

Selective Amplification of Start codon Polymorphic Loci (SASPL): a new PCR-based molecular marker in olive

Alsamman M. Alsamman*, S. S. Adawy, S. D. Ibrahim, B. A. Hussein and E. H. A. Hussein

Table S1. Primers names (PN), primers sequences (PS), total number of amplicons (TA), monomorphic amplicons (MA), polymorphic amplicons (PA), percentage of polymorphism (%P) and polymorphism information content (PIC) as revealed by RAPD markers among the 10 olive varieties.

PN	PS(5'-3')	TA	MA	PA	%P	PIC
B17	AGGGAACGAG	21	15	6	28.6%	0.3162
A20	GTTGCGATCC	12	9	3	25.0%	0.221
K7	AGCGAGCAAG	11	6	5	45.5%	0.2091
G9	CTGACGTCAC	11	7	4	36.4%	0.206
P1	GTAGCACTCC	22	12	10	45.5%	0.3146
B2	TGATCCCTGG	11	6	5	45.5%	0.2106
B13	TTCCCCGCT	13	2	11	84.6%	0.1815
O3	CTGTTGCTAC	10	4	6	60.0%	0.1915
A14	TCTGTGCTGG	12	9	3	25.0%	0.1638
C2	GTGAGGCGTC	8	4	4	50.0%	0.2268
C16	CACACTCCAG	14	11	3	21.4%	0.2558
A18	AGGTGACCGT	16	11	5	31.2%	0.2744
H18	GAATCGGCCA	17	11	6	35.3%	0.2879
A6	GGTCCCTGAC	22	5	17	77.3%	0.3029
A17	GACCGCTTGT	10	6	4	40.0%	0.1948
G13	CTCTCCGCCA	7	7	0	0.0%	0.1601
C13	AAGCCTCGTC	21	6	15	71.4%	0.2947
A16	AGCCAGCGAA	24	5	19	79.2%	0.0972
G12	CAGCTCACGA	5	3	2	40.0%	0.2839
K6	CACCTTCCC	16	8	8	50.0%	0.3081
A13	CAGCACCCAC	21	11	10	47.6%	0.2665
A12	TCGGCGATAG	22	10	12	54.5%	0.3012
E5	TCAGGGAGGT	18	11	7	38.9%	0.2889
K4	CCGCCCAAAC	15	10	5	33.3%	0.2571
Total	-	359	189	170	-	-
Average	-	14.95	7.9	7.1	47.4%	0.2423

Table S2. Primers name (PN), primers sequences (PS), total number of amplicons (TA), monomorphic amplicons (MA), polymorphic amplicons (PA), percentage of polymorphism (%P) and polymorphism information content (PIC) as revealed by SCoT markers among the 10 olive varieties.

PN	PS (5'-3')	TA	MA	PA	%P	PIC
SCoT-01	ACGACATGGCGACCACGC	17	9	8	47.10%	0.2116
SCoT-02	ACCATGGCTACCACCGGC	7	4	3	42.90%	0.1036
SCoT-03	ACGACATGGCGACCCACA	11	2	9	81.80%	0.1387
SCoT-06	CAATGGCTACC ACTACAG	25	6	19	76.00%	0.2558
SCoT-08	ACAATGGCTACC ACTGAG	11	9	2	18.20%	0.1730
SCoT-09	ACAATGGCTACC ACTGCC	10	6	4	40.00%	0.1445
SCoT-10	ACAATGGCTACC ACCAGC	16	9	7	43.80%	0.2127
SCoT-11	ACAATGGCTACC ACTACC	13	7	6	46.20%	0.1845
SCoT-12	CAACAATGGCTACC ACCG	20	9	11	55.00%	0.2379
SCoT-13	ACCATGGCTACC ACGGCA	17	3	14	82.40%	0.1990
SCoT-14	ACCATGGCTACC ACGCGC	12	3	9	75.00%	0.1543
SCoT-15	CCATGGCTACC ACCGGCT	17	7	10	58.80%	0.1990
SCoT-16	CCATGGCTACC ACCGGCA	8	7	1	12.50%	0.1372
SCoT-17	CCATGGCTACC ACTACC	14	6	8	57.10%	0.1782
SCoT-18	CCATGGCTACC ACTAGCA	13	3	10	76.90%	0.1756
SCoT-21	CCATGGCTACC ACCGGCC	13	11	2	15.40%	0.2013
SCoT-23	CATGGCTACC ACCGGCCC	17	7	10	58.80%	0.2116
SCoT-25	ACGACATGGCGACC GCGA	19	10	9	47.40%	0.2466
SCoT-26	ACGACATGGCGACC ACGT	13	4	9	69.20%	0.1704
SCoT-27	ACCATGGCTACC ACCGTC	15	11	4	26.70%	0.2048
SCoT-28	CAACAATGGCTACC ACCA	12	9	3	25.00%	0.1832
SCoT-29	CAACAATGGCTACC ACCC	10	7	3	30.00%	0.1529
SCoT-30	CAACAATGGCTACC ACCT	15	2	13	86.70%	0.1978
SCoT-31	CAACAATGGCTACC ACGA	30	8	22	73.30%	0.2846
SCoT-32	CAACAATGGCTACC ACGC	22	7	15	68.20%	0.2475
SCoT-33	AAGCAATGGCTACC ACCA	16	5	11	68.80%	0.2138
SCoT-34	ACGACATGGCGACC AACG	21	6	15	71.40%	0.2399
SCoT-35	AACCATGGCTACC ACCAC	12	6	6	50.00%	0.1691
SCoT-36	CACCATGGCTACC ACCAT	20	11	9	45.00%	0.2437
SCoT-37	GCAACAATGGCTACC ACC	22	12	10	45.50%	0.2408
SCoT-38	ACCATGGCTACC ACCGGG	16	3	13	81.30%	0.1882
SCoT-40	CAACAATGGCTACC ACGT	23	6	17	73.90%	0.2339
SCoT-41	CAACAATGGCTACC AGCA	22	5	17	77.30%	0.2558
SCoT-43	ACGACATGGCGACC ATCG	21	8	13	61.90%	0.2288
SCoT-45	ACCATGGCTACC ACCGAG	13	3	10	76.90%	0.1769
SCoT-46	ACCATGGCTACC ACCGCC	17	6	11	64.70%	0.2203
SCoT-47	ACCATGGCTACC ACCGCG	17	6	11	64.70%	0.2288
SCoT-48	CACCATGGCTACC ACCAG	20	13	7	35.00%	0.2428
SCoT-49	ACCATGGCTACC ACCGTC	25	4	21	84.00%	0.2585
Total	-	642	260	382	-	-
Average	-	16.46	6.66	9.7	59.50%	0.2038

Table S3. Primers name (PN), forward primers sequences (FPS), reverse primers sequences (RPS), total number of amplicons (TA), monomorphic amplicons (MA), polymorphic amplicons (PA), percentage of polymorphism (%P) and polymorphism information content (PIC) as revealed by SAMPL marker combinations among the 10 olive varieties.

PN	FPS (5'-3')	RPS	TA	MA	PA	%P	PIC
SAMPL-01	(CT)8T	M-CAA	30	12	18	60.00%	0.2072
SAMPL-02	(CT)8T	M-CAC	41	25	16	39.00%	0.2644
SAMPL-03	(CT)8T	M-CAT	45	23	22	48.90%	0.2594
SAMPL-04	(GT)8C	M-CAA	49	36	13	26.50%	0.2976
SAMPL-05	(AGAC)4GC	M-CAT	33	15	18	54.50%	0.2239
SAMPL-06	(GATA)4GC	M-CAC	39	24	15	38.50%	0.2570
SAMPL-07	(GATA)4GC	M-CAT	68	39	29	42.60%	0.3350
SAMPL-08	(GACA)4AT	M-CAT	53	44	9	17.00%	0.3186
SAMPL-09	(AG)8YT	M-CAA	79	58	21	26.60%	0.3628
SAMPL-10	(AG)8YT	M-CTC	59	39	20	33.90%	0.3305
SAMPL-11	(CTC)5TT	M-CAA	38	25	13	34.20%	0.2504
SAMPL-12	(CTC)5TT	M-CTC	37	14	23	62.20%	0.2266
Total	-	-	571	354	217	-	-
Average	-	-	47.6	29.5	18.1	38.00%	0.2778

Table S4. Primers name (PN), forward primers sequences (FPS), reverse primers sequences (RPS), total number of amplicons (TA), monomorphic amplicons (MA), polymorphic amplicons (PA), percentage of polymorphism (%P) and polymorphism information content (PIC) as revealed by SASPL marker combinations among the 10 olive varieties.

PN	FPS (5'-3')	RPS	TA	MA	PA	%P	PIC
SASPL-01	ACGACATGGCGACCCACA	M-CAC	42	15	27	64.30%	0.3224
SASPL-02	ACAATGGCTACCACTACC	M-CAA	33	12	21	63.60%	0.2721
SASPL-03	ACAATGGCTACCACTACC	M-CTG	40	16	24	60.00%	0.3110
SASPL-04	ACCATGGCTACCAGCGCG	M-CAA	36	14	22	61.10%	0.2997
SASPL-05	ACCATGGCTACCAGCGCG	M-CTG	22	12	10	45.50%	0.2371
SASPL-06	CCATGGCTACCACCGCAC	M-CAA	40	30	10	25.00%	0.3302
SASPL-07	CCATGGCTACCACCGCAC	M-CAC	29	26	3	10.30%	0.2890
SASPL-08	ACCATGGCTACCACCGCG	M-CAA	27	14	13	48.10%	0.2661
Total	-	-	269	139	130	-	-
Average	-	-	33.6	17.4	16.2	48.30%	0.2909

Table S5. Variety name (VN), primer name (PN), marker band Size (MBS), total number of markers per cultivar (TMC) and grand total markers (GT) for the 10 olive varieties characterized by unique positive and/or negative RAPD markers.

VN	Unique negative markers			Unique positive markers			GT
	PN	MBS	TMC	PN	MBS	TMC	
Kronaki	C13	550	8			0	8
	H18	410,250,150					
	A12	350					
	K7	470					
	B2	920					
	A14	340					
Tofahi	A16	470	5			0	5
	B17	675					
	K6	320,290					
	P1	700					
Calamata	A18	1250	4			0	4
	H18	600					
	E5	550					
Manzanillo	G9	150	1			0	1
	P1	430					
OjaziShami	A12	550	2			0	2
	E5	250					
Koratina	A16	750	4			0	4
	A13	525					
	P1	650					
	C2	150					
Dolci	C13	440	2			1	3
	A13	410					
Maraki	A12	600,150	6			1	7
	A16						
	O3						
	K4						
Pekoal	A13	250	3			0	3
	B2	500					
	A14	500					
Khodari	C13	560	9			0	9
	B17	650					
	A18	450					
	A6	830,575,410,290					
	K7	390					
Total	C2	310	44			2	46

Table S6. Variety name (VN), primer name (PN), marker band size (MBS), total number of markers per cultivar (TMC) and grand total markers (GT) for the 10 olive varieties characterized by unique positive and/or negative SCoT markers.

VN	Unique negative markers			Unique positive markers			GT
	PN	MBS	TMC	PN	TMC	TMC	
				SCoT-10	470		
				SCoT-01	350		
Kronaki	SCoT-41	900,830,570					
	SCoT-48	1800					
	SCoT-13	1500					
	SCoT-40	700					
	SCoT-37	790	15			2	17
	SCoT-17	730					
	SCoT-29	430					
	SCoT-30	650,580					
	SCoT-23	350					
	SCoT-01	1,030,470					
Tofahi	SCoT-06	410					
	SCoT-18	460					
	SCoT-25	680					
	SCoT-45	1350	6			0	6
	SCoT-40	470					
	SCoT-26	450					
Calamata				SCoT-36	440		
	SCoT-18	300					
	SCoT-03	800					
	SCoT-34	930,800,730					
	SCoT-21	1,400,500	13			1	14
	SCoT-29	420					
	SCoT-46	700,650					
	SCoT-33	750,650					
SCoT-06	870						
Manzanillo				SCoT-37	200		
				SCoT-12	310		
			2	SCoT-48	1300	3	5
	SCoT-18	750					
	SCoT-06	530					
Ojazi Shami	SCoT-18	500					
	SCoT-12	440					
	SCoT-49	3000					
	SCoT-31	630					
	SCoT-25	580	9			0	9
	SCoT-38	630					
	SCoT-47	570					
	SCoT-17	410					
SCoT-33	670						
Cont.							
Table S6.Cont.							
Koratina	SCoT-41	150					
	SCoT-18	1100					
	SCoT-03	600					
	SCoT-34	1450					
	SCoT-12	500					
	SCoT-49	380					
	SCoT-45	730					
	SCoT-38	1100					
	SCoT-47	13,501,100					
	SCoT-37	2500					
	SCoT-23	220	12			0	12
	SCoT-41	200					
	SCoT-18	420					
SCoT-13	350						
Dolci	SCoT-45	800					
	SCoT-47	740					
	SCoT-30	930	10			0	10

	SCoT-10	1500					
	SCoT-43	420					
	SCoT-26	680,580					
				SCoT-40	310		
	SCoT-41	1150					
	SCoT-28	450					
	SCoT-13	550					
	SCoT-45	830					
	SCoT-38	450					
	SCoT-14	630,600					
	SCoT-47	1,250,780					
	SCoT-30	700					
	SCoT-36	1,350,790					
	SCoT-11	730					
Maraki	SCoT-16	290	16			1	17
				SCoT-02	200		
	SCoT-37	750					
	SCoT-30	730					
	SCoT-09	730					
	SCoT-43	480					
Pekoal	SCoT-13	750	5			1	6
	SCoT-34	880					
	SCoT-12	650					
	SCoT-49	650,150					
	SCoT-38	1125					
	SCoT-14	500					
	SCoT-47	600					
	SCoT-32	1,450,950					
	SCoT-30	790					
	SCoT-46	400					
	SCoT-11	750					
	SCoT-33	880,550					
Khodari	SCoT-01	917	15			0	15
Total			103			8	111

Table S7. Variety name (VN), primer name (PN), marker band size (MBS), total number of markers per cultivar (TMC) and grand total markers (GT) for the 10 olive varieties characterized by unique positive and/or negative SAMPL markers.

VN	PN	Unique negative markers		Unique positive markers			GT
		MBS	TMC	PN	BS	TMC	
Kronaki	SAMPL-02	395					
	SAMPL-07	248	3			0	3
	SAMPL-05	443					
Tofahi	SAMPL-08	480	1			0	1
					SAMPL-12	365	
Calamata	SAMPL-12	315					
	SAMPL-07	203	7			1	8
	SAMPL-09	640					
	SAMPL-05	900					
Manzanillo	SAMPL-08	300,295,285					
	SAMPL-10	175,173	2			0	2
OjaziShami	SAMPL-01	335,330,325					
	SAMPL-05	230	4			0	4
Koratina	SAMPL-02	595					
	SAMPL-01	660	5			0	5
	SAMPL-11	315,250					
	SAMPL-08	275					
Dolci	SAMPL-04	310					
	SAMPL-07	180,175					
	SAMPL-09	300,295,265	7			0	7
Maraki	SAMPL-08	543					
	SAMPL-12	690,320,280					
	SAMPL-02	360	38			0	38
	SAMPL-09	347					

	SAMPL-11	330			
	SAMPL-05	680,440,400,395,390			
	SAMPL-06	440,435,425,420,410,405,230			
	SAMPL-04	315,290,98			
	SAMPL-10	460,410,400,363,350,348,310,308,275,218			
	SAMPL-07	375,330,255,235			
	SAMPL-01	375			
	SAMPL-03	460,320			
Pekoal	SAMPL-02	465	1	0	1
	SAMPL-10	150			
Khodari	SAMPL-02	345	6	0	6
	SAMPL-01	900,460			
	SAMPL-09	600,390			
Total			74	1	75

Table S8. Variety name (VN), primer name (PN), marker band Size (MBS), total number of markers per cultivar (TMC) and grand total Markers (GT) for the 10 olive varieties characterized by unique positive and/or negative SASPL markers.

Variety name	Unique negative markers			Unique positive markers			GT
	PN	MBS	TMC	PN	BS	TMC	
Kronaki	SASPL-01	670					
	SASPL-08	310	6			0	6
Tofahi	SASPL-03	500,480,180,150					
	SASPL-04	1,220,480	3			0	3
	SASPL-01	355					
Calamata				SASPL-04	250		
				SASPL-06	140		
			3	SASPL-02	380,375	4	7
	SASPL-04	150					
Manzanillo	SASPL-01	600					
	SASPL-02	400					
			0			0	0
Ojazi Shami	SASPL-04	1,010,660					
	SASPL-06	80	5			0	5
	SASPL-02	490,320					
Koratina	SASPL-05	1,500,580					
	SASPL-01	500	5			0	5
	SASPL-08	405					
Dolci	SASPL-03	560					
	SASPL-01	356	1			0	1
Maraki	SASPL-05	750,600,550,480					
	SASPL-04	600,360					
	SASPL-01	725,680,220	12			0	12
	SASPL-06	395					
	SASPL-08	400					
Pekoal	SASPL-03	325					
	SASPL-08	340	1			0	1

Khodari	SASPL-06	245	3	0	3
	SASPL-03	645,255			
Total			39	4	43

Table S9. Similarity table for the 10 olive varieties constructed from the RAPD data using UPGMA and similarity matrices computed according to Dice coefficient.

Variety Name	Kronaki	Tofahi	Calamata	Ojazi Shami	Manzanillo	Koratina	Maraki	Dolici	Pekoal	Khodari
Kronaki	100%									
Tofahi	88%	100%								
Calamata	87%	89%	100%							
OjaziShami	89%	92%	89%	100%						
Manzanillo	91%	91%	89%	93%	100%					
Koratina	89%	88%	88%	88%	89%	100%				
Maraki	89%	90%	88%	90%	91%	88%	100%			
Dolici	88%	88%	88%	91%	92%	88%	90%	100%		
Pekoal	91%	90%	90%	91%	93%	90%	92%	91%	100%	
Khodari	88%	87%	88%	88%	89%	88%	88%	91%	91%	100%

Table S10. Similarity table for the 10olive varieties constructed from the SCoTs data using UPGMA and similarity matrices computed according to Dice coefficient.

Variety Name	Khodari	Calamata	Kronaki	Ojazi Shami	Dolci	Manzanillo	Pekoal	Koratina	Tofahi	Maraki
Khodari	100%	-	-	-	-	-	-	-	-	-
Calamata	85%	100%	-	-	-	-	-	-	-	-
Kronaki	84%	83%	100%	-	-	-	-	-	-	-
OjaziShami	86%	87%	86%	100%	-	-	-	-	-	-
Dolci	87%	86%	86%	87%	100%	-	-	-	-	-
Manzanillo	85%	87%	87%	87%	89%	100%	-	-	-	-
Pekoal	86%	87%	87%	88%	88%	90%	100%	-	-	-
Koratina	83%	84%	85%	85%	85%	87%	86%	100%	-	-
Tofahi	86%	85%	86%	89%	89%	88%	88%	86%	100%	-
Maraki	83%	83%	83%	85%	86%	87%	86%	83%	86%	100%

Table S11. Similarity table for the 10olive varieties constructed from the SAMPL data using UPGMA and similarity matrices computed according to Dice coefficient.

Variety Name	Kronaki	Tofahi	Calamata	Ojazi Shami	Manzanillo	Koratina	Maraki	Dolci	Pekoal	Khodari
Kronaki	100%									
Tofahi	93%	100%								
Calamata	94%	93%	100%							
OjaziShami	93%	94%	92%	100%						
Manzanillo	94%	94%	93%	94%	100%					
Koratina	93%	93%	91%	94%	93%	100%				
Maraki	87%	88%	86%	88%	88%	88%	100%			
Dolci	93%	93%	92%	94%	96%	92%	86%	100%		
Pekoal	93%	93%	92%	93%	96%	93%	89%	94%	100%	
Khodari	93%	94%	95%	92%	94%	91%	86%	93%	93%	100%

Table S12. Similarity table for the 10olive varieties constructed from the SASPL data using UPGMA and similarity matrices computed according to Dice coefficient.

Variety Name	Kronaki	Tofahi	Calamata	Ojazi Shami	Manzanillo	Koratina	Maraki	Dolci	Pekoal	Khodari
Kronaki	100%									
Tofahi	89%	100%								
Calamata	92%	89%	100%							
OjaziShami	86%	90%	87%	100%						
Manzanillo	91%	92%	91%	91%	100%					
Koratina	89%	90%	88%	89%	91%	100%				
Maraki	85%	87%	84%	87%	88%	87%	100%			
Dolci	91%	91%	90%	89%	93%	92%	87%	100%		
Pekoal	87%	89%	87%	89%	93%	91%	88%	90%	100%	
Khodari	89%	91%	92%	89%	93%	90%	86%	91%	90%	100%

Table S13. Similarity table for the 10olive varieties constructed from the combined data using UPGMA and similarity matrices computed according to Dice coefficient.

Variety Name	Dolci	Pekoal	Kronaki	Khodari	Maraki	Koratina	Ojazi Shami	Manzanillo	Calamata	Tofahi
Dolci	100.0%									
Pekoal	91.0%	100.0%								
Kronaki	89.5%	89.6%	100.0%							
Khodari	90.4%	89.6%	88.6%	100.0%						
Maraki	87.0%	88.2%	85.6%	85.6%	100.0%					
Koratina	88.7%	89.9%	88.8%	87.7%	86.0%	100.0%				
OjaziShami	90.6%	90.3%	89.0%	88.9%	87.1%	88.8%	100.0%			
Manzanillo	92.1%	92.7%	90.7%	89.6%	88.0%	89.9%	90.7%	100.0%		
Calamata	88.8%	89.1%	88.7%	89.8%	85.2%	87.5%	88.8%	90.1%	100.0%	
Tofahi	90.4%	90.2%	89.4%	89.4%	87.7%	89.4%	91.3%	91.1%	89.2%	100.0%