A new challenge in melon resistance breeding: the ToLCNDV case

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Abstract Tomato leaf curl New Dehli virus (ToLCNDV) is a whitefly transmitted plant virus that is affecting European melon cultivation for over a decade. Since its first introduction in the Mediterranean basin the virus has been associated with significant economic losses including lower yields and cracked non-marketable fruits in Spain and other key cucurbits production areas. Since there is no chemical application against viral pathogens the focus is geared towards resistance breeding. Various QTLs associated with ToLCNDV resistance have been reported over the recent years in melon and other cucurbits. In the current review we summarize the latest advances in melon breeding for ToLCNDV resistance and present all relevant loci known so far in cucurbits. As a way forward in the future we propose an alternative to traditional resistance gene introgression breeding by exploiting the knowledge on genes that confer susceptibility to the virus in melon and other cucurbits.

Keywords Cucumis melo · Melon · Tomato leaf curl New Dehli virus · Resistance · Breeding

Introduction

Melon (Cucumis melo L.), also known as muskmelon, is an important dicotyledonous annual fruit crop (Jeffrey 1980; Pitrat 2008, 2016). It belongs to the Cucurbitaceae family, which also includes many economic important crops, such as cucumber (Cucumis sativus), watermelon (Citrus lanatus), zucchini (Cucurbita pepo), pumpkin (Cucurbita moschata) and bitter melon (Momordica charantia). The global melon production was 28.5 million tons in 2020 (www.fao.org/faostat), with China being the leading producer (13.8 million tons), followed by Turkey (1.7 million tons), India (1.3 million tons) and Kazakhstan (1.2 million tons). Melon cultivation is favored by high temperatures ideally between 21 °C and 35 °C under long days and high light intensities, conditions which lead to high sugar accumulation (Pitrat 2008). It is cultivated for the consumption of fruits that are harvested either immature (not sweet) or most often mature (high sugar content and thus sweet) (Pitrat 2008, 2016).

Compared with wild species, cultivated melons have increased their sizes of fruits with thicker flesh, larger leaves and seeds, and loss of bitterness during the domestication (Pitrat 2016; Zhao et al. 2019). Although East Africa was regarded as the place of
origin, recent data suggests that melon and cucumber may originate from India and Australia (Sebastian et al. 2010; Garcia-Mas et al. 2012; Chomicki et al. 2020). Cultivated melon grown today can be traced back to two wild lineages and is believed to have been first domesticated in Asia as early as 4 thousand years ago. A second area of domestication is in Africa (Chomicki et al. 2020). The Asian lineage (C. melo subsp. melo) gave rise to all the commercially important cultivars and their market types including the most widely cultivated and consumed ‘Galia’, ‘Cantaloupe’, and ‘Honeydew’ melons (Chomicki et al. 2020), while the wild African lineage (C. melo subsp. meloides) gave rise to the ‘Tibish’ and ‘Fadasi’ melons, landraces grown in the Sudanian region (Endl et al. 2018).

Melon’s genome sequence of a doubled-haploid line (DHL92), derived from the cross between PI161375 (kachri type) and Piel de sapo T111 (inodorus type), was obtained, assembled and released in 2012. This assembled genome sequence is 375-Mb in size with 27,427 predicted protein-coding genes (Garcia-Mas et al. 2012). The number of predicted disease resistance genes has been estimated as 396 or 459 depending on different studies (Garcia-Mas et al. 2012; Qin et al. 2021), and 81 or 60 of them encode the nucleotide-binding site and leucine-rich repeat (NLR) type of proteins, while, the rest codes for receptor-like kinases (205 or 359) or receptor-like proteins (110 or 40) (Table 1). In cucumber (C. sativus var. sativus) and its wild species (C. hystrix), 57 and 77 annotated NLR genes have been identified, respectively (Table 1) (Qin et al. 2021). In watermelon (C. lanatus subsp. vulgaris) and its semi-wild and wild species (subsp. Mucosospermus and subsp. lanatus), 44 and 6 (semi-wild and wild species) annotated NLR genes have been identified (Table 1) (Guo et al. 2013). The numbers of NLR genes identified in cucurbit crops are relatively low when compared with Arabidopsis (149), tomato (260), grape (302), poplar (398), and rice (600) (Andolfo et al. 2014; Casacuberta et al. 2016; Tuskan et al. 2006; Wang et al. 2008; Meyers et al. 2003).

The published melon reference genome assembly has still 40-Mb of unassigned sequences primarily due to the high percentage of repetitive sequences and 50-Mb of sequencing gaps. Different approaches have been undertaken to improve the melon genome assembly and annotation in recent years (Castanera et al. 2020; Ruggieri et al. 2018). By incorporating the available melon RNA-Seq data collections, a new annotation has been released consisting of 29,980 protein-coding genes roughly 1,500 more than the original annotation.

Apart from melon, the genomes of other important cultivated cucurbits have become available over the last 20 years. The first breakthrough in cucurbits research occurred in 2009 with the sequencing of cucumber genome Chinese long inbred line 9930 (Huang et al. 2009). Due to its high agricultural and research interest newer versions of the cucumber genome came out in 2012 (Yang et al. 2012) namely of Gy14, an inbred line with excellent horticultural traits in North America. A more recent 2019 Chinese Long version followed (Li et al. 2019). The first genome of watermelon occurring from 20 watermelon

| Resistance gene | Arabidopsis | Cucumber | Watermelon | Melon |
|-----------------|-------------|-----------|------------|-------|
|                 | Cucumis sativus | Cucumis hystrix | Citrullus lanatus | Cucumis melo |
| NBS-LRR         | 149         | 57        | 44         | 60−81 |
| RLP             | 57          | 49        | 35         | 40−110|
| RLK             | 239         | 436       | 162        | 205−359|

*aNBS-LRR, nucleotide-binding site-leucine-rich repeat; RLK, receptor-like kinase; RLP, receptor-like protein

*bNumber of resistance genes in Arabidopsis (Wang et al. 2008; Meyers et al. 2003)

cCucumber and wild species, Cucumis sativus and C. hystrix (Huang et al. 2009; Qin et al. 2021*; Wan et al. 2013);

dWatermelon, semiwild, and wild species Citrullus lanatus subsp. vulgaris, subsp. mucosospermus, and subsp. lanatus (Guo et al. 2013);

eNumbers of resistance genes in melon, Cucumis melo are taken from Qin et al. (2021)

*Numbers are taken from Garcia-Mas et al. (2012)
resequencing data became publicly available in 2013 (Guo et al. 2013). The genome of Charleston Gray watermelon, which is a popular American cultivar resistant to *Fusarium* and anthracnose disease, was published in 2019. Finally, *C. moschata* and *Cucurbia maxima* genomes became available in 2017 (Sun et al. 2017), followed by *C. pepo* genome in 2018 (Montero-Pau et al. 2018). Due to the collinearity of the aforementioned genomes mapping of resistance in one cucurbit crop could be very fruitful for another one.

Melon, cucumber, and watermelon which are major crop species in the *Cucurbitaceae* family, but distant from *Cucurbita* species, have different chromosome numbers (melon, $2n=2x=24$; cucumber, $2n=2x=14$; watermelon, $2n=2x=22$). The current assembly of cucumber and watermelon genomes span 243.5 Mb containing 26,682 predicted genes and 353.5 Mb containing 23,400 predicted protein-coding genes, respectively (Guo et al. 2013; Huang et al. 2009). These genome sequences offer many opportunities to understand the genome structure/size and cucurbit genome evolution (Garcia-Mas et al. 2012; Guo et al. 2013). There is high synteny among these genomes (Huang et al. 2009; Li et al. 2011). In addition to several intra- and inter-chromosomal rearrangements, there are likely ancestral fusions and fissions of melon chromosome pairs in cucumber and watermelon (Garcia-Mas et al. 2012; Guo et al. 2013). The increased size of the melon/watermelon genome compared with cucumber is suggested to be partly caused by the accumulation of transposable elements (Garcia-Mas et al. 2012; Guo et al. 2013). Regarding the more distant cucurbits, *C. maxima* and *C. moschata* and *C. pepo* have genome sizes of 271, 270 and 263 Mb respectively. Interestingly, the genomes of *C. pepo*, *C. maxima* and *C. moschata* are the result of a genome duplication that occurred 30–40 million years ago which is not observed in the sequenced genera of *Cucumis* and *Citrullus* (Montero-Pau et al., 2018).

Within melon, genetic variability at the whole-genome level has been analyzed regarding single nucleotide polymorphisms (SNPs), structural variation (SV) and transposon insertion polymorphisms (Demirci et al. 2020; Sanseverino et al. 2015). This variability was found to be greatly reduced among elite varieties, likely due to selection during breeding. In the elite cultivars, some chromosomal regions particularly associated with agronomic traits such as fruit ripening and stress response showing a high differentiation indicates these are the candidate regions under strong selection (Demirci et al. 2020; Sanseverino et al. 2015).

Because cultivated melon is highly susceptible to various diseases, knowing the progenitors and (closest) wild relatives of melon is important for the introgression of resistance genes. Furthermore, through genome-wide association studies (GWAS), genes or quantitative trait loci (QTLs) that underlie complex traits related to domestication such as fruit mass and quality have been uncovered which provides valuable resources for melon breeding (Zhao et al. 2019).

**Tomato leaf curl New Dehli virus**

Melon is affected by many important diseases caused not only by fungi and oomycetes but also by viruses. ToLCNDV is a relatively newly emerging virus in the European continent threatening cucurbit crops (Fortes et al., 2016). The virus is prevalent in India where it infects mainly solanaceous species and causes significant economic losses in tomato cultivation (Moriones et al. 2017). The virus however, has been expanding its genetic diversity, infecting new hosts and occurring in new territories. In 2012, it was first detected in the Mediterranean basin affecting zucchini crops in Spain (Juarez et al. 2014), and since then ToLCNDV is a major problem for open field and greenhouse grown cucurbits. Sequence analysis of the strain that was spreading in the Mediterranean territory showed a new isolate named ToLCNDV-ES which originated from recombination of ToLCNDV strains. ToLCNDV-ES is better adapted to cucurbits than its Indian relative and it poses a danger for European cucurbit production (Fortes et al. 2016). ToLCNDV is a whitefly transmitted bipartite Geminivirus. It’s highly flexible genomic organization is thought to be a contributing factor to its wide distribution in hosts (43 dicotyledonous species so far) and areas around the world (Pakistan, India, Bangladesh, Iran, Sri Lanka, Malaysia, Taiwan, Thailand, Indonesia, Tunisia, Spain, Italy, Morocco, Algeria, Portugal, Estonia, Greece) (Zaidi et al. 2017; Bragard et al. 2020). The virus belongs to *Geminiviridae* (genus: *Begomovirus*) a family of circular single stranded DNA viruses and has two genome components DNA-A and DNA-B.
DNA-A codes for a replication enhancer protein, a transcriptional activator protein, a coat protein AV1, an AV2 protein involved in viral movement and AC4 protein. DNA-B encodes a movement protein and a nuclear shuttle protein (Zaidi et al. 2017) (Table 2). Finally, the viral components can easily interact with beta satellites often in mixed infections enhancing the viral pathogenesis and virulence (Sivalingam et al. 2012).

Disease cycle and symptoms

ToLCNDV, like other begomoviruses is transmitted through viruliferous whiteflies in the field/greenhouse which feed on the host plant. The whitefly acquires virus particles from the host plant’s phloem upon herbivory through its stylet. After 10–60 min of herbivory the whitefly becomes viruliferous and after ingestion there is a latency period for the virus to be transmissible to other plants. This latency period varies between begomoviruses between 8 and 19 h (Rosen et al. 2015). The virus circulates in the insect moving from the midgut to the epithelial cells and hemolymph to the saliva. From the saliva during herbivory it can be passed on to a new healthy plant. Besides vectors, ToLCNDV has been reported to be transmitted mechanically (Lopez et al. 2015) in melon and through seeds in zucchini (Kil et al. 2020) and chayote (Saneeetha et al. 2018) plants.

The symptoms of ToLCNDV in several hosts include curling of the leaves, vein thickening, darkening of leaf margins, reduction of leaf area, short internodes and severely stunted plants (Zaidi et al. 2017). In melon symptoms have been reported 9 days post inoculation with whiteflies (Ruiz et al. 2017). Melon exhibits severe symptoms including curled leaves with yellow mosaic and most importantly, cracking of the fruits (Fig. 1) (Sáez et al. 2017).

Research on the identification of ToLCNDV resistant cucurbit cultivars is limited. Therefore, measures of integrated management that would require a knowledge of the ecology and the variability of the virus are crucial. In 2016, four years after the first detection of the virus in the Mediterranean basin Fortes and coworkers conducted a genetic diversity and phylogenetic analysis study of ToLCNDV that helped to distinguish the Mediterranean strain from the Asian/Indian (Fortes et al. 2016). The study

| Genome component | ORF/Encoded protein | Function |
|------------------|---------------------|----------|
| DNA-A            | AC1/Replication-associated protein (Rep) | Initiates replication of the viral genome by binding to the viral DNA (Fondong et al. 2013) |
|                  | AC2/Transcriptional Activator Protein (TrAP) | Virus pathogenicity, gene activation, suppression of gene silencing, activation of the transcription of the genes coding for coat protein and movement protein, alter host responses including jasmonate signaling (Fondong et al. 2013), counter hypersensitive response (Hussain et al. 2007) |
|                  | AC3/Replication enhancer protein (REN) | Enhances viral DNA accumulation and symptom development in plants. Interacts with Rep. (Fondong et al. 2013) |
|                  | AC4/Viral effector | Interacts with host AGO4 and precludes viral DNA methylation (Fondong et al. 2013) |
|                  | AV1/Coat protein (CP) | Structural protein of ToLCNDV particles, involved in viral movement (Fondong et al. 2013) |
|                  | AV2/Pre-coat protein | Pre-coat protein. Involved in viral movement. Main pathogenicity factor involved in suppressing plant antiviral RNAi mechanisms (Basu et al., 2018). Mutations in this protein result in low ToLCNDV accumulation (Fondong et al. 2013) |
| DNA-B            | BV1/Nuclear shuttle protein (NSP) | Directs viral DNA to the nucleus, involved into cell to cell movement, pathogenicity determinant (Fondong et al., 2013; Hussain et al. 2007) |
|                  | BV2/Movement protein (MP) | Required for virus cell-to-cell and long-distance movement, interacts with the NSP in bipartite begomoviruses. (Fondong et al. 2013) |
showed a uniform viral population throughout Spain that could be explained by the population bottleneck of moving of the virus to a new area. Furthermore, the similarity of four regions of DNA-A between the Spanish and the Indian isolates were low as well as most of the regions of DNA-B which showed less than 90% similarity. Begomoviruses are very prone to recombination and pseudo-recombination events that can alter their pathogenicity and virulence significantly (Juarez et al. 2019). The mixed infections where two viruses share the same niche can buffer these events. ToLCNDV-ES is hypothesized to be the result of such a recombination event (Fortes et al. 2016). Juarez et al. conducted in 2019 a similar genetic diversity study and confirmed that the ToLCNDV population in Spain was genetically very homogeneous and clearly different from the Indian ones. Panno et al. in the same year also found a low genetic diversity of ToLCNDV in Italy among the Italian isolates and high similarity of these isolates to the ones from Spain, Morocco and Tunisia. This further suggests that the common origin is indeed ToLCNDV-ES (Panno et al. 2019). These data show that the virus is currently still at an introductory phase in the European continent and why its genetic variability is still low in Europe.

Geminiviruses can exploit the host mechanisms in order to promote their proliferation and virulence. Viruses can (i) alter host gene expression, (ii) interfere with hormonal signaling, (iii) change host protein degradation pathways and (iv) interfere with cellular metabolism, and (v) counter antiviral gene silencing (Ramesh et al. 2017) Antiviral gene silencing is an important plant defense mechanism against viruses since plants lack the ability to produce antibodies. Upon viral infection double stranded RNA (dsRNA) of the virus is cleaved into 21–22 nt small interfering RNAs (siRNAs) or 19–24 nt microRNAs (miRNAs). During post transcriptional gene silencing (PTGS) these siRNAs form a complex with Argonaute proteins and cleave cognate mRNAs in the cytoplasm. During transcriptional gene silencing 24nt siRNAs are interacting withAGO or methyltransferases in order to induce viral DNA methylation in the nucleus (Schwab et al. 2006). ToLCNDV has also been shown to possess mechanisms for evading antiviral defenses. For example AC2 protein of the virus has been shown to act as silencing suppressor on the host miRNA machinery. This is happening by directly interacting with the plant’s Argonaute protein 1 (AGO1) which can cleave viral mRNAs during antiviral defense, inhibiting AGO1 action (Kumar et al. 2016). Apart from PTGS, ToLCNDV can suppress also TGS by interaction of its AC4 protein with AGO4 that is involved in DNA methylation (Vimutha et al. 2018). Finally, ToLCNDV besides suppressing TGS and PTGS, the main plant antiviral mechanism, should also be competent for mediating in plant physiology in various ways mentioned above similar to other Geminiviruses.

Yield loss and control methods

Open field melon cultivation in central Spain has been reported to reach losses up to 20% due to the virus (Saez et al. 2017). The cracking of the melon fruits is an important factor since it makes them non-marketable, moreover apart of the cracks, fruits don’t reach marketable size and internal quality is very poor as they don’t ripe properly. The European
Plant Protection Organization has placed the virus in its alert list in order to prevent the spreading in the rest of Europe [https://www.eppo.int/]. The main way that the virus is spreading in nature is through whiteflies (Bemisia tabaci). Therefore, limiting the populations of these insects is crucial for controlling the virus. Isolated nurseries for growing plants such as greenhouses and screenhouses that are insect free and insecticide applications could limit the contact of the plants with potential viruliferous whiteflies. Removing plants that are infected with the virus quickly or to remove plant remainders and other plant hosts around the area that whiteflies can use as shelters could also be helpful to reduce epidemics. An integrated pest management strategy suggested monitoring of insect populations and planning of the cultivation periods outside of flight activity periods of B. tabaci (Bragard et al. 2020). Eventually, large efforts are carried out by the plant breeding industry for introducing tolerant or resistant cucurbit varieties that could be widely adopted to limit the economic loss due to ToLCNDV in Europe.

**Host resistance to ToLCNDV**

Genetic resources of resistance

Germplasm screenings have resulted in the identification of resistant cucurbits sources against ToLCNDV. In total, 36 resistant/tolerant accessions were identified in C. melo groups corresponding to C. melo (26 accessions, of which taxonomy groups were not shown in the corresponding reports), C. melo var. acidulus (2), C. melo var. kachri (2), C. melo var. ibericus (1), C. melo var. momordica (4), and C. melo var. tibish (1) (Table 3) (López et al. 2015; Romay et al. 2019; Rosa et al. 2018; Sáez et al. 2017). Resistant accessions belonging to C. melo var. momordica (i.e., Kharbuja, PI 124,112, and PI1417223), C. melo var. kachri (i.e., WM9 and WM7), and C. melo (i.e., IC-274014, PI 282,448, AM 87 and PI 179,901) were asymptomatic or exhibited mild symptoms. Virus accumulation was either not detectable or diagnosed at a low amount (López et al. 2015; Romay et al. 2019; Sáez et al. 2017). Accession Kharbuja exhibited no or mild viral symptoms, but contained high virus accumulation which makes it a tolerant source to ToLCNDV (López et al. 2015; Sáez et al., 2017). Accessions PI 164,723 and PI 313,970 showed a recovery phenotype after infection and it was confirmed by Polymerase Chain Reaction (PCR) analysis that the virus content gradually decreased (Romay et al., 2019). In addition, we summarized susceptible accessions to avoid redundant screening efforts. The list includes 90 C. melo accessions (76 of them were susceptible to the isolate ND2014-1 V, the other 14 to ES13-35) and C. melo var. cantalupensis (3 to ES13-35) (Table 3) (Supplementary Table 1) (Romay et al. 2019; Rosa et al. 2018).

**Genetics of ToLCNDV resistance**

To support breeding programs, decent knowledge on the genetic basis of the resistance to ToLCNDV in a few promising melon accessions has been

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### Table 3: Summary of previously identified sources of *Cucumis melo* groups species resistant to tomato leaf curl New Delhi virus

| Isolate       | Botanical group         | Resistance accessions                                                                 | References                             |
|---------------|-------------------------|--------------------------------------------------------------------------------------|----------------------------------------|
| ND2014-1 V    | *C. melo*               | LM47, LM96, LM78, LM77, LM8, LM66, LM98, LM89, LM93, LM60, LM95, LM62, LM52, LM57, LM55, LM75, LM70, LM73, LM91, LM90 | Rosa et al. (2018)                      |
| Spanish isolate | *C. melo var. momordica* | Kharbuja, PI 124,112, PI 414,723                                                    | López et al. (2015); Sáez et al. 2017  |
|               | *C. melo var. kachri*    | WM9, WM7                                                                           |                                        |
|               | *C. melo var. ibericus*  | Pinonet Piel de sapo                                                                 |                                        |
| ES13-35       | *C. melo*               | AM 87, IC-274014, PI 282,448, PI 179,901, HSD 2445–005, HSD 2458 B, PI 414,723, MR-1, PI 124,112 | Romay et al. (2019)                     |
|               | *C. melo var. momordica* |                                                                                     |                                        |
|               | *C. melo var. tibish*    | Tibish Kordofan                                                                    |                                        |
|               | *C. melo var. kachri*    | WM9, WM7                                                                           |                                        |
|               | *C. melo var. acidulus*  | PI 313,970 (90,625), PI 164,723                                                    |                                        |
established through quantitative trait loci (QTL) mapping approaches (Romay et al. 2019; Sáez et al. 2017). Segregating populations (F2 and BC1) were developed by crossing parental lines WM 7 (resistant) and C. melo cultivar C. melo subsp. melo Piñonet Piel de sapo (PS) (susceptible) (Sáez et al. 2017). Mapping analysis resulted in the identification of one major dominant locus on chromosome 11 (three overlapping QTLs, ToLCNDV Sy15_11, ToLCNDV Sy30_11, and ToLCNDV VT30_11), and two minor QTLs on chromosomes 2 (ToLCNDV VT30_2) and 12 (ToLCNDV Sy15_12, ToLCNDV Sy30_12, ToLCNDV VT30_12) (Fig. 2). The SNP marker D16 which is closely linked to the resistance gene on chromosome 11 can be used in marker-assisted breeding for ToLCNDV resistance in melon (Sáez et al. 2017) (Fig. 2). Using the same resistant and susceptible lines, two candidate genes CmARP4 and CmNAC were identified and their transcript amount was differentially higher in the inoculated susceptible genotype (Piel de Sapo) when compared to the inoculated resistant line WM 7 (Román et al. 2019). Overexpression of NAC domain protein in tomato (SlNAC1) resulted in substantial increase in TYLCV accumulation, and CmNAC, which play a function similar to SlNAC1, located on chromosome 7 (Selth et al. 2005) (Fig. 2). The co-location of CmARP4 with the minor QTL on chromosome 2 indicates that the accumulation of transcripts of this gene might be involved in ToLCNDV accumulation and degree of symptoms development (Fig. 2).

Accessions IC 274,014 possessed the highest resistance level because all the tested individuals were asymptomatic with no detectable virus accumulation (Romay et al. 2019). ToLCNDV resistance is explained by one recessive locus begomovirus resistance-1 (bgm-1) and two dominant genes Begomovirus resistance-2 (Bgm-2), and ToLCNDV, according to phenotypic results of F2 population (Romay et al. 2019).

Recently, four patents were issued on ToLCNDV resistant fragments (five QTLs) in C. melo accessions NCIMB 42,585 (QTL-5), NCIMB 42,506 (QTL-11), NCIMB 42,705 (QTL-1) and NCIMB 42,625 (QTL11 & QTL12), respectively. The resistance QTL-5 on chromosome 5 is flanked by markers KASP06 and KASP01 and it was successfully introgressed into commercial susceptible cultivars (Nunhems B.V. Patent: US20190225983A1) (Fig. 2). The QTL-11 is located on chromosome 11 between markers melon_sbg_617_42 and melon_sbg_16835_17, and has been implemented in breeding programs as well (Vilmorin

![Fig. 2](image-url)
SA Patent: US20200178485A1) (Fig. 2). Moreover, QTL-1 is identified on chromosome 11 and flanked between makers SED ID No.1 and SED ID No.9 (Rijk Zwaan Patent: WO2018219861A1) (Fig. 2). The ToLCNDV resistant plants have an introgression fragment on chromosome 11 comprises QTL11 acting in a recessive manner and/or on chromosome 12 contains QTL12 presenting in a partially dominant manner (Nunhems B.V. Patent: US2020040355) (Fig. 2).

Understanding the genetic control of ToLCNDV resistance in relative species could be helpful for resistance gene identification in melon. An extensive Cucurbita spp. collection was screened in 2016 for ToLCNDV in Spain including C. pepo, C. moschata and C. maxima and wild species using mechanical and whitely inoculation. From all these species examined only C. moschata accessions were found resistant to the virus (Saez et al. 2016). QTL analysis of two resistant Cucurbita moschata accessions coming from this screening namely PI 604,506 and PI 381,814 yielded the same monogenic recessive factor on chromosome 8, controlling both symptom expression and virus accumulation (Sáez et al. 2020). The chromosome 8 candidate region of C. moschata is syntenic to the region in chromosome 11 of melon, previously described as responsible for ToLCNDV resistance with defined QTLs (Fig. 2) (Vilmorin SA Patent: US20200178485A1; Sáez et al. 2017). Transcriptomic analysis that was performed in the WM-7 melon which carried the chromosome 11 QTL indicated genes that were differentially expressed after ToLCNDV infection and thus might play a role in ToLCNDV susceptibility (Saez et al. 2021). These top candidate genes are involved in important functions for antiviral defense such as jasmonic acid signaling pathways, photosynthesis, RNA silencing, sugar transport (Saez et al. 2021).

Among melon and C. moschata resistance against ToLCNDV has also been reported in cucumber. Three cucumber accessions originating from India CGN23089, CGN23423 and CGN23633 were found highly resistant to the virus after mechanical inoculation. After examining the inheritance of the resistance found in CGN23089 by crossings with susceptible accessions the authors concluded in a recessive monogenic resistance on chromosome 2. The resistance includes a significant sharp reduction in viral titres and asymptomatic or mild symptomatic plants (Sáez et al. 2020). Finally, more recent research in Cucurbita spp. revealed a single dominant gene for ToLCNDV resistance in the C. moschata accession BSUAL-252 that originates from Japan after screening with the virus (Masegosa et al. 2020).

Susceptibility gene impairment as a new way to control diseases

Breeding for dominant versus recessive resistance

Traditionally, resistance has been introgressed into varieties by using resistance genes (R-genes). The procedure initiates with interspecific hybridization between the cultivated variety and wild relatives progressing with successive backcrossing generations of the progeny with the elite line. This method has been widely adopted in plant breeding and used to confer resistance to various plant pathogens despite the drawbacks that are associated with it such as the limited durability of the resistance in many cases. Multiple backcrossing generations accompanied with the respective genotyping/phenotyping screenings for each generation delay significantly the final product which is often falling behind to the evolutionary arms race with the ever-evolving pathogens. As a consequence, the newly introduced resistance lasts only for a short while on the field before it is overcome by new pathogen races with the need of constantly introducing new resistance genes (Sun et al. 2014). Nevertheless, there are examples of durable R-gene resistance and also against viruses such as the Tm-22 which is used for many decades in tomato against Tobacco Mosaic Virus (Lanfermeijer et al. 2005). A few R-genes have been identified for dominant resistance against TYLCV in tomato such as the Ty-1/Ty-3 loci that code for RNA-dependent RNA polymerases nad Ty-2 which codes for a NLR protein as well as Ty-6 and Ty-4 loci (Caro et al. 2015; Shen et al. 2020; Gill et al. 2019). Similarly several QTLs for ToLCNDV resistance described above could harbour R-genes that should be identified in order to be exploited by plant breeding.

An alternative approach for obtaining resistance is deployment of impaired susceptibility genes (S-genes) in the host (Pavan et al. 2010), also known as recessive resistance breeding. S-genes are hijacked by the pathogen virulence factors upon several stages
of infection in order to promote its establishment and sustenance in the host plant. Van Schie and Takken (2014) distinguished three different temporal stages on which susceptibility genes act contributing to the successful disease establishment: (a) early infection process, (b) regulation of host defenses and (c) later pathogen sustenance.

S-genes that are exploited by viruses have also been identified with one of the most known categories of them being the translation initiation factors for Potyvirus susceptibility. These proteins are binding to the 5’ end cap of mRNAs and play an important role in the initiation of their translation. Translation initiation factors interact with the genome linked viral protein of the virus (VPg) which is located to the 5’ end of viral RNA and is crucial for viral infection (Wang et al. 2012). For Begomoviruses, such as TYLCV, the recessive locus ty-5 is one of the best known recessive loci for resistance. The locus was generated in Solanum peruvianum by a natural mutation in PELOTA (Lapidot et al. 2015). PELOTA contributes to Geminivirus susceptibility also in pepper apart from tomato. A loss of function protein that was identified in pepper random mutagenesis population was found to be resistant to other Geminiviruses such as Pepper Yellow Leaf Curl Indonesia Virus and Pepper Leaf Curl Virus (KEYGENE N.V. Patent: WO2019122374A1) (Koeda et al. 2021). As far as ToLCNDV is concerned there is no research currently specifically identifying S-genes but only speculation regarding candidate genes (Roman et al. 2019; Saez et al. 2017). For example, the aforementioned identified recessive ToLCNDV resistance QTL in chromosome 11 of melon as well as the respective recessive QTL in chromosome 2 of cucumber should both harbour impaired susceptibility genes. More research is needed e.g fine mapping to identify those genes.

Recessive resistance by disrupting S-genes

Natural or artificial disruption of the genes mentioned above can confer durable and broad spectrum resistance in melon. A characteristic example of the durability of this resistance is the powdery mildew resistance in barley, based on the disrupted mlo-gene, which is used for over 70 years (Sun et al. 2014). Artificial disruption of S-genes in plant breeding industry has been mainly focused on Random Mutagenesis and TILLING (Targeting Induced Local Lesion IN Genomes). Random mutagenesis includes the exposure of plants under x-rays, gamma-rays or chemicals like ethyl-methane sulfonate (EMS) in order to induce random mutations in their genome (Tadele et al. 2016). Detection of the random mutations follows with TILLING which is a DNA-screening technique that identifies point mutations occurring in a specific gene. The method is based on the formation of heteroduplexes between alleles during PCR and subsequent recognition and cleavage of their mismatch point by nucleases (Tadele et al. 2016). TILLING is a quite popular reverse genetics technique as it can be applied to a large array of plant species with genome size or ploidy not being limiting factors (Kurowska et al. 2011). However random mutagenesis can have various drawbacks. Detection of the desired mutants in an EMS population is a laborious and time consuming procedure. Furthermore, since the mutations are happening randomly the method can result into many unwanted off target mutations that can affect other crop traits (fitness, yield etc.) (Liu et al. 2017).

Clustered regularly interspaced short palindromic repeats/CAS (CRISPR/CAS) is one of the latest advances on genome editing technology which allows disruption of S-alleles (Zaidi et al. 2017). The method is based on the procaryotic immunity that can degrade invading DNA whether is originating from attacking bacteriophages or plasmids. CRISPR/Cas utilizes two components to cleave target DNA, single guide RNAs (sgRNAs) and Cas enzyme which is a DNA endonuclease coming from bacteria. SgRNA is a ~ 100nt synthetic RNA which on its 5’ has a 20nt sequence that is complementary to the target genomic DNA sequence and adjacent to this gRNA target site, should be a PAM site (protospacer adjacent motif) of a few nt to allow the DNA double stranded breaking activity by the Cas enzyme. The sgRNA forms a loop that binds to the Cas enzyme and creates a complex in order to guide the Cas enzyme to the cleavage site. Cas has then the ability to induce double stranded breaks (DSB). After the DSB the cell initiates the repair of the DNA through non-homologous end joining (NHEJ) or homology directed repair (HDR). NHEJ which is the most common repair mechanism can result in small insertions, deletions and single nucleotide polymorphisms (SNPs) that can lead to gene knock-outs (Liu et al. 2017).
The CRISPR/Cas complex is usually introduced stably into the plant using a plasmid through Agrobacterium mediated transformation which can later be crossed out in order obtain non-transgenic plants. Also methods for transient introduction of the CRISPR-Cas complex exist. The main advantage of the method compared to TILLING is the prevention of off targets mutations that are occurring through random mutagenesis. In this way we can add important agronomic traits to cultivar while at the same time we secure its existing genetic background.

CRISPR/Cas9 has already been applied in cucumber, melon and watermelon (Chandrasekaran et al. 2016; Hoogvorst et al. 2019; Tian et al. 2017). However, research is still limited due to the fairly recent introduction of CRISPR/Cas9 and to the fact that cucurbits are recalcitrant plants for agrobacterium mediated transformation needed for CRISPR/CAS9 editing. In cucumber Chandrasekaran et al. in 2016 edited eIF4E (eukaryotic translation initiation factor 4E) gene which was a known S-gene (Nicaise et al. 2003; Julio et al. 2015) for potyviruses in other plant species and conferred resistance to Zucchini yellow mosaic virus, Papaya ring spot mosaic virus-W, and the Ipomovirus Cucumber vein yellowing virus. In the same way Pechar et al. in 2021 edited the melon eIF4E in order to achieve resistance to potyviruses. Very recently, another group (Wan et al. 2020) using Crispr/Cas9 edited Clpsk1 previously known to attenuate immunity against Botrytis cinerea and Hyaloperonospora arabidopsidis in arabidopsis and achieved Fusarium oxysporum f.sp. niveum resistance in watermelon.

CRISPR/Cas9 wide application in plants for disruption of S-genes and disease resistance begun the previous decade. CRISPR/Cas in melon is still in an initial phase probably due to the difficulty in Agrobacterium mediated transformation. Many already proven S-genes for resistance have been published in the literature for various plants species. Knocking out the homologues of those genes in melon and other cucurbits using CRISPR/Cas9 could confer resistance to these pathogens. The role of genes located in recessive resistance QTLs for these diseases could also be elucidated by testing impaired function mutants. Finally, it must be noted that editing susceptibility genes with Crispr/Cas in order to create these mutants can lead to pleiotrophic phenotypes and can very often be lethal as these genes most of the times have key roles in plant physiology.

Conclusion

Here, we have presented a compilation of the potential ToLCNDV resistance in cucurbits based on available literature. Host resistance is generally the most favourable control method when considering environmental, economic, and social reasons. The key to the success of melon breeding aiming at durable and broad-spectrum resistance relies on (1) the development of high-throughput and accurate artificial inoculation method per disease; (2) consistent disease scores implemented to indicate the resistance level for each disease; (3) broad-spectrum resistant cultivars to multiple diseases; (4) taking into account resistance durability through resistance genes pyramiding; (5) in view of resistance-breaking and lack of resistance sources, utilization of impaired susceptibility genes offers a novel alternative strategy for melon resistance breeding.

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Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

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