

COMPARISON OF DIFFERENT CLASSICAL AND MOLECULAR METHODS FOR IDENTIFICATION OF SELF-COMPATIBLE AND SELF-INCOMPATIBLE ALMOND PROGENIES

'G-2-25' (S₁₁S_f)
 (S₁₁S₁₂) ' (S₁S₂₅) '
 (FM) S PCR (NEpHGE)
 (FM) PCR (NEpHGE)
 'G-2-25' S
 (:)
 S₁₁S₂₅ (%) S₂₅S_f (%) S₁S_f (%) S_fS₁₁ 'G-2-25'
 S
 :
 .PCR
Prunus dulcis *Prunus amygdalus* Batch *Amygdalus communis*
 n= x= Pronoidea Rosaceae (Mill.)
 ()

// :

// :

()

()

S-
)

S-locus

RNases

()

SF-BOX

(

)

(

PCR

()

'G-2-25'

S_f

'Marcona'

Bagging

Glycoprotein -

Ribonuclease -

Gamethophytic
'Desmyo Largueta'

'G-2-25' (S₁₁S_f)

() (S₁₁S₁₂) ' () (S₁S₂₅) ' ,

S

'G-2-25'

(S₆S₁₁) ' ,

(S₈SF) ' ,

PCR

NEpHGE(NonEquilibrium pH Gradient Electro Focusing)

(D)

%

% /

() DNA
PCR PCR ()
PCR

PCR PCR

PCR AS1II-AmyC5R , ConF-ConR , SfF-SfR
SfF AS1II AS1II- SfF- AmyC5R : PCR
ConF, SfF- ConR AmyC5R
ConF-ConR ConR ConF-SfF
ConR S3F, ConF S3F
) PCR
SfF-SfR .(
Sf

()

Reverse primer

Forward primer

Gept and Clegg

Table 1. Used specific primers and recognizable S-alleles of almond by PCR method.

Primer	Primer sequence	Recognizable alleles
AS1II(F)	TATTTTCAATTTGTGCAACAATGG	S ₁ , S _f , S ₃ , S ₁₂ , S ₁₁
AmyC5R(R)	CAAAATACCACTTCATGTAACAAC	
ConF(F)	GTGCAACAATGGCCACCGAC	S ₁ , S _f , S ₃ , S ₁₁
ConR(R)	TACCACTTCATGTAACAACACTGAG	
SfF(F)	GTGCCCTATCTAATTTGTTGAC	S _f
SfR(R)	GACATTTTTTTAGAAAGAGTG	
S3F(F)	CTTCTGCGCTTACGAGAGGTT	S ₃
S3R1(R)	AAAACGTAAGGGATAGTTTCT	
S3F(F)	CTTCTGCGCTTACGAGAGGTT	S ₃
S3R2(R)	TGTGATTTCCACATGTCT	

Table 2. Buffer components (in 50 ml) for extraction of style ribonucleases.

% ()	Material
	(DMSO)
	10 3 pH
	%

rpm

()

NEpHGE

- DL - /
()

pH

()

()

()

pH

/) RNA

RNA

RNA

RNA

RNA

RNA

/

%

/

)

(

Tampon

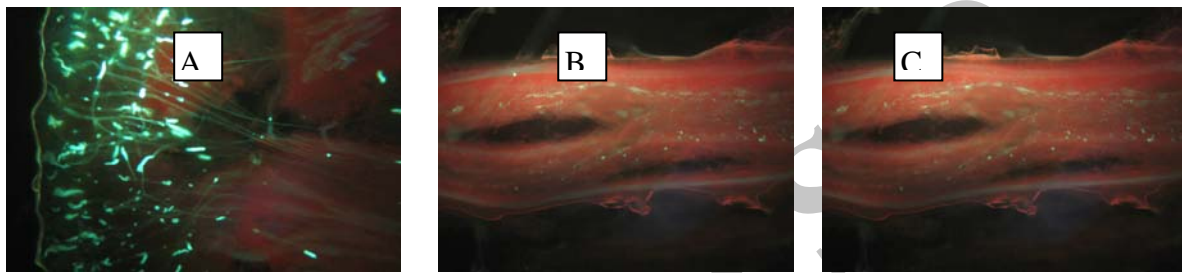


Fig. 1. Absence of pollen tube growth in self-incompatible cultivars.

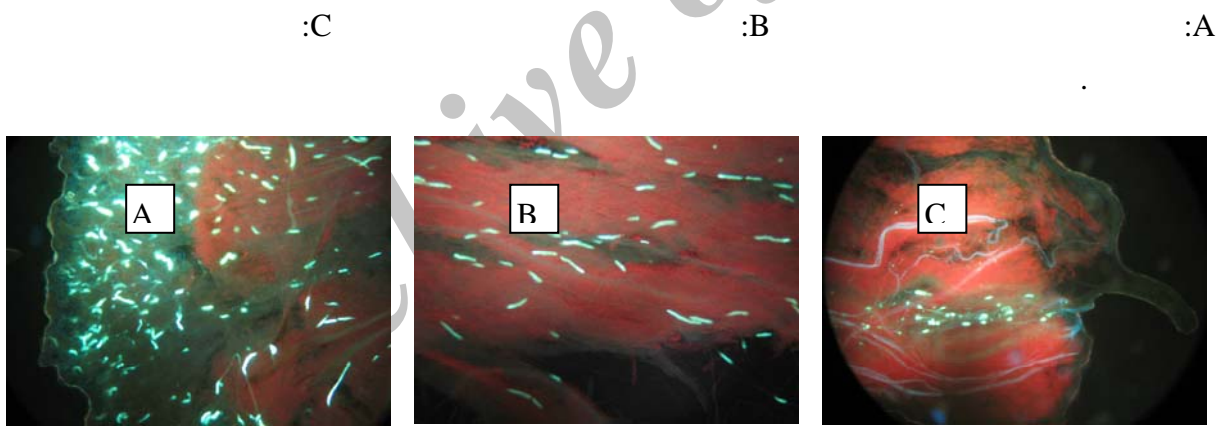


Fig. 2. pollen tube growth to base of style in self-compatible cultivars.

		PCR	S
$S_{25}S_f$	S_1S_f	SfF-SfR	
	(S_1S_{25})		'G-2-25' ($S_{11}S_f$)
'G-2-25'		S_1S_{11}	
$S_{12}S_f$		$S_{11}S_{12}$	$(S_{11}S_{12})$
		$()$	
	$(S_{11}S_{12})$		'G-2-25' ($S_{11}S_f$)
% $S_{11}S_{12}$			(χ^2)
			$S_{12}S_f$
	'G-2-25'		
	(S_{11})		
S_{11}	S_f		S_{11}
			S_{12}
$S_{11}S_{25}$	S_1S_{11}	$(S_{11}S_f)$	'G-2-25' ($S_1S_{25})$
%			'G-2-25'
S_{25}		$S_{11}S_{25}$	$S_{25}S_f$
			% S_1S_f % S_1S_{11}
	% S_{25}	S_1	%
	$S_{11}S_{25}$		
S_{12}			
		ConF-ConR	
			AS1II-AmyC5R
SfF-SfR			
	S_f		S_f SfF-SfR
	AS1II-AmyC5R	ConF-ConR	
			S_f
	$()$	$()$	$()$
S_3	$()$	ConF-ConR	
		S_f	
	S_f	$()$	AS1II-AmyC5R
		$()$	

Chi-Square Test

(S₁₁S₁₂) ‘ , (S₁S₂₅) ‘ , ‘G-2-25’ (S₁₁S_f)

Table 3- Fluorescence microscopy method for identification of self-compatible genotypes in crossing of female parent of ‘G-2-25’ (S₁₁S_f) with male parents of ‘Desmayo Margeuta’ (S₁S₂₅) and ‘Marcona’ (S₁₁S₁₂).

Average pollen tube number at the base of each style	Total pollen tube number at the base of 12 styles	Maximum pollen tube number at the base of each style	Minimum pollen tube number at the base of each style	Total number of pistils (style)	Seedling (progeny)	Population or cultivar	
5.5	66	9	2	12	P-3-27	‘Desmayo Margeuta’ * ‘G-2-25’	
0.08	1	1	0	12	P-3-28		
0.17	2	2	0	12	P-3-29		
0.33	4	1	0	12	P-3-30		
0	0	0	0	12	P-3-31		
6.33	76	9	5	12	P-3-32		
2.67	32	8	0	12	P-3-33		
4.42	53	8	2	12	P-3-34		
2.42	29	8	0	12	P-3-35		
3.33	40	8	1	12	P-3-36		
3.08	37	4	2	12	P-3-37		
1.92	23	3	1	12	P-3-38		
2.17	26	4	0	12	P-3-39		
4.17	50	8	2	12	P-3-40		
5.92	71	9	3	12	P-3-41		
0.92	11	2	0	12	P-3-42		
3.92	47	6	1	12	P-3-43		
6	72	9	4	12	P-3-44		
4.75	51	8	1	12	P-3-45		
0.25	3	1	0	12	P-5-20		‘Marcona’ * ‘G-2-25’
5.50	66	8	1	12	P-5-21		
5.92	71	8	4	12	P-5-22		
1.42	17	6	0	12	P-5-23		
5.17	66	8	2	12	P-5-24		
0	0	0	0	12	P-5-25		
7.75	93	9	5	12	P-5-26		
0.08	1	1	0	12	P-5-27		
0	0	0	0	12	P-5-28		
1	12	3	0	12	P-5-29		
6.50	78	9	3	12	P-5-30		
0	0	0	0	12	P-5-31		
4.42	49	8	3	12	G-2-25		
7.76	92	9	5	12	‘Jinko’	Control cvs. self-compatible	
5.50	66	8	3	12	‘Superano’		
7	84	9	4	12	‘Tono’		

'G-2-25'

Table 4. Comparison of mean pollen tube number at the base of style in crossing of female parent of 'G-2-25' (S₁₁S_f) with male parents of 'Desmayo Largeuta'(S₁S₂₅) and 'Marcona' (S₁₁S₁₂).

Seedling	Number of studied style in each progeny	Mean of pollen tube number at the base of each style
P-5-25	12	0 a
P-5-31	12	0 a
P-5-25	12	0 a
P-3-31	12	0 a
P-5-27	12	0.08 a
P-3-28	12	0.08 a
P-3-29	12	0.17 a
P-5-20	12	0.25 a
P-3-30	12	0.33 a
P-3-42	12	0.92 ab
P-5-29	12	1 abc
P-5-23	12	1.42 abcd
P-3-38	12	1.92 bcd
P-3-39	12	2.17 bcd
P-3-35	12	2.42 cd
P-3-33	12	2.67 cd
P-3-37	12	3.83 ef
P-3-36	12	3.33 efg
P-3-43	12	3.92 efgh
P-3-40	12	4.17 fghi
G-2-25	12	4.42 fghij
P-3-34	12	4.42 fghij
P-3-45	12	4.75 ghij
P-5-24	12	5.17 hijkl
P-5-21	12	5.50 ijkl
'Suprano'	12	5.50 ijkl
P-3-27	12	5.50 ijkl
P-5-22	12	5.92 jkl
P-3-41	12	5.92 jkl
P-3-44	12	6 kl
P-3-32	12	6.33 lmn
P-5-30	12	6.50 lmn
'Tono'	12	7 mn
'Jinko'	12	7.67 n
P-5-26	12	7.75 n

† Means followed with the same leffers in each column are not significant at 5% probability using DNMRT.

%

†

'G-2-25'

Table 5. The percentage of Obtained Progeny from 'G-2-25' ($S_{11}S_f$) X 'Desmayo Langueta' (S_1S_{25}).

Compatibility situation	Number of crossing	%	Genotypes
Self-compatible	10	52.6	S_1S_f
Self-compatible	5	26.3	$S_{25}S_f$
Self-incompatible	4	21.05	S_1S_{11}
Self-incompatible	0	0	$S_{11}S_{25}$

G-2-25

Table 6. The percentage of Obtained Progeny from 'G-2-25' ($S_{11}S_f$) X 'Marcona' ($S_{11}S_{12}$)

Compatibility situation	Number of crossing	%	Genotype
Self-incompatible	5	41.6	$S_{11}S_{12}$
Self-compatible	7	58.33	$S_{12}S_f$

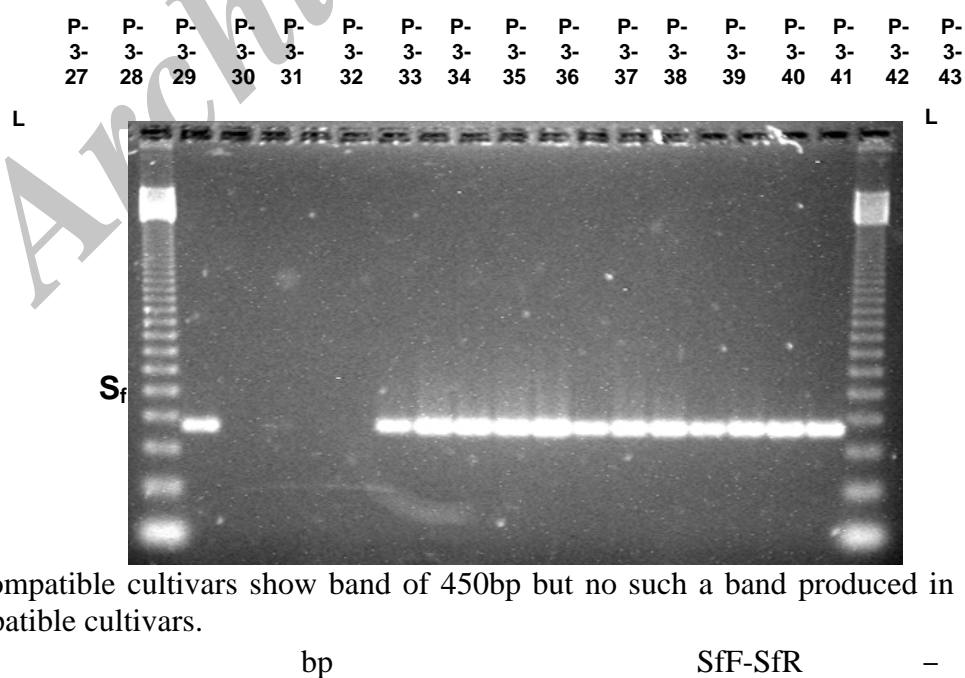


Fig. 3. Self-compatible cultivars show band of 450bp but no such a band produced in self-incompatible cultivars.

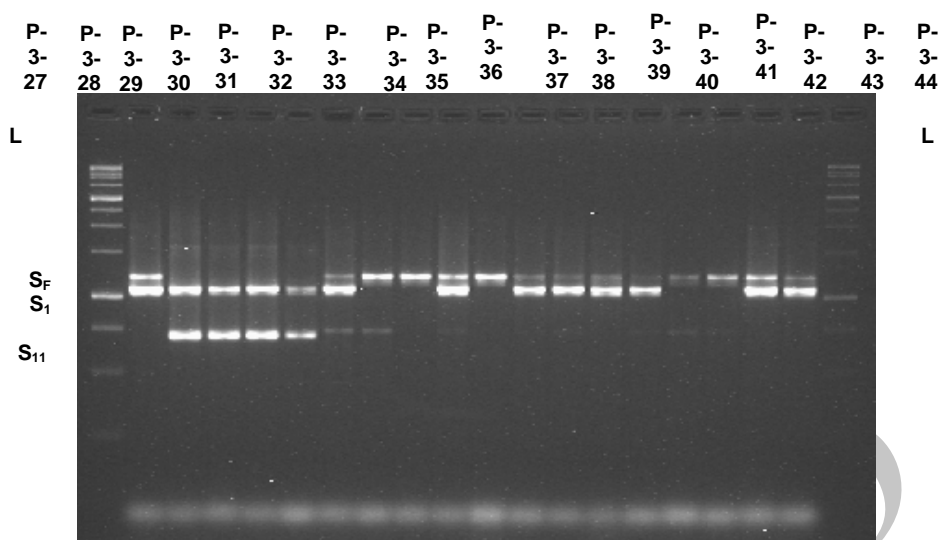


Fig. 4. Alleles of S₁₁, S₁, S_f have been produced by ConF-ConR primer.

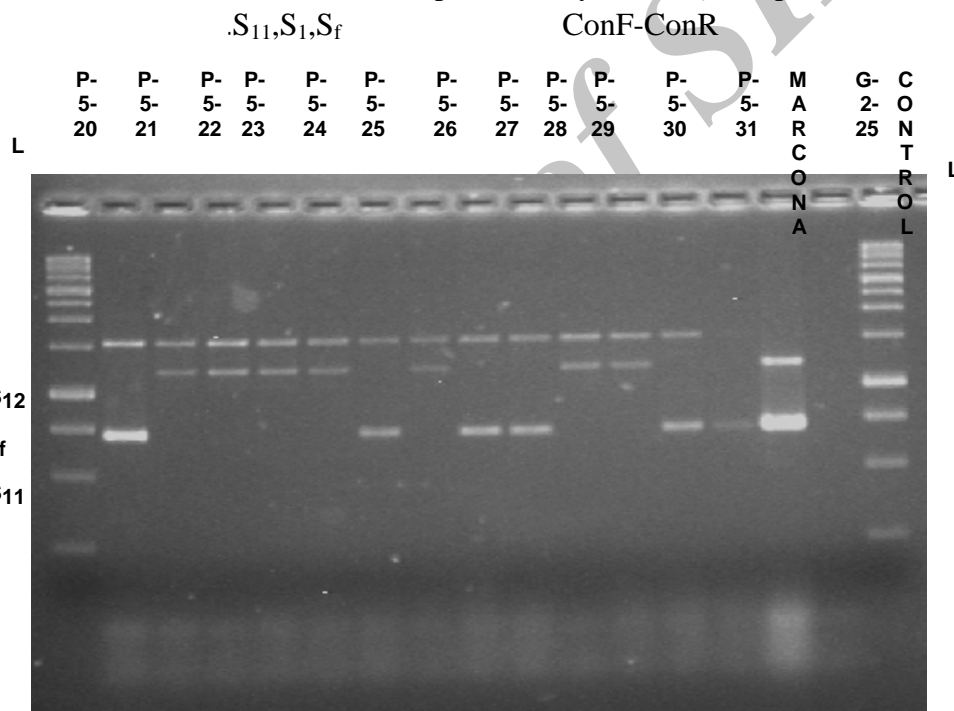
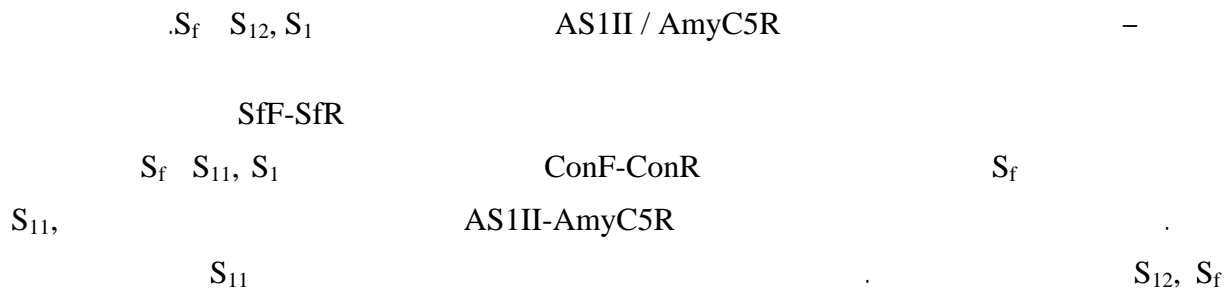


Fig. 5. Identification of S₁, S₁₂ and S_f by AS1II / AmyC5R primers.



S-RNases () NEpHGE ()
pH
()
% NEpHGE
()

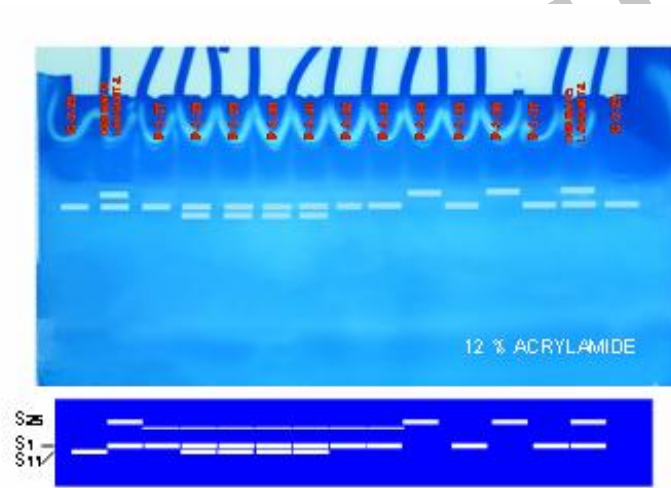


Fig. 6. Identification of almond S alleles by NEpHGE method.

NEpHGE S
PCR NEpHGE
(S₁S₂₅) 'G-2-25', (S₁₁S_f) (S₁₁S₁₂)
PCR

‘G-2-25’ (S₁₁S_f)

(S₁S₂₅) ‘ (S₁₁S₁₂)

Table 7. Comparison of different classic and molecular methods in discrimination of self-compatible and self-incompatible genotypes in controlled crossing of female parent of ‘G-2-25’ (S₁₁S_f) with male parents of ‘Desmayo Largeuta’(S₁S₂₅) and ‘Marcona’ (S₁₁S₁₂).

Florescence Microscopy	PCR Method		PCR	NEpHGE Method	Seedling	Population
	ConF- ConR	ASIII- Amy5CR				
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-27	1
	S ₁ S ₁₁	S ₁ S ₁₁		S ₁ S ₁₁	P-3-28	2
Self- incompatible	S ₁ S ₁₁	S ₁ S ₁₁	Self-incompatible	S ₁ S ₁₁	P-3-29	3
Self- incompatible			Self-incompatible	S ₁ S ₁₁	P-3-30	4
Doubtful	S ₁ S ₁₁	S ₁ S ₁₁	Self-compatible	S ₁ S ₁₁ S	P-3-31	5
Self- incompatible	S ₁ S _f	S ₁ S _f	Self-incompatible	S ₁ S _f	P-3-32	6
Self-compatible			Self-compatible	S ₂₅ S _f	P-3-33	7
Self-compatible	S ₂₅ S _f	S ₂₅ S _f	Self-compatible	S ₂₅ S _f	P-3-34	8
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-35	9
Self-compatible	S ₂₅ S _f	S ₂₅ S _f	Self-compatible	S ₂₅ S _f	P-3-36	10
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-37	11
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-38	12
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-39	13
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-40	14
Self-compatible	S ₂₅ S _f	S ₂₅ S _f	Self-compatible	S ₂₅ S _f	P-3-41	15
Self-compatible	S ₂₅ S _f	S ₂₅ S _f	Self-compatible	S ₂₅ S _f	P-3-42	16
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-43	17
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-44	18
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-45	19
Self-compatible	S ₁ S _f	S ₁ S _f	Self-compatible	S ₁ S _f	P-3-45	19

‘G-2-25’ x
‘Desmayo Largeuta’ ‘G-2-25’

Table 7. Continued						
	$S_{11}S_{12}$	$S_{11}S_{12}$		$S_{11}S_{12}$	P-5-20	20
Doubtful			Self-incompatible			
	$S_{12}S_f$	$S_{12}S_f$		$S_{12}S_f$	P-5-21	21
Self-compatible			Self-compatible			
	$S_{12}S_f$	$S_{12}S_f$		$S_{12}S_f$	P-5-22	22
Self-compatible			Self-compatible			
	$S_{12}S_f$	$S_{12}S_f$		$S_{12}S_f$	P-5-23	23
Self-compatible			Self-compatible			
	$S_{12}S_f$	$S_{12}S_f$		$S_{12}S_f$	P-5-24	24
Self-compatible			Self-compatible			
	$S_{11}S_{12}$	$S_{11}S_{12}$		$S_{11}S_{12}$	P-5-25	25
Self-compatible			Self-incompatible			
	$S_{12}S_f$	$S_{12}S_f$		$S_{12}S_f$	P-5-26	26
Self-compatible			Self-compatible			
	$S_{11}S_{12}$	$S_{11}S_{12}$		$S_{11}S_{12}$	P-5-27	27
Self-incompatible			Self-incompatible			
	$S_{11}S_{12}$	$S_{11}S_{12}$		$S_{11}S_{12}$	P-5-28	28
Self-incompatible			Self-incompatible			
	$S_{12}S_f$	$S_{12}S_f$		$S_{12}S_f$	P-5-29	29
Self-compatible			Self-compatible			
	$S_{12}S_f$	$S_{12}S_f$		$S_{12}S_f$	P-5-30	30
Self-compatible			Self-compatible			
	$S_{11}S_{12}$	$S_{11}S_{12}$		$S_{11}S_{12}$	P-5-31	31
Self-incompatible			Self-incompatible			
	S_1S_{25}	S_1S_{25}		S_1S_{25}		32
Self-incompatible			Self-incompatible			
	$S_{11}S_f$	$S_{11}S_f$		$S_{11}S_f$	'G-2-25'	33
Self-compatible			Self-compatible			
	$S_{11}S_{12}$	$S_{11}S_{12}$		$S_{11}S_{12}$		34
Self-incompatible			Self-incompatible			

"G-2-25" ×
"Marcona" 'G-2-25' ×

Parents

NepHGE

PCR

S

NEpHGE

PCR

NEpHGE

S

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