Use of stylar ribonucleases in almond breeding

Batlle I., Romero M.A., Vargas F.J., Tanriver E., Lakhal H.

in

Ak B.E. (ed.).
XI GREMPA Seminar on Pistachios and Almonds

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 56

2001
pages 111-115

Use of stylar ribonucleases in almond breeding

I. Batlle*, E. Tanriver, H. Lakhal***, M.A. Romero* and F.J. Vargas***
*Dept. d'Arboricultura Mediterrània, Centre de Mas Bové, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Apartat 415, 43280 Reus, Spain
**Visiting postgraduate student from Dept. of Horticulture, Faculty of Agriculture, Çukurova University, Adana 01330, Turkey
***Visiting student from Dept. d'Arboriculture et Viticulture, Ecole Nationale d'Agriculture de Meknès, BP S-40 Meknès, Morocco

SUMMARY – There is a correlation between the incompatibility (*S*) alleles in almond and the stylar ribonucleases revealed electrophoretically. This correspondance between the *S* alleles and the pattern of bands of RNases is being used since 1996 at IRTA's breeding programme to breed self-compatible cultivars. This technique is also used in practical breeding as a tool both for designing crosses and selecting self-compatible seedlings. Cultivars, selections and seedlings are analysed for stylar ribonucleases using Non-Equilibrium pH Gradient Electrofocusing and staining for RNase activity which allows their genotyping. Crosses are planned with cultivars or selections of known *S* genotypes. Some crosses are semi-compatible, the two parents having one *S* allele in common and the self-compatible parent is used as the male parent. In a few cases, known S_f homozygous genotypes have been used. In both approaches only self-compatible seedlings are obtained. The inheritance of stylar ribonucleases in almond progenies and their correlation with self-compatibility has also been established. In addition, this technique is also used to assign *S* genotypes to seedlings and selections raised. The rapidity with which results can be obtained and the moderate cost of equipment and consumables has made possible its practical use in our breeding programme.

Key words: Almond, *Prunus dulcis*, breeding, incompatibility, electrofocusing, ribonuclease.

RESUME – *"Utilisation des ribonucléases stylaires pour l'amélioration génétique de l'amandier". Il existe une corrélation entre les allèles (*S*) d'incompatibilité de l'amandier et les ribonucléases stylaires révélées par l'électrophorèse. Cette correspondance entre les allèles* S *et le patron des bandes des RNases a été utilisée dès 1996 dans le programme d'amélioration de l'IRTA pour l'autocompatibilité. Cette technique est aussi utilisée dans l'amélioration pratique comme un outil pour la désignation des croisements et la sélection des descendants autocompatibles. L'utilisation de la technique NepHge (Non-Equilibrium pH Gradient Electrofocusing) et la coloration postérieure du gel pour révéler l'activité des RNases, permet l'attribution des génotypes* S *aux cultivars, sélections et descendance. Les croisements sont planifiés en utilisant des cultivars ou sélections dont les génotypes sont connus. Certains croisements sont semi-compatibles, c'est le cas où les deux parents montrent un allèle* S *en commun et où le parent autocompatible est utilisé comme géniteur mâle. Dans peu de cas, des génotypes homozygotes pour l'allèle* S_f *ont été utilisés. Dans ces deux cas, on obtient une progéniture totalement autocompatible. L'hérédité des ribonucléases stylaires pour l'amandier et leur corrélation avec l'autocompatibilité a été aussi bien établie. En plus, cette technique est utilisée pour l'assignation des génotypes* S *des descendants et des sélections obtenues. La rapidité avec laquelle s'obtiennent les résultats et le coût modéré de l'équipement et des produits utilisés a rendu possible l'utilisation de cette technique dans nos programmes d'amélioration.*

Mots-clés : *Amandier,* Prunus dulcis*, amélioration variétale, incompatibilité, électrofocalisation, ribonucléases stylaires.*

Introduction

Along with classical breeding of almond leading to successful cultivar release at IRTA (Vargas and Romero, 1994) we are developing molecular markers for about 12 years. The first development was the detection of linkages among 10 isoenzyme loci in F_1 segregating progenies (Arús *et al.*, 1994). Second was the construction of an almond linkage map using a 'Ferragnes' x 'Tuono' population with 7 isoenzyme genes and 120 RFLPs (Viruel *et al.*, 1995). And later, mapping using an interspecific F_2 peach x almond ('Texas' x 'Earlygold') population has resulted in a saturated marker linkage map for *Prunus* with 235 markers comprising 11 isoenzymes and 224 RFLPs (Arús, 1996; Joobeur *et al.*, 1998).

Fruit and nut trees have developed different genetic mechanisms to prevent self-fertility. Almond is known to exhibit gametophytic self-incompatibility controlled by a single locus, the *S* locus, with multiple alleles. Gametophytic self-incompatibility allows the pistil to distinguish between own and alien pollen. Thus almond cultivars will not set fruit unless they are pollinated with cultivars from a genetically distinct pollination group. Most almond breeding programmes aim to obtain self-compatible cultivars suitable for single cultivar orchards and less dependent on bee activity for pollination, combined of course with other desirable traits. Currently at Mas Bové early selection in segregating progenies for a range of characters is conducted in the nursery which is useful to handle large populations (Vargas *et al.*, 1997, 1998). We are now starting to use markers to assist cross design and selection in our breeding programme. Recently some American and European cultivars and selections have been characterized for their incompatibility groups by a European team (Boskovic *et al.*, 1997) using stylar ribonucleases. Stylar protein extracts were separated electrophoretically and stained for ribonuclease (RNase) activity which reveals bands corresponding to *S* alleles. This technique can also predict which seedlings are self-compatible and has shown good agreement with field records of fruit set after selfing and with self pollen tube growth scores (Boskovic *et al.*, 1998, 1999). The correspondence demonstrated in almond between the *S* alleles and the pattern of bands of RNases (Boskovic *et al.*, 1997; Tao *et al.*, 1997; Duval *et al.*, 1998), as in other *Prunus* species like sweet cherry (Boskovic and Tobutt, 1996), has hastened the possibilities of using markers. Ribonucleases are the product of the *S* gene in *Prunus*. In practical plant breeding it can be used as a tool for more efficient almond breeding both for designing crosses and selecting seedlings. In addition, this correlation has been used to locate the self-incompatibility gene in group 6 of the almond linkage map (Ballester *et al.*, 1998) and has opened the possibilities of cloning this gene.

The determination of the *S* constitution of cultivars and selections is of practical importance to breeders and growers. The *S* genotypes determined by stylar RNases can be used for: (i) breeding (crossing design, parental choice and seedling selection); and (ii) planting commercial orchards (cultivar choice and pollination design) (Batlle *et al.*, 1997). In this paper a revision of the work on RNases carried out between 1995-99 involving IRTA is given.

Cross design and parental choice

When two almond cultivars are intercrossed the combination may be one of the three types:

(i) Incompatible (both *S* alleles in common): S_xS_y x S_xS_y.
(ii) Semi-compatible (one *S* allele in common): S_xS_y x S_xS_z.
(iii) Fully-compatible (no *S* allele in common): S_xS_y x S_zS_w.

Before each crossing season at Mas Bové (February-March), crosses are planned with cultivars of known *S* genotypes (Boskovic *et al.*, 1997) as presented in Table 1. Each cross is semi-compatible (i.e. the two parents have one S_x allele, S_1 or S_9, in common) and the self-compatible parent is used as the male parent. Only pollen carrying the S_f allele should succeed in these circumstances and thus all the resulting seedlings should be self-compatible. This approach was already suggested by Grasselly *et al.* (1981) and Dicenta and García (1993), but, hitherto, relatively few suitable combinations of parents have been known. In addition, seven crosses have been designed using as male parent two selections (12-1 and 12-21) homozygous for the S_f allele.

Seedling selection

Apart from using RNases for cross design this technique was also used to assign *S* genotypes to 4 self-compatible and 1 self-incompatible GREMPA selection and 15 IRTA selections derived from earlier crosses made to obtain self-compatible seedlings. Although only French and Greek selections were used here, GREMPA material from breeding programmes in various countries (France, Italy, Greece and Spain) was exchanged through the group in 1985/86 for trialling and further use in breeding (Romero and Vargas, 1992). Table 2 shows the genotypes of promising selections and their parents. All cultivar genotypes have been already reported by Boskovic *et al.* (1997, 1998). IRTA selections were RNase genotyped either at HRI East Malling or at IRTA Cabrils or Mas Bové. GREMPA selections were genotyped at Cabrils or Mas Bové. The genotype of GREMPA selection ('Tuono' x 'Ai') 6 was deduced after knowing its inter-incompatibility with 'Ferragnes' (Ch. Grasselly, pers. comm.).

Table 1. Crosses designed considering *S* genotype of parents to obtain wholly self-compatible progenies

Cross	Stylar RNase alleles assigned (*S* genotype)	Expected seedling *S* genotype	No. of flowers pollinated	No. of seeds sown
'Anxaneta' x 12-2	S_2S_9 x S_fS_f	S_2S_f or S_9S_f	113	5
'Anxaneta' x 12-21	S_2S_9 x S_fS_f	S_2S_f or S_9S_f	71	2
'Anxaneta' x 21-133	S_2S_9 x S_9S_f	S_2S_f or S_9S_f	83	9
'Anxaneta' x 21-323	S_2S_9 x S_9S_f	S_2S_f or S_9S_f	733	10
'Glorieta' x 'Falsa Barese'	S_1S_5 x S_1S_f	S_1S_f or S_5S_f	417	47
'Glorieta' x 'Filippo Ceo'	S_1S_5 x S_1S_f	S_1S_f or S_5S_f	169	22
'Glorieta' x 'Tuono'	S_1S_5 x S_1S_f	S_1S_f or S_5S_f	618	73
'Glorieta' x ('Ferralise' x 'Tuono') 18	S_1S_5 x S_1S_f	S_1S_f or S_5S_f	285	40
'Glorieta' x 12-2	S_1S_5 x S_fS_f	S_1S_f or S_5S_f	313	25
'Glorieta' x 12-21	S_1S_5 x S_fS_f	S_1S_f or S_5S_f	212	20
'Masbovera' x 'Genco'	S_1S_9 x S_1S_f	S_1S_f or S_9S_f	700	200
'Masbovera' x 'Tuono'	S_1S_9 x S_1S_f	S_1S_f or S_9S_f	383	60
'Masbovera' x ('Ferralise' x 'Tuono') 18	S_1S_9 x S_1S_f	S_1S_f or S_9S_f	469	85
'Masbovera' x 12-2	S_1S_9 x S_fS_f	S_1S_f or S_9S_f	227	46
'Masbovera' x 12-21	S_1S_9 x S_fS_f	S_1S_f or S_9S_f	361	96
'Masbovera' x 21-133	S_1S_9 x S_9S_f	S_1S_f or S_9S_f	250	51
'Masbovera' x 21-323	S_1S_9 x S_9S_f	S_1S_f or S_9S_f	62	16
'Tarragonés' x 12-2	S_2S_9 x S_fS_f	S_2S_f or S_9S_f	181	52
'Tarragonés' x 21-133	S_2S_9 x S_9S_f	S_2S_f or S_9S_f	352	143
'Tarragonés' x 21-323	S_2S_9 x S_9S_f	S_2S_f or S_9S_f	199	–

Table 2. Promising selections analysed for stylar ribonucleases and *S* genotypes assigned

Selection	Parentage	Parental *S* genotype	Assigned *S* genotype
GREMPA			
INRA	('Ferragnes' x 'Tuono') 36	S_1S_3 x S_1S_f	S_3S_f
INRA	('Ferralise' x 'Tuono') 18	S_1S_3 x S_1S_f	S_1S_f
NAGREF	('Ferragnes' x 'Troito') 13	S_1S_3 x S_1S_f	S_3S_f
NAGREF	('Ferragnes' x 'Troito') 30	S_1S_3 x S_1S_f	S_1S_f
INRA	('Tuono' x 'Ai') 6	S_1S_f x S_3S_f	S_1S_3
IRTA			
12-2	'Lauranne' x 'Desmayo Largueta'	S_3S_f x S_1S_5	S_f S_f
12-21	'Lauranne' x 'Desmayo Largueta'	S_3S_f x S_1S_5	S_f S_f
12-161	'Francolí' x 'Lauranne'	$S_?S_?$ x S_3S_f	S_1S_3
12-221	'Genco' x 'Masbovera'	S_1S_f x S_1S_9	S_9S_f
12-350	'Lauranne' x 'Marcona'	S_3S_f x $S_{11}S_{12}$	$S_{12}S_f$
12-477	'Genco' x 'Masbovera'	S_1S_f x S_1S_9	S_9S_f
12 643	'Genco' x 'Masbovera'	S_1S_f x S_1S_9	S_9S_f
12-645	'Genco' x 'Masbovera'	S_1S_f x S_1S_9	S_9S_f
12-665	'Glorieta' x 'Lauranne'	S_1S_5 x S_3S_f	S_5S_f
12-786	'Genco' x 'Masbovera'	S_1S_f x S_1S_9	S_9S_f
12-798	'Glorieta' x 'Lauranne'	S_1S_5 x S_3S_f	S_1S_f
12-1021	'Glorieta' x 'Lauranne'	S_1S_5 x S_3S_f	S_5S_f
21-133	('Ferralise' x 'Tuono') 18 x 'Anxaneta'	S_1S_f x $S_?S_?$	S_9 S_f
21-323	4-665 ('Primorskiy' x 'Cristomorto') x 'Lauranne'	$S_?S_9$ x S_3S_f	S_9S_f
21-332	4-665 ('Primorskiy' x 'Cristomorto') x 'Lauranne'	$S_?S_9$ x S_3S_f	S_3S_9

Regarding RNase genotyping to detect self-compatible seedlings in almond progenies it is interesting to note that from a cross 'Lauranne' (S_3S_f) x 'Desmayo Largueta' (S_1S_5), from which 38 seedlings were raised, 7 were presumably selfings of the 'Lauranne' parent (S_3S_f) (Boskovic *et al.*, 1999). Two of these S_fS_f seedlings, having a few interesting agronomic characters, have been used for crossing as reported before.

Concluding remarks

A successful almond cultivar must combine a high number of desirable characters, some monogenic and some polygenic. Thus large progenies are needed to produce sufficient seedlings having most of the targeted genes. Almond, although it has a shorter juvenile period before cropping when grown on own roots (3-4 years) than most nut and fruit crops, except peach, has required a large amount of land before selection for vigour, habit, resistance, cropping and, eventually for nut quality (Vargas *et al.*, 1997, 1998). In addition, the limited knowledge of the genetics of most of the important characters slows the process (Socias i Company, 1997, 1998).

Rapid screening of large progenies to eliminate inferior seedlings at the earliest possible stage is essential for efficient breeding programmes. Early selection techniques have been developed for late flowering (Kester *et al.*, 1977), as have preselection methods for tree type and vigour which can be applied in the first two years after germination (Vargas *et al.*, 1997, 1998). Seedling selection is conducted in the nursery (at 12 months for vigour and at 15 months for time of bud break). Assessment of self-pollination is made in the field or in the laboratory after cropping (3-4 years), along with evaluation of tree architecture (growth habit and branching), vigour, production, disease susceptibility and nut quality (shelling percentage, double kernels, kernel appearance and taste). Final testing is in orchard trials (4-6 years) before final assessment. From a large initial population of over 1000 seedlings one new cultivar may be chosen 8-12 years after germination. However, there is scope for improvement in early selection and preselection techniques used.

The use of the *S* genotypes of cultivars and selections, determined by RNase analysis, for cross design would save land and time in cultivar improvement programmes. In addition, commercial cultivar choice regarding cross compatible cultivars for planting new orchards would avoid cropping failures. Apart from bloom overlapping, main cultivar and pollinators, should cross-pollinate satisfactorily. Checking *S* genotypes of desirable cultivars will prevent mistakes when establishing orchards, one case being 'Glorieta' (S_1S_5) and 'Texas' (S_1S_5) which are inter-incompatible.

The use of RNases as a preselection technique is likely to be most appropriate on seedling progenies that have already been reduced in numbers to a few promising seedlings. Although the stylar ribonuclease assay is used to indicate genetically self-compatible seedlings, they should still be field tested to show they are self-fertile enough in practice (Boskovic *et al.*, 1998). This useful technique uses only about 5-10 flowers and is a quicker method for genotyping cultivars than making a series of crosses and assessing pollen tube growth or fruit set, for which more flowers are required. As just explained, it is proving very useful for cross design and genotyping seedlings. The rapidity with which results can be obtained, and the moderate cost of equipment and consumables (except ribonucleic acid) has made possible its effective use in our almond breeding programme though the technique of non-equilibrium pH gradient electrofocusing is not as simple as starch or polyacrylamide gel electrophoresis. In the near future we hope to develop other molecular markers linked to agronomically important characters which would help to speed the process of developing new self-fertile almond varieties.

Acknowledgements

Almond breeding at IRTA Mas Bové is funded by the Ministry of Agriculture, Fisheries and Food (INIA Project SC 97-049). Both Emine Tanriver and Hicham Lakhal are grateful for studentships granted by International Centre for Advanced Mediterranean Agronomic Studies of Zaragoza (CIHEAM-IAMZ).

References

Arús, P. (1996). Developing tools for a more efficient fruit breeding: The European *Prunus* mapping project. *Nucis*, 5: 6-8.

Arús, P., Olarte, C., Romero, M. and Vargas, F.J. (1994). Linkage analysis of ten isozyme genes in F_1 segregating almond progenies. *J. Amer. Soc. Hort. Sci*., 119: 339-344.

Ballester, J., Boskovic, R., Batlle, I., Arús, P., Vargas, F.J. and de Vicente, M.C. (1998). Location of the self-compatibility gene in the almond linkage map. *Plant Breed.*, 117: 69-72.

Batlle, I., Ballester, J., Boskovic, R., Romero, M.A., Tobutt, K.R. and Vargas, F.J. (1997). Use of stylar ribonucleases in almond breeding to design crosses and select self-compatible seedlings. *Nucis*, 6: 12-14.

Boskovic, R., Duval, H., Rovira, M., Tobutt, K.R., Romero, M.A., Dicenta, F. and Batlle, I. (1998). Inheritance of stylar ribonucleases in two almond progenies and their correlation with self-compatibility. In: II International Symposium on Pistachios and Almonds, California (USA), 24-29 August 1997. *Acta Horticulturae*, 470: 118-122.

Boskovic, R. and Tobutt, K.R. (1996). Correlation of stylar ribonuclease zymograms with incompatibility alleles in sweet cherry. *Euphytica*, 90: 245-250.

Boskovic, R., Tobutt, K.R., Batlle, I. and Duval, H. (1997). Correlation of ribonuclease zymograms and incompatibility genotypes in almond. *Euphytica*, 97: 167-176.

Boskovic, R., Tobutt, K.R., Duval, H., Batlle, I., Dicenta, F. and Vargas, F.J. (1999). A stylar ribonuclease assay to detect self-compatible seedlings in almond progenies. *Theor. Appl. Genet*., 99: 800-810.

Dicenta, F. and García, J.E. (1993). Inheritance of self-compatibility in almond. *Heredity*, 70: 313-317.

Duval, H., Batlle, I., Boskovic, R. and Tobutt, K.R. (1998). Confirmation of incompatibility alleles in almond cultivars predicted by analysis of stylar ribonucleases. *Options Méditerranéennes* (in press).

Grasselly, Ch., Crossa Raynaud, P., Olivier, G. and Gall, H. (1981). Transmission du caractère d'autocompatibilité chez l'amandier (*Amygdalus communis*). *Options Méditerranéennes, Series Etudes*, 1: 71-75.

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. and Arús, P. (1998). Construction of a saturated marker linkage map for *Prunus* using an almond x peach F_2 progeny. *Theor. Appl. Genet*., 97: 1034-1041.

Kester, D.E., Raddi, P. and Asay, R.D. (1977). Correlations among chilling requirements for germination, blooming and leafing in almond (*Prunus amygdalus* Batsch). *J. Amer. Soc. Hort. Sci*., 87: 214-221.

Romero, M.A. and Vargas, F.J. (1992). Notas sobre el comportamiento en colección de selecciones de almendro del GREMPA. In: IIX Colloque GREMPA, Nîmes (France), 26-27 June 1990. *Rapport EUR 14081*: 121-125.

Socias i Company, R. (1997). Qualitative traits in almond. *Nucis*, 6: 6-9.

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

Tao, R., Yamane, H., Sassa, H., Mori, H., Gradziel, T.M., Dandekar, A.M. and Sugiura, A. (1997). Inheritance of stylar RNases associated with gametophytic self-compatibility in almond (*Prunus dulcis*). *Plant Cell Physiol*., 38: 304-311.

Vargas, F.J. and Romero, M.A. (1994). 'Masbovera', 'Glorieta' and 'Francolí', three new almond varieties from IRTA. *Acta Horticulturae*, 373: 75-82.

Vargas, F.J., Romero, M.A., Batlle, I. and Clavé, J. (1998). Early selection in almond progenies. In: X GREMPA Seminar, Meknès (Morocco), 1996. *Options Méditerranéennes*, *Series Cahiers*, 33: 171-176.

Vargas, F.J., Romero, M.A., Clavé, J. and Batlle, I. (1997). Early selection in almond breeding. *Nucis*, 6: 9-2.

Viruel, M.A., Messeguer, R., de Vicente, M.C., García Mas, J., Puigdomènech, P., Vargas, F.J. and Arús, P. (1995). A linkage map with RFLP and isozyme markers for almond. *Theor. Appl. Genet*., 91: 964-971.