Breeding Program for the Introgression of Resistance to Viral and Fungal Diseases in Melon Landraces: Evaluation of Advanced Generations

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Introduction

Melon (*Cucumis melo* L.) production is near 30 million metric tons harvested worldwide. Production in Spain is characterized by high yields (35 t/ha in 2024), reaching approximately 594,000 tons annually, of which more than 397,000 tons are exported. This places Spain as the leading producer and exporter within the European Union. The melon types most widely grown in Spain belong to the *ibericus* group, which includes Piel de Sapo, Amarillo, Tendral, Rochet, and Blanco types. Efforts are currently underway to revive the cultivation of other melon landraces, such as snake melons. These non-sweet melons belong to the *flexuosus* group, and are given different names such as *alficoz*, *alficòs* or *cogombre*.

Biotic stresses currently represent the main limiting factor in melon (*Cucumis melo* L.) production. Among them, viral and fungal diseases are particularly important. In this context, watermelon mosaic virus (WMV) is among the most significant threats in Spain and in major melon-producing regions worldwide (Desbiez et al., 2020; López-Martín et al., 2024a; Pérez-de-Castro et al., 2019). WMV is a potyvirus transmitted by several aphid species and it induces symptoms such as mosaic, leaf deformation, and chlorosis, often accompanied by growth arrest, yield reduction and negative effect on fruit quality. Powdery mildew, mainly caused by the fungus *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff, is also widely distributed and causes significant economic losses worldwide. This fungus affects aerial plant tissues and is characterized by powdery spore masses on leaves, petioles, and stems. Infected leaves frequently wilt and plants undergo premature senescence. The globalization of agriculture and changes in cropping systems facilitate the spread of these pathogens, while current control strategies often provide limited effectiveness. Consequently, the development of resistant cultivars remains a key objective. However, despite the global importance of these pathogens, no cultivars providing a definitive solution are currently available.

In melon breeding, the genetic variability exploited is mainly intraspecific. Strong crossability barriers between wild species of the genus *Cucumis* and cultivated melon limit the use of interspecific variability. Fortunately, substantial genetic diversity exists within *C. melo* for many agronomic traits. For disease resistance, the main sources are exotic or wild accessions belonging to the *agrestis*, *momordica*, or *acidulus* groups, originating from regions such as India, the Far East, and Africa. Many of these accessions exhibit multiple disease resistances, as is the case for the accessions TGR-1551 (PI 482420) and PI 414723.

The Zimbabwean accession TGR-1551 belongs to the *acidulus* group. TGR-1551 exhibits resistance to WMV, cucurbit yellow stunting disorder virus (CYSDV), and powdery mildew. In addition, it is resistant to cucurbit aphid-borne yellow virus (CABYV) and to aphids of the species *Aphis gossypii* Glover, and shows tolerance to the whitefly *Bemisia tabaci* Gennadius. Although partial resistance to WMV has been identified in other accessions, TGR-1551 displays the highest level of resistance reported so far, which is effective against several pathogen isolates. This resistance involves both reduced vector-mediated transmission and resistance to the virus itself. It is mainly controlled by a recessive gene, although additional modifying genes have also been described (Díaz-Pendón et al., 2005; Pérez-de-Castro et al., 2019). With respect to powdery mildew, TGR-1551 is resistant to races 1, 2, and 5, with resistance controlled by two genes showing dominant–recessive epistatic interaction (Yuste-Lisbona et al., 2011; López-Martín et al., 2022).

PI 414723 is an Indian accession, of the *momordica* group, showing resistance to WMV, zucchini yellow mosaic virus (ZYMV), tomato leaf curl New Delhi virus (ToLCNDV) and different races of powdery mildew (Anagnostou et al., 2000; Fazza et al., 2013; Sáez et al., 2017). Resistance to WMV is conferred by a dominant gene, *Wmv* (Gilbert et al., 1994). Resistance to ZYMV is also monogenic dominant, and both genes are linked, facilitating their introgression (Anagnostou et al., 2000). Regarding powdery mildew, *Pm-7* gene confers resistance to race 1, and *Pm-x* confers resistance to race 2; this

accession is also resistant to races 3 and 5, although the genetic control is unknown (Fazza et al., 2013).

A breeding program for the introgression of resistance into the main Spanish melon landraces was initiated using TGR-1551 and PI 414723, among other resistance sources (López-Martín, 2023). In this context, the objective of this work was the evaluation of the resistances to powdery mildew and to viral diseases in advanced materials derived from these two sources.

Materials and Methods

Advanced generations of the breeding program derived from initial crosses between different melon landraces and two resistance sources were tested. The two multi-resistant exotic melons used were the PI 414723 and the TGR-1551. The recurrent parents were accessions of the main Spanish melon types: 'Amarillo' (22AM-GO-BGV016451), 'Blanco' (29BL-BGV015753 and 26BL-BGV000444), 'Piel de Sapo' (11PS-BGV013188) and a snake melon, 'Alficoz' (*flexuosus* group, 05AL-BGV004853). The populations used corresponded to selfed generations from backcross populations ranging from BC3 to BC5, depending on the family. The families evaluated for resistance to powdery mildew included fixed lines and segregating generations. All the families tested for resistance to viruses were segregating generations. For fixed lines, 4 to 8 plants were tested, while around 30 plants were evaluated for the segregating generations. Plants were genotyped using the markers previously described as linked to the candidate regions for the resistance derived from the different sources (Pérez-de-Castro et al., 2019; López-Martín et al., 2022; López-Martín, 2023).

The assay for resistance to powdery mildew was carried out in greenhouse conditions in early spring season in Valencia. Plants were inoculated artificially by depositing a small amount of conidia at three spots on the second true leaf of each plant. The inoculum came from infected leaves collected in greenhouses in the same cultivation area, thus representing the races present in natural conditions. Symptoms were visually evaluated at 15, 30 and 45 days post-inoculation (dpi), according to the level of sporulation of the fungus, using a scale from 0 (no conidia germination) to 7 (profuse sporulation).

Evaluation for resistance to viral diseases was carried out in open field conditions, in Museros and Alcàsser (Valencia, Spain) in the spring-summer season, under natural infection conditions. Symptoms were evaluated at 30 and 45 days post-transplant (dpt), using a scale from 0 (asymptomatic plant) to 3 (plant showing severe symptoms). Samples were collected at these same dates and viral accumulation was evaluated using qPCR or RT-qPCR, as previously described (López-Martín et al., 2024a).

Statistical analyses were performed using the Statgraphics Centurion XVII software (Statpoint Technolgies, Inc).

Results and Discussion

Resistance to powdery mildew was confirmed in the families fixed for the candidate regions from each of the sources, TGR-1551 and PI 414723. Symptoms were slight in most of the families throughout the assay. Even in the cases of families which showed higher mean symptoms in early evaluation dates, means were lower at 45 dpi, which indicated the control of the infection in the resistant genotypes (Figure 1).

In segregating generations, there was a positive significant low-to-moderate correlation (range 0.24-0.55) between the genotype for the candidate region (marker alleles) and the phenotype (symptom score) for the resistance to powdery mildew at the different evaluation dates. Correlation was higher at 45 dpi for both resistance sources. In families derived from each resistant parent, symptom scores were significantly lower in plants homozygous for the allele of the resistant parent when compared with those homozygous for the allele of the susceptible parent (Figure 2). The behavior of the heterozygotes differed depending on the resistance source. In generations derived from PI 414723, symptom scores in heterozygotes were not significantly different from homozygotes at 15 and 30 dpi, while at 45 dpi symptom scores were intermediate between the susceptible and resistant homozygotes. Resistance to different races derived from PI 414723 has been previously reported as dominant (Fazza et al., 2013). Results obtained here suggest incomplete dominance. In the case of generations derived from TGR-1551, symptom scores at 15 and 30 dpi in heterozygotes were comparable to those in homozygotes for the allele of the susceptible parent; at 45 dpi, symptom scores in heterozygotes did not significantly differ from those of the homozygotes for the allele of the resistant parent, as previously reported (López-Martín et al., 2022).

WMV was the only virus detected in the open field virus assays. ZYMV, CYSDV and ToLCNDV, were not detected in any of the samples. In previous studies by this group, WMV was the virus most prevalent (López-Martín et al., 2024a). Again, the genotypes were confirmed using the markers previously described as associated to the different candidate regions. Symptoms were evaluated at 30 and 45 dpi. Viral accumulation measured by qPCR was also determined at the same evaluation dates.

In the case of segregating generations with resistance derived from PI 414723 most of the homozygote resistant plants remained asymptomatic at 30 dpt, and those with

symptoms showed only slight mosaic (Figure 3). Similarly, symptoms in heterozygotes were mild. Mosaic and leaf deformation was more severe from 30 dpt in homozygotes susceptible. In any case, differences were not significant at this date (Figure 4). At 46 dpt mean symptom scores remained low in homozygote resistant and heterozygote plants, while in homozygotes susceptible symptoms increased, being significantly higher (Figures 3 and 4). The ability to recover from the infection in plant materials with resistance derived from PI 414723 has previously been reported, supporting these results (Anagnostou et al., 2000; Gilbert et al., 1994). Positive moderate significant correlation between the genotype and viral accumulation was identified at 30 dpt (0.52) and 45 dpt (0.56), with a gradient in the viral accumulation detected in homozygotes resistant, heterozygotes and homozygotes susceptible. These results are consistent with those previously reported for WMV PI 414723-derived resistance (Anagnostou et al., 2000); lines derived from this source exhibited dominant monogenic resistance to WMV, resulting in a reduction in symptom expression in plants carrying the allele. Subsequent studies confirmed that this accession acts by restricting the systemic spread of the virus and reducing viral accumulation (Adler-Berke et al., 2021; Anagnostou et al., 2000). Results presented here seem to indicate that viral accumulation in heterozygotes is intermediate between the parents, but sufficient to reduce the symptom severity ratings to the level of "resistant."

Resistance to WMV in generations derived from TGR-1551 was also evaluated. Heterozygotes for the candidate region were grouped with the homozygotes susceptible, given the recessive nature of the resistance to WMV derived from this source. Plants homozygote for the allele of the resistant parent either remained asymptomatic or showed only slight symptoms at 30 dpt, while mean symptoms in plants classified as susceptible were significantly higher (Figures 3 and 4). At 46 dpt, symptoms increased in plants of both groups, but in the resistant plants were still significantly lower (Figure 4). This delay in symptom manifestation has relevant agronomic implications, as it may contribute to preserving fruit quality during critical stages of crop development (Díaz-Pendón et al., 2005; Pérez-de-Castro et al., 2019; López-Martín et al., 2024). There was a positive moderate significant correlation between the genotype and viral accumulation at 30 dpt (0.48), with lower viral accumulation in the homozygotes for the allele of the resistant parent. At 46 dpt, the correlation was not significant, probably due to higher variability in viral accumulation at later dates. Virus accumulation in asymptomatic plants has been described in lines with TGR-1551-derived resistance (López-Martín et al., 2024b). Thus, although the defensive response effectively limits replication of the virus in early stages, the virus accumulates at higher rates at later dates, explaining the lack of significant correlation between genotype and viral accumulation.

In conclusion, the effectiveness of the markers previously described as associated with resistances derived from TGR-1551 and PI 414723 for assisted selection in breeding programs for the introgression of these resistances has been confirmed. Additionally, the evaluated materials constitute advanced materials for the development of resistant varieties in different landrace backgrounds belonging to the most appreciated Spanish melon types. Recovery of the fruit quality after the backcrossing program has been confirmed in similar families (López-Martín, 2023). Future works will deal with the pyramidalization of resistances and evaluation of the resistance in lines combining resistance from both sources.

Acknowledgements

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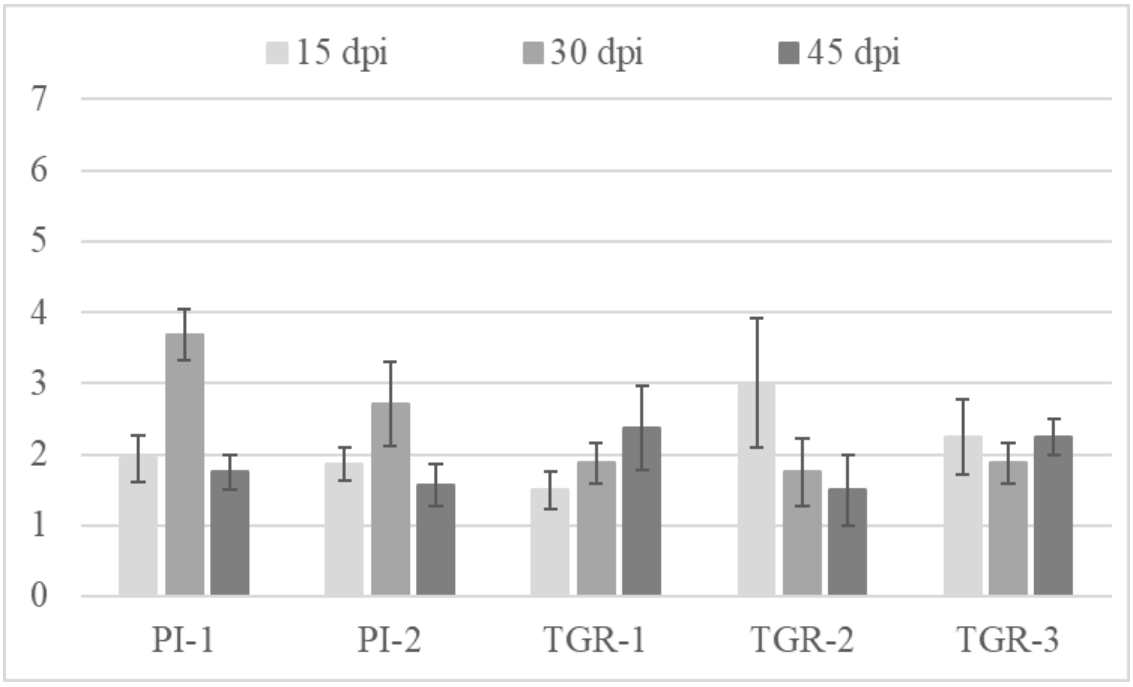

Figure 1. Mean powdery mildew (*Podosphaera xanthii*) symptom scores (scale from 1 to 7 - see text for description) after inoculation in families fixed for resistances derived from PI 414723 (PI-1 and PI-2) and TGR- 1551 (TGR-1, TGR-2, and TGR-3), at different days post-inoculation (dpi). Four to 8 plants per family were evaluated. Vertical bars represent ± the standard errors of the means.

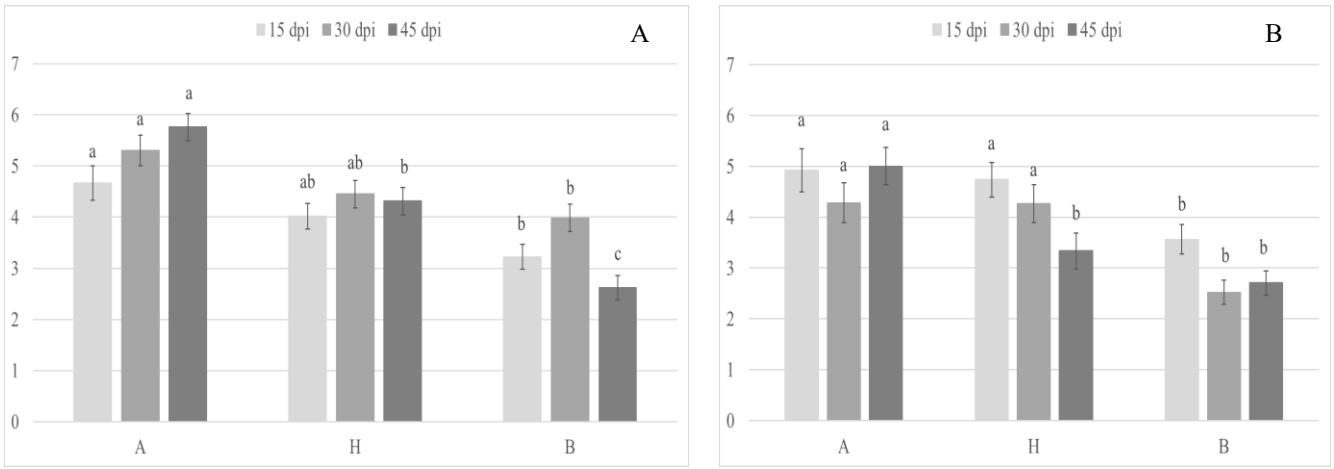

Figure 2. Mean powdery mildew (*Podosphaera xanthii*) symptom scores (scale from 1 to 7 - see text for description) after inoculation in families segregating for resistances derived from PI 414723 (A) and TGR-1551 (B) at different days post-inoculation (dpi). The genotype for the region associated with reaction to powdery mildew is indicated (A: homozygous for the susceptible parent allele; H: heterozygous; B: homozygous for the resistant parent allele). Approximately 30 plants per genotype were evaluated. Vertical bars represent ± the standard errors of the means. Different letters for the same evaluation date indicate statistically significant differences (LSD, $p<0.05$).

Figure 3. Resistant (R) and susceptible (S) plants in the field, 30 days post-transplant (dpt) (upper row) and 45 dpt (lower row), derived from PI 414723 (left panel) and TGR-1551 (right panel).

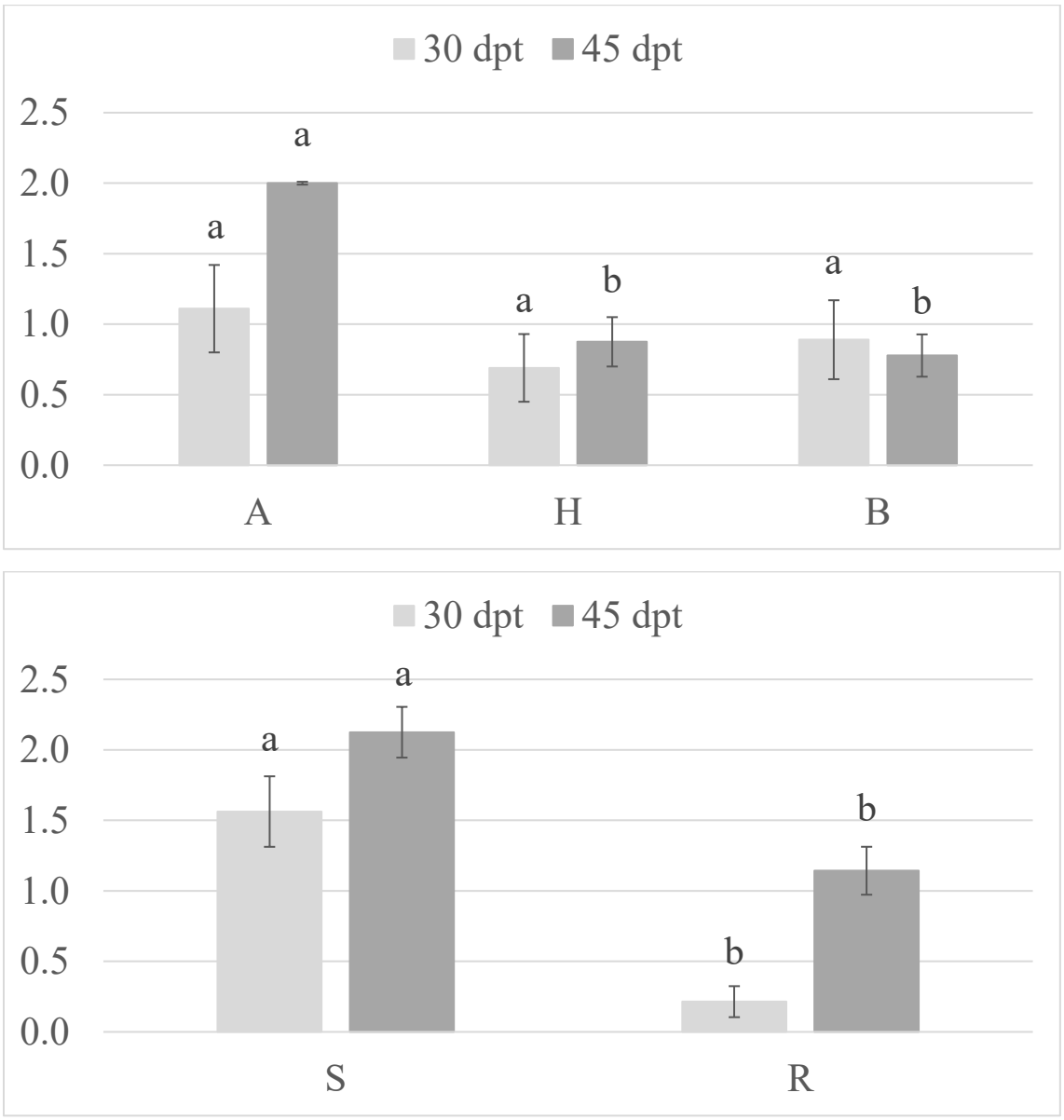

Figure 4. Average score of virus-like symptoms (scale from 0 to 3 - see text for description) after natural infection in field conditions in families segregating for resistance to viral diseases derived from PI 414723 (upper graph) and TGR-1551 (lower graph) at different days post-transplant dates (dpt). The genotype for the region associated with resistance to watermelon mosaic virus (WMV) is indicated (A: homozygous for the susceptible parent allele; H: heterozygous; B: homozygous for the resistant parent allele; S: homozygous for the susceptible parent allele or heterozygous; R: homozygous for the resistant parent allele). Approximately 30 plants per genotype were evaluated. Vertical bars represent ± the standard errors of the means. Different letters for the same evaluation date indicate statistically significant differences (LSD, $p<0.05$).