Advances in resistance breeding in tomato against viral diseases like TBRFV, TLCV, etc

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Abstract

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop cultivated in the tropical and sub-tropical regions of the world. It is rich in vitamins, antioxidants and minerals. Low productivity in India is due to occurrence of both biotic and abiotic stresses. Among the biotic stresses, tomato leaf curl disease, tomato brown rugose fruit virus, tomato spotted wilt virus, tomato mosaic virus and tomato pepino virus have become serious production constraints causing considerable yield loss in the major tomato growing areas of the country. Conventional breeding methods are a laborious process, so advanced breeding techniques can be used for controlling diseases. Some of the advanced breeding techniques are genome editing, gene pyramiding and marker assisted backcross selection. By using these techniques, resistant varieties can be created. Some more research works need to be done to adopt these technologies. Viral diseases and there symptoms, advanced breeding techniques and also some research studies are discussed here.

Key words : Tomato, Viral diseases, Genome editing, Gene pyramiding, Marker Assisted Selection

1. Introduction

Tomato (*Solanum lycopersicum* L.) is self-pollinated crop belongs to family Solanaceae. It is native to Central and South America (Vavilov 1951). It is important vegetable crop grown throughout the world due to its wider adaptation under different climate and soil conditions, higher yield and suitability of various varieties for fresh as well as processed food industries. Tomato is also considered as nutritional crop because it contains vitamin A and C, minerals, sugar (glucose and fructose), organic acids (malic and citric acid) and anti-oxidant pigments such as lycopene (Rana *et al*. 2014).

In India, tomato is cultivated in all agro-climatic regions under an area about 8.63 lakh hectares with a production of 20,621.43 thousand tonnes and the productivity is 23.87 metric ton per hectare. The major tomato producing states of India are Andhra Pradesh, Madhya Pradesh, West Bengal, Tamil Nadu, Bihar, Maharashtra, Andhra Pradesh, Karnataka, Gujarat, Odisha, Uttar Pradesh, Telangana and Haryana.

Plant viruses are among the most significant agricultural pathogens since they cause a considerable portion of crop diseases and are highly challenging to control due to the lack of effective treatments. As demonstrated by large epidemics of tospoviruses (Prins and Goldbach, 1998) and Gemini viruses (Fargette *et al*., 2006) and pepino mosaic virus outbreaks in tomato crops in Europe and North America, viruses are the primary source of the majority of new infectious illnesses of plants. Food quality can be impacted by infectious diseases, which can also lower

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production, although pesticide spraying to eradicate insect vectors can also have an impact. Utilising genetically resistant crop cultivars or types is an alternate viral control technique. These virus-resistant genotypes possess a heritable characteristic or group of traits that prevent virus replication or distribution even in environments that encourage virus infection in the relevant species. Because it can offer efficient protection without adding to the producer's costs throughout the growing season, the adoption of genetic resistance is desirable. Additionally, it is safe for consumers and beneficial to the environment. Many cultivars with varied degrees of virus resistance have commercial status and most crop species have virus resistant characteristics available.

Unfortunately, some important traits, such as resistance to biotic and abiotic factors, which existed in the wild tomatoes, have been compromised during the domestication. As a result, conventional breeding has resulted in improved traits accompanied by loss of fitness and genetic diversity. However, it is a highly time-consuming and laborious task due to backcrosses (Meyer *et al.,* 2013). Technological advancement in genomics improved the genetic engineering of crops in the last two decades. The genetic engineering also referred to as recombinant DNA technology involves the transfer of desired gene from one species to another, thereby broadening the chances for crop improvement (Parmar *et al.*, 2017). The transgenic plants developed using this technology are named as genetically modified organisms (GMOs). However, the regulatory approval of GMOs is a major drawback as the release of GM crops to the public market is costly and often delayed (Bawa *et al.,* 2013).

2. Top 10 Plant viral diseases

Tobacco mosaic virus, tomato spotted wilt virus, tomato yellow leaf curl virus, cucumber mosaic virus, potato virus Y, cauliflower mosaic virus, african cassava mosaic virus, plum pox virus, brome mosaic virus and potato virus X.

2.1 Tomato virus diseases

Tomato leaf curl virus, tomato brown rugose fruit virus, tomato mosaic virus, tomato spotted wilt virus and tomato peppino mosaic virus.

2.1.1 Tomato leaf curl disease

The most prevalent virus in the world is the tomato leaf curl virus disease (ToLCVD) (Moriones and Navas, 2000). Whitefly (*Bemisia tabaci*) serves as the vector for tomato leaf curl virus (ToLCV), which is a member of the genus *Begomovirus* and family Geminiviridae (Mehta *et al*., 1994). All the information necessary for viral DNA replication, transcription, and transmission throughout the plant cell is encoded in the 2.7 kb DNA genome of the virus (Pandey *et al*., 2009). *Begomovirus* species associated with tomato leaf curl disease in India are tomato leaf curl kerala virus, tomato leaf curl ranchi virus, tomato leaf curl patna virus, tomato leaf curl rajasthan virus, tomato leaf curl karnataka virus, tomato leaf curl joydebpur virus, tomato leaf curl bangalore virus, tomato leaf curl new delhi virus, tomato leaf curl palampur virus and tomato leaf curl gujarat virus.

When a plant is infected with the virus, leaves produce leaflets cupped downward and inward in a hook-like shape and become yellow and then become mis-shapen and smaller, showing interveinal and marginal chlorosis and upward curling the leaf border (Zhang *et al*., 2008). It is evident that ToLCV resistance sources are available in some of the wild tomato species. Resistance genes *Ty-1* and *Ty-2* have been identified in *Solanum chilense* (Zamir *et al*., 1994) and *Solanum habrochaites f. glabratum*, whereas *Ty-3* and *Ty-4* have been identified in *Solanum chilense*. A resistance gene *Ty-5* was reported in *Solanum peruvianum* and *Ty-6* has been identified in *Solanum chilense*.

2.1.2 Tomato brown rugose fruit virus

The genus *Tobamovirus* (family: Virgaviridae), which contains the tomato brown rugose fruit virus, is one of the largest genera in its family due to the abundance of viral species. *Tobamoviruses* have an undivided genome, which sets them apart from other members of this family. ToBRFV has the normal *tobamovirus* genus genomic structure. Four open reading frames (ORFs) in a single-stranded positive-sense RNA (+ssRNA) molecule of about 6400 nucleotides each encode the following proteins: the movement protein (MP), which is about 30 kDa and the coat protein, which is about 17.5 kDa and is expressed by the 30 identical sub-genomic RNAs.

Due to their genetic similarity, all ToBRFV isolates that have been discovered in various impacted regions likely have a single common ancestor. This hypothetical situation supports the belief that seed transmission from one nation to another was the primary factor in the spread of this virus. Until now, there have been no research studies that confirm ToBRFV seed-transmission, because this virus appears to be confined only in the seed coat and not in the endosperm or embryo (Oladokun., *et al* 2019). ToBRFV is primarily spread mechanically, although it can also travel great distances on contaminated seeds or fruits, just as other widespread *tobamoviruses*. Through direct contact with infected plants or infected sap from various surfaces (operator, clothing, pots, packaging, consumption of tomatoes from a different crop, transport equipment, working tools, nutrient solutions), propagation materials (grafts and cuttings), bumblebees, and seeds, this new pathogen can be mechanically transmitted within crops (Levitzky *et al*., 2019). After harvesting, ToBRFV inoculum may also be present in a greenhouse's wires, glass, concrete, soil and other surfaces and materials. The symptoms caused by ToBRFV infection consist of tomato leaves interveinal yellowing, deformation and mosaic staining, young leaves deformation and necrosis, sepal necrosis and deformation and young fruits discolouration, deformation, marbling and necrosis.

2.1.3 Tomato mosaic virus

Among these viruses, Tomato Mosaic Virus (ToMV), a rigid rod particle (ssRNA genome) of the genus *Tobamovirus*, is one of the most widespread and destructive viruses in tomato globally**.** The tomato variety infected, the period of infection, the viral isolate involved and the local environmental factors all affect the yield losses brought on by ToMV. ToMV infection can reduce yield by up to 25%, however early infection can result in larger production losses. Common methods of ToMV transmission include mechanical injection, grafting, and most crucially contaminated seeds. Yellowing, mosaic, mottling, deformation, necrosis, fern-leaf and shoestring signs are present in tomato plants that have been infected.

2.1.4 Tomato spotted wilt virus

The tomato spotted wilt virus (TSWV) belongs to the broad family of RNA viruses known as Bunyaviridae and is the type species of the genus *Tospovirus*. Most members of this family infect vertebrate and/or invertebrate hosts. One of the plant viruses that causes the most damage is TSWV. TSWV is one of the plant viruses that has been the subject of the most research because of its commercial significance as well as its biological and molecular characteristics. TSWV virions are spherical, 80–120 nm in diameter, enclosed and covered in surface projections made of the two glycoproteins G1 and G2. In a virion, there are 5 % RNA, 70 % protein, 5 % carbohydrate and 20 % fat.

At least eight different species of thrips are known to circulate and spread TSWV in nature. Recently, *Frankliniella occidentalis* and *Thrips tabaci* vector competence-determining factors were discovered. The sap of naturally infected plants can easily mechanically spread TSWV. Because the distinctive brown local lesion appears quickly, typically within 2-4 days of inoculation, *Petunia hybrida* is one of the most helpful diagnostic species. When manually inoculated, *Nicotiana glutinosa, Nicotiana benthamiana, and Nicotiana tabacum* all exhibit massive necrotic local lesions, systemic mosaic and necrosis, which in the case of *Nicotiana benthamiana* can be fatal. Because it develops chlorotic local lesions on cotyledons 4-5 days after inoculation, *Cucumis sativus* is a dependable test host. Small, orange-colored flecks on some middle or lower leaves or on the calyx are typically the first symptoms. Older leaves become brown, die and droop while new spots emerge. The stems and petioles have a similar pattern of dots or streaks. The entire plant shrinks in size, and the drooping leaves give it the appearance of a plant that has wilted. Yellowish dots up to 10 mm in diameter, typically with prominent concentric zones of hues of brown or yellow alternating with green and later with pink or red, develop on the green fruits.

2.1.5 Tomato pepino virus

Pepino mosaic virus (PepMV) was only discovered in tomato (*Solanum lycopersicum*) crops for the first time in the Netherlands ten years ago and it is now a significant problem of glasshouse tomato crops globally. Pepino (*Solanum muricatum*), which displayed indications of a yellow leaf mosaic, was the plant from which PepMV was first isolated in Peru (1974), as its name suggests. In a survey in central and southern Peru, the virus has been identified in natural infections of the wild tomato species *S. chilense, S. chmielewskii, S. parviflorum and S. peruvianu.* Furthermore, by performing mechanical inoculations, the host range of PepMV has been shown to contain eggplant (*Solanum melongena*), potato (*Solanum tuberosum*) and species from the genera *Nicotiana* (*N. benthamiana*), Datura (*D. stramonium*), Capsicum (*C. annuum*) and Physalis (*P. floridana*). The Potato virus X is the type species of the potexviruses, which include the positive-sense single-stranded RNA virus known as PepMV.

PepMV is mechanically conveyed effectively. In tomato, the virus is extremely contagious and spreads quickly through direct plant-to-plant contact, contaminated tools, hands, and clothing, as well as through routine crop handling techniques in a greenhouse. When PepMV infects tomatoes, a variety of symptoms have been reported, including leaf mosaic, leaf deformities, nettle heads, and stunting. Other than these, fruit discolouration, which is typically seen as marbling or burning and produced by erroneous lycopene distribution, is thought to be the most disastrous effect of PepMV infection since it lowers the fruit's market value. In some tomato cultivation areas up to 90 % of the greenhouse tomato crops are infected with PepMV, leading to up to 40 % production losses. Since no resistant varieties are available and no curative measures exist, prevention of PepMV infection by hygienic measures is important.

3. Advanced breeding methods

3.1 Genome editing - Zinc Finger Nucleases (ZFNs), (TALENs) Transcription activator like effector nucleases and CRISPR/Cas9 clustered regularly interspaced short palindromic repeats,

3.2 Gene pyramiding

3.3 Marker assisted backcross selection

In genome editing the popular method is CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats.

3.1 Genome editing, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/CRISPR-associated (Cas) systems, which have bacterial and archaeal origins, perform as a crucial kind of adaptive defense against foreign nucleic acids. The rapid evolution of CRISPR/Cas systems has resulted in them becoming useful tools for editing exogenous and endogenous DNA or RNA sequences in various organisms. A condensed CRISPR system as of now just consists of a Cas effector protein and a guide RNA (gRNA) that is simple to build.

CRISPR/Cas systems are divided into two main groups based on the variations in the sequence, structure and function of Cas proteins. Types I, III and IV of the CRISPR/Cas systems, which employ several proteins as effectors, are included in class 1. On the other hand, type II, type V and type VI CRISPR/Cas systems can only achieve target editing with a single effector. As a result, class 2 CRISPR/Cas systems are frequently used for manipulating and detecting nucleic acids. Among them, the type II, type V and type VI Cas proteins are used for editing DNA and RNA, respectively. Plant genetic engineering has quickly adopted CRISPR/Cas system-mediated gene editing technologies in recent years to create virus and bacterial resistance.There are primarily two methods for using CRISPR/Cas technology to manage plant viruses. To prevent the reproduction and infection of invasive viruses, one strategy is to target, eradicate and interfere with the viral genome. The second method involves modifying host susceptibility elements necessary for viral infection and life cycle in order to improve plant immunity and prevent virus invasion.

3.2 Gene pyramiding

Gene pyramiding refers to the process of stacking multiple genes into a single genotype to combine desirable traits through recombinant DNA technology or conventional breeding.

In a gene pyramiding scheme, the goal is to combine genes found in several parents into a single genotype. The pace of the pyramiding process is accelerated by the introduction of DNA markers, which enables complete gene identification of the progeny at each generation. Gene pyramiding generally seeks to create a homozygous genotype for the advantageous alleles at all n loci, which is the optimal genotype. It is possible to divide the gene pyramiding scheme into two components. The first section, referred to as a pedigree, seeks to combine all target genes into a single genotype known as the root genotype. This range of intermediate genotypes all have resistance. Pyramiding might also enhance becoming a parent in the next cross. The intermediate genotypes aren't only randomly chosen offspring of a particular cross; rather, they are a specific genotype chosen among the offspring in which both sets of parental target genes are present. Even though the pedigree phase in gene pyramiding may be typical, there are a variety of other approaches that can be applied.

3.3 Marker assisted backcross selection

The backcrossing technique has been widely employed in plant breeding to introduce one or a few genes from a plant with a distinctive attribute (donor parent) into a desirable adapted or elite variety (recurrent parent) that lacks a few qualities, like disease resistance. The parent that is used for backcrossing typically possesses a lot of excellent qualities but is lacking in only a few traits. The effectiveness of selection has risen as a result of the use of molecular markers in backcrossing. Molecular markers are used in marker-assisted backcrossing to follow either the target locus or the ancestry of the recurrent parent. A cultivar produced by such a technique retains the genome of the recurrent parent but only includes the primary gene that was taken from the donor parent.

4. Some of the research works are studied below;

4.1 Introgression of tomato leaf curl virus (ToLCV) resistant gene into tomato (*Solanum lycopersicum*) cultivars through marker assisted backcross breeding studied by Sowjanya (2019).

Using two F_2 populations of the crosses GPBT-08 × CLN2768A and DMT-2 × CLN2777H, research was done to validate three markers (TG0302, P1-16 and TES0344) associated to *Ty-2* providing resistance to ToLCV illness. In both populations, the three molecular markers separated in the ratios 1:2:1 (genotypic) and 3:1 (phenotypic). *Ty-2* significant correlation with leaf curl resistance via SMA accounted for 11.67 % and 9.09 %, respectively, of the phenotypic variance in the crosses GPBT-08 × CLN2768A and DMT-2 × CLN2777H. P1-16 and TG0302, two verified markers, were utilised by MABB to introduce *Ty-2* into cultivated cultivars. *Ty-2* confers resistance to ToLCV in backcross populations created by mating GPBT-08 and DMT-2 as recurrent parents with CLN2768A and CLN2777H as donor parents, respectively.

In BC_2F_4, GA-27-1, GA-37-9 and GA-40-1 lines of the cross GPBT-08 × CLN2768A and DH-4-8, DH-12-8 and DH-14-5 lines of the cross DMT-2 × CLN2777H recovered highest RPG, exhibited superior performance for yield traits with moderate resistant reaction. In BC_3F_3 GA-8-5 and GA-12-3 lines of the cross GPBT-08 × CLN2768A and DH-5-6, DH-12-3 and DH-13-3 lines of the cross DMT-2 × CLN2777H recovered highest RPG with superior yield performance and with moderate resistant reaction. In southern India's leaf curl-affected regions or seasons, these lines seem potential for increasing tomato yield.

Through single marker analysis, the target gene demonstrated a substantial correlation with leaf curl resistance, explaining the significant phenotypic variance for all three markers employed in the study in both F_2 populations. It also suggests that there is a strong possibility of using all three markers in MAS to confer resistance to ToLCV illness. Introduction of the ToLCV disease resistance gene (*Ty-2*) by marker-assisted backcross breeding into commercial tomato varieties. Through the use of a validated marker, the elite tomato cultivars GPBT-08 and DMT-2 were selected for the introduction of *Ty-2*, which confers ToLCV disease resistance, from resistant donor parents CLN2768A and CLN2777H. Foreground selection using validated markers that were closely linked to the trait allowed for precise monitoring of the transfer of resistance genes altering the expression of targeted traits in each generation. By regularly backcrossing to the respective recurrent parents (GPBT-08 and DMT-2), near isogenic lines (NILs) harbouring *Ty-2* giving resistance to ToLCV illness were created, yielding two BC_1F_1, BC_2F_1, and BC_3F_1 populations. With the exception of the targeted region, markers were spread on all non-carrier chromosomes and carrier chromosomes during background selection against the donor parent alleles in each backcross generation.

Two F_2 populations derived from the crosses GPBT-08 × CLN2768A and DMT-2 × CLN2777H validated the molecular markers TG0302, P1-16 and TES0344 linked to targeted gene *Ty-2* mapped on long arm of chromosome 11, which confers resistance to monopartite begomovirus, which is highly prevalent in southern India. While CLN2768A and CLN2777H showed a mild resistant reaction, the parents GPBT-08 and DMT-2 showed a susceptible reaction. The segregation ratio of 1:2:1 was found in F_2 population of both the crosses for all the three markers following Mendelian segregation pattern of the gene, indicating dominant gene governing the resistance. In BC_2F_4, GA-27-7, GA-37-9 and GA-40-1 lines derived from cross GPBT-08 × CLN2768A and DH-4-8, DH-12-8 and DH-14-5 lines from cross DMT-2 × CLN2777H performed better under disease stress condition with minimum per cent disease incidence showing moderately resistant reaction. BC_3F_3 lines, GA-8-5 and GA-12-3 derived from the cross GPBT-08 × CLN2768A and lines DH-5-6, DH-12-3 and DH-13-3 from the cross DMT-2 × CLN2777H superior for most of the yield related traits

studied under disease stress condition with minimum per cent disease incidence showing moderate resistant reaction. In recent years, studies were conducted by Frisch *et al*. (1999), Hospital *et al*. (1992) and Visscher *et al*. (1996) on computer simulation to develop and optimize the MABB.

4.2 Study conducted by Martinez *et al*. (2020) on UMH1400 and UMH1401: New Cherry tomato breeding lines resistant to virus.

The objective was to develop the breeding line UMH1401 without TYLCV resistance. The commercial cultivar Anastasia F_1 was crossed with the cherry cultivar accession cherry pera, which had previously been chosen for fruit morphological traits, uniformity and good quality. This produced the breeding lines UMH1400 and UMH1401. The *Tm2a, Sw-5* and *Ty-1* genes, which give resistance to ToMV, TSWV and TYLCV, were used as the donor parent of "Anastasia F_1" (Perez *et al*., 2007).

Using Cleaved Amplified Polymorphic Sequences (CAPS) markers, twelve generations of backcrossing to the cultivar Cherry Pera were carried out in order to aid in the selection of viral resistance genes. Several studies were conducted to check for the existence of resistance alleles in the first backcross (BC) generations and to evaluate the efficacy of the molecular markers under various infection environments. The *Tm-2a*, *Sw-5* and *Ty-1* genes were used as molecular markers to check the progeny of each backcross generation. Only plants with the three resistance genes were transplanted and then crossed with the recurrent parent cherry pera to create each BC progeny (typically between 5 and 10 plants). Usually between two and four of the best plants per generation were chosen for further backcrossing.

This selection was made based on the high quality (soluble solid content and titratable acidity), good agronomic behaviour (correct fruit set, sufficient uniformity among fruits and yields) and desirable cherry traits (fruit shape and size, low sensitivity to blossom-end rot). Using molecular markers, the pure-breeding lines UMH1400 (homozygous for *Tm-2a, Sw-5* and *Ty-1*) and UMH1401 (homozygous for *Tm-2a* and *Sw-5*) were chosen after the selfing of one BC_{12} triple heterozygous plant, followed by two generations of selfing and selection. These lines were subsequently multiplied in a greenhouse under controlled circumstances by self-pollination.

UMH1400 and UMH1401 breeding lines have an indeterminate growth habit with intermediate foliage density and small-sized fruits (8–16 g) in a bell shape. UMH1400 is homozygous for the *Tm-2a, Sw-5* and *Ty-1* resistance genes, while UMH1401 is homozygous for the Tm-2a and Sw-5 resistance genes only.

Between 2013 and 2015, cherry pera and the breeding lines UMH1400 and UMH1401 were grown in a mesh-covered net house during the spring-summer crop cycle. In comparison to cultivar cherry pera, UMH1400 exhibits significant declines in marketable yield (between 35 % and 48 %), average fruit weight (except for 2013), fruit number per plant (between 18 % and 40 %), titratable acidity (TA) (between 17 % and 35 %) and soluble solid content (SSC) (about 15 %). The breeding lines, UMH1400 shows significant decreases with respect to UMH1401 in marketable yield (ranging between 48 % and 65 %), average fruit weight (ranging between 15 % and 33 %), fruit number per plant (ranging between 35 % and 63 %), TA (ranging between 19 % and 26 %) and SSC (ranging between 10 % and 17 %). Therefore UMH1400 can be used for further breeding programme.

The introgressed genes themselves or the linkage drag connected to the *Ty-1* gene, which confers resistance to TYLCV, are to blame for these reductions (Rubio *et al*., 2016). In the past, tobacco (Lewis *et al*., 2007), tomatoes for industrial use (Tanksley *et a*l., 1998), and tomatoes for fresh consumption (Tanksley *et al*., 1998) have all been subject to the detrimental effects of resistance gene introduction from wild relatives. Marketable yield (between 15 % and 35 %) and average fruit weight (between 18 % and 39 %) of UMH1401 are much higher than those of the cultivar cherry pera. For the typical fruit weight, TA and SSC, no appreciable changes were discovered.

4.3 Study was conducted on tomato brown rugose fruit virus resistance generated by quadruple knockout of homologs of *tobamovirus* multiplication in tomato by ishikawa *et al*. (2022).

The objective was to develop resistance to tomato brown rugose fruit virus (ToBRFV) in tomato plants by targeted mutagenesis of TOM1 homolog.

In order to provide ToBRFV resistance, the tomato TOM1 genes were knocked out using the clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) technique. Five genes known as SlTOM1ae were discovered by a BLAST search of the tomato genome sequence database (ITAG release 4.0) using the *Arabidopsis* TOM1 amino acid sequence as a query. These three genes (SlTOM1ac) were the targets, along with SlTOM1d, another expressed homolog. SlTOM1e's expression level in the public data was so low that it was not examined. The CRISPR-P programme was used to provide the guide RNA sequences needed for the SlTOM1ad genes to be specifically altered by the CRISPR/Cas9 system. built a vector to express three single guide RNA (sgRNA) sequences that target the SlTOM1a-d genes and Streptococcus pyogenes Cas9. The gene cassette was introduced to tomato cultivar Craigella GCR26, which is susceptible to ToMV and ToBRFV, by Agrobacterium-mediated transformation.

When measured at 7 dpi, the level of CP accumulation was in the following order: wt > Sltom1bcd > Sltom1abd 4 > Sltom1abc > Sltom1acd. This implies that SlTOM1 genes contribute to ToBRFV proliferation in the following order: SlTOM1a > SlTOM1c > SlTOM1d > SlTOM1b. At 21 dpi, neither ToBRFV CP buildup nor disease symptoms were seen in Sltom1abcd quadruple-mutant plants. ToBRFV was inoculated onto the cotyledons of Sltom1 mutant plants, and the accumulation of viral coat protein (CP) in the inoculated leaves at 7 days after infection (dpi) was investigated using SDS-PAGE and Coomassie blue staining. ToBRFV CP accumulates to virtually wt levels in Sltom1 single or double

mutants. ToBRFV CP buildup was marginally lessened in several double mutants, such as Sltom1ac, when compared to wild-type. After longer incubation, ToBRFV-inoculated single and double mutants systemically accumulated viral CP and showed disease symptoms. In Sltom1 triple mutants, ToBRFV CP accumulation was reduced compared with that in wt plants. At 7 dpi, the level of CP accumulation was in the following order: 4 Sltom1bcd, 5 Sltom1abd, 4 Sltom1abc and 4 Sltom1acd. This shows that SlTOM1 genes contribute to ToBRFV proliferation in the following order: SlTOM1a, 5, SlTOM1c, 4, SlTOM1d and 4, SlTOM1b. The relative mRNA accumulation levels of the various SlTOM1 genes revealed a limited correlation with the contribution of the SlTOM1 genes to ToBRFV proliferation. SlTOM1b's contribution to ToBRFV multiplication is insignificant since ToBRFV CP accumulation was greater in the Sltom1ac double mutant than the Sltom1abc triple mutant. At 7 dpi, CP in ToBRFV-infected Sltom1acd mutant plants was hardly detectable, but after further incubation (for example, 21 d), a low level of ToBRFV CP was found not only in the inoculated leaves but also in systemic leaves and some plants displayed symptoms of the illness. The other triple mutants (Sltom1bcd, abd and abc) displayed clinical signs and systemically accumulated viral CP. At 521 dpi, neither ToBRFV CP buildup nor disease symptoms were seen in Sltom1abcd quadruple-mutant plants.

Similar results were obtained when ToMV was inoculated onto Sltom1 mutant plants. Importantly, ToMV CP accumulation was not detected in Sltom1 quadruple-mutant plants. The Sltom1 quadruple-mutant plants did not show CP accumulation when inoculated with other tobamoviruses These results indicated that simultaneous loss-of-function mutations in SlTOM1ad genes confer tobamovirus resistance to tomato.

4.4 Prasanna *et al*. (2015) pyramided *Ty-2* and *Ty-3* genes for resistance to monopartite and bipartite tomato leaf curl viruses of India.

The objective of this study was to combine *Ty-2* and *Ty-3* and to determine the effect of pyramiding on infection of tomato by three diverse *begomovirus* species. So far, resistance to *begomoviruses* in tomato has been achieved using wild species and at least five resistance genes (*Ty* genes) have been studied. In order to create pyramided tomato lines from crosses between *Ty* stocks, the diagnostic potential of the markers connected to *Ty* genes was evaluated. There have been established five stable pyramided tomato lines with various fruit morphologies and yield potential. Pyramided lines horticultural performance in field tests demonstrated that the plants yield and horticultural features are well preserved. Agroinoculation and field testing were used to evaluate how well these lines responded in a disease hotspot. The monopartite and two bipartite *begomoviruses* examined showed a high level of resistance to the pyramided lines and *Ty*-3-carrying lines. The created pyramided tomato lines may be crucial genetic resources for long-term tomato production.

A group of tomato lines expressing several *Ty* genes were utilised as markers: LA3473 (*Ty-1*), CLN 2585D (*Ty-2*), CA4 (*Ty-3*), GC171 (*Ty-3*) and TY172 (*Ty-5*). The *Ty-1* introgression derived from *S. chilense* (LA1969) is present in line LA3473 and the *Ty-2* introgression derived from *S. habrochaites* is present in line CLN2585D. *Ty-3* was present in two lines, CA4 and GC171. Line CA4 includes a longer fragment derived from *S. chilense* than GC171. The plants that tested positive for the employed *Ty-4*-linked marker were separated from the GC171 seed stock for *ty-4*. Four separate S. peruvianum accessions were used in the convoluted pedigree of the *Ty-5*-carrying tomato line TY-172, which was included. For pyramiding purposes, two F_2 populations of a cross between FLA478-6-1-11 9 × CLN2498C and FLA478-6-1- 11 9 × CLN1621E used as a base population. Line FLA478- 6-1-11 carries the *Ty-3* introgression, whereas the lines CLN2498C and CLN1621E carry the *Ty-2* introgression. Punjab Chhuhara and Kashi Vishesh cultivars were used as susceptible controls in both agroinoculation tests and field trials.

Tomato lines carrying different *Ty* genes were genotyped using several *Ty*-linked SCAR and CAPS markers. Tomato-infecting *begomovirus* species used in this study was comprised bipartite Tomato leaf curl New Delhi virus (ToLCNDV), Tomato leaf curl Palampur virus (ToLCPalV) and a monopartite Tomato leaf curl Bangalore virus (ToLCBV). Disease resistance tests were performed by agroinoculation of 4-5-week-old tomato seedlings with *Agrobacterium tumefaciens*. To evaluate disease resistance and horticultural performance, five pyramided lines, three lines each carrying *Ty-1, Ty-2* and *Ty-3* and the two susceptible cultivars Kashi Vishesh and Punjab Chhuhara, were planted. *Ty-1* gene was effective against ToLCBV and ToLCNDV but was ineffective against ToLCPalV. *Ty-2* conferred moderate resistance to the monopartite virus but was not effective against two bipartite *begomoviruses*. *Ty-3* was highly resistant to both monopartite (ToLCBV) and two bipartite viruses (ToLCNDV and ToLCPalV). Level of resistance was high in pyramided lines and *Ty-3* lines. Therefore the ToLCD management through reistance breeding could benefit from using the *Ty-3* gene. Pyramiding of resistance genes has been successful in different plant pathogen systems. In most cases, combining two or three resistance genes resulted in a broader resistance spectrum and/or higher levels of resistance. Similar findings were confirmed by Kelly *et al*. (1995), Caranta *et al*. (1996), Werner *et al*. (2005) and Shi *et al*. (2009).

4.5 Martinez *et al*. (2016) conducted research on the UMH 1353 and UMH 1354 breeding lines of the 'De la Pera' tomato variety that are resistant to tomato mosaic virus and tomato spotted wilt virus.

In a small region in southeast Spain, a tomato landrace known as "De la Pera" is highly well-liked. The fruits feature a high percentage of mucilage and seeds, are solid and juicy, and have a robust flavour. The fruit is elongated-oval to bell-shaped, weighs between 75 and 125 g and has no ribs and dark green shoulders. The viruses tomato mosaic virus (ToMV), tomato spotted wilt virus (TSWV), and tomato yellow leaf curl virus (TYLCV) are particularly dangerous to De la Pera varieties.

Medium-sized fruits (70-90 g) with a bell shape and green shoulders grow on indeterminate plants (UMH 1353 and UMH 1354) with intermediate foliage density. The *Tm-2a* and *Sw-5* resistance genes are homozygous in both lines. Between 2011 and 2014, the UMH 1353 and UMH 1354 breeding lines were grown in conjunction with three previously created breeding lines, the cultivar P21, and under various conditions (open field and mesh-covered net houses) during the spring-summer crop cycle, which is the most popular cycle in the traditional area of cultivation for the 'De la pera' tomato. UMH 1353 and UMH 1354 outperformed the ToMV, TYLCV, and TSWV resistance-carrying UMH 1203 breeding line in terms of desirable agronomic features.

The UMH 1353 and UMH 1354 lines, which generated nearly twice the marketable yields of the UMH 1203 line, were particularly notable for the increase in marketable yield. In three of the four investigated cycles, the UMH 1353 and UMH 1354 breeding lines produced marketable yields that were higher than those of the previously developed breeding lines. These novel lines produced exceptional yields for a tomato landrace, ranging from 4.21 kg to 5.73 kg per plant in marketable yields. The gains in the new breeding lines compared to the previously developed lines for fruit production were comparable to increases in marketable yield (about 50%). However, disparities between the lines were less noticeable when it came to the average fruit weight, with the new lines showing increases of around 15 % to 20 % with respect to the other lines studied.

Additionally, the titratable acidity values achieved by UMH 1353 and UMH 1354 were on par with or even greater than those of the other breeding lines. Only two of the four cycles under study showed substantial variations in soluble solids content, with the traditional cultivar and the previously released breeding lines displaying similar or lower values than the UMH1353 and UMH1354 breeding lines, respectively. It is possible to see the minor variations between the breeding lines UMH1353 and UMH1354. To boost production by utilising genetic resistance to ToMV and TSWV, UMH 1353 and UMH 1354 breeding lines can be utilised to create F_1 hybrids by mating them with other 'De la pera' landraces. Furthermore, these new lines can also be used in breeding programs to facilitate the introgression of these resistance genes into other landraces.

5. Conclusion

With the advent of sequencing technologies, functional genomics has revolutionized with new editing tools to create novel allelic series of mutants for crop improvement. Among them, CRISPR-Cas technology has gained much attention over the other genome editing techniques because designing specific nuclease domains each time is a tedious task. Further studies are required to enhance the essential traits such as improvement in the resistance to pathogens and abiotic stresses, yield, resistant varieties and nutritional aspects in tomato and other agronomic crops.

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