Rootstock breeding program for apricot through interspecific crosses of Myrobalan x apricot: Significant effect of accidental pollinations.

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Abstract

A rootstock breeding program for apricot is developed through interspecific crosses of the Myrobalan plum (*Prunus cerasifera* Ehrh.) and the Moniquí and Moniquí Borde (*P. armeniaca* L.) apricot cultivars. In this work the incidence of accidental pollinations on these low setting hybridizations was assessed. Progeny was originated through hand pollination of emasculated flowers of three Myrobalan clones, in three consecutive years. Fruit set was low and variable among years (1.8-8.0%), but higher than the level of accidental pollination measured with emasculated and non-pollinated flowers (1.2%). The molecular characterization of the progeny was performed with three SSR markers. The results show that, from the 278 genotypes evaluated, 73 appeared to be hybrids and 205 did not. The procedure to obtain hybrid plants is discussed here in terms of the loss of hybrid clones along the process from pollination to field growing .

INTRODUCTION

Moniquí is one of the most interesting apricot cultivars in the Ebro Valley region due to its organoleptic characteristics which make it a highly valuable crop (Rodrigo et al., 2002). A rootstock breeding program for apricot is being developed through interspecific crosses of the Myrobalan plum rootstock (*Prunus cerasifera* Ehrh.) and the apricot cultivars Moniquí and Moniquí Borde (*P. armeniaca* L.) in order to achieve good scion compatibility, rusticity and water logging resistance. Interspecific crosses often present incompatibility barriers resulting, in most cases, in low seed setting rates. Besides, the process to obtain true hybrid seeds should be carefully checked because of the presence of accidental pollinations with undesired pollen. The rate of fertilization with undesired pollen could be assessed using morphological traits, although nowadays, molecular techniques like SSR markers are an important tool for paternity assessment (Wünsch and Hormaza, 2002).

In this work, we studied how low seed settings and accidental pollinations affect the process of obtaining hybrid seeds, following interspecific crosses. In vitro germination and propagation were used in order to overcome embryo abortion (Arbeloa et al., 2003) and SSR markers were used to know the extent of fortuitous pollinations.

MATERIALS AND METHODS

Myrobalan flowers from three different clones (Mb1, Mb2 and Mb3) were pollinated with pollen from ‘Moniquí’ (M) and ‘Moniquí Borde’ (MB) apricot cultivars, in three consecutive years. Flowers were emasculated at balloon stage and pistils were hand-pollinated the following day in a variable number (Table 1). Since emasculated

flowers were unattractive to insects (Free, 1964), no bagging was applied to pollinated branches. Fruit set was assessed weekly for each of the six crossing combinations. Immature embryos, 12 weeks after pollination, were cultured for germination and later shoot apices were multiplied in vitro (Arbeloa et al., 2003). Germination and survival rates were recorded for each treatment. Shoots were later rooted in vitro and acclimatized in the greenhouse (Marín, 2003).

In order to assess the level of accidental pollinations of the three Myrobalan clones, fruit set was determined in 1) emasculated non-pollinated flowers, 2) emasculated and self-pollinated flowers and 3) non-emasculated open-pollinated flowers. An average of 500 flowers was controlled for each treatment and clone.

The parental genotype clones as well as 278 putative hybrids were analysed with SSRs to identify the hybrid status of the progeny. Genomic DNA isolation and PCR reaction were carried out according to Hormaza (2002). The parental genotypes were initially screened with eight SSR loci previously developed in peach (Cipriani et al., 1999; Sosinski et al., 2000). Three SSR loci (pchgms2, UDP96-003 and UDP96-008), polymorphic among the parental genotypes, were selected to confirm the hybrid origin of progeny. Each of the progeny genotypes analysed was considered to be a hybrid when one of the two SSR amplified alleles of each locus was the same as one of the two alleles in the maternal genotype, and the other was the same as one of the SSR alleles found in the paternal genotype (Figure 1).

RESULTS

Fruit set following interspecific hand pollinations were low and very variable among years and treatments (Table 1). A total of 16,466 flowers were emasculated and pollinated to obtain 667 fruits (an average of 4% of fruit set). Among all the seeds sowed in vitro, an average of 78% germinated resulting in 535 seedlings, which were multiplied in vitro (Table 2). During the post-germination growth and the multiplication phase, an average of 20% of them was lost throughout the first year (Table 2). Some of them died soon after germination and some died along the multiplication phase.

The assessment of the level of accidental pollinations was performed on emasculated flowers. The average fruit set of the three maternal clones in emasculated and non-pollinated flowers was 1.1%, similar to that of self-pollinated flowers (0.9%), whereas it rose up to 11.5% in open-pollination conditions.

The SSR loci pchgms2, UDP96-003 and UDP96-008, polymorphic for the Myrobalan and apricot clones, were used to confirm the hybrid origin of the progenies (Figure 1). From the 278 genotypes evaluated, 73 showed hybrid origin and 205 did not. The percentage of hybrids, confirmed by microsatellites, among the progeny in the three years was 37.1% in 1998, 26% in 1999 and 20.3% in 2000.

DISCUSSION

In this work we have emphasized the high incidence of low fruit settings and accidental pollinations in a rootstock breeding program for apricot. Thus, in interspecific crosses the number of initial pollinated flowers must be increased in order to get an acceptable number of hybrid seeds to carry out the selection program. The low fruit settings may be due to incongruity between the applied apricot pollen and Myrobalan (Perez and Moore, 1985). As a consequence, fertilization of Myrobalan ovules by the accidental pollen that manages to reach the stigmas will be favoured against the applied apricot pollen. We have found a high proportion of non-hybrid plants derived from these fortuitous pollinations. Since the Myrobalan clones appeared to be self-incompatible (0.9 % fruit set after self-pollination), pollen for these accidental

pollinations probably arrived by wind or gravity from adjacent trees, as in other fruit tree species (Visser and Verhaegh, 1980). The small rate of pollinated flowers with foreign pollen observed in emasculated but non-pollinated flowers (1.1%) compared with open-pollinated ones (11.5%) would be similar to those obtained in interspecific pollinations. This rate is, however, magnified along the developmental process due to the disadvantageous development of hybrid versus non-hybrid embryos or seeds.

Accidental fertilization of the Myrobalan ovules with non-desired pollen is probably favoured because of the incompatibility barriers present in interspecific crosses. Besides, once the ovules are fertilized, incompatibility may produce early embryo or endosperm abortion reducing the proportion of hybrid seed. On the other hand, in vitro germination of hybrid seed also took place in a lower rate (Daorden et al., 2004). Finally, interspecific hybrid seedlings are less adapted to in vitro culture. An average of 20% of the seedlings was lost during the year they spent in in vitro culture. As a consequence, we observed a low percentaje of hybrids among progeny .

Since escapes are also present even in bagged flowers (de la Rosa et al., 2004) a study of the hybrid condition of the descendants is always highly advisable. The screening of hybrids with molecular markers at a very early stage, even at embryo level (Hormaza, 1999), to eliminate all non-desired seeds, could be an alternative method.

A large number of flowers must be pollinated to obtain a sufficient number of hybrid progenies in fruit tree breeding programs, while accidental pollinations should be carefully prevented through the bagging of flowers and/or by early molecular identification of hybrids. This approach would reduce the number of clones for the later morphological characterization, which is time consuming and expensive.

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Tables

Table 1. -Number of pollinated flowers, harvested fruits and fruit set rate in 1998,1999 and 2000

	1998			**1999**			**2000**		
	Flowers	**Fruits**	**Fruit set**	**Flowers**	**Fruits**	**Fruit set**	**Flowers**	**Fruits**	**Fruit set**
Mb1 x M	1072	13	1.2	912	40	4.4	1175	32	2.7
Mb1 x MB				870	33	3.8	1069	47	4.4
Mb2 x M	567	26	4.6	630	21	3.3	1397	47	3.4
Mb2 x MB	1130	19	1.7	859	8	0.9	1325	23	1.7
Mb3 X M	1200	13	1.1	1202	159	13.2	1055	9	0.9
Mb3 x MB				909	170	18.7	1096	7	0.6
Total	**3969**	**71**	**1.8**	**5382**	**431**	**8.0**	**7117**	**165**	**2.3**

Table 2. -Number of germinated seeds (A), germination rate (B) and growing seedlings one year after germination (C) in 1998, 1999 and 2000

	1998			**1999**			**2000**		
	A	B	C	A	B	C	A	B	C
Mb1 x M	10	83	8	36	90	30	22	69	17
Mb1 x MB				30	88	24	36	77	30
Mb2 x M	18	72	15	9	64	3	33	70	15
Mb2 x MB	13	76	13	5	71	5	14	61	11
Mb3 X M	8	62	7	142	88	96	9	100	5
Mb3 x MB				144	86	132	6	86	5
Total	**49**	**78**	**43**	**366**	**85**	**290**	**120**	**73**	**83**

Figures

Figure 1. - Amplification of the SSR locus UDP96-003 in the Myrobalan (Mb) and Moniquí (Mo) parental genotypes and 23 progeny genotypes. Hybrid progenies are marked with *.