Evaluation of Resistance to Moroccan Watermelon Mosaic Virus (MWMV) in a Melon Recombinant Inbred line (RIL) Population Derived from TGR-1551

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Introduction

Cucurbit crops constitute an economically important horticultural group around the world but are severely affected by various viral diseases, particularly those caused by potyviruses (Martín-Hernández & Picó, 2020). Among them, the Moroccan watermelon mosaic virus (MWMV), a member of the genus *Potyvirus* (family *Potyviridae*), has emerged as a significant threat in the Mediterranean Basin, Africa, and several European countries, where its incidence and geographic range have steadily expanded (Miras et al., 2019).

MWMV was initially discovered in Morocco in 1972, but at that time, it was considered a watermelon mosaic virus (WMV) strain (Fischer & Lockhart, 1974); it was subsequently recognized as a distinct member (Lecoq et al., 2001). Symptoms associated with MWMV are characterized by mosaic, severe leaf and fruit deformations (Fischer & Lockhart, 1974), as well as severe vein clearing (De Moya-Ruiz et al., 2021). However, these symptoms can be exacerbated under natural conditions by mixed infection with other potyviruses (Jagunić et al., 2025).

Resistance to MWMV has been identified in previous works carried out by our research group in melon (*Cucumis melo* L.) accessions TGR-1551, PI 414723, and IC 274006 (personal communication). In melon, the source of virus resistance is usually found in accessions of the Asian Conomon, Momordica, and Acidulus Groups (Martín-Hernández & Picó, 2020). When these accessions present a multi-resistant profile, breeding programs are simplified, as a single donor parent can be used to introgress multiple resistance traits into a single genetic background (López Martín, 2023). The African accession TGR-1551 (acidulus group) has been reported as resistant to WMV, cucurbit yellow stunting disorder virus (CYSDV), cucurbit aphid-borne yellows virus (CABYV), MWMV, and powdery mildew (*Podosphaera xanthii*) races 1, 2, and 5 (Díaz-Pendón et al., 2005; Lecoq & Desbiez, 2012; López-Sesé & Gómez-Guillamón, 2000; Kassem et al., 2015, Palomares-Ríus et al., 2011, 2016; Yuste-Lisbona et al., 2010, 2011a, 2011b).

TGR-1551-derived resistance to the *potyvirus* WMV has been reported to provoke a drastic and significant reduction in virus titer, with infected plants being asymptomatic or showing only mild symptoms of the disease (Díaz-Pendón et al., 2005), with a strong early transcriptional defense response, despite the resistance mechanism being recessive (López Martín et al., 2024). A population of recombinant inbred lines (RILs) derived from the cross between the resistant genotype TGR-1551, and the susceptible Spanish cultivar 'Bola de Oro' (BO) has enabled significant advances in the detection of QTLs linked to WMV resistance. A major QTL on chromosome 11 and another minor QTL on chromosome 5 have been reported, revealing a complex genetic architecture influenced by environmental conditions (Palomares-Rius et al., 2011; Pérez-de-Castro et al., 2019).

A close genomic and biological relationship between WMV and MWMV has been previously reported (Jagunić et al., 2025). In this context, the objective of this study was to evaluate a population of RILs (TGR-1551 x BO) mechanically inoculated with MWMV to identify resistant lines and generate phenotypic data that will subsequently allow mapping of resistance and identification of candidate genes.

Materials and Methods

A recombinant inbred lines (RILs) population (F_7/F_8; 61 lines) derived from the cross TGR-1551 × BO was evaluated for resistance to MWMV. This set of 61 lines, previously genotyped by sequencing (GBS) covered 99.6% of TGR-1551 genome (Pérez-de-Castro et al., 2019). Plants (1–5 per line) were mechanically inoculated with a Mediterranean MWMV isolate, using infected tissue homogenized in inoculation buffer (1% PVP-10, 1% PEG-6000, 10% KH_2PO_4, pH 8). The inoculation was performed mechanically by rubbing one cotyledon and the first genuine leaf, previously sprinkled with carborundum. The plants were reinoculated 7 days later.

Symptoms were scored at 15, 21, and 28 days post-inoculation (dpi) on a 0–4 severity scale, where 0 indicates the plant does not show any disease symptoms, and 4 indicates

the plant is highly affected (Figure 1, adapted from the symptoms described for MWMV infections (Miras et al., 2019)). Based on this scale, each line was classified as resistant (R) or susceptible (S) according to the maximum severity value recorded during the experiment. Lines were considered resistant if symptom score was lower than 2, whereas those reaching scores of 2 or greater than 2 at any evaluation date were classified as susceptible.

The lines identified as resistant in this first assay, based on symptom scoring, were subsequently re-evaluated, together with both the resistant and the susceptible parents, TGR-1551 and BO. Leaf tissue was collected at 21 and 28 dpi, and stored at −80 °C until used to quantify MWMV accumulation.

Total RNA was extracted from leaf tissue using EXTRAzol® (Blirt). Samples were homogenized in 700 µL of EXTRAzol®, phase-separated with chloroform, and the aqueous phase was recovered. RNA was precipitated with isopropanol, washed twice with 70% ethanol, air-dried, and resuspended in 30 µL of nuclease-free water. Then, the RNA was quantified using a NanoDrop™ 1000 spectrophotometer (Waltham, Massachusetts, United States).

Once RNA quality and concentration were confirmed, samples were diluted to 100 ng/µL. RNA was then reverse-transcribed using the RevertAid™ Reverse Transcription Kit (Thermo Fisher Scientific) with random hexamer primers, following the manufacturer's protocol. The resulting cDNA was stored at −20 °C until RT-qPCR analysis.

Relative quantification of MWMV accumulation was performed by RT-qPCR using the FastStart Essential DNA Green Master (Roche). Each 15 µL reaction contained 7.5 µL of 2× Green Master Mix, 1.5 µL of each primer (10 µM), 3 µL of nuclease-free water, and 1.5 µL of cDNA. Two primer sets were used: one targeting the MWMV coat protein (CP). (F:5′CAACACCAGGGCAACTCAGA3′ and R:5′TGCACCACACCATGAAACCA3′, MWMV-CP primers designed in this study), and one targeting the endogenous reference gene Cyclophilin CYP7 (F:5′CGATGTGGAAATTGACGGAA3′ and R:5′CGGTGCATAATGCTCGGAA3′) (Sáez et al. 2022).

Ct values were used to calculate ΔCt, ΔΔCt, $2^{-\Delta\Delta Ct}$, and its logarithmic transformation log ($2^{-\Delta\Delta Ct}$) to estimate viral load. Statistical analyses (ANOVA and LSD 95%) were performed using GraphPad Prism.

Results and Discussion

A total of 61 recombinant inbred lines (RILs) were mechanically inoculated with MWMV. Symptom severity was evaluated at 15, 21, and 28 dpi using a 0–4 scale. According to the symptom score, each line was classified as susceptible (S) or resistant (R) (Table 1). Fifty-seven out of the 61 lines showed symptoms typically associated with MWMV (Miras et al., 2019), including leaf deformation, blistering, and severe mosaic, with severity scores of 3 or 4 during the evaluation period. These lines were therefore classified as susceptible. Four of the lines (195, 55, 60, 68) showed very low symptoms (range 0-1) during the evaluation period and were therefore classified as resistant (R). This finding is consistent with the previous works of the group reporting resistance to MWMV in accession TGR-1551.

A new trial was conducted with the previously classified resistant lines. Consistently, these lines either remained asymptomatic or showed only slight symptoms (range 0–1) similarly to the first evaluation. The accession TGR-1551, on the other hand, showed variability in its response: some plants remained practically asymptomatic (0–1), while others developed slightly more noticeable symptoms. Notably, some of the RILs included in this study were resistant, indicating that the RILs population was derived from a resistant TGR-1551 plant. Viral load was assessed at 21 dpi and 28 dpi. MWMV accumulation increased from 21 to 28 dpi, indicating a progressive rise in viral replication over time. This temporal trend is consistent with previous findings showing that viral load after inoculation with an infectious MWMV clone in melon plants steadily increased throughout the infection period, specifically at 6, 12, 18, and 24 dpi (De Moya-Ruiz et al., 2021).

In our study, differences in viral accumulation at 21 dpi were highly significant ($p = 0.021$). The four resistant lines (RILs) as well as the resistant control TGR-1551 showed virus accumulation lower than the susceptible parent BO (Figure 2A). At 28 dpi, viral accumulation in RILs 68, 60, and 195 was significantly lower than in BO, while viral accumulation in RIL 55 did not significantly differ from either that of TGR-1551 or BO (Figure 2B). The results indicated that the resistant lines exhibit reduced MWMV accumulation, consistent with their lack of symptoms. Previous studies on TGR-1551-derived resistance to the closely related potyvirus WMV, reported that this resistance consisted of a substantial decrease in viral titer and milder symptom expression compared with susceptible genotypes (Díaz-Pendón et al. 2005), thus, similar to resistance to MWMV characterized in the present study.

As previously stated, a GBS was available for the RILs population (Pérez de Castro et al., 2019). A preliminary haplotype analysis of the 61 evaluated lines, comparing resistant and susceptible lines, identified a TGR-1551 introgression on chromosome 11 shared by the four resistant lines. In contrast, susceptible lines predominantly carry the BO-derived allele for this region. The identified region, although longer, included the candidate region for the major QTL conferring resistance to WMV (López-Martín et al., 2024; Pérez-de-Castro et al., 2019). However, preliminary results by the research group with lines segregating for candidate region

for TGR-derived WMV resistance suggest that both resistances have different genetic control. The QTL responsible to TGR-derived resistance to MWMV could be in the candidate region on chromosome 11 identified here, but is different from the QTL for WMV resistance. In this sense, the study by Rodríguez-Hernández et al. (2012) showed that silencing the translation initiation factor eIF4E in melon conferred resistance to MWMV but not to WMV, suggesting the resistance to each of these two potyviruses is the result of two separate mechanisms.

TGR-1551 is a widely recognized multi-resistant accession, against various viruses and fungi affecting melon. Molecular markers are available for marker assisted selection for some of these resistances, including CYSDV (Pérez de Castro et al., 2020), WMV (Pérez de Castro et al., 2019) or powdery mildew (López-Martín et al., 2022). Thus, its use in breeding programs facilitate the introgression of multiple resistances in a common genetic background. In this report, we present evidence that TGR-1551 also confers resistance to MWMV. As a next step, segregating generation studies derived from the resistant RILs 195, 55, 60, and 68, will be conducted to confirm the candidate region conferring resistance to MWMV.

Acknowledgements

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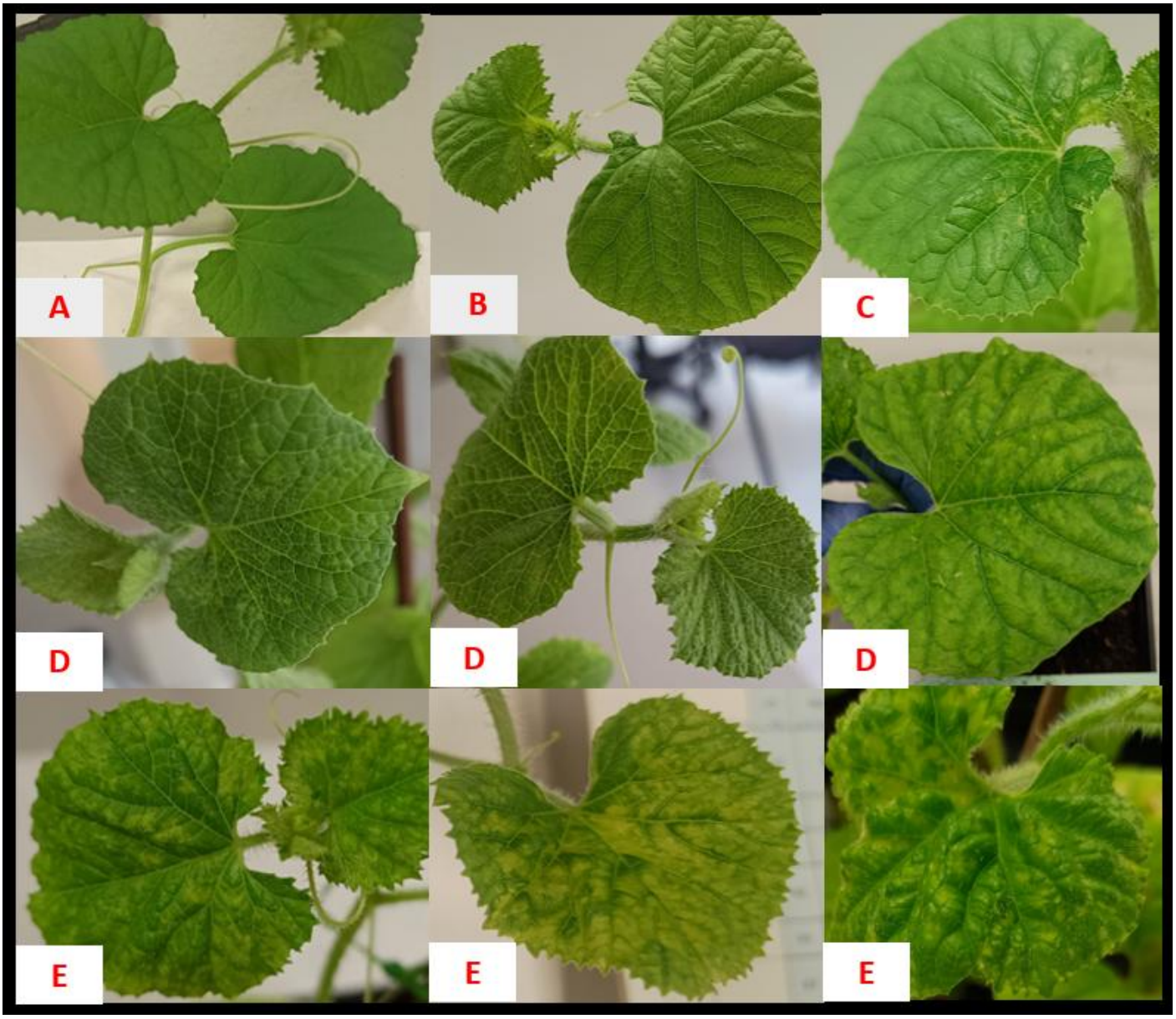

Figure 1 Symptoms after inoculation with Moroccan watermelon mosaic virus (MWMV) on a scale from 0 to 4. (A) No symptoms (0). (B) Mild symptoms (1). (C)Moderate symptoms (2). (D) Severe symptoms (3). (E) Very severe symptoms (4).

Table 1. Symptom severity ranges at 15, 21 and 28 days post inoculation (dpi) after MWMV inoculation in lines of the TGR-1551-derived recombinant inbred lines (RILs) population. Classification of the lines as resistant (R) or susceptible (S) (see text for description). N indicates the number of plants evaluated per line.

Line	N	Symptoms 15-dpi	Symptoms 21-dpi	Symptoms 28-dpi	Maximum severity	Classification
BO	5	(2 - 3)	(3 - 4)	(3 - 4)	4	S
TGR	5	(0 - 1)	(0 - 1)	(0 - 1)	1	R
7	3	(2 - 3)	(3 - 4)	(1 - 3)	4	S
9	3	(3 - 3)	(1 - 3)	(1 - 3)	3	S
17	3	(1 - 2)	(0 - 4)	(1 - 3)	4	S
21	2	(0 - 3)	(2 - 3)	(2 - 4)	4	S
28	3	(1 - 2)	(2 - 3)	(2 - 4)	4	S
35	3	(3 - 4)	(3 - 4)	(1 - 4)	4	S

49	1	3	3	3	3	S
50	3	(0 - 4)	(2 - 4)	(3 - 4)	4	S
55	5	(0 - 1)	(1 - 1)	(0 - 1)	1	R
60	5	(0 - 0)	(0 - 0)	(0 - 0)	1	R
67	3	(0 - 0)	(1 - 1)	(1 - 4)	4	S
68	5	(1 - 1)	(0 - 1)	(0 - 1)	1	R
69	4	(1 - 4)	(2 - 4)	(2 - 4)	4	S
73	4	(1 - 4)	(1 - 4)	(1 - 4)	4	S
85	2	(1 - 3)	(3 - 3)	(4 - 4)	4	S
87	5	(0 - 4)	(1 - 3)	(1 - 3)	4	S
101	1	3	4	4	4	S
104	2	(4 - 4)	(2 - 3)	(3 - 4)	4	S
109	5	(0 - 2)	(2 - 3)	(4 - 4)	4	S
110	2	(0 - 1)	(0 - 2)	(1 - 3)	3	S
114	1	2	4	2	4	S
131	4	(1 - 1)	(0 - 4)	(0 - 4)	4	S
143	1	1	1	4	4	S
147	3	(1 - 4)	(1 - 4)	(2 - 4)	4	S
151	5	(3 - 4)	(3 - 4)	(4 - 4)	4	S
156	3	(1 - 3)	(2 - 4)	(0 - 4)	4	S
157	2	(0 - 2)	(1 - 4)	(1 - 4)	4	S
158	5	(0 - 4)	(0 - 3)	(0 - 2)	4	S
159	2	(3 - 3)	(3 - 4)	(2 - 3)	4	S
161	3	(0 - 4)	(2 - 4)	(1 - 3)	4	S
164	2	(4 - 4)	(3 - 4)	(3 - 4)	4	S
165	4	(1 - 3)	(2 - 3)	(1 - 4)	4	S
167	4	(0 - 4)	(2 - 4)	(0 - 4)	4	S
172	2	(0 - 1)	(1 - 2)	(2 - 3)	3	S
172	2	(0 - 1)	(1 - 2)	(2 - 3)	3	S
176	3	(4 - 4)	(0 - 4)	(0 - 1)	4	S
182	3	(1 - 3)	(1 - 2)	(2 - 3)	3	S
183	4	(4 - 4)	(3 - 4)	(2 - 4)	4	S
186	3	(2 - 4)	(2 - 2)	(0 - 2)	4	S
192	3	(3 - 4)	(1 - 4)	(3 - 4)	4	S
193	3	(1 - 3)	(0 - 3)	(1 - 4)	4	S
195	5	(0 - 0)	(0 - 1)	(1 - 1)	1	R
215	5	(1 - 1)	(2 - 4)	(1 - 4)	4	S
246	5	(0 - 3)	(0 - 3)	(0 - 4)	4	S
253	4	(0 - 4)	(0 - 2)	(2 - 4)	4	S
258	1	4	4	2	4	S
263	5	(0 - 3)	(1 - 4)	(1 - 4)	4	S
271	1	3	4	4	4	S
272	3	(1 - 3)	(2 - 3)	(2 - 3)	3	S
275	3	(2 - 2)	(1 - 1)	(1 - 3)	3	S
278	2	(0 - 3)	(1 - 2)	(2 - 3)	3	S
313	1	4	4	4	4	S
398	1	0	3	3	3	S
401	3	(1 - 2)	(2 - 3)	(2 - 3)	3	S
435	2	(0 - 3)	(1 - 4)	(3 - 4)	3	S
478	5	(1 - 4)	(2 - 4)	(2 - 4)	4	S
513	3	(4 - 4)	(4 - 4)	(2 - 4)	4	S
520	3	(0 - 2)	(0 - 4)	(1 - 4)	4	S
556	3	(2 - 3)	(1 - 2)	(2 - 3)	3	S
562	5	(2 - 4)	(2 - 4)	(2 - 4)	4	S
579	5	(0 - 3)	(3 - 4)	(0 - 1)	4	S

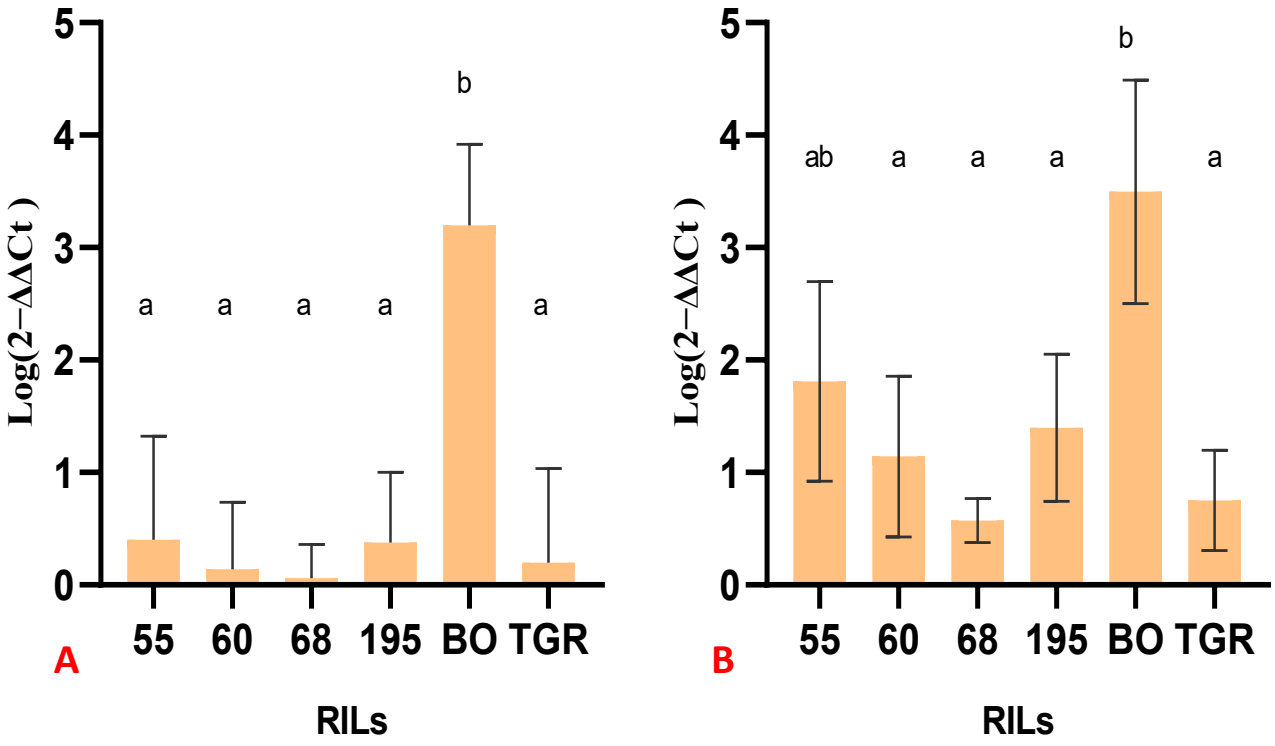

Figure 2. Mean viral accumulation log ($2^{-\Delta\Delta Ct}$) in melon recombinant inbred lines (RILs) derived from TGR-1551 (TGR) in 'Bola de Oro' (BO) genetic background, inoculated with Moroccan watermelon mosaic virus (MWMV) at 21 days post inoculation (dpi) (A) and 28 dpi (B). Vertical bars represent ± the standard error. Different letters indicate significant differences ($p < 0.05$, LSD test).