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Full Length Research Paper

Verifying the reliability of hybrid issued from the cross "Picholine marocaine clones X Picholine du Languedoc"

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In order to verify the reliability of hybrid population issued from crossing between 3 clones of "Picholine marocaine" cultivar and the "Picholine du Languedoc" cultivar, the descendants and their parents were analysed using 35 microsatellite loci. No offspring resulted from self crossing of "Picholine marocaine" cultivar and 218 descendants among 220 analysed are legitimate. This study showed clearly a segregating population and may be used as a genetic material for linkage map construction and for phenotyping resistance traits related to *Spilocaea oleagina* disease.

Key words: *Olea europaea*, segregating population, microsatellite markers, QTL, *Spilocaea oleagina*.

INTRODUCTION

The olive tree is widely cultivated in Morocco. It has a major socio economical role since it contributes to the maintenance of the rural populations. The "Picholine marocaine" is the most dominant cultivar; more than 98% of the olive growing orchards are planted by this cultivar (Boulouha et al., 1992; Bamouh, 1998). Nevertheless, this variety is sensitive to the peacock spot disease attributed to the fungus *Spilocaea oleagina*. This cryptogam parasite is frequent in the most humid areas of olive tree culture and high attacks may result in complete leaves falling. In Morocco, during the most humid years, the defoliation of olive trees can reach 98% (Tajnari, 1998).

Several varieties are considered as resistant to *S. oleagina* like Leccino", "Picholine de Languedoc", "Koroneiki", and "Piangente". Other varieties are sensitive such as "Arbequine" and "Frantoïo" (Barranco et al., 2002). To introduce resistance to *S. oleagina* in the Moroccan varieties, the genetic improvement program requires the development of genetic knowledge of the resistance traits. Constructing the olive genetic map based on segregating population is essential to detect and to identify loci related to resistance to *S. oleagina*.

The contaminations by the foreign pollen are possible in F1 populations issued from crossing between 2 cultivars (La Rosa et al., 2003). Butcher et al. (1999) reported that the microsatellite markers may be used to detect illegitimate offsprings, even by using few SSR loci (Butcher et al., 1999). The present study consists to verify the reliability of the hybrid population issued from crossing between three clones of "Picholine marocaine" ("Menara", "Haouzia" and "M26") sensitive to the *S. oleagina*, and the "Picholine de Languedoc" resistant to this pathogen. This study will be considered as the prerequisite of the olive genetic mapping and of the *QTL* (Quantitative Trait Loci) detection linked to *S. oleagina* resistance.

MATERIALS AND METHODS

Molecular analyses were carried out on a F1 population issued from crossing between 3 clones selected from the cultivar "Picholine marocaine" (PM), ("Menara", "Haouzia", "M26") and the "Picholine

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du Languedoc" cultivar (PL): “M26” x PL, “Haouzia” x PL and “Menara” x PL. Crosses were performed in 1991 at INRA (Insitute of Agronomic Research) Marrakech. Fructiferous branches with long internodes were chosen and the flowers were castrated and bagged to avoid the self-fertilizations or foreign pollen. These flowers were pollinated manually while bringing "Picholine du Languedoc" pollen during one week. The seeds germination was realized in aseptic conditions while using a substrate appropriate in the darkness at 13°C. At the end of the year 1993, 235 individuals were planted at INRA. More than half of this hybrid population have started to produce olives since 1999.

The three "Picholine marocaine" clones are considered as having same genotype on the basis of RAPD and SSR markers analysis (Khadari and Bervillé, 2001; Khadari et al., 2007). The descendants issued from the 3 crosses are considered as only one hybrid population in segregation. A total of 218 descendants plus the 4 parents were analysed.

DNA extraction, SSR reaction and analysis were carried out following Khadari et al. (2007). For the indirect fluorescence, 5 pmol of “Reverse” primer (or “Forward”) and 4 pmol of tail fluore-scent sequence were used in the PCR reaction (Ganache et al., 2001).

The data analysis is carried out as follows: or each primer used in the progeny analysis, the expected proportion of the genotypes in segregation is defined on the basis of two parent genotypes. From this proportion, a χ^2 test was calculated to test the null hypothesis.

To estimate the reliability degree of discrimination between the descendants, the probability that one genotype is confused with a given genotype is calculated. Under the hypothesis of the band absence between markers, this probability is defined as the product of the presence or absence frequency of each bands fj of the molecular profile i: $Pi = \prod fj$.

RESULTS AND DISCUSSION

As a prerequisite to the genetic mapping, it is necessary to verify the validity of the F_1 population resulting from a cross between “Picholine marocaine” and “Picholine de Languedoc”. Our primary objective is to analyze rapidly the reliability of the large F_1 population and to choose a number of individuals sufficient for the genetic mapping.

Firstly, among the 35 primers which revealed polymorphism between the two parents, 6 were used for genotyping all of the descendants (Table 1). The alleles number detected by locus varied from 2 (GAPU 59 and UDO99-012) to 4 alleles (GAPU 71B and UDO99-017) with an average of 3 alleles by locus (GAPU 89 and DCA3). With the exception of only two individuals who have genotype 156/168 in the UDO99-017 locus, 218 analyzed descendants have parental alleles. This data indicate that the presumed F1 individuals are truly descendants from the crossing between the "Picholine du Languedoc" and the 3 clones of the "Picholine marocaine" except for 2 individuals. The presence of the 18 alleles in the two parents is confirmed in the segregating F_1 population. Furthermore, no offspring was resulted from self- fertilization of one of the 3 "Picholine marocaine" clones.

Nevertheless, few SSR loci examined in the first part of this study (6 loci) and missing data obtained would not us excluded to have illegitimate individuals, resulting from a contamination by pollen of other varieties than the "Picholine du Languedoc" which have genotypes identical to hybrid forms. This hypothesis is not very plausible since the probability to have a genotype by chance among descendants of this crossing varied from 5.98×10^{-5} to 2.37×10^{-6}. All precautions were taken during the crossing between the clones of the "Picholine marocaine" and the "Picholine du Languedoc" (see Material and Methods). However, other varieties ("Arbequine", "Manzanilla" and "Olivière") planted in the experimental station of the Marrakech INRA may contaminate the crosses. Some alleles detected in these varieties were different to those present in “Picholine du Languedoc” (Khadari et al., unpublished data). This data exclude the hypothesis of an outside contribution of pollen and display therefore that the analyzed individuals are real descendants of the crossing PL x PM ("Menara", "Haouzia", M26).

In the second part of the study, we reduced randomly the number of descendants to 140 individuals. This part of pedigree underwent a larger verification by using 29 other SSR loci (Table 2). In opposition to the first analysis, few missing data were obtained. Detected alleles varied among 2 for 12 loci, 3 for 18 loci and 4 alleles for 5 loci (Table 2). Using 35 SSR loci, any illegitimate descendant was detected on the 140 selected individuals. According to the 3 segregate Mendelian models (1:1, 1:2:1 and 1:1:1:1) and to the χ^2 statistical test, all SSR loci displayed mendelian allelic segregation except for 5. The null hypothesis is rejected at 5% for the locus UDO99-005 and a very distorted level was observed at 4 loci (DCA7, UDO99-015, UDO99-025 and UDO99-042; Table 2).

Luro et al. (1995) display the existence of the distortion to the segregation for intraspecific cross. In olive-tree, 19.6 % of AFLP markers have showed distorted segregation in the hybrid population "Leccino" x "Dolce Agogia" (Baldoni et al., 1999). In apple tree, 24% of RAPD and isoenzymatic markers have shown distorted segregation in the descendants of two different crossings (Conner et al., 1997). This distorted segregation can be explained by physical association between markers with a lethal or semi-lethal locus (Bradshaw and Stettler, 1994; Echt et al., 1994), with the locus of incompatibility (allelic incompatible combinations; Gebhardt et al., 1991; Wricke and Wehling, 1985; Paterson et al., 1988) or with chromosomal rearrangements (Kianan and Quiros, 1992; Fauré et al., 1994).

This study shows that the hybrid population resulting from the crossings between the 3 clones of the "Picholine marocaine" and "Picholine du Languedoc" is a true F1 population in segregation. The phenotyping of the characters related to *S. oleagina* resistance in a segregate population will support the QTL resistance detection and characterization. This works will be of high importance to selection programs assisted by molecular markers.

Table 1. Number of descendants, allelic segregation and the test of segregation distortion of six SSR primers used to reduce population size.

Primer	Parents genotype PM x PL	Number of descendants[1]	Number of descendants[2]	Number of Illegitimate individuals[3]	segregation Type	observed χ^2	P
GAPU89[a]	160/205, 160/175	200	18	-	1 :1 :1 :1	6.286	0.1
GAPU71B[b]	127/118, 141/121	207	11	-	1 :1 :1 :1	1.95	n.s.*
GAPU59[a]	211/220, 211/211	176	42	-	1 :1	0.364	n.s.*
DCA3[b]	237/251, 230/251	205	13	-	1 :1 :1 :1	1.46	n.s.*
UDO99-012[b]	154/154, 154/164	208	10	-	1 :1	4.92	0,05
UDO99-017[b]	152/154, 156/160	210	8	2 (156/168)	1 :1 :1 :1	0.73	n.s.*

[a]With M13 or T7 adapter.
[b]Marked directly in fluorescence.
[1]Analyzed.
[2]Not analyzed.
n.s.: The null hypothesis is not rejected in bold genotype of presumed wrong descendants.

Table 2. Parents' genotype, allelic segregation and the test of segregation distortion of 140 selected F1 individuals analysed by 35 SSR primers.

Locus	Parents genotype PM x PL	Missing data	segregation Type	χ^2	P
DCA1	204/212, 204/264		1 :1 :1 :1	2.971	n.s.
DCA11	141/161, 131/179		1 :1 :1 :1	5.886	n.s.
DCA14	199/209, 209/209		1 :1	0.257	n.s.
DCA16	153/172, 144/172		1 :1 :1 :1	0.571	n.s.
DCA17	113/113, 102/113		1 :1	0.457	n.s.
DCA18	169/176, 166/176		1 :1 :1 :1	2.800	n.s.
DCA3*	237/251, 230/251		1 :1 :1 :1	2.400	n.s.
DCA5	203/203, 199/203		1 :1	1.829	n.s.
DCA7*	149/165, 149/165		1:2:1	17.857	D
EMO2	203/222, 203/245		1 :1 :1 :1	6.229	n.s.
EMO90	185/187, 185/192		1 :1 :1 :1	4.743	n.s.
GAPU101	218/237, 220/226	5	1 :1 :1 :1	3.971	n.s.
GAPU103A	134/150, 150/190		1 :1 :1 :1	3.371	n.s.
GAPU11e17	214 /220, 214/214		1 :1	1.829	n.s.
GAPU45	181/184, 182/182		1 :1 :1 :1	3.886	n.s.
GAPU47	181/ 209, 181/204		1 :1 :1 :1	4.800	n.s.
GAPU59*	211/220, 211/211	1	1 :1	0.180	n.s.
GAPU71B*	127/118, 141/121		1 :1	1.029	n.s.
GAPU89*	160/205, 160/175	1	1 :1 :1 :1	3.800	n.s.
UDO99-011	121/127, 109/121		1 :1 :1 :1	1.314	n.s.
UDO99-012*	154/154, 154/164		1 :1	2.314	n.s.
UDO99-015	102/102, 102/105		1:2:1	14.238	D
UDO99-017*	152/154, 156/160	1	1 :1 :1 :1	0.714	n.s.
UDO99-024	188/209, 207/209		1 :1 :1 :1	1.771	n.s.
UDO99-025	171/-, 171/176		1:2:1	19.038	D
UDO99-027	119/191, 191/191		1 :1	0.714	n.s.
UDO99-031	111/151, 107/151		1 :1 :1 :1	3.600	n.s.
UDO99-036	141/147, 147/151		1 :1 :1 :1	0.171	n.s.

Table 2. Contd.

UDO99-042	142/148, 142/150	5	1 :1 :1 :1	62.600	D
UDO99-043	175/213, 210/219	1	1 :1 :1 :1	1.629	n.s.
UDO99-044	146/146, 126/146		1 :1	0.714	n.s.
UDO99-005	157/159, 157/159		1:2:1	7,724	0.05
ME30MS	216/233, 212/216	1	1 :1 :1 :1	1,971	n.s.
PA(ATT)2	103/121, 109/121		1 :1 :1 :1	0,971	n.s.
PA(GA)2	115/125, 112/115		1 :1 :1 :1	3,771	n.s.

n.s.: The null hypothesis is not rejected.
D: Very distorted data (null hypothesis is rejected).
*SSR loci used in the analysis of the complete segregate population (Table 1).

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