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REPRODUCTIVE BIOLOGY OF A DIVERSE APRICOT (*PRUNUS ARMENIACA* L.) GERMPLASM FROM THE REGIONS OF HODNA AND AURÈS IN ALGERIA.

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Abstract

Description of the subject: Gametophytic self-incompatibility, governed by the S-locus, functions in Prunus species including the apricot to protect self-pollination of flowers. Knowledge of the S-genotypes in apricot cultivars is essential to establishing productive orchards, defining combinations of compatible cultivars.

Objective : In this study, reproductive biology of 92 apricot cultivated accessions from the regions of Aurés (semi-arid) and Hodna (semi-arid to arid) in Algeria has been evaluated.

Methods : The cultivated accessions assayed included local Algerian cultivars and French and Spanish introduced cultivars with kwon S-alleles. Percentage of fertilized flowers and fruit set was evaluated in field conditions by bagging. To identify the S-alleles associated with self-incompatibility, two pairs of specific primers were tested using PCR. This PCR-based molecular tool allows S-genotypes to be characterized quickly and efficiently.

Results : The origin of the apricot accessions does not influence the percentage of fertilized flowers and fruit set. Location and Year, however, have a significant influence. On the other hand, results showed a great diversity in terms of S-alleles in the Algerian germplasm. Some of these apricot cultivars are self-incompatible, and so to obtain a yield they need to be pollinated with cross-compatible cultivars.

Conclusion : Results indicate that Algerian apricots represent an interesting source of new self-compatible germplasm adapted to dry conditions for Mediterranean apricot breeding programs. Determination of the S-genotype might be considered as an important addition to a set of identity markers of apricot genetic resources.

Keywords : Apricot, Fruit sets, Self-incompatibility, Crosspollination, germplasm.

REPRODUCTION BIOLOGIQUE DE DIVERS GERMOPLASM D'ABRICOTIER (*PRUNUS ARMENIACA* L.) DES RÉGIONS DU HODNA ET DES AURÈS, ALGÉRIE

Résumé

Description du sujet : L'auto-incompatibilité gamétophytique, régie par le locus S, fonctionne chez les espèces Prunus, y compris l'abricot, pour protéger l'autopollinisation des fleurs. La connaissance des génotypes S dans les cultivars d'abricot est essentielle à l'établissement de vergers productifs, définissant des combinaisons de cultivars compatibles.

Objectifs : Dans cette étude, la biologie de la reproduction de 92 accessions d'abricotier des régions d'Aurés (semi-aride) et Hodna (semi-aride à aride) en Algérie ont été évaluées.

Méthodes : Ces accessions cultivées comprenaient des cultivars locaux algériens et des cultivars introduits par la France et l'Espagne avec des allèles S. Le pourcentage de fleurs fertilisées et de nouaison a été évalué dans les conditions du champ par ensachage. Pour identifier les allèles S associés à l'auto-incompatibilité, deux paires d'amorces spécifiques ont été testées par PCR. Cet outil moléculaire basé sur la PCR permet de caractériser rapidement et efficacement les génotypes *S*.

Résultats : L'origine des accesions d'abricot n'influence pas le pourcentage de fleurs fertilisées et de nouaison. Cependant, le lieu et l'année ont une influence significative. Les résultats ont montré une grande diversité en termes d'allèles S dans le matériel génétique algérien. Certains de ces cultivars d'abricot sont auto-incompatibles, et donc pour obtenir un rendement, ils doivent être pollinisés avec des cultivars compatibles croisés.

Conclusion : Les résultats indiquent que les abricots algériens représentent une source intéressante de nouveau matériel génétique auto-compatible adapté aux conditions sèches pour les programmes de sélection d'abricots méditerranéens. La détermination du génotype S pourrait être considérée comme un ajout important à un ensemble de marqueurs d'identité des ressources génétiques d'abricot.

Mots clés: Abricot, nouaison, auto-incompatibilité, pollinisation croisée, germoplasme.

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INTRODUCTION

Apricot (*Prunus armeniaca* L.) is one of the most important *Prunus* species after peach and plum with an annual production of 4.1 million tons in 2019 [1]. Main producers are Turkey (0.85 million t), Iran (0.32 million t), Uzbekistan (0.53 million t) and Algeria (0.21 million t) in the case of apricot and China, Serbia, Romania, Chile and Iran in the case of plum. In Algeria, most important production areas are the regions of Hodna (semiarid area) and Ares (slightly arid area) [2, 3] (Figure 1). Despite the new apricot cultivars released from French and Spanish breeding programs are displacing the autochthonous Algerian cultivars in each area, there are a lot of local cultivars that still have a great interest and they are cultivated in the different areas. This autochthonous germplasm from North of Africa presents interesting characteristics from the breeding point of view including low chilling requirements and high fruit quality [4].

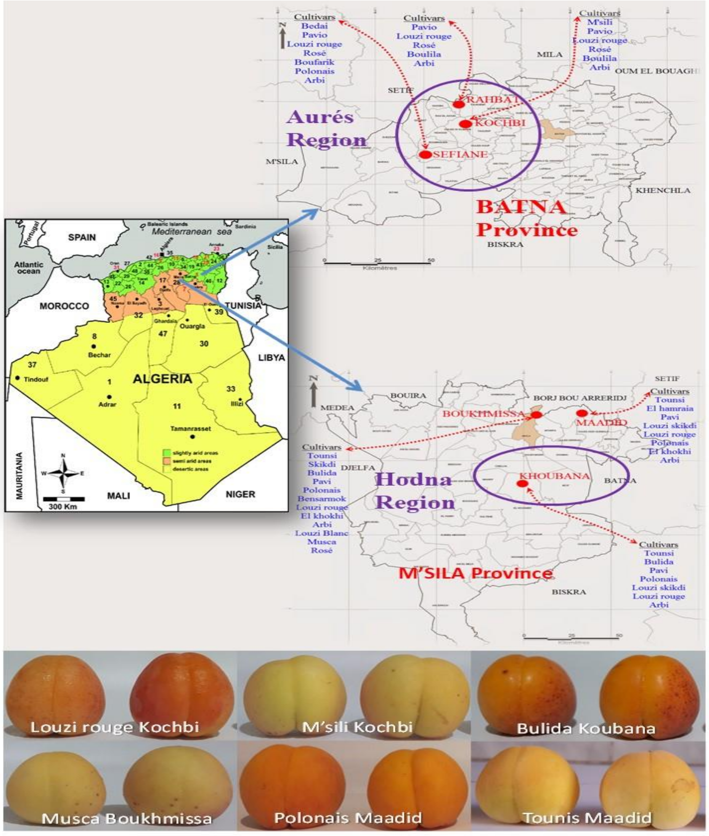

Figure 1: Bioclimatic map of Algeria with the location of the apricot germplasm evaluated in this study (above) and an illustration of the pomological diversity of the apricot germplasm assayed (below).

These accessions were selected over the years for its adaptation to warm Mediterranean climate and they are a valuable plant genetic resource that must be protected. However, some of them are difficult to evaluate reproductive biology of the cultivated accessions regarding productivity and inter-incompatibility problems thorough a gametophytic system. Gametophytic self-incompatibility, governed by the *S*-locus, functions in *Prunus* species including the apricot to protect self-pollination of flowers. Knowledge of the S-genotypes in apricot cultivars is essential to establishing productive orchards, defining combinations of compatible cultivars [5]. The objective of this work is the analysis of identification of reproductive biology of a diverse apricot germplasm from the regions of Hodna and Aurés in Algeria evaluating the percentage of pollinated flowers and fruit set and the *S* alleles by using PCR markers.

MATERIAL AND METHODS

1.Plant material

Ninety-two apricot accessions from the regions of Aurés and Hodna in Algeria in five different locations (Boukhmissa, Kochbi, Khoubana, Sefiane and Maadid) have been evaluated for two years (Figure 1). These cultivated accessions included local Algerian and Tunisian cultivars and French and Spanish introduced cultivars (Table 1).

Table 1: Apricot cultivars assayed, code of reference (Figure 5) and alleles observed. In brackets the locations of the cultivars: B: Boukhmissa; M: Maadid; Kh: Khoubana; E: Errahbat; K: Kochbi; S: Sefiane. Type of material: S (Spontaneus), A (Autochthonous), I (Introduced).

Code	Genotype	Alleles	Type	Code	Genotype	Alleles	Type	Code	Genotype	Alleles	Type
1	Arbi 1 (B)	S_c	S	32	Pavi 6 (Kh)	--	I	63	Louzi R (S)	S_8	I
2	Arbi 2 (B)	S_c	S	33	Pavi 7 (Kh)	--	I	64	Louzi R (B)	--	I
3	Arbi 3(B)	S_c	S	34	Pavi 8 (Kh)	S_c	I	65	Rosé (E)	S_c	A
4	Arbi 1 (M)	S_8	S	35	Pavi 9 (Kh)	S_c	I	66	Rosé 1 (K)	--	A
5	Arbi 2 (M)	S_8	S	36	Pavio 1 (E)	S_c	I	67	Rosé 2 (K)	--	A
6	Arbi1 (Kh)	S_8	S	37	Pavio 2 (E)	S_c	I	68	Rosé 1 (S)	--	A
7	Arbi2 (Kh)	--	S	38	Pavio (K)	S_8	I	69	Rosé (B)	--	A
8	Arbi 1 (E)	--	S	39	Pavio 1 (S)	S_8	I	70	Rosé 2 (B)	--	A
9	Arbi 2 (E)	--	S	40	Pavio 2 (S)	S_8	I	71	El Ham (M)	--	A
10	Arbi 3 (E)	--	S	41	Pavio 3 (S)	S_8	I	72	M´Sili (K)	S_c	A
11	Arbi 4 (E)	--	S	42	Pavi 2 (B)	--	I	73	Polonais (B)	S_c	I
12	Arbi 1 (K)	S_8	S	43	Pavi 3 (B)	S_c	I	74	Polonai 1 (K)	S_c	I
13	Arbi 2 (K)	S_8	S	44	ElKhok (B)	S_8	A	75	Polonai2 (K)	S_c	I
14	Arbi 3 (K)	S_8	S	45	ElKhok (M)	S_8	A	76	Polonai3 (K)	S_c	I
15	Arbi 4 (K)	S_c	S	46	Tounsi (B)	--	I	77	Polonais (S)	S_c	I
16	Arbi 5 (K)	--	S	47	Tounsi (M)	--	I	78	Polonais (M)	S_c	I
17	Arbi 6 (K)	--	S	48	Tounsi (K)	--	I	79	Bulida (B)	--	I
18	Arbi 7 (K)	S_c	S	49	Bedai 1 (S)	--	A	80	Bulida (K)	--	I
19	Arbi 8 (K)	S_c	S	50	Bedai 2 (S)	--	A	81	Bulida (Sp)	S_c	I
20	Arbi 1 (S)	S_c	S	51	Bedai 3 (S)	S_c	A	82	Boulila (E)	$S_c S_8$	I
21	Arbi 2 (S)	S_8	S	52	Bedai 4 (S)	S_c	A	83	Boulila (K)	S_c	I
22	Arbi 3 (S)	--	S	53	Bedai 5 (S)	--	A	84	Skikdi (B)	--	A
23	Bensam(B)	S_8	A	54	Bedai 6 (S)	--	A	85	Musca (B)	S_8	A
24	Boufarn(S)	S_8	A	55	Bedai 7 (S)	--	A	86	Canino (Sp)	S_c	I
25	Pavi 1 (B)	S_c	I	56	Louzi R (B)	S_8	I	87	Currot (Sp)	S_c	I
26	Pavi (M)	S_c	I	57	LouR 2 (M)	S_8	I	88	OrangR (Sp)	--	I
27	Pavi1 (Kh)	S_c	I	58	LouR 2 (M)	S_8	I	89	M. Nar (Sp)	S_c	A
28	Pavi2 (Kh)	S_c	I	59	Louzi R (K)	S_c	I	90	Berger (Sp)	S_c	A
29	Pavi3 (Kh)	S_c	I	60	LouzR (Kh)	S_8	I	91	Estrella (Sp)	--	I
30	Pavi4 (Kh)	S_c	I	61	Louz iR (E)	S_8	I	92	Colorao (Sp)	--	I
31	Pavi5 (Kh)	S_8	I	62	Louzi R (K)	S_8	I				

2. Percentage of fertilized flowers and fruit set

Percentage of fertilized flowers and fruit set was evaluated in field conditions by bagging. During two different seasons, years 2018 and 2020, three branches were bagged in each apricot tree of each cultivar evaluation the percentage of fruit set in absence of natural pollination by bees.

3. S alleles identification

Total DNA was isolated using the procedure described by Doyle and Doyle [6]. Identification of *S*-allele was performed using a PCR using SFBc/SFB8 specific primers, APRFBC8-F and APRFBC8-R, for the identification and differentiation of the Sc (≈500 bp) and S8 (≈ 150 bp) alleles using as DNA Marker the 1kb+ DNA ladder [7]. The negative controls (absence of *Sc*) are shown in bold and the positive controls (presence of *Sc*) are also shown in the gel.

4. Statistical analysis

Data were analysed using the R software v.4.0.1 [8]. Packages *car* [9] and *emmeans* [10] were used to perform the analysis of variance (ANOVA) and the multiple comparisons, respectively.

RESULTS

1. Percentage of fertilized flowers and fruit set

Regarding ANOVA results, interestingly, origin does not influence the percentage of fertilized flowers and fruit set. Location and year, however, have a significant influence (Table 2).

In this sense, apparently, no differences among the 3 groups of material, spontaneous, autochthonous and introduced. When analysing the interaction Origin x Location, we find a low p-value ($9.33\cdot10^{-5}$) which explains that no clear patterns Origin-Location can be found e.g. spontaneous show the highest values in some locations and the lowest in others (Figure 2).

Table 2: Analysis of Variance (ANOVA) of Percentage of fertilized flowers and fruit set.

Source	Sum Square	Df	F value	Pr (>F)
Origin	14	2	0.0271	0.9732299
Location	5577	5	4.3729	0.0009823 ***
Year	16728	1	65.5775	2.225e-13 ***
Origin x Location	9501	9	4.1384	9.335e-05 ***
Origin x Year	1124	2	2.2026	0.1142478
Location x Year	4241	5	3.3254	0.0071587 **
Residuals	36478	143		

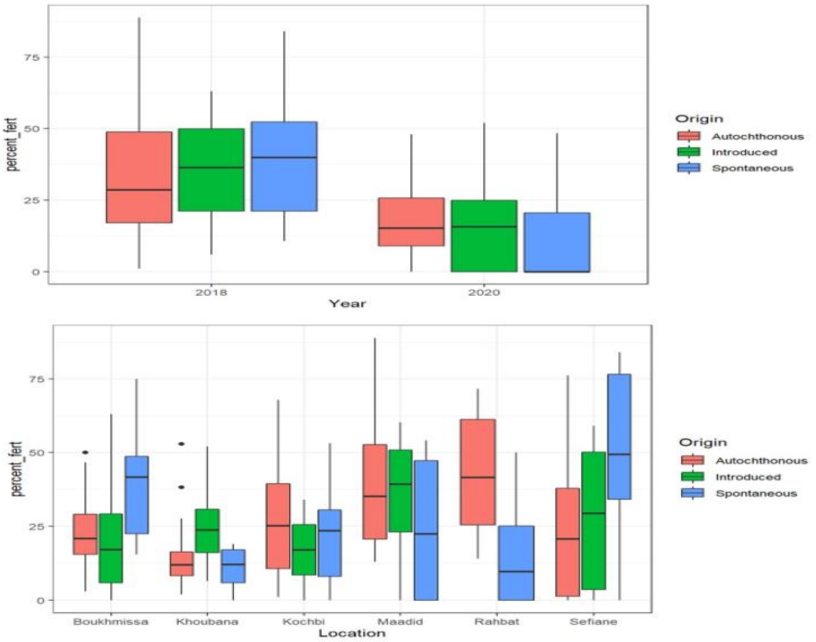

Figure 2: Box plot graphs showing the percentage of fertilized flowers and fruit set. Top: Considering origin. Bottom: Considering the interaction Location x Origin.

However, fruit set analysis showed important differences in the assayed cultivars ranging percentage between 0 to 75% (Figure 2). Additionally, a new ANOVA model was formulated ignoring the Location factor to analyse the influence of Location and Year (ANOVA Table not shown). Both factors have a separate influence, specially Year, as expected (p-value = 6.3×10^{-12}). The interaction has a moderate influence in the percentage of fertilized flowers and fruit set (p-value = 0.012). Figure 3 analyses the interaction between these two factors by visualizing the confidence interval for each pair of locations in the years 2018 and 2020. If two intervals overlap, it means that these two locations can be considered in the same group.

Following this procedure, we can conclude that no differences among locations are found in 2020. In 2018, Maadid presents the highest percentage of fertilized flowers (group A) while Khoubana presents the lowest one (group C) Rahbat and Sefiane: AB Kochbi: ABC Boukhmissa: BC (Figure 3). Finally, ANOVA hypotheses checking was carried out. Residual *vs.* fitted values plot (Figure 4, left) does not show any pattern, thus we can consider homogeneity of variance. The QQ plot (Figure 4, right) shows a linear trend, which suggests that the residuals are normal. This was confirmed applying the Kolmogorov-Smirnov test (p-value = 0.332).

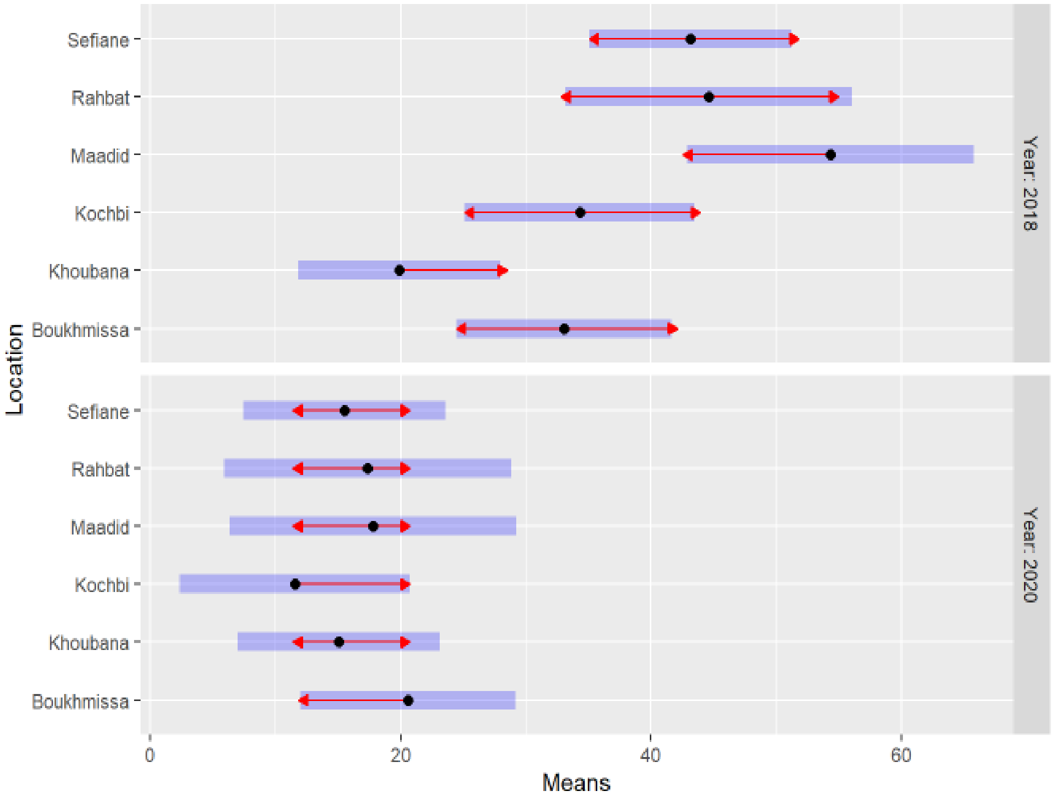

Figure 3: Box plot graphs showing the percentage of fertilized flowers and fruit set. Top: Considering origin. Bottom: Considering the interaction Location x Origin.

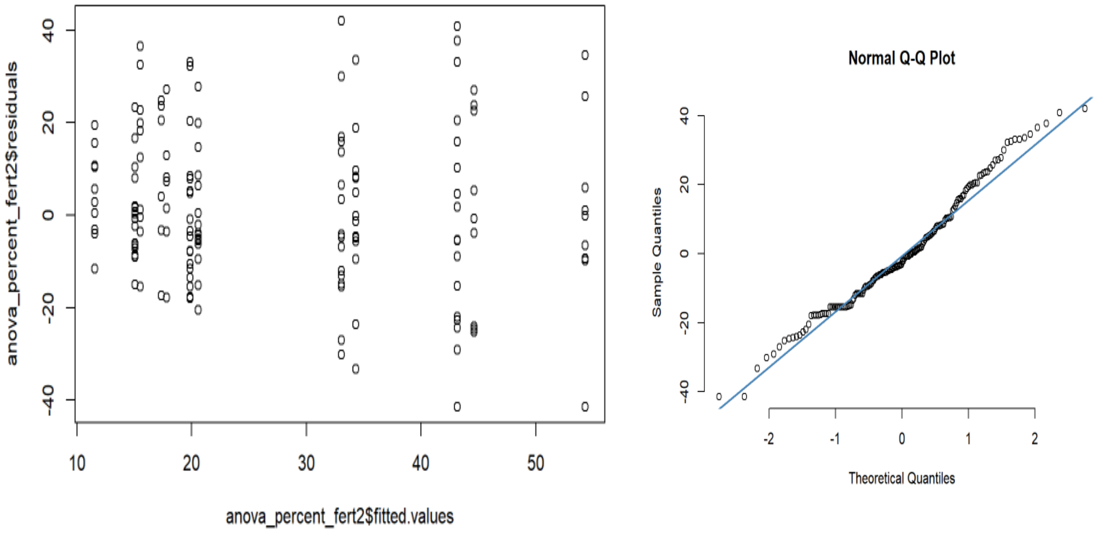

Figure 4: ANOVA hypothesis testing: Left: residuals vs. fitted values. Right: Q-Q plot.

2. Self-compatibility allele evaluation

Results showed that the assayed PCR using specific primers allowed the identification of *S*-genotypes to be characterized quickly and efficiently. In addition, these results showed an extended self-compatibility in the assayed Algerian cultivars with the presence of the self-compatibility (Sc) allele (Figure 5; Table 2). The results showed the suitability of SFBc/SFB8 specific primer pair and APRFBC8-F and APRFBC8-R primer pair, for the identification and differentiation of the *Sc* (≈500 bp) and *S8* (≈ 150 bp) alleles using as DNA Marker the 1kb+ DNA ladder.

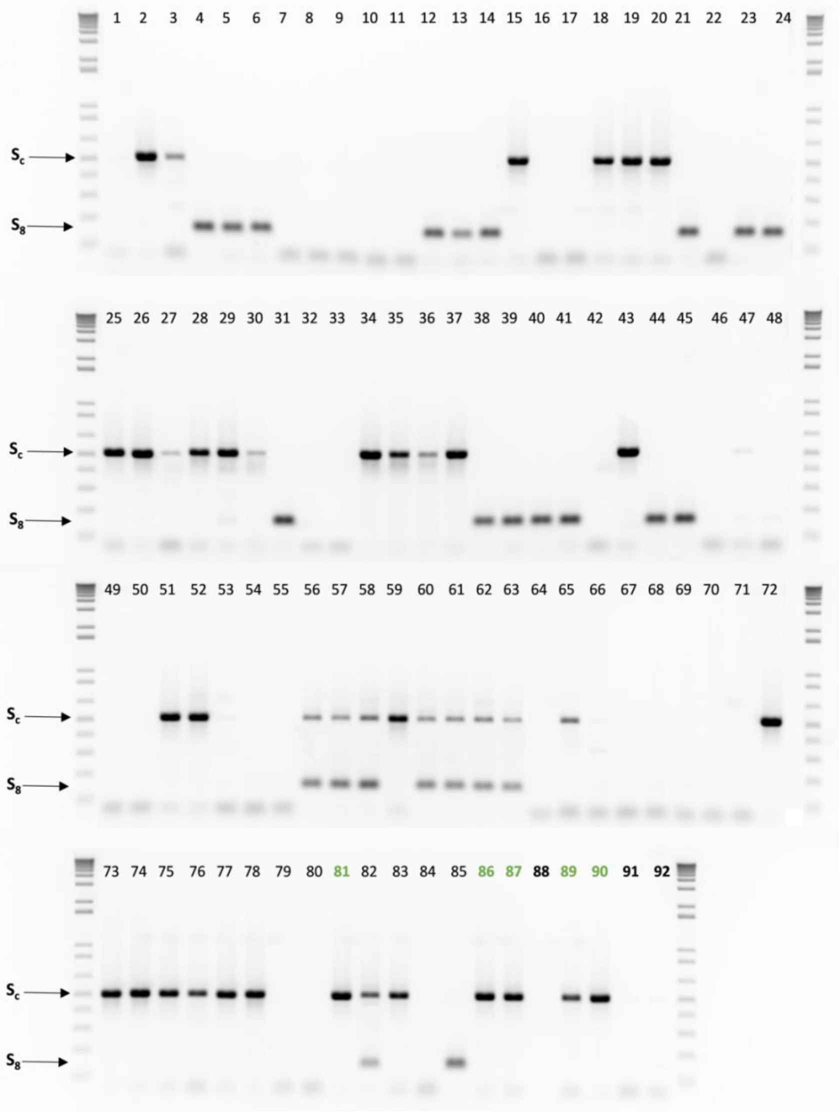

Figure 5: PCR amplification in 84 Algerian genotypes and 8 control apricot cultivars using SFBc/SFB8 specific primers, APRFBC8-F and APRFBC8-R, for the identification and differentiation of the Sc (≈500 bp) and S8 (≈ 150 bp) alleles. Marker: 1kb+ DNA ladder. The negative controls (absence of Sc) are shown in bold and the positive controls (presence of Sc) are shown in bold and green. Codes refer to cultivars showed in Table 1.

DISCUSSION

The origin of the apricot accessions does not influence the percentage of fertilized flowers and fruit set. Location and Year, however, have a significant influence. However, fruit set analysis showed important differences in the assayed cultivars ranging percentage between 0 to 75%. The reasons of these differences are by one side the presence of self-incompatibility alleles and by other side the lack of suitable cross pollination for two reasons, lack of bees or lack of pollinizer [11]. On the other hand, results showed a great diversity in terms of *S*-alleles in the assayed Algerian germplasm. In addition, some of these apricot cultivars are self-incompatible, and so to obtain a yield they need to be pollinated with cross-compatible cultivars.

These results, as far as we known represent the first molecular characterization of apricot germplasm for self-compatibility/self-incompatibility alleles to complete reproductive biology studies. The self-compatibility observed by PCR was also corroborated by the percentage of fruit in the evaluation in field conditions in absence of natural pollination by bees in many cases. This extended self-compatibility is also present in local cultivars in Spain and North of Africa [5, 12]. The self-incompatibility allele S8 was the most common incompatibility allele in the Algerian germplasm. This allele is shared by many cultivars and transmitted to the Spontaneous materials. This allele is also presented in the autochthonous Tunisian apricot cultivars [12].

However, other self-incompatibility alleles are presented which must be clarified using new PCRs with other specific primer pair [7, 12]. Results obtained have allowed establishing the pollination requirements of some commercial Algerian apricot cultivars. For the coming years, it will be necessary to continue determining the self-(in) compatibility and the inter-incompatibility relationships of the new releases to stabilize apricot production in the main apricot production areas of Algeria [11].

CONCLUSION

Results indicate that Algerian apricots represent an interesting source of new self-compatible germplasm adapted to dry conditions for Mediterranean apricot breeding programs with the presence of the self-compatibility (*Sc*) allele. The evaluation of the percentage of fertilized flowers and fruit set showed very productive accessions. However, fruit set analysis showed important problems related to the low percentage of fruit set. The reasons of these reproductive problems are by one side the presence of self-incompatibility alleles and by other side the lack of suitable cross pollination for two reasons, the lack of bees or the lack of pollinizer. It will be necessary to determine the self-(in) compatibility and the inter-incompatibility relationships of the problematic cultivars to stabilize apricot production in the main apricot production areas of Algeria of Hodna and Ares. On the other hand, determination of the S-genotype might be considered as an important addition to a set of identity markers of apricot genetic resources, what is of particular interest in the characterization of the germplasm diversity. Further studies about phenological and pomological variation of local Algerian apricot cultivars must be completed to establish their breeding value for drought resistance and low chilling breeding programs.

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