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Candidate gene analysis of quantitative trait variation in flowering time in almond [*Prunus dulcis* (Mill.) D.A. Webb]

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SUMMARY – The identification of genes involved in flower development and blooming time could be important to assist breeders in creating new late flowering cultivars as well as to understand the complex process of flower induction in a fruit species like almond. A major gene and some quantitative trait loci (QTLs) for blooming time were previously detected and localized on the *Prunus* genome. The aim of this work was to associate these QTLs to known genes using a candidate gene approach. Two cDNAs putatively encoding protein homologs to *Arabidopsis* flower genes were isolated from almond flower buds. A *Prunus* EST database was screened and ten ESTs were selected by their matches with genes known to be involved in flower development in other plant species. Molecular markers were developed from these candidate genes for mapping on the almond 'Texas' x peach 'Earlygold' F_2 population. Twelve candidate genes were assigned to seven linkage groups in the *Prunus* genome.

Key words: Almond, blooming time, CAPS, candidate genes, EST.

RESUME *– "Analyse de gènes candidats pour la variation de caractères quantitatifs concernant la période de floraison chez l'amandier [*Prunus dulcis *(Mill.) D.A. Webb]". L'identification de gènes impliqués dans le temps de floraison et le développement de la fleur peut être importante pour aider les créateurs à sélectionner de nouveaux cultivars à floraison tardive, ainsi qu'à comprendre le processus complexe d'induction florale dans une espèce fruitière comme l'amandier. Un gène important et quelques quantitative trait loci (QTLs) pour le temps de floraison ont été précédemment détectés et localisés sur le génome de* Prunus*. Le but du travail était d'associer ces QTLs à des gènes connus en utilisant une approche de gènes candidats. Deux cDNAs codant putativement pour des homologues de protéines de gènes de fleur d'*Arabidopsis *ont été isolées dans des bourgeons de fleur d'amande. Une base de données d'EST de* Prunus *a été examinée et dix ESTs ont été choisis pour leur homologie avec des gènes impliqués dans le développement floral d'autres espèces. Des marqueurs moléculaires ont été développés à partir de ces gènes candidats pour les placer sur la carte génétique de la population F_2 du croisement 'Texas' (amandier) x 'Earlygold' (pêcher). Douze gènes candidats ont été assignés à sept groupes de linkage sur le génome de* Prunus.

Mots-clés : *Amandier, temps de floraison, CAPS, gènes candidats, EST.*

Introduction

Among temperate fruit crops, almond [*Prunus dulcis* (Mill.) D.A. Webb; *Syn. Prunus amygdalus*] has the earliest blooming time. Early blooming is a disadvantage because pollination (essential for fruit production) depends on meteorological conditions and also because flower damage by frost has an obvious negative effect on year production (Kester and Asay, 1975). Because of all the problems associated with early booming, the Late Blooming trait has been a main target in almond breeding programs.

Blooming time in almond seems to be determined by the *Lb* gene (with late bloom dominant over early bloom) and by quantitatively inherited genes (Socias i Company *et al.*, 1998a,b; Socias i Company, 1998). The *Lb* gene was mapped using the progeny from a cross between 'D35' (a *Lb* heterozygous almond variety) and 'Bertina' (a variety with unknown origin) (Ballester *et al.,* 2001). Furthermore, the interspecific cross between the almond variety 'Texas' and the peach variety 'Earlygold', which was segregating for various agronomic traits, was used to map QTLs (quantitative

trait loci) controlling blooming time (Joobeur, 1998). To date the sequences of both, the *Lb* gene and the genes controlling QTLs for blooming time remain unknown.

Among the possible strategies used to identify these genes, the "Candidate Gene" (CGs) approach to genetic mapping and QTL analysis (de Vienne *et al.*, 1999) still appears to be the simplest one for *Prunus*. CGs are already sequenced genes of known or presumed function that could correspond to major loci or QTLs. In this context, Expressed Sequences Tags (ESTs) with homology to genes with known function in model plants can also be a source of CGs. An efficient strategy, which has been extensively tested in humans, relies on the use of ESTs to discover Single Nucleotide Polymorphisms (SNPs). SNPs can be used to map the candidate gene.

The "Prunus Genome Database" (http://www.genome.clemson.edu/) provides access to the genetics and genomic data of peach and almond, including ESTs from these two species and constitutes a very useful source of information for studies on *Prunus* species.

This database contains 9984 ESTs isolated from a peach mesocarp cDNA library and 2794 ESTs from an almond developing seed cDNA library. All sequences were compared against the GenBank nr protein database and significant matches ($EXP < 1^{e-9}$) were recorded.

The purpose of this study was to map potential candidate genes with homology to flowering genes in an effort to understand the molecular basis for quantitative trait variation in flowering time. In this paper we report the map location of 2 genes and 10 ESTs, homologous to genes presumably involved in flowering in other species, on a *Prunus* saturated linkage map (Joobeur *et al.*, 1998). The same F_2 population used for QTL was used for segregation analysis of the candidate genes showing polymorphisms.

Materials and methods

Search of candidate genes

In a preliminary step, we used degenerated primers to isolate two CND's, from almond flower buds, that showed sequence homology to the *Arabidopsis* flower genes *AGAMOUS*-like 11 and *LEAFY*. Additionally, among the peach and almond dbEST (http://www.genome.clemson.edu/), 8 peach and 2 almond ESTs (see Table 1) were chosen for their significant match with genes involved in the molecular control of flowering transition.

Table 1. Almond and peach ESTs selected as CGs based on their homologies

Locus	Species	Clone length	Sequence identity (%)	Putative ID	Organism
PpAp1	Peach	796	81	MADS-box protein 2	*Malus x domestica*
PpFT	Peach	605	86	hd3a	*Oryza sativa*
PpAgl2	Peach	657	73	Agamous-like MADS box protein AGL2.	*Arabidopsis thaliana*
PpFLC	Peach	622	50	Flowering Locus C protein (MADS box protein FLOWER)	*Arabidopsis thaliana*
PpFRI	Peach	599	48	Frigida protein	*Arabidopsis thaliana*
PdTFL	Almond	609	75	Terminal flower 1 (tfl1) putative	*Arabidopsis thaliana*
PdGA20	Almond	636	88	Gibberellin 20-oxidase	*Malus x domestica*
PpLD	Peach	604	41	Homeobox protein LUMINIDEPENDENS	*Arabidopsis thaliana*
PpAP2	Peach	1075	60	Apetala 2-like protein	*Pisum sativum*
PpCO	Peach	642	86	Constans-like protein 1	*Malus x domestica*

Pd = *Prunus dulcis*; Pp = *Prunus persica.*

Mapping of candidate genes

The highly polymorphic F_2 population, developed from the interspecific cross 'Texas' x 'Earlygold', was used for segregation analysis of the candidate genes. For all candidate genes, specific primers were designed, and genomic fragments from both parents were PCR amplified. All fragments were sequenced and homologies were confirmed by BLAST analysis. Pairwise sequence alignments between the parental lines revealed DNA polymorphisms, mainly single nucleotide changes (SNPs). SNPs were used to develop co-dominant cleaved amplified polymorphic sequence (CAPS) markers for mapping.

The segregation data obtained were added to the corresponding data set of markers and linkage analysis was performed using the MAPMAKER/EXP v. 3.0 software (Lander *et al.*, 1987). Recombination units were converted into map distances using the Kosambi function.

Results

All 10 ESTs, as well as the 2 almond cDNAs, were polymorphic for the mapping population, which allowed assigning them to 7 linkage groups in the *Prunus* genome.

Polymorphisms observed were mainly SNPs (7), although 4 INDELS and 1 SSR were also detected. As expected, polymorphisms were mainly localized in introns (Table 2). It was possible to use SNPs to map CGs as CAPS markers. INDELS were mapped based on differences in fragment size, except for PdFRI that had an insertion of only 1 bp in the 'Texas' parent, which generated a differential restriction site.

Table 2. Characterization of polymorphisms observed for each candidate gene, restriction nucleases used in each case for CAPS markers and linkage groups where each CG was located

Locus	Polymorphism T/E characterization			Restriction nuclease	Linkage group
PdLFY	INDEL	+65 bp in E	Intron	-	V
PdMADS1	SNP	G/C	Intron	Alu I	I
PdAp1	SNP	G/T	Intron	Taq I	V
PdFT	INDEL	+40 bp in T	Intron	-	VI
PdAgl2	SNP	T/G	Intron	HpA I	III
PdFLC	SNP	C/T	Cod. Reg.	Hae III	VII
PdFRI	INDEL	+1 bp in T	Intron	Msl I	VIII
PdTFL	INDEL	+7 bp in E	Intron	-	V
PdGA20	SNP	T/C & C/T	Intron	Hinf I	II
PdLD	SNP	T/C	Cod. Reg.	Bsu36 I	I
PdAP2	SSR	$(TA)^7/(TA)^4$	Intron	-	VI
PdCO	SNP	A/G	Cod. Reg.	Alw I	I

Candidate genes in shadow background refer to almond cDNAs cloned from flower buds using degenerated primers. All the other CGs genes refer to *Prunus* sp. ESTs.

Data on map co-segregation between candidate genes and QTLs is still under analysis. From preliminary results two associations are observed, as indicated in Fig. 1.

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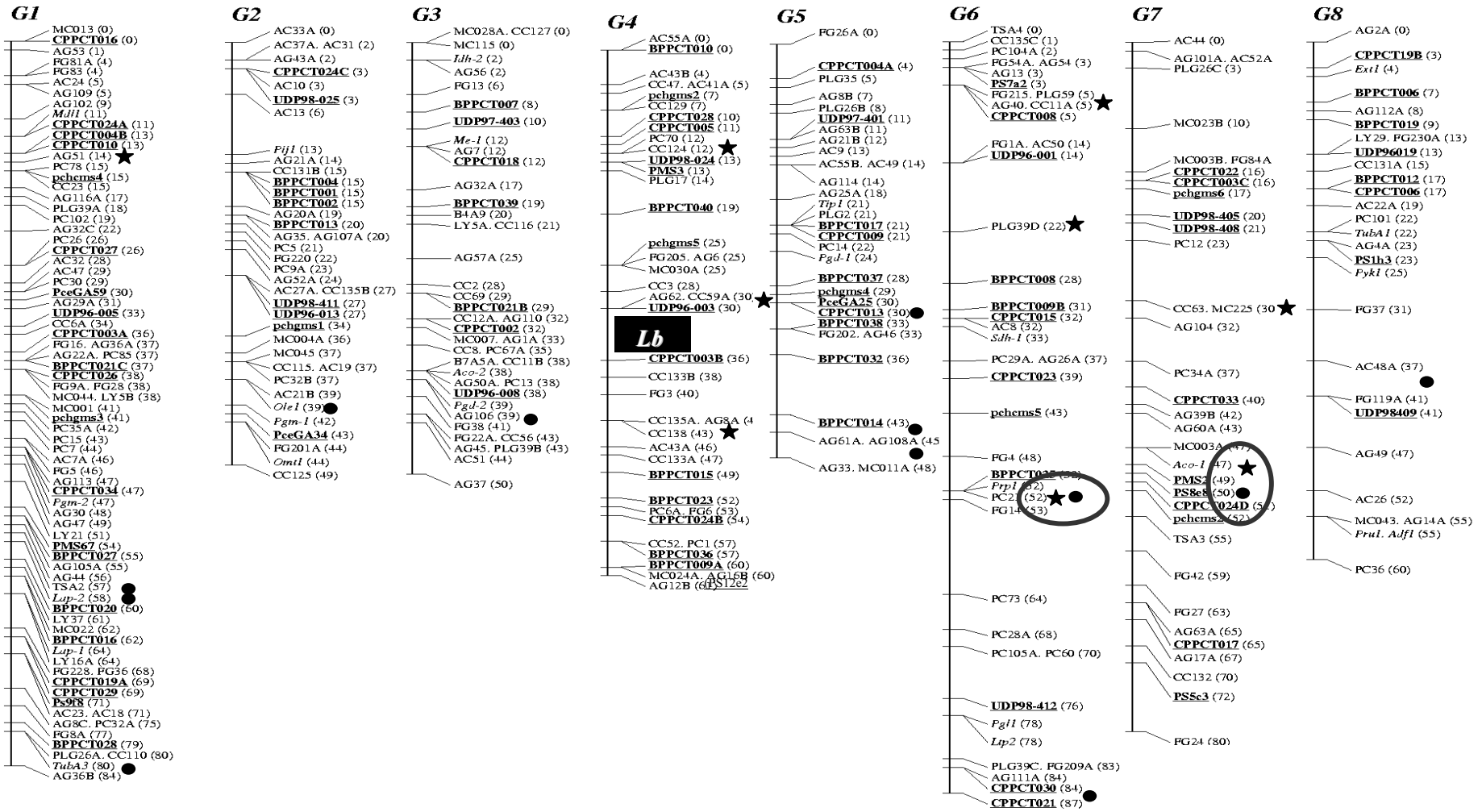

Fig. 1. Map of the 'Texas' x 'Earlygold' F_2 population with the markers located by Joobeur *et al*. (1998) plus the SSRs mapped by Aranzana *et al.* (2003). Dots indicate location of floral candidate genes and stars QTLs for blooming time (Joobeur, 1998). The major gene Lb is located in group 4. Possible associations between CGs and QTLs are highlighted with circles.

References

Aranzana, M.J., Pineda, A., Cosson, P., Dirlewanger, E., Ascasibar, J., Cipriani, G., Rider, C.D., Testolin, R., Abbott, A., King, G.J., Iezzoni, A.F. and Arús, P. (2003). A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. *Theor. Appl. Genet.*, 106: 819-825.

Ballester, J., Socias i Company, R., Arús, P., de Vicente, M.C. (2001). Genetic mapping of a major gene delaying blooming time in almond. *Plant Breed.*, 120: 268-270.

de Vienne, D., Leonardi, A., Damerval, C. and Zivy, M. (1999). Genetics of proteome variation for QTL characterization: application to drought-stress responses in maize. *J. Exp. Bot.*, 50: 303-309.

Joobeur, T. (1998). *Construcción de un mapa de marcadores moleculares y análisis genético de caracteres agronónicos en* Prunus*.* Tesis Doctoral. Universitat de Lleida, Lleida, Spain.

Joobeur, T., Viruel, M.A., de Vicente, M.C., Jáuregui, B., Ballester, J., Dettori, M.T., Verde, I., Truco, M.J., Messeguer, R., Batlle, I., Quarta, R., Dirlewanger, E., Arús, P. (1998). Construction of a saturated linkage map in *Prunus* using an almond x peach F_2 progeny. *Theor. Appl. Genet.*, 97: 1034-1041.

Kester, D.E. and Asay, R. (1975). Almonds. In: *Advances in Fruit Breeding*, Janick, J. and Moore, J.N. (eds). Purdue University Press, West Lafayette, Indiana, pp. 387-419.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Newburg, L. (1987). MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics,* 1: 174-181.

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

Socias i Company, R., Felipe, A.J. and Gómez Aparisi, J. (1998a). Genetics of late blooming in almond. *Acta Horticulturae (ISHS),* 484: 261-266.

Socias, R., Felipe, A.J. and Gómez Aparisi, J. (1998b). Approche génétique de la floraison tardive chez l'amandier. *Cahiers Options Méditerranéennes,* 33: 163-169.