The regulation of MADS-box gene expression during ripening of banana and their regulatory interaction with ethylene

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Table 1. Primers for cloning and expression analysis of *MaMADS-box* genes. Primers pairs used in successive PCR reactions to clone the full lengths of each of the genes. Each letter denoted for one reaction in which the first primer is the forward and the second is the reverse. Race technology was used to clone the full length sequence of all the genes. Open reading frame (ORF) describes the pair of primers used for cloning of the full length of the genes. RT PCR describes the primers used for Real Time PCR reactions for expression quantization of the genes.

Amplified cDNA		Primers	Amplified cDNA		Primers
MaMADS1 (705bp) EU869307	A	5'-GGATCGAGAACWMVAYMAACCGSC-3' 5'-TTCCAGCCATGCAGGCATA-3'	*MaMADS3* (728bp) EU869308	A	5'-GGATCGAGAACWMVAYMAACCGSC-3' 5'-ACCAGACAAGGAGAGTGAGACTT-3'
	B	5'-GTCTCAGAAGAAGGTTGGAGGAG-3'		B	5'-AATGCATTTGCTGCACAGCCA-3'
	C	5'-CGGTCAGTTGGTCAAGCATTTGT-3' 5'-GTGCTTTCTGACCCTCCATAG-3'		ORF	5'-ATGGGGAGGGGACGAGTTGAGCTG-3' 5'-GGGAACCCATTCCAGCATGAAATT-3'
	ORF	5'-ATGGGCAGGGGAAGGGTGGAGCTA-3' 5'-TTCCAGCCATGCAGGCATATAAG-3'		RT PCR	5'-TTGATCCTGGAGCAGATGGAA-3' 5'-GCTTTCAAGGTGGCACCTTCTA-3'
	RT PCR	5'-ACAACTGGACATGTCACTGAAGG-3' 5'-GCTGGATGGGCACTGTTTTC-3'	*MaMADS4* (728bp) EU869309	A/ORF	5'-ATGGGGAGGGGAAGGGTGGAG-3' 5'-CATGCAAGCCACCCGGGCATGTAA-3'
MaMADS2 (729bp) EU869306	A	5'-GATGAAGGAAACCAGGCCAA-3' 5'-CGCAATCATCAGCACAAGAAAT-3'		RT PCR	5'-TCCCAACACTCATGCTGTAGCT-3' 5'-CGCCATTTGATCTGGATGGT-3'
	B	5'-GATGAAGGAAACCAGGCCAA-3'	*MaMADS5* (705bp) EU869310	A/ORF	5'-ATGGGAAGGGGTAAGATTGAGATCA-3' 5'-GTCGGTATATGCATATATGCAACA-3'
	C	5'-CGATTTGAAGAGTAGGTTCGCATT-3' 5'-GAGCTTCAAGTACTCCTGTTGGCT-3' 5'-CCGCTTCGCGAACGTTACCTGCCG-3'		RT PCR	5'-CCATTGTGGACGTCAATTCTCA-3' 5'-AAAGCGTCGCCCATCAAGT-3'
	ORF	5'-ATGGGGAGGGGGAGAGTGGAGCTG-3' 5'-AGCAAGCCATCCTGGCACGTA-3'	*MaMADS6* (633bp) EU869311	A	5'-CGGATCGAGAACTCAACCAACCGG-3' 5'-TCATTTGTTTTCCTGCAAGTTGGG-3'
	RT PCR	5'-CAGGTGACGGGTTCTTCCAA-3' 5'-CGATTTGAAGAGTAGGTTCGCATT-3'		C	5'-CTCGCACAGGATGCTGATCT-3' 5'-CTGGCCTTCTTGATGATCCCGTTG-3' 5'-CTGCCGGTTGGAGTTCTC-3'
				ORF	5'-ATGGGTCGAGGAAAGATCGAGATC-3' 5'-TCATTTGTTTTCCTGCAAGTTGGG-3'
				RT PCR	5'-TGGAGATCTGGAAGTTGCACAA-3' 5'-TTCCATCCATCGCCAGTTG-3'

Table 2. Determination of primers specificity for RT- PCR reactions. Numbers describe the cycle threshold (CT) at the initiation of fluorescent linear step. In bold are the specific cycles threshold for each of the genes. Non-specific reactions and reaction with water show much higher cycle thresholds. Note that the CT obtained with the *MaMADS1* primers and *MaMADS2* DNA is also low, however the product obtained show two peaks while that of the specific reaction only one sharp peak.

Clones	**Primers**						
	MaMADS1	*MaMADS2*	*MaMADS3*	*MaMADS4*	*MaMADS5*	*MaMADS6*	ddw
MaMADS1	**9.26**	17.03	12.08	22.56	15.55	19.59	20.16
MaMADS2	10.63	**5.19**	13.11	17.91	21.68	21.39	19.05
MaMADS3	20.9	21.07	**4.89**	21.27	16.45	21.14	14.27
MaMADS4	14.73	16.78	11.75	**3.45**	21.94	21.42	24.29
MaMADS5	19.52	19.53	14.49	24.39	**4.12**	18.54	19.39
MaMADS6	21.69	21.48	17.38	25.97	16.34	**5.99**	21.17