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Confirmation of incompatibility alleles in almond cultivars predicted by analysis of stylar ribonucleases

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SUMMARY - It had been demonstrated previously at East Malling that the various incompatibility (*S*) alleles of almond cultivars correlate with the stylar ribonucleases revealed electrophoretically. This work showed that 'Desmayo Largueta', an old Spanish cultivar, and 'Glorieta', a cultivar bred at Mas Bové, neither of which had previously been genotyped, have the same pattern of stylar ribonucleases as the French 'Languedoc' and the American cultivar 'Texas'. To check the hypothesis that these cultivars have the same *S* genotype, test crosses were made; two at Mas Bové, 'Glorieta' x 'Desmayo Largueta' and 'Texas' x 'Glorieta', and one at Avignon, 'Glorieta' x 'Texas', in addition to 'Texas' x 'Languedoc' which was known to be incompatible. No fruit was set. This incompatibility suggests that 'Desmayo Largueta' and 'Glorieta' indeed have the same *S* genotype as 'Texas' and 'Languedoc', which was formerly designated S_aS_b, and is now relabelled S_1S_5. This work validates the practical use of stylar ribonuclease analysis for predicting incompatibility alleles of almond cultivars.

Key words: Almond, *Prunus dulcis*, incompatibility, isoelectric focusing, ribonuclease, fruit set.

RESUME - *"Confirmation des allèles d'incompatibilité chez des cultivars d'amandier, prédite par analyse des ribonucléases du style". Il avait été démontré au préalable à East Malling que les différents allèles d'incompatibilité (S) chez les cultivars d'amandier étaient corrélés aux ribonucléases du style, révélées par électrophorèse. Ce travail a montré que 'Desmayo Largueta', un vieux cultivar espagnol, et 'Glorieta', un cultivar planté à Mas Bové, dont aucun n'avait fait l'objet d'étude de génotype, présentent la même disposition pour les ribonucléases du style que la variété française 'Languedoc' et que le cultivar américain 'Texas'. Afin de vérifier l'hypothèse selon laquelle ces cultivars auraient le même génotype S, des tests de croisement ont été réalisés ; deux à Mas Bové, 'Glorieta' x 'Desmayo Largueta' et 'Texas' x 'Glorieta', et un en Avignon, 'Glorieta' x 'Texas', en plus de 'Texas' x 'Languedoc' dont on savait qu'il était incompatible. Il n'y eut pas de mise à fruit. Cette incompatibilité suggère que 'Desmayo Largueta' et 'Glorieta' ont bien le même génotype S que 'Texas' et 'Languedoc', qui était auparavant dénommé S_aS_b et qui s'appelle désormais S_1S_5. Ce travail valide l'utilisation pratique de l'analyse des ribonucléases du style pour la prédiction des allèles d'incompatibilité des cultivars d'amandier.*

Mots-clés : *Amandier,* Prunus dulcis, *incompatibilité, "isoelectric focusing", ribonucléase, mise à fruit.*

Introduction

It is well known that most cultivars of almond (*Prunus dulcis*) are self-incompatible and need cross pollination to set a crop, and that some cultivars are cross-incompatible. Incompatibility in almond is gametophytic and governed by incompatibility (*S*) alleles. Determining the incompatibility relationships of almond cultivars and assigning *S* alleles to them, traditionally needs a series of test crosses in the field. Boskovic *et al.* (1997) at East Malling have recently shown a correlation of *S* alleles with stylar ribonucleases revealed by non-equilibrium pH gradient electrofocusing; under suitable conditions each cultivar produced two principal bands corresponding to the two *S* alleles. The cultivars 'Desmayo Largueta' and 'Glorieta' had the same pattern of stylar ribonucleases as 'Texas' and 'Languedoc' and we predicted they would have the same *S* alleles and therefore be cross-incompatible. The *S* genotype of 'Texas' (syn. 'Mission') and 'Languedoc' was reported by Kester *et al.* (1994) as S_aS_b and relabelled by Boskovic *et al.* (1997) S_1S_5. Test crosses involving these

cultivars were made to test the prediction of cross-incompatibility and are the subject of this paper. (Two of these crosses are already mentioned in Boskovic *et al.* (1997), having been included when that paper was redrafted).

Materials and methods

Two Spanish cultivars, 'Desmayo Largueta' and 'Glorieta', the old French cultivar 'Languedoc' (INRA, R 759) and the American cultivar 'Texas' were used for the test crosses to check the *S* genotypes assigned on the basis of stylar ribonuclease zymograms. 'Desmayo Largueta' is a traditional cultivar widely grown in Spain which produces valued kernels but blooms early, 'Glorieta' ('Primorski' x 'Cristomorto') is a highly productive cultivar recently released from IRTA Mas Bové which blooms late and produces good quality kernels (Vargas and Romero, 1994). 'Texas' is a late blooming, highly productive cultivar grown in California, and has intermediate kernel quality.

The test crosses, 'Glorieta' x 'Desmayo Largueta' and 'Texas' x 'Glorieta', were made at IRTA Mas Bové and, 'Glorieta' x 'Texas' at INRA Avignon. The cross 'Texas' x 'Languedoc', known to be incompatible, was also made at Avignon. Flowers were collected at the balloon stage and anthers were removed and placed in a Petri dish to dehisce. Pollen was stored in vials in a desiccator at 4ºC for a few days until use. The viability of the collected pollen at Mas Bové was established by observation of germination on 10% sucrose in 5% agar with 50 ppm of boric acid, and pollen of all three male parents gave successful fruit set in some other crosses. For each cross around 100 flowers were de-petalled, and open flowers and the remaining flower buds were removed. Pollen was applied to the stigma of prepared flowers with a paint brush. Pollinated pistils were left unbagged. Each branch used for crossing was tagged with an indelible written label. Two months after crossing, fruit set was recorded.

Results and discussion

The results of the four test crosses are presented in Table 1. 'Glorieta' x 'Desmayo Largueta' and 'Glorieta' x 'Texas' set no fruit, as already reported by Boskovic *et al.* (1997); 'Texas' x 'Languedoc' failed to set and 'Texas' x 'Glorieta' set only one almond. These results indicate that 'Desmayo Largueta' and 'Glorieta' indeed have the same incompatibility genotype as 'Texas', and 'Languedoc'. The failure of the cross 'Glorieta' x 'Texas', and its reciprocal, confirm the identity of the S_1 allele in 'Glorieta', derived from 'Cristomorto', (Crossa-Raynaud and Grasselly, 1985) with the S_b allele from 'Texas' (Kester *et al.*, 1994), as explained by Boskovic *et al.* (1997). Thus all four cultivars which failed to intercross are considered to be S_1S_5.

Mutual incompatibility of almond cultivars has been attributed to relatedness resulting from a narrow range of cultivars being used for breeding (Socias i Company, 1990). However 'Desmayo Largueta', 'Languedoc' and 'Texas', which are chance seedlings, and the newly bred cultivar 'Glorieta' are not known to be related.

Table 1. Test crosses made

Parentage	No. of pollinated flowers	No. fruit set	Assigned stylar RNase alleles (*S*-genotype)†
'Glorieta' x 'Texas'	146	0	S_1S_5 x S_1S_5
'Texas' x 'Glorieta'	89	1	S_1S_5 x S_1S_5
'Glorieta' x 'Desmayo Largueta'	87	0	S_1S_5 x S_1S_5
'Texas' x 'Languedoc'	85	0	S_1S_5 x S_1S_5

†Incompatibility genotypes according to Boskovic *et al.* (1997)

The assignment of incompatibility alleles to almond cultivars can guide the selection of cultivar combinations both for breeding and for orchard planting. Our results show the practical use of stylar

ribonuclease analysis for predicting incompatibility alleles of almond cultivars. And even if the individual alleles are not identified, similar ribonuclease patterns predict cross-incompatibility. However, confirmation of ribonuclease predictions of cross-incompatibility by making test crosses is essential, as it is possible that two different *S* alleles may code for ribonucleases that are electropheretically indistinguishable. Checking semi-compatible combinations by test crossing is more difficult, even with such methods as observation of pollen tube growth by ultra-violet fluorescence microscopy. However, crosses predicted to be semi-compatible could be checked by analysing the segregation of stylar ribonucleases in the seedlings once they flower.

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