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(71) Applicants: TIERRA SEED SCIENCE PVT. LTD

[IN/IN]; Tierra Seed Science Pvt. Ltd., Malaxmi Courtyard, Khajaguda, Golconda Post, Hyderabad, Telangana 500008 (IN). SEQ ID 1.0 INC [CA/CA]; 388, Armadale Ave, Toronto, Ontario M6S3X8 (CA).

(72) Inventors: BHATTACHARYA, Parthasarathi; Tierra

Seed Science Pvt. Ltd., Malaxmi Courtyard, Khajaguda, Golconda Post, Hyderabad, Telangana 500008 (IN). ATLURI, Suresh; Tierra Seed Science Pvt. Ltd., Malaxmi Courtyard, Khajaguda, Golconda Post, Hyderabad, Telangana 500008 (IN). TIKOO, Surinder, Kumar; Tierra Seed Science Pvt. Ltd., Malaxmi Courtyard, Khajaguda, Golconda Post, Hyderabad, Telangana 500008 (IN). PICK, Charles; 388, Armadale Ave, Toronto, Ontario M6S3X8 (CA).

(74) Agent: BHATTACHARYYA, Goutam et al.; K&S Part-

ners, Intellectual Property Attorneys, 101, Ivy Terrace, Plot. No. 119, Road no. 44, Kavuri Hills, Madhapur, Hyderabad, Telangana 500 033 (IN).

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(54) Title: TOSPOVIRUS RESISTANT PLANTS AND METHODS THEREOF

(57) Abstract: The present invention provides tomato plants exhibiting resistance to TOSPO (GBNV) Virus exhibiting the traits of healthy and evenly ripened fruits without brown spots, reduced wilting of the top portion of the tomato plants and reduced dark spots on the leaves and stems of the tomato plant, and materials useful for improving tomato yield. In particular, the present invention provides a path to development of hybrid tomato plants exhibiting resistance to TOSPO virus infection, seed of hybrid tomato plants exhibiting TOSPO virus resistance, and methods of producing the same. Methods described herein involve the use of marker assisted selection and marker assisted backcrossing utilizing molecular markers associated with a tomato TOSPO Resistance phenotype.

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“Tospovirus Resistant Plants and Methods Thereof”

FIELD OF INVENTION:

The present invention relates to the field of agricultural biotechnology more specifically to the development of disease resistant tomato lines.

BACKGROUND:

Viruses of the genus TOSPO cause significant worldwide crop losses. Tospoviruses have a tripartite RNA genome of ambisense polarity. The Tospovirus genus includes plant viruses that infect a wide range of plant species including vegetable, fruit and ornamental crops. The viruses belonging to this genus are popularly described as tospovirus and tomato spotted wilt virus is a type of species belonging to this genus. Tospoviruses are distributed in tropical, subtropical and temperate regions throughout the Northern Hemisphere, Western Europe and Asia. Ground nut bud necrosis virus (GBNV) is currently recognized as the most economically important tospovirus in South Asia & South East Asia. Losses due to GBNV alone have been estimated at more than US\$89 million per annum in Asia.

The transmission of the disease is by the vector *Thrips palmi*. The disease is affecting tomato crop cultivation to the tune of 350,000 hectares per year in India, especially in Kharif season. Control of Tospoviruses remains problematic. Cultural practices and varietal selection have proven effective in minimizing losses due to tomato spotted wilt virus (TSWV) in some field crops. A series of risk factors including prior history, planting date, cultivar selection and plant and row spacing have been identified as critical factors in peanuts. In other high-risk areas, such as Hawaii, highly susceptible crops cannot be grown profitably. In greenhouse grown crops, such as floral crops, extreme measures including screening of production areas with fine-meshed cloth, preventative thrips management strategies and use of propagation material shown to be free of TSWV and impatiens necrosis spot virus (INSV) are necessary for control of these viruses. While forms of resistance have been introduced into various crops, they have

nearly always been overcome by the rapid occurrence of resistance-breaking strains of the virus. TSWV is thought to exist in nature as a complex heterogeneous mixture of distinct isolates that can exchange genetic information through re-assortment of genome segments. This provides a readily available reservoir of genetic information to facilitate adaptation. For the reasons stated above control of the disease is a challenge so far. Various resistant genes were identified and designated as *Sw1*, *Swb1*, *Sw2*, *Sw3*, and *Sw4*, *Sw-5*, *Sw6*, *Sw7* from wild source of tomato (*S. peruvianum*) against TOSPO virus. However, these resistance genes are not effective to control the GBNV disease in India. Chemical control measures have not been able to contain the disease and farmers are suffering yield loss on account of the disease outbreak. The estimated loss (Kunkalikal, S. R. et al (2011) is around 80% per season of the crop. The calculated loss to the farmer in India is approximate of US\$25 million if approximately 50% of acreage gets affected by the disease i.e. 175,000 hectares. As known genetic resistance and chemical measures are not effective in control of the disease, there is a need to develop an edible and cultivable tomato line resistant to GBNV.

OBJECT OF THE INVENTION:

The object of the invention is for an edible and cultivable tomato line resistant to GBNV, and related method for developing such line.

The invention also relates to a cultivable & edible F1 hybrid variety resistant to GBNV, and related method for developing such hybrid line.

The invention relates to the identification of DNA markers linked to GBNV resistance trait and use of marker assisted breeding for selection of resistant edible and cultivable tomato line or F1 hybrid variety

BRIEF DESCRIPTION OF DRAWINGS AND FIGURES:

Figure 1 shows the flow chart of breeding interspecific cross up to BC2F2.

Figure 2 shows the flow chart of breeding BC2F3 to current status.

Figure 3 depicting the frequency distribution of TOSPO (GBNV) disease over generations.

Figure 4 photomicrographs of the disease scoring chart.

Figure 5 depicts the distribution of BC2F3 lines with different recipient allele frequency based on 2455 SNP.

Figure 6 showing BC2F6 & BC2F7 families (RP x SP1) x SP2 indicating trait linked markers in resistant progenies.

DETAILED DESCRIPTION OF THE INVENTION:

Accordingly, the present invention provides an edible and cultivable tomato line resistant to GBNV, and related method for developing such line.

The invention also relates to development of cultivable & edible F1 hybrid variety resistant to GBNV, and related method thereof.

The invention relates to the identification of markers and use of marker assisted breeding for selection of GBNV resistant edible and cultivable tomato line.

The donor is selected from *Solanum peruvianum* line obtained from World Vegetable Centre - Asian Vegetable Research and Development Center (AVRDC), Taiwan.

Susceptible cultivated lines of tomato used in this work are collections made by Tierra Seed Science from known Indian sources following proper legal process

Cultivated lines used were SP-1 (Susceptible parent 1 – Sel 22, purchased from Indian Institute of Horticultural Research Bengaluru (IIHR), a commercial variety since 1985). SP-2 (Susceptible line) – collected from AVRDC carrying Ty 2 gene that gives partial resistance against TyLCV (Tomato Yellow Leaf Curl Virus) but susceptible to TOSPO (GBNV).

The invention provides a method for producing a tomato plant, wherein the tomato plant exhibits TOSPO Virus resistance, comprising:

- (a) providing a donor tomato plant, wherein the donor plant exhibits TOSPO virus resistance;
- (b) transferring at least one nucleic acid from the first tomato plant to one or more tomato plants, wherein the recipient tomato plants do not exhibit TOSPO virus resistance, and wherein the transfer results in the introduction of genomic material from the donor tomato plant in a corresponding region of one or more recipient tomato plants; and
- (c) identifying and selecting, from the one or more recipient tomato plants, a tomato plant that comprises within its genome at least one quantitative trait locus (QTL) for TOSPO virus resistance, wherein the QTL is selected from the group consisting of: QTL1, QTL2 and TOSPO viral resistance related traits thereof, and wherein QTL1 is indicated by a genomic region on chromosome 1, QTL2 is indicated by a genomic region on chromosome 2

The method for producing the disease resistant tomato plant further comprising the step of selecting a recipient tomato plant which is originally susceptible to TOSPO Viruses

Some of the post infection common characteristics traits of the recipient tomato plant which is susceptible to TOSPO are deformed shape and uneven ripening of the fruit, wilting of the top portion of the plant, dark brown spots on the leaves and dark brown patches on stems of the plants.

The transfer of one or more nucleic acids is performed by crossing the donor tomato plant with a recipient tomato plant to produce progeny tomato plants comprising at least one QTL for TOSPO virus resistance as in introgression, and wherein identification and selection is performed on one or more progeny plants.

The method of transfer of one or more nucleic acids is based on selecting the TOSPO (GBNV) resistant plants from progenies of an interspecific cross. The crossing was made possible using embryo rescue technique to overcome incompatibility.

Further, the method of the invention is performed by detecting the marker in DNA isolated from the recipient tomato plants or from one or more of progeny plants.

The method of the invention comprises selecting a recipient tomato plant that comprises within its genome at least two QTLs selected from the group consisting of QTL1, QTL 2 and virus-resistance related parts thereof.

The method of the invention comprises selecting a recipient tomato plant that comprises QTL1 and QTL2 within its genome.

The method of the invention comprises selecting a recipient tomato plant that comprises within its genome a QTL or a viral resistance related part thereof, and at least one QTL selected from the group consisting of QTL1, QTL2 and related parts thereof.

The method of the invention further comprises a step of crossing a recipient tomato plant with a plant of a cultivated variety of tomato to produce progeny plants.

The method of the invention comprises the recipient tomato plant as same as that of the plant of the cultivated variety of tomato to which the recipient tomato plant is crossed.

The resistance provided against by the method of the invention is for a Groundnut Bud Necrosis Virus (GBNV) which is a TOSPO virus.

The invention is for a tomato plant exhibiting TOSPO virus resistance, and the said tomato plant or part thereof comprises a QTL selected from the group consisting of QTL1, QTL2 and TOSPO virus related parts thereof, wherein the QTLs or viral resistance-related parts thereof are not present in the natural genetic background of the edible tomato plants.

The tomato plant exhibiting TOSPO virus resistance, is produced by back crossing the tomato plant BC 1 (back cross 1) with a tomato plant that exhibits commercially desirable characteristics, wherein the resulting hybrid plant comprises at least one QTL selected from the group consisting of QTL1, QTL2 and TOSPO virus resistance related parts thereof, wherein at

least one QTL is not present in the natural background of either the tomato plant selected in step 1 (c) or the tomato plant exhibiting commercially desirable characteristics.

Standard embryo rescue method was employed to overcome sexual incompatibility between *S. peruvianum* & *S. lycopersicum*, and to raise the seven day old fertilized ovules on tissue culture medium.

Scoring of the disease symptoms (phenotyping) was performed in a hot spot location for TOSPO (GBNV) disease and were categorized into 5 types (1) whole plant diseased (score 1); (2) diseased with few green stem and leaves (score 2); (3) 50% plant covered-may have 1 or 2 fruit (score 3); (4) Except for the top leaves, stem and other plant parts are healthy (score 4); (5) Healthy plants (score 5) using the phenotypic symptoms observed in the field at young and adult plant stages, e.g 40, 60, 80 days after transplantation in the field.

EXAMPLES:

The following examples are for the purpose of illustration of the invention and are not intended in any way to limit the scope of the invention.

Materials:

Wild type *Solanum peruvianum* line was obtained from World Vegetable Centre - Asian Vegetable Research and Development Center (AVRDC), Taiwan. Susceptible cultivated lines of tomato used in this work are collected from known germplasm resources. The cultivated lines used were SP-1 (Susceptible parent 1 – Sel 22, purchased from Indian Institute of Horticultural Research Bengaluru (IIHR), a commercial line since 1985). SP-2 (Susceptible line) – A Ty 2 line collected from AVRDC but susceptible to TOSPO.

Example 1:

Development and determination of disease resistant phenotype:

The *S. peruvianum* accession RP (used as donor), (*S. peruvianum* accession from AVRDC - Resistant to GBNV), was crossed with a GBNV susceptible *S. lycopersicum* line (SP1- that

responds well to tissue culture). To overcome sexual incompatibility between *S. peruvianum* & *S. lycopersicum*, standard embryo rescue method was employed to raise the seven day old fertilized ovules on (Figure 1 detailing the crossing and backcrossing) tissue culture medium. More than 5000 ovules were subjected to embryo rescue method and only two embryos were able to develop into true hybrid plants (F1) (seen phenotypically). These F1s were crossed with another GBNV susceptible line of *S. lycopersicum* as it was agronomically better & had more firm fruits (SP2- Susceptible parent 2 (Square Round)) and obtained BC1 seeds. The crossed ovules from these BC1 pollinations, using the inter-specific F1 as pollen source, were subjected to embryo rescue to get BC1 plants that were grown to fruiting. BC1F1 progeny was grown in the field in rainy season under high thrips pressure. Pollen from plants, that showed no GBNV symptoms were used to pollinate the SP1 recipient parent to obtain BC2F1 seeds. BC2F1 plants were grown and selfed to obtained BC2F2 seeds. Selected BC2F2 plants were grown to obtain BC2F3 (Figure 2). Selected BC2F3 families were planted in hot spot location along with the parents (donor as well as recipient) in an augmented design where a susceptible check variety was planted repeatedly after every 10 rows in each bed. Frequency distribution of the disease in various generations and resistant (RP) and susceptible (SP) parent shown in Figure 3.

EXAMPLE 2:

Development of field screening for disease and disease rating scores:

Using the phenotypic symptoms observed in the field at young and adult plant stages, e.g 40, 60, 80 days after transplantation in the field, the disease symptoms were categorized into 5 types as in Figure 4 as (1) whole plant diseased (score 1); (2) diseased with few green stem and leaves (score 2); (3) 50% plant covered-may have one or two fruit (score 3); (4) Except for the top leaves, stem and other parts healthy (score 4); (5) Healthy plants (score 5).

Based on data on GBNV scores at 40, 60 & 80 days after transplanting (DAT) the families were grouped into three categories of: 1) Resistant (scores 4, 5); 2) intermediates (score 3) and 3) susceptible (scores 1 and 2). Three individual plants per category were harvested for obtaining

BC2F4 seeds. BC2F4 families were again raised and BC2F5 progenies harvested from same groups of families.

The frequency distribution for disease score 1 to 5 for GBNV incidence on resistant parent, susceptible recipient, BC2F3 & BC2F4 progenies are provided in Figure 3. Disease pressure during the screening has been very high as can be seen by the over 65 percent of susceptible parent shows susceptibility rating of 1 and 2 and another 15 percent having score of 3. The resistant parent on the other hand showed 90 percent plants scoring rating 5 and another 10 percent scoring a rating of 4, confirming high resistance in this parent (*S. peruvianum*). BC2F3 distribution for the disease rating shows a normal distribution (Figure 3: bottom left) indicative of an incomplete dominance for resistance and involvement of more than one gene for its inheritance. BC2 F4 progenies derived from the susceptible and resistant families of BC2F3 showed the population moving towards resistant & susceptible groups (Figure 3: bottom right). This indicates that it was possible to select high resistant genotypes with a disease rating scale of 4 & 5.

The mean disease ratings and their standard deviation in case of the BC2F3, BC2F4 & BC2F5 families versus resistant & susceptible parents as well as intermediates are presented in Figure 3 below. These data represents three seasons of screening. BC2F5 progenies show an overall stable resistance which is closer to the donor parent while as the susceptible (recipient parent) and a super susceptible line as well as the progenies of intermediate resistance show clear susceptibility over all three seasons of testing. The BC2F5 progenies have further planted and the top stable lines for resistance to GBNV has been selected.

Example 3:

Genotyping, Linkage and QTL analysis- Identification of trait linked molecular markers: To understand precisely the location of resistance genes, 181 leaf samples consisting of 174 BC2F3 families (bulks of 4-20 plants) and 3 parental lines were used. DNA was extracted from each sample using standard extraction method.

For Marker identification SNP Panel was used

- Solcap 7K Infinium SNP panel
- 6,007 SNPs resulted in genotypes with <10% missing datapoints
- 1,767 showed segregation in the population
- 556 SNPs selected for linkage construction and QTL analysis based on: Missing datapoints <20%
- SNPs were observed to be polymorphic between SP2 and RP1 (original parents)

Trait linked molecular markers by way of multiple QTLs in specified chromosomes is identified. 7000 SNPs were generated and used to screen and identify the SNPs that were closely linked with the resistant trait. Out of 7000 SNPs that were used for the screening on the segregating materials, 17 SNPs were found to be associated with the resistance to GBNV trait.

The following were used for Linkage construction

- 556 SNPs used for linkage construction
- 330 SNPs were non-redundant (not co-segregated)
- Mapped to 12 linkage groups
- Linkage groups ranged from 79.2 cM to 174.9 cM
- Total length 1,567.2 cM
- Based on physical coordinates, linkage groups covered most of the genome

Out of 17 SNPs, 5 SNPs were found to closely linked to the resistance trait associated with two major QTLs present in chromosome 1 & 2.

Example 4:

Production of TOPSO virus resistant tomato line by non-transgenic method:

The BC₂F₆ progenies, resulting from individual plant selections (pedigree selection) since when the BC₂F₃ families were first screened in the rainy season in Hyderabad have shown high resistance levels to TOSPO during Kharif 16 at the hot spot locations. A summary data on the TOSPO scores of these progenies in K 16 vs checks and historically in their parent families are shown in table 1 below.

Table 1: TOSPO Resistant lines at BC₂F₆ vs susceptible (SP 1 & 2) parents, Resistant (RP) parent and a general susceptible check along with historical TOSPO scores over seasons

TOSPO scores over seasons							
Kharif(K)16 code	Source # in K15	TOSPO Scr K 16 MEAN±SD	K 16 Fruit Wt (g)	TOSPO Scr K 16 (Lost due Flood)	TOSPO Scr K15	TOSPO Scr K 15 Fruit Wt (g)	TOSPO Scr K14
GB0001	H2-7-2-1	4.69±1.11	30	4.21±0.97	3±1.87	15	3.71±1.43
GB0024	H17-5-1	4.83±0.41	25	3.67±1.12	1.6±1.01	31	2.50±1.46
GB0027	GB0049-1-SB	4.75±0.45	35	3.75±0.71	5±0	20	4.10±1.16
GB0038	GB0720-1-2	4.36±0.67	120	4±0.76	5±0		
GB0039	GB0279-2-1	4.31±0.48	50	4.15±0.69	4.25±1.50	50	
SP1	SP1	1.5±0.22	60	1.6±0.2	1.2±0.18	55	1.5±0.15
SP2	SP2	1.6±0.18	65	1.3±0.5	1.4±0.28	60	1.6±0.2
RP	RP	5±0	5	4.9±0.22	4.9±0.24	5	4.8±0.15
GBE002	GB0134-1	3.77±1.17	80	4.27±0.65	4±0	15	4
GBE003	GB0134-3	4.73±0.47	60	4±0.77	4±0	15	4
GBE004	GB0135-1	4±1.11	20	4.80±0.45	4.5±0.57	20	4
GBE009	GB0202-4	3.91±0.70	35	3.6±1.12	5±0	23	4
GBE034	GB0074-1	4.69±0.48	100	4.07±1.14	1.57±1.51		5
GBE035	GB0074-2	4.29±0.47	90	4.27±1.19	1.57±1.51		5
GBE036	GB0075-1	3.50±1.07	35	5±0	4±2	15	5
GBE040	GB0196-1	4.46±0.52	150	4±1.58	1±0		4
GBE062	GB0703-2	3.88±1.81	120	3.79±1.72	5±0		5
GBE064	GB0704-1	4.64±0.50	120	3.92±1.31			5
GBE073	GB0708-1	4.86±0.36	100	4.21±0.97	4±0		5
GBE074	GB0709-1	4.62±0.51	150	4.43±1.09		5	5
GBE075	GB0710-1	4.64±0.50	120	3.50±0.84	5±0		5
GBE083	GB0782-1	4.55±0.69	90	3.67±0.50	5±0		5
GBE084	GB0782-BK	4.83±0.39	30	4.46±0.66	5±0		5
GBE085	GB0783-1	4.85±0.38	90	3.93±1.21			5
GBE086	GB0783-BK	4.90±0.32	30	4.33±0.98			5
GBE087	GB0784-BK	4.08±1.44	25	4.80±0.42			5
GBE088	GB0785-BK	4.43±0.51	25	4±0.67			5
SEL-4 (S check)	SEL-4	1.71±0.49	50	1.8±0.56	2±0.47		1.7±0.56

In Figure 8 a few TOSPO resistant lines in field vs susceptible checks are presented.

Example 5:**Production of TOPSO virus resistant tomato line by marker assisted breeding**

Following table show results of SNP marker analysis showing linkage with the TOSPO resistance trait.

Table 2: SNP association with resistance at 40 or 60 days

	SNP	Mean Resistance Index			F-Test			T-Test		
		A	B	H	A/B	A/H	B/H	A/B	A/H	B/H
QTL 1	SNP527	4.01 (209)	4.44 (209)	3.85 (117)	0.0237	0.6354	0.0164	1E-06	0.1729	3E-07
	SNP001	4.01 (215)	4.43 (215)	3.83 (116)	0.0153	0.6749	0.0145	7E-07	0.1248	1E-07
	SNP540	3.91 (267)	4.52 (267)	3.92 (103)	2E-07	0.6044	0.001	8E-14	0.9208	4E-07
	SNP066	3.98 (239)	4.47 (239)	3.82 (110)	0.0064	0.2713	0.0008	2E-09	0.1754	7E-08
	SNP521	3.82 (242)	4.51 (242)	3.79 (73)	1E-05	0.2667	3E-05	3E-17	0.8699	2E-06
	SNP434	3.89 (260)	4.53 (260)	3.7 (71)	2E-08	0.2066	2E-07	8E-17	0.3148	4E-07
QTL 2	SNP044	3.52 (223)	4.56 (223)	4.04 (47)	2E-15	0.0534	6E-14	3E-30	0.0034	0.0019
	SNP017	3.5 (222)	4.56 (222)	4.13 (48)	3E-15	0.4657	0.0004	5E-31	0.0004	0.0057
	SNP304	3.5 (222)	4.56 (222)	4.13 (48)	3E-15	0.4657	0.0004	5E-31	0.0004	0.0057
	SNP131	3.51 (225)	4.56 (225)	4.18 (51)	3E-15	0.3954	0.0004	2E-30	0.0001	0.0111
	SNP155	3.71 (264)	4.53 (264)	4.26 (61)	1E-15	0.1956	0.001	2E-22	0.0006	0.0474
	SNP151	3.71 (263)	4.53 (263)	4.25 (57)	2E-15	0.2413	0.0011	3E-22	0.0013	0.0432
	SaNP193	3.69 (254)	4.53 (254)	4.2 (64)	3E-16	0.1546	0.0007	1E-22	0.0012	0.0128
	SNP097	3.69 (254)	4.53 (254)	4.2 (64)	3E-16	0.1546	0.0007	1E-22	0.0012	0.0128
	SNP034	3.5 (222)	4.56 (222)	4.13 (48)	2E-15	0.4657	0.0003	4E-31	0.0004	0.0056

Random SNP		Mean Resistance Index			F-Test			T-Test		
	SNP	A	B	H	A/B	A/H	B/H	A/B	A/H	B/H
	SNP506	3.62 (23)	2.96 (8)	3.48 (14)	0.2265	0.2554	0.8112	0.0448	0.5999	0.2201
	SNP507	3.32 (16)	3.53 (12)	3.6 (16)	0.4254	0.9966	0.4276	0.5264	0.3218	0.8441
	SNP508	3.38 (16)	3.41 (18)	3.59 (11)	0.9498	0.3004	0.2685	0.9134	0.4894	0.5577

- SNP flanking the QTL1 and QTL2 were significantly associated with resistance index
- Random SNP were not associated with the resistance index

Table 3 provides the impact of either or both QTLs

	Resistance index	F-test			T-test		
QTL		QTL1	QTL2	QTL1/QTL2	QTL1	QTL2	QTL1/QTL2
-	3.59 (86)	0.12418 9557	0.3561910 85	1.11696E -07	0.0293882 01	0.0011598 54	6.99241E -14
QTL1	3.24 (129)		0.0070126 33	5.22526E -18		1.55039E- 08	2.58405E -24
QTL2	4.06 (112)			9.24693E -06			5.14217E -08
QTL1/QTL2	4.61 (438)						

- Lines carrying QTL2 had significantly higher resistance index than the lines carrying QTL1
- Lines carrying both QTL had significantly higher resistance index than either QTL

The claims:

1. A method for producing a tomato plant, wherein the tomato plant exhibits TOSPO Virus resistance, comprising:

(a) providing a donor tomato plant, wherein the donor plant exhibits TOSPO virus resistance;

(b) transferring at least one nucleic acid from the first tomato plant to one or more tomato plants, wherein the recipient tomato plants do not exhibit TOSPO virus resistance, and wherein the transfer results in the introduction of genomic material from the donor tomato plant in a corresponding region of one or more recipient tomato plants; and

(c) identifying and selecting, from the one or more recipient tomato plants, a tomato plant that comprises within its genome at least one quantitative trait locus (QTL) for TOSPO virus resistance, wherein the QTL is selected from the group consisting of: QTL1, QTL2 and TOSPO viral resistance related traits thereof, and wherein QTL1 is indicated by a genomic region on chromosome 1 and QTL2 is indicated by a genomic region on chromosome 2.

2. The method for producing the tomato plant of claim 1, further comprising the step of selecting a recipient tomato plant which is infected by TOSPO Viruses.

3. The method of claims 1 and 2, wherein the recipient tomato plant exhibits one or more of the traits selected from deformed shape and uneven ripening / yellow patch formation on the fruit, wilting of the top portion of the plant, dark brown spots on the leaves and dark brown patches on stems of the plants.

4. The method of claim 1, wherein the transfer of one or more nucleic acids is performed by crossing the donor tomato plant with a recipient tomato plant to produce progeny tomato plants comprising at least one QTL for TOSPO virus resistance as in introgression, and wherein step (c) is performed on one or more progeny plants.

5. The method of claim 1, wherein step (c) is performed by detecting the marker in DNA isolated from the recipient tomato plants.
6. The method of claims 1 and 4, wherein step 1 (c) is performed by detecting the marker in DNA isolated from one or more progeny plants.
7. The method of claims 1, 4, 5, 6 and 7, wherein step 1(c) comprises selecting a recipient tomato plant that comprises within its genome at least two QTLs selected from the group consisting of QTL1, QTL2 and virus-resistance related parts thereof.
8. The method of claims 1, 4, 5, 6 and 7, wherein step 1(c) comprises selecting a recipient tomato plant that comprises within its genome at least two QTLs selected from the group consisting of QTL1, QTL2 and virus-resistance related parts thereof.
9. The method of claims 1, 4, 5, 6 and 7, wherein step (c) comprises selecting a recipient tomato plant that comprises QTL1 and QTL2 within its genome.
10. The method of any one of claims 1, 4, 5, 6, 7 and 10, wherein step (c) comprises selecting a recipient tomato plant that comprises within its genome QTL or a viral resistance related part thereof, and at least one QTL selected from the group consisting of QTL1, QTL2 and related parts thereof.
11. The method of claim 1, wherein the method further comprises a step of crossing a recipient tomato plant selected in step (c) with a plant of a cultivated variety of tomato to produce progeny plants.
12. The method of claim 12, wherein the recipient tomato plant selected in step (c) is of a same variety as the plant of the cultivated variety of tomato to which the recipient tomato plant is crossed.
13. The method of claims 1-13, wherein the TOSPO virus is a Groundnut bud necrosis virus (GBNV).

14. A tomato plant exhibiting TOSPO (GBNV) virus resistance, produced by a method of any one of claims 1-14, wherein the tomato plant or part thereof comprises a QTL selected from the group consisting of QTL1, QTL2 and TOSPO (GBNV) virus related parts thereof, wherein the QTLs or viral resistance-related parts thereof are not present in the natural genetic background of the tomato plants.

15. A tomato plant exhibiting TOSPO (GBNV) virus resistance, produced by crossing the tomato plant selected in step 1(c) of claim 1 with a tomato plant that exhibits commercially desirable characteristics, wherein the resulting hybrid plant comprises at least one QTL selected from the group consisting of QTL1, QTL2 and TOSPO virus resistance related parts thereof, wherein at least one QTL is not present in the natural background of either the tomato plant selected in step 1 (c) or the tomato plant exhibiting commercially desirable characteristics.

16. The tomato plant of claim 16, wherein at least one resistance-related QTL is from *Solanum peruvianum*

18. A seed of the plant of claims 15-17.

19. The tomato plant of claims 15-17, wherein the TOSPO virus is a Groundnut bud necrosis virus (GBNV).

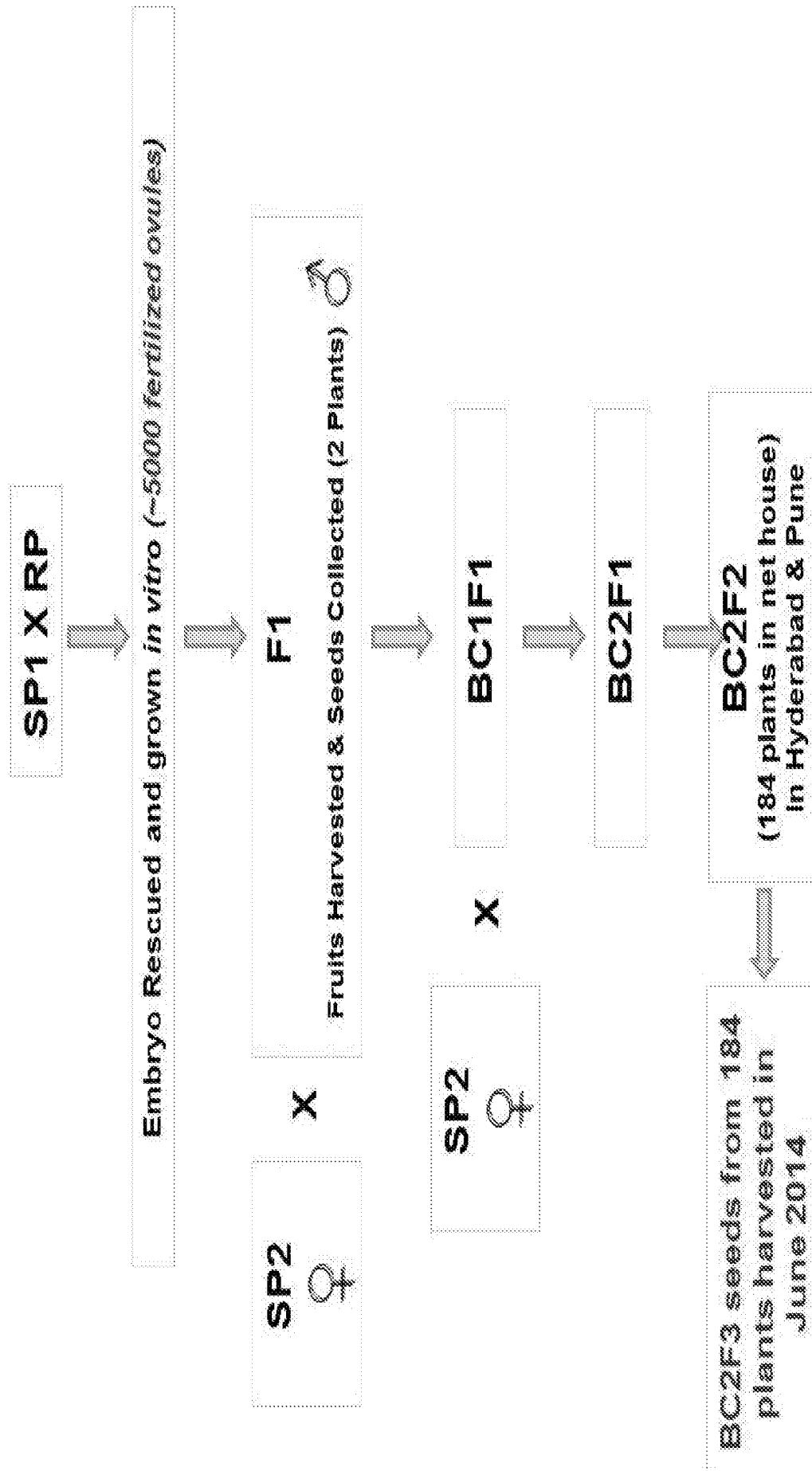


FIGURE 1

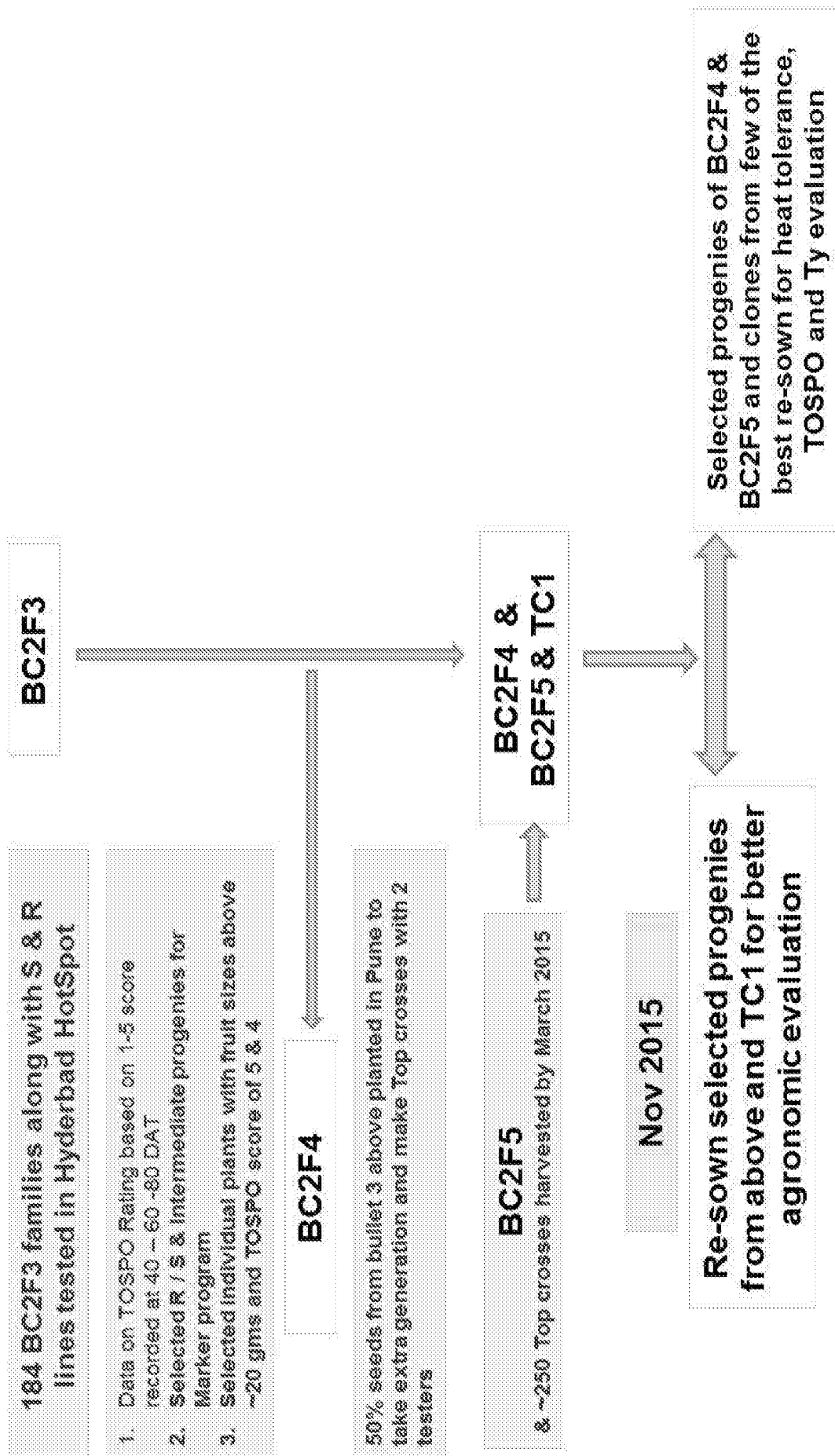


FIGURE 2

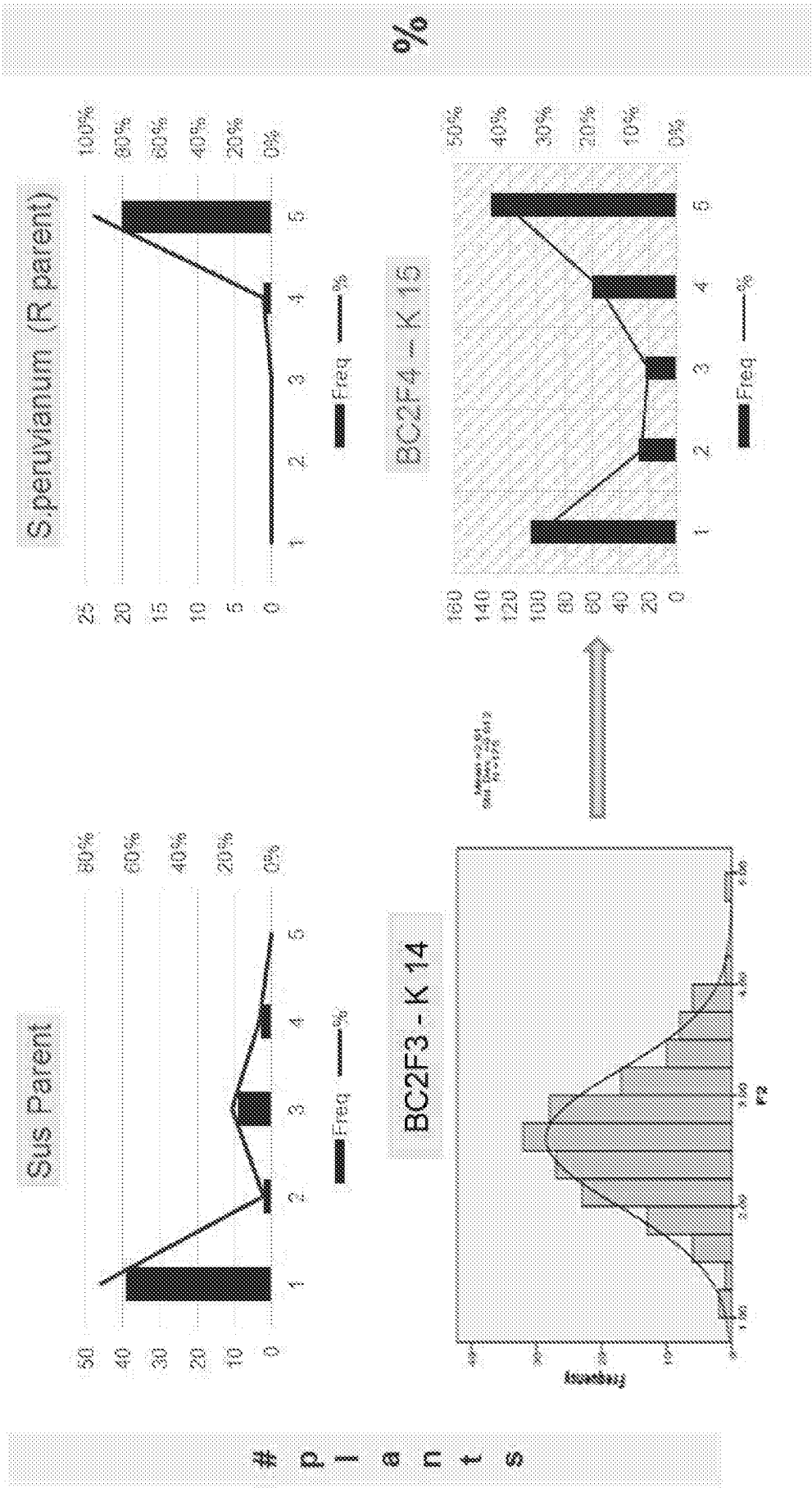
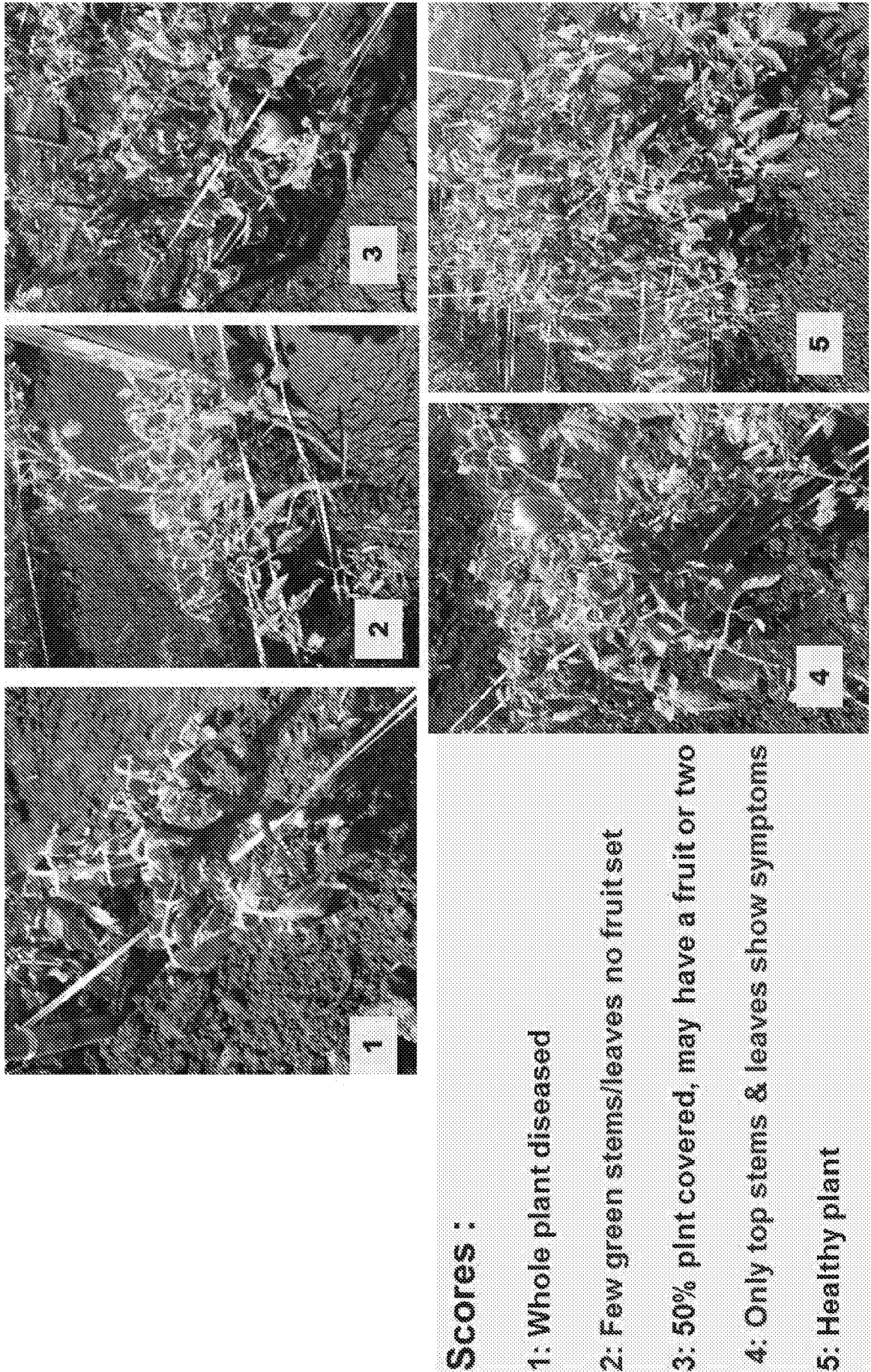


FIGURE 3

**FIGURE 4**

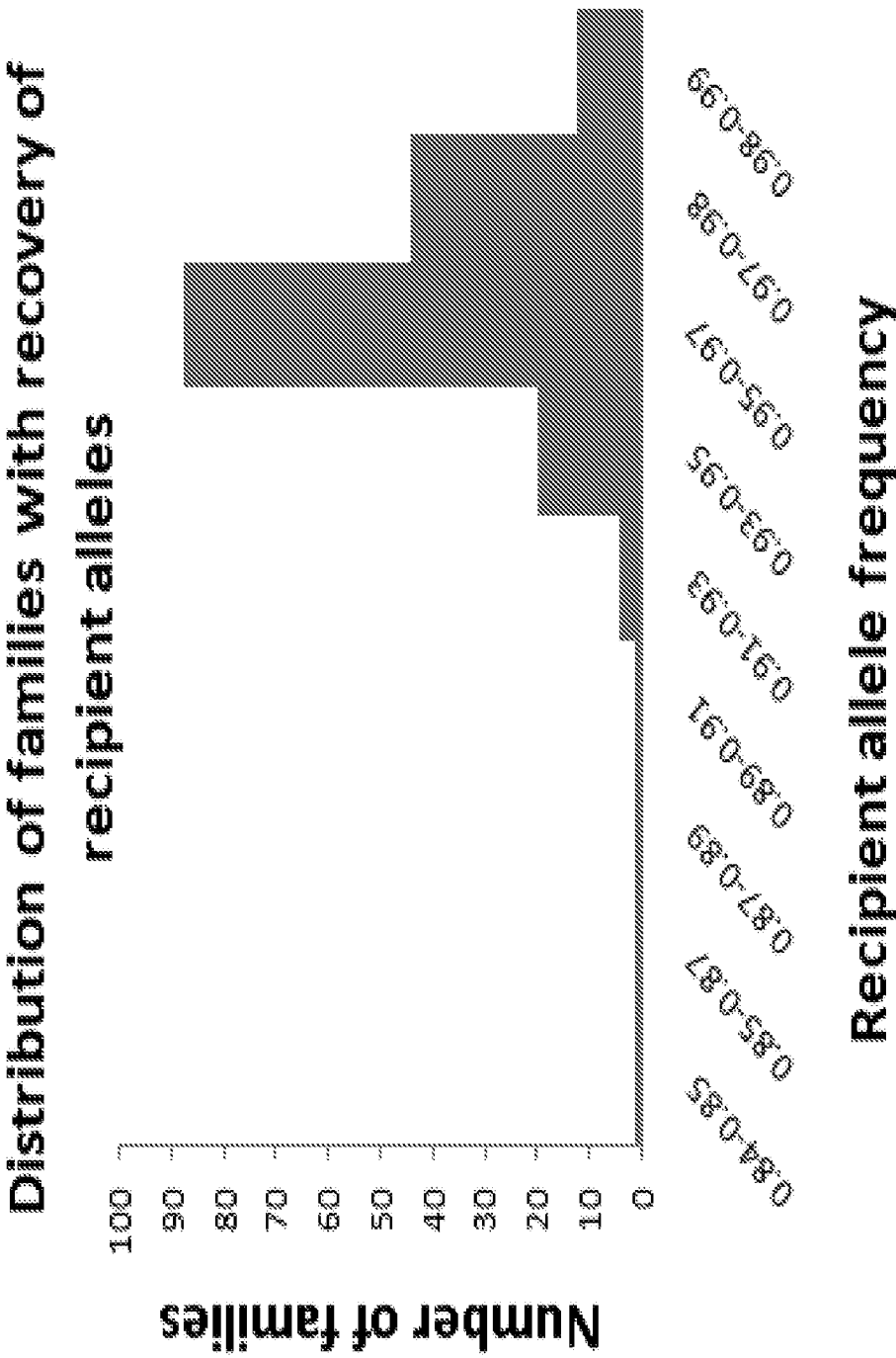


FIGURE 5

Sample_ID	QTL1										QTL2										% of recipient parent genome
	SN1	SN1	SN1	SN1	SN1	SN1	SN1	SN1	SN1	SN1	SN	SN	SN	SN	SN	SN	SN	SN	SN	SN	
RPI (zygosity)	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	RES P
SP1 (zygosity)	B	B	A	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	SUS P
SP2 (zygosity)	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	SUS P
GB0720_1_Plate10_H10	B	B	A	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	BC2F7
H41_1_Plate12_A12	B	B	A	B	B	B	B	B	B	B	H	H	H	H	H	H	H	H	H	H	93.8
H2_7_3_Plate2_A04	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	91.9
H179_15_7_Plate7_A06	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	94
H1_1_2_Plate1_A05	B	B	B	B	B	B	B	B	B	B	H	H	H	H	H	H	H	H	H	H	94.1
GB0152_1_Plate10_H12	B	B	B	B	B	B	B	B	B	B	A	A	A	H	H	H	A	A	A	A	69.1
GB0109_3_Plate10_H06	A	A	A	B	A	B	A	H	A	B	B	B	B	B	B	B	B	B	B	B	94
GB0089_4_Plate10_A05	A	A	A	B	A	B	A	H	A	B	B	B	B	B	B	B	B	B	B	B	95.8
GB0071_3_Plate10_H07	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	94
GB0048_1_Plate10_B04	B	B	B	B	B	B	B	B	B	B	B	H	H	A	A	A	A	H	QTL1, QTL2	4.8	84.2
GB0044_3_Plate10_G08	A	A	A	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	QTL1	3.58	
GB0023_9_Plate10_C03	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	QTL1, QTL2	4.27	
H153_3_9_Plate5_B06	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	QTL1, QTL2	4.1	
																					94.1

- Families consistently showing resistance in multiple seasons
- Families bearing fruit size greater than 28 grams
- Recipient parent genome in selected families: from 69 to 97%
- Two selected families might carrying QTL other than QTL1 & QTL2
- Resistance is highest when all 5 SNPs for the 2 QTL are homozygous R

FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2017/055151

A. CLASSIFICATION OF SUBJECT MATTER
A01H1/00, A01H4/00, A01H5/00, A01H5/10 Version=2018.01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01H

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)

PatSeer, IPO-internal, Patentscope, Espacenet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Sohrab SS et al.: "Development of interspecific Solanum lycopersicum and screening for Tospovirus resistance", SAUDI JOURNAL OF BIOLOGICAL SCIENCES, 2015 Nov 30, vol. 22, no. 6, pages 730-738; Published online 2014 Nov 20. doi: 10.1016/j.sjbs.2014.11.009. Abstract, page 732, left coln., 1st- 3rd para.; page 733, left coln., 2nd & 3rd para.; Fig. 2 & 3	1-4, 7-10, 13-16, 18, 19
Y	Abstract, page 732, left coln., 1st- 3rd para.; page 733, left coln., 2nd & 3rd para.; Fig. 2 & 3	5, 6, 11, 12
Y	WO 2013/127988 A1, NUNHEMS B.V., 06 September 2013 (06-09-2013). paragraphs [0061, 0099-0120]; claims 1, 2, 8	5, 6, 11, 12
Y	US 2012/0054905 A1, THE OHIO STATE UNIVERSITY, 01 March 2012 (01-03-2012). Abstract, para. [0134]	5, 6, 11, 12
A	Nascimento IR et al.: "Marker assisted identification of tospovirus resistant tomato genotypes in segregating progenies", SCIENTIA	1-16, 18, 19



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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"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

15-01-2018

Date of mailing of the international search report

15-01-2018

Name and mailing address of the ISA/

Indian Patent Office
Plot No.32, Sector 14, Dwarka, New Delhi-110075
Facsimile No.

Authorized officer

Sunil Kumar

Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2017/055151

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	AGRICOLA, 2009 Jun, vol. 66, no. 3, pages 298-303. The whole document	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2017/055151

Citation	Pub.Date	Family	Pub.Date
WO 2013/127988 A1	06-09-2013	CN 104582473 A	29-04-2015
		EP 2819505 A1	07-01-2015
		US 9603319 B2	28-03-2017