# QTL Analysis of Fruit Quality Traits in Peach [*Prunus persica* (L.) Batsch] using Dense SNP Maps

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## ABSTRACT

**Fruit quality is the main criterion used for selection of new varieties in peach, and it is usually defined by the conjunction of organoleptic and nutritional traits and postharvest behavior. The aim of this study was the identification of quantitative trait loci (QTL) for several fruit quality traits using an F1 segregating population of 75 seedlings derived from the cross between 'Venus' and 'Big Top' nectarine cultivars. The progeny was evaluated over several years for agronomic and pomological characteristics (including basic quality traits and antioxidant compounds content) and also genotyped using SNPs included in the 'IPSC 9K peach SNP array v1' developed by the International Peach SNP Consortium, which carries 8,144 SNPs. Two preliminary dense genetic linkage maps were constructed for 'Venus' and 'Big Top', with 160 and 208 markers placed onto 11 linkage groups, respectively. A second round was used to identify QTLs that were mapped over twelve LG representing seven peach chromosomes. Some of the QTLs mapped in the same position of previously reported QTLs, interestingly QTLs for fructose in LG 6 and phenolic compounds in LG2 were detected for the first time. LG4 in 'Venus' and LG5 in 'Big Top' maps presented the highest density of QTLs controlling several traits. This work represents the first study identifying QTLs for fruit quality traits using the high-density SNP array 'IPSC 9K peach SNP array v1' in an F1 nectarine family.**

## INTRODUCTION

The peach [*Prunus persica* (L.) Batsch] is one of the most important economically fruit crop in the world after apples and pears (FAO, 2012). In 2010, Spain was the third main producer country in the world after China and Italy, with around 1.4 million tons in 73,000 ha.

The external quality of fruits is determined by shape, colour and size, while the internal quality is determined by the texture, sugars, organic acids and antioxidant compounds contents, which contribute significantly to the taste and aroma of the fruit (Hudina et al., 2012). The last decade has seen the proliferation of an enormous number of scientific studies focused on the activity of antioxidant compounds present in our diet because they contribute to prevent the occurrence of degenerative diseases (Russo et al., 2012). Biochemical and genetic studies on the mechanisms of action of phytochemicals provide a functional explanation of how and why a diet rich in fruits and vegetables is considered healthy (Russo et al., 2012). It is now believed that polyphenols may exert their beneficial action through the modulation of gene expression and the activity of a wide range of enzymes and cell receptors (Chagné et al., 2012, and references therein). However, the health effects of dietary antioxidant compounds depend on the total amount consumed and on their bioavailability. In addition, the content in antioxidant compounds can vary according to the location within the

fruit (skin vs flesh), the stage of fruit maturity and even the location of the fruit within the tree (Chagné et al., 2012).

Most of traits related to fruit quality are quantitatively inherited and their genetic control are still unknown (Eduardo et al., 2011). Dissection of the genetic components underlying complex agricultural traits in plants has so far used mainly experimental bi-parental crosses and a limited number of genetic markers (Verde et al., 2012). Over the last two decades, availability of genetic knowledge of peach as the model for *Prunus* and the Rosaceae has accelerated with the development of molecular markers, linkage and physical maps, comparative genomics studies, databases, and the very recent release of the full genome sequence of a dihaploid peach genotype (Arús et al., 2012). Also the development of Illumina's Infinium BeadArray Technology platform, an extremely high-throughput single nucleotide polymorphism (SNPs) genotyping system, allows the detection of up to 2.5 million SNPs per single DNA sample (Verde et al., 2012). In this context, the IPSC have developed, characterized and validated the first version of an array in peach which carries 8,144 SNPs.

In peach, several Mendelian characters involved in fruit quality have been already studied and mapped (see Arús et al., 2012, for a recent review), such as peach/nectarine, polycarpel and flesh color (Bliss et al., 2002), melting/non melting flesh (Warburton et al., 1996), and freestone/clingstone (Dettori et al., 2001). Moreover, several authors (Dirlewanger et al., 1998; Etienne et al., 2002; Quilot et al., 2004; Cantín et al., 2010) have localized QTLs involved in the control of physico-chemical components of different fruit quality traits, such as sugars and organic acid contents on linkage groups 4, 5 and 6. Regarding antioxidant compounds, Dirlewanger et al. (2006) analyzed the genetic control of fruit phenolics in the peach F2 population ('Ferjalou-Jalousia'® × 'Fantasia') and detected QTLs involved in phenolic compounds on LGs 1, 2, 4 and 6.

The aim of the present research was to analyze the genetic control of the main organoleptic fruit quality traits evaluated for four years in an F1 population derived from the cross of 'Venus' × 'Big Top' nectarines. In this paper we presented preliminary results for the first identification of genomic regions that regulate the main fruit quality traits using the IPSC 9K peach SNP array. These results will contribute to define the peach antioxidant compounds map that can be useful for breeding and Marker Assisted Selection (MAS) purposes.

## MATERIALS AND METHODS

### Plant material and quality traits evaluated

The progeny assayed was a segregant F1 population of 75 seedlings obtained from a controlled cross, between *Prunus persica* cvs. 'Venus' (female parent) and 'Big Top' (male parent). 'Venus' is a freestone, melting and yellow flesh nectarine cultivar, whereas 'Big Top' is a clingstone, melting and yellow flesh nectarine cultivar. The segregant population is entirely melting flesh, either cling- or freestone. The resulting seedlings were budded on the same rootstock (GF 677) and established (one tree per genotype) at the Experimental Station of Aula Dei-CSIC (northern Spain, Zaragoza) in 2002. Trees were grown under standard conditions of irrigation, fertilization and pest and disease control. Fruits were harvested over four years at commercial maturity (2007-2010).

During four years, agronomic and biochemical fruit quality traits were measured individually in each seedling tree. Annual yield, fruit weight, flesh firmness, soluble solids content (SSC), titratable acidity, pH, vitamin C, total phenolics, flavonoids, anthocyanins, relative antioxidant capacity (RAC) and sugars were evaluated in each independent seedling as reported by Abidi et al. (2011).

**Phytochemical extraction and analysis**

For the biochemical analyses, samples of 5 grams of fruit flesh were used, as flesh is usually consumed in peaches. All samples were frozen in liquid nitrogen and kept at -20ºC until analysis. For vitamin C analysis, samples were kept in 5 mL of 5 % metaphosphoric acid for preservation of ascorbic acid, frozen in liquid nitrogen and stored at -20 ºC until analyses. Then, samples were homogenized, centrifuged and supernatant was recovered as described by Cantín et al. (2009b) and Abidi et al. (2011). Vitamin C, total phenolics, flavonoids, anthocyanins and RAC were evaluated with colorimetric methods and measured using a spectrophotometer (Beckman Coulter DU 800) as described by Abidi et al. (2011). For sugar profile, the sugar composition and quantification were analyzed by HPLC as described by Cantín et al. (2009a) with some modifications described in Abidi et al. (2011).

**Genotyping, Map construction and QTL analysis**

For genotyping, DNA was extracted from young leaves of 'Venus', 'Big Top' and all the progeny (75 genotypes) by using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA), following manufacturer's instructions. For SNPs marker analysis, concentration and quality of DNA was checked using PicoGreen. Samples were genotyped using the *RosBREED_Peach_10k_11494376_A* chip from Illumina which includes more than 8,144 SNPs peach markers (Verde et al., 2012) following the single base extension assay (Steemers, et al., 2006) and manufacturer conditions included in the Illumina® Infinium® HD Assay Ultra protocol.

Individuals that showed the same genotype as the female parent 'Venus' in all the markers were identified as self-pollinated seedlings. All polymorphic, non-skewed and non-repetitive markers were selected. For map construction, pseudo-testcross strategy was used (Grattapaglia and Sederoff, 1994) using JoinMap® 4.0 software (Van Ooijen, 2006). To facilitate the mapping process all SNP markers were codified adding the scaffold numbers as a prefix ahead the name of each marker. For practical reasons this codification were maintained in the results and discussion section. Two mapping rounds were performed. In the first round, the preliminary number of groups (linkage groups) was established using the recombination fraction criterion. The value where most of the nodes had markers with one or the minimum number of different prefixes was used. The preliminary order for markers was established using the Regression Mapping option and map distances in centimorgans (cM) were calculated in all linkage groups using the Haldane's mapping function. The order was compared to its known physical position inside the scaffold, in this moment; all the markers initially excluded because of their identical segregation pattern were considered. At this point, only markers following a correlative physical order attending the scaffold number and position in Mbp were selected. A second mapping round was done to map the QTLs. In this round, the order of each linkage group and map distances in centimorgans (cM) were established following manufacturer's instructions. QTL analysis was performed with R/qtl software using multiple-QTL-Model (MQM) in the R platform (Broman et al., 2003).

## RESULTS AND DISCUSSION

**Phenotyping and marker selection**

The results for agronomical and fruit quality traits evaluated in the 2007-2010 year period were summarized in Table 1 (Zeballos, 2012). The mean values were obtained from 69 seedlings. A wide phenotypic variation was found for most of the traits studied in this progeny. These variations supported the quantitative nature of these traits.

Out of the 8,144 SNPs markers, 64% were non polymorphic, 22% showed the same segregation pattern and 4% presented a distorted segregation. A total of 675 SNPs were

informative with Gentrain Scores ranging from 0.35 to 0.92 and 405 markers were used for both maps.

**Genetic linkage maps of 'Venus' and 'Big Top' and QTL analyses**

In the first mapping round 160 SNP markers were mapped onto 11 linkage groups in the 'Venus' parent (Fig. 1). Nine groups included markers with the same prefix but two LGs included markers with more than one prefix. Nine scaffolds were represented in the map (1, 2, 3, 4, 5, 6, 7, 8 and 17). The length of the LGs ranged from less than 5 cM to around 60 cM. In nine groups out of 11, all markers shared the same prefix inside each linkage group (Fig. 1). Linkage groups 3 and 4 included markers with more than one prefix, LG3 had two over twenty (2/20) and LG4 eight over eighteen (8/18) markers.

For the 'Big Top' parent we followed the same strategy as in the 'Venus' map. In the first mapping round, 208 SNP markers were grouped on 11 linkage groups (Zeballos, 2012). Five linkage groups (LG4, LG6, LG7, LG10 and LG11) showed markers with the same prefix (Fig. 2). Three linkage groups (LG2, LG8 and LG9) included only one marker with different prefix (1/37, 1/13 and 1/9), LG3 and LG5 had two markers with different prefixes (2/35 and 2/18) and LG1 included 8 markers with different prefixes (8/39).(Fig. 2). Ten scaffolds were represented in this map (1, 2, 3, 4, 5, 6, 7, 8, 10 and 13).

The second mapping round included 102 SNPs and 5 SSRs (data not shown) in the 'Venus' parent, and 123 markers on the 'Big Top' parent, on nine and ten LGs respectively. The second round was used to identify QTLs that were mapped over twelve LG representing seven peach chromosomes (data not shown). Some of the QTLs were mapped in the same position of previously reported for antioxidant compounds and soluble solids content found in the same population (Cantín et al., 2010) and other unrelated peach progeny populations (Quilot et al., 2004; Dirlewanger et al., 2006; Arús et al., 2012). QTLs for fructose in LG 6 and phenolic compounds in LG2 were detected for the first time. LG4 in 'Venus' and LG5 in 'Big Top' maps presented the highest density of QTLs controlling several traits.

**CONCLUSIONS**

This study represents the first study identifying QTLs for fruit quality traits using the high-density SNP array 'IPSC 9K peach SNP array v1' in an F1 nectarine family. These results will contribute to a better understanding of the genetic control of the most important nutritional quality traits of peach and nectarine fruit.

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## Tables

Table 1. Units, minimum, maximum and mean values for the pomological traits evaluated in the 'Venus' × 'Big Top' progeny. Data are mean ± SE (n=69 genotypes).

| Trait | Units | Minimum | Maximum | Mean | S.E |
|---|---|---|---|---|---|
| Production/yield | Kg . tree$^{-1}$ | 0.83 | 19.70 | 7.09 | ± 0.29 |
| Fruit weight | Grams (g) | 69.44 | 375.87 | 185.22 | ± 3.30 |
| Flesh firmness | Newton (kg.cm$^{-2}$) | 6.23 | 60.76 | 40.78 | ± 0.68 |
| Soluble Solids Content | ºBrix | 9.20 | 20.20 | 13.36 | ± 0.13 |
| pH | pH units | 3.00 | 4.40 | 3.68 | ± 0.02 |
| Titratable acidity (TA) | g malic acid . (100 g FW)$^{-1}$ | 0.25 | 1.86 | 0.68 | ± 0.02 |
| Ripening index (RI) | SSC/TA | 7.55 | 66.98 | 25.60 | ± 0.84 |
| Total sugars | g . (kg FW)$^{-1}$ | 45.34 | 205.18 | 89.10 | ± 1.52 |
| Sucrose | g . (kg FW)$^{-1}$ | 23.16 | 125.33 | 58.50 | ± 1.04 |
| Glucose | g . (kg FW)$^{-1}$ | 6.59 | 40.91 | 12.09 | ± 0.29 |
| Sorbitol | g . (kg FW)$^{-1}$ | 0.99 | 28.28 | 6.39 | ± 0.31 |
| Fructose | g . (kg FW)$^{-1}$ | 7.43 | 27.75 | 12.32 | ± 0.21 |
| Vitamin C | (mg AsA) . (100 g FW) $^{-1}$ | 1.17 | 12.11 | 4.11 | ± 0.13 |
| Total phenolics | (mg GAE) . (100 g FW)$^{-1}$ | 12.10 | 58.85 | 32.25 | ± 0.90 |
| Flavonoids | (mg CE) . (100 g FW)$^{-1}$ | 1.58 | 60.13 | 12.64 | ± 0.61 |
| Anthocyanins | (mg C3GE). (kg FW)$^{-1}$ | 0.32 | 25.72 | 3.16 | ± 0.22 |
| RAC | (µg TE) . (g FW)$^{-1}$ | 125.30 | 1099.60 | 451.4 | ± 11.76 |

*AsA*: ascorbic acid, *FW:* fresh weight, *GAE:* gallic acid equivalents, *CE:* catechin equivalents, *C3GE:* cyanidin-3-glucoside equivalents, *TE:* trolox equivalents, *RAC:* Relative Antioxidant Capacity

## Figures

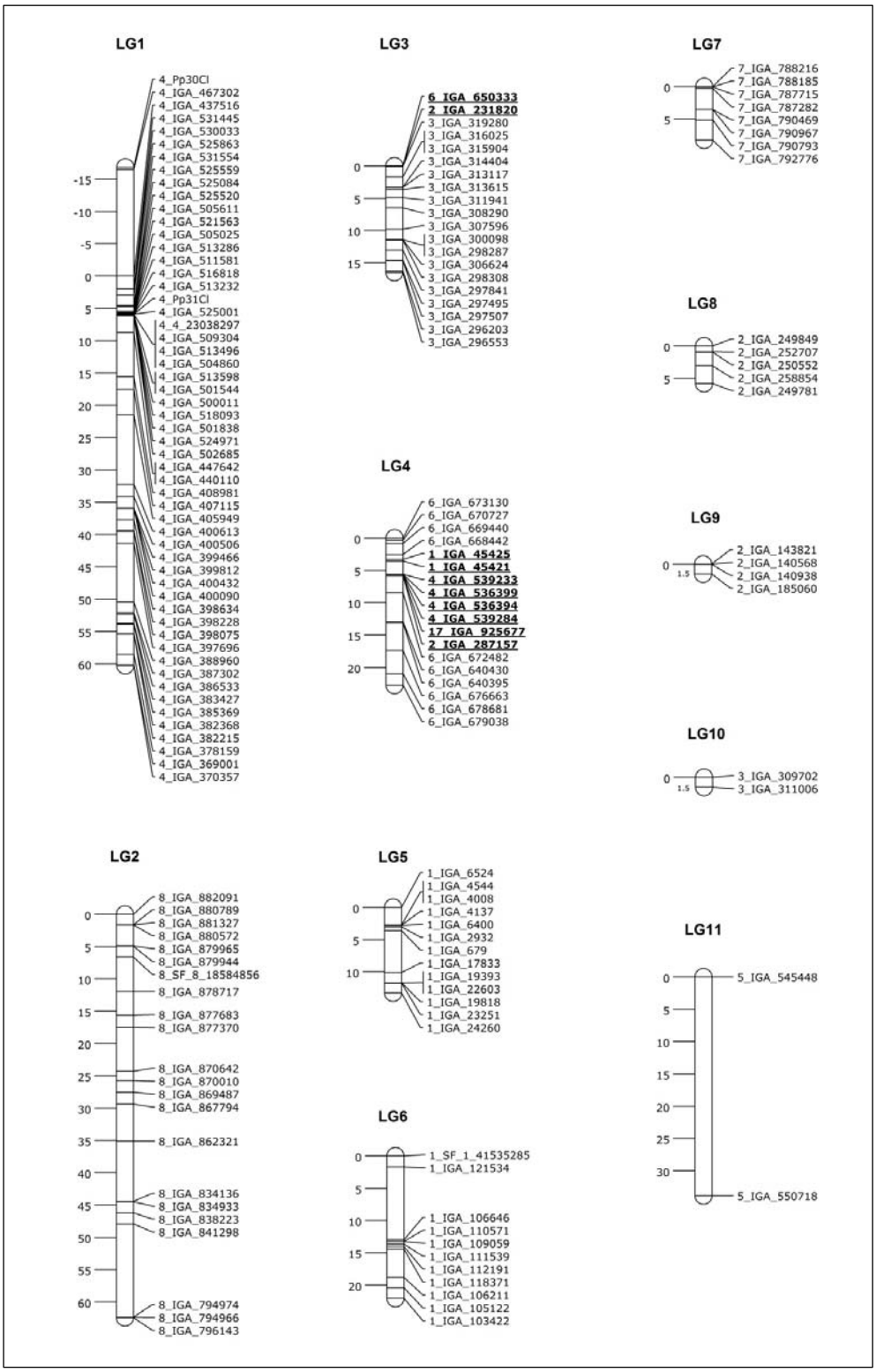


Fig. 1. Preliminary 'Venus' genetic linkage map showing the position of SNP markers (right side) and genetic distances in cM (left side). Eleven linkage groups have included nine scaffolds. Markers that do not share the same prefix inside a LG are in bold and underlined.

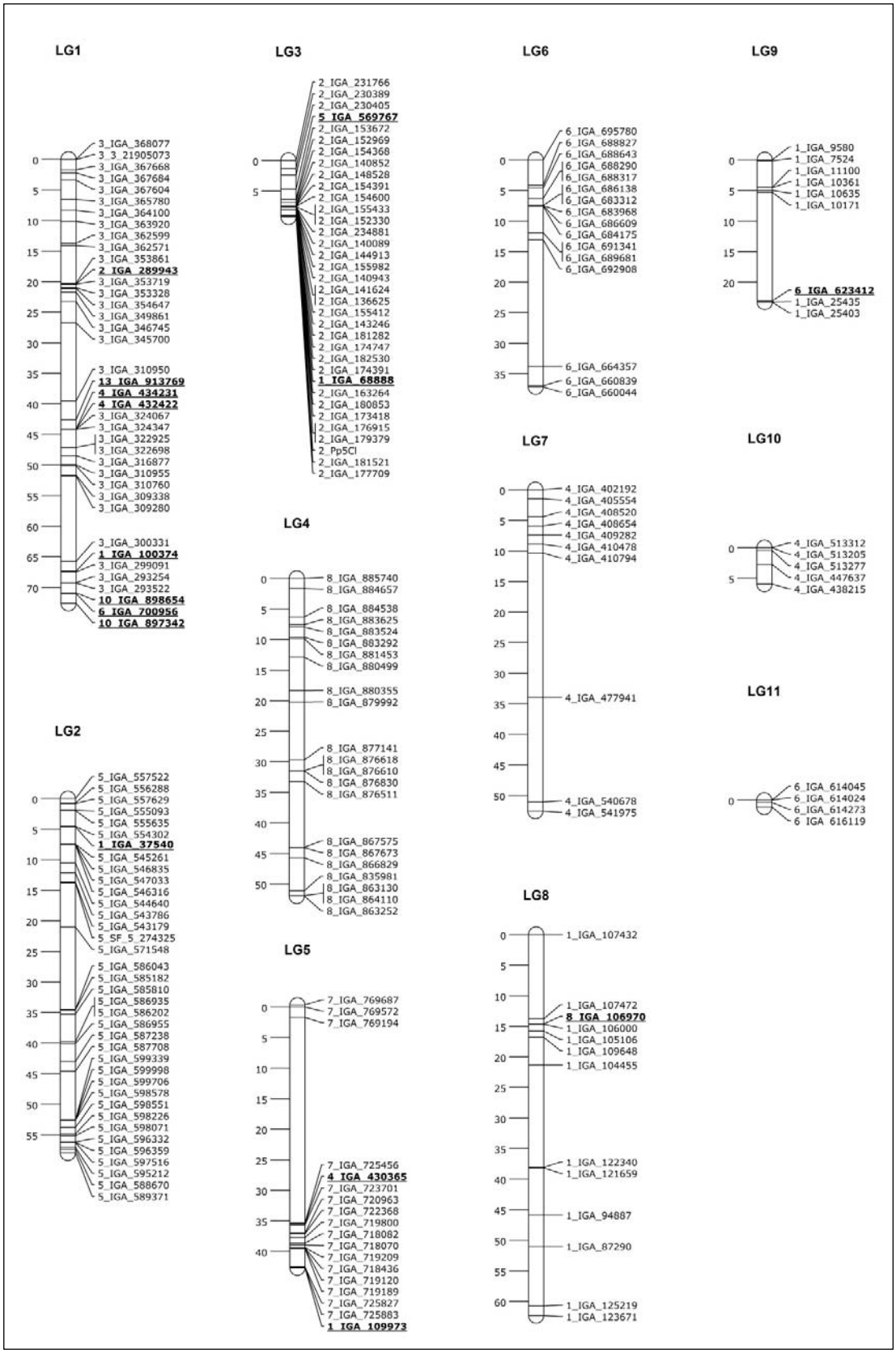


Fig. 2. Preliminary ‘Big Top’ genetic linkage map showing the position of SNP markers (right side) and genetic distances in cM (left side). Eleven linkage groups have included ten scaffolds. Markers that do not share the same prefix inside a LG are in bold and underlined.