ERASMUS MUNDUS

MASTER IN PLANT BREEDING

UniLaSalle Terre & Sciences

Universitat Politècnica de València

Institut Valencià d'Investigacions Agràries

Analysis of the inheritance patterns in tetraploid pummelo 'Chandler' and 'Carrizo' citrange

Final Master's Thesis presented by
Ana Cristina Benedict Villagrán

to qualify for the academic degree of Master's Degree in
Plant Breeding
2019-2021

Tutor UPV: Dra. María José Díez

Tutor IVIA: Dr. Pablo Aleza Gil

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UNIVERSITAT
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Instituto de Conservación y Mejora
de la Agrodiversidad Valenciana

El/los Doctor/es D./Dª. María José Díez Niclós, Pablo Aleza Gil, Andrés García Lor profesor/es del Master Oficial en Mejora Genética Vegetal, en calidad de director/es del Trabajo de Fin de Máster, por la Presente,

RECONOCE/N

Que el Trabajo Fin de Máster realizado por el/la alumno/a

D./Dª.: Ana Cristina Benedict Villagrán, con el título: "Analysis of the inheritance patterns in tetraploid pummelo 'Chandler' and 'Carrizo' citrange" y realizado bajo mi/nuestra dirección, reúne las condiciones necesarias para completar la formación del alumno y por tanto,

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La presentación del citado Trabajo Final de Máster para su defensa ante el correspondiente Tribunal.

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Máster Oficial en Mejora Genética Vegetal

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ABSTRACT

Citrus fruits is one of the most expansively and important fruit-tree crop, where approximately 80% of the production is destined to the fresh-fruit market. In the European Union, Spain in the number one ranking citrus producing country followed by Italy, Greece and Portugal. The Valencian Community is one of the main producing regions for oranges, mandarins, lemons and grapefruits in Spain. One of the main demands of the citrus fresh fruit market, from the point of view of consumers, is seedlessness in fruit. This characteristic can be attainted by using triploid hybrids since they are usually sterile, resulting in the production of seedless fruit as well as in the inability to induce seed formation in other citrus fruit varieties by cross-pollination. There are various approaches to produce these desired triploid hybrids: through sexual hybridization of two diploid parents (2x X 2x) and by sexual interploid hybridization (4x X 2x or 2x X 4x). The tetraploid parent can be obtained by selection of tetraploid individuals that arise from spontaneous somatic chromosome doubling of the nucellar cells or through the induction of somatic chromosome doubling with chemical agents, such as colchicine, also called double-diploid genotypes. When working with tetraploid genotypes for the production of triploid hybrids it is important to determine the chromosome segregation model of the tetraploid parent genotypes for the production of triploid hybrids to be able to comprehend how markers or genes, possibly related to resistance to abiotic and biotic factors, may segregate in a given hybridization. There are two extreme chromosome segregation models: the disomic segregation model and the tetrasomic segregation model. However, an intermediate inheritance has also been reported and can be a possibility. Therefore, the objective of this study was to determine the chromosome segregation model of tetraploid 'Chandler' pummelo and 'Carrizo' citrange. In order to achieve these objectives the triploid progeny of two interploid hybridizations – diploid 'Tomatera' clementine as female parent X tetraploid 'Chandler' pummelo as male parent and diploid 'Fina' clementine X tetraploid 'Carrizo' citrange – were analyzed with molecular markers, Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs) markers, distributed homogenously among the nine citrus linkage groups (LG), of the reference Clementine genetic map, to be able to determine the genetic structure of the diploid gametes that result from the tetraploid parents. Once the genotypes of each diploid gamete was identified it was possible to estimate the percentage of heterozygosity restitution for each marker conclude that tetraploid 'Chandler' pummelo presents tetrasomic iniheritance while tetraploid 'Carrizo' citrange predominantly presented disomic inheritance. This was determined by using a maximum-likelihood approach from the analysis of the marker closest to the centromere for each LG. The results obtained from this study will be of great value for breeding programs with the objective to produce triploid hybrids for the production of seedless fruits to be able to analyze the segregation pattern of markers involved in resistance to abiotic and biotic factors. They will also be of use for rootstock breeding to be able to compile multilocus structures from different rootstocks to end up with a genotype that presents all the desired complementary characteristics.

Key words: Citrus, Triploid, SNPs and SSR markers, Disomic inheritance, Tetrasomic inheritance, Interspecificity.

RESUMEN

Los cítricos son uno de los cultivos frutales más importantes y ampliamente cultivados en el mundo, donde aproximadamente el 80% de la producción se destina al consumo en fresco. En la Unión Europea, España es el primer país productor seguido por Italia, Grecia y Portugal. La Comunidad Valenciana es una de las principales regiones españolas productoras de naranjas, mandarinas, limones y pomelos. Una de las principales demandas del mercado de los cítricos para consumo en fresco, es la producción de frutos sin semillas ya que los consumidores no aceptan la presencia de estas en los frutos. Esta característica se puede lograr mediante la producción de híbridos triploides ya que estos generalmente son estériles, resultando en la producción de frutos sin semillas, y evitando la formación de semillas en otras variedades de cítricos por polinización cruzada. Hay varias estrategias para la obtención de híbridos triploides: a través de la hibridación sexual entre dos parentales diploides (2x X 2x) y mediante la hibridación sexual interploide (4x X 2x o 2x X 4x). El parental tetraploide puede obtenerse mediante la selección de individuos tetraploides que surgen de la duplicación espontánea del número de cromosomas en las células nucelares o mediante la inducción de la duplicación del número de cromosómas utilizando agentes químicos, como por ejemplo la colchicina. Cuando se trabaja con genotipos tetraploides para la producción de híbridos triploides, es importante determinar el modelo de segregación cromosómica del genotipo tetraploide. Hay dos modelos extremos de segregación cromosómica: el modelo de segregación disómica y el modelo de segregación tetrasómica. Sin embargo, también se ha demosrado la posibilidad de herencia intermedia. Es importante determinar el modelo de segregación de los parentales tetraploides para la producción de híbridos triploides para poder comprender cómo marcadores o genes, posiblemente relacionados con la resistencia a factores abióticos y bióticos, pueden segregar en una hibridación determinada. Por lo tanto, el objetivo de este estudio fue determinar el modelo de segregación cromosómica de los genotipos tetraploides 'Chandler' pummelo y citrange 'Carrizo'. Con el fin de lograr estos objetivos, se obtuvieron dos poblaciones de híbridos triploides a partir de dos hibridaciones interploides entre clementina 'Tomatera' diploide como parental femenino X 'Chandler' pummelo tetraploide como parental masculino y clementina 'Fina' diploide como parental femenino X citrange 'Carrizo' tetraploide como parental masculino. Las dos poblaciones de híbridos triploides se analizaron con marcadores moleculares, Single Nucleotide Polymorphisms (SNPs) y marcadores Simple Sequence Repeats (SSRs), distribuidos homogéneamente en los nueve grupos de ligamiento del mapa genético de referencia de Clementina, para poder determinar la estructura genética de los gametos diploides que resultan de los parentales tetraploides. Una vez que se identificaron los genotipos de cada gameto diploide, fue posible estimar el porcentaje de restitución de heterocigosidad para cada marcador y concluir que tetraploide 'Chandler' pummelo presenta herencia tetrasómica mientras que el 'Carrizo' citrange tetraploide muestra principalmente herencia disómica. Esto se determinó utilizando un enfoque de máxima verosimilitud a partir del análisis del marcador más cercano al centrómero para cada LG. Los resultados obtenidos de este estudio serán de gran valor para programas de mejoramiento con el objetivo de producir híbridos triploides para la producción de frutos sin semillas para poder analizar el patrón de segregación de marcadores involucrados en la resistencia a factores abióticos y bióticos. También serán de utilidad para el mejoramiento de portainjertos para poder recopilar estructuras multilocus de diferentes portainjertos para terminar con un genotipo que presente todas las características complementarias deseadas.

Palabras clave: Cítricos, Triploides, Marcadores SNP y SSR, Herencia disómica, Herencia tetrasómica, Interespecificidad.

RESUM

Els cítrics són un dels cultius fruiters més importants i àmpliament cultivats en el món, on aproximadament el 80% de la producció es destina al consum en fresc. A la Unió Europea, Espanya és el primer país productor seguit per Itàlia, Grècia i Portugal. La Comunitat Valenciana és una de les principals regions espanyoles productores de taronges, mandarines, llimes i aranges. Una de les principals demandes del mercat dels cítrics per a consum en fresc, és la producció de fruits sense llavors ja que els consumidors no accepten la presència de llavors en els fruits cítrics. Aquesta característica es pot aconseguir mitjançant la producció d'híbrids triploides ja que aquests generalment són estèrils, resultant en la producció de fruits sense llavors, a més d'evitar la formació de llavors en altres varietats de cítrics per pol·linització creuada. Hi ha diverses estratègies per a l'obtenció d'híbrids triploides: a través de la hibridació sexual entre dues parentals diploides (2x X 2x) i mitjançant la hibridació sexual interploide (4x X 2x o 2x X 4x). El parental tetraploide pot obtindre's mitjançant la selecció d'individus tetraploides que sorgeixen de la duplicació espontània del nombre de cromosomes en les cèl·lules de la nucela o mitjançant la inducció de la duplicació del número de cromosomes utilitzant agents químics. Quan es treballa amb genotips tetraploides per a la producció d'híbrids triploides, és important determinar el model de segregació cromosòmica del genotip tetraploide. Hi ha dos models extrems de segregació cromosòmica: el model de segregació disómica i el model de segregació tetrasómica. No obstant això, també s'ha reportat la possibilitat d'herència intermèdia. És important determinar el model de segregació dels parentals tetraploides per a la producció d'híbrids triploides per a poder comprendre com marcadors o gens, possiblement relacionats amb la resistència a factors abiòtics i biòtics, poden segregar en una hibridació determinada. Per tant, l'objectiu d'aquest estudi va ser determinar el model de segregació cromosòmica dels genotips tetraploides 'Chandler' pummelo i 'Carrizo' citranje. Amb la finalitat d'aconseguir aquests objectius, es van obtindre dues poblacions d'híbrids triploides a partir de dues hibridacions interploides entre clementina 'Tomatera' diploide com a parental femení X 'Chandler' pummelo tetraploide com a parental masculí i clementina 'Fina' diploide com a parental femení X citrange 'Carrizo' tetraploide com a parental masculí. Les dues poblacions d'híbrids triploides es van analitzar amb marcadors moleculars, Single Nucleotide Polymorphisms (SNPs) i marcadors Simple Sequence Repeats (SSRs), distribuïts homogèniament en els nou grups de lligament del mapa genètic de referència de Clementina, per a poder determinar l'estructura genètica dels gàmetes diploides que resulten dels parentals tetraploides. Una vegada que es van identificar els genotips de cada gàmeta diploide, va ser possible estimar el percentatge de restitució de heterocigosidad per a cada marcador i concloure que el tetraploide 'Chandler' pummelo presenta herència tetrasómica mentre que el tetraploide 'Carrizo' citrange presenta principalment herència disómica. Això es va determinar utilitzant un enfocament de màxima versemblança a partir de l'anàlisi del marcador més pròxim al centròmer per a cada LG. Els resultats obtinguts d'aquest estudi seran de gran valor per a programes de millorament amb l'objectiu de produir híbrids triploides per a la producció de fruits sense llavors per a poder analitzar el patró de segregació de marcadors involucrats en la resistència a factors abiòtics i biòtics. També seran d'utilitat per al millorament de portainjertos per a poder recopilar estructures multilocus de diferents portainjertos per a acabar amb un genotip que presente totes les característiques complementàries.

Paraules clau: Cítrics, Triploides, Marcadors SNP i SSR, Herència disómica, Herència tetrasómica, Interespecificidad.

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LIST OF ABBREVIATIONS

1. **SNPs:** Single Nucleotide Polymorphisms
2. **SSRs:** Simple Sequence Repeats
3. **LG:** Linkage groups
4. **SDR:** Second Division Restitution
5. **FDR:** First Division Restitution
6. **PMD:** Post-Meiotic Doubling
7. **PEG:** Polyethylene Glycol
8. **PHR:** Parental Heterozygosity Restitution
9. **PP:** Preferential Pairing
10. **DR:** Double Reduction
11. **IVIA:** Institut Valencià d'Investigacions Agràries
12. **CCP:** diploid 'Tomatera' clementine as female parent X tetraploid 'Chandler' pummelo as male parent
13. **CCC:** diploid 'Fina'clementine as female parent X tetraploid 'Carrizo' citrange as male parent
14. **FRET:** Fluorescence Resonance Energy Transfer
15. **PCR:** Polimerase Chain Reaction
16. **GBS:** Genotyping by Sequencing

I.   INTRODUCTION

A. Citrus Economic Importance

Citrus is one of the most expansively produced fruit-tree crops ("FAOSTAT," 2019). Citrus are mainly produced for the fresh-fruit market in the Mediterranean basin, being Spain the main producing country with a reported production of 6,010 thousand tons in 2019 (FAOSTAT, 2020).   When considering the European Union (EU), the main producing countries are Spain, Italy, Greece and Portugal, (Figure 1) (European Commission, 2019).
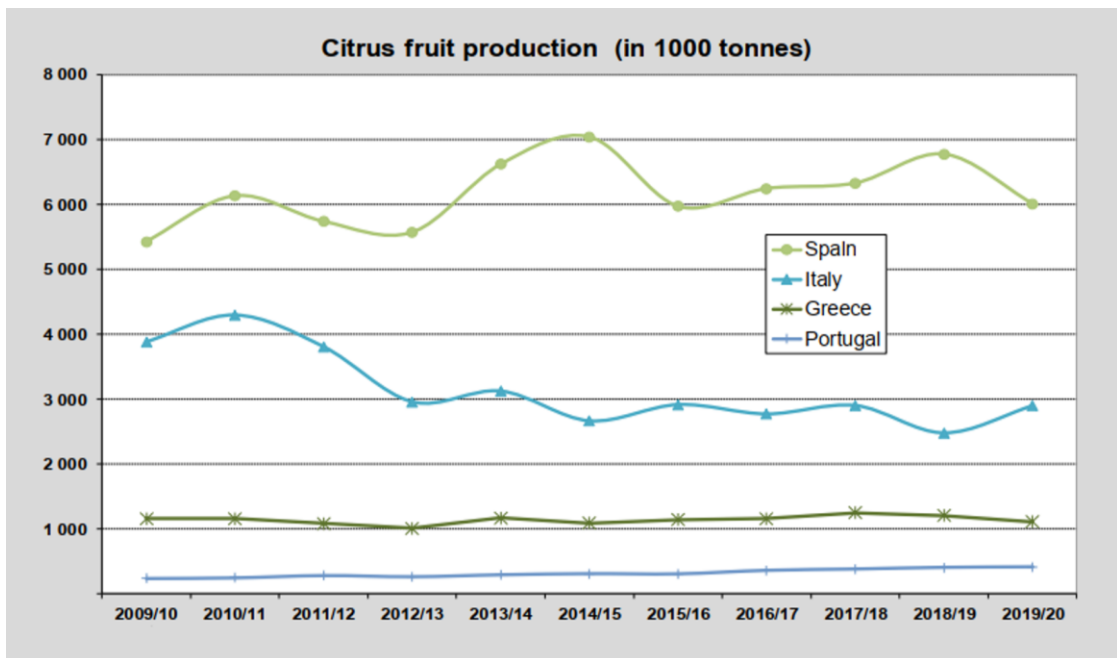
Figure 1. Citrus fruit production in the Mediterranean countries of the EU (European Commission, 2019)

1

The main citrus fruits produced in Spain are oranges, mandarins, lemons and grapefruits – the orange production being the most important. The production surface (or area) of oranges amounted to 129,926 hectares, 93,733 hectares of mandarins, 41,985 hectares of lemons and 2,274 hectares of grapefruits. However, it is important to mention that the production in hectares varied drastically when compared to the production in tons when considering losses. The production in tons of oranges was 167,112 tons, 252,741 tons of mandarins, 650,938 tons of lemons and 33,926 tons of grapefruits (Gobierno de España: Ministerio de Agricultura, n.d.).
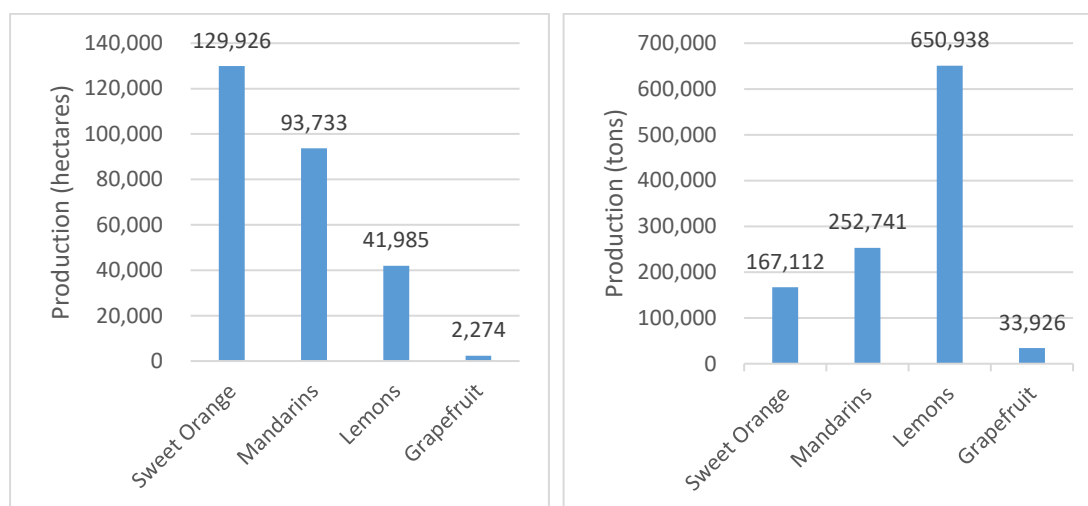
Figure 2. Citrus fruit production in Spain a) Production in hectares and b) Production in tons (Gobierno de España: Ministerio de Agricultura, n.d.)

Spain and Italy produce 80% of the total orange production in the European Union. Spain is ranked top sixth as a global citrus producing country and is ranked first as the global citrus exporter. Roughly 90% of the orange production comes from the main producing regions, the Valentian Community and Andalusia. It is important to note that of the oranges produced by Spain, 32% is destined for domestic fresh consumption, 18% for processing and 45% are destined for exportation. Of the oranges exported, 90% of them are destined to markets in the European Union – mainly Germany, France, the Netherlands, Italy and the United Kingdom – , while only 10% are destined to countries not within the European Union (USDA, Valverde, & Clever, 2019).

Considering the mandarin production, Spain is the leading producer and exporter in the European Union, with the main producing regions being the Valentian Community, Andalusia and Catalonia. In the mandarin market, the production of seedless varieties is the most important characteristic desired by consumers (USDA et al., 2019).

As for the lemon market, Spain ranks second, being Argentina the largest lemon producer in the world. However, Spain ranks first as the exporter of lemons destined to the fresh fruit consumption market with its main importing countries being Switzerland, Serbia, Canada and Norway. Spain also ranks second as the global producer of processed lemons, where approximately 20-25% of the lemons

produced are destined for processing. Its main producing regions are Murcia, the Valencia Community, Malaga and Almeria (USDA et al., 2019).

Lastly, considering the grapefruit market, Spain and Cyprus are the main producing countries in the European Union. The production in the European Union is however significantly lower than the demand, therefore requiring import from countries such as China, South Africa and Turkey among other countries (USDA et al., 2019).

B. Botanical classification, origin and diffusion of citrus

Carl Linneaus established the genus *Citrus* in 1753 (Swinge & Reece, 1967). The genus *Citrus* is found under the order *Geraniales*, the suborder *Geraniineae*, the family *Rutaceae*, the subfamily *Aurantioideae*, the tribe *Citreae* and the subtribe *Citrinae*. The subtribe *Citrinae* contains various genus including *Fortunella*, *Eremocitrus*, *Poncirus*, *Clymenia*, *Microcitrus* and *Citrus* which are considered as the true citrus (Swingle & Reece 1967).

Genotypes from the genus *Fortunella, Poncirus* and *Citrus* are commonly used as citrus cultivars or as rootstocks. *Fortunella* is of interest in breeding programs due to its cold hardiness and cold tolerance as well as resistance to citrus canker and *Phytophtora* (Krueger & Navarro, 2007). The genus *Poncirus* only contains one species, *Poncirus trifoliata* (L.) Raf, which is also characterized by its cold hardiness, ability to grow in acidic soils and resistance to the citrus tristeza virus. Therefore, *P. trifoliata* is commonly used for rootstock breeding programs to produce citranges (*C. sinesis* (L.) Osbeck X *P. trifoliata*), citrumelos (*C. paradisi* Macfad. x *P. trifoliata*) and citrandarins (*C. reticulata* Blanco x *P. trifoliata*) (Swinge and Reece, 1967), which are used extensively as rootstocks. Lastly, the genus *Citrus* has the greatest economic importance as citrus cultivars. There are two taxonomic systems that are used for the botanical classification of this genus: the Swingle's system and Tanaka's system (Swingle & Reece, 1967; Tanaka, 1977). These two systems remain in place since classifying *Citrus* taxa is difficult due to its broad morphological diversity, total sexual interspecific compatibility and the partial apomixis of many cultivars (Scora, 1975; Curk et al., 2016). The Swingle system (Swingle, 1943; Swingle & Reece, 1967) recognizes 16 species for the genus *Citrus* whereas Tanaka (1977) includes 162 species.

All citrus fruits and their ancestral relatives originated in South-east Asia, New Caledonia and Australia (Swingle & Reece, 1976; Webber 1967; Calabrese 1992). Though various biochemical studies (Malik et al., 1974; Scora, 1975) numerical taxonomy (Barrett & Rhodes, 1976) and recent molecular marker analysis (Barkley, Roose, Krueger, & Federici, 2006; Curk et al., 2016; Garcia-Lor et al., 2013; Garcia-Lor et al., 2012; Ollitrault et al., 2012) and genome sequencing (Wu et al., 2014; 2018) it has been established that the cultivated citrus varieties are a result from intricate interspecific admixtures of four different ancestral taxa; *C. medica* L. (citron), *C. reticulata* (mandarin), *C. maxima* (L.) Osb. (pummelo) and *C. micrantha* Wester (a Papeda cv. Wild citrus) (Curk et al., 2016; Garavello et al., 2020; Wu et al., 2018). These four taxa have originated in four different geographic zones: citron in northeastern India; mandarins in Vietnam, southern China and Japan; pummelo in the Malay Archipelago

and Indonesia; and *C. micrantha* in the Philippines (Cuenca et al., 2018). It is important to mention the secondary species present in the genus *Citrus* have resulted from reticulation events between the four ancestral species followed by a few interspecific recombinations and by subsequent natural mutations (Cuenca et al., 2018). These secondary species include: *C. sinensis* (sweet orange), *C. aurantium* (L.) (sour orange), *C. paradisi* (grapefruit), *C. limon* (L.) Osbeck (lemon) and *C. aurantiifolia* (Christm.) Swingle (lime). The four taxa and some of the secondary species found in the genus *Citrus* can be seen in Figure 3.
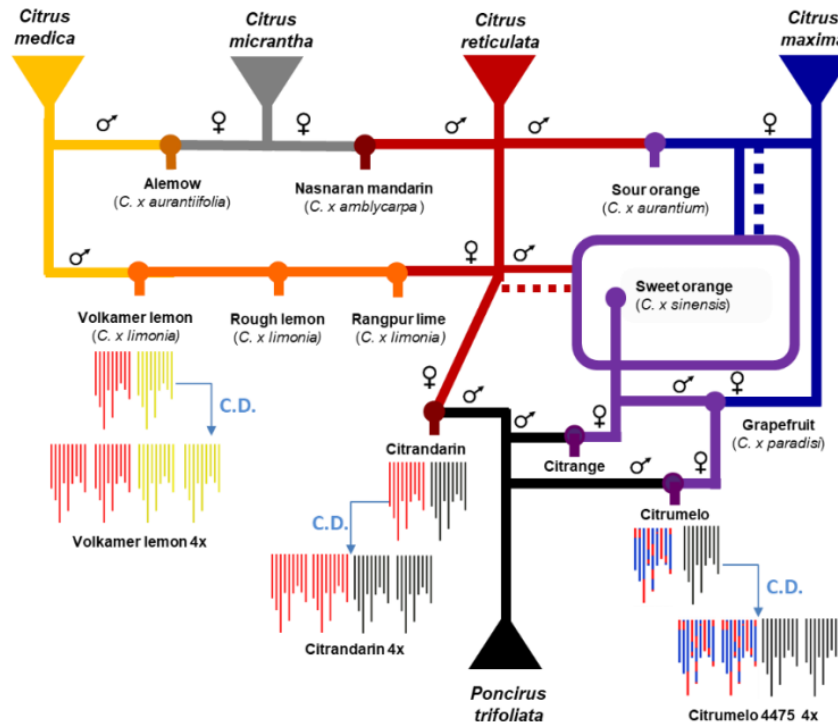
Figure 3. Citrus fruits and their ancestral taxa (Calvez et al., 2020)

C. Genome characteristics

Citrus have a diploid genome composed of nine chromosomes ($2n = 2x = 18$) (Krug, 1943) and has a genome size of approximately 367 Mb (Terol et al., 2008; Rouiss et al., 2018; Inglese et al., 2019). It is important to mention that most citrus are diploid, however a few triploid and tetraploid genotypes have been identified among citrus germplasm (Longley, 1925; Lee, 1988).

D. Triploid and tetraploid citrus and implications in breeding programs

Polyploidy is a valuable resource for citrus breeding (Ollitrault et al. 2008; Cuenca et al. 2015) and there are two main mechanisms; somatic doubling of chromosome number (somatic polyploidization) and meiotic nuclear restitution leading to unreduced gametes (sexual polyploidization).

Triploid citrus genotypes are being developed in breeding programs to satisfy the consumers demand for seedless citrus (Cameron & Frost 1968; Aleza et al., 2010; Aleza et al., 2012). There are two characteristics which play an important rol in the production of seedless triploid citrus fruits: parthenocarpy and sterility. Parthenocarpy is the ability to produce fruits without the need of pollination (Ahmed et al., 2020; Aleza al., 2012; Ollitrault et al., 2008; Terol et al., 2008). The second characteristic of importance is that triploid hybrids are usually sterile resulting in the production of seedless fruit and the inability to induce seed formation in fruits of other genotypes by cross-pollinations (Ollitrault et al., 2008). Triploid sterility is caused by (1) aneuploidy gametes – due to trivalent, bivalent and univalent associations – which results in very low fertility (Aleza et al., 2012a; Cameron & Frost 1968) and (2) the abortion of megasporogenesis (Aleza et al., 2010; Aleza et al., 2012a, Fatta Del Bosco et al, 1992). However, even with these two contributing factors that usually result in sterile genotypes there have been some rare cases of triploids producing fruit with few seeds as well as reports of their ability to induce seed formation in some citrus cultivars (Aleza et al., 2012).

Tetraploid genotypes, are not valuable for citrus fresh fruit production, due to characteristics such as being thornier, slower growth, less fruit production and poor fruit quality (Ruiz et al., 2020). The most common use for tetraploid genotypes is as parents in triploid breeding programs (Aleza et al. 2012a, 2012b) and as rootstocks since tetraploid genotypes can be more tolerant to abiotic stresses such as salt and water stress, as well as cold tolerance, when compared to their parental diploids (Aleza et al., 2011; Allario et al., 2013; De Souza et al., 2017; Garavello et al., 2020; Mouhaya et al., 2010; Oustric et al., 2018). Other studies have also demonstrated that tetraploid rootstocks tend to cause a reduction in the canopy size, which is also a desired trait in modern orchards since it reduces the costs associated to harvesting, treating with chemicals and pruning (Aleza et al., 2011; Barrett & Hutchingson, 1978; Lee, 1988). Therefore, selection of tetraploid genotypes from the most common rootstocks used seems to improve tolerance to abiotic stress without modifying allelic constitution, with high probabilities of transmitting traits related to disease resistance (Aleza et al., 2011).

E. Production of triploid hybrids

1. Sexual hybridization 2x X 2x

As first described by Cameron and Frost 1968, one way to produce triploid hybrids is through sexual hybridization with two diploid parents (2x X 2x) in which one of the gametes of either parent remains diploid, as it does not suffer chromosome reduction (Aleza et al., 2010; Esen & Soost, 1971; Garavello et al., 2020; Geraci et al., 1975) resulting in triploid individuals (Aleza et al., 2011). Triploids are usually obtained from the fertilization of a megagametophyte (2n), resulting from abortion of the second division of meiosis (SDR), and a haploid pollen (n) (Ahmed et al., 2020; Aleza, et al., 2012; Aleza et al., 2009; Esen & Soost, 1971; Geraci et al., 1975) although other mechanisms like FDR (First Division Restitution) and post-meiotic genome doubling (PMD) have also been reported with less frequency (Rouiss et al. 2017a; 2017b). Nevertheless, it is important to mention that the frequency of the production of 2n gametes is influenced by the genotype, ranging

below 1% to over 20% (Aleza et al., 2012a; Aleza et al., 2012b; Esen & Soost, 1971; Geraci et al., 1975; Cameron & Frost, 1968). Additionally, as first indicated by Esen and Soost 1971, most of the spontaneous triploids are present in seeds that are smaller – between 1/3 to 1/6 smaller than normal seeds – and abnormal in appearance and thus are unlikely to germinate naturally. This size reduction is caused by the 3:5 ratio found between the ploidy level of the embryo and that of the endosperm (Aleza et al., 2011; Esen & Soost, 1971).

Therefore, in order to be able to select and recover the small percentage of triploid individuals two important methodologies that must be considered: (1) an efficient method, based on in vitro embryo rescue, to recover the triploid embryos that are not able to germinate under greenhouse conditions and (2) an accurate and fast method to determine the ploidy level of the recovered plants (Aleza et al., 2010; Aleza et al., 2012; Aleza et al., 2011; Ollitrault et al., 1996).

2. Interploid sexual hybridization 2x X 4x and 4x X 2x

Another way to produce triploid hybrids is through sexual interploid hybridizations where one parent is a tetraploid and the other is a diploid: 2x female X 4x male hybridizations (Esen & Soost 1971; Oiyama et al.1981; Starrantino & Recupero 1981) or 4x female X 2x male hybridizations (Cameron & Burnett 1978; Esen et al. 1978; Aleza et al., 2010; Aleza et al., 2009; Garavello et al., 2020).

In the cross 2x X 4x, the female parent is diploid and the male parent is tetraploid, resulting in a 3:4 ratio between the embryo and the endosperm ploidy which causes endosperm failure and embryo abortion. As a result, to obtain the triploid progeny from 2x X 4x hybridization an efficient embryo rescue technique must be established (Aleza et al., 2009). In order to implement the cross 2x X 4x to mass produce triploids the methodologies of embryo rescue and ploidy evaluation, previously mentioned, must be established as well as the production of tetraploid genotypes to be used as the male parents for the cross (Aleza et al., 2012b).

In contrast, in the 4x X 2x, the female parent is tetraploid and the male parent is diploid. This results in a ratio between the embryo and the endosperm ploidy of 3:5 leading to a smaller seed but still favors appropriate seed and embryo development (Aleza et al., 2009). Therefore, 4x X 2x hybridizations for triploid production is more efficient than 2x X 4x hybridizations. However, it is important to establish that the tetraploid parent must be a non-apomictic genotype because it guarantees that the unique embryo within each seed is the triploid sexual embryo and these fully developed embryos have the advantage that they are able to germinate not only invitro but also in vivo in the greenhouse, although with lower germination rates than normal sized citrus seeds (Aleza et al., 2012b; Aleza et al., 2009).

F. Production of tetraploid citrus

Tetraploids may be produced through three different methods: (1) spontaneous somatic chromosome doubling observed in nucellar cells, (2) the induction of somatic chromosome doubling with chemical agents like colchicine and oryzaline (3) and somatic hybridization by protoplast fusion (Garavello et al., 2020).

1. Spontaneous production of tetraploids

Citrus germplasm can be either apomictic or non-apomictic. Most of the citrus germplasm is apomictic, although some genotypes are non-apomictic. These non-apomictic genotypes include pummelo (*C. maxima* (L.) Osb.), all citron (*C. medica* L.), clementime (*C. clementina* Hort. Ex Tan.) and some mandarins (Aleza et al., 2012a; Aleza et al., 2009). Apomictic genotypes present polyembryony, where one embryo is produced through sexual fertilization of the ovule while the other embryos develop from somatic embryogenesis from nucellar cells (Ollitrault et al., 2008). The nucellar embryos may begin growing before the sexual embryo, competing with it for space and nutrients, and usually resulting in the failed development of the sexual embryo (Aleza et al., 2009). In citrus, the nucellar cells that give rise to apomictic embryos frequently suffer chromosome doubling resulting in tetraploid genotypes which are commonly used as male parents in breeding programs (Aleza et al., 2012a). In non-apomictic genotypes the seeds are monoembryonic with the single embryo resulting from sexual fertilization (Ollitrault et al., 2008).

When considering apomitic genotypes, there must only be a selection of the spontaneous production of tetraploid embryos that arise. These tetraploids are denoted nucellar doubled-diploids and result from spontaneous somatic chromosome doubling of nucellar cells (Aleza et al., 2011; Cameron & Frost, 1968). The chromosome doubling takes place in individual nucellar primordium cells of the nucellus and therefore results in one solid tetraploid embryo (Aleza et al., 2011). The spontaneous production of tetraploids is affected not only by genotype or variety, but also by environmental factors such as inter-annual climatic variation (Aleza et al., 2011).

2. Induced production of autotetraploids

To produce tetraploids when considering non-apomictic genotypes, different artificial methods have been developed to induce somatic chromosome doubling with chemical agents such as colchicine or orizaline (Garavello et al., 2020).

Colchicine ($C_{22}H_{25}NO_6$) in having affinity for tubulin, inhibits the role of the spindles, therefore inhibiting cell division and nuclear division during mitosis or meiosis (Aleza et al., 2009). There are various strategies that have been used for chromosome doubling with colchicine, however, the most reliable way to produce tetraploid plants that are nonchimeric is through colchicine treatments while in vitro (Gmitter & Ling, 1991; Juarez 2004; Wakana, 2005). In 2009, Aleza et al., reported

an efficient method to obtain tetraploids from non-apomictic citrus genotypes considering: (1) in vitro micrografting of shoot tip containing the apical meristem and two to three leaf primordia, (2) treating the micrografted shoot-tips with colchicine or oryzalin for chromosome doubling and (3) identification of the ploidy through flow cytometry (Ollitrault & Michaux-Ferriere 1992; Ollitrault et al. 1996, Aleza et al., 2010; Aleza et al., 2009; Navarro et al., 2003).

Oryzalin (3,5-dinitro-N4,N4-dipropylsulfanilamide) is a chemical agent that is used less commonly than colchicine and it can be applied in vivo or in vitro. It is a herbicide presenting high antimitotic activity since it binds to tubulin, preventing the polymerization of microtubules, resulting in chromosome doubling (Aleza et al., 2009).

3. Production of allotetraploids through protoplast fusion

Tetraploid somatic hybrids, also denoted allotetraploids, can be obtained through protoplast fusion (Ohgawara et al., 1985). Somatic hybridization is a useful tool to cumulate the whole nuclear genome of two different parents, this is done without recombination and can potentially combine the complementary beneficial dominant traits of both parents (Calvez et al., 2020). This is a powerful tool than can be used to obtain progeny from sterile or sexually incompatible cultivars, including hybridization of citrus varieties with secondary citrus species, that otherwise could never be obtained through sexual hybridization. In consequence, over the years, protoplast fusion has been used to: (1) improve scion varieties by hybridizing elite varieties, (2) to obtain tetraploids from sterile cultivars (Ollitrault et al., 2008), (3) to combine sexually incompatible citrus to increase variability of germplasm, (4) to directly produce triploids by the fusion of a haploid and a diploid, not commonly used, (5) to create highly heterozygous tetraploid parents that can be exploited for triploid breeding or that have potential as rootstocks to improve disease resistance, size control, horticultural performance and (6) to produce cybrids which may be of use for scion and rootstock improvement (Grosser et al., 2000).

There are various approaches for protoplast fusion – the callus protoplast and the leaf protoplast – from the two parents: chemically induced fusion, electrically induced fusion, or electrochemical induced fusion. For chemically induced fusion polyethylene glycol (PEG) is used to aggregate the cells and fuse them together (Ohgawara et al., 1985; Grosser & Gmitter 1990; Olivares-Fuster et al., 2005). For electrically induced fusion an electric pulse is used (Saito et al., 1991, Ling & Iwamasa, 1994; Hidaka et al., 1995; Ollitrault et al., 1996). This approach has the advantage that it has a higher reproducibility than PEG-mediated fusion (Olivares-Fuster et al., 2005). Lastly, a combination of electro-chemical fusion integrates chemical protoplast aggregation and electrical pulses resulting in very high consistency (Olivares-Fuster et al., 2005).

G. Chromosome segregation models of citrus tetraploid genotypes

Tetraploid plants produce diploid gametes and it is important to determine the way that these gametes are formed. To explain chromosome segregation there are two extreme models: the disomic segregation model and the tetrasomic segregation model.

The disomic segregation model applies to allotetraploids, where there is the fusion of two species and therefore contains two sets of homologous chromosomes. When meiosis takes place to produce gametes, each chromosome is paired with its homologous chromosome resulting only in the formation of bivalents. As a result, all gametes produced are heterozygous (AB), with 100% of interspecific heterozygosity transmitted by each gamete (Garavello et al., 2020; Stift et al., 2008).

The tetrasomic segregation model applies to autotetraploids and the presence of four homologous chromosomes instead of two results in equal opportunities to pair at meiosis, leading to multivalent formation and tetrasomic inheritance. For doubled diploids, it hypothetically leads to 66 % of parental heterozygosity restitution (PHR) of the diploid that led to the tetraploid (Sanford, 1983; Aleza et al., 2016). Allo- and autotetraploids (with disomic and tetrasomic inheritance, respectively) are the extremes of the range. In cases where parents are divergent but have retained enough homology to prevent exclusive preferential pairing, intermediate inheritance patterns between di- and tetrasomic can be expected (Rouiss et al. 2018; Garavello et al., 2020).

Stift et al. (2008) developed a likelihood-based approach to decipher whether disomic, intermediate or tetrasomic inheritance patters best fitted with the segregation of genetic markers and to estimate preferential pairing (PP) and double reduction (DR) rates. PP is defined as the proportion gametes resulting from exclusive pairing of homologous chromosomes and allow to identify between disomic, intermediate and tetrasomic inheritance. DR takes place when sister alleles migrate to the same gamete during meiosis (Ollitrault et al., 2008). Later, Aleza et al. (2016) simplified this approach, using a maximum likelihood approach for centromeric loci.

Molecular marker analysis is a powerful tool to calculate PP as its impact on genome fragment inheritance and recombination. The inference of diploid gamete genetic structure produced by the tetraploid plant requires identifying the allelic doses of the triploid hybrids. This methodology was validated for citrus by Cuenca et al. (2011; 2013).

It is also important to mention that molecular marker analysis permits to calculate the genetic structure of diploid gamete populations and to determine their origin through the PHR in combination with PP and DR. PHR is defined as the percentage of gametes with heterozygosity. When considering diploid gametes (2n) PHR is therefore a function of the genetic distance to the centromere (Aleza et al., 2016).

The aim of this work is to analyze how the phylogenetic origin impacts on inheritance patterns of two tetraploid genotypes with very different genetic backgrounds. We analyzed the preferential chromosome pairing and inheritance of the interspecific (*C. sinensis* / *P. trifoliata*) doubled-diploid 'Carrizo' citrange and the monospecific (*C. maxima* / *C. maxima*) 'Chandler' pummelo. Doubled-diploid 'Carrizo' citrange can be considered as allotetraploid produced from the merger of two divergent and genetic distance species, whereas doubled-diploid 'Chandler' pummelo can be considered as autotetraploid recovered from only one specie.

II. OBJECTIVES

In the framework of the triploid breeding program carried out at IVIA based on interploid sexual hybridizations, the objectives of this work include:

1. Identify the genetic composition of the diploid gametes obtained when using tetraploid 'Chandler' pummelo and tetraploid 'Carrizo' citrange as male parents in 2x X 4x sexual hybridizations.

2. Study the inheritance pattern of tetraploid 'Chandler' pummelo and tetraploid 'Carrizo' citrange using SNP and SSR molecular markers distributed homogenously along the nine linkage groups of the reference clementine genetic map.

3. Analyze how interspecificity impacts on tetraploid inheritance patterns and its implication in breeding programs based on sexual interploid hybridizations.

III.   MATERIALS AND METHODS

A. Study site

The present study took place in the Institut Valencià d'Investigacions Agràries (IVIA), Moncada, Valencia, Spain in the Tissue Culture Unit, Center for Citriculture and Plant Production.

B. Plant material

'Chandler' pummelo is a synthetic variety that was created as a hybrid of 'Siamese Pink' pummelo and 'Siamese Sweet' pummelo. This variety was released in 1961 by the University of California, Citrus Research Center, Riverside (Cameron & Soost, 1961; Siebert, 2002a). The parental origin of both 'Siamese Pink' pummelo and 'Siamese Sweet' pummelo are unknown (Siebert, 2002c, 2002b).

'Carrizo' citrange is a variety that was created as a hybrid of *C. sinensis* 'Washington' navel and *P. trifoliata*. This variety resulted from a cross made in 1909 under the direction of W.T. Swingle of the U.S. Department of Agriculture and it was later named 'Carrizo' in 1938. 'Carrizo' citrange is the most extensively rootstock used in our citriculture in the last decades for its excellent agronomical behavior. (Calvez et al., 2020; Siebert, 2002d).

Tetraploid 'Chandler' pummelo is a variety that was obtained in the IVIA through the treatment of in vitro micrografting of diploid 'Chandler' pummelo shoot tips, containing the apical meristems and two to three leaf primordia, with colchicine to induce chromosome doubling. From the apical meristems treated, tetraploid 'Chandler' pummelo was selected by flow cytometry and regenerated in the years 2017 and 2015, respectively. This work was carried out by the research team in the years 2015-2017.

On the other hand, 'Carrizo' citrange is an apomictic variety that has nucellar embryos that can frequently suffer spontaneous chromosome doubling. Therefore, tetraploid 'Carrizo' citrange was selected from nucellar embryos which presented spontaneous chromosome doubling as described by Aleza *et al*. (2011).

Various interploid hybridizations were established between diploid 'Tomatera' clementines as female parent X tetraploid 'Chandler' pummelo as male parent (hereinafter referred as CCP) as well as between diploid 'Fina' clementine as female parent X tetraploid 'Carrizo' citrange as male parent (hereinafter referred as CCC). 'Tomatera' and 'Fina' clementines are non-apomictic genotypes and therefore were used as females. The use of non-apomictic genotypes as females in both hybridizations guarantees the presence of only one embryo per seed, and that the embryo is the one resulting from the

hybridization. These hybridizations were carried out in the IVIA in the years 2016/2017 (CCP) and 2014/2015 (CCC). In CCP sexual hybridization, 85 triploid hybrids were recovered whereas in CCC sexual hybridization, 88 triploid hybrids were obtained. Ploidy-level analysis by flow cytometry and triploid hybrid recovery was performed following the methodology described by Aleza et al. (2012). This work was carried out by the research team in the years 2015-2017.

C. Genotyping of triploid hybrids with SNP and SSR molecular markers

In order to study the genetic structure of the diploid gametes produced by the tetraploid genotypes, triploid hybrids of each cross along with the parents, a series of molecular markers were used, SNPs and SSRs , distributed homogenously along the nine linkage groups (LG) of the clementine genetic map (Ollitrault et al., 2012). The SNPs and SSRs used for each cross can be seen in Table 1 and Table 2. A minimum of three molecular markers were used for each LG, with one near the centromere and the other two at both telomeric extremes. It is important to mention that all molecular markers used in the analysis of tetraploid 'Chandler' pummelo and tetraploid 'Carrizo' citrange were heterozygous for the tetraploid genotype and polymorphic between the parents involved in each cross, diploid 'Tomatera' clementine and diploid 'Fina' clementine, respectively. A total of 39 molecular markers were used to genotype and analyze the CCP triploid hybrids while a total of 29 molecular markers were used for the CCC triploid hybrids.

DNA was extracted from all parents and progenies using a DNeasy Plant Qiagen Kit. After DNA extraction, the concentration of each sample was determined using a NanoDrop spectrophotometer. Dilutions at 10 ng/µL were prepared for each sample, according to the initial concentration of each sample.

1. SNP markers

A total of 15 SNP molecular markers were used for CCP and one SNP was used for CCC. All 15 SNPs used to analyze the triploid hybrids from CCP have been developed from a Genotyping-by-Sequencing (GBS) diversity analysis performed by Dr. Patrick Ollitrault (unpublished data). Primers were designed by LGC Genomics based on the SNP locus flanking sequence (~50 nt on either side of the SNP). SNPs were analyzed using KASPar™ technology developed by LGC Genomics. The KASPar™ genotyping system is a competitive, allele-specific dual fluorescence resonance energy transfer (FRET)-based assay for SNP genotyping (http://www.lgcgenomics.com). This technology is based on the extension and FRET as a signal of allele-specific oligonucleotides (Semagn et al., 2014). It combines the use of a 5'-3'exonuclease-deleted Taq DNA polymerase that is highly specific along with two allele-specific forward primers, which compete between each other, and one common reverse primer. This genotyping system is simple and cost-effective when compared to other SNP genotyping technologies (Cuenca et al., 2013). The results from this allele-specific polymerase chain reaction (PCR) were then analyzed with the KlusterCaller™ software. An Eppendorf ® Thermocycler was used for the PCR which had a final volume of 10 µL containing

2 µL of citrus DNA (10 ng/µL), 0.14µL of the mix of two dye-labeled forward primers and one unlabeled backward primer, 5µL of PACE 2x Genotyping Master Mix and 3µL of DEPC water. The PCR protocol used was as follows: denaturation at 94°C for 15 min, 10 cycles of 20 s at 94°C, 1 min at 61°C (with a gradient -0.6°C) followed by 26 cycles of 20 s at 94°C, 1 min at 55°C. A first reading was done at this time followed by two other readings after a two short recycling protocols each consisting of 5 cycles of 20 s at 94°C, 1 min at 57°C. The KlusterCaller™ software used for analysis displays the results in a two-dimensional graph with X and Y axis, each indicating the floursecense signal of the X and Y alleles. Depending on the angle of the dispersion of the data of the genotypes the software is able to characterize and genotype a triploid individual on the allelic dosage for a certain allele. The SNPs used to analyze the inheritance pattern of tetraploid 'Chandler' pummelo and tetraploid 'Carrizo' citrange are listed in the **Table 1** and **Table 2**, respectively.

2. SSR markers

A total of 24 SSR molecular markers were used to analyze CCP triploids and 28 SSRs for the CCC triploids. An Eppendorf ® Thermocycler was used for the PCR which had a final volume of 15 µL containing 0.2 µl 5U/µL of NZY Taq II DNA polymerase, 1.5 µL of citrus DNA (10 ng/µL), 1.875µL of 2µM of dye-labeled forward primer, 1.875µL of 2 µM of non-dye-labeled reverse primer, 0.4 mM of each dNTP, 1.5 µL of 10X reaction buffer (nzytech), and 0.75µL of 50mM $MgCl_2$ (nzytech). The PCR protocol used was as follows: denaturation at 94°C for 5 min followed by 40 cycles of 30 s at 94°C, 1 min at 50 or 55°C, and 45 s at 72 °C; and a final elongation step of 4 min at 72°C.

A CEQ™ 8000 Genetic Analysis Beckman Coulter automatic sequencer was used to separate the amplified PCR fragments through capillary electrophoresis. The PCR products were initially denatured at 90°C for 2 minutes, loaded at 2 kV for 30 seconds, and separated at 6 kV for 35 minutes. Alleles were sized based on a DNA size standard of 400 bp. The results of the electrophoresis were collected, visualized and analyzed using the GenomeLab™ GeXP v.10.0 (Beckman Coulter ®) software. The Microsatelite DNA Allele Counting Peak Ratio (MAC-PR) methodology was used to calculate the allelic dosage for each triploid hybrid (Cuenca et al., 2011; Esselink et al., 2004). The SRRs used to analyze the inheritance pattern of tetraploid 'Chandler' pummelo and tetraploid 'Carrizo' citrange are described in **Table 1** and **Table 2**, respectively.

Table 1. SNP and SSR molecular markers used to analyze the inheritance pattern of tetraploid 'Chandler' pummelo in the CCP hybridization.

LG	Locus	Marker type	Primer		Alleles 'Chandler' pummelo 4x	Alleles 'Tomatera' clementine 2x	Reference
1	CI02G08	SSR	F	CATGCAATGTTCCACTT	249-257	245-249	Froelicher et al. (2008)
			R	AGGCAGTTGTTAGACCC			
	1_14053816	SNP	F	--	C:G	C:C	Unpublished data
			R	--			
	MEST321	SSR	F	ATAACCGTTTCTCTCAATTTTCA	108-112	105-108	Ollitrault et al., (2012)
			R	AAGAAAGAAGAGATCGCTGGC			
2	2_60291	SNP	F	--	A:G	A:A	Unpublished Data
			R	--			
	CX2004	SSR	F	AAACCGCCGAAACTTT	175-183	183-186	Chen et al., (2008)
			R	GGAATCCCAAAGAAAGTTGG			
	CI01C07	SSR	F	GTCACTCACTCTCGCTCTTG	259-277	243-280	Froelicher et al., (2008)
			R	TTGCTAGCTGCTTTAACTTT			
	2_36363713	SNP	F	--	G:C	G :G	Unpublished data
			R	--			
3	3_17626	SNP	F	--	C:A	C :C	Unpublished data
			R	--			
	CX0124	SSR	F	CACTGTGGCGTCCTTT	169-171	162-171	Chen et al. In Preparation
			R	CCAACAGCAACAAGAAAGCG			
	CI02G02	SSR	F	CAATAAGAAAACGCAGG	110-122	112-122	Luro et al., (2012)
			R	TGGTAGAGAAACAGAGGTG			
	3_51024515	SNP	F	--	A:G	A :A	Unpublished data
			R	--			
4	CF-CA31	SSR	F	GTCGAAGCCTTAGTTATACAGT	211-229	211	Ollitrault et al., (2012)
			R	TTTTGGTTTTAGAGTTGCACCA			
	CI07D06	SSR	F	CCTTTTCACAGTTTGCTAT	163-166	166-188	Froelicher et al., (2007)
			R	TCAATTCCTCTAGTGTGTGT			
	CIBE3255	SSR	F	GCACAACAAAGAGAAACAGAG	205-210	211-215	Ollitrault et al., (2010)
			R	CAACTCCAACACCTCAAGTAA			
5	5_27500244	SNP	F	--	G:C	G:G	Unpublished data
			R	--			
	CMS30	SSR	F	AACACCCCTTGGAGGGAG	149-160	151-155	Ahmad et al., (2003)
			R	GCTGTTCACACACACAACCC			
	MEST56	SSR	F	AGTCCGCCTTTGCTTTTTCT	155-165	135-139	Aleza et al., (2009)
			R	GGTGCAAAAGAGAGCGAGAG			
6	6_10554	SNP	F	--	A:G	A:A	Unpublished data
			R	--			
	6_5996116	SNP	F	--	T:A	T:T	Unpublished data
			R	--			
	TAA1	SSR	F	GACAACATCAACAACAGCAAGAGC	163-167	160-163	Kijas et al., (1997)
			R	AAGAAGAAGAGCCCCCATTAGC			
7	CI07E05	SSR	F	GGAGAACAAAACACAATG	116-118	114-116	Luro et al., (2012)
			R	ATCTTTCGGACAATCTT			
	7_20255	SNP	F	--	A:G	A:A	Unpublished data
			R	--			
	Ci03B07	SSR	F	CACCTTTCCCTTCCA	263-265	276-278	Froelicher et al., (2008)
			R	TGAGGGACTAAACAGCA			
	7_15708026	SNP	F	--	T:C	T:T	Unpublished data
			R	--			

Table 1. – Cont. SNP and SSR molecular markers used to analyze the inheritance pattern of tetraploid 'Chandler' pummelo in the CCP hybridization.

LG	Locus	Marker type	Primer		Alleles 'Chandler' pummelo 4x	Alleles 'Tomatera' clementine 2x	Reference
8	CI01F04a	SSR	F	AAGCATTTAGGGAGGGTCACT	191-199	186-202	Froelicher et al., (2008)
			R	TGCTGCTGCTGTTGTTGTTCT			
	CI07B05	SSR	F	TTTGTTCTTTTTGGTCTTTT	200-219	202-222	Froelicher et al., (2008)
			R	CTTTTCTTTCCTAGTTTCCC			
	MEST830	SSR	F	TTCATGGCAGCTTGAGTTTC	205-214	196-200	Luro et al., (2012)
			R	TTGGTTTCTTTTGGGGATCA			
	8_15415145	SNP	F	--	C:A	C:C	Unpublished data
			R	--			
	CI02C09	SSR	F	TACTGACTGACCCCACC	236-238	249-254	Froelicher et al., (2008)
			R	TCCCCGTCTCCTACC			
	8_25026006	SNP	F	--	C:T	C:C	Unpublished data
			R	--			
9	MEST330	SSR	F	TCCTTGTGAATTTGGGAACC	269-287	261-270	Guo et al., (2015)
			R	GTTGCATAGCCATATTGCCC			
	CI07F11	SSR	F	ACTATGATTACTTTGCTTTGAG	155-158	152-160	Froelicher et al., (2008)
			R	GAAGAAACAAGAAAAAAAAAT			
	MEST308	SSR	F	CCTCTTCATTTTTCTCTGAAACTAA	241-244	253-259	Ollitrault et al., (2012)
			R	TTGCAACATCGTTCCTCTTG			
	CI08C05	SSR	F	TCCACAGATTGCCCATTA	143-153	153-174	Froelicher et al., (2008)
			R	CCCTAAAAACCAAGTGACA			
	9_9543785	SNP	F	--	G:A	G:G	Unpublished data
			R	--			
	9_26016472	SNP	F	--	G:T	G:G	Unpublished data
			R	--			
	9_31288895	SNP	F	--	T:C	T:T	Unpublished data
			R	--			

LG: Linkage Group; F: Forward primer; R: Reverse primer

Table 2. SNP and SSR molecular markers used to analyze the inheritance pattern of tetraploid 'Carrizo' citrange in CCC hybridization.

LG	Locus	Marker Type	Primer		Alleles 'Carrizo' citrange 4x	Alleles 'Fina' clementine 2x	Reference
1	CIBE6126	SSR	F*	TTTACTTCATCGTTCATCCTT	222-226	226-228	Ollitrault et al., (2010)
			R*	GGACTACTTGAGATTTGCTGA			
	MEST539	SSR	F	GGACGGTTACTCCACGTTGT	97-103	103-107	Ollitrault et al., (2010)
			R	AAAAAGGGGTTCTCACTTCTCTT			
	TAA15	SSR	F	GAAAGGGTTACTTGACCAGGC	143-189	191-194	Kijas et al., (1997)
			R	CTTCCCAGCTGCACAAGC			
2	SOS1-M50	SNP	X*	GGTTTAGTACTGAGTAAGTTACTTGC	A:G	A:A	Garcia-Lor et al., (2013)
			Y*	AAATGGTTTAGTACTGAGTAAGTTACTTGT			
	CX6F23	SSR	F	TTTTAGGAAATTTCAATCACCCGA	143-156	149-161	Chen et al., (2008)
			R	TCATTGTCATCACCATCACCATC			
	CX2004	SSR	F	AAACCGCCGAAACTTT	167-180	183-187	Chen et al., (2008)
			R	GGAATCCCAAAGAAAGTTGG			
	CI02D09	SSR	F	AATGATGAGGGTAAAGATG	227-231	231-239	Froelicher et al., (2008)
			R	ACCCATCACAAAACAGA			
3	ID/CIBE4721	SSR	F	GGCACAAAGATGGCTGTTGG	284-289	284-293	Chen et al., (2008)
			R	AGCCTGTTGGGGTATGTGCC			
	ID0039-380	SSR	F	AATCCTGACATCCAGACAAGG	193-202	200-206	Ollitrault et al., (2010)
			R	AGCCTCCAGAATCACAGTCG			
	MEST131	SSR	F	TACCTCCACGTGTCAAACCA	110-146	140-146	Ollitrault et al., (2012)
			R	GCTGTCACGTTGGGTGTATG			

LG	Locus	Marker Type	Primer		Alleles 'Carrizo' citrange 4x	Alleles 'Fina' clementine 2x	Reference
4	CI07D06	SSR	F	CCTTTTCACAGTTTGCTAT	162-188	166-188	Froelicher et al., (2007)
			R	TCAATTCCTCTAGTGTGTGT			
	CI02D04B	SSR	F	CTCTCTTTCCCCATTAGA	196-210	200-210	Ollitrault et al., (2012)
			R	AGCAAACCCCACAAC			
	CI03D12A	SSR	F	GCCATAAGCCCTTTCT	247-261	251-261	Froelicher et al., (2008)
			R	CCCACAACCATCACC			
5	mCr-CI07G11	SSR	F	ACATCAAGATTCAAGACCAT	194-202	202-210	Froelicher et al., (2008)
			R	CAAAAAGTGAAAAAATGAAA			
	CMS30	SSR	F	AACACCCCTTGGAGGGAG	148-156	152-156	Ahmed et al., (2003)
			R	GCTGTTCACACACACAACCC			
	ID5485-526	SSR	F	CAAAGCAAACACGCA	221-235	228-235	Ollitrault et al., (2012)
			R	TCCAGGAGGTGGTGCCATTT			
6	LG6-TTA6	SSR	F	--	342-345	345	Unpublished data
			R	--			
	MEST191	SSR	F	AAACACAGGAACCCACTTCG	235-238	242-245	Ollitrault, et al., (2012)
			R	ACCACGAAAGAACAACACCC			
	MEST123	SSR	F	GGGATGGACTCCCAGTGTTA	246-250	252-280	Aleza et al., (2011)
			R	AAGAAAGATTTGCTGGCAGAG			
7	MEST107	SSR	F	GCTGAGATGGGGATGAAAGA	171-174	174-182	Garcia-Lor et al., (2012)
			R	CCCCATCCTTTCAACTTGTG			
	CI07E05	SSR	F	GGAGAACAAAACACAATG	118-128	114-116	Luro et al., (2012)
			R	ATCTTTCGGACAATCTT			
	CI03B07	SSR	F	CACCTTTCCCTTCCA	266-280	264-266	Froelicher et al., (2008)
			R	TGAGGGACTAAACAGCA			
	CID0591	SSR	F	GGTAAGGGGCTGGGCAAAAA	350-364	347-350	Ollitrault et al., (2012)
			R	CAGCATCACATATGCAGGCTTGT			
8	CI01F04a	SSR	F	AAGCATTTAGGGAGGGTCACT	182-198	186-202	Froelicher et al., (2008)
			R	TGCTGCTGCTGTTGTTGTTCT			
	MEST502	SSR	F	TCAGCAGAAGAAGGAGACTCG	159-174	174	Ollitrault et al., (2012)
			R	CGGACATGGATGTAATCAGG			
	CI02C09	SSR	F	TACTGACTGACCCCACC	236-238	248-253	Froelicher et al., (2008)
			R	TCCCCGTCTCCTACC			
9	LG9-GA15	SSR	F	--	204-215	213-215	Unpublished data
			R	--			
	JI-TCT01	SSR	F	TCAG…AAAACAATCAA	155-167	149-155	Ollitrault et al., (2012)
			R	AGCAGGCTGACTACATCAGT			
	CI02B07	SSR	F	CAGCTCAACATGAAAGG	164-168	161-164	Froelicher et al., (2008)
			R	TTGGAGAACAGGATGG			

LG: Linkage Group; F: Forward primer; R: Reverse primer

D. Data analysis

1. Identification of the parent producing the diploid gamete and the genetic configuration of the diploid gamete

For all triploid hybrids it is necessary to determine the parent in the cross that gave rise to the diploid gamete (2x) and the genetic configuration of the diploid gamete. When considering a locus where the allelic configurations of both parents are completely different (female parent – A_1A_2 – and male parent – A_3A_4), the parent that produced the diploid gamete as well as the genotype of the diploid gamete can be directly identified when looking at the genotype of the triploid hybrid. However, when considering a locus in which both female and male parents share an allele (A_1A_2 x A_2A_2 or A_1A_2 x A_2A_3), the parent that produced the diploid gamete and the genotype of the diploid

gamete must be estimated calculating the ratios of the allelic dosage as was validated in citrus by Cuenca et al. (2011; 2013).

2. Determination of Parental Heterozygosity Restitution

For each of the molecular markers, the parental heterozygosity restitution (PHR) was calculated as the percentage of diploid gametes that presented the same heterozygotic allelic configuration as the tetraploid parent, 'Chandler' pummelo or 'Carrizo' citrange:

$$PHR\ (\%) = \frac{N^{\underline{o}}\ of\ diploid\ gametes\ in\ heterozygosity}{Total\ number\ of\ diploid\ gametes\ analyzed} \times 100$$

The average of PHR was also calculated for each of the nine LGs:

$$Average\ PHR\ (\%) = \frac{\sum PHR_{LG}}{Number\ of\ molecular\ markers\ analyzed\ per\ LG}$$

Lastly, the heterozygosity of each triploid hybrid was calculated considering all the molecular markers analyzed:

$$Tiploid\ hybrid's\ PHR\ (\%)$$
$$= \frac{Number\ of\ molecular\ markers\ displaying\ diploid\ heterozygous\ gametes}{Total\ number\ of\ molecular\ markers\ used\ to\ analyze\ the\ diploid\ gamete}$$
$$\times 100$$

3. Determination of preferential association frequency and maximum double reduction rate

It is important to calculate the rate of preferential pairing (PP) of the chromosomes in order to determine which chromosome segregation model – disomic, tetrasomic or intermediate – best describes the inheritance patterns of each of the tetraploid genotypes analyzed. The expected gamete frequencies depend on the PP parameter, corresponding to the proportion of gametes formed by random meiotic chromosome associations (i.e., random bivalent or quadrivalent pairing) and the double reduction parameter (DR) representing the frequency of double reductions relative to the total frequency of random (quadrivalent or bivalent) meiotic associations (Aleza et al., 2016).

Considering centromeric loci, where there is no probability of DR, the expected proportions for each gamete are:

$$P\ (aa) = \frac{1 - PP}{6}$$

$$P\ (ab) = 1 - \frac{1 - PP}{3}$$

$$P\ (bb) = \frac{1 - PP}{6}$$

The probability of obtaining the observed gamete population can be expressed with respect to PP as described by Aleza et al., (2016):

$$L(PP) = C \, x \left[\frac{1}{6}(1-pp)\right]^{x1+x2} \times \left[1 - \frac{1}{3}(1-PP)\right]^{x3}$$

Where *C* is a constant combinatory coefficient for the observed data and *x1*, *x2* and *x3* are the number of *aa*, *bb* and *ab* gametes observed in the population, respectively.

The estimation of the value of PP is calculated using a maximum likelihood approach. A molecular marker located closest to the centromere was selected for each LG and the probability L(PP) functions were analyzed varying from 0 – 1 with intervals of 0.005. In this way, the estimated PP value was the one that maximized the probability of obtaining the observed gamete population. In order to establish the confidence intervals (CIs), values above or below the estimated PP that corresponded to a tenfold decrease in probability were selected (Aleza et al., 2016).

After estimating PP for each chromosome, it was possible to estimate the DR for markers located on the same LG. This was done by using the maximum likelihood approach and considering a fixed PP value estimated from the loci closest to the centromere (the one previously determined) and varying the DR in intervals of 0.005 increments. The following probability equation is used to determine the observed gamete population as a function of DR (Aleza et al., 2016):

$$P(DR) = C \times \left[(1+2DR)(\frac{PP}{6})\right]^{x1+x2} \times \left[1 - \frac{1}{3}(1-PP) - \frac{2}{3}DR(1-PP)\right]^{x3}$$

Where *C* is a combinatory coefficient constant for the data observed and where *x1*, *x2* and *x3* are the number of observed A_1A_1, A_2A_2 and A_1A_2 gametes.

4. **Organization of the population diversity**

The software DARwin6 (Perrier & Jacquemoud-Collet, 2018) was used to estimate the genetic differences between the individuals within the same population with Neighbor-Joining statistical analysis using the Simple Matching Dissimilarity Index:

$$d_{i-j} = 1 - \frac{1}{L}\sum_{l=1}^{L}\frac{m_l}{\pi}$$

Where d_{i-j} represents the dissimilarity between the locus i and j; L is the number of loci; and m_l is the number of matching alleles for the locus *l*, and π is the ploidy level. From the dissimilarity matrix obtained, a weighted Neighbor-Joining tree was then computed, including 1000 bootstrap.

A Chi-square test (χ^2), along with the Bonferroni correction (Goeman & Solari, 2014; Bonferroni, 1963; Holm, 1979) for multiple testing, was used to analyze the potential distortion in allelic segregation.

IV.   RESULTS & DISCUSSION

A. Triploid genotyping

75 of the 79 triploid hybrids obtained from the cross CCP were analyzed with 39 codominant molecular markers, 15 SNPs and 24 SSRs. All 15 SNPs are new molecular markers that were developed from a genotyping by sequencing (GBS) analysis (unpublished data). On the other hand, 88 of triploid hybrids obtained from the cross CCC were analyzed with 29 codominant molecular markers, 1 SNP and 28 SSRs. The molecular marker analysis permitted to identify the origin of diploid gametes, by either observing the triallelic configuration of the triploid hybrids or from the dosage estimation as proposed by Cuenca et al. (2015).

Determining the triallelic configuration of the triploid hybrid as well as the origin of the diploid gamete by observation and dosage estimation can be exemplified with the SSR CMS30**,** used for the cross considering 'Chandler' pummelo as male parent (**Figure 4)**. For this SSR, pummelo has the alleles of 149 nt and 160 nt, (**Figure 4a)** and clementine has two distinct alleles at 151nt and 155nt, (**Figure 4b)**. In **Figure 4c**, considering the triploid hybrid 1, it is possible to determine the genotype by observing the alleles at 149nt, 151nt, and 160nt. Since the allele at 151nt can only be inherited from clementine, and the alleles at 149nt and 160nt can only be inherited from tetraploid 'Chandler' pummelo, it is confirmed that the diploid gamete derives from tetraploid 'Chandler' pummelo and that its genetic constitution is 149-160. However, in **Figure 4d**, considering the triploid hybrid 2, where only two alleles are present at 149nt and 151nt it can be assumed that the diploid gamete has the configuration 149-149, since only this allele can be inherited from tetraploid 'Chandler' pummelo. However, it must be confirmed that the allele 149 is present twice, to ensure that the diploid gamete in fact is derived from 'Chandler' pummelo and not clementine. This can only be determined through dosage estimation. In this case, the genotype of the diploid gamete is one of these two options: (1) 149-149, confirming the diploid gamete is derived from tetraploid 'Chandler' pummelo or (2) 151-151, indicating the diploid gamete is derived from unreduced gamete of clementine. The dosage estimation is claculated considering the relationship between the alleles 149-151 of the triallelic triploid hybrid 1 as a baseline (R1) and dividing the ratio value of the triploid number 2 (149 and 151 alleles) by the R1 baseline. For instance, the ratio between alleles of triploid hybrids number 2 (R2=1.22) and the baseline (R1=2.07) is approximately 0.5, indicating that the genetic configuration of the diploid gamete is 149-149 and in fact is derived from tetraploid 'Chandler' pummelo.
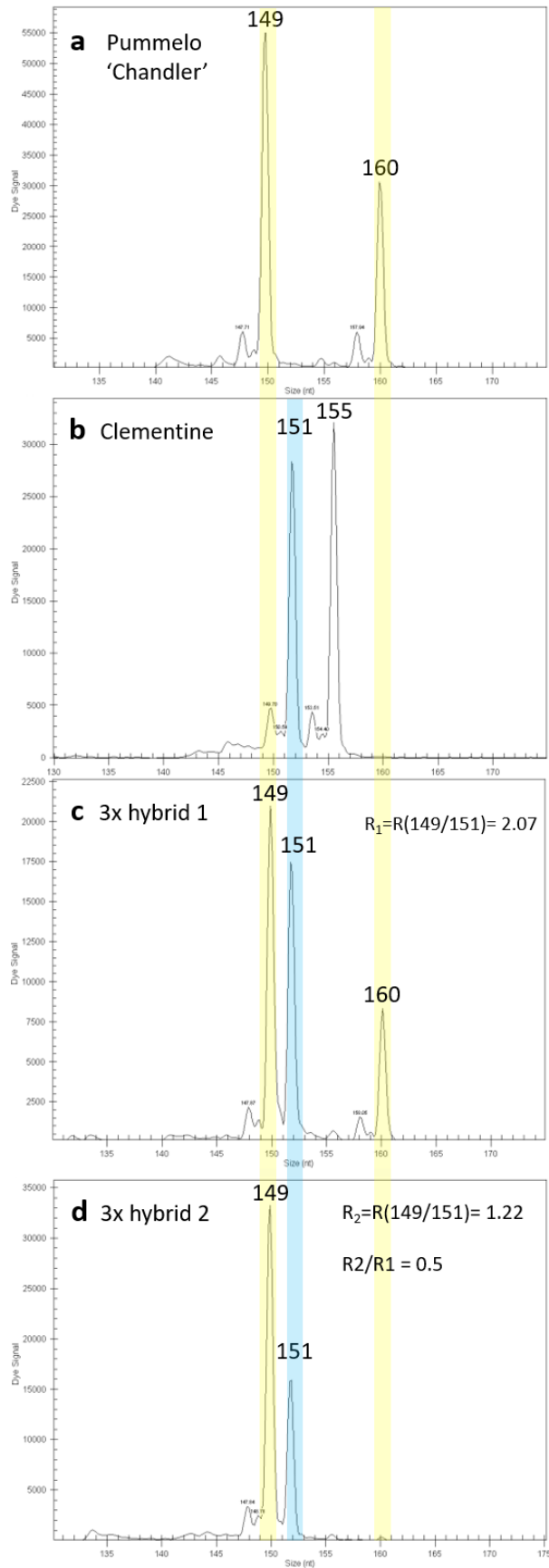
Figure 4. Analysis of triploid hybrids, from the cross CCP, with SSR marker CMS30; a) diploid 'Chandler' pummelo, b) diploid clementine, c) triploid hybrid 1 and d) triploid hybrid 2.

Determining the triallelic configuration of the triploid hybrid is also accomplished through SNPs using the KASPar™ technology. **Figure 5**, presents the visualization of the results obtained from this technology, specifically for SNP 9_26016472 used to analyze CCP triploid progenies of 'Chandler' pummelo. Tetraploid 'Chandler' pummelo is heterozygote thymine:guanine (T:G) for this SNP while clementine is homozygote guanine (G:G). In the population of triploid hybrids analyzed, different allelic configurations can be observed for this SNP, with clementine (dark blue square) and 'Chandler' pummelo (green triangle) used as controls. As for the population of triploid hybrids, the angle and location of each group indicate the specific allelic configuration, determined in relation to the controls previously mentioned. The light blue group, closest to the G:G clementine control, presents three 'G' alleles; two 'G's' which form part of the diploid gamete inherited from the male tetraploid parent 'Chandler' pummelo (G:G), while the third 'G' is inherited from the female diploid clementine parent (G), resulting in the allelic configuration (G:G – G). The orange group, located between 'Chandler' pummelo T:G control and the Clementine G:G control, presents one 'T' allele and two 'G' alleles; the 'T' and one of the 'G's coming from the diploid gamete inherited from tetraploid 'Chandler' pummelo (T:G) and the other 'G' inherited from the female diploid clementine (G), resulting in the allelic configuration (T:G – G) coming from 'Chandler' pummelo heterozygosity restitution. Lastly, the yellow group, closest to the 'Chandler' pummelo T:G control, presents two 'T' alleles and one 'G' allele; the two 'T's which form part of the diploid gamete inherited from tetraploid 'Chandler' pummelo (T:T), while the 'G' is inherited from the female diploid parent (G), resulting in the allelic configuration (T:T – G).

The genetic analysis with these molecular markers confirmed that all of the triploid hybrids obtained from both 2x X 4x crosses were formed by the fusion of a diploid gamete coming from the tetraploid parent and a haploid gamete coming from the diploid parent and paves the way for identifying the genetic configuration of diploid gametes produced by tetraploids genotypes, 'Chandler' pummelo and 'Carrizo' citrange (**Tables 11 and 12 of Annexes**). This type of analysis and results are in agreement with other studies that determined the origin of the diploid gamete in a triploid population in 2x X 4x crosses (Aleza et al., 2012; Garavello et al., 2020)
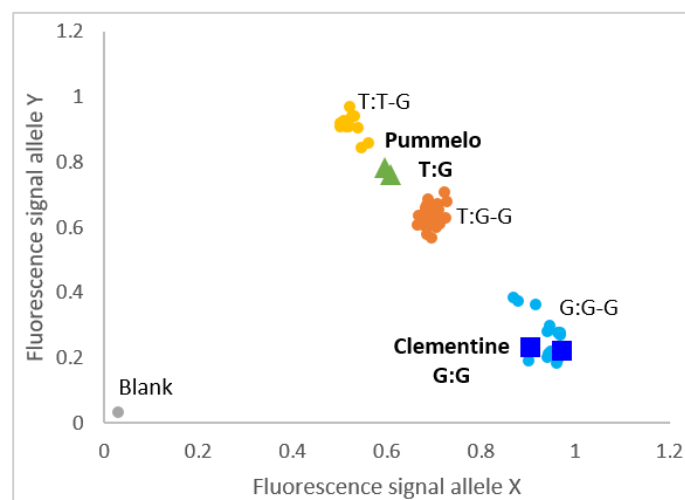
Figure 5. Representation of the results obtained from the analysis of SNP 9_26016472 for the cross CCP. Each dot plotted represents parents and triploid hybrids analyzed. The different colors represent groups of triploid hybrids with different allelic configuration: yellow being T:T-G, orange being T:G-G and light blue being G:G-G.

The allelic segregation distortion was analyzed for both populations of triploid hybrids and for each molecular marker used by performing the chi-squared analysis (χ^2) with the Bonferroni correction, (**Tables 3 and 4**). The value resulting from the chi-squared analysis indicates the molecular markers whose frequency results differ from the expected frequency. With tetraploid 'Chandler' pummelo as male parent, the following molecular markers presented significant allelic segregation distortion (p-value < 0.001): 3_51024515 (LG3), 6_10554 (LG6), 8_25026006 (LG8), and CI08C05 (LG9). With tetraploid 'Carrizo', no molecular markers presented significant allelic segregation distortion (p-value < α 0.001).

Other citrus studies also showed segregation distortions for molecular markers distributed homogenously among the nine LGs. A study analyzing the inheritance patterns of reciprocal crosses between 'Fortune' mandarin and 'Chandler' pummelo, significant allelic segregation distortions were found in both populations (Bernet et al., 2010). Similarly, Ollitrault et al. (2012) observed significant segregation distortions in male and female 'Clemenules' clementine gametes. Aleza et al. (2016) analyzed the inheritance patterns in double-diploid clementine and molecular marker CiC4993-03 (LG6) showed significant allelic segregation distortions (p-value < α 0.001). Garavello et al. (2020) analyzed the inheritance patterns in tetraploid 'Moncada' mandarin as female and male parent. When used as female parent, 'Moncada' mandarin showed significant allelic segregation distortions (p-value < α 0.001) for the molecular marker MEST256 (LG3). When used as male parent, 'Moncada' mandarin showed signiicant allelic segregation distortions (p-value < α 0.001) for molecular markers: CHSM183 (LG3), MEST 123 (LG6) and FLSM400 (LG7).

Segregation distortion is a phenomenon commonly reported in vegetable species that indicates a deviation from Mendelian deviations. The allelic segregation distortions are due to having greater frequencies of one homozygous gamete when compared to the alternative homozygous gamete. Segregation distortion reduces the probability of being able to obtain certain allelic combinations and is one disadvantage of using double diploids to produce tetraploid genotypes in breeding strategies (Bélanger et al., 2016; Germanà, 2011; Taylor & Ingvarsson, 2003; Xu et al., 1997). It seems that allelic segregation distortion is caused by the presence of selective pressure over certain genomic regions, usually reported in the production of microspores, regeneration of plants and spontaneous diplodization of haploid plants. Various studies have analyzed this subject, among them, the study of allelic segregation distortion of barley that occurs during embryogenesis and plant regeneration (Bélanger et al., 2016; Dreissig et al., 2017).

Table 3. Estimation of parental heterozygosity restitution frequency (PHR) by diploid 'Chandler' pummelo pollen for each marker in triploid hybrids obtained from CCP hybridization.

Locus	LG	GP (cM)	PHR	χ2	P value
Ci02G08	1	16.76-	0.59	1.58	0.209
SNP 2	1	57.51	0.68	6.00	0.014
MEST321	1	118.48	0.63	0.14	0.705
SNP 3	2	1	0.55	1.48	0.223
CX2004	2	56.757	0.60	1.20	0.273
CI01C07	2	125.61	0.63	0.57	0.450
SNP 4	2	150.13	0.63	0.00	1.000
SNP 5	3	7.38	0.61	1.69	0.194
CX0124	3	110.3	0.61	7.76	0.005
CI02G02	3	149.15	0.67	0.36	0.549
SNP 7	3	192.02	0.79	16.00	0.000*
CF-CA31	4	12.22	0.48	0.64	0.423
CI07D06	4	16.33	0.60	0.53	0.465
CIBE3255	4	89.19	0.63	0.14	0.705
SNP 10	5	25.23	0.68	0.17	0.683
CMS30	5	31.35	0.61	0.03	0.853
MEST56	5	110.16	0.61	4.17	0.041
SNP 11	6	1.53	0.00	75.00	0.000*
SNP 12	6	5.07	0.67	9.00	0.003
CIBE4818	6	28.317	0.59	3.90	0.048
CI02F12	6	60.92	0.59	0.81	0.369
TAA1	6	93.48	0.61	0.03	0.853
CI07E05	7	14.37	0.53	0.03	0.866
SNP 13	7	10.89	0.56	0.27	0.602
CI03B07	7	83.89	0.59	0.81	0.369
SNP 14	7	105.01	0.61	0.86	0.353
CI01F04a	8	5.91	0.55	1.06	0.303
CI07B05	8	31.7	0.69	0.04	0.835
MEST830	8	41.65	0.88	9.00	0.003
SNP 16	8	50.78	0.68	2.67	0.102
CI02C09	8	95.03	0.57	0.13	0.724
SNP 17	8	113.52	0.00	75.00	0.000*
MEST330	9	5.97	0.39	5.57	0.018
CI07F11	9	49.56	0.67	0.36	0.549
MEST308	9	50.41	0.63	0.14	0.705
Ci08C05	9	52.23	0.80	15.00	0.000*
SNP 18	9	55.14	1.00	NA	NA
SNP 19	9	54.88	0.59	0.03	0.857
SNP 20	9	98.18	0.52	3.46	0.063

* Statistically significant (p<0.001)

Table 4. Estimation of parental heterozygosity restitution frequency (PHR) by diploid 'Carrizo' citrange pollen for each marker in triploid hybrids obtained from CCC hybridization.

Locus	LG	GP (cM)	PHR	χ2	P value
CIBE6126	1	6.29	0.91	8.00	0.005
MEST539	1	61.82	0.90	5.44	0.020
TAA15	1	119.73	0.78	0.47	0.491
SOS1-M50	2	78.51	0.90	0.11	0.739
CX6F23	2	59.35	0.83	0.60	0.439
CX2004	2	56.76	0.89	0.40	0.527
CI02D09	2	133.78	0.79	2.00	0.157
ID4721	3	5.81	0.81	0.06	0.808
ID0039-380	3	87.92	0.92	0.14	0.705
MEST131	3	179.33	0.80	0.00	1.000
CI07D06	4	16.35	0.91	2.00	0.157
CI02D04B	4	85.84	0.87	0.09	0.763
CI03D12A	4	90.06	0.87	0.09	0.763
CI07G11	5	14.66	0.92	0.14	0.705
CMS30	5	31.35	0.90	0.11	0.739
ID5485	5	102.01	0.77	0.80	0.371
LG6-TTA6	6	79.95	0.79	8.00	0.005
MEST191	6	10.79	0.94	0.20	0.655
MEST123	6	91.88	0.86	1.33	0.248
MEST107	7	8.90	0.89	0.40	0.527
CI07E05	7	14.37	0.86	0.33	0.564
CI03B07	7	83.39	0.86	5.33	0.021
ID0591	7	115.59	0.76	1.80	0.180
CI01F04A	8	5.92	0.95	4.00	0.046
MEST502	8	43.13	1.00	NA	NA
CI02C09	8	95.03	0.95	0.00	1.000
LG9-GA15	9	61.95	0.81	4.00	0.046
JI-TCT01	9	55.11	0.91	0.00	1.000
CI02B07	9	0.01	0.86	1.33	0.248

GP, genetic position in cM; PHR, percentage of parental heterozygosity restitution; χ2, chi square value; NA, not applicable.

B. Genetic structure of diploid gamete populations arising from tetraploid 'Chandler' pummelo and 'Carrizo' citrange as male parents

Variability of PHR

The PHR was calculated for each LG and at gamete and marker level when using tetraploid 'Chandler' pummelo and 'Carrizo' citrange as male parents. When considering tetraploid 'Chandler' pummelo the PHR for all the LG ranged between 56.9% (LG4) and 70.0% (LG9), with an average PHR of 63.0% (**Table 5**). These values most likely indicate tetrasomic inheritance as they approximate the hypothetical PHR value of 66% reported for double diploids (Sanford, 1983; Aleza et al., 2016), however it must be confirmed with the PP value. In contrast, when considering tetraploid 'Carrizo' citrange, the PHR for all LG ranged between 84.1% (LG3) and 96.9% (LG8), with an average PHR of 87.0% (**Table 5**). Again, these values most likely indicate disomic inheritance as they approximate the hypothetical PHR value of 100% (Garavello et al., 2020; Stift et al., 2008).

Table 5. Parental heterozygosity restitution of diploid gametes produced by tetraploid 'Chandler' pummelo and 'Carrizo' citrange.

	'Chandler' pummelo		'Carrizo' citrange	
LG	PHR	PHR SD	PHR	PHR SD
1	63.1	4.7	86.2	7.2
2	60.2	3.4	85.0	4.9
3	66.9	8.3	84.1	6.9
4	56.9	7.8	88.3	2.1
5	63.6	3.8	86.1	8.2
6	61.3	3.8	86.4	7.6
7	57.3	3.4	84.3	5.4
8	67.5	13.2	96.9	2.7
9	70.0	17.4	86.2	4.7
Total	63.0	12.1	87.0	5.5

PHR, percentage of parental heterozygosity restitution; PHR SD, parental heterozygosity restitution standard deviation percentage.

Genetic structure at the individual marker level of the triploid hybrid population from tetraploid 'Chandler' pummelo and 'Carrizo' citrange as male parents

The PHR value for each marker was determined for both tetraploid 'Chandler' pummelo and 'Carrizo' citrange as male parents, **Tables 3 and 4**, respectively. The PHR value for molecular markers used to analyze 'Chandler' pummelo ranged from 0% PHR at markers 6_10554 (LG6) and 8_25026006 (G8), to 100% PHR at marker 9_9543785 (LG9). For the other LGs, the PHR values of the other markers along the chromosome remain mostly constant. The PHR value for molecular markers used to analyze 'Carrizo' citrange ranged from 76% PHR at marker ID0591 (LG7), to 100% PHR at marker MEST502 (LG8). For the other LGs, the PHR values of the other markers along the chromosome remain mostly constant.

Parental heterozygosity restitution (PHR) was calculated for each molecular marker and used to analyze the triploid hybrid populations obtained from genotypes under study. This allowed to determine the heterozygosity distribution, **Figure 6**. Both populations displayed unimodal distribution of PHR for the molecular markers used. However, the diploid gamete population obtained from tetraploid 'Chandler' pummelo (red) showed lower PHR values, with most of the markers presenting a PHR value between 55%-65%. In comparison, the diploid gamete population obtained from tetraploid 'Carrizo' citrange (blue) showed higher PHR values, most markers presenting a PHR value between 80%-90%.
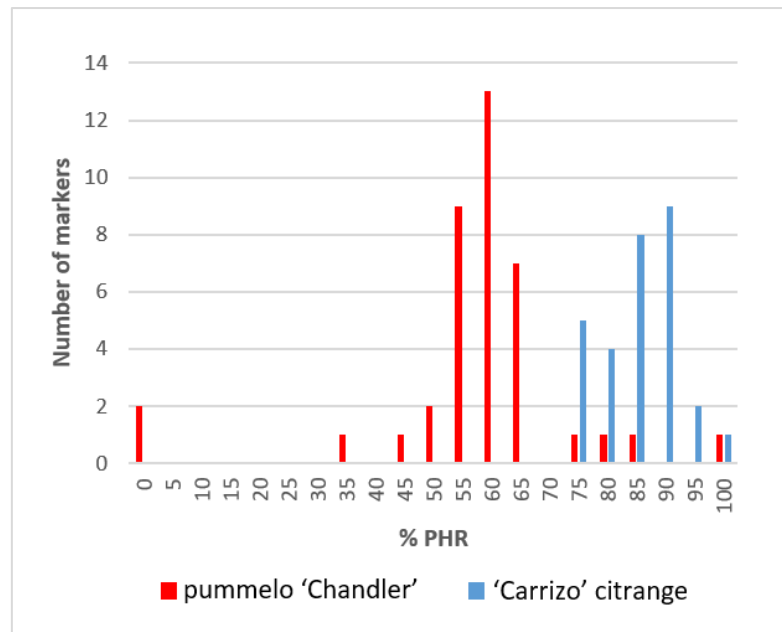
Figure 6. Distribution of the parental heterozygosity restitution (PHR) at the marker level of diploid gametes produced by tetraploids 'Chandler' pummelo (red) and 'Carrizo' citrange (blue)

Genetic structure at the gamete level of the triploid hybrid population from tetraploid 'Chandler' pummelo and 'Carrizo' citrange as male parents

At gamete level (**Figure 7**), both populations displayed a unimodal distribution of PHR. However, the diploid gamete population obtained from tetraploid 'Chandler' pummelo (red) showed lower PHR values, most of the markers presenting a PHR value between 50%-65% (average PHR 59.9 $\pm$ 12.1%), when compared to the diploid gamete population obtained from tetraploid 'Carrizo' citrange (blue), most markers presenting a PHR value between 75%-90% (average PHR 87 $\pm$ 5.5%).
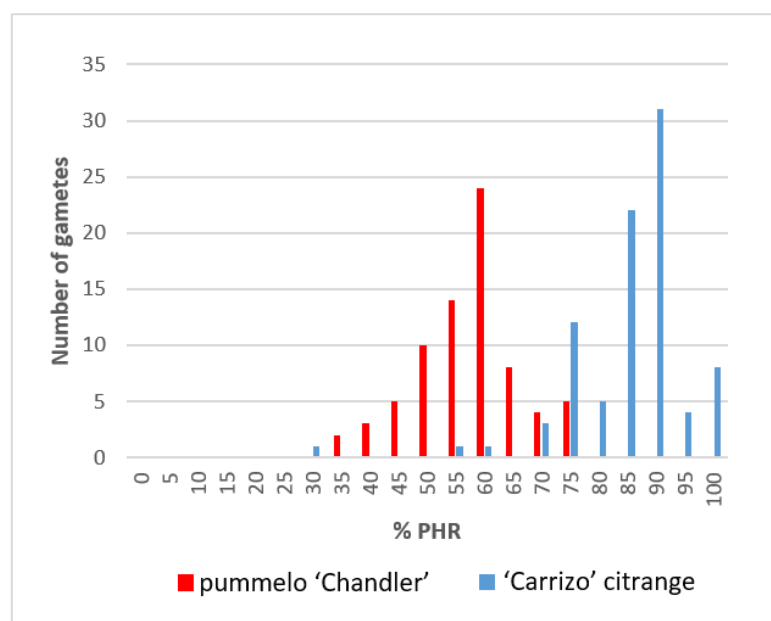
Figure 7. Distribution of the parental heterozygosity restitution (PHR) at the gamete level of diploid gametes produced by tetraploids 'Chandler' pummelo (red) and 'Carrizo' citrange (blue)

Genotypic Variability

The genetic structure was calculated by neighbor-joining analysis for both triploid hybrids populations obtained when using tetraploid 'Chandler' pummelo and 'Carrizo' citrange as male parents. This analysis allows the differentiation of triploid hybrid groups within each family and to measure the genetic distance between them. The molecular markers used to analyze the gamete population of tetraploid 'Chandler' pummelo, as well as the ones used to analyze the gamete population of tetraploid 'Carrizo' citrange allows the differentiation of all triploid hybrids within each progeny (**Figure 8**). The average genetic distance between diploid gametes of 'Chandler' pummelo and those of 'Carrizo' citrange are 0.280 $\pm$ 0.060 and 0.121 $\pm$ 0.063, respectively. Additionally, the genetic structure of diploid 'Chandler' gametes is more heterogeneous and dispersed in comparison with the genetic structure of 'Carrizo' citrange diploid gametes.

The genetic distance of 'Chandler' pummelo and clementine to the diploid gamete population of tetraploid 'Chandler' pummelo is 0.062 ± 0.069 and 0.383 ± 0.029, respectively (**Figure 8a**). On the other hand, the average genetic distance of clementine and 'Carrizo' citrange to the diploid gamete population of tetraploid 'Carrizo' citrange is 0.571 ± 0.047 and 0.075 ± 0.081, respectively (**Figure 8b**). This low genetic distance of 'Carrizo' citrange to diploid gametes is due to 'Carrizo' being a direct hybrid of *C. sinensis* and *P. trifoliata*. In fact, the genetic distance of *P. trifoliata* to the diploid gamete population is more or less similar to that calculated for clementine. If we compare both diploid gametes populations, the neighbor-joining tree analysis displayed a different pattern of genetic diversity, 'Chandler' pummelo diploid gametes being more diverse than 'Carrizo' diploid gametes. In a previous work performed by Calvez et al. (2020), the authors analyzed the genetic diversity of diploid gametes produced for two intergeneric *Citrus* x *Poncirus* hybrids, and the corresponding dendograms fitted perfectly with the calculated for 'Carrizo' citrange, with the gamete population centered on the diploid 'Carrizo' citrange.
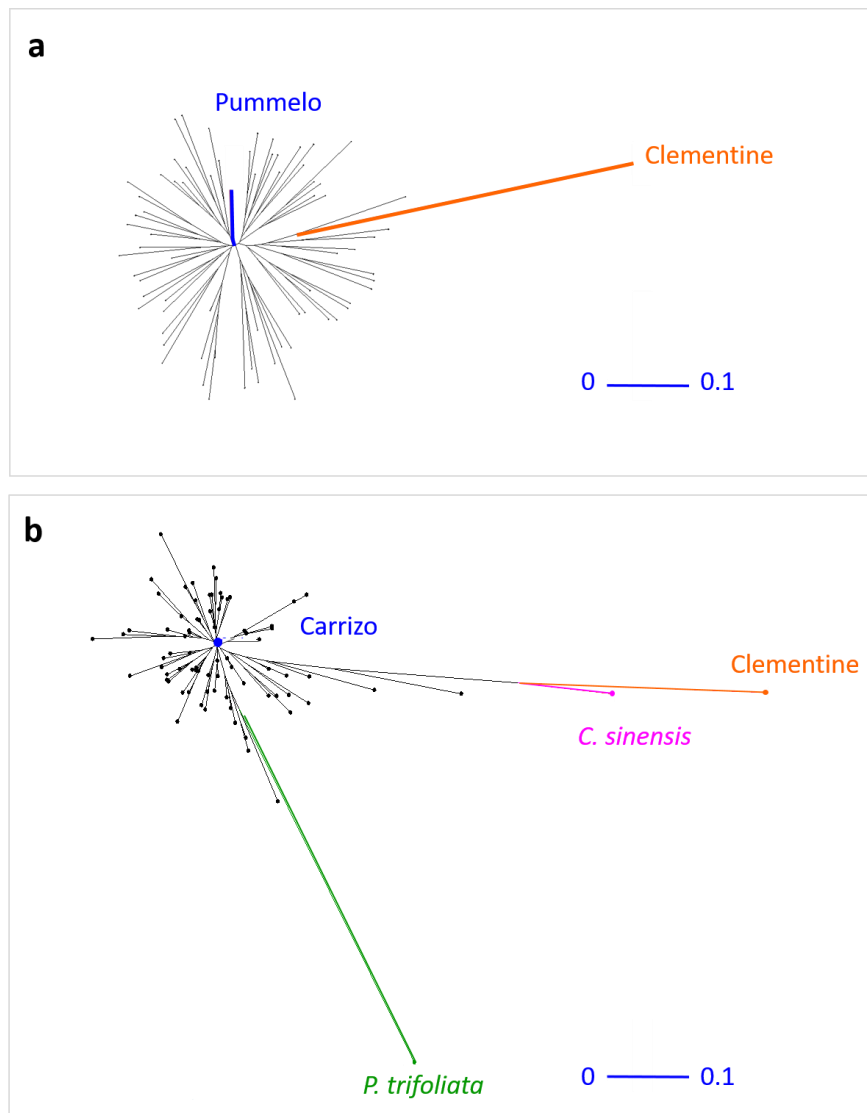
Figure 8. Dendrograms corresponding to the genetic analysis performed with SSR and SNP markers obtained by calculating the Simple Matching Dissimilarity Index and tree construction by weighted neighbor-joining of a diploid gamete population produced by a) tetraploid 'Chandler' pummelo and b) tetraploid 'Carrizo' citrange.

C. Estimation of the preferential pairing coefficient (PP)

The PP coefficient for tetraploid 'Chandler' pummelo and 'Carrizo' citrange was calculated using the allelic frequencies (aa, ab and bb) of the molecular marker closest to the centromere, **Table 6** and **Table 7**, respectively. For tetraploid 'Chandler' pummelo, complete tetrasomic inheritance was observed for all LGs. The PP coefficient was 0 for LGs 2, 3, 4, 6, 7, and 9, and almost zero (0.04) for LGs 1, 5 and 8 (**Table 6**).

The genome of many cultivated citrus is composed of mosaics of the ancestral species (Curk et al. 2014, 2015; Wu et al. 2014, 2018). The works carried out on citrus phylogeny (Oueslati et al. 2017; Wu et al. 2014, 2018) have shown that 'Chandler' pummelo is constituted by monospecific pummelo/pummelo structure with very few interspecific mandarin/pummelo structure in heterozygosity (0.4%). Therefore, 'Chandler' pummelo can be considered as a *C. maxima* specie along its chromosomes. For this reason, the genomic structure of its chromosomes can be considered homogeneous with no interspecificity, leading to tetrasomic inheritance pattern for all LGs. Similarly, 'Cleopatra' mandarin, which is a genotype widely used as rootstock, has been shown to be completely mandarin in its genomic constitution, with very few introgression from *C. maxima* (Wu et al. 2018). The inheritance pattern of tetraploid 'Cleopatra' mandarin has been analyzed using the same methodology described above and also displayed tetrasomic inheritance like 'Chandler' pummelo (data not shown). This information indicates that homogeneous phylogenomic structure of chromosomes pave the way for tetrasomic inheritance in all LGs. On the other hand, genetic analysis of tetraploid citrus genotypes with mandarin/pummelo interspecific phylogenetic structure produced preferential tetrasomic inheritance. For example, Aleza et al. (2016) revealed that all LGs of tetraploid *C. clementina* presented tetrasomic inheritance, evidenced by low PP values (0.000 < PP < 0.115), except for LG 4 (PP = 0.545), which presented intermediate inheritance. Recently, Garavello et al. (2020) analyzed the inheritance patterns of tetraploid 'Moncada' mandarin (which is constituted by an interspecific mandarin/pummelo mosaic structure) used either as female and male parent. In the case of tetraploid 'Moncada' mandarin as female, most of the LGs showed a low PP value (0.000 < PP < 0.085) indicating tetrasomic inheritance. However, LG4 (PP = 0.5) presented clear intermediate inheritance while LG9 (PP = 0.375) presented intermediate inheritance with a tendency towards tetrasomic inheritance.

Allotetraploids typically present strict preferential pairing which results in disomic inheritance, with no interspecific recombination. However, it is important to mention that non-homologous chromosome pairing can occur occasionally in allopolyploids leading to non-strict disomic inheritance and therefore to inter-genomic recombination (Kamiri et al., 2018; Rouiss et al., 2018; Soltis & Soltis, 2000; Stift et al., 2008). 'Carrizo' citrange is a hybrid between *C. sinensis* and *P. trifoliata*, therefore the resulting tetraploid 'Carrizo' citrange is an allotetraploid that results from the hybridization event of the genomes of these two divergent species. Wu et al. (2018) pointed out clear differentiation between *Poncirus* and *Citrus* genera. In **Table 7** we displayed the estimated preferential pairing (PP) values for tetraploid

'Carrizo' citrange. High preferential pairing values were observed for LG6 (PP = 0.825) and LG8 (PP = 0.995) indicating disomic inheritance. Intermediate inheritance with a tendency to disomy can be seen for LGs 1, 2, 3, 4, 5, and 9 with values between (0.655 < PP < 0.76). Lastly, intermediate inheritance can be seen for LG7 (PP = 0.570). These results are similar to those obtained by Calvez et al. (2020) in which intermediate inheritance with a disomic tendency was observed for both tetraploid citrumelo (*C. paradisi* x *P. trifoliata*) and tetraploid Citrandarin (*C. reticulata* x *P. trifoliata*). This was expected as both species are a result of interspecific hybridizations. In the case of citrandarin, LGs 5, 6 and 9 presented intermediate inheritance, PP not significantly different from 0.5). While LGs 1, 2, 3, 4, 5, 7, 8 and 9 in citrumelo and LGs 1, 2, 3, 7 and 8 in citrandarin presented intermediate behavior with preferential disomic tendency (0.5 < PP < 0.9). Lastly, LG 6 in citrumelo and LG 4 in citrandarin were close to disomic inheritance (PP > 0.9). However, sexual compatibility between *Poncirus* and *Citrus* genera shows that the remaining homology is still sufficient to allow chromosome pairing between both genera explaining why Citrange, Citrumelo and Citrandarin intergeneric hybrids do not display strict disomic inheritance at tetraploid level (Calvez, et al. 2020).

Kamiri el al. (2011) studied the inheritance patterns of an intergeneric tetraploid somatic hybrid of *C. reticulata* + *P. trifoliata*. Through molecular marker analysis, strong but incomplete preferential pairing was observed between homologous chromosomes of the same ancestral genome, therefore indicating intermediate inheritance with tendency for disomic inheritance for most LGs resulting in a high level of intergeneric heterozygosity of the resulting diploid gametes.

Rouiss et al. (2017) studied the inheritance pattern of tetraploid 'Mexican' lime, a direct interspecific *C. micrantha* x *C. medica* hybrid. For three LGs, disomic inheritance was observed; for five LGs intermediate inheritance with a tendency for disomic inheritance was observed, and one LG presented intermediate inheritance. These results indicate that double-diploid 'Mexican' lime primarily presents disomic segregation.

In conclusion, the varying results presented from our work and the previously described studies reveal that the PP of tetraploid citrus genotypes is highly dependent on the origins and the constitutive genomes of the genotype.

Estimation of the coefficient of double reduction (DR)

Tetraploid 'Chandler' pummelo can be considered as autotetraploid (doubled-diploid) because its phylogenomic structure came from one ancestral specie, *C. maxima*. DR requires multivalent formation and crossing over between the locus and its centromere and results in loci of two sister chromatids to segregate to a single gamete (Haynes & Douches, 1992). If during meiosis of autotetraploid quadrivalents are produced and an effective crossing over takes place between considered locus and centromere, the maximum frequency of DR is 1/6 (Haynes & Douches, 1992). For this tetraploid genotype, after estimating PP for each chromosome, it was possible to estimate the DR for markers

located on the same LG that were farthest from the centromere. This was done by using the maximum likelihood approach and considering a fixed PP value, the one previously determined, and varying the DR interval in 0.005 increments. The highest DR values were observed for LGs 7, 8, 9 (0.105 < DR < 0.22) and all LGs, except for LG 3, present DR values that when considering the confidence interval (CI) include the maximum value 1/6 (**Table 6**).

It is important to highlight that since tetraploid 'Chandler' pummelo is a double-diploid, high DR values implicate greater homozygosis frequency which leads to a decrease in PHR, indicating reduced genetic variability of the gametes or an increased inbreeding. As a result, 'Chandler' pummelo's triploid progeny will be less likely to resemble the characteristics of tetraploid 'Chandler' pummelo.

Different studies have performed the estimation of DR frequencies and showed values ranging between 0 to almost 0.30 (Welch, 1960; Wu et al., 2001). DR values can differ between loci according to the tetrasomic inheritance model. DR values depend on the chromosome in which the molecular marker used to determine the DR value is located on, as well as the position of the molecular marker within the chromosome. Chromosomes with a greater tendency for tetrasomic inheritance give rise to higher DR values (Butruille & Boiteux, 2000). However, it is important to mention that a more accurate estimation of DR can always be accomplished by evaluation of larger populations. DR has a greater tendency to occur in molecular markers present in telomeric regions, in comparison to molecular markers located near the centromere where recombination events have a probability close to zero (Butruille & Boiteux, 2000).

Table 6. Estimated preferential pairing (PP) and double reduction (DR) values for tetraploid 'Chandler' pummelo

LG	Molecular marker	dC	aa	bb	ab	PP	CI	DR	CI
1	1_14053816	3.15	18	51	6	0.040	0-0.365		
	MEST321	57.82	13	47	15			0.085	0-0.28
2	CX2004	0.113	18	45	12	0.000	0-0.195		
	2_36363713	93.26	14	47	14			0.060	0-0.245
3	CX0124	19.71	22	46	7	0.000	0-0.215		
	3_51024515	101.43	16	59	0			0.000	0-0.075
4	CI07D06	0.19	17	45	13	0.000	0-0.195		
	CIBE3255	73.05	15	47	13			0.060	0-0.245
5	5_27500244	2.11	13	51	11	0.040	0-0.365		
	MEST56	87.04	20	46	9			0.105	0-0.3
6	6_5996116	1.13	20	50	5	0.000	0-0.33		
	TAA1	87.28	14	46	15			0.080	0-0.27
7	7_15708026	8.58	17	46	12	0.000	0-0.215		
	7_20255	85.54	15	42	18			0.160	0-0.35
8	8_15415145	3.43	8	51	16	0.040	0-0.365		
	CI02C09		17	43	15			0.165	0-0.36
9	MEST308	1.75	13	47	15	0.000	0-0.24		
	9_31288895	46.02	23	38	12			0.22	0.04-0.41

dC, distance from centromere in cM; aa, bb and ab number of diploid gametes with that allelic configuration; PP, preferential pairing; DR, double reduction rate; CI, confidence interval.

LG	Molecular marker	dC	aa	bb	ab	PP	CI
1	MEST539	1.2	8	79	1	0.695	0.45-0.86
2	CX2004	0.1	6	77	4	0.655	0.4-0.835
3	ID0039-380	2.7	3	80	4	0.760	0.53-0.905
4	CI07D06	0.2	6	78	2	0.720	0.48-0.88
5	CMS30	8.2	4	78	5	0.690	0.44-0.86
6	MEST191	4.6	2	80	3	0.825	0.165-0.945
7	CI03B07	13.0	2	72	10	0.570	0.29-0.78
8	MEST502	11.1	0	87	0	0.995	0.915-1
9	JI-TCT01	2.9	4	79	4	0.725	0.480-0.885

dC, distance from centromere in cM; aa, bb and ab number of diploid gametes with that allelic configuration; PP, preferential pairing; CI, confidence interval.

D. Phylogenomic structure of 'Carrizo' citrange diploid gametes

A more precise analysis of the data at the gamete level was obtained when using tetraploid 'Carrizo' citrange as male parent **Table 8** and **Table 13 of Annexes**, respectively. 'Carrizo' is an intergeneric hybrid of *C. sinensis* and *P. trifoliata,* two genera that are highly genetically distanced, allowing the analysis of the phylogenetic structure of the population. Genetic analysis of each gamete per LG was determined which made it possible to identify the origin of alleles inherited by the diploid gamete that produced the triploid hybrid: (1) both alleles inherited from *P. trifoliata* (PT) (2) both alleles inherited from *C. sinensis* (CS) (3) one allele inherited from *P. trifoliata* and the other from C. sinensis (HE) (**Annexes Table 12**). Based on the allele configuration of each molecular marker, it was possible to visualize the phylogenetic structure of the whole population. For instance, it was possible to classify each LG as (1) full *P. trifoliata* - all molecular markers of that LG indicated allelic configurations inherited from *P. trifoliata* (PT) (2) full *C. sinensis* - all molecular markers of that LG indicated allelic configuration inherited from *C. sinensis* (CS) (3) full heterozygous - all molecular markers of that LG indicated that for each molecular marker one allele came from *P. trifoliata* and the other from *C. sinensis* (HE) and (4) mixed where molecular markers of that LG indicated that there was a mixture of the allelic configuration (PT, CS, and/or HE). Lastly, a LG was homozygous at the centromere if both the molecular markers flanking the centromere of the LG presented allelic configurations of *C. sinensis* or both presented allelic configurations of *P. trifoliata*.

This analysis revealed that on average 72.9% of the individual LGs of the different hybrids present full heterozygosity. On the contrary, on average 0.5% and 0.8% fully homozygous LGs at the population level were observed for *C. sinensis* and *P. trifoliata*, respectively. A total of 23.1% of all the LGs showed a mixed structure with homozygosity and heterozygosity. The presence of homozygous and mixed LGs indicate pairing of chromosomes between *C. sinensis* and *P. trifoliata* and the presence of intergeneric recombination. Lastly, a 2.8% average of all the LGs present homozygosity for both markers at either side of the centromere.

Considering each diploid gamete individually, **Table 9 and Figure 9**, eight gametes were completely heterozygous for all 9 LGs, and 34% of the diploid gametes presented a PHR $\geq$ 90% ($\geq$8 LGs that are completely heterozygous). Additionally, an average of 72%, 6.5 out of 9 LGs in each gamete are full heterozygous. It is also important to highlight that for LG2, there is a 10.2% of homozygosity on both sides of the centromere, which suggests higher homology between *C. sinensis* and *P. trifoliata* for this chromosome when compared to the other LGs.

Table 8. Interspecific structures of 'Carrizo' citrange diploid gametes by LGs

	FH	F*sin*	F*tri*	Mixed	Hcent
LG1	69.3	0.0	2.3	26.1	2.3
LG2	69.3	0.0	0.0	20.5	10.2
LG3	64.8	0.0	0.0	34.1	1.1
LG4	80.7	1.1	1.1	17.1	0.0
LG5	73.9	1.1	2.3	20.5	2.3
LG6	70.5	0.0	0.0	29.6	0.0
LG7	64.8	2.3	0.0	27.3	5.7
LG8	90.9	0.0	0.0	9.1	0.0
LG9	71.6	0.0	1.1	23.9	3.4
Total	72.9	0.5	0.8	23.1	2.8

FH, percentage of fully heterozygous gametes; F*sin*, percentage of fully *C. sinensis* homozygous gametes; F*tri*, percentage of fully *P. trifoliata* homozygous gametes; Mixed, percentage of gametes with mixed heterozygosity and homozygosity; Hcent, homozygous at the centromere for either *C. sinensis* or *P. trifoliata*.

Table 9. Interspecific structures of 'Carrizo' citrange diploid gametes

Total no. gametes	Gametes 100% heterozygous for all loci	Gametes with PHR >90
88	8	30
100%	9.1%	34.1%
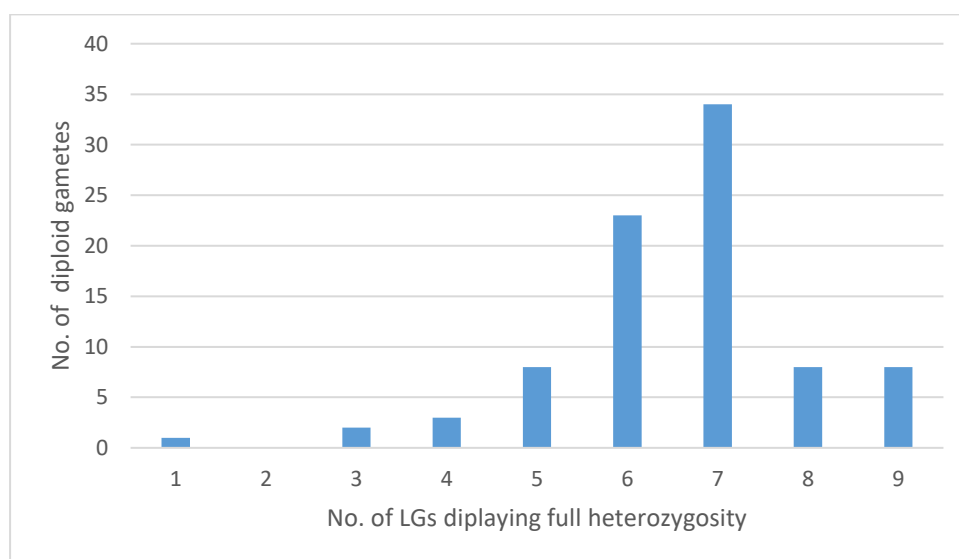
Figure 9. Distribution of parental heterozygosity restitution (PHR) among diploid gametes of tetraploid 'Carrizo' citrange

E. Implications in citrus breeding programs

Citrus breeding is difficult because of the complex genome structures, long juvenile phase and the existence of apomictic genotypes. Most breeding strategies generally only carry out one cycle of breeding to produce variability followed by direct selection of cultivars or rootstocks. Different breeding strategies have been developed including the use of tetraploid genotypes with two different objectives: as parents in breeding for varieties or as rootstocks themselves. Therefore, genotyping as well as understanding of chromosome segregation models is essential to give insight on the best crosses to optimize the transfer of desired traits to the progenies (Grosser & Gmitter, 2011).

This study concluded that 'Chandler' pummelo presents tetrasomic inheritance and would therefore be of great use in increasing variability to produce new triploid hybrids. In breeding for varieties, Aleza et al., (2016), studied the inheritance patterns of tetraploid clementine and made a comparative study with the second division restitution (SDR) unreduced gametes produced by the same diploid clementime (2x X 2x sexual hybridization). It was reported that tetraploid clementime displays primarily tetrasomic inheritance when used as female parent, correlated with high PHR values (0.65), whereas SDR unreduced gametes presented lower values of PHR (0.42). Therefore, interploid 4x X 2x hybridizations produces triploid hybrids that are genotypically closer to the diploid parent from which tetraploid clementine was developed. In contrast, SDR unreduced gametes has a greater potential to produce new varieties.

It is also important to mention that significant DR results in a decrease of PHR and thereby an increased inbreeding (Haynes and Douches, 1993; Garavello *et al*., 2020), which could have the ability of revealing deleterious alleles to selection as well as increase the accumulation of rare but favorable allelic combinations with the use of molecular markers (Bourke et al., 2015). It has been reported that chromosomes with a greater tendency for tetrasomic inheritance give rise to higher DR values (Butruille & Boiteux, 2000). This advantage could be exploited when using tetraploid 'Chandler' pummelo as male parent since this study concluded that it presents tetrasomic inheritance and high DR values.

On the other hand, male tetraploid genotypes that present preferently disomic inheritance, such as Mexican Lime (Rouiss et al. 2018), are mainly used to benefit from the relatively high transmission of parental heterozygosity (0.9 as an average), resulting in a high number of allelic combinations that are like the diploid genotype (used to create the tetraploid) in the resulting triploid hybrid population. In other words, using tetraploid genotypes with disomic inheritance is a useful strategy to develop new varieties that are genetically closer to the diploid genotype that gave rise to the tetraploid genotype in comparison with tetrasomic inheritance displayed by the genotypes mentioned above.

In breeding for rootstocks, vegetative propagation including apomictic seeds is an exploited citrus characteristic. It is also important to mention that tetraploid genotypes are being used in rootstock breeding programs (Calvez, et al. 2020). It has been reported that tetraploid genotypes can be more tolerant to abiotic stresses such as salt and water stress, as well as cold tolerance, when compared to their parental diploids (Aleza et al., 2011; Allario et al., 2013; De Souza et al., 2017; Garavello et al., 2020;

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Mouhaya et al., 2010; Oustric et al., 2018). Other studies have also demonstrated that tetraploid rootstocks tend to cause a reduction in the canopy size, which is also a desired trait in modern orchards since it reduces the costs associated to harvesting, treating with chemicals and pruning (Aleza et al., 2011; Barrett & Hutchingson, 1978; Lee, 1988). Therefore, selection of double-diploid genotypes from the most common rootstocks used seems to be a way to improve the tolerance to biotic or abiotic stress. More specifically, *Citrus-Poncirus* intergeneric polyploid breeding is able to combine many favorable traits from both genomic backgrounds. The *Citrus* genomic background contributes to tolerance of abiotic stresses, such as salinity, water deficit, and calcareous soils, while the *Poncirus* genomic background contributes to cold tolerance as well as resistance and tolerance to diseases and pests such as tristeza virus, Phytophthora, nematodes (Grosser & Gmitter, 2011; Calvez et al., 2020). 'Carrizo' citrange is one of the single most important rootstocks used for citrus plants in the Spanish citriculture because it has a very good adaptation to calcareous soils, induces good fruit quality and resistance and tolerance to various citrus diseases such as citrus tristeza virus and foot rot (Savage & Gardner, 1965; Belknap et al., 2011; Folimonova et al., 2009; Calvez *et al*., 2020).

Disease and pest resistance is usually a result of the additive properties of various complex multilocus structures and multiple genes. In this study we have observed that 'Carrizo' citrange presented disomic inheritance which can be useful in breeding strategies since it indicates that it transmits a large part of its parental intergeneric heterozygocity to its progeny, helping to avoid disruption of complex multilocus structures responsible for its resistance (Grosser & Gmitter, 2011). The use of tetraploid rootstock genotypes that present disomic inheritance is an effective breeding strategy to be able to compile multilocus structures from different rootstocks to end up with a genotype that presents all the complementary characteristics.

V. CONCLUSIONS

The analysis of codominant marker segregation distributed homogenously over the nine citrus chromosomes of both tetraploid 'Chandler' pummelo and 'Carrizo' citrange allowed to determine the inheritance patterns of both genotypes when used as male parents. Tetraploid 'Chandler' pummelo as male parent presented tetrasomic inheritance for all nine LGs and significant DR values for LGs 5, 7, 8, 9. In contrast, tetraploid 'Carrizo' citrange as male parent predominantly presented disomic inheritance: specifically disomic inheritance for LG 6 and 8, intermediate inheritance with a tendency for disomic inheritance for LGs 1, 2, 3, 4, 5, and 9, and clear intermediate inheritance for LG7. These new insights on the inheritance patterns of tetraploid 'Chandler' pummelo and 'Carrizo' citrange will be of use to define future crossing strategies in triploid citrus breeding programs of great importance in the global market of fresh consumed citrus, as well as to efficiently develop new rootstocks with improved traits.

XI.   REFERENCES

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XII. ANNEXES

Table 10. Interspecific structures of tetraploid 'Carrizo' citrange diploid gametes

	Full Heterozygous	Full *C. sinensis*	Full *P. trifoliata*	Mixed	Homozygous at centromere
CLEMxCA 2013 -01	9				
CLEMxCA 2013 -02	7			2	
CLEMxCA 2013 -03	7			1	1
CLEMxCA 2013 -04	7			2	
CLEMxCA 2013 -05	6			3	
CLEMxCA 2013 -06	7			2	
CLEMxCA 2013 -07	7			2	
CLEMxCA 2013 -08	9				
CLEMxCA 2013 -09	9				
CLEMxCA 2013 -10	6		1	2	
CLEMxCA 2013 -11	7			2	
CLEMxCA 2013 -12	5			3	1
CLEMxCA 2013 -13	6			3	
CLEMxCA 2013 -14	7			2	
CLEMxCA 2013 -15	7			2	
CLEMxCA 2013 -16	5			4	
CLEMxCA 2013 -17	8			1	
CLEMxCA 2013 -18	6			2	1
CLEMxCA 2013 -19	6			3	
CLEMxCA 2013 -20	9				
CLEMxCA 2013 -21	6			3	
CLEMxCA 2013 -22	3		1	3	2
CLEMxCA 2013 -23	7		1	1	
CLEMxCA 2013 -24	7			2	
CLEMxCA 2013 -25	5			4	
CLEMxCA 2013 -26	6	1		2	
CLEMxCA 2013 -27	8			1	
CLEMxCA 2013 -28	7			2	
CLEMxCA 2013 -29	7			2	
CLEMxCA 2013 -30	6			2	1
CLEMxCA 2013 -31	7			2	
CLEMxCA 2013 -32	7			2	
CLEMxCA 2013 -33	7			2	
CLEMxCA 2013 -34	8			1	
CLEMxCA 2013 -35	6			3	
CLEMxCA 2013 -36	5		1	2	1
CLEMxCA 2013 -37	7		1	1	
CLEMxCA 2013 -38	9				
CLEMxCA 2013 -39	7			2	
CLEMxCA 2013 -40	5			4	
CLEMxCA 2013 -41	6			3	
CLEMxCA 2013 -42	7			2	
CLEMxCA 2013 -43	7			2	
CLEMxCA 2013 -44	6			3	
CLEMxCA 2013 -45	3			6	
CLEMxCA 2013 -46	7			2	
CLEMxCA 2013 -47	1	2		4	2
CLEMxCA 2013 -48	9				
CLEMxCA 2013 -49	9				
CLEMxCA 2013 -50	6			3	
CLEMxCA 2013 -51	8			1	
CLEMxCA 2013 -52	7			1	1
CLEMxCA 2013 -53	7			2	
CLEMxCA 2013 -54	7			1	1
CLEMxCA 2013 -55	6			3	
CLEMxCA 2013 -56	7		1		1
CLEMxCA 2013 -57	6			2	1
CLEMxCA 2013 -58	7			1	1
CLEMxCA 2013 -59		6		2	1
CLEMxCA 2013 -60	8				1
CLEMxCA 2013 -61	7			2	
CLEMxCA 2013 -62	6			3	
CLEMxCA 2013 -63	7			2	
CLEMxCA 2013 -64	6			3	
CLEMxCA 2013 -65	6		1	2	
CLEMxCA 2013 -66	9				
CLEMxCA 2013 -67	6			3	
CLEMxCA 2013 -68	4			5	
CLEMxCA 2013 -69	8			1	
CLEMxCA 2013 -70	6			3	
CLEMxCA 2013 -71	5			3	1
CLEMxCA 2013 -72	7			2	
CLEMxCA 2013 -73	8				1
CLEMxCA 2013 -74	6			3	
CLEMxCA 2013 -75	8				1
CLEMxCA 2013 -76	7			1	1
CLEMxCA 2013 -77	7			2	
CLEMxCA 2013 -78	6			2	1
CLEMxCA 2013 -79	5			4	
CLEMxCA 2013 -80	4	1		4	
CLEMxCA 2013 -81	7			2	
CLEMxCA 2013 -82	7			2	
CLEMxCA 2013 -83	4			5	
CLEMxCA 2013 -84	6			3	
CLEMxCA 2013 -85	6			3	
CLEMxCA 2013 -86	7			2	
CLEMxCA 2013 -87	5			4	
CLEMxCA 2013 -88	7			2	

Table 11. Tetraploid pummelo 'Chandler' diploid gamete population analyzed with 39 molecular markers.

	LG 1			LG 2				LG 3			
	16.76	57.51	118.48	1	56.757	125.61	150.13	7.38	110.3	149.15	192.02
Marker/Genotype	Ci02G08	1_14053816	MEST321	2_60291	CX2004	CI01C07	2_36363713	3_17626	CX0124	CI02G02	3_51024515
CLEMxCH 2013 -01	249 257	CC	108 112		175 175	259 277	GC	CC	169 171	110 110	AG
CLEMxCH 2013 -02	249 257	CG	112 112	AG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -03	249 257	CG	108 112	AA	175 175	259 259	CC	AA	169 169	110 122	AG
CLEMxCH 2013 -04	249 257	CC	108 112	AG	175 183	259 277	GC	AA	169 171	110 122	AG
CLEMxCH 2013 -05	257 257	CG	108 112	AG	175 183	259 277	GC	CC	169 169	110 122	AG
CLEMxCH 2013 -06	249 257	CG	108 112	AG	183 183	259 277	GC	AA	171 171	110 122	AG
CLEMxCH 2013 -07	257 257	CG	108 112	AA	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -08	249 257	CC	112 112	GG	175 175	277 277	GG	CA	169 171	110 110	AA
CLEMxCH 2013 -09	257 257	GG	108 112	AG	175 183	259 259	CC	CC	169 171	110 122	AG
CLEMxCH 2013 -10	249 257	CG	112 112	AG	175 183	259 259	GC	AA	169 169	110 122	AG
CLEMxCH 2013 -11	249 257	CG	108 108	AG	175 175	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -12	249 257	CG	108 112	AG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -13	249 257	CG	112 112	GG	175 175	259 277	GC	AA	169 171	110 122	AG
CLEMxCH 2013 -14	257 257	CG	108 112	AG	175 183	259 277	GC	CC	169 169	110 110	AG
CLEMxCH 2013 -15	249 257	CG	108 112	AG	175 183	277 277	GG	CA	169 169	110 122	AG
CLEMxCH 2013 -16	249 257	CG	108 112	AG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -17	249 257	CC	108 112	AG	175 183	277 277	GC	AA	169 171	122 122	AG
CLEMxCH 2013 -18	257 257	CG	108 112	AA	175 183	259 277	GC	CA	169 169	122 122	AG
CLEMxCH 2013 -19	257 257	CC	108 112	AG	175 183	277 277	GG	CA	169 169	110 122	AG
CLEMxCH 2013 -20	249 257	CC	108 112	AG	175 183	259 259	CC	AA	171 171	110 122	AA
CLEMxCH 2013 -21	249 257	CC	108 112	AG	175 183	259 277	GC	CC	169 171	110 122	AA
CLEMxCH 2013 -22	249 249	CG	108 112	AG	175 175	259 277	GC	CC	169 169	122 122	AG
CLEMxCH 2013 -23	249 257	CG	108 112	AG	175 175	259 259	GC	CA	171 171	110 122	AG
CLEMxCH 2013 -24	249 257	CG	108 112	GG	175 175	259 277	GC		169 171	110 110	AG
CLEMxCH 2013 -25	249 249	CC	108 112	GG	175 183	259 277	GC	CA	169 169	110 110	AA
CLEMxCH 2013 -26	249 257	CC	112 112	AA	175 183	277 277	GG	CA	169 171	122 122	AG
CLEMxCH 2013 -27	249 257	CC	112 112	AA	175 183	277 277	GG	CA	169 171	122 122	AG
CLEMxCH 2013 -28	249 257	CC	108 108	AA	183 183	259 277	GC	CA	169 169	110 122	AG
CLEMxCH 2013 -29	249 257	CC	108 108	AA	183 183	259 277	GC	CA	169 169	110 122	AG
CLEMxCH 2013 -30	249 257	CG	108 112	AA	183 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -31	249 257	CG	108 112	GG	175 175	259 277	GC	CA	171 171	110 122	AG
CLEMxCH 2013 -32	257 257	CG	108 112	AG	175 183	259 277	GC	CC	169 169	110 122	AG
CLEMxCH 2013 -33	249 257	CG	108 112	GG	183 183	259 277	GG	CA	169 169	110 122	AG
CLEMxCH 2013 -34	249 257	CG	112 112	GG	175 183	277 277	GG	CA	169 169	110 122	AG
CLEMxCH 2013 -35	249 257	CG	112 112	GG	175 183	277 277	GG	CC	169 171	110 110	AG
CLEMxCH 2013 -36	249 257	CG	108 112	AA	175 183	259 259	CC	CA	169 169	122 122	AG
CLEMxCH 2013 -37	257 257	CG	108 112	AA	183 183	259 259	CC	CA	169 171	110 122	AG
CLEMxCH 2013 -38	249 257	CG	108 108	GG	175 183	259 277	GC	CA	169 171	110 122	AA
CLEMxCH 2013 -39	249 249	CG	108 108	GG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -40	249 257	CG	108 108	AG	175 183	259 277	GC	CA	169 171	122 122	AG
CLEMxCH 2013 -41	257 257	CG	108 112	AG	175 175	259 277	GC	CA	169 171	122 122	AG
CLEMxCH 2013 -42	249 249	CG	108 108	AG	175 175	277 277	GC	CC	169 171	110 122	AG
CLEMxCH 2013 -43	257 257	GG	108 112	AG	175 183	259 277	GC	CC	169 171	110 122	AA
CLEMxCH 2013 -44	249 257	CC	108 108	AG	183 183	259 277	GC	CC	169 171	122 122	AG
CLEMxCH 2013 -45	257 257	CG	112 112	AG	175 183	259 259	CC	CA	169 171	122 122	AA
CLEMxCH 2013 -46	249 257	CC	108 108	AA	175 183	259 259	CC	CA	169 171	110 110	AG
CLEMxCH 2013 -47	249 257	CG	108 112	GG	175 183	259 277	GC	CA	169 169	122 122	AG
CLEMxCH 2013 -48	257 257	GG	112 112	AG	183 183	259 277	GC	CA	169 171	110 110	AG
CLEMxCH 2013 -49	249 249	CG	108 112	AA	183 183	259 277	GC	CA	171 171	122 122	AG
CLEMxCH 2013 -50	249 249	CC	112 112	AG	175 183	277 277	GG	CA	169 169	110 110	AG
CLEMxCH 2013 -51	249 257	CG	108 112	GG	175 183	259 277	CC	AA	169 169	110 122	AG
CLEMxCH 2013 -52	257 257	CG	108 108	AG	175 175	259 277	GC	AA	169 171	110 122	AG
CLEMxCH 2013 -53	249 257	GG	108 112	AG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -54	249 257	CG	112 112	AG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -55	257 257	CG	112 112	GG	175 183	259 259	CC	CA	169 171	110 122	AG
CLEMxCH 2013 -56	257 257	CG	112 112	AG	175 183	259 277	GC	AA	169 169	110 122	AA
CLEMxCH 2013 -57	249 249	CC	108 108	AA	175 183	259 277	CC	CC	169 171	110 122	AA
CLEMxCH 2013 -58	249 257	CG	108 112	GG	183 183	277 277	GG	CA	169 171	110 122	AA
CLEMxCH 2013 -59	249 257	CG	108 112	AG	183 183	277 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -60	249 249	CC	108 108	AG	175 183	277 277	GG	CC	169 171	122 122	AG
CLEMxCH 2013 -61	249 257	CG	108 108	GG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -62	257 257	CG	112 112	AG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -63	257 257	GG	108 112	GG	175 183	259 277	CC	CC	169 171	110 110	AG
CLEMxCH 2013 -64	249 249	CG	108 112	GG	175 175	259 277	GC	CA	171 171	110 122	AG
CLEMxCH 2013 -65	249 257	CG	108 112	AG	175 183	259 277	GC	CC	169 171	110 122	AA
CLEMxCH 2013 -66	257 257	CG	108 112	GG	175 183	259 277	GC	CA	169 171	110 110	AA
CLEMxCH 2013 -67	249 257	CG	108 112	AG	175 183	259 277	GC	AA	169 169	110 122	AA
CLEMxCH 2013 -68	249 257	CG	108 112	AG	175 183	259 259	CC	CA	169 171	122 122	AG
CLEMxCH 2013 -69	249 257	CG	108 112	GG	175 175	277 277	GG	CC	169 171	110 122	AA
CLEMxCH 2013 -70	249 257	CG	108 112	GG	175 175	277 277	GG	CA	171 171	110 122	AA
CLEMxCH 2013 -71	249 257	CG	108 112	AG	175 183	259 277	GC	CA	169 171	110 122	AG
CLEMxCH 2013 -72	249 257	CC	108 112	AG	175 175	259 277	GC	CC	169 169	110 122	AG
CLEMxCH 2013 -73	249 249	CG	108 112	AG	175 175	259 277	CC	CA	169 169	110 122	AA
CLEMxCH 2013 -74	249 249	CG	108 112	AG	183 183	277 277	GG	CC	169 171	110 122	AG
CLEMxCH 2013 -75	257 257	GG	108 112	AG	175 183	259 259	CC	CA	169 171	110 122	AG
Hetero ab	44	51	47	41	45	47	47	45	46	50	59
Homo aa	12	18	13	13	18	12	14	18	22	11	16
Homo bb	19	0	15	0	12	16	0	0	7	14	0
Total Genotypes	75	69	75	54	75	75	61	63	75	75	75

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Table 11.- Cont. *Tetraploid pummelo 'Chandler' diploid gamete population analyzed with 39 molecular markers.*

| | LG 4 | | | LG 5 | | | LG 6 | | | | |
| | 12.22 | 16.33 | 89.19 | 25.23 | 31.35 | 110.16 | 1.53 | 5.07 | 28.317 | 60.92 | 93.48 |
Marker/Genotype	CF-CA31	CI07D06	CIBE 3255	5_275 00244	CMS30	MEST56	6_10 554	6_599 6116	CIBE4818	CI02F12	TAA1
CLEMxCH 2013 -01	211 229	163 166	211 211	GC	149 160	155 165	AA	TT	151 151	119 119	163 167
CLEMxCH 2013 -02	229 229	163 163	211 215	GC	149 160	155 165	AA	TA	141 151	123 123	163 163
CLEMxCH 2013 -03	211 211	166 166	211 211	GC	149 160	155 165	AA	TA	141 141	123 123	163 163
CLEMxCH 2013 -04	211 229	163 166	211 215	CC	149 160	155 155	AA	AA	141 141	119 123	163 167
CLEMxCH 2013 -05	229 229	163 163	211 215	GC	149 160	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -06	211 229	163 163	211 215	CC	149 149	155 165	AA	TA	141 151	119 119	163 167
CLEMxCH 2013 -07	211 229	163 166	211 215	GC	149 160	155 165	AA	TT	151 151	119 119	163 167
CLEMxCH 2013 -08	211 229	166 166	211 215	GC	149 160	155 165	AA	AA	141 141	119 123	163 167
CLEMxCH 2013 -09	211 229	163 166	211 215	GC	149 160	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -10	211 229	163 166	211 211	GC	149 149	155 155	AA	TA	151 151	119 119	167 167
CLEMxCH 2013 -11	229 229	163 163	211 211	CC	149 149	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -12	211 229	163 166	211 215	GC	160 160	165 165	AA	TA	151 151	119 119	167 167
CLEMxCH 2013 -13	211 211	163 166	211 215	GC	149 160	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -14	211 211	166 166	211 215	GC	149 160	155 165	AA	TT	151 151	119 123	163 167
CLEMxCH 2013 -15	211 229	163 166	211 215	GC	149 160	155 165	AA	TT	151 151	119 119	163 163
CLEMxCH 2013 -16	229 229	163 163	211 215	GG	160 160	165 165	AA	TT	151 151	119 119	167 167
CLEMxCH 2013 -17	211 229	163 166	215 215	GC	149 160	155 165	AA	TT	151 151	119 123	163 167
CLEMxCH 2013 -18	211 229	163 166	211 215	GC	160 160	155 165	AA	TT	141 151	119 119	167 167
CLEMxCH 2013 -19	229 229	163 166	215 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -20	211 229	163 166	211 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -21	211 211	163 166	211 215	GC	149 160	155 155	AA	AA	141 141	119 123	163 167
CLEMxCH 2013 -22	211 229	163 166	215 215	GC	149 149	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -23	229 229	163 163	215 215	GG	160 160	155 155	AA	TA	141 151	123 123	163 163
CLEMxCH 2013 -24	211 229	163 166	211 215	GC	149 160	155 165	AA	TT	151 151	119 119	167 167
CLEMxCH 2013 -25	229 229	163 163	211 215	CC	149 149	155 155	AA	TA	141 151	119 123	167 167
CLEMxCH 2013 -26	211 211	163 166	211 215	GG	160 160	165 165	AA	TT	151 151	123 123	163 163
CLEMxCH 2013 -27	211 211	163 166	211 215	GG	160 160	165 165	AA	TT	151 151	123 123	163 163
CLEMxCH 2013 -28	229 229	163 166	211 215	GC	149 160	155 165	AA	TA	141 151	123 123	163 167
CLEMxCH 2013 -29	211 229	163 166	211 215	GC	149 160	155 165	AA	TA	141 151	123 123	163 167
CLEMxCH 2013 -30	211 229	163 166	215 215	GC	149 149	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -31	211 229	163 166	211 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -32	229 229	163 163	211 215	GC	149 160	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -33	211 229	163 166	215 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -34	229 229	163 163	211 211	GC	149 160	165 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -35	211 229	163 166	211 215	CC	149 149	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -36	229 229	163 166	211 215	GC	149 160	165 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -37	211 229	163 166	215 215	GC	149 160	165 165	AA	TA	141 151	119 123	163 163
CLEMxCH 2013 -38	229 229	163 163	211 215	GG	160 160	155 165	AA	TA	141 141	123 123	163 163
CLEMxCH 2013 -39	211 229	163 166	211 211	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -40	211 229	163 166	211 215	GC	149 149	155 155	AA	TA	141 151	119 123	167 167
CLEMxCH 2013 -41	211 229	163 166	211 215	GC	149 160	155 155	AA	TA	151 151	119 119	163 167
CLEMxCH 2013 -42	211 229	166 166	211 211	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -43	211 229	163 166	211 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -44	211 211	166 166	211 215	CC	149 149	155 165	AA	TT	141 141	119 119	163 167
CLEMxCH 2013 -45	211 211	166 166	211 215	GC	149 160	155 155	AA	AA	141 151	119 123	163 167
CLEMxCH 2013 -46	211 229	163 166	211 215	CC	149 160	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -47	211 229	163 166	211 215	GC	149 149	155 155	AA	TT	141 151	119 123	163 167
CLEMxCH 2013 -48	211 211	166 166	211 211	GC	149 160	165 165	AA	TT	141 151	119 123	163 163
CLEMxCH 2013 -49	229 229	163 163	215 215	GG	160 160	155 165	AA	TT	151 151	119 119	163 167
CLEMxCH 2013 -50	211 211	166 166	211 211	GC	149 160	155 165	AA	TA	141 141	119 123	163 167
CLEMxCH 2013 -51	211 229	163 166	211 215	CC	149 149	165 165	AA	TA	141 151	123 123	163 163
CLEMxCH 2013 -52	211 211	163 166	211 215	CC	160 160	155 165	AA	TA	151 151	119 123	163 167
CLEMxCH 2013 -53	229 229	163 163	211 215	GG	149 160	155 165	AA	TT	151 151	119 119	167 167
CLEMxCH 2013 -54	229 229	163 163	211 215	GC	149 160	155 165	AA	TA	141 151	123 123	163 163
CLEMxCH 2013 -55	211 229	163 166	215 215	GC	149 160	155 155	AA	TA	141 151	119 123	163 163
CLEMxCH 2013 -56	211 229	163 166	215 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -57	211 211	163 166	211 215	GC	149 160	155 165	AA	TT	151 151	119 123	167 167
CLEMxCH 2013 -58	229 229	163 163	211 215	GG	160 160	155 165	AA	AA	141 141	123 123	163 163
CLEMxCH 2013 -59	229 229	163 163	211 215	GG	160 160	155 165	AA	TA	141 141	123 123	163 163
CLEMxCH 2013 -60	211 211	166 166	215 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -61	211 229	163 166	211 211	GG	149 160	155 165	AA	TA	141 151	119 123	167 167
CLEMxCH 2013 -62	229 229	163 163	211 215	GC	149 160	155 165	AA	TT	151 151	119 119	167 167
CLEMxCH 2013 -63	211 211	166 166	211 211	GC	149 160	155 165	AA	TA	151 151	119 119	167 167
CLEMxCH 2013 -64	211 229	163 166	211 215	GC	149 160	155 165	AA	TA	141 151	119 123	167 167
CLEMxCH 2013 -65	211 229	163 166	211 215	GC	149 160	155 165	AA	TA	141 151	119 123	167 167
CLEMxCH 2013 -66	211 229	163 166	215 215	CC	149 149	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -67	211 229	163 166	211 211	GC	149 160	155 165	AA	TA	141 141	123 123	163 167
CLEMxCH 2013 -68	211 211	166 166	211 215	GG	160 160	155 155	AA	TT	151 151	119 119	163 167
CLEMxCH 2013 -69	229 229	163 166	211 215	GG	160 160	155 155	AA	TT	151 151	119 119	163 167
CLEMxCH 2013 -70	211 211	166 166	211 211	GG	160 160	155 165	AA	TA	141 151	119 123	167 167
CLEMxCH 2013 -71	229 229	163 163	211 211	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -72	211 211	166 166	211 211	GC	149 160	155 155	AA	TA	141 151	119 123	163 167
CLEMxCH 2013 -73	229 229	163 163	211 215	GC	149 160	155 165	AA	TT	151 151	119 123	163 167
CLEMxCH 2013 -74	211 229	163 166	211 215	CC	149 149	155 155	AA	TA	141 151	119 119	163 163
CLEMxCH 2013 -75	211 229	163 166	215 215	GC	149 160	155 165	AA	TA	141 151	119 123	163 167
Hetero ab	36	45	47	51	46	46	0	50	44	44	46
Homo aa	17	17	15	13	15	20	75	20	10	18	14
Homo bb	22	13	13	0	14	9	0	0	21	13	15
Total Genotypes	75	75	75	64	75	75	75	70	75	75	75

Table 11.- Cont. Tetraploid pummelo 'Chandler' diploid gamete population analyzed with 39 molecular markers.

	LG 7				LG 8					
	14.37	10.89	83.89	105.01	5.91	31.7	41.65	50.78	95.03	113.52
Marker/Genotype	CI07E05	7_20 255	CI03B07	7_1570 8026	CI01F04a	CI07B05	MEST830	8_154 15145	CI02C09	8_2502 6006
CLEMxCH 2013 -01	116 118	AG	276 276	TC	191 191	219 219	205 205	CA	236 238	CC
CLEMxCH 2013 -02	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -03	116 118	AG	276 276	TC	191 191	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -04	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -05	116 116	GG	276 276	TT	191 199	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -06	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -07	118 118	AA	276 278	TC	191 199	200 200	205 214	CA	236 238	CC
CLEMxCH 2013 -08	116 118	AG	276 276	TT	191 199	219 219	205 205	CC	236 236	CC
CLEMxCH 2013 -09	116 116	GG	276 276	TC	191 191	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -10	118 118	AA	278 278	CC	199 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -11	116 118	GG	276 278	TC	191 191	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -12	116 118	AA	276 278	TC	199 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -13	116 118	AG	276 278	TC	191 199	200 200	205 214	AA	236 236	CC
CLEMxCH 2013 -14	116 118	AG	276 278	TC	199 199	200 200	205 214	CC	236 236	CC
CLEMxCH 2013 -15	116 116	GG	276 276	TT	199 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -16	116 118	AG	276 278	CC	199 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -17	116 116	GG	276 278	TC	191 191	219 219	205 214	CA	238 238	CC
CLEMxCH 2013 -18	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -19	116 116	GG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -20	116 118	AG	276 278	TC	199 199	200 200	205 214	CA	236 238	CC
CLEMxCH 2013 -21	118 118	AA	276 278	CC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -22	118 118	AA	276 278	TC	191 199	200 200	205 214	AA	236 238	CC
CLEMxCH 2013 -23	118 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -24	116 118	AG	276 278	TC	199 199	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -25	116 116	GG	276 278	TC	191 191	200 219	205 214	CC	236 238	CC
CLEMxCH 2013 -26	118 118	AG	278 278	CC	191 191	200 219	205 214	AA	236 238	CC
CLEMxCH 2013 -27	118 118	AG	278 278	CC	191 191	200 219	205 214	AA	236 238	CC
CLEMxCH 2013 -28	118 118	AA	276 278	TC	191 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -29	118 118	AA	276 278	TC	191 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -30	118 118	AA	276 278	TT	191 191	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -31	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -32	116 116	GG	276 276	TT	191 199	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -33	116 118	AG	276 278	CC	191 191	219 219	205 214	CA	236 238	CC
CLEMxCH 2013 -34	116 118	AG	276 276	TT	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -35	116 116	AG	276 276	TT	191 199	200 219	205 214	CC	236 238	CC
CLEMxCH 2013 -36	116 118	AG	278 278	CC	191 191	219 219	205 205	AA	236 238	CC
CLEMxCH 2013 -37	116 118	AG	276 276	TT	199 199	200 219	205 214	CC	238 238	CC
CLEMxCH 2013 -38	118 118	AA	276 278	TC	199 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -39	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -40	116 118	AG	278 278	CC	191 191	200 200	205 214	AA	238 238	CC
CLEMxCH 2013 -41	116 118	AG	276 276	TT	199 199	219 219	205 205	AA	236 238	CC
CLEMxCH 2013 -42	116 116	AA	276 278	TC	191 199	200 219	205 214	AA	236 238	CC
CLEMxCH 2013 -43	116 116	GG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -44	116 116	GG	276 276	TT	191 199	200 219	205 214	AA	236 238	CC
CLEMxCH 2013 -45	116 116	GG	276 276	TT	191 191	200 200	205 214	AA	238 238	CC
CLEMxCH 2013 -46	116 118	AG	276 278	CC	191 199	219 219	205 205	CC	236 236	CC
CLEMxCH 2013 -47	116 118	AG	278 278	CC	199 199	200 200	205 214	AA	236 238	CC
CLEMxCH 2013 -48	116 118	AG	278 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -49	118 118	AA	276 278	TT	191 199	200 200	205 214	AA	238 238	CC
CLEMxCH 2013 -50	116 118	GG	276 278	TC	191 191	219 219	205 205	CA	236 236	CC
CLEMxCH 2013 -51	118 118	AA	278 278	TC	191 191	200 219	205 214	AA	236 238	CC
CLEMxCH 2013 -52	118 118	AG	278 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -53	116 116	GG	276 276	TT	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -54	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -55	118 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -56	116 116	GG	276 278	TC	191 191	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -57	116 116	GG	276 278	TC	191 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -58	116 118	AG	278 278	CC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -59	116 118	AG	278 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -60	116 118	AG	276 278	TC	191 191	219 219	205 214	AA	238 238	CC
CLEMxCH 2013 -61	116 118	AG	276 278	TC	199 199	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -62	116 118	AG	276 276	TT	191 191	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -63	116 118	AG	276 276	TC	191 199	219 219	205 205	CC	236 236	CC
CLEMxCH 2013 -64	118 118	AA	278 278	TC	199 199	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -65	116 118	AG	276 276	TT	191 191	219 219	205 205	CA	236 236	CC
CLEMxCH 2013 -66	118 118	AA	276 278	TC	191 199	219 219	205 205	CC	236 236	CC
CLEMxCH 2013 -67	116 118	GG	276 278	TC	191 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -68	116 116	GG	276 278	TC	199 199	200 200	205 214	AA	236 238	CC
CLEMxCH 2013 -69	116 118	AG	278 278	CC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -70	118 118	AA	276 278	TC	191 191	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -71	116 118	AG	276 276	TT	191 199	200 219	205 214	AA	236 238	CC
CLEMxCH 2013 -72	116 116	GG	276 278	TC	191 199	200 219	205 214	CA	238 238	CC
CLEMxCH 2013 -73	116 118	AG	276 278	TC	191 199	200 219	205 214	CA	236 238	CC
CLEMxCH 2013 -74	116 118	AG	276 276	TT	191 199	200 219	205 214	CA	236 236	CC
CLEMxCH 2013 -75	116 118	AG	276 278	TC	199 199	200 200	205 214	CA	236 236	CC
Hetero ab	40	42	44	46	41	52	66	51	43	0
Homo aa	17	15	18	17	20	11	9	8	17	75
Homo bb	18	0	13	0	14	12	0	0	15	0
Total Genotypes	75	57	75	63	75	75	75	59	75	75

Table 11.- Cont. Tetraploid pummelo 'Chandler' diploid gamete population analyzed with 39 molecular markers.

	LG 9						
	5.97	**49.56**	**50.41**	**52.23**	**55.14**	**54.88**	**98.18**
Marker/Genotype	**MEST330**	**CI07F11**	**MEST308**	**Ci08C05**	**9_9543785**	**9_26016472**	**9_31288895**
CLEMxCH 2013 -01	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -02	269 269	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -03	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -04	269 287	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -05	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -06	269 269	155 158	244 244	143 153	GA	TT	TT
CLEMxCH 2013 -07	287 287	158 158	241 241	143 153	GA	GG	CC
CLEMxCH 2013 -08	269 287	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -09	269 269	155 155	241 244	143 153	GA	GT	CC
CLEMxCH 2013 -10	269 269	155 158	241 244	143 153	GA	GG	CC
CLEMxCH 2013 -11	269 269	155 155	244 244	143 153	GA	GT	TT
CLEMxCH 2013 -12	269 287	155 158	241 244	143 143	GA	GT	TC
CLEMxCH 2013 -13	287 287	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -14	269 269	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -15	287 287	158 158	241 244	143 153	GA	GG	TC
CLEMxCH 2013 -16	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -17	269 287	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -18	269 269	155 158	241 244	143 153	GA	GT	
CLEMxCH 2013 -19	287 287	155 158	241 244	143 153	GA	GG	TC
CLEMxCH 2013 -20	269 269	155 155	244 244	143 153	GA	GT	TC
CLEMxCH 2013 -21	269 269	155 155	244 244	143 153	GA	GT	TC
CLEMxCH 2013 -22	269 269	158 158	241 241	143 153	GA	GG	TC
CLEMxCH 2013 -23	269 269	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -24	269 287	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -25	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -26	287 287	158 158	241 241	143 153	GA	GG	TC
CLEMxCH 2013 -27	287 287	158 158	241 241	143 153	GA	GG	TC
CLEMxCH 2013 -28	269 287	158 158	241 241	143 153	GA	GG	TC
CLEMxCH 2013 -29	269 287	158 158	241 241	143 153	GA	GG	TC
CLEMxCH 2013 -30	269 269	155 155	244 244	143 153	GA	GT	CC
CLEMxCH 2013 -31	269 269	158 158	241 241	143 153	GA	GT	CC
CLEMxCH 2013 -32	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -33	287 287	155 158	241 244	143 143	GA	GT	CC
CLEMxCH 2013 -34	269 287	158 158	241 241	143 143	GA	GG	TT
CLEMxCH 2013 -35	269 269	158 158	241 241	143 153	GA	GG	TC
CLEMxCH 2013 -36	269 287	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -37	269 287	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -38	269 269	155 155	244 244	143 153	GA	GT	
CLEMxCH 2013 -39	269 287	155 155	244 244	143 143	GA	GT	CC
CLEMxCH 2013 -40	287 287	155 158	241 244	143 143	GA	GT	CC
CLEMxCH 2013 -41	269 269	155 158	241 244	143 143	GA	GT	TC
CLEMxCH 2013 -42	269 287	155 155	241 244	143 143	GA	GG	TC
CLEMxCH 2013 -43	269 287	155 158	241 244	143 143	GA	GT	TC
CLEMxCH 2013 -44	287 287	155 158	241 244	143 143	GA	GT	TC
CLEMxCH 2013 -45	269 287	155 158	241 244	143 143	GA	GT	TC
CLEMxCH 2013 -46	287 287	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -47	287 287	158 158	241 241	143 153	GA	GG	TT
CLEMxCH 2013 -48	269 287	155 158	241 244	143 153	GA	TT	TC
CLEMxCH 2013 -49	269 269	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -50	287 287	158 158	241 241	143 153	GA	GG	TC
CLEMxCH 2013 -51	287 287	155 158	244 244	143 153	GA	TT	TC
CLEMxCH 2013 -52	269 287	155 158	241 244	143 153	GA	TT	TT
CLEMxCH 2013 -53	269 287	158 158	241 241	143 153	GA	GG	TT
CLEMxCH 2013 -54	269 287	155 158	241 244	143 153	GA	GT	TT
CLEMxCH 2013 -55	269 287	155 158	241 244	143 153	GA	GT	CC
CLEMxCH 2013 -56	269 269	155 158	241 244	143 153	GA	TT	TT
CLEMxCH 2013 -57	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -58	269 287	155 158	241 244	143 143	GA	TT	TT
CLEMxCH 2013 -59	269 287	155 158	241 244	143 143	GA	GT	TT
CLEMxCH 2013 -60	269 287	155 158	241 244	143 153	GA	TT	TC
CLEMxCH 2013 -61	269 287	155 158	241 244	143 143	GA	TT	TT
CLEMxCH 2013 -62	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -63	269 269	155 155	244 244	143 153	GA	GT	TC
CLEMxCH 2013 -64	269 287	155 158	244 244	143 153	GA	TT	TC
CLEMxCH 2013 -65	269 269	155 155	244 244	143 153	GA	TT	TC
CLEMxCH 2013 -66	269 287	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -67	269 287	155 158	244 244	143 153	GA	TT	TT
CLEMxCH 2013 -68	269 287	155 158	244 244	143 143	GA	TT	TC
CLEMxCH 2013 -69	287 287	155 158	241 244	143 153	GA	GT	CC
CLEMxCH 2013 -70	269 269	155 158	241 244	143 153	GA	GT	CC
CLEMxCH 2013 -71	269 287	158 158	241 241	143 153	GA	GG	CC
CLEMxCH 2013 -72	269 269	155 158	244 244	143 153	GA	TT	TT
CLEMxCH 2013 -73	269 269	155 158	241 244	143 153	GA	GT	TC
CLEMxCH 2013 -74	269 287	155 158	241 244	143 153	GA	TT	TT
CLEMxCH 2013 -75	287 287	155 155	244 244	143 153	GA	TT	TT
Hetero ab	29	50	47	60	75	44	38
Homo aa	31	11	13	15	0	16	23
Homo bb	15	14	15	0	0	0	0
Total Genotypes	75	75	75	75	75	60	61

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Table 12. Tetraploid 'Carrizo' citrange diploid gamete population analyzed with 29 molecular markers.

	LG 1			LG 2				LG 3		
	6.288	61.822	119.734	78.511	59.354	56.757	133.78	5.807	87.915	179.329
Marker/ Genotype	CIBE6126	MEST539	TAA15	SOS1-M50	CX6F23	CX2004	CI02D09	ID4721	ID0039-380	MEST131
CLEMxCA 2013 -01	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -02	222226	97103	143189	AG	143156	167180	231231	284289	193202	110146
CLEMxCA 2013 -03	222226	97103	143189	AG	143156	180180	231231	284289	193202	110146
CLEMxCA 2013 -04	222226	97103	143143	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -05	222226	97103	143189	AG	143156	167180	227227	284289	193193	110110
CLEMxCA 2013 -06	222226	97103	143189	AG	143156	167180	227231	284289	193202	110110
CLEMxCA 2013 -07	222226	9797	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -08	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -09	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -10	222222	9797	143143	AG	143156	167180	227231	289289	193202	110110
CLEMxCA 2013 -11	222226	97103	189189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -12	222226	97103	143189	GG	143143	167167	227231	284289	193202	110146
CLEMxCA 2013 -13	222226	97103	143189	AG	143156	167180	231231	284289	193202	110146
CLEMxCA 2013 -14	222226	97103	143189	AG	143156	167180	227231	284284	202202	110146
CLEMxCA 2013 -15	222226	97103	143189	AG	143156	167180	227231	284289	193202	146146
CLEMxCA 2013 -16	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -17	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -18	222226	97103	143189	AG	156156	180180	231231	284284	193202	110146
CLEMxCA 2013 -19	222226	97103	143189	AG	156156	167180	227231	289289	193202	110146
CLEMxCA 2013 -20	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -21	222226	97103	189189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -22	222226	97103	189189	AG	156156	180180	231231	284284	193202	110146
CLEMxCA 2013 -23	222226	97103	143143	AG	143143	167167	227227	284289	193202	110146
CLEMxCA 2013 -24	222226	97103	143189	AG	143156	167180	227231	289289	193202	110146
CLEMxCA 2013 -25	222226	97103	143189	AA	156156	167180	231231	284284	193202	110146
CLEMxCA 2013 -26	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -27	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -28	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -29	222226	97103	143189	AG	143156	167180	227231	284289	202202	110110
CLEMxCA 2013 -30	222222	9797	143189	AG	143156	167180	227231	284289	193202	146146
CLEMxCA 2013 -31	222222	97103	143189	AG	143156	167180	227231	284289	193202	110110
CLEMxCA 2013 -32	222226	97103	143189	AG			227231	284289		110146
CLEMxCA 2013 -33	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -34	222226	97103	143189	AG	156156	167180	227231	284289	193202	110146
CLEMxCA 2013 -35	222226	97103	189189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -36	222226	103103	189189	AG	143143	167167	227227	284289	193202	110146
CLEMxCA 2013 -37	222222	9797	143143	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -38	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -39	222226	97103	143189	AG	143156	167180	227231	284289	193202	146146
CLEMxCA 2013 -40	222226	97103	189189	AG	143156	167180	227231	284284	193202	110110
CLEMxCA 2013 -41	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -42	222226	97103	143189	AG	156156	167180	227231	284289	193202	110146
CLEMxCA 2013 -43	222226	97103	143189	AG	143156	167180	227231	284289	193202	146146
CLEMxCA 2013 -44	222226	97103	143189	AG	143156	167180	227227	284289	193202	110146
CLEMxCA 2013 -45	222226	9797	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -46	222226	97103	189189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -47	222226	97103		AA	156156	180180	231231	284289	202202	146146
CLEMxCA 2013 -48	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -49	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -50	222226	97103	143189	GG	143156	167180		289289	202202	110146
CLEMxCA 2013 -51	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -52	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -53	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -54	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -55	222226	97103	143189	AG	143156	167180	227231	284289	193202	146146
CLEMxCA 2013 -56	222226	97103	143189	GG	143143	167167	227231	284289	193202	110146
CLEMxCA 2013 -57	222226	97103	143189		143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -58	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -59	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -60	222226	97103	143189	AG	143143	167167	231231	284289	193202	110146
CLEMxCA 2013 -61	222226	97539	143189	AG	143156	167180	227231	289289	ID193202	110146
CLEMxCA 2013 -62	222222	97103	143189	AG	143156	167180	227231	284289	193202	110110
CLEMxCA 2013 -63	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -64	222226	97103	189189	AG	143156	167180	227231	284284	193202	146146
CLEMxCA 2013 -65	222226	97103	143189	GG	143156	167180	227231	284284	193202	110146
CLEMxCA 2013 -66	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -67	222226	97103	143189	AA	156156	167180	231231	284289	193202	110146
CLEMxCA 2013 -68	222226	9797	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -69	222226	97103	143143	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -70	222226	97103	189189	AG	143156	167180	231231	284289	193202	110146
CLEMxCA 2013 -71	222222	97103	143143	AG	143156	167180	231231	284284	193202	110146
CLEMxCA 2013 -72	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -73	222222	9797	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -74	222222	97103	143189	AG	143156	167180	227231	284289	193202	110110
CLEMxCA 2013 -75	222226	97103	143189	AG	143156	167180	227231	284289	193202	110110
CLEMxCA 2013 -76	222226	97103	143189	AG	143156	167180	227227	284289	193202	110146
CLEMxCA 2013 -77	222226	97103	143189	GG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -78	222226	97103	143189	AG	143143	167167	227227	284289	193202	110146
CLEMxCA 2013 -79	222226	97103	189189	AG	143156	167180	227231	284289	193202	146146
CLEMxCA 2013 -80	222226	97103	143189	AG	143156	167180	231231	284289	193202	146146
CLEMxCA 2013 -81	222226	97103	143189	AG	143156	167180	227231	289289	193202	110146
CLEMxCA 2013 -82	222226	97103	143189	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -83	222226	97103	143143	AA	156156	167180	227231	289289	193193	110110
CLEMxCA 2013 -84	222226	97103		AG		167180	227231	284289	193202	110146
CLEMxCA 2013 -85	222226	9797	143143		143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -86	222226	97103	143143	AG	143156	167180	227231	284289	193202	110146
CLEMxCA 2013 -87	222226	97103	143189	AG	143156	167180	227231	284284	193193	110146
CLEMxCA 2013 -88	222226	97103	143189	AG	143156	167180	227231	289289	193202	110146
Hetero ab	80	79	67	77	71	77	69	71	80	70
Homo aa	8	8	8	5	6	6	6	9	3	9
Homo bb	0	1	11	4	9	4	12	8	4	9
Total Genotypes	88	88	86	86	86	87	87	88	87	88

Table 12. – Cont. Tetraploid 'Carrizo' citrange diploid gamete population analyzed with 29 molecular markers.

	LG 4			LG 5			LG 6		
	16.354	85.835	90.063	14.66	31.35	102.01	79.95	10.792	91.877
Marker/Genotype	CI07D06	CI02D04B	CI03D12A	CI07G11	CMS30	ID5485	LG6-TTA6	MEST191	MEST123
CLEMxCA 2013 -01	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -02	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -03	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -04	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -05	162188	196210	247261	194202	148156	235235	342345	235238	246250
CLEMxCA 2013 -06	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -07	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -08	162188	196210	247261		148156	221235	342345	235238	246250
CLEMxCA 2013 -09	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -10	162188	196210	247261	194202	148156	221235	345345	235238	250250
CLEMxCA 2013 -11	162188	196210	247261	194202	148156	221235	345345	235238	250250
CLEMxCA 2013 -12	162188	196210	247261	194202	148148	221221	342345	235238	246250
CLEMxCA 2013 -13	162188	210210	261261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -14	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -15	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -16	188188		261261	194202	148156	221235	345345	235238	246250
CLEMxCA 2013 -17	162188	196210	247261	194202	148156	221221	342345	235238	246250
CLEMxCA 2013 -18	162162	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -19	162188	196210	247261	194202	148156	221221	342345	235238	246250
CLEMxCA 2013 -20	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -21	162162	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -22	162188	210210	261261	194194	148148	221221	342345	235238	246250
CLEMxCA 2013 -23	162162	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -24	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -25	162188	196196	247247	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -26	162188	196196	247247	194202	148156	221235	342342	235238	246246
CLEMxCA 2013 -27	162188	196210	247261	194202	148156	235235	342345	235238	246250
CLEMxCA 2013 -28	162188	196210	247261	194202	148156	235235	342345	235238	246250
CLEMxCA 2013 -29	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -30	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -31	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -32	162188	196210	247261	194202	148156	221235	342345	235238	250250
CLEMxCA 2013 -33	162188	196210	247261	194202	148156	221221	342345	235238	246250
CLEMxCA 2013 -34	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -35		196210	247261	194202	148156	221235	342345	235238	246246
CLEMxCA 2013 -36	162188	196210	247261	194194	156156	235235	342345	235238	246250
CLEMxCA 2013 -37	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -38	162188			194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -39	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -40	162188	196210	247261	194202	148156	221235	342345		246250
CLEMxCA 2013 -41	162188	196210	247261	194202	148156	235235	342345	235238	246250
CLEMxCA 2013 -42	162188	196210	247261	194202	148156	235235	342345	235238	246250
CLEMxCA 2013 -43	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -44	162188	196210	247261	194202	148156	221235	345345	235238	250250
CLEMxCA 2013 -45	162188	196196	247247	194202	148156	221235	342345	235238	246246
CLEMxCA 2013 -46	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -47	188188	210210	261261	202202	148156		345345	235238	
CLEMxCA 2013 -48		196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -49	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -50	162188	210210	261261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -51	162188	196210	247261	194202	148156	221235		235235	250250
CLEMxCA 2013 -52	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -53	162188	196210	247261	194202	148156	221235		235238	250250
CLEMxCA 2013 -54	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -55	162188		247261	194202	148156	221235		235238	250250
CLEMxCA 2013 -56	162188	196210	247261	194194	148148	221221	342345	235238	246250
CLEMxCA 2013 -57	162188	196210	247261		148156	221235	345345	235238	246246
CLEMxCA 2013 -58	162188	196210	247261	194202	148156		342345	235238	246250
CLEMxCA 2013 -59	162162	196210	247261	194202	148156	221221	342345	235238	246250
CLEMxCA 2013 -60	162188	196210	247261	194202		221235	342345	235238	246250
CLEMxCA 2013 -61	162188	CI96210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -62	162188	196210	247261	194202	148156	235235	342345	235238	246250
CLEMxCA 2013 -63	162188	196210	247261	194202	148156	221235	342345	235235	246250
CLEMxCA 2013 -64	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -65	162162	196196	247247	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -66	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -67	162188	210210	261261	194202	156156	235235	342345	235238	246250
CLEMxCA 2013 -68	162188	196196	247247	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -69	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -70	162188	196210	247261	194202	148156	221221	342345	235238	246250
CLEMxCA 2013 -71	162188	196210	247261	202202	156156	221235	342345	235238	246250
CLEMxCA 2013 -72	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -73	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -74	162188	196210	247261	194202	148156	221235	342342	235238	246250
CLEMxCA 2013 -75	162188	196210	247261	194194	148148	235235	342345	235238	246250
CLEMxCA 2013 -76	162188	196210	247261	194202	148156	221235	342345	235238	246250
CLEMxCA 2013 -77	162188	196210	247261	194202	148156	235235	342345	235238	246250
CLEMxCA 2013 -78	162188	196210	247261	194202	148156	221235	342342	235238	246250
CLEMxCA 2013 -79	162188	196210	247261	194202	148156	221235	345345	238238	250250
CLEMxCA 2013 -80	162188	196210	247261	202202	156156	235235	345345	235238	246250
CLEMxCA 2013 -81	162188	196210	247261	194202	148156	221235	345345	235238	246250
CLEMxCA 2013 -82	162188	196210	247261	194202	148156	221235	345345	235238	246250
CLEMxCA 2013 -83	162188	196210	247261	194202	148148	221235	345345	238238	246250
CLEMxCA 2013 -84	162188	210210		194202	148156	221235	345345		246250
CLEMxCA 2013 -85	162188	196210	247261	194202	148156	221235	345345	238238	246250
CLEMxCA 2013 -86	162188	196210	247261	194202	148156	221235	345345		246250
CLEMxCA 2013 -87	162162	196210	247261	194202	148156	235235	345345	235238	246250
CLEMxCA 2013 -88	162188	196210	247261	194202	148156	221235	345345	235238	246250
Hetero ab	78	74	75	79	78	66	67	80	75
Homo aa	6	5	5	4	4	8	3	2	4
Homo bb	2	6	6	3	5	12	15	3	8
Total Genotypes	86	85	86	86	87	86	85	85	87

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Table 12. – Cont. Tetraploid 'Carrizo' citrange diploid gamete population analyzed with 29 molecular markers.

	LG 7				LG 8			LG 9		
	8.899	14.365	83.39	115.594	5.917	43.126	95.032	61.95	55.108	0.01
Marker/Genotype	MEST107	CI07E05	CI03B07	ID0591	CI01F04A	MEST502	CI02C09	LG9-GA15	JI-TCT01	CI02B07
CLEMxCA 2013 -01	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -02	171174	118128	266280	350364	182198	159174	236238	215215	155167	164164
CLEMxCA 2013 -03	171174	118128	266280	350364	182198	159174	236238		155155	164164
CLEMxCA 2013 -04	171174	118128	266280	350364	182198	159174	236238	204204	167167	164168
CLEMxCA 2013 -05	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -06	174174	118118	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -07	171174	118128	266280	350364	182198	159174	236238	204204	155167	164168
CLEMxCA 2013 -08	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -09	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -10	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -11	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -12	171174	118128	266280	350364	182198	159174	236236	204215	155167	164168
CLEMxCA 2013 -13	171174	118128	266280	364364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -14	171174	118128	266280	350364	182198	159174	236238	204215	155167	168168
CLEMxCA 2013 -15	171174	118128	266280	350350	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -16	171174	118128	266280	350350	182198	159174	236238	204204	155167	168168
CLEMxCA 2013 -17	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -18	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -19	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -20	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -21	171174	118128	280280	364364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -22	171174	118128	266280	350364	182198	159174	236238	204215	155155	164164
CLEMxCA 2013 -23	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -24	171174	118128	266280	350364	182198	159174	236238	204215	155167	164164
CLEMxCA 2013 -25	171174	118128	266280	350350	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -26	174174	118118	280280	350350	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -27	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -28	171171	128128	266280	350364	182198	159174	236238	204215		164168
CLEMxCA 2013 -29	171174	118128	266280	350350	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -30	171174	118128	266280	350364	182198	159174	238238	204215	155167	164168
CLEMxCA 2013 -31	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -32	171174	128128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -33	171174	118128	266280	350364	182198	159174	236238	204215	167167	164168
CLEMxCA 2013 -34	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -35	171174	128128	266280	350364	182198	159174		204215	155167	164168
CLEMxCA 2013 -36	171174	118128	266280	350364	182198	159174	236238	204204	167167	168168
CLEMxCA 2013 -37	171174	118128	266280	364364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -38	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -39	174174	118118	266280	350350	182198	159174		204215	155167	164168
CLEMxCA 2013 -40	171174	118128		350364	182198	159174	238238	215215	155155	164168
CLEMxCA 2013 -41	171174	118128	266280	350364	182182	159174	236238	204215	167167	164168
CLEMxCA 2013 -42	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -43	171174	118128	280280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -44	171174	118128	280280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -45	171174	118128	266280	364364	182182	159174	236238	204204	155167	164168
CLEMxCA 2013 -46	171174	118128	280280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -47	174174	118118	280280	350350	182198	159174	236236	215215	155167	164168
CLEMxCA 2013 -48	171174	118128	266280	350364	182198	159174		204215	155167	164168
CLEMxCA 2013 -49	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -50	171174	118128	266280		182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -51	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -52	171174	118128	280280	350350	182198	159174	236238	204204	155167	164168
CLEMxCA 2013 -53	171174	118128	266280	350364	182198	159174	236238	204215	155167	164164
CLEMxCA 2013 -54	171174	118128	280280	350350	182198	159174	236238	204204	155167	164168
CLEMxCA 2013 -55	171174	118128	266280	350364	182198		236238		155167	164164
CLEMxCA 2013 -56	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -57	171174	118128	280280	350350	182198	159174	236238	204204	155167	164168
CLEMxCA 2013 -58	171174	118128	280280	350350	182198	159174	236238	204204	155167	164168
CLEMxCA 2013 -59	171174	118128	266280	350364	182198	159174	236238	204215	155155	164164
CLEMxCA 2013 -60	171174	118128		350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -61	171171	128128	266280	350350	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -62	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -63	171171	128128	266266	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -64	171174	118128			182182	159174	236238	204215	155167	164168
CLEMxCA 2013 -65	171174	118128			182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -66	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -67	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -68	171174	118128	266280	364364	182182	159174	236238	204204	155167	164168
CLEMxCA 2013 -69	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -70	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -71	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -72	171171	128128	266280	350364	182198	159174	236238	204204	155167	168168
CLEMxCA 2013 -73	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -74	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -75	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -76	174174	118128	266266	364364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -77	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -78	171174	118128	266280	364364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -79	171174	118128	266280	350364	182198	159174	236238	204204	155167	164168
CLEMxCA 2013 -80	171174	128128	266280	350350	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -81	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -82	171174	118118	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -83	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -84	174174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -85	171174	118128	266280	350364	182198	159174	236238	215215	155167	164164
CLEMxCA 2013 -86	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -87	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
CLEMxCA 2013 -88	171174	118128	266280	350364	182198	159174	236238	204215	155167	164168
Hetero ab	78	76	72	65	84	87	81	70	79	76
Homo aa	6	7	2	7	0	0	2	12	4	8
Homo bb	4	5	10	13	4	0	2	4	4	4
Total Genotypes	88	88	84	85	88	87	85	86	87	88

Table 13. Phylogenetic structure for all LG of diploid gametes obtained from male tetraploid 'Carrizo' citrange, where allele combination is indicated in color: heterozygous C. sinensis *and* P. trifoliata *(HE – green),* C. sinensis *(CS – red) and* P. trifoliata *(PT – yellow). The blue column indicates the location of the centromere and white cells are NA.*

	LG1				LG2					LG3				LG4				LG5				LG6				LG7					LG8				LG9			
Position	6,288	60.660	61,822	119,734	78.511	56.757	56.870	59.354	133.780	5.807	87.915	90.590	179.329	16.140	16.354	85.835	90.063	14.658	23.120	31.350	102.011	6.200	10.792	79.950	91.877	8.899	14.365	83.390	96.430	115.594	5.917	43.126	54.210	95.032	0.010	52.160	55.108	61.950
Marker	CIBE6126		MEST539	JK-taa15	SOS1-M50	CX2004		CX6F23	CI02D09	ID4721	ID0039-380		MEST131		CI07D06	CI02D04B	CI03D12A	CI07G11		CMS30	ID5485		MEST191	LG6-TTA6	MEST123	MEST107	CI07E05	CI03B07		ID0591	CI01F04A	MEST502		CI02C09	CI02B07		JI-TCT01	LG9-GA15