

Molecular markers flanking the corky root resistance gene, *cor*

Description:

PCR-based molecular markers linked to *cor* that can be used for marker-assisted selection in a broad spectrum of lettuce cultivars have been identified and developed (Moreno-Vazquez *et al.*, 2003). These markers are co-dominant, easy to score, and inexpensive. They can be analyzed by PCR using standard conditions and without the use of restriction enzymes. Marker SCO07 has been the most commonly used PCR-based marker for introgressing *cor* into lettuce cultivars.

Methods:

Three different *cor* markers and the PCR primers used to amplify each, along with the sizes of the PCR products produced as a result of the amplifications, are shown in Table 1. More than two primers are listed for each marker because primers that specifically amplify the resistant allele are used in conjunction (“multiplexed”) with primers that specifically amplify the susceptible allele; these combinations of primers produce bands of different sizes (Table 1) that can be distinguished on agarose gels (Figure 1).

Table 1. Details of molecular markers flanking the *cor* locus.

Marker	Primer Name	Sequence 5'>3'	<i>Cor Cor</i> Band size (bp)	<i>cor cor</i> Band size (bp)
<i>SCY15</i>	Y15F1 Y15GRNPAKR1 Y15DIAKR2	AGCGTTATATCTCTCCTCTC (A) ₂₀ TGTGTAGTACTCCTCATAGAT ATATTCCAGACAAGTGATTA	160	94
<i>SCO07</i>	O07GRNKF4 O07DIAKF7 O07R	AGAGTTGACAGAGCAACACG ATGGGCTAAAACACTCACAG ATGTGGCTATGACTTCAGA	134	284
<i>SC853</i>	53DIAF1 853GRNF2 853R4	TAGTAGCAAAGAAGAGAG GGTTTTCGCCATTACTTT CTAGAAAAC TGAGATGAG	192	141

PCR amplifications for each marker are carried out as follows (as per Moreno-Vazquez *et al.*, 2003). Genomic DNA (approximately 30 ng) from each lettuce line is used as template in 20 µl reactions containing: 1x PCR buffer (10 mmol Tris-HCl/L pH 8.3, 50 mmol KCl/L, 0.01% w/v gelatin), 125 µmol each dNTP/L, 0.5 µmol (each) primer/L, 2 mmol MgCl₂/L, and 1 U *Taq* polymerase. Initial denaturation is for 30 s at 94°C, followed by three cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C; 32 cycles of 10 s at 94°C, 30 s at 35°C, and 1 min at 72°C; final extension is for 5 min at 72°C.

PCR products are then visualized following electrophoresis through an agarose gel and staining with ethidium bromide (Figure 1). For resolving these small fragments (less than 300 bp), a mixture of 0.70% w/v agarose and 1.25% w/v Synergel (Research Products International Corp., Mount Prospect, IL) gives good results.

Figure 1. PCR markers *SCO07* (b), and *SCY15* (c) amplified from parental lines 'Green Lake' (GRN) and 'Diana' (DIA) and from 22 homozygous resistant $F_{2:3}$ families. One recombinant is observed for *SCO07* (line 13). (See Table 1 for primer sequences and fragment sizes.) Numbers on the left show molecular sizes in bases pairs.

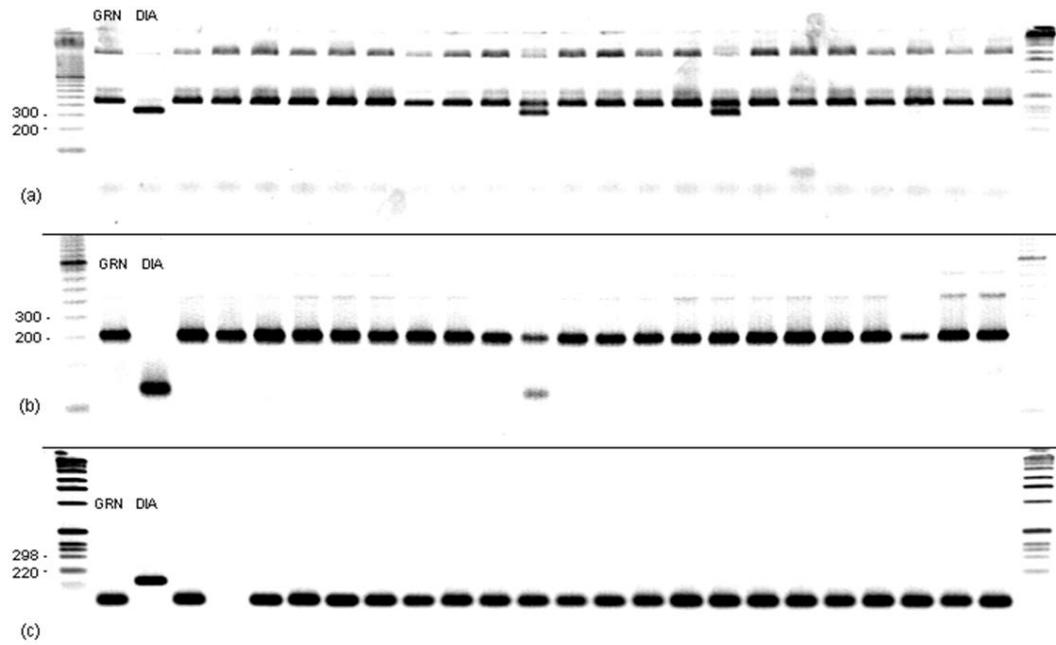


Table 1. Six SCAR markers flanking around the cor/Cor locus. The name of each primer is composed by the marker name, followed by the abbreviated name of the cultivar for what the primer is specific (DIA for Diana, GRN for Green Lake), then followed by letter 'K' whenever it contains an internal artificial mismatch and finally by followed by an 'F' or 'R' for a forward or reverse orientation, respectively. Underlined inside the primer sequences are the nucleotides mispriming specifically one of the alleles (most commonly the 3' terminal nucleotide) or both alleles (internal unspecific mispriming sites). In the fourth column, for each primer the mismatch types are specified starting above with the most 5' mismatch. Each mismatch is described by two letters separated by the colon mark, the first letter refers to the primer base, the second to the mismatching base at the template sequence. In the fifth column the alleles affected by the mismatch are cited.

Marker	Allele specific PCR ^a		Mismatches		Band size (bp)		Notes
	Primer name ^b	Primer sequence (5' - 3')	Type	Allele	CorCor (S)	corcor (R)	
SCAJ12	AJ12GRNKF2b	AAC AC <u>A</u> TAC TGGGCG A <u>A</u> C	A:G G:G C:A	DIA GRN and DIA DIA	596	105	Two PCRs per sample. PCR1 with primers AJ12GRNKF2b and AJ12R; PCR2 with primers AJ12F2 and AJ12DIAKR22. PCR products are mixed and loaded in the same well before electrophoresis.
	AJ12R	TCA GTT CCC GTA TGGTGA TAA					
	AJ12F2 AJ12DIAKR22	TCT ACC CAA GCA TCGTGT TCG CCG AGT AGT CGA CC <u>A</u> T <u>A</u>	A:G A:C	GRN and DIA GRN			
SC853	853DIAF1	TAG TAG CAA AGA AGA GA <u>G</u>	G:A	GRN	192	141	
	853GRNF2	GGT TTT CGC CAT TAC TT <u>T</u>	T:G	DIA			
	853R4	CTA GAA AAC TGA GAT GAG					
SCY15	Y15F1	AGC GTT ATA TCT CTC CTC TC			160	94	
	Y15GRNPAKR1	(A) ₂₀ TGT GTA GTA CTC CTC ATA <u>GAT</u>	G:G T:C	GRN and DIA DIA			
	Y15DIAKR2	ATA TTC CAG ACA AGT <u>GAT</u> T <u>A</u>	A:A A:A	GRN and DIA GRN			
SCO07	O07GRNKF4	AGA GTT GAC AGA GCA AC <u>A</u> C <u>G</u>	A:A G:T	GRN and DIA DIA	134	284	
	O07DIAKF7	ATG GGC TAA AAC ACT CA <u>C</u> A <u>G</u>	C:T G:G	GRN and DIA GRN			
	O07R	ATG TGG CTA TGA CTT CAGA					
SC268	268GRNF3	<u>GAG CTC GAG CTT GGT TAG G</u>	InDel	DIA	262	>506	Additional bands of high molecular weight may also be amplified
	268DIAF4	TGA TGT <u>TGC</u> ATC AAA AAT <u>T</u>	T:G T:G T:G C:A	GRN GRN DIA DIA			
	268GRNR2	CTC TCG C <u>TG</u> T <u>GC</u> TGT C					
	268R2	ACC TGT GAT TTT GAG TTT GC					
SC074	74F1				315	418	Additional bands of high molecular weight may also be amplified
	74DIAR7	<u>ACA</u> ACT AAC AAA AA <u>A</u> GGG A <u>G</u>	A:C A:C G:A G:T G:G G:A	GRN GRN GRN GRN and DIA DIA			
	74GRNKF13	GTC CCA TTG CTC TCA <u>GCG</u>					
	74R2	ATT CCT TGA GAT TCC ATA GT					

Reference:

Moreno-Vazquez, S., Ochoa, O., Faber, N., Chao, S., Jacobs, J.M.E., Maisonneuve, B., Kesseli, R., Michelmore, R.W. (2003). SNP-based co-dominant markers for a recessive gene conferring resistance to corky root rot (*Rhizomonas suberifaciens*) in lettuce (*Lactuca sativa*). *Genome* **46**:1059-1069.

Sequences of genes tightly linked to cor.

Gene 1 has sequence similarity to a transcription factor.

Gene 2 has sequence similarity to SH3_domain-containing_protein-1.

Gene 3 has sequence similarity to Nucleotide-diphospho-sugar_transferase_family_protein-1.

Gene 4 has sequence similarity to an unknown protein.

>Salinas_Gene1

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>Diana_Gene1

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>Diana_Gene2

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