Genetic Control and Location of QTLs Involved in Antioxidant Capacity and Fruit Quality Traits in Peach [*Prunus persica* (L.) Batsch]

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Abstract

In peach fruits, phenolic compounds serve as a major source of potential antioxidants which are known to play a significant role in fruit quality and in human wellbeing. This study was conducted in a F1 population derived from the cross 'Venus' x 'Big Top' nectarines in order to investigate the variability in the fruit antioxidant content and to study the genetic control and location of QTLs involved in fruit quality traits. Biochemical analyses have been performed to measure L-ascorbic acid (vitamin C), total phenolics, flavonoids, anthocyanins contents and antioxidant capacity.

INTRODUCTION

Peach and nectarine fruit [*Prunus persica* (L.) Batch] are the second most important fruit crop in the European Union (EU) after apples (Iglesias and Echeverría, 2008). Peach, a member of the *Prunoideae* subfamily, is one of the most widely grown and the best genetically characterized species in the *Rosaceae* family, it is a self-fertile and naturally self-pollinating fruit species with very low genetic variability (Scorza et al., 1985). As a result of its small genome size, the developing genomic resources, and the colinearity of the peach genome within *Prunus*, diploid peach has become a model genome for these fruit crops, providing a platform for comparative studies within the entire *Rosaceae* (Abbott et al., 2002).

Peach fruit contain ascorbic acid (vitamin C), carotenoids (provitamin A), and phenolic compounds that are good sources of antioxidants (Byrne, 2002). Phenolic compounds represent the major sources of antioxidant capacity in peaches (Cantin et al., 2009; Chang et al., 2000); although vitamin C also contributes to antioxidant capacity (Gil et al., 2002). These naturally occurring substances not only have a role in the visual appearance (pigmentation and browning) and taste (astringency) of fruit, but also have health-promoting properties, acting as antioxidants by scavenging harmful free radicals which are implicated in most degenerative diseases (Rice-Evans et al., 1996; Cantín et al., 2009). These benefits make phenolic compounds an interesting target for breeding programmes.

The development of molecular markers and linkage maps provides efficient tools to locate genes or quantitative trait loci (QTLs) involved in agronomical characters and could be helpful for monitoring of breeding programs (Young, 1996). QTLs for traits, which determine fruit quality in peach, have been localized on maps (Dirlewanger et al., 1998). Most of the QTLs affecting fruit quality characters have been localized in few linkage groups. In linkage group 6, QTLs associated with sucrose, sorbitol, soluble solid content, malic acid and citric acid have been identified (Dirlewanger et al., 1999; Etienne et al., 2002).

The purpose of the present research was to measure the variation of major antioxidant compounds in peach fruits of a total of 75 genotypes derived from the cross 'Venus' x 'Big Top' nectarines, to evaluate the antioxidant capacity and to search for QTLs involved in these fruit quality traits.

MATERIALS AND METHODS

Plant Material

The progeny assayed was a segregant F1 population of 75 seedlings obtained from a controlled cross made in 2000-2001, between *P. persica* cvs. 'Venus' (female parent) and 'BigTop' (male parent), in collaboration with Agromillora Catalana S.A. 'Venus' is a FMF (freestone melting flesh) nectarine cultivar, whereas 'BigTop' is a CMF (clingstone melting flesh) nectarine cultivar. The segregant population is entirely melting flesh, either cling- or freestone. Progenies were established during 2001 in a plot at the Experimental Station of Aula Dei (Zaragoza, Spain).

DNA Extraction and Molecular Analysis

DNA was extracted from leaves of 'Venus', 'Big Top' and all the progeny (75 genotypes) by using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA), following manufactured instructions. For initial polymorphism testing in LG 6, eleven markers were used (Table 1) and assays were performed on 'Venus', 'Big Top', and six progenies. For the PCR reactions, 10 ng of genomic DNA was amplified in a final volume of 15 µl containing 1X biotools buffer, 2.0 mM $MgCl_2$, 0.20 mM of dNTPs, 0.15 µM of each primer pair and 0.5 U Taq DNA polymerase (Biotools, Madrid, Spain). The PCR conditions were as follows: preliminary denaturation (3 min at 94 ºC), followed by 35 cycles consisting of denaturation (1 min at 94 ºC), 45 s at the corresponding annealing temperature and extension (1 min at 72 ºC), and a final extension after the last cycle (7 min at 72 ºC).

Biochemical Analyses

Five representative fruits were selected at harvest. They were peeled, cut in pieces, and 5 g samples were prepared, which were frozen in liquid nitrogen and stored at -20 ºC until analyses. Fruit samples were analyzed for vitamin C, total phenolics, flavonoids and anthocyanins content, and antioxidant capacity as described by Cantín et al. (2009). For the determination of ascorbic acid the samples were kept frozen in 5% metaphosphoric acid and homogenized at 4 ºC. Then, they were centrifuged at 16,000g for 20 min at 4 ºC, and the supernatant was immediately used for vitamin C analysis. Absorbance was measured at 525 nm and the amount of vitamin C was expressed as mg of ascorbic acid (AsA) per 100 g fresh weight (FW) (Zaharieva and Abadía, 2003).

Mapping and QTL analysis

Polymorphic microsatellite markers were used to construct a partial map of the linkage group 6 with JoinMap 4.0 software (Van Ooijen, 2006). QTL analysis was implemented with MapQTL 5.0 (Van Ooijen, 2004) by interval mapping.

RESULTS AND DISCUSSION

The results obtained for all biochemical traits studied showed a normal distribution typical of quantitative characters (Fig. 1). Considerable differences were

found in the content of all antioxidant compounds in the parents of the population, 'Venus' and 'Big Top' (Table 2).

Vitamin C

Average ascorbic acid content ranged from 1.3 to 9.5 mg of AsA/100g of FW with a mean value of 3.9 mg of AsA/100 g FW (Table 2) and it showed high variation between genotypes (Fig. 1). Values were in the same range as previously reported for vitamin C contents in peach flesh, namely, 1-14 mg of AsA/100 g of FW (Tavarini et al., 2008). Cantín et al. (2009) reported that the total ascorbic acid (vitamin C) content greatly varied from approximately 1 to 9 mg of AsA/100 g of FW, with a mean value of 3.7 mg of AsA/100 g of FW in several peach genotypes. Gil et al. (2002) reported that the total ascorbic acid contents (mg/100 g FW) of nectarine and peach cultivars from California, were from 6 to 8 in yellow-flesh nectarines and from 5 to 14 in white-flesh nectarines. This variation is justified by many pre- and postharvest factors which influence the vitamin C content of horticultural crops. Maturity at harvest, harvesting method, and postharvest handling conditions also affect the vitamin C content of fruits and vegetable (Kader, 1988). It is also been reported that the production of vitamin C increases at ripening stage to balance a decrease in polyphenol concentration in order to protect fruits against damages and oxidation.

Phenolic Compounds

Total phenolics ranged from 23 to 51 mg GAE/100g of FW (Table 2), and showed less variation between genotypes. The amounts of total phenolics fell within the range reported in the literature for peach fruits, namely 14-77 mg GA/100 g of FW (Proteggente et al., 2002). Cantín et al. (2009) reported that total phenolics varied among genotypes with values in the range of 12.7-71.3 mg of GAE/100 g of FW. Gil et al. (2002) reported that total phenolics (in mg CGA /100 g of FW) were 14-102 in white-flesh nectarines and 18-54 in yellow-flesh nectarines. Marinova et al. (2005), characterizing the content of total phenolics in fruit reported that in peaches the values were 50.9 mg GAE/100g of FW. Tavarini et al. (2008) reported that the content of phenols ranged between 14 and 50 mg GA/100 g of FW.

Flavonoids ranged from 5 to 47 mg CE/100g of FW (Table 2) with an average of 21 mg of CE/100 g of FW. Cantín et al. (2009) reported that total flavonoids content ranged from 1.8 to 30.9 mg of CE/100 g of FW, with an average of 8.8 mg of CE / 100 g of FW. Marinova et al (2005), characterizing the content of flavonoids in fruit reported that in peaches the values were 15.0 mg CE/100 g of FW. The anthocyanins content showed a normal distribution and ranged from 0.3 to 5 mg C3G eq/kg of FW (Table 2), but presented less variation between genotypes as compared to ascorbic acid. Cantín et al (2009) reported that total anthocyanins greatly varied among genotypes [0.1-26.7 mg of cyanidin-3-glucoside equivalents (C3GE) per kg of FW] depending on the red pigmentation of the flesh. Lavelli et al. (2009) reported that the cyanidin 3-O-glucoside in peach samples ranged from 0 to 9.4 mg/kg.

Antioxidant capacity (RAC) ranged from 255 to 1099 (μg Trolox/g of FW) (Table 2). Cantín et al. (2009) reported that the relative antiradical capacity (RAC) varied among genotypes, with values ranging from 227.3 to 629.9 μg of Trolox/g of FW, with an average of 405 μg of Trolox/g of FW. Cevallos-Casals et al (2002) reported that the red-fleshed peaches had lower levels of antioxidant activity (440 to 1784 μg equivalent Trolox/ g of tissue). The antioxidant content in peaches varied greatly depending on the

type of the fruit (melting, non melting and nectarine), cultivar, genetic and environmental factors. Furthermore, as it happens for other climacteric fruits that are often picked before the full ripe stage, peach antioxidant content may be affected by the ripening stage at harvest. The antioxidant capacity of fruits varies in relation to the antioxidant molecules present in the different species (Wang et al., 1996) but variations can also occur within the genotype of a single species (Gil et al., 2002).

Mapping and QTL Analysis

For QTLs detection, the partial map of the linkage group 6 was constructed containing six polymorphic SSR markers (Fig. 2). However, significant QTLs have not been identified until now for the control of the biochemical traits analyzed.

CONCLUSIONS

In the current study, the variability in the fruit antioxidant content in the 'Venus' x 'Big Top' progeny are presented. All the traits analyzed showed a normal distribution and a high variability between genotypes. QTLs involved in the control of biochemical traits and antioxidant capacity were not localized in LG6. The addition of new polymorphic markers to this linkage group is being implemented to complete the map in order to find significant QTLs affecting these traits.

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Table 1. SSR markers tested for polymorphism in the progeny

Locus code	Reference	Polymorphism	Tra Annealing (ºC)	Size range (bp)
pchcms5	Abbott et al., 2000	polymorphic	52ºC	240-260
BPPCT025	Dirlewanger et al., 2002	polymorphic	57ºC	155–175
CPPCT030	Aranzana et al., 2002	polymorphic	52 ºC	170 – 200
BPPCT008	Dirlewanger et al., 2002	monomorphic	59 ºC	140-160
UDP98-407	Testolin et al., 2000	polymorphic	60 ºC	180-190
UDP98-412	Testolin et al., 2000	polymorphic	57 ºC	95–140
UDP98-416	Testolin et al., 2000	monomorphic	57 ºC	80–90
UDP96-001	Testolin et al., 2000	monomorphic	57 ºC	120
UDP96-010	Testolin et al., 2000	monomorphic	57 ºC	131
Chill1	Ogundiwin et al., in press	polymorphic	60 ºC	-
Chill2	Ogundiwin et al., in press	polymorphic	60 ºC	-

Table 2. Variation of the amounts of antioxidants compounds in the studied progeny and the parents.

	Progenitors		Progeny		
Traits	**'Venus'**	**'Big Top'**	**Min**	**Max**	**Means ± SD**
Vitamin C (mg AsA /100 g of FW)	3.3	5.6	1.3	9.5	3.9 ± 1.7
Total phenolics (mg GAE/100 g of FW)	36.5	46.6	23.0	51.2	44.0 ± 6.1
Flavonoids (mg CE/100g of FW)	13.7	16.9	5.0	47.0	21.2 ± 9.4
Anthocyanins (mg C3G eq /kg of FW)	1.9	1.4	0.3	5.0	1.7 ± 0.7
Antioxidant Capacity (µg Trolox/g of FW)	423.0	926.2	255.0	1099.5	617.0 ± 200

AsA= Ascorbic acid; C3G eq = Cyanidin-3-glucoside equivalents; CE= catechin equivalents; GAE= gallic acid equivalents

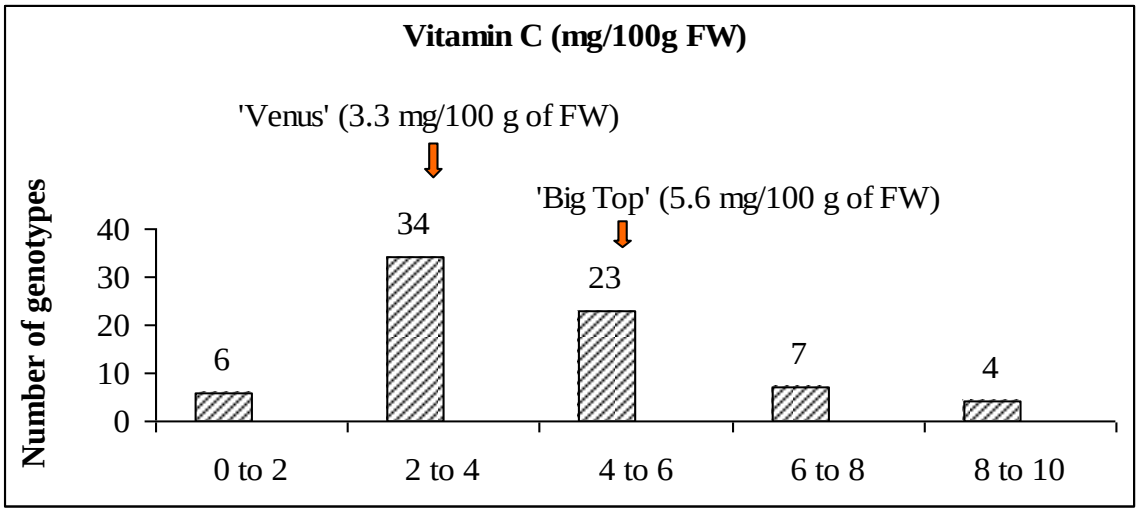

Fig. 1. Segregation of contents of vitamin C in `Venus´ x `Big Top´ progeny

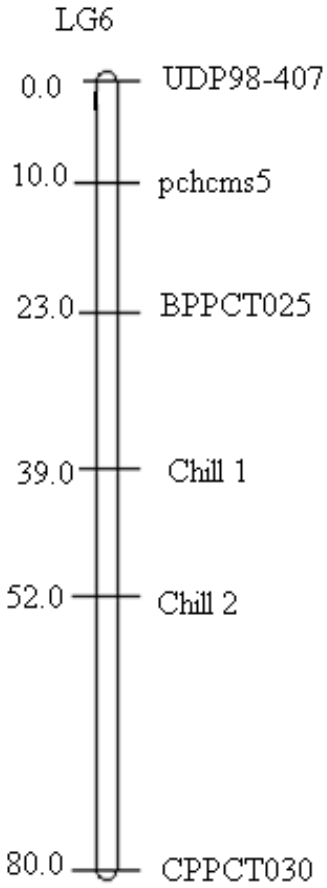

Fig. 2. Linkage map of peach chromosome 6 (LG 6) in the 'Venus' x 'Big Top' progeny.