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(54) Title: GENE LEADING TO TOBRFV RESISTANCE IN S. LYCOPERSICUM

(57) Abstract: The invention relates to a *Solanum lycopersicum* plant that is resistant to ToBRFV, which plant comprises a QTL on chromosome 8 between SEQ ID No. 1 and 4. The presence of the QTL on chromosome 8 is identified by use of at least one of the markers selected from the group 5 consisting of the SNP presented in SEQ ID Nos. 1 to 42. The QTL is present in the genome of a *Solanum lycopersicum* plant representative seed of which was deposited with the NCIMB under deposit number NCIMB 43637.

GENE LEADING TO ToBRFV RESISTANCE IN S. LYCOPERSICUM

The present invention relates to a tomato (*Solanum lycopersicum*) plant comprising a QTL which comprises an allele of a gene that leads to *Tomato brown rugose fruit virus* (ToBRFV) resistance. The present invention further relates to an allele of a gene that confers ToBRFV resistance. The invention further relates to a method for producing a ToBRFV resistant *Solanum lycopersicum* plant, and methods for identification and selection of such a plant comprising the allele of the gene. The invention also relates to progeny, seed and fruit of the *Tomato brown rugose fruit virus* resistant *Solanum lycopersicum* plant, to propagation material suitable for producing the *Solanum lycopersicum* plant, and to a food product comprising such tomato fruit or part thereof. The invention further relates to a cell or a tissue culture that results from or can be regenerated into a *Tomato brown rugose fruit virus* resistant *Solanum lycopersicum* plant. The invention also relates to a marker for identification of the allele of the gene that leads to *Tomato brown rugose fruit virus* resistance in *Solanum lycopersicum,* and to use of said marker.

Viral diseases pose one of the major threats vegetable growers have to deal with, both in protected and open field crop cultivation. Once a crop is infected, spread of the virus can occur rapidly through hard-to-control vectors, usually insects. In addition, cultivation methods often contribute to a further spread of the virus, by sap transmission through tools and fieldworkers.

The best protection against virus infection is the use of a resistant variety. Resistances against many known viruses have been identified, which resistances are incorporated in suitable tomato varieties through breeding, allowing the growers to obtain a good yield even under virus pressure. Resistances can usually be categorized into different types, the most common ones being tolerance and resistance. Tolerance does not necessarily mean the resistance has a lower level, but it indicates there are no or reduced symptoms in the plant, even though the virus can still replicate within the host. Resistance describes a mechanism in which not only symptoms are absent or strongly reduced, but also virus replication within the plant is restricted, i.e. it is absent or strongly reduced.

In 2015 the occurrence of a new tobamovirus in tomato was published (Salem et al: *A new tobamovirus infecting tomato crops in Jordan.* Arch Virol. 2016 Feb; 161(2):503-6. Epub 2015 Nov 19). This virus was shown to be related to the known tobamoviruses *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), and *Tomato mild mottle virus* (ToMMV), with sequence identities of around 80% to 90% for the closest related sequences of ToMMV and ToMV. Symptoms were rather mild on the plant, but very severe brown rugose symptoms were present on almost all fruits. The virus was observed to break the resistance of the commonly used resistance genes against ToMV: *Tm-1, Tm-2,* and *Tm-2*^{2}, of which the latter one is also known as

Tm-2^a. A later publication showed that the virus was also found in Israel, and it was established that the virus can also infect pepper (*Capsicum annuum)* plants (Luria et al (2017): *A new Israeli tobamovirus isolate infects tomato plants harboring Tm-2*^{2} *resistance genes.* PLoS ONE 12(1):e0170429. Doi:10.1371/journal.pone.0170429). Symptoms appeared to vary based on the affected variety, and in certain instances symptoms were mainly found on the vegetative parts in the form of severe or mild mosaic, necrosis, leaf distortion, or other symptoms. As the virus was clearly different from the known tobamoviruses it was assigned a new designation: *Tomato brown rugose fruit virus* (TBRFV). In the meantime, the commonly used abbreviation for this virus is ToBRFV, which is therefore now also used in the present application.

Over the past few years ToBRFV has quickly spread to many countries and has a major impact on tomato fruit production in a rapidly increasing number of areas of vegetable farming. Because of the severity of the symptoms on the fruits, the impact of the presence of ToBRFV for tomato growers is very high as it leaves the fruits basically unmarketable. The virus is at least transmitted mechanically, which makes the spread easy and rapid, and difficult to control. Transmission of the virus is also likely to occur through infected seed.

It is an object of the present invention to provide a tomato plant of the species *Solanum lycopersicum* that shows true resistance to *Tomato brown rugose fruit virus* (ToBRFV).

It is a further object of the present invention to provide an allele of a gene that leads to ToBRFV resistance.

Because the problems with the new ToBRFV spread very quickly and had a major effect on tomato production in certain areas, the urgency to obtain resistant tomato plants was very high. In addition, the virus was expected to be able to spread rapidly to other areas due to its very effective transmission. A large germplasm screen was therefore organized to get an insight in the presence of possible sources. Initially, several resistant accessions of the species *Solanum pimpinellifolium* were identified, and QTLs on chromosomes 6, 11, and 12 were found through fine mapping and phenotyping of populations made between these sources and *S. lycopersicum* lines, as described in co-pending applications WO2019110130 and WO2019110821. Introgression of these QTLs into *S. lycopersicum* led to useful phenotypic resistance levels, but in lab experiments it was found that virus titer was still relatively high, which demonstrated that the virus could still replicate in the plant.

Therefore, for one of the used *S. pimpinellifolium* sources, GNL.3951, further populations were developed by combining it with internal breeding lines for additional QTL mapping, to acquire plants with a more robust true resistance based on restriction of virus replication. On plants of all generations bio-assays were done, but also qPCR analysis was performed to determine the presence and level of virus titer in the plants **(Example 2)**.

The identification and characterization of a QTL through molecular markers gives the opportunity to use genetically linked markers to identify the presence of the QTL and therefore the presence of the resistance, which is obviously much more efficient than the use of a bio-assay in combination with qPCR analysis. For this purpose, new QTL mapping studies were performed on F2 populations, backcrosses, and F1 hybrids with the source. These observations resulted in the identification of a new QTL region on chromosome 8 **(Example 1)**.

The present invention provides a *Solanum lycopersicum* plant that is resistant to *Tomato brown rugose fruit virus* (ToBRFV), which plant comprises a QTL comprising an allele of a gene on chromosome 8 that restricts virus replication in the plant. The allele of the gene on chromosome 8 is in particular an allele of a gene derived from or introgressed from the species *S. pimpinellifolium*.

The first QTL region that was identified on chromosome 8 was located between SEQ ID No. 1 and SEQ ID No. 42. The presence of this QTL on chromosome 8 can be identified by a genetically linked marker selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 42, preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 14, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No, 34, and SEQ ID No. 42. It was determined that the QTL was tightly linked to a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 29, and SEQ ID No. 30. A genetically linked marker for identification of the QTL is a marker represented by any one of the sequences listed in **Figure 1** that is present in the QTL.

The initially identified QTL region on chromosome 8 was further finemapped to determine the position of the gene within the QTL that confers the resistance. A first finemapping exercise resulted in a smaller QTL region, that was positioned between SEQ ID No. 22 and SEQ ID No. 34. This smaller QTL region comprised seven genes. Five markers from the first mapping, including the three that were determined to be highly linked, were present in the sequence of one of those genes, the *Tom2a* gene, indicating the presence of polymorphisms in *Tom2a* as compared to the ToBRFV susceptible reference genome, version SL3_00. Because the best-linked markers were present in this gene, *Tom2a* appeared to be the most likely candidate gene for the ToBRFV resistance of the present invention. Sequencing of this gene, and subsequent alignment with the public reference genome, resulted in the determination of two more polymorphisms. The polymorphisms between the reference genome sequence SL3_00 and the resistant sequence of *Tom2a* can be identified by any one of the markers presented as SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, or SEQ ID No. 44. The resistant sequence of *Tom2a* constitutes the allele of the invention.

Further research and observations were done to confirm the involvement of the *Tom2a* gene in the ToBRFV resistance. In this process, populations that had recombinations in the QTL region were developed. Through marker analysis and phenotyping of these recombinant lines, the other genes that were present in the QTL region were eliminated as potential contributors to the ToBRFV resistance, and the *Tom2a* gene could be confirmed as the causal gene for the ToBRFV resistance of the present invention (**Example 4; Figure 4).**

The invention relates to a *Solanum lycopersicum* plant comprising a QTL on chromosome 8 which comprises a resistance conferring *Tom2a* allele, wherein the presence of the QTL on chromosome 8 is genetically linked to at least one of the markers selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 14, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No, 34, SEQ ID No. 42, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44.

The present invention provides a resistance conferring *Tom2a* allele on chromosome 8 of *Solanum lycopersicum*, which resistance conferring *Tom2a* allele comprises SEQ ID No. 45, or comprises a homologous *Tom2a* sequence having at least 70% sequence identity to SEQ ID No. 45. The presence of said resistance conferring *Tom2a* allele or homologous *Tom2a* sequence in a *S. lycopersicum* plant leads to ToBRFV resistance. SEQ ID No. 45 presents the coding sequence (CDS) of a resistance conferring allele of *Tom2a.* As used herein, a resistance conferring allele of *Tom2a* is a version of the *Tom2a* gene that leads to ToBRFV resistance.

SEQ ID No. 45 encodes a protein comprising SEQ ID No. 46. The present invention relates to a Tom2a protein comprising SEQ ID No. 46, or comprising a homologous Tom2a protein having at least 70% sequence identity to SEQ ID No. 46, which leads to ToBRFV resistance.

The wildtype sequence of the *Tom2a* gene comprises SEQ ID No. 47, and encodes a protein comprising SEQ ID No. 48. Polymorphisms in the genomic sequence of this gene, which can be used to identify the presence of the resistance conferring allele of the *Tom2a* gene, comprise a SNP as presented in the sequences of SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, or SEQ ID No. 44. The resistance conferring allele of *Tom2a* can be identified by the use of any one of these SNPs as a marker, by determining the presence of the SNP.

A homologous *Tom2a* gene comprises a homologous sequence, which is a sequence having at least 70% sequence identity to SEQ ID No. 45, preferably at least 75%, 77%,

80%, 83%, 85%, 87%, 90%, 93%, 95%, 96%, 97%, 98%, or 99% sequence identity. A homologous Tom2a protein comprises a homologous sequence, which is a sequence having at least 70% sequence identity to SEQ ID No. 46, preferably at least 75%, 77%, 80%, 83%, 85%, 87%, 90%, 93%, 95%, 96%, 97%, 98%, or 99% sequence identity.

In one embodiment, a resistance conferring homologous *Tom2a* allele has retained a polymorphism that can be identified by SEQ ID No. 26, or by SEQ ID No. 28, or by SEQ ID No. 43, or by SEQ ID No. 44. In one embodiment, a resistance conferring *Tom2a* allele, or a homologous allele thereof, has retained a combination of polymorphisms that can be identified by SEQ ID No. 26 and SEQ ID No. 28; SEQ ID No. 26 and SEQ ID No. 43; SEQ ID No. 26 and SEQ ID No. 44; SEQ ID No. 28 and SEQ ID No. 43; SEQ ID No. 28 and SEQ ID No. 44; SEQ ID No. 43 and SEQ ID No. 44; SEQ ID No. 26 and SEQ ID No. 28 and SEQ ID No. 43; SEQ ID No. 26 and SEQ ID No. 28 and SEQ ID No. 44; SEQ ID No. 26 and SEQ ID No. 43 and SEQ ID No. 44; SEQ ID No. 28 and SEQ ID No. 43 and SEQ ID No. 44; or SEQ ID No. 26 and SEQ ID No. 28 and SEQ ID No. 43 and SEQ ID No. 44.

Because the gene is present on the minus-strand in the public genome, SEQ ID No. 26 identifies an A to G conversion on position 559 of SEQ ID No. 47; SEQ ID No. 28 identifies a GGC deletion on positions 312-314 of SEQ ID No. 47; SEQ ID No. 43 identifies a G to A conversion on position 673 of SEQ ID No. 47, and SEQ ID No. 44 identifies an A to G conversion on position 844 of SEQ ID No. 47.

In one embodiment, the resistance conferring homologous *Tom2a* allele has retained at least one of an A to G conversion on position 559 of SEQ ID No. 47; a GGC deletion on positions 312-314 of SEQ ID No. 47; a G to A conversion on position 673 of SEQ ID No. 47, an A to G conversion on position 844 of SEQ ID No. 47, or any of those polymorphisms on a corresponding position of the homologous *Tom2a* gene. In a further embodiment, the resistance conferring homologous *Tom2a* allele has retained a combination of two, three, or all four of these polymorphisms. **Figure 5** shows the CDS sequences of the *Tom2a* wildtype and the resistance conferring *Tom2a* allele individually and in an alignment.

In one embodiment, the homologous *Tom2a* allele sequence encodes a protein comprising at least one of a deletion of A on position 105 of SEQ ID No. 48, a conversion to G on position 187 of SEQ ID No. 48, a conversion to S on position 225 of SEQ ID No. 48, or a conversion to A on position 282 of SEQ ID No. 48.

In one embodiment, the protein encoded by the resistance conferring *Tom2a* allele, or a homologous protein thereof, has retained an Ala deletion on position 105 of SEQ ID No. 48, a Arg to Gly substitution on position 187 of SEQ ID No. 48, a Gly to Ser substitution on position 225 of SEQ ID No. 48, or a Thr to Ala substitution on position 282 of SEQ ID No. 48, or a modification on a corresponding position of an homologous protein. In one embodiment, the

protein encoded by the resistance conferring *Tom2a* allele has retained a combination of two, three, or all four of these polymorphisms. **Figure 6** shows the Tom2a protein sequences of the wildtype and the resistance conferring *Tom2a* allele individually and in an alignment.

As used herein, sequence identity is the percentage of nucleotides or amino acids that is identical between two sequences after proper alignment of those sequences. The person skilled in the art is aware of how to align sequences, for example by using a sequence alignment tool such as BLAST®, which can be used for both nucleotide sequences and protein sequences. To obtain the most significant result, the best possible alignment that gives the highest sequence identity score should be obtained. The percentage sequence identity is calculated through comparison over the length of the shortest sequence in the assessment, whereby in the present case a sequence represents a gene that at least comprises a start codon and a stop codon, or a complete protein encoded by such a gene.

The Tom2a protein is a tetraspanin protein, which is a protein having a tetraspanin/peripherin domain. The protein comprises an N-terminal and a C-terminal tail, and four transmembrane domains which are connected by two non-cytoplasmic and one very short cytoplasmic loop **(Figure 8)**. The observed mutations were present at the end of the second transmembrane domain (TM2), which is in particular the Ala deletion of position 105, and in the C-terminal tail of the protein. The deletion of Ala on position 105 affects the TM2 domain, and might also have an effect on the short cytoplasmic loop that follows it. The present invention relates to a mutation in a *Tom2a* gene having an effect on the TM2 domain, or a mutation having an effect on the cytoplasmic loop, or a mutation having an effect on the C-terminal tail of the protein, or a combination of said mutations.

In one embodiment, the resistance conferring *Tom2a* allele encodes a non-functional protein.

Figure 1 provides the sequences of the SEQ ID Nos. that can be used as markers, or used to develop markers, to identify the presence of the QTL comprising the allele of the *Tom2a* gene of the invention on chromosome 8 leading to ToBRFV resistance in a tomato plant. **Table 4** shows the nucleotide in the sequence that identifies the presence of the QTL, and therefore a resistant plant, as well as the position of the SNP in the sequence of **Figure 1.** As used herein, the 'SNP presented in' a certain SEQ ID No., is the nucleotide within the sequence that is indicative of resistance, as given in the column 'Nucleotide of the SNP in **Figure 1**, to be used as marker of the invention' of **Table 4.** When the sequences of the markers are positioned on version SL3_00 of the publicly available genome reference sequence for *S. lycopersicum,* the physical position to which the SNP polymorphism in said marker sequence corresponds can be derived. This position is also presented in **Table 4**. Version SL3_00 of the public *S. lycopersicum* genome reference sequence can for example be accessed at the Solgenomics website (solgenomics.net) and is the reference for

'the public tomato genome' as used herein. The positions of the markers of the invention are derivable from a public map and these positions are relative to said physical positions.

Identifying the presence of the QTL, or of the resistance conferring *Tom2a* allele, by using a marker is in particular done by identifying the presence of the nucleotide at the position of the SNP within the marker sequence that is indicative for the resistance. The wildtype nucleotide is the nucleotide that is present on that position in the public genome.

As used herein, a tomato plant is a plant of the species *Solanum lycopersicum*.

As used herein, resistance to the *Tomato brown rugose fruit virus* is resistance to the virus as described in Salem et al (2016, *supra*), which virus was assigned NCBI Taxonomy ID 1761477.

As used herein, a marker is genetically linked to, and can therefore be used for the identification of, the QTL which comprises the allele of the *Tom2a* gene of the invention, when the marker and the ToBRFV resistance co-segregate in a segregating population resulting from a cross between a plant comprising the QTL of the invention and a plant lacking the QTL. A marker that is genetically linked to a QTL can be used for identification of that QTL because a linked marker is present in said QTL. Markers that are present in the resistance conferring *Tom2a* allele of the invention are completely linked, and are therefore directly indicative of the presence of the resistance conferring allele of the gene.

The ToBRFV resistance of the present invention inherits in a recessive manner. This means that when the allele of the *Tom2a* gene of the invention is homozygously present, virus replication in the plant is absent or strongly reduced, when compared to a plant in which the allele of the invention is absent or heterozygously present. However, heterozygous plants can be used for development of homozygous plants through crossing and selection, and heterozygous plants therefore also form a part of this invention.

Virus replication, and thereby the ToBRFV resistance of the invention, is suitably determined through a qPCR test, as described in **Example 2**. Confirmation of the resistance can be determined through a bioassay, for example using a standard sap-mechanical inoculation technique for tobamoviruses, which is known to the skilled person, and is also for example described in Luria et al (2017, *supra*).

To determine resistance phenotypically, seeds of the accessions to be tested are sown in standard seedling trays and at least 10 seedlings are inoculated 4 weeks after sowing. Inoculum is prepared by grounding leaves of tomato plants that were infected with ToBRFV in a 0.01 M phosphate buffer (pH 7.0) mixed with celite. The seedlings are then dusted with carborundum powder prior to gently rubbing the leaf with inoculum. Resistance is scored on a scale of 0-5; the description of the scales of the scores can be found in **Table 3.** Observation of the symptoms on the young tomato plants in the bio-assay is done 14-21 days after inoculation (dai).

ToBRFV resistance is determined by comparison to a control variety known to be ToBRFV susceptible. Examples of ToBRFV susceptible tomato varieties that do not have the resistance conferring *Tom2a* allele of the invention on chromosome 8, to be used as susceptible control, are Livento F1 and Adventure F1. Since no tomato varieties with the ToBRFV resistance of the invention were known before the invention was done, it was not possible to include a resistant control that was known before the present invention was done. However, as a resistant control a plant grown from seed deposited as NCIMB 43637 can be used; a plant grown from this deposit comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8. The test is performed with 10 plants of a certain line, and the average score is taken. The test is performed properly when susceptible (S) controls have an average score that is higher than 3.0, preferably higher than 3.5. Once this average is reached is a correct moment to score the assay.

Table 3: scales ToBRFV resistance scores

Score	Symptoms
0	No symptoms
1	Not clean, a single spot, some minor discoloration
2	Mosaic, clear visible symptoms
3	Severe mosaic, starting deformation in the head
4	Severe mosaic, necrosis on the stem, serious deformation in the head, spots in blisters
5	Dead plant

As used herein, a ToBRFV resistant tomato plant homozygously comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 has an average score of 1.5 or lower than 1.5, preferably a score lower than 1.0, when scoring according to **Table 3** is used.

As used herein, resistance means that replication of the virus is reduced or absent in a plant that is infected with ToBRFV. Reduction of virus replication can be measured by a qPCR test. To determine if a line has reduction or absence of ToBRFV virus replication, the virus titer is determined in leaf samples which are taken from at least 5 plants of that line that are ToBRFV infected. From each plant a leaf punch of 6 mm in diameter is taken and subsequently ground in 500 µl of PBS buffer solution. 50 µl of the resulting suspension is used in a 96-well KingFisher Flex isolation protocol, whereby isolation of the leaf material is done using the innuPREP DNA/RNA virus PLUS Kit. The samples are then analysed in a 96CFX qPCR thermocycler (Biorad) to get a Cq_ToBRFV value, which represents the number of cycles needed to obtain the virus PCR product, using a programme of 5 minutes on 50 ^{0}C and 20 sec. on 95 ^{0}C, followed by 40 cycles of 10 sec. on 95 ^{0}C and 60 sec. on 60 ^{0}C.

To be able to compare the values of samples of different sizes and backgrounds, the *S. lycopersicum* PHD reference gene, a tomato housekeeping gene, is included in the qPCR

assay, which corrects any variation in the amount of sample material, and then yields a Cq_PHD value. The primers used in the assay are given in **Table 1.** The PCR volumes that are used in the assay are given in **Table 2.**

Table 1: ToBRFV and PHD primers for qPCR assay

Primers	Sequence
TBFRV FW 3 (SEQ ID No.49)	GTACATCTTGCTGGTCTTGT
TBFRV RV 3 (SEQ ID No.50)	CGCTCTCTCCATTCTCTTATC
TBFRV P3 (SEQ ID No.51)	FAM-TGGTGGTGTCAGTGTCTGTTTGGT-BHQ1
PHD FW1 (SEQ ID No.52)	TACCAACTCTTCCTCCCATAG
PHD RV1 (SEQ ID No. 53)	TGGATCGCACTGTGAGTA
PHD P1 (SEQ ID No. 54)	TxR 5'-TTCAAAGACTTCTCCGCCCGTCAC-3' BHQ2

Table 2: qPCR assay volumes

PCR-volumes	1x (µl)
4x TaqMan fast virus1-step (Applied Biosystems)	6.25
TBRFV FW3 (20pmol/µl)	0.30
TBRFV RV3 (20pmol/µl)	0.30
TBRFV P3 (20pmol/µl)	0.20
PHD FW1 (20pmol/µl)	0.30
PHD RV1 (20pmol/µl)	0.30
PHD P1 (20pmol/µl)	0.20
Milli Q	15.15
RNA	2.00
Total volume	25

To accurately determine the final value for virus titer the delta-delta Ct method is used, with PHD as a housekeeping gene and ToBRFV as the gene of interest. The final value is the Cq_corr, which is calculated as Cq_ToBRFV – Cq_PHD. A plant is determined to have a reduction of ToBRFV virus replication when the average Cq_corr of at least 5 plant samples is higher than -11.00, or when the average Cq-corr is at least 5.00 higher than the average Cq_corr of a susceptible control (**Example 2, Table 5).**

In one embodiment a resistant plant of the invention that comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein has an average Cq_corr score that is in order of increased preference higher than -11.00 -10.50, -10.00, -9.50, -9.00, -8.50, -8.00, -7.50, or -7.00.

In one embodiment a resistant plant of the invention that comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein has an average Cq_corr score that is in order of increased preference at least 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00 higher than the average value of a susceptible control.

Calculation of an average value as described above is preferably done as an average taken of at least 2 trials. Plants are properly infected with ToBRFV when the susceptible control in a test has an average Cq_corr value of -12 or lower, preferably -13 or lower, most preferably -14 or lower.

A *S. lycopersicum* plant that has the resistance conferring *Tom2a* allele of the invention that leads to ToBRFV resistance can be grown from seed deposited as NCIMB 43637.

NCIMB 43637 has the ToBRFV resistance of the invention and comprises the QTL of the invention on chromosome 8 that is located between SEQ ID Nos. 1 and 42, which comprises the resistance conferring *Tom2a* allele having SEQ ID No. 45. The QTL, and thereby the resistance conferring *Tom2a* allele, is present in deposit NCIMB 43637 in homozygous form. The QTL in NCIMB 43637 is linked to any of the markers represented by the SNP presented in SEQ ID Nos. 1 to 44, and can therefore be identified by any one of the markers represented by the SNP presented in SEQ ID Nos. 1 to 44. The resistance conferring *Tom2a* allele in NCIMB 43637 comprises the polymorphisms presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No, 43, and SEQ ID No. 44. A resistance conferring *Tom2a* allele can in particular be identified by determining the presence of a SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 43, or SEQ ID No. 44.

A plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 can be used as a resistant control variety in a ToBRFV bio-assay or qPCR test. When a plant, line, or population to be assessed shows the same resistance as NCIMB 43637, and this plant, line or population comprises the resistance conferring *Tom2a* alleleas described herein on chromosome 8, this plant, line, or population is considered to have the ToBRFV resistance of the invention and is therefore a plant of the invention.

A plant of the present invention is optionally a cultivated *S. lycopersicum* plant having improved agronomic characteristics that make it suitable for commercial cultivation. The plant is thus an agronomically elite plant.

In one embodiment, a ToBRFV resistant *S. lycopersicum* plant of the present invention does not require a resistance conferring allele of the *Tm-1* gene. In one embodiment, a ToBRFV resistant *S.lycopersicum* plant of the present invention does not require a resistance conferring *TOM1* gene or *TOM3* gene. A ToBRFV resistant *S. lycopersicum* plant of the invention comprising a resistance conferring *Tom2a* allele does not require the presence of a tobamo-resistance-conferring *Tm-1* gene, *TOM1* gene, or *TOM3* gene to show resistance.

In one embodiment, a ToBRFV resistant *S. lycopersicum* plant of the present invention comprises a resistance conferring *Tom2a* allele and a resistance conferring QTL on chromosome 11, as described in co-pending application WO2019110821. Said resistance conferring QTL on chromosome 11 in particular comprises a resistance conferring CCA gene or a resistance conferring *Albino3-like* gene as described in WO2021110855 and co-pending application PCT/EP2021/055008, respectively. The combination of these QTLs, in particular of a resistance conferring *Tom2a* allele with either of said genes, results in a stronger and more durable ToBRFV resistance in *S. lycopersicum*.

The invention also relates to a tomato fruit harvested from a plant of the invention, wherein the tomato fruit comprises the resistance conferring *Tom2a* allele of the invention in its genome, which leads to ToBRFV resistance in the plant. This tomato fruit is also referred to herein as 'the fruit of the invention' or 'the tomato fruit of the invention'. As used herein, a 'tomato fruit' is a fruit produced by a plant of the species *Solanum lycopersicum.*

The present invention relates to a method for producing a ToBRFV resistant *S. lycopersicum* plant comprising introducing a QTL comprising a resistance conferring *Tom2a* allele on chromosome 8 in a *S. lycopersicum* plant lacking said QTL, wherein the QTL region is located between SEQ ID No. 1 and SEQ ID No. 42, and is linked to any of the markers selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably to a marker selected from the group consisting of the SNP presented in SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 14, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No, 34, SEQ ID No. 42, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44.

The present invention relates to a method for producing a ToBRFV resistant *S. lycopersicum* plant comprising introducing a resistance conferring *Tom2a* allele as described herein in a *S. lycopersicum* plant lacking said allele.

The resistance conferring *Tom2a* allele of the invention can be introduced from another plant, which comprises the resistance conferring *Tom2a* allele, through commonly used breeding techniques, such as crossing and selection, when the plants are sexually compatible. Such introduction can be from a plant of the same species, that usually can be crossed easily, or from a plant of a related species. Difficulties in crossing can be overcome through techniques known in the art such as embryo rescue, or cis-genesis can be applied. Suitably markers as described herein are used to follow the incorporation of the resistance conferring *Tom2a* allele into another plant.

The above method can in particular be used to introduce the resistance conferring *Tom2a* allele of the invention into a plant species that is suitable for incorporation of such genetic

information. In a particular embodiment, said resistance conferring *Tom2a* allele can be introduced from a *Solanum pimpinellifolium* plant comprising the resistance conferring *Tom2a* allele, or from another *Solanum* species that is sexually compatible to *S. lycopersicum,* into a *Solanum lycopersicum* plant lacking the resistance conferring *Tom2a* allele, for example by using standard breeding methods comprising crossing and selection. In another embodiment said resistance conferring *Tom2a* allele can be introduced from a *Solanum lycopersicum* plant comprising the resistance conferring *Tom2a* allele into a *Solanum lycopersicum* plant lacking the resistance conferring *Tom2a* allele using standard breeding methods.

The resistance conferring *Tom2a* allele on chromosome 8 can be introduced from a *Solanum lycopersicum* plant representative seed of which was deposited with the NCIMB under deposit number NCIMB 43637, or from the deposited seed of NCIMB 43637, or from progeny of the deposit, which are sexual or vegetative descendants thereof. Introduction of the resistance conferring *Tom2a* allele on chromosome 8 in *Solanum lycopersicum* leads to ToBRFV resistance.

Alternatively, the resistance conferring *Tom2a* allele of the invention can be transferred or introduced from another, sexually incompatible, plant, for example by using a transgenic approach. Techniques that can suitably be used comprise general plant transformation techniques known to the skilled person, such as the use of an *Agrobacterium*-mediated transformation method. Genome editing methods such as the use of a CRISPR/Cas system might also be employed to obtain a plant of the invention, for example by editing an endogenous susceptible *Tom2a* gene to modify it into a resistance conferring *Tom2a* allele. A susceptible *Tom2a* gene can in particular be targeted to induce a mutation resulting in a deletion of Ala on position 105 of SEQ ID No. 48, a conversion of Arg to Gly on position 187 of SEQ ID No. 48, a conversion of Gly to Serine on position 225 of SEQ ID No. 48, or a conversion of Thr to Ala on position 282 of SEQ ID No. 48, or any of those modifications on a corresponding position of a homologous protein. A targeted modification resulting in a combination of those modifications also forms part of the invention. A susceptible *Tom2a* gene can further be edited to obtain a non-functional *Tom2a* protein, which is part of the invention.

The invention further relates to a plant of the invention comprising the resistance conferring *Tom2a* allele of the invention leading to ToBRFV resistance either homozygously or heterozygously. The plant is a plant of an inbred line, a hybrid, a doubled haploid, or a plant of a segregating population. Preferably, the plant of the invention is a non-transgenic plant.

The invention also relates to a *Solanum lycopersicum* seed comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8, wherein the plant grown from the seed is a plant of the invention. In a preferred embodiment, the resistance conferring *Tom2a* allele of the invention is homozygously present in the seed and the plant grown from the seed is resistant to ToBRFV. The invention also relates to seed produced by a plant of the

invention wherein the seed harbors the resistance conferring *Tom2a* allele of the invention, and as such, a plant grown from said seed is a plant of the invention. The invention also relates to use of said seed for the production of a plant of the invention, by growing said seed into a plant. The invention also relates to a plant part of a plant of the invention, which comprises a fruit or a seed, wherein the plant part comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 in its genome.

Moreover, the invention also relates to a food product or a processed food product comprising the tomato fruit of the invention or part thereof. The food product may have undergone one or more processing steps. Such a processing step might comprise but is not limited to any one of the following treatments or combinations thereof: peeling, cutting, washing, juicing, cooking, cooling or a salad mixture comprising the fruit of the invention. The processed form that is obtained is also part of this invention.

The invention also relates to propagation material suitable for producing a *Solanum lycopersicum* plant of the invention, wherein the propagation material is suitable for sexual reproduction, and is in particular selected from a microspore, pollen, an ovary, an ovule, an embryo sac, and an egg cell; or is suitable for vegetative reproduction, and is in particular selected from a cutting, a root, a stem, a cell, a protoplast; or is suitable for tissue culture of regenerable cells, and is in particular selected from a leaf, pollen, an embryo, a cotyledon, a hypocotyl, a meristematic cell, a root, a root tip, an anther, a flower, a seed, and a stem; wherein the plant produced from the propagation material comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein that confers ToBRFV resistance. A plant of the invention may be used as a source of the propagation material.

The invention further relates to a cell comprising the resistance conferring *Tom2a* allele of the invention as defined herein. A cell of the invention can be obtained from, or be present in, a plant of the invention. Such a cell may either be in isolated form, or a part of a complete plant, or from a part thereof, and still constitutes a cell of the invention because such a cell comprises the genetic information that determines the resistance conferring *Tom2a* allele as described herein that leads to ToBRFV resistance of a cultivated *S. lycopersicum* plant. Each cell of a plant of the invention carries the genetic information that leads to ToBRFV resistance. A cell of the invention may also be a regenerable cell that can regenerate into a new plant of the invention. The presence of the genetic information in this context is the presence of the resistance conferring *Tom2a* allele of the invention on chromosome 8, wherein the resistance conferring *Tom2a* allele is as defined herein.

The invention further relates to plant tissue of a plant of the invention, which comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein. The tissue can be undifferentiated tissue or already differentiated tissue. Undifferentiated

tissue is for example a stem tip, an anther, a petal, or pollen, and can be used in micropropagation to obtain new plantlets that are grown into new plants of the invention. The tissue can also be grown from a cell of the invention.

The invention moreover relates to progeny of a plant, a cell, a tissue, or a seed of the invention, which progeny comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein, the presence of which resistance conferring *Tom2a* allele, preferably in homozygous form, leads to ToBRFV resistance. Such progeny can in itself be a plant, a cutting, a seed, a cell, or a tissue.

As used herein, 'progeny' is intended to mean the first and all further descendants, such as an F1, F2, or further generation, from a cross with a plant of the invention, wherein a cross comprises a cross with itself or a cross with another plant, and wherein a descendant that is determined to be progeny comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein that leads to resistance to ToBRFV. The plant of the invention that is used in this cross is optionally a plant grown from seed of deposit NCIMB 43637, or from progeny seed thereof which is a direct or further descendant through crossing a plant grown from the deposited seed with itself or with another plant for one or more subsequent generations, wherein the progeny seed has retained the resistance conferring *Tom2a* allele of the invention on chromosome 8.

Progeny also encompasses a *S. lycopersicum* plant that carries the resistance conferring *Tom2a* allele of the invention on chromosome 8 and is resistant to ToBRFV, and is obtained from the plant, or progeny of a plant, of the invention by vegetative propagation or another form of multiplication.

The invention further relates to a part of a *S. lycopersicum* plant of the invention that is suitable for sexual reproduction, which plant part comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8, which resistance conferring *Tom2a* allele is as defined herein. Such a part is for example selected from the group consisting of a microspore, a pollen, an ovary, an ovule, an embryo sac, and an egg cell.

Additionally, the invention relates to a part of a *S. lycopersicum* plant of the invention that is suitable for vegetative reproduction, which is in particular a cutting, a root, a stem, a cell, or a protoplast that comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8, which resistance conferring *Tom2a* allele is as defined herein. A part of a plant as previously mentioned is considered propagation material. The plant that is produced from the propagation material comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein, the presence of which resistance conferring *Tom2a* allele leads to ToBRFV resistance.

The invention further relates to tissue culture of a plant of the invention, which is also propagation material and which comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8 in its genome, which resistance conferring *Tom2a* allele is as defined herein. The tissue culture comprises regenerable cells. Such tissue culture can be selected or derived from any part of the plant, in particular from a leaf, pollen, an embryo, a cotyledon, a hypocotyl, a meristematic cell, a root, a root tip, an anther, a flower, a seed, or a stem. The tissue culture can be regenerated into a *S. lycopersicum* plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein, wherein the regenerated *S. lycopersicum* plant expresses ToBRFV resistance and is also part of the invention.

The invention additionally relates to the use of a plant of the invention in plant breeding. The invention thus also relates to a breeding method for the development of a cultivated *S. lycopersicum* plant that is resistant to ToBRFV, wherein a plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein is used for conferring said resistance to another plant. Seed being representative of a plant that can be used in plant breeding to develop another plant with ToBRFV resistance was deposited with the NCIMB under accession number NCIMB 43637.

The invention also relates to the use of the QTL of the invention on chromosome 8, in particular to the use of a resistance conferring *Tom2a* allele as defined herein, for the development of a *Solanum lycopersicum* plant that has resistance to ToBRFV.

The invention also relates to a marker for the identification of ToBRFV resistance in a *Solanum lycopersicum* plant, or in a *Solanum pimpinellifolium* plant, or in a plant of another *Solanum* species that is sexually compatible with *Solanum lycopersicum*, which marker is selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 43, and SEQ ID No. 44. Any other marker that is developed based on a polymorphism in the region defined above between SEQ ID No. 1 and SEQ ID No. 42, in particular based on a polymorphism compared to SEQ ID No. 47, is also part of the invention.

The use of any of the markers from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 43, and SEQ ID No. 44, for identification of ToBRFV resistance in a *Solanum lycopersicum* plant, or in a *Solanum pimpinellifolium* plant, or in a plant of another *Solanum* species that is sexually compatible with *Solanum lycopersicum,* is also

part of the invention. Any use of these markers to develop other markers for the identification of the QTL on chromosome 8, in particular for the identification of a resistance conferring *Tom2a* allele leading to ToBRFV resistance, comprising determining any other polymorphism in the region between SEQ ID Nos. 22 and 34, is also part of the present invention.

The present invention also relates to a method for selecting a ToBRFV resistant *Solanum lycopersicum* plant, or a ToBRFV resistant *Solanum pimpinellifolium* plant, or a plant of another *Solanum* species that is sexually compatible with *Solanum lycopersicum* that is ToBRFV resistant, comprising identifying the presence of the resistance conferring *Tom2a* allele of the invention on chromosome 8, and selecting a plant that comprises said resistance conferring *Tom2a* allele as a ToBRFV resistant plant.

Identifying the presence of the resistance conferring *Tom2a* allele of the invention on chromosome 8 is suitably done using at least one of the markers selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 43, and SEQ ID No. 44.

The invention also relates to a method of testing a *Solanum lycopersicum* plant, a *Solanum pimpinellifolium* plant, or a plant of another *Solanum* species that is sexually compatible with *Solanum lycopersicum,* for the presence of the resistance conferring *Tom2a* allele of the invention that confers ToBRFV resistance, comprising detecting the presence of a marker sequence selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 43, and SEQ ID No. 44, in the genome of the plant.

The method of testing a *Solanum lycopersicum* plant, a *Solanum pimpinellifolium* plant, or a plant of another *Solanum* species that is sexually compatible with *Solanum lycopersicum,* for the presence of the resistance conferring *Tom2a* allele of the invention that confers ToBRFV resistance optionally further comprises selecting a *Solanum lycopersicum* plant, a *Solanum pimpinellifolium* plant, or a plant of another *Solanum* species that is sexually compatible with *Solanum lycopersicum,* that comprises said resistance conferring *Tom2a* allele as a ToBRFV resistant plant. The *Solanum lycopersicum* plant, *Solanum pimpinellifolium* plant, or a plant of another *Solanum* species that is sexually compatible with *Solanum lycopersicum,* that is thus selected can subsequently be used as a source for introgressing the ToBRFV resistance conferring *Tom2a* allele into a *S. lycopersicum* plant lacking the resistance conferring *Tom2a* allele.

The invention also relates to a method for the production of a *Solanum lycopersicum* plant which is resistant to ToBRFV, said method comprising:

a) crossing a plant of the invention, which comprises the resistance conferring *Tom2a* allele of the invention on chromosome 8, with another plant;

b) optionally performing one or more rounds of selfing and/or crossing of the plant resulting from the cross to obtain a further generation population;

c) selecting from the population resulting from the cross of step a), or from the further generation population of step b), a plant that comprises the resistance conferring *Tom2a* allele on chromosome 8 as defined herein, which plant is resistant against ToBRFV when the resistance conferring *Tom2a* allele is homozygously present.

The invention also relates to a method for the production of a *Solanum lycopersicum* plant which is resistant to ToBRFV, said method comprising:

a) crossing a first parent plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 with a second parent plant, which is a plant not comprising the resistance conferring *Tom2a* allele of the invention;

b) backcrossing the plant resulting from step a) with the second parent plant for at least three generations;

c) selecting from the third or higher backcross population a plant that comprises at least the resistance conferring *Tom2a* allele on chromosome 8 of the first parent plant of step a).

Selection of a plant comprising the resistance conferring *Tom2a* allele on chromosome 8 is suitably done by using a molecular marker genetically linked to the resistance conferring *Tom2a* allele, which marker is selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 43, and SEQ ID No. 44, for the identification of the resistance conferring *Tom2a* allele on chromosome 8. The plant can alternatively, or in addition, be confirmed to have resistance to ToBRFV, in particular by performing a qPCR test for determining the absence or strong reduction of virus titer in the plant after ToBRFV infection.

In one embodiment, the plant of the invention used in the method for the production of a *Solanum lycopersicum* plant which is resistant against ToBRFV is a plant grown from seed deposited under NCIMB accession numbers NCIMB 43637, or a progeny plant thereof.

The invention additionally provides for a method of introducing another desired trait into a *Solanum lycopersicum* plant comprising ToBRFV resistance, comprising:

a) crossing a *Solanum lycopersicum* plant of the invention comprising the resistance conferring *Tom2a* allele on chromosome 8 with a second *Solanum lycopersicum* plant that comprises the other desired trait to produce F1 progeny;

b) optionally selecting in the F1 for a plant that comprises the resistance conferring *Tom2a* allele and the other desired trait;

c) crossing the optionally selected F1 progeny with either parent, to produce backcross progeny;

d) selecting backcross progeny comprising ToBRFV resistance and the other desired trait; and

e) optionally repeating steps c) and d) one or more times in succession to produce selected fourth or higher backcross progeny that comprises the other desired trait and has resistance to ToBRFV. Backcrossing is optionally done until the backcross progeny is stable and can be used as a parent line, which can be reached after 3 up to 10 backcrosses.

In one embodiment, the plant of the invention used in the method of introducing another desired trait into a *Solanum lycopersicum* plant comprising resistance to ToBRFV is a plant grown from seed deposited under NCIMB accession number NCIMB 43637, or a progeny plant thereof.

Optionally, selfing steps are performed after any of the crossing or backcrossing steps in above described methods. Selection of a plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 that leads to ToBRFV resistance and the other desired trait can alternatively be done following any crossing or selfing step of the method. The other desired trait can be selected from, but is not limited to, the following group: resistance to bacterial, fungal or viral diseases, insect or pest resistance, improved germination, plant size, plant type, improved yield, improved shelf-life, tolerance to water stress, tolerance to salt stress, tolerance to heat stress, and male sterility. The invention includes a *Solanum lycopersicum* plant produced by this method and a tomato fruit obtained therefrom.

The invention further relates to a method for the production of a *Solanum lycopersicum* plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8, wherein the homozygous presence of the resistance conferring *Tom2a* allele leads to resistance to ToBRFV, by using tissue culture or by using vegetative propagation.

The invention further provides a method for the production of a *Solanum lycopersicum* plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 and having resistance to ToBRFV as defined herein by using a doubled haploid generation technique to generate a doubled haploid line that homozygously comprises the resistance conferring *Tom2a* allele of the invention and is resistant against ToBRFV.

The invention further relates to a method for the production of a *Solanum lycopersicum* plant comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein, wherein the presence of said resistance conferring *Tom2a* allele leads to ToBRFV resistance, which method comprises growing a seed comprising said resistance conferring *Tom2a* allele into the said *Solanum lycopersicum* plant. In one embodiment, the seed used in the method is seed deposited with the NCIMB under deposit number NCIMB 43637, or progeny seed thereof.

The invention further relates to a method for seed production comprising growing a *Solanum lycopersicum* plant from a seed of the invention, allowing the plant to produce a tomato fruit with seed, harvesting the tomato fruit, and extracting those seed. Production of the seed is suitably done by crossing with itself or with another plant that is optionally also a plant of the invention. The seed that is so produced has the capability to grow into a plant that comprises the resistance conferring *Tom2a* allele of the invention. In a preferred embodiment in the plants used in seed production the resistance conferring *Tom2a* allele is homozygously present.

The invention further relates to hybrid seed and to a method for producing said hybrid seed, comprising crossing a first parent plant with a second parent plant and harvesting the resultant hybrid seed, wherein the first parent plant and/or the second parent plant are a plant of the invention comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 as defined herein. The resulting hybrid seed and the hybrid plant that can be grown from the hybrid seed is also a part of the invention. In a preferred embodiment both parents plants comprise the resistance conferring *Tom2a* allele of the invention and the hybrid seed comprises the resistance conferring *Tom2a* allele of the invention homozygously.

Introgression of the resistance conferring *Tom2a* allele of the invention on chromosome 8 as used herein comprises introduction of the resistance conferring *Tom2a* allele from a donor plant comprising said resistance conferring *Tom2a* allele into a recipient plant not carrying said resistance conferring *Tom2a* allele, or carrying the resistance conferring *Tom2a* allele heterozygously, by standard breeding techniques. Breeding methods such as crossing and selection, backcrossing, recombinant selection, or other breeding methods that result in the transfer of a genetic sequence from a resistant plant to a susceptible plant can be used. Selection for plants comprising the resistance conferring *Tom2a* allele of the invention can be performed by doing a qPCR test, or by doing a bio-assay by means of observation of the resistance to ToBRFV, or selection can be performed with the use of markers as defined herein, preferably a marker selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No.

43, and SEQ ID No. 44, through marker assisted breeding, or combinations of these selection methods. The donor plant can be a *Solanum lycopersicum* plant, a *Solanum pimpinellifolium* plant, or a plant of another *Solanum* species that is sexually compatible with *S. lycopersicum*. Selection is started in the F1 or any further generation from an initial cross between the recipient plant and the donor plant, followed by either further crossing with itself or with another plant, suitably by using markers as identified and defined herein.

The present invention will be further illustrated in the Examples that follow and that are for illustration purposes only. The Examples are not intended to limit the invention in any way. In the Examples and the application reference is made to the following figures.

FIGURES

Figure 1 - Nucleotide sequences of **SEQ ID Nos. 1 to 44**, including the SNP indicative for ToBRFV resistance.

Figure 2 – variation in virus titer in susceptible lines, the resistant donor, and F2 populations based on qPCR observation

Figure 3 – qPCR results of the deposits

Figure 4 – identification of *Tom2a* as the causal gene for ToBRFV resistance, by marker analysis combined with phenotyping of recombinant lines.

Figure 5 – CDS sequences *Tom2a* genes **SEQ ID No. 45:** GNL_R (resistant), resistance conferring allele from GNL.3951, as present in NCIMB 43637; **SEQ ID No. 47:** reference genome SL3_00 (susceptible); individually and alignment

Figure 6 – Tom2a protein sequences **SEQ ID No. 46:** protein encoded by resistance conferring *Tom2a* allele; **SEQ ID No. 48:** wildtype protein, susceptible; encoded by reference sequence of SL3_00; individually and alignment

Figure 7 – Average qPCR results showing virus titer (Cq_corr) of plants having the susceptible wildtype of a line used as susceptible parent in a cross developing isogenic lines, and plants of these isogenic lines having a QTL on chr 11, having the QTL that comprises the resistance conferring *Tom2a* allele of the present invention on chr 8, having the combination of QTLs on chr 8 and 11, and lacking both QTLs

Figure 8 – Prediction of domains and membranes of the Tom2a protein

DEPOSIT

Seeds of tomato *Solanum lycopersicum* population 20R.1552Q08_04, comprising the resistance conferring *Tom2a* allele of the invention on chromosome 8 homozygously, were deposited with NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, UK on 14 July, 2020 under deposit accession number NCIMB 43637.

EXAMPLES

EXAMPLE 1

Mapping of the ToBRFV *resistance QTL on chromosome 8*

It was observed that BC3F2 populations that were earlier made with *S. pimpinellifolium* source GNL.3951 and an internal breeding line TB2 had a good phenotypic resistance level, but they were segregating for virus titer level when a qPCR was done. The source itself had a low virus titer after infection (**Example 2, Figure 2, Table 5**), so apparently not the complete resistance mechanism was transferred during the breeding process. Since the resistance was expected to be more reliable when virus replication is restricted, it was decided to cross the BC3F2 again with the donor plant to obtain a new F1. Subsequently F2 populations were made from 3 F1 plants, and again a qPCR observation was done to determine virus titer in the plants after infection with ToBRFV. Two of the populations were observed to have a virus titer level comparable to the source, which meant the plants did not only have no symptoms, but also virus replication in the plant was strongly reduced. However, the third population showed a lot of segregation in the qPCR test **(Example 3, Figure 2)**.

This observation was followed up with an extensive marker analysis to determine which genetic regions were still heterozygous, and to identify differences between the three populations. Through this, it appeared that a region on chromosome 8 might be responsible for the restriction in virus replication, and the better resistance level. This region was fixed for the two F2 populations that showed low virus titer, but was still segregating for the population that segregated for virus presence in the plant after infection. Through further fine-mapping, this QTL region on chromosome 8 could be further narrowed down. Identification of recombinants within the original QTL initially resulted in a smaller region of approximately 1.5 Mbp, located between SEQ ID No. 1 and SEQ ID No. 42. Within this region, a number of polymorphic SNP markers that were linked to the QTL were identified.

Polymorphic SNP markers that were identified in this analysis and that are present in the QTL region are presented in **Table 4**. The exact physical positions of the SNPs as based on the public SL3_00 tomato map can also be found in **Table 4.** The sequences of these markers, i.e. the SNP that indicates resistance combined with a number of surrounding nucleotides to be used in marker design, are given in **Figure 1**. These markers, in particular the SNPs in these markers, were determined to co-segregate with the QTL. The markers can be used to identify the presence of the QTL in plants grown from the deposits or in progeny thereof. These markers can further be used to identify the presence of the QTL of the invention for ToBRFV resistance on chromosome 8 in any other *S. lycopersicum* population that comprises the QTL. In addition, the markers can be used to identify resistance in *S. pimpinellifolium* or another *Solanum* species.

Further observations were done on F2 and F3 plants that were selected to have the QTL homozygously, which confirmed that the presence of the QTL resulted in a disease score averaging 0 – 1, occasionally towards 1.5, according to the scale described in **Table 3**, and a low virus titer when observed through a qPCR analysis. Further backcrosses were made with the breeding lines as recurrent parents, and also in subsequent selfed populations of the backcross generations the homozygous presence of the QTL was found to result in plants that were resistant to ToBRFV.

Table 4

SNP markers – nucleotides and physical positions

Marker sequence (Fig. 1)	Nucleotide(s) of the SNP in Fig. 1, to be used as marker of the invention	Nucleotide(s) of the SNP in the wildtype (susceptible allele)	physical position of the SNP on the public SL3_00 genome map (wildtype) (in bp)	Type of polymorphism [wildtype/resistance]
SEQ ID No. 1	**C**	T	60.940.159	Substitution [T/C]
SEQ ID No. 2	**T**	C	60.974.766	Substitution [C/T]
SEQ ID No. 3	**A**	G	60.975.712	Substitution [G/A]
SEQ ID No. 4	**T**	C	60.977.552	Substitution [C/T]
SEQ ID No. 5	**A**	G	60.982.084	Substitution [G/A]
SEQ ID No. 6	**A**	C	61.034.283	Substitution [C/A]
SEQ ID No. 7	**T**	G	61.080.755	Substitution [G/T]
SEQ ID No. 8	**C**	T	61.080.770	Substitution [T/C]
SEQ ID No. 9	**C**	G	61.080.791	Substitution [G/C]
SEQ ID No. 10	**T**	G	61.081.880	Substitution [G/T]
SEQ ID No. 11	**G**	C	61.081.909	Substitution [C/G]
SEQ ID No. 12	**G**	A	61.098.941	Substitution [A/G]
SEQ ID No. 13	**C**	G	61.124.509	Substitution [G/C]
SEQ ID No. 14	**A**	G	61.124.521	Substitution [G/A]
SEQ ID No. 15	**G**	C	61.124.616	Substitution [C/G]
SEQ ID No. 16	**T**	C	61.136.984	Substitution [C/T]
SEQ ID No. 17	**G**	A	61.153.365	Substitution [A/G]
SEQ ID No. 18	**G**	T	61.153.405	Substitution [T/G]
SEQ ID No. 19	**A**	G	61.154.280	Substitution [G/A]
SEQ ID No. 20	**A**	C	61.167.805	Substitution [C/A]
SEQ ID No. 21	**A**	G	61.167.956	Substitution [G/A]
SEQ ID No. 22	**T**	C	61.175.582	Substitution [C/T]
SEQ ID No. 23	**G**	A	61.187.909	Substitution [A/G]
SEQ ID No. 24	**T**	G	61.192.735	Substitution [G/T]
SEQ ID No. 25	**C**	G	61.202.144	Substitution [G/C]
SEQ ID No. 26	**C**	T	61.251.554	Substitution [T/C]
SEQ ID No. 27	**C**	A	61.252.348	Substitution [A/C]

SEQ ID No. 28	**TGCT**	TGCCGCT	61253155 - 61253161	Deletion [GCC/***]
SEQ ID No. 29	**C**	T	61.253.498	Substitution [T/C]
SEQ ID No. 30	**TGA**	TGGA	61254440 - 61254443	Deletion [G/*]
SEQ ID No. 31	**T**	C	61.257.969	Substitution [C/T]
SEQ ID No. 32	**T**	C	61.258.594	Substitution [C/T]
SEQ ID No. 33	**T**	A	61.258.680	Substitution [A/T]
SEQ ID No. 34	**C**	T	61.268.020	Substitution [T/C]
SEQ ID No. 35	**G**	A	61.319.325	Substitution [A/G]
SEQ ID No. 36	**G**	A	61.320.559	Substitution [A/G]
SEQ ID No. 37	**T**	A	61.330.646	Substitution [A/T]
SEQ ID No. 38	**G**	T	61.331.331	Substitution [T/G]
SEQ ID No. 39	**A**	G	61.339.395	Substitution [G/A]
SEQ ID No. 40	**A**	T	61.341.448	Substitution [T/A]
SEQ ID No. 41	**T**	A	61.380.617	Substitution [A/T]
SEQ ID No. 42	**G**	A	61.388.756	Substitution [A/G]
SEQ ID No. 43	**C**	T	61.250.851	Substitution [T/C]
SEQ ID No. 44	**T**	C	61.251.440	Substitution [C/T]

The positions of the SNPs are present on position 101 of the SEQ ID Nos., as found in **Figure 1**, where they are bold and underscored. For SEQ ID No. 28 the polymorphism to be used as marker is on positions 101-104. For SEQ ID No. 30 the polymorphism to be used as marker is on positions 101-103. In this way, a deletion of GCC and a deletion of G, respectively, after position 101 when compared to the wildtype sequence can be identified.

SEQ ID Nos. 28 and 30 are technically speaking not SNPs, as they are not Single Nucleotide Polymorphisms, but InDels. SEQ ID No. 28 has a deletion of nucleotides TGCC starting on position 61.253.155 of the sequence of the wildtype in the public reference genome, which is replaced by a T, leading to an actual deletion of GCC. Positions corresponding to 61.253.155 to 61.253.161 in the wildtype sequence are TGCCGCT, whereas the resistant line has TGCT, i.e. there is a deletion of GCC, as found on positions 101-104 of SEQ ID No. 28. SEQ ID No. 30 has a deletion of nucleotides TG starting on position 61.254.440 of the sequence of the wildtype in the public reference genome, which is replaced by a T, leading to an actual deletion of G. Positions corresponding to 61.254.440 to 61.254.443 in the wildtype sequence are TGGA, whereas the resistant line has TGA, i.e. there is a deletion of G, as found on positions 101-103 of SEQ ID No. 30. For reasons of simplicity, these InDel polymorphisms are also referred to as SNPs in the context of this invention.

EXAMPLE 2

qPCR test of ToBRFV resistant sources

Sources of phenotypic ToBRFV resistance were infected with ToBRFV and subsequently observed for virus titer. This was done to determine if the resistance was only phenotypic, so there were no symptoms but the virus was still replicating, and the source could therefore be called tolerant, or if the virus titer was really strongly reduced or even absent, and the source was therefore really resistant.

Measuring the virus titer was done through carrying out a qPCR test. In this test, leaf punch samples having around 6 mm in diameter were taken from ToBRFV infected plants. These samples were ground in 500 µl of PBS buffer solution, and 50 µl of the resulting suspension was used in a 96-well KingFisher Flex isolation protocol. Isolation of the leaf material was done using the innuPREP DNA/RNA virus PLUS Kit. The samples were analysed in a 96CFX qPCR thermocycler (Biorad), using a programme of 5 minutes on 50°C and 20 sec. on 95°C, followed by 40 cycles of 10 sec. on 95°C and 60 sec. on 60°C. The number of cycles that is necessary to obtain the PCR product is a measurement for the amount of virus that is present in the sample (Cq_ToBRFV). To be able to compare samples having different genetic backgrounds and having different sample sizes, a tomato reference gene, the PHD gene, was included in each PCR run to correct the outcome (Cq_PHD). The final value for each sample, the Cq_corr, is calculated as Cq_ToBRFV – Cq_PHD = Cq_corr. Because the Cq_PHD is usually between 25 and 29, a sample with a very high virus titer that has for example a Cq_ToBRFV of 7 would result in a final Cq_corr of around -20. A sample with a low virus titer would result in a Cq_corr of around -10 to 0.

The primers used in the assay are given in **Table 1.** The PCR volumes that are used in the assay are given in **Table 2.**

From five different trials samples of two internal breeding lines (TO1 and TB2), and two sources having phenotypic resistance, i.e. they had no symptoms in bio-assays, were collected. In each trial 5 to 10 plants were included per line. A qPCR analysis was done on each of these samples to determine the virus titer after infection. The first trial, Jordan_Winter, was a field trial which was actively inoculated. The other trials were done under controlled conditions, whereby seedlings were inoculated and scored. Some variation was observed between the results of each genotype in different trials, but there was an extremely good correlation between virus titer and genotypes (**Table 5**). The two tolerant lines scored comparable and had average Cq_corr values of between -14.94 and -19.94. The symptomless source GNL.3919 clearly had to be considered as a tolerant source, since the virus titer was not much lower than in the susceptible breeding lines, with values of between -11.70 and -17.19. The other source, GNL.3951 however scored very convincing and consistent low levels of virus titer in all trials, with average Cq_corr values per trial of between -0.40 and -8.75. It was therefore confirmed that for true resistance,

having reduced replication of the virus in the plant, the genetics of GNL.3951 would have to be used.

Table 5 – Average Cq_corr scores over trials

Trial	Jordan_Winter	19TBRFV34	19TBRFV46	20TBRFV05	20ToBRFV12
Line	Av. Cq_Corr	Av. Cq_Corr	Av. Cq_Corr	Av. Cq_Corr	Av. Cq_Corr
TO1	-16.07	-14.94	-15.43	nd	-18.86
TB2	-17.95	-15.13	-15.40	-15.69	-19.94
GNL.3951	-1.15	-5.38	-8.75	-0.40	-4.47
GNL.3919	-17.19	-11.70	-14.01	-14.78	nd

EXAMPLE 3

qPCR test of ToBRFV backcross and F2 populations and the deposits

As described in Example 1, initially BC3F2 populations were made between GNL.3951 and susceptible *S. lycopersicum* line TB2. Virus titer was determined in samples from bio-assays for ToBRFV on these populations, and it was found the titer was not comparably to the GNL.3951 source. Therefore a new cross was made between the BC3F2 and GNL.3951, and this F1 was selfed to obtain F2 populations.

Again, three of the F2 populations were infected with ToBRFV to observe virus symptoms. In these three F2 populations, resulting from three F1 plants, phenotypic virus resistance was found to be present. However, when a qPCR assay was done to determine virus titer, one of the F2 populations showed clear segregation in virus titer, while the other two had virus titer levels comparable to the resistant source (**Figure 2**). It was concluded that the true resistance, i.e. the reduction of virus replication, could not necessarily be determined through a bio-assay, but had to be confirmed with a test determining virus titer. It was also confirmed that it was possible to obtain true resistance, i.e. reduction of virus titer, in *S. lycopersicum* plants.

From one of the F2 populations that did not segregate for virus titer in the qPCR, F3 populations were obtained through selfing. Using the parallel developed markers from the QTL analysis, as described in **Example 1**, plants were confirmed to have the QTL on chromosome 8 linked to the markers of **Table 4**. Again, plants were infected with ToBRFV and a qPCR analysis for virus titer was done. It was confirmed that also the F3 populations had a reduction of virus replication, so the resistance was maintained in these plants (**Figure 3**). The markers developed for the QTL on chromosome 8 were found to co-segregate with the reduction of virus titer, and can therefore be used to identify the reduction in virus titer. One of these F3 populations, 20R.1552Q08_4, was subsequently deposited for this invention, as NCIMB 43637.

EXAMPLE 4

Identification of Tom2a as the causal gene for ToBRFV resistance

To determine the actual gene within the QTL region on chromosome 8 that conferred the ToBRFV resistance, first a further fine-mapping step was carried out with recombinants, i.e. lines that had recombinations in the QTL region, derived from the originally used lines. After phenotyping of the recombinants, the involved QTL region was shortened to a region between the markers presented as SEQ ID No. 22 and SEQ ID No. 34. Within this region, seven putative genes were present (**Figure 4**).

Initially the markers having SEQ ID No, 26, SEQ ID No. 29, and SEQ ID NO. 30 were identified as the most preferred, and these markers were present within one of those genes, identified as Solyc08g077220.3 in the SI3_00 genome, which is named *Tom2a.* The goal was to confirm this gene as the resistance conferring gene in the material. The resistance is conferred by the allele of the gene as disclosed herein.

In the newly developed set of lines, again selfings were made to obtain further recombinants. These recombinants were analyzed with 13 markers that were present in the region (SL001 – SL013 of **Figure 4**), and it was found that the population included plants having recombinations on both sides of the *Tom2a* gene. A recombination between SL004 and SL005 separated the genes left of Solyc08g077220.3 from the region. A recombination between SL009 and SL010 separated Solyc08g077220.3 from Solyc08g077230.3. Phenotyping of these recombinants was done to determine if indeed the resistant phenotype could be linked to the presence of the *Tom2a* gene.

Figure 4 shows the marker profiles and phenotyping scores of the recombinant lines. The indicated genes within the region are identified based on the annotation of the public ITAG3.2 reference, which can for example be found on the solgenomics website. The phenotypes of all 12 recombinants, except 07, deviated from what would be expected if the cluster of genes left from SL005 would contain the causal gene. These genes could therefore be ruled out. The phenotype of recombinant 07 was in line with the expectation of Solyc08g077220.3, i.e. *Tom2a,* being the causal gene for the ToBRFV resistance. Through these observations, it was indeed confirmed that the allele of the *Tom2a* gene in the resistant material was causing the ToBRFV resistance in the tomato lines.

EXAMPLE 5

Virus titer of material with the QTL on chr 8 and a QTL on chr 11

Isogenic lines were made through development of a BC2F3 population. A line having the QTL of the invention which comprises the resistance conferring *Tom2a* allele, a line having a QTL on chromosome 11 as described in WO2019110821, a line having the combination

of the QTLs on chromosomes 8 and 11, and a line having the susceptible version of the QTLs on chromosomes 8 and 11, were inoculated and analysed, together with the susceptible parent that was used in the cross for the development of the isogenic BC2F3 lines. The QTL on chromosome 11 comprised a resistance conferring CCA gene and/or a resistance conferring *Albino3-like* gene. After inoculation, qPCR analysis for virus titer was performed, as described in **Example 2**.

Results are presented in **Figure 7**. It was found that the reduction of virus titer in the line having the resistance conferring *Tom2a* allele (4_IL_QTL08) was even further reduced when it was combined with the QTL on chromosome 11 (5_IL_QTL08+11). This was a very surprising effect, since a line with only the QTL on chromosome 11 (3_IL_QTL11) had limited reduction of virus titer. The isogenic line lacking both resistance QTLs (2_IL_Wildtype) had a similar virus titer as the susceptible parent (1_Original line). Therefore, it was concluded that a stronger and more durable resistance in tomato can be obtained by combining the QTLs of chromosomes 8 and 11. A stronger and more durable ToBRFV resistance can in particular be obtained through a combination of a resistance conferring *Tom2a* allele combined with a resistance conferring CCA gene or combined with a resistance conferring *Albino3-like* gene.

CLAIMS

1. A *Solanum lycopersicum* plant comprising a QTL on chromosome 8, wherein the QTL on chromosome 8 is located between SEQ ID No. 1 and SEQ ID No. 42, which QTL confers ToBRFV resistance when homozygously present.

2. A *Solanum lycopersicum* plant as claimed in claim 1, wherein the QTL comprises a resistance conferring *Tom2a* allele, which resistance conferring *Tom2a* allele comprises SEQ ID No. 45, or comprises a homologous *Tom2a* sequence having at least 70% sequence identity to SEQ ID No. 45.

3. A *Solanum lycopersicum* plant as claimed in claim 1 or 2, wherein the presence of the QTL on chromosome 8 is genetically linked to at least one of the markers selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 14, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No, 34, SEQ ID No. 42, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44.

4. A *Solanum lycopersicum* plant as claimed in claim 1, 2 or 3, wherein the QTL is as present in the genome of a *Solanum lycopersicum* plant representative seed of which was deposited with the NCIMB under deposit number NCIMB 43637.

5. A *Solanum lycopersicum* plant as claimed in claim 1, 2 or 3, wherein the QTL is introgressed from NCIMB 43637 or from a progeny plant thereof.

6. A *Solanum lycopersicum* plant as claimed in claim 1, 2 or 3, wherein the QTL is introgressed from a plant of the species *Solanum pimpinellifolium,* or from a plant of another *Solanum* species that is sexually compatible with *S. lycopersicum.*

7. A *Solanum lycopersicum* plant as claimed in any of the claims 1-6, wherein the QTL is present homozygously.

8. A cell of a *Solanum lycopersicum* plant as claimed in any one of the claims 1-7, which cell comprises the QTL on chromosome 8 as defined in any one of the claims 1-3 in its genome.

9. A *Solanum lycopersicum* seed comprising the QTL on chromosome 8 as defined in any of the claims 1-4, wherein a plant grown from the seed is a plant as claimed in any one of the plants 1-7.

10. Propagation material suitable for producing a *Solanum lycopersicum* plant as claimed in any one of the claims 1-7, wherein the propagation material is suitable for sexual

reproduction, and is in particular selected from the group consisting of a microspore, pollen, an ovary, an ovule, an embryo sac, and an egg cell; or is suitable for vegetative reproduction, and is in particular selected from the group consisting of a cutting, a root, a stem, a cell, and a protoplast; or is suitable for tissue culture of regenerable cells, and is in particular selected from the group consisting of a leaf, pollen, an embryo, a cotyledon, a hypocotyl, a meristematic cell, a root, a root tip, an anther, a flower, a seed, and a stem; wherein the plant produced from the propagation material comprises the QTL on chromosome 8 that leads to ToBRFV resistance as defined in any one of the claims 1-4.

11. Marker for the identification of ToBRFV resistance in a *Solanum lycopersicum* plant, or in a *Solanum pimpinellifolium* plant, or in a plant of another *Solanum* species that is sexually compatible with *S. lycopersicum*, which marker is selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 28, SEQ ID No. 43, and SEQ ID No. 44.

12. Marker for the identification of a QTL on chromosome 8 that leads to ToBRFV resistance in a *Solanum lycopersicum* plant, or in a *Solanum pimpinellifolium* plant, or in a plant of another *Solanum* species that is sexually compatible with *S. lycopersicum*, which marker is selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 29, and SEQ ID No. 30.

13. Use of a marker as claimed in claim 11 or 12 for identification of ToBRFV resistance in a *Solanum lycopersicum* plant, or in a *Solanum pimpinellifolium* plant, or in a plant of another *Solanum* species that is sexually compatible with *S. lycopersicum*.

14. Method for producing a ToBRFV resistant *Solanum lycopersicum* plant comprising introgressing a QTL on chromosome 8 as defined in any of the claims 1-4 into a *S. lycopersicum* plant.

15. Method for selecting a ToBRFV resistant *Solanum lycopersicum* plant, or a *Solanum pimpinellifolium* plant, or a plant of another *Solanum* species that is sexually compatible with *S. lycopersicum*, comprising identifying the presence of a QTL on chromosome 8 as defined in any of the claims 1-4, and selecting a plant that comprises said QTL as a ToBRFV resistant plant.

16. Method as claimed in claim 15, wherein identifying the presence of the QTL on chromosome 8 is done by using a marker selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 14, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No, 34, SEQ ID No. 42, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44.

17. A method for the production of a *Solanum lycopersicum* plant which is resistant to ToBRFV, said method comprising:

a) crossing a plant as claimed in any one of the claims 1-6 comprising the QTL on chromosome 8 with another plant;

b) optionally performing one or more rounds of selfing and/or crossing of the plant resulting from the cross in step a) to obtain a further generation population;

c) selecting from the plant resulting from the cross in step a), or from the further generation population of step b), a plant that comprises the QTL on chromosome 8, as defined in any one of the claims 1-4, which plant is resistant against ToBRFV when the QTL is homozygously present.

18. Method as claimed in claim 17, wherein selection of a plant comprising the QTL on chromosome 8 is done by using a molecular marker genetically linked to the QTL, which marker is selected from the group consisting of the SNP presented in SEQ ID Nos. 1 to 44, preferably from the group consisting of the SNP presented in SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 14, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No, 34, SEQ ID No. 42, SEQ ID No. 43, and SEQ ID No. 44, most preferably by a marker selected from the group consisting of the SNP presented in SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 43, and SEQ ID No. 44.

19. Method as claimed in claim 17, wherein the plant which is resistant to ToBRFV is phenotypically selected, in particular by using a bio-assay for ToBRFV resistance, or is selected using a qPCR test.

20. Method as claimed in any one of the claims 17-19, wherein the plant as claimed in any one of the claims 1-7 is a plant grown from seed deposited under NCIMB accession number NCIMB 43637, or a progeny plant thereof.

21. Method for the production of a *Solanum lycopersicum* hybrid seed comprising crossing a first parent plant with a second parent plant and harvesting the resultant hybrid seed,

wherein the first parent plant and the second parent plant are a plant of the invention that is resistant to ToBRFV comprising the QTL on chromosome 8 as defined in any of the claims 1-4, wherein the presence of the QTL leads to resistance to ToBRFV in the hybrid plant that is grown from the seed.

22. The hybrid seed produced by the method of claim 21.

23. Resistance conferring *Tom2a* allele comprising SEQ ID No. 45, or comprising a homologous *Tom2a* sequence having at least 70% sequence identity to SEQ ID No. 45.

24. Resistance conferring *Tom2a* allele as claimed in claim 23 which encodes a protein comprising SEQ ID No. 46, or encodes a homologous protein having at least 70% sequence identity to SEQ ID No. 46.

25. Resistance conferring *Tom2a* allele as claimed in claim 23 or 24, wherein the homologous sequence encodes a protein comprising at least one of a deletion of Ala on position 105 of SEQ ID No. 48, a conversion from Arg to Gly on position 187 of SEQ ID No. 48, a conversion from Gly to Ser on position 225 of SEQ ID No. 48, or a conversion from Thr to Ala on position 282 of SEQ ID No. 48.

Fig. 1-1

SEQ ID No. 1

TGTAAACTTAACATTAATGTTTACTTAGATATTTTAACTAGATTTAGACACCTAATGTCAGATTTCAGTATGTTAT
TTTGACACTTTTTTACACCATCAA**C**AAAAATATATAATGTAAATATGTTATACTTATTAATAACGTGTAAAATATC
AAACTAAATGATAACACTTATCATTTTGTGTCCAAAATAATACTAAAAT

SEQ ID No. 2

TTTTAYATATTTCACTTAAGGCACCAATGGATCAACACCTTCATATGTATATCACCCAAATTTCTCAATTGACGGC
GGTGAATATTTGCACTTTAGACCT**T**TGCAAGTCATTTCCTTATTCTGTYGACACTACATTAAAAATGATTTTTAGT
GTCAATTAATTAACGGTAATACTTTCGAAATATATATTTTTAGCAGCAA

SEQ ID No. 3

TTCATCGTATTAATATGCAAAAAAAKTAAATTTATAATACTTTTTCTATAATTTTTGAAAATCTAATTATATTTTTT
AAATATCAAATTAATATAATCTA**A**TTTAATTTTGAAAAATTAATCAAATTGACTTTYAATAAGTGCAACATGATA
CTTAAAAAGTGGATGGAAGAAGTAATCAACTGCAACGGTATGTTTGGAA

SEQ ID No. 4

GATTACTTGTCTAATTAATTATTGATATAATTTTAAGTAGTTTACGAACTAGAGAGTATATTTAAAATAAATAAT
TATTTACTAAAAGAGATTCACTCAA**T**ATAAACTATAAATAACAAGATTACAACATGAAGACGTGCAAAGATGTT
AGGCCTTATGATAAAGAATAAGGAATAAAATCTTTTTTAGCATTTCGTTTAG

SEQ ID No. 5

TTAACTAAACAMWTAAAATGTATTTAAAACTAGCTATTCCTTTTAATCAATGTATAATATATGTGACAGTATTA
AACTGGACATAAGATATAAGAAATAT**A**AAAARACTTAGCAATGTTTTAAAATATCTTTAAAAGAATATATAAAT
ATTTGTGATTATTTTGAGCAAATAAATGGTAAACATAGATGGAATATTGATAA

SEQ ID No. 6

AATCAAAATTTTATAAAATTGAACCGAAGAATCAAAAGTCCACTCCTAGTCTTAGGGTCTAAAGARTTTTTTGTA
GGGGAGAATTTGTGGGAGTGAATCT**A**TTTGTACTTCGAGCGAATTGTGTGAGCTTAYTCCTTTTTATCATTTGG
ACTTTTTTTAATAAAGTGGATTGTTCATCTCCTCTYATCAAGTGACCGTATC

SEQ ID No. 7

ARTCATTTTGTTTTATGAGATATAGAAATAAAATTTAATTATATTTTATTCTTCTCARATTCTACGTGTAAAATTA
CCATAAATATAACTATTATTAAGA**T**TGTTTAAGAGAAGAYGTAATGACTTTGCACAAAGTSGTTGTTAATATAA
GGATCCACTTGTCCTCGAACGTAATTCATTGTCAAAATTTTGTCTCTATTT

SEQ ID No. 8

TGAGATATAGAAATAAAATTTAATTATATTTTATTCTTCTCARATTCTACGTGTAAAATTACCATAAATATAACTA
TTATTAAGAKTGTTTAAGAGAAGA**C**GTAATGACTTTGCACAAAGTSGTTGTTAATATAAGGATCCACTTGTCCTC
GAACGTAATTCATTGTCAAAATTTTGTCTCTATTTTCAAACGCGCTTAGA

SEQ ID No. 9

AATTATATTTTATTCTTCTCARATTCTACGTGTAAAATTACCATAAATATAACTATTATTAAGAKTGTTTAAGAGA
AGAYGTAATGACTTTGCACAAAGT**C**GTTGTTAATATAAGGATCCACTTGTCCTCGAACGTAATTCATTGTCAAA
ATTTTGTCTCTATTTTCAAACGCGCTTAGATGCRAGTTTAATTTACCAACT

Fig. 1-2

SEQ ID No. 10

TCATTTTTTAAAATAATATTTATATATTTRTAAATTATGTAAAAAGATAATATAAGTCAAAATAATTGGCAATTCA
AAATGGTTGAAAGATATATAAAAA**T**TTCACGATAAAAATAATAATTGAAATCTSAACATCCAAAAAAAATCACA
TAATTAAGACGGAGGAGAAAATATCATCTTTTTAAATTTCAATTATATAAC

SEQ ID No. 11

RTAAATTATGTAAAAAGATAATATAAGTCAAAATAATTGGCAATTCAAAATGGTTGAAAGATATATAAAAAKTT
CACGATAAAAATAATAATTGAAATCT**G**AACATCCAAAAAAAATCACATAATTAAGACGGAGGAGAAAATATCA
TCTTTTTAAATTTCAATTATATAACTATACTGAATATATTATTATTACCACGGT

SEQ ID No. 12

TTTGTTTTGATTGTATCGTATAGTATTATAATTTATAAATTTASTAAAATATCGTTAATCATTCTAGGGAATGAGG
TTTGACTAGATTTAAATAATTAAG**G**TAAAGGGTAAAATAGTATTTTGAAATATTACGTAAAGATATAATTGGAA
AAAGAAATTAAGTAACAATGCGAAAACACCAAATCGGTTGTTCCATAAAAT

SEQ ID No. 13

AAAAATTCTTTGAATGAAGTAGAATTCTAWATGATAAAAATTTAAAATAATTAAATTTTAATGAAATACACTGA
CAAATAGGGCTGTGTATCGATCGATT**C**GGTTTAATTTTRAAGTTTATCGGATTGGCTTATTGGTTATYGGTTTAT
AATGATGCTAAATCATTATAGAACCATTAARATATTGACTTATCGGTTATTG

SEQ ID No. 14

AATGAAGTAGAATTCTAWATGATAAAAATTTAAAATAATTAAATTTTAATGAAATACACTGACAAATAGGGCT
GTGTATCGATCGATTSGGTTTAATTTT**A**AAGTTTATCGGATTGGCTTATTGGTTATYGGTTTATAATGATGCTAA
ATCATTATAGAACCATTAARATATTGACTTATCGGTTATTGGTTTATSGGTTT

SEQ ID No. 15

ATTTTRAAGTTTATCGGATTGGCTTATTGGTTATYGGTTTATAATGATGCTAAATCATTATAGAACCATTAARAT
ATTGACTTATCGGTTATTGGTTTAT**G**GGTTTTTGATCGTTATCGGTTTAACCGTTAAGATTTGAMTCAAAARAA
AAAATATTGAAAATCACTTAGAAACAAGGTGACAAATCAAATAAACCATGCA

SEQ ID No. 16

TTTTAATTTAATATTGGTATATATAAAAGTTGGACCTCCTAAAATAATTTAGTAAGACACAATAGAGTTGGCAAT
AGCTAGGGCATCCCTACACTTTGTC**T**GGGTTTGATTTCCCGCGGGATGTTTCATTTTTCTTTAATTATTTTTGACA
ATTTTATTATTAAAAAAACATGCTTASTCTTAACATAAACATTTAGTAGT

SEQ ID No. 17

CACAAATTATTAGATTCATTTCTGAACTATTAACAGCCTTGAAAACACCTCTCTACTAAYATAGCAAGCGATCTC
AAACTCTTATATGAAYATGAGACTT**G**TAATAAAAAGCTGAGAGAAGTGTTGAAAACACTCCTAAAKTAGTGAT
GTACTGCATTCATGATGCAAGATCGGRGATGTATTTTATCCTTCAGGGTTGYC

SEQ ID No. 18

GAAAACACCTCTCTACTAAYATAGCAAGCGATCTCAAACTCTTATATGAAYATGAGACTTRTAATAAAAAGCTG
AGAGAAGTGTTGAAAACACTCCTAAA**G**TAGTGATGTACTGCATTCATGATGCAAGATCGGRGATGTATTTTATC
CTTCAGGGTTGYCCAGTGTTACCTCGCCTATTTAGTTTGCGCTCTATTGAGTA

Fig. 1-3

SEQ ID No. 19

AATAAARTAGTACACATTGTTTGGTCAAACAATTTGGTGATTTTTTGAAAATATATCTTCAAAAATTAATCAAAC
TTCAAAATCTGGTCRTGAAATATCA**A**TTTCATTCATAGAATTTTAATTTATTTGCAGAGAAAATGTAGGGATAAG
AGTYTGAAAAATATCTCAATTTTGATCGAATTTGTTCTTGCGATACTTAAC

SEQ ID No. 20

GCCAGATGACAGACTTTGTGGATGCTTACTCTCAGTTGTGTCCTACTGCAAGGGCGACGATGCAGATAAGGTC
CTTGCTTGTCTACAACAAGCTAACCCA**A**GATTGGTAACCTTTGTCAAGATGCTAGAAGATGAGAGCACTAGCTA
TGACATTGTGAAAGAAGAGTTCAGGTCCATACTGACTAATACATCAGATGATGC

SEQ ID No. 21

ATTGTGAAAGAAGAGTTCAGGTCCATACTGACTAATACATCAGATGATGCTAGAAGGCCCTTCTGTAATTGTCT
AATTGATATATGCAGAAAGAGAAATC**A**CGCAGAGAGGGCTCACGAGCTTCTATACTTGGGAACTGTATATGGA
CTATACCCAGGTTTACATACTAAGACACCAGAAGAATGGCGATTRAACGTCCGA

SEQ ID No. 22

TAAATATAAATAAATCGTTCAAGTTTGTTACGATCCAAAAAYGAGGCTTGARTGRCACTCACACTTATCCTACTA
TGTGAGCGAACCAACCCATCTAAAY**T**CCAACATTTCAAAACATAATAGACAGAATATAATGYGGAAGACTTAAA
ACTCATTAACGAAGTCAATAATTAACTTCTAAAACTCAAAACTTATCATTAT

SEQ ID No. 23

TTAATARGACTCCTTACAAGCGTTGTTTCTATCTACTATTATCTAAAAATAATCAAGTTATTAATGACCGGACGA
AACAAAGAAATAACCCCTCATGTGC**G**AAATTATAGAAGATCCCCTTTAAGATCAAACAATTCCATMRAATTGA
GTATGATTGTATGTGTGATAGCATCTACTATACCAGGAATATCAATGAACCCG

SEQ ID No. 24

AAGAGGCCGTGGTAGAGGTCGTGGGAGGACGTCCTCTAGAGGAAGGGGACGAGCACCTAGCCCATCTGATA
CTAGGGCGGTGACTCCTCCACCGACTGAA**T**AAGTAGTAATAGAAGGGGAGGATGGGGAAAATGAACAAGTAC
AGAATGAGGAATTACCACCCCAACCTACCCCAGAAATGATCAATCAGGTTCTTGCTTA

SEQ ID No. 25

TAACTTATATTTGTAAATAAATCTCATGTTTAACTCAAACTCAAAAAGCAACSACACTATCATAAAAATAAAAAG
TCACTCATCCTTAAAGGGACAATTC**C**GGACTCATTAAATTCCTCGAACTCAATCTGAATCTAGACATCTAATGCA
TAAACAATTTAATATAAAAAGTCCTACACCAAAAAATAAAAAATGGAATAA

SEQ ID No. 26

GCTGGTGGCCTATTGTTGATCAGTGGCTGTCGGATTTGTTGTCTGGGACCACCTATGTACTCATCATCACTATC
ATARTCTGCTGGTCTGTTTGCTGCCC**C**TACTACGAGGGCCAATAAGAATATAAGAGCCTGCAACTCATGTAAAC
RGACGAAACTGAGAGACTYGGAGAAAATTAAGACAAACAATTTTCAGGTNNNN

SEQ ID No. 27

TCGTTTCAAAGTTGCCCGTTTTATCCCTTGGAATTTCCTGACAAAGACTTTCATCACTTTTCTGACAACAAAGTAT
AGTWTGAAAGTGAAGTCGTGAGTG**C**GAAAACACTTACATCTTTCCAGCTTTTATCAAAGAATATAAAACCAGC
AGCACCTAGCTCTACCAAGATCAACAAGAAAATCAACATGGAGTACTGTTCC

Fig. 1-4

SEQ ID No. 28 - InDel

TATGTGGGCTGTATATTTTTTAAATTTTCCCATAAATAATAAATCAAGAAGACTGAACAAATGATAAGGATACA
CAACTCAGGCAGCAACCATTCCTTGT**TGCT**CCAATACAACCACAGCAAGATACAACTACAAGAACTGCTCCAAT
ACCAATAAACAAATATATGAACCTGCGACATAACAAATGGGTCAATGCTTCAT

SEQ ID No. 29

TRCAAATCATGTAGTCAAGTTCTGAGAATTCACACCCACTGACCACTCAAAACTTGGAAAATATTATGAGACTC
TGTTAATAATTCAATAWTGGACAAGC**C**CAAACAGGTGAAGAMCATATATAGTTCCTTTTTCTTATTTATACTTC
CATGTTTCTTTAGCTCTACACCTRGGAAACATATGATTCAACCTTCGCATATA

SEQ ID No. 30 - InDel

AGAGGATGTAAYAGAATGAACTCTCAGTATTTTTAACTGGTTCAAGACTACTTTGGRTTTTCAGAGATTTTAACT
CAATAAGTTCCCTAGGGAAAAGAGA**TGA**ATGTGATTCTTTATCTAGTGTAATAGTTCTTGGAGATTTTATTGAT
TATCCGTTGACTGATGATAAGTTCTCCAAATCAAGGAGTCAAAGTGAAGAT

SEQ ID No. 31

TGTATATCTRGMGAGCGAGATTAAGAGAGGGATGAAAGAGGCAAGTGTGATTGGGAGAGAGAGGAGAGAG
ACTAATTGTATTTGTATATCTTTCATATAA**T**TGTATGTATAAATGTACAATTTGTATTTGTATATGGATTAGCAAC
CTAATTACAACTAAAATTAAGTTGTGAGTCGTAATTAATTCAAACTATAGCTACA

SEQ ID No. 32

AATTTGTATAAAATGAGAAAGAGAGAAAGATAAAAGAGACTTGACAAGGAATATACAATTRAATCGAATTGTA
TAAAATGAGAAAGAGAGAAAGTAAATA**T**AWTTTGAAAATTGTATAAGACGAGAAAGAGAGAAGGGCAAAAG
AAASTGGGYAGAGGAGTATTTTTATTGTATAATTATAATTGTWTAGGACGAAAATAT

SEQ ID No. 33

AGAGAAAGTAAATAYAWTTTGAAAATTGTATAAGACGAGAAAGAGAGAAGGGCAAAAGAAASTGGGYAGAG
GAGTATTTTTATTGTATAATTATAATTGT**T**TAGGACGAAAATATATGTACGTGCATGTGTATATACAATTTTCTC
ACGCTTTATAYAAACAGAAAYGCAATTTATACATTTCGCTTCTGTTTGTACAAGT

SEQ ID No. 34

AATATACATCAATTTTTTATATTAYTTTTCATATAGTGTTTTTAAATATTTTTATTTTCAATRATATTTCTCGTAACA
TAGCCACATATCTAATAAAATA**C**TTTTATTTTTGTAATTTTTAATTTTTAAATACGAGGGTTTGTGACTGACCAAC
RTACSGACTACTCTACCACGTATATACAACATAGTTGGAAAGAATTT

SEQ ID No. 35

CAAAGCTAAATCCTCCCCACTACTCCATCCACTCTCCACCGCCTTTTTYGCCTCCACATTTTCGAAATCAGCCTCA
TTTCCACCCTCAGCTCTCTTATCA**G**CYGTTTTCCTGTCCTTCAAAAACCTCTGATAGGAATCCCCATCACTCATTTT
CTTCTCACCCAATTCTTTAGATTTCTTAATCACACTTTCCACTTTGTA

SEQ ID No. 36

TCTTGTTGGTTGTTATATTTTCAACATTGTTTTCTTTTTCTTTGTACTTGAATTTGTTGTTATTAATCGAACATCTTT
GAAAAACAATCTATCTACCTCG**G**CAAGGTAATGATATGATTCGTCTATATTCTACCATCTTCGAATCGTGCTTAT
GGAATTTCACGCTAAGGAAACCTTTTCAATACATGAGAAGCGCAGTGT

Fig. 1-5

SEQ ID No. 37

TTTCTGGCAGGGCTGGTGGTTCTTTTGATGATAGGAGGTTTCTAGACGATAGATTTTCAAGGGATGGTGTTTAT
CCCCGGGGTGCCTATCACCGTGATAT**T**CTGGATGGAGAGCACTACCCTCATCCACCAGCAGCAGTTGGACACT
GGCCTCAGACAAGGAGGAGAAGTTATGAAGAAGTGTACCCTGTGGAGAGGGATT

SEQ ID No. 38

CCGATACGATAGAAGTGAGAGACGAAGAGATCGTGATGATAAAAGATACCATGACAATTATTCTGTGGTATGT
TTCCTGTTTCTTATGTTGCACTTCCCA**G**TTGCTTTTATGTTTGGAGGGGCTCATGGCTTCTGTTATTTTAATGCAG
GCTCCTTCAGCGACTGTTGTTGTCAAAGGCCTTTCACAAAAAACTACTGAAG

SEQ ID No. 39

TATTATTGCTTGGGCTTCAAGATCGGGACTCAACATTCAGAAGAGGTGTTTTTGATCCAACTCCTTTCCCCCCTG
GTGTYGGAGGTGGACGTAGTGCAGA**A**GCGAATAGTCAGAGCTTTGAGGTTATCACGGCTGACAGGGCTATAG
ACGAGAGTAAYGTGGGCAATCGTATGCTTCGCAACATGGGATGGCAGGAGGGCT

SEQ ID No. 40

TTCAACACCCTTTAGCTGCAGGTAGAGTTATGATGAAGCTCTGTTCTTCACAATCCACTTTTCCTCATGCTGTAA
AGATTTTTGAGAATCTTGAATACCC**A**GATGCTTTTATTTGTAATACAGTTATGAAATGTTATGTGAATTTCGATG
ACCCGGAAAAGGGTTTGGTGTTTTATTCTGATCAGATGGTTAAAAATGGGA

SEQ ID No. 41

TACAAATAATGTCGTGTATGGAATAAACTTGCTTACAATTCAATGGTTCTATGAAACAGAAATTAGGTAAAATC
TGTTCTRTAGTTTGTTAAGTATATTG**T**AAAAATAAAATTTCTTTATATGTGGTTAATAGTYATTAGTTTGGCCGTC
CTAATGCATTTTATTCGTTTGATTAAGATTTTCTTTTCATGTATTTGTATA

SEQ ID No. 42

CCAATTTTATAAAATGAGATAGTTCCTCAGTATTCCGTCACTGCTTTTGTTTAAGCTTCAGTTTCCACTAGCAAAC
TTTACATTGTTATGCTCCATATTC**G**TAATAATCCATGAACTTAACTGTTTACCGCTCTTTCCTCATTGAGAAATGG
TTTAAAACATATAACTTGTACTTTTCGCTGCAAACATATTACATTGTTT

Fig. 2

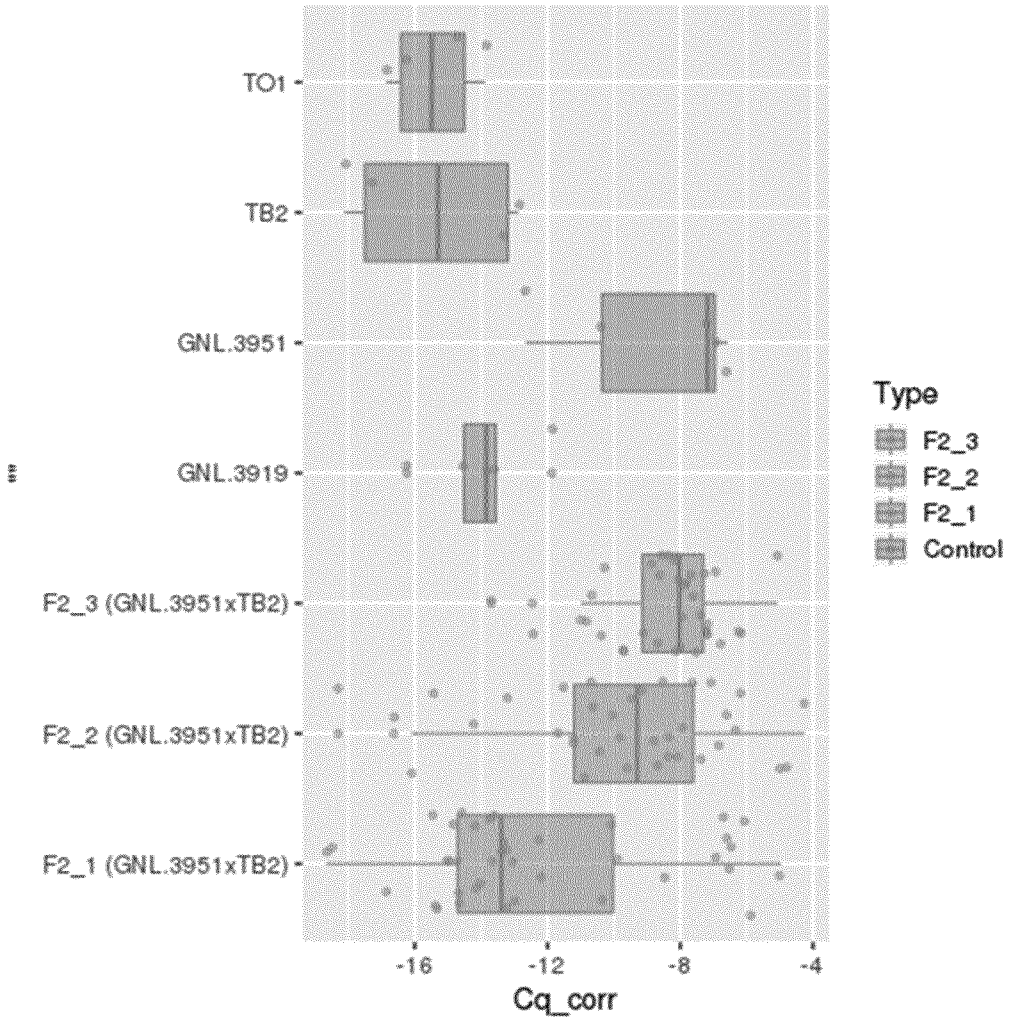
TO1
TB2
GNL.3951
GNL.3919
F2_3 (GNL.3951xTB2)
F2_2 (GNL.3951xTB2)
F2_1 (GNL.3951xTB2)
-16
-12
-8
-4
Cq_corr
Type
F2_3
F2_2
F2_1
Control

Fig. 3

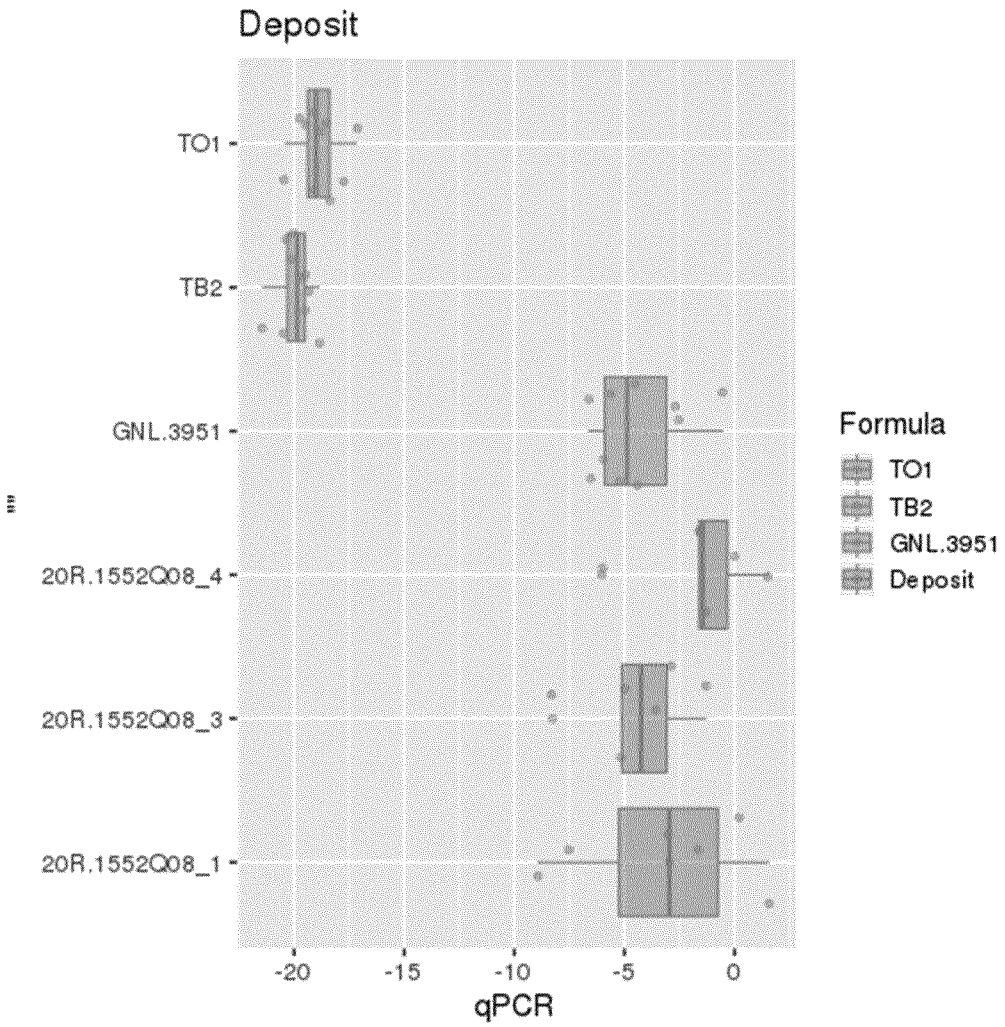
Deposit
TO1
TB2
GNL.3951
20R.1552Q08_4
20R.1552Q08_3
20R.1552Q08_1
-20
-15
-10
-5
0
qPCR
Formula
TO1
TB2
GNL.3951
Deposit

Fig. 4

Figure 4 – Marker haplotypes and phenotypes of recombination lines

					Chromosome	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
					SL3.00 >	61.175.582	61187788..61188051	61201639..61208886	61.202.144	61.208.138	61208597..61215801	61217182..61217481	61222622..61228579	61229146..61234179	61.235.008	61.245.722	61250475..61256857	61.251.554	61.253.498	61.257.969	61.258.594	61.262.045	61.262.164	61261820..61267676	61.268.020	61.279.928
Pedigree	**Expected if cluster left of SL005 contains causal gene**	**Expected if Tom2a is causal gene**	**Expected if Solyc08g077230.3.1 is causal gene**	**OBSERVED ToBRFV phenotype**	**Generation**	SL001 - SEQ ID No. 22	Solyc08g077160.2	Solyc08g077170.3	SL002 - SEQ ID No. 25	SL003	Solyc08g077180.3	Solyc08g077190.1	Solyc08g077200.3	Solyc08g077210.3	SL004	SL005	Solyc08g077220.3	SL006 - SEQ ID No. 26	SL007 - SEQ ID No. 29	SL008 - SEQ ID No. 31	SL009 - SEQ ID No. 32	SL010	SL011	Solyc08g077230.3	SL012 - SEQ ID No. 34	SL013
(TB2*GNL3951) - 01	Fixed R	Segr	Segr	Segr	F2	B			B	B					B	H		H	H	H	H	H	H		H	H
(TB2*GNL3951) - 02	Segr	Fixed R	Fixed R	Fixed R	F2	H			H	H					H	B		B	B	B	B	B	B		B	B
(TB2*GNL3951) - 03	Segr	Fixed R	Fixed R	Fixed R	F2	H			H	H					H	H		B	B	B	B	B	B		B	B
(TB2*GNL3951) - 04	Segr	Fixed S	Fixed S	Fixed S	F2	B			B	H					H	A		A	A	A	A	A	A		A	A
(TB2*GNL3951) - 05	Segr	Fixed S	Fixed S	Fixed S	F2	H			H	H					H	A		A	A	A	A	A	A		A	A
(TB2*GNL3951) - 06	Segr	Fixed R	Fixed R	Fixed R	F3	H			H	H					H	B		B	B	B	B	B	B		B	B
(TB2*GNL3951) - 07	Segr	Segr	Fixed R	Segr	F3	H			H	H					H	H		H	H	H	H	B	B		B	B
(TB2*GNL3951) - 08	Fixed R	Segr	Segr	Segr	F3	B			B	B					B	H		H	H	H	H	H	H		H	H
(TB2*GNL3951) - 09	Fixed R	Segr	Segr	Segr	F3	B			B	B					B	H		H	H	H	H	H	H		H	H
(TB2*GNL3951) - 10	Segr	Fixed R	Fixed R	Fixed R	F3	H			H	H					H	B		B	B	B	B	B	B		B	B
(TB2*GNL3951) - 11	Fixed R	Segr	Segr	Segr	F3	B			B	B					B	B		H	H	H	H	H	H		H	H
(TB2*GNL3951) - 12	Fixed R	Segr	Segr	Segr	F3	B			B	B					B	H		H	H	H	H	H	H		H	H

Fig. 5-1

Figure 5 – Tom2a CDS sequences

SEQ ID No. 45

>Tom2a_GNL.3951_R 43637 minus-strand

ATGGCGTGCAAAGGGTTTTGGGAGTGCTTGTTGAAGCTCTTGAACTTTTTGTTGACCCTTGTTGGTTTGAC
AATGGTGGGGTATGGTATTTATCTATTTGTTGAGTACAAAAATCATTCACACTCCGGAGATGATTACCCAG
TTGCACCACCTATGAGTGGTGACATGATAGAGTTTGGTCGTCCAATGCTGATGGCTGTATCGTTGGCTGAA
AACATATTTGATAAACTTCCAAAACCTTGGTTCATATATTTGTTTATTGGTATTGGAGCAGTTCTTGTAGT
TGTATCTTGCTGTGGTTGTATTGGAGCAACAAGGAATGGTTGCTGCCTGAGTTGTTACTCCATGTTGATTT
TCTTGTTGATCTTGGTAGAGCTAGGTGCTGCTGGTTTTATATTCTTTGATAAAAGCTGGAAAGATGAAATT
CCAAGGGATAAAACGGGCAACTTTGAAACGATCTATGACTTTCTGGATGACCACTGGAAGATTATTAAGTG
GGTTGCCCTTGGTGCTGTTATATTCGAGGCTCTTATATTCTTATTGGCCCTCGTAGTAGGGGCAGCAAACA
GACCAGCAGATTATGATAGTGATGATGAGTACATAGGTGGTCCCAGACAACAAATCCGACAGCCACTGATC
AACAATAGGCCACCAGCAAATCCTGCAACTAGTGTCCCTGTTACTGCTACTCTTGATAATCGTCCAAGTAG
AAATGATGCATGGAGTACACGTATGAGGGAAAAGTATGGACTTGACACATCAGAGTTTACTTACAACCCAT
CGGAGTCGAACAGATATCCGCCAACAGCCGCACAGCCGCAAGAGGAAAGGAAGGGTTGTGCCATAATGTGA

SEQ ID No. 47

>tom2a_SL3_00_S minus-strand

ATGGCGTGCAAAGGGTTTTGGGAGTGCTTGTTGAAGCTCTTGAACTTTTTGTTGACCCTTGTTGGTTT
GACAATGGTGGGGTATGGTATTTATCTATTTGTTGAGTACAAAAATCATTCACACTCCGGAGATGATT
ACCCAGTTGCACCACCTATGAGTGGTGACATGATAGAGTTTGGTCGTCCAATGCTGATGGCTGTATCG
TTGGCTGAAAACATATTTGATAAACTTCCAAAACCTTGGTTCATATATTTGTTTATTGGTATTGGAGC
AGTTCTTGTAGTTGTATCTTGCTGTGGTTGTATTGGAGCGGCAACAAGGAATGGTTGCTGCCTGAGTT
GTTACTCCATGTTGATTTTCTTGTTGATCTTGGTAGAGCTAGGTGCTGCTGGTTTTATATTCTTTGAT
AAAAGCTGGAAAGATGAAATTCCAAGGGATAAAACGGGCAACTTTGAAACGATCTATGACTTTCTGGA
TGACCACTGGAAGATTATTAAGTGGGTTGCCCTTGGTGCTGTTATATTCGAGGCTCTTATATTCTTAT
TGGCCCTCGTAGTAAGGGCAGCAAACAGACCAGCAGACTATGATAGTGATGATGAGTACATAGGTGGT
CCCAGACAACAAATCCGACAGCCACTGATCAACAATAGGCCACCAGCAAATCCTGCAACTGGTGTCCC
TGTTACTGCTACTCTTGATAATCGTCCAAGTAGAAATGATGCGTGGAGTACACGTATGAGGGAAAAGT
ATGGACTTGACACATCAGAGTTTACTTACAACCCATCGGAGTCGAACAGATATCCGCCAACTGCCGCA
CAGCCGCAAGAGGAAAGGAAGGGTTGTACCATAATGTGA

Fig. 5-2

CDS Alignment

```
Query: Tom2a_S CDS Length: 855
Sequence ID: Tom2a_R CDS Length: 852
Range 1: 1 to 852

Score:1526 bits(826), Expect:0.0,
Identities:846/855(99%),  Gaps:3/855(0%)

SL3_00 1    ATGGCGTGCAAAGGGTTTTGGGAGTGCTTGTTGAAGCTCTTGAACTTTTTGTTGACCCTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  1    ATGGCGTGCAAAGGGTTTTGGGAGTGCTTGTTGAAGCTCTTGAACTTTTTGTTGACCCTT  60

SL3_00 61   GTTGGTTTGACAATGGTGGGGTATGGTATTTATCTATTTGTTGAGTACAAAAATCATTCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  61   GTTGGTTTGACAATGGTGGGGTATGGTATTTATCTATTTGTTGAGTACAAAAATCATTCA  120

SL3_00 121  CACTCCGGAGATGATTACCCAGTTGCACCACCTATGAGTGGTGACATGATAGAGTTTGGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  121  CACTCCGGAGATGATTACCCAGTTGCACCACCTATGAGTGGTGACATGATAGAGTTTGGT  180

SL3_00 181  CGTCCAATGCTGATGGCTGTATCGTTGGCTGAAAACATATTTGATAAACTTCCAAAACCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  181  CGTCCAATGCTGATGGCTGTATCGTTGGCTGAAAACATATTTGATAAACTTCCAAAACCT  240

SL3_00 241  TGGTTCATATATTTGTTTATTGGTATTGGAGCAGTTCTTGTAGTTGTATCTTGCTGTGGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  241  TGGTTCATATATTTGTTTATTGGTATTGGAGCAGTTCTTGTAGTTGTATCTTGCTGTGGT  300

SL3_00 301  TGTATTGGAGCGGCAACAAGGAATGGTTGCTGCCTGAGTTGTTACTCCATGTTGATTTTC  360
            ||||||||||||   ||||||||||||||||||||||||||||||||||||||||||||
GNL_R  301  TGTATTGGAGC---AACAAGGAATGGTTGCTGCCTGAGTTGTTACTCCATGTTGATTTTC  357

SL3_00 361  TTGTTGATCTTGGTAGAGCTAGGTGCTGCTGGTTTTATATTCTTTGATAAAAGCTGGAAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  358  TTGTTGATCTTGGTAGAGCTAGGTGCTGCTGGTTTTATATTCTTTGATAAAAGCTGGAAA  417

SL3_00 421  GATGAAATTCCAAGGGATAAAACGGGCAACTTTGAAACGATCTATGACTTTCTGGATGAC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  418  GATGAAATTCCAAGGGATAAAACGGGCAACTTTGAAACGATCTATGACTTTCTGGATGAC  477

SL3_00 481  CACTGGAAGATTATTAAGTGGGTTGCCCTTGGTGCTGTTATATTCGAGGCTCTTATATTC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  478  CACTGGAAGATTATTAAGTGGGTTGCCCTTGGTGCTGTTATATTCGAGGCTCTTATATTC  537

SL3_00 541  TTATTGGCCCTCGTAGTAAGGGCAGCAAACAGACCAGCAGACTATGATAGTGATGATGAG  600
            |||||||||||||||||| |||||||||||||||||||| ||||||||||||||||||||
GNL_R  538  TTATTGGCCCTCGTAGTAGGGGCAGCAAACAGACCAGCAGATTATGATAGTGATGATGAG  597

SL3_00 601  TACATAGGTGGTCCCAGACAACAAATCCGACAGCCACTGATCAACAATAGGCCACCAGCA  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  598  TACATAGGTGGTCCCAGACAACAAATCCGACAGCCACTGATCAACAATAGGCCACCAGCA  657

SL3_00 661  AATCCTGCAACTGGTGTCCCTGTTACTGCTACTCTTGATAATCGTCCAAGTAGAAATGAT  720
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  658  AATCCTGCAACTAGTGTCCCTGTTACTGCTACTCTTGATAATCGTCCAAGTAGAAATGAT  717

SL3_00 721  GCGTGGAGTACACGTATGAGGGAAAAGTATGGACTTGACACATCAGAGTTTACTTACAAC  780
            || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GNL_R  718  GCATGGAGTACACGTATGAGGGAAAAGTATGGACTTGACACATCAGAGTTTACTTACAAC  777

SL3_00 781  CCATCGGAGTCGAACAGATATCCGCCAACTGCCGCACAGCCGCAAGAGGAAAGGAAGGGT  840
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
GNL_R  778  CCATCGGAGTCGAACAGATATCCGCCAACAGCCGCACAGCCGCAAGAGGAAAGGAAGGGT  837
```

Fig. 5-3

```
SL3_00 841  TGTACCATAATGTGA  855
            ||| ||||||||||
GNL_R  838  TGTGCCATAATGTGA  852
```

Fig. 6

Figure 6 - Tom2a protein sequences

SEQ ID No. 46
>Tom2a_GNL.3951_R 43637
MACKGFWECLLKLLNFLLTLVGLTMVGYGIYLFVEYKNHSHSGDDYPVAPPMSGDMIEFGRPMLMAVSLAE
NIFDKLPKPWFIYLFIGIGAVLVVVSCCGCIGATRNGCCLSCYSMLIFLLILVELGAAGFIFFDKSWKDEI
PRDKTGNFETIYDFLDDHWKIIKWVALGAVIFEALIFLLALVVGAANRPADYDSDDEYIGGPRQQIRQPLI
NNRPPANPATSVPVTATLDNRPSRNDAWSTRMREKYGLDTSEFTYNPSESNRYPPTAAQPQEERKGCAIM

SEQ ID No. 48
>Tom2a_SL3_00_S
MACKGFWECLLKLLNFLLTLVGLTMVGYGIYLFVEYKNHSHSGDDYPVAPPMSGDMIEFGRPMLMAVSLAE
NIFDKLPKPWFIYLFIGIGAVLVVVSCCGCIGAATRNGCCLSCYSMLIFLLILVELGAAGFIFFDKSWKDE
IPRDKTGNFETIYDFLDDHWKIIKWVALGAVIFEALIFLLALVVRAANRPADYDSDDEYIGGPRQQIRQPL
INNRPPANPATGVPVTATLDNRPSRNDAWSTRMREKYGLDTSEFTYNPSESNRYPPTAAQPQEERKGCTIM

Protein alignment

Query: Tom2a proteins Length: 284

>unnamed protein product
Sequence ID: Query_10407 Length: 283
Range 1: 1 to 283

Score:577 bits(1486), Expect:0.0,
Method:Compositional matrix adjust.,
Identities:280/284(99%), Positives:280/284(98%), Gaps:1/284(0%)

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SL3_00 1    MACKGFWECLLKLLNFLLTLVGLTMVGYGIYLFVEYKNHSHSGDDYPVAPPMSGDMIEFG  60
            MACKGFWECLLKLLNFLLTLVGLTMVGYGIYLFVEYKNHSHSGDDYPVAPPMSGDMIEFG
GNL_R  1    MACKGFWECLLKLLNFLLTLVGLTMVGYGIYLFVEYKNHSHSGDDYPVAPPMSGDMIEFG  60

SL3_00 61   RPMLMAVSLAENIFDKLPKPWFIYLFIGIGAVLVVVSCCGCIGAATRNGCCLSCYSMLIF  120
            RPMLMAVSLAENIFDKLPKPWFIYLFIGIGAVLVVVSCCGCIGA TRNGCCLSCYSMLIF
GNL_R  61   RPMLMAVSLAENIFDKLPKPWFIYLFIGIGAVLVVVSCCGCIGA-TRNGCCLSCYSMLIF  119

SL3_00 121  LLILVELGAAGFIFFDKSWKDEIPRDKTGNFETIYDFLDDHWKIIKWVALGAVIFEALIF  180
            LLILVELGAAGFIFFDKSWKDEIPRDKTGNFETIYDFLDDHWKIIKWVALGAVIFEALIF
GNL_R  120  LLILVELGAAGFIFFDKSWKDEIPRDKTGNFETIYDFLDDHWKIIKWVALGAVIFEALIF  179

SL3_00 181  LLALVVRAANRPADYDSDDEYIGGPRQQIRQPLINNRPPANPATGVPVTATLDNRPSRND  240
            LLALVV AANRPADYDSDDEYIGGPRQQIRQPLINNRPPANPAT VPVTATLDNRPSRND
GNL_R  180  LLALVVGAANRPADYDSDDEYIGGPRQQIRQPLINNRPPANPATSVPVTATLDNRPSRND  239

SL3_00 241  AWSTRMREKYGLDTSEFTYNPSESNRYPPTAAQPQEERKGCTIM  284
            AWSTRMREKYGLDTSEFTYNPSESNRYPPTAAQPQEERKGC IM
GNL_R  240  AWSTRMREKYGLDTSEFTYNPSESNRYPPTAAQPQEERKGCAIM  283
```

Fig. 7

Figure 7 – qPCR results showing virus titer of material with the individual QTLs of chr 8 and 11, and the combination.

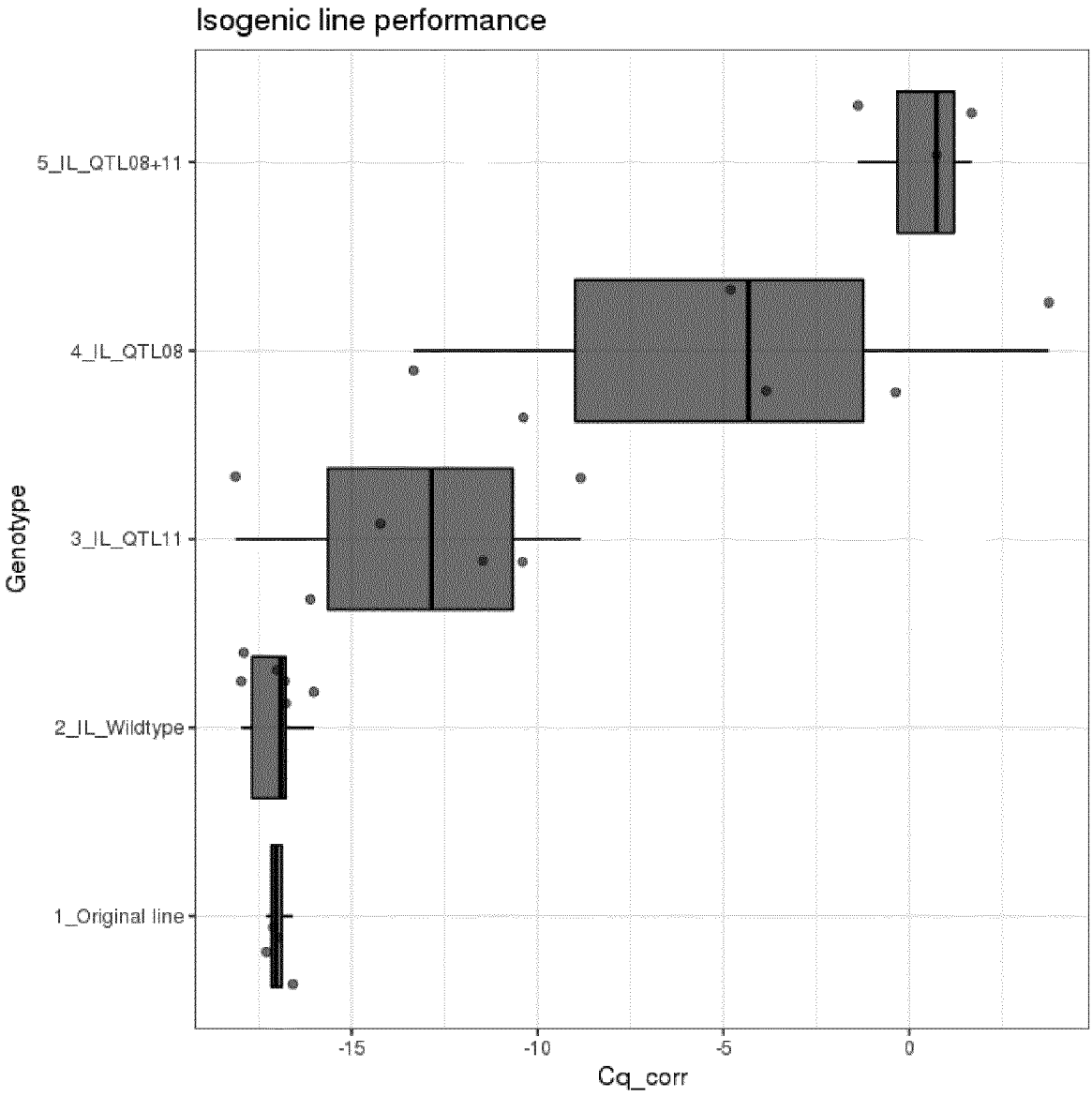
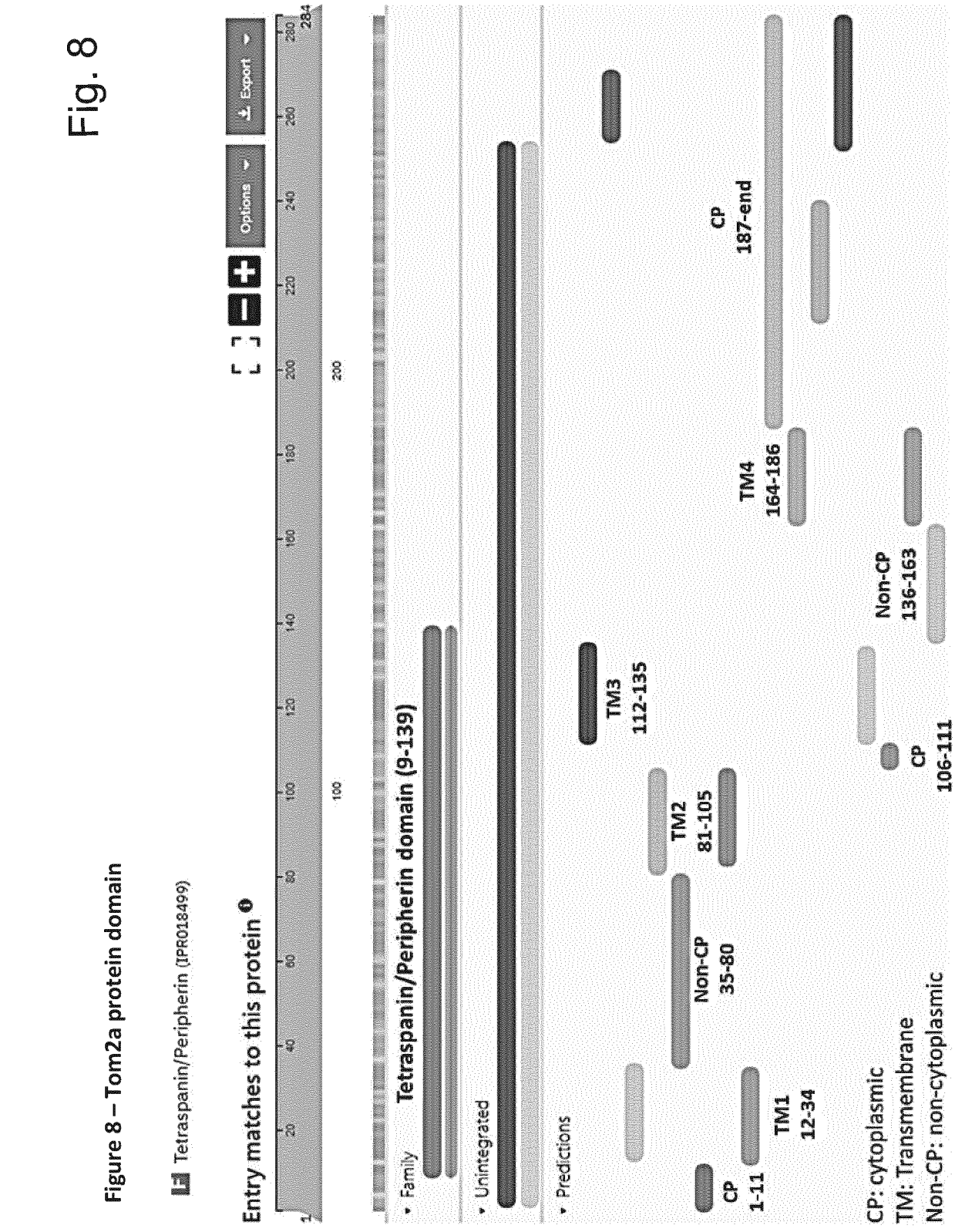


Fig. 8

Figure 8 – Tom2a protein domain



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/070104

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H1/04 A01H1/08 C12N15/82 C12Q1/6895 A01H6/82
A01H5/08
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/110130 A1 (RIJK ZWAAN ZAADTEELT EN ZAADHANDEL BV [NL]) 13 June 2019 (2019-06-13) cited in the application examples 1, 2; tables 2, 3	1-9, 16-21
A	US 2020/077614 A1 (ASHKENAZI VARDA [IL] ET AL) 12 March 2020 (2020-03-12) paragraph [0071]; claim 1; table 3	1-21

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
8 November 2021	19/11/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Krüger, Julia

3

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. O. OLADOKUN ET AL: "Tomato brown rugose fruit disease: current distribution, knowledge and future prospects", PLANT PATHOLOGY, vol. 68, no. 9, 16 October 2019 (2019-10-16), pages 1579-1586, XP055729464, GB ISSN: 0032-0862, DOI: 10.1111/ppa.13096	1-21
X,P	WO 2020/148021 A1 (ENZA ZADEN BEHEER BV [NL]) 23 July 2020 (2020-07-23) page 9 - page 10	2-10,21
X	DATABASE GenPept [Online] NCBI; 28 January 2019 (2019-01-28), Anonymous: "tobamovirus multiplication protein 2A [Solanum pennellii] - Protein - NCBI", XP55855393, Database accession no. XP_015085284 sequence	23-25
A	WO 2019/110821 A1 (RIJK ZWAAN ZAADTEELT EN ZAADHANDEL BV [NL]) 13 June 2019 (2019-06-13) cited in the application claims 1-3; example 2	1-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2021/070104

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

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2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2021/070104

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019110130 A1	13-06-2019	CA 3078275 A1	13-06-2019
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		US 2020399652 A1	24-12-2020
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		EP 3911147 A1	24-11-2021
		KR 20210113675 A	16-09-2021
		PE 20211661 A1	26-08-2021
		US 2021238627 A1	05-08-2021
		WO 2020147921 A1	23-07-2020
		WO 2020148021 A1	23-07-2020
WO 2019110821 A1	13-06-2019	CA 3078275 A1	13-06-2019
		CA 3092499 A1	13-06-2019
		CN 111432630 A	17-07-2020
		EP 3720272 A1	14-10-2020
		EP 3735125 A1	11-11-2020
		JP 2021505165 A	18-02-2021
		JP 2021516548 A	08-07-2021
		US 2020399652 A1	24-12-2020
		WO 2019110130 A1	13-06-2019
		WO 2019110821 A1	13-06-2019