,68			
			qP-ISC1	2013	8,33	SNP_IGA_88751	22,6	59,9	1,40			
	Gimenells	T1E	qP-ISC1	2011	3,38	SNP_IGA_23251	5,5	25,1	0,91			
			qP-ISC1	2012	6,33	SNP_IGA_91583	23,3	25,2	0,92			
			qP-ISC1	2013	4,61	EPPCU5331	12,2	23,3	0,83			
Percentage skin color	Cabrils	T1E	qP-PSC1	2011	3,16	SNP_IGA_123719	53,6	25,5	1,03			
			qP-PSC4		4,60	M12a	31,6	33,0	-1,13			
			qP-PSC1	2012	6,63	SNP_IGA_126668	52,8	30,3	0,98			
			qP-PSC4		2,93	SNP_IGA_412380	33,4	14,5	-0,68			
			qP-PSC1	2013	8,08	BPPCT028	51,2	57,9	1,52			
			qP-PSC4		2,50	M12a	31,6	23,5	-0,95			
	Gimenells	T1E	qP-PSC1	2011	2,55	SNP_IGA_126668	52,8	19,9	0,88			
			qP-PSC1	2012	7,99	BPPCT028	51,2	30,5	1,09			
			qP-PSC4		6,25	M12a	31,6	24,8	-0,98			
			qP-PSC1	2013	3,96	SNP_IGA_123719	53,6	22,1	1,01			
			qP-PSC4		6,63	M12a	31,6	32,1	-1,21			
	Gimenells	TE	qP-PSC3	2011	3,99	SNP_IGA_317001	18,2	57,6	-1,50	0,35	-0,23	A
			qP-PSC3	2012	4,26	SNP_IGA_887061	24,7	41,7	-1,32	0,57	-0,43	AD
			qP-PSC3	2013	3,00	SNP_IGA_321565	21,5	37,4	-1,33	1,23	-0,93	D
Fruit weight	Gimenells	T1E	qP-FW6	2011	2,71	CPP21413	0,0	21,3	24,80			
			qP-FW6	2012	5,30	CPP21413	0,0	21,7	28,60			
			qP-FW6	2013	3,58	CPP21413	0,0	18,9	28,08			
	Gimenells	TE	qP-FW4	2011	6,56	SNP_IGA_410265	31,7	66,6	-13,87	24,80	-1,79	U
			qP-FW4	2012	6,93	SNP_IGA_410265	31,7	57,2	-15,68	26,76	-1,71	U
			qP-FW4	2013	4,65	SNP_IGA_410265	31,7	48,5	-10,73	33,94	-3,16	U

QTLs fruit quality (continued)

Stone weight	Cabrils	T1E	qP-SW6	2011	3,61	SNP_IGA_682735	28,8	28,1	2,78			
			qP-SW6	2012	9,35	CPPCT047	27,6	37,4	2,98			
	Gimenells	T1E	qP-SW6	2011	4,90	CPPCT047	27,6	37,5	3,44			
			qP-SW6	2012	8,99	CPPCT047	27,6	35,3	3,32			
			qP-SW6	2013	7,84	CPPCT047	27,6	37,1	3,82			
Flesh weight	Gimenells	T1E	qP-FlW7	2011	3,34	SNP_IGA_790122	38,3	27,5	-25,17			
			qP-FlW7	2012	2,88	SNP_IGA_769687	13,9	13,3	-21,03			
			qP-FlW7	2013	2,86	CPPCT047	18,0	16,3	-24,75			
	Gimenells	TE	qP-FlW4	2011	6,56	SNP_IGA_410265	31,7	66,6	-13,40	23,94	-1,79	U
			qP-FlW4	2012	6,70	SNP_IGA_410265	31,7	58,0	-15,60	26,35	-1,69	U
			qP-FlW4	2013	4,78	SNP_IGA_410265	31,7	49,4	-10,29	31,86	-3,09	U
Fruit polar diameter	Gimenells	TE	qP-FpD4	2011	5,72	SNP_IGA_410265	31,7	61,7	-0,61	0,78	-1,28	U
			qP-FpD4	2012	5,62	SNP_IGA_410265	31,7	50,0	-0,47	0,79	-1,69	U
			qP-FpD4	2013	2,90	SNP_IGA_410265	31,7	35,8	-0,31	0,85	-2,73	U
Fruit cheek diameter	Gimenells	T1E	qP-FcD7	2011	3,31	EPPCU5176	30,6	23,4	-0,71			
			qP-FcD7	2012	3,56	SNP_IGA_769687	13,9	15,5	-0,55			
			qP-FcD7	2013	3,66	CPPCT057	18,0	20,3	-0,61			
	Gimenells	TE	qP-FcD4	2011	7,94	SNP_IGA_410265	31,7	73,3	-0,74	1,08	-1,45	U
			qP-FcD4	2012	8,36	SNP_IGA_410265	31,7	64,0	-0,64	1,03	-1,60	U
			qP-FcD4	2013	5,29	SNP_IGA_410265	31,7	55,1	-0,53	1,07	-2,01	U
Fruit suture diameter	Gimenells	TE	qP-FsD4	2011	8,04	SNP_IGA_410265	31,7	73,9	-0,65	0,85	-1,31	U
			qP-FsD4	2012	8,16	SNP_IGA_410265	31,7	63,2	-0,64	0,85	-1,33	U
			qP-FsD4	2013	4,42	SNP_IGA_410265	31,7	48,9	-0,49	0,91	-1,87	U
Stone cheek diameter	Cabrils	T1E	qP-ScD6	2011	4,14	SNP_IGA_629177	14,5	31,6	0,39			
			qP-ScD6	2012	7,64	SNP_IGA_680864	28,2	31,7	0,39			
			qP-ScD6	2013	2,77	BPPCT025	27,3	27,3	0,28			
	Gimenells	T1E	qP-ScD6	2011	4,17	BPPCT008	16,0	34,0	0,47			
			qP-ScD6	2012	11,44	CPPCT047	27,6	41,3	0,46			
			qP-ScD6	2013	10,58	CPPCT047	27,6	46,0	0,54			
Stone suture diameter	Cabrils	T1E	qP-SsD6	2011	3,86	SNP_IGA_682735	28,8	30,9	0,38			
			qP-SsD6	2012	8,95	CPPCT047	27,6	35,8	0,39			
			qP-SsD6	2013	3,60	EPPCU4092	37,0	34,0	0,30			
	Gimenells	T1E	qP-SsD6	2011	4,41	pchcms5	20,7	33,4	0,33			
			qP-SsD6	2012	10,24	SNP_IGA_683956	30,6	38,4	0,38			
			qP-SsD6	2013	10,17	SNP_IGA_683956	30,6	45,1	0,40			
Flesh polar diameter	Gimenells	TE	qP-FlpD4	2011	7,17	SNP_IGA_410955	33,8	69,3	-0,60	0,63	-1,05	D
			qP-FlpD4	2012	8,25	EPPCU2000	36,9	65,2	-0,62	0,56	-0,90	D
			qP-FlpD4	2013	4,54	SNP_IGA_410955	32,2	49,1	-0,36	0,62	-1,72	U
Flesh cheek diameter	Gimenells	TE	qP-FlcD4	2011	8,67	SNP_IGA_412380	34,3	77,5	-0,88	1,02	-1,17	D
			qP-FlcD4	2012	8,28	SNP_IGA_410955	32,6	66,5	-0,60	1,01	-1,67	U
			qP-FlcD4	2013	5,78	SNP_IGA_410265	31,7	58,1	-0,46	0,84	-1,82	U
Flesh suture diameter	Gimenells	TE	qP-FlsD4	2011	8,18	SNP_IGA_412380	34,3	76,9	-0,75	0,84	-1,12	D
			qP-FlsD4	2012	7,72	SNP_IGA_412380	34,3	63,2	-0,58	0,78	-1,36	U
			qP-FlsD4	2013	4,12	SNP_IGA_410265	31,7	46,5	-0,43	0,78	-1,83	U

QTLs Leaf

Trait	Location	Population	QTL name	Year	LOD	Nearest marker	Position (cM)	R^2	*a* [a]	*d*	*d / a*	Genic action[b]
Leaf perimeter	Cabrils	T1E	qP-LP1	2012	5,02	CPPCT004	7,9	16,9	-353,18			
			qP-LP1	2013	7,14	CPPCT004	7,9	23,4	-393,61			
			qP-LP8		5,10	SNP_IGA_799291	3,1	16,7	326,32			
	Gimenells	T1E	qP-LP8	2012	4,12	SNP_IGA_795756	0,8	13,1	215,69			
			qP-LP1	2013	2,79	CPPCT004	7,9	9,0	-177,85			
			qP-LP8		5,70	SNP_IGA_796755	1,6	17,0	243,57			

QTLs Leaf (continued)

Leaf surface	Cabrils	T1E	qP-LS1	2012	5,57	SNP_IGA_9197	3,4	20,4	-48,85			
			qP-LS1	2013	6,77	SNP_IGA_23351	5,5	21,8	-71,49			
			qP-LS7		3,04	CPPCT017	39,1	10,3	46,57			
			qP-LS8		3,50	SNP_IGA_800634	4,7	11,8	50,41			
	Gimenells	T1E	qP-LS7	2012	3,30	EPDCU3392	41,9	10,7	33,27			
			qP-LS8		3,35	SNP_IGA_795756	0,8	10,8	33,74			
			qP-LS1	2013	3,71	SNP_IGA_2651	0,0	11,5	-35,53			
			qP-LS7		2,82	EPPCU5176	30,6	8,8	30,83			
			qP-LS8		3,99	SNP_IGA_796755	1,6	12,2	36,60			
Leaf blade width	Cabrils	T1E	qP-LBW1	2012	2,74	SNP_IGA_2651	0,0	9,5	-23,32			
			qP-LBW6		3,10	pchcms5	20,7	10,6	23,68			
			qP-LBW1	2013	4,21	EPDCU3122	0,8	13,8	-35,36			
			qP-LBW6		4,57	BPPCT025	24,1	14,9	34,91			
	Gimenells	T1E	qP-LBW1	2013	3,14	SNP_IGA_2651	0,0	9,8	-21,64			
			qP-LBW6		4,63	pchcms5	20,7	14,0	25,66			
Leaf length	Cabrils	T1E	qP-LL1	2012	7,35	CPPCT004	7,9	26,4	-149,18			
			qP-LL1	2013	6,40	CPPCT004	7,9	21,3	-165,32			
			qP-LL8		5,45	SNP_IGA_799291	3,1	17,7	146,99			
	Gimenells	T1E	qP-LL8	2012	4,01	SNP_IGA_795756	0,8	12,8	87,39			
			qP-LL8	2013	5,68	SNP_IGA_796755	1,6	17	100,44			
Leaf blade length	Cabrils	T1E	qP-LBL1	2012	7,91	CPPCT004	7,9	25,3	-133,56			
			qP-LBL1	2013	8,05	CPPCT004	7,9	25,9	-167,89			
			qP-LBL8		4,13	SNP_IGA_799291	3,1	13,8	119,29			
	Gimenells	T1E	qP-LBL8	2012	3,47	SNP_IGA_795756	0,8	11,4	76,11			
			qP-LBL1	2013	2,78	CPPCT004	7,9	8,9	-71,43			
			qP-LBL8		4,27	SNP_IGA_796755	1,6	13,0	85,91			
Petiole length	Cabrils	T1E	qP-PL5	2012	4,13	SNP_IGA_598784	27,4	13,8	20,22			
			qP-PL6		3,37	MA14a	34,9	11,4	18,39			
			qP-PL7		2,68	MA20a	25,8	9,2	16,53			
			qP-PL8		4,48	SNP_IGA_859354	12,2	14,9	21,25			
			qP-PL5	2013	3,44	SNP_IGA_590546	13,5	11,6	18,15			
			qP-PL6		4,10	MA14a	34,9	13,5	19,63			
			qP-PL7		4,16	CPPCT033	24,1	13,8	19,79			
			qP-PL8		13,61	SNP_IGA_870509	28,3	39	34,07			
	Gimenells	T1E	qP-PL5	2012	2,66	SNP_IGA_590546	13,5	8,9	15,34			
			qP-PL7		3,12	PMS02	27,9	10,3	16,55			
			qP-PL8		6,78	SNP_IGA_869040	23,1	22,6	25,47			
			qP-PL5	2013	3,94	SNP_IGA_589750	12,9	12,4	18,09			
			qP-PL6		2,64	MA040a	34,4	8,3	14,75			
			qP-PL7		3,82	CPPCT033	24,1	11,7	17,59			
			qP-PL8		10,61	SNP_IGA_869040	23,1	29,9	29,01			
	Gimenells	TE	qP-PL5	2012	3,06	SNP_IGA_594745	28,3	16,7	23,73	5,68	0,24	A
			qP-PL8		2,66	SNP_IGA_876610	36,3	15,0	18,74	20,10	1,07	R
			qP-PL5	2013	4,83	SNP_IGA_584315	14,8	28,1	29,22	14,09	0,48	AR
			qP-PL8		7,35	SNP_IGA_881120	44,8	35,8	33,09	5,94	0,18	A
Leaf weight	Cabrils	T1E	qP-LW1	2012	3,89	SNP_IGA_9197	3,4	14,4	-0,06			
			qP-LW6		3,35	pchcms5	20,7	12,3	0,05			
			qP-LW1	2013	4,81	SNP_IGA_23251	5,5	15,9	-0,08			
			qP-LW6		3,71	pchcms5	20,7	12,3	0,06			
			qP-LW8		3,58	SNP_IGA_800634	4,7	11,9	0,06			
	Gimenells	T1E	qP-LW1	2012	3,89	SNP_IGA_18927	5,5	13,5	-0,04			
			qP-LW6		2,83	SNP_IGA_687583	33,8	9,5	0,04			
			qP-LW8		4,02	CPSCT018	0,0	13,2	0,04			
			qP-LW1	2013	3,59	SNP_IGA_17419	4,8	11,3	-0,04			
			qP-LW8		6,21	SNP_IGA_796755	1,6	18,4	0,06			

QTLs Leaf (continued)

Chlorophyll content	Cabrils	T1E	qP-CC6	2011	5,27	SNP_IGA_695974	42,0	14,5	-4,09
			qP-CC6	2012	5,40	CPPCT021	43,0	15,8	-3,82
			qP-CC6	2013	5,35	SNP_IGA_695974	42,0	15,7	-3,49

[a] In TxE is ((A+B)/2), being the A the almond allele and in T1E is A-H, being A the homozygous almond allele

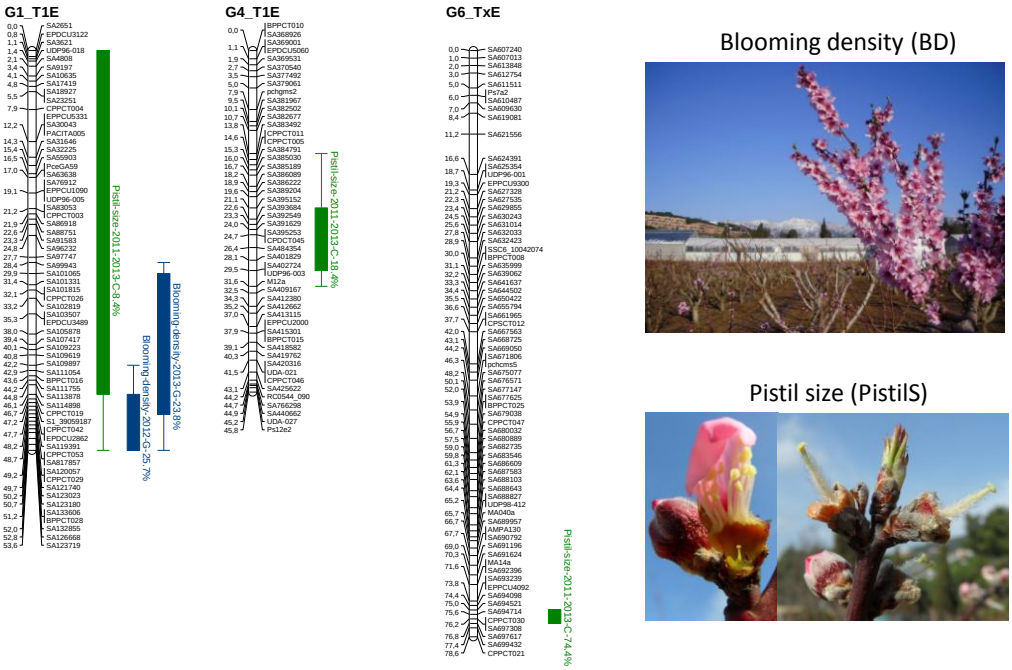
[b] U: underdominant, D: dominance for peach allele; AD: partial dominance for peach allele; A: additive, AR: partial dominance for a allele; R: dominant for peach allele, O: overdominant.

4.3.3.2 Phenology traits

A total of 23 consistent QTLs (10 major QTLs) were detected for eight of the nine traits studied: 15 in T1E, two in E and six in TxE; three were in both T1E and TxE. The most significant QTLs were for MD and FDP in both populations in the same region of G4 (qP-MD4 and qP-FDP4), explaining from 32.9 to 81.9% of the phenotypic variance for MD and 28.3 to 79.5% for FDP. All of them peaked between markers SNP_IGA_409167 and SNP_IGA_420306. The presence of the almond allele in this locus in T1E population increases the MD in an average of 32 days. In TxE the almond allele present an additive effect that increases MD in an average of 42 days.

Consistent QTLs for beginning of flowering time were identified in G1, G2 and G8 in T1E and only in G1 in TxE. The QTL (qP-BF1) located in T1E and TxE at the beginning of G1 was identified in all the datasets except in Cabrils in 2011. These QTL explained 19.1-26.3% of the phenotypic variance in Gimenells and around the 12% in Cabrils. The second QTL (qP-BFT2), located at the beginning of G2, was identified in T1E both years in Gimenells and only in 2011 in Cabrils. The third QTL was located in G8 (qP-BFT8) and was detected in Cabrils but not in Gimenells and explained 11.5 and 16.0% of the phenotypic variance. In TxE the major QTL identified for BFT in G1 explained 19.8-23.8% of the phenotypic variance and in T1E 13.1-23.3%. Although in TxE is more at the beginning of the group they have overlapping regions in some years, therefore we considered that was the same and it is called qP-BFT1. In this same region a consistent QTL for End of flowering was identified in T1E (qP-EFT1) explaining 13.1 and 23.3% of the phenotypic variance. For end of flowering time, three QTLs were identified in T1E, two co-localizing with those described for beginning of flowering time in G1 (qP-EFT1) and G8 (qP-EFT1), and another in the central part of G7 found only in the Earlygold map (qP-EFT7). For beginning of shooting, four QTLs were identified: three at similar positions than those described before for the flowering time characters, two in G2 (qP-BS2) and G8 (qP-BS8) in T1E and one in G7 (qP-BS7) mapped in E explaining similar proportions of the phenotypic variance as before. A different QTL was found in G6 of TxE (qP-BS6) with R^2=13.4-16.6%.

QTLs Flower traits



QTLs Phenology traits

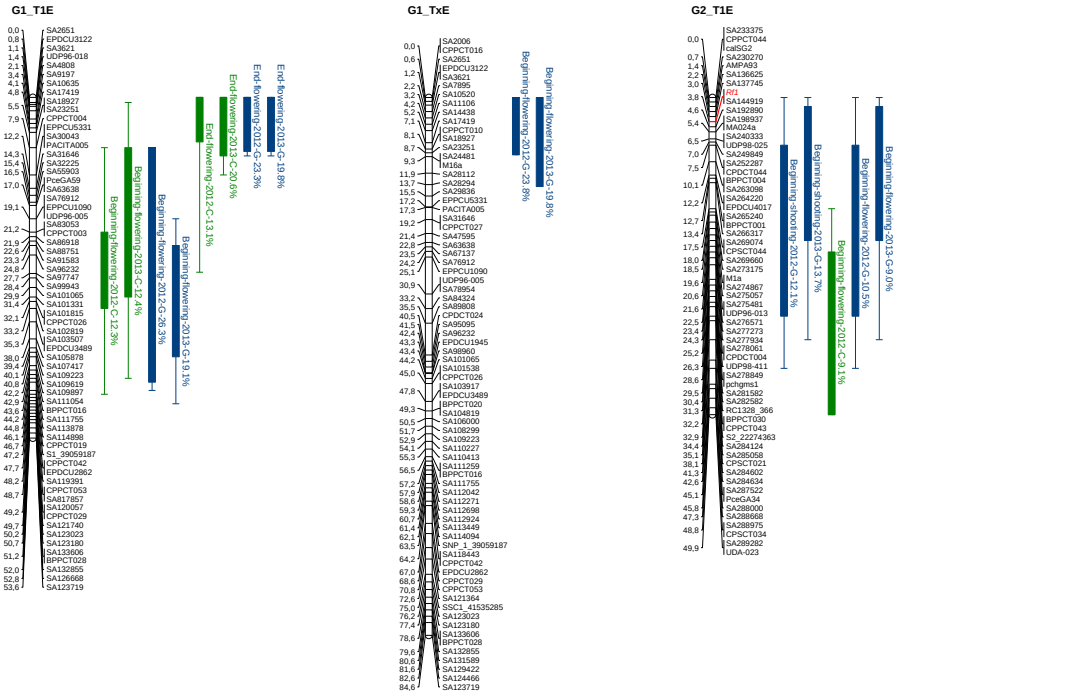
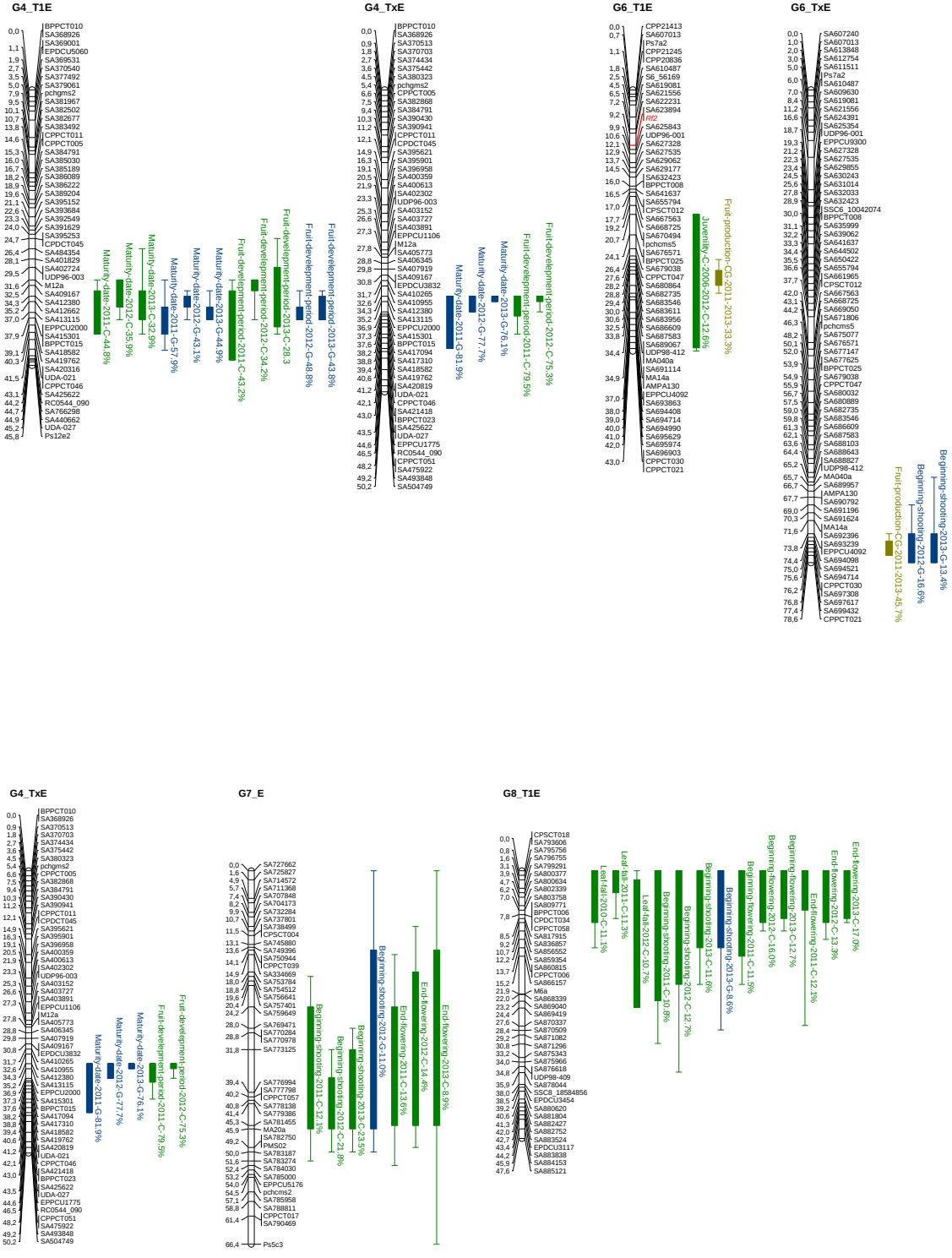
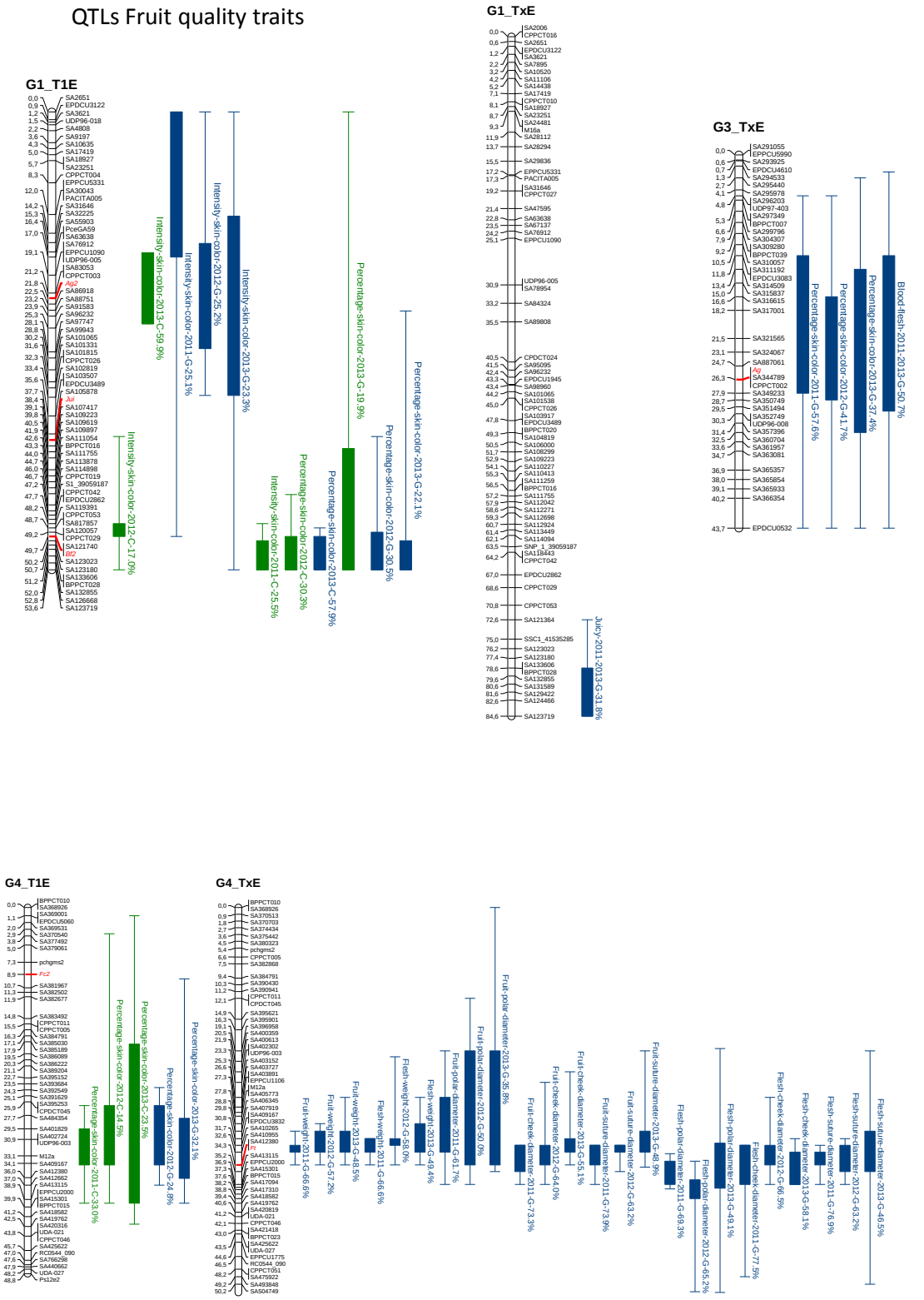


Figura 12. Location of putative QTLs controlling flower, phenology, fruit quality and leaf morphology traits on TxE, T1E and E maps. At the top of each linkage group followed by the number according to the reference map is the parent used for mapping. The color of the bars indicates the place of assessment (green = Cabrils; = Gimenells blue, yellow = Cabrils-Gimenells). The thick parts of the vertical bars next to the linkage groups (G) indicate minus one logarithm of the odds (LOD) intervals and the thin bars represent minus two-LOD intervals. (The figure 12 follows in the next pages).

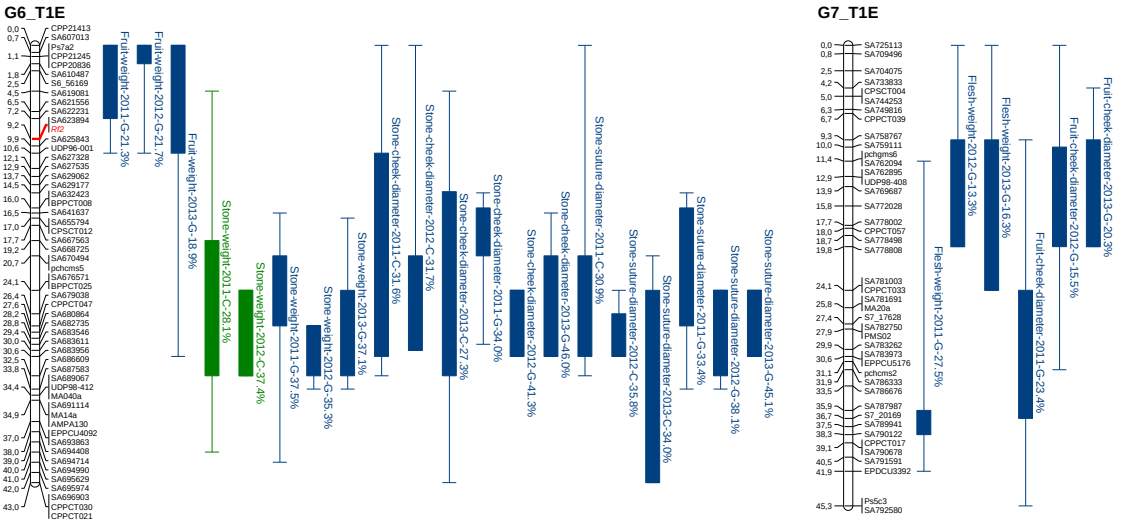
QTLs Phenology traits



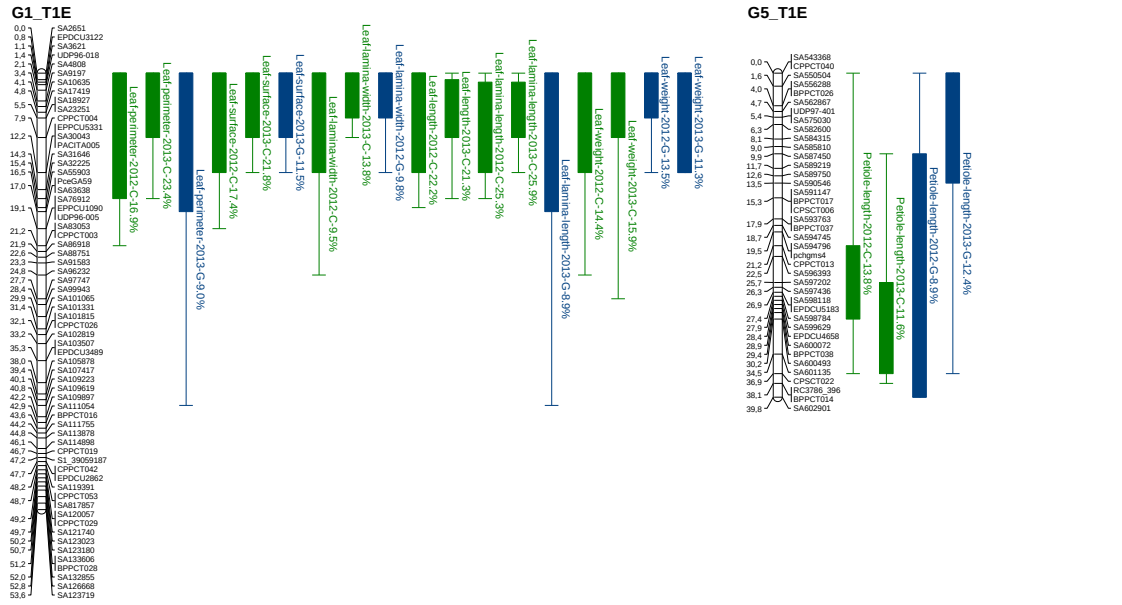
QTLs Fruit quality traits



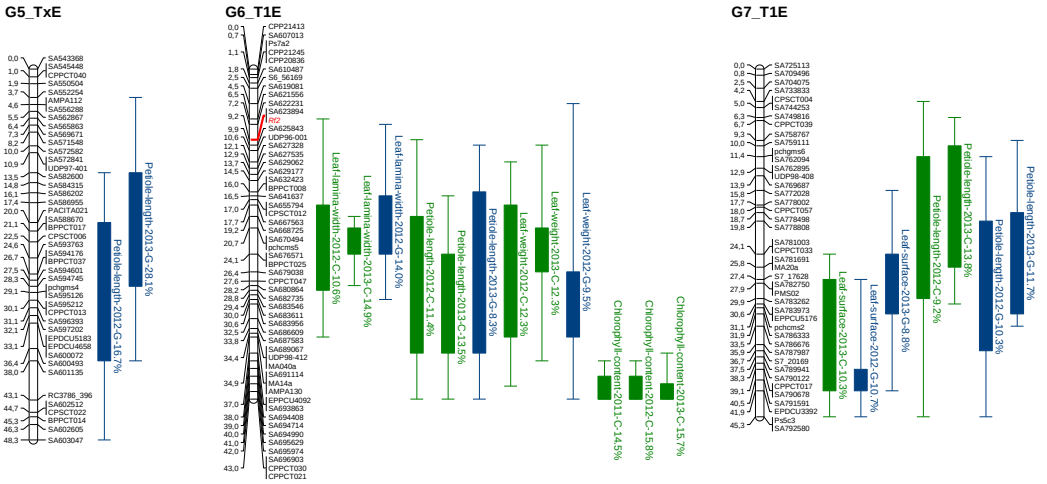
QTLs Fruit quality traits

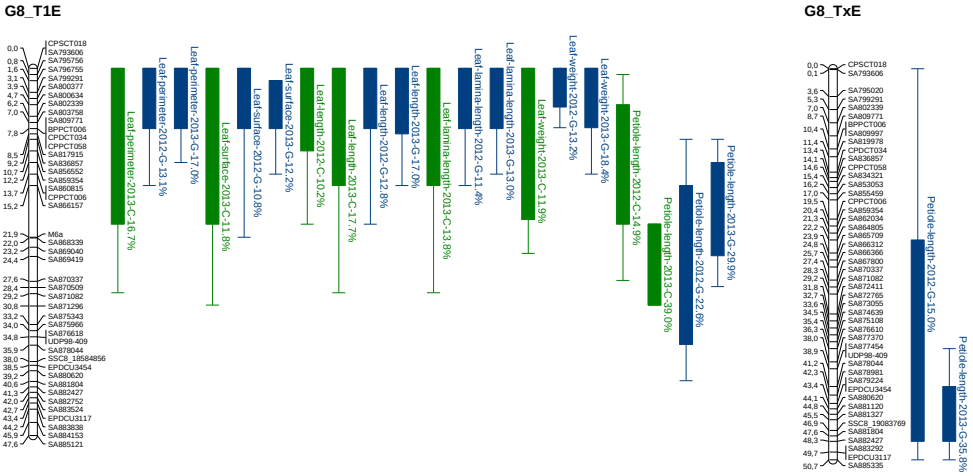


QTLs Leaf traits



QTLs Leaf traits





A major QTL for fruit production (qP-FP6) was detected in the central part of G6 explaining 45.7% of the phenotypic variance in TxE and 33.3% in T1E. This QTL colocalized also with a QTL for Juvenility in T1E (qP-Juv6) explaining a 13.3% of the phenotypic variance. A QTL for leaf fall date was detected in G8 (qP-LF8) in T1E at Cabrils that was the only place where it was measured.

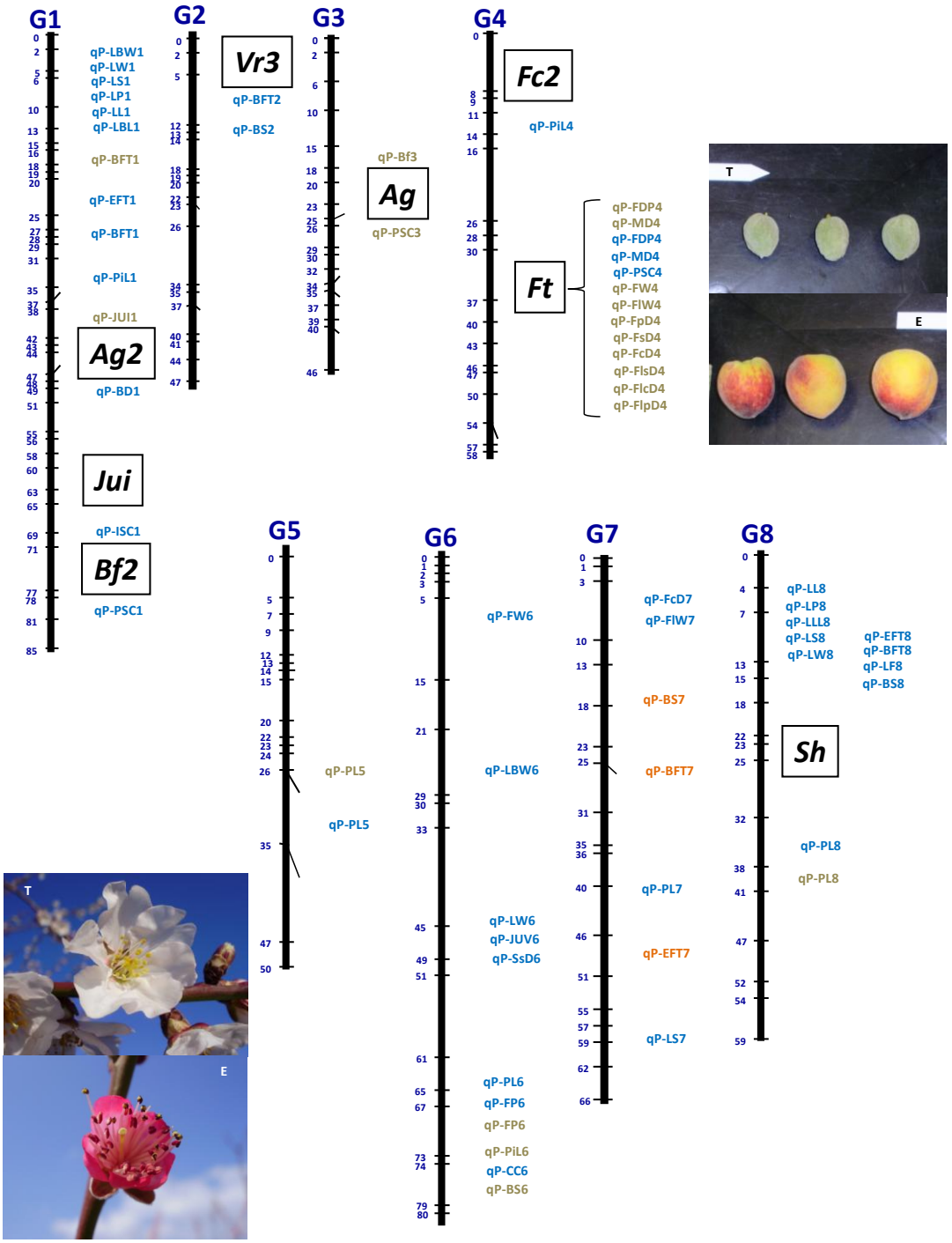


Figure 13. Genes and consistent QTLs mapped in this work in the TxE and T1E progenies and represented in the *Prunus* reference map. Genes are in text box. QTLs in blue, red and green were identified using the maps of T1E, E and TxE respectively.

4.3.3.3 Fruit traits

A total of 20 consistent QTLs were detected for fruit quality traits: 9 in T1E and 11 in TxE. Individual QTLs explained between 17.0 % and 77.5% of the phenotypic variance. A major QTL for juiciness explaining a 31.8% of the phenotypic variance was found in TxE (qP-Jui1) at the end of G1 collocating with the position of the *Jui* gene mapped in T1E. For the blood flesh trait one QTL was detected in the central region of G3 in TxE (qP-Bf3) explaining a 50.7% of the phenotypic variance and at a different position than the gene *Bf2* mapping in the distal part of G1 in T1E. The variability of intensity of skin color in T1E was explained by a major QTL in the middle of G1 (qP-ISC1) with R^2 ranging between a 17.0 and a 59.9%. Two major QTLs were detected in T1E for the percentage of skin color: one was detected in both locations at the end of G1 (qP-PSC1) and explaining between a 19.9 and a 57.9% of the phenotypic variance, and the other QTL was detected in the middle of G4 (qP-PSC4) and explained between a 14.5 and a 33.0% of the phenotypic variance. In TxE a major QTL was detected in G3 (qP-PSC3) explaining between the 18.2 and the 24.7% of the phenotypic variance.

For fruit weight a consistent QTL was detected in T1E at the beginning of G6 (qP-FW6) explaining around the 20% of the phenotypic variance. In TxE most of the variance of this trait (40-62.6%) is explained by a major QTL found in G4 (qP-FW4). The same occurred for other characters related with fruit dimensions in this population where a major QTL was found in G4 for flesh weight (qP-FlW4), fruit and flesh polar diameters (qP-FpD4 and qP-FlpD4), fruit and flesh cheek diameter (qP-FcD4, qP-FlcD4) and fruit suture diameter (qP-FsD4, qP-FlD4), all of them explaining between 41 and 76% of the phenotypic variance. In TxE a major QTL for stone weight was detected in both locations and all years in the central part of G6 close to the marker CPPCT047 (qP-SW6). In this genomic region other QTLs were also detected in T1E for stone cheek diameter (qP-ScD6) and stone suture diameter (qP-SsD6), explaining between 28.1 and 46.0% of the phenotypic variance. Finally, we also identified major QTLs for flesh weight and fruit cheek diameter in T1E in G7 only in Gimenells (qP-FlW7, qP-FcD7) explaining 13.8-27.5 and 15.5-23.4% of the phenotypic variance.

4.3.3.4 Leaf traits

A total of 19 consistent QTLs were detected for leaf traits in both populations: 17 only in T1E and two in both T1E and TxE. Individual QTLs explained between 8.3 % and 39.0% of the

phenotypic variance, seven being major QTLs. QTLs for leaf traits were detected in all chromosomes except G2, G3 and G4.

For leaf perimeter two QTLs were detected in T1E, a major QTL at the beginning of G1 (qP-LP1) and a QTL at the beginning of G8 (qP-LP8). At the same position of G1 and G8 we also detected QTLs for leaf surface (qP-LS1, qP-LS8), leaf length (qP-LL1 and qP-LL8), leaf blade length (qP-LBL1 and qP-LBL8), and leaf weight (qP-LW1 and qP-LW8). Leaf blade width had a QTL in G1 (qP-LBW1), another one in G6 (qP-LBW6) but none in G8. Petiole length variability identified four QTLs: two of them present in all populations years and locations, one in the proximal end of G5 (qP-PL5) with R^2=8.9-28.1%, and another one located in the middle of G8 (qP-PL8), explaining from 14.9 to 39.0% of the phenotypic variance. Other QTLs for PL were detected in G6 and G7 of T1E (qP-PL6, qP-PL7). Other QTLs were also detected for leaf surface at the end of G7 (qP-LS7), for leaf blade width and for leaf weight on G6 (qP-LBW6 and qP-LW6). For chlorophyll content a QTL was identified in G6 in T1E (qP-*CC6*) that explained during the three years around the 15% of the phenotypic variance. Any QTL was detected for this trait in TxE.

4.4 DISCUSSION

We have mapped eight major genes and 64 QTLs across seasons using two almond x peach interspecific progenies. From the QTLs identified, 44 were in the backcross one population T1E (43 mapped in the map of the almond x peach interspecific hybrid used as female parent and 2 in the peach 'Earlygold' male parent), and 19 in the F_2 progeny TxE. We expected a higher segregation in the TxE than in the T1E progeny, because the characters with dominant gene action in the peach parent would not be detected in the BC_1 population, but we found a lower number of QTLs in TxE. The most important reason is that the stringent criterion used for declaring a consistent QTL favored the populations with higher size (N=88 in TxE vs. N=178 in T1E), particularly for loci that explained low proportions of the observed phenotypic variation (R^2=8-15%), because more numerous populations have more statistical power to identify QTLs with low effect. In addition, the fact that T1E was in two locations and that QTLs were accepted as consistent when they were found all years in one of them was also important. Only two QTLs were detected with the E map, which we attribute to different reasons: a) the lower level of genetic variability within peach, b) the fact that a large part of the genome of 'Earlygold' is identical by descent (Chapter 3) and c) the phenotypic effects within the peach gene pool could be often masked by larger effects from the almond allele.

Only a six of the 20 QTLs detected in TxE were found in T1E, while one could expect this occurring more frequently. These were beginning of flowering time (qP-Bf1), Maturity date (qP-MD4), Fruit development period (qP-FDP4), Juiciness (qP-Jui1) and two QTLs of petiole length (qP-PL5 and qP-PL8). These characters may be less affected by the environment, the genetic background or both than others, resulting in a more heritable behavior. The genetic background may explain a good part of this low correspondence between QTLs, one example is the fruit dimension traits that in the case of TxE are strongly affected by the presence of individuals with almond-like fruit type, determined by a the major gene *Ft* that are not present in the T1E progeny. All dimension traits identify only a major QTL in G4 that correspond to the position of the *Ft* gene and no more QTLs are detected, whereas the T1E progeny does not detect any QTL on G4 and two different QTLs in G6 and G7.

The analysis of T1E in two locations is an opportunity to detect possible genotype x environmental interactions. These locations have contrasted climates: Cabrils, located on the Mediterranean coast North of Barcelona has a very mild wheather, whereas Gimenells lying in central Catalonia, 150 Km west of Barcelona, has a continental climate. In addition, the Cabrils plants were planted on their own roots and at a tighter density than the copy of Gimenells that was grafted and submitted to a more standard agricultural practice. We identified six QTLs that were only found in one location and not in the other; two for beginning of flowering time (qP-BFT8) and leaf length (qP-LL1) were only present in Cabrils and four, determining blooming density (qP-BD1), fruit weight (qP-FW6), flesh weight (qP-FlW7) and fruit cheek diameter (qP-FcD7) were detected in Gimenells but not in Cabrils. For qP-BFT8 it may respond to climatic differences between the two locations, and the characters related with fruit dimensions may be associated with the cultural practices that determined that fruit in Gimenells had higher sizes than in Cabrils (Annex 2 and 3).

A QTL analysis was previously done in the TxE population of Cabrils in 1996 and 1997 using a map with 172 markers (162 RFLPs, 11 isoenzymes and two morphological traits) and less individuals and a lower LOD score threshold for the QTL analysis (Joobeur 1998). Some of the traits analyzed were also analyzed in this work, allowing the comparison of the results. These traits were three phenological traits (BFT, EFT and FD), and five leaf traits (LS, LBL, LBW, PL and CC). For flowering time a coincident QTL was identified on G1, being the only one detected by Joobeur (1998) with a LOD score threshold higher than 3. For flowering duration different QTLs were found in the two studies but none of them was consistent in both works. Any coincident

QTL in both works was neither identified for leaf surface, leaf weight nor chlorophyll content. For the other traits another situation was found, for example Joobeur (1998) identified one QTL for leaf blade width that in our study we were not able to identify in TxE but it was identified in T1E. For leaf length, a QTL in G3 was detected in both works but other detected in G2 and G4 were not. Finally for petiole length coincident QTLs were detected in G5, G7 and G8 but not in G3. The differences between both studies could be due to the population size, genetic map and LOD threshold differences, or by the presence of genotype x environment interactions.

Interspecific crosses allow the analysis of characters that present contrasted phenotypes between the species involved. Some of these characters have a simple inheritance and allow the identification of major genes or major QTLs that explain a large part of the observed variability. In our almond x peach crosses we have been able to map ten major genes, eight described here and two more corresponding to two fertility restorer genes on G2 and G6 described in the Chapter 3. In addition, several characters resolved in QTLs explaining more than 50% of R, such as maturity date (and fruit development period that we consider the same QTL), pistil length in TxE or blood flesh in G3 of T1E. Some of these major genes or QTLs corresponded to traits of unknown genetics, such as fruit type (almond vs. peach), anther color (red vs. yellow), pistil length (short vs. long) or juiciness (juicy vs. non-juicy) never described before and corresponding to characters that are clearly distinctive between peach and almond. Others, as fruit flesh and skin anthocyanic color, flower color or powdery mildew resistance, corresponded to characters segregating within peach, but that interestingly were encoded in most cases by genes at chromosome positions different from those described so far, indicating that the variability supplied by almond is often in different genes than that found at the intraspecific level. This is important not only to identify different genes that cause similar phenotypes, but also as a source of novel variability that can be combined to that already available to produce cultivars with added value. One obvious example is that of the resistance to powdery mildew in G2 that can be pyramided with others found before (Dabov, 1983; Foulogne et al. 2003b; Pascal et al. 2010) to obtain cultivars with a broader or more durable resistance.

4.4.1 Flower traits

The *Sh* gene, responsible for the type of flower in peach was initially mapped on G8 by Ogundiwin et al. (2009) and later by Fan et al. (2010) and Eduardo et al. (2011) in two peach

intra-specific crosses. This gene was heterozygous in 'Earlygold' and placed at the expected position of its map, confirming its inheritance and position on the *Prunus* genome.

For pistil length a major QTL has been identified in TxE in G6. In this population this trait is highly correlated with fruit production for which a major QTL was also detected in the same region. The same chromosomal region of G6 contains one or two tightly linked genes that affect fruit shape (*S/s*) and fruit abortion (*Af/af*), traits that are also associated to the length of the pistil and fruit production (Dirlewanger et al. 2006; Picañol et al. 2013). In T1E, a QTL for fruit production was also detected in the same region, but no QTLs were found for pistil length, that instead mapped to G1 and G4, suggesting that at least in this population fruit production was not associated with pistil length.

For Blooming density we identified a major QTL on G1 where the peach allele decreased flower density. This is to our knowledge the first time that a QTL has been reported for this trait in *Prunus*, an important one as it determines the needs of flower or fruit thinning during the growing season, one of the most expensive operations of peach cultivation.

4.4.2 Phenology traits

Six consistent QTLs on different linkage groups, G1, G2, G4, G6, G7 and G8, were detected for three phenology traits related with the time of shooting and blooming (beginning and end) in two progenies. None was found in all locations, populations and characters. Only one at the proximal end of G8 was detected in T1E for all characters, although the QTL for beginning of flowering was not detected in Gimenells. The QTL on G6 appeared only in TxE and the observed on G7 only in the E map. These characters are highly heritable and appear to be related as suggested by the high correlations between their data of different environments, but there are examples in the literature that do not support this hypothesis. In almond, a low correlation between leafing and blooming dates was observed in a large sample of materials (Kester et al. 1973), and later on Dicenta et al. (1993) identified that the level of correlation between these traits was not strong enough for early selection of blooming date based on the leafing date. Data from almond (Ballester et al. 2001; Sánchez-Pérez et al. 2007; Sánchez-Pérez et al. 2012), peach (Quilot et al. 2004; Fan et al. 2010; Zhebentyayeva et al. 2014; Romeu et al. 2014) apricot (Olukolu et al. 2009; Campoy et al. 2011; Socquet-Juglard et al. 2013), sour cherry (Wang et al. 2000) and integrating several *Prunus* species for comparing the inheritance of this trait throughout the genus (Dirlewanger et al. 2012), are similar to those we found,

supporting a complex inheritance of these traits in peach and other *Prunus* with many genes involved in each trait and different sets of these genes, partly overlapping between traits, segregating in each mapping population (Dirlewanger et al. 2012).

For MD and FDP a major QTL was found on G4 in both populations (T1E and TxE). This locus has been already detected using several populations from different *Prunus* species as peach, apricot and sweet cherry (Dirlewanger et al. 2012). This fact suggests that a common mechanism may control fruit maturation in different *Prunu*s species. In peach this locus has been fine mapped using a progeny where it was segregating as a codominant trait (Eduardo et al. 2011; Pirona et al. 2013).

The juvenile reproductive phase refers to the period when young plants are unable to respond to inductive environmental signals to induce flowering (Basheer, 2007), or to produce fruits as it has been interpreted in this study. The transition to maturity stage is influenced by environmental as well as genetic factors (Hackett, 1985). We scored this character as the number of years required by each plant of T1E to produce fruit for the first time, with values that ranged from three to six and identified a QTL (qP-Juv1) that explained of 13.3% of the phenotypic variance and with the almond allele increasing the juvenility period. No other results on genetic analysis of this character are to our knowledge available for *Prunus*, but in citrus Raga et al. (2012) detected four QTLs related to juvenility that jointly explained 39.2% of the phenotypic variance. Knowledge of the inheritance for this character may be helpful to breed lines with short intergeneration periods that would speed up the breeding process.

4.4.3 Fruit traits

Several studies have been carried out to decipher fruit quality genetics in peach (de Souza et al. 1998; Dirlewanger et al. 1999; Yamamoto et al. 2001; Etienne et al. 2002; Quilot et al. 2004; Eduardo et al. 2011; Eduardo et al. 2013; Martínez-García et al. 2013) and in other *Prunus* species (Wang et al. 2000; Zhang et al. 2010; Rosyara et al. 2013; Salazar et al. 2013), but few of them have used interspecific populations (Quarta et al. 2000; Quilot et al. 2004). In this study we have identified several loci involved in important fruit quality traits in two interspecific progenies between almond and peach.

The fruit type trait has been mapped as a morphological marker in a 2 cM region of the TxE map, the same region were the MD and the slow ripening locus have been recently located

(Eduardo et al. 2011; Pirona et al. 2013; Eduardo et al. 2014 in preparation). These authors proposed a NAC-like transcription factor gene (ppa008301) as a candidate gene for both traits. It could be that when almond alleles from this locus are in homozygosis, trees do not have the ability to start the ripening process, and if the almond allele is in heterozygosis the effect is the delay of the maturity date. Some authors suggested that a dry, splitting mesocarp is likely to be ancestral for the common ancestor of subg. *Amygdalus*, and the fleshy, non-splitting mesocarp found in peaches was probably developed in China (Yazbek et al. 2013) from wild peach species such as *P. davidiana* (Carrière) Franch., *P. kansuensis* Rehder, and *P. mira* Koehne, that are still cultivated locally in northern and northwestern regions from China (Wang 1985). Our results suggest that another scenario could be possible and that the common ancestor presented a fleshy mesocarp and almond lost the ability to ripen by a loss of function the NAC-like gene ppa008301. Therefore, the *Fruit type* locus described here could have been important during the domestication process of the actual fleshy peaches and during the speciation process between almond and peach. Sequencing the homologue of ppa008301 in almond could shed light into this matter.

Blood flesh color has been mapped in T1E as a morphological trait (*Bf2*) at the end on G1. However, in the TxE population this same trait detected only a major QTL on G3 (R2=50.7), in the same genomic region were the anthocyanic anther color (*Ag*) has been located. Previously, two loci implicated in this trait have been described, the blood-flesh phenotype determined by a single recessive locus (*Bf*) located in G4 (Gillen and Bliss, 2005) present in 'Harrow Blood', a cultivar showing blood-flesh in both immature and mature fruits, and the dominant blood flesh phenotype coming from the 'Wu Yue Xian' cultivar that has been recently mapped by Shen et al. (2013) at the top of G5 and that is controlled by a single dominant locus (*DBF*). Therefore, these loci are not allelic to *Bf2*.

One major QTL was detected for the intensity of skin color on G1 in the same region where one of the anther color genes was located (*Ag2*). For the percentage of skin color two major QTLs were identified: one at the end of G1, in the same region of *Bf2*, and another on G4, in the region of the *Ft* locus. Considering that TxE and T1E have been developed from the same parents, this scenario reflects a complex genetic regulation of anthocyanic traits. A possible explanation for this phenomenon is the relationship that could exist between the traits related to the anthocyanin pigments in different parts of the plant. For example, the three loci involved with the anther color (*Ag* and *Ag2*) and the blood flesh (*Bf2*) loci could have a pleiotropic effect over other traits differentially expressed in both populations as the intensity

or percentage of red skin color. Another possible factor involved, is the ripening process that could also modulate the ability to enable the expression of the percentage of external red color. Frett et al. (2014) found a major and three minor QTLs for red blush in peach. The major QTL is located in the same region of the QTL detected in TxE in G3. In T1E, two minor QTL were detected on G4, one of which in the same region where the locus Flower color 2 (*Fc2*) has been mapped.

QTLs cluster for Fruit weight and Fruit dimensions were detected on G6 and on G7 in T1E and on G4 in TxE. The QTL observed on G4 in TxE presented a dominant effect from peach and for this reason was not possible to identify it in the T1E population. Furthermore as we have explained previously the cluster of QTLs located in G4 of TxE is probably a pleiotropic effect of the Fruit type trait. In T1E, the cluster of QTLs from G6 includes QTLs for Fruit weight and Stone weight and dimensions, and in G7 for Flesh weight and Fruit cheek Diameter, suggesting that Fruit weight can be dissected in Flesh and Stone weights and that both are controlled by different loci. Several QTLs have been described for FW in *Prunus* species, in G6 (Dirlewanger et al. 1999) and in G4 and G6 (Yamamoto et al. 2001; Eduardo et al. 2011), in F_2 intraspecific populations in peach, and in G4 in a BC_2 inter-specific population between *P davidiana* x *P. persica* (Quilot et al. 2004). The locus from G4 has been detected in all the populations except in JxF, where the fruit shape trait is segregating and could be masking the effects of the G4 locus. We must keep in mind that most populations where this locus has been associated with FW are interespecific populations. Except in Yamamoto et al. (2001) that used two peach parentals but one is an ornamental cultivar and has been selected for flower traits and not for fruit size, and in CxA (Eduardo et al. 2011) where it was associated to a pleiotropic effect of the MD, being bigger the fruits with longer FDP. The locus from G6 is associated with the locus fruit shape (*S*) in the FJ (flat) x F (round). In CxA, where *S* is not segregating the QTL for fruit weight was also detected. This could indicate that the locus controlling fruit shape is also controlling the fruit weight in populations where *S* is not segregating. Looking at the position where *S* has been mapped (Dirlewanger et al. 2006; Picañol et al. 2013) and to our cluster of QTLs it is not clear if it is the same locus or not. These results suggest that these loci from G4 and G6 would have a broad effect in the peach germplasm during domestication.

For TA QTLs were detected in two out the three years of evaluation on G6 in both populations. Although these QTLs were not consistent all years is important to note the coincidence with other studies that presented QTLs in this group (Dirlewanger et al. 1999; Quilot et al. 2004).

4.4.4 Leaf traits

The location of the different QTLs identified for most of the leaf traits suggest that there are at least two loci controlling most of the phenotypic variance and that are located in G1 and G8. Another important locus also was identified on G6 for Leaf blade width and leaf weight. Almost no data has been published for leaf traits neither in *Prunus* nor in other species (Chitwood et al. 2013). As far as we know the only reported result for leaf dimensions in peach is the *Nl* locus, a major gene for leaf shape (narrow / wide) identified by Yamamoto et al. (2001). This locus was located in a genomic region coincident with the QTLs located in our study on G6, suggesting that both loci could be allelic. Another leaf trait previously analyzed is the chlorophyll content. In *Prunus* this trait has been studied in assessments associated with the problematic of iron chlorosis in a population with a complex genetic background of *Prunus cerasifera*, *P. dulcis* and *P. persica*, where two QTLs (G4 and G6) were identified for this trait (Gonzalo et al. 2012). While our results are consistent in identifying QTLs in G6, the location of these suggests they are not allelic. To interpret the results of leaf traits we must keep in mind that are traits difficult to phenotype because although we tried to take representative leaves, in each tree there is a wide variance of leaves in different stages of development. Although this complexity, in this study we set out to discover some loci that contribute to the phenotypic differences in leaf morphology between peach and almond.

4.4.5 Resistance to powdery mildew

Powdery mildew control in peach requires several fungicide applications per year with the associated negative ecological and economical consequences. Most commercial peach cultivars are susceptible to the disease, and although some sources of tolerance have been found in peach (Pacheco et al. 2009) or close species as *P. davidiana* (Foulogne et al. 2003b) or *P. ferganensis* (Dabov, 1983; Verde et al. 2002) mapping in G6, G7 and G8, only a source of complete resistance has been described recently in the peach rootstock cultivar Pamirskij 5 (Pascal et al. 2010) and mapped as a single dominant gene in G6 (Pascal et al. 2010). Therefore, the major gene *Vr3* described in this study is a new source of full resistance to powdery mildew in peach. The availability of different sources of resistance and molecular markers linked to them allow pyramidizing them to obtain cultivars with more durable resistance.

4.4.6 Implications for breeding and conclusions

Despite the low genetic diversity of occidental peach commercial cultivars (Scorza et al. 1985), phenotypic variability has been observed for several fruit traits as flesh color (white/yellow/blood), flesh adhesion (cling/freestone), fruit shape (flat/round), acidity (acid/sub-acid) and skin hairiness (nectarine/peach) between others. Some of these traits have been mapped (Arús et al. 2012; Salazar et al. 2014) and are currently being used in breeding programs for marker assisted selection (Eduardo et al. 2013). However, peach variability could be further enriched using oriental germplasm (Li et al. 2013) or using close species as almond as donors. This last approach has been mainly used for rootstock development (Byrne et al. 2012) or for introgression of resistance (Foulogne et al. 2003a), but introgression for improving fruit quality traits has not been reported. Some reasons that can explain why introgression in peach is not a common strategy are the long intergenerational period and genetic linkage drag of undesired traits. In this work we have identified interesting alleles from almond that could be introgressed in peach, and although it is a long process, a possible strategy could be generating pre-breeding collections of introgresión lines as the one that is being develop using the plant material described here (Picañol et al. 2007). This strategy could allow to introgress interesting traits in peach in a more efficient way. Some of these interesting alleles could be the resistance to powdery mildew (*Vr3*), the blood flesh (*Bf2*) locus and the major QTLs for skin color in G1 and G4. Another trait that could be further explored is the fruit type. Heterozygous individuals for this character behaved as overdominant, producing fruits with higher weight than those of either parent. Finally, given that at least one of the stone and flesh QTLs are different it could also be interesting to investigate if certain allele combinations of these two loci could allow produce commercial cultivars with a higher relationship flesh/stone.

5. MARKER-ASSISTED INTROGRESSION OF ALMOND GENES INTO THE PEACH BACKGROUND: A FIRST STEP TOWARDS THE CONSTRUCTION OF A NIL COLLECTION OF ALMOND CHROMOSOME FRAGMENTS INTO PEACH.

5.1 INTRODUCTION

Peach is a species with a low level of variability compared to other crops of the same genus such as almond, apricot, plum or cherry (Byrne 1990; Mnejja et al. 2010). Considering only the peach gene pool, occidental commercial germplasm has a lower level of variability than the oriental materials, as estimated by Li al. (2013). This is a consequence of the absence in peach of a functional self-incompatibility system, unlike most other *Prunus*, and of important bottlenecks in its recent history since domestication 6.000 years ago in China (Faust and Timon 1995). Low levels of variability are a limit to the progress towards some of the main challenges of current breeding programs that include longer shelf life, improved fruit quality, a higher level of disease resistance and adaptation to climate change.

One way to increase genetic variation is by using the vast reservoir of novel alleles found in landraces, cultivated relatives, and wild germplasm (Tanksley and McCouch 1997). New sources of variability for peach varieties grown in the western countries may arise from other sources known to be more variable such as local European cultivars (Aranzana et al. 2010), Chinese accessions (Li et al. 2013) or other species of the genus that are compatible to peach, such as other cultivated *Prunus* such as almond (*P. dulcis*) and Japanese plum (*P. salicina*) or the wild relatives closer to peach, such as *P. mira*, *P. kansuensis*, *P. davidiana*, *P. ferganensis*, and *P. cerasifera*. Crosses between these species are possible and some of the interspecific hybrids have been developed and are currently used as rootstocks for peach (Martínez-Gómez and Gradziel 2002; Gradziel 2003; Moreno 2004; Zarrouk et al. 2005; Felipe 2009, Pinochet 2009). Some of the first molecular marker maps of peach were developed using interspecific populations between peach and almond (Foolad et al. 1995; Joobeur et al. 1998) and peach crosses with *P. davidiana* (Foulongne et al. 2002). Given the low level of variability of peach, these populations were a guarantee of a high marker polymorphism that facilitated the development of highly populated maps with coverage of the entire genome. One of these maps, an F_2 between 'Texas' almond and 'Earlygold' peach was accepted by the scientific community as the reference map for the genus and was taken as the basis for linkage group assignment and direction and provided data on the position of a large number of transferable markers to be used for the construction of maps in other populations (Dirlewanger et al. 2004).

Genes from compatible species have been a major source of useful variability in the cultivated tomato, a species that shares with peach a low level of variability and is intercompatible with many other wild relatives. These genes include mainly disease resistances (Zamir et al. 1994; Griffiths and Scott 2001; Bai et al. 2003; Verlaan et al. 2013), but also characters important to enhance favorable traits such as soluble solids content, yield, early fruit ripening, color, and viscosity (Eshed and Zamir 1995; Tanksley and Nelson 1996; Fulton et al. 2000; Frary et al. 2002). Another example is apple (*Malus* x *domestica*) where the resistance to apple scab (*Venturia inaequalis*) has been introgressed from *M. floribunda* (Crandall 1926; Crosby et al. 1992; Gessler et al 2006) and *M. sieversii* (Bus et al. 2005) into susceptible commercial apple materials. In peach, while several nematode resistance genes have been identified and characterized coming from *P. cerasifera*, almond and other peach cultivars and are being introduced into rootstocks (Fernández et al. 1994; Felipe et al. 1997, Gomez-Aparisi et al. 2001), there is only one recent example of a resistance to powdery mildew that has been introgressed into peach scions coming from *P. davidiana* (Foulongne et al. 2003a).

The study of quantitative characters using their co-segregation with markers from a linkage map using markers of linkage maps of traditional populations presents various drawbacks. If the number of individuals is low, typically below several hundred only one part of the QTLs can be identified (Tanksley 1993), their position on the maps is estimated with low precision (Lebreton *et al.* 1998) and their effects tend to be overestimated (Melchinger *et al.* 1998). In all, this results in a partial or biased understanding of the genetic determinism of the character under study. Eshed and Zamir (1994) proposed the construction of near-isogenic lines (NILs) as a way to increase the efficiency of the genetic analysis of any character, particularly if polygenic, and to detect new major genes and QTLs of interest provided by exotic or wild genetic sources (Tanksley and Futon 2007). A NIL collection is composed by a low number of individuals (usually 40-60), where each presents a unique introgression of the donor parent (a wild or exotic line) on the background of the recurrent parent (an elite line of the parent with economic interest), so that the sum of the fragments of the each individual of the collection cover all the donor genome. Differences between the phenotypes of a given NIL and the recurrent line are produced by genes that occur in the introgressed fragment. Since the NILs and the recurrent parent can be replicated, phenotyping is done very accurately and the effects of each introgressed fragment can be estimated in a homogeneous genotypic background. This allows identifying and characterizing more QTLs and a more precise mapping of these (Eshed and Zamir, 1994, Eduardo et al. 2007, Fernández-Silva et al. 2010). NIL

collections are also adequate to study the QTL x QTL (Grandillo et al. 2007; Fernandez-Silva *et al* 2010), QTL x environment (Bernachi *et al.* 1998, Frary *et al*. 2000, Eduardo *et al.* 2005) and QTL x genotype (Monforte *et al*. 2001, Swamy and Sarla 2008, Fernandez *et al*. 2009) interactions.

Our long term objective is to create a NIL collection of almond chromosome fragments of 'Texas' into the background of 'Earlygold' peach. This will allow us a twofold objective: a) to develop a resource for genetic analysis in peach, and b) to evaluate the capability of almond to supply genes of interest for peach and to produce individuals with single fragments of almond containing genes of interest for peach that are in the commercial peach background and thus can be readily incorporated into commercial breeding programmes. So far NIL populations have been created for annual species such as tomato (Eshed and Zamir 1995; Monforte *et al*. 2000), wheat (Pestsova et al. 2001), lettuce (Jeuken *et al*. 2004), rice (Sobriza *et al*. 2004), melon (Eduardo *et al* 2005), diploid strawberry (Bonet 2010) or the model plant Arabidopsis (Koumproglou et al. 2002; Keurentjes et al. 2007; Torjek et al. 2008; Fletcher et al. 2013) where the number of generations is not a limiting factor in their progress. Nevertheless, this is a critical question in peach due to its long intergeneration period (3-4 years). In this paper we provide our results on the creation of a large backcross one population ('Texas' x 'Earlygold') x 'Earlygold' (T1E) and the selection from it of individuals with a low number of introgressions as the primary step towards the controlled introgression of almond chromosomal fragments. With the help of markers as a selection tool we propose a strategy that would allow the production of commercial lines with one introgression in only two or three generations, allowing for the incorporation of interesting almond alleles at the commercial peach breeding operation.

5.2 MATERIALS AND METHODS

5.2.1 Plant materials and crosses

We used the hybrid plant 'MB1.37' coming from the cross between 'Texas' almond as female parent and 'Earlygold' peach as pollen donor using the latter as recurrent parent to obtain a backcross one generation (T1E). Crosses were performed during the spring of 2006, 2007 and 2008. Due to the high number of crosses performed, pollen from 'Earlygold' was kept in Parafilm sealed Petri dishes at -20ºC, and used for the pollination of the following year. To

check for pollen viability we used the method described by Asma (2008) with an additional 15% of sucrose.

5.2.2 NIL development strategy

Given the long intergeneration period of *Prunus*, we chose a strategy that minimized the number of generations. For that we produced a large BC_1 progeny from which we selected as many lines as possible with three or less introgressions. These lines would require only one additional selfing generation (BC_1S_1) to obtain homozygous NILs with sufficient frequency. The number of offspring required to obtain homozygous NILs would be 46 and 190 seedlings in lines with two or three introgressions, respectively (calculated as n= log α /log (1-p), where n= number of individuals, α = 0.05 and p= probability of finding a specific homozygous NIL, which is $(¼)^k$, with k=number of introgressions of the parental line). Individuals with more introgressions would require an additional backcross generation to have heterozygous lines with three or less introgressions that could be selfed and used to extract homozygous NILs. Selecting in the first backcross generation has the additional advantage that the introgressed fragments are large, reducing the probability of undetected introgressions with the marker density used for this analysis.

5.2.3 Genotyping

Genomic DNA was extracted from young leaves using the CTAB method (Doyle and Doyle, 1990) omitting the final RNAse treatment step. Plants of T1E were selected using a three-step procedure with the objective of reducing the cost of the operation. In the first step all plants were analyzed with a set of eight SSRs (set 1; Table 8) located each at one of the extremes of the eight *Prunus* linkage groups selected from the TxE map of Dirlewanger et al. (2004). Only plants having three or less of these markers heterozygous for the almond allele were selected. In the second step, the selected plants were then genotyped for a second SSR set (set 2; Table 8) with eight more SSRs located at the opposite extreme of the linkage group than those of set 1. Only the plants with three or less heterozygous loci for sets 1 and 2 were selected. Finally, the selected plants were genotyped for 89 additional SSRs with coverage of the complete genome. The eight markers of set 1 were selected to be multiplexed at an annealing temperature (Ta) of 57ºC. Marker assisted selection (MAS) in the step two had to be multiplexed in two groups of four markers, one (2a) at Ta=55ºC and the other (2b) at Ta=50ºC (Table 8).

Table 8. The two sets of SSR markers used for the selection of the T1E lines with low numbers of introgressions. Sets with the same reference (1,2a and 2b) were multiplexed

Linkage group	Set	Marker	Annealing Temp. (^{o}C)	Origin
G1	1	BPPCT028	57	Dirlewanger et al. (2002)
G2	1	CPPCT044	57	Aranzana et al. (2002)
G3	1	BPPCT007	57	Dirlewanger et al. (2002)
G4	1	EPDCU5060	57	Howad et al. (2005)
G5	1	CPPCT040	57	Aranzana et al. (2002)
G6	1	CPPCT021	57	Aranzana et al. (2002)
G7	1	CPSCT004	57	Mnejja et al. (2005)
G8	1	EPPCU3117	57	Howad et al. (2005)
G1	2a	UDP96-018	55	Testolin et al. (2000)
G2	2b	UDA-023	50	Messina et al. (2004)
G3	2a	EPDCU0532	55	Howad et al. (2005)
G4	2a	CPPCT051	55	Aranzana et al. (2002)
G5	2a	BPPCT014	55	Dirlewanger et al. (2002)
G6	2b	Ps7a2	50	Joobeur et al. (2000)
G7	2b	Ps5c3	50	Cantini et al. (2001)
G8	2b	CPSCT018	50	Mnejja et al. (2005)

All markers of the two first steps were analyzed in an ABI Prism® 3130*xl* (Applied Biosystems) capillary sequencer. The PCR amplification conditions for the multiplex of eight SSRs were: 1.55 µl of HPLC water (Panreac), 1µl of Lab1X buffer (Lab 10X: KCl 50mM, Tris-HCl 10mM pH 8.3, gelatine 0.0001%), 0.6µl $MgCl_2$ 25mM (Applied Biosystems), 1µl of 2mM dNTP mix (Applied Biosystems), 0.25µl of each forward primer labeled with fluorochrome 8µM (Applied Biosystems), 0.2µl of each reverse primer 10µM (Applied Biosystems or Operon), 0.3µl of Taq polymerase (Applied Biosystems).

The individuals selected that had three or less introgressions detected with the 16 SSRs tested in the two before steps were genotyped with 93 additional SSRs. The SSRs used were the same studied in T1E population (Chapter 3) with some exceptions: 14 markers (CPP8026, MA024a, UDP98-025, M1a, CPPCT005, UDA-027, Ps12e2, BPPCT026, BPPCT038, CPP21413, CPP21245, CPP20836, UDP98-408, BPPCT006) were excluded and 10 (CPPCT027, PceGA59, AMPA93,

BPPCT024, BPPCT023, CPPTC051, AMPA112, Pacita021, EPPCU9300, UDAp-423) were added, resulting in similar genome coverage. In six markers (AMPA93, M14a, CPPCT017, CPPCT057, CPPCT039 and M6a) was not possible interpret all genotypic information due to presented preferential amplification in favour of peach genome therefore when the genotype was homozygous for allele of 'Earlygold' we considered as missing data.

5.2.4 The preNIL set: phenotyping and genetic analysis

At the end of the selection process we chose a set of lines that had a low number of introgressions and contained the whole almond genome so it was possible to use them as parents towards the extraction of a complete set of NILs. This set of selected lines, to which we will refer as preNILs, were phenotyped as described in Chapter 4. Our objective was to perform a proof of concept for the genetic analysis of major genes using only this set of preNILs and show that at this stage it is possible to determine their map positions. For that we studied all major genes segregating in T1E, implying that they were additive or dominant for the almond allele. We assayed also two major QTLs (i.e., those reproducible across years and with an explained proportion of the variability higher than 20% each year) that were detected in the T1E progeny and to check to what extent genetic analysis in the preNIL set was also possible with certain QTLs.

For their genetic analysis, the lines of the preNIL set were divided into the two phenotypic classes and we compared their graphical genotypes. Chromosomal regions having one genotype in all individuals of one phenotypic class (the homozygote for the peach allele) and the alternative (the heterozygote almond/peach) in the other were identified as those that contained the gene under study.

5.3 RESULTS

5.3.1 Selection process in the backcross one generation

During years 2006 to 2008 we performed approximately 8,500 controlled pollinations that yielded 1,720 seeds. These seeds were stratified and produced a total of 1,080 seedlings, to which we added 15 BC_1 plants obtained previously, with a total number of 1,095 analyzed individuals. The first step of the process of marker-assisted selection (MAS) with 8 SSRs allowed identifying 213 (19.5%) plants that did not belong to the BC_1 population: they originated from self-pollination (14.2%) or from cross-pollination (5.3%) with other neighboring trees, which gave a final number of 882 BC_1 plants. For this first set of markers, 3,899 (55.2%) were heterozygous with one allele of almond and another of peach, which we interpreted as introgressions (Table 9). In all, G2 had the lowest percentage of heterozygous individuals (48.7%) and G8 had the highest (66.3%). After marker examination, 226 plants (26% of the initial 882) with 3 or less detected introgressions were carried to the next stage of selection.

Table 9. Percentage of individuals having at least one introgression in each chromosome and on average in the different stages of selection in the BC1 generation of 'Texas' almond x 'Earlygold' peach.

Selection stage	Nº of plants[1]	Linkage group								
		G1	G2	G3	G4	G5	G6	G7	G8	Average
Stage 1	882	57.2	48.7	54.4	58.5	54.2	50.4	52.6	66.3	55.2
(8 SSRs)	226	37.4	25.2	28.5	37.5	28.5	33.8	28.6	44.5	33.0
Stage 2	226	45.3	38.0	37.7	46.4	41.8	39.7	42.3	50.3	42.6
(16 SSRs)	22	45.4	31.8	18.1	63.6	22.7	31.8	40.9	40.9	36.3
Stage 3	22	54.5	45.5	27.3	72.7	31.8	36.4	45.5	45.5	45.5
(109 SSRs)	9	66.7	11.1	11.1	77.8	33.3	11.1	33.3	44.4	33.3

[1]On the top line, the initial number of plants, the bottom line shows the number of plants selected at the end of each stage to obtain plants with three or less introgressions.

Eight additional SSRs were genotyped in these 226 plants where we found 1,084 heterozygous data, 59% of the total analyzed. Considering the 16 markers together and assuming that individuals with both markers in heterozygosis for the almond allele in a particular linkage group had a single introgression, the proportion of individuals with at least one introgression ranged from 50.3% in G8 to 37.7% in G3 (Table 9). The plants selected to have three or less

introgressions at this stage were 22 (9.7% of the 226, or 2.5% of the 882), one with two introgressions and the other 21 with three.

In a third step, 93 additional SSRs were analyzed in the 22 selected plants that allowed the identification of undetected introgressions and genotyping errors in the previous steps leading to the final selection of 9 plants with three or less introgressions (8 plants with three and one plant with two). Three of these plants had very low vigor and were discarded for further use. As a consequence, we had to change our original plan of having a set of preNILs with three or less introgressions and completed these six plants with 12 individuals with four introgressions to obtain a final set of 18 plants: one with two introgressions (preNIL 67), five with three introgressions (preNILs 87, 147, 306, 630, 695) and 12 with four introgressions (preNILs: 3, 27, 31, 79, 110, 193, 333, 412, 498, 505, 694, 769). This group of plants (Table 10) covers at least threefold the almond genome in all linkage groups except of G5 where coverage is more than twice (Figure 14).

Table 10. Genotypes of the set of selected preNILs. To facilitate the overall interpretation the background has been highlighted with orange for heterozygous peach/almond genotypes (H), and with yellow for peach homozygous (B) genotypes.

Marker (SSR)	cM[2]	preNILs collections																	
		3	27	31	67	79	87	110	147	193	306	333	412	498	505	630	694	695	769
G1																			
EPDCU3122	0.8	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
UDP96-018	1.4	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
CPPCT004	7.9	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
EPPCU5331	12.2	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
PACITA005	12.2	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
EPPCU1090	19.1	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
UDP96-005	19.1	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
CPPCT003	21.2	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
CPPCT026	32.1	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
EPDCU3489	35.3	B	B	H	H	B	H	B	B	B	B	B	B	B	B	B	B	H	B
BPPCT016	43.6	B	B	H	H	B	H	B	B	B	B	H	H	B	B	B	H	H	B
CPPCT019	46.7	B	B	H	H	B	H	B	B	B	B	H	H	B	B	B	H	H	B
CPPCT042	47.7	B	B	H	H	B	H	B	B	B	B	H	H	B	B	B	H	H	B
EPDCU2862	47.7	B	B	H	H	B	H	B	B	B	B	H	H	B	B	B	H	H	B
CPPCT053	48.7	B	B	H	H	B	H	B	B	B	B	H	H	B	B	B	H	H	B
CPPCT029	49.2	B	B	H	H	B	H	B	B	B	B	H	H	B	B	B	H	H	B
BPPCT028	51.2	B	B	H	H	B	H	B	B	B	B	H	H	B	B	B	H	H	B
G2																			
CPPCT044	0.0	H	H	H	B	B	B	B	B	B	B	H	H	B	B	B	B	B	H
CPP8062	0.0	H	H	H	B	B	B	B	B	B	B	H	H	B	B	B	B	B	H
AMPA93	1.4	H	H	-	B	-	B	-	B	B	-	H	-	B	B	-	B	-	B
MA024a	5.4	H	H	H	B	B	B	B	B	B	B	H	H	B	B	B	B	B	B
UDP98-025	6.5	H	H	H	B	B	B	B	B	B	B	H	H	B	B	B	B	B	B
CPDCT044	7.5	H	H	H	B	B	B	B	B	B	B	H	H	B	B	B	B	B	B
BPPCT004	10.1	H	H	H	B	B	B	B	B	B	B	H	H	B	B	B	B	B	B
EPDCU4017	12.2	H	H	H	B	B	B	B	B	B	B	H	H	H	B	B	B	B	B
BPPCT001	12.7	H	H	H	B	B	B	B	B	B	B	H	H	H	B	B	B	B	B
CPSCT044	17.5	H	H	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
M1a	19.6	H	H	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
UDP96-013	21.6	H	H	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
CPDCT004	25.2	H	H	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
UDP98-411	26.3	H	H	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
pchgms1	28.6	H	B	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
BPPCT030	32.2	H	B	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
CPPCT043	32.2	H	B	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
CPSCT021	38.1	B	B	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
PceGA34	45.1	B	B	H	B	B	B	B	B	B	B	H	H	H	B	B	H	-	B
CPSCT034	48.8	B	B	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
UDA-023	49.9	B	B	H	B	B	B	B	B	B	B	H	H	H	B	B	H	B	B
G3																			
EPPCU5990	0.0	B	H	B	B	H	B	B	B	B	B	B	B	B	B	H	B	B	B
EPPCU4610	0.0	B	H	B	B	H	B	B	B	B	B	B	B	B	B	H	B	B	B
UDP97-403	6.5	B	H	B	B	H	B	B	B	B	B	B	B	B	B	H	B	B	B
BPPCT007	6.5	B	H	B	B	H	B	B	B	B	B	B	B	B	B	H	B	B	B
BPPCT039	15.5	B	H	B	B	H	B	H	B	B	B	B	B	B	B	H	B	B	B
EPDCU3083	16.6	B	H	B	B	H	B	H	B	B	B	B	B	B	B	H	B	B	B
CPPCT002	28.2	B	H	B	B	H	B	H	B	B	B	B	B	B	B	H	B	B	B
UDP96-008	30.6	B	H	B	B	H	B	H	B	B	B	B	B	B	B	H	B	B	B
EPDCU0532	42.6	B	H	B	B	H	B	H	B	B	B	H	B	B	B	H	B	B	B

[1]"-" no data; these genotypes occurred in SSRs where the peach alleles were dominant and heterozygous for the 'Earlygold' parent resulting in segregations with two of the four classes that were indistinguishable

[2]The distances of the genetic map correspond at the map described in Chapter 1 in cM.

Table 3 (continued)

G4																			
BPPCT010	0.0	B	H	H	B	H	H	B	B	B	B	B	H	H	B	H	B	H	B
EPDCU5060	1.1	B	H	H	B	H	H	B	B	B	B	B	H	H	B	H	B	H	B
pchgms2	7.9	H	H	H	B	H	H	H	B	B	B	B	H	H	B	H	B	H	B
CPPCT011	14.6	H	H	H	B	H	H	H	H	H	B	B	H	H	B	H	B	H	B
CPPCT005	14.6	H	H	H	B	H	H	H	H	H	B	B	H	H	B	H	B	H	B
CPDCT045	24.7	H	H	H	H	H	H	H	H	H	B	H	H	H	B	H	B	H	H
UDP96-003	29.5	H	H	H	H	H	H	H	H	H	B	H	H	H	B	H	B	H	H
M12a	31.6	H	H	H	H	H	H	H	H	H	B	H	H	H	B	H	B	H	H
EPPCU2000	37.0	H	H	H	H	H	B	H	H	H	B	H	H	H	B	H	B	H	H
BPPCT015	37.0	-	-	H	H	H	-	-	H	-	B	H	-	H	B	-	B	H	-
UDA-021	40.6	H	B	H	H	B	B	H	H	H	B	H	H	H	B	H	B	H	H
CPPCT046	40.6	H	B	H	H	B	B	H	H	H	B	H	H	H	B	H	B	H	H
UDA-027	44.3	H	B	H	H	B	B	H	H	H	B	H	H	H	B	H	B	B	H
Ps12e2	44.9	H	B	H	H	B	B	H	H	H	B	H	H	H	B	H	B	B	H
G5																			
CPPCT040	0.0	B	B	H	B	B	B	B	B	B	H	B	B	H	H	B	H	H	B
BPPCT026	4.0	B	B	H	B	B	B	B	B	B	H	B	B	-	H	B	H	H	B
UDP97-401	5.4	B	B	H	B	B	B	B	B	B	H	B	B	H	H	B	H	H	B
BPPCT017	15.3	B	B	H	B	B	B	B	B	H	H	B	B	B	H	B	H	B	B
CPSCT006	15.3	B	B	H	B	B	B	B	B	H	H	B	B	B	H	B	H	B	B
BPPCT037	17.9	B	B	H	B	B	B	B	B	H	H	B	B	B	H	B	H	B	B
pchgms4	19.5	B	B	H	B	B	B	B	B	H	H	B	B	B	H	B	H	B	B
CPPCT013	21.2	B	B	H	B	B	B	B	B	H	H	B	B	B	H	B	H	B	B
EPDCU5183	26.9	B	B	H	B	B	B	B	B	H	H	B	B	B	H	B	B	B	B
EPDCU4658	28.4	B	B	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	B
BPPCT038	29.4	B	B	B	B	B	B	B	B	H	B	B	B	B	H	B	B	B	B
CPSCT022	36.9	B	B	B	B	B	B	B	B	H	B	B	B	B	H	B	B	B	B
BPPCT014	38.1	B	B	B	B	B	B	B	B	H	B	B	B	B	H	B	B	B	B
G6																			
CPP21413	0.0	H	B	B	B	H	B	B	B	B	B	B	H	B	B	B	B	B	H
Ps7a2	1.1	H	B	B	B	H	B	B	B	B	B	B	H	B	B	B	B	B	H
CPP21245	1.1	H	B	B	B	H	B	B	B	B	B	B	H	B	B	B	B	B	H
CPP20836	1.1	H	B	B	B	H	B	B	B	B	B	B	H	B	B	B	B	B	H
UDP96-001	10.6	H	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	H
BPPCT008	16.0	H	H	B	B	H	B	B	B	B	H	B	B	B	H	B	B	B	H
CPSCT012	17.0	H	H	B	B	H	B	B	B	H	H	B	B	B	H	B	B	B	H
pchcms5	20.7	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
BPPCT025	24.1	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
CPPCT047	27.6	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
UDP98-412	34.4	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
MA040a	34.4	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
AMPA130	34.9	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
MA14a	34.9	-	H	-	-	B	-	-	B	H	-	B	B	B	H	-	B	B	-
EPPCU4092	37.0	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
CPPCT030	43.0	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
CPPCT021	43.0	H	H	B	B	B	B	B	B	H	H	B	B	B	H	B	B	B	H
G7																			
CPSCT004	5.0	H	B	B	B	B	B	H	B	B	B	B	B	B	H	B	B	B	B
CPPCT039	6.7	-	-	-	B	-	B	-	-	-	B	-	-	-	H	B	-	B	-
pchgms6	11.4	H	B	B	B	B	B	H	-	B	B	B	B	B	H	B	B	B	B
UDP98-408	12.9	H	B	B	B	B	B	H	B	B	B	B	B	B	H	B	B	B	B
CPPCT057	18.0	-	-	B	-	B	B	-	-	B	B	-	-	B	-	-	-	-	B
CPPCT033	24.1	H	B	B	B	B	B	B	H	B	B	B	B	B	H	B	B	B	B
MA20a	25.8	H	B	B	B	B	B	B	H	B	B	B	B	B	H	B	B	B	B
PMS02	27.9	H	B	B	B	B	B	B	H	B	B	B	B	B	H	H	B	B	B
EPPCU5176	30.6	H	B	B	B	B	B	B	H	B	B	B	B	B	H	H	B	B	B
pchcms2	31.1	H	B	B	B	B	B	B	H	B	B	B	B	B	H	H	B	B	B
CPPCT017	39.1	-	-	B	-	B	B	-	-	H	B	-	-	B	-	-	-	-	B
EPDCU3392	41.9	H	B	B	B	B	B	B	H	H	B	B	B	B	B	H	B	B	B
Ps5c3	45.3	H	B	B	B	B	B	B	H	H	B	B	B	B	B	H	B	B	B
G8																			
CPSCT018	0.0	B	B	B	B	H	B	H	H	B	B	H	B	H	B	B	H	B	H
BPPCT006	7.8	B	B	B	B	H	B	B	H	B	B	H	B	H	H	B	H	B	B
CPPCT058	7.8	B	B	B	B	H	B	B	H	B	B	H	B	H	H	B	H	B	B
CPDCT034	7.8	-	-	B	B	-	B	-	-	B	B	-	B	H	-	B	-	B	B
CPPCT006	13.7	B	B	B	B	H	B	B	H	B	B	B	B	H	H	B	H	B	B
M6a	21.9	-	B	B	-	H	B	-	-	-	-	-	-	H	H	-	-	B	-
UDP98-409	34.7	B	B	B	B	B	B	B	H	B	H	B	B	H	H	B	H	B	B
EPDCU3454	38.4	B	B	B	B	B	H	B	H	B	H	B	B	H	H	B	H	B	B
EPDCU3117	43.3	B	B	B	B	B	H	B	H	B	H	B	B	H	H	B	H	B	B

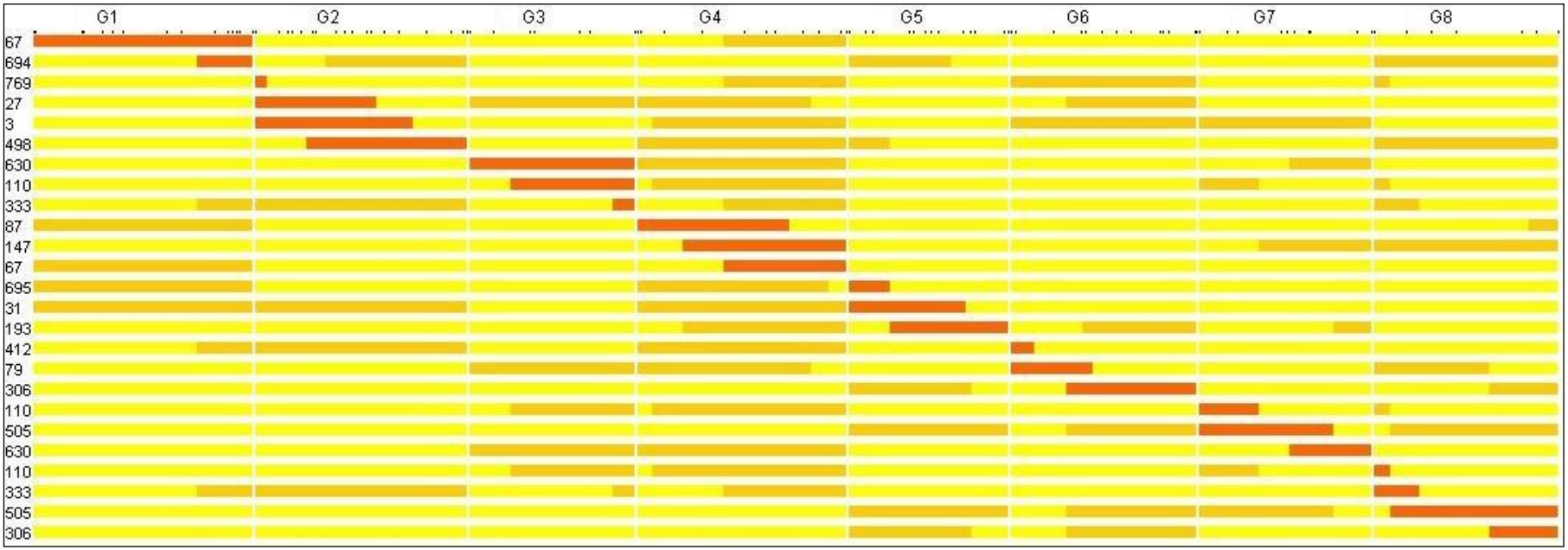


Figure 14. Graphical genotypes of 18 backcross 1 lines that cover the whole peach genome with almond introgressions. Horizontal bars represent the eight chromosomes of *Prunus*. The chromosomes are segmented by each SSR used in this study. The genome of peach is represented by yellow color while the almond genome is noted by orange-red color. The red color highlights the introgressions that have allowed covering the whole genome with almond introgressions.

In this set of plants were found 66 introgressed fragments from almond in the peach genetic background (Table 11): a third of them (22 introgressions) correspond to the entire chromosome (at least one case per chromosome), and the remaining two thirds (44 introgressions) exhibited a single recombination event. In no case more than one recombination event occurred in a given chromosome. The distribution of the different fragments varied from 5 in G3 till 15 in G4, although most of them showed 6 to 8 introgressions. The average length of the introgression was 31.2 cM, representing an average of 67.8 % of the total length of a linkage group. Three small introgressions were found: one in G6 (preNIL 412) of 0.7 cM and two in G8 (preNILs 110 and 769) of 3.9 cM each.

Table 11. Characteristics of the introgressions of the 18 preNILs selected in the T1E offspring.

	Linkage group								
	G1	G2	G3	G4	G5	G6	G7	G8	Average
Number of introgressions	7	7	5	15	7	8	6	10	8.1
Average length (cM) per introgression	35.3	35.7	34.3	37.2	24.3	27.2	25.3	30.5	31.2
% introgression[1]	68.9	71.6	76.1	81.2	61.0	63.2	55.9	64.1	67.8

[1]Average proportion of the genetic map of each linkage group covered by the almond introgression.

5.3.2 Genetic analysis

The phenotypic and genetic analysis on the preNILs was focused in the major genes identified in the T1E population: resistance to powdery mildew (*Vr3*), flesh color (*Bf2*), flower color (*Fc2*), Juiciness (*Jui*) and male sterility (*Rf1* and *Rf2*). Anther color was not included in this analysis because male-sterile plants, the majority in this group of plants (11), did not exhibit the phenotype associated with the major gene (*Ag2*) described in the Chapter 2. Two major QTLs, maturity date (*qMD.G4*) and stone weight (*qSW.G6*), were also evaluated.

For the resistance to powdery mildew, five preNILs (3, 27, 31, 333, and 412) were phenotyped as resistant to the disease. These plants were all heterozygous almond/peach in a region of G2 (Figure 15). In contrast, the rest of the preNILs were susceptible to the disease and homozygous for the peach allele at this region. The presence of the introgressions of the preNILs 498 and 769 in G2 allowed reducing the position of the resistance gene to a region of

11.2 cM between markers AMPA93 and EPDCU4017. This genomic region coincides with the location of the gene described in Chapter 2 between BPPCT004 and CPDCT044 in both TxE and T1E, although the resolution was lower (11.2 vs. 2.7 cM) in the preNIL collection.

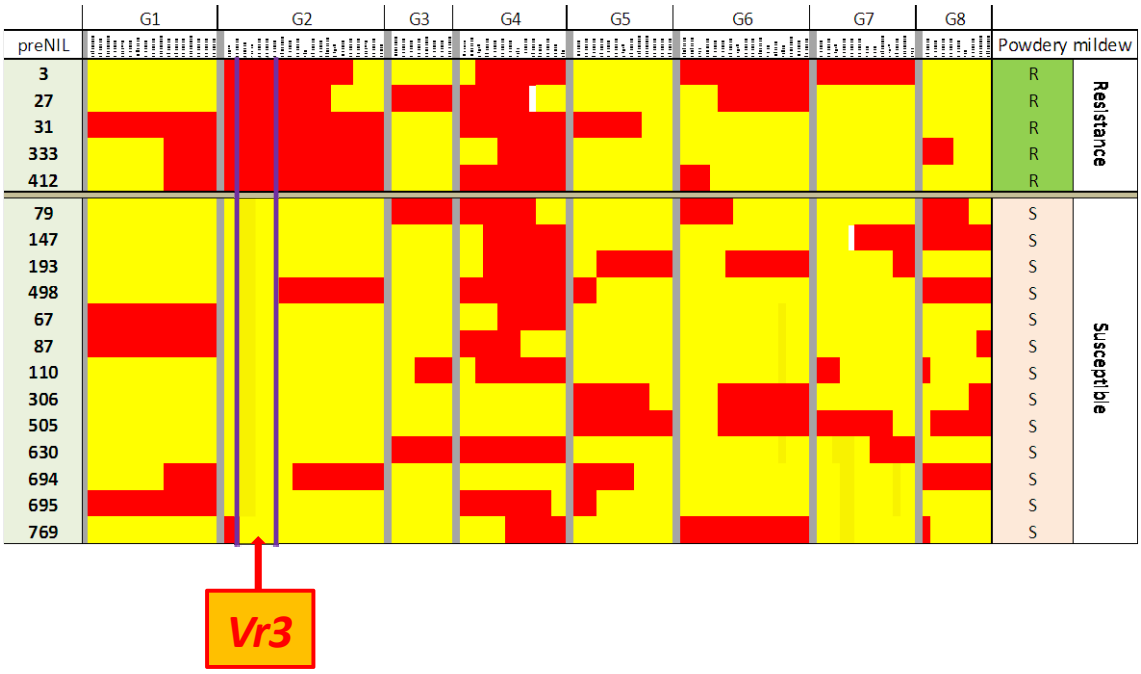


Figure 15. Identification of the map position of the powdery mildew resistance gene (*Vr3*) with the preNIL set (between violet bars). The red color corresponds to the almond/peach heterozygote and the yellow color to the peach homozygote. The red arrow indicates Vr3 position according to Chapter 4.

For the other major genes studied in the preNILs it was also possible to identify a specific area of the genome including the gene responsible for the trait (Table 12), confirming the possibility to study and identify major genes with this set of plants. For the fertility restorers *Rf1* and *Rf2*, where a single phenotype classification defined two genes, it was also possible to identify their position on the map: in the subset of male sterile lines only the expected two fragments in G2 and G6 were homozygous for the peach allele whereas the fertile lines had heterozygous and homozygous individuals (Figure 16).

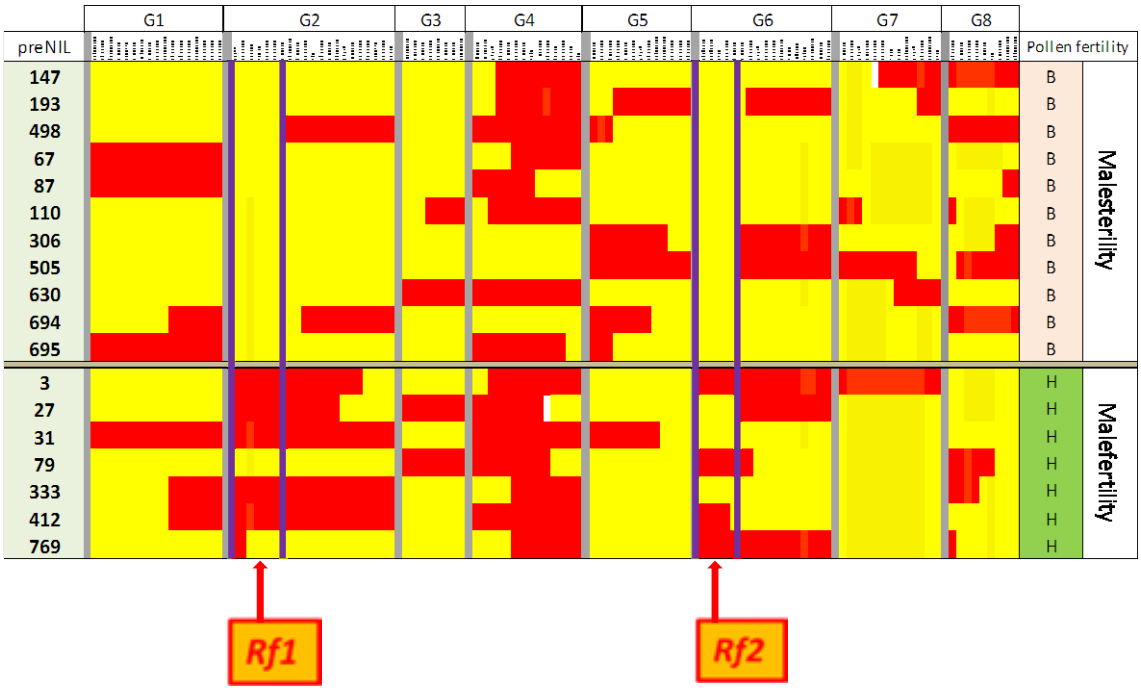


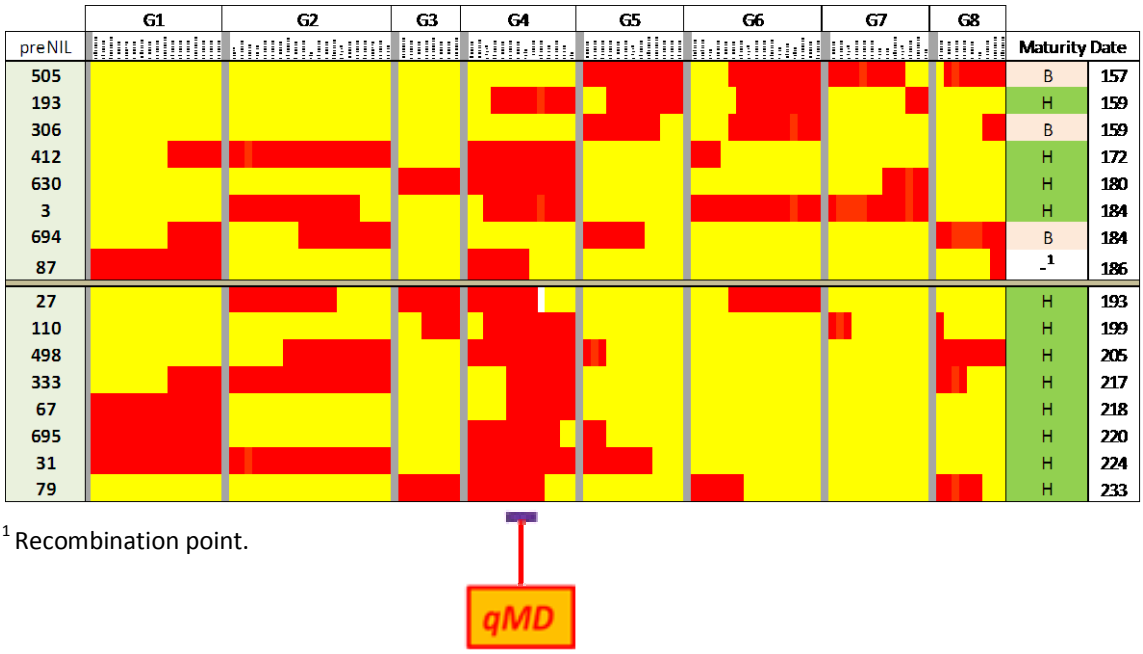
Figure 16. Identification of the map position of the fertility restores genes with the preNIL set. The red color corresponds to the almond/peach heterozygote and the yellow color to the peach homozygote. The violet bars indicate the position of the genes according to the preNIL set of plants. The arrow indicates the position of the gene in the T1E map (Chapter 3)

For complex traits, we studied two major QTLs, one for maturity date and another for stone weight. The other major QTLs detected in T1E belonged to traits affected by two or more consistent QTLs as the case of petiole length and percentage of external color (Chapter 4). In the case of maturity date the analysis did not permit to identify consistently the position of *qMD.G4*. Looking specifically at the region of G4 where the QTL occurs, we found that all lines with late maturation presented the almond genotype in the region of G4, which is consistent with what we would have expected, but the group with early maturation showed lines with both genotypes (Figure 17).

Table 12. Mapping of major genes segregating in the T1E offspring using the 18 preNIL set and comparison with results with the entire T1E mapping population.

Major gene/QTL	symbol	LG	marker interval	cM preNIL set[1]	cM T1E[2]
Powdery mildew resistance	*Vr3*	G2	CPPCT044-EPPCU4017	11.2	2.7
Fruit flesh color	*Bf2*	G1	EPDCU3122-BPPCT016	39.6	2.0
Juiciness	*Jui*	G1	EPDCU3122-BPPCT016	39.6	8.3
Flower color	*Fc*	G4	pchgms2-CPDCT045	16.8	6.0
Fertility restorer 1	*Rf1*	G2	CPPCT044-EPPCU4017	11.2	1.1
Fertility restorer 2	*Rf2*	G6	CPP21413-BPPCT008	13.3	5.4
Maturity date	*qMD*	G4	-[3]	-	6.3[4]
Stone weight	*qSW*	G6	CPP20836-CPPCT021	27.0	15.2[4]

[1] Distance of flanking markers in the preNIL set.
[2] Distance of flanking markers in the T1E map.
[3] Not determined.
[4] According the QTL analysis with - 1 LOD criteria.



[1] Recombination point.

Figure 17. Identification of the map position of the maturity date QTL with the preNIL set. The red color corresponds to the almond/peach heterozygote and the yellow color to the peach homozygote. The purple horizontal line indicates the qMD position interval according to Chapter 4.

On the other hand, the analysis of stone weight was successful. When the preNILs were ordered by stone weight we found that the set of lines with stones of 4.1 g or lighter had associated a fragment of G6 with the peach genotype, whereas the heavier stones had this fragment heterozygous for the almond allele (Figure 18).

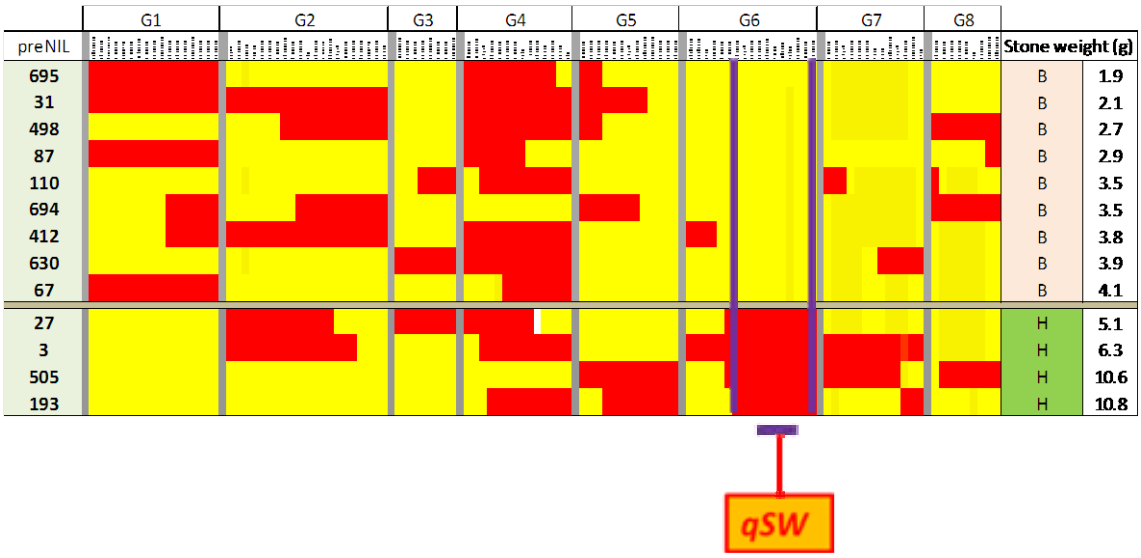


Figure 18. Identification of the map position of the stone weight QTL with the preNIL set. The red color corresponds to the almond/peach heterozygote and the yellow color to the peach homozygote. The violet bars indicate the position of the QTL according to the preNIL set of plants. The purple horizontal line indicates the *qSW* position interval according to Chapter 4.

5.4 DISCUSSION

In Chapter 4 we described what kind of variability can be incorporated into peach coming from almond and have discovered interesting genes of fruit quality and disease resistance that could enrich the peach genome. Here, we try to evaluate a breeding strategy based on marker-assisted selection that would allow introgressing these genes into the peach commercial gene pool in the shortest possible period of time, using also this effort to select a collection of NILs that could be a useful resource for genetic analysis in the *Prunus* genus. In the following paragraphs we discuss the results of the selection with markers in a large backcross one population of almond and peach and their consequences on various aspects of the process of gene introgression.

5.4.1 NIL development strategy

Nine individuals with three or less introgressions were selected representing the 1.02% of the total of 882 offspring belonging to backcross one. Assuming that a single crossover is produced in every pair of homologous chromosomes during meiosis in the almond x peach hybrid

individual used, the expected proportion of individuals with one, two or three introgressions is 2.73%, implying that 24 plants were expected from the 882 original, a significantly different number than the 9 plants (χ^2=9.7; P<0.01) observed. One possible explanation is that individuals with homozygous fragments of the peach genome would have produced seed or germinated less frequently than others or had resulted in weak or deleterious phenotypes, causing them to die or be discarded for further analysis. This would be more frequent in the individuals that we sought, as they were selected to have most of its genome homozygous for the peach alleles. Examples of this are the three plants known to have three introgressions that we discarded because of their low vigor, or the fact that certain genomic regions appeared much more frequently as heterozygous than others, particularly the central region of G4 or the proximal region of G8. Another explanation is that more than one recombination per chromosome per meiosis occurred, although in this case our scenario seems realistic based on the mapping data of the T1E population (Chapter 3).

The six preNIL survivors with three or less introgressions allowed full coverage of the peach genome in 6 out the 8 linkage groups of the genus (G1, G2, G3, G4, G7, and G8) and 64.5% of coverage in the remaining two groups (G5 and G6), representing a coverage over the whole genome of 92,3%. Nevertheless, to complete the whole coverage of the genome; to facilitate phenotypic characterization and genetic analysis and to continue the development of a second backcrossing process 18 individuals were finally selected, including the six original preNILs plus 12 plants from BC_1 with four introgressions.

The three-stage plan of selection with SSR markers produced the expected results. In total we obtained a total of 13,451 individual data (including 213 markers analyzed in plants that were dismissed as selfing or open pollinated), 10,568 in the first two steps and 2,883 in the third step. If we had chosen to run 16 markers in the first step, we would have generated a total of 17,520 individual data, 60.3% more than that we have produced, meaning that our strategy led to considerable savings of time and effort without loss of data quality. Full genotyping of the preNILs improved the precision on the definition of the boundaries of the introgressed fragments of each line. The average size of the introgressions was very high, 42.2 cM for introgression, equivalent to 11.8% of total genetic map distance of T1E. In 1/3 of the cases the introgression comprised the entire chromosome. These data are not surprising because the selection was made in the first backcross generation, representing a single meiosis, whereas

smaller introgressions would be expected if it had occurred in later generations. Another possible reason could be the low recombination rate of the almond x peach hybrid compared to that of peach (Chapter 3) also observed in other interspecific crosses in *Prunus* (Arús et al. 2005) and in other species (Gebhardt et al. 1991; Tanksley et al. 1992). The advantage of an early selection is that unidentified small introgressed fragments are unlikely, improving the quality of the final NIL set. Moreover, the offspring of heterozygous NILs with large introgressions or covering the whole genome may produce series of sub-NILs suitable for more detailed genetic analysis and identification of genes in small chromosomal fragments (Koumproglou et al. 2002). Nevertheless, it has the disadvantage that the phenotype of each line is affected by many almond genes simultaneously, which in some cases may complicate genetic analysis.

5.4.2 Choice of parental lines

NIL collections developed in different crops have been obtained on the genome of an inbred line of the recurrent parent (Eshed and Zamir, 1995, Pestsova et al. 2001, Eduardo et al. 2005, Monforte and Tanksley, 2000, Keurentjes et al. 2007, Fletcher et al. 2013). In our case the individual we chose as recurrent parent, 'Earlygold', is a regular peach commercial cultivar being partially heterozygous. Although peach is a species with a low level of variability (Aranzana et al. 2010; Li et al. 2013), and as we have seen in Chapter 3 approximately half of its physical distance is homozygous in 'Earlygold', this fact introduces an element of distortion in the genetic analysis of a NIL collection because the genetic background of peach in each preNIL (or later in the NIL) will not be completely fixed due to the segregation of the alleles present in 'Earlygold'. This problem could have been avoided if the selected line would have been homozygous, as in the few dihaploid lines that exist in this species (Monet et al. 1993) or highly homozygous, as in traditional non-melting Spanish varieties (Aranzana et al. 2010). However, the construction the NIL collection would have been delayed for a long time, since it would require the development of the hybrid and then 3-5 additional years in order to begin the process as we did with the hybrid 'MB 1.37'. This decided us to start, knowing this limitation and considering that a) many of the important genes that almond may bring into peach would have a clear qualitative effect, and b) that we can estimate in part the effects of the variation in the 'Earlygold' background using its map derived from the T1E population as we did in Chapter 4.

5.4.3 Mapping major genes and QTLs

This study demonstrates that the preNIL collection can be used for mapping simple morphological traits and for identifying molecular markers flanking them. Analysis of Mendelian trait loci in the preNIL collection has allowed locating a set of genes (*Vr3*, *Fc2*, *Bf2*, *Jui*, *Rf1* and *Rf2*) in the same genome positions as in the full T1E map, although as expected with lower precision, using the phenotype and the graphical genotype of these lines. This proof of concept is important because it means that this approach can be used in other preNIL collections without previous knowledge on the genetics of the characters under study. PreNILs carrying the character of interest may be advanced to further selection generations of introgression allowing MAS of the fragment of interest given that its genome position is known including that of markers nearby.

When we performed this analysis with major QTLs the outcome was less effective than with major genes. For one of the characters we were able to identify the position of the QTL (seed weight) but not for the other (maturity date). A reason for the latter result may be that phenotypes could be masked by the segregation in the 'Earlygold' background. A significant QTL explaining 18.5% of the phenotypic variance was identified on the ‘Earlygold’ map at this region at Cabrils in 2012 (Chapter 4), in agreement with this hypothesis and suggesting that ‘Earlygold’ may be heterozygous at this locus for alleles that produce a minor effect compared to the ‘Texas’ allele.

In all these results indicate that part of the major genes and major QTLs generated by the new variability introduced in peach by the almond genome can be identified and mapped in a preNIL collection of only 18 plants. Excluded from this analysis are all major genes/QTLs where the allele of the donor parent is recessive and the characters determined by various QTLs or by one that has minor effects. This genetic analysis, although partial, is worthwhile performing during the process of introgression assisted by markers, because it provides useful data on the inheritance of the traits analyzed and it requires a minimal expenditure of resources and time as the number of plants studied is small. The information acquired allows implementing more targeted and efficient strategies in further steps of the process that conclude with the breeding of improved varieties incorporating one or more valuable genes from the donor parent.

5.4.4 A model for marker-assisted introgression (MAI)

Two basic results of our research allow us to propose a model for introgressing genes from exotic germplasm or close wild or cultivated relatives of woody perennials. The first is that marker selection in a sufficiently large BC_1 generation is enough to identify individuals with a low number of introgressions covering most of the donor genome allowing to obtain in one or two more generations either lines with elite genome background of the recurrent species that could be introduced in a commercial breeding program or a set of homozygous NILs. In our case, six plants selected from 882 BC_1 offspring had three or less introgressions, contained 92.3% of the almond genome and were vigorous enough to perform adequately as parents for the next generation. The second is that a small (15-25) set of preNILs with a short number of introgressions allows mapping a part of the major genes/QTLs segregating as a consequence of the wide cross. This facilitates the understanding of the variability incorporated by the donor parent and enables the use of markers for their selection in the following steps of the process. In summary these results indicate that the introgression process can be efficiently done in a much shorter period of time with the aid of marker loci. Indeed, the usual 5-8 generations needed to extract a collection of NILs (Eshed and Zamir 1994; Monforte and Tanksley 2000; Eduardo et al. 2005) or to obtain a new cultivar with the backcross method (Allard et al. 1961) in herbaceous species, would make the process practically unfeasible in a species of long intergenerational period as the peach that would require 20-30 years (three years per generation plus five of evaluation at the end of the process). This can be performed over a period of 10-15 years with MAI.

The model proposed in this work is based in part on those developed by Tanksley and Nelson (1996), Eshed and Zamir (1994, 1995), and Tanksley and Fulton (2007), and adapted to woody species with long intergenerational period. The version for MAI in a breeding program and the NIL extraction is summarized in Figure 19. It consists essentially of three steps: 1) the creation of a large BC_1 population (>1,000 individuals) from where 15-30 individuals (preNILs) with ideally three or less introgressions are selected with markers, 2) the analysis of the preNIL collection for the traits of interest and the characterization of segregating major genes/QTLs that determine their expression, and 3) the advancement to a next generation of backcross or selfing to obtain a collection of individuals with a single introgression in the background of a peach elite commercial germplasm. The whole process could be done for peach or species of similar chromosome numbers (x=7-10) with a minimum of two backcross generations. The genetic evaluation performed in step 2 does not necessarily interfere or slow the process, as

crosses to advance to the BC_2 or BC_1S_1 (step 3) could be done at the same time or before the phenotyping for the traits is performed.

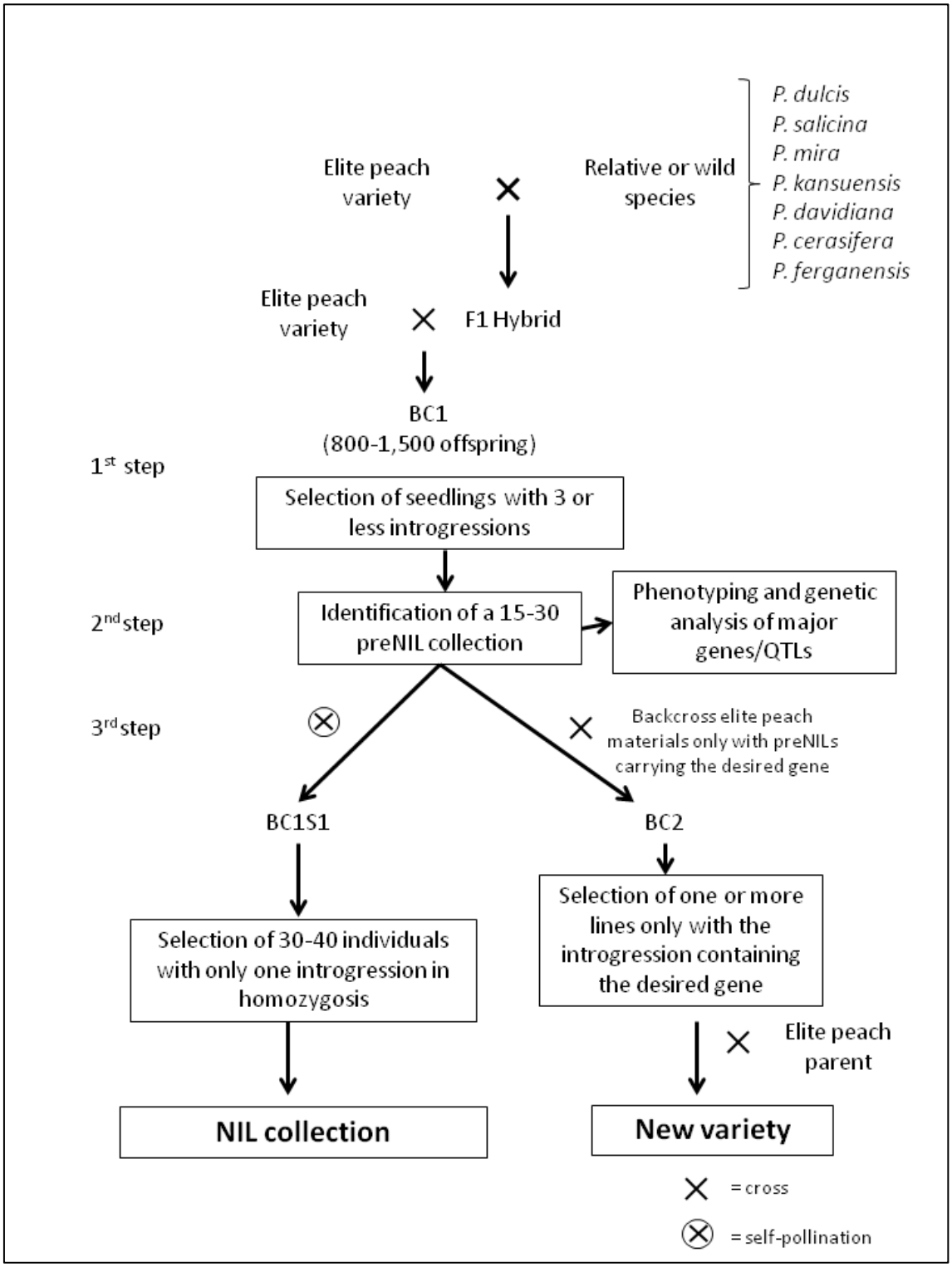


Figure 19. Scheme of the method for marker-assisted introgression from relatives or wild species into peach to obtain a near-isogenic line (NIL) collection or to develop a new cultivar.

Several circumstances may make the process longer or more complex or laborious. The number of individuals to be analyzed in the BC_1 generation to reach the objective of preNILs with ≥3 introgressions increases with chromosome number. For species such as apple (x=17) or grape (x=19) the number of BC_1 plants needed for having a reasonable preNIL set would be too high, implying that at least two backcross generations would be needed. Unforeseen circumstances, as in the case of almond x peach hybrids the discovery of a cytoplasmic male sterility system (Chapter 3), implied the addition of one generation to recover the peach cytoplasm and allow for selfed seed from all preNILs to be obtained. This could be avoided by starting the process with a hybrid individual coming from the reciprocal cross (peach x almond). For backcross breeding, the large size of the introgression may result in linkage drag, i.e. the unwanted effects of other genes included in the introgressed fragment. These may be eliminated by reducing the size of these fragments (Tanksley et al 1989), which requires additional backcross steps that could be monitored with markers developed within the introgressed fragment.

The essential reason why peach breeders have almost never used other *Prunus* species for the development of new varieties is the extremely high cost in time and effort needed to integrate the exotic alleles. The generalization of molecular markers for genetic analysis and the steady improvement of their quality and decreased costs provide an opportunity for using them in areas that were difficult to imagine only a few years ago. Here we propose a strategy (MAI) to make introgressed fragments available for peach breeding in a short period of time, with an approach that allows monitoring what part of the peach and what of the almond genome are present in each line. When considering its use as a source of novel variability for peach, MAI can be expanded to other species different for almond. Interspecific hybrids are available between peach and other species (*P. davidiana*, *P. salicina*, *P. cerasifera*, *P. kansuensis*, *P. mira* and others) that could be backcrossed with peach and generate a large offspring that could be selected with markers and individuals with low numbers of introgressions identified. The discovery of genes of step 2 of our model provides the information to allow only individuals carrying major genes of interest for a specific breeding program to be advanced to the next generation. In this fashion, a wave of materials with foreign DNA fragments, but in the background of the cultivated species, will be available for breeding and may represent a new source of variability that may be essential to incorporate needed genes of pest and disease resistance and fruit quality in peach for the next decades.

6. DISCUSIÓN GENERAL

6.1 Estudios genéticos y genómicos en melocotonero

El melocotonero es uno de los árboles frutales mejor caracterizados desde el punto de vista genético. Los estudios desarrollados desde principios del siglo XX habían permitido identificar un conjunto de 30 genes mayores descritos y revisados por Monet et al. (1996). Posteriormente, el desarrollo y uso generalizado de los marcadores moleculares tipo RFLP permitió la construcción del primer mapa genético saturado descrito en *Prunus*, mediante la utilización de una población interespecífica entre almendro y melocotonero (Joobeur et al. 1998). Este mapa conocido como TxE terminaría siendo aceptado por la comunidad científica como el mapa de referencia del género *Prunu*s. En 2003, Aranzana et al. reportaron el aumento de densidad del mapa de TxE con la inclusión de un nuevo tipo de marcadores altamente polimórfico y transferible: los microsatélites. Ello se tradujo en la construcción de nuevos mapas genéticos por otros grupos de trabajo, que utilizaron marcadores situados en el mapa de TxE en diferentes especies del genero *Prunus.* La ubicación de los loci del mapa de TxE fue comparada posteriormente con la de estos mismos marcadores en otros mapas desarrollados en *P. dulcis* (Joobeur et al. 2000), *P. ferganensis* (Dettori et al. 2001), *P. armeniaca* (Lambert et al. 2004), *P. davidiana* (Foulongne et al. 2003) y *P. avium* (Dirlewanger et al. 2004); observando en todos ellos los ocho grupos de ligamiento representativos del género y un alto nivel de sintenia et alinealidad entre marcadores, lo que sugería según Arús et al. (2005) que las diferentes especies de *Prunus* estudiadas compartían el mismo genoma. En estudios posteriores Illa et al. (2011) y Jung et al. (2012) utilizaron la información existente en los mapas y las secuencias de *Prunus, Fragaria* y *Malus*, encontrando una conjunto de bloques sinténicos conservados en los tres géneros, lo que permitió proponer un modelo de evolución cromosómica de estos tres genomas a partir de un genoma ancestral compuesto de nueve cromosomas para la familia *Rosaceae*.

Paralelamente al desarrollo de los mapas genéticos, el fenotipado de diversos caracteres en poblaciones de mapeo en *Prunus* permitió localizar en el mapa de referencia un conjunto de 28 genes mayores (Dirlewanger et al. 2004), entre los que se encontraban muchos de los recopilados por Monet et al. (1996). Además, una característica importante del conjunto de genes identificados es que varios de ellos son responsables de variantes fenotípicas con tremendo impacto para la industria: afectando entre otros al fruto (melocotón vs. nectarina, fruto plano vs. redondo, tipo pavía vs. prisco, fundente vs. no fundente, sabor sub-ácido vs. ácido, pulpa amarilla vs. blanca), la arquitectura del árbol (árbol columnar vs. normal), y la resistencia a enfermedades (nematodo agallador de la raíz) (Arús et al. 2012). Posteriormente,

otros genes mayores han sido mapeados en poblaciones de melocotonero y localizados en el mapa de referencia: uno que afecta la morfología de la flor (campanulácea vs. rosacea; *Sh/sh*) mapeado en G8 (Ogundiwin et al. 2010; Fan et al. 2010); un gen que afecta el desarrollo del fruto y causa aborto temprano (fruto que no aborta vs. fruto que aborta; *Af/af*) mapeado en G6 (Dirlewanger et al. 2006), co-segregando con el locus de la forma del fruto (*S/s*). Otro gen identificado afecta la coloración de la pulpa (antociánico vs. no antociánico; *DBF/dbf*), produciendo un fenotipo de color rojo-violáceo que ha sido mapeado en el G5 (Shen et al. 2013) y podría tener grandes implicaciones en la mejora ya que generaría una tipología de fruto no disponible en el mercado actualmente. El locus responsable del período de maduración de la fruta (*MD*) fue mapeado en G4 por Eduardo et al. (2011). Otro gen interesante es una fuente de resistencia (*Vr2/vr2*) al oídio identificada por Pascal et al. (2010) localizado cerca del punto de rotura de una translocación recíproca entre los grupos de ligamiento seis y ocho (Jáuregui et al. 2001; Yamamoto et al. 2005). La identificación de estos genes provenientes de diferentes especies y de cruzamientos interespecíficos y su posterior localización en el mapa de TxE estaría posibilitada, por la similitud de los genomas de las especies estudiadas (Arús et al. 2012). Diversos estudios filogenéticos entre diferentes especies del género *Prunus* (Badenes y Parfitt, 1995; Bortiti et al. 2001; Chin et al. 2014) y el análisis de la resecuenciación de *P. ferganensis*, *P. kansuensis*, *P. mira*, *P. davidiana* y nueve variedades de melocotonero (Verde et al. 2013; material suplementario 7) han corroborado la estrecha relación genética entre ellos.

El uso del chip de SNPs desarrollado por el IPSC (International Peach SNP consortium) (Verde et al. 2012) en las poblaciones TxE y T1E, juntamente con un juego de un centenar de SSRs de posición conocida, nos ha permitido obtener mapas de alta densidad y calidad en ambas poblaciones. El elevado número de marcadores obtenido en cada mapa (~2.000) nos indica que el sesgo debido a la selección de estos SNPs en colecciones de melocotonero no ha impedido detectarlos en el almendro, lo que facilitará su uso en otros cruzamientos entre estas dos especies, permite considerar su uso para el estudio de la variabilidad en almendro e indica que la proximidad entre almendro y melocotonero a nivel de secuencia génomica es muy grande.

La comparación entre los mapas obtenidos en TxE, T1E y E, este último basado en la recombinación ocurrida en el melocotonero 'Earlygold', han permitido también verificar que el nivel de recombinación a nivel interespecífico es inferior al que ocurre dentro de la misma especie, tal como se había observado en *Prunus* y en otras especies (Tanksley et al. 1992; Arús

et al. 2005). El descubrimiento de amplias regiones monomórficas en aproximadamente la mitad del mapa de 'Earlygold' indica que una parte de su genoma es idéntico por descendencia, lo que implica que está fijado tanto para los marcadores como para cualquier otro gen que allí se encuentre. Ya que esta parece ser la situación generalizada en las variedades comerciales de melocotonero (Eduardo et al. 2013; Martínez-García et al. 2013), la conclusión es que el conocimiento de cuáles son estas regiones en los parentales usados, juntamente con la información ya existente sobre la herencia y situación de genes y QTLs importantes, puede ser muy útil en los programas de mejora para caracterizar los parentales a cruzar y poder predecir que caracteres van a segregar y cuáles no en las poblaciones de mejora.

En nuestro estudio hemos identificado diez genes mayores mediante la caracterización genotípica y fenotípica de dos poblaciones interespecificas y en tres mapas genéticos (TxE, T1E y E), lo que es un importante avance en el conocimiento genético en *Prunus*. La posición en el mapa de dos genes ya descritos con anterioridad, la forma de la flor (*Sh*) y el color de la antera (*Ag*), se estableció con mayor precisión. Los otros ocho genes han sido identificados por primera vez, su descripción y ubicación se presenta detalladamente en el Capitulo 4, aunque dos de ellos merecen principal atención. En G2 se encuentra *Vr3* una nueva fuente de resistencia al oídio no alélica del gen *Vr2* previamente descrito por Pascal et al. (2010) en G6. El color antociánico de la pulpa (*Bf2*) mapea en G1 y presenta una tipología similar al gen *DBF* descrito en G5 por Shen et al. (2013). *Bf2* difiere también por su acción génica y posición del gen *bf* localizado en G4 (*Bf2* es dominante mientras que *bf* es recesivo) que determina también el color rojo de la carne (Shen et al. 2013). Otro genes con potencial interés es el *Ft* que determina el tipo de fruto (almendro vs. melocotonero) y se encuentra en G4. *Ft* tiene un efecto pleiotrópico sobre el carácter peso del fruto que se comporta como superdominante en TxE, siendo el heterocigoto de mayor peso que cualquiera de los homocigotos. En la región de G4 donde se halla *Ft* se concentra un grupo de genes, como el *MD* (época de maduración; Eduardo et al. 2013), el *Lb* (época de floración en almendro; Ballester et al. 2001) y dos genes relacionados con la consistencia de la carne (fundente vs. no fundente; *M*) y la adherencia del hueso al fruto (clingstone vs. freestone; *F*) Peace et al. (2005). Estos genes, particularmente el primero, podrían tener un efecto adicional en el carácter por lo que creemos que conviene un estudio en mayor detalle que permita establecer su posible interés comercial. Finalmente, los QTLs mayores involucrados en el porcentaje e intensidad de coloración antociánica en la epidermis del fruto presentan interesantes opciones para su utilización en la mejora de variedades de melocotonero.

Con respecto a los QTLs, Arús et al. en el año 2003 describían un conjunto de 28 QTLs consistentes identificados en el mapa de referencia. Más de una década después se ha incrementado grandemente el número de QTL identificados: Salazar et al. (2014) sitúan el número de QTLs descritos en *Prunus* en 760, aunque muchos de ellos sean redundantes, es decir, el mismo QTL localizado en diferentes poblaciones o años. Este aumento es lógico teniendo en cuenta que los muchos de los caracteres agronómicos importantes como productividad, resistencia a estreses abióticos, duración postcosecha y calidad nutricional son de herencia cuantitativa (Tanksley, 1993; Tanksley y Fulton, 2007). Para ello ha sido necesario crear mapas en nuevas poblaciones segregantes y fenotipar y diseccionar genéticamente estos caracteres usando el software apropiado. La disponibilidad de marcadores de posición conocida en el genoma y altamente reproducibles, como los contenidos en el mapa de referencia y otros mapas relacionados, la facilidad de transferencia de algunos de estos marcadores, particularmente los SSR, entre diferentes especies del género *Prunus* (Mnneja et al. 2010) y la accesibilidad a esta información mediante la base de datos de las rosáceas (http://www.rosaceae.org/), han sido sin duda elementos favorables al rápido crecimiento de la información en este ámbito no tan solo en melocotonero si no también en otros frutales de hueso.

La mayoría de estudios de análisis de caracteres agronómicos están hechos con pocos individuos y en mapas construidos con pocos marcadores, suficientes para la localización de genes mayores o QTLs pero con una resolución baja si de lo que se trata es de identificar los genes causales y entender su función. Por ello, se torna cada vez más importante el desarrollo de mapas genéticos de alta densidad y el análisis de colecciones de materiales más amplias para la identificación de regiones genómicas estrechas que incluyan unos pocos genes candidatos. Durante esta Tesis hemos construido tres mapas genéticos de alta densidad mediante la saturación de mapas desarrollados previamente con marcadores SSRs, a los que hemos añadido los SNPs del chip 9k de Illumina. Los datos obtenidos nos han permitido situar los genes mayores que hemos descubierto en intervalos cromosómicos en algunos casos bastante pequeños, lo que nos permite la localización de candidatos para dichos genes con mayor facilidad. Un ejemplo es el de los PPRs que hemos localizado en las regiones donde se encuentran los genes responsables de la restauración de la fertilidad (Capítulo 3). Desarrollando una estrategia similar, Pirona et al. (2013) saturaron dos mapas genéticos lo que les permitió reducir la distancia de los marcadores flanqueantes al locus putativo responsable de la época de maduración de la fruta (*MD*) de 3.56 Mb a 220 kb, identificando que

correspondería a un factor de transcripción de la familia NAC. La región genómica donde hemos localizado un gen mayor en TxE y un QTL mayor en T1E para este carácter contiene también este factor de transcripción.

En el corto plazo, será posible llevar a cabo el proceso de caracterización genética mediante la resecuenciación de individuos o poblaciones completas. La utilización de la información proporcionada por estos proyectos permitirá obtener una mayor cantidad de marcadores que con cualquier otro método anteriormente empleado, lo que sustentará la construcción de mapas genéticos de resolución superior capaces de detectar con mayor exactitud los puntos de recombinación. En este momento se están resecuenciando los individuos de la población de mapeo T1E (Alexiou et al. 2014) con el objetivo de estudiar con inusual precisión las regiones cromosómicas de interés detectadas en esta investigación y el desarrollo de marcadores para aplicarlos eficazmente en la mejora genética del melocotonero.

6.2 Creación de una colección de NILs de almendro en el fondo genético del melocotonero

Al mismo tiempo que los cambios producidos en las plantas domesticadas son beneficiosos para el hombre, principalmente en términos de productividad (Grandillo et al., 2007), los procesos de domesticación de las plantas cultivadas han producido una serie de cuellos de botella, de manera que sólo una pequeña porción de la variabilidad genética que se encuentra en las especies silvestres está representada en los cultivares actuales (Torres et al. 2012). En el caso del melocotonero este problema ha sido acentuado por la condición autógama de la especie y por la utilización de muy pocos parentales en los programas de mejora que dieron origen a las principales variedades cultivadas en América y Europa durante el siglo pasado (Faust y Timon, 1995; Scorza et al. 1985). Estas son las principales causas que sitúan al melocotonero como el frutal cultivado del género *Prunus* con menor variabilidad genética (Byrne et al. 1990; Mnejja et al. 2010). Considerando estos antecedentes como una limitante de la mejora genética del melocotonero, el grupo del IRTA se propuso la construcción de una colección de NILs en el fondo genético del melocotonero cultivado con la finalidad de utilizar especialmente las ventajas de este tipo de poblaciones en el estudio y mapeo de QTLs y en la introgresión de genes exóticos en las variedades comerciales.

El primer elemento de análisis para dar inicio a un proyecto de esta envergadura se basó en la elección de la especie que se utilizaría como parental donante. Esta elección se produjo inicialmente en 1990, cuando el objetivo era crear un mapa genético saturado para el melocotonero, y conociendo ya la poca variabilidad genética de esta especie. Se optó, tal como hicieran antes en tomate - otra especie poco variable - Tanksley et al. (1986) por utilizar un cruzamiento interespecífico que garantizaba el polimorfismo de los marcadores usados (en aquel momento RFLPs) en la totalidad del genoma. El híbrido 'Texas' x 'Earlygold' (MB1.37) seleccionado era parte del programa de mejora de patrones de melocotonero del IRTA dirigido por Francisco J. Vargas y se eligió por ser una planta vigorosa y muy fértil. Más adelante, y dado que la F_2 de este híbrido produjo un número reducido de plantas capaces de producir fruto, se decidió generar una descendencia BC_1 que de acuerdo con los datos entonces disponibles sería más fértil, como así ocurrió. La especie utilizada como donante es importante por muchos motivos, de una manera particular por su capacidad para donar caracteres interesantes y también porque su comportamiento con respecto a su fertilidad en el híbrido y generaciones subsiguientes es crucial para el éxito de un proyecto como el que se pretendía iniciar. Mientras que no existían dudas sobre el interés del almendro como posible donante dado su alto polimorfismo y su mayor resistencia a diversas enfermedades, la falta de

conocimientos sobre el comportamiento de los híbridos almendro x melocotonero en diferentes generaciones introducía un elemento de riesgo en el proyecto. Anderson y Weir (1967), presentan uno de los primeros registros en los que se detalla de forma sistemática la producción de híbridos interespecíficos entre especies del género *Prunus*. Los cruzamientos de melocotonero con *P. tenella*, *P. domestica*, *P. besseyi* y *P. davidiana*, generaron descendientes que presentaban en su mayoría un alto grado de rusticidad y resistencia, pero a la vez, una alta proporción de aberraciones de las estructuras florales como pistilos pequeños con tendencia al aborto; escasez o ausencia de polen e incapacidad para producir fruta, exceptuando el cruce realizado con *P. davidiana*, que produjo híbridos con características intermedias entre ambas especies mientras que los frutos se presentaban un mesocarpo muy delgado que al madurar se separaba del hueso. La posibilidad de cruzar exitosamente melocotonero y *P. davidiana* concuerda con los resultados presentados por Foulongne et al. (2003), que encontraron regiones genómicas asociadas a la resistencia al oídio en dicha descendencia. Por otra parte, las descripciones de Anderson y Weir (1967) sobre el tamaño de los pistilos son congruentes con los resultados de nuestro trabajo donde fue posible identificar plantas con pistilos pequeños en ambas poblaciones de estudio. Específicamente, un QTL que explicó el 76,2% de la variación fenotípica se mapeo en G6 de TxE, en una región genómica que incluye también el gen *Af* (fruit abortion) descrito por Dirlewanger et al. (2006) responsable del tamaño de los pistilos y el aborto prematuro de los frutos. En T1E dos QTLs menores responsables del tamaño del pistilo fueron también mapeados en otras posiciones (G1 y G4) que en TxE, sugiriendo que diferentes loci en *Prunus* estarían involucrados en la determinación del tamaño y funcionalidad del pistilo. También en relación con la capacidad productiva, Anderson y Weir (1967) señalan que en los 14 años de estudio los híbridos del cruzamiento de melocotonero con ciruelo europeo sólo produjeron frutos una temporada, situación extrapolable a la diferente capacidad productiva de los individuos de TxE y T1E, y al hecho que algunas plantas no produjeran fruta todos los años del estudio.

El segundo elemento determinante del proyecto fue el diseño del proceso de introgresión. Se trata de una parte crítica porque la colección de NILs debía obtenerse con un número de generaciones mínimo, dado que el largo período intergeneracional del melocotonero (3-4 años hasta que puede obtenerse un número aceptable de frutos) prolongaría excesivamente el proceso con estrategias parecidas a las usadas en especies herbáceas como el melón (Eduardo et al. 2005), la lechuga (Jeuken y Lindhout, 2004), el tomate (Eshed y Zamir 1994) o el trigo (Pestova et al. 2001) que necesitaron entre 4-5 (trigo) y 8-12 (tomate) generaciones. El modelo propuesto para melocotonero consiste en tres fases: 1) Creación de una numerosa población

BC1 (>1.000 individuos) y selección en la misma de individuos con tres o menos introgresiones, 2) creación de una colección de líneas (15-25) con pocas introgresiones (preNILs) y análisis genético de los caracteres segregantes, 3) autofecundación (BC_1S_1) o retrocruzamiento (BC_2) de las preNILs y obtención de la colección de NILs homocigoticas o heterocigoticas, respectivamente. La fase 2 pretende recolectar información genética que sirva para seleccionar los parentales más interesantes y facilitar el proceso de selección en generaciones posteriores, pero no debería retardar el proceso al poderse hacer en paralelo con la fase 3. El modelo sería también aplicable al proceso de introgresión de un fragmento de almendro en el fondo de variedades elite de melocotonero en un programa de mejora, en el que las líneas obtenidas en la fase 3 podrían ser usadas ya como parentales para la obtención de nuevas variedades.

En la fase 1 se obtuvieron 1.080 individuos de los que 882 eran efectivamente de la población BC_1. Al final del proceso quedaron 9 líneas con 3 o menos introgresiones. El bajo vigor de tres de ellas obligó a descartarlas, pero las seis líneas restantes presentaron una cobertura prácticamente completa (92,7%) del genoma del almendro. Complementando, por tanto, esta colección con una línea adicional con cuatro introgresiones que cubriera las regiones faltantes sería suficiente para continuar el proceso. Nuestros datos demuestran que esta primera fase es factible, a pesar de que el número de líneas con tres o menos introgresiones encontradas estuviera por debajo de las esperadas, lo que fue probablemente debido a la selección en contra de homocigotos en determinadas regiones genómicas.

Para el desarrollo de la fase 2, seleccionamos un grupo de 18 preNILs que incluían las seis inicialmente seleccionadas más 12 con cuatro introgresiones. El estudio de la comparación entre su genotipo y su fenotipo nos permitió situar en el mapa todos los genes mayores ya identificados y mapeados anteriormente (Capítulo 4), lógicamente con menor precisión, pero en las zonas genómicas esperadas. Los resultados con dos QTLs mayores en los que habíamos encontrado un solo QTL por carácter produjeron resultados diversos, pudiendo ser mapeados en un caso (peso del hueso) pero no en el otro (época de maduración). En general, nuestra conclusión es que en una situación real, sin información previa, en la que quisiéramos estudiar genes o QTLs mayores responsables de la variación de algunos de los caracteres segregando cualitativamente en una colección de preNILs, seríamos capaces de situar en el mapa una buena parte de ellos. Los más interesantes en estas poblaciones, como las resistencias dominantes a enfermedades, podrían ser sin duda mapeados. Los que no podrían encontrarse serían todos aquellos que fueran dominantes para el alelo del melocotonero, o una fracción de

los QTLs posiblemente aquellos que expliquen una parte más pequeña de la variabilidad o que interaccionen de alguna manera con otros genes segregantes en la población.

La tercera etapa está en proceso de desarrollo, pero por la información que disponemos actualmente sabemos que requerirá cambios importantes ya que nos encontramos con un escollo de gran magnitud. En las población T1E y, de manera especial, en la sub-colección de preNILs, se identificó un gran número de plantas androestériles, que se manifestaron por la ausencia total de polen en sus tecas. El estudio genético de esta población nos permitió identificar un sistema de androesterilidad citoplasmática con dos genes provenientes del almendro capaces individual o conjuntamente de restaurar la fertilidad de los individuos (Capítulo 3). Una primera consecuencia importante es que muchas de las preNILs son androestériles (11 de las 18), por lo que no pueden autofecundarse para producir NILs en homocigosis ni tampoco cruzarse con 'Earlygold' como parental femenino para obtener líneas con pocas introgresiones y con el citoplasma del melocotonero. Esto significa que han sido ya descartadas para la producción de NILs homocigóticas y han sido substituidas por otras, la mayoría con cuatro, pero algunas con cinco introgresiones. La segunda consecuencia es que es imprescindible la recuperación del citoplasma del melocotonero, lo que obliga a una nueva generación de retrocruzamiento entre las preNILs androfértiles finalmente seleccionadas y 'Earlygold' como parental femenino. Todo ello supondrá retrasar una generación el final del proceso, es decir, retrasar el proyecto 3-4 años más, aunque tenga la compensación parcial de que los fragmentos introgresados serán menores de promedio. Un importante corolario de estos resultados es que para evitar estos problemas de androesterilidad hay que procurar que el citoplasma de los individuos de los diferentes retrocruzamientos sea el del parental recurrente. Sin embargo, consideramos que la estrategia diseñada para la creación de una colección de NILs de melocotonero ha sido globalmente exitosa y nos está permitiendo avanzar en un ámbito en el que no existía prácticamente información alguna.

6.3 Perspectivas de futuro

La actividad realizada dentro de este trabajo abre nuevas perspectivas dentro de la investigación en frutales dentro del grupo del IRTA. En primer lugar, nuestros resultados han permitido establecer con bastante precisión la posición en el genoma de varios genes mayores, algunos especialmente interesantes y no encontrados anteriormente en el melocotonero (*Jui*, *Bf2*, *Ft*, *Vr3*, entre otros), que sería interesante explorar en más detalle y clonar en un futuro próximo. Quedan muchos caracteres por evaluar, en una primera instancia resistencias a enfermedades, pero también otros relacionados con el contenido en nutrientes del fruto, la calidad postcosecha u otros que podrían inicialmente evaluarse en la colección de preNILs y detectar otros genes de interés. El desarrollo de la colección de NILs está actualmente en el BC_2 y algunos individuos con una sola introgresión en heterocigosis han sido ya extraídos. La colección completa de NILs en homocigosis, que esperamos en 5-6 años, permitirá disponer de una herramienta de gran valor para el estudio de caracteres de herencia compleja y que finalmente contribuirá al enriquecimiento del pool genético del melocotonero con genes procedentes del almendro. En efecto, algunos genes y QTLs (*Bf2*, *Vr3* y los QTL de color de la piel, entre otros) presentan un interés evidente en mejora. Las líneas actuales suponen ya un avance notable en nuestras posibilidades de integrarlos en variedades comerciales, para lo cual habría que cruzarlas con parentales de programas de mejora, seleccionar luego con marcadores los fragmentos de interés y evaluar el efecto de los nuevos genes así como el arrastre de ligamiento que otros genes ligados pueda producir.

El almendro es una de las muchas especies de *Prunus* que pueden intercruzarse con el melocotonero. Existen híbridos entre el melocotonero y *P. cerasifera*, *P. mira*, *P. ferganensis*, *P. kansuensis*, *P. davidiana* o *P. salicina*, algunos de ellos usados como portainjertos. Hay también híbridos con otros individuos de almendro que, dada la gran variabilidad existente en esta especie (Mnneja et al, 2010), aportarán muy probablemente nueva variación interesante y útil. La misma estrategia usada con el almendro podría aplicarse a estos híbridos, generando colecciones de preNILs que permitieran evaluar algunos de los genes valiosos que estas especies puedan contener y avanzar solamente a generaciones ulteriores aquellas que prometan la incorporación de un carácter interesante. Es evidente que pueden surgir dificultades inesperadas, empezando por la fertilidad de los híbridos disponibles, que seguramente serán diferentes en cada especie. Sin embargo, la única manera de avanzar en este ámbito es empezar proyectos parecidos asumiendo la existencia de riesgos e intentar implicar en ellos a los mejoradores que son unos de los primeros beneficiarios de esta

búsqueda de genes. Una ventaja de este proceso es que cada paso adelante (cada generación de retrocruzamiento y selección con marcadores queda consolidado y supone un avance hasta el objetivo final que es la introducción en el germoplasma élite de melocotonero de genes de almendro de interés. Nuestra experiencia en almendro abre un camino, del que solo hemos recorrido algunas etapas, pero que ofrece premios tanto en aspecto científico como en el comercial.

7. CONCLUSIONES

1. La utilización del chip IPSC de 9K juntamente con 131 marcadores microsatélite facilitó el desarrollo de tres mapas genéticos de alta densidad: el primero de ellos en la población F_2 (TxE) y los otros dos correspondientes a los parentales de la población BC_1 (T1E y E), permitiendo mejorar el conocimiento del genoma de melocotonero al identificar segmentos mal ensamblados y reorientar algunos 'supercontigs' descritos en la primera versión del genoma.

2. La calidad de los mapas obtenidos fue alta considerando la colinealidad, sintenia y presencia de marcadores comunes entre TxE y T1E (>92% de coincidencia). El elevado número de marcadores segregando en estas poblaciones sugiere que el chip IPSC de 9K de melocotonero puede ser usado con éxito en otras poblaciones interespecíficas de melocotonero por almendro.

3. El mayor nivel de recombinación observado en el mapa de E comparado con el de los mapas de TxE y T1E confirma la tasa de recombinación es mayor en cruzamientos intraespecíficos que en cruzamientos interespecíficos.

4. La presencia de grandes zonas (>2 Mb) sin marcadores en el mapa genético de E (103.0 Mb del mapa físico de T1E) sugiere que dichos fragmentos son idénticos por descendencia representado el 50% del mapa físico cubierto por T1E.

5. Se estableció que la presencia de individuos androestériles en las poblaciones genéticas de TxE y T1E se debe a un mecanismo de esterilidad citoplasmática causada por la interacción del citoplasma del almendro y el genoma nuclear del melocotonero. Los alelos dominantes, *Rf1* y *Rf2*, de dos genes independientes son capaces de restaurar la fertilidad. La posición de *Rf1* y *Rf2* se estimó en 3.4 Mb en el grupo de ligamiento dos y en 1.4 Mb en el grupo de ligamiento seis, respectivamente. En ambos fragmentos cromosómicos se localizaron genes codificando para proteínas PPRs (pentatricopeptidos), que suelen estar asociadas a la restauración de la fertilidad masculina en otras especies.

6. El análisis genético de los fenotipos estudiados ha permitido identificar un total de ocho genes mayores además de los dos restauradores de fertilidad. Seis de ellos corresponden a genes descritos por primera vez en el genoma de *Prunus* (*Fc2, Ag2, Bf2, Jui, Ft, Vr3*), mientras que los otros dos (*Ag* y *Sh*) eran conocidos con anterioridad habiendo sido mapeados con mayor precisión en esta tesis.

7. Se identificó un conjunto de 336 QTLs totales en los tres mapas analizados para el conjunto de caracteres evaluados en los tres años de duración del estudio. De manera consistente se detectaron 43, 2 y 19 QTLs en los mapas de T1E, E y TxE, respectivamente.

8. La identificación de un mayor número de QTLs en el mapa de T1E con respecto al mapa de TxE estaría determinado principalmente por el estricto criterio para considerar valido un QTL favoreciendo a la población más numerosa, especialmente para aquellos loci que explican una baja proporción de la variación fenotípica observada

9. El bajo número de QTLs identificados en el mapa de E es consistente con la escasa variabilidad del melocotonero y al hecho de que 'Earlygold' presentaría grandes zonas del genoma idénticas por descendencia. Sin embargo el efecto de los alelos del almendro habría podido enmascarar la detección de algunos QTLs en E.

10. Después del análisis de 1.095 genotipos en el BC_1 'T1E' identificamos nueve plantas con tres ó menos fragmentos cromosómicos de almendro. Se seleccionaron seis de estos individuos, con una cobertura del 92,3% en el fondo genético del melocotonero, y 12 adicionales con cuatro introgresiones que constituyeron una colección de 18 líneas (a las que llamamos preNILs, con una cobertura completa del genoma.

11. La colección de preNILs permitió la identificación de la posición de los genes mayores que segregan en esta población. En cambio, el análisis de los caracteres cuantitativos fue menos fiable y solo uno de los dos QTLs estudiados pudo ser mapeado. Esto demuestra que se puede obtener información relevante sobre la herencia de algunos caracteres a nivel de primer BC, lo que es útil para realizar una selección de los mismos en posteriores generaciones con la ayuda de marcadores.

12. La presencia de la androesterilidad citoplásmica obliga a introducir el citoplasma de melocotonero en las líneas seleccionadas obteniendo descendientes entre las 'Earlygold' como parental femenino y las preNILs androfértiles, así como a redefinir el juego de preNILs de modo que sean androfértiles y contengan en conjunto todo el genoma del almendro. En conjunto esto supone el retraso de una generación en el proceso de obtención de las NILs.

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9. ANEXOS

Annex 1. Characteristics of the microsatellites used for the construction of the maps with the almond x peach F_2 (TxE) and backcross 1 (T1E) populations.

	Map position[1]			Mapped in			Map reference[4]
Marker	LG	cM[2]	Physical position (bp)[3]	TxE	T1E	E	
CPPCT016	G1	1.3	579,334	x			Dirlewanger et al. (2004)
EPDCU3122	G1	2.5	1,207,146	x	x	x	Dirlewanger et al. (2004)
UDP96-018	G1	1.4	1,298,998		x		Present work
CPPCT010	G1	11.6	1,298,998	x			Dirlewanger et al. (2004)
CPPCT004	G1	11.6	?		x		Dirlewanger et al. (2004)
M16a	G1	1:14	8,491,350	x			Howad et al. (2005)
EPPCU5331	G1	1:26	11,172,303	x	x		Howad et al. (2005)
PaCITA005	G1	17.3	11,321,910	x	x		Present work
CPPCT027	G1	23.1	12,409,450	x		x	Dirlewanger et al. (2004)
EPPCU1090	G1	1:34	22,653,653	x	x	x	Howad et al. (2005)
UDP96-005	G1	29.2	?	x	x		Dirlewanger et al. (2004)
CPPCT003	G1	33.2	?		x		Howad et al. (2005)
CPDCT024	G1	37.2	27,190,581	x			Dirlewanger et al. (2004)
EPPCU1945	G1	1:50	29,356,873	x			Present work
CPPCT026	G1	33.9	31,792,329	x	x		Dirlewanger et al. (2004)
EPDCU3489	G1	46.7	33,027,380	x	x		Dirlewanger et al. (2004)
BPPCT020	G1	52.6	33,281,418	x			Dirlewanger et al. (2004)
BPPCT016	G1	55.2	36,075,308	x	x		Dirlewanger et al. (2004)
CPPCT019	G1	46.7	38,032,452		x		Present work
CPPCT042	G1	62.5	39,307,938	x	x		Dirlewanger et al. (2004)
EPDCU2862	G1	66.5	39,371,824	x	x		Dirlewanger et al. (2004)
CPPCT029	G1	65.1	40,195,426	x	x		Dirlewanger et al. (2004)
CPPCT053	G1	70.8	?	x	x		Present work
BPPCT028	G1	77.4	45,685,797	x	x		Dirlewanger et al. (2004)
CPPCT044	G2	7.2	9,968,143	x	x	x	Dirlewanger et al. (2004)
CPP8062	G2	0.0	10,246,874		x		Present work
UDP98-025	G2	9.6	10,872,238	x			Dirlewanger et al. (2004)
AMPA93	G2	1.4	9,100,912		x	x	Present work
MA024a	G2	2:13	7,279,019		x	x	Howad et al. (2005)
CPDCT044	G2	12.5	?	x	x		Dirlewanger et al. (2004)
BPPCT004	G2	20.2	11,922,357	x	x		Dirlewanger et al. (2004)
EPDCU4017	G2	20.9	15,974,268	x	x		Dirlewanger et al. (2004)
BPPCT001	G2	20.9	16,133,969	x	x		Dirlewanger et al. (2004)
CPSCT044	G2	23.6	17,217,107	x	x		Dirlewanger et al. (2004)
M1a	G2	2:25	18,412,524		x		Howad et al. (2005)
UDP96-013	G2	27.8	18,895,941	x	x		Dirlewanger et al. (2004)
CPDCT004	G2	27.8	19,912,907	x	x		Dirlewanger et al. (2004)
UDP98-411	G2	27.8	20,172,861	x	x		Dirlewanger et al. (2004)
pchgms1	G2	35.1	21,255,417	x	x		Dirlewanger et al. (2004)
BPPCT030	G2	38.0	22,248,468	x	x		Dirlewanger et al. (2004)
CPPCT043	G2	38.0	22,248,469	x	x		Dirlewanger et al. (2004)
CPSCT021	G2	39.4	23,734,599	x	x		Dirlewanger et al. (2004)
PceGA34	G2	43.9	25,199,001	x	x		Dirlewanger et al. (2004)
CPSCT034	G2	48.6	26,348,777	x	x		Dirlewanger et al. (2004)
UDA-023	G2	2:50	26,348,970	x	x		Howad et al. (2005)

Annex 1 (continued)

EPPCU5990	G3	3:04	527,238	x	x		Howad et al. (2005)
EPDCU4610	G3	0.7	?	x			Present work
UDP97-403	G3	11.9	2,557,069	x	x		Dirlewanger et al. (2004)
BPPCT007	G3	11.2	2,741,897	x	x	x	Dirlewanger et al. (2004)
BPPCT039	G3	18.0	5,802,960	x	x	x	Dirlewanger et al. (2004)
EPDCU3083	G3	19.8	6,458,850	x	x		Dirlewanger et al. (2004)
CPPCT002	G3	31.9	16,205,250	x	x	x	Dirlewanger et al. (2004)
UDP96-008	G3	36.4	16,946,780	x	x		Dirlewanger et al. (2004)
EPDCU0532	G3	43.7	?	x	x	x	Present work
BPPCT010	G4	2.1	227,708	x	x		Dirlewanger et al. (2004)
EPDCU5060	G4	1.8	484,115		x	x	Dirlewanger et al. (2004)
pchgms2	G4	7.0	2,086,474	x	x	x	Dirlewanger et al. (2004)
CPPCT005	G4	10.4	?	x	x	x	Dirlewanger et al. (2004)
CPPCT011	G4	12.1	?	x	x	x	Present work
CPDCT045	G4	16.8	6,205,903	x	x	x	Dirlewanger et al. (2004)
UDP96-003	G4	28.3	8,757,479	x	x		Dirlewanger et al. (2004)
EPPCU1106	G4	4:46	9,586,328	x			Howad et al. (2005)
M12a	G4	4:46	9,208,608	x	x	x	Howad et al. (2005)
EPDCU3832	G4	4:46	10,362,508	x			Howad et al. (2005)
EPPCU2000	G4	4:46	12,467,623	x	x		Howad et al. (2005)
BPPCT015	G4	44.0	12,546,880	x	x	x	Dirlewanger et al. (2004)
UDA-021	G4	4:46	14,074,659	x	x		Howad et al. (2005)
CPPCT046	G4	45.4	14,476,745	x	x		Dirlewanger et al. (2004)
BPPCT023	G4	45.4	14,731,686	x			Dirlewanger et al. (2004)
UDA-027	G4	4:63	18,639,915	x	x		Howad et al. (2005)
EPPCU1775	G4	4:63	22,684,552	x			Howad et al. (2005)
CPPCT051	G4	48.2	?	x			Present work
Ps12e2	G4	45.8	?		x		Howad et al. (2005)
CPPCT040	G5	1.5	993,617	x	x		Dirlewanger et al. (2004)
AMPA112	G5	4.6	2,761,133	x			Present work
BPPCT026	G5	5.2	4,388,739		x		Dirlewanger et al. (2004)
UDP97-401	G5	11.0	5,940,393	x	x		Dirlewanger et al. (2004)
PaCITA021	G5	20.0	10,778,022	x			Present work
BPPCT017	G5	20.1	11,174,144	x	x		Dirlewanger et al. (2004)
CPSCT006	G5	21.7	11,533,644	x	x		Dirlewanger et al. (2004)
BPPCT037	G5	25.6	12,312,049	x	x	x	Dirlewanger et al. (2004)
pchgms4	G5	26.7	12,665,818	x	x		Dirlewanger et al. (2004)
CPPCT013	G5	29.2	12,835,904	x	x		Dirlewanger et al. (2004)
EPDCU5183	G5	35.2	13,859,578	x	x		Dirlewanger et al. (2004)
EPDCU4658	G5	33.1	14,489,300	x	x	x	Present work
BPPCT038	G5	32.9	14,658,198		x	x	Dirlewanger et al. (2004)
CPSCT022	G5	40.7	16,626,112	x	x		Dirlewanger et al. (2004)
BPPCT014	G5	44.0	16,626,126	x	x		Dirlewanger et al. (2004)

Annex 1 (continued)

CPP21413	G6	0.0	4,150,445		x	x	Present work
Ps7a2	G6	7.0	2,165,426	x	x		Dirlewanger et al. (2004)
CPP21245	G6	1.1	3,108,930		x	x	Present work
CPP20836	G6	1.1	1,141,321		x	x	Present work
UDP96-001	G6	17.5	7,040,897	x	x		Dirlewanger et al. (2004)
EPPCU9300	G6	6:25	7,282,736	x			Howad et al. (2005)
BPPCT008	G6	30.1	10,280,037	x	x		Dirlewanger et al. (2004)
CPSCT012	G6	36.2	16,098,467	x	x		Dirlewanger et al. (2004)
pchcms5	G6	44.7	19,166,407	x	x		Dirlewanger et al. (2004)
BPPCT025	G6	56.4	21,129,944	x	x	x	Dirlewanger et al. (2004)
CPPCT047	G6	58.9	21,583,770	x	x		Dirlewanger et al. (2004)
UDP98-412	G6	72.0	24,753,513	x	x		Dirlewanger et al. (2004)
MA040a	G6	6:74	24,857,835	x	x	x	Howad et al. (2005)
AMPA130	G6	67.7	25,322,083	x	x		Present work
MA14a	G6	6:74	?	x	x	x	Howad et al. (2005)
EPPCU4092	G6	6:80	25,943,449	x	x		Howad et al. (2005)
CPPCT030	G6	80.2	26,851,012	x	x		Dirlewanger et al. (2004)
CPPCT021	G6	83.7	27,604,841	x	x		Dirlewanger et al. (2004)
CPSCT004	G7	9.5	6,681,998	x	x	x	Dirlewanger et al. (2004)
CPPCT039	G7	14.1	8,338,791	x	x	x	Dirlewanger et al. (2004)
CPPCT022	G7	18.6	10225365	x			Dirlewanger et al. (2004)
pchgms6	G7	10.7	10,439,275	x	x		Present work
UDP98-408	G7	23.7	12,216,663	x	x		Dirlewanger et al. (2004)
UDAp-432	G7	7:31	13,499,331	x			Howad et al. (2005)
UDAp-407	G7	21.4	?	x			Present work
CPPCT057	G7	21.4	15,854,584	x	x	x	Present work
CPPCT033	G7	38.9	16,846,940	x	x		Dirlewanger et al. (2004)
MA20a	G7	7:41	17,253,269		x	x	Howad et al. (2005)
PMS02	G7	7:48	18,106,108	x	x	x	Howad et al. (2005)
EPPCU5176	G7	7:56	18,772,878	x	x	x	Howad et al. (2005)
pchcms2	G7	51.4	19,089,997	x	x	x	Dirlewanger et al. (2004)
CPPCT017	G7	61.8	20,899,157	x	x	x	Dirlewanger et al. (2004)
EPDCU3392	G7	64.7	21,681,912	x	x		Dirlewanger et al. (2004)
Ps5c3	G7	70.6	22,285,603	x	x	x	Dirlewanger et al. (2004)
CPSCT018	G8	0.0	123,201	x	x		Dirlewanger et al. (2004)
BPPCT006	G8	14.1	?	x	x	x	Dirlewanger et al. (2004)
CPPCT058	G8	14.6	7,943,526	x	x	x	Present work
CPDCT034	G8	16.8	?	x	x	x	Dirlewanger et al. (2004)
CPPCT006	G8	24.8	13,659,021	x	x	x	Dirlewanger et al. (2004)
M6a	G8	8:41	15,033,895		x	x	Howad et al. (2005)
UDP98-409	G8	44.5	17,783,855	x	x	x	Dirlewanger et al. (2004)
EPDCU3454	G8	46.7	18,639,388	x	x		Dirlewanger et al. (2004)
EPDCU3117	G8	54.7	20,218,634	x	x		Dirlewanger et al. (2004)

[1]As in Dirlewanger et al (2004) or Howad et al. (2005). In the latter case, the position corresponds to the bin where the marker was found.

[2] map position as in the map reference. Markers are ordered according to their position in the maps presented in this paper.

[3] According to the peach sequence v1.0 (http://www.rosaceae.org/species/prunus_persica/genome_v1.0)

[4] Reference where the marker was mapped in the TxE map. For the markers mapped here, their position is that of the TxE map and if not mapped in TxE in T1E map

Annex 2. Summary of phenotypic data of 'Texas', 'Earlygold', their F1 hybrid MB1.37 and the TxE population (mean, maximum, minimum, standard deviation and Shapiro-Wilk test). The table includes trait names and acronyms, year, location and number of individuals (N) for which data was obtained.

Trait family	Trait	Acronym	Location	Year	N	Texas	Earlygold	MB 1.37	Mean	Max	Min	Std Dev	Shapiro-Wilk
Flower	Pistil length	PiL	Gimenells	2011-2013	86	3.00	3.00	3.00	2.28	3.00	1.00	0.86	<,0001*
	Blooming density	BD	Gimenells	2012	71	5.00	3.00	4.00	3.32	5.00	1.00	1.26	<,0001*
				2013	83	5.00	3.00	4.00	3.05	5.00	1.00	1.47	<,0001*
Phenology	Beginning of shooting	BS	Gimenells	2012	80	74.00	79.00	78.00	72.96	81.00	65.00	2.68	0,0037*
				2013	81	71.00	69.00	68.00	69.11	75.00	58.00	2.87	0,0001*
	Begining of flowering	BFT	Gimenells	2012	77	71.00	72.00	70.00	69.38	75.00	64.00	2.93	0,0049*
				2013	77	70.00	66.00	68.00	65.27	73.00	57.00	3.28	0,0662
	End of flowering	EFT	Gimenells	2012	77	80.00	81.00	77.00	76.47	84.00	72.00	2.82	0,0032*
				2013	75	78.00	75.00	74.00	72.79	83.00	65.00	3.67	0,0043*
	Flowering duration	FD	Gimenells	2012	77	9.00	9.00	7.00	7.10	11.00	4.00	1.66	0,0031*
				2013	73	8.00	9.00	6.00	10.90	17.00	6.00	2.55	0,0757
	Fruit production	FP	Gimenells	2011-2013	86	4.00	4.00	4.00	2.16	4.00	1.00	1.16	<,0001*
	Madurity date	MD	Gimenells	2011	29	-	160.00	214.00	222.03	270.00	156.00	36.48	0,0222*
				2012	38	259.00	164.00	226.00	227.74	261.00	164.00	27.53	0,0074*
				2013	33	263.00	167.00	224.00	232.70	274.00	168.00	27.78	0.3049
	Fruit developme	FDP	Gimenells	2012	36	191.00	90.00	162.00	151.97	187.00	87.00	28.23	0,0223*
				2013	29	185.00	89.00	139.00	159.03	204.00	95.00	29.84	0.3191
Fruit	Juiciness	Jui	Gimenells	2011-2013	39	2.00	1.00	2.00	1.74	2.00	1.00	0.44	<,0001*
	Blood flesh	Bf	Gimenells	2011-2013	37	-	1.00	2.00	1.32	2.00	1.00	0.47	<,0001*
	Intensity skin color	ISC	Gimenells	2011	21	-	2.00	3.00	1.48	3.00	1.00	0.58	0,0007*
				2012	30	-	2.00	3.00	1.90	3.00	1.00	0.82	0,0001*
				2013	27	-	2.00	3.00	1.81	3.00	1.00	0.75	0,0004*
	Percentage skin color	PSC	Gimenells	2011	23	1.00	2.00	3.00	2.35	4.00	1.00	1.23	0,0005*
				2012	37	1.00	3.00	4.00	2.43	4.00	1.00	1.39	<,0001*
				2013	31	1.00	2.00	4.00	2.56	4.00	1.00	1.37	<,0001*
	Soluble solid content	SSC	Gimenells	2011	16	-	15.40	no juicy	14.23	17.60	10.40	2.04	0,6659
				2012	24	-	16.00	no juicy	14.08	17.40	9.50	1.81	0,7435
				2013	28	-	12.40	no juicy	12.83	18.00	8.50	2.59	0,6361
	Titratable acidity	TA	Gimenells	2011	14	-	6.39	10.99	8.23	15.21	4.95	2.73	0,0181*
				2012	24	-	6.50	11.42	9.04	18.48	3.95	3.48	0,0017*
				2013	28	-	7.26	12.06	12.15	24.85	5.63	5.25	0,0257
	Fruit weight	FW	Gimenells	2011	28	-	127.80	87.50	42.34	91.95	9.70	21.56	0,2680
				2012	38	14.25	132,1	76.49	47.81	124.80	11.57	26.07	0,0879
				2013	33	-	141.54	128.18	58.30	135.07	15.11	28.65	0,1856
	Stone weight	SW	Gimenells	2011	28	-	7.57	7.51	4.97	7.41	2.40	1.29	0,8876
				2012	36	5.17	7.90	5.72	5.56	11.24	1.99	2.13	0,0929
				2013	33	-	7.12	9.65	6.33	17.06	2.20	2.90	0,0003*
	Flesh weight	FlW	Gimenells	2011	28	-	120.23	79.99	37.37	84.74	6.49	20.83	0,2179
				2012	36	9.08	124.20	70.77	41.36	114.19	6.32	25.82	0,0741
				2013	33	-	134.42	118.54	51.97	125.30	10.34	26.76	0.1739
	Fruit polar diameter	FpD	Gimenells	2011	28	-	5.42	6.11	4.70	5.87	2.76	0.83	0,4323
				2012	38	3.78	5.39	5.85	4.85	6.59	3.26	0.83	0,8557
				2013	31	-	6.05	6.95	5.13	7.33	3.54	0.87	0,9353
	Fruit cheeck diameter	FcD	Gimenells	2011	28	-	5.53	5.33	4.00	5.56	2.28	0.99	0,0553
				2012	38	2.72	5.50	5.49	4.27	6.53	2.42	0.97	0,1993
				2013	31	-	6.71	6.23	4.51	6.46	2.37	0.96	0,3139
	Fruit suture diameter	FsD	Gimenells	2011	28	-	5.78	5.09	4.00	5.32	2.41	0.82	0,1112
				2012	38	-	5.80	5.36	4.30	6.31	2.73	0.88	0,5114
				2013	31	2.82	6.24	5.98	4.56	6.21	2.57	0.90	0.8684
	Stone polar diameter	SpD	Gimenells	2011	28	-	3.27	4.03	3.34	4.68	2.42	0.45	0,4104
				2012	36	3.18	3.30	3.71	3.49	4.49	2.83	0.45	0,2149
				2013	32	-	3.10	4.27	3.58	4.78	2.78	0.48	0,4070
	Stone check diameter	ScD	Gimenells	2011	28	-	2.47	2.47	2.14	2.98	1.67	0.28	0,1338
				2012	36	2.07	2.49	2.46	2.20	2.73	1.66	0.27	0,4583
				2013	32	-	2.15	2.75	2.24	3.15	1.59	0.33	0,6521
	Stone suture diameter	SsD	Gimenells	2011	28	-	1.93	1.70	1.54	1.99	1.14	0.17	0,6238
				2012	36	1.65	1.82	1.80	1.65	2.34	1.25	0.25	0,0229
				2013	32	-	1.97	1.92	1.67	2.45	1.16	0.29	0,1009
	Flesh polar diameter	FlpD	Gimenells	2011	28	-	2.15	2.08	1.36	2.36	0.27	0.64	0.1014
				2012	36	0.60	2.09	2.14	1.38	2.64	0.34	0.63	0.1719
				2013	31	-	2.95	2.68	1.54	2.62	0.24	0.63	0.5553
	Flesh check diameter	FlcD	Gimenells	2011	28	-	3.06	2.86	1.88	3.39	0.33	0.95	0,0117*
				2012	36	0.65	3.01	3.03	2.10	3.95	0.28	0.90	0.4769
				2013	31	-	4.56	3.48	2.27	3.89	0.76	0.77	0.8519
	Flesh suture diameter	FlsD	Gimenells	2011	28	-	3.85	3.39	2.46	3.52	0.93	0.79	0,0290*
				2012	36	1.17	3.98	3.56	2.68	3.97	1.33	0.80	0.0562
				2013	31	-	4.27	4.07	2.88	4.24	0.41	0.79	0.1513
Leaf	Leaf perimeter	LP	Gimenells	2012	77	3071.13	3169.17	2906.91	2574.64	3322.92	1584.39	362.81	0,1774
				2013	77	3109.05	3147.63	2784.93	2654.34	3383.95	1786.95	323.29	0,1724
	Leaf surface	LS	Gimenells	2012	77	290.29	279.26	190.84	182.00	295.93	67.26	49.60	0,3470
				2013	77	296.94	275.64	195.80	197.41	306.84	87.20	48.07	0,2158
	Leaf blade width	LBW	Gimenells	2012	77	394.00	331.00	259.00	285.91	368.67	174.00	40.27	0,2787
				2013	77	389.00	340.25	272.88	297.42	402.75	190.00	37.86	0,3244
	Leaf length	LL	Gimenells	2012	77	1310.00	1369.00	1258.00	1088.60	1395.40	672.50	153.12	0,1208
				2013	77	1314.00	1362.75	1228.75	1139.41	1451.71	766.67	145.13	0,4721
	Leaf blade length	LBL	Gimenells	2012	77	1096.00	1241.00	1089.00	946.92	1234.25	579.00	137.42	0,3187
				2013	77	1112.14	1201.25	1053.38	975.21	1262.00	620.67	130.38	0,7166
	Petiole length	PL	Gimenells	2012	77	214.00	128.00	169.00	141.68	255.25	71.33	39.46	0,0188*
				2013	77	201.86	161.50	175.38	164.21	244.57	90.67	35.92	0,3607
	Leaf weight	LW	Gimenells	2012	79	0.28	0.28	0.31	0.23	0.57	0.09	0.07	<,0001*
				2013	75	0.37	0.33	0.24	0.26	0.44	0.12	0.07	0,1818
	Chlorophyll content	CC	Gimenells	2011	83	39.60	37.60	42.00	39.22	48.70	28.10	3.96	0,9476
				2012	79	39.00	40.70	37.40	37.92	45.70	29.50	3.10	0,9992
				2013	81	39.20	42.20	46.00	37.51	45.60	27.10	3.97	0,2963

Annex 3. Summary of phenotypic data of 'Texas', 'Earlygold', their F1 hybrid MB1.37 and the T1E population (mean, maximum, minimum, standard deviation and Shapiro-Wilk test). The table includes trait names and acronyms, year, location and number of individuals (N) for which data was obtained.

Trait family	Trait	Acronym	Location	Year	N	Texas	Earlygold	MB 1.37	Mean	Max	Min	Std Dev	Shapiro-Wilk
Flower	Pistil length	PiL	Gimenells	2011-2013	161	3.00	3.00	3.00	2.81	3.00	1.00	0.52	<,0001*
	Bloming density	BD	Cabrils	2012	149	5.00	3.00	4.00	3.17	5.00	1.00	1.22	<,0001*
				2013	137	5.00	3.00	4.00	3.28	5.00	1.00	1.32	<,0001*
			Gimenells	2012	104	5.00	3.00	4.00	3.53	5.00	1.00	1.07	<,0001*
				2013	128	5.00	3.00	4.00	3.14	5.00	1.00	1.27	<,0001*
Phenology	Beginning of shooting	BS	Cabrils	2011	159	67.00	76.00	71.00	60.25	80.00	50.00	5.37	<,0001*
				2012	150	73.00	80.00	72.00	71.60	81.00	62.00	4.00	0,0377*
				2013	141	72.00	62.00	65.00	63.40	79.00	48.00	7.28	<,0001*
			Gimenells	2012	131	74.00	79.00	78.00	72.54	80.00	66.00	3.44	<,0001*
				2013	145	71.00	69.00	68.00	68.76	73.00	59.00	2.68	<,0001*
	Begining of flowering time	BFT	Cabrils	2011	137	41.00	55.00	50.00	54.09	63.00	39.00	4.84	<,0001*
				2012	150	65.00	70.00	70.00	68.19	79.00	57.00	4.37	0,0329*
				2013	137	50.00	58.00	59.00	53.31	70.00	35.00	9.01	0,0001*
			Gimenells	2012	130	71.00	72.00	70.00	69.56	78.00	59.00	3.20	0,0043*
				2013	139	70.00	66.00	68.00	65.62	72.00	55.00	3.24	<,0001*
	End of flowering time	EFT	Cabrils	2011	131	61.00	69.00	63.00	65.34	74.00	52.00	4.93	<,0001*
				2012	150	74.00	81.00	78.00	76.57	86.00	64.00	4.08	0,0082*
				2013	136	68.00	74.00	64.00	68.19	82.00	46.00	7.11	0,0001*
			Gimenells	2012	120	80.00	81.00	77.00	76.70	84.00	71.00	2.49	0,0016*
				2013	138	78.00	75.00	74.00	72.67	82.00	66.00	3.80	<,0001*
	Flowering duration	FD	Cabrils	2011	113	20.00	14.00	13.00	11.61	24.00	2.00	3.75	0,0309*
				2012	149	9.00	11.00	8.00	8.43	14.00	4.00	1.87	0,0038*
				2013	135	18.00	16.00	5.00	15.04	25.00	7.00	3.84	0,0024*
			Gimenells	2012	113	9.00	9.00	7.00	7.43	13.00	3.00	1.80	0,0493*
				2013	137	8.00	9.00	6.00	6.93	13.00	3.00	2.18	<,0001*
	Fruit production	FP	Gimenells	2011-2013	161	4.00	4.00	4.00	2.42	4.00	1.00	1.03	<,0001*
	Madurity date	MD	Cabrils	2011	65	-	145.00	210.00	174.48	223.00	138.00	23.24	0,0147*
				2012	125	-	159.00	211.00	191.43	241.00	152.00	23.13	0,0041*
				2013	54	-	164.00	213.00	181.28	225.00	161.00	15.50	0,0010*
			Gimenells	2011	58	-	160.00	214.00	196.41	242.00	156.00	26.50	<,0001*
				2012	100	259.00	164.00	226.00	200.81	252.00	167.00	24.30	0,0036*
				2013	75	263.00	167.00	224.00	203.61	257.00	158.00	24.51	0,0057*
	Fruit developme nt period	FDP	Cabrils	2011	58	-	76.00	147.00	108.90	156.00	76.00	22.56	0,0124*
				2012	123	-	79.00	136.00	115.18	167.00	73.00	23.10	0,0036*
				2013	52	-	78.00	134.00	113.44	157.00	85.00	17.12	0,2373
			Gimenells	2012	83	191.00	90.00	162.00	125.71	176.00	86.00	24.63	0,0004*
				2013	72	185.00	89.00	139.00	131.76	187.00	84.00	25.02	0,0097*
	Leaf fall	LF	Cabrils	2011	151	359.00	341.00	324.00	327.52	358.00	293.00	18.36	<,0001*
				2012	165	369.00	348.00	348.00	345.67	381.00	292.00	17.84	<,0001*
				2013	149	365.00	339.00	341.00	344.64	365.00	295.00	14.64	<,0001*
	Juvenility	Juv	Cabrils	2008-2013	135	-	-	-	3.61	6.00	3.00	0.86	<,0001*
Fruit	Intensity skin color	ISC	Cabrils	2011	49	-	2.00	3.00	1.84	4.00	1.00	0.83	<,0001*
				2012	90	-	2.00	3.00	1.87	4.00	1.00	0.83	<,0001*
				2013	42	-	2.00	3.00	1.92	3.50	1.00	0.85	<,0001*
			Gimenells	2011	54	-	2.00	3	2.10	4.00	1.00	0.89	<,0001*
				2012	101	-	2.00	3	2.11	4.00	1.00	0.93	0,0001*
				2013	80	-	2.00	3.00	1.96	4.00	1.00	0.86	<,0001*
	Percentage skin color	PSC	Cabrils	2011	53	1	3.00	3.00	2.82	4.00	1.00	1.00	<,0001*
				2012	87	1	2.00	4.00	3.25	4.00	1.00	0.89	<,0001*
				2013	43	1	2.00	4.00	3.16	4.00	1.00	1.00	<,0001*
			Gimenells	2011	54	1	2	3	3.18	4.00	1.00	0.97	<,0001*
				2012	101	1	3	4	3.19	4.00	1.00	0.98	<,0001*
				2013	79	1	2.00	4.00	3.13	4.00	1.00	1.04	<,0001*
	Soluble solid content	SSC	Cabrils	2011	51	-	17.00	no juicy	13.37	19.20	6.80	2.99	0,7514
				2012	75	-	14.20	no juicy	14.76	23.10	7.10	3.64	0,4727
				2013	36	-	16.30	no juicy	13.46	16.20	9.40	1.91	0,0519
			Gimenells	2011	55	-	15.40	no juicy	13.96	21.20	8.00	2.67	0,0711
				2012	88	-	16.00	no juicy	14.28	23.95	8.20	2.62	0,0335
				2013	62	-	12.40	no juicy	12.95	17.40	7.40	2.18	0.5410
	Titratable acidity	TA	Cabrils	2011	51	-	4.10	9.67	8.13	17.70	3.00	3.00	0,0136*
				2012	76	-	6.66	10.50	9.83	25.08	3.69	4.50	<,0001*
				2013	46	-	6.20	11.66	8.65	23.40	4.60	3.72	<,0001*
			Gimenells	2011	53	-	6.39	10.99	7.95	18.48	2.57	3.97	0,0004*
				2012	82	-	6.50	11.42	11.14	29.97	3.49	4.96	<,0001*
				2013	77	-	7.26	12.06	10.38	24.15	4.29	4.28	0,0009*
	Fruit weight	FW	Cabrils	2011	53	-	100.28	82.31	59.96	120.30	8.86	25.68	0,9297
				2012	96	-	130.31	68.16	61.28	140.10	9.70	26.47	0,2184
				2013	42	-	121.23	106.86	86.74	167.87	28.05	34.10	0,2716
			Gimenells	2011	52	-	127.80	87.50	75.47	134.98	21.87	26.80	0,1327
				2012	100	14.25	132.10	76.49	82.94	156.92	21.61	30.74	0,3952
				2013	79	-	141.54	128.18	99.69	184.71	33.78	32.22	0,7564
	Stone weight	SW	Cabrils	2011	51	-	3.73	7.35	5.06	9.90	1.07	2.25	0,0853
				2012	92	-	6.43	6.44	5.01	12.11	1.81	2.45	<,0001*
				2013	40	-	6.48	11.30	5.37	12.49	1.64	2.34	0,0167*
			Gimenells	2011	48	-	7.57	7.51	6.37	11.03	2.35	2.39	0,1416
				2012	95	5.17	7.90	5.72	5.71	12.19	1.37	2.73	0,0002*
				2013	78	-	7.12	9.65	7.17	13.65	2.62	2.95	0,0115*
	Flesh weight	FlW	Cabrils	2011	51	-	96.56	74.96	55.76	112.00	7.79	24.26	0,9456
				2012	92	-	123.88	61.72	55.72	135.82	10.67	24.13	0,1028
				2013	40	-	114.75	95.57	79.43	159.50	23.75	32.20	0,2295
			Gimenells	2011	48	-	120.23	79.99	72.06	124.08	35.84	23.94	0,0579
				2012	95	9.08	124.20	70.77	76.60	145.64	19.85	28.94	0,3483
				2013	78	-	134.42	118.54	92.17	173.46	30.37	30.27	0,6791

Annex 3 (continued)

Trait family	Trait	Acronym	Location	Year	N	Texas	Earlygold	MB 1.37	Mean	Max	Min	Std Dev	Shapiro-Wilk
Fruit	Fruit polar diameter	FpD	Cabrils	2011	52	-	5.23	6.23	5.02	6.35	2.85	0.87	0,0103*
				2012	95	-	6.56	5.41	5.24	7.19	3.02	0.83	0.7440
				2013	41	-	6.41	5.56	5.68	7.35	4.12	0.79	0,6973
			Gimenells	2011	54	-	5.42	6.11	5.33	7.21	3.37	0.79	0,9174
				2012	100	3.78	5.39	5.85	5.56	7.36	3.30	0.71	0.1060
				2013	78	-	6.05	6.95	5.83	7.21	4.23	0.72	0.0780
	Fruit cheek diameter	FcD	Cabrils	2011	52	-	5.13	5.79	4.49	6.35	2.05	0.90	0,0851
				2012	95	-	6.35	5.13	4.64	6.30	2.34	0.80	0,1332
				2013	42	-	6.24	5.04	5.27	7.04	3.31	0.83	0,9685
			Gimenells	2011	54	-	5.53	5.33	4.95	6.29	2.97	0.74	0,3477
				2012	100	2.72	5.50	5.49	5.29	6.61	3.17	0.71	0,0644
				2013	77	-	6.71	6.23	5.53	6.91	4.01	0.67	0,5005
	Fruit suture diameter	FsD	Cabrils	2011	52	-	5.15	5.55	4.56	5.99	2.38	0.87	0,0279*
				2012	95	-	5.91	4.95	4.68	6.21	2.47	0.78	0.1930
				2013	41	-	5.63	5.39	5.26	6.71	3.81	0.73	0,3269
			Gimenells	2011	54	-	5.78	5.09	4.91	6.88	3.00	0.80	0,8886
				2012	100	2.82	5.80	5.36	5.25	6.56	3.30	0.67	0,1429
				2013	78	-	6.24	5.98	5.54	6.95	3.75	0.68	0,7643
	Stone polar diameter	SpD	Cabrils	2011	50	-	2.36	3.94	3.12	4.26	1.70	0.51	0,0716
				2012	93	-	3.49	3.81	3.29	4.58	2.26	0.51	0,1507
				2013	40	-	3.54	3.39	3.22	4.23	2.27	0.40	0,9797
			Gimenells	2011	50	-	3.27	4.03	3.43	4.64	2.20	0.49	0,9964
				2012	99	3.18	3.30	3.71	3.43	4.57	2.08	0.53	0,6013
				2013	79	-	3.10	4.27	3.49	4.82	2.38	0.53	0,4505
	Stone cheek diameter	ScD	Cabrils	2011	50	-	1.83	2.32	2.01	2.78	1.20	0.34	0,3834
				2012	93	-	1.94	2.20	2.01	2.95	1.25	0.35	0,3324
				2013	40	-	2.37	2.45	2.17	2.71	1.59	0.28	0,736
			Gimenells	2011	50	-	2.47	2.47	2.27	2.94	1.41	0.37	0,0806
				2012	99	2.07	2.49	2.46	2.22	3.02	1.43	0.36	0,1523
				2013	79	-	2.15	2.75	2.37	3.44	1.55	0.38	0,7578
	Stone suture diameter	SsD	Cabrils	2011	49	-	1.59	1.83	1.53	2.15	0.80	0.31	0,8288
				2012	93	-	2.24	1.75	1.65	2.64	1.13	0.33	0,0023*
				2013	40	-	1.67	1.83	1.69	2.27	1.28	0.26	0,0258*
			Gimenells	2011	50	-	1.93	1.70	1.61	2.03	1.02	0.26	0,1811
				2012	99	1.65	1.82	1.80	1.68	2.32	0.89	0.31	0,0547
				2013	79	-	1.97	1.92	1.72	2.40	1.26	0.28	0.0590
	Flesh polar diameter	FlpD	Cabrils	2011	50	-	2.87	2.29	1.93	3.11	0.97	0.57	0,0063*
				2012	92	-	3.07	1.60	1.92	2.97	0.25	0.56	0.5399
				2013	39	-	2.87	2.17	2.40	3.68	1.53	0.51	0.5787
			Gimenells	2011	50	-	2.15	2.08	1.99	2.70	1.14	0.35	0.7612
				2012	99	0.60	2.09	2.14	2.14	3.40	1.15	0.39	0.1311
				2013	78	-	2.95	2.68	2.33	3.66	1.25	0.47	0.7429
	Flesh cheek diameter	FlcD	Cabrils	2011	50	-	3.30	3.47	2.49	3.68	0.70	0.75	0.0285
				2012	92	-	4.41	2.93	2.59	4.02	0.40	0.64	0.0314
				2013	39	-	3.87	2.59	3.05	4.41	1.19	0.68	0.9064
			Gimenells	2011	50	-	3.06	2.86	2.77	3.56	1.15	0.45	0.0501
				2012	99	0.65	3.01	3.03	3.09	4.49	1.74	0.52	0.1244
				2013	77	-	4.56	3.48	3.16	4.27	2.03	0.49	0.6347
	Flesh suture diameter	FlsD	Cabrils	2011	49	-	3.56	3.72	3.04	4.54	1.57	0.73	0.1527
				2012	92	-	3.67	3.20	2.98	4.53	0.83	0.65	0.1018
				2013	39	-	3.96	3.56	3.55	5.13	2.22	0.67	0.4121
			Gimenells	2011	50	-	3.85	3.39	3.40	4.85	2.13	0.56	0.9510
				2012	99	1.17	3.98	3.56	3.57	4.61	2.20	0.50	0.1357
				2013	78	-	4.27	4.07	3.81	5.15	2.27	0.54	0.8620
Leaf	Leaf perimeter	LP	Cabrils	2012	128	2659.85	2768.26	3091.12	2900.48	4504.56	1925.89	395.92	0,0008*
				2013	130	2824.36	2836.30	2861.35	3108.87	4343.02	2041.08	394.81	0,4011
			Gimenells	2012	135	3071.13	3169.17	2906.91	3119.58	4378.58	2515.62	295.68	0,0065*
				2013	141	3109.05	3147.63	2784.93	3022.84	3755.84	2382.69	294.15	0,632
	Leaf surface	LS	Cabrils	2012	128	178.83	208.92	221.45	234.26	369.87	115.98	52.05	0,2974
				2013	130	189.44	246.01	215.38	296.10	578.41	95.13	72.63	0,0361*
			Gimenells	2012	135	290.29	279.26	190.84	267.04	408.68	160.78	50.99	0,1203
				2013	141	296.94	275.64	195.80	269.73	436.96	122.33	52.10	0,1114
	Leaf blade width	LBW	Cabrils	2012	128	292.00	285.00	284.00	317.31	411.00	217.25	36.47	0,8563
				2013	130	285.50	315.25	289.50	355.11	503.33	175.25	45.15	0,0154*
			Gimenells	2012	135	394.00	331.00	259.00	335.77	455.50	242.00	36.94	0,1756
				2013	141	389.00	340.25	272.88	339.67	439.33	257.50	34.33	0,0076*
	Leaf length	LL	Cabrils	2012	128	1127.75	1194.86	1354.00	1204.99	1496.75	818.80	142.78	0,4331
				2013	130	1229.83	1252.25	1261.88	1340.68	1846.50	873.25	172.54	0,4766
			Gimenells	2012	135	1310.00	1369.00	1258.00	1316.12	1783.50	1064.00	121.42	0,0175*
				2013	141	1314.00	1362.75	1228.75	1302.61	1608.83	940.00	131.10	0,8946
	Leaf blade length	LBL	Cabrils	2012	128	903.50	1111.00	1148.00	1096.81	1373.00	758.20	128.64	0,6056
				2013	130	961.50	1172.71	1087.63	1215.48	1657.25	778.75	158.87	0,8313
			Gimenells	2012	132	1096.00	1241.00	1089.00	1185.62	1649.00	931.67	111.99	0,0309*
				2013	141	1112.14	1201.25	1053.38	1172.61	1482.83	881.50	118.45	0,9197
	Petiole length	PL	Cabrils	2012	128	224.25	83.86	206.00	108.18	191.25	59.90	27.31	0,0325*
				2013	130	268.33	89.16	174.25	125.21	192.00	33.00	26.75	0,2933
			Gimenells	2012	132	214.00	128.00	169.00	129.51	196.50	58.60	25.69	0,4593
				2013	141	201.86	161.50	175.38	130.00	240.75	58.50	25.75	0,0170*
	Leaf weight	LW	Cabrils	2012	117	0.25	0.23	0.24	0.32	0.51	0.17	0.08	0,0028*
				2013	130	0.32	0.30	0.29	0.38	0.75	0.12	0.10	0,0644
			Gimenells	2012	131	0.28	0.28	0.31	0.33	0.50	0.19	0.07	0,1088
				2013	141	0.37	0.33	0.24	0.33	0.65	0.18	0.07	<,0001*
	Chlorophyll content	CC	Cabrils	2011	157	40.10	38.20	43.10	40.98	51.00	23.10	5.39	<,0001*
				2012	145	35.90	39.80	40.70	41.43	51.40	23.60	4.81	<,0001*
				2013	146	41.60	37.60	39.10	42.35	51.60	27.60	4.40	0,0013*
			Gimenells	2011	143	39.60	37.60	42.00	38.42	47.50	24.80	3.64	<,0001*
				2012	150	39.00	40.70	37.40	38.32	46.20	24.80	3.61	<,0001*
				2013	159	39.20	42.20	46.00	36.84	47.60	26.30	3.65	0,3237

Annex 4. Pictures of flowers, fruits, stones and leaves of the parentals ('Texas', 'Earlygold' and the hybrid 'MB1.37') and some individuals of TxE and T1E progenies.

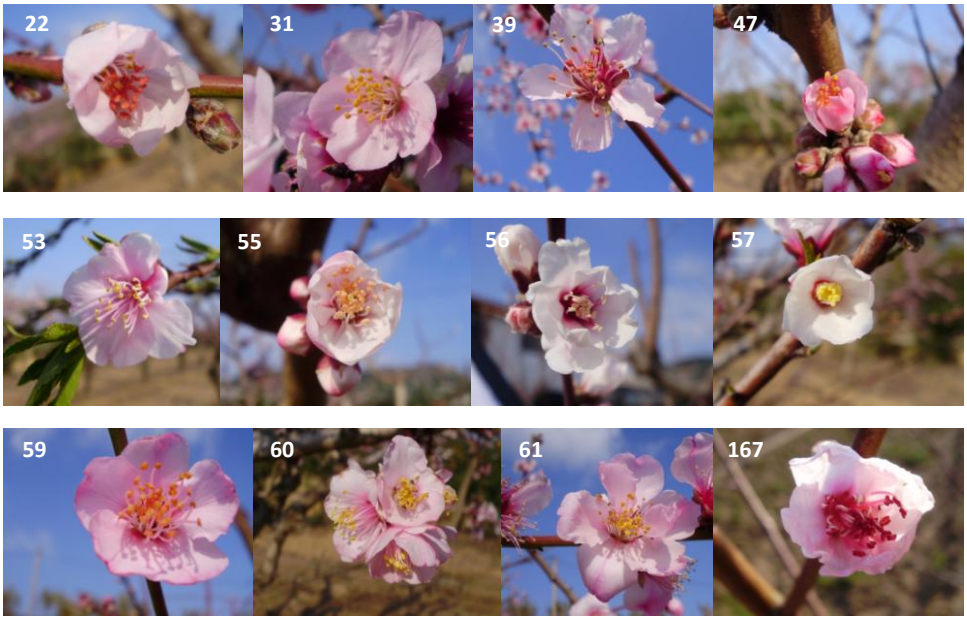
Parental Flowers

Texas

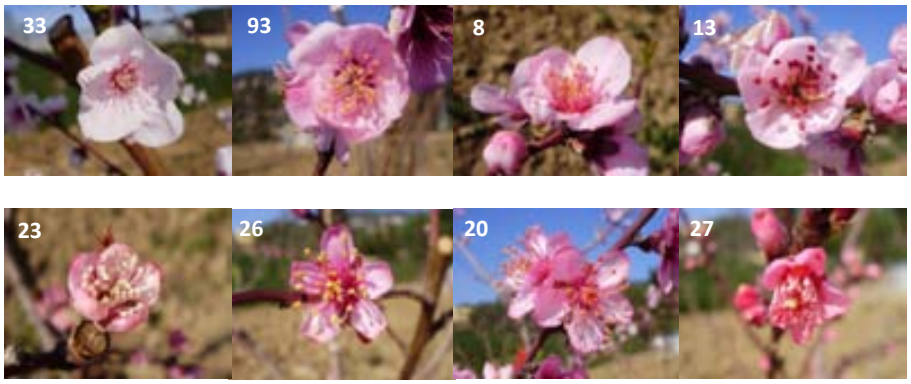
EG

F1

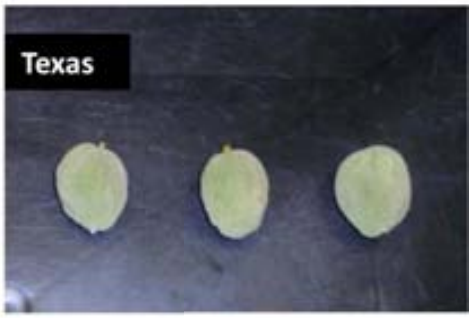
TxE - Flowers



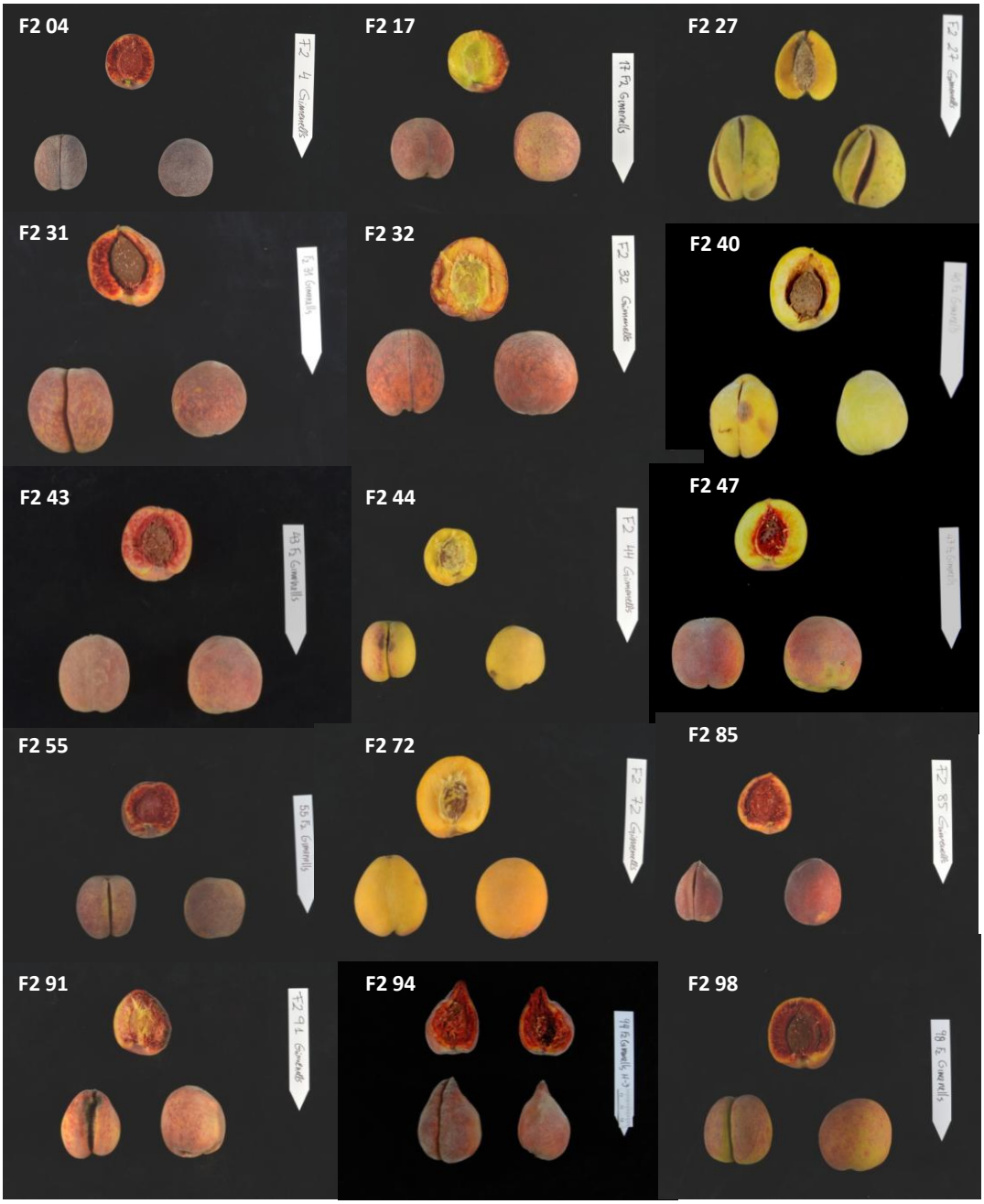
T1E - Flowers



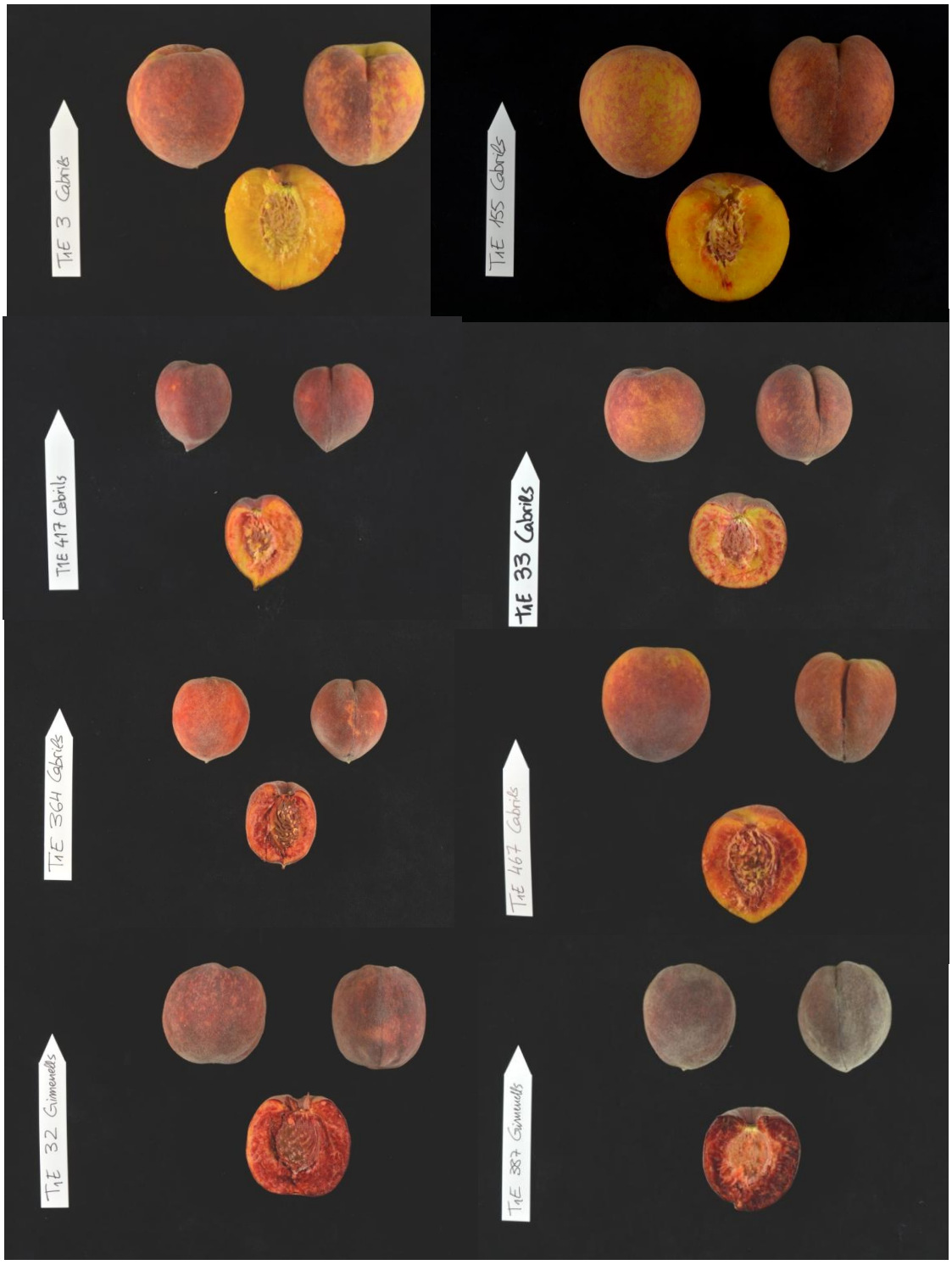
Parental fruits



TxE - Fruits

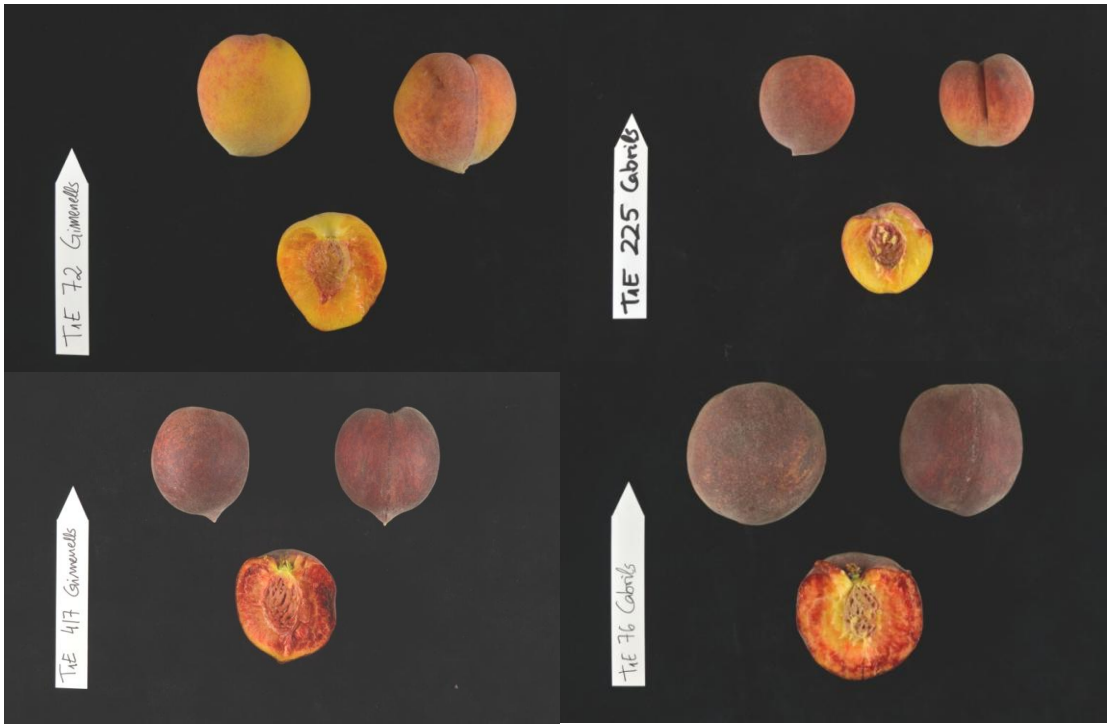

F2 04
F2 17
F2 27
F2 31
F2 32
F2 40
F2 43
F2 44
F2 47
F2 55
F2 72
F2 85
F2 91
F2 94
F2 98

T1E - Fruits

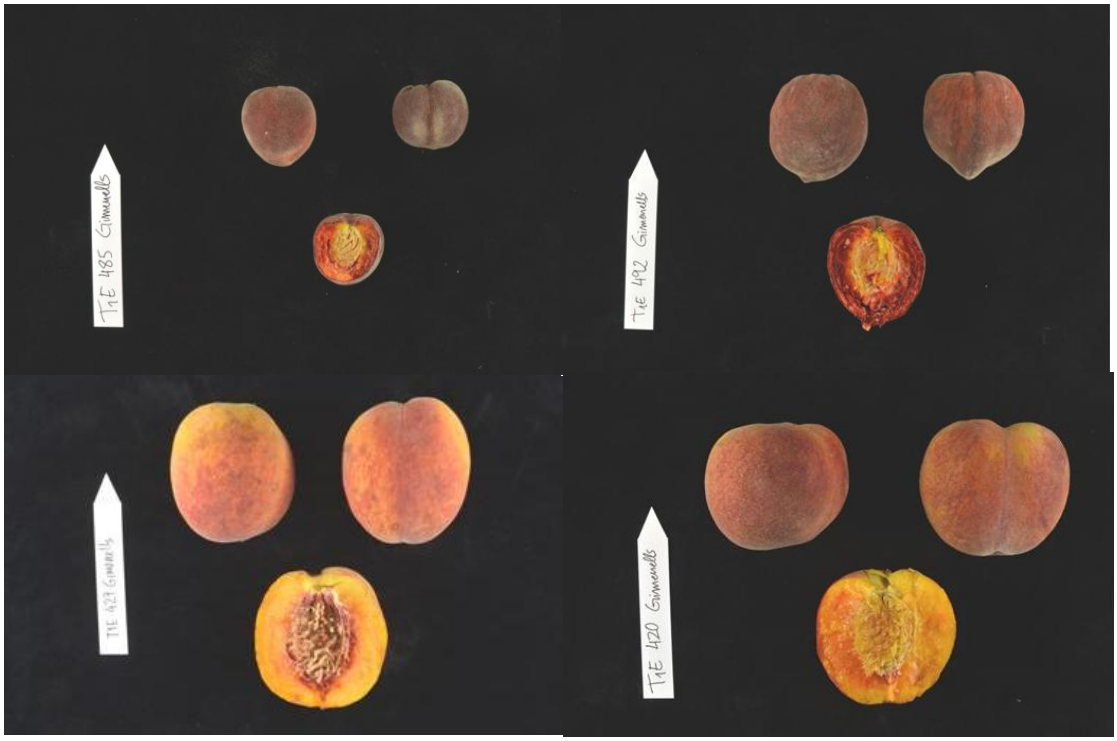

T1E 3 Cabrils
T1E 155 Cabrils
T1E 417 Cabrils
T1E 33 Cabrils
T1E 364 Cabrils
T1E 467 Cabrils
T1E 32 Guinevells
T1E 387 Guinevells

T1E- Percent skin color (PSC)

T1E- Intensity skin color (IEC)



T1E - Fruit weight (FW)



T1E - Stones 2010 (84 individuals, Texas, Earlygold and MB1.37)

Parental Leaves

Texas

F1

EG

T1E - Leaves

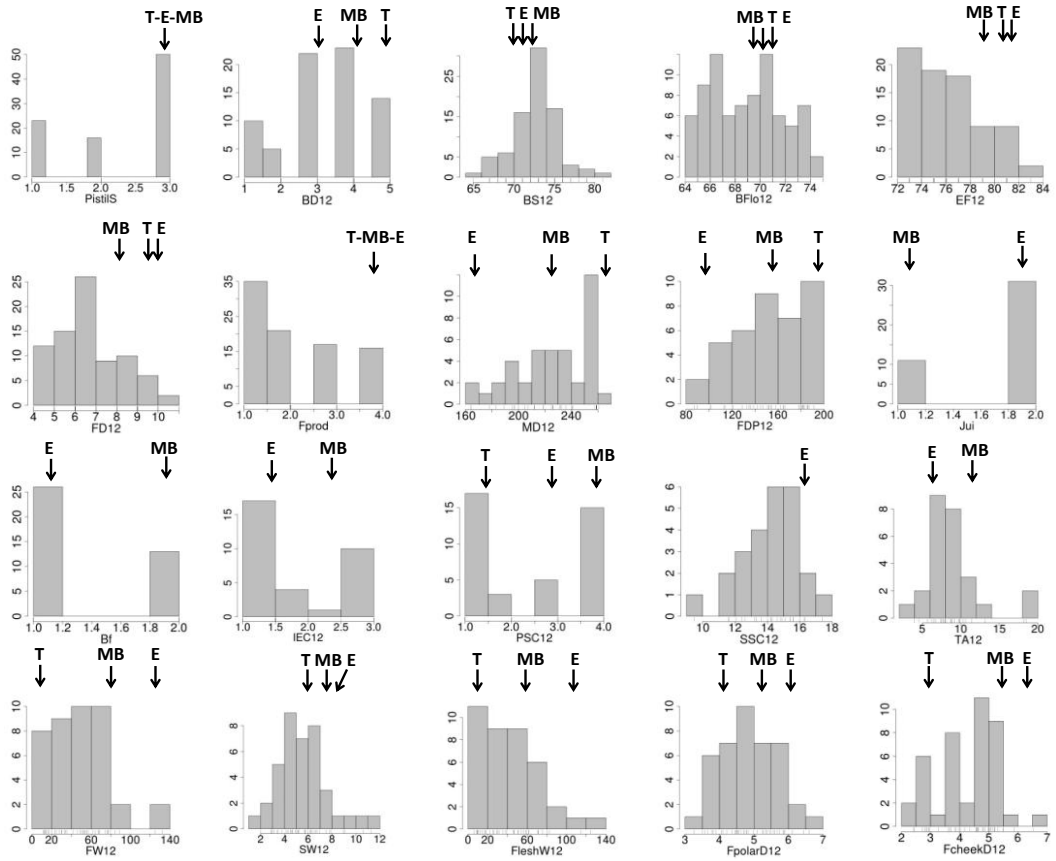
TxE - Leaves


20 F2 Gimenells
53 F2 Gimenells
95 F2 Gimenells
82 F2 Gimenells
108 F2 Gimenells
84 F2 Gimenells
70 F2 Gimenells
72 F2 Gimenells

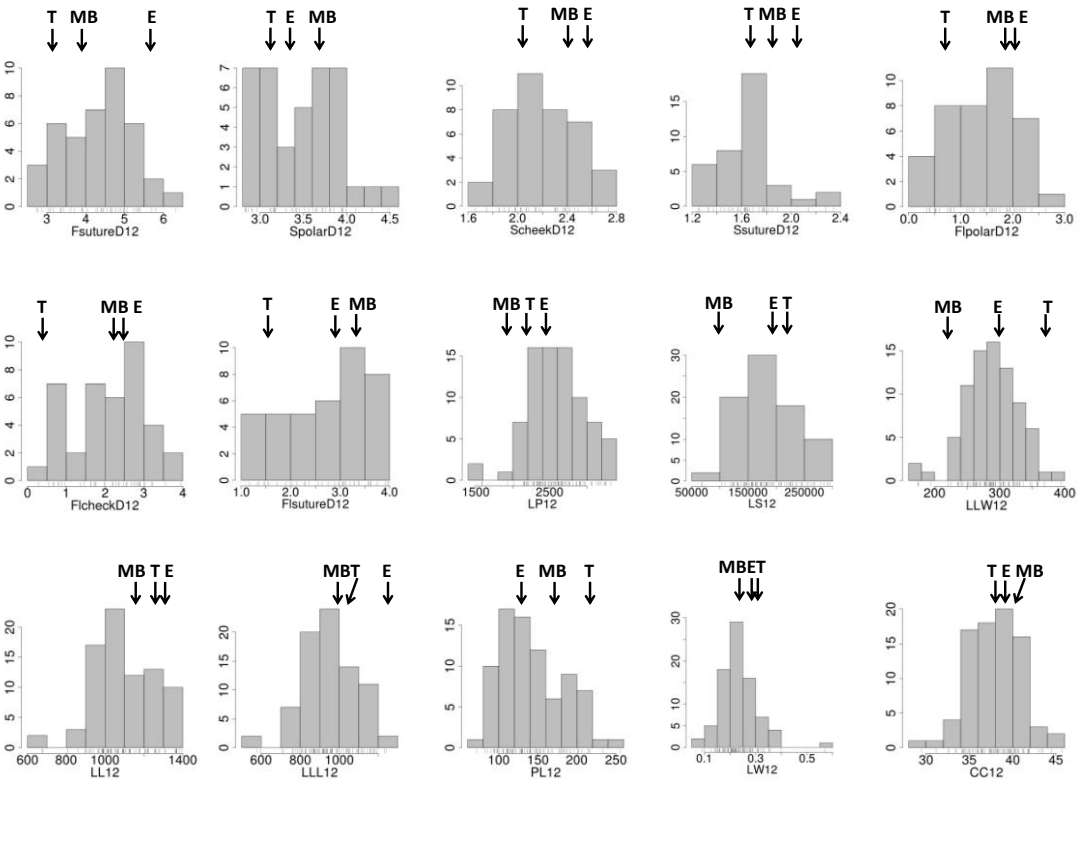
Annex 5.

Distributions of all traits evaluated in TxE and T1E progenies during 2012 in Gimenells represented as histograms. Arrows indicate the position of values detected for 'Texas'(T), 'Earlygold'(E) and 'MB1.37'(MB) .

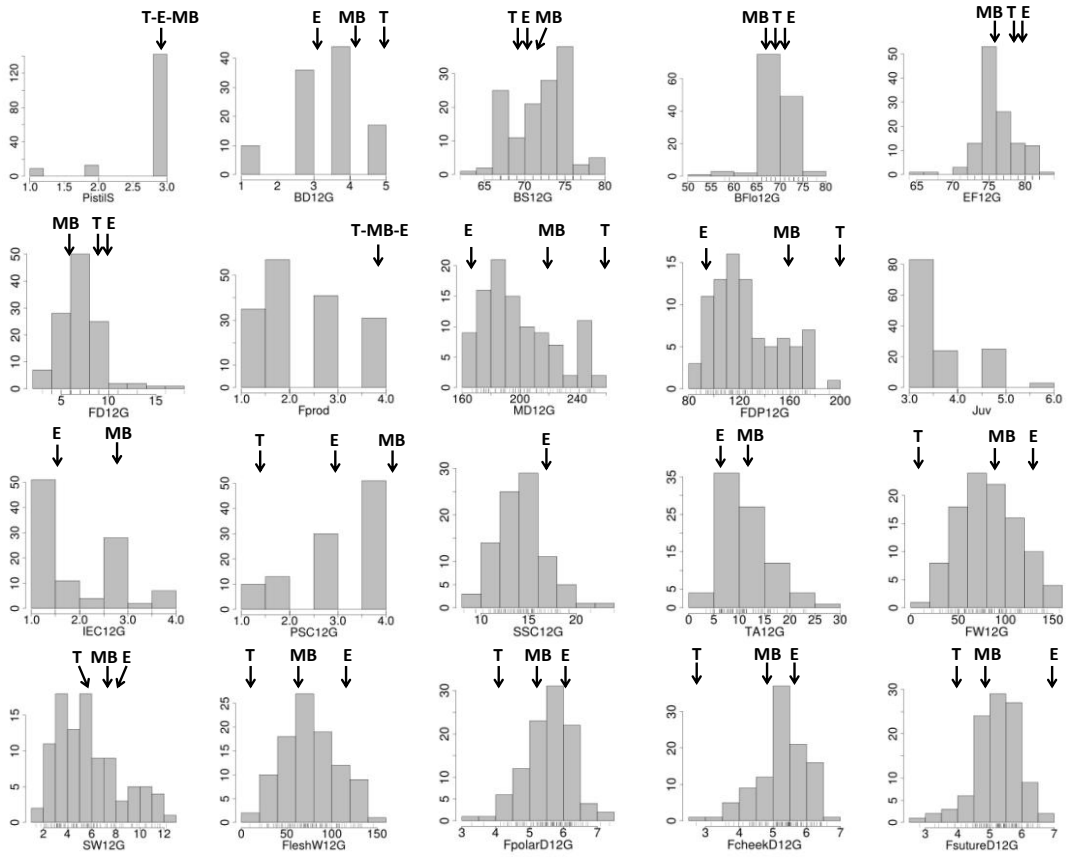
TxE histograms



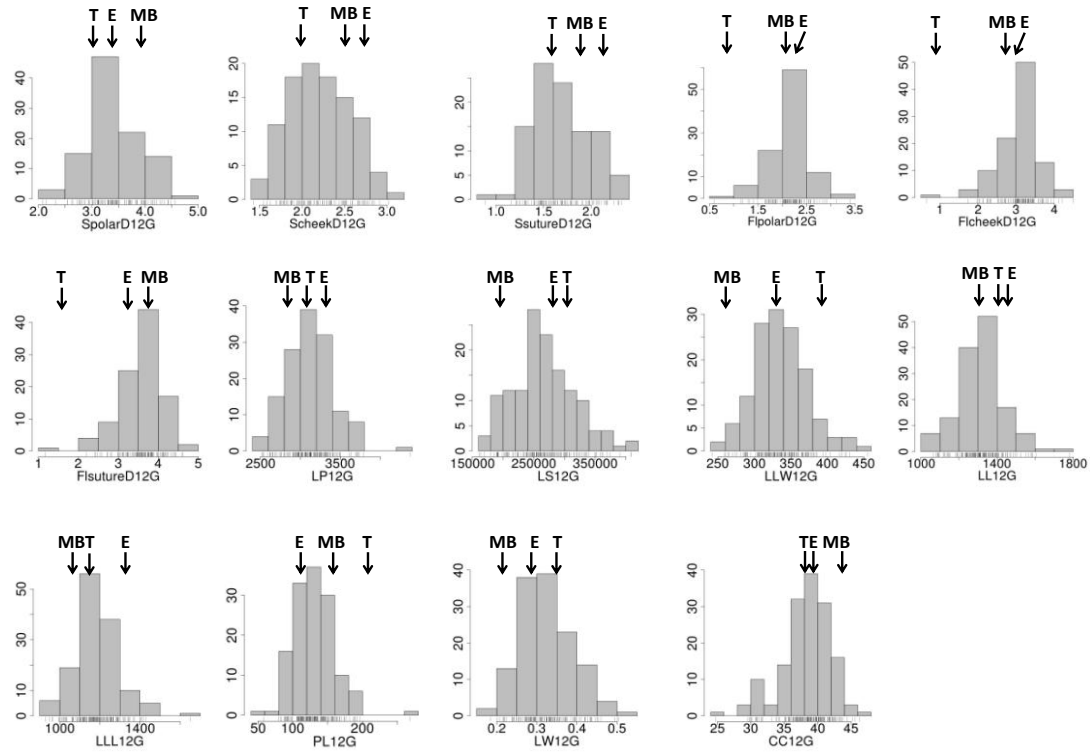
TxE histograms



T1E histograms



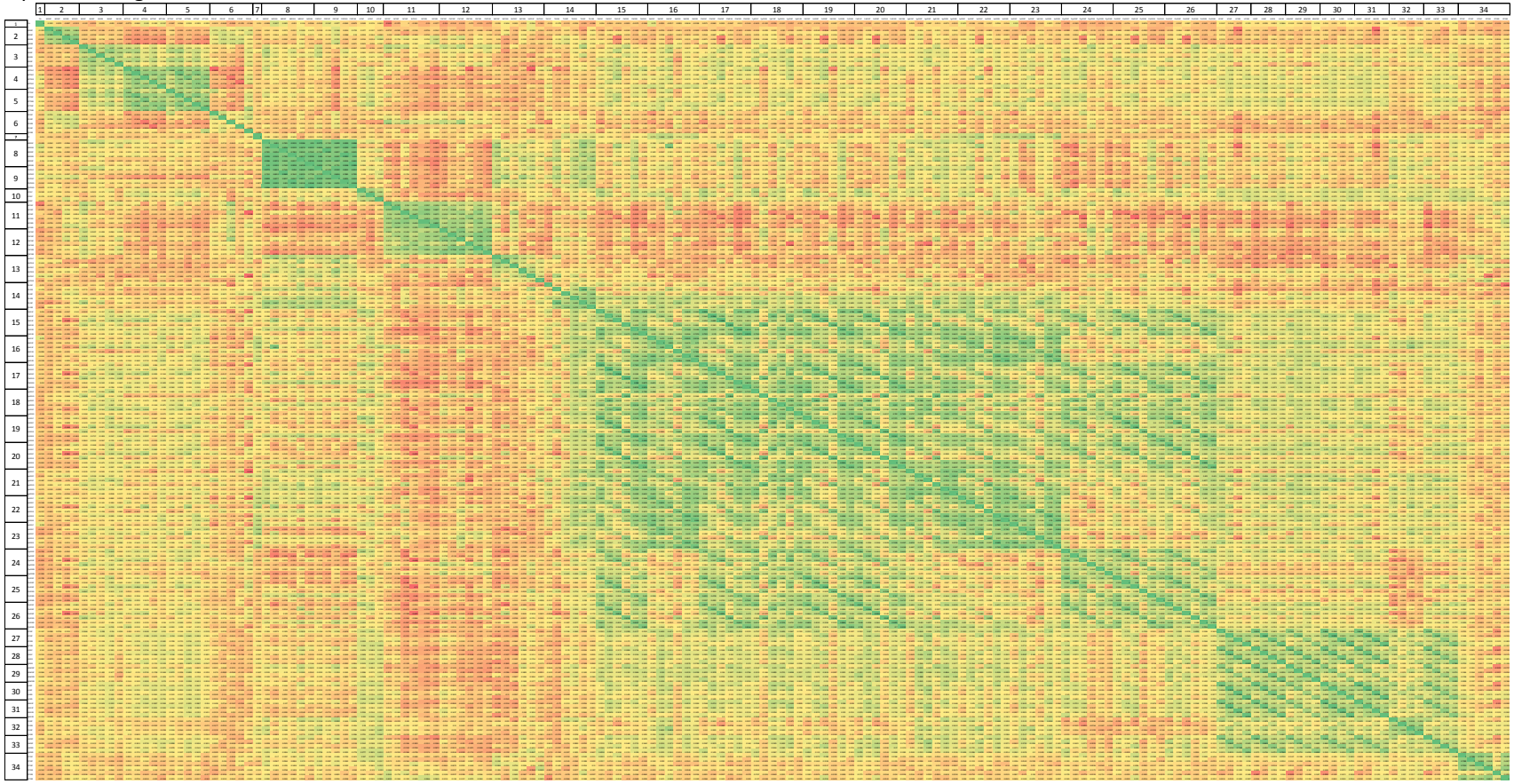
T1E histograms



Annex 6. Correlation coefficients between all quantitative traits measured during 2011, 2012 and 2013 for TxE progeny. The correlation coefficients were calculated with one mean value per genotype by Pearson test. The green color represents positive correlation values, the yellow color shows uncorrelated values and the red-orange color represents negative correlation.

1 = Pistil Size
2 = Blooming density
3 = Beginning of shooting
4 = Beginning of
5 = End of flowering
6 = Flowering duration
7 = Fruit production
8 = Maturity date
9 = Fruit development period
10 = Juiciness
11 = Blood flesh
12 = Intensity skin color
13 = Percentage skin color
14 = Solid soluble content
15 = Titratable acidity
16 = Fruit weight
17 = Stone weight
18 = Flesh weight
19 = Fruit polar diameter
20 = Fruit cheek diameter
21 = Fruit suture diameter
22 = Stone polar diameter
23 = Stone cheek
24 = Stone suture
25 = Flesh polar diameter
26 = Flesh cheek diameter
27 = Flesh suture
28 = Leaf perimeter
29 = Leaf surface
30 = Leaf blade width
31 = Leaf length
32 = Leaf blade length
33 = Petiole length
34 = Leaf weight
35 = Chlorophyll content

Annex 7. Correlation coefficients between all quantitative traits measured during 2011, 2012 and 2013 for T1E progeny. The correlation coefficients were calculated with one mean value per genotype by Pearson test. The green color represents positive correlation values, the yellow color shows uncorrelated values and the red-orange color represents negative correlation.



Annex 8

All putative QTLs identified in this study using the TxE and T1E progenies, including trait name, location, population where it was identified, QTL name (according to the GDR recommendations), year when phenotypic data was obtained, LOD score of the maximum peak, nearest marker, position of the maximum peak, and parameters for genic action estimation (*a, d, d/a*) and gene action. In TxE is ((A+B)/2), being the A the almond allele and in T1E is A-H, being A the homozygous almond allele. U: underdominant, D: dominance for peach allele; AD: partial dominance for peach allele; A: additive, AR: partial dominance for a allele; R: dominant for peach allele, O: overdominant.

QTLs Flower

Trait	Acronym	Population	Location	Year	G	LOD	Nearest marker	Position (cM)	R2	a	d	d / a	Genic action
Pistil length	PiL	T1E	Cabrils - Gimenells	2011-2013	1	3.05	SNP_IGA_107417	39.4	8.4	0.29			
					4	7.13	CPDCT045	24.7	18.4	-0.47			
		TxE	Gimenells	2011-2013	6	25.44	CPPCT030	76.2	74.4	0.28	1.21	4.39	O
Bloming density	BD	T1E	Gimenells	2012	1	6.70	CPPCT029	49.2	25.7	-1.14			
				2013	1	7.54	SNP_IGA_109897	42.2	23.8	-1.23			
		TxE	Gimenells	2013	1	2.54	SNP_IGA_129422	81.6	14.50	0.94	0.08	0.09	A

QTLs Phenology

Trait	Code	Population	Location	Year	G	LOD	Nearest marker	Position (cM)	R2	a	d	d / a	Genic action
Beginning of shooting	BS	T1E	Cabrils	2011	1	2.59	SNP_IGA_123719	53.6	7.4	2.94			
					8	3.93	CPPCT006	13.7	10.8	3.54			
				2012	4	4.38	SNP_IGA_383492	13.8	12.7	2.97			
					8	4.38	SNP_IGA_803758	7.0	12.7	2.84			
				2013	8	3.75	SNP_IGA_796755	1.6	11.6	4.93			
			Gimenells	2012	2	3.61	SNP_IGA_144919	3.8	12.1	2.38			
					8	2.55	SNP_IGA_802339	6.2	8.6	2.02			
				2013	2	4.54	CPSCT044	19.0	13.5	1.97			
		TxE	Gimenells	2012	6	3.14	SNP_IGA_694098	74.4	16.6	-0.73	2.81	-3.85	U
				2013	6	2.53	CPPCT030	76.2	13.4	-1.47	3.57	-2.43	U
		E	Cabrils	2011	7	4.24	SNP_IGA_779386	41.4	12.1	3.73			
				2012	4	3.11	SNP_IGA_370357	6.3	9.2	2.41			
					7	7.51	SNP_IGA_779386	41.4	21.8	3.72			
				2013	7	8.13	SNP_IGA_779386	41.4	23.5	7.03			
			Gimenells	2012	7	3.28	SNP_IGA_781455	45.3	11.0	2.30			
Begining of flowering time	BFT	T1E	Cabrils	2011	2	2.77	SNP_IGA_288668	47.3	9.1	-2.94			
					8	3.63	SNP_IGA_802339	6.2	11.5	3.26			
				2012	1	4.27	SNP_IGA_99943	28.4	12.3	-3.06			
					5	4.80	SNP_IGA_601135	34.5	13.8	-3.24			
					8	5.66	SNP_IGA_803758	7.0	16.0	-3.48			
				2013	1	3.95	SNP_IGA_31646	14.3	12.4	-6.52			
					5	3.95	SNP_IGA_601135	34.5	12.7	-6.39			
					8	4.02	SNP_IGA_796755	1.6	12.7	6.40			
			Gimenells	2012	1	8.62	SNP_IGA_109897	42.2	26.3	-3.31			
					2	3.14	pchgms1	28.6	10.5	-2.07			
				2013	1	6.39	SNP_IGA_101331	31.4	19.1	-2.82			
					2	2.86	CPPCT044	7.5	9.0	-1.94			
		TxE	Gimenells	2012	1	4.47	CPPCT010	80.1	23.8	-2.12	0.10	-0.05	A
				2013	1	3.68	CPPCT010	80.1	19.8	-2.32	0.56	-0.24	A
		E	Cabrils	2011	7	2.50	SNP_IGA_769471	45.3	11.0	2.30			
				2012	7	3.36	SNP_IGA_769471	28	8.7	2.84			
Endl of flowering time	EFT	T1E	Cabrils	2011	8	3.08	SNP_IGA_796755	1.6	12.1	3.42			
				2012	1	4.53	SNP_IGA_91583	23.3	13.1	-2.95			
					2	2.65	UDA-023	49.9	7.8	-2.32			
					5	2.57	SNP_IGA_601135	34.5	7.7	-2.25			
					8	4.63	CPSCT018	0.0	13.3	2.96			
				2013	1	6.70	SNP_IGA_96232	24.8	20.6	-6.45			
					2	2.85	SNP_IGA_288668	47.3	9.4	-4.38			
					8	5.44	SNP_IGA_796755	1.6	17.0	5.86			
			Gimenells	2012	1	6.79	SNP_IGA_10635	4.1	23.3	-2.42			
					2	3.41	SNP_IGA_275057	20.6	12.3	-1.74			
				2013	1	6.37	SNP_IGA_91583	23.3	19.8	-3.37			
		TxE	Gimenells	2012	1	4.29	SNP_IGA_10520	3.2	23.0	-1.95	0.27	-0.14	A
					3	4.26	EPDCU0532	43.7	24.0	2.02	-0.54	-0.27	A
		E	Cabrils	2011	7	3.62	SNP_IGA_776994	36.8	13.6	3.61			
				2012	7	4.65	SNP_IGA_778138	40.8	14.3	3.07			
				2013	7	2.50	SNP_IGA_776994	36.8	8.9	4.24			
Flowering duration	FD	T1E	Cabrils	2012	5	3.05	EPDCU4658	28.8	9.0	1.11			
				2013	5	2.53	CPSCT022	36.9	8.3	2.23			
			Gimenells	2012	1	6.24	SNP_IGA_109223	40.1	22.5	1.72			
		TxE	Gimenells	2012	7	3.08	SNP_IGA_782427	40.9	19.7	-1.08	-0.43	0.40	AR
Fruit production	FP	T1E	Cabrils - Gimenells	2011-2013	6	13.94	SNP_IGA_683956	30.6	33.3	1.19			
		TxE	Gimenells	2011-2013	6	11.39	CPPCT030	76.2	45.7	0.36	1.21	3.40	O
Maturity date	MD	T1E	Cabrils	2011	4	8.16	SNP_IGA_413115	36.1	44.8	30.99			
					8	3.06	SNP_IGA_859354	12.2	20.0	20.93			
				2012	4	12.06	SNP_IGA_409167	31.6	35.9	29.38			
				2013	4	4.47	SNP_IGA_412662	34.3	32.9	17.67			
			Gimenells	2011	4	10.91	SNP_IGA_420316	40.6	57.9	40.01			
				2012	4	11.81	SNP_IGA_413115	36.1	43.1	32.08			
				2013	4	9.71	EPPCU2000	37.0	44.9	33.49			
		TxE	Gimenells	2011	4	10.05	SNP_IGA_413115	35.2	81.9	45.31	-2.79	-0.06	A
				2012	4	11.41	SNP_IGA_412380	34.3	77.7	36.71	1.02	0.03	A
				2013	4	9.28	SNP_IGA_412380	34.3	76.1	37.05	4.18	0.11	A
		E	Cabrils	2012	4	4.92	BPPCT015	52.6	18.6	-20.01			
Fruit develepment period	FDP	T1E	Cabrils	2011	4	6.96	SNP_IGA_413115	36.1	43.2	29.43			
				2012	4	11.36	SNP_IGA_412380	31.6	34.2	29.07			
				2013	4	3.74	SNP_IGA_412380	33.4	28.3	18.15			
			Gimenells	2012	4	11.89	SNP_IGA_413115	36.1	48.8	34.63			
				2013	4	9.02	EPPCU2000	37.0	43.8	33.74			
		TxE	Gimenells	2012	4	11.52	SNP_IGA_412380	34.3	79.5	37.48	3.88	0.10	A
				2013	4	7.91	SNP_IGA_412380	34.3	75.3	38.11	6.67	0.18	A
		E	Cabrils	2012	4	4.29	BPPCT015	52.6	16.9	-18.98			
Leaf fall	LF	T1E	Cabrils	2010-2011	8	3.78	SNP_IGA_796755	1.6	11.1	12.20			
				2011-2012	1	3.29	SNP_IGA_55903	16.5	8.8	10.66			
					3	3.53	SNP_IGA_364833	39.4	9.6	11.05			
					8	4.27	SNP_IGA_796755	1.6	11.3	11.95			
				2012-2013	3	2.96	SNP_IGA_364833	39.4	9.0	8.81			
					8	3.53	SNP_IGA_869040	23.1	10.7	9.68			
Juvenility period	Juv	T1E	Cabrils	2006-2012	6	3.94	BPPCT025	24.1	12.6	-0.61			

QTLs fruit quality

Trait	Code	Population	Location	Year	G	LOD	Nearest marker	Position (cM)	R2	a	d	d / a	Genic action
Blood flesh	Bf	TxE	Gimenells	2011-2013	3	3.95	SNP_IGA_317001	18.0	50.7	-0.50	-0.14	0.29	AR
Juiciness	Jui	TxE	Gimenells	2011-2013	1	2.76	SNP_IGA_129422	81.6	31.8	0.40	0.21	0.53	AR
Intensity skin color	ISC	T1E	Cabrils	2012	1	3.64	CPPCT053	48.7	17.0	0.68			
					4	3.24	M12a	31.6	15.3	-0.66			
				2013	1	8.33	SNP_IGA_88751	22.6	59.9	1.40			
			Gimenells	2011	1	3.38	SNP_IGA_23251	5.5	25.1	0.91			
				2012	1	6.33	SNP_IGA_91583	23.3	25.2	0.92			
					4	5.55	SNP_IGA_409167	31.6	22.4	-0.88			
				2013	1	4.61	EPPCU5331	12.2	23.3	0.83			
					4	3.53	M12a	31.6	18.4	-0.76			
Percentage skin color	PSC	T1E	Cabrils	2011	1	3.16	SNP_IGA_123719	53.6	25.5	1.03			
					4	4.60	M12a	31.6	33.0	-1.13			
				2012	1	6.63	SNP_IGA_126668	52.8	30.3	0.98			
					4	2.93	SNP_IGA_412380	33.4	14.5	-0.68			
					8	3.07	CPPCT006	13.7	15.0	0.69			
				2013	1	8.08	BPPCT028	51.2	57.9	1.52			
					4	2.50	M12a	31.6	23.5	-0.95			
			Gimenells	2011	1	2.55	SNP_IGA_126668	52.8	19.9	0.88			
				2012	1	7.99	BPPCT028	51.2	30.5	1.09			
					4	6.25	M12a	31.6	24.8	-0.98			
				2013	1	3.96	SNP_IGA_123719	53.6	22.1	1.01			
					4	6.63	M12a	31.6	32.1	-1.21			
					8	3.12	SNP_IGA_885121	47.5	18.5	0.92			
		TxE	Gimenells	2011	3	3.99	SNP_IGA_317001	18.2	57.6	-1.50	0.35	-0.23	A
				2012	3	4.26	SNP_IGA_887061	24.7	41.7	-1.32	0.57	-0.43	AD
					4	3.75	SNP_IGA_418582	39.4	37.4	-1.12	0.34	-0.30	AD
				2013	3	3.00	SNP_IGA_321565	21.5	37.4	-1.33	1.23	-0.93	D
					4	3.22	SNP_IGA_412380	34.3	39.6	-0.81	-1.90	2.35	U
Soluble solid content	SSC	T1E	Cabrils	2011	1	2.55	CPPCT004	7.9	20.6	2.68			
				2012	1	4.98	EPPCU5331	12.2	26.3	3.94			
			Gimenells	2012	4	3.09	UDA-021	40.6	15.0	2.01			
Titratable acidity	TA	T1E	Cabrils	2013	1	3.17	EPDCU3489	35.3	27.2	3.89			
			Gimenells	2011	1	2.80	CPPCT003	21.2	21.6	3.65			
				2012	6	3.55	CPPCT21245	0	18.1	4.20			
				2013	1	3.58	CPPCT029	49.2	19.3	3.94			
					6	2.75	SNP_IGA_623894	9.2	15.2	3.37			
		TxE	Gimenells	2011	2	3.29	SNP_IGA_290243	49.9	67.7	3.78	-1.90	-0.50	AD
				2012	6	3.21	MA14a	71.6	46	6.09	-3.11	-0.51	AD
				2013	6	3.31	SNP_IGA_691196	69	43.6	7.37	-2.32	-0.31	AD
Fruit weight	FW	T1E	Cabrils	2012	1	3.20	SNP_IGA_17419	4.8	14.4	-20.74			
					2	4.98	UDA-023	49.9	21.3	-25.08			
			Gimenells	2011	6	2.71	CPP21413	0.0	21.3	24.80			
				2012	6	5.30	CPP21413	0.0	21.7	28.60			
					7	3.09	SNP_IGA_769687	13.9	13.5	22.47			
					8	3.11	CPSCT018	0.0	13.4	22.52			
				2013	6	3.58	CPP21413	0.0	18.9	28.08			
					7	3.18	PPCT057	18	17.7	-27.42			
		TxE	Gimenells	2011	4	6.56	SNP_IGA_410265	31.7	66.6	-13.87	24.80	-1.79	U
				2012	4	6.93	SNP_IGA_410265	31.7	57.2	-15.68	26.76	-1.71	U
				2013	4	4.65	SNP_IGA_410265	31.7	48.5	-10.73	33.94	-3.16	U
Stone weight	SW	T1E	Cabrils	2011	6	3.61	SNP_IGA_682735	28.8	28.1	-2.78			
				2012	2	4.29	PceGA34	45.1	19.3	2.21			
					6	9.35	CPPCT047	27.6	37.4	-2.98			
					8	3.24	SNP_IGA_796755	1.6	15.7	-1.92			
				2013	8	3.56	BPPCT006	7.8	33.7	-2.76			
			Gimenells	2011	2	2.69	SNP_IGA_284602	41.3	22.9	2.46			
					6	4.90	CPPCT047	27.6	37.5	-3.44			
					7	3.04	SNP_IGA_783262	29.9	25.3	2.38			
				2012	6	8.99	CPPCT047	27.6	35.3	-3.32			
					8	3.95	CPSCT018	0.0	17.4	-2.28			
				2013	2	3.63	UDP98-411	26.3	19.3	2.70			
					6	7.84	CPPCT047	27.6	37.1	-3.82			
					7	3.38	UDP98-408	12.9	18.1	2.52			
					8	3.90	SNP_IGA_795756	0.8	20.7	-2.69			
		TxE	Gimenells	2012	2	5.08	pchgms1	33.7	47.8	-2.11	-1.69	0.80	R
					6	4.05	SNP_IGA_686609	61.3	40.6	2.61	-0.79	-0.30	AD
				2013	2	3.80	pchgms1	33.7	41.2	-2.80	-1.13	0.40	AR
					6	4.69	MA14a	71.6	48.1	6.30	-4.55	-0.72	AD
Flesh weight	FlW	T1E	Cabrils	2012	2	3.75	UDA-023	49.9	17.1	-20.57			
			Gimenells	2011	7	3.34	SNP_IGA_790122	38.3	27.5	-25.17			
				2012	1	3.06	EPDCU3122	0.8	13.8	-21.53			
					4	3.28	SNP_IGA_409167	31.6	14.7	22.44			
					6	5.06	CPP21413	0.0	21.8	26.93			
					7	2.88	SNP_IGA_769687	13.9	13.3	-21.03			
				2013	6	3.59	CPP21413	0.0	19.1	26.59			
					7	2.86	CPPCT047	18.0	16.3	-24.75			
		TxE	Gimenells	2011	4	6.56	SNP_IGA_410265	31.7	66.6	-13.40	23.94	-1.79	U
				2012	4	6.70	SNP_IGA_410265	31.7	58.0	-15.60	26.35	-1.69	U
					5	3.24	SNP_IGA_586202	16.1	35.9	-12.11	-26.77	2.21	O
					6	3.09	SNP_IGA_613848	2.0	35.1	-26.45	-15.59	0.59	AR
				2013	4	4.78	SNP_IGA_410265	31.7	49.4	-10.29	31.86	-3.09	U
					5	2.85	SNP_IGA_586202	16.1	37.3	-19.29	-18.21	0.94	R

Fruit polar diameter	FpD	T1E	Cabrils	2012	1	3.15	SNP_IGA_17419	4.8	14.5	-0.65				
			Gimenells	2012	6	4.05	CPP21413	0.0	17	0.58				
				2013	6	3.72	CPP21413	0.0	19.7	0.64				
					7	3.17	pchgms6	11.4	17.1	-0.59				
		TxE	Gimenells	2011	4	5.72	SNP_IGA_410265	31.7	61.7	-0.61	0.78	-1.28	U	
				2012	4	5.62	SNP_IGA_410265	31.7	50.0	-0.47	0.79	-1.69	U	
				2013	4	2.90	SNP_IGA_410265	31.7	35.8	-0.31	0.85	-2.73	U	
Fruit cheek diameter	FcD	T1E	Cabrils	2012	2	3.68	SNP_IGA_288668	47.3	16.3	-0.66				
			Gimenells	2011	7	3.31	EPPCU5176	30.6	23.4	-0.71				
				2012	6	3.94	Ps7a2	1.1	16.6	0.57				
					7	3.56	SNP_IGA_769687	13.9	15.5	-0.55				
					8	3.22	CPSCT018	0.0	13.8	0.52				
				2013	7	3.66	CPPCT057	18.0	20.3	-0.61				
		TxE	Gimenells	2011	4	7.94	SNP_IGA_410265	31.7	73.3	-0.74	1.08	-1.45	U	
				2012	4	8.36	SNP_IGA_410265	31.7	64	-0.64	1.03	-1.60	U	
				2013	4	5.29	SNP_IGA_410265	31.7	55.1	-0.53	1.07	-2.01	U	
Fruit suture diameter	FsD	T1E	Cabrils	2012	1	3.35	SNP_IGA_17419	4.8	15.2	-0.63				
					2	3.30	UDA-023	49.9	14.8	-0.61				
				2012	6	5.86	Ps7a2	1.1	23.6	0.64				
			Gimenells		7	3.51	SNP_IGA_769687	13.9	15.1	-0.51				
					8	3.21	CPSCT018	0.0	13.8	0.49				
				2013	6	4.01	Ps7a2	1.1	21.1	0.62				
					7	3.01	pchgms6	11.4	16.3	-0.55				
		TxE	Gimenells	2011	4	8.04	SNP_IGA_410265	31.7	73.9	-0.65	0.85	-1.31	U	
				2012	4	8.16	SNP_IGA_410265	31.7	63.2	-0.64	0.85	-1.33	U	
				2013	4	4.42	SNP_IGA_410265	31.7	48.9	-0.49	0.91	-1.87	U	
Stone polar diameter	SpD	T1E	Cabrils	2012	5	3.78	EPDCU5183	26.9	17.1	0.42				
					6	5.39	CPP21413	0.0	23.4	0.49				
			Gimenells	2012	5	5.68	BPPCT037	17.9	23.2	0.51				
					6	7.80	SNP_IGA_623894	9.20	30.5	0.58				
				2013	5	7.24	BPPCT037	17.9	34.4	0.61				
					6	4.48	SNP_IGA_623894	9.2	23.3	0.51				
		TxE	Gimenells	2011	5	3.33	SNP_IGA_5947445	28.3	42.2	0.42	-0.05	-0.12	A	
				2012	5	3.44	SNP_IGA_5947445	28.3	35.6	0.44	-0.08	-0.18	A	
				2013	2	3.47	pchgms1	33.7	39.3	-0.37	-0.34	0.92	R	
					6	3.08	SNP_IGA_676571	50.1	40.7	0.50	-0.38	-0.76	D	
Stone cheek diameter	ScD	T1E	Cabrils	2011	6	4.14	SNP_IGA_629177	14.5	31.6	0.39				
				2012	6	7.64	SNP_IGA_680864	28.2	31.7	0.39				
				2013	6	2.77	BPPCT025	27.3	27.3	0.28				
			Gimenells	2011	6	4.17	BPPCT008	16.0	34.0	0.47				
				2012	6	11.44	CPPCT047	27.6	41.3	0.46				
					8	4.74	CPSCT018	0.0	19.8	0.32				
				2013	6	10.58	CPPCT047	27.6	46.0	0.54				
					7	3.35	pchgms6	11.4	17.7	-0.32				
					8	3.45	SNP_IGA_803758	7.0	18.6	0.32				
		TxE	Gimenells	2012	2	3.91	pchgms1	33.7	39.4	-0.23	-0.23	1.00	R	
					6	3.38	SNP_IGA_680032	56.7	35.2	0.29	-0.05	-0.17	A	
				2013	2	3.33	pchgms1	33.7	38.1	-0.29	-0.16	0.57	AR	
Stone suture diameter	FsD	T1E	Cabrils	2011	6	3.86	SNP_IGA_682735	28.8	30.9	0.38				
				2012	2	3.12	SNP_IGA_285058	35.1	14.8	-0.25				
					6	8.95	CPPCT047	27.6	35.8	0.39				
				2013	6	3.60	EPPCU4092	37.0	34.0	0.30				
			Gimenells	2011	6	4.41	pchcms5	20.7	33.4	0.33				
				2012	6	10.24	SNP_IGA_683956	30.6	38.4	0.38				
					8	4.31	CPSCT018	0.0	18.2	0.26				
				2013	2	3.12	SNP_IGA_284602	41.3	16.7	-0.25				
					6	10.17	SNP_IGA_683956	30.6	45.1	0.40				
					7	3.12	pchgms6	11.4	16.7	-0.23				
					8	3.10	SNP_IGA_7965756	0.8	16.7	0.23				
		TxE	Gimenells	2012	2	3.56	pchgms1	33.7	36.6	-0.21	-0.19	0.89	R	
					6	3.81	AMPA130	67.7	38.6	0.31	-0.10	-0.31	AD	
Flesh polar diameter	FlpD	TxE	Gimenells	2011	4	7.17	SNP_IGA_410955	33.8	69.3	-0.60	0.63	-1.05	D	
					6	3.32	SNP_IGA_612754	3.1	42.1	-0.67	-0.12	0.18	A	
				2012	4	8.25	EPPCU2000	36.9	65.2	-0.62	0.56	-0.90	D	
					5	4	SNP_IGA_571548	8.0	40.0	-0.20	-0.78	3.90	O	
				2013	4	4.54	SNP_IGA_410955	32.2	49.1	-0.36	0.62	-1.72	U	
					5	2.75	SNP_IGA_586202	16.1	33.5	-0.36	-0.63	1.75	O	
Flesh cheek diameter	FlcD	T1E	Cabrils	2012	1	3.45	CPPCT004	7.9	16.0	-0.52				
			Gimenells	2012	7	3.69	SNP_IGA_778002	17.7	15.9	-0.41				
		TxE	Gimenells	2011	4	8.67	SNP_IGA_412380	34.3	77.5	-0.88	1.02	-1.17	D	
				2012	4	8.28	SNP_IGA_410955	32.6	66.5	-0.60	1.01	-1.67	U	
					6	3.28	SNP_IGA_611511	5.0	35.2	-0.87	-0.20	0.23	A	
				2013	4	5.78	SNP_IGA_410265	31.7	58.1	-0.46	0.84	-1.82	U	
Flesh suture diameter	FlsD	T1E	Gimenells	2012	6	3.66	CPP21413	0.0	15.6	0.39				
					7	3.72	SNP_IGA_769687	13.9	16.4	-0.40				
				2013	6	2.53	SNP_IGA_607013	0.7	13.9	0.40				
		TxE	Gimenells	2011	4	8.18	SNP_IGA_412380	34.3	76.9	-0.75	0.84	-1.12	D	
				2012	4	7.72	SNP_IGA_412380	34.3	63.2	-0.58	0.78	-1.36	U	
					5	3.00	SNP_IGA_571548	8.2	32.2	-0.16	-0.87	5.26	O	
				2013	4	4.12	SNP_IGA_410265	31.7	46.5	-0.43	0.78	-1.83	U	

QTLs Leaf

Trait	Code	Population	Location	Year	G	LOD	Nearest marker	Position (cM)	R2	a	d	d / a	Genic action
Leaf perimeter	LP	T1E	Cabrils	2012	1	5.02	CPPCT004	7.9	16.9	-353.18			
				2013	1	7.14	CPPCT004	7.9	23.4	-393.61			
					8	5.10	SNP_IGA_799291	3.1	16.7	326.32			
			Gimenells	2012	8	4.12	SNP_IGA_795756	0.8	13.1	215.69			
				2013	1	2.79	CPPCT004	7.9	9.0	-177.85			
					8	5.70	SNP_IGA_796755	1.6	17.0	243.57			
		TxE	Gimenells	2012	1	3.40	CPPCT026	45.0	18.5	-228.65	3.64	-0.02	A
					3	4.48	EPPCU5990	0.0	24.4	-228.89	-198.03	0.87	R
Leaf surface	LS	T1E	Cabrils	2012	1	5.25	SNP_IGA_9197	3.4	17.4	-45506.30			
				2013	1	6.77	SNP_IGA_23351	5.5	21.8	-71488.70			
					7	3.04	CPPCT017	39.1	10.3	46565.50			
					8	3.50	SNP_IGA_800634	4.7	11.8	50407.50			
			Gimenells	2012	2	3.32	CPSCT021	38.1	10.7	-33464.50			
					7	3.30	EPDCU3392	41.9	10.7	33266.00			
					8	3.35	SNP_IGA_795756	0.8	10.8	33737.40			
				2013	1	3.71	SNP_IGA_2651	0.0	11.5	-35530.10			
					7	2.82	EPPCU5176	30.6	8.8	30825.00			
					8	3.99	SNP_IGA_796755	1.6	12.2	36602.50			
		TxE	Gimenells	2013	1	3.27	CPPCT026	45.0	18.2	-31120.70	2587.07	-0.08	A
					3	5.13	EPPCU5990	0.0	27.1	-33901.10	-26779.80	0.79	R
Leaf blade width	LBW	T1E	Cabrils	2012	1	2.74	SNP_IGA_2651	0.0	9.5	-23.32			
					6	3.10	pchcms5	20.7	10.6	23.68			
				2013	1	4.21	EPDCU3122	0.8	13.8	-35.36			
					6	4.57	BPPCT025	24.1	14.9	34.91			
			Gimenells	2013	1	3.14	SNP_IGA_2651	0.0	9.8	-21.64			
					6	4.63	pchcms5	20.7	14.0	25.66			
		TxE	Gimenells	2012	3	3.45	EPPCU5990	0.0	19.0	-20.49	-22.90	1.12	R
				2013	2	3.17	SNP_IGA_279439	29.5	17.5	-27.37	6.11	-0.22	A
Leaf length	LL	T1E	Cabrils	2012	1	6.78	CPPCT004	7.9	22.2	-138.87			
					8	2.89	SNP_IGA_800634	4.7	10.2	91.56			
				2013	1	6.40	CPPCT004	7.9	21.3	-165.32			
					8	5.45	SNP_IGA_799291	3.1	17.7	146.99			
			Gimenells	2012	8	4.01	SNP_IGA_795756	0.8	12.8	87.39			
				2013	8	5.68	SNP_IGA_796755	1.6	17.0	100.44			
		TxE	Gimenells	2012	1	3.62	CPPCT026	45.0	19.7	-98.52	-10.64	0.11	A
					3	4.26	EPPCU5990	0.0	22.7	-94.95	-78.30	0.82	R
Leaf blade length	LBL	T1E	Cabrils	2012	1	7.91	CPPCT004	7.9	25.3	-133.56			
				2013	1	8.05	CPPCT004	7.9	25.9	-167.89			
					8	4.13	SNP_IGA_799291	3.1	13.8	119.29			
			Gimenells	2012	8	3.47	SNP_IGA_795756	0.8	11.4	76.11			
				2013	1	2.78	CPPCT004	7.9	8.9	-71.43			
					8	4.27	SNP_IGA_796755	1.6	13.0	85.91			
		TxE	Gimenells	2012	3	4.05	EPPCU5990	0.0	21.8	-88.43	-57.88	0.65	AR
Petiole length	PL	T1E	Cabrils	2012	5	4.13	SNP_IGA_598784	27.4	13.8	20.22			
					6	3.37	MA14a	34.9	11.4	18.39			
					7	2.68	MA20a	25.8	9.2	16.53			
					8	4.48	SNP_IGA_859354	12.2	14.9	21.25			
				2013	5	3.44	SNP_IGA_590546	13.5	11.6	18.15			
					6	4.10	MA14a	34.9	13.5	19.63			
					7	4.16	CPPCT033	24.1	13.8	19.79			
					8	13.61	SNP_IGA_870509	28.3	39	34.07			
			Gimenells	2012	5	2.66	SNP_IGA_590546	13.5	8.9	15.34			
					7	3.12	PMS02	27.9	10.3	16.55			
					8	6.78	SNP_IGA_869040	23.1	22.6	25.47			
				2013	5	3.94	SNP_IGA_589750	12.9	12.4	18.09			
					6	2.64	MA040a	34.4	8.3	14.75			
					7	3.82	CPPCT033	24.1	11.7	17.59			
					8	10.61	SNP_IGA_869040	23.1	29.9	29.01			
		TxE	Gimenells	2012	5	3.06	SNP_IGA_594745	28.3	16.7	23.73	5.68	0.24	A
					7	3.32	SNP_IGA_781352	33.6	18.4	38.70	-36.47	-0.94	D
					8	2.66	SNP_IGA_876610	36.3	15	18.74	20.10	1.07	R
				2013	5	4.83	SNP_IGA_584315	14.8	28.1	29.22	14.09	0.48	AR
					8	7.35	SNP_IGA_881120	44.8	35.8	33.09	5.94	0.18	A
Leaf weight	LW	T1E	Cabrils	2012	1	3.89	SNP_IGA_9197	3.4	14.4	-0.06			
					6	3.35	pchcms5	20.7	12.3	0.05			
				2013	1	4.81	SNP_IGA_23251	5.5	15.9	-0.08			
					6	3.71	pchcms5	20.7	12.3	0.06			
					7	3.38	EPPCU5176	30.6	11.3	0.06			
					8	3.58	SNP_IGA_800634	4.7	11.9	0.06			
			Gimenells	2012	1	3.89	SNP_IGA_18927	5.5	13.5	-0.04			
					6	2.83	SNP_IGA_687583	33.8	9.5	0.04			
					7	3.38	EPDCU3392	41.9	11.2	0.04			
					8	4.02	CPSCT018	0.0	13.2	0.04			
				2013	1	3.59	SNP_IGA_17419	4.8	11.3	-0.04			
					8	6.21	SNP_IGA_796755	1.6	18.4	0.06			
		TxE	Gimenells	2012	1	3.32	PaCITA005	17.3	17	-0.04	-0.01	0.12	A
Chlorophyll content	CC	T1E	Cabrils	2011	1	3.32	SNP_IGA_121740	49.7	9.3	3.33			
					2	3.33	UDP96-013	21.6	8.5	-3.13			
					6	5.27	SNP_IGA_695974	42.0	14.5	-4.09			
					8	4.65	BPPCT006	7.8	12.8	3.85			
				2012	6	5.40	CPPCT021	43.0	15.8	-3.82			
				2013	1	3.51	SNP_IGA_2651	0.0	10.6	2.93			
					6	5.35	SNP_IGA_695974	42.0	15.7	-3.49			
					8	2.87	SNP_IGA_802339	6.2	8.7	2.59			
			Gimenells	2011	1	3.14	CPPCT053	48.7	9.6	2.20			
					8	2.72	SNP_IGA_800377	3.9	8.4	2.11			
				2013	1	4.45	CPPCT019	46.7	12.1	2.54			
					8	3.47	CPSCT018	0.0	9.6	2.25			
		TxE	Gimenells	2013	5	3.20	CPPCT013	30.1	16.7	-2.52	-1.03	0.41	AR

ABREVIACIONES

ADN: Deoxyribonucleic acid. Ácido desoxirribonucleico.
AFLP: Amplified Fragment Length Polymorphism. Polimorfismo de longitud de fragmentos amplificados.
ANOVA: Analysis of variance. Análisis de varianza.
BC: Backcross. Retrocruzamiento
BD: Blooming density Densidad de la floración.
BFT: Beginning of flowering time. Comienzo del período de floración.
BS: Beginning of shooting. Comienzo del período de brotación.
CC: Chlorophyll content. Contenido de clorofila.
CIM: Composite interval mapping. Mapeo por intervalos compuesto.
cM: centimorgan. centimorgan.
CRAG: Centre de Recerca en Agrigenòmica. Centro de investigación en Agrigenómica.
CTAB: Cetrimonium bromide. Bromuro de hexadeciltrimetilamonio
DH: Double Haploid Line. Línea doble haploide.
E: Genetic map of 'Earlygold' from T1E. Mapa genético del parental 'Earlygold' en T1E.
EFT: End of flowering time. Final del período de floración.
FAO: Food and Agricultural Organization of the United Nations. Organización para los alimentos y la agricultura de las Naciones Unidas.
FcD: **F**ruit cheek diameter. Diámetro mejilla del fruto
FD: Flowering duration. Duración de la floración.
FDP: Fruit development period. Período de desarrollo del fruto.
FlpD: Flesh polar diameter. Diámetro polar de la pulpa
FlcD: Flesh cheek diameter. Diámetro mejilla de la pulpa
FlsD: **F**lesh suture diameter. Diámetro sutura de la pulpa
FlW: Flesh weight. Peso de la pulpa
FpD: Fruit polar diameter. Diámetro polar del fruto
FP: Fruit production. Producción de fruta.
FsD: Fruit suture diameter. Diámetro sutura del fruto
FW: Fruit weight. Peso de la fruta
G: Linkage group. Grupo de ligamiento.
GDR: Genome database for Rosaceae. Base de datos genómica para Rosaceae.
IRTA: Institut de Recerca i Tecnologia Agroalimentàries. Instituto de Investigación y Tecnología Agroalimentarias.
ISC: Intensity of skin color. Intensidad del color de la piel.
Juv: Juvenility period. Período juvenil.
Jui: Juiciness. Jugosidad.
LBL: leaf blade length. Longitud de la lamina foliar.
LBW: leaf blade width. Anchura de la hoja
LD: Linkage disequilibrium. Desequilibrio de ligamiento.
LF: Leaf fall. Período de caída de hoja.
LL: Leaf length. Longitud de la hoja.
LS: Leaf surface. Área de la hoja

LP: Leaf perimeter. Perímetro de la hoja.
LOD: Log of Odds. Logaritmo de probabilidades.
LW: Leaf dry weight. Peso seco de la hoja.
MAI: Marker Assisted Introgression. Introgresión asistida por marcadores.
MAS: Marker Assisted Selection. Selección asistida por marcadores.
Mb: Mega pairs base. Mega pares de bases.
MD: Maturity date. Fecha de maduración de la fruta.
NIL: Near Isogenic Line. Línea casi isogénica.
PCR: Polymerase Chain Reaction. Reacción en cadena de la polimerasa.
PL: Petiole length. Longitud del peciolo.
PiL: Pistil length. Longitud de pistilo.
PPR: Pentatricopeptide. Pentatricopeptidos
PSC: percentage of surface covered by anthocyanin coloration. Porcentaje de coloración antociánico en la piel de los frutos.
QTL: Quantitative Trait locus. Locus de carácter cuantitativo.
RAPD: Random Amplified Polymorphic DNA. DNA polimórfico amplificado aleatoriamente.
RIL: Recombinant Inbred Line. Línea recombinante consanguínea.
RNase: Ribonuclease. Ribonucleasa.
Rf: Restorer of fertility. Restauradores de la fertilidad.
RFLP: Restriction Fragment Length Polymorphism. Polimorfismo de longitud de fragmentos de restricción.
ScD: Stone cheek diameter. Diámetro mejilla del hueso.
SIM: Simple interval maping. Mapeo simple por intervalo.
SNP: Single Nucleotide Polymorphism. Polimorfismo de un sólo nucleótido.
SpD: Stone polar diameter. Diámetro polar del hueso
SSC: Soluble solid content. Contenido de sólidos soluble.
SsD: Stone suture diameter. Diámetro sutura del hueso
SSR: Simple Sequence Repeat. Secuencia repetida en tandem, (microsatélite).
SW: Stone weight. Peso del hueso
TA: Titratable acidity. Acidez titulable.

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