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Analysis of stylar ribonucleases (S-Rnases) in an almond progeny of 'Ferralise x Tuono'

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SUMMARY – Recent studies have demonstrated the interest of stylar ribonucleases electrophoretic patterns for predicting incompatibility S-alleles in almond cultivars. In this work, we have analysed a progeny of 'Ferralise x Tuono'. 'Ferralise' shares the S1 allele with 'Tuono' (S1Sf) and has the same S1S3 genotype than 'Ferragnès'. All tested progeny seedlings, except one, were genotyped S1Sf or S3Sf, and were considered self-compatible. The transmission of self-compatibility from 'Tuono', is similar in the two progenies 'Ferralise x Tuono' and 'Ferragnès x Tuono'.

Key words: Almond, *Prunus dulcis*, compatibility, ribonuclease, NEpHGE.

RESUME – "Analyse des ribonucléases stylaires (S-Rnases) chez la descendance de l'amandier 'Ferralise x Tuono'". Des études récentes ont montré l'intérêt de l'analyse des ribonucléases stylaires par électrophorèse, pour prédire les allèles du gène S, dans différents cultivars d'amandier. Dans cette étude, nous avons analysé une descendance de 'Ferralise x Tuono'. 'Ferralise' a en commun l'allèle S1 avec 'Tuono' (S1Sf) et a le même génotype, S1S3, que 'Ferragnès'. Tous les hybrides analysés de cette descendance, sauf un, ont été trouvés S1Sf ou S3Sf, et donc considérés autocompatibles. La transmission de l'autocompatibilité à partir de 'Tuono', est similaire dans les deux descendance 'Ferralise x Tuono' et 'Ferragnès x Tuono'.

Mots-clés : Amandier, *Prunus dulcis*, compatibilité, ribonucléase, NEpHGE.

Introduction

Boskovic *et al.* (1997) have assigned S-genotypes to almond cultivars by analysis of stylar ribonucleases which are the S-proteins encoded by the S gametophytic-incompatibility gene. The two cultivars 'Cristomorto' and 'Ai' were found S1S2 and S3S4; 'Ferragnès' and 'Ferraduel' ('Cristomorto x Ai') were found as expected, S1S3 and S1S4, and 'Ferralise' ('Ferragnès x Ferraduel') S1S3. The self-compatible variety 'Tuono' was determined S1Sf. The allele of self-compatibility Sf was deduced by the lack of RNase activity. We know 'Ferralise' and 'Ferragnès' had the same S-genotype, because they are inter-incompatible (Crossa Raynaud and Grasselly, 1985). However, Grasselly *et al.* (1985) published field-data which showed that the progeny transmission of the self-compatibility trait was different for the two varieties, when pollinated by 'Tuono': (i) 100% self-compatible with 'Ferragnès'; and (ii) 58% self-compatible with 'Ferralise'. For the cross 'Ferralise x Tuono', the field data were effected on young trees, and were not repeated a second year. So, to be sure of the stability of these results, we have made a new progeny of 'Ferralise x Tuono' and we have determined the S-genotype of the seedlings by analysis of stylar ribonucleases.

Material and methods

A controlled cross, 'Ferralise x Tuono' was made in 1996 on a tree of the INRA Collection in Bellegarde (Gard). After stratification and germination of the seeds, 60 seedlings were obtained. They were grown in greenhouse in 1997 and planted in the field in January 1998. In spring 1999, we collected flowers in the balloon stage, from the seedlings which gave more than 20 flowers. The styles (15 to 25) were frozen until use. Stylar ribonucleases of seedlings and parents were extracted, and analysed by Non-Equilibrium pH Gradient Electrofocusing (NEpHGE) on vertical acrylamide gels, in according to Boskovic *et al.* (1997).

Results

The Fig. 1 represents the zymograms of the parents 'Tuono' and 'Ferralise', and of some seedlings. The zymogram of the parent 'Ferralise' showed two ribonuclease bands corresponding to the S1 and S3 alleles. The self-compatible parent 'Tuono' had one main ribonuclease band S1 corresponding to the S1 allele. We observed an other slight band, at the same level than S3. This faint band was more and less present in the other 'Tuono' zymograms, it had always a very weak RNase activity compared to the other S1 band and we did not record it. For the 33 tested seedlings, 19 showed a zymogram with one band corresponding to the S1 allele (Fig. 1: 18, 31, 34, 36), 11 with one band corresponding to the S3 allele (Fig. 1: 35, 39, 47, 49) and one seedling with the two bands S1 and S3, like the 'Ferralise' parent. Two seedlings did not show any RNase activity.

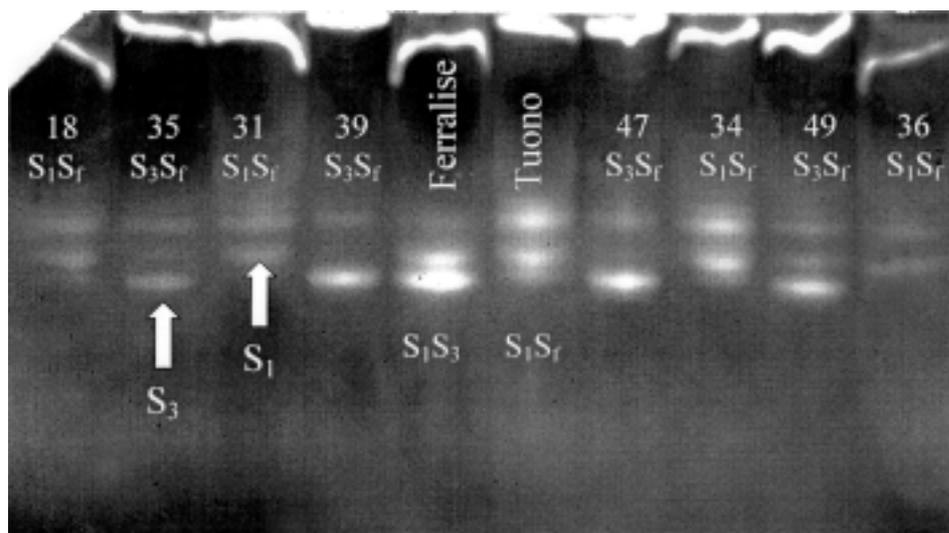


Fig. 1. Zymogram of stylar ribonucleases in some seedlings of the progeny 'Ferralise x Tuono' and the two parents 'Ferralise' and 'Tuono'.

Discussion

As the seedling trees are yet too young, the field records of fruit set after selfing are missing. However, Boskovic *et al.* (1998, 1999) have demonstrated the high correlation between the self-compatibility and the absence of ribonuclease band for the Sf allele. Consequently, we can determine the S-genotype of the 19 seedlings with one S1 band as S1Sf and the S-genotype of the 11 seedlings with one S3 band as S3Sf. With the Sf allele, these 30 seedlings (97%) are self-compatible. Only the seedling S1S3 is not self-compatible (3%).

These results show, that the fields records of Grasselly *et al.* (1985) giving a proportion of 42% of seedlings which set less than 1% after bagging, were not sufficient to conclude these 42% of seedlings were self-incompatible. These observations were effected in 1985 on four years aged trees. To be sure that seedlings are self-incompatible, it would be necessary to repeat the field test at least a second year (Gradziel and Kester, 1998), on older trees. In fact, some trees set very few fruit, in the first planting years.

Our results are similar to those obtained for the cross 'Ferragnès x Tuono' (Grasselly *et al.*, 1985; Dicenta and García, 1993; Ballester *et al.*, 1998). Thus, it is improbable that other genetic factors affect the transmission of self-compatibility (Socias i Company and Felipe, 1994).

The presence of a S1S3 seedling means that a S1 pollen of 'Tuono' has achieved fertilisation of a S3 'Ferralise' ovule. In this case, the S1 tube growth was not interrupted by the S1 stylar ribonucleases. We suppose either a mentor effect of compatible S3 pollen (Villar and Gaget-Faurobert, 1996), or an effect of high temperatures, during the pollination; 'Ferralise' having a late

flowering. For the two seedlings with no RNase activity, a probably bad conservation of the styles could cause S-proteins denaturation before extraction. These samples, with the other genotypes of the progeny will be analysed next year, to increase the seedling number and to get a better reliability. Then, when the trees will be enough productive, we will test the self-compatibility in the fields, to check again its correspondence with the Sf allele, determined by the lack of RNase activity.

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