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QTL analysis of yield and seed number in *Citrus*

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Abstract Amount, regularity and low seed content of the crop are important properties of scion citrus cultivars. The genetic control of these traits was studied in a progeny derived from the cross *Citrus volkameriana* × *Poncirus trifoliata* using molecular marker analysis. Since the traits were not normally distributed, the Kruskal-Wallis non-parametric test was used for quantitative trait loci (QTLs) detection. Most of the QTLs detected correspond to the trait "number of fruits per tree", in agreement with its known physiological complexity. Related traits (fruit number, fruit size and seed number) are controlled by QTLs some of which are located in the same genomic regions, suggesting that undesired associations could be broken to some degree by recombination. QTL analysis over years revealed important effects of genotype-by-environment interaction on QTL detection. This result agrees with the differences found for the trait means among years, which was found to be related, among other causes, to the alternate bearing of some genotypes and the amount of rain before harvest.

Key words Fruit breeding · GxE interaction · Yield components · Seedless fruits · Parthenocarpy · Molecular markers

Introduction

Genetic improvement in *Citrus* by hybridisation has been much hampered by several factors: the heterozygosity of parental genotypes that translates into wide segregations; reproduction by nucellar embryony (apomixis), sterility, cross- and self-incompatibility limiting progeny size; a long juvenility delaying the evaluation of fruit traits and the high costs involved in citrus culture due to their large size. These characteristics commonly imply the requirement of large areas to grow the seedlings and by necessity reduce the number of individuals that can be handled. In addition, inheritance studies on which to base selection or, in general, to improve methodology are very scarce in contrast to our genetic knowledge of other fruit species such as apple, peach and almond (reviewed by Socias 1998). All these facts explain why citrus improvement has been largely the result of selection of naturally occurring somatic mutants; to date only a relatively small number of cultivars of widespread significance have originated from breeding programmes. The amount of genetic variation that arises through spontaneous mutation is quite poor compared to that obtained by sexual hybridisation, making citrus improvement progress very limited. The main breeding aims in programmes for improving scion citrus cultivars are the amount and regularity of the crop; fruit of a good size, high quality, attractive appearance and colour; very low seed content, easy peeling; and a high adaptation to maintenance of fruit on tree. These agronomic traits are more quantitative than qualitative. Molecular markers have become very efficient and powerful tools in plant breeding for the genetic dissection of quantitative traits and the early screening of desired genotypes, thereby offering new possibilities for genetic improvement in citrus.

Genetic control of quantitative traits has been studied in many annual crops by means of molecular markers (quantitative trait loci or QTL analysis), and attention is now being directed towards for forest and fruit trees. Seedling height, tree growth and wood quality in *Eucalyptus* (Grattapaglia et al. 1996; Byrne et al. 1997), growth, wood specific gravity in *Pinus taeda* (Kaya et al. 1999; Knott et al. 1998), growth in *Pinus radiata* (Emeberi et al. 1998), tree growth and seedling height in *Populus* (Wu 1998; Wu et al. 1998), flowering traits in almond (Asíns et al, 1994), powdery mildew resistance in *Prunus* (Dirlewanger et al. 1996), growth and development in apple (Conner et al. 1998), apomictic reproduction in *Citrus* (Garcia et al. 1999) and many more quantitative traits have already been studied.

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A major concern in citrus, as in any tree breeding, is yield stability across years and environments (i.e. genotype-by-environment interaction, GxE). GxE or differential genotype expression reduces the association between phenotype and genotype values and may result in a selection from one environment to perform poorly in another, thereby forcing plant breeders to examine genotype adaptation. Thus, efforts should be made to study this interaction in depth. One way to accomplish this is by QTL analysis based on genetic markers.

Separated genetic maps with isozymes, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs) and microsatellite markers were developed for *Citrus volkameriana* and *Poncirus trifoliata* by analysing a 80-tree progeny derived from its cross (García et al. 1999). In the present paper, we report our investigation of linkages between these molecular markers and quantitative traits related to yield (fruit number, fruit weight and fruit size) and to fruit quality (seed number). Marker loci were used to estimate the number and direction of the gene effects. Since these traits were measured over a 2- or 3-year period, QTL stability across years was also studied. Associated markers will help citrus breeding by increasing our understanding of the inheritance of these characters and the prospects for marker-assisted selection.

Materials and methods

Plant material

A progeny of 80 trees from an intergeneric cross (CxP) between *Citrus volkameriana* Ten. (Volkamer lemon, the female parent) and *Poncirus trifoliata* (L.) Raf. var. Rubidoux was used for QTL analysis. The CxP population was obtained and grafted on citrange "Carrizo" more than 20 years ago by Dr. J. Forner at an IVIA field plot. Only 50 trees within the progeny yielded fruits between 1995 and 1997 although all but 3 flowered.

Evaluation of traits

Three yield components were evaluated: fruit number (FN), average fruit weight (FW) and average fruit size (FS) measured as the mean fruit perimeter in centimeters. A fruit quality trait, average seed number per fruit (SN), was also studied. Fruit number was estimated in February in 1995, 1996 and 1997, with the maximum-recorded number per tree being 200. Average fruit weight, average fruit size and average seed number were evaluated in 1996 and 1997, over roughly 25 fruits per tree.

Marker analysis

Segregation of a total of 69 markers [eight microsatellites, 43 RAPDs, 13 RFLPs, one cleaved amplified polymorphic sequence (CAPS) and four isozymatic loci] was analysed in the whole CxP population as described by García et al. (1999).

Statistical analysis

Fruit number was analysed by two approaches: as a categorical variable ("yield" at any degree versus "no yield") and as a continuous one (measured as number of fruits per tree) excluding those trees that yielded no fruit. For the categorical approach, the χ^2 test of independence between each marker locus and phenotype for "yield" versus "no yield" was computed. Yate's *P* values were used for the significance level.

QTL analysis was based on the individual linkage maps for *C. volkameriana* (V) and *P. trifoliata* (P). Putative QTLs for average fruit weight, average fruit size, average seed number and fruit number as a continuous variable were studied using the MapQTL 3.0 computer programme (Van Ooijen and Maliepaard 1996). Since all the traits studied were not normally distributed, a non-parametric test based on the Kruskal-Wallis methodology was also considered. Bonferroni adjustment was used to obtain an overall protection level of 0.05.

QTLs were named as *Fn* (fruit number), *Fw* (average fruit weight), *Fs* (average fruit size) and *Sn* (average seed number) followed by a number. Genomic regions were named as V, for the female map, or P, for the male map, plus the linkage group number. Unlinked markers were named as NL followed by a number.

Since QTLs associated with different traits were sometimes detected in the same genomic region, a correlation analysis between traits was performed for every year. The stability across years was studied by correlation analysis between years for every trait.

Results

Only 33 trees yielded fruit in all 3 years 1995, 1996, and 1997, whereas 14 did not produce fruit at all. The percentage of non-producing trees per year was 31, 43 and 34, respectively. Mean values of fruit number (FN), average fruit size (FS), average fruit weight (FW) and average seed number (SN) in the progeny across the years and in the parents *C. volkameriana* and *P. trifoliata* in 1997 are shown in Table 1. Fruit number and average fruit weight means varied over the 3 years, both being maximum at 1996.

Significant results of χ^2 tests of independence between each marker locus and phenotypes for "yield" versus "no yield" are shown in Table 2. One putative QTL (*Fn1*) for the female parent and two for the male parent (*Fn2* and *Fn3*) were identified.

Table 1 Means and standard errors for fruit number, average seed number, average fruit weight and average fruit size in the progeny and parental lines

Trait	Code	Progeny			Parentals (1997)	
		1995	1996	1997	Poncirus	Volkamer
Fruit number	FN	30.04±0.57	92.89±2.40	34.40±0.93	80	100
Average seed number	SN		12.5±0.9	11.6±0.2	28±1.2	21.8±1.2
Average fruit weight	FW (g)		55.0±1.5	33.6±0.3	17.9±0.5	146.7±6.1
Average fruit size	FS (cm)		15.2±0.1	13.3±0.1	11±0.1	21.8±0.4

When we considered fruit number as a continuous variable and excluded the non-yielding trees, several QTLs were found (Table 3). Eight genomic regions were identified as being associated with FN in the female parent (NL_1, NL_2, NL_3, NL_4, V3, V4, V7 and V9) and 8 in the male parent (NL_5, NL_6, NL_7, NL_8, NL_9, P3, P4 and P5). Two of the three genomic regions identified in the previous analysis (containing *Fn1* and *Fn3*) were also found in this analysis. Only 1 QTL per linkage group is reported except for P4 and P5 where linked marker alleles were associated with gene effects of opposite direction. Only 4 QTLs (*Fn*1, *Fn9*, *Fn3* and *Fn17*) were detected in more than 1 year. *Fn3* showed opposite gene effects depending on the year.

Table 2 QTL analysis by χ^2 test. LG: linkage group

LG	Marker	QTL	χ2 (1995)	χ2 (1996)
C. volkameriana				
NL_1	OPG13190	*Fn1*	15.6	
P. trifoliata				
P2	OPO13140	*Fn2*	12.2	
P4	OPD07060	*Fn3*	16.2	9.5

Table 4 shows the significant associations of markers with FS, FW and SN. Three genomic regions showed an association with FS in the female parent (V2, V7 and V8) and 2 in the male parent (NL_{10} and NL_{11}). Two linkage groups were identified affecting FW in the female parent (V2 and V7) and 1 genomic region in the male parent (NL_{12}). Five genomic regions were identified associated with SN in the female parent (NL_{13}, NL_{14}, NL_{15}, V7 and V8) and 3 genomic regions in the male parent (NL_{16}, NL_{17} and P1). Only 3 putative QTLs involved in the number of seeds per fruit were detected in 1996 and 1997. QTLs for fruit weight were detected only in 1996, the year of the heaviest fruits.

Most RAPD markers were scored as presence versus absence of a given band. Genetic interpretation of the isoenzymatic markers, GOT-2 and PGI-2; microsatellite markers, TAA1, TAA45, CAC23 and TAA52; and the RFLPs markers, pRLc3 and pRLc11 is shown in Fig. 1. These markers were associated with QTLs for the traits fruit number, average fruit size, average fruit weight and average seed number. Their distribution through the genome is quite scattered (Fig. 2). Two genomic positions (CAC23 at V7 and OPG19400) involve both fruit number and size QTLs. Marker alleles associated with increased number of fruits at V7 (*Fn9*) are also associated

Table 3 Detection of QTLs for fruit number by the Kruskal-Wallis methodology. Means and standard errors for this trait are calculated by averaging all individuals having the allele shown in parenthesis

Linkage group	QTL	Marker	1995 (mean ± SE)[a]		SL[b]	1996 (mean ± SE)[a]		SL[b]	1997 (mean ± SE)[a]		SL[b]
C. volkameriana											
NL_1	*Fn1*	OPG13190	22.0±1.1(p)	34.8±1.0(n)	**	65.9±4.1(p)	109.4±3.3(n)	*	23.1±1.9(p)	45.3±1.9(n)	*
NL_2	*Fn4*	OPD07086	22.9±1.5(n)	31.7±0.9(p)	*						
NL_3	*Fn5*	OPK16120	23.1±1.3(n)	33.7±0.9(p)	*						
NL_4	*Fn6*	TAA1				74.7±3.6(b)	105.7±3.5(a)	*			
		OPB05087				52.1±4.2(p)	101.7±2.8(n)	*			
V3	*Fn7*	Egp47				84.3±10.5(b)	96.3±2.7(a)	**			
		Idh				71.5±6.9(b)	101.3±2.5(c)	*			
V4	*Fn8*	OPB05050							49.5±2.3(p)	16.0±2.7(n)	*
		TAA45							49.8±1.8(b)	11.6±0.5(c)	**
		Got2							31.6±1.5(a)	37.7±2.2(b)	*
		CAC23	26.8±0.8(a)	54.2±1.3(b)	*				27.6±1.5(a)	31.5±0.9(b)	*
V7	*Fn9*	OPD07070	27.6±1.4(n)	31.5±0.9(p)	*				22.8±1.4(n)	42.2±1.8(p)	*
		OPE04180							22.5±4.0(p)	48.1± 0.9(n)	*
		OPD07090	31.6±3.4(p)	36.0±1.7(n)	*				19.6±2.6 (p)	39.8±2.6 (n)	*
V9	*Fn10*	Pgi2							32.1±5.5 (a)	49.0±3.5 (b)	*
P. trifoliata											
NL_5	*Fn11*	OPB05040	29.7±1.1(b)	31.0±1.5(c)	*						
NL_6	*Fn12*	OPG09060							24.1±1.5(p)	39.8±2.7(n)	*
NL_7	*Fn13*	OPG09130	28.1±0.91(p)	21.3±1.1(n)	*						
NL_8	*Fn14*	OPG19400	24.2±0.9(p)	36.2±1.3(n)	*						
NL_9	*Fn15*	OPO13150	31.8±0.7(p)	21.9±2.5(n)	**						
P3	*Fn16*	OPG09125				101.3±2.9(p)	75.7±6.2(n)	*			
		OPG09120				101.0±3.1(n)	63.1±6.9(p)	*			
P4	*Fn3*	OPD07060	33.8±1.9(p)	27.1±0.8(n)	**	55.8±4.6(p)	111.3±2.9(n)	**			
	Fn17	PRLc3	26.8±1.2(b)	32.0±1.0(a)	*	63.9±4.8(b)	106.9±2.9(a)	**			
	Fn18	OPD07087				68.5±10.8(p)	55.1±1.9(n)	**			
P5	*Fn19*	gp47				70.2±14.4(a)	95.7±2.1(b)	*			
		OPG19050				61.0±9.1(p)	98.4±2.7(n)	*			

[a] RAPD alleles, n, null allele; p, preserence; a, b, c, d, alleles
[b] Significane level. * $P \leq 0.05$, ** $P \leq 0.01$

Table 4 Similar information than Table 3. QTLs named *Fs* are for average fruit size, *Fw* for average fruit weight, and *Sn* for average seed number

Linkage group	QTL	Marker	1996 (mean ± SE)		SL	1997 (mean ± SE)		SL
C. volkameriana								
V2	*Fs1*	pRLc11	16.6±0.3(a)	14.4±0.2(b)	*			
		CAC23	16.2±0.2(a)	14.6±0.1(b)	*			
V7	*Fs2*	OPD07070	16.2±0.2(n)	14.6±0.2(p)	*			
		OPE04180	16.4±0.2(p)	14.6±0.1(n)	*			
V8	*Fs3*	OPF14070				13.8±0.2(a)	13.5±0.2(b)	*
V2	*Fw1*	PRLc11	53.9±1.1(a)	44.8±2.1(b)	*			
		CAC23	83.0±7.1(a)	41.6±0.8(b)	**			
V7	*Fw2*	OPD07070	83.0±7.1(n)	41.6±0.8(p)	**			
		OPE4180	86.6±8.8(p)	42.6±0.9(n)	**			
NL_{13}	*Sn1*	OPE04100				15.1±0.5(p)	10.0±0.3(n)	**
NL_{14}	*Sn2*	OPF14040	25.9±4.7(p)	5.5±0.3(n)	**	15.4±0.5(p)	10.4±0.2(n)	*
NL_{15}	*Sn3*	OPG13190	1.8±0.1(p)	17.8±1.6(n)	**	9.4±0.3(p)	13.1±0.3(n)	*
V7	*Sn4*	OPD07090	12.4±1.1(p)	6.1±0.5(n)	*			
V8	*Sn5*	OPG13110	27.1±5.4(p)	6.1±0.3(n)	**			
P. trifoliata								
NL_{10}	*Fs4*	OPG19400	14.3±0.2(p)	15.8±0.2(n)	*			
NL_{11}	*Fs5*	TAA1				12.0±0.2(b)	14.5±0.33(c)	*
NL_{12}	*Fw3*	OPO13160	69.2±2.1(p)	40.1±0.8(n)	*			
NL_{16}	*Sn6*	OPG13130	10.5±0.6(p)	13.5±0.8(n)	*			
NL_{17}	*Sn7*	OPG13090	22.5±3.7(p)	5.5±0.3(n)	*			
P1	*Sn8*	TAA52	21.0±2.6(n)	5.3±0.3(b)	*			
		cG13				14.9±0.4(c)	10.7±0.6(d)	*

with more seeds per fruit (*Sn4*). On the contrary, the marker allele at OPG19400 (linked to *Fn14* and *Fs4*) associated with increased fruit number is also associated with a larger size of the fruit although these QTLs were detected only in *P. trifoliata*. Two of the three putative fruit weight QTLs are located in similar positions as 2 fruit size QTLs. In these cases, the same marker alleles are associated with both increased fruit weight and fruit size.

Correlation analysis between traits per year was significant only between FS and FW in the 2 years studied. Significant correlation values between years were only found for FN and FW. Fruit number showed a significant correlation across the 3 years, but only when all trees (including the non-yielding trees) were considered. If those trees were not included, no significant correlation was found between 1996 and 1997.

Discussion

Segregating populations derived from two pure lines are the most convenient experimental designs for QTL detection, and the most commonly used statistical analysis are suited for them. On the other hand, fruit tree progenies usually arise from crosses between heterozygous genotypes where linkage phases are unknown and more than two alleles are usually segregating per polymorphic locus. The number of polymorphic loci depends primarily on the level of heterozygosity of the parental lines limiting the number of genomic regions to be studied. In addition, dominant markers such as RAPDs are poorly informative in this type of experimental design (Conner et al. 1998). Another limitation of the experimental design is that the additivity/dominance ratio of a QTL cannot be estimated even using codominant markers if the parental lines present four alleles because homozygous classes are lacking. With respect to the agronomic traits we evaluated, other limitations of the study were the number of fruit-yielding trees of the CxP progeny and the non-normal distribution of the traits. This is the reason why a non-parametric test was used. The results were compared with those using standard procedures based on normal theory: single marker analysis comparing trait means within male- or female-derived haplotypes; by phenotype on marker regression and composite interval mapping for only those markers segregating as a backcross (like in the pseudo-test-cross design described by Grattapaglia et al. 1996; M_iM_j x M_iM_i for the *C. volkameriana* genetic map, or M_iM_i x M_iM_j for *P. trifoliata*). Some QTLs detected with those methodologies were also detected with the non-parametric approach. These QTLs are *Fn1, Fn2, Fn3, Fn8, Fn9, Fw2, Sn4* and *Sn5*. Therefore, important differences in the number and distribution of QTLs were found, depending on the statistical methodology used, when the traits are not normally distributed.

Nineteen putative QTLs (8 in *C. volkameriana* and 11 in *P. trifoliata*) controlling the number of fruits per tree were detected, indicating the complexity of this trait in the CxP progeny. There are several well-known factors contributing to the complexity of fruit number in citrus –

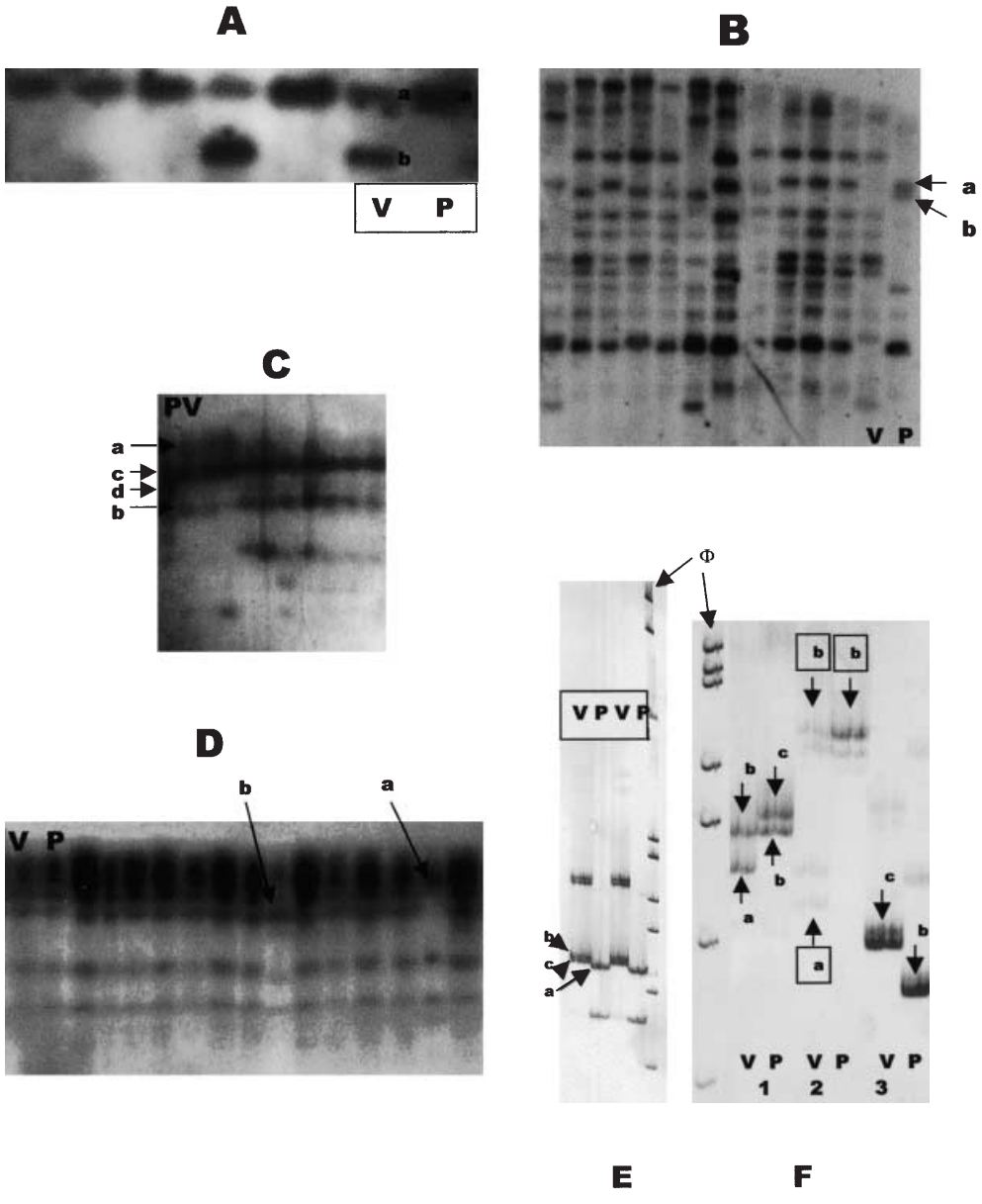

Fig. 1A–F Allele codification (*a, b, c, d*) at marker loci (other than RAPDs) associated with QTLs (Tables 3 and 4). *V C. volkameriana*, *P P. trifoliata*. **A, B** correspond to RFLPs pRLc11 and pRLc03, respectively. **C, D** are GOT and PGI zymograms. **E, F** are microsatellites TAA45 (**E**), TAA1 (**1F**), CAC23 (**2F**) and TAA52 (**3F**). The genotype of *C. volkameriana*, following the order from **A** to **F**, is *ab nn ab ab bc ab ab cn*; the genotype for *P. trifoliata* is *aa ab cd ab aa bc bb bn*. The letter *n* stands for null allele. For gp47 and Egp47 and cG13 see García et al. 1999. Φ Molecular weight marker (*HaeIII* fragments of Φ174 RF)

autonomic parthenocarpy (setting of fruit without pollen stimulation), fruit-set after pollination, fruitlet abscission and maintenance of fruit on the tree, etc. (Spiegel-Roy and Goldschmidt 1996). It is commonly known in citrus that the presence of seeds in the fruit is associated with a larger fruit size and less fruitlet abscission. Marker alleles related to increased FN are also related to increased seed number. Two genomic regions in *C. volkameriana* contain QTLs governing both traits, number of fruits and number of seeds per fruit: V7 (*Fn9* and *Sn4*) and OPG13190 (*Fn1* and *Sn3*). Given that most non-yielding CxP trees flowered, juvenility is not involved and, hence, they must be sterile and non-parthenocarpic. So, fruit number QTLs in Table 2 must be related to these characteristics. The *p* allele at OPG13190 is associated with no fruit setting (*Fn1* in Table 2), low fruit number (*Fn1* in Table 3) and very low seed number (*Sn3* in Table 4); therefore, this marker must be related to female sterility. On the other hand, *Fn2* and/or especially *Fn3* (from Table 2) might be involved in parthenocarpy since no QTL for seed number is located nearby.

Regarding the direct relationship between SN and FS, marker alleles at V7 and V8 associated with increased FS are also associated with increased SN. Also at V7, marker alleles associated with increased FN are associated with smaller FS. Therefore, there exists a genetic basis for the association among these three traits that is against the objectives of the breeders (abundant, large and seedless fruits). Recombination between FS and SN QTLs could break this association, at least in the cases of V7 and V8.

Most QTL analysis over years in forest and fruit trees have revealed important effects of GxE interaction on QTL detection: in almond (Asíns et al. 1994), apple

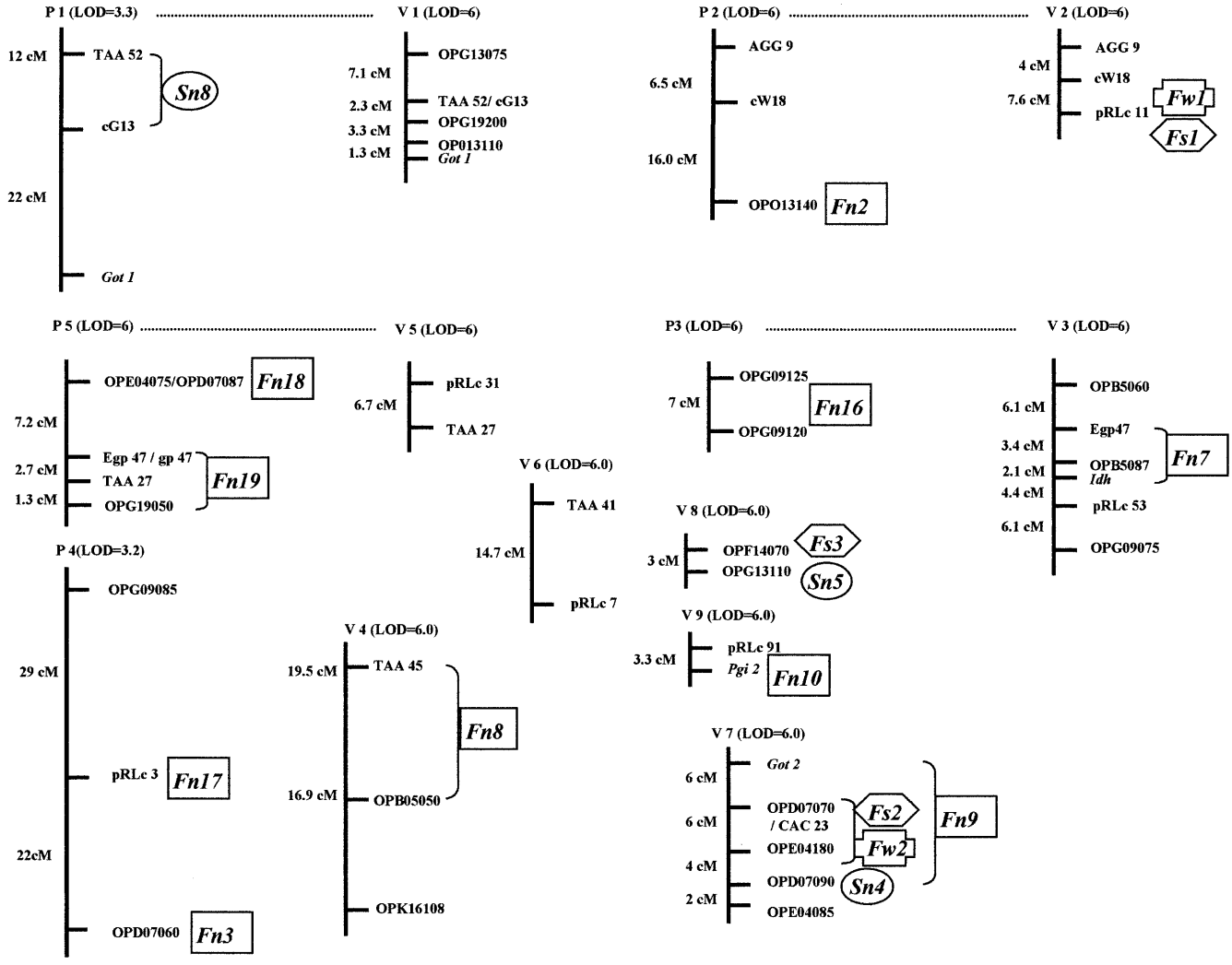

Fig. 2 Approximate locations of QTLs in the linkage maps for *C. volkameriana* and *P. trifoliata*. QTLs associated with unlinked markers are not shown

(Conner et al. 1998), *Pinus* (Emeberi et al. 1998; Kaya et al. 1999). This differential QTL detection depending on the environment is normally referred as a lack of stability in the expression of the majority of the identified QTLs across years. This is generally, and wrongly, interpreted by some authors as false QTLs instead of a possible cause of GxE interaction at the molecular level. In our case, although some FN and SN QTLs are consistent through years, most of them are not. CxP means for FN, FW and FS are also markedly different among years and due to the presence of alternate-bearing trees – the CxP population is not exactly the same over the years for fruit yield. Therefore, besides random fluctuations because of the low number of fruit-yielding trees, differences in QTL detection over years might be explained by the alternate-bearing of some genotypes, which is not uncommon in fruit trees. Contrary to FN, for which the maximum number of QTLs were found for the year in which most trees yielded fruits (1995) and therefore QTL detection had better statistical power, most FS and all FW QTLs were detected the year of the largest fruits (1996). Temperature has a considerable effect on pollination efficiency, influencing the rate of pollen-tube growth as well as bee activity (Spiegel-Roy and Goldschmidt 1996), but no important differences regarding temperature or seed number means were found among years. On the other hand, differences regarding the amount of rain recorded during the 3 months prior to harvesting were found between 1996 (174.4 mm) and 1997 (147.4 mm). In 1996 fruits were much heavier and slightly larger than in 1997 (Table 1). Therefore, the scarceness of water in the field must have limited the final growth of fruits, probably limiting the expression of FW QTLs in the 1997 harvest. Following the nomenclature by Monforte et al. (1997), these QTLs could be considered drought "response-sensitive" QTLs. Another case of the effect of the year on QTL detection is a "stable" QTL, *Fn3* (Table 3), where opposite directions of gene effects were found depending on the year.

From the practical point of view, QTL analysis is a valuable methodology by which to study the complexity of quantitative agronomic traits. However, the low number of fruit-yielding trees per segregating population and the experimental design (cross between heterozygous ge-

notypes) greatly limit QTL detection, providing only a minimum number of QTLs actually involved in the expression of the trait. Due to the non-normal distribution of traits, the contribution of the QTLs detected was not estimated by the statistical test used here but it is reasonable to assume that we have detected those with the strongest effect. It is clear from the present results that the improvement of citrus cultivars by hybridisation can result in certain correlated responses between fruit number and size, and seed number being disassociated through recombination, thereby allowing citrus breeders to better obtain their objectives than by simply using mutational breeding. Nevertheless, the utilisation of QTL-linked markers to assist selection must be taken with care. The alleles and their effects at QTLs in the *Citrus* species of horticultural value, such as *C. sinensis* (L.) Osb. or *C. clementina* Hort. ex Tan., can be different in our parental genotypes (*C. volkameriana* and *P. trifoliata*), even though the inheritance of the traits (number and position of QTLs) and the influence of the environment on QTL expression are similar.

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References

Asíns MJ, Mestre PF, García JE, Dicenta F, Carbonell EA (1994) Genotype x environment interaction in QTL analysis of an intervarietal almond cross by means of genetic markers. Theor Appl Genet 89:358–364

Byrne M, Murell JC, Owen JV, Kriedemann P, Williams ER, Moran GF (1997) Identification and mode of action of quantitative trait loci affecting seedling height and leaf area in *Eucalyptus nitens*. Theor Appl Genet 94:674–681

Conner PJ, Brown SK, Weeden NF (1998) Molecular-maker analysis of quantitative traits for growth and development in juvenile apple trees. Theor Appl Genet 96:1027–1035

Dirlewanger E, Pascal T, Zuger C, Kervella J (1996) Analysis of molecular markers associated with powdery mildew resistance genes in peach (*Prunus persica* (L) Batsch) × *Prunus davidiana* hybrids. Theor Appl Genet 93: 909–919

Emeberi LC, Devey ME, Matheson AC, Slee MU (1998) Age-related changes in the expression of QTLs for growth in radiata pine seedlings. Theor Appl Genet 97: 1053–1061

García R, Asíns MJ, Forner J, Carbonell E (1999) Genetic analysis of apomixis in *Citrus* and *Poncirus* by molecular markers. Theor Appl Genet 99: 511–518

Grattapaglia D, Bertolucci FLG, Penchel R, Sederoff R (1996) Genetic mapping of Quantitative Trait Loci controlling growth and wood quality traits in *Eucalyptus grandis* using a maternal half-sib family and RAPD markers. Genetics 144: 1205–1214

Kaya Z, Sewell MM, Neale DB (1999) Identification of quantitative trait loci influencing annual height- and diameter-increment growth in loblolly pine (*Pinus tadea* L.) Theor Appl Genet 98: 586–592

Knott SA, Neale DB, Sewell MM, Haley CS (1998) Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. Theor Appl Genet 94: 810–820

Monforte AJ, Asíns MJ, Carbonell EA (1997) Salt tolerance in *Lycopersicon* species. VI. Genotype-by-salinity interaction in quantitative trait loci detection: constitutive and response QTLs. Theor Appl Genet 95: 706–713

Socias i Company R (1998) Fruit tree genetics at a turning point: the almond example. Theor Appl Genet 96: 588–601

Spiegel-Roy P, Goldschmidt EE (1996) Biology of citrus. Cambridge University Press, Cambridge, UK

Van Ooijen JW, Maliepaard C (1996) MapQTL(tm) version 3: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen, the Neterlands

Wu RL (1998) Genetic mapping of QTLs affecting tree growth and architecture in *Populus*: implication for ideotype breeding. Theor Appl Genet 96: 447–457

Wu R, Bradshaw HD, Stettler RF (1998) Developmental quantitative genetics of growth *in Populus*. Theor Appl Genet 97: 1110–1119