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Fine-mapping of the bitterness locus in almond

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Abstract. During domestication, almond kernels have been selected for low content of amygdalin, a cyanogenic glucoside responsible for bitterness. The taste of the almond kernel is under monogenic control, with the dominant sweet kernel (*Sk*) allele associated to sweetness and the recessive *sk* allele associated to bitterness. Since most of the cultivated almonds are heterozygous, new bitter almond seedlings are usually obtained during breeding programs and it is valuable to develop molecular markers enabling assisted selection. Although its biochemical function remains unidentified, the *SK* gene has been localised on linkage group five (G5) in the almond genetic linkage map obtained from the cross between the two heterozygous genotypes ‘R1000’ and ‘Desmayo Largueta’ (RxD). We report here the identification of several SNPs over a 3.7 Mb peach (*Prunus persica* L.) physical region, which is synthenic to the one containing the *Sk* locus in almond. Some of them were converted into cleaved amplified polymorphic DNA (CAPS) markers suitable for assisted selection. Further studies are in progress aiming to the functional characterization of candidate genes for controlling the phenotype.

Keywords. Almond – Fine mapping – Bitterness – Amygdalin – SNPs – HCN.

Cartographie fine du locus de l’amertume dans l’amande

Résumé. *Au cours de la domestication, les amandes ont été sélectionnées pour une faible teneur en amygdaline, un glucoside cyanogène responsable de l’amertume. Le goût de l’amande est sous un contrôle monogénique, avec l’allèle dominant (Sk) associé à la douceur du noyau et l’allèle récessif de (sk) associée à l’amertume. Comme la plupart des amandes cultivées sont hétérozygotes, de nouveaux plants d’amandes amères sont habituellement obtenus au cours des programmes de sélection et il est utile de développer des marqueurs moléculaires permettant la sélection assistée. Bien que sa fonction biochimique reste non identifié, le gène SK a été localisé sur le groupe de liaison cinq (G5) dans la carte d’amande obtenue à partir d’un croisement entre les deux génotypes hétérozygotes ‘R1000’ et ‘Desmayo Largueta’ (RxD). Nous rapportons ici l’identification de plusieurs SNP plus de 3,7 Mb de pêche* (Prunus persica *L.) de région physique, qui est synthenic à celui contenant le locus Sk en amande. Certains d’entre eux ont été convertis en (CAPS) (cleaved amplified polymorphic DNA) marqueurs appropriés pour la sélection assistée. Des études supplémentaires sont en cours visant à la caractérisation fonctionnelle des gènes candidats pour commander le phénotype.*

Mots-clés. *Amandier – Cartographie génetique – Amertume – Amygdaline – SNPs – HCN.*

I – Introduction

Bitterness is one of the most studied traits in almond (*Prunus dulcis* Miller D.A. Webb syn. *Prunus amygdalus* Batsch). This is due to the degradation of amygdalin, a cyanogenic diglucoside, which also releases cyanide, toxic for animals and humans (McCarty *et al*., 1952; Conn, 1980; Poulton, 1990; Swain *et al*., 1992 and Sánchez-Pérez *et al*., 2008).

The bitter or sweet taste has a monogenic control in almond, the *sweet kernel* (*Sk*) allele being dominant over the bitter kernel (*sk*) allele (Heppner, 1923; Dicenta and García, 1993 and Sánchez-Pérez *et al*., 2010). Bitter is the original taste of kernels of wild almond species. Due to an unknown mutation and human domestication, most of the cultivated almonds are sweet and heterozygous at the *Sk* locus. The inheritance of bitterness in almond is controlled by the seed mother genotype (Heppner, 1923; Kester and Assay, 1975; Kester and Gradziel, 1996; Dicenta and Garcia, 1993; Dicenta *et al*., 2000 and 2007; and Sánchez-Pérez *et al*., 2010). Therefore, in practical breeding programs, in which two heterozygous varieties are crossed, 25% of the seedlings will be bitter and therefore need to be removed (Grasselly and Crossa-Raynaud, 1980; Dicenta and Garcia, 1993).

The selection process is complicated by the almond long juvenile period, as breeders have to wait three to four years to analyze traits related to reproductive organs. For this reason, it would very useful to develop molecular markers to distinguish between sweet and bitter kernelled seedlings in the nursery during the first year, just after the germination of seedlings (Sánchez-Pérez *et al.,* 2010). Previous studies have shown that the *Sk* locus is localised in almond linkage group five (G5) (Joobeur *et al*., 1998, Sánchez-Pérez *et al*., 2008). Almond G5 was saturated with SSRs and six of them (UDA-045, EPDCU2584, CPDCT028, BPPCT037, PceGA025 and CPDCT016) were found very close to the *Sk* locus.

In this work we try to shorten this region with new CAPS molecular markers in order to fine mapping the G5.

II – Materials and methods

Aiming to find a *Sk*-ortholog region in the peach genome, a BLAST analysis against the Genomic Database of Rosaceae (www.rosaceae.org) (Jung *et al*., 2008) was carried out, using sequences of *Sk*-linked SSR markers as query. Peach genes included in this region were sequenced on the two genotypes 'R1000' (R) and 'Desmayo Largueta' (D), aiming to identify single nucleotide polymorphisms (SNPs).

Afterwards, five new cleaved amplified polymorphic sequence (CAPS) markers linked to the *Sk* locus were designed by means of the CAPS designer tool available at the SolGenomics website (http://solgenomics.net/).

DNA of a large F_1 population (550 individuals), originating from the RxD cross, was isolated according to a CTAB method (Doyle and Doyle, 1990), and used as template for marker analysis with the CAPS above mentioned and the *Sk*-linked SSRs UDA-045, EPDCU2584, BPPCT037 and CPDCT028 (Sánchez-Pérez *et al*., 2010). Marker scoring was performed for CAPS using Metaphor® agarose gel electrophoresis (Lonza), and for SSR with the ABI Prism 3500 Genetic Analyzer (AB HITACHI).

Mapping was performed through the JoinMap 4.1 software (Van Ooijen, 2001), using the ML algorithm and a LOD score threshold value of 3.0.

III – Results and discussion

BLAST search, using sequences of *Sk*-linked almond markers, resulted in hits all localizing on the peach LG5 chromosomal region, suggesting the identification of a synthenic region containing the *Sk* ortholog.

Two SSR markers previously shown to flank the *Sk* locus (EPDCU2584 and CPDCT028) were found to delimit a peach genomic region of about 800 Kb. We used five genes included in this region to identify SNP polymorphisms between the R and D genotypes and develop CAPS markers saturating the *Sk* region. An example of CAPS, requiring simple gel electrophoresis is shown in Fig. 1.

Notably, mapping of newly developed CAPS markers and four previously reported SSR markers revealed full overlap with the peach physical map (Fig. 2), thus substantiating the notion that *Prunus* genomes are colinear (Dirlewanger *et al*., 2004).

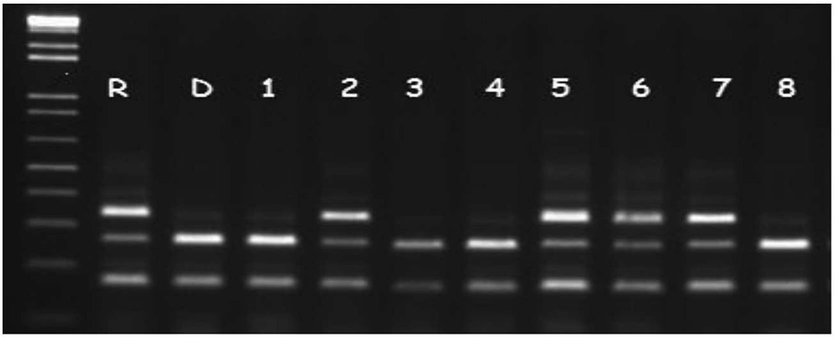

Fig. 1. Segregation analysis of R1000 (R), Desmayo Largueta (D) and 8 segregant F_1 individuals by means of one of the newly developed CAPS markers.

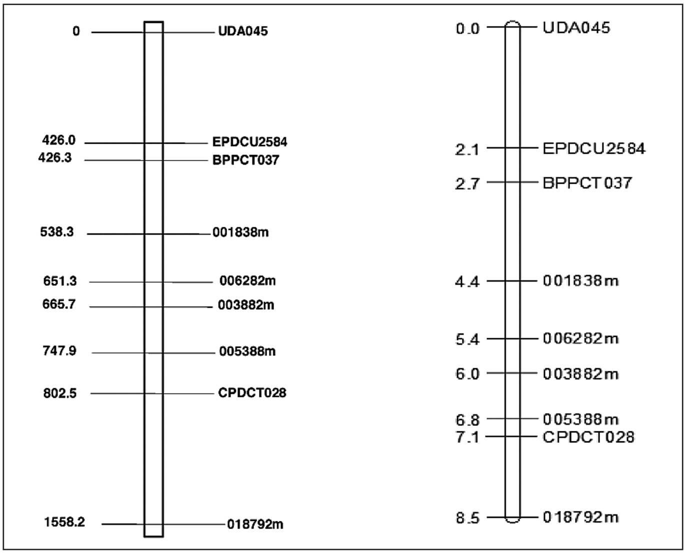

Fig. 2. Co-linearity between the physical map of peach GL5 (left) and the genetic map of almond (right). Markers distances are indicated in Kb and cM, respectively.

At the moment, we are assaying the phenotyping of the F_1 RxD population, aiming to fine-map the *Sk* locus. We expect that our work will be of significant value for marker-assisted selection of bitterness in almond, thus by-passing limitations due to phenotypic screenings. Moreover, this is expected to provide useful information for the positional isolation of the *Sk* gene and reveal molecular mechanisms underlying bitterness in almond.

IV – Perspectives

Currently, we are characterizing the F1 population with respect to the kernel taste. In the meantime, we are carrying out a de novo sequencing of the almond *Sk* genomic region and transcriptome analysis of the tegument in sweet and bitter almonds.

Our results, based on syntheny between peach and almond, pave the way to the positional cloning of the *sk* gene. Moreover, they could be useful to assist the selection of sweet or bitter almonds in practical breeding programs.

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References

Conn E., 1980. Cyanogenic compounds. In: *Annu. Rev. Plant Physiol.,* 31, p. 433-451.

Dicenta F. and García J.E., 1993. Inheritance of the kernel flavor in almond. In: *Heredity,* 70, p. 308-312.

Dicenta F., Martínez-Gómez P. and Ortega E., 2000. Cultivar Pollinizer Does Not Affect Almond Flavor. In: *HortScience,* 35, p. 1153-1154.

Dicenta F., Ortega E. and Martinez-Gomez P., 2007. Use of recessive homozygous genotypes to assess the genetic control of kernel bitterness in almond. In: *Euphytica,* 153, p. 221-225.

Doyle J.J. and Doyle J.L., 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12, p. 13-15.

Dirlewanger E., Graziano E., Joobeur T., Garriga-Calderé F., Cosson P., Howad W. and Arús P., 2004. Comparative mapping and marker-assisted selection. In: *Rosaceae Fruit Crops*, vol. 101, no. 26.

Grasselly C. and Crossa-Raynaud P., 1980. *L’amandier*. Maisonneuve et Larose, Paris, 446 p.

Heppner J., 1923. The factor for bitterness in the sweet almond. In: *Genetics,* 8, p. 390-392.

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

Jung S., Staton M., Lee T., Blenda A., Svancara R., Abbott A. and Main D., 2008. GDR (Genome Database for Rosaceae): integrated web-database for Rosaceae genomics and genetics data. Nucleic Acids Research, 36 (Database Issue) – D1034 – D1040.

Kester D.E. and Asay R.N., 1975. Almonds. In: *Advances in fruit breeding*. Editors: Janick J. and Moore J.N. Purdue University Press, West Lafayette. Indiana, p. 387-419.

Kester D.E. and Gradziel T.M., 1996. Almonds (Prunus) In: *Fruit Breeding*. Editors: Moore, J.N. and Janick, J. New York: Wiley, p. 1-97.

McCarty C.D., Leslie J.W. and Frost H.B., 1952. Bitterness of kernels of almond x peach hybrids and their parents. In: *Proc. Am. Soc. Hortic. Sci.*, 59, p. 254-258.

Poulton J.E., 1990. Cyanogenesis in plants. In: *Plant. Physiol.*, 94, p. 401-405.

Sánchez-Pérez R., Jørgensen K., Erik Olsen C., Dicenta F. and Møller B.L., 2008. Bitterness in almonds. In: *Plant Physiol.,* 146, p. 1040-1052.

Sánchez-Pérez R., Howad W., Garcia-Mas Jordi., Arús P., Martínez-Gómez P. and Dicenta F., 2010. Molecular markers for kernel bitterness in almond. In: *Tree Genetics and Genomes,* 6, p. 237-245.

Swain E., Li C.P. and Poulton J.E., 1992. Development of the potential for cyanogenesis in maturing black cherry (*Prunus serotina* Ehrh.) fruits. In: *Plant Physio.,* 98, p. 1423-1428.

Van Ooijen J.W., 2001. JoinMap4, Software for the calculation of genetic linkage. Plant research international. Wageningen, The Nederlands.