(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 July 2023 (20.07.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/135335 A1

(51) International Patent Classification:
C07K 14/415 (2006.01) *A01H 6/34* (2018.01)
C12N 15/82 (2006.01) *A01H 6/82* (2018.01)
A01H 1/00 (2006.01) *C12N 15/00* (2006.01)

(21) International Application Number:
PCT/EP2023/051031

(22) International Filing Date:
17 January 2023 (17.01.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/EP2022/050893
17 January 2022 (17.01.2022) EP
PCT/EP2022/064821
31 May 2022 (31.05.2022) EP

(71) Applicant: **RIJK ZWAAN ZAADTEELT EN ZAADHANDEL B.V.** [NL/NL]; Burgemeester Crezéelaan 40, 2678 KX De Lier (NL).

(72) Inventors: **KALISVAART, Jonathan**; Burgemeester Crezéelaan 40, 2678 KX De Lier (NL). **LUDEKING, Daniël Johannes Wilhelmus**; Burgemeester Crezéelaan 40, 2678 KX De Lier (NL). **ROOVERS, Alwin Johannes Marinus**; Burgemeester Crezéelaan 40, 2678 KX De Lier (NL). **VAN DUN, Cornelis Maria Petrus**; Burgemeester Crezéelaan 40, 2678 KX De Lier (NL). **BASTIAANSEN, Ellen Anna Johanna**; Burgemeester Crezéelaan 40, 2678 KX De Lier (NL). **HUIJBREGTS-DOORDUIN, Lena Johanna**; Burgemeester Crezéelaan 40, 2678 KX De Lier (NL).

(74) Agent: **VAN SOMEREN, Petronella Francisca Hendrika Maria** et al.; Arnold & Siedsma, Bezuidenhoutseweg 57, 2594 AC THE HAGUE (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: MODIFIED TOM2A GENE INVOLVED IN TOBAMOVIRUS RESISTANCE

(57) Abstract: The present invention relates to a plant comprising a modified Tom2a gene, the wildtype of which comprises SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, or SEQ ID No. 6, or comprises another homologous sequence having at least 70% sequence identity to SEQ ID No. 1, which modified gene leads to increased resistance to a Tobamovirus. The modification in the Tom2a gene leads to a modified Tom2a protein that has a deletion, a substitution, or an insertion of at least one amino acid when compared to SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12, or compared to another homologous sequence having at least 70% sequence similarity to SEQ ID No. 7. The plant is preferably a plant of the family Cucurbitaceae or Solanaceae. The invention further relates to a modified Tom2a gene encoding a Tom2a protein comprising a deletion, a substitution, or an insertion of at least one amino acid when compared to SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12, or compared to another homologous sequence having at least 70% sequence similarity to SEQ ID No. 7.

WO 2023/135335 A1

MODIFIED TOM2A GENE INVOLVED IN TOBAMOVIRUS RESISTANCE

The present invention relates to a plant comprising a modified gene which leads to resistance against a Tobamovirus. The invention further relates to methods for producing such a plant, methods for identification of the modified gene, and methods for selection of such a plant. The invention also relates to the modified gene, and the use of said gene to confer or increase Tobamovirus resistance to a plant. The invention is also directed to a marker for identification of a modified gene in a plant, and to use of said marker.

Wherever agriculture is practiced, viral diseases pose one of the major threats that vegetable growers are facing, both in protected as well as open field cultivation. Initial infection can occur through the use of infected seed, or by mechanical or insect transmission. Once a crop is infected, spread of the virus can occur rapidly by mechanical sap transmission through the use of tools and cultivation practices, or through hard-to-control vectors such as insects.

Viruses belonging to the genus Tobamovirus are often seed-borne, and are thereafter mostly spread by mechanical transmission; insects are not particularly involved in the transmission of Tobamoviruses. Common symptoms of Tobamovirus infection include yellowing through leaf chlorosis, severe or mild mosaic, leaf distortion, and fruit symptoms. Tobamoviruses are positive-strand RNA viruses; their replication takes place in highly specialized replication compartments, that are formed as spherules or vesicles in certain membranes. Viral positive-strand RNA that is synthesized within these compartments, whereby negative-strand RNA is used as a template, is released into the cytoplasm through a narrow channel that connects the inner space of the compartment to the cytoplasm. In a standard situation, the existence of this very narrow channel appears to prevent contact between the components that are involved in viral synthesis, and antiviral defense mechanisms that are available to the host plant.

Numerous genes have been recognized for their involvement in virus resistance in plants, for example because a gene is known to be part of the viral replication process that the virus requires to infect its host. Virus resistance can be based on various mechanisms, and many different phases of plant development, along with different plant defense pathways, can be involved. However, even when a gene is known to be part of the viral replication mechanism, it is not necessarily clear how that gene should or could be modified to ensure its original role is lost to the virus.

It is an object of the present invention to provide a plant showing resistance against a virus belonging to the genus of Tobamoviruses, in particular to Cucumber Green Mottle Mosaic Virus (CGMMV) or Tomato Brown Rugose Fruit Virus (ToBRFV).

The search for new genetic resources for virus resistance is permanently ongoing in plant breeding research. Developing new varieties that help the growers cope with the ever-

present disease challenges requires constant adaptation to new or stronger virus variants. Research leading to the present invention focused on identifying and developing new genetics for resistance to Tobamoviruses, which affect various crops, in particular of the *Cucurbitaceae* and *Solanaceae* families. In cucumber (*Cucumis sativus)*, the Tobamovirus species CGMMV is an important disease, and even though resistance is present, it is not absolute and stacking of various resistant backgrounds is a common practice. In the search for new resistance, a large EMS population was developed, and various mutants in a number of genes were identified by applying a TILLING approach. Mutations resulting in stop mutants, splice sites, and amino acid changes that were predicted to be deleterious when a SIFT prediction was applied, were selected.

Tom2a was one of the genes for which a number of potentially interesting EMS TILLING mutants were obtained (**Example 1**). Two mutants resulted in a premature stop codon, which in turn generated a truncated Tom2a protein that missed a number of domains thought to be essential for proper functioning of the protein. In addition, four mutants resulting in amino acid changes that were predicted to be deleterious, i.e. it could be expected that the function of the protein would be affected, were identified. The stop mutants were anticipated to be especially interesting, since at least part of the crucial C-terminal tail of the protein was missing. One of those, the mutant resulting in a Q268stop mutation in the encoded cucumber Tom2a protein, was phenotyped first (**Example 2**), to get an idea of the potential of *Tom2a* mutants for virus resistance in general.

In co-pending application PCT/EP2021/070104 the identification of several modifications in the *Tom2a* gene of *Solanum pimpinellifolium* is described. PCT/EP2021/070104 discloses a GGC312-314del modification, an A559G substitution, a G673A substitution, and an A844G substitution in the *Tom2a* gene of *Solanum lycopersicum.* Transfer of these modifications to *Solanum lycopersicum* is described, and it is shown that the presence of one or more of these modifications in the *Tom2a* gene in *S. lycopersicum* leads to increased ToBRFV resistance. The combination of the findings of CGMMV resistance due to a *Tom2a* modification in cucumber, and ToBRFV resistance due to a *Tom2a* modification in tomato, is considered to form a good basis for extrapolation of the presence of these or similar modifications to the homologous *Tom2a* genes of other crops, in particular crops of the Cucurbitaceae, related to cucumber, and of the Solanaceae, related to tomato, to achieve increased Tobamovirus resistance, in particular CGMMV in *Cucurbitaceae* or ToBRFV resistance in *Solanaceae*.

The present invention provides a plant comprising a modified *Tom2a* gene, which modified *Tom2a* gene leads to increased resistance against a Tobamovirus. The presence of the modified *Tom2a* gene in particular leads to increased Tobamovirus resistance in a plant belonging to the family *Solanaceae* or in a plant belonging to the family *Cucurbitaceae*. The plant comprising the modified *Tom2a* gene is preferably a plant of the *Cucurbitaceae* family or a plant of the

Solanaceae family, in particular a plant of the species *Cucumis sativus, Cucumis melo, Citrullus lanatus, Cucurbita pepo, Solanum lycopersicum,* or *Capsicum annuum.* A plant of the invention comprising a modified *Tom2a* gene is most preferably a plant of the species *Cucumis sativus* or of the species *Solanum lycopersicum.* A plant of the invention is preferably a cultivated plant, which is non-wild and has agronomical value, and is in particular agronomically elite. The presence of the modified *Tom2a* gene preferably leads to increased resistance against Cucumber Green Mottle Mosaic Virus (CGMMV) or Tomato Brown Rugose Fruit Virus (ToBRFV), optionally in combination with resistance against another virus, in particular another Tobamovirus. Resistance to CGMMV is preferably increased in a plant of the *Cucurbitaceae* family, in particular in *Cucumis sativus*. Resistance to ToBRFV is preferably increased in a plant of the *Solanaceae* family, in particular in *Solanum lycopersicum.*

As used herein, a Tobamovirus is in particular Cucumber Green Mottle Mosaic Virus (CGMMV) or Tomato Brown Rugose Fruit Virus (ToBRFV), but optionally also includes any other virus belonging to the Tobamoviruses. Other viruses belonging to the Tobamoviruses are for example Pepper Mild Mottle Virus (PMMoV) in *Capsicum annuum*, TMV, ToMV, ToMMV, BPMoV, TMGMV, PaMMV, WGMMV, ZGMMV, and KGMMV.

Phenotyping of a cucumber plant having a modified *Tom2a* gene for increased CGMMV resistance is performed by sowing seeds of an accession to be tested in a standard seedling tray at 23°C. After 5 days, at least 10 seedlings are transplanted to a larger pot. One week after sowing, the transplants are inoculated and transferred to a temperature regime of 20°C by day and 18°C by night. Inoculum is prepared by grounding leaves of cucumber plants that were infected with CGMMV in a 0.01 M phosphate buffer (pH 7.0). The seedlings are then dusted with carborundum powder prior to gently rubbing the leaf with inoculum. Resistance is scored on a scale of 1-5; the description of the scales of the scores can be found in **Table 1.** Observation of the symptoms on the young cucumber plants in the bio-assay is done 14-21 days after inoculation (dai). A susceptible control that does not comprise a modified *Tom2a* gene has to be included, an example of which is standard variety Ventura F1. A test is properly performed when a susceptible (S) control, in particular Ventura F1, has an average score that is higher than 4.9, preferably 5.0. Once this average is reached, it is a proper moment to score the assay. As is a standard prerequisite for all experiments, a test is adequately performed when plants are tested in at least 2 repetitions.

As used herein, a plant that has increased CGMMV resistance due to the presence of a modified *Tom2a* gene has an average score lower than 4.0, preferably an average score lower than 3.5, more preferably an average score lower than 3.0, most preferably an average score lower than 2.8, when scoring according to **Table 1** is used.

Table 1: scales CGMMV resistance scores

Score	Symptoms
1	No symptoms
2	Healthy but not clean, some small spots or discolored patches
3	Clear visible symptoms, minor in severity. Some mosaic or blistering, no deformation
4	Severe mosaic and leaf mottling in old and young leaves; starting deformation in the head
5	Severe mosaic and mottling; plants are strongly deformed

Phenotyping of ToBRFV resistance is performed by sowing seeds of the accessions to be tested in standard seedling trays. After 14-21 days, seedlings are transplanted into larger pots. Preferably at least 10 seedlings, optimally at least 20 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 7.** 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, and can therefore be used as susceptible control, are Livento F1, Adventure F1, or Eclipse F1. The test is performed with at least 10 plants of a certain line, and the average score is taken. The test is performed properly when the susceptible (S) control has 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.

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 7** is used (**Example 6, Figure 6**).

As used herein, ToBRFV 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. To accurately determine the final value for virus titer the Delta Cq 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.

In one embodiment a ToBRFV resistant *S. lycopersicum* plant of the invention comprises a knockout of the *Tom2a* gene of the invention on chromosome 8 and has an average Cq_corr score that is in order of increased preference higher than -6.00, -5.50, -5.00, -4.50, -4.00, -3.50, -3.00, -2.50, -2.00, -1.50, -1.00, -0.50, or higher than 0.00. The knockout of the *Tom2a* gene leads to a loss-of-function, wherein the encoded protein is absent, has a reduced function, or is non-functional. The average Cq_corr score is preferably taken from at least 20 plants comprising the same knockout event (**Example 6, Figure 7**).

A plant of the invention comprises a modified *Tom2a* gene homozygously or heterozygously, i.e. a modified *Tom2a* gene can be present on both chromosomes of a chromosome pair in the genome of a plant, or on only one chromosome of a chromosome pair. A plant of the invention comprises a plant of an inbred line, an F1 cross, a hybrid variety, an open pollinated variety, a doubled haploid, or a plant of a segregating population.

A plant of the invention that has increased Tobamovirus resistance due to the presence of a modified *Tom2a* gene does not require the presence of a Tobamovirus-resistance-conferring allele of a *Tm-1* gene, *TOM1* gene, or *TOM3* gene to show resistance.

The wildtype CDS sequence of the *Tom2a* gene of the invention comprises SEQ ID No. 1 for *Cucumis sativus*, or comprises a homologous sequence of a *Tom2a* gene in another crop having at least 70% sequence identity to SEQ ID No. 1, in particular SEQ ID No. 2 for *Cucumis melo,* SEQ ID No. 3 for *Citrullus lanatus,* SEQ ID No. 4 for *Cucurbita pepo*, SEQ ID No. 5 for *Solanum lycopersicum*, SEQ ID No. 6 for *Capsicum annuum* (**Figure 1**).

The wildtype *Tom2a* gene encodes a protein comprising SEQ ID No. 7 in *Cucumis sativus*, or encodes a Tom2a protein comprising a homologous sequence in another crop having at least 70% sequence similarity to SEQ ID No. 7, in particular SEQ ID No. 8 in *Cucumis melo,* SEQ ID No. 9 in *Citrullus lanatus,* SEQ ID No. 10 in *Cucurbita pepo*, SEQ ID No. 11 in *Solanum lycopersicum*, SEQ ID No. 12 in *Capsicum annuum* (**Figure 2**).

The present invention relates to a modified *Tom2a* gene that has a modification that leads to a modified Tom2a protein. As used herein, a Tom2a protein is a protein comprising SEQ ID No. 7, or comprising a sequence having at least 70% sequence similarity to SEQ ID No. 7.

As used herein, a *Tom2a* gene is a gene encoding a Tom2a protein. As used herein, a *Tom2a* gene is a gene comprising a wildtype CDS sequence represented by SEQ ID No. 1 in *Cucumis sativus*, or a homologous gene in another crop comprising a sequence having at least 70% sequence identity to SEQ ID No. 1; or a gene encoding a Tom2a protein comprising SEQ ID No. 7 in *Cucumis sativus*; or a gene encoding a homologous Tom2a protein in another crop comprising a sequence having at least 70% sequence similarity to SEQ ID No. 7. As used herein, a gene optionally comprises the 5'-UTR sequence, the promoter, and the 3'-UTR sequence of that gene.

As used herein, a homologous *Tom2a* gene in another crop than *Cucumis sativus* comprises a homologous CDS sequence, which is a sequence having at least 70% sequence identity to SEQ ID No. 1, preferably at least 71%, 73%, 74%, 75%, 77%, 80%, 83%, 85%, 87%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. A homologous Tom2a protein in another crop than *Cucumis sativus* comprises a homologous protein sequence, which is a sequence having at least 70% sequence similarity to SEQ ID No. 7, preferably at least 72%, 75%, 77%, 80%, 81%, 83%, 85%, 87%, 90%, 93%, 95%, 96%, 97%, 98%, 99%, or 100%. A homologous *Tom2a* gene or protein is preferably a gene or protein present in a plant belonging to the Solanaceae family or in a plant belonging to the Cucurbitaceae family, in particular in *Cucumis melo*, *Citrullus lanatus, Cucurbita pepo, Solanum lycopersicum,* or *Capsicum annuum.*

As used herein, sequence identity or sequence similarity is the percentage of nucleotides or amino acids that is identical or similar 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 or similarity score should be obtained. The percentage sequence identity or similarity is calculated through comparison over the length of the shortest sequence in the assessment, whereby in the present case a sequence that is included in such assessment represents a gene that at least comprises a start codon and a stop codon, or a complete protein encoded by such a gene. Sequence identity is used for comparison of nucleotide sequences. Sequence similarity is used to compare amino acid sequences, whereby conservative amino acid substitutions are deemed to be similar and is calculated herein based on the BLOSUM62 scoring matrix.

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 (TM1-4) which are connected by two non-cytoplasmic loops and one very

short cytoplasmic loop (**Figure 4**). The second non-cytoplasmic, extracellular loop (EC2) harbours various conserved regions, and this loop is thought to play an essential role in tetraspanin function of both animal and plant cells. The polar residues of the transmembrane domains appear to be essential for stabilization of the tertiary protein structure. The C-terminal tail of the protein is also described as a crucial region for proper tetraspanin function.

In one embodiment, the modification in a *Tom2a* gene leads to a modified Tom2a protein that has a deletion, a substitution, or an insertion of at least one amino acid when compared to SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12, or when compared to another homologous wildtype sequence of SEQ ID No. 7.

The modification in the *Tom2a* gene that leads to Tobamovirus resistance, in particular to resistance against CGMMV or ToBRFV, will change the activity and/or function of the encoded protein. The modification in the *Tom2a* gene of the invention comprises a modification resulting in an amino acid substitution in the encoded protein, a modification resulting in a premature stop codon, which results in truncation of the encoded protein, or a modification resulting in a frameshift. A modification resulting in a frameshift is a modification comprising an insertion or a deletion of at least one nucleotide. A frameshift mutation usually leads to a knockout of the gene, rendering it non-functional. Due to the modification, the encoded protein is absent, has a changed function, a reduced function, or it is non-functional. The modification of the present invention is in particular an induced modification. An induced modification is not naturally present, and leads to a non-natural modified *Tom2a* gene, and thereby to a non-natural modified Tom2a protein.

In one embodiment, the modified Tom2a protein has a modification in the second non-cytoplasmic, extracellular loop. In one embodiment, the modified Tom2a protein has a modification in a transmembrane domain, in particular in the TM2 domain. In one embodiment, the modified Tom2a protein has a modification in the cytoplasmic loop. In one embodiment the modified Tom2a protein has a modification in the C-terminal tail. A modification in the C-terminal tail is optionally resulting in a truncated protein, which leads to the absence or shortening of the C-terminal tail, which in that situation cannot perform its function. In one embodiment the modified Tom2a protein has a modification in the Non-CP EC1 loop or the Non-CP EC2 loop. In one embodiment, the modified Tom2a protein comprises a combination of said modifications.

In one embodiment the modified Tom2a protein is truncated and comprises only amino acids 1-267 of SEQ ID No. 7 or less, or the corresponding number of amino acids of a homologous Tom2a protein sequence, or the modified Tom2a protein comprises a modification in amino acids 268-279 of SEQ ID No. 7 that leads to a changed function, a reduced function, or a non-functional protein, or in the corresponding amino acids of a homologous Tom2a protein

sequence, in particular of SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12.

In one embodiment the modified *Tom2a* gene is a knockout. In one embodiment the knockout leads to a protein that lacks the C-terminal tail; lacks the TM4 domain and the C-terminal tail; lacks the Non-CP EC2 loop and the TM4 domain and the C-terminal tail; lacks the TM3 domain and the Non-CP EC2 loop and the TM4 domain and the C-terminal tail; lacks the CP loop and the TM3 domain and the Non-CP EC2 loop and the TM4 domain and the C-terminal tail; or lacks the TM2 domain and the CP loop and the TM3 domain and the Non-CP EC2 loop and the TM4 domain and the C-terminal tail. In one embodiment the knockout leads to a protein that is truncated in the Non-CP EC1 loop. The knockout gene can be caused by a frameshift mutation, or by a mutation that leads to an early stop codon in another way, or by another mutation that leads to the loss-of-function of the gene or the encoded protein.

In one embodiment, a modification resulting in a modified Tom2a protein that increases Tobamovirus resistance comprises a C802T modification in SEQ ID No. 1 of cucumber, or a modification on a corresponding position in a homologous sequence of SEQ ID No. 1. Said nucleotide change results in a Q268stop modification in SEQ ID No. 7 of cucumber, or in a modification at the corresponding position of a homologous protein sequence of SEQ ID No. 7.

A modified Tom2a protein increasing Tobamovirus resistance comprises a protein having a Q268stop modification in SEQ ID No. 7 in cucumber, or a modification at the corresponding position of a homologous sequence having at least 70% sequence similarity to SEQ ID No. 7. This modification is a representative of a modification in the C-terminal tail of a Tom2a protein.

For *Solanum lycopersicum,* the *Tom2a* modifications GGC312-314del, A559G substitution, G673A, and A844G are described in co-pending application PCT/EP2021/070104. These modifications respectively lead to an A105del modification, an R187G substitution, a G225S substitution, and a T282A substitution in the *S. lycopersicum* Tom2a protein sequence of SEQ ID No. 11.

The present invention relates to extrapolated modifications of said *S. lycopersicum* modifications to the corresponding positions on other *Tom2a* genes, which lead to increased Tobamovirus resistance, in particular increased CGMMV or ToBRFV resistance. Extrapolation of amino acid positions can be done using a protein sequence alignment, which is presented for particular Tom2a proteins mentioned herein in **Figure 2**.

In one embodiment, a modification resulting in a modified Tom2a protein that increases Tobamovirus resistance comprises one or more of a TGC306-308del modification, a C553G modification, a G658A modification, or a A829G modification in SEQ ID No. 1 of cucumber, or one or more modifications on corresponding positions in a homologous sequence of

SEQ ID No. 1. Said nucleotide changes respectively result in a A103del modification, a R185G amino acid substitution, a G220S amino acid substitution, or a T277A amino acid substitution in SEQ ID No. 7 of cucumber, or in a modification at the corresponding position of a homologous protein sequence of SEQ ID No. 7.

A modified Tom2a protein increasing Tobamovirus resistance comprises a protein having an A103del modification, a R185G amino acid substitution, a G220S amino acid substitution, or a T277A amino acid substitution in SEQ ID No. 7 in cucumber, or a modification at the corresponding position of a homologous sequence having at least 70% sequence similarity to SEQ ID No. 7. An A103del modification is a representative of a modification in a transmembrane domain, in particular in the TM2 domain. This modification potentially also impacts the short cytoplasmic loop. The R185G, G220S, and T277A modifications are representatives of a modification in the C-terminal tail of a Tom2a protein (see **Figure 4**).

The CDS of a modified *Tom2a* gene in cucumber, resulting in a modified Tom2a protein, that increases Tobamovirus resistance, having a Q268stop modification is presented in SEQ ID No. 13, and the resulting truncated protein is presented in SEQ ID No. 14 (**Figure 3**).

In one embodiment, the modified *Tom2a* gene encodes a modified Tom2a protein, comprising a combination of herein disclosed modifications on any of the corresponding positions in SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12, or on another homologous sequence having at least 70% sequence similarity to SEQ ID No. 7. In one embodiment, the protein encoded by the modified *Tom2a* gene has retained a combination of two, three, or four of these modifications. **Table 2** gives an overview of a number of said nucleotide and resulting amino acid modifications.

Table 2: Certain Tom2a modifications correlating with Tobamovirus resistance

Modifications based on SEQ ID No. 1 (CDS) and SEQ ID No. 7 (protein) positions	**Tom2a**	**Tom2a**	**Tom2a**	**Tom2a**	**Tom2a**
CDS SNP modification	C802T	TGC306-308del	C553G	G658A	A829G
Susceptible wildtype nucleotide (SEQ ID No. 1)	C	TGC	C	G	A
Resistant nucleotide	T	deleted	G	A	G

Tom2a protein modification	Q268stop	A103del	R185G	G220S	T277A

As used herein, at least 70% sequence identity relates to a sequence having in order of increased preference at least 70%, 71%, 73%, 74%, 75%, 77%, 80%, 81%, 83%, 85%, 87%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity.

As used herein, at least 70% sequence similarity relates to a sequence having in order of increased preference at least 70%, 72%, 75%, 77%, 80%, 81%, 83%, 85%, 87%, 90%, 93%, 95%, 96%, 97%, 98%, or 99% sequence similarity.

As used herein, an 'X000Y' mutation, modification, SNP, or substitution means that the wildtype sequence has a nucleotide or amino acid X on position 000, which is changed to nucleotide or amino acid Y in the modified sequence. The indication 'X000del' means that the modification is a deletion of the nucleotide or amino acid on that position or positions; the indication 'X000stop' means that the modification comprises the protein being truncated starting from that position, which is the result of the gene having a mutation leading to a premature stop codon.

The invention further relates to a seed that comprises a modified *Tom2a* gene of the invention, wherein a plant grown from said seed is a plant of the invention. In a preferred embodiment, the modified *Tom2a* gene of the invention is homozygously present in the seed. The invention also relates to seed produced by a plant of the invention, wherein the seed harbors the modified *Tom2a* gene 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 a modified *Tom2a* gene in its genome.

The invention also relates to a fruit harvested from a plant of the invention, wherein the fruit comprises the modified *Tom2a* gene of the invention in its genome. This fruit is also referred to herein as 'the fruit of the invention'. Moreover, the invention also relates to a food product or a processed food product comprising a fruit of a plant of the invention, or part of said fruit. 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 preparing a salad mixture comprising the fruit of the invention. The processed form that is obtained is also part of this invention

The invention further relates to a method for seed production, comprising growing a plant from a seed of the invention that comprises a modified *Tom2a* gene of the invention, allowing the plant to produce a fruit with seed, harvesting the 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 will grow into a plant that comprises the modified *Tom2a* gene of the invention. In a preferred embodiment the modified *Tom2a* gene is homozygously present in a plant used in seed production. The method in particular relates to production of a *Cucumis sativus* or a *Solanum lycopersicum* seed.

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 is a plant of the invention comprising a modified *Tom2a* gene of the invention. 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 parent plants comprise the modified *Tom2a* gene of the invention homozygously, and the hybrid seed comprises the modified *Tom2a* gene of the invention homozygously. The hybrid seed is in particular seed of *Cucumis sativus* or of *Solanum lycopersicum*.

The present invention also relates to a method for producing a plant that has increased resistance to a virus of the genus Tobamovirus, in particular to CGMMV or to ToBRFV, comprising introducing a modification in a *Tom2a* gene, which modification leads to increased resistance. The introduced modification comprises a deletion, a substitution, or an insertion in the coding sequence of a *Tom2a* gene. Such modifications can for example lead to a codon change, a premature stop codon, or a frameshift. The introduced modification preferably leads to a modified Tom2a protein, which comprises an amino acid substitution, a truncated protein due to a premature stop codon, a deletion of one or more amino acids, or a changed amino acid sequence due to a frameshift. The introduction of such a modification can be done by a mutagenesis approach using a chemical compound, such as ethyl methane sulphonate (EMS); or by using physical means, such as UV-irradiation, fast neutron exposure, or other irradiation techniques.

Introduction of a modification can also be done using a more specific, targeted approach including targeted genome editing by means of homologous recombination, oligonucleotide-based mutation introduction, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs) or Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) systems, which comprises CRISPR/Cas systems. An endogenous susceptible *Tom2a* gene can thus be edited and modified into a *Tom2a* gene that confers increased Tobamovirus resistance.

The invention in particular relates to a method for producing a plant of the family *Cucurbitaceae* or *Solanaceae* that has increased Tobamovirus resistance, in particular increased CGMMV for the *Cucurbitaceae* or ToBRFV resistance for the *Solanaceae*, comprising introducing a modification in a *Tom2a* gene comprising SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID

No. 4, SEQ ID No. 5, or SEQ ID No. 6, or in another homologous sequence having at least 70% sequence identity to SEQ ID No. 1. The method in particular comprises the introduction of a modification that leads to a Tom2a protein that has a changed function, a reduced function, or is non-functional. In one embodiment, the introduced modification leads to a truncated protein, wherein the truncated protein comprises the first 267 remaining amino acids of SEQ ID No. 7 or less, or the corresponding number of amino acids of a homologous Tom2a protein sequence, which in particular comprises SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12. In one embodiment, the introduced modification leads to a modification in one or more of the amino acids 268-279 of SEQ ID No. 7, which results in a changed function, a reduced function, or a non-functional protein, or in the corresponding amino acids of a homologous Tom2a protein sequence, in particular SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12. The method in particular relates to producing a *Cucumis sativus* plant having increased CGMMV resistance.

Introduction of a modified *Tom2a* gene of the invention can be done through introgression from a donor plant comprising said modified *Tom2a* gene, in particular from another plant that is resistant to a Tobamovirus and in which a modified *Tom2a* gene of the invention was identified, into a recipient plant that does not carry a modified *Tom2a* gene, or which carries a modified *Tom2a* gene heterozygously. 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 donor plant to a recipient plant can be used. A donor plant, which is preferably a resistant plant, can be of the same species or of a different and/or wild species. Difficulties in crossing between species can be overcome through techniques known in the art such as embryo rescue, or cis-genesis can be applied. A plant produced by such method is also a part of the invention.

Alternatively, the modified *Tom2a* gene 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.

The present invention relates to the use of a modified *Tom2a* gene for increasing Tobamovirus resistance in a plant. The invention also relates to the use of a modified *Tom2a* gene for the development of a plant of the *Solanaceae* or *Cucurbitaceae* that has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV. The use of the modified *Tom2a* gene comprises introducing said gene into a plant.

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, preferably agronomically elite, plant that has increased resistance to a Tobamovirus, in particular

to CGMMV or ToBRFV, wherein a plant comprising the modified *Tom2a* gene of the invention is used for conferring said resistance to another plant, by transferring the modified *Tom2a* gene.

The invention also relates to a method for the production of a plant which has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV, said method comprising:

a) crossing a plant of the invention, which comprises a modified *Tom2a* gene of the invention, with another plant;

b) optionally performing one or more rounds of selfing and/or crossing of the plant resulting from the cross of step a) 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 modified *Tom2a* gene as defined herein, which plant has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV.

In a preferred embodiment, above method relates to the production of a *Cucumis sativus* plant with increased CGMMV resistance or a *Solanum lycopersicum* plant with increased ToBRFV resistance.

The invention also relates to a method for the production of a plant which has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV, said method comprising:

a) crossing a first parent plant comprising a modified *Tom2a* gene of the invention with a second parent plant, which is a plant not comprising a modified *Tom2a* gene 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 modified *Tom2a* gene of the first parent plant of step a).

In a preferred embodiment, the above method relates to the production of a *Cucumis sativus* plant with increased CGMMV resistance or a *Solanum lycopersicum* plant with increased ToBRFV resistance.

The invention additionally provides for a backcrossing method for introducing another desired trait into a plant that has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV, comprising:

a) crossing a plant comprising a modified *Tom2a* gene of the invention with a second plant that comprises the other desired trait to produce F1 progeny;

b) optionally selecting in the F1 for a plant that comprises the modified *Tom2a* gene and the other desired trait;

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

d) selecting backcross progeny comprising the increased Tobamovirus 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 increased Tobamovirus resistance and the other desired trait.

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 other desired trait is resistance to the same Tobamovirus for which resistance is increased through the presence of the modified *Tom2a* gene, but the resistance of the other desired trait is caused by a different gene then the *Tom2a* gene. This approach, known as stacking of resistances, leads to a stronger and more durable Tobamovirus resistance in a plant of the invention, in particular to stronger and more durable CGMMV or ToBRFV resistance.

In a preferred embodiment, the above backcrossing method relates to the introduction of another desired trait in a *Cucumis sativus* plant that has increased CGMMV resistance or in a *Solanum lycopersicum* plant that has increased ToBRFV resistance.

Optionally, selfing steps are performed after any of the crossing or backcrossing steps in above described methods. Selection of a plant comprising the modified *Tom2a* gene of the invention 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, plant vigor, improved shelf-life, larger fruit size, smaller fruit size, improved fruit quality, parthenocarpic fruit set, water stress tolerance, heat stress tolerance, cold stress tolerance, and male sterility. The invention includes a plant produced by this method and a fruit obtained therefrom.

The invention further relates to a method for the production of a plant comprising a modified *Tom2a* gene of the invention, by using tissue culture or by using vegetative propagation.

The invention further provides a method for the production of a plant comprising the modified *Tom2a* gene of the invention by using a doubled haploid generation technique to generate a doubled haploid line that is completely homozygous, and therefore homozygously comprises the modified *Tom2a* gene of the invention, and has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV.

The invention further relates to a method for the production of a plant comprising the modified *Tom2a* gene of the invention, wherein the presence of said modified *Tom2a* gene

leads to increased Tobamovirus resistance, which method comprises growing a seed comprising said modified *Tom2a* gene into the said plant.

The present invention relates to a method for identification of a plant comprising a modified *Tom2a* gene of the invention, wherein the identification comprises determining the presence of a modification in a *Tom2a* gene comprising SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, or in a homologous sequence of SEQ ID No. 1, and optionally analyzing if the plant comprising the modification has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV. Said method relates to the identification of a plant of a species belonging to the *Cucurbitaceae* or *Solanaceae,* and in particular to the identification of a plant of a species belonging to the genus *Cucumis* or the genus *Solanum.* The method preferably relates to the identification of a *Cucumis sativus* plant comprising a modified *Tom2a* gene, or of a wild tomato species, in particular of the species *Solanum pimpinellifolium* comprising a modified *Tom2a* gene, or to the identification of a plant of the species *Solanum lycopersicum* comprising a modified *Tom2a* gene.

Determining the presence of a modification in a *Tom2a* gene comprises identification of any modification in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, or in another homologous sequence of SEQ ID No. 1, that leads to increased Tobamovirus resistance, in particular increased CGMMV or ToBRFV resistance. Determining the presence of a modification includes any of the modifications as described herein, in particular the modifications C802T, TGC306-308del, C553G, G658A, or A829G in SEQ ID No. 1, as presented in **Table 2.** Determining the presence of any modification can be done through sequence comparison between the wildtype *Tom2a* sequence and the *Tom2a* sequence of a plant to be analysed, whereby methods for performing sequence comparison are known to the skilled person. Determining the presence of a specific modification is suitably done by using a marker that is designed to identify such modification, as its sequence comprises that particular modification in relation to the wildtype sequence.

The present invention further relates to a method for selection of a plant, which has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV, the method comprising identification of a modified *Tom2a* gene of the invention in a plant, and subsequently selecting said plant as a plant which has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV. Optionally, the increased virus resistance can be confirmed by performing a bio-assay as described herein. The selected plant obtained by such method is also a part of this invention.

The invention also relates to a method of testing a plant for the presence of the modified *Tom2a* gene of the invention that increases Tobamovirus resistance, in particular CGMMV or ToBRFV resistance, comprising detecting the presence of a polymorphism that leads

to said increased resistance in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, or in another homologous sequence of SEQ ID No. 1, in the genome of the plant. The method of testing a plant for the presence of the modified *Tom2a* gene of the invention optionally further comprises selecting a plant that comprises said modified *Tom2a* gene as a plant having increased Tobamovirus resistance, in particular increased CGMMV or ToBRFV resistance. The plant that is thus selected can subsequently be used as a source for introgressing the modified *Tom2a* gene into a plant lacking said allele.

The invention also relates to propagation material suitable for producing a 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 cell, and 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 modified *Tom2a* gene of the invention as defined herein that provides increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV. A plant of the invention may be used as a source of the propagation material.

The invention further relates to a cell comprising the modified *Tom2a* gene 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 the 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 modified *Tom2a* gene as described herein. Each cell of a plant of the invention carries the modified *Tom2a* gene of the invention, and thereby the genetic information that leads to increased Tobamovirus 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 modified *Tom2a* gene of the invention, wherein the modified *Tom2a* gene is as defined herein.

The invention further relates to plant tissue of a plant of the invention, which comprises the modified *Tom2a* gene of the invention. 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 modified *Tom2a* gene of the invention. Such progeny can in itself be a plant, a cutting, a cell, a tissue, or a seed.

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 modified *Tom2a* gene of the invention that leads to increased Tobamovirus resistance. Progeny also encompasses a plant or plant material that comprises the modified *Tom2a* gene of the invention, and that is obtained from a 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 plant of the invention that is suitable for sexual reproduction, which plant part comprises the modified *Tom2a* gene of the invention 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 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 modified *Tom2a* gene of the invention 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 modified *Tom2a* gene of the invention, the presence of which leads to increased Tobamovirus resistance.

The invention further relates to tissue culture of a plant of the invention, which is also propagation material, and which comprises the modified *Tom2a* gene of the invention in its genome. 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 plant comprising the modified *Tom2a* gene of the invention, wherein the regenerated plant has increased Tobamovirus resistance and is also part of the invention.

The invention also relates to a marker for the identification of a modified *Tom2a* gene in a plant, which marker comprises any of the modifications in a *Tom2a* gene as described herein and can thereby identify said modifications. Such marker for identification comprises a nucleotide sequence that includes a particular polymorphism in its sequence, when compared to the same sequence stretch in a wildtype *Tom2a* gene, which polymorphism leads to a modification in the encoded protein that changes the function or activity of the Tom2a protein.

A marker of the invention is in particular a marker comprising in its sequence any one of the following: a T at position 802, a deletion at positions 306-308, a G at position 553, an A at position 658, and a G at position 829, wherein the positions are relative to SEQ ID No. 1, and wherein the nucleotide is representative of a C802T, TGC306-308del, C553G, G658A, or A829G modification in SEQ ID No. 1, or comprises in its sequence nucleotides representative of a

polymorphism that leads to a modification on a corresponding position in a homologous sequence thereof, and is thereby suitable for identifying any of those modifications in a *Tom2a* gene.

Nucleotide sequences comprising said polymorphisms, that are thereby suitable to identify said polymorphisms in SEQ ID No. 1, are presented as SEQ ID No. 15 for identifying a C802T modification, SEQ ID No. 16 for identifying a TGC306-308del modification, SEQ ID No. 17 for identifying a C553G modification, SEQ ID No. 18 for identifying a G658A modification, and SEQ ID No. 19 for identifying a A829G modification. Nucleotide sequences comprising said polymorphisms, that are suitable to identify corresponding polymorphisms on the corresponding positions in SEQ ID No. 5, are presented as SEQ ID No. 20 for identifying a C817T modification, SEQ ID No. 21 for identifying a GGC312-314del modification, SEQ ID No. 22 for identifying an A559G modification, SEQ ID No. 23 for identifying a G673A modification, and SEQ ID No. 24 for identifying an A844G modification. Because the Tom2a gene is present on the minus-strand in the public *S. lycopersicum* genome, the marker sequences of SEQ ID No. 20 – 24 are presented as reversed complement sequences in relation to SEQ ID No. 5.

Optionally, the sequence to be used as a marker can be longer on either side of the modification, to ensure the sequence is unique in the genome and locates to the *Tom2a* gene. The sequences surrounding the polymorphisms are according to SEQ ID No. 1 for *Cucumis sativus*, according to SEQ ID No. 5 for *Solanum lycopersicum* or, for other crops, according to a sequence that has at least 70% sequence identity therewith, in particular SEQ ID No. 2, SEQ ID No. 3, SEQ ID NO. 4, or SEQ ID No. 6.

The invention also relates to the use of a marker for identification of a modified *Tom2a* gene, particularly a marker represented by any one of SEQ ID Nos. 15-24. The invention further relates to the use of a marker, in particular a marker as described herein, for identification of a modified *Tom2a* gene that leads to increased Tobamovirus resistance, in particular in a *Cucumis sativus* or a *Solanum lycopersicum* or a *Solanum pimpinellifolium* plant, and/or for selection of a *Cucumis sativus* or *Solanum lycopersicum* or *Solanum pimpinellifolium* plant comprising a modified *Tom2a* gene that leads to increased Tobamovirus resistance, in particular increased CGMMV or ToBRFV resistance. The invention also relates to selection of a plant identified by a marker as described herein and to the plant thus selected.

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 in the application, reference is made to the following figures.

FIGURES

Figure 1 – CDS sequences of wildtype *Tom2a* genes of *Cucumis sativus* (**SEQ ID No. 1**), *Cucumis melo* (**SEQ ID No. 2**), *Citrullus lanatus* (**SEQ ID No. 3**), *Cucurbita pepo* (**SEQ ID No.**

4), *Solanum lycopersicum* (**SEQ ID No. 5**), *Capsicum annuum* (**SEQ ID No. 6**), and alignment of SEQ ID Nos. 1-6.

Figure 2 – protein sequences of wildtype *Tom2a* genes of *Cucumis sativus* (**SEQ ID No. 7**), *Cucumis melo* (**SEQ ID No. 8**), *Citrullus lanatus* (**SEQ ID No. 9**), *Cucurbita pepo* (**SEQ ID No. 10**), *Solanum lycopersicum* (**SEQ ID No. 11**), *Capsicum annuum* (**SEQ ID No. 12**), and alignment of SEQ ID Nos. 7-12 .

Figure 3 – Modified CsTom2a CDS (**SEQ ID No. 13**) and protein (**SEQ ID No. 14**) sequence leading to CGMMV resistance.

Figure 4 – prediction of the transmembrane protein sections and domains of the Tom2a protein of a. *Cucumis sativus* and b. *Solanum lycopersicum.*

Figure 5 – Nucleotide sequences representing marker sequences SEQ ID Nos. 15 – 24.

Figure 6 – Phenotyping results of Tom2a knockout mutants in *S. lycopersicum.*

Figure 7 – Virus titer measurements of Tom2a knockout mutants in *S. lycopersicum.*

EXAMPLES

EXAMPLE 1

Tom2a TILLING mutants in Cucumis sativus

To develop new variation for various traits, a large TILLING population was developed in *Cucumis sativus*, using ethyl methane sulphonate (EMS) as the mutagenic agent. In the search for new resistances, a TILLING approach was applied for various genes, and multiple screens were performed in more than 3000 plants over the years. In a screen performed in 2016, three mutants in the *Tom2a* gene were identified; in 2017 two more were found, and in 2019 a sixth mutant was identified. All of these mutants were selected for follow-up observation, since they resulted in potentially interesting amino acid changes or premature stop codons (**Table 3**).

To obtain plants that had the mutation of interest, while other mutations resulting from the EMS treatment would be removed, first several rounds of selfing were performed that focused on maintaining the specifically identified mutations in the *Tom2a* gene.

Table 3: Tom2a TILLING mutants in C. sativus

Tom2a TILLING modifications obtained in C. sativus	**Tom2a**	**Tom2a**	**Tom2a**	**Tom2a**	**Tom2a**	**Tom2a**
CDS Tom2a SNP modification	G11A	G64A	G411A	G751A	C802T	G824A

Tom2a protein modification	R4K	G22S	W137stop	E251K	Q268stop	R275H

The screen from 2016 included the very interesting C802T mutant, which resulted in a truncated protein that only comprised the first 267 amino acids of the wildtype protein. This mutant, which was assigned the code 'mutant 2720', was among the first to have gone through several selfing steps, and was selected for a first phenotyping observation round for virus resistance. In addition, backcrosses with the line used for developing the EMS population was done, designated 'KK1', and segregating populations were developed. In a segregating population, good phenotypic comparison of plants with and without the specific mutation can be done.

EXAMPLE 2

Phenotyping of Q268stop mutant for CGMMV resistance in Cucumis sativus

A population of cucumber plants of Q268stop mutant '2720' was selfed two times, while selecting for the presence of the C802T mutation. After these rounds of selfing, a phenotyping assay for CGMMV resistance was performed.

To do the bio-assay, seeds of the mutant plants were sown, and the CGMMV susceptible KK1 line and susceptible variety Ventura F1 were included as controls. Sowing was done in a standard seedling tray at 23°C. After 5 days, 30 seedlings of the mutant and 30 seedlings of the controls were transplanted to a larger tray. The plants of the mutant line and the controls were divided over three replicates. Unfortunately, only 17 plants of the mutant line survived; 7 in the first replicate, 4 in the second, an 6 in the third replicate. One week after sowing, the transplants were inoculated and transferred to a temperature regime of 20°C by day and 18°C by night. Inoculum was prepared by grounding leaves of cucumber plants that were infected with CGMMV in a 0.01 M phosphate buffer (pH 7.0). The seedlings were then dusted with carborundum powder prior to gently rubbing the leaf with inoculum.

Resistance was scored on a scale of 1-5, according to the description of the scales of the scores in **Table 1.** Observation of the symptoms on the young cucumber plants in the bio-assay was done 14-21 days after inoculation (dai). Scores are presented in **Table 4**; a/b/c/d/e corresponds to a score in scale 1/2/3/4/5**.** The mutant showed a clearly increased CGMMV resistance over the susceptible controls.

Table 4: phenotyping of the Q268stop mutant for CGMMV resistance

CGMMV phenotyping	Total score over all replicates	Score rep 1	Score rep 2	Score rep 3	Average over all replicates
Ventura	0/0/0/0/30	0/0/0/0/10	0/0/0/0/10	0/0/0/0/10	5.0
KK1	0/0/1/9/20	0/0/0/0/10	0/0/1/9/0	0/0/0/0/10	4.6
Q268stop mutant	0/11/0/6/0	0/7/0/0/0	0/4/0/0/0	0/0/0/6/0	2.7

EXAMPLE 3

Modification of a Tom2a gene to obtain resistance to a Tobamovirus

Modifications are introduced in seed of a plant of interest in which resistance to a Tobamovirus is needed, in particular to CGMMV in a plant of the *Cucurbitaceae,* or to ToBRFV in a plant of the *Solanaceae.* The modification is introduced through mutagenesis, such as an EMS treatment, through radiation means, or through a specific targeted approach, such as a CRISPR/Cas system. When a non-targeted approach such as EMS is used, this is combined with an identification technique such as TILLING. In this way, both for mutagenesis as well as a targeted modification means, a modification in a *Tom2a* gene can be generated and identified. The skilled person is familiar with these means for introducing modifications into the genome of a plant of interest.

Modified seed is then germinated and plants are grown, which are crossed or selfed to generate M2 or further generation seed. Subsequently a plant screen is performed to identify the modifications in a *Tom2a* gene, based on comparison to the wildtype sequence of the *Tom2a* gene of that species. For *Cucumis sativus* for example, comparison to SEQ ID No. 1 should be done; for *Solanum lycopersicum,* comparison to SEQ ID No. 5 should be done. The skilled person is familiar with TILLING to identify mutations in specific genes (McCallum et. al. (2000) Nature Biotechnology, 18: 455-457), and with techniques for identifying nucleotide changes such as DNA sequencing, amongst others.

Plants with a modified *Tom2a* gene are homozygous or made homozygous by selfing, crossing, or the use of doubled haploid techniques which are familiar to the skilled person. Plants identified and selected on the basis of a modification in a *Tom2a* gene can then be tested for resistance to a Tobamovirus, in particular for resistance to CGMMV or ToBRFV. A plant that is produced, identified and selected in this way is confirmed to have their virus resistance as a result from one or more modifications in the *Tom2a* gene.

EXAMPLE 4

Tom2a TILLING mutants in Solanum lycopersicum

To develop new variation for various traits, a large TILLING population was developed in *Solanum lycopersicum*, using ethyl methane sulphonate (EMS) as the mutagenic agent. In the search for new Tobamovirus resistance, a TILLING approach was applied for various genes, and multiple screens were performed over the years. In the *Tom2a* gene, a total of ten mutants that led to protein changes were found. According to a SIFT prediction, four of those mutants were predicted to be tolerated, and the others were either deleterious or resulted in a truncated protein. These mutants, in particular the ones that are predicted to be deleterious or resulted in a truncated protein, were selected for follow-up phenotype observation, since they resulted in potentially interesting amino acid changes or premature stop codons (**Table 5**).

To obtain plants with only the mutation of interest, several rounds of selfing and backcrossing are being performed to get the suitable generation for doing a bio-assay on Tobamovirus resistance, in particular on ToBRFV resistance.

Table 5: Tom2a TILLING mutants in S. lycopersicum

Tom2a TILLING mutants obtained in S. lycopersicum	**Result SIFT prediction**
W7stop	
R191K	TOLERATED
A172V	TOLERATED
A91T	TOLERATED
A2V	DELETERIOUS
P144L	DELETERIOUS
P227L	DELETERIOUS
C3Y	DELETERIOUS
P80L	DELETERIOUS

D45Y	TOLERATED

EXAMPLE 5

Generation of Tom2a CRISPR mutants in Solanum lycopersicum

An internal ToBRFV susceptible *S. lycopersicum* breeding line was used for developing targeted knockouts of the *Tom2a* gene, using a CRISPR/Cas9 editing system, to observe the effect on ToBRFV resistance. The susceptible *S. lycopersicum* line was determined to have the wildtype *Tom2a* sequence, represented by SEQ ID No. 5. The annotated gene sequence was used to design a large number of possible single guide RNAs (sgRNAs). Subsequently two sgRNAs were chosen that had a high on-target score and a low off-target score. The *Tom2a* gene comprises 6 exons; one of the sgRNAs targeted a location in exon 1. The other sgRNA targeted a location in exon 5. The used sgRNAs are presented in **Table 6**.

Table 6: sgRNA sequences

sgRNA nr	SEQ ID No.	#RGEN Target (5' to 3') sgRNA-PAM	Gene name
1	SEQ ID No. 25	TGCGTGGAGTACACGTATGA	Tom2a
2	SEQ ID No. 26	CCAGTTGCACCACCTATGAG	Tom2a

The two selected sgRNAs were used in *Agrobacterium tumefaciens*-mediated transformation with the CRISPR/Cas9 construct, according to the protocol as described in Pan, C. et al, CRISPR/Cas9-mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations. *Sci. Rep.* 6, 24765 (2016).

The use of the combination of the two sgRNAs resulted in multiplex events in the Tom2a gene. Mutant plants were obtained that were affected in both targeted locations. Two plants with mutations in the Tom2a gene were selected. Both mutants comprised a 1bp insertion in exon 1, at location 157 of SEQ ID No. 5, which led to a frameshift. One of the mutants comprised another event, a 1 bp insertion in exon 5, at location 737 of SEQ ID No. 5. The other mutant comprised a different second event, namely a 2 bp deletion in exon 5, of positions 736 and 737 of SEQ ID No. 5. The targeted mutations led to knockouts of the Tom2a gene. Seeds of the mutant plants were increased to the T2 generation. The mutations were homozygously present in the T2 generation, which generation was tested for ToBRFV resistance. Eight T2 lines having a combination of a mutation in exon 1 and exon 5 were selected for phenotyping.

EXAMPLE 6

Phenotyping for symptoms and virus titer of Tom2a knockouts in S. lycopersicum.

Eight T2 lines obtained from plants having homozygous knockouts of the *Tom2a* gene, that were obtained by the CRISPR/Cas9 editing method described in Example 5, were phenotyped in a ToBRFV bio-assay. All plants had the described 1 bp insertion in exon 1; lines 3.71, 3.64, and 3.32 also had the described 1bp insertion in exon 5. Lines 3.62, 3.54, 3.38, 3.28, and 3.14 had next to the exon 1 insertion also the described 2bp deletion in exon 5.

From all numbers, 20 seeds were sown in standard seedling trays and seedlings were transplanted to larger pots after 2-3 weeks. The transplanted seedlings were inoculated 4 weeks after sowing. As a susceptible control, the variety Eclipse F1 was included. From some lines, not all seeds were germinated. From lines 3.14 and 3.71, only 11 seedlings could be inoculated; from line 3.38, 10 seedlings were inoculated; and from line 3.64, only 6 seedlings were inoculated. Inoculum was 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 were then dusted with carborundum powder prior to gently rubbing the leaf with inoculum. Resistance was scored on a scale of 0-5; the description of the scales of the scores can be found in **Table 7.** Observation of the symptoms on the young tomato plants in the bio-assay was done 14-21 days after inoculation (dai).

Table 7: 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

All plants of the susceptible control nicely showed a score 4 during phenotyping, which indicated a well-performed experiment. The *Tom2a* knockout mutants that were included in the assay showed an extremely good level of resistance. Average scores were all 1.0 or lower; for line 3.64, which scored 1.0, only a limited number of plants could be included. Scores of the phenotypes are presented in **Figure 6.**

Apart from the visual symptoms, also the measurement of the virus titer is a very relevant aspect of virus resistance. Virus titer was determined in ToBRFV infected leaf samples from 20 plants of each T2 line that was obtained as described in Example 5. As a susceptible control, the variety Eclipse F1 was used. From each plant a leaf punch of 6 mm in diameter was taken, and subsequently ground in 500 µl of PBS buffer solution. 50 µl of the resulting suspension was used in a 96-well KingFisher Flex isolation protocol, whereby isolation of the leaf material

was done using the innuPREP DNA/RNA virus PLUS Kit. The samples were 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, was included in the qPCR assay, which corrects any variation in the amount of sample material, and then yields a Cq_PHD value. To accurately determine the final value for virus titer the Delta Cq method was 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 was higher than -11.00, or when the average Cq-corr was at least 5.00 higher than the average Cq_corr of the susceptible control.

Virus titer results of all knockout mutants were very convincing. The average Cq_corr value of the susceptible control was -16.21. All mutants had an average score that was higher than -5.00, and most of them even had an average score higher than 0.00. Results of virus titer measurements are presented in **Figure 7**.

It was concluded that *Tom2a* knockout mutants in *S. lycopersicum* result in a very high level of ToBRFV resistance, for both visual symptoms as well as virus titer in the plants.

CLAIMS

1. A plant comprising a modified *Tom2a* gene, the wildtype of which comprises SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, or SEQ ID No. 6, or comprises another homologous sequence having at least 70% sequence identity to SEQ ID No. 1, which modified gene leads to increased resistance to a Tobamovirus.

2. A plant as claimed in claim 1, which comprises a modification in the *Tom2a* gene that leads to a modified Tom2a protein that has a deletion, a substitution, or an insertion of at least one amino acid when compared to SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12, or compared to another homologous sequence having at least 70% sequence similarity to SEQ ID No. 7.

3. A plant as claimed in claim 1 or 2, wherein the modification results in a premature stop codon which leads to a truncated protein.

4. A plant as claimed in any one of the claims 1-3, wherein the Tom2a protein encoded by the modified *Tom2a* gene is absent, has a changed function, a reduced function, or it is non-functional.

5. A plant as claimed in any of the claims 1-4, wherein the modification in the Tom2a protein is present in a transmembrane domain or in the C-terminal tail.

6. A plant as claimed in any of the claims 1-4, wherein the modification in the Tom2a protein is present in Non-CP EC1 or Non-CP EC2.

7. A plant as claimed in any of the claims 1-6, wherein the modified Tom2a protein is truncated and comprises only amino acids 1-267 of SEQ ID No. 7 or less, or the corresponding number of amino acids of a homologous Tom2a protein sequence, or the modified Tom2a protein comprises a modification in amino acids 268-279 of SEQ ID No. 7 that leads to a changed function, a reduced function, or a non-functional protein, or in the corresponding amino acids of a homologous Tom2a protein sequence, in particular of SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12.

8. A plant as claimed in any of the claims 1-7, wherein the modified Tom2a protein comprises SEQ ID No. 14.

9. A plant as claimed in any one of the claims 1-8, which is a plant of the family *Cucurbitaceae* or *Solanaceae.*

10. A plant as claimed in claim 9, which is a plant of the species *Cucumis sativus, Cucumis melo, Citrullus lanatus, Cucurbita pepo, Solanum lycopersicum,* or *Capsicum annuum,* preferably a plant of the species *Cucumis sativus* or *Solanum lycopersicum.*

11. A plant as claimed in claim 9 or 10, which is a plant of the family *Cucurbitaceae* and has increased resistance to CGMMV.

12. A plant as claimed in claim 9 or 10, which is a plant of the family *Solanacae* and has increased resistance to ToBRFV.

13. A modified *Tom2a* gene encoding a Tom2a protein comprising a deletion, a substitution, or an insertion of at least one amino acid when compared to SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12, or compared to another homologous sequence having at least 70% sequence similarity to SEQ ID No. 7.

14. A modified *Tom2a* gene as claimed in claim 13, which modified gene comprises a premature stop codon and which encodes a truncated Tom2a protein

15. A modified *Tom2a* gene as claimed in claim 13 or 14, wherein the Tom2a protein encoded by the modified *Tom2a* gene is absent, has a changed function, a reduced function, or it is non-functional.

16. A modified *Tom2a* gene as claimed in any of the claims 13-15, wherein the encoded modified Tom2a protein is truncated and comprises only amino acids 1-267 of SEQ ID No. 7 or less, or the corresponding number of amino acids of a homologous Tom2a protein sequence, or the modified Tom2a protein comprises a modification in amino acids 268-279 of SEQ ID No. 7 that leads to a changed function, a reduced function, or a non-functional protein, or in the corresponding amino acids of a homologous Tom2a protein sequence, in particular of SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, or SEQ ID No. 12.

17. A modified *Tom2a* gene as claimed in any of the claims 13-16, wherein the presence of the modified gene in a plant leads to increased Tobamovirus resistance, in particular to increased CGMMV resistance in a plant of the family *Cucurbitaceae*, or to increased ToBRFV resistance in a plant of the family *Solanaceae*.

18. Seed, comprising a modified *Tom2a* gene as defined in any of the claims 13-17, wherein the plant grown from the seed is a plant as claimed in any one of claims 1-12.

19. Use of a marker for identification of a modified *Tom2a* gene that leads to increased Tobamovirus resistance, in particular a marker comprising any one of SEQ ID Nos. 15-24.

20. Use of a marker as claimed in claim 19 for identification of a plant having a modified *Tom2a* gene.

21. Use of a marker as claimed in claim 19 or 20 for identification of increased Tobamovirus resistance in a plant.

22. Method for increasing Tobamovirus resistance in a plant, in particular CGMMV or ToBRFV resistance, comprising introducing a modification in a *Tom2a* gene, wherein the modification leads to a *Tom2a* gene as defined in any of the claims 13-17.

23. Method for increasing Tobamovirus resistance in a plant, comprising introducing a modified *Tom2a* gene as claimed in any one of the claims 13-17 into a plant.

24. Method for producing a plant of the family *Cucurbitaceae* or *Solanaceae* that has increased Tobamovirus resistance, in particular increased CGMMV or ToBRFV resistance, comprising introducing a modification in a *Tom2a* gene comprising SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, or SEQ ID No. 6, or in another homologous sequence having at least 70% sequence identity to SEQ ID No. 1.

25. Method as claimed in claim 24 wherein the modification leads to a *Tom2a* gene as defined in any of the claims 13-17.

26. Method for the production of a plant which has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV, comprising:

a) crossing a plant as claimed in any of the claims 1-12, with another plant;

b) optionally performing one or more rounds of selfing and/or crossing of the plant resulting from the cross of step a) 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 modified *Tom2a* gene as defined in any of the claims 13-17.

27. Method for the production of a 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 is a plant as claimed in any of the claims 1-12, and wherein the hybrid plant that is grown from the seed has increased Tobamovirus resistance, in particular increased CGMMV or ToBRFV resistance.

28. Method as claimed in claim 27 for the production of a hybrid *Cucumis sativus* seed that has increased CGMMV resistance.

29. Method as claimed in claim 27 for the production of a hybrid *Solanum lycopersicum* seed that has increased ToBRFV resistance.

30. The hybrid seed produced by the method of claim 27 or 28 or 29.

31. Method for identification of a plant comprising a modified *Tom2a* gene as claimed in any of the claims 13-17, wherein the identification comprises determining the presence of a modification in a *Tom2a* gene comprising SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, or in another homologous sequence of SEQ ID No. 1, and optionally analyzing if the plant comprising the modification has increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV.

32. Propagation material suitable for producing a plant as claimed in any of the claims 1-12, 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 cell, and 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 modified *Tom2a* gene as claimed in any of the claims 13-17 that provides increased resistance to a Tobamovirus, in particular to CGMMV or ToBRFV.

33. Method for seed production, comprising growing a plant from a seed as claimed in claim 18 that comprises a modified *Tom2a* gene as claimed in any of the claims 13-17, allowing the plant to produce a fruit with seed, harvesting the fruit, and extracting those seed.

Fig. 1

Tom2a CDS sequences

SEQ ID No. 1 - *Cucumis sativus* (cucumber) CDS (Csa3G904130.1)

ATGGCCTGCAGAGGGTGCTTTGAGTGCCTATTGAAGCTTTTGAACTTCTTCTTGTCCCTGCTGGGTCTTGCCATGGT
GGGCTATGGGATTTACTTATTGGTTGAGTACTTGCAATCTCATAGTGATGTTCCAGGACCTTCGTTGAGTGGTGATC
ATGATCTGGTCCAGCTTGGTCGACCAATGCTAATGGCTGTGTCTCTGTCTTCTAGCATCTTTGACAACCTTCCAAAA
GCCTGGTTCATATATTTGTTCATTGCTACGGGAGTCACTATCTTTGTTGTCTCTTGTTTTGGCTGTATTGGAGCTGCA
ACACGTAGTGGATGCTGTTTAAGTTGTTATTCGATTTTATTGCTTCTACTGATTTTGGTTCAACTAGGATGTGGTGCC
TTCATATTCTTTGACAAAAATTGGAGAGATGAAATTCCTGGGGACAGAACAGGAAACTTTGATAAGATCTATGAAC
TTCTGGAAAGCAAGTGGGAAATCATCAGATGGGTTGCACTAGGAACTATAATTTTTGAGGCTCTCCTTTTCTTGTTG
GCTCTTGTGGTTCGTGCAGCAAACAGACCTGTAGACTATGACAGTGATGATGAATACATTGCTCCAAGGCAACAAA
TCCGACAACCGTTGATCAATAGGCCCGTTGCTCCAGCAACAGGTGTGCCTGTTGCTGGGACACTTGATCAACGACC
AAGTCGAAATGATGCTTGGAGTACACGAATGAGGGAAAAGTATGGGCTGGATACTTCGGAGTTCACGTACAACCC
ATCTGAGTCTCACAGGTTTCAGCAAGTTGCTCCTCAGCCAGCCGAAGAAAAGAGCCGCTGCACAATCATGTGA

SEQ ID No. 2 - *Cucumis melo* (melon) CDS (MELO3C003376)

ATGGCCTGCAGAGGGTGCTTTGAGTGCCTATTGAAGCTTTTGAACTTCTTCTTATCTCTGCTGGGTCTTGCCATGGT
GGGCTATGGGATTTACTTGTTGGTTGAGTACTTGCAATCTTCTAGTGATGTTCCAGGACCTTCCTTGAGTGGTGATC
ATGATCTGGTCCAGCTTGGTCGACCAATGCTAATGGCCGTGTCTCTGTCTTCTAACATCTTTGATAACCTTCCAAAAG
CCTGGTTCATATATTTGTTCATTGCTACGGGAGTCATTGTCTTTGTTGTCTCTTGTTTTGGCTGTATTGGAGCTGCAA
CACGTAGTGGATGCTGTTTAAGTTGTTATTCGATTTTATTGCTTCTACTGATTTTGGTGCAACTAGGATGTGCTGCCT
TCATATTCTTTGACAAAAATTGGAGAGATGAAATTCCTGGGGACAAAACAGGAAACTTTGATAAGATCTATGAACT
TCTGGAGAACAAGTGGAAAATCATCAGATGGGTTGCATTAGGAACTATAATTTTTGAGGCTCTCCTTTTCTTGTTGG
CTCTTGTGGTTCGTGCAGCAAACAGACCTGTAGACTATGACAGTGATGATGAGTACATTGCTCCAAGGCAACAAAT
CCGACAACCGTTGATCAATAGGCCCGTTGCTCCAGCAACTGGTGTGCCTGTTGCTGGGACACTTGATCAACGACCA
AGTCGAAATGATGCTTGGAGTGCACGAATGAGGGAAAAGTATGGGCTGGATACTTCGGAGTTCACGTACAACCCA
TCCGAGTCTCACAGGTTTCAGCAAGTTGCTCCTCAGGCAGCCGAAGAAAAGAGCCGCTGCACCATCATGTGA

SEQ ID No. 3 - *Citrullus lanatus* (watermelon) CDS (Cla97C09G175160)

ATGGCCTGCAGAGGGTGCTTTGAGTGCCTATTGAAGCTTCTGAACTTCTTCTTATCCCTGCTGGGTCTTGCCATGGT
GGGCTATGGGATTTACTTGTTGGTTGAGTATTTGCAATCTCCTAGTGATGTCCCAGGACCTTCGTCGAGTGGTGATC
ATGATCTGGTCCAGCTTGGTCGACCAATGCTAATGGCCGTGTCTCTGTCTTCTAATATCTTTGATAACCTTCCAAAAG
CCTGGTTCATATACTTGTTCATTGCTGTGGGAGTCATTATCTTTGTTGTCTCTTGTTTTGGGTGTATTGGAGCTGCAA
CACGTAATGGATGCTGTTTAAGTTGTTATTCAGTTTTGCTGCTTCTACTGATTTTGGTGCAACTAGGATGTGCTGCCT
TCATATTCTTTGACAAAACTTGGAGAGATGAAATTCCTGGGGACAAAACAGGAAACTTTGATAAGATCTATGAACT
CCTTGAAGACAAGTGGGAAATCATCAGATGGGTTGCATTGGGAGCTGTAATTTTTGAGGCCCTCCTTTTCTTGTTGG
CTCTTGTGGTTCGTGCAGCAAACAGACCTGCAGACTATGACAGCGATGATGAGTACATTGCTCCAAGGCAACAAAT
CAGACAACCATTGATCAATAGGCCTGCTGCTCCTGCAACAGGTGTGCCTGTGGCTGGGACACTTGATCAACGACCA
AGTCGAAATGATGCTTGGAGTACACGAATGAGGGAAAAGTATGGGCTGGATACTTCAGAGTTCACATACAACCCA
TCTGAGTCTCACAGGTTTCAGCAAGTTGCCCCTCAGCCAGCCGAAGAAAAGAGCCGCTGCACCATCATGTGA

Fig. 1 (cont.)

SEQ ID No. 4 - *Cucurbita pepo* (squash) CDS (Cp4.1LG05g01990)

ATGGCCTGCAGAGGGTGCTTTGAGTGCCTATTGAAGCTTCTGAACTTCTTCTTATCCCTGCTGGGTCTTGCCATGGT
GGGCTATGGGATTTACTTGTTAGTTGAGTACTTGCAATCTTCTAGTGATGTTCCAAGACCTCCGTTGAGTGGTGATC
ATGATGTGATTCAGCTTGGTCGACCAATGCTAATGGCCGTGTCTCTGTCTTCTAACATCTTTGATAACCTTCCAAGA
GCCTGGTTCATATACTTGTTCATTGCTGTGGGAGTCATTATCTTTGTTGTCTCTTGTTTTGGGTGTATTGGAGCTGCC
ACACGTAATGGATGCTGTTTAAGTTGTTATTCAGTTTTGGTGCTTCTACTGATTTTGGTGCAACTAGGATGTGCAGC
ATTCATATTCTTTGACAAACATTGGAGAGATGAAATTCCTGGGGATAGAACTGGGAACTTTGATAAAATCTATGAA
CTCCTGGAAGACAAGTGGGAAATCATCAGATGGGTTGCATTAGGAGCTGTAATTTTTGAGGCTCTCCTTTTCTTGTT
GGCCCTTGTGGTTCGTGCAGCAAACAGACCTTTAGACTATGACAGTGATGATGAGTACATTGCTCCAAGGCAACAA
ATCCGACAACCGTTGATCAATAGGCCTGTTGCTCCTGCAACTGGTGTGCCTGTTGCTGGGACACTTGATCAACGACC
AAGCCGAAATGATGCTTGGAGTACACGAATGAGGGAAAAGTATGGGCTGGATACTTCCGAGTTCACATACAACCC
ATCTGAGTCTCACAGGTTTCAGCAAGTTGCCCCACAGCCAGCAGAAGAAAAGAGCCGCTGCACCATCATGTGA

SEQ ID No. 5 - *Solanum lycopersicum* (tomato CDS) (Solyc08g077220.2.1)

ATGGCGTGCAAAGGGTTTTGGGAGTGCTTGTTGAAGCTCTTGAACTTTTTGTTGACCCTTGTTGGTTTGACAATGGT
GGGGTATGGTATTTATCTATTTGTTGAGTACAAAAATCATTCACACTCCGGAGATGATTACCCAGTTGCACCACCTA
TGAGTGGTGACATGATAGAGTTTGGTCGTCCAATGCTGATGGCTGTATCGTTGGCTGAAAACATATTTGATAAACT
TCCAAAACCTTGGTTCATATATTTGTTTATTGGTATTGGAGCAGTTCTTGTAGTTGTATCTTGCTGTGGTTGTATTGG
AGCGGCAACAAGGAATGGTTGCTGCCTGAGTTGTTACTCCATGTTGATTTTCTTGTTGATCTTGGTAGAGCTAGGTG
CTGCTGGTTTTATATTCTTTGATAAAAGCTGGAAAGATGAAATTCCAAGGGATAAAACGGGCAACTTTGAAACGAT
CTATGACTTTCTGGATGACCACTGGAAGATTATTAAGTGGGTTGCCCTTGGTGCTGTTATATTCGAGGCTCTTATAT
TCTTATTGGCCCTCGTAGTAAGGGCAGCAAACAGACCAGCAGACTATGATAGTGATGATGAGTACATAGGTGGTCC
CAGACAACAAATCCGACAGCCACTGATCAACAATAGGCCACCAGCAAATCCTGCAACTGGTGTCCCTGTTACTGCT
ACTCTTGATAATCGTCCAAGTAGAAATGATGCGTGGAGTACACGTATGAGGGAAAAGTATGGACTTGACACATCA
GAGTTTACTTACAACCCATCGGAGTCGAACAGATATCCGCCAACTGCCGCACAGCCGCAAGAGGAAAGGAAGGGT
TGTACCATAATGTGA

SEQ ID No. 6 - *Capsicum annuum* (pepper) CDS (XP_016571245.1)

ATGGCGTGCAAAGGTTTTTGGGAGTGTTTGTTGAAGCTCTTGAACTTCTTGTTGACCCTTGTTGGTTTGGCAATGGT
GGGGTATGGTATTTATCTATTTGTTGAATACAAAAATCATTCATCCTCCGGGGATGATCACCCTGTTGCACCACCTA
CAAGTGCTGCTGATGTGATAGAGTTTGGTCGTCCAATGCTTATGGCTGTATCGTTGGCTGATAACATCTTTGATAAA
CTTCCGAAACCTTGGTTCATATATTTGTTTATTGGTGTTGGAGCAGTTCTTGTAATTGTGTCTTGCTGTGGTTGCATT
GGAGCGGCAACAAGGAACGGCTGTTGCCTGAGTTGTTACTCCGTGTTGATTTTCTTGTTGATCCTGGTAGAGCTAG
GTGCTGCTGGTTTTATGTTCTTTGATAAAAGCTGGAGAGAGGAAATTCCGACTGACAAAACGGGTAACTTTGATAC
GATCTATGGATTTTTGGATGAACACTGGAAGATTGTCAAGTGGGTTGCCCTTGGTGCTGTTATTTTAGAGGCTCTTA
TATTCTTATTGGCCCTCATAGTAAGGGCGGCAAACAGACCAGCAGACTATGATAGTGATGATGAGTACATAGGTGG
TCCCAGACAACAAATCCGACAGCCACTGATCAGCAATAGGGCACCACCAAATGCTGCAACTGGTGTCCCTGTTACT
GGTACCCTTGATAATCGTCCAAGTAGAAATGACGCATGGAGTACACGTATGAGAGAAAAGTATGGGCTCGATACA
TCGGAGTTCACTTACAACCCGTCAGAGTCCAACAGATATCCACAAACAGCCGTACAGCCGCAAGAGGAAAAGAAG
GGTTGTGCCATAATGTGA

Fig. 1 (cont.)

CDS alignment SEQ ID Nos. 1-6

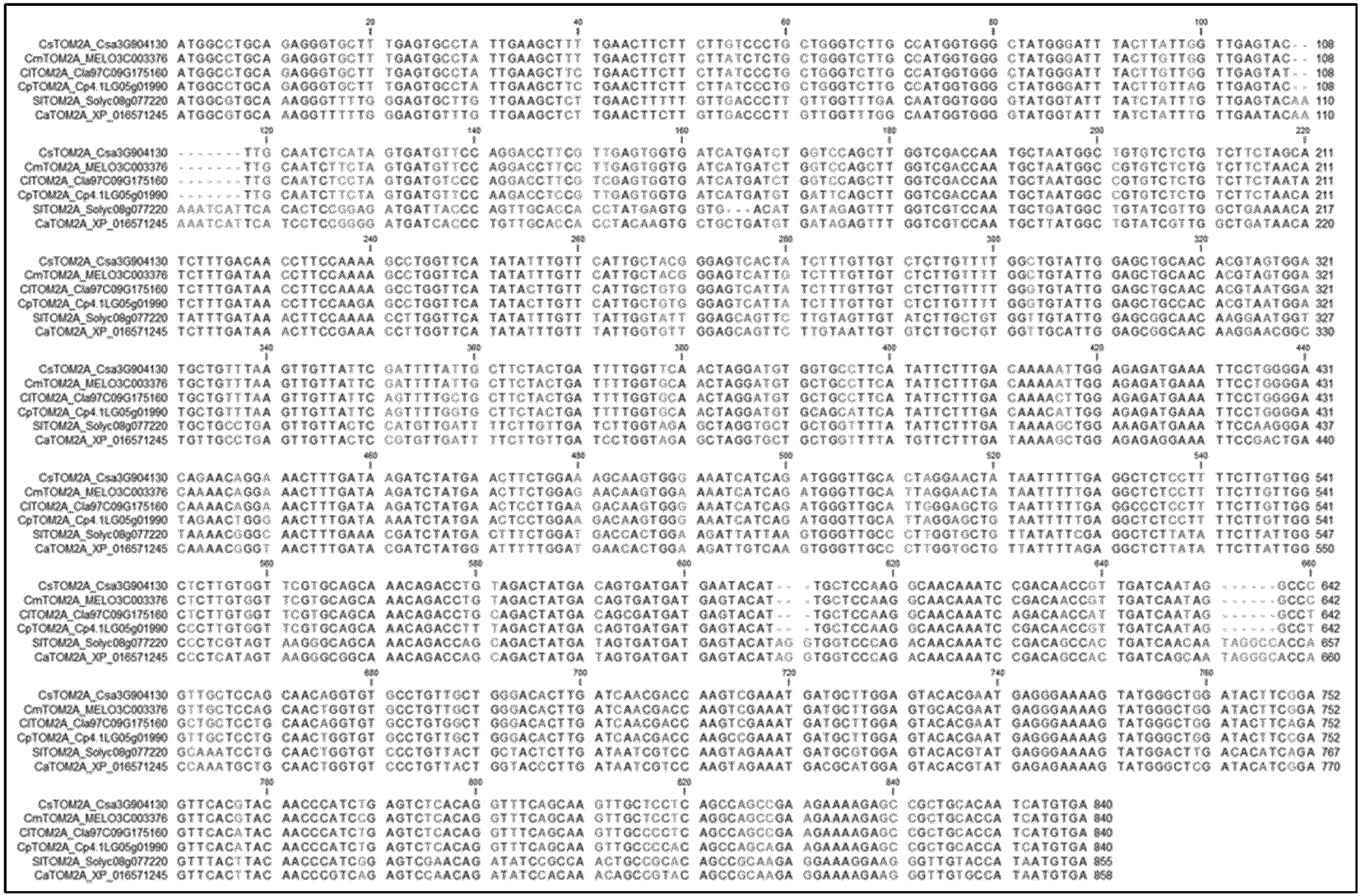


Fig. 2

Tom2a protein sequences

SEQ ID No. 7 - *Cucumis sativus* (cucumber) protein (Csa3G904130.1)
MACRGCFECLLKLLNFFLSLLGLAMVGYGIYLLVEYLQSHSDVPGPSLSGDHDLVQLGRPMLMAVSLSSSIFDNLPKAWF
IYLFIATGVTIFVVSCFGCIGAATRSGCCLSCYSILLLLLILVQLGCGAFIFFDKNWRDEIPGDRTGNFDKIYELLESKWEIIRW
VALGTIIFEALLFLLALVVRAANRPVDYDSDDEYIAPRQQIRQPLINRPVAPATGVPVAGTLDQRPSRNDAWSTRMREKY
GLDTSEFTYNPSESHRFQQVAPQPAEEKSRCTIM

SEQ ID No. 8 – *Cucumis melo* (melon) protein (MELO3C003376)
MACRGCFECLLKLLNFFLSLLGLAMVGYGIYLLVEYLQSSSDVPGPSLSGDHDLVQLGRPMLMAVSLSSNIFDNLPKAWF
IYLFIATGVIVFVVSCFGCIGAATRSGCCLSCYSILLLLLILVQLGCAAFIFFDKNWRDEIPGDKTGNFDKIYELLENKWKIIRW
VALGTIIFEALLFLLALVVRAANRPVDYDSDDEYIAPRQQIRQPLINRPVAPATGVPVAGTLDQRPSRNDAWSARMREKY
GLDTSEFTYNPSESHRFQQVAPQAAEEKSRCTIM

SEQ ID No. 9 – *Citrullus lanatus* (watermelon) protein (Cla97C09G175160)
MACRGCFECLLKLLNFFLSLLGLAMVGYGIYLLVEYLQSPSDVPGPSSSGDHDLVQLGRPMLMAVSLSSNIFDNLPKAWF
IYLFIAVGVIIFVVSCFGCIGAATRNGCCLSCYSVLLLLLILVQLGCAAFIFFDKTWRDEIPGDKTGNFDKIYELLEDKWEIIRW
VALGAVIFEALLFLLALVVRAANRPADYDSDDEYIAPRQQIRQPLINRPAAPATGVPVAGTLDQRPSRNDAWSTRMREK
YGLDTSEFTYNPSESHRFQQVAPQPAEEKSRCTIM

SEQ ID No. 10 – *Cucurbita pepo* (squash) protein (Cp4.1LG05g01990)
MACRGCFECLLKLLNFFLSLLGLAMVGYGIYLLVEYLQSSSDVPRPPLSGDHDVIQLGRPMLMAVSLSSNIFDNLPRAWFI
YLFIAVGVIIFVVSCFGCIGAATRNGCCLSCYSVLVLLLILVQLGCAAFIFFDKHWRDEIPGDRTGNFDKIYELLEDKWEIIR
WVALGAVIFEALLFLLALVVRAANRPLDYDSDDEYIAPRQQIRQPLINRPVAPATGVPVAGTLDQRPSRNDAWSTRMRE
KYGLDTSEFTYNPSESHRFQQVAPQPAEEKSRCTIM

SEQ ID No. 11 – *Solanum lycopersicum* (tomato) protein (Solyc08g077220.2.1)
MACKGFWECLLKLLNFLLTLVGLTMVGYGIYLFVEYKNHSHSGDDYPVAPPMSGDMIEFGRPMLMAVSLAENIFDKLP
KPWFIYLFIGIGAVLVVVSCCGCIGAATRNGCCLSCYSMLIFLLILVELGAAGFIFFDKSWKDEIPRDKTGNFETIYDFLDDH
WKIIKWVALGAVIFEALIFLLALVVRAANRPADYDSDDEYIGGPRQQIRQPLINNRPPANPATGVPVTATLDNRPSRNDA
WSTRMREKYGLDTSEFTYNPSESNRYPPTAAQPQEERKGCTIM

SEQ ID No. 12 – *Capsicum annuum* (pepper) protein (XP_016571245.1)
MACKGFWECLLKLLNFLLTLVGLAMVGYGIYLFVEYKNHSSSGDDHPVAPPTSAADVIEFGRPMLMAVSLADNIFDKLP
KPWFIYLFIGVGAVLVIVSCCGCIGAATRNGCCLSCYSVLIFLLILVELGAAGFMFFDKSWREEIPTDKTGNFDTIYGFLDEH
WKIVKWVALGAVILEALIFLLALIVRAANRPADYDSDDEYIGGPRQQIRQPLISNRAPPNAATGVPVTGTLDNRPSRNDA
WSTRMREKYGLDTSEFTYNPSESNRYPQTAVQPQEEKKGCAIM

Fig. 2 (cont.)

Protein alignment SEQ ID Nos. 7-12

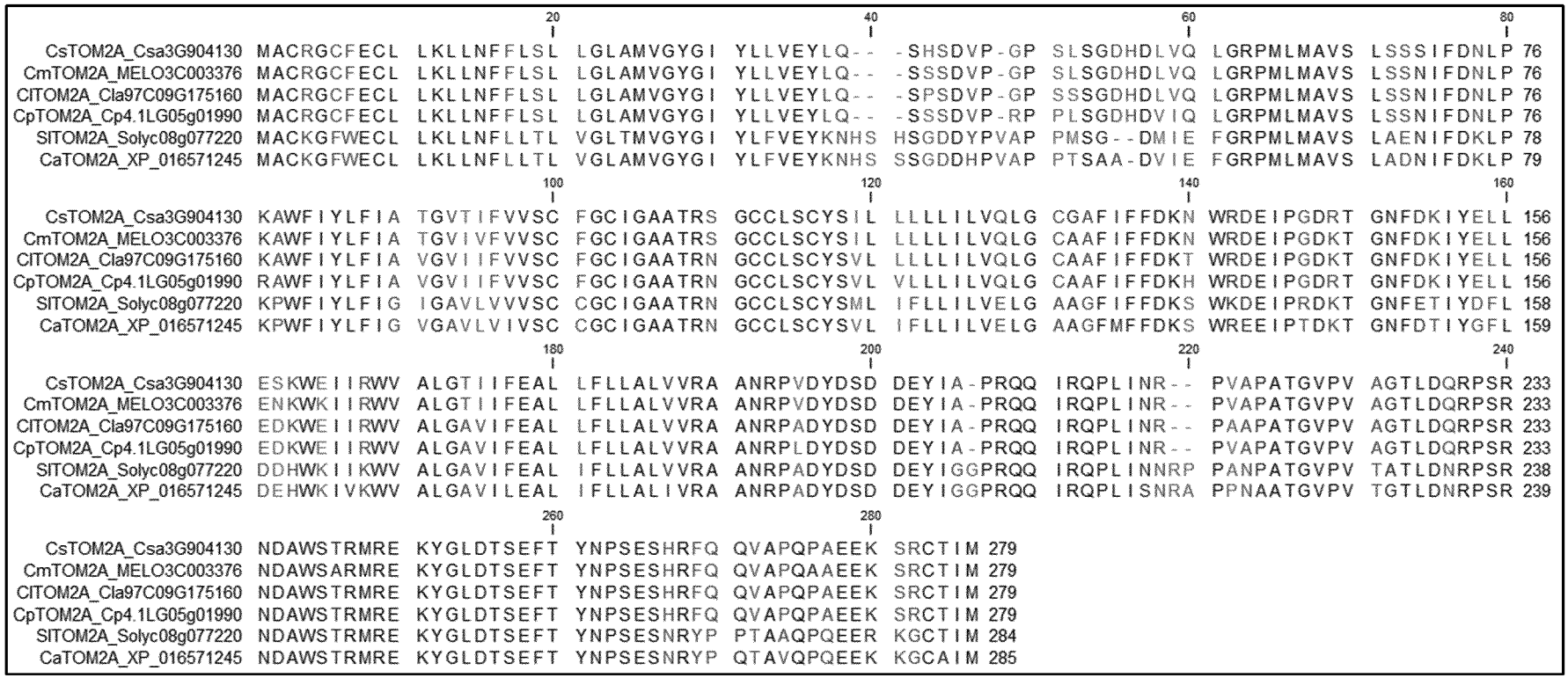


Fig. 3

Modified Tom2a gene and protein

SEQ ID No. 13 - Modified *Cucumis sativus* (cucumber) Tom2a CDS (mutant nr 2720)

ATGGCCTGCAGAGGGTGCTTTGAGTGCCTATTGAAGCTTTTGAACTTCTTCTTGTCCCTGCTGGGTCTTGCCATGGT
GGGCTATGGGATTTACTTATTGGTTGAGTACTTGCAATCTCATAGTGATGTTCCAGGACCTTCGTTGAGTGGTGATC
ATGATCTGGTCCAGCTTGGTCGACCAATGCTAATGGCTGTGTCTCTGTCTTCTAGCATCTTTGACAACCTTCCAAAA
GCCTGGTTCATATATTTGTTCATTGCTACGGGAGTCACTATCTTTGTTGTCTCTTGTTTTGGCTGTATTGGAGCTGCA
ACACGTAGTGGATGCTGTTTAAGTTGTTATTCGATTTTATTGCTTCTACTGATTTTGGTTCAACTAGGATGTGGTGCC
TTCATATTCTTTGACAAAAATTGGAGAGATGAAATTCCTGGGGACAGAACAGGAAACTTTGATAAGATCTATGAAC
TTCTGGAAAGCAAGTGGGAAATCATCAGATGGGTTGCACTAGGAACTATAATTTTTGAGGCTCTCCTTTTCTTGTTG
GCTCTTGTGGTTCGTGCAGCAAACAGACCTGTAGACTATGACAGTGATGATGAATACATTGCTCCAAGGCAACAAA
TCCGACAACCGTTGATCAATAGGCCCGTTGCTCCAGCAACAGGTGTGCCTGTTGCTGGGACACTTGATCAACGACC
AAGTCGAAATGATGCTTGGAGTACACGAATGAGGGAAAAGTATGGGCTGGATACTTCGGAGTTCACGTACAACCC
ATCTGAGTCTCACAGGTTTCAGCAAGTTGCTCCTTAG

SEQ ID No. 14 - Modified *Cucumis sativus* (cucumber) Tom2a protein (mutant nr 2720)

MACRGCFECLLKLLNFFLSLLGLAMVGYGIYLLVEYLQSHSDVPGPSLSGDHDLVQLGRPMLMAVSLSSSIFDNLPKAWF
IYLFIATGVTIFVVSCFGCIGAATRSGCCLSCYSILLLLLILVQLGCGAFIFFDKNWRDEIPGDRTGNFDKIYELLESKWEIIRW
VALGTIIFEALLFLLALVVRAANRPVDYDSDDEYIAPRQQIRQPLINRPVAPATGVPVAGTLDQRPSRNDAWSTRMREKY
GLDTSEFTYNPSESHRFQQVAP

Fig. 4

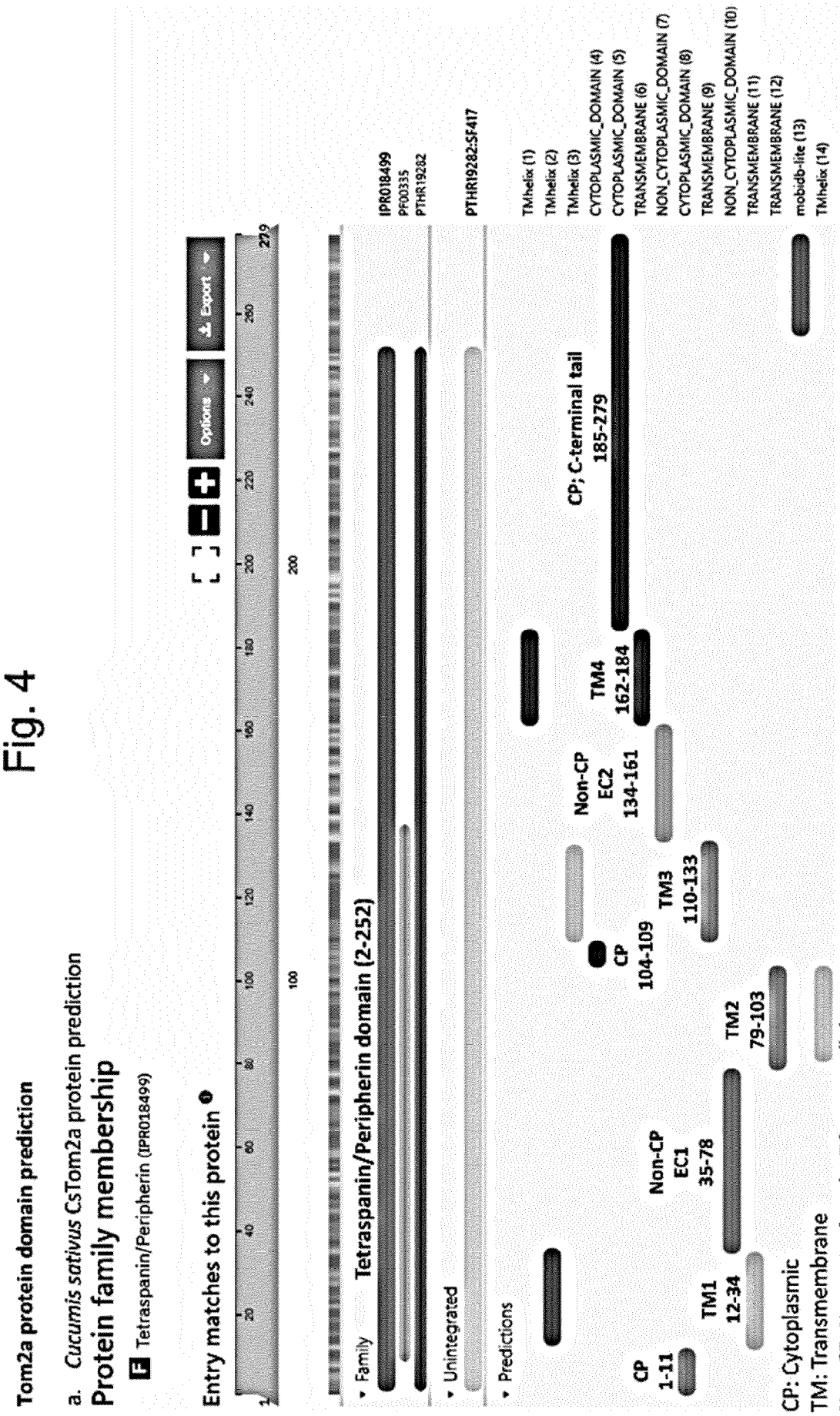

Tom2a protein domain prediction
a. Cucumis sativus CsTom2a protein prediction
Protein family membership
Tetraspanin/Peripherin (IPR018499)
Entry matches to this protein
Options
Export
Family
Tetraspanin/Peripherin domain (2-252)
IPR018499
PF00335
PTHR19282
Unintegrated
PTHR19282:SF417
Predictions
CP
1-11
TM1
12-34
Non-CP
EC1
35-78
TM2
79-103
CP
104-109
Non-CP
EC2
110-133
TM3
134-161
TM4
162-184
CP; C-terminal tail
185-279
TMhelix (1)
TMhelix (2)
TMhelix (3)
CYTOPLASMIC_DOMAIN (4)
CYTOPLASMIC_DOMAIN (5)
TRANSMEMBRANE (6)
NON_CYTOPLASMIC_DOMAIN (7)
CYTOPLASMIC_DOMAIN (8)
TRANSMEMBRANE (9)
NON_CYTOPLASMIC_DOMAIN (10)
TRANSMEMBRANE (11)
TRANSMEMBRANE (12)
mobidb-lite (13)
TMhelix (14)
CP: Cytoplasmic
TM: Transmembrane
Non-CP: Non-cytoplasmic; EC: extracellular

Fig. 4 (cont.)

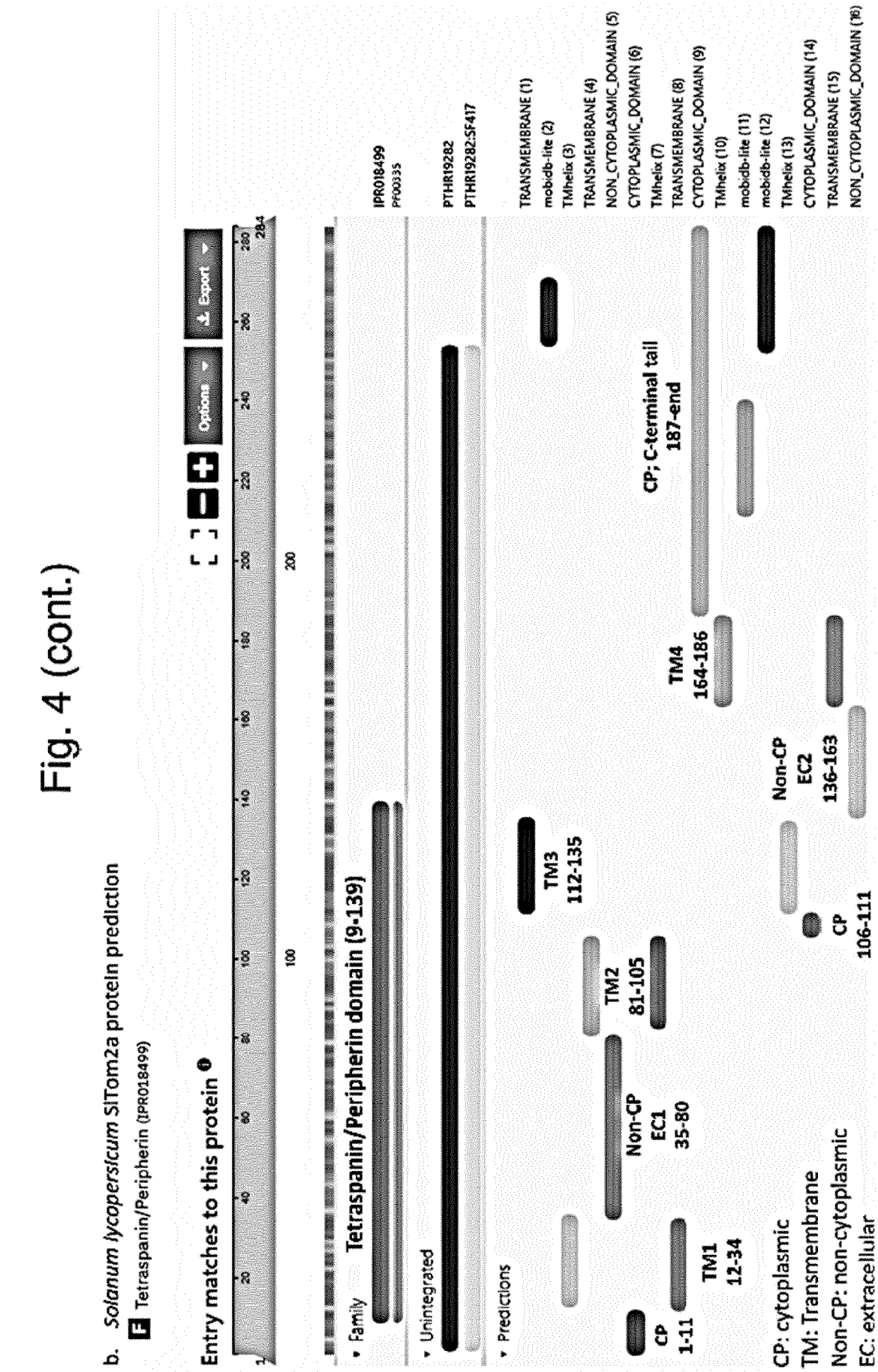

b. Solanum lycopersicum SlTom2a protein prediction
Tetraspanin/Peripherin (IPR018499)
Entry matches to this protein
Options
Export
Family
Tetraspanin/Peripherin domain (9-139)
IPR018499
PF00335
Unintegrated
PTHR19282
PTHR19282:SF417
Predictions
TRANSMEMBRANE (1)
mobidb-lite (2)
TMhelix (3)
TRANSMEMBRANE (4)
NON_CYTOPLASMIC_DOMAIN (5)
CYTOPLASMIC_DOMAIN (6)
TMhelix (7)
TRANSMEMBRANE (8)
CYTOPLASMIC_DOMAIN (9)
TMhelix (10)
mobidb-lite (11)
mobidb-lite (12)
TMhelix (13)
CYTOPLASMIC_DOMAIN (14)
TRANSMEMBRANE (15)
NON_CYTOPLASMIC_DOMAIN (16)
CP
1-11
TM1
12-34
Non-CP
EC1
35-80
TM2
81-105
TM3
112-135
CP
106-111
Non-CP
EC2
136-163
TM4
164-186
CP; C-terminal tail
187-end
CP: cytoplasmic
TM: Transmembrane
Non-CP: non-cytoplasmic
EC: extracellular

Fig. 5

Tom2a marker sequences

SEQ ID No. 15 -
GATCTGATGGCATGTTCTTTTCACAATTTTTTTCGAGTCTGACTTTGTTCTTTTTATTTTTGAAACAGTATGGGC
TGGATACTTCGGAGTTCACGTACAACCCATCTGAGTCTCACAGGTTTCAGCAAGTTGCTCCT**T**AGCCAGCCG
AAGAAAAGAGCCGCTGCACAATCATGTGATAAAAGTATGCCTTGTACTATTTGTTTCTCACTCCACTGTAGTG
TTTTAGCGGGTCCGAA

SEQ ID No. 16 - TGTTTTGGCTGTATTGG**AGCA**ACACGTAGTGGATGCTGTT

SEQ ID No. 17 - TCTTGTTGGCTCTTGTGGTT**G**GTGCAGCAAACAGACCTGTA

SEQ ID No. 18 – GGCCCGTTGCTCCAGCAACA**A**GTGTGCCTGTTGCTGGGACA

SEQ ID No. 19 – CCTCAGCCAGCCGAAGAAAA**G**AGCCGCTGCGCAATCATGTGA

SEQ ID No. 20 – CTTCCTTTCCTCTTGCGGCT**A**TGCGGCAGTTGGCGGATATC

SEQ ID No. 21 – GGCAGCAACCATTCCTTGT**TGCT**CCAATACAACCACAGCAA

SEQ ID No. 22 – TGCTGGTCTGTTTGCTGCCC**C**TACTACGAGGGCCAATAAGA

SEQ ID No. 23 – ACCATATCATCACATTATGG**C**ACAACCCTTCCTTTCCTCTT

SEQ ID No. 24 – AGTAGCAGTAACAGGGACAC**T**AGTTGCAGGATTTGCTGGTG

Fig. 6

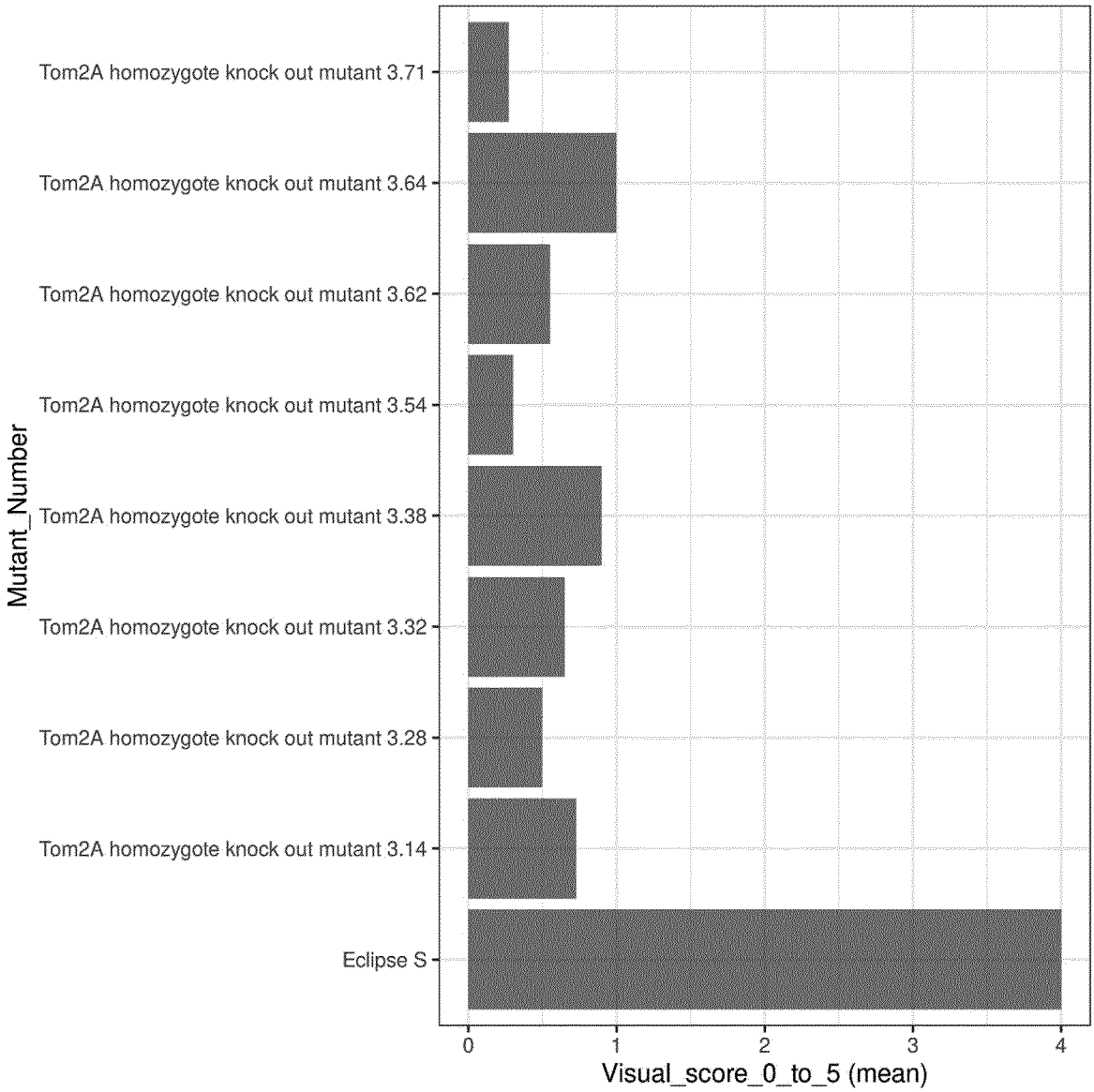
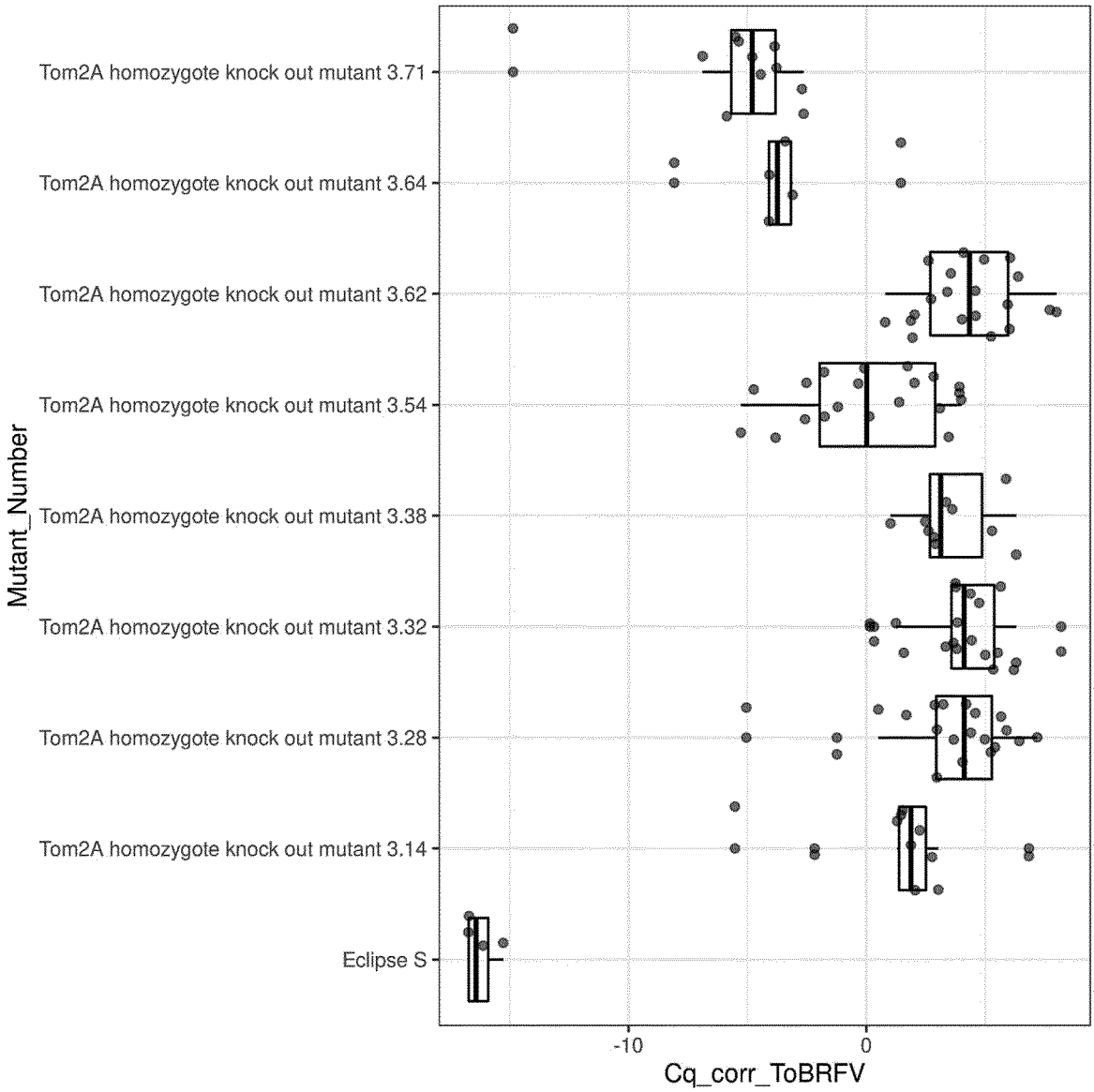

Tom2A homozygote knock out mutant 3.71
Tom2A homozygote knock out mutant 3.64
Tom2A homozygote knock out mutant 3.62
Tom2A homozygote knock out mutant 3.54
Tom2A homozygote knock out mutant 3.38
Tom2A homozygote knock out mutant 3.32
Tom2A homozygote knock out mutant 3.28
Tom2A homozygote knock out mutant 3.14
Eclipse S
Mutant_Number
0
1
2
3
4
Visual_score_0_to_5 (mean)

Fig. 7


Tom2A homozygote knock out mutant 3.71
Tom2A homozygote knock out mutant 3.64
Tom2A homozygote knock out mutant 3.62
Tom2A homozygote knock out mutant 3.54
Tom2A homozygote knock out mutant 3.38
Tom2A homozygote knock out mutant 3.32
Tom2A homozygote knock out mutant 3.28
Tom2A homozygote knock out mutant 3.14
Eclipse S
Mutant_Number
-10
0
Cq_corr_ToBRFV

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/051031

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/415 C12N15/82 A01H1/00 A01H6/34 A01H6/82
C12N15/00
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)
C07K C12N A01H

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, BIOSIS, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2001 161365 A (UNIV KYOTO) 19 June 2001 (2001-06-19)	1-7,13, 15-18, 22,23
Y	the whole document	8-12,14, 19-21, 24-33

-/--

[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
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2023	20/04/2023

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	Bilang, Jürg

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TSUJIMOTO Y.: "Arabidopsis TOBAMOVIRUS MULTIPLICATION (TOM) 2 locus encodes a transmembrane protein that interacts with TOM1", THE EMBO JOURNAL / EUROPEAN MOLECULAR BIOLOGY ORGANIZATION, vol. 22, no. 2, 15 January 2003 (2003-01-15), pages 335-343, XP055955781, Oxford ISSN: 0261-4189, DOI: 10.1093/emboj/cdg034	1-7,13, 15-18, 22,23
Y	the whole document	8-12,14, 19-21, 24-33

X	HU QUN ET AL: "Two TOBAMOVIRUS MULTIPLICATION 2A homologs in tobacco control asymptomatic response to tobacco mosaic virus", PLANT PHYSIOLOGY , vol. 187, no. 4 4 December 2021 (2021-12-04), pages 2674-2690, XP055955933, Rockville, Md, USA ISSN: 0032-0889, DOI: 10.1093/plphys/kiab448 Retrieved from the Internet: URL:https://academic.oup.com/plphys/article-pdf/187/4/2674/41521184/kiab448.pdf the whole document	1-33

Y	BLANCA J ET AL: "Transcriptome characterization and high throughput SSRs and SNPs discovery in Cucurbita pepo (Cucurbitaceae)", BMC GENOMICS, BIOMED CENTRAL LTD, LONDON, UK; BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US, vol. 12, 1 February 2011 (2011-02-01), XP002662299, ISSN: 1471-2164, DOI: 10.1186/1471-2164-12-104 page 7, left-hand column	8-12,14, 19-21, 24-33

Y	DATABASE GenPept [Online] NCBI; 28 January 2019 (2019-01-28), Anonymous: "tobamovirus multiplication protein 2A [Solanum pennellii] - Protein - NCBI", XP055855393, Database accession no. XP_015085284 abstract	8-12,14, 19-21, 24-33

	-/--	

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE EMBL [Online] 3 November 2021 (2021-11-03), "Citrullus lanatus tobamovirus multiplication protein 2A mRNA, complete cds.", XP002807415, retrieved from EBI accession no. EM_STD:MW465335 Database accession no. MW465335 sequence -----	8-12,14, 19-21, 24-33
A	NISHIKIORI MASAKI ET AL: "Membrane-Bound Tomato Mosaic Virus Replication Proteins Participate in RNA Synthesis and Are Associated with Host Proteins in a Pattern Distinct from Those That Are Not Membrane Bound", JOURNAL OF VIROLOGY , vol. 80, no. 17 1 September 2006 (2006-09-01), pages 8459-8468, XP055956004, US ISSN: 0022-538X, DOI: 10.1128/JVI.00545-06 Retrieved from the Internet: URL:https://journals.asm.org/doi/pdf/10.1128/JVI.00545-06 page 8459, left-hand column, paragraph 1 - page 8460, left-hand column, paragraph 3 page 8466, right-hand column, paragraph 2; figure 8 page 8467, right-hand column, paragraph 2 -----	1-18, 22-33
A	Y. HAGIWARA ET AL: "Subcellular localization of host and viral proteins associated with tobamovirus RNA replication", THE EMBO JOURNAL, vol. 22, no. 2, 15 January 2003 (2003-01-15), pages 344-353, XP055109833, DOI: 10.1093/emboj/cdg033 abstract ----- -/--	1-18, 22-33

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/051031

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ALI MD EMRAN ET AL: "Conferring virus resistance in tomato by independent RNA silencing of three tomato homologs ofArabidopsis TOM1", ARCHIVES OF VIROLOGY, SPRINGER WIEN, AT, vol. 163, no. 5, 6 February 2018 (2018-02-06), pages 1357-1362, XP036473633, ISSN: 0304-8608, DOI: 10.1007/S00705-018-3747-4 [retrieved on 2018-02-06] abstract ----- WO 2022/013452 A1 (RIJK ZWAAN ZAADTEELT EN ZAADHANDEL BV [NL]) 20 January 2022 (2022-01-20) cited in the application page 3, line 23 - page 6, line 24 -----	1-18, 22-33 1-5,9, 10,12, 13,15, 17,18, 26,27, 32,33
X,P		

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2023/051031

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.

   b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      ☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2023/051031

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2001161365 A	19-06-2001	JP 3286732 B2	27-05-2002
		JP 2001161365 A	19-06-2001
WO 2022013452 A1	20-01-2022	AU 2021309723 A1	09-02-2023
		CA 3183549 A1	20-01-2022
		WO 2022013452 A1	20-01-2022