QTL mapping for flower characters using 'Guara' × 'Nurlu' F_1 population in almond

A. Paizila[1], S. Kafkas[1], K. Ercik[2], N.E. Kafkas[1], E.Z. Motalebipour[1], I. Acar[3] and N. Turemis[1]

[1]Department of Horticulture, Faculty of Agriculture, University of Çukurova, 01330 Sarıçam, Adana, Turkey; [2]Alata Horticultural Research Institute, Mersin, Turkey; [3]University of Harran, Department of Horticulture Faculty of Agriculture, Sanliurfa, Turkey.

Abstract

Almond is the only nut tree in *Rosaceae* family and it is one of the most important nut crops in Turkey. Construction of genetic linkage maps and quantitative trait locus (QTLs) analysis are important tools to develop markers linked to economically important phenotypic characters. Simple sequence repeat (SSR) based linkage map in almond using 'Guara' × 'Nurlu' F_1 population was constructed and flower characters such as color, full blooming date and position of anthers were phenotypically characterized in the population during three consecutive years. The consensus map included a total of 153 SSR markers in eight linkage groups (LGs). The number of mapped markers changed between 15 (LG4) and 25 (LG2), and the length of the LGs varied from 49.8 (LG6) cm to 85.1 cm (LG1). An average number of SSR markers per linkage group was 19. The average distance between markers was 3.2. Two QTLs for full blooming in LG1 and LG7, two QTLs for the position of anthers in LG3 and in LG8, and one QTL for flower color in LG6 were detected.

Keywords: almond, SSR, QTL, flower

INTRODUCTION

Almond (*Prunus dulcis* (Mill) D.A.Webb) along with other fruit crops, such as peach, sweet cherry and apricot belongs to the *Prunoideae* subfamily of *Rosaceae*, valued for its health benefits and high nutritional value. The importance of the crop is increasing in the human diet, and consequently its production and commercial value are growing worldwide.

Almond is considered as one of oldest tree crops that has been domesticated (Spiegel-Roy, 1986) and has been grown in the Mediterranean Basin at least for the last 2,500 years (Grasselly and Crossa-Raynaud, 1980). Being one of the major producers in the region, almond is very important in Turkey. However, most of the almond cultivars grown in Turkey are local cultivars which are self-incompatible, early blooming, and low in productivity. So, it is necessary to develop new almond cultivars that are well adapted to local growing conditions.

Like other fruit trees, conventional breeding takes long time. Genetic and genomic tools can help us to overcome these challenges by improving efficiency and reducing labor and time. Well-assessed almond populations and saturated genetic maps are essential for breeding programs in order to investigate the molecular and genetic controls of the important agronomical traits. Most of the important agronomical traits are quantitatively inherited, so determining their genetic basis is a difficult task (Brem and Kruglyak, 2005).

The first genetic map in almond was constructed with a 'Ferranges' × 'Tuono' F_1 population using restriction fragment length polymorphism (RFLP) and isozyme markers (Arus et al., 1994). Then several authors constructed genetic linkage maps of almonds using different F_1 populations and several molecular marker techniques such as ISSR, SSR and SNP. After that several genetic maps were constructed in almond (Sánchez-Pérez et al., 2007a; Tavassolian et al., 2010; Donoso et al., 2016; Goonetilleke et al., 2018). There is also a reference genetic linkage map for *Prunus* using an inter-specific F_2 population (Dirlewanger et al., 2004).

Based on the genetic linkage maps mentioned above a few quantitative trait loci (QTLs) were identified. Most of the studies were focused on the important traits of almond, such as

self-incompatibility, flowering time, kernel quality, tree productivity, active compounds in the nut (Socias i Company and Felipe, 1999; Sánchez-Pérez et al., 2007a).

In this study, an F_1 population derived from a cross between 'Guara' × 'Nurlu' cultivars was genotyped using SSR markers. Main objectives of this study are: (i) to develop relatively well-saturated linkage maps; and (ii) to identify flowering-related QTLs, such as full blooming date, flower color and relative position of anther to the stigma and to provide powerful tools for further breeding applications in almond.

MATERIALS AND METHODS

In this study, a total of 105 F_1 progenies derived from a cross between 'Guara' × 'Nurlu' population were used. 'Nurlu' is a local cultivar, and is mainly grown in Aegean region of Turkey. The F_1 plants were planted during 2011-2012 winter in Pistachio Research Institute in Gaziantep province of Turkey.

Fresh young leaves were collected from the progenies as well as from the parents for DNA extraction and SSR analysis. The fresh leaves were lyophilized in a freeze-dryer, and stored at +4°C until DNA extraction. DNA was extracted by CTAB method (Doyle and Doyle, 1987) with some minor modifications (Kafkas et al., 2005).

During the years 2015, 2016 and 2017 the following flowering characteristics were evaluated in 'Guara' × 'Nurlu' F_1 population: (i) full blooming date (natural days from 1 January) until 95% of the flowers were open; (ii) flower color (white = 1, bright pink = 2, pink = 3); (iii) relative position of stigma to anther (below = 1, equal = 3, above = 5).

275 SSR markers were selected for this study from previous genetic maps in *Prunus* mostly from Dirlewanger et al. (2004) and Howad et al., (2005). We firstly conducted gradient PCR reactions to determine optimum annealing temperatures of the primer pairs, and PCR products were evaluated after 1.5% agarose gel electrophoresis. SSR-PCR were carried out using a three-primer strategy according to Schuelke (2000) with some modifications (Motalebipour et al., 2016). The PCR products were analyzed by capillary electrophoresis in ABI 3130xl genetic analyzer (Applied Biosystems Inc., Foster City, Calif.). The fragments were resolved using ABI data collection software 3.0, and SSR fragment analysis was performed with GeneScan Analysis Software 4.0 (ABI).

For the genetic linkage map construction, five different segregation patterns were scored ('ab × cd' and 'ef × eg' having 1:1:1:1 segregation, 'hk × hk' having 1:2:1 segregation, 'lm × ll' and 'nn × np' having 1:1 segregation). The consensus and parental linkage maps were constructed using the JoinMap v4.1 software (Van Ooijen, 2011). The markers were tested for segregation distortion using the chi-square test before they were used for linkage mapping. A LOD threshold of 5 was chosen to construct the linkage groups of almond. The final version of the genetic maps was drawn using the Map Chart 2.2 Software (Voorrips, 2002). Interval mapping function of MapQTL (version 5.0) software was used in QTL analysis (Van Ooijen, 2004). The value of the limit of detection (LOD) above 2 was used for determination of significant QTLs.

RESULTS AND DISCUSSION

Of tested 275 SSR primer pairs, 159 segregated and the consensus linkage map was constructed with 153 SSR markers along 8 linkage groups of almond. The number of mapped common markers was 94 (61.4%), and the total map length was 493.9 cm, with an average marker distance of 3.2 cm between markers. The LG length varied from 49.8 cm (LG5) to 85.1 cm (LG1). The average length of the linkage groups was 64.1 cm. The average marker distance varied from 2.3 cm (LG2) to 4.3 cm (LG7). The number of markers in 8 LGs were changed between 15 (LG4) and 25 (LG2). The average marker density (marker cm^{-1}) was between 0.24 (LG7) and 0.43 (LG2) (Table 1).

The parental maps were also constructed and they used in the QTL analysis. From a total of three traits, 5 QTLs were detected in the parental maps (Figure 1). Blooming date is a very important agronomic trait in almond, because it is susceptible to early frost damage that effects the productivity. Blooming date in almond is a polygenic trait (Kester et al., 1977), which can be explained by a major Late blooming (Lb) gene and quantitatively

modified by other minor genes. Lb gene was mapped in LG4 by Ballester et al. (2001) in 'Tardy' × 'Nonpareil' population. Silva et al. (2005) mapped QTLs related to flowering time in an interspecific cross F_1 population of almond × peach and mapped in LG1, LG2, LG3, LG5, LG6, and LG7. (Sánchez-Pérez et al., 2007b; Martínez-Gómez et al., 2012) has mapped the flowering time QTLs in LG1, LG6, and LG7. In this study, we found QTLs for full blooming date in LG1 in 2015 and 2017, and in LG7 in three consecutive years in both of our parental maps (Figure 1).

Table 1. Mapped SSR markers in consensus map of 'Guara' × 'Nurlu' F_1 population.

LG	No of SSR loci	Length (cm)	Distance between markers	Marker cm^{-1}
1	22	85.1	3.9	0.26
2	25	57.9	2.3	0.43
3	22	64.2	2.9	0.34
4	15	53.4	3.6	0.28
5	16	49.8	3.1	0.32
6	20	51.4	2.6	0.39
7	17	72.3	4.3	0.24
8	16	59.9	3.7	0.27
Total	153	493.9		
Average	19.1	61.7	3.2	0.31

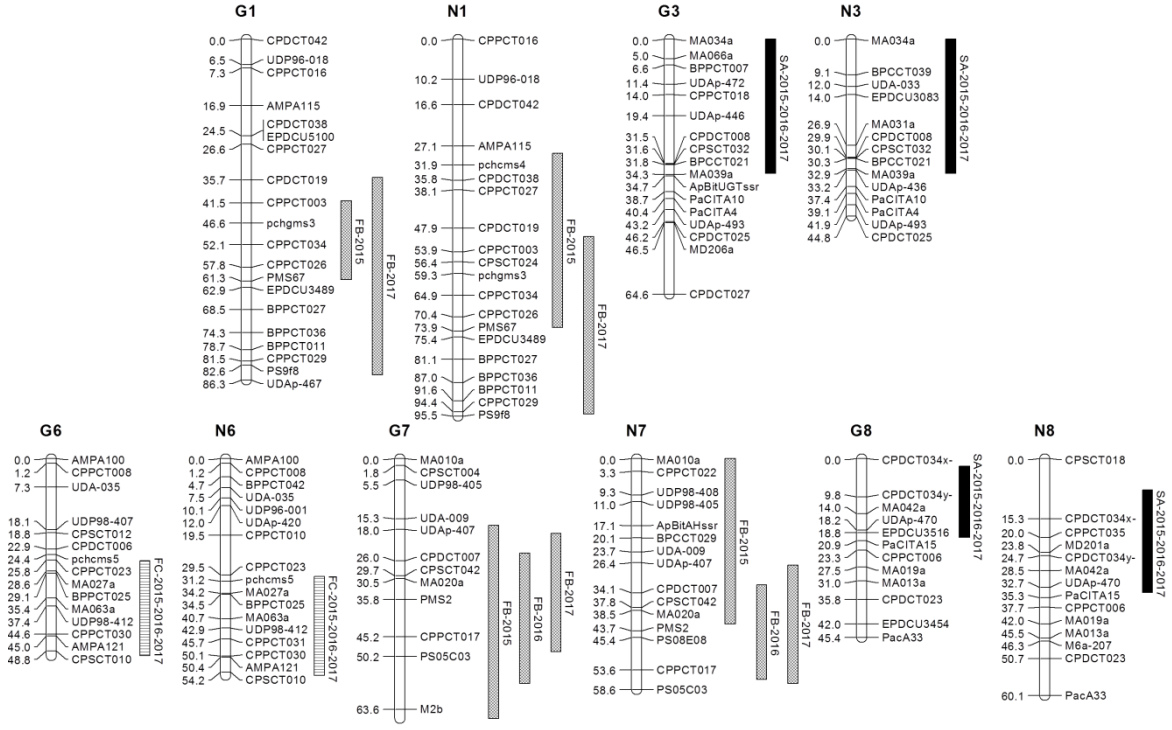

Figure 1. QTLs detected in the parental maps of 'Guara' (G) × 'Nurlu' (N) F_1 population in almond.

Flower color and relative position of stigma to anther have not been studied in almond. The relative position of stigma to the anther is important for successful pollination of

self-compatible almonds. Both characters were stable during three consecutive years. The flower color was mapped in LG6, and the relative position of stigma to the anthers was mapped in LG3 and LG8 in both of parental linkage maps (Figure 1).

CONCLUSIONS

In this study, we constructed genetic linkage maps of almond using 'Guara' × 'Nurlu' F_1 population. A total of 153 SSR markers were mapped. We detected five QTLs for the three flower characters using phenotypic data obtained during three consecutive years. The linkage maps can be used in QTL analysis of other economically important phenotypic traits in almond in the future.

ACKNOWLEDGEMENTS

The authors thank Republic of Turkey Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policies (TAGEM/14/ARGE/01) and Çukurova University Scientific Research Projects Unit (Project ID. FYL-2015-3640, FUK-2015-3628) for the financial supports.

Literature cited

Arus, P., Messeguer, R., Viruell, M., Tobutt, K., Dirlewanger, E., Santi, F., Quartas, R., and Ritter, E. (1994). The European *Prunus* mapping project Progress in the almond linkage map. Euphytica *77* (*1-2*), 97–100 https://doi.org/10.1007/BF02551469.

Ballester, J., Socias i Company, R., Arus, P., and De Vicente, M.C. (2001). Genetic mapping of a major gene delaying blooming time in almond. Plant Breed. *120* (*3*), 268–270 https://doi.org/10.1046/j.1439-0523.2001.00604.x.

Brem, R.B., and Kruglyak, L. (2005). The landscape of genetic complexity across 5,700 gene expression traits in yeast. Proc. Natl. Acad. Sci. U.S.A. *102* (*5*), 1572–1577 https://doi.org/10.1073/pnas.0408709102. PubMed

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. Proc. Natl. Acad. Sci. U.S.A. *101* (*26*), 9891–9896 https://doi.org/10.1073/pnas.0307937101. PubMed

Donoso, J.M., Picanol, R., Serra, O., Howad, W., Alegre, S., Arus, P., and Eduardo, I. (2016). Exploring almond genetic variability useful for peach improvement: mapping major genes and QTLs in two interspecific almond × peach populations. Mol. Breed. *36* (*2*), 16 https://doi.org/10.1007/s11032-016-0441-7.

Doyle, J.J., and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. *19*, 11–15.

Goonetilleke, S.N., March, T.J., Wirthensohn, M.G., Arús, P., Walker, A.R., and Mather, D.E. (2018). Genotyping by sequencing in almond: SNP discovery, linkage mapping and marker design. G3 (Bethesda) *8* (*1*), 161–172 https://doi.org/10.1534/g3.117.300376. PubMed

Grasselly, C., and Crossa-Raynaud, P. (1980). L'Amandier (Paris, France: G.P. Maisonneuve et Larosa), pp.446.

Howad, W., Yamamoto, T., Dirlewanger, E., Testolin, R., Cosson, P., Cipriani, G., Monforte, A.J., Georgi, L., Abbott, A.G., and Arús, P. (2005). Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. Genetics *171* (*3*), 1305–1309 https://doi.org/10.1534/genetics.105.043661. PubMed

Kafkas, S., Özkan, H., and Sütyemez, M. (2005). DNA polymorphism and assessment of genetic relationships in walnut genotypes based on AFLP and SAMPL markers. J. Amer. Soc. Hort. Sci. *130* (*4*), 585–590.

Kester, D.E., Hansche, P.E., Bres, W., and Asay, R.N. (1977). Variance components and heritability of nut and kernel traits in almond. J. Am. Soc. Hortic. Sci. *102*, 264–266.

Martínez-Gómez, P., Sánchez-Pérez, R., and Rubio, M. (2012). Clarifying omics concepts, challenges, and opportunities for *Prunus* breeding in the postgenomic era. OMICS *16* (*5*), 268–283 https://doi.org/10.1089/omi.2011.0133. PubMed

Motalebipour, E.Z., Kafkas, S., Khodaeiaminjan, M., Çoban, N., and Gözel, H. (2016). Genome survey of pistachio (*Pistacia vera* L.) by next generation sequencing: development of novel SSR markers and genetic diversity in *Pistacia* species. BMC Genomics *17* (*1*), 998 https://doi.org/10.1186/s12864-016-3359-x. PubMed

Sánchez-Pérez, R., Ortega, E., Duval, H., Martínez-Gómez, P., and Dicenta, F. (2007a). Inheritance and relationships of important agronomic traits in almond. Euphytica *155* (*3*), 381–391 https://doi.org/10.1007/s10681-006-9339-5.

Sánchez-Pérez, R., Howad, W., Dicenta, F., Arus, P., and Martinez-Gomez, P. (2007b). Mapping major genes and

quantitative trait loci controlling agronomic traits in almond. Plant Breed. *126* (*3*), 310–318 https://doi.org/10.1111/j.1439-0523.2007.01329.x.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. *18* (*2*), 233–234 https://doi.org/10.1038/72708. PubMed

Silva, C., Garcia-Mas, J., Sánchez, A.M., Arús, P., and Oliveira, M.M. (2005). Looking into flowering time in almond (*Prunus dulcis* (Mill) D.A. Webb): the candidate gene approach. Theor. Appl. Genet. *110* (*5*), 959–968 https://doi.org/10.1007/s00122-004-1918-z. PubMed

Socias i Company, R., and Felipe, A.J. (1999). 'Blanquerna', 'Cambra' y 'Felisia': tres nuevos cultivares autógamos de almendro. Inf. Técn. Econ. Agrar. *95*, 111–117.

Spiegel-Roy, P. (1986). Domestication of fruit trees. In The Origin and Domestication of Cultivated Plants, C. Barigozzi, ed. (Amsterdam, Netherlands: Elsevier), p.210–211.

Tavassolian, I., Rabiei, G., Gregory, D., Mnejja, M., Wirthensohn, M.G., Hunt, P.W., Gibson, J.P., Ford, C.M., Sedgley, M., and Wu, S.B. (2010). Construction of an almond linkage map in an Australian population Nonpareil x Lauranne. BMC Genomics *11* (*1*), 551–561 https://doi.org/10.1186/1471-2164-11-551. PubMed

Van Ooijen, J.W. (2004). MapQTL 5.0, software for the mapping of quantitative trait loci in experimental populations (Wageningen, Netherlands: Kyazma B.V.).

Van Ooijen, J.W. (2011). JoinMap® 4.1, software for the calculation of genetic linkage maps in experimental populations. (Wageningen, Netherlands: Kyazma B.V.).

Voorrips, R.E. (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. *93* (*1*), 77–78 https://doi.org/10.1093/jhered/93.1.77. PubMed