ANNEXES

7. ANNEXES

7.1 Annex I: Tissue printing membranes

Table I. Tissue printing buffer composition.

Buffer	Composition
Buffer 2x SSC/0.1% SDS	SSC 20x and SDS 20%
Buffer 0.5x SSC/0.1% SDS	SSC 20x and SDS 20%
Buffer T1	0.1M maleic acid, 0.15M NaCl, pH 7.5 NaOH
Washing buffer TL	0.3% Tween 20, T1 buffer
Blocking buffer T2	Blocking 10%, T1 buffer
Buffer T3	0.1M Tris-HCl pH 9.5, 0.1M NaCl
SSC 20x	3M NaCl, 0.3M sodium citrate 2 H_2O pH 7 with diluted HCl
10% SLS	SLS, sterile water
20% SDS	SDS, sterile water

CO-002:3	CO-002:4	CO-002:7	CO-002:6	CO-002:9	CO-003:3	CO-003:8	CO-003:1	CO-003:7	CO-003:6
CO-006:2	CO-006:6	CO-006:5	CO-006:4	CO-010:2	CO-010:6	CO-010:5	CO-010:4	CO-010:7	CO-013:1
CO-013:5	CO-013:4	CO-013:3	CO-013:2	CO-030:2	CO-030:7	CO-030:5	CO-030:9	CO-030:8	CO-031:2
CO-031:3	CO-031:10	CO-031:8	CO-031:9	CO-033:4	CO-033:3	CO-037:3	CO-033:2	CO-033:5	CO-033:1
CO-037:6	CO-037:4	CO-037:1	CO-037:2	CO-050:2	CO-050:6	CO-050:5	CO-050:1	CO-050:3	CO-053:2
CO-053:1	CO-053:3	CO-053:4	CO-054:3	CO-054:1	CO-054:4	CO-054:5	CO-054:2	CO-060:1	CO-061:5
CO-061:3	CO-061:2	CO-061:1	CO-061:4	CO-064:2	CO-064:1	CO-064:4	CO-064:06	CO-064:03	CO-068:4
CO-068:2	CO-068:3	CO-068:1	CO-068:8	CO-078:2	CO-078:8	CO-078:10	CO-078:1	CO-078:6	CO-095:2
CO-095:3	CO-095:4	CO-095:5	CO-095:1		p318	MU-CU-16			

Figure I. Tissue printing membrane for the first batch of plant samples, corresponding to week 2 after MWMV inoculation, with the corresponding negative and positive controls, p318 and MU-CU-16, respectively.

CO-053:7	CO-046:1	CO-046:2	CO-046:3	CO-046:4	CO-046:7	CO-042:1
CO-042:2	CO-092:1	CO-092:3	CO-092:4	CO-092:5	CO-092:6	CO-040:4
CO-040:2	CO-040:3	CO-039:1	CO-039:2	CO-039:3	CO-039:4	CO-039:5
CO-043:1	CO-043:6	CO-043:5	CO-043:8	CO-043:9	CO-006:1	CO-056:1
CO-055:1	CO-055:2	CO-055:3	CO-055:4	CO-055:5	p318	MU-CU-16

Figure II. Tissue printing membrane for the second batch of plant samples, corresponding to week 2 after MWMV inoculation, with the corresponding negative and positive controls, p318 and MU-CU-16, respectively.

7.2 Annex II: Evaluation of disease symptoms

Table I. MWMV symptoms on the *Cucurbita* accessions studied at week 2, 3 and 4 after inoculation. * Yellowing, * Stunting, * Lack of leaves and * Early flowering.

Accession	Plant	Symptoms		
		Week 2 (15 dpi)	Week 3 (21 dpi)	Week 4 (28 dpi)
CO-2	3	1.5	2	3
	4	2	1.5	1.5
	6	2	3	3.5
	7	4	4	4
	9	3	4	4
CO-3	1	0.5	4	4
	3	0.5	4	3
	6	2.5	4	4
	7	2.5	4	4
	8	0	4	4
CO-6	2	2.5-3	4	4
	4	3	2*	4
	5	2	3-3.5	4
	6	2	3.5	4
	1	2	4	4
CO-10	2	3	3	4
	4	4	4	4
	5	4	4	4
	6	3.5	2.5	4
	7	3-3.5	4	4
CO-13	1	2	3.5	4
	2	2.5	4	4
	3	3	4	4
	4	3	4	4
	5	2.5	4	4
CO-30	2	0	0*	1*
	5	1	1*	0.5*
	7	1.5	0.5	0.5*
	8	0.5	0.5	0**
	9	1	1	1*

Table II. MWMV symptoms on the *Cucurbita* accessions studied at week 2, 3 and 4 after inoculation. * Yellowing, * Stunting, * Lack of leaves and * Early flowering.

Accession	Plant	Symptoms		
		Week 2 (15 dpi)	Week 3 (21 dpi)	Week 4 (28 dpi)
CO-31	2	3	1.5	1
	3	1.5	2	1.5
	8	2.5	2	1*
	9	1	1	1*
	10	2.5	2	2.5*
CO-33	1	4	4	4*
	2	3	3	4
	3	1	4	4*
	4	3.5	4	4*
	5	4	4	4*
CO-37	1	2.5	4	4
	2	2	4	4
	3	4	4	4
	4	2	4	4
	6	3	4	4
CO-50	1	4	4	4
	2	4	4	4
	3	4	4	4
	5	4	4	4
	6	4	4	4
CO-53	1	4	3.5	3,5
	2	4	4	2.5*
	3	2	3.5	4
	4	3	0.5	4
	7	4	4	4
CO-54	1	4	4	4
	2	4	4	4
	3	4	4	4
	4	4	4	4
	5	4	4	4
CO-60	1	4	4	4

Table III. MWMV symptoms on the *Cucurbita* accessions studied at week 2, 3 and 4 after inoculation. * Yellowing, * Stunting, * Lack of leaves and * Early flowering.

Accession	Plant	Symptoms		
		Week 2 (15 dpi)	Week 3 (21 dpi)	Week 4 (28 dpi)
CO-61	1	4	4	4
	2	4	4	4
	3	4	4	4
	4	4	4	4
	5	4	4	4
CO-64	1	2*	0*	4*
	2	4	4	4
	3	4	4*	4
	4	4	0	4*
	6	4	4	4
CO-68	1	3	2.5	4
	2	3	4	4
	3	1.5	2-2.5	4
	4	4	4	4
	8	3	4	4
CO-78	1	4	4	4
	2	0*	1.5*	4*
	6	4	4	4
	8	4	4	4
	10	4	4	4
CO-95	1	4	4	4
	2	4	4	4
	3	4	4	4
	4	4	4	4
	5	4	4	4
CO-39	1	2	3	3.5
	2	2	2	0.5-1
	3	1.5	2.5	2
	4	3	3.5	2.5-3
	5	1.5*	3	2
CO-40	2	2	2.5	3
	3	2.5	2.5	3
	4	1.5	3	4

Table IV. MWMV symptoms on the *Cucurbita* accessions studied at week 2, 3 and 4 after inoculation. * Yellowing, * Stunting, * Lack of leaves and * Early flowering.

Accession	Plant	Symptoms		
		Week 2 (15 dpi)	Week 3 (21 dpi)	Week 4 (28dpi)
CO-42	1	2	3.5	4
	2	1.5	3.5	4
CO-43	1	3	2***	0***
	5	0.5*	0**	4**
	6	0.5*	0**	0***
	8	3	0*	0**
	9	0*	0***	4**
CO-46	1	4	4	4
	2	0.5-1*	4	4
	3	4	4	4
	4	4	4	4*
	7	3	4	4
CO-55	1	4	4	4
	2	4	4	4
	3	4	4	4
	4	4	4	4
	5	4	4	4
CO-56	1	3.5	4	4
CO-92	1	0	0.5	0.5
	3	0	0.5	0.5*
	4	0	0.5	1.5
	5	0	0.5*	0
	6	0	0	0*

7.3 Annex III: RNA extraction and NanoDrop quantification

Table I. NanoDrop data for RNA samples at week 3 after MWMV inoculation. Yellow cells indicate the samples used for the qPCR reaction.

SAMPLE	ng/µL	A260	A280	260/280	260/230
MWMV-CO-2:3	898.85	22.471	10.91	2.06	1.87
MWMV-CO-2:4	855.79	21.395	10.276	2.08	2.04
MWMV-CO-2:6	176.46	4.411	2.2	2.01	1.38
MWMV-CO-2:6_2	270.03	6.751	3.362	2.01	1.06
MWMV-CO-2:7	755.92	18.898	9.221	2.05	1.83
MWMV-CO-2:9	3945.11	98.628	52.584	1.88	1.85
MWMV-CO-3:1	378.16	9.454	4.692	2.01	1.13
MWMV-CO-3:6	2144.98	53.624	25.867	2.07	2.24
MWMV-CO-6:1	161.78	4.045	2.184	1.85	0.67
MWMV-CO-6:1_2	189.38	4.734	2.566	1.85	0.78
MWMV-CO-6:2	1866.95	46.674	22.499	2.07	1.67
MWMV-CO-6:4	1955.68	48.892	23.712	2.06	2.06
MWMV-CO-6:5	3600.62	90.015	45.838	1.96	2.08
MWMV-CO-6:6	2019.75	50.494	24.962	2.02	1.87
MWMV-CO-10:2	1370.14	34.253	16.925	2.02	1.58
MWMV-CO-10:4	356.32	8.908	4.429	2.01	1.23
MWMV-CO-10:5	238.82	5.971	3.32	1.8	0.49
MWMV-CO-10:5	266.01	6.65	3.726	1.78	0.52
MWMV-CO-10:6	260.6	6.515	3.338	1.95	0.8
MWMV-CO-10:7	641.17	16.029	8.322	1.93	1.22
MWMV-CO-13:3	1443.33	36.083	17.506	2.06	2.02
MWMV-CO-13:4	850.74	21.268	10.628	2	1.79
MWMV-CO-30:2	2413.58	60.339	29.954	2.01	1.87
MWMV-CO-30:5	668.17	16.704	8.187	2.04	1.72
MWMV-CO-30:7	3959.74	98.993	55.565	1.78	1.72
MWMV-CO-30:8	402.77	10.069	5.02	2.01	1.38
MWMV-CO-30:9	474.47	11.862	5.984	1.98	1.33
MWMV-CO-31:2	290.14	7.253	3.756	1.93	0.65
MWMV-CO-31:3	1059.46	26.487	12.986	2.04	1.82
MWMV-CO-31:8	372.93	9.323	4.761	1.96	1.07
MWMV-CO-31:9	177.21	4.43	2.374	1.87	0.65
MWMV-CO-31:9_2	176.99	4.425	2.412	1.83	0.65
MWMV-CO-31:10	1977.73	49.443	23.971	2.06	2.08
MWMV-CO-33:3	357.26	8.932	4.632	1.93	1.07
MWMV-CO-33:4	340.93	8.523	4.345	1.96	0.8
MWMV-CO-37:1	3093.59	77.34	38.073	2.03	2.14
MWMV-CO-37:2	1465.9	36.648	17.868	2.05	1.95
MWMV-CO-50:2	234.74	5.868	3.074	1.91	0.72
MWMV-CO-53:1	275.24	6.881	3.694	1.86	0.68
MWMV-CO-53:2	97.83	2.446	1.442	1.7	0.41
MWMV-CO-53:2_2	91.67	2.292	1.38	1.66	0.38
MWMV-CO-53:3	266	6.65	3.493	1.9	0.73
MWMV-CO-53:4	644.73	16.118	7.719	2.09	1.84
MWMV-CO-53:7	1450.6	36.265	17.843	2.03	1.74
MWMV-CO-54:1	857.71	21.443	10.674	2.01	1.44
MWMV-CO-54:2	604.83	15.121	7.423	2.04	1.62
MWMV-CO-60:1	275.35	6.884	3.543	1.94	0.81

Table II. NanoDrop data for RNA samples at week 3 after MWMV inoculation. Yellow cells indicate the samples used for the qPCR reaction.

SAMPLE	ng/µL	A260	A280	260/280	260/230
MWMV-CO-61:1	242.01	6.05	3.377	1.79	0.47
MWMV-CO-61:1_2	416.53	10.413	5.974	1.74	0.73
MWMV-CO-64:2	685.07	17.127	8.416	2.03	1.43
MWMV-CO-64:3	369.63	9.241	4.593	2.01	1.24
MWMV-CO-68:1	104.96	2.624	1.47	1.78	0.61
MWMV-CO-68:1_2	81.69	2.042	1.196	1.71	0.53
MWMV-CO-68:2	243.7	6.092	3.271	1.86	0.67
MWMV-CO-68:3	1965.35	49.134	24.283	2.02	1.73
MWMV-CO-68-4	692.03	17.301	8.76	1.98	1.35
MWMV-CO-68-8	216.67	5.417	3.033	1.79	0.52
MWMV-CO-68-8_2	184.64	4.616	2.563	1.8	0.49
MWMV-CO-78:6	590.66	14.767	7.236	2.04	1.45
MWMV-CO-78:8	1388.52	34.713	17.124	2.03	1.68
MWMV-CO-95:1	180.14	4.504	2.391	1.88	0.7
MWMV-CO-39:1	910.62	22.766	11.094	2.05	1.86
MWMV-CO-39:2	4262.85	106.571	63.058	1.69	1.75
MWMV-CO-39:3	292.74	7.318	3.831	1.91	0.97
MWMV-CO-39:4	1012.12	25.303	12.507	2.02	1.78
MWMV-CO-39:5	1971.11	49.278	23.718	2.08	2.07
MWMV-CO-40:2	122.67	3.067	1.704	1.8	0.59
MWMV-CO-40:2_2	248.21	6.205	3.36	1.85	0.95
MWMV-CO-40:3	329.53	8.238	4.277	1.93	1.27
MWMV-CO-40:4	186.35	4.659	2.373	1.96	1.36
MWMV-CO-42:1	564.62	14.116	6.896	2.05	1.71
MWMV-CO-42:2	162.77	4.069	2.202	1.85	1.01
MWMV-CO-43:1	356.19	8.905	4.884	1.82	0.74
MWMV-CO-43:5	512.26	12.806	6.317	2.03	1.62
MWMV-CO-43:6	1471.96	36.799	22.343	1.65	0.48
MWMV-CO-43:8	426.45	10.661	5.426	1.96	0.9
MWMV-CO-43:9	125.54	3.138	1.943	1.62	0.44
MWMV-CO-43:9_2	140.62	3.515	2.192	1.6	0.47
MWMV-CO-46:1	1580	39.5	22.892	1.73	0.52
MWMV-CO-46:2	489.08	12.227	5.663	2.16	1.98
MWMV-CO-55:1	526.11	13.153	8.571	1.53	0.47
MWMV-CO-92:5	1164.05	29.101	14.439	2.02	1.67
MWMV-CO-56:1	426.1	10.652	5.451	1.95	1.09
MWMV-CO-92:1	3210.38	80.259	39.776	2.02	2.17
MWMV-CO-92:3	565.24	14.131	6.855	2.06	1.69
MWMV-CO-92:4	4291.83	107.296	67.215	1.6	1.69
MWMV-CO-92:4_2	4282.25	107.056	73.423	1.46	1.58
MWMV-CO-92:6	273.41	6.835	3.459	1.98	1.31
MWMV-CO-33:5	176.95	4.424	2.324	1.9	0.81
MWMV-CO-33:5_2	114.61	2.865	1.574	1.82	0.61
MWMV-CO-3:3	566.47	14.162	7.079	2	1.47
MWMV-CO-13:2	336.87	8.422	4.273	1.97	1.59
MWMV-CO-10:6	1691.42	42.286	24.677	1.71	0.56
MWMV-CO-50:1	265.87	6.647	3.45	1.93	0.71
MWMV-CO-3:7	687.12	17.178	8.508	2.02	1.58
MWMV-CO-13:5	379.62	9.491	4.7	2.02	1.37

Table III. NanoDrop data for RNA samples at week 3 after MWMV inoculation. Yellow cells indicate the samples used for the qPCR reaction.

SAMPLE	ng/µL	A260	A280	260/280	260/230
MWMV-CO-55:5	224.56	5.614	2.974	1.89	0.73
MWMV-CO-50:3	954.12	23.853	11.403	2.09	1.98
MWMV-CO-55:2	924.62	23.116	11.435	2.02	1.74
MWMV-CO-61:2	159.36	3.984	2.000	1.99	0.84
MWMV-CO-95:2	220.12	5.503	2.931	1.88	1.14
MWMV-CO-55:4	487.29	2.182	6.201	1.96	1.49
MWMV-CO-61:3	273.37	6.834	3.635	1.88	0.84
MWMV-CO-78:10	222.88	5.572	3.010	1.85	1.25

7.4 Annex IV: Correlation symptoms/tissue printing/dot-Blot

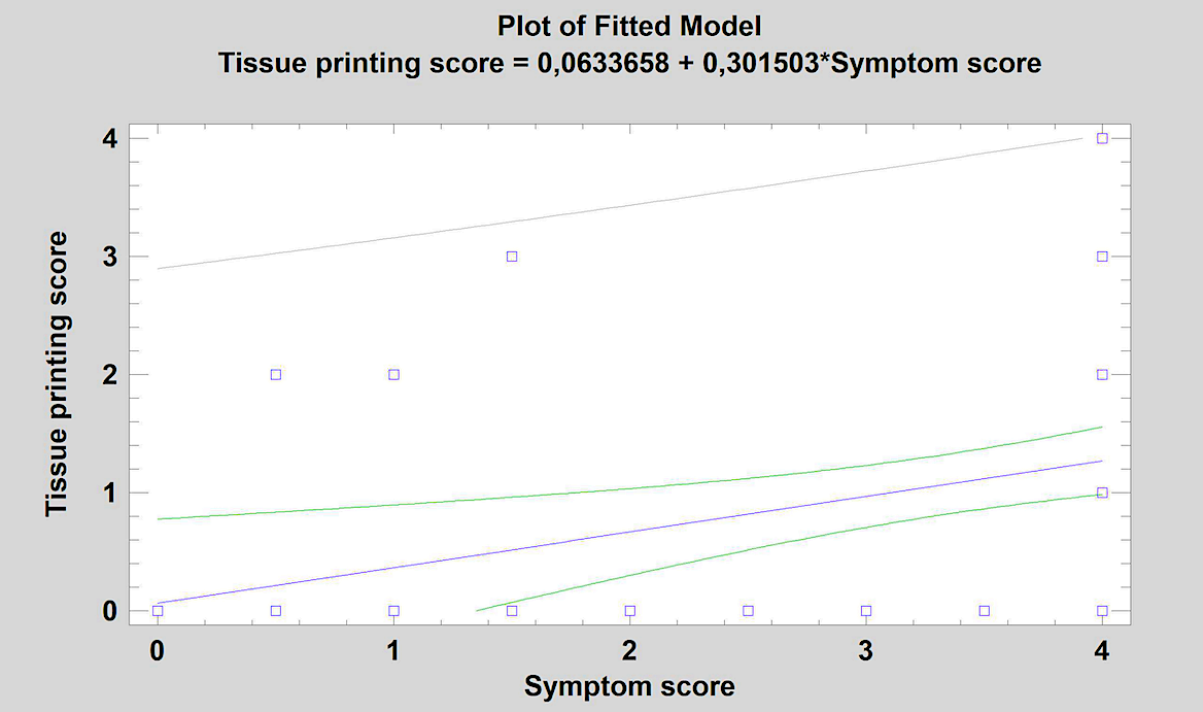

Figure I. Linear regression between symptoms (independent variable) and tissue printing (dependent variable) at 28 dpi. Correlation coefficient = 0.268633. P-value = 0.0034.

Figure II. Linear regression between symptoms (independent variable) and dot-Blot (dependent variable) at 21 dpi. Correlation coefficient = 0.481114. P-value = 0.

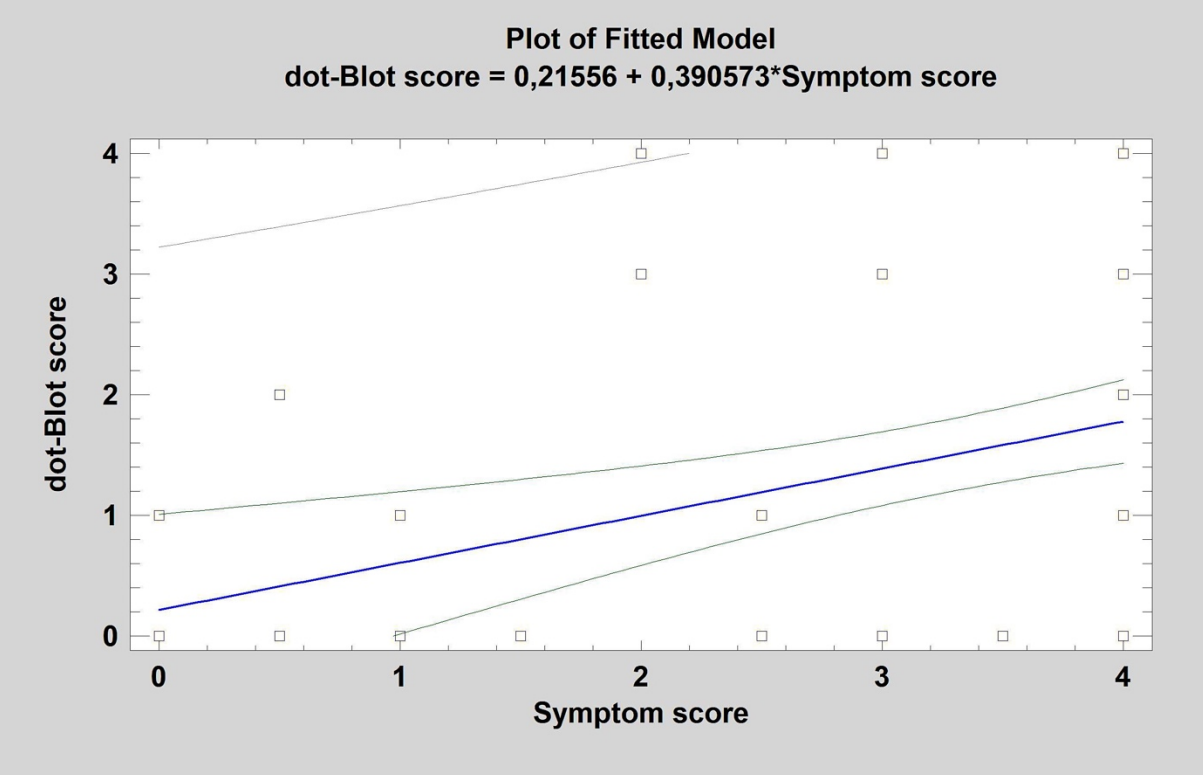

Figure III. Linear regression between symptoms (independent variable) and dot-Blot (dependent variable) at 28 dpi. Correlation coefficient = 0.338649. P-value = 0.0009.

7.5 Annex V: Primer design

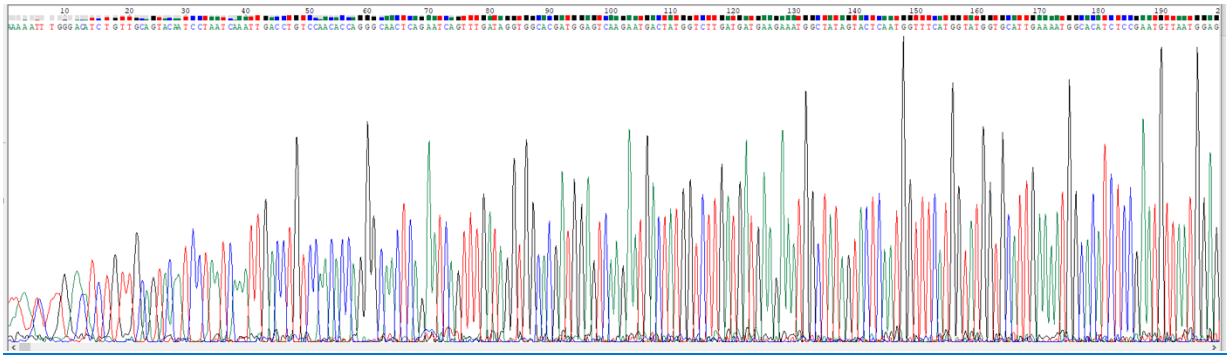

Figure I. Electropherogram of a CPF sequence region, visualized on Chromas 2.6.6 software.

Moroccan watermelon mosaic virus polyprotein gene, partial cds
Sequence ID: AF305545.1 Length: 1693 Number of Matches: 1

Range 1: 888 to 1481 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1075 bits(582)	0.0	590/594(99%)	0/594(0%)	Plus/Plus

```
Query  3     AAATTTGGGACATCTGTTGCAGTACAATCCTAATCAAATTGACCTGTCCAACACCAGGGC
             ||||| | | ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888   AAATCTTGAACATCTGTTGCAGTACAATCCTAATCAAATTGACCTGTCCAACACCAGGGC

Query  63    AACTCAGAATCAGTTTGATAGGTGGCACGATGGAGTCAAGAATGACTATGGTCTTGATGA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  948   AACTCAGAATCAGTTTGATAGGTGGCACGATGGAGTCAAGAATGACTATGGTCTTGATGA

Query  123   TGAAGAAATGGCTATAGTACTCAATGGTTTCATGGTATGGTGCATTGAAAATGGCACATC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1008  TGAAGAAATGGCTATAGTACTCAATGGTTTCATGGTATGGTGCATTGAAAATGGCACATC

Query  183   TCCGAATGTTAATGGAGTTTGGACCATGATGGACAATGGGGAGCAGGTGGAGTACTTACT
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1068  TCCGAATGTTAATGGAGTTTGGACCATGATGGACAATGGGGAGCAGGTGGAGTACTTACT

Query  243   GAAGCCAATGATAGAACATGCATCTCCGACTCTGCGACAGATTATGGCTCATTATAGCAA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1128  GAAGCCAATGATAGAACATGCATCTCCGACTCTGCGACAGATTATGGCTCATTATAGCAA

Query  303   TGCAGCAGAGGCGTACATTGCTAAGAGAAATGCAACGGAGCGTTACATGCCTCGATATGG
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1188  TGCAGCAGAGGCGTACATTGCTAAGAGAAATGCAACGGAGCGTTACATGCCTCGATATGG

Query  363   ACAAAAACGAAACCTCAGGGACATCAGTTTGGCCAGATATGCTTTCGATTTCTATGAGAT
             |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  1248  ACAAAAACGAAACCTCAGGGACATCAGTTTGGCCAGGTATGCTTTCGATTTCTATGAGAT

Query  423   GACTTCCAAGACTCCTGAGAGAGCGCGAGAAGCACACATGCAGATGAAGGCAGCAGCAAT
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1308  GACTTCCAAGACTCCTGAGAGAGCGCGAGAAGCACACATGCAGATGAAGGCAGCAGCAAT

Query  483   TAGAGGTGCGAACACTCGATTGTTTGGTATTGATGGAAATGTTGGTGGGGGAGAAGAGAA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1368  TAGAGGTGCGAACACTCGATTGTTTGGTATTGATGGAAATGTTGGTGGGGGAGAAGAGAA

Query  543   CACGGAGAGACACACTGTTGATGATGTTGAGCGCGATATGCATAGCCTCCTGGG  596
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1428  CACGGAGAGACACACTGTTGATGATGTTGAGCGCGATATGCATAGCCTCCTGGG  1481
```

Figure II. BLAST alignment of cpf sequence and MWMV.

Moroccan watermelon mosaic virus polyprotein gene, partial cds
Sequence ID: AF305545.1 Length: 1693 Number of Matches: 1

Range 1: 868 to 1452 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1075 bits(582)	0.0	584/585(99%)	0/585(0%)	Plus/Minus

```
Query  10    ATCATCAACAGTGTGTCTCTCCGTGTTCTCTTCTCCCCCACCAACATTTCCATCAATACC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1452  ATCATCAACAGTGTGTCTCTCCGTGTTCTCTTCTCCCCCACCAACATTTCCATCAATACC

Query  70    AAACAATCGAGTGTTCGCACCTCTAATTGCTGCTGCCTTCATCTGCATGTGTGCTTCTCG
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1392  AAACAATCGAGTGTTCGCACCTCTAATTGCTGCTGCCTTCATCTGCATGTGTGCTTCTCG

Query  130   CGCTCTCTCAGGAGTCTTGGAAGTCATCTCATAGAAATCGAAAGCATATCTGGCCAAACT
             |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  1332  CGCTCTCTCAGGAGTCTTGGAAGTCATCTCATAGAAATCGAAAGCATACCTGGCCAAACT

Query  190   GATGTCCCTGAGGTTTCGTTTTTGTCCATATCGAGGCATGTAACGCTCCGTTGCATTTCT
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1272  GATGTCCCTGAGGTTTCGTTTTTGTCCATATCGAGGCATGTAACGCTCCGTTGCATTTCT

Query  250   CTTAGCAATGTACGCCTCTGCTGCATTGCTATAATGAGCCATAATCTGTCGCAGAGTCGG
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1212  CTTAGCAATGTACGCCTCTGCTGCATTGCTATAATGAGCCATAATCTGTCGCAGAGTCGG

Query  310   AGATGCATGTTCTATCATTGGCTTCAGTAAGTACTCCACCTGCTCCCCATTGTCCATCAT
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1152  AGATGCATGTTCTATCATTGGCTTCAGTAAGTACTCCACCTGCTCCCCATTGTCCATCAT

Query  370   GGTCCAAACTCCATTAACATTCGGAGATGTGCCATTTTCAATGCACCATACCATGAAACC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1092  GGTCCAAACTCCATTAACATTCGGAGATGTGCCATTTTCAATGCACCATACCATGAAACC

Query  430   ATTGAGTACTATAGCCATTTCTTCATCATCAAGACCATAGTCATTCTTGACTCCATCGTG
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1032  ATTGAGTACTATAGCCATTTCTTCATCATCAAGACCATAGTCATTCTTGACTCCATCGTG

Query  490   CCACCTATCAAACTGATTCTGAGTTGCCCTGGTGTTGGACAGGTCAATTTGATTAGGATT
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972   CCACCTATCAAACTGATTCTGAGTTGCCCTGGTGTTGGACAGGTCAATTTGATTAGGATT

Query  550   GTACTGCAACAGATGTTCAAGATTTAATGCAATTTTACCTCTGAC  594
             ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  912   GTACTGCAACAGATGTTCAAGATTTAATGCAATTTTACCTCTGAC  868
```

Figure III. BLAST alignment of cpr sequence and MWMV.

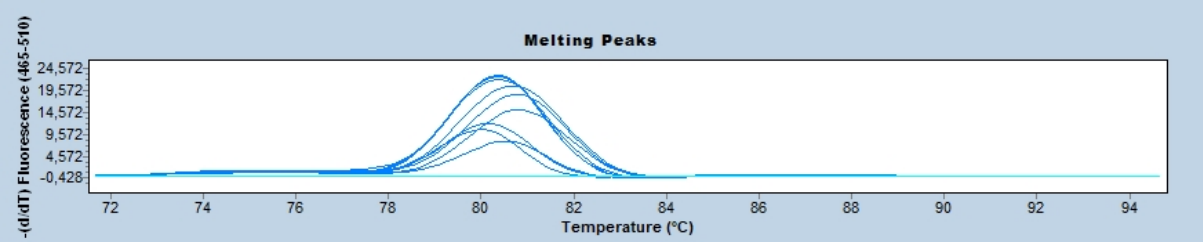

Figure IV. Melt curve genotyping for qPCR_MWMV_F and qPCR_MWMV_R primers.

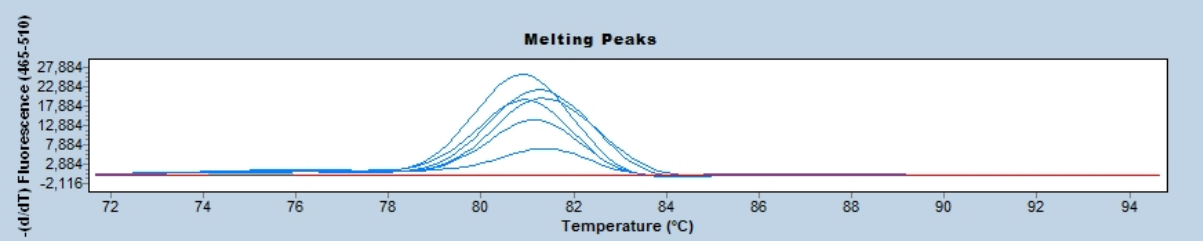

Figure V. Melt curve genotyping qPCR_MWMV_F2 and qPCR_MWMV_R2 primers.

7.6 Annex VI: qPCR

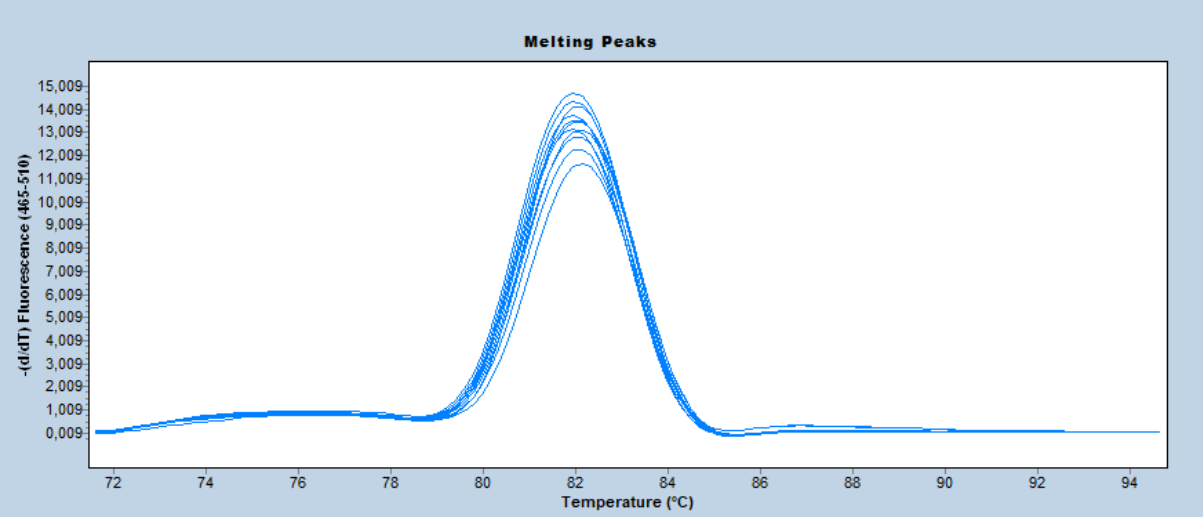

Figure I. Typical melting curve peaks for qPCR amplification.

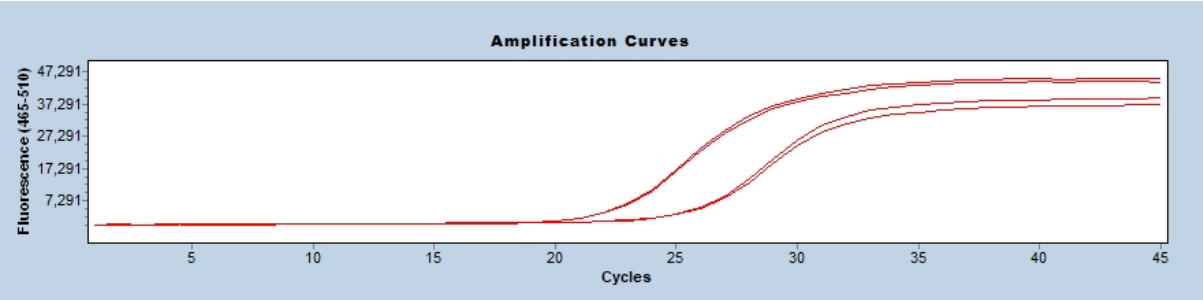

Figure II. Technical replicates for MWMV (lower curves) and UFP (upper curves) primer set amplification through qPCR.

Table I. qPCR disposition, amplification cycles and calculation of ΔCt, average (orange) and standard deviation (green) for each sample of plate 1. Controls are excluded from the calculation. Colors indicate: MWMV mix (yellow), UFP (blue), negative control of amplification (orange) and positive control of amplification (green).

	1	2	3	4	5	6	7	8	9	10	11	12
A	2:7	2:7	3:6	3:6	13:2	13:2	50:1	50:1	p318	H20	Mucu16	54:2
B	2:7	2:7	3:6	3:6	13:2	13:2	50:1	50:1	p318	H20	Mucu16	54:2

	1	2	3	4	5	6	7	8	9	10	11	12
Ct_{MWMV}	12.62	12.63	13.76	13.95	11.00	10.89	10.99	11.19	33.29	-	36.86	10.66
Ct_{UFP}	25.68	25.63	25.49	25.32	20.17	20.31	22.63	22.66	27.80	-	21.79	21.88

	1	2	3	4	5	6	7	8	9	10	11	12
ΔCt	-13.06	-13.00	-11.73	-11.37	-9.17	-9.42	-11.64	-11.47				
ΔCt	-13.01	-13.05	-11.56	-11.54	-9.31	-9.28	-11.67	-11.44				
$\bar{x} \pm \sigma$	-13.03	0.03	-11.55	0.15	-9.30	0.10	-11.56	0.12				

Table II. qPCR disposition, amplification cycles and calculation of ΔCt, average (orange) and standard deviation (green) for each sample of plate 2. Controls are excluded from the calculation. Colors indicate: MWMV mix (yellow), UFP (blue), negative control of amplification (orange) and positive control of amplification (green).

	1	2	3	4	5	6	7	8	9	10	11	12
A	2:9	2:9	6:4	6:4	3:3	3:3	6:5	6:5	10:2	10:2	10:4	10:4
B	2:9	2:9	6:4	6:4	3:3	3:3	6:5	6:5	10:2	10:2	10:4	10:4
C	37:1	37:1	13:5	13:5	30:2	30:2	30:5	30:5	31:2	31:2	31:3	31:3
D	37:1	37:1	13:5	13:5	30:2	30:2	30:5	30:5	31:2	31:2	31:3	31:3
E	33:3	33:3	33:4	33:4	37:2	37:2	39:1	39:1	40:2	40:2	40:3	40:3
F	33:3	33:3	33:4	33:4	37:2	37:2	39:1	39:1	40:2	40:2	40:3	40:3
G	42:1	42:1	42:2	42:2	43:1	43:1	43:5	43:5	PI38	mucu	H_2O	54:2
H	42:1	42:1	42:2	42:2	43:1	43:1	43:5	43:5	PI38	mucu	H_2O	54:2

	1	2	3	4	5	6	7	8	9	10	11	12
Ct_{MWMV}	15.47	15.99	13.27	13.48	12.91	13.02	15.22	15.49	14.16	14.42	14.62	14.77
Ct_{UFP}	29.43	29.61	23.63	26.47	19.92	19.88	25.74	25.81	24.68	24.31	25.84	25.64
Ct_{MWMV}	18.73	18.97	12.61	12.70	18.23	18.32	14.54	14.76	14.22	14.14	19.14	19.45
Ct_{UFP}	30.21	32.65	20.43	20.49	26.62	26.67	18.92	18.90	19.87	19.95	21.15	21.21
Ct_{MWMV}	21.19	21.46	17.31	17.24	16.89	16.95	21.79	21.85	15.69	15.76	14.02	14.23
Ct_{UFP}	29.92	30.01	27.89	27.69	27.23	27.15	31.90	30.72	26.06	26.13	23.72	23.29
Ct_{MWMV}	17.07	17.16	13.83	13.88	12.49	12.55	15.27	15.45	-	34.75	-	11.01
Ct_{UFP}	23.51	23.57	25.03	25.01	19.97	19.94	26.02	25.96	19.68	21.28	-	21.94

	2:9		6:4		3:3		6:5		10:2		10:4	
ΔCt	-13.96	-13.62	-10.36	-12.99	-7.01	-6.86	-10.52	-10.32	-10.52	-9.89	-11.22	-10.87
ΔCt	-14.14	-13.44	-13.20	-10.15	-6.97	-6.90	-10.59	-10.25	-10.15	-10.26	-11.02	-11.07
$\bar{x} \pm \sigma$	-13.79	0.32	-11.68	1.64	-6.94	0.07	-10.42	0.16	-10.21	0.26	-11.05	0.14
	37:1		13:5		30:2		30:5		31:2		31:3	
ΔCt	-11.48	-13.68	-7.82	-7.79	-8.39	-8.35	-4.38	-4.14	-5.65	-5.81	-2.01	-1.76
ΔCt	-13.92	-11.24	-7.88	-7.73	-8.44	-8.30	-4.36	-4.16	-5.73	-5.73	-2.07	-1.70
$\bar{x} \pm \sigma$	-12.58	1.42	-7.81	0.06	-8.37	0.06	-4.26	0.13	-5.73	0.07	-1.89	0.18
	33:3		33:4		37:2		39:1		40:2		40:3	
ΔCt	-8.73	-8.55	-10.58	-10.45	-10.34	-10.20	-10.11	-8.87	-10.37	-10.37	-9.70	-9.06
ΔCt	-8.82	-8.46	-10.38	-10.65	-10.26	-10.28	-8.93	-10.05	-10.44	-10.30	-9.27	-9.49
$\bar{x} \pm \sigma$	-8.64	0.16	-10.52	0.12	-10.27	0.06	-9.49	0.68	-10.37	0.06	-9.38	0.28
	42:1		42:2		43:1		43:5					
ΔCt	-6.44	-6.41	-11.20	-11.13	-7.48	-7.39	-10.75	-10.51				
ΔCt	-6.50	-6.35	-11.18	-11.15	-7.45	-7.42	-10.69	-10.57				
$\bar{x} \pm \sigma$	-6.43	0.06	-11.17	0.03	-7.44	0.04	-10.63	0.11				

Table III. qPCR disposition, amplification cycles and calculation of ΔCt, average (orange) and standard deviation (green) for each sample of plate 3. Controls are excluded from the calculation. Colors indicate: MWMV mix (yellow), UFP (blue), negative control of amplification (orange) and positive control of amplification (green).

	1	2	3	4	5	6	7	8	9	10	11	12
A	46:1	46:1	46:2	46:2	95:2	95:2	50:3	50:3	53:1	53:1	53:7	53:7
B	46:1	46:1	46:2	46:2	95:2	95:2	50:3	50:3	53:1	53:1	53:7	53:7
C	54:1	54:1	39:4	39:4	55:2	55:2	55:4	55:4	56:1	56:1	61:2	61:2
D	54:1	54:1	39:4	39:4	55:2	55:2	55:4	55:4	56:1	56:1	61:2	61:2
E	61:3	61:3	64:2	64:2	64:3	64:3	68:2	68:2	68:3	68:3	78:8	78:8
F	61:3	61:3	64:2	64:2	64:3	64:3	68:2	68:2	68:3	68:3	78:8	78:8
G	78:10	78:10	92:1	92:1	92:5	92:5	95:1	95:1	PI38	H_2O	54:2	
H	78:10	78:10	92:1	92:1	92:5	92:5	95:1	95:1	PI38	H_2O	54:2	

	1	2	3	4	5	6	7	8	9	10	11	12
Ct_{MWMV}	12.14	11.99	12.81	12.98	12.25	12.32	14.87	14.76	12.10	12.48	16.05	16.14
Ct_{UFP}	21.03	20.77	21.81	21.90	21.84	21.77	24.78	25.08	22.91	22.92	23.94	23.82
Ct_{MWMV}	13.27	13.48	16.44	16.57	13.32	13.45	13.99	14.06	11.46	11.60	10.86	11.15
Ct_{UFP}	23.23	23.09	25.45	25.52	22.27	22.54	23.94	23.83	23.60	23.44	20.63	20.55
Ct_{MWMV}	13.79	13.89	12.78	12.87	12.45	12.83	13.76	13.83	14.66	14.76	12.84	12.89
Ct_{UFP}	20.27	20.49	23.92	24.05	24.55	24.19	23.98	24.02	25.89	25.88	24.49	24.31
Ct_{MWMV}	13.57	13.58	22.66	22.89	25.54	25.54	9.58	9.77	40.00	40.00	11.13	
Ct_{UFP}	22.84	22.89	24.12	24.23	22.21	22.16	20.65	20.67	19.88	-	22.18	

	46:1		46:2		95:2		50:3		53:1		53:7	
ΔCt	-8.89	-8.78	-9.00	-8.92	-9.59	-9.45	-9.91	-10.32	-10.81	-10.44	-7.89	-7.68
ΔCt	-8.63	-9.04	-9.09	-8.83	-9.52	-9.52	-10.21	-10.02	-10.82	-10.43	-7.77	-7.80
$\bar{x} \pm \sigma$	-8.84	0.17	-8.96	0.11	-9.52	0.06	-10.12	0.18	-10.63	0.22	-7.79	0.09
	54:1		39:4		55:2		55:4		56:1		61:2	
ΔCt	-9.96	-9.61	-9.01	-8.95	-8.95	-9.09	-9.95	-9.77	-12.14	-11.84	-9.77	-9.40
ΔCt	-9.82	-9.75	-9.08	-8.88	-9.22	-8.82	-9.84	-9.88	-11.98	-12.00	-9.69	-9.48
$\bar{x} \pm \sigma$	-9.79	0.15	-8.98	0.09	-9.02	0.17	-9.86	0.08	-11.99	0.12	-9.59	0.17
	61:3		64:2		64:3		68:2		68:3		78:8	
ΔCt	-6.48	-6.60	-11.14	-11.18	-12.10	-11.36	-10.22	-10.19	-11.23	-11.12	-11.65	-11.42
ΔCt	-6.70	-6.38	-11.27	-11.05	-11.74	-11.72	-10.26	-10.15	-11.22	-11.13	-11.47	-11.60
$\bar{x} \pm \sigma$	-6.54	0.14	-11.16	0.09	-11.73	0.30	-10.21	0.05	-11.18	0.06	-11.54	0.11
	78:10		92:1		92:5		95:1					
ΔCt	-9.27	-9.31	-1.46	-1.34	3.33	3.38	-11.07	-10.90				
ΔCt	-9.32	-9.26	-1.57	-1.23	3.38	3.33	-11.09	-10.88				
$\bar{x} \pm \sigma$	-9.29	0.03	-1.40	0.15	3.36	0.03	-10.99	0.11				

Table IV. qPCR disposition, amplification cycles and calculation of ΔCt, average (orange) and standard deviation (green) for each sample of plate 4. Controls are excluded from the calculation. Colors indicate: MWMV mix (yellow), UFP (blue), negative control of amplification (orange) and positive control of amplification (green).

	1	2	3	4	5	6	7	8	9	10	11	12
A	54:2	54:2	39:4	39:4	92:3	92:3	92:4	92:4	92:6	92:6	PI38	H_2O
B	54:2	54:2	39:4	39:4	92:3	92:3	92:4	92:4	92:6	92:6	PI38	H_2O
C	54:2											
D	54:2											

	1	2	3	4	5	6	7	8	9	10	11	12
Ct_{MWMV}	12.30	12.12	11.81	12.14	20.58	20.68	25.92	25.21	18.82	18.91	-	-
Ct_{UFP}	23.49	23.27	20.30	20.26	22.90	29.23	29.13	21.92	21.77	19.85	-	-
Ct_{MWMV}	11.06											
Ct_{UFP}	21.94											

	54:2		39:4		92:3		92:4		92:6			
ΔCt	-11.19	-11.15	-8.49	-8.12	-2.32	-8.55	-3.21	3.29	-2.95	-0.94		
ΔCt	-10.97	-11.37	-8.45	-8.16	-8.65	-2.22	4.00	-3.92	-1.03	-2.86		
$\bar{x} \pm \sigma$	-11.17	0.16	-8.31	0.19	-5.44	3.66	0.04	4.18	-1.95	1.11		

Table V. qPCR disposition, amplification cycles and calculation of ΔCt, average (orange) and standard deviation (green) for each sample of plate 5. Controls are excluded from the calculation. Colors indicate: MWMV mix (yellow), UFP (blue), negative control of amplification (orange) and positive control of amplification (green).

	1	2	3	4	5	6	7	8	9	10	11	12
E	31:2	31:2	42:2	42:2	78:10	78:10	53:1	53:1	54:2	54:2	60:1	60:1
F	31:2	31:2	42:2	42:2	78:10	78:10	53:1	53:1	54:2	54:2	60:1	60:1
G	92:3	92:3	PI38	H_2O	54:2							
H	92:3	92:3	PI38	H_2O	54:2							

	1	2	3	4	5	6	7	8	9	10	11	12
Ct_{MWMV}	14.05	14.05	13.75	13.94	13.47	13.69	12.51	12.51	12.04	22.01	11.52	11.87
Ct_{UFP}	20.31	20.32	25.29	25.12	22.64	22.78	22.65	22.78	23.53	23.78	20.24	20.46
Ct_{MWMV}	20.46	20.84	-	40.00	11.15							
Ct_{UFP}	22.98	23.39	19.95	-	26.89							

	31:2		42:2		78:10		53:1		54:2		60:1	
ΔCt	-6.26	-6.27	-11.54	-11.18	-9.17	-9.09	-10.14	-10.27	-11.49	-1.77	-8.72	-8.59
ΔCt	-6.27	-6.26	-11.37	-11.35	-9.31	-8.95	-10.27	-10.14	-11.74	-1.52	-8.94	-8.37
$\bar{x} \pm \sigma$	-6.27	0.01	-11.36	0.15	-9.13	0.15	-10.21	0.08	-6.63	5.76	-8.66	0.24
	92:3											
ΔCt	-2.52	-2.55										
ΔCt	-2.93	-2.14										
$\bar{x} \pm \sigma$	-2.54	0.32										

Table VI. qPCR disposition, amplification cycles and calculation of ΔCt, average (orange) and standard deviation (green) for each sample of plate 6. Controls are excluded from the calculation. Colors indicate: MWMV mix (yellow), UFP (blue), negative control of amplification (orange) and positive control of amplification (green).

	1	2	3	4	5	6	7	8	9	10	11	12
A	6:4	6:4	37:1	37:1	92:6	92:6	92:3	92:3	78:10	78:10	92:4	92:4
B	6:4	6:4	37:1	37:1	92:6	92:6	92:3	92:3	78:10	78:10	92:4	92:4
C	53:1	53:1	54:2	54:2	PI38	H_2O	54:2					
D	53:1	53:1	54:2	54:2	PI38	H_2O	54:2					

	1	2	3	4	5	6	7	8	9	10	11	12
Ct_{MWMV}	17.18	17.34	21.48	21.67	19.58	20.53	23.43	23.27	15.51	15.62	25.19	25.19
Ct_{UFP}	27.55	27.45	33.36	33.35	22.55	22.82	25.27	25.62	24.31	24.65	29.20	29.18
Ct_{MWMV}	12.91	13.12	15.06	15.16	40.00	40.00	15.82					
Ct_{UFP}	23.11	23.45	25.70	25.58	19.77	-	27.84					

	6:4		37:1		92:6		92:3		78:10		92:4	
ΔCt	-10.37	-10.11	-11.88	-11.68	-2.97	-2.29	-1.84	-2.35	-8.80	-9.03	-4.01	-3.99
ΔCt	-10.27	-10.21	-11.87	-11.69	-3.24	-2.02	-2.19	-2.00	-9.14	-8.69	-3.99	-4.01
$\bar{x} \pm \sigma$	-10.24	0.11	-11.78	0.11	-2.63	0.57	-2.10	0.22	-8.92	0.21	-4.00	0.01
	53:1		54:2									
ΔCt	-10.20	-10.33	-10.64	-10.42								
ΔCt	-10.54	-9.99	-10.52	-10.54								
$\bar{x} \pm \sigma$	-10.27	0.23	-10.53	0.09								

Table VI. Multiple range test Turkey HSD. Homogeneous groups indicate statistical significance between the different accession means. Coincidence of a X letter indicates no significance between the groups.

Accession	Cases	Mean	Homogeneous groups
CO-002	2	-13.41	X
CO-056	1	-11.99	XX
CO-064	2	-11.445	XX
CO-037	2	-11.025	XX
CO-050	2	-10.84	XX
CO-068	2	-10.695	XX
CO-010	2	-10.63	XX
CO-006	2	-10.33	XX
CO-095	2	-10.255	XX
CO-078	2	-10.23	XX
CO-054	2	-10.16	XX
CO-040	2	-9.875	XX
CO-033	2	-9.58	XX
CO-055	2	-9.44	XX
CO-003	2	-9.245	XX
CO-039	2	-9.235	XX
CO-043	2	-9.035	XX
CO-053	2	-9.0	XX
CO-046	2	-8.9	XX
CO-042	2	-8.895	XX
CO-060	1	-8.66	XXX
CO-013	2	-8.555	XX
CO-061	2	-8.065	XX
CO-030	2	-6.315	XXX
CO-031	2	-4.08	XX
CO-092	5	-1.354	X

Table VII qPCR results for the 26 accessions studied, with two samples analysed per entry, except for CO-092 in which all samples have been used. ΔCt (yellow) and standard deviation (green) are shown for each sample.

CO-002:7	**CO-002:9**	**CO-003:3**	**CO-003:6**	**CO-006:4**	**CO-006:5**	**CO-010:2**	**CO-010:4**
-13.03	-13.79	-6.94	-11.55	-10.24	-10.42	-10.21	-11.05
0.03	0.32	0.07	0.15	0.11	0.16	0.26	0.14
CO-013:2	**CO-013:5**	**CO-030:2**	**CO-030:5**	**CO-031:2**	**CO-031:3**	**CO-033:3**	**CO-033:4**
-9.30	-7.81	-8.37	-4.26	-6.27	-1.89	-8.64	-10.52
0.10	0.06	0.06	0.13	0.01	0.18	0.16	0.12
CO-037:1	**CO-037:2**	**CO-039:1**	**CO-039:4**	**CO-040:2**	**CO-040:3**	**CO-042:1**	**CO-042:2**
-11.78	-10.27	-9.49	-8.98	-10.37	-9.38	-6.43	-11.36
0.11	0.06	0.68	0.09	0.06	0.28	0.06	0.15
CO-043:1	**CO-043:5**	**CO-046:1**	**CO-046:2**	**CO-050:1**	**CO-050:3**	**CO-053:1**	**CO-053:7**
-7.44	-10.63	-8.84	-8.96	-11.56	-10.12	-10.21	-7.79
0.04	0.11	0.17	0.11	0.12	0.18	0.08	0.09
CO-054:1	**CO-054:2**	**CO-055:2**	**CO-055:4**	**CO-056:1**	**CO-060:1**	**CO-061:2**	**CO-061:3**
-9.79	-10.53	-9.02	-9.86	-11.99	-8.66	-9.59	-6.54
0.15	0.09	0.17	0.08	0.12	0.24	0.17	0.14
CO-064:2	**CO-064:3**	**CO-068:2**	**CO-068:3**	**CO-078:8**	**CO-78:10**	**CO-092:1**	**CO-092:3**
-11.16	-11.73	-10.21	-11.18	-11.54	-8.92	-1.40	-2.10
0.09	0.30	0.05	0.06	0.11	0.21	0.15	0.22
CO-092:4	**CO-092:5**	**CO-092:6**	**CO-095:1**	**CO-095:2**			
-4.00	3.36	-2.63	-10.99	-9.52			
0.01	0.03	0.57	0.11	0.06			

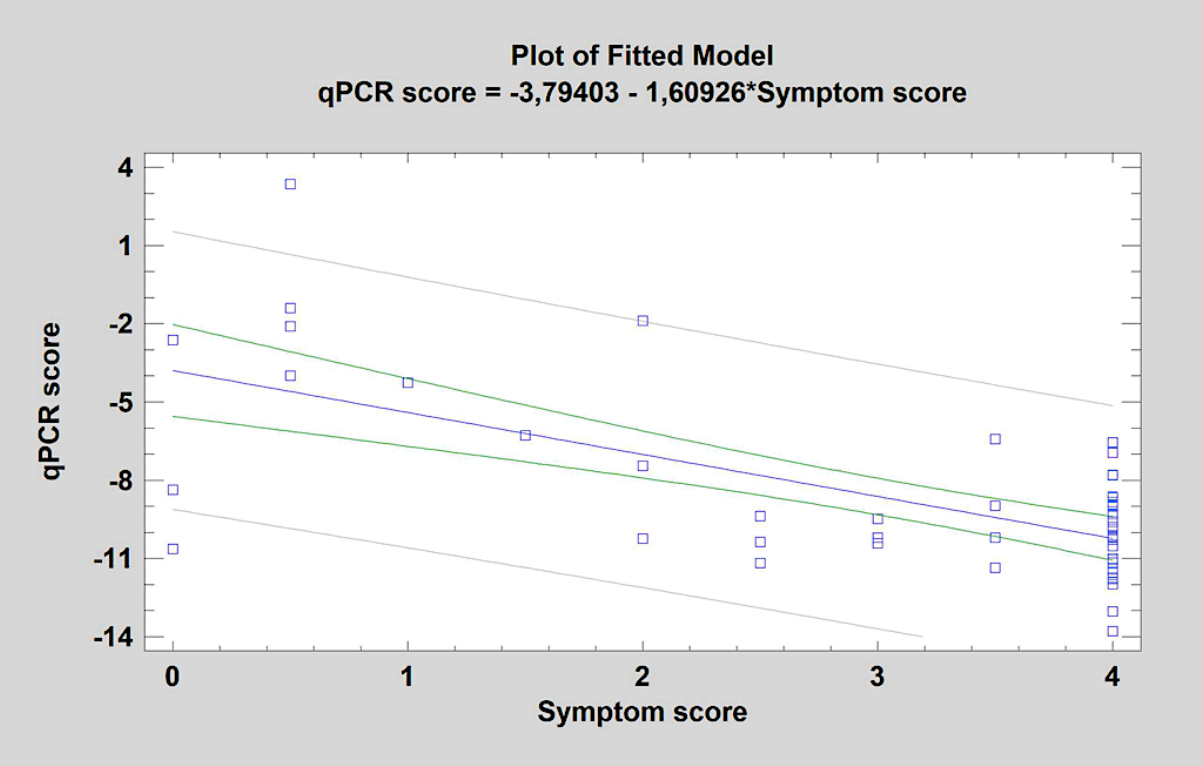

Figure III. Linear regression between symptoms (independent variable) and qPCR ΔCt (dependent variable) at 21 dpi. Correlation coefficient = -0.658451. P-value = 0.