UNIVERSIDADE FEDERAL DE LAVRAS

MARCELA CARVALHO ANDRADE

GENETIC CONTROL OF GLANDULAR TRICHOME DENSITIES AND THEIR ASSOCIATION WITH WHITEFLY RESISTANCE FROM *Solanum galapagense* accession LA1401

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Orientador
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MARCELA CARVALHO ANDRADE

GENETIC CONTROL OF GLANDULAR TRICHOME DENSITIES AND THEIR ASSOCIATION WITH WHITEFLY RESISTANCE FROM *Solanum galapagense* accession LA1401

(CONTROLE GENÉTICO DA DENSIDADE DE TRICOMAS GLANDULARES E SUA ASSOCIAÇÃO COM RESISTÊNCIA A MOSCA BRANCA EM *Solanum galapagense* acesso LA1401)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de plantas, área de concentração em Genética e Melhoramento de Plantas, para a obtenção do título de Doutor.

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RESUMO

O tomateiro é atacado por um grande número de artrópodes-pragas, sendo a mosca branca (*Bemisia tabaci*) considerada uma das mais destrutivas. Devido à ineficiência do controle por meio de produtos químicos, programas de melhoramento tem focado no desenvolvimento de cultivares resistentes por meio de introgressão de resistência proveniente de espécies selvagens. Em *S. galapagense* a resistência à mosca branca tem sido associada à presença de tricomas glandulares tipo IV. O conhecimento sobre os fatores genéticos envolvidos na presença de tricomas glandulares tipo IV auxiliaria no processo de introgressão gênica. Nosso estudo objetivou estudar a herança da densidade de tricomas glandulares tipo IV, sua associação a resistência à mosca branca e identificar QTL (Quantitative trait loci) associados à presença desses tricomas em populações derivadas do cruzamento interespecífico entre *S. lycopersicum* TOM-684 x *S. galapagense* acesso LA1401. Estimativas altas para herdabilidade, tanto no sentido amplo quanto no sentido restrito, para densidade de tricomas glandulares tipo IV, sugerem que esse caráter possui herança relativamente simples. A resistência à mosca branca esteve associada à maior densidade de tricomas glandulares tipo IV. As plantas da população F_2 (*S. lycopersicum* x *S. galapagense*) selecionadas para maiores densidades de tricomas tipo IV apresentaram o mesmo nível de resistência encontrado no genitor LA1401, considerado resistente à mosca branca. Dois QTLs foram detectados para tricomas tipo IV, um QTL de efeito maior (gal.IV-2), responsável por 35, 22% da variação fenotípica, e localizado no cromossomo 2, e outro QTL de efeito menor (gal.IV-3) localizado no cromossomo 3. O QTL gal.IV-2 aparenta ser o principal responsável pela presença de tricomas tipo IV em *S. galapagense*.

Palavras-chave: Quantitative trait loci. Composite Interval Mapping. Marcadores Indel. Binomial negativo. Poisson inflacionado de zeros.

ABSTRACT

Tomato is affected by a large number of arthropod pests, among which the whitefly (*Bemisia tabaci*) is considered to be one of the most destructive. Arthropod pest management by chemical control is often inefficient. Therefore, tomato breeding programs have been trying to develop resistant cultivars by introgression of resistance present in wild species. In *S. galapagense* the resistance to whitefly has been associated with the presence of type IV glandular trichomes. Knowledge about the genetic control of type IV glandular trichome densities would facilitate trait introgression. Our research aimed to study the inheritance of type IV glandular trichome densities, their association with resistance to whitefly, and to identify quantitative trait loci (QTL) associated with the presence of these trichomes in populations derived from the interspecific cross *Solanum lycopersicum* TOM-684 x *Solanum galapagense* accession LA1401. High estimates for both broad and narrow sense heritabilities of type IV glandular trichome densities suggest that inheritance of this trait is not complex. Whitefly resistance was associated with high density of type IV glandular trichomes. F_2 (*S. galapagense* x *S. lycopersicum*) progeny selected for high densities of type IV glandular trichomes showed similar levels of resistance to those found in LA1401, considered resistant to whitefly. Two QTLs were detected as associated with type IV trichomes: one major QTL (gal.IV-2), responsible for 35.22% of phenotypic variation, located on chromosome 2, and another minor QTL (gal.IV-3) located on chromosome 3. QTL gal.IV-2 appears to be mainly responsible for the presence of type IV trichomes in *S. galapagense*.

Key words: Quantitative trait loci. Composite Interval Mapping. Indel markers. Negative binomial. Zero-inflated Poisson.

SUMMARY

CHAPTER 1

1 INTRODUCTION

Tomato is affected by a large number of arthropod-pests. Among these, whitefly is considered one of the most important as it is responsible for decreasing yields and fruit quality. This insect can cause both direct and indirect damage. The main cause of direct damage is physiological disorders that lead to irregular fruit maturation; Indirect damage is caused by transmission of geminiviruses that seriously impair fruit yields.

Chemicals have been deployed as the main method to control arthropod pests in order to mitigate the losses they cause. However, efficiency of this practice has been decreasing due to the indiscriminate use of these products, combined with the insect's ability to develop resistance to them. Breeding programs have therefore been trying to introduce arthropod resistance known to be present in wild tomato species into commercially acceptable tomato lines.

Resistance sources to arthropod-pests, including whitefly (*Bemisia* ssp.), have been reported in several wild species (*S. pennellii*, *S. habrochaites*, *S. pimpinellifolium* and *S. galapagense*) (HARTMAN; ST. CLAIR, 1998; MALUF: CAMPOS; CARDOSO, 2001; MUIGAI et al., 2003; MACIEL et al., 2011; FIRDAUS et al., 2012; 2013; LUCATTI et al., 2013; 2014; LUCINI et al., 2015). This resistance is often associated with the presence of glandular trichomes and the allelochemicals they contain, which may confer antibiosis and/or antixenosis against the pests.

Among these wild species, *S. galapagense* is one of the closest relatives to *S. lycopersicum* phylogenetically (PERALTA; SPOONER; KNAPP, 2008). Recent studies have shown accessions of *S. galapagense* presenting superior levels of resistance to whitefly in comparison to other wild species, although these levels varied among different accessions of *S. galapagense* (FIRDAUS et al., 2012; LUCATTI et al., 2013). Presence of type IV glandular trichomes and the acylsugar content have been consistently associated with high levels of resistance in *S. galapagense* therefore accessions of this species are important sources of resistance for breeding programs (LUCATTI et al., 2013; FIRDAUS et al., 2012; 2013).

Knowledge about the genetic control of type IV glandular trichome densities and genomic regions associated with the densities is essential for successful trait introgression. The present work was carried out with the purpose of understanding the inheritance of type IV glandular trichome densities, their association with resistance to whitefly and to identify quantitative trait loci (QTL) associated with the presence of type IV trichomes in populations derived from the interspecific cross *Solanum lycopersicum* TOM-684 x *Solanum galapagense* accession LA1401.

2 REVIEW OF THE LITERATURE

2.1 Centre of origin of tomato and of the wild species *Solanum galapagense*

The *Solanaceae* family contains many important crop plants. Tomatoes, eggplants, potatoes, scarlet (African) eggplants and peppers are some examples of important horticultural plants, whereas mandrake, tobacco and belladonna are used for medical purposes. In addition, some *Solanum* species as well as tobacco and petunias are traded as ornamental plants. *Solanaceae* family members can be found throughout the world; however the Neotropics present the greatest diversity of species (DARWIN, KNAPP; PERALTA, 2003).

The genus *Solanum* contains more than one thousand five hundred species. It is not only the largest genus of this family, but also one of the most numerous of angiosperm species. There is also a great morphological diversity among its members, which include a range from very small herbs to large trees in every existing habitat (DARWIN, KNAPP; PERALTA, 2003).

Tomato plants belong to the genus *Solanum*. They are native to the occidental part of South America, from Colombia to the north of Chile. Two wild species are endemic to the Galapagos Islands: *S. cheesmanii* and *S. galapagense* (DARWIN, KNAPP; PERALTA, 2003; PERALTA; SPOONER, 2005). Thirteen tomato species are currently recognized, including the cultivated tomato (*Solanum lycopersicum*) (Table 1) (PERALTA; KNAPP; SPOONER, 2006).

Even though Linnaeus (1753) had already classified *S. lycopersicum, S. peruvianum L.* and *S. pimpinellifolium* as members of the genus *Solanum*, Miller (1754) created a new genus, called *Lycopersicon*, to group tomatoes and other species. (LINNAEUS, 1753; MILLER, 1754; cited by DARWIN; KNAPP; PERALTA, 2003).

Table 1 List of tomato species using the *Solanum* nomenclature, which is frequently employed nowadays, and their corresponding denominations as *Lycopersicon*.

Current Nomenclature (*Solanum*)	**Previous Nomenclature (*Lycopersicon*)**
Solanum pennellii Correll	*Lycopersicon pennellii* (Correll) D'Arcy
Solanum habrochaites S. Knapp and D.M Spooner	*Lycopersicon hirsutum* Dunal
Solanum chilense (Dunal) Reiche	*Lycopersicon chilense* Dunal
Solanum huaylasense Peralta and S. Knapp	Part of *Lycopersicon peruvianum* (L.) Miller
Solanum peruvianum L.	*Lycopersicon peruvianum* (L.) Miller
Solanum corneliomuelleri J.F. Macbr.	Part of *Lycopersicon peruvianum* (L.) Miller; Also known as *L. glandulosum* C.F. Müll.
Solanum arcanum Peralta	Part of *Lycopersicon peruvianum* (L.) Miller
Solanum chmielewskii (C.M. Rick, Kesicki, Fobes and M. Holle) D.M. Spooner, G.J. Anderson and R.K. Jansen	*Lycopersicon chmielewskii* C.M. Rick, Kesicki, Fobes and M. Holle
Solanum neorickii D.M. Spooner, G.J. Anderson and R.K. Jansen	*Lycopersicon parviflorum* C.M. Rick, Kesicki, Fobes and M. Holle
Solanum pimpinellifolium L.	*Lycopersicon pimpinellifolium* (L.) Miller
Solanum lycopersicum L.	*Lycopersicon esculentum* Miller
Solanum cheesmaniii (L. Riley) Fosberg	*Lycopersicon cheesmanii* L. Riley
Solanum galapagense S.C. Darwin and Peralta	Part of *Lycopersicon cheesmanii* var. minor L. Riley

Adapted from Peralta et al. in press, cited by PERALTA; KNAPP, S.; SPOONER 2006.

The classification of tomatoes as *Lycopersicon* was supported by several botanists and plant breeders until recently. However, both taxonomists and an increasing number of plant breeders agree now that tomatoes belong to the genus *Solanum*. Such acceptance is based on evidence from phylogenetic studies with DNA sequences and also in-depth studies on morphology and geographical distribution (PERALTA; KNAPP; SPOONER, 2006).

These species inhabit a wide range of habitats: the arid Pacific coast at sea level; regions in the Andes that may reach altitudes of 3.300m; and even in desert areas as the Atacama Desert in Chile. This fact has contributed to the great diversity of wild tomato species (PERALTA; SPOONER, 2005).

All wild tomato species are diploid (2n = 2x = 24) and can be crossed with the cultivated form, although this may be sometimes difficult. Wild species provide several useful traits, which may be used by breeding programs as resistance sources to pests and diseases (PERALTA; SPOONER, 2005).

S. galapagense is native to the Galapagos Islands, which is a volcanic region located at approximately 1000 km from the west coast of South America in the Republic of Ecuador. It is often found at the coastline of the occidental and south islands, but occasionally it can be seen at inland areas as in Isabela and Fernandina (Figure 1) (DARWIN; KNAPP; PERALTA, 2003).

S. galapagense tomatoes are known as “Tomatillo” or “Galapagos tomato” in the Galapagos Islands. The herbaceous plants have a citrus aroma. Trichomes of different sizes can be observed on their stems and leaves, which are densely pubescent. The length of the internodes is short. The leaves of *S. galapagense* are characterized by their serrated margins and the presence of trichomes in both abaxial and adaxial surfaces. Its fruit is small and edible varying in size from 0.6 to 1.1 cm. Other peculiarities of the ripe fruit include an orange color, a pronounced flavor and two locules (DARWIN; KNAPP; PERALTA, 2003).

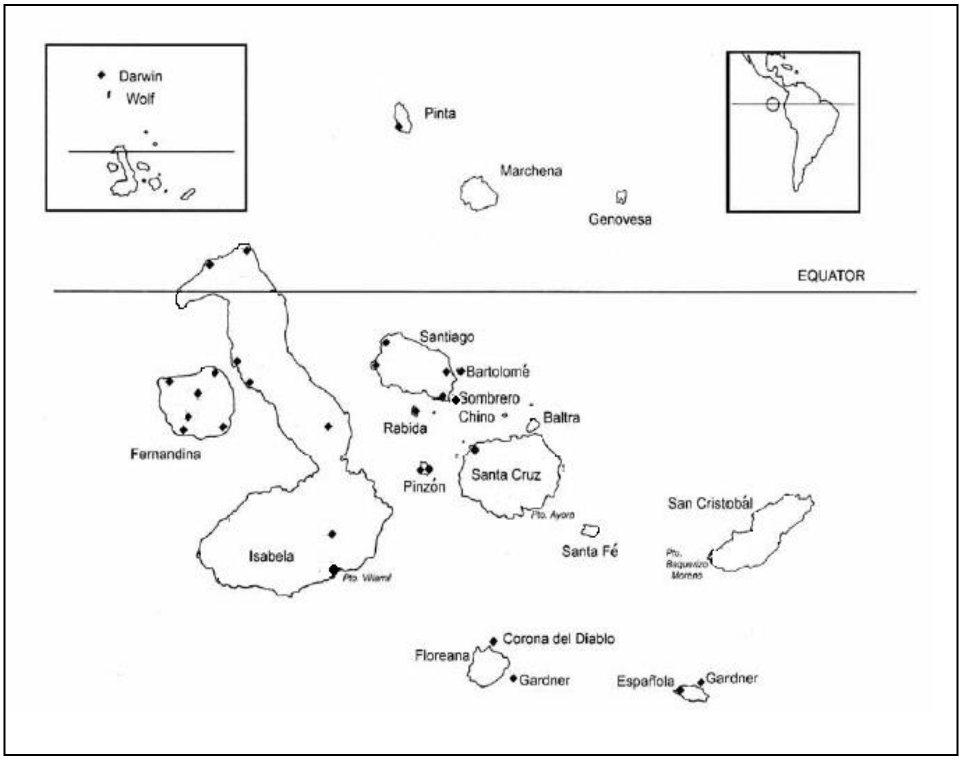

Figure 1 Map of the *S. galapagense* distribution in the Galapagos Islands. The occurrences of *S. galapagense* are represented by black dots
Fonte: Adapted from Darwin et al. (2003).

Seeds of *S. galapagense* present physical dormancy, which might be related to an evolutionary mechanism. Rick and Bowman (1961) observed that seed dormancy was overcome whenever the seeds passed through the digestive tract of a giant tortoise which is endemic to the Galapagos Islands. This mechanism would, in fact, favor both species, since the tomato fruit provide nutrients to the giant tortoise, which in turn contributes to seed dissemination. Alternatively, dormancy could also be suppressed by treating the seeds with a 2.7% sodium hypochlorite solution (TOMATO GENETICS RESOURCE CENTER, 2015).

At least three other important characteristics of *S. galapagense* have been reported. First, this species presents high tolerance to salinity, hopefully allowing the development of cultivars, which are more adapted to soils with higher salt concentrations (RUSH; EPSTEIN, 1980). Second, its fruit show a high soluble solids content (GARVEY; HEWITT, 1991). Third, this plant is resistant to pests such as the whitefly (*Bemisia* spp.) and leafminer (*Liriomyza trifolii*) (JOUY; BORDAT; BESSIERE, 1992; FIRDAUS et al., 2012; LUCATTI et al., 2013). Through breeding programs, these traits could be introgressed into the cultivated tomato.

2.2 Tomato crops: social and economic importance

Tomato is the most widely produced and consumed vegetable in Brazil, with the largest contribution to the country's economy. In 2014, the national production of tomatoes demanded 65,178 hectares to yield 4,275,070 tons. Five states were responsible for 70% of the total production: Minas Gerais, São Paulo, Rio de Janeiro, Espírito Santo, and Goiás. There are only eight nations in the world that produce more tomatoes than Brazil. China is the largest producer. India and United States rank second and third, respectively (AGRIANUAL, 2015).

Part of the production supplies the retail chains, where the fruit will be traded for fresh-market consumption. The other part is directed towards the processing tomato industry. Tomato's market share is quite significant for the Brazilian agribusiness, since it represents about 16% of the total income from the horticultural market, which amounts to over R$2 billion yearly. In addition, the tomato business is an important source of jobs and income (ASSOCIAÇÃO BRASILEIRA DO COMÉRCIO DE SEMENTES E MUDAS, ABCSEM, 2010). Estimates point out that the production of fresh-market tomato employs five or six workers for each cultivated hectare every year. Indirect jobs are also created at the same rate. These numbers are even greater if the production chain is considered since several other sectors must be involved before consumers' needs are fulfilled (MELO; VILELA; BOITEUX, 2010).

High-level technology and pesticides are deployed for tomato crops, due to the high incidence of pests and diseases. As a result, costs and risks are increased. Pest control is one of the main aspects to be managed in tomato culture. Losses caused by pests and diseases may reach 35% of total production. If no chemicals were used, damage could be as high as 78% (ZALOM, 2003). This problem is even worse in tropical countries, like Brazil, because temperature conditions and high humidity favor the appearance and development of pests (MALUF; CAMPOS; CARDOSO, 2001; FIRDAUS et al., 2012).

Nevertheless, the use of pesticides is not always successful. Therefore, tomato growers often use agricultural chemicals preventively as an attempt to increase efficiency and guarantee high yield. This practice not only may cause adverse health effects to workers who spray pesticides, but also to consumers' health, which may be affected due to the excess of chemical residuals in the fruit. In addition, large amounts of such pesticide compounds are likely to cause negative impacts to the environment (FREITAS et al., 2002; PERES; MOREIRA; DUBOIS, 2003).

The facts indicate the necessity of developing cultivars which are resistant to pests. The use of resistant cultivars would contribute to reduce the use of pesticides and to develop more sustainable agricultural practices throughout the year. In order to obtain pest resistant tomato cultivars, studies have emphasized the introgression of resistance found in wild tomato species into commercially accepted lines of cultivated tomato (MALIEPAARD et al., 1995; FREITAS et al., 2002; RESENDE et al., 2002; MOMOTAZ; SCOTT; SHUSTER, 2010; FIRDAUS et al., 2013).

2.3 Trichomes and their association with resistance to arthropod-pests in tomato

Foliar trichomes may help to protect plants from insects by acting as physical barriers, particularly because of the density, length and structure of the different trichome types. They can also provide the plant with a source of allelochemicals, which are toxic to insects (LARA, 1991; SIMMONS; GURR, 2005). Tomato plants present a diversity of trichomes, with variable densities depending on the species or cultivars. Luckwill (1943) identified seven types of trichomes (types I to VII) in *Solanum lycopersicum*, and classified them into non-glandular (II, III and V) and glandular (I, IV, VI, VII) types.

Wild species of tomato (*S. pennellii, S. habrochaites, S. pimpinellifolium* and *S. galapagense*) have shown to be resistant to arthropod-pests such as whitefly (*Bemisia tabaci*.), South American tomato pinworm (*Tuta absoluta)*, and two spotted spider mite (*Tetranychus urticae*), among others. The resistance has been correlated with the presence of glandular trichomes and their association with allelochemical content suggesting a mechanism (MALUF; CAMPOS; CARDOSO, 2001; MUIGAI et al., 2003; FIRDAUS et al., 2012; LUCATTI et al., 2013; LUCINI et al., 2015).

Exudates of glandular trichomes contain allelochemicals which may confer antibiosis and/or antixenosis response against the pests. As a consequence, increased pest mortality may be observed. Other possible effects include reduction of oviposition as well as deleterious effects in certain phases of the insect development. The main allelochemicals found in these exudates are acylsugar, 2-tridecanone (methyl-ketones) and zingiberene (sesquiterpene) (SIMMONS; GURR, 2005).

Type IV and VI glandular trichomes are predominantly associated with negative effects towards arthropod pests (SIMMONS; GURR, 2005). Resistance present in *S. habrochaites* is associated with presence of type VI glandular trichomes and content of either 2-tridecanone or zingiberene (CHATZIVASILEIADIS; BOON; SABELIS, 1999; FREITAS et al., 2002; SIMMONS; GURR, 2005). On the other hand, in *S. pennellii*, *S. pimpinellifolium*, *S. galapagense*, the resistance to arthropod-pests is mainly associated with type IV trichomes. Exudate from this type of trichome consists mostly of acylsugars (GOFFREDA et al., 1989; BLAUTH; CHURCHILL; MUTSCHLER, 1998; MUIGAI et al., 2002; RODRÍGUEZ-LÓPEZ et al., 2011; FIRDAUS et al., 2012; LUCATTI et al., 2013; FIRDAUS et al., 2013; LUCINI et al., 2015).

Neves et al. (2003) evaluated the behavior of tomato populations derived from the cross between *S. lycopersicum* (cv. IPA-6) and *S. habrochaites* (PI 134418), which were infested with South American tomato pinworm. The researchers observed negative phenotypic correlations between the foliar area consumed by the insects, and the density of type VI trichomes on the leaves. This result indicates that resistance is related to higher quantities of type VI foliar trichomes. Freitas et al. (2002) observed a positive correlation between zingiberene and two different types of trichomes (IV and VI) in populations derived from *S. habrochaites* accession PI 127826. Plants with high levels of zingiberene presented resistance to whitefly.

Several studies have reported the association between type IV trichomes and acylsugar content as the cause of resistance to arthropod-pests (GOFFREDA et al., 1989; MUIGAI et al., 2002; RODRÍGUEZ-LÓPEZ et al., 2011; LUCATTI et al., 2013; FIRDAUS et al., 2012; 2013; LUCINI et al., 2015). In *S. pennelli*, the relation between acylsugar content and densities of glandular trichomes were initially reported by Fobes, Mudd and Marsden (1985) and

Goffreda et al. (1989). Lucini et al. (2015) verified that genotypes with high density of type IV trichomes also presented high levels of acylsugars. In addition, the highest mortality and lowest oviposition of two spotted spider mites (*Tetranychus urticae*) were observed in these genotypes. In this study, the populations were obtained through the cross between *S. lycopersicum* (cv. ‘Redenção’) and *S. pennellii* (accession LA0716).

Gonçalves (2006) and Costa (2013) did not find any correlation between glandular trichomes and acylsugar accumulation. Both authors evaluated advanced populations, selected for high level of acylsugar, from the cross *S. lycopersicum* x *S. pennellii* accession LA0716. Because these advanced populations were selected solely for their acylsugar content, but not for densities of glandular trichomes, the authors suggest that acylsugar may also be present in other parts of the plant, such as the cells of leaf epidermis.

Firdaus et al. (2012) evaluated the resistance to whitefly in several different wild tomato species. They reported that, in the resistant wild accessions of *S. galapagense* and *S. habrochaites*, resistance was associated with acylsugar content and the presence of type IV trichomes. Similar results were also found in *S. galapagense* by Lucatti et al. (2013).

Trichome-based host-plant resistance may therefore be considered a promising mechanism for pest control, provided by different types of glandular trichomes, combined with the toxicity of allelochemicals causing antibiosis and/or antixenosis responses. The selection of tomato genotypes with high density of type IV trichomes may result in higher resistance to pests. Thus introgression of glandular trichomes is a strategy to transfer resistance from wild species into the cultivated tomato. (SIMMONS; GURR, 2005).

2.4 *Solanum galapagense* as a source of resistance to the whitefly

Whitefly (*Bemisia tabaci*) is considered one of the most destructive pests to horticultural and ornamental plants. Whitefly infestation may cause direct damage due to insect feeding on the phloem, resulting in physiological disorders such as irregular fruit maturation. Whiteflies can also vector viruses (geminiviruses), which may cause even worse problems to the tomato crop (BYRNE; BELLOWS, 1991; MORALES; JONES, 2004; RODRÍGUEZ-LÓPEZ et al., 2011).

Pesticides have often failed to control whitefly efficiently. In fact, these polyphagous insects ovoposit and feed mainly on the abaxial surface of the leaves allowing them to escape from spray application. They have also developed resistance to most of the classes of chemicals, which are employed to control their outbreak (PALUMBO; HOROWITZ; PRABHAKER, 2001; JONES, 2003; MOMOTAZ; SCOTT; SCHUSTER, 2010). Alternatively, resistant cultivars could offer an alternative solution to help to control whitefly infestation.

Recent studies point out that *S. galapagense* is highly resistant to whitefly (FIRDAUS et al., 2012; 2013; LUCATTI et al., 2013). Lucatti et al. (2013) evaluated whitefly resistance in the wild species *S. galapagense* and *S. cheesmanii*, which are endemic to the Galapagos Islands. All the evaluated accessions of *S. galapagense* were resistant. In addition, they had high density of type IV trichomes as well as elevated levels of acylsugars. In contrast, *S. cheesmanii* accessions were susceptible, even though they contained acylsugar levels similar to those of *S. galapagense.* This observation could probably be explained by the absence of type IV trichomes in *S. cheesmanii*. In conclusion, the authors suggest that a minimum level of acylsugars and the presence of type IV glandular trichomes are needed to achieve an effective level of resistance.

Firdaus et al. (2012) examined the whitefly resistance of different accessions of several wild tomato species. They found that the resistance levels varied among the evaluated accessions of *S. galapagense.* In addition, the accession PRI95004/PY-8027 (*S. galapagense*) was the most resistant and had the lowest oviposition and adult survival rates. A high correlation was recorded between resistance and high density of type IV trichomes. Such resistance was confirmed in segregating populations, obtained through the cross between the cv. Moneymaker^{tmvR} PRI91117 (*S. lycopersicum*) and accession PRI95004 (*S. galapagense*) (FIRDAUS et al., 2013).

Accession LA1401 stood out from all other accessions of *S. galapagense*, not only because of its superior whitefly resistance, but also because of its salinity tolerance and soluble solid contents (LUCATTI et al., 2013; MAHMOUD; JONES; EL-BELTAGY, 1986; SHANNON; WRONA, 1992; RAZDAN; MATTOO, 2006). It is therefore an important resistance source that could be incorporated into plant breeding programs.

2.5 Inheritance of type IV glandular trichome

In tomato breeding, knowledge about the genetic control of type IV glandular trichome densities and the associated genomic regions is helpful for successful trait introgression. Freitas et al. (2002), studying populations derived from interspecific cross between *S. lycopersicum* and *S. habrochaites* (PI 127826), reported that a major locus could explain the variation of densities for trichome types IV, VI and VII, although types IV and VI trichomes are also subject to the action of other epistatic loci. Lenke and Mutscheler (1984) reported that the inheritance of type IV glandular trichome in *S. pennelli* was not complex and two independent genes were possibly involved. Saeidi (2012) found similar results to those of Lenke and Mutscheler (1984) and suggested that two independent dominant genes were responsible for the presence of type IV trichomes in *S. pennelli.*

Studies have been carried out in order to identify genomic regions which are associated with trichome presence. QTLs, associated with density of type IV glandular trichomes, were identified in *S. pennelli* (accession LA716), *S. galapagense* (accession PRI95004) and *S. habrochaites* (accessions CGN1.1561 and LA1777) (MALIEPAARD et al., 1995; BLAUTH; CHURCHILL; MUTSCHLER, 1998; MOMOTAZ; SCOTT; SCHUSTER, 2010; FIRDAUS et al., 2013; LUCATTI et al., 2014).

Firdaus et al. (2013) identified QTLs associated with whitefly resistance as well as the traits associated with this resistance. The researchers evaluated a segregating population derived from a cross between the cultivated tomato and an accession of *S. galapagense.* Two QTLs were associated with the presence of type IV glandular trichomes and adult survival. One major QTL (Wf-1) on chromosome 2 and a minor QTL (Wf-2) on chromosome 9. These authors indicate an association of whitefly resistance in *S. galapagense* with type IV trichomes. In addition, they suggest that their inheritance is relatively simple, which favors their use in breeding programs (FIRDAUS et al., 2013).

These studies suggest that the presence of type IV glandular trichomes is controlled by few genes, which would facilitate the introgression of this trait in tomato cultivars. Moreover, *S. galapagense* can be considered as a promising source of resistance to arthropods. Nevertheless, further studies on specific accessions are necessary to better understand the resistance mechanism endowed by glandular trichomes, because resistance levels may vary among different accessions of this species.

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CHAPTER 2: ARTICLES

ARTICLE 1: Inheritance of type IV glandular trichome density and its association with whitefly resistance from *Solanum galapagense* accession LA1401.

ABSTRACT

Several accessions of the wild species of *S. galapagense*, including accession LA1401, are considered resistant to whitefly (*Bemisia tabaci*). This resistance has been associated with the presence of type IV glandular trichomes on the leaf surface. Our research aimed to study the inheritance of type IV glandular trichome density and its association with resistance to whitefly in populations derived from the interspecific cross *Solanum lycopersicum* x *Solanum galapagense* 'LA1401'. High estimates for both broad sense and narrow sense heritabilities of type IV glandular trichome densities suggest that inheritance of this trait is not complex. Whitefly resistance was associated with high density of type IV glandular trichomes. F_2 (*S. galapagense* x *S. lycopersicum*) population plants selected for highest densities of type IV glandular trichomes showed similar levels of resistance to those found in the donor of resistance LA1401.

Key words: Heritability, negative binomial, zero-inflated Poisson.

RESUMO

Diversos acessos da espécie selvagem *S. galapagense*, entre eles o acesso LA1401, são considerados resistentes à mosca branca (*Bemisia tabaci*), e essa resistência tem sido associada à presença de tricomas glandulares tipo IV na superfície foliar. Nosso trabalho objetivou estudar a herança da densidade de tricomas glandulares tipo IV e a sua associação à resistência à mosca branca em populações derivadas do cruzamento interespecífico entre *Solanum lycopersicum* x *Solanum galapagense* 'LA1401'. Estimativas altas para herdabilidade, tanto no sentido amplo quanto no sentido restrito, da densidade de tricomas glandulares tipo IV sugerem que esse caráter possui herança relativamente simples. A resistência à mosca branca esteve associada à maior densidade de tricomas glandulares tipo IV. As plantas da população F_2 (*S. galapagense* x *S. lycopersicum*) selecionadas para maiores densidades de tricomas tipo IV apresentaram o mesmo nível de resistência encontrado no genitor resistente LA1401.

Palavras-chave: Herdabilidade, binomial negativo, Poisson inflacionado de zeros.

1 INTRODUCTION

Whitefly (*Bemisia tabaci* biotype B) is considered one of the most important arthropod pests in tomato and in other vegetable crops. It affects tomato production directly (resulting in physiological disorders leading to irregular fruit maturation) and indirectly (virus transmission) resulting in yield losses (BYRNE; BELLOWS, 1991; MORALES; JONES, 2004).

Pest management in tomato through chemical products has often been inefficient, and can result in chemical-resistant pest populations. Alternatively, breeding programs have been trying to develop resistant cultivars by transferring the resistance present in wild species to the cultivated tomato. Sources of resistance to whitefly and other arthropod pest have been reported in *S. pennellii*, *S. habrochaites, S. pimpinellifolium e S. galapagense* (MALUF; CAMPOS; CARDOSO, 2001; MUIGAI et al., 2003; MACIEL et al., 2011; FIRDAUS et al., 2012; LUCATTI et al., 2013; 2014; LUCINI et al., 2015).

In wild species, whitefly resistance is often associated with the presence of high densities of glandular trichomes (type IV and VI) and with the presence of allelochemicals (FREITAS et al., 2002; MUIGAI et al., 2003; RODRÍGUEZ-LÓPES et al., 2011; FIRDAUS et al., 2012). In *S. pennellii, S. pimpinellifolium*, and *S. galapagense* the acylsugar content is associated with the presence of type IV trichomes, which play an important role in whitefly resistance (GOFFREDA et al., 1989; RODRÍGUEZ-LÓPES et al., 2011; LUCATTI et al., 2013; FIRDAUS et al., 2013; LUCINI et al., 2015). Among these wild species, *S. galapagense* is one of the closest related to *S. lycopersicum* phylogenetically (PERALTA; SPOONER; KNAPP, 2008). Recent studies have shown its accessions present high levels of resistance to whitefly. This resistance was associated with the presence of type IV glandular trichomes and acylsugar content (FIRDAUS et al., 2012; 2013; LUCATTI et al., 2013). Those factors make the *S. galapagense* accessions important sources of resistance to be deployed in breeding programs.

Further studies using specific accessions of *S. galapagense* would be helpful to understand the genetic factors involved in the presence of trichomes and their relation to whitefly resistance, which provide important information to accomplish a successful trait introgression. Among the accessions of *S. galapagense*, the accession LA1401 can be highlighted because it bears at least three traits of economic importance: resistance to whitefly, salt tolerance and high soluble solids content. (LUCATTI et al., 2013; MAHMOUD; JONES; EL-BELTAGY, 1986; SHANNON; WRONA, 1992; RAZDAN; MATTOO, 2006). In this work, we aimed to study the inheritance of type IV glandular trichome densities and their association with resistance to whitefly in populations derived from the interspecific cross *Solanum lycopersicum* 'TOM-684' x *Solanum galapagense* 'LA1401'.

2 MATERIALS AND METHODS

This study was developed through a partnership between the Universidade Federal de Lavras (UFLA), in Lavras, and the Hortiagro Sementes S.A. seed company, in Ijaci, both in the State of Minas Gerais, Brazil. The field experiments were carried out at the Hortiagro Sementes S.A. seed company. The analyses for identification and quantification of trichomes were performed at the Plant Anatomy laboratory at the Biology Departament, UFLA.

2.1 Plant material

Segregating populations were obtained from the interspecific cross *Solanum lycopersicum* 'TOM-684' x *Solanum galapagense* 'LA1401'. LA1401

(=P2) is a wild accession characterized by a high level of acylsugar, the presence of type IV trichomes, and resistance to insects (JOUY; BORDAT; BESSIERE, 1992, LUCATTI et al., 2013). TOM-684 (=P1) is susceptible to insects and has low acylsugar content. It is a proprietary fresh-market tomato inbred line from Hortiagro Sementes S.A. The parents LA1401 and TOM-684 were crossed to obtain the F_1 generation. F_1 plants were self-pollinated and backcrossed with both parents (accession LA1401 and line TOM-684) obtaining respectively the F_2 generation and the backcrosses: $F_1BC_{1(1)}$ [=(F_1 x TOM-684)] and $F_1BC_{1(2)}$ [=(F_1 x LA1401)].

Parents (P1 and P2) and the populations F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ were sown in flat 128-weel seed trays with commercial potting mix. After one month, the seedlings were transplanted to the greenhouse in a completely randomized design in order to be exposed to natural whitefly infestation. The experiment consisted of 40 plants from each parental line, 40 plants from the F_1, 268 individuals from the F_2 and 120 individuals from each backcross. Identification and quantification of trichomes types were made in a random sample of plants obtained from the populations. The sampled population consisted of 20 plants from each parental line, 20 plants from the F_1, 145 individuals from the F_2, and 60 individuals from each backcross.

2.2 Resistance to whitefly

Whitefly infestation occurred naturally in the greenhouse. The plants were evaluated relative to traits related to whitefly resistance three weeks after the infestation. This three-week period was necessary to allow the assessment of traits related to whitefly resistance in all stages of plant development. Insect pest management was not performed during the whitefly resistance experiment.

Incidence of whitefly was evaluated by measuring the number of eggs and the number of nymphs in the sampled leaf area. Number of eggs and number of nymphs were quantified on the abaxial surfaces of the fully-expanded fourth leaf in five areas of $1cm^2$ using a 10x magnifying glass with a millimeter scale. The total number of eggs and total number of nymphs per $5cm^2$ leaf surface were considered for statistical analyses.

2.3 Identification and quantification of trichomes

Two months after transplanting plants to the greenhouse, the fully-expanded fourth leaf from each plant was collected and stored in 70% ethanol in a beaker for 48 hours. After storage, paradermal sections were prepared from the abaxial and adaxial surfaces of leaflets. The sections were cleared in 50% sodium hypochlorite for 20 seconds and washed three times in water to remove the excess of hypochlorite. After that, sections were placed in 0.1% safranin dye for 20 seconds and then washed twice in water to remove the excess of dye. Six paradermal sections per surface were used per plant to make slides. Semi-permanent slides were prepared as mounts in glycerinated water (FREITAS et al., 2002).

The slides were viewed under a 10x optical microscope (Carl Zeiss, model AXILl) with an attached camera (AxionCam ERc5s). Images were captured using the Axio Vision Rel. 4.8 program and analyzed using the Image Tool v. 3.00 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, Texas). Type IV and type II+III trichome densities were quantified in three different areas of 1 mm^2 in each epidermal surface of the leaf (abaxial and adaxial) for each trichome types. Trichome types were identified and classified based on Luckwill (1943). Because number of trichomes on abaxial and adaxial surfaces were highly correlated for each type of trichome, the sum of the trichomes of both surfaces for each type of trichome was considered for statistical analyses. Therefore, trichome numbers were expressed in 6mm^2 per leaf surface.

2.4 Statistical analysis

2.4.1 Statistical Models

The traits trichome density, number of eggs and number of nymphs result in count data that do not follow a normal distribution. Poisson distribution is the natural choice for modeling count data, but such distribution imposes equality of mean and variance. In this study, the count outcomes exhibit overdispersion, that is, variance greater than the mean. In order to model this extra variability and obtain reliable estimates for the variances of each population and trait, negative binomial (NB) (HILBE, 2011) and zero-inflated Poisson (ZIP) (ZUUR et al., 2009) models were fitted.

A random variable Y follows a negative binomial distribution if its probability function is given by

$$\Pr(Y = y) = \frac{\Gamma(k^{-1} + y)}{\Gamma(k^{-1})y!} \left(\frac{k\mu}{1 + k\mu}\right)^{y} \left(\frac{1}{(1 + k\mu)}\right)^{1/k},$$

where k is the dispersion parameter of the distribution. This distribution approaches Poisson as $k \to 0$.

Negative binomial models were fitted to data on trichome density and number of eggs, considering each population separately. In all cases, there is only an overall effect, that is, no treatment effect. Then, assuming the logarithmic link function, we have that

$$y_{ij} = \mu_i + \varepsilon_{ij},$$
$$\mathrm{E}(Y_i) = \mu_i = e^{\beta_i},$$
$$\mathrm{Var}(Y_i) = \mu_i + k_i\mu_i^2 = e^{\beta_i} + k_i e^{2\beta_i},$$

where y_{ij} is the count outcome of the jth plant in the ith population, $i = 1, \dots, 6$ and $j = 1, \dots, J$; $\mathrm{E}(Y_i)$ and $\mathrm{Var}(Y_i)$ are the mean and the variance, respectively, of the ith population. The β_i and k_i estimates are obtained using, for example, the maximum likelihood method, which is available in statistical programs like R and SAS.

Zero-inflated Poisson models were fitted to number of nymphs, due to high incidence of zeros in this trait. The probability function of a random variable *Y*, which follows a ZIP distribution is

$$\Pr(Y = y) = \begin{cases} \pi + (1-\pi)\exp(-\lambda) & y = 0 \\ \dfrac{(1-\pi)\exp(-\lambda)\,\lambda^y}{y!} & y > 0 \end{cases}$$

In this case, the population is considered to have two types of individuals: the first type has count data always equal to zero and the second type gives Poisson count data, which can be zero. In the probability function expression, λ refers to the mean of the Poisson distribution and π (zero-inflation probability) represents the probability of zero counts.

As before, there is only an overall effect and each population was considered separately. In this case,

$$\lambda_i = e^{\beta_i},$$

that is, the mean of the Poisson count data, and

$$\pi_i = \frac{e^{\alpha_i}}{1 + e^{\alpha_i}}.$$

From these terms, we have

$$\mathrm{E}(Y_i) = \mu_i = (1 - \pi_i)\lambda_i,$$
$$\mathrm{Var}(Y_i) = \mu_i + \left(\frac{\pi_i}{1 - \pi_i}\right)\mu_i^2,$$

where $\mathrm{E}(Y_i)$ and $\mathrm{Var}(Y_i)$ are the mean and variance, respectively, of the ith population, $i = 1, \dots, 6$. The β_i and α_i estimates are obtained through maximum likelihood method, implemented in statistical programs.

The aforementioned models were fitted to the trichomes and whitefly (eggs and nymphs) count data using the NLMIXED procedure of SAS 9.3 program (SAS INSTITUTE, 2011) . Some codes are presented in Appendix A. In all traits, the outcomes refer to the total values per plant instead of average, because these models are used for count data.

2.4.2 Variance estimate and heritability

From the parameters estimates of the fitted model, the variances of parents (P1 e P2), populations F_1, F_2, backcrosses $F_1BC_{1(1)}$ [=(F_1xTOM-684)] and $F_1BC_{1(2)}$ [=(F_1xLA1401)] were estimated considering each trait. The heritability values were then estimated by ratios of genotypic variance $\mathrm{V(G)}$ or additive variance $\mathrm{V(A)}$ and phenotypic variance $\mathrm{V(P)}$ (WRIGHT, 1968):

$$\mathrm{V\,(P)} = \mathrm{VF_2}$$

$$\mathrm{V\,(E)} = \frac{\mathrm{VP_1} + \mathrm{VP_2} + (2 \times \mathrm{VF_1})}{4}$$

$$\mathrm{V\,(G)} = \mathrm{V(P)} - \mathrm{V(E)}$$

$$\mathrm{V\,(A)} = (2 \times \mathrm{VF_2}) - (\mathrm{VF_1BC_{1(1)}} + \mathrm{VF_1BC_{1(2)}}),$$

where $\mathrm{V(E)}$ is environmental variance; $\mathrm{VP_1}$ is the environmental variance among plants of parent TOM-684; $\mathrm{VP_2}$ is the environmental variance among plants of parent LA1401; $\mathrm{VF_1}$ is the environmental variance among plants of F_1 population; $\mathrm{VF_2}$ is the variance among individuals of F_2 population; $\mathrm{VF_1BC_{1(1)}}$ is the variance among individuals of the first backcross to the parent TOM-684; $\mathrm{VF_1BC_{1(2)}}$ is the variance among individuals of the first backcross to the parent LA1401.

2.4.3 Associative analysis between type IV glandular trichomes and whitefly resistance

The 20 plants with the highest densities and the 20 plants with the lowest densities of type IV trichomes were selected from F_2 sampled population. These two groups, as well as the parents (P_1 and P_2) and the F_1 population were considered as covariates (treatment effects) in the estimation of contrasts. Then, the negative binomial model is

$$y_{ij} = \mu_i + \varepsilon_{ij},$$

$$\mu_i = e^{\beta_0 + \beta_i},$$

where y_{ij} is the count outcome of the jth plant in the ith group (High F_2, low F_2, P_1, P_2 and F_1), $i = 1, \dots, 5$, and $j = 1, \dots, J$, where $J = 20, 20, 20, 20, 20$, for the groups high F_2, low F_2, P_1, P_2 and F_1, respectively.

The model specification is similar for the ZIP model, where

$$\lambda_i = e^{\beta_0+\beta_i},$$

and

$$\pi_i = \frac{e^{\alpha_0+\alpha_i}}{1+e^{\alpha_0+\alpha_i}}.$$

Contrasts of means between the groups were tested based on the asymptotic chi-square distribution of the likelihood ratio statistic using the GENMOD procedure of SAS 9.3 program (SAS INSTITUTE, 2011).

Spearman correlations between the traits type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs were calculated using the F_2 population.

3 RESULTS

Trichome densities and whitefly incidence

The average total values for density of type IV glandular trichomes, types II+III non- glandular trichomes, number of eggs and number of nymphs for P1, P2, F_1, F_2, $F_1BC_{1(1)}$ e $F_1BC_{1(2)}$ are shown in Table 1. Type IV glandular trichomes were found in high density in the parent LA1401 (*S. galapagense*), absent in the parent TOM-684 (*S. lycopersicum*) and in low density in the F_1 population. Contrasting results were observed for types II+III non-glandular trichomes, for which the density in parent LA1401 was close to zero (0.85)

(Table 1). The backcross $F_1BC_{1(1)}$ [=(F_1 x TOM-684)] had low mean density of type IV trichomes. In the backcross $F_1BC_{1(2)}$ [=(F_1 x LA1401)] the mean densities of type IV trichomes were comparable to the densities present in the F_2 population.

The parental accession LA1401, considered resistant to whitefly, had a lower number of eggs and nymphs for both the total population and the sampled population of the experiment (Table 1). The F_1 population had values for number of eggs and number of nymphs close to those of the susceptible parent TOM-684. In the sampled population used for quantification of trichome densities, the F_2 plants selected for highest density of type IV glandular trichomes had a lower number of eggs and nymphs than the F_2 plants selected for lowest density of type IV glandular trichomes. In addition, the number of eggs and nymphs in the plants selected for highest density of type IV trichomes were similar to the ones found in LA1401 (Table 1).

Heritability

The negative binomial model was fitted for type IV glandular trichomes, types II+III non-glandular trichomes and number of eggs, because count data with overdispersion was observed for these traits. The number of nymphs had count data with overdispersion due the high incidence of zero, therefore the best statistical model fitted was a zero-inflated Poisson. After fitting the appropriate model for each trait, the variances were estimated for the parents (LA1401 and TOM-684) and each of the populations F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ (Table 2).

The density of type IV glandular trichomes had a high estimate for both broad sense heritability (0.93) and narrow sense heritability (0.60). For this type of trichome, additive variance estimate represented 0.64 of the genotypic variance and for non-additive variance represented 0.36. The estimates of broad sense heritability were relatively high for types II+III trichome density (h^2_a=0.81), number of eggs (h^2_a>0.42) and number of nymphs (h^2_a>0.61) (Table 2).

Association between glandular trichomes type IV and whitefly resistance

The measured traits were significantly correlated (Table 3). Type IV glandular trichome densities were negatively correlated with type II+III non-glandular trichomes (r = -0.56), number of eggs (r = -0.22) and number of nymphs (r = -0.24). Positive correlations were found with type II+III non-glandular trichomes, number of eggs and number of nymphs (Table 3).

Parents LA1401 and TOM-684 were statistically different ($p < 0.05$) for type IV trichome densities, type II+III trichome densities and number of eggs (Table 4). Even though the parents were not significantly different for number of nymphs (Table 4), the number of nymphs was nonetheless higher in the TOM-684 and lower to LA1401 (Table 1).

F_2 plants with highest average of type IV trichome density had number of eggs and number of nymphs significantly lower than the F_2 plants with lowest average of type IV trichome density (Table 4). Considering the traits type IV glandular trichomes, number of eggs and number of nymphs, the contrasts between LA1401 and F_2 plants with highest density of type IV trichomes were not significant. Neither were the contrasts between TOM-684 and F_2 plants with lowest density of type IV trichomes.

4 DISCUSSION

Inheritance of type IV glandular trichome density

Type IV glandular trichomes were observed in abundance in the parent LA1401 (*S. galapagense*) and in the plants selected for highest density of type IV trichomes in the F_2 population. *S. galapagense* accessions are characterized by having high densities of this type of trichome (SIMMONS; GURR, 2004; FIRDAUS et al., 2012; LUCATTI et. al., 2013). The average total values for the density of type IV glandular trichomes found in the populations F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ are indicative that high density of type IV trichomes may be under control of incompletely recessive alleles from LA1401. In fact, the F_2 population had average total values for density of type IV glandular trichomes approximately four times lower than the density found in the parent LA1401 (Table 1). Apparently, F_2 plants that are heterozygous and homozygous for the TOM-684 alleles negatively affect the average of the F_2 population since a large proportion of those plants probably have type IV trichomes either in lower density or absent, respectively. Their behavior were similarly to what was expected in the F_1 population and in the parent TOM-684. F_2 plants selected for the highest density of type IV glandular trichomes are probably recessive homozygous for the alleles inherited from LA1401 and they had average values similar to those found in LA1401 for type IV trichomes (Table 4). In addition, the F_1 population and the backcross towards TOM-684 [=(F_1xTOM-684)] had low densities of type IV trichomes, with values close to zero, which was the value found for TOM-684 (Table 1).

Additive and non-additive effects are involved in the expression of type IV trichomes. Furthermore, the high estimate for the additive variance suggests that it should be rather easy to practice selection for this trait. High estimates for both broad sense and narrow sense heritabilities are indicative that inheritance of type IV glandular trichome may not be complex. High broad sense heritability for type IV trichomes was also reported in *S. galapagense* by Firdaus et al. (2013). Freitas et al. (2002) studied the inheritance of glandular trichomes in a segregating population from the interspecific cross *S. habrochaites* accession PI 127826 and *S. lycopersicum* TOM-556. For *S. habrochaites*, high broad sense heritability for type IV trichomes was also demonstrated. Furthermore, research into the *S. habrochaites* source shows that densities of type IV glandular trichomes appear to be under control of incompletely recessive allele at a major locus. In *S. pennelli*, Saeidi (2012) suggested that two independent dominant genes were responsible for the presence of type IV trichomes.

Types II+III non-glandular trichomes, number of eggs and number of nymphs also had high estimates of broad sense heritability (Table 2), which indicates that genetic effects are more important than environmental effects in the expression of these traits.

Resistance to whitefly

A negative correlation among type IV glandular trichomes and the traits number of eggs and number of nymphs is indicative that this trichome type is associated with the resistance to whitefly, because populations that had higher numbers of type IV trichomes had low incidence of number of eggs and nymphs (Table 1). Firdaus et al. (2012) evaluated whitefly resistance in different accessions of several wild tomato species. They reported that resistant accessions showed a high correlation between type IV trichomes and whitefly resistance components. Types II+III non-glandular trichomes were positively correlated to number of eggs and nymphs, which suggests that their presence are associated to an increase in the rate of eggs and nymphs. Hence, we can infer that types II+III non-glandular trichomes do not confer resistance to whiteflies. Types II+III and type IV trichomes were negatively correlated, therefore, the selection of plants with high density of type IV trichomes result in an indirect selection of plants resistant to whitefly with a low density of types II+III trichomes. A negative correlation between type III and type IV trichomes was also found by Firdaus et al. (2013).

In fact, this association between type IV trichomes and whitefly resistance is supported by the results of the estimated contrasts (Table 4). F_2 plants selected for highest density of type IV trichomes behaved similarly to the resistant parent LA1401, whereas in the F_2 plants selected for lowest density of type IV trichomes, the incidences of eggs and nymphs were similar to those found in the susceptible parent TOM-684. The relation between whitefly resistance and type IV trichomes has been reported in several wild species of tomato, such as *S. galapagense*, *S. habrochaites*, *S. pennelli* (MUIGAI et al., 2002; FREITAS et al., 2002; SAEIDI, 2012; FIRDAUS et al., 2012; 2013; LUCATTI et al., 2013). Presence of type IV trichomes has also been associated with resistance to other arthropod pests such as spider mite and aphids (GOFFREDA et al., 1989; MALUF et al., 2007; SAEIDI, 2012; LUCINI et al., 2015).

The resistance provided by glandular trichomes seems to be associated with allelochemical content that are present inside of the trichome glands (GOFFREDA et al., 1989; MALUF; CAMPOS; CARDOSO, 2001; MUIGAI et al., 2003; FIRDAUS et al., 2012; FIRDAUS et al., 2013; 2013; LUCINI et al., 2015). These allelochemicals may provide antibiosis and/or antixenosis plant response to the pests (SIMMONS; GURR, 2005). Lucatti et al. (2013) evaluated whitefly resistance in accessions of the *S. galapagense* and *S. cheesmanii*. In their study, all the *S. galapagense* accessions showed resistance to whitefly. This resistance was accompanied by high density of type IV trichomes as well as high acylsugar accumulation. In contrast, *S. cheesmanii* accessions were susceptible, even though they had acylsugar levels comparable to those found in *S. galapagense*. This fact could probably be explained by the absence of type IV trichomes in *S. cheesmanii*. In conclusion, the authors suggest that a minimum level of acylsugars and the presence of type IV glandular trichomes are needed to achieve an effective level of resistance. Firdaus et al. (2013) also found that the presence of type IV trichomes and acylsugar content were related to whitefly resistance.

In *S. pennelli*, the relation between acylsugar content and densities of glandular trichomes were initially reported by Fobes, Mudd and Marsden (1985) and Goffreda et al. (1989). In contrast, Gonçalves (2006) and Costa (2013) did not find any correlation between glandular trichomes and acylsugar accumulation. Both authors evaluated advanced populations, selected for high level of acylsugar, from the cross *S. lycopersicum* x *S. pennellii* accession LA0716. Because these advanced populations were selected solely for their acylsugar content, but not for densities of glandular trichomes, the authors suggest that acylsugar may also be present in other parts of the plant, such as the cells of leaf epidermis.

Our study suggests that the inheritance of type IV trichomes is relatively simple and it appears to be associated with the resistance to whitefly in *S. galapagense*. However, other mechanisms which were not evaluated in our study, as the acylsugar content, also seem to be involved in this resistance. Based on the progenies from the plants selected for highest and lowest densities of type IV trichomes, further studies can be carried out to confirm the relation between type IV trichomes and acylsugar content in populations derived from *S. lycopersicum* ‘TOM-684’ x *S. galapagense* ‘LA1401’.

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Table 1: Average total values for density of type IV glandular trichomes, density of types II+III non-glandular trichomes, number of eggs and number of nymphs per leaf of the populations derived from interspecific cross *S. galapagense* LA1401 x *S. Lycopersicum* TOM-684.

	Sampled population				**Total population**	
Populations	**Type IV Trichomes** $^{(a)}$	**Type II+III trichomes** $^{(a)}$	**Eggs**$^{(b)}$	**Nymphs**$^{(b)}$	**Eggs**$^{(b)}$	**Nymphs**$^{(b)}$
TOM-684 (P1)	0 (± 0)	313.9 (± 14.41)	11.95 (± 2.58)	0.75 (± 0.19)	10.74 (± 1.65)	0.59 (± 0.13)
LA1401 (P2)	82.3 (± 5.69)	0.85 (± 0.30)	1.55 (± 0.55)	0.15 (± 0.11)	2.89 (±1.22)	0.11 (± 0.07)
F_1	4.11 (± 1.09)	207.33 (± 7.51)	7.67 (± 2.03)	0.56 (± 0.29)	12.90 (± 2.37)	0.59 (± 0.22)
F_2	20.99 (± 2.81)	150.35 (± 6.74)	8.86 (± 1.42)	0.88 (± 0.14)	11.38 (± 1.29)	1.14 (± 0.27)
F_2 plants selected:						
F_2 highest density (IV)	94.05	57.55	1.15	0.05	-	-
F_2 lowest density (IV)	0.00	194.10	5.75	0.70	-	-
F_1RC_{11} (F_1xTOM-684)	0.39 (± 0.21)	283.68 (± 9.20)	8.15 (± 1.18)	0.95 (± 0.19)	12.24 (± 1.56)	1.13 (± 0.18)
F_1RC_{12} (F_1xLA1401)	25.25 (±5.31)	160.35 (±13.32)	8.67 (± 1.38)	0.90 (± 0.24)	9.28 (± 1.22)	0.87 (± 0.19)

Mean followed by standard error of mean.

$^{(a)}$: Average total density of trichomes per 6mm^2.

$^{(b)}$: Average total number of eggs or nymphs per 5cm^2.

F_2 highest density (IV): Average total density of the 20 plants which showed the highest density of type IV glandular trichomes selected in the F_2 population sampled.

F_2 lowest density (IV): Average total density of the 20 plants which showed the lowest density of type IV glandular trichomes selected in the F_2 population sampled.

Table 2: Estimates of variance in the populations derived from interspecific cross *S. galapagense* LA1401 x *S. Lycopersicum* TOM-684 and heritability to the type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs.

	Sampled population				**Total population**	
Populations	**Type IV trichome**	**Types II+III trichome**	**Eggs**	**Nymphs**	**Eggs**	**Nymphs**
TOM-684 (P1)	0.00	4129.31	121.93	0.75	87.76	0.60
LA1401 (P2)	532.68	2.02	6.54	0.26	32.01	0.17
F_1	21.09	984.92	104.68	1.13	215.92	1.60
F_2	1940.80	8017.47	155.11	2.11	235.84	4.05
F_1BC_{11}(F_1xTOM-684)	2.76	5126.96	71.57	1.75	172.63	2.08
F_1BC_{12}(F_1xLA1401)	2723.91	16735.75	118.37	2.63	145.85	2.53
Broad sense heritability	0.93	0.81	0.46	0.61	0.42	0.76
Narrow sense heritability	0.60	- (a)	- (a)	- (a)	- (a)	- (a)
s (h^2_a)	0.07	0.09	0.13	0.10	0.09	0.06

s (h^2_a): Estimate of associate error to broad sense heritability by Vello and Vencovsky (1974).

(a): Estimate of the narrow sense heritability higher than Estimate of the broad sense heritability, or negative estimate of the narrow sense heritability, which the real estimate probably is zero.

Table 3: Correlation between the traits type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs in the F_2 sampled population.

	Type IV trichomes	**Types II+III trichomes**	**Number of eggs**
Type II+III trichomes	-0.56**		
Number of eggs	-0.22**	0.24**	
Number of nymphs	-0.24**	0.34**	0.37**

**Significant at the 0.01 level.

Table 4: Contrasts between populations with different densities of type IV glandular trichomes to the traits type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs.

Estimated contrasts	Type IV Trichome		Types II+III Trichome		Eggs		Nymphs	
	Contrast	Pr > ChiSq	Contrast	Pr > ChiSq	Contrast	Pr > ChiSq	Contrast	Pr > ChiSq
P1(TOM-684) - P2(LA1401)	-82.30	<.0001	313.05	<.0001	10.40	<.0001	0.60	0.0855
F_2 lowest density (IV) - F_2 highest density (IV)	-94.05	<.0001	136.55	<.0001	4.60	0.0004	0.65	0.0006
P1(TOM-684) - F_1	-4.11	<.0001	106.57	0.0007	4.28	0.2634	0.19	0.7134
P2(LA1401) - F_1	78.19	<.0001	-206.48	<.0001	-6.12	0.0004	-0.41	0.0597
P1(TOM-684) - F_2 lowest density (IV)	0.00	1	119.80	<.0001	6.20	0.0626	0.05	0.1454
P2(LA1401) - F_2 lowest density (IV)	82.30	<.0001	-193.25	<.0001	-4.20	0.0026	-0.55	0.0096
P1(TOM-684) - F_2 highest density (IV)	-94.05	<.0001	256.35	<.0001	10.80	<.0001	0.70	0.0073
P2(LA1401) - F_2 highest density (IV)	-11.75	0.236	-56.70	<.0001	0.40	0.5158	0.10	0.3116

(a): Contrasts using the observed values.

(b): Probability considering the test of likelihood ratio.

F_2 highest density (IV): Average total density of the 20 plants which showed the highest density of type IV glandular trichomes selected in the F_2 population sampled.

F_2 lowest density (IV): Average total density of the 20 plants which showed the lowest density of type IV glandular trichomes selected in the F_2 population sampled.

APPENDIX A

The SAS programs, using the procedure NLMIXED are as follows. The program below fits the Negative Binomial model to trichomes type IV counted in F_1 population. The codes for other populations are similar.

```
proc nlmixed data=tricF1 tech=NRRIDG  qpoints=50;
title 'Type_IV: F1 Negative Binomial';
eta = beta0;
lambda = exp(eta);
ll= lgamma(Type_IV + (1/k)) - lgamma(Type_IV+1) - lgamma(1/k) +
Type_IV*log(k*lambda) - (Type_IV+(1/k))*log(1+k*lambda);
model Type_IV ~ general(ll);
run;
```

The overall effect, beta0, and k estimates are provided as output. From these estimates, the mean and variance are easily obtained using the expressions presented in Section 2.4.1.

The program for the ZIP model is below. In this example, we fit such model to the number of nymphs in F1 population. The estimates of alpha0 and beta0 are provided as output and used in the mean and variance calculation.

```
proc nlmixed data=mtF1 tech=NRRIDG  qpoints=50;
title 'nymphs: Pop total - F1 ZIP';
eta0 = alpha0;
exp_eta0 = exp(eta0);
p0 = exp_eta0 / (1 + exp_eta0);
etap = beta0;
exp_etap = exp(etap);
if nymphs = 0 then ll = log(p0 + (1 - p0) * exp(- xp_etap));
else ll= log(1 - p0) + nymphs * etap - exp_etap - lgamma(nymphs +
1);
model nymphs ~ general(ll);
run;
```

In the associative analysis, the contrasts were estimated through the GENMOD procedure as follows. In the codes, numtrat refers to the covariate (treatment) effect, as specified in Section 2.4.3.

```
proc genmod data=contrast20;
class numtrat;
model Type_IV=numtrat /dist=nb;
contrast 'P1 vs P2' numtrat 1 -1 0 0 0;
contrast 'F2 lowest vs F2 highest' numtrat 0 0 0 1 -1;
contrast 'P1 vs F1' numtrat 1 0 -1 0 0;
contrast 'P2 vs F1' numtrat 0 1 -1 0 0;
contrast 'P1 vs F2 lowest' numtrat 1 0 0 -1 0;
contrast 'P2 vs F2 lowest' numtrat 0 1 0 -1 0;
contrast 'P1 vs F2 highest' numtrat 1 0 0 0 -1;
contrast 'P2 vs F2 highest' numtrat 0 1 0 0 -1;
run;
```

ARTICLE 2: Quantitative trait loci associated with trichomes in the *Solanum galapagense* accession LA1401

ABSTRACT

Pest resistance in *Solanum galapagense* has been associated with the presence of type IV glandular trichomes. Knowledge about the genetic factors involved with the presence of trichomes may assist in the process of gene introgression for development of pest resistant tomato cultivars. In this study, we sought to identify QTLs associated with the presence of type IV trichomes in the F_2 population derived from the interspecific cross of *Solanum lycopersicum* TOM-684 x *Solanum galapagense* accession LA1401. Two QTLs contributing to type IV trichome occurrence and density were detected, one major QTL (gal.IV-2), responsible for 35.22% of phenotypic variation, was located on chromosome 2. The other QTL (gal.IV-3) located on chromosome 3, and explained 23.35% of the phenotypic variation in the F_2 population. These QTLs were responsible for 23.4% and 3.4% of the variation observed in a backcross population. Our results indicate that the inheritance for type IV trichome seems to be not complex, and the QTL gal.IV-2 appears to be the main responsible for the presence of type IV trichomes in *S. galapagense.*

Key words: Single nucleotide polymorphism, Indel markers, Composite Interval Mapping

RESUMO

Em *Solanum galapagense* a resistência a pragas tem sido associada à presença de tricomas glandulares tipo IV. O conhecimento dos fatores genéticos envolvidos na presença desses tricomas podem auxiliar no processo de introgressão gênica para o desenvolvimento de cultivares de tomateiro resistentes a pragas. Nosso estudo objetivou identificar QTLs associados à presença de tricomas tipo IV em população F_2 proveniente do cruzamento interespecífico *Solanum lycopersicum* TOM-684 x *Solanum galapagense* acesso LA1401. Dois QTLs foram detectados contribuindo para presença e densidade de tricomas tipo IV, um QTL de efeito maior (gal.IV-2), responsável por 35.22% da variação fenotípica, foi localizado no cromossomo 2. Outro QTL (gal.IV-3) foi localizado no cromossomo 3 e explicou 23.35% da variação fenotípica na população F_2. Esses QTLs foram responsáveis respectivamente por 26.44% e 3.37% da variação observada no retrocruzamento. Nossos resultados indicam que o tricoma tipo IV possui herança relativamente simples sendo que o QTL gal.IV-2 aparentemente é o principal responsável pela presença de tricomas tipo IV em *S. galapagense.*

Palavras-chave: Single nucleotide polymorphism, marcadores Indel , Composite Interval Mapping.

1 INTRODUCTION

The tomato plant may be attacked by a large number of pests that cause damage to plant and fruits, thus reducing yield. The use of chemical products has been the main way of control; however, their indiscriminate use, as well as the ability of pests to develop resistance, have reduced the efficiency of these products (MUTSCHLER et al., 1996; MOMOTAZ; SCOTT; SCHUSTER, 2010; LUCINI et al., 2015).

A promising alternative for control is the use of pest resistant cultivars (BROEKGAARDEN et al., 2011; LUCATTI et al., 2014). To develop such resistant cultivars, it is first necessary to identify the sources of resistance (BROEKGAARDEN et al., 2011; LUCINI et al., 2015). However, knowledge about the cause and mechanism of resistance can help to improve selection efficiency. Further, an understanding of the underlying genetic basis for pest resistance can lead to augmented selection strategies. Wild tomato species (*S. pennellii, S. habrochaites, S. pimpinellifolium, S. galapagense*) are reported sources of resistance to pests such as whitefly (*Bemisia* ssp.), South American tomato pinworm (*Tuta absoluta*), spider mites (*Tetranychus urticae*), among others (MALUF; CAMPOS; CARDOSO, 2001; MUIGAI et al., 2003; MACIEL et al., 2011; FIRDAUS et al., 2012; LUCATTI et al., 2013; 2014; LUCINI et al., 2015).

In wild species, the mechanism of resistance has often been related to the presence of glandular trichomes and to their association with production of allelochemicals (GOFFREDA et al., 1989; MALUF; CAMPOS; CARDOSO, 2001; SIMMONS; GURR, 2005; MALUF et al., 2007). Trichomes are categorized in seven types (type I to type VII) and classified as either glandular (types I, IV, VI, and VII) or non-glandular (types II, III, and V) (LUCKWILL, 1943). In *S. galapagense, S. pennellii,* and *S. pimpinellifolium*, a high density of type IV trichomes is also associated with the synthesis of acylsugars (GOFFREDA et al., 1989, RODRÍGUEZ-LÓPES et al., 2011; FIRDAUS et al., 2013; LUCINI et al., 2015). The acylsugar and trichomes cause negative effects on arthropod pests, including increased mortality, reduction in oviposition and reduced adult survival (SIMMONS et al., 2004; SIMMONS; GURR, 2005).

Knowledge about the genetic factors involved with the presence of trichomes and their role in pest resistance is important in order to facilitate and ensure the success of introgression of this resistance in the cultivated tomato plant. Studies have been undertaken in order to identify the genomic regions, which are associated with the presence of trichomes. QTLs associated with the density of type IV glandular trichomes have been identified for the species *S. pennelli* (accession LA0716), *S. galapagense* (accession PRI95004), and *S. habrochaites* (accessions CGN1.1561 and LA1777) (MALIEPAARD et al., 1995; BLAUTH; CHURCHILL; MUTSCHLER et al., 1998; MOMOTAZ; SCOTT; SCHUSTER, 2010; FIRDAUS et al., 2013; LUCATTI et al., 2014). Firdaus et al. (2013), evaluating populations derived from the accession PRI95004 (*S. galapagense*), identified two QTLs associated with resistance to whitefly and type IV glandular trichome. One major QTL (Wf-1) on chromosome 2 and a minor QTL (Wf-2) on chromosome 9. In addition, they suggest that inheritance for presence of type IV trichomes is relatively simple, which favors their use in breeding programs.

The species *S. galapagense* has high resistance to whitefly, associated with the presence of type IV trichomes, and it constitutes an important source of resistance to be used in breeding programs (LUCATTI et al., 2013). However, not all the accessions of *S. galapagense* have the same performance in relation to pest resistance, especially to whitefly, as shown by Firdaus et al. (2012); and Lucatti et al. (2013), uncovering the need for more in-depth studies with different accessions. Among the accessions of *S. galapagense* evaluated by

Lucatti et al. (2013), LA1401 is among the accessions with high level of resistance to whitefly accompanied by high density of type IV trichomes as well as high acylsugar accumulation. Jouy, Bordat and Bessiere (1992) reported resistance of this accession to *Liriomyza trifolii* (leafminer), and they associated this resistance with production of acylsugars and glandular trichomes. The accession LA1401 has also been described as a source of resistance to salinity and high soluble solids content in fruits (MAHMOUD; JONES; EL-BELTAGY, 1986; SHANNON; WRONA, 1992; RAZDAN; MATTOO, 2006).

Studies with the purpose of identifying genomic regions associated with the presence of type IV glandular trichomes and their relation to pest resistance would, in different *S. galapagense* accessions, assist in clarification of this resistance mechanism. In this study, we sought to identify QTLs associated with type IV glandular trichomes in a population derived from the interspecific cross between *S. lycopersicum* and *S. galapagense* accession LA1401.

2 MATERIALS AND METHODS

This study was developed through a partnership among the Universidade Federal de Lavras (UFLA) in Lavras, Hortiagro Sementes S.A. seed company in Ijaci, both in the State of Minas Gerais, Brazil, and The Ohio State University - Ohio Agricultural Research and Development Center (OSU-OARDC), United Sates. Phenotypic data were collected at UFLA/Hortiagro, while genotype data collection and subsequent analyses were performed at the OSU-OARDC.

2.1 Plant material

Segregating populations were developed for the study described. The parents used to develop these populations were *S. galapagense* (LA1401) and *S. lycopersicum* (TOM-684). LA1401 is a wild accession characterized by a high level of acylsugars, the presence of type IV trichomes, and resistance to insects (JOUY, BORDAT; BESSIERE, 1992, LUCATTI et al., 2013). Previous studies at UFLA/Hortiagro demonstrated resistance of LA1401 to the silverleaf whitefly *Bemisia tabaci* biotype B (= *Bemisia argentifolii*) (data not shown). TOM-684 (susceptible to insects) is a proprietary fresh-market tomato inbred line from Hortiagro Sementes S.A. A cross was initially obtained between the line TOM-684 (female parent) and the accession LA1401 (male parent), thus obtaining the F_1 generation. F_1 plants were self-pollinated to obtain the F_2 generation and backcrossed with the parental accession TOM-684 to obtain the backcross BC_1 [=(F_1 x TOM-684)]. Segregating progeny and parental accessions were phenotyped for the density and type of trichomes (LUCKWILL, 1943). The F_2 population was used to identify QTLs associated with trichomes, and the F_1BC_{11} backcross population was used to validate these QTLs. Tomato plants were grown in a greenhouse in a completely randomized design, with parents included as replicated checks. Experiments consisted of 20 plants from each parental line, 20 plants from the F_1, 140 individuals from the F_2. For the backcross120 individuals were included.

2.2 Identification and quantification of trichomes

When tomato plants were three months old, the fully-expanded fourth leaf from each plant was collected and stored in 70% ethanol in a beaker for 48 hours. After storage, paradermal sections were prepared from the abaxial and adaxial surfaces of leaflets. The sections were cleared in 50% sodium hypochlorite for 20 seconds and washed three times in water to remove the excess hypochlorite. After that, sections were placed in 0.1% safranin dye for 20 seconds and then washed twice in water to remove the excess of dye. Six paradermal sections per surface were used per plant to make slides. Semi-permanent slides were prepared as mounts in glycerinated water (FREITAS et al. 2002).

The slides were viewed under a 10x optical microscope (Carl Zeiss, model Axil l) with an attached camera (AxionCam ERc5s). Images were captured using the Axio Vision Rel. 4.8 program and analyzed using the Image Tool v. 3.00 software (WILCOX et al. 2002). Trichome densities were quantified (number of trichomes per mm^2) on abaxial and adaxial surfaces of the leaflets for each of the trichome types. Trichome types were identified and classified based on Luckwill (1943).

2.3 Phenotypic analysis

Means, standard errors, and broad-sense heritabilities were calculated for each type of trichome. The total number of trichomes within each trichome type were obtained by the sum of abaxial and adaxial trichomes. Pearson correlation coefficients among densities of trichome types were calculated. All analyses were performed in R 3.1.1 (R CORE TEAM, 2014). Broad-sense heritabilities were estimated using the ratio of genotypic to phenotypic variances. Phenotypic (P), environmental (E), and genotypic (G) variances were estimated from generation variances as follows (WRIGHT, 1968):

$$V\,(P) = VF_2$$

$$V\,(E) = \frac{VP_1 + VP_2 + (2 \times VF_1)}{4}$$

$$V\,(G) = V(P) - V(E)$$

2.4 Genotyping

The DNA, from the F_2 and parent (LA1401 and TOM-684) plants, was extracted from young leaves using the CTAB procedure (FERREIRA; GRATAPAGLIA, 1998). The DNA was resuspended in TE (1 mM Tris to 0.1 mM EDTA) and sent to the Horticulture and Crop Science Department at the OSU-OARDC/USA. DNA quality was checked using a Nano drop and by running samples on a 1% agarose gel and visually inspecting the ethidium bromide stained aliquots. The concentration was adjusted to 50 ng/µl, and 20 µl of each sample was used for genotyping. A total of 384 SNPs (Supplementary Table 1) previously optimized for fresh-market tomatoes, based on the SolCAP Infinium SNP Array (SIM et al., 2012), were used for genotyping. Genotyping was carried out using the *Kompetitive Allele* Specific (KASP) assay (LGC Genomics, United States).

2.5 Genetic map and QTL identification

A genetic map was developed using the polymorphic SNPs. Analyses were performed using r/QTL 1.36-6 (BROMAN et al., 2003). The SNPs that showed distorted segregation patterns based on a chi-square test with the threshold at 5% and/or more than 10% missing data, were removed. The linkage groups were built based on maximum recombination frequency of 0.35 and minimum LOD score 4. Map quality was verified through reference to the physical map (SIM et al., 2012).

The association between trichome type and density was established using Simple Interval Mapping (SIM) and Composite Interval Mapping (CIM). Phenotypic distributions of trichome density were non-normal and, thus, several approaches were conducted to establish marker linkage to QTL. In SIM, both parametric and non-parametric models were considered. Significant thresholds for each trait were determined by permutation tests at the 5% level with 1000 replicates each.

Single marker-trait regression and Kruskal-Wallis tests were used to confirm SNP markers that were significantly associated in the interval mapping. In addition, these analyses provided an estimate of how much of phenotypic variation was explained by each QTL, as well as the proportion of additive or dominance contribution to variances. Interaction analyses between significant SNPs were conducted to explore the potential interaction (epistatic) effects between QTLs. Analyses were performed using R 3.1.1(R CORE TEAM, 2014). The genetic linkage map, physical map, and QTL maps were drawn using Mapchart 2.3 (VOORRIPS, 2002).

2.6 Validation of QTLs in backcross population

Indel Markers were developed for validation of QTLs in a backcross population [BC_1 =(F_1 x TOM-684)] with 120 individuals. Genome sequence data were used to find Indels which segregated in the crosses. Tomato now has extensive sequence data available for approximately 440 genomes (AFLITOS et al., 2014; LIN et al., 2014). Among the 85 unique genomes available from the high-quality data generated through the 100 Tomato Genome Sequencing Consortium (AFLITOS et al., 2014), there are three Galapagos Island accessions

[LA1401 (*S. galapagense*), LA0483 and LA1044 (*S. cheesmaniae*)] and seven fresh-market accessions with descriptors similar to TOM-684 [EA00465, CGN20815, LYC 1410, TR00022, N020212, LYC 1365 (all *S. lycopersicum*), and PI 406760 (*S. lycopersicum var. cerasiforme*)]. The DNA sequences for these accessions were used to identify new markers near QTLs identified with SNPs.

Among the Galapagos accessions, LA1401 (*S. galapagense*) was a direct parent in our study. DNA sequences, of approximately 15 Kp inside the QTL regions identified, were obtained from the reference Genome Browser (SGN - FERNANDEZ-POZO et al., 2015). We then obtained matching sequences from the three Galapagos accessions and seven *S. lycopersicum* varieties using a custom BASH script which uses BLAST to identify top hits in FASTA formatted sequences and extracts of the top match. Sequences were then aligned using MUSCLE (EDGAR, 2004). Indel Markers were identified by visual inspection of the resulting alignment. The criteria for identifying these markers were find Indel which cluster S. galapagense separately from all the S. lycopersicum accession and also were at least 10 bp of length.. Flanking primers were designed using Primer3 (UNTERGRASSER et al., 2012).

DNA extraction was performed for the backcross population and the parental accessions as previously described. Polymerase Chain Reaction (PCR) conditions were standardized to INDELS markers and included denaturation at 94°C for 45 s, annealing at 56°C for 45 s, and elongation at 72°C for 45 s, repeated for 40 cycles. PCR products were separated on 4% agarose gel at 180 V run for 4 hours. Confirmation of QTL was based on regression between Indel marker genotypes and phenotypic data on trichomes obtained as previously described.

3 RESULTS

Type, density, and correlation among trichomes

The types and densities of trichomes found in the population derived from the cross between *S. lycopersicum* and *S. galapagense* are shown in Table 1. The parent LA1401 (*S. galapagense*) had high density of the type IV glandular trichomes. The type I glandular trichomes, as well as the type II+III non-glandular trichomes, are present in LA1401, however, at low densities with count values near zero. The susceptible parent *S. lycopersicum* (TOM-684) has high density of type II+III non-glandular trichomes and does not have either type I or IV glandular trichomes. Type V non-glandular trichomes are present at similar densities in both parents. Type I, VI, and VII glandular trichomes occurred at low density in the parents (when present) and in the F_1 and F_2 populations; therefore, they were not used for identification of QTLs (Table 1).

For type IV trichomes, heritability was 0.85 and the F_2 population had a mean of 7.0 trichomes per mm^2 and range from 0 to 51.33 trichomes per mm^2, with some individual plants at a density greater than the mean of the parent LA1401 (Table 1). High heritability was also found for type II+III non-glandular trichomes.

There was a high negative correlation between the type IV glandular trichome and type II+III non-glandular trichomes. A significant positive correlation was found between the type I and IV glandular trichomes, and both are inherited from the wild parent LA1401. The type V non-glandular trichomes, present in both parents, did not show significant correlation with any of the other trichomes (Table 2).

Linkage map

The genetic map was constructed based on 161 SNPs polymorphic for the F_2 population, consisting of 103 individuals, and it had a total length of 950.0 cM (Fig. 1). Thirteen linkage groups were formed with chromosome 1 represented by two linkage groups (1a and 1b). The physical position of each SNP was obtained (SIM et al., 2012) according to tomato genome assembly SL2.40 from the Solanaceae Genome Network (SGN, solgenomics.net).

Trichome QTLs

QTLs for the type IV glandular trichomes, and type V and type II+III non-glandular trichomes were identified through analyses of Composite Interval Mapping, Simple Interval Mapping, Single Marker-Trait Regression, and the Kruskal-Wallis test (Table 3). The QTLs detected using different techniques for analyses were in agreement. It shows even though phenotypic distributions of trichome density were non-normal, all the approaches applied (parametric and non-parametric models) identified the same QTLs.

For the type IV trichomes, two QTLs were identified: one major QTL, called gal.IV-2, located on chromosome 2, responsible for 35.22% of phenotypic variation. A second QTL, called gal.IV-3, is located on chromosome 3 and explained 23.35% of the variation.

There was a significant difference between homozygous and heterozygous individuals for both QTLs (gal.IV-2 and gal.IV-3) (Fig. 3). Individuals that are homozygous for the alleles from *S. galapagense* have high density of type IV trichomes, whereas the homozygotes for the parent *S. lycopersicum* lack the type IV trichomes. Heterozygotes have type IV trichomes, but at a lower density than homozygotes for the *S. galapagense* alleles (Fig. 3).

The additive effects for both QTLs were of greater magnitude than the effects of dominance (Table 3). The negative values and the magnitudes of the effects of dominance (Table 3) associated with the average density observed for the parents and populations for type IV trichome (Table 1) are indicative that the alleles for high density of type IV trichomes may be incompletely recessive.

Significant interaction was found between the QTL gal.IV-2 and QTL gal.IV-3 and it is responsible for 53.68% of phenotypic variation (Table 3). The interaction occurred in the direction of increasing the density of type IV trichomes for the individuals that are (a) homozygotes for the allele of the *S. galapagense* parent for both QTLs (gal^2/gal^2 – gal^3/gal^3), (b) heterozygotes for QTL gal.IV-2 and homozygotes for parent *S. galapagense* QTL gal.IV-3 (gal^2/lyc^2 – gal^3/gal^3), (c) homozygotes for parent *S. galapagense* QTL gal.IV-2 and heterozygotes for QTL gal.IV-3 (gal^2/gal^2 – gal^3/lyc^3), and (d) heterozygotes for both the QTLs (gal^2/lyc^2 – gal^3/lyc^3) (Fig. 4).

The QTLs gal.IV-2 and gal.IV-3 were also associated with type II+III non-glandular trichomes, but with lower values of the LOD score in Composite Interval Mapping (5.52 and 2.93, respectively). These QTLs were responsible for a lower phenotypic variation for this type of trichome (Table 3). At the Fig. 2 it is possible to verify the significance of QTL gal.IV-2 and QTL gal.IV-3 for both type IV glandular trichome and type II+III non-glandular trichomes.

Two other minor QTLs were detected for type II+III trichomes, one located on chromosome 4 and the other on chromosome 5. These QTLs were responsible for 10.46% and 6% of phenotypic variation, respectively (Table 3). One QTL on chromosome 10 was detected for the type V non-glandular trichome.

Validation of QTL trichome type IV in backcross population

As a validation strategy, InDel markers were developed for regions within the LOD confidence interval for the QTLs gal.IV-2 and gal.IV-3. Primers are described in Table 4. The presence of both QTLs was confirmed in the backcross population [(*S. lycopersicum* x *S. galapagense*) x *S. lycopersicum*] for type IV trichomes, but with a lesser effect than that found in the F_2 population. The QTL gal.IV-2 explained 26.44% of phenotypic variation, whereas the QTL gal.IV-3 explained only 3.37% of the variation in BC_1 progeny (Table 5).

Just as in the F_2 population, in the backcross population there was significant interaction between the QTLs gal.IV-2 and gal.IV-3 (Fig. 5). The density of type IV trichomes is increased when the individual is heterozygous for both QTLs (gal^2/lyc^2 – gal^3/lyc^3). In homozygous individuals for the cultivated allele at QTL gal.IV-2 (lyc^2/lyc^2), the presence of the QTL gal^3 was ineffective, and the presence of type IV trichomes was practically suppressed, just as it occurs in the parent *S. lycopersicum* (Fig. 5).

4 DISCUSSION

The trichomes found in the greatest density in *S. galapagense*, accession LA1401, were the type IV glandular trichome (Table 1). The accession LA1401 is considered resistant to whitefly, and this resistance is associated with the density of type IV trichomes and with the accumulation of high levels of acylsugars (LUCATTI et al., 2013). Resistance of the accession LA1401 to whitefly (*Bemisia argentifolii*) was also found in one of our previous studies, with a significant association between type IV trichomes and resistance to whitefly (data not shown). Various studies of different wild species of tomato have reported the importance of the presence of the type IV trichomes and an association with allelochemical content that confers pest resistance (GOFFREDA et al., 1989; MUIGAI et al., 2002; SIMMONS; GURR, 2005; FIRDAUS et al., 2012; LUCATTI et al., 2013). In our populations, the type IV trichome density showed high heritability, an indication that this trait may be easily selected and introgressed. High heritability for type IV trichomes has previously been reported for *S. galapagense* and other wild tomato species (MALIEPAARD et al., 1995; FREITAS et al., 2002; FIRDAUS et al., 2013).

QTL on chromosome 2 (gal.IV-2) and on chromosome 3 (gal.IV-3) were associated with the type IV trichome densities. In the F_2 population, these QTLs played an important role for the occurrence of these trichomes. Individuals homozygous for the alleles of the susceptible parent TOM-684 (*S. lycopersicum*) do not have type IV trichomes, whereas individuals homozygous for the alleles of the resistant parent LA1401 (*S. galapagense*) have trichome density similar to the parent LA1401 (Fig. 3). Partial dominance action in the sense of lower density of type IV glandular trichomes was found for both QTLs, which explains the fact of the number of trichomes in the heterozygotes are less than the number of trichomes found in the homozygous individuals with the alleles from the parent LA1401.

In *S. galapagense*, accession PRI195004, a major QTL (Wf-1) on chromosome 2 and another minor QTL on chromosome 9 (Wf-2) were associated with resistance to whitefly, with density of type IV trichomes, and with production of acylsugars (FIRDAUS et al., 2013). These authors showed that the QTL Wf-1 has an effect on adult survival and the presence of type IV trichomes. Whiteflies raised on F_2 plants homozygous for the QTL from *S. galapagense* (Wf-1*gal*) exhibited a low rate of adult survival, just as observed for the parent *S. galapagense* PRI195004. The markers used to identify QTL Wf-1 lie approximately between 47.35 and 49.45 Mb, a region near the QTL gal.IV-2 found in our population for the presence of type IV trichomes (45.67 to

46.99 Mb) (SL2.40 - SGN - Fernandez-Pozo et al., 2015). The identification of these QTLs (Wf-1 and gal.IV-2) emphasizes the importance of this region on chromosome 2 for the presence of genes controlling type IV trichome presence in *S. galapagense*. The lack of overlap of the physical regions identified for Wf-1 and gal.IV-2 may be due to marker choice and inexact mapping of QTL position. These QTLs may be co-located and perhaps represent the same locus responsible for providing resistance to whitefly and the presence of type IV trichomes inherited from *S. galapagense*. Firdaus et al. (2013) also detected the presence of a QTL (Wf-2) on chromosome 9 associated with adult survival and the presence of type IV trichomes; however, the presence of this QTL was not confirmed by these authors in the F_3 population. No QTL associated with trichomes was detected on chromosome 9 in our F_2 population derived from the accession LA1401. Nevertheless, we identified the QTL gal.IV-3 on chromosome 3, a locus not detected in *S. galapagense* accession PRI195004 by Firdaus et al. (2013). These results may indicate the presence of different modifier genes in different accessions of *S. galapagense*.

The QTLs found for type IV trichome density in *S. galapagense* appear to be different from those found in other wild species of tomato. In *S. habrochaites*, Maliepaard et al. (1995) identified, in the accession CGN1.1561, a QTL on chromosome 5 (*TriIV*) and another on chromosome 9 *(TriIV*), located in a different region from the QTL Wf-2 identified by Firdaus et al. (2013). For the accession LA1777 (*S. habrochaites*), QTLs on chromosomes 9, 10, and 11 were associated with type IV trichome density and with the oviposition rate of whitefly (MOMOTAZ et al., 2010). In *S. pennelli*, seven QTLs, located on chromosomes 2, 4, 5, 6, 7, 10, and 11, were associated with the density of type IV trichomes and with production of acylsugars (BLAUTH; CHURCHILL; MUTSCHLER, 1998).

The significant interaction between the QTLs gal.IV-2 and gal.IV-3 suggests that the action of the QTL gal.IV-3 is dependent on QTL gal.IV-2. This interaction indicates that QTL gal.IV-2 is responsible for the presence of type IV trichomes, while QTL gal.IV-3 acts in the sense of increasing the density of type IV trichomes. However, for the QTL gal.IV-3 to express itself, it is necessary that there is at least one allele of the *S. galapagense* parent for the QTL gal.IV-2 (Fig. 4). Thus, individuals that have alleles of the parent *S. galapagense* only for the QTL gal.IV-3, even in the homozygous state, probably would not have type IV trichomes. In contrast, individuals with alleles from *S. galapagense* only at QTL gal.IV-2 had type IV trichomes, even though at low densities. Thus, it may be inferred that there is epistasis in the sense of an increase in the density of type IV trichomes whenever there is simultaneous presence of at least one allele derived from *S. galapagense* (gal^2 and gal^3) in each one of the two loci in question.

The QTLs gal.IV-2 and gal.IV-3 were also associated with type II+III non-glandular trichomes, with a negative correlation between the type IV and the type II+III trichomes. QTL gal.IV-2 was responsible for most of the variation, just as it occurs for the type IV trichome. The co-located QTL for type IV trichome and type III non-glandular trichome was also described by Firdaus et al. (2013). The identification of the same QTLs for type IV glandular trichomes and type II+III non-glandular trichomes, and the negative correlation between these types of trichomes suggest that the loci found may be pleiotropic for the presence of these trichomes in the plants. Thus, in making selection for greater densities of type IV trichomes, one automatically selects for lower density of type II+III trichomes. The negative correlation between the density of type IV and type II+III trichomes, and the reported effect of the loci gal.IV-2 and gal.IV-3 in opposite directions for type IV and type II+III trichomes suggest an ontogenetic relationship between these types of trichomes, where the alleles gal^2 and gal^3 would be involved in the development of glandular structure at the tip of the trichomes.

Two other QTLs, one on chromosome 4 and another on chromosome 5, were detected, indicating that the QTLs on chromosome 2 and 3 are not solely responsible for the presence of type II+III trichomes.

The type V non-glandular trichome was found in accession LA1401 at low density and did not show correlation with any other type of trichome. Simmons and Gurr (2004) also reported the presence of type V trichomes in *S. galapagense*. Different results were found by Firdaus et al. (2013), where type V trichomes were not identified in the accession *S. galapagense* PRI195004; in the F_2 population, they observed negative correlation for the presence of type IV and type V trichomes. The authors showed that the presence of type IV trichomes resulted in a low number of adults surviving and reduced the oviposition rate for whitefly. However, the presence of type IV trichomes combined with the presence of the type V trichomes caused an increase in the number of adults surviving and in the oviposition rate. For the type V non-glandular trichome, only one QTL was detected on chromosome 10 in our population, and no QTL was found to be co-located with the QTLs for type IV and type II+III trichomes.

Confirmation of QTLs for type IV trichomes

The QTLs gal.IV-2 and gal.IV-3 were also detected in the backcross population. The same type of action exhibited by the QTLs in the F_2 population was observed in the backcross progeny, though of lesser magnitude. The QTL gal.IV-2 has a major effect and is responsible for the presence of trichomes, while the QTL gal.IV-3 increases the density of type IV trichomes as long as it is associated with the presence of the QTL gal.IV-2. The markers developed for the QTLs will be able to be used for indirect selection, assisting in the gene introgression process.

The results indicate that the inheritance of type IV trichomes from *S. galapagense* accession LA 1401 is not complex, and appears to be mainly controlled by one major locus with partially dominant action which apparently positively interacts with another minor locus on chromosome 3. Firdaus et al. (2013) also concluded that the presence of type IV trichomes in *S. galapagense* (accession PRI195004) is predominantly controlled by a single QTL and that this locus perhaps interacts with other genes. Similar results were found in *S. habrochaites*, for which Freitas et al. (2002) reported that the density of type IV trichomes was predominantly controlled by a major locus and that there was evidence of the action of another epistatic locus.

Our study confirms that the QTL identified on chromosome 2 is the main locus responsible for the presence of type IV trichomes. In *S. galapagense*, only studies relating the action of the type IV trichomes to resistance against whitefly have been carried out. However, based on studies on other wild tomato species, in which glandular trichomes, especially those of type IV, are associated with resistance to pests such as whitefly, mites, and aphids (GOFFREDA et al., 1989; MALUF; CAMPOS; CARDOSO, 2001; FREITAS et al., 2002; MUIGAI et al., 2003; LUCATTI et al., 2013; LUCINI et al., 2015), it may be expected that the mechanism of resistance present in the type IV trichomes in *S. galapagense* may also act on other pests.

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Table 1: Mean of trichome types in populations derived from the interspecific cross of *S. lycopersicum* with *S. galapagense*.

Population	**Glandular**				**Non-glandular**	
	Type I	**Type IV**	**Type VI**	**Type VII**	**Type II + III**	**Type V**
P1 (*S. lycopersicum*)	0 ± 0	0 ± 0	0.05 ± 0.03	0.97 ± 0.19	104.28 ± 4.8	4.53 ± 0.52
P2 (*S. galapagense*)	0.03 ± 0.02	27.43 ± 1.90	0.08 ± 0.06	0.17 ± 0.07	0.28 ± 0.10	5.37 ± 0.47
F_1	0.04 ± 0.02	1.38 ± 0.36	0.07 ± 0.03	0.35 ± 0.11	69.11 ± 2.50	3.22 ± 0.37
F_2	0.10 ± 0.02	7.00 ± 0.94	0.10 ± 0.02	0.44 ± 0.04	50.12 ± 2.25	4.66 ± 0.23
F_2 variation amplitude	0 - 2	0 - 51.33	0 - 2.33	0 - 2.33	0 - 144.67	0 - 16.67
Heritability	0.90	0.85	0.64	-0.02	0.77	0.52

Mean (followed by standard error of mean) and heritability of trichome types to parents, F_1, F_2.
Variation amplitude in F_2 population.

Table 2: Correlation between densities of trichome types.

	Type I	**Type IV**	**Type VI**	**Type VII**	**Type V**
Type IV	0.19*				
Type VI	0.09	-0.07			
Type VII	-0.07	0.00	0.06		
Type V	-0.06	0.07	0.09	0.01	
Type II + III	-0.10	-0.56***	0.05	-0.08	-0.14

*** Significant correlation at the 0.00 level.
*Significant correlation at the 0.05 level.

Table 3: SNPs associated with trichome density based on Composite Interval Mapping Simple Interval Mapping, single marker-trait regression, and Kruskal-Wallis analyses in the F_2 mapping population derived from the interspecific cross of *S. lycopersicum* with *S. galapagense*.

Trichomes	QTL Analysis										
	QTL location	Composite interval mapping		SNPs associated	Simple Interval Mapping			Single marker-trait regression			Kruskal-Wallis
		Position (cM)	LOD (peak)		Position (cM)	LOD score	F-value	% Expl. (R^2)	Additive	Dominance	chi.squared
Type II+III	chr#2 (gal.IV-2)	97.2	5.52	solcap_snp_sl_10796	92.09	4.32	10.57***	15.81%	-14.68	4.84	19.90***
				solcap_snp_sl_36224	97.2	4.95	13.19***	19.30%	-16.18	3.56	22.81***
	chr#3 (gal.IV-3)	91	2.93	CL017416.0406	98.11	3.4	6.71***	10.07%	-11.97	3.27	15.70***
	chr#4	0	3.2	solcap_snp_sl_9856	0	2.49	6.89***	10.46%	-11.76	4.06	11.23***
	chr#5	73	4.31	X2719_1_118_b	76.35	1.51	4.2*	6.00%	-8.44	-8.64	6.95*
Type IV	chr#2 (gal.IV-2)	96	9.08	solcap_snp_sl_10796	92.09	7.68	26.76***	33.57%	8.95	-4.62	35.37***
				solcap_snp_sl_36224	97.2	9.02	28.73***	35.22%	9.27	-3.99	41.57***
	chr#3 (gal.IV-3)	91	7.43	CL017416.0406	98.11	5.48	16.44***	23.35%	7.55	-2.73	25.25***
Type V	chr#10	12	5.89	solcap_snp_sl_46386	7.17	4.39	14.16***	20.67%	1.48	-1.86	20.07***
				solcap_snp_sl_34365	23.87	3.21	8.16***	12.42%	1.46	-1.15	14.74***
				solcap_snp_sl_5191	26.90	3.60	8.41***	12.69%	1.45	-1.06	16.56***
				SGNNAU317657_...	40.19	3.59	8.29***	12.50%	1.24	-1.43	16.50***
QTL Interaction: gal.IV-2 x gal.IV-3							15.77***		53.68%		

Composite interval mapping - the significant LOD score is 3.0, 7.0, and 4.0 for trichomes type II+III, type IV, and type V, respectively; Simple interval mapping - the significant LOD score is 3.0 for trichomes type II+III, type IV, and type V.
*** Significant at the 0.00 level; *Significant at the 0.05 level; %Expl. is percent of variance explained by phenotypic variation.
SGNNAU317657_... is the SNP marker SGNNAU317657_C2_At3g47930_snp417.
QTL Interaction : Interaction effect between the QTL gal.IV-2 and QTL gal.IV-3 for trichome type IV in an F_2 population.

Table 4: InDel markers developed for validation of QTL mapping on chromosome 2 and 3 for type IV trichome.

QTL name	chr	Physical position	InDel Primer	Primer sequence	InDel in *S. gal*	InDel sequence	Fragment size (bp)
gal.IV-2	2	51097140	IND2.3Tri_IV	CGAATTGTGGCCCTCTCTCT ACGGGATCAAAGTGGGAGAC	Insertion of 19 bp	AATTGCCACTAAAACCATA	243 (*S. gal*) 224 (*S. lyc*)
gal.IV-3	3	63224262	IND3.2Tri_IV	TGATCAGTTTAAGACAGTACGGT CCGTCCAATTGCAAATCACA	deletion of 10 bp	TACATTAGAG	117 (*S. gal*) 127 (*S. lyc*)

chr: chromosome.
bp: base pars.
S. gal: *S. galapagense.*
S. lyc: S. lycopersicum.

Table 5: Single marker-trait regression and Kruskal-Wallis test for validation of QTL mapping of type IV trichome density on chromosome 2 and 3 in the backcross population.

QTL	chromosome	Single marker-trait regression		Kruskal-Wallis
		F-value	% Expl. (R^2)	chi.squared
gal.IV-2	2	37.66***	26.44%	45.97***
gal.IV-3	3	4.60*	3.37%	4.06*

*** Significant at the 0.00 level.
*Significant at the 0.05 level.
%Expl. is percentage of variance explained by phenotypic variation.

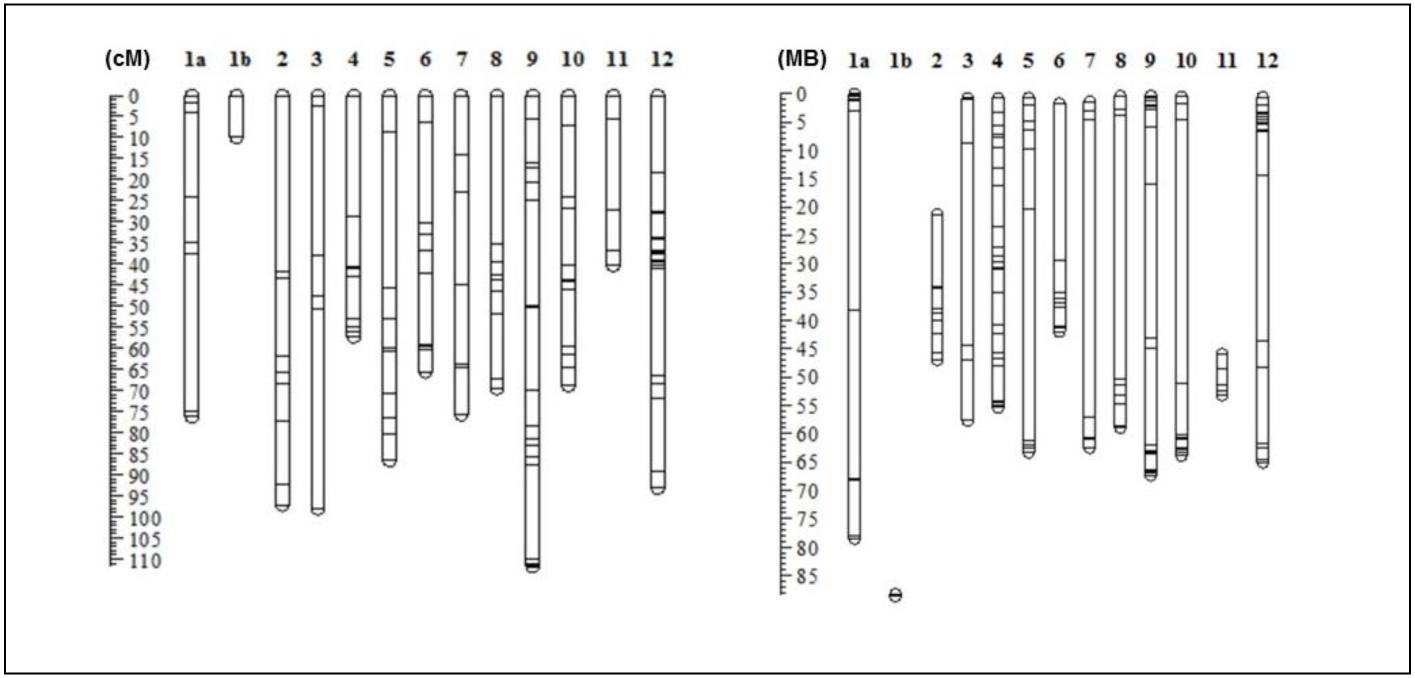

Fig. 1: Genetic map based on an F_2 population (*S. lycopersicum* x *S. galapagense*) and built using 161 SNP markers in the r/qtl - R program (left). Physical map based on physical map location of the SNPs in the tomato database at SGN (SL2.40) (Sim et al. 2012) (right).

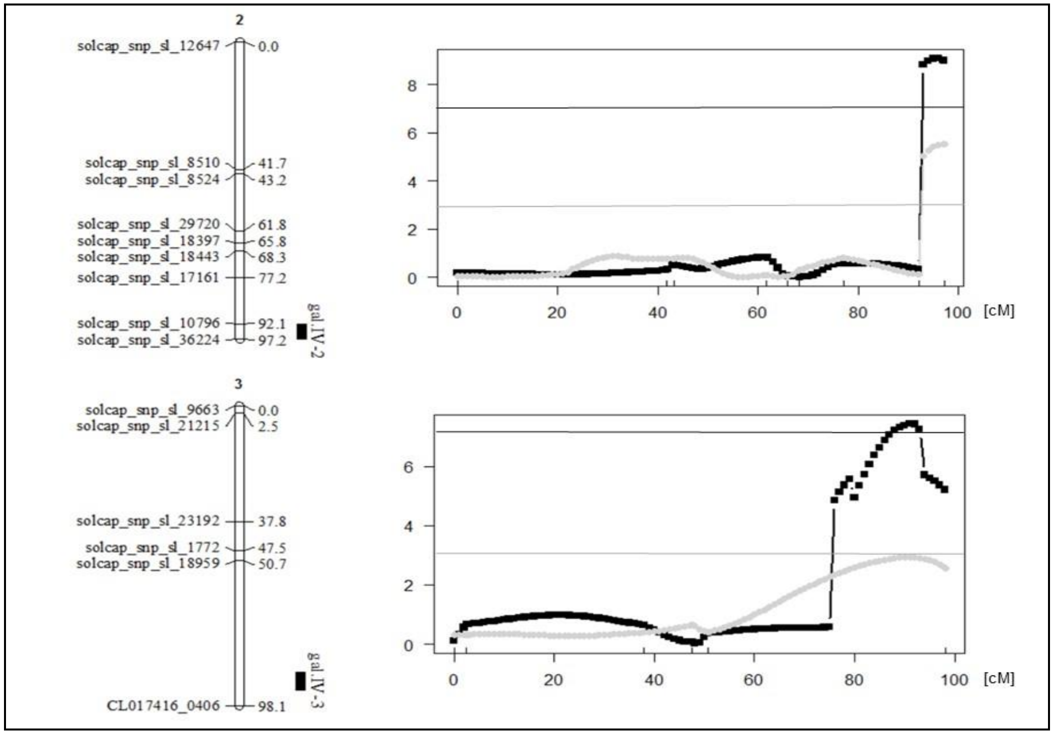

Fig. 2: Interval mapping of QTL affecting Type IV and Type II+III trichomes on chromosome 2 (upper) and 3 (lower). The schematic chromosome shows the name and the genetic distance in centiMorgan of the SNP markers and the position of the QTL. The Composite Interval Mapping graphs show the significant QTL for Type IV (black) and Type II+III (gray) trichomes on chromosome 2 (upper) and chromosome 3 (lower). Significant LOD score (y-axis) is 7.0 for trichome type IV and 3.0 for trichome type II+III.

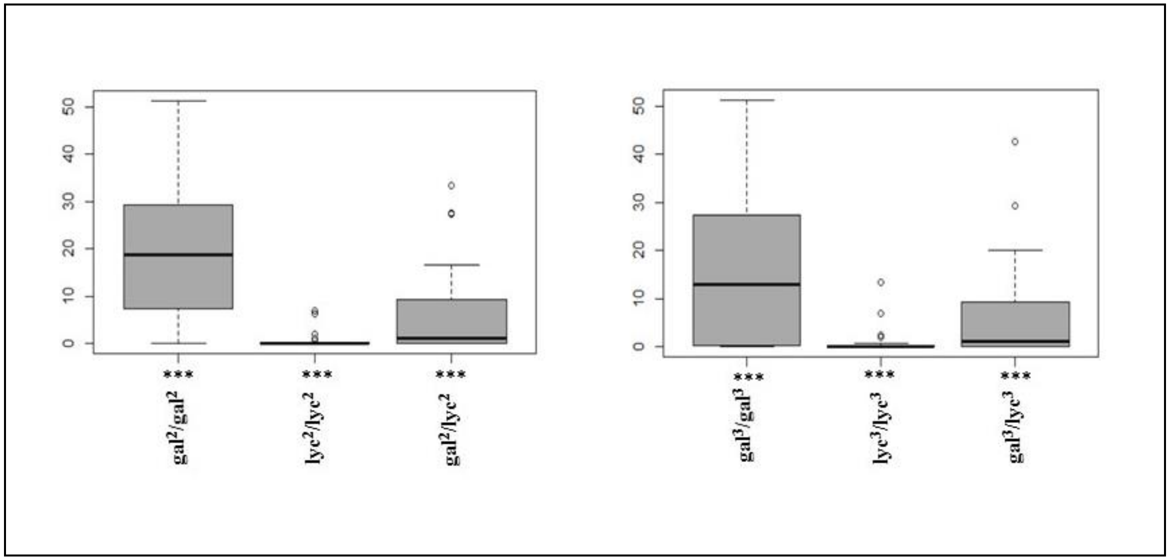

Fig. 3: Type IV trichome density for homozygous and heterozygous genotypes for QTL gal.IV-2 on chromosome 2 (right) and QTL gal.IV-3 on chromosome 3 (left).

The allele gal^2 is from *S. galapagense* for QTL on chromosome 2; lyc^2 is the *S. lycopersicum* allele for QTL at chromosome 2; gal^3 is the *S. galapagense* allele for QTL on chromosome 3; lyc^3 is the *S. lycopersicum* allele for QTL at chromosome 3.

*** Significant at the 0.00 level.

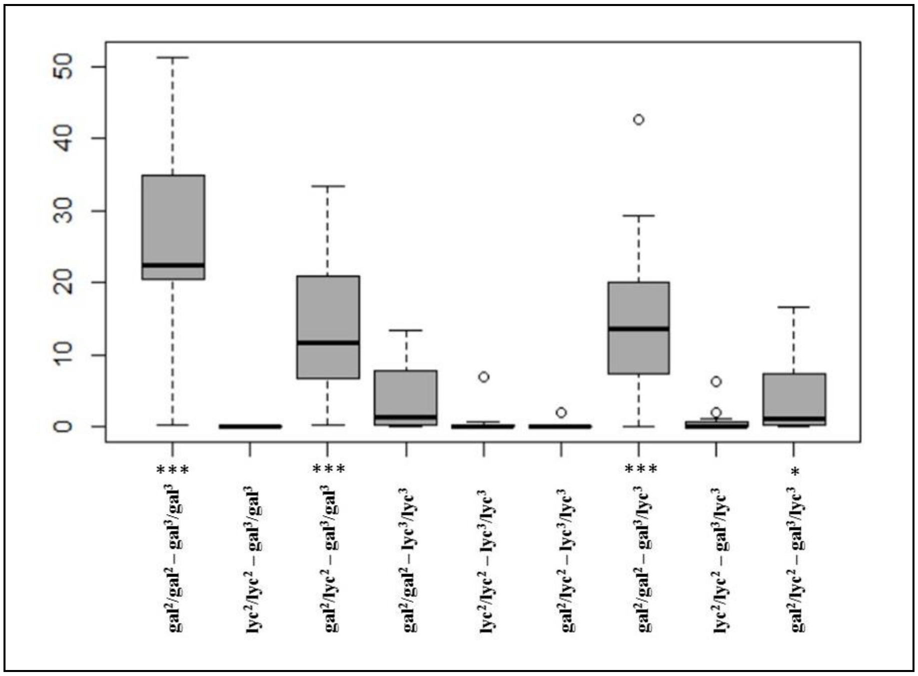

Fig 4: Interaction effect between the QTL gal.IV-2 (chromosome 2) and QTL gal.IV-3 (chromosome 3) for type IV trichome density in the F_2 population (*S. lycopersicum x S. galapagense*).

The allele gal^2 is the *S. galapagense* QTL on chromosome 2; lyc^2 is the *S. lycopersicum* allele for QTL on chromosome 2; gal^3 is the *S. galapagense* allele for QTL on chromosome 3; lyc^3 is the *S. lycopersicum* allele for QTL on chromosome 3.

*** Significant interaction at the 0.00 level.

* Significant interaction at the 0.05 level.

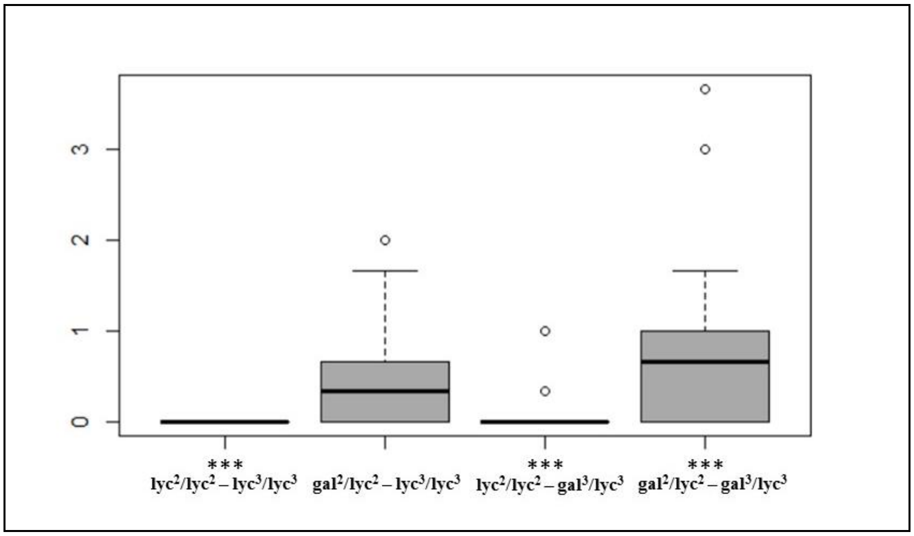

Fig 5: Interaction effect between the QTL gal.IV-2 (chromosome 2) and QTL gal.IV-3 (chromosome 3) for type IV trichome density in a backcross population [(*S. galapagense x S. lycopersicum*) x *S. lycopersicum*].

gal^2 is the *S. galapagense* allele for QTL at chromosome 2; lyc^2 is the *S. lycopersicum* allele for QTL at chromosome 2; gal^3 is the *S. galapagense* allele for QTL at chromosome 3; lyc^3 is the *S. lycopersicum* allele for QTL at chromosome 3.

*** Significant interaction at the 0.00 level.

Supplementary Table 1: 384 SNPs used for genotyping previously optimized for fresh-market tomatoes, based on the SolCAP Infinium SNP Array (SIM et al., 2012) (continua)

n^O	**SNP name**	**Chr**	**n^O**	**SNP name**	**Chr**
1	solcap_snp_sl_15058	1	**193**	solcap_snp_sl_15515	6
2	solcap_snp_sl_15051	1	**194**	solcap_snp_sl_65677	6
3	solcap_snp_sl_60559	1	**195**	solcap_snp_sl_25160	6
4	solcap_snp_sl_60557	1	**196**	solcap_snp_sl_11281	6
5	solcap_snp_sl_15046	1	**197**	solcap_snp_sl_2622	6
6	solcap_snp_sl_15039	1	**198**	solcap_snp_sl_2629	6
7	solcap_snp_sl_33745	1	**199**	solcap_snp_sl_14458	6
8	solcap_snp_sl_60417	1	**200**	solcap_snp_sl_1337	6
9	solcap_snp_sl_60360	1	**201**	solcap_snp_sl_12638	6
10	solcap_snp_sl_8697	1	**202**	solcap_snp_sl_12749	6
11	solcap_snp_sl_33701	1	**203**	solcap_snp_sl_12757	6
12	solcap_snp_sl_8669	1	**204**	solcap_snp_sl_27197	6
13	solcap_snp_sl_100164	1	**205**	solcap_snp_sl_17019	6
14	solcap_snp_sl_15013	1	**206**	solcap_snp_sl_24454	6
15	solcap_snp_sl_60078	1	**207**	solcap_snp_sl_101067	6
16	solcap_snp_sl_15000	1	**208**	solcap_snp_sl_24440	6
17	solcap_snp_sl_20440	1	**209**	solcap_snp_sl_24437	6
18	solcap_snp_sl_59771	1	**210**	solcap_snp_sl_24428	6
19	solcap_snp_sl_14042	1	**211**	solcap_snp_sl_11231	7
20	solcap_snp_sl_18641	1	**212**	solcap_snp_sl_11205	7
21	solcap_snp_sl_13762	1	**213**	solcap_snp_sl_22109	7
22	solcap_snp_sl_18619	1	**214**	solcap_snp_sl_15789	7
23	solcap_snp_sl_4963	1	**215**	solcap_snp_sl_15785	7
24	solcap_snp_sl_531	1	**216**	solcap_snp_sl_15780	7
25	solcap_snp_sl_12826	1	**217**	solcap_snp_sl_11092	7
26	solcap_snp_sl_43632	1	**218**	solcap_snp_sl_22065	7
27	solcap_snp_sl_2234	1	**219**	solcap_snp_sl_15765	7
28	solcap_snp_sl_14323	1	**220**	solcap_snp_sl_12421	7
29	solcap_snp_sl_13404	1	**221**	solcap_snp_sl_13868	7
30	solcap_snp_sl_13399	1	**222**	solcap_snp_sl_10686	7
31	solcap_snp_sl_13398	1	**223**	solcap_snp_sl_5863	7

Table 1, continua

nº	**SNP name**	**Chr**	**nº**	**SNP name**	**Chr**
32	solcap_snp_sl_4283	1	**224**	solcap_snp_sl_5861	7
33	solcap_snp_sl_48121	1	**225**	solcap_snp_sl_6291	7
34	solcap_snp_sl_48097	1	**226**	solcap_snp_sl_7025	7
35	solcap_snp_sl_7410	2	**227**	solcap_snp_sl_37097	7
36	solcap_snp_sl_6255	2	**228**	solcap_snp_sl_101085	7
37	solcap_snp_sl_6196	2	**229**	solcap_snp_sl_7232	8
38	solcap_snp_sl_13842	2	**230**	solcap_snp_sl_19759	8
39	solcap_snp_sl_12647	2	**231**	solcap_snp_sl_7305	8
40	solcap_snp_sl_12372	2	**232**	solcap_snp_sl_14530	8
41	solcap_snp_sl_15693	2	**233**	solcap_snp_sl_7386	8
42	solcap_snp_sl_15688	2	**234**	solcap_snp_sl_7388	8
43	solcap_snp_sl_20344	2	**235**	solcap_snp_sl_13899	8
44	solcap_snp_sl_8464	2	**236**	solcap_snp_sl_11588	8
45	solcap_snp_sl_8505	2	**237**	solcap_snp_sl_13725	8
46	solcap_snp_sl_8510	2	**238**	solcap_snp_sl_4431	8
47	solcap_snp_sl_8524	2	**239**	solcap_snp_sl_13464	8
48	solcap_snp_sl_15574	2	**240**	solcap_snp_sl_13458	8
49	solcap_snp_sl_23850	2	**241**	solcap_snp_sl_4374	8
50	solcap_snp_sl_13625	2	**242**	solcap_snp_sl_48426	8
51	solcap_snp_sl_29720	2	**243**	solcap_snp_sl_48414	8
52	solcap_snp_sl_18397	2	**244**	solcap_snp_sl_21394	8
53	solcap_snp_sl_18443	2	**245**	solcap_snp_sl_21461	8
54	solcap_snp_sl_18519	2	**246**	solcap_snp_sl_34761	8
55	solcap_snp_sl_17161	2	**247**	solcap_snp_sl_21473	8
56	solcap_snp_sl_10796	2	**248**	solcap_snp_sl_25111	8
57	solcap_snp_sl_36224	2	**249**	solcap_snp_sl_15432	8
58	solcap_snp_sl_36192	2	**250**	solcap_snp_sl_10246	8
59	solcap_snp_sl_21862	2	**251**	solcap_snp_sl_17547	9
60	solcap_snp_sl_9663	3	**252**	solcap_snp_sl_17525	9
61	solcap_snp_sl_21215	3	**253**	solcap_snp_sl_17502	9
62	solcap_snp_sl_9681	3	**254**	solcap_snp_sl_17481	9
63	solcap_snp_sl_9689	3	**255**	solcap_snp_sl_7775	9
64	solcap_snp_sl_9703	3	**256**	solcap_snp_sl_14653	9

Table 1, continua

nº	SNP name	Chr	nº	SNP name	Chr
65	solcap_snp_sl_19643	3	**257**	solcap_snp_sl_19983	9
66	solcap_snp_sl_14355	3	**258**	solcap_snp_sl_19982	9
67	solcap_snp_sl_14354	3	**259**	solcap_snp_sl_32342	9
68	solcap_snp_sl_55037	3	**260**	solcap_snp_sl_16654	9
69	solcap_snp_sl_19514	3	**261**	solcap_snp_sl_16648	9
70	solcap_snp_sl_23192	3	**262**	solcap_snp_sl_100372	9
71	solcap_snp_sl_12718	3	**263**	solcap_snp_sl_26683	9
72	solcap_snp_sl_12414	3	**264**	solcap_snp_sl_22830	9
73	solcap_snp_sl_4926	3	**265**	solcap_snp_sl_12536	9
74	solcap_snp_sl_5547	3	**266**	solcap_snp_sl_12535	9
75	solcap_snp_sl_1779	3	**267**	solcap_snp_sl_39804	9
76	solcap_snp_sl_12799	3	**268**	solcap_snp_sl_12501	9
77	solcap_snp_sl_1772	3	**269**	solcap_snp_sl_39506	9
78	solcap_snp_sl_5761	3	**270**	solcap_snp_sl_39457	9
79	solcap_snp_sl_5656	3	**271**	solcap_snp_sl_39452	9
80	solcap_snp_sl_18959	3	**272**	solcap_snp_sl_45076	9
81	solcap_snp_sl_18985	3	**273**	solcap_snp_sl_45159	9
82	solcap_snp_sl_10372	3	**274**	solcap_snp_sl_41458	9
83	solcap_snp_sl_7942	3	**275**	solcap_snp_sl_30380	9
84	solcap_snp_sl_7939	3	**276**	solcap_snp_sl_30379	9
85	solcap_snp_sl_7919	3	**277**	solcap_snp_sl_29188	9
86	solcap_snp_sl_9379	3	**278**	solcap_snp_sl_46840	9
87	solcap_snp_sl_62495	3	**279**	solcap_snp_sl_29222	9
88	solcap_snp_sl_62409	3	**280**	solcap_snp_sl_3430	9
89	solcap_snp_sl_100561	3	**281**	solcap_snp_sl_58143	9
90	solcap_snp_sl_21317	4	**282**	solcap_snp_sl_7829	9
91	solcap_snp_sl_9856	4	**283**	solcap_snp_sl_58180	9
92	solcap_snp_sl_63869	4	**284**	solcap_snp_sl_69787	9
93	solcap_snp_sl_21335	4	**285**	solcap_snp_sl_69874	9
94	solcap_snp_sl_21372	4	**286**	solcap_snp_sl_69978	9
95	solcap_snp_sl_21385	4	**287**	solcap_snp_sl_63704	9
96	solcap_snp_sl_27167	4	**288**	solcap_snp_sl_100810	9
97	solcap_snp_sl_16978	4	**289**	solcap_snp_sl_63641	9

Table 1, continua

nº	**SNP name**	**Chr**	**nº**	**SNP name**	**Chr**
98	solcap_snp_sl_16982	4	**290**	solcap_snp_sl_63588	9
99	solcap_snp_sl_16985	4	**291**	solcap_snp_sl_100269	10
100	solcap_snp_sl_1698	4	**292**	solcap_snp_sl_13202	10
101	solcap_snp_sl_17756	4	**293**	solcap_snp_sl_46386	10
102	solcap_snp_sl_17765	4	**294**	solcap_snp_sl_17851	10
103	solcap_snp_sl_17771	4	**295**	solcap_snp_sl_30515	10
104	solcap_snp_sl_18763	4	**296**	solcap_snp_sl_34373	10
105	solcap_snp_sl_18759	4	**297**	solcap_snp_sl_9598	10
106	solcap_snp_sl_5211	4	**298**	solcap_snp_sl_34365	10
107	solcap_snp_sl_18755	4	**299**	solcap_snp_sl_5191	10
108	solcap_snp_sl_51382	4	**300**	solcap_snp_sl_100743	10
109	solcap_snp_sl_51338	4	**301**	solcap_snp_sl_59159	10
110	solcap_snp_sl_51332	4	**302**	solcap_snp_sl_100240	10
111	solcap_snp_sl_51325	4	**303**	solcap_snp_sl_14868	10
112	solcap_snp_sl_18739	4	**304**	solcap_snp_sl_14890	10
113	solcap_snp_sl_56956	4	**305**	solcap_snp_sl_33168	10
114	solcap_snp_sl_100205	4	**306**	solcap_snp_sl_61192	10
115	solcap_snp_sl_56978	4	**307**	solcap_snp_sl_61131	10
116	solcap_snp_sl_100154	4	**308**	solcap_snp_sl_61108	10
117	solcap_snp_sl_17655	4	**309**	solcap_snp_sl_100516	10
118	solcap_snp_sl_101035	4	**310**	solcap_snp_sl_8859	10
119	solcap_snp_sl_17649	4	**311**	solcap_snp_sl_8858	10
120	solcap_snp_sl_100246	4	**312**	solcap_snp_sl_8855	10
121	solcap_snp_sl_45495	4	**313**	solcap_snp_sl_8835	10
122	solcap_snp_sl_17645	4	**314**	solcap_snp_sl_15094	10
123	solcap_snp_sl_45469	4	**315**	solcap_snp_sl_8807	10
124	solcap_snp_sl_17641	4	**316**	solcap_snp_sl_100691	10
125	solcap_snp_sl_45448	4	**317**	solcap_snp_sl_8787	10
126	solcap_snp_sl_45432	4	**318**	solcap_snp_sl_8774	10
127	solcap_snp_sl_45412	4	**319**	solcap_snp_sl_21829	11
128	solcap_snp_sl_53136	4	**320**	solcap_snp_sl_21035	11
129	solcap_snp_sl_24148	4	**321**	solcap_snp_sl_9444	11
130	solcap_snp_sl_58945	4	**322**	solcap_snp_sl_62702	11

Table 1, continua

nº	**SNP name**	**Chr**	**nº**	**SNP name**	**Chr**
131	solcap_snp_sl_24606	4	**323**	solcap_snp_sl_9510	11
132	solcap_snp_sl_24577	4	**324**	solcap_snp_sl_6905	11
133	solcap_snp_sl_24575	4	**325**	solcap_snp_sl_16424	11
134	solcap_snp_sl_58916	4	**326**	solcap_snp_sl_719	11
135	solcap_snp_sl_8223	4	**327**	solcap_snp_sl_706	11
136	solcap_snp_sl_3066	4	**328**	solcap_snp_sl_2983	11
137	solcap_snp_sl_3067	4	**329**	solcap_snp_sl_3017	11
138	solcap_snp_sl_23480	4	**330**	solcap_snp_sl_36504	11
139	solcap_snp_sl_3096	4	**331**	solcap_snp_sl_10958	11
140	solcap_snp_sl_28824	4	**332**	solcap_snp_sl_53055	11
141	solcap_snp_sl_2191	4	**333**	solcap_snp_sl_100995	11
142	solcap_snp_sl_69262	4	**334**	solcap_snp_sl_100959	11
143	solcap_snp_sl_11543	4	**335**	solcap_snp_sl_101068	11
144	solcap_snp_sl_36809	4	**336**	solcap_snp_sl_2671	11
145	solcap_snp_sl_3480	4	**337**	solcap_snp_sl_100981	11
146	solcap_snp_sl_47762	4	**338**	solcap_snp_sl_100987	11
147	solcap_snp_sl_3977	4	**339**	solcap_snp_sl_101009	11
148	solcap_snp_sl_4000	4	**340**	solcap_snp_sl_3159	12
149	solcap_snp_sl_4005	4	**341**	solcap_snp_sl_3163	12
150	solcap_snp_sl_4021	4	**342**	solcap_snp_sl_12656	12
151	solcap_snp_sl_23589	4	**343**	solcap_snp_sl_12664	12
152	solcap_snp_sl_4038	4	**344**	solcap_snp_sl_1490	12
153	solcap_snp_sl_4042	4	**345**	solcap_snp_sl_41220	12
154	solcap_snp_sl_47843	4	**346**	solcap_snp_sl_1498	12
155	solcap_snp_sl_4139	4	**347**	solcap_snp_sl_1504	12
156	solcap_snp_sl_15889	5	**348**	solcap_snp_sl_100197	12
157	solcap_snp_sl_19102	5	**349**	solcap_snp_sl_1525	12
158	solcap_snp_sl_18306	5	**350**	solcap_snp_sl_1568	12
159	solcap_snp_sl_18272	5	**351**	solcap_snp_sl_1572	12
160	solcap_snp_sl_13481	5	**352**	solcap_snp_sl_14758	12
161	solcap_snp_sl_23724	5	**353**	solcap_snp_sl_58807	12
162	solcap_snp_sl_29477	5	**354**	solcap_snp_sl_58869	12
163	solcap_snp_sl_29473	5	**355**	solcap_snp_sl_8064	12

Table 1, continua

nº	SNP name	Chr	nº	SNP name	Chr
164	solcap_snp_sl_23974	5	**356**	solcap_snp_sl_65964	12
165	solcap_snp_sl_5050	5	**357**	solcap_snp_sl_9707	12
166	solcap_snp_sl_100056	5	**358**	solcap_snp_sl_8549	12
167	solcap_snp_sl_50895	5	**359**	solcap_snp_sl_8547	12
168	solcap_snp_sl_50902	5	**360**	solcap_snp_sl_20409	12
169	solcap_snp_sl_50925	5	**361**	solcap_snp_sl_26884	12
170	solcap_snp_sl_50932	5	**362**	solcap_snp_sl_16794	12
171	solcap_snp_sl_5094	5	**363**	solcap_snp_sl_16795	12
172	solcap_snp_sl_5095	5	**364**	solcap_snp_sl_16796	12
173	solcap_snp_sl_5110	5	**365**	solcap_snp_sl_16803	12
174	solcap_snp_sl_51601	5	**366**	solcap_snp_sl_19574	12
175	solcap_snp_sl_22562	5	**367**	solcap_snp_sl_19570	12
176	solcap_snp_sl_22565	5	**368**	solcap_snp_sl_19569	12
177	solcap_snp_sl_22567	5	**369**	solcap_snp_sl_18996	12
178	solcap_snp_sl_22572	5	**370**	solcap_snp_sl_18995	12
179	solcap_snp_sl_74	5	**371**	solcap_snp_sl_20216	12
180	solcap_snp_sl_75	5	**372**	solcap_snp_sl_100037	12
181	solcap_snp_sl_22603	5	**373**	solcap_snp_sl_22748	12
182	solcap_snp_sl_22620	5	**374**	solcap_snp_sl_3112	12
183	solcap_snp_sl_37209	5	**375**	solcap_snp_sl_19345	12
184	solcap_snp_sl_12200	5	**376**	solcap_snp_sl_12856	12
185	solcap_snp_sl_12201	5	**377**	solcap_snp_sl_23507	12
186	solcap_snp_sl_37265	5	**378**	solcap_snp_sl_14415	12
187	solcap_snp_sl_12212	5	**379**	solcap_snp_sl_7042	12
188	solcap_snp_sl_12213	5	**380**	solcap_snp_sl_7045	12
189	solcap_snp_sl_16177	5	**381**	solcap_snp_sl_19630	12
190	solcap_snp_sl_100022	5	**382**	solcap_snp_sl_31973	12
191	solcap_snp_sl_22649	5	**383**	solcap_snp_sl_6568	12
192	solcap_snp_sl_231	5	**384**	solcap_snp_sl_6526	12

Chr: chromosome.