

Effect of long-term storage on the change in the expression of selected *Mal d 1* gene isoforms in the apple cultivar Opal®

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Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

Table S1. Statistical analysis performed for each *Mal d 1* gene isoform using Student's *t*-test

Mal d 1.01			Opal						Gala							
			Cool storage			ULO storage			Cool storage			ULO storage				
			Fresh	4 months	6 months	9 months	4 months	6 months	9 months	Fresh	4 months	6 months	9 months	4 months	6 months	9 months
Opal	Cool storage	Fresh														
		4 months	2.086E-06													
		6 months	4.597E-07	9.474E-04												
	ULO storage	9 months	1.284E-06	0.431	4.059E-04											
		4 months	2.470E-08	1.187E-06	1.412E-06	5.863E-07										
		6 months	5.381E-08	1.561E-07	1.723E-07	1.368E-07	4.336E-07									
		9 months	1.243E-07	1.992E-06	2.923E-06	1.381E-06	8.922E-04	8.867E-07								
		Fresh	5.493E-08	5.914E-07	7.027E-07	4.097E-07	1.392E-05	1.691E-06	2.379E-04							
		4 months	4.186E-08	6.228E-07	7.245E-07	3.979E-07	2.811E-05	1.096E-06	1.538E-03	3.977E-03						
Gala	Cool storage	6 months	9.639E-08	3.683E-07	4.306E-07	3.152E-07	1.642E-06	1.027E-04	4.255E-06	1.266E-05	6.481E-06					
		9 months	2.349E-10	3.768E-10	3.408E-10	3.283E-10	3.730E-10	3.830E-09	6.259E-10	6.714E-10	5.497E-10	2.384E-09				
		4 months	1.569E-04	5.902E-07	6.798E-07	5.584E-07	4.036E-04	7.653E-04	2.166E-06	3.527E-06	2.701E-06	3.866E-05	2.606E-08			
	ULO storage	6 months	3.220E-10	7.924E-10	6.308E-10	5.976E-10	6.942E-10	4.656E-08	1.982E-09	2.251E-09	1.535E-09	1.942E-08	1.688E-08	6.764E-07		
		9 months	1.169E-05	2.356E-09	2.363E-09	2.261E-09	1.575E-05	8.932E-09	3.271E-09	3.545E-09	3.260E-09	6.526E-09	5.009E-06	2.620E-08	3.888E-08	
Mal d 1.02			Opal						Gala							
			Cool storage			ULO storage			Cool storage			ULO storage				
			Fresh	4 months	6 months	9 months	4 months	6 months	9 months	Fresh	4 months	6 months	9 months	4 months	6 months	9 months
Opal	Cool storage	Fresh														
		4 months	1.464E-05													
		6 months	3.860E-08	6.196E-06												
	ULO storage	9 months	1.667E-05	2.185E-03	2.152E-04											
		4 months	5.327E-03	0.462	1.400E-04	1.491E-02										
		6 months	1.125E-08	2.789E-07	4.696E-07	2.490E-06	4.449E-06									
		9 months	1.578E-08	6.462E-07	2.248E-06	7.636E-06	1.156E-05	3.439E-05								
		Fresh	4.857E-07	0.630	3.508E-07	1.069E-03	0.373	3.378E-08	6.092E-08							
		4 months	8.598E-09	1.417E-07	1.691E-07	1.027E-06	2.016E-06	4.390E-05	2.532E-06	2.158E-08						
Gala	Cool storage	6 months	1.909E-08	4.983E-08	6.542E-08	1.206E-07	1.963E-07	2.428E-07	1.422E-07	3.012E-08	4.606E-07					
		9 months	7.968E-05	1.070E-07	1.575E-07	1.848E-07	2.365E-07	4.310E-07	2.878E-07	9.773E-05	6.902E-07	2.901E-04				
		4 months	1.342E-07	8.205E-06	0.228	2.107E-04	1.350E-04	1.113E-06	6.246E-06	1.160E-06	3.559E-07	7.876E-08	1.728E-07			
	ULO storage	6 months	2.325E-04	2.338E-06	1.078E-05	7.151E-06	7.675E-06	5.685E-03	1.772E-04	3.906E-04	4.423E-02	1.561E-06	1.285E-06	1.317E-05		
		9 months	8.449E-03	1.119E-02	1.805E-02	1.289E-02	1.072E-02	4.244E-02	2.959E-02	1.130E-02	0.067	0.100	3.376E-02	1.831E-02	0.055	

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Table S1 to be continued

Mal d 1.03D			Opal						Gala							
			Cool storage			ULO storage			Cool storage			ULO storage				
			Fresh	4 months	6 months	9 months	4 months	6 months	9 months	Fresh	4 months	6 months	9 months	4 months	6 months	9 months
Opal	Fresh	4 months	5.838E-09													
		6 months	1.512E-06	3.153E-08												
		9 months	2.236E-02	3.282E-03	0.377											
	Cool storage	4 months	9.469E-05	1.983E-05	4.517E-04	3.183E-02										
		6 months	4.637E-08	3.761E-08	5.521E-06	0.120	1.493E-08									
		9 months	1.373E-03	6.863E-09	2.830E-06	2.823E-02	1.097E-02	6.985E-08								
	ULO storage	Fresh	2.046E-04	8.138E-09	4.939E-06	3.373E-02	4.586E-02	1.065E-07	6.314E-03							
		4 months	5.420E-04	1.686E-05	1.489E-04	1.199E-02	2.407E-09	1.449E-05	3.431E-04	3.000E-04						
		6 months	9.709E-05	1.408E-05	3.937E-07	1.697E-02	6.675E-08	6.321E-09	1.314E-05	6.218E-06	1.685E-07					
9 months		7.532E-08	8.668E-03	2.885E-07	1.138E-04	1.080E-04	6.203E-07	8.728E-08	9.871E-08	8.102E-05	8.619E-05					
4 months		2.885E-06	9.171E-09	4.906E-05	0.081	2.688E-06	1.857E-07	8.746E-06	2.603E-05	5.238E-08	1.948E-07	1.363E-07				
6 months		2.264E-08	3.462E-02	8.756E-08	7.021E-05	5.376E-05	1.467E-07	2.609E-08	2.966E-08	4.222E-05	4.242E-05	2.849E-03	3.784E-08			
Gala	Fresh	4 months	2.249E-04	1.992E-03	1.401E-06	5.386E-05	3.131E-04	5.770E-04	2.348E-04	2.354E-04	2.420E-04	2.652E-04	8.422E-04	3.168E-04	5.139E-03	
	Cool storage	Fresh	2.046E-04	8.138E-09	4.939E-06	3.373E-02	4.586E-02	1.065E-07	6.314E-03							
		4 months	5.420E-04	1.686E-05	1.489E-04	1.199E-02	2.407E-09	1.449E-05	3.431E-04	3.000E-04						
		6 months	9.709E-05	1.408E-05	3.937E-07	1.697E-02	6.675E-08	6.321E-09	1.314E-05	6.218E-06	1.685E-07					
	ULO storage	4 months	2.885E-06	9.171E-09	4.906E-05	0.081	2.688E-06	1.857E-07	8.746E-06	2.603E-05	5.238E-08	1.948E-07	1.363E-07			
		6 months	2.264E-08	3.462E-02	8.756E-08	7.021E-05	5.376E-05	1.467E-07	2.609E-08	2.966E-08	4.222E-05	4.242E-05	2.849E-03	3.784E-08		
		9 months	2.249E-04	1.992E-03	1.401E-06	5.386E-05	3.131E-04	5.770E-04	2.348E-04	2.354E-04	2.420E-04	2.652E-04	8.422E-04	3.168E-04	5.139E-03	
Mal d 1.03G			Opal						Gala							
Opal	Fresh	4 months	4.985E-07													
		6 months	2.351E-05	0.745												
		9 months	4.948E-05	2.508E-03	6.546E-04											
	Cool storage	4 months	4.751E-09	1.199E-09	4.719E-07	3.199E-08										
		6 months	2.759E-05	4.277E-05	1.445E-08	1.108E-08	5.385E-05									
		9 months	1.113E-07	1.827E-04	4.612E-07	1.949E-07	8.249E-04	6.110E-08								
	ULO storage	Fresh	7.428E-04	1.089E-07	1.047E-05	1.144E-05	2.094E-09	3.029E-05	9.225E-08							
		4 months	0.116	5.747E-08	1.684E-05	2.364E-05	1.120E-09	3.436E-05	8.854E-05	6.429E-04						
		6 months	2.713E-06	3.717E-06	1.947E-03	3.411E-03	1.759E-09	3.905E-05	1.363E-04	5.187E-07	4.567E-07					
9 months		6.793E-09	2.912E-05	9.212E-08	1.754E-08	2.951E-07	4.095E-08	0.919	4.862E-09	4.469E-09	7.246E-09					
4 months		2.582E-04	2.883E-04	2.479E-04	2.634E-04	3.659E-04	2.705E-04	1.294E-06	2.560E-04	2.615E-04	2.798E-04	4.258E-04				
6 months		1.909E-05	2.258E-05	3.377E-09	1.703E-05	2.484E-05	5.028E-08	5.903E-09	1.973E-05	2.069E-05	2.180E-05	2.058E-05	1.500E-06			
Gala	Fresh	4 months	3.689E-06	5.537E-06	2.371E-10	1.975E-10	5.549E-06	3.675E-09	4.344E-10	4.165E-06	4.733E-06	5.129E-06	3.014E-10	2.167E-07	1.888E-06	
	Cool storage	Fresh	2.046E-04	8.138E-09	4.939E-06	3.373E-02	4.586E-02	1.065E-07	6.314E-03							
		4 months	5.420E-04	1.686E-05	1.489E-04	1.199E-02	2.407E-09	1.449E-05	3.431E-04	3.000E-04						
		6 months	9.709E-05	1.408E-05	3.937E-07	1.697E-02	6.675E-08	6.321E-09	1.314E-05	6.218E-06	1.685E-07					
	ULO storage	4 months	2.885E-06	9.171E-09	4.906E-05	0.081	2.688E-06	1.857E-07	8.746E-06	2.603E-05	5.238E-08	1.948E-07	1.363E-07			
		6 months	2.264E-08	3.462E-02	8.756E-08	7.021E-05	5.376E-05	1.467E-07	2.609E-08	2.966E-08	4.222E-05	4.242E-05	2.849E-03	3.784E-08		
		9 months	2.249E-04	1.992E-03	1.401E-06	5.386E-05	3.131E-04	5.770E-04	2.348E-04	2.354E-04	2.420E-04	2.652E-04	8.422E-04	3.168E-04	5.139E-03	

Table S1 to be continued

<i>Mal d 1.06A</i>		Opal						Gala					
		Cool storage			ULO storage			Cool storage			ULO storage		
		Fresh	4 months	6 months	9 months	4 months	6 months	9 months	Fresh	4 months	6 months	9 months	6 months
Opal	Fresh												
	4 months		2.013E-02										
	6 months		8.629E-06	0.743									
	9 months		7.235E-08	2.233E-02	4.760E-04								
	4 months		4.213E-07	8.967E-06	1.884E-07	8.826E-05							
	6 months		1.540E-08	1.754E-06	1.098E-08	1.617E-05	5.565E-06						
	9 months		2.826E-03	2.885E-02	6.978E-06	1.712E-09	1.772E-04	8.151E-09					
	Fresh		9.366E-05	1.053E-03	2.697E-05	1.096E-02	1.559E-04	8.538E-06	2.231E-02				
	4 months		5.574E-05	2.415E-04	2.900E-05	1.548E-02	0.994	3.346E-04	2.303E-02	5.379E-03			
Gala	6 months		9.186E-06	3.744E-04	1.119E-05	1.993E-07	2.856E-04	9.060E-04	8.930E-07	7.586E-03	2.694E-02		
	9 months		1.531E-06	5.217E-06	8.100E-07	2.519E-04	2.158E-04	2.082E-02	4.471E-04	1.686E-04	2.861E-03	5.199E-03	
	4 months		2.126E-05	4.805E-04	9.515E-06	7.812E-03	5.490E-04	1.283E-05	1.304E-02	1.208E-02	1.278E-02	7.739E-03	3.235E-04
	6 months		3.883E-06	8.581E-05	7.055E-07	9.755E-05	6.227E-06	1.376E-07	1.704E-06	0.673	3.515E-04	5.029E-05	6.876E-06
	9 months		1.33E-04	5.41E-05	5.42E-05	1.42E-04	7.66E-04	2.18E-03	2.59E-04	1.08E-04	5.28E-03	1.38E-03	1.96E-02
													2.34E-04
													1.146E-03
													7.80E-05
<i>Mal d 1.06B</i>		Opal						Gala					
		Cool storage			ULO storage			Cool storage			ULO storage		
		Fresh	4 months	6 months	9 months	4 months	6 months	9 months	Fresh	4 months	6 months	9 months	6 months
Opal	Fresh												
	4 months		2.393E-04										
	6 months		8.478E-07	8.243E-03									
	9 months		3.268E-06	1.032E-02	1.479E-05								
	4 months		2.183E-04	5.496E-03	3.440E-05	2.315E-06							
	6 months		3.131E-04	9.609E-03	1.866E-02	1.366E-05	3.040E-06						
	9 months		2.341E-04	4.676E-03	5.472E-06	1.193E-06	9.412E-05	3.236E-07					
	Fresh		3.284E-04	1.175E-02	3.349E-04	3.157E-05	1.239E-06	2.335E-04	1.919E-07				
	4 months		2.371E-04	4.016E-03	4.140E-04	3.190E-04	3.345E-06	5.059E-08	9.745E-06	4.057E-08			
Gala	6 months		6.689E-07	7.576E-03	2.606E-03	5.560E-06	1.985E-05	4.614E-05	8.994E-07	6.414E-06	7.835E-08		
	9 months		0.140	1.782E-04	8.629E-07	3.059E-06	2.306E-04	3.230E-04	2.439E-04	3.386E-04	2.452E-04	3.031E-04	
	4 months		2.094E-04	3.798E-03	1.415E-06	6.548E-07	3.580E-06	9.464E-08	1.185E-05	6.842E-08	1.419E-03	1.731E-07	2.192E-04
	6 months		5.516E-04	3.495E-02	1.476E-06	0.113	4.250E-08	1.521E-07	6.179E-09	5.326E-07	6.552E-10	3.687E-08	5.483E-04
	9 months		3.890E-04	1.111E-02	1.026E-02	1.825E-03	3.048E-04	6.695E-03	6.965E-05	2.725E-02	4.649E-06	5.271E-04	3.931E-04
													4.022E-05
													5.488E-05

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Table S1 to be continued

<i>Mal d 1.06D</i>		Opal						Gala					
		Cool storage			ULO storage			Cool storage			ULO storage		
		Fresh	4 months	6 months	9 months	4 months	6 months	9 months	Fresh	4 months	6 months	9 months	6 months
Opal	Fresh												
	4 months	1.347E-04											
	6 months	2.023E-04	2.610E-06										
	9 months	2.728E-04	2.074E-03	5.809E-06									
	4 months	1.703E-04	1.260E-06	1.722E-03	2.453E-06								
	6 months	1.502E-04	3.801E-07	4.317E-06	5.149E-07	9.298E-06							
	9 months	3.155E-04	3.405E-06	9.747E-04	5.579E-06	0.056	2.280E-05						
	Fresh	8.821E-05	1.395E-04	2.014E-04	2.720E-04	1.697E-04	1.497E-04	3.134E-04					
	4 months	1.580E-03	1.958E-03	2.669E-03	2.006E-03	2.961E-03	5.464E-03	2.885E-03	1.575E-03				
Gala	6 months	1.604E-05	1.271E-05	3.230E-09	2.099E-09	3.597E-09	8.110E-09	4.962E-09	1.604E-05	4.613E-06			
	9 months	1.116E-04	1.174E-04	1.967E-07	1.010E-04	2.315E-07	6.717E-07	2.861E-07	1.114E-04	1.057E-03	4.877E-07		
	4 months	1.565E-05	7.938E-09	5.071E-07	2.008E-08	1.232E-06	0.925	6.063E-06	1.609E-05	6.260E-03	4.381E-09	4.956E-07	
	6 months	1.020E-04	1.478E-07	1.068E-06	1.937E-07	1.857E-06	3.236E-04	4.067E-06	1.018E-04	8.753E-03	9.962E-09	1.098E-06	9.698E-05
	9 months	2.641E-03	3.956E-03	8.182E-03	4.177E-03	1.081E-02	0.138	1.154E-03	2.626E-03	8.492E-04	4.598E-07	1.548E-05	0.194
<i>Mal d 1.07</i>		Opal						Gala					
		Cool storage			ULO storage			Cool storage			ULO storage		
		Fresh	4 months	6 months	9 months	4 months	6 months	9 months	Fresh	4 months	6 months	9 months	6 months
Opal	Fresh												
	4 months	2.938E-05											
	6 months	7.119E-08	2.518E-07										
	9 months	1.173E-04	2.459E-04	1.796E-06									
	4 months	1.023E-07	3.927E-07	1.631E-03	6.722E-05								
	6 months	8.764E-07	1.865E-05	2.847E-08	3.483E-08	1.332E-07							
	9 months	1.154E-07	4.381E-07	1.306E-02	9.018E-05	0.152	2.341E-07						
	Fresh	7.060E-07	5.956E-06	3.391E-06	1.544E-03	7.871E-06	5.066E-05	8.434E-06					
	4 months	3.704E-08	9.919E-08	1.045E-06	9.941E-08	1.464E-06	1.327E-08	3.230E-06	4.878E-07				
Gala	6 months	6.091E-08	1.898E-07	4.457E-05	1.133E-06	2.637E-05	4.323E-08	6.548E-05	1.584E-06	2.586E-05			
	9 months	1.144E-04	4.498E-06	6.866E-08	1.162E-04	8.932E-08	4.493E-07	9.654E-08	3.821E-07	3.947E-08	5.882E-08		
	4 months	8.287E-05	1.564E-04	0.079	2.700E-07	2.283E-03	1.434E-08	2.773E-02	5.463E-04	5.377E-07	2.229E-05	6.633E-08	
	6 months	4.998E-03	2.708E-05	6.706E-09	7.458E-09	1.572E-08	1.169E-07	2.365E-08	2.806E-07	4.464E-09	8.852E-09	2.247E-05	4.929E-09
	9 months	2.054E-05	6.530E-04	1.314E-03	2.517E-03	2.102E-05	0.227	1.998E-05	8.185E-04	3.575E-06	7.716E-06	5.379E-06	1.511E-03
													2.420E-05

Table S1 to be continued

Mal d 1.13A			Opal						Gala					
			Fresh			Cool storage			ULO storage			Fresh		
			4 months	6 months	9 months	4 months	6 months	9 months	4 months	6 months	9 months	4 months	6 months	9 months
Opal	Cool storage	Fresh												
		4 months	2.393E-06											
		6 months	1.413E-02	1.668E-08										
	ULO storage	9 months	3.497E-03	4.909E-08	8.825E-07									
		4 months	0.096	1.155E-06	5.837E-03	1.910E-03								
		6 months	2.226E-02	2.578E-03	0.751	3.874E-02	6.627E-03							
Gala	Cool storage	9 months	0.862	1.983E-05	2.542E-07	1.271E-08	0.093	0.051						
		Fresh	4.092E-02	2.714E-09	4.839E-08	5.340E-09	0.168	2.067E-02	5.372E-08					
		4 months	0.096	3.181E-09	8.774E-08	7.580E-09	0.931	2.813E-02	3.702E-07	4.169E-06				
	ULO storage	6 months	1.148E-02	1.937E-08	7.142E-03	1.513E-06	4.957E-03	0.489	2.034E-07	4.395E-08	7.659E-08			
		9 months	2.390E-07	4.769E-07	7.916E-05	1.004E-04	1.637E-07	1.662E-06	8.447E-05	7.559E-05	7.853E-05	7.891E-05		
		4 months	4.194E-02	9.508E-09	2.246E-05	1.475E-07	1.277E-02	0.263	9.855E-07	8.090E-08	1.860E-07	1.027E-05	5.554E-08	
	ULO storage	6 months	3.366E-06	0.345	4.102E-07	1.239E-06	1.787E-06	5.368E-05	1.550E-04	1.206E-04	1.311E-04	4.589E-07	2.693E-07	
		9 months	3.111E-05	6.144E-07	1.311E-06	4.645E-05	1.220E-05	1.376E-02	1.360E-04	7.911E-05	9.263E-05	1.746E-06	1.518E-07	4.892E-06

ULO – ultra-low oxygen; cells marked green contain values that are not significant. ($P > 0.05$)

<https://doi.org/10.17221/102/2022-CJGPB>

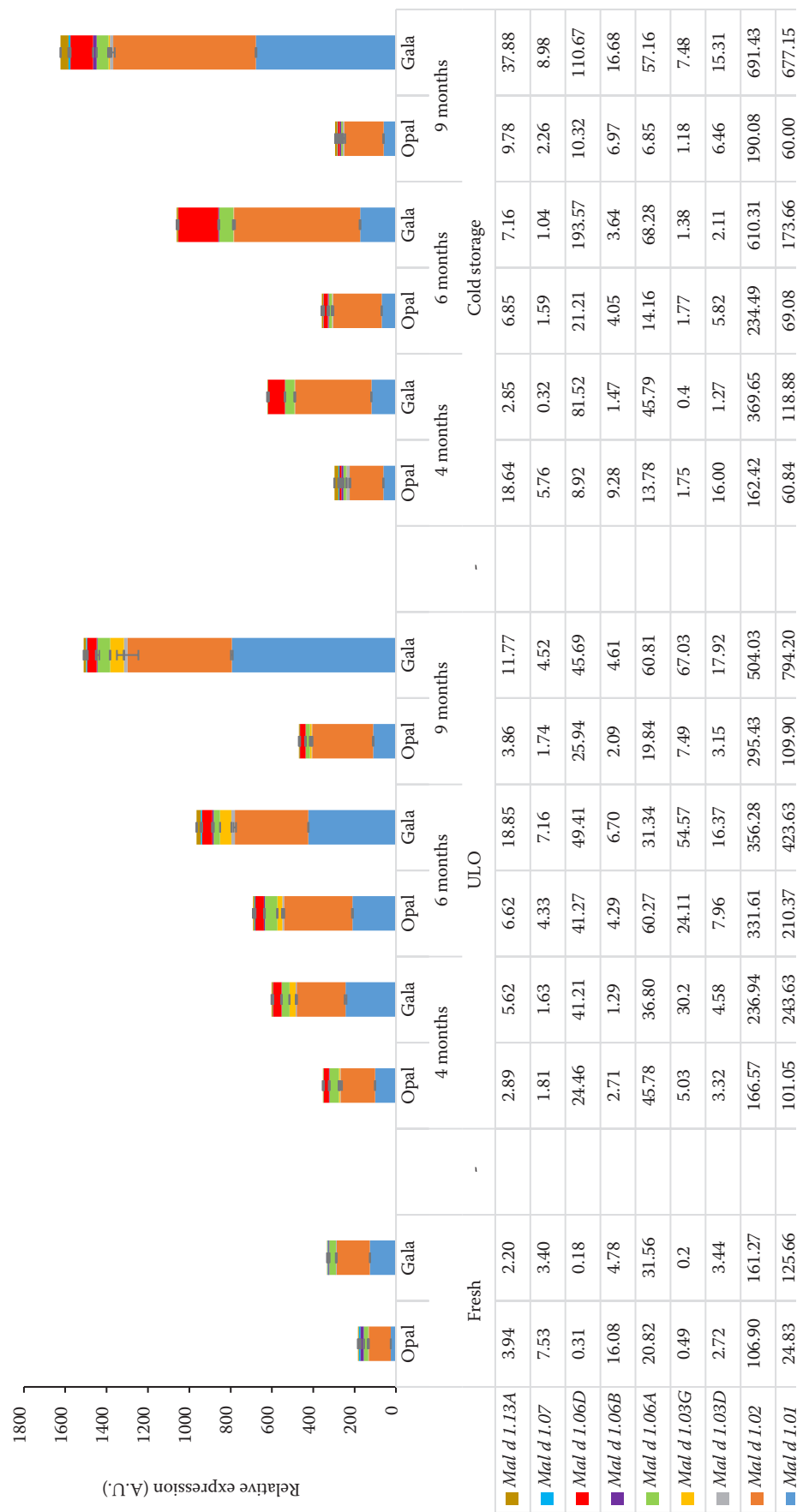


Figure S1. Effect of individual isoforms on total gene expression of the *Mal d 1* allergen in cv. Gala Brookfield and Opal®; the chart shows the change in gene expression of individual isoforms during storage in controlled atmosphere ULO and in cold storage and compares it to fresh fruits
Error bars represent the standard deviation of technical triplicates

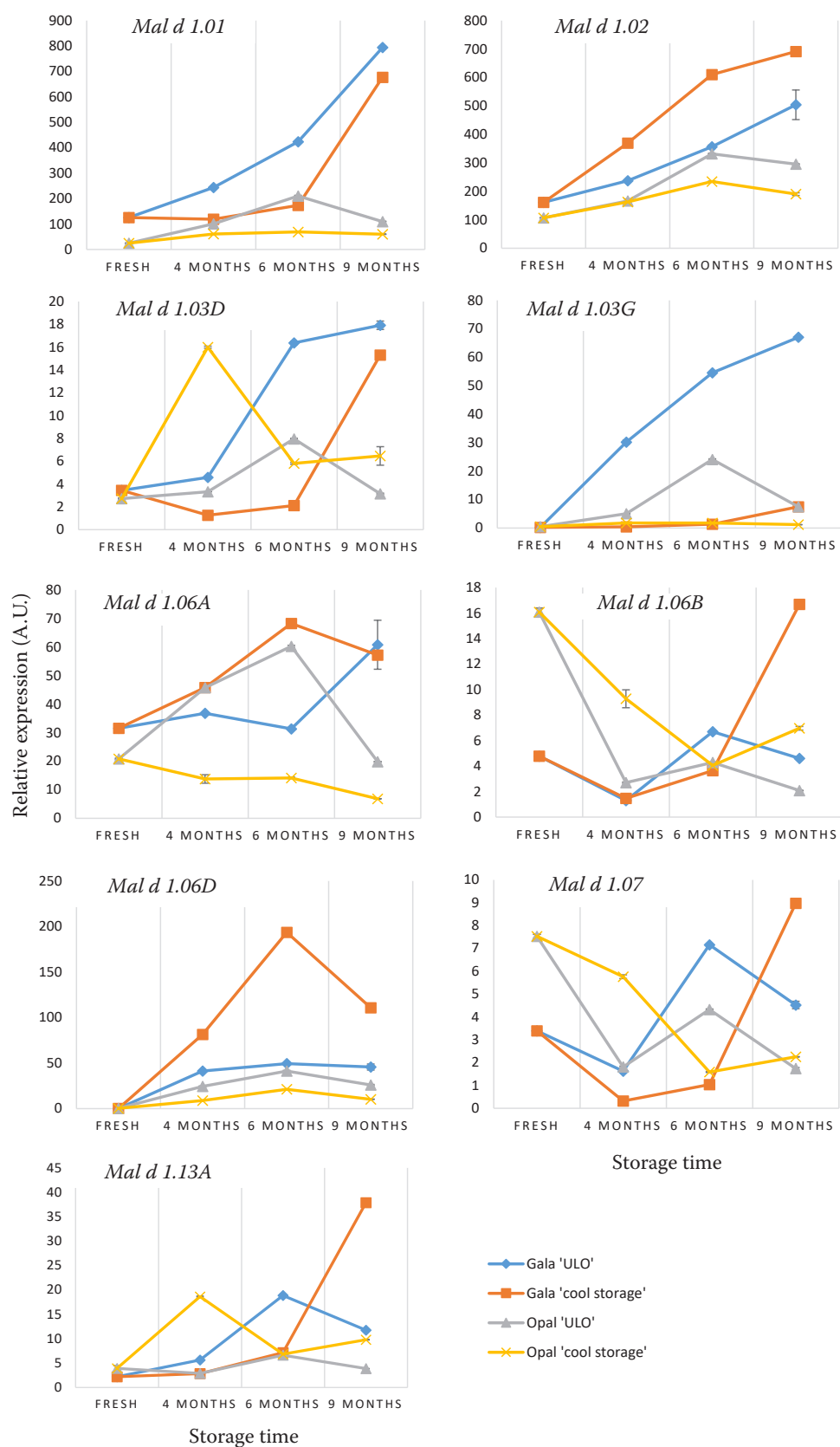


Figure S2. Expression profiles of the selected *Mal d 1* genes isoforms that illustrate the change in gene expression during storage under controlled atmosphere ULO and in cold storage compared to the fresh fruit in cv. Gala Brookfield and Opal®. Error bars represent the standard deviation of technical triplicates.

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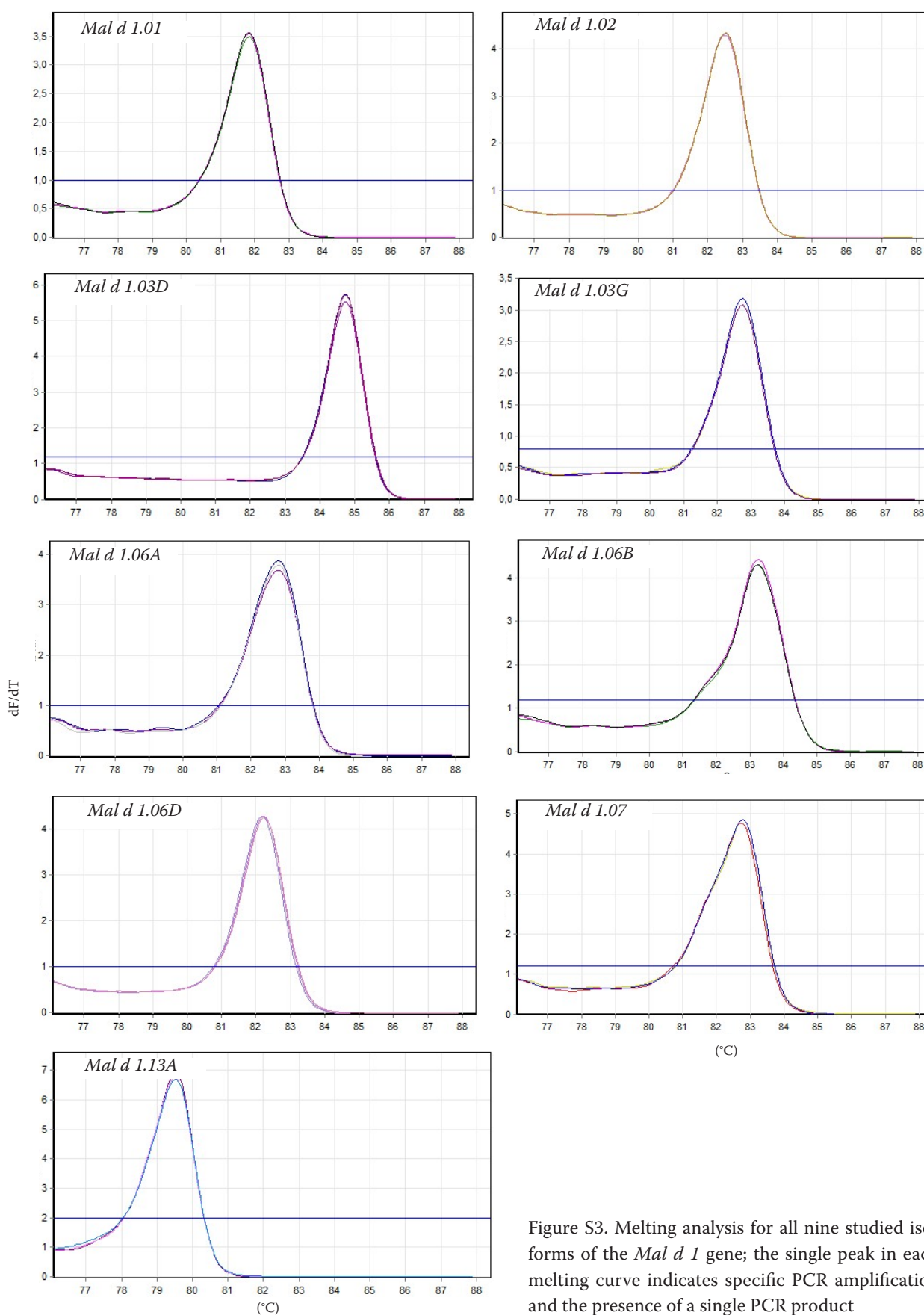


Figure S3. Melting analysis for all nine studied isoforms of the *Mal d 1* gene; the single peak in each melting curve indicates specific PCR amplification and the presence of a single PCR product