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**Copy number variant leading to virus resistance.**

(57)

The present invention relates to a genetic determinant comprising at least two copies of a combination of two closely linked *RDR1* genes, which two closely linked *RDR1* genes are inversely oriented, and which genetic determinant leads to virus resistance when present in a plant. In one embodiment, of the *RDR1* genes in the combination is represented by **SEQ ID No. 1** or has a sequence identity of at least 70% thereto, and one of the *RDR1* genes in the combination is represented by **SEQ ID No. 3** or has a sequence identity of at least 70% thereto; or one of the *RDR1* genes in the combination encodes a protein represented by **SEQ ID No. 2** or a protein that has a sequence identity of at least 70% thereto, and one of the *RDR1* genes encodes a protein represented by **SEQ ID No. 4** or a protein that has a sequence identity of at least 70% thereto.

## COPY NUMBER VARIANT LEADING TO VIRUS RESISTANCE

The present invention relates to a genetic determinant which leads to virus resistance in a plant. The invention further relates to a method for producing such a plant and a method for selecting such a plant. The invention also relates to a virus resistant plant or seed comprising the genetic determinant.

Viruses constitute a major group of pathogens that infect plants, resulting in negative effects that influence aspects of crop cultivation such as plant growth, plant vigour, product quality, and yield potential. Like most eukaryotes, plants have established a general defense response against invading pathogens, such as viruses. Pathogenic viruses however are able to evade such defense response by using different suppressor mechanisms. By consequence, within the host plant species, specific defense responses have evolved to counteract the suppressor mechanism of the pathogenic viruses.

When a plant or crop is affected by disease, in many cases this will not just be by a single virus, but by a combination of two or more viruses or other pathogens, which only enlarges the problem. Many professionally cultivated crops have resistances against several pathogens by which they can be affected. One of the challenges of a breeding programme is to efficiently combine resistances that are most relevant to that specific crop, or that are for example relevant for a specific cultivation season or area of that crop.

In co-pending application PCT/EP2015/057409 a modified *RDR1* gene is described which confers resistance to viruses, in particular against viruses of certain families and specifically against viruses of the *Potyviridae*, *Bromoviridae* and/or the *Virgaviridae*. The presence of this modified *RDR1* gene in a *Cucumis sativus* plant results in resistance against Cucumber Vein Yellowing Virus (CVYV), and may contribute to resistance against other viruses, such as for instance Cucumber Green Mottle Mosaic Virus (CGMMV), Cucumber Mosaic Virus (CMV) and Zucchini Yellow Mosaic Virus (ZYMV).

Further research in *Cucumis sativus* that led to the invention showed however that resistance against CVYV and CGMMV were often present together, but that there were also recombinants that were either only resistant to CVYV or only resistant to CGMMV. Modification of the *RDR1* gene alone was therefore not a guarantee to acquire resistance against both CVYV and CGMMV, and potentially other virus infections. Also, identification of the presence of the modified *RDR1* gene was always predictive for resistance against CVYV, but was not always predictive for resistance against CGMMV. Something similar can be expected for some of the other viruses, and in some of the other crops in which the presence of the modified *RDR1* gene contributes to virus resistance.

It was then established that in the genome of *Cucumis sativus* two closely linked *RDR1* genes are located on chromosome 5. These two *RDR1* genes are not exact copies, but are very similar, and are designated herein as *CsRDR1\_I* or 14137, and *CsRDR1\_II* or 14138.

Furthermore, the two closely linked *RDR1* genes are inversely oriented, more specifically meaning that they are linked to each other through their 3'-ends.

It was surprisingly found that when this combination of two closely linked inversely oriented *RDR1* genes was duplicated, resulting in two or more copies of the combination, and thus a total of four or more *RDR1* genes, this duplication resulted in increased virus resistance in a plant. The presence of two or more copies of this combination is designated as a copy number variant, the presence of which copy number variant influences virus resistance.

The present invention thus provides a genetic determinant comprising at least two copies of a combination of two closely linked *RDR1* genes, which two closely linked *RDR1* genes are inversely oriented, and which genetic determinant leads to virus resistance when present in a plant.

The combination of two closely linked inversely oriented *RDR1* genes is defined herein as 'the combination' or 'the *RDR1* locus'. As used herein, 'closely linked' in relation to the two *RDR1* genes that are present in the combination means that no recombination takes place between these two *RDR1* genes. In one embodiment, the distance between the two *RDR1* genes within the combination comprises not more than 3000 nucleotides. The nucleotides between the genes are not part of the sequences of said genes. No other genes are located between the two *RDR1* genes that are present in the combination. The terms 'copy' and 'duplicate' represent the same and are used interchangeably herein. 'Duplication' and 'copied' as used herein comprise multiplication to two or more copies of the combination. Two or more copies are therefore different copy number variants of the combination of two *RDR1* genes.

The genetic determinant of the invention can comprise two copies of the combination of two closely linked, inversely oriented, *RDR1* genes. The genetic determinant can also comprise three copies of the combination, or four copies, or more than four copies. The presence of at least two copies of the combination leads to virus resistance in a plant. A plant may become resistant to a certain virus due to the presence of the genetic determinant of the invention, or the resistance of an already resistant plant may be increased. The level of resistance is as compared to an isogenic plant that has only a single version of the combination of two closely linked, inversely oriented, *RDR1* genes. The presence of each additional copy of the combination in the genetic determinant may lead to an additional increase in the level of virus resistance. This means that for example a plant having four copies is more resistant than a plant having three copies, which in turn is more resistant than a plant having two copies.

The *RDR1* locus that is duplicated in the genetic determinant of the invention optionally comprises one additional gene adjacent to one of the inversely oriented *RDR1* genes, which additional gene is also duplicated as part of the combination to form a further version of the genetic determinant of the invention.

5                   The two or more copies, or duplicates, of the combination of *RDR1* genes in the genetic determinant of the invention can be present as tandem duplicates, meaning that the duplicates are located directly adjacent to each other on the chromosome. The duplicates are directly adjacent when no, or a maximum of 10, nucleotides are present in between the copies. Each duplicate optionally comprises one additional gene adjacent to one of the *RDR1* genes. The  
10                   duplicates can also be present as interspersed duplicates, which means that about one thousand, two thousand, or even six thousand nucleotides are present in between the duplicated copies. A larger chromosome segment can also be present between copies. Optionally, one of the duplicates can even be positioned on a different chromosome. The presence of multiple copies of the described combination as such within the genome of the plant is sufficient to form the genetic  
15                   determinant of the invention.

                  Copy number variants (CNVs) are relatively recently identified as one of the major potential sources of genetic variation. The approach for determining the presence of CNVs in a genome to be able to identify their effect is however very different from identification of other variations within genes, such as modifications that are present within genes. As for the latter,  
20                   usually analysis of sequences leads to the identification of differences between the sequences in the comparison. These differences are subsequently used for the development of markers that are linked to a genomic region that comprises a modification, or markers that comprise the mutation itself. This is however not feasible for establishing the presence of CNVs, since these are not based on differences in the nucleotide sequence of a gene. A copy number variant has to be identified by  
25                   determining the repetition of specific sequences within a genome, and especially sequences that form genes or parts of genes. These variations in copy number can be present close to each other, for example on the same chromosome, but they can also be positioned on different locations in the genome. The majority of genetic variation that is caused by CNVs, and especially their impact on and relation to specific phenotypic traits, is not yet revealed.

30                   In one embodiment the sequence of at least one of the *RDR1* genes of the combination is represented by **SEQ ID No. 1**, which is the sequence of *CsRDR1\_II*, or has a sequence with a sequence identity of, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% to **SEQ ID No. 1**. Said similar sequence should underlie a functionally homologous gene of the *CsRDR1\_II* gene. Alternatively, at least one of the *RDR1*  
35                   genes of the combination has a sequence that encodes a protein that is represented by **SEQ ID No.**

2, or encodes a protein that has a sequence identity of, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% to **SEQ ID No. 2**.

In one embodiment, the sequence of at least one of the *RDR1* genes of the combination is represented by **SEQ ID No. 3**, which is the sequence of *CsRDR1\_I*, or has a  
 5 sequence with a sequence identity of, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% to **SEQ ID No. 3**. Said similar sequence should underlie a functionally homologous gene of the *CsRDR1\_I* gene. Alternatively, at least one of the *RDR1* genes of the combination has a sequence that encodes a protein that is represented by **SEQ ID No. 4**, or encodes a protein that has a sequence identity of, in order of increased preference, at least  
 10 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% to **SEQ ID No. 4**.

In a particular embodiment the combination comprises one *RDR1* gene represented by **SEQ ID No. 1** or by a sequence having, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity thereto, and one *RDR1* gene represented by **SEQ ID No. 3** or by a sequence having, in order of increased preference, at least 70%, 75%, 80%,  
 15 85%, 90%, 95%, 98%, or 99% sequence identity thereto.

In an alternative embodiment the combination comprises one *RDR1* gene that encodes a protein as represented by **SEQ ID No. 2** or encodes a protein having, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity thereto, and one *RDR1* gene that encodes a protein as represented by **SEQ ID No. 4** or encodes a  
 20 protein having, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity thereto. A genetic determinant comprising the particular combination of **SEQ ID No. 1** and **SEQ ID No. 3** leads to resistance to Cucumber Green Mottle Mosaic Virus when present in a *Cucumis sativus* plant.

In one embodiment the combination comprises one *RDR1* gene represented by  
 25 **SEQ ID No. 1** or by a sequence having, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity thereto, and one *RDR1* gene that encodes a protein as represented by **SEQ ID No. 4** or encodes a protein having, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity thereto.

In one embodiment the combination comprises one *RDR1* gene that encodes a  
 30 protein as represented by **SEQ ID No. 2** or encodes a protein having, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity thereto, and one *RDR1* gene represented by **SEQ ID No. 3** or by a sequence having, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity thereto.

**SEQ ID Nos. 1 and 3** represent the wild-types of the *CsRDR1\_II* and *CsRDR1\_I*  
 35 genes of *Cucumis sativus*, and the corresponding proteins represent the wild-type proteins. Genes

that are the functional homologue of *CsRDR1\_I* or *CsRDR1\_II* in other crops have at least 70% up to 99% sequence identity with one of these *RDR1* genes of *Cucumis sativus*.

In certain instances the expression of at least one of the *RDR1* genes of the combination in the genetic determinant can be increased as compared to the expression when only a single version of the wild-type is present. The expression of one or both of the *RDR1* genes of the combination can for example be increased due to the presence of at least two copies, optionally three, four, or more copies of the combination in the genetic determinant.

The expression of at least one of the *RDR1* genes can alternatively be increased due to a modification in the wild-type nucleotide sequence of said gene. Such a modification comprises for example a modification upstream of the start codon of the gene, in particular a modification in the promoter or the 5' - UTR.

The increased expression can be an increase of the mRNA level of the *RDR1* gene, or an increase of the level of the RDR1 protein, or an increase of the activity of the RDR1 protein.

Increased expression of a gene that is present in a plant can be measured in steady state situation, which in relation to the function of this gene means a situation wherein no virus infection is present in the plant. Alternatively increased expression of a gene that is incorporated in a plant can be measured in an infected state situation, whereby a virus infection is present in the plant.

In a specific embodiment the modification upstream of the start codon of one of the *RDR1* genes in the combination resulting in increased expression and virus resistance is an indel. The indel that leads to increased expression is suitably an indel resulting in a modified gene sequence represented by **SEQ ID No. 5**. Downstream from the start codon ATG, **SEQ ID No. 5** has the same sequence as **SEQ ID No. 1**. The invention also relates to a genetic determinant whereby the sequence upstream of the start codon of one of the *CsRDR1* genes in the combination has, in order of increased preference, at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the sequence upstream of the start codon of **SEQ ID No. 5**.

In a particular embodiment the genetic determinant comprises one *CsRDR1* gene represented by **SEQ ID No. 3** or having, in order of increased preference, at least 90%, 95%, 98%, 99% sequence identity thereto, and one *CsRDR1* gene represented by **SEQ ID No. 5** or having, in order of increased preference, at least 90%, 95%, 98%, 99% sequence identity thereto. A genetic determinant comprising the particular combination of **SEQ ID No. 3** and **SEQ ID No. 5** leads to resistance to Cucumber Green Mottle Mosaic Virus and to Cucumber Vein Yellowing Virus when present in a *Cucumis sativus* plant. The *CsRDR1* genes represented by **SEQ ID No. 3** and **SEQ ID No. 5** need not necessarily have the exact sequence of these SEQ ID's in said resistant *Cucumis sativus* plant, but can also show at least 90%, 95%, 98%, 99% sequence identity thereto.

As used herein, the percentage 'sequence identity' is the percentage of nucleotides or amino acids that is identical between two sequences after proper alignment of those sequences. The person skilled in the art is aware of how to align sequences. To obtain the most significant result, the best possible alignment that gives the highest sequence identity score should be obtained. The sequences are compared over the length of the shortest sequence in the assessment.

A high percentage of sequence identity is commonly assumed to point to a homologous sequence. A genetic determinant comprising *RDR1* genes having a sequence identity percentage as claimed is part of the invention if said similar sequence is functionally homologous. Functionally homologous means that it is a gene sequence that leads to a protein that has a similar function as the *RDR1* genes that were identified in *Cucumis sativus*. A similar sequence is a sequence has at least 70%, up to 99%, sequence identity to **SEQ ID No. 1** and/or **SEQ ID No. 3** and/or **SEQ ID No. 5**. For this invention 'functionally homologous' means that the gene or protein is involved in virus resistance.

An 'indel' as used herein can represent an insertion, a deletion, or a combination of both. Preferably, the indel in one of the *RDR1* genes in the combination, resulting in increased expression, comprises at least a deletion.

The presence of the genetic determinant of the invention in a plant suitably leads to resistance to a virus of the family *Potyviridae*, *Bromoviridae*, and/or *Virgaviridae*. Virus species belonging to these families that cause major problems by infecting a large number of cultivated crops are for example, but not limited to, Cucumber Vein Yellowing Virus (CVYV), Cucumber Mosaic Virus (CMV), Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ringspot Virus (PRSV), Watermelon Mosaic Virus (WMV), Cucumber Green Mottle Mosaic Virus (CGMMV), Tobacco Mosaic Virus (TMV), Tomato Mosaic Virus (ToMV), Pepper Mild Mottle Virus (PMMoV), Pepper Mottle Virus (PepMoV), Potato Virus Y (PVY), Soybean Mosaic Virus (SMV), and Maize Dwarf Mosaic Virus (MDMV).

Plant species that have in their genome *RDR1* genes that are homologous to **SEQ ID No. 1** and/or **SEQ ID No. 3**, and are therefore particularly suitable for acquiring a genetic determinant of the invention, belong to various plant families such as *Cucurbitaceae*, *Solanaceae*, *Brassicaceae*, *Apiaceae*, *Fabaceae*, *Amaranthaceae*, and *Asteraceae*. Crop species suitable for acquiring a genetic determinant of the invention can specifically be selected from any of the following: *Phaseolus vulgaris*, *Beta vulgaris*, *Brassica oleracea*, *Daucus carota*, *Lactuca sativa*, *Cucumis melo*, *Cucumis sativus*, *Cucumis pepo*, *Spinacia oleracea*, *Solanum lycopersicum*, *Capsicum annuum*, and *Citrullus lanatus*.

The present invention relates to a method for producing a virus resistant plant comprising introducing a genetic determinant that has at least two copies of a combination of two closely linked *RDR1* genes, which two closely linked *RDR1* genes are inversely oriented, in a

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

The above method can in particular be used to introduce the genetic determinant of the invention into a plant species that is suitable for incorporation of such genetic determinant. In a particular embodiment the genetic determinant of the invention can be introduced from a *Cucumis sativus* plant comprising the genetic determinant into a *Cucumis sativus* plant lacking the genetic determinant using standard breeding methods. In *Cucumis sativus* the genetic determinant can comprise two, three, four or more copies of the combination of two inversely oriented *RDRI* genes. Introduction of the genetic determinant in *Cucumis sativus* leads to resistance to Cucumber Green Mottle Mosaic Virus. When one of the *RDRI* genes in the combination is represented by **SEQ ID No. 5**, the presence of the genetic determinant in *Cucumis sativus* leads to resistance to Cucumber Green Mottle Mosaic Virus and to resistance to Cucumber Vein Yellowing Virus.

Alternatively the genetic determinant of the invention can be introduced by increasing the copy number of closely linked inversely oriented *RDRI* genes that are already present in the genome of a plant, or they can be transferred from another, sexually incompatible, plant, for example by using transgenic modification. Techniques that can suitably be used for modification of the copy number of a gene or a combination of genes, or for the transfer of multiple copies of *RDRI* genes from other plants, comprise general plant transformation techniques known to the skilled person, such as the use of an *Agrobacterium*-mediated transformation method. Other genome editing methods such as the use of a CRISPR/Cas system might also be employed.

The invention further provides a plant comprising the genetic determinant of the invention, which plant is resistant to one or more viruses due to the presence of the genetic determinant. A plant of the invention is preferably resistant to one or more viruses of the family *Potyviridae*, *Bromoviridae*, and/or of the family *Virgaviridae*.

The invention also relates to a seed comprising the genetic determinant of the invention, wherein the plant grown from the seed is resistant to one or more viruses, in particular to one or more viruses of the family *Potyviridae*, *Bromoviridae*, and/or the family *Virgaviridae*.

A plant or a seed of the invention is a plant or a seed in which two or more copies of a combination of two inversely oriented *RDRI* genes are present, which presence results in virus resistance, for example a plant or a seed of a species selected from the group consisting of

*Phaseolus vulgaris*, *Beta vulgaris*, *Brassica oleracea*, *Daucus carota*, *Lactuca sativa*, *Cucumis melo*, *Cucumis sativus*, *Spinacia oleracea*, *Solanum lycopersicum*, and *Citrullus lanatus*.

A *Cucumis sativus* plant comprising the genetic determinant of the invention preferably is resistant against Cucumber Green Mottle Mosaic Virus, optionally in combination with resistance to Cucumber Vein Yellowing Virus.

The present invention also relates to a method for selecting a virus resistant plant, comprising determining the copy number of a combination of two *RDRI* genes that are inversely present, and selecting a plant that comprises at least two copies of said combination as a virus resistant plant. A plant comprising at least two copies of said combination comprises the genetic determinant of the invention.

Various methods based on sequencing of the genome have been developed to identify copy number variants (CNVs), and it is known to the person skilled in the art how to establish the presence of copy number variants within a genome of a plant. Straightforward strategies for CNV detection based on next generation sequencing data are for example (1) read depth analysis, (2) split-read analysis, (3) discordantly mapped read pair analysis, and (4) *de novo* genome assembly.

In the present research a tool was used to detect local copy number variation of the combination of the two *RDRI* genes that were identified in the region of relevance in *Cucumis sativus*. Subsequently qPCR was used to also determine the expression level of the genes in the combination. Results of both the variation in copy number that was identified, and of the expression analysis, were linked to virus resistance of the plants (**Example 1**). In this way the relation between genotype and phenotype was established.

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

## FIGURES

**Figure 1** – Genomic sequences of **SEQ ID No. 1**, **SEQ ID No. 3**, and **SEQ ID No. 5**, including the coding sequence (CDS), which starts with the start codon **ATG** (bold in the sequence), and the sequence about 2 kb upstream of the start codon including the 5'-UTR and the promoter. Sequences are of *Cucumis sativus* *CsRDRI\_II*, *CS RDRI\_I*, and *CsRDRI\_II* having an indel upstream of the start codon respectively.

**Figure 2** – Protein sequences of **SEQ ID No. 2** and **SEQ ID No. 4**, generated by the CDS's of *CsRDRI\_II* and *CsRDRI\_I* respectively, whereby *CsRDRI\_II* with the indel, represented by **SEQ ID No. 5**, codes for the same protein as *CsRDRI\_II* since the CDS is the same.

**Figure 3** – Read depth analysis of sequencing data from various lines that were susceptible to both CVYV and CGMMV, were resistant to CGMMV and susceptible to CVYV, or were resistant to both viruses.

A: WGS read mapping of Geno3 to reference genome (pacbio);

5 B: WGS read mapping to BF11 reference (pacbio), CGMMV susceptible (S) and CVYV susceptible (S) lines;

C: WGS read mapping to BF11 reference (pacbio), CGMMV resistant (R) and CVYV susceptible (S) lines;

10 D: WGS read mapping to BF11 reference (pacbio), CGMMV resistant (R) and CVYV resistant (R) lines.

**Figure 4** – Possible locations of copies of the combination of *RDR1* genes within the genome, in relation to each other. For ‘Geno4’ a genetic determinant with 3 copies is depicted, and for ‘Geno2’ and ‘Geno3’ genetic determinants having 2 copies are depicted.

## 15 **EXAMPLES**

### **EXAMPLE 1**

*Identification of copy number variants for RDR1 genes in Cucumis sativus related to virus resistance*

20 In previous research two closely linked, inversely oriented, *RDR1* genes were identified on chromosome 5 of the *Cucumis sativus* genome. At least one of these *RDR1* genes was, when modified, determined to be involved in conferring resistance to CVYV. Although there appeared to be a linkage with resistance to CGMMV, recombinants having only CGMMV or only CVYV resistance could be observed. Markers that were developed for the modified *RDR1* gene were predictive for the CVYV resistance, but not always for the CGMMV resistance.

25 Whole genome sequencing (WGS) was subsequently done on a number of lines that were susceptible, or had one or both resistances. More than 250 additional markers were developed based on sequence differences in the region of interest. Again, also the analysis of these data and the application of the new markers did not result in better markers for the CGMMV resistance. This led to the assumption that something different than a modification in or around the  
30 gene on the chromosome 5 region might be leading to the CGMMV resistance, and it was decided to take a different approach.

The WGS data of the various lines were then mapped and subsequently analyzed using WGS read alignment visualization tools, and compared against an internally generated reference genome sequence of *Cucumis sativus*. Using this, the read depth of the sequence with the  
35 two *RDR1* genes was observed. It was highly interestingly found that the read-depth of the specific

sequence that has the two *RDR1* genes was around two times or even three times higher for certain material when compared to the reference genome (**Figure 3**).

To confirm this information a large number of 61 lines having CGMMV and CVYV resistance in various combinations, or being susceptible to both, were resequenced and a qPCR was performed using the available data. The qPCR data were analyzed using the ‘delta delta Cq’ method (ddCq), with efficiency correction. The threshold was calculated using suitable software, which is commonly known, for both target genes *CsRDR1\_I* and *CsRDR1\_II*, as well as for the reference gene. The following formula was used to calculate the eventual fold changes,

which also indicates copy number variation: 
$$\frac{\text{Sample A}}{\text{Sample B}} = \frac{(1 + E_{ref})^{CqA_{ref} - CqB_{ref}}}{(1 + E_{tar})^{CqA_{tar} - CqB_{tar}}}$$

The different methods eventually led to the same result, and confirmed the presence of copy number variants for the combination of the two closely linked, inversely oriented, *RDR1* genes in the genome of *Cucumis sativus*.

## EXAMPLE 2

### Determination of the location of the different copies

The location within the *Cucumis sativus* genome of the different copies was determined by a combined strategy, which includes split-read analysis, discordantly mapped read pair analysis, and *de novo* genome assembly. Based on this analysis, it was determined that the multiple copies of the *RDR1* combination in the *Cucumis sativus* genome in some backgrounds are present as tandem repeats. This was determined by split-read analysis, but also verified with the use of an MGB assay that confirmed the presence of overlapping sequences. Other backgrounds showed that the copies were present on the same chromosome, but with around 1000 up to 6000 bp in between. This was done through *de novo* genome assembly. A third result showed that the copies can be even further away, possibly with around 200 kb in between, but a location on a different chromosome is also still feasible. To obtain this result, split-read analysis was combined with discordantly mapped read pair analysis. The combined results are visualized in **Figure 4**.

## EXAMPLE 3

### Linking resistance to the presence of copy number variation

The lines that were analyzed in Examples 1 and 2 were also phenotyped for resistance to CVYV and to CGMMV. A bio-assay was performed using commonly known inoculation and observation methods for evaluating the resistance. For CGMMV two repetitions were carried out. CVYV resistance score was based on several bio-assays in different years.

Subsequently for each line the genotypic data indicating the copy number and the presence or absence of an indel, and the phenotypes indicating virus resistance were compared

with each other to be able to draw conclusions. Results of certain representative lines are presented in **Table 1**.

‘Geno1’ refers to plants in which only one version of the combination of two closely linked inversely oriented *RDR1* genes is present, so there are no multiple copies. ‘Geno2’ is a genetic background in which 2 copies are present, but they are located far from each other in the genome, probably around 200 kb or even more in between. ‘Geno3’ is a genetic background that does not have the indel in *CsRDR1\_II* that is known to lead to CVYV. The copies in this background are located at a distance of between 1000 and 6000 bp from each other. ‘Geno4’ is a genetic background in which the copies of the combination of *RDR1* genes are tandem duplications. Also, *CsRDR1\_II* in this background has the indel that leads to CVYV resistance.

‘R’ means that in that test all plants were resistant. A score of 8/2/0 means that 8 plants are resistant, i.e. without symptoms, 2 plants show light symptoms, and 0 plants are susceptible.

**Table 1**

Copy number in relation to CVYV and CGMMV resistance, and gene expression

|           | COPY NUMBER        |                     |             | EXPRESSION |       | RESISTANCE |          |          |
|-----------|--------------------|---------------------|-------------|------------|-------|------------|----------|----------|
| Reference | calculated cnv WGS | Calculated CNV qPCR | copy number | 14138      | 14137 | CVYV       | CGMMV-t1 | CGMMV-t2 |
| Geno1_1   | 1,1                | 0,87                |             | -1,31      | -0,81 | S          | S        | S        |
| Geno1_2   | 1,11               | 0,93                |             | -0,64      | -0,55 | S          | S        | S        |
| Geno2_1   | 2,19               | 1,92                | 2x          | 0,9        | -1,32 | S          | 8/2/0    | 8/2/0    |
| Geno3_1   | 2,02               | 1,86                | 2x          | 1,05       | -0,33 | S          | R        | R        |
| Geno4_1   | 1,08               | 1,02                |             | 2,29       | -0,68 | R          | S        | S        |
| Geno4_2   | 2,13               | 1,99                | 2x          | 3,73       | -1,14 | R          | R        | R        |
| Geno4_3   | 3,21               | 3,13                | 3x          | 3,56       | -0,47 | R          | R        | R        |
| Geno4_4   | 3,03               | 3,2                 | 3x          | 4,54       | -0,22 | R          | R        | 3/7/0    |

Based on these results it was concluded that the presence of multiple copies of the combination of *RDR1* genes leads to resistance to CGMMV. When the indel in one of the two *RDR1* genes is present (Geno4) it gives only CVYV resistance when just 1 version is present

(Geno4\_1). Only when two or more copies are present there is resistance to both CVYV and CGMMV. No copies and no indel gives susceptibility to both viruses (Geno1). Geno2\_1 and Geno3\_1 show that CGMMV resistance can be present independent of CVYV resistance.

## CONCLUSIES

1. Genetische determinant omvattende ten minste twee kopieën van een combinatie van twee nauw gekoppelde *RDR1* genen, welke twee nauw gekoppelde *RDR1* genen omgekeerd georiënteerd zijn, en welk genetische determinant leidt tot virusresistentie wanneer aanwezig in een plant.

2. Genetische determinant zoals geclaimd in conclusie 1, omvattende drie, vier, of meerdere kopieën van de combinatie van twee nauw gekoppelde omgekeerd georiënteerde *RDR1* genen.

3. Genetische determinant zoals geclaimd in conclusie 1 of 2, waarbij  
 a) ten minste een van de *RDR1* genen in de combinatie wordt voorgesteld door **SEQ ID No. 1** of een sequentie-identiteit heeft van ten minste 70% daaraan; of  
 b) ten minste een van de *RDR1* genen in de combinatie een eiwit codeert voorgesteld door **SEQ ID No. 2** of een eiwit codeert dat een sequentie-identiteit heeft van ten minste 70% met **SEQ ID No. 2**.

4. Genetische determinant zoals geclaimd in conclusie 1 of 2, waarbij  
 a) ten minste een van de *RDR1* genen in de combinatie wordt voorgesteld door **SEQ ID No. 3** of een sequentie-identiteit heeft van ten minste 70% daaraan; of  
 b) ten minste een van de *RDR1* genen in de combinatie een eiwit codeert voorgesteld door **SEQ ID No. 4** of een eiwit codeert dat een sequentie-identiteit heeft van ten minste 70% met **SEQ ID No. 4**.

5. Genetische determinant zoals geclaimd in conclusie 3 of 4, waarbij  
 a) een van de *RDR1* genen in de combinatie wordt voorgesteld door **SEQ ID No. 1** of een sequentie-identiteit heeft van ten minste 70% daaraan, en een van de *RDR1* genen in de combinatie wordt voorgesteld door **SEQ ID No. 3** of een sequentie-identiteit heeft van ten minste 70% daaraan; of  
 b) een van de *RDR1* genen in de combinatie een eiwit codeert voorgesteld door **SEQ ID No. 2** of een eiwit dat een sequentie-identiteit heeft van ten minste 70% daaraan, en een van de *RDR1* genen een eiwit codeert voorgesteld door **SEQ ID No. 4** of een eiwit dat een sequentie-identiteit heeft van ten minste 70% daaraan.

6. Genetische determinant zoals geclaimd in een der conclusies 1-5, waarbij ten minste een van de *RDR1* genen in de combinatie een indel upstream van het startcodon heeft.

7. Genetische determinant zoals geclaimd in een der conclusies 1-6, waarbij de afstand tussen de twee *RDR1* genen die omgekeerd aanwezig zijn niet meer dan 3000 bp is.

8. Genetische determinant zoals geclaimd in een der conclusies 1-7, waarbij de afstand tussen kopieën van combinaties van twee *RDR1* genen niet meer is dan 6000 bp, bij voorkeur niet meer dan 1000 bp, meest bij voorkeur 0 bp.

9. Genetische determinant zoals geclaimd in een der conclusies 1-8, waarbij een van de *RDR1* genen *CsRDR1\_II* is, voorgesteld door **SEQ ID No. 1**, of ten minste 90% sequentie-identiteit heeft daaraan, en een van de *RDR1* genen *CsRDR1\_I* is, voorgesteld door **SEQ ID No. 3**, of ten minste 90% sequentie-identiteit heeft daaraan, waarbij de aanwezigheid van de genetische determinant in een *Cucumis sativus* plant leidt tot virusresistentie tegen Cucumber Green Mottle Mosaic Virus.

10. Genetische determinant zoals geclaimd in een der conclusies 6-8, waarbij een van de *RDR1* genen *CsRDR1\_I* is, voorgesteld door **SEQ ID No. 3**, of ten minste 90% sequentie-identiteit heeft daaraan, en een van de *RDR1* genen een gemodificeerd *CsRDR1\_II* is, voorgesteld door **SEQ ID No. 5**, of ten minste 90% sequentie-identiteit heeft daaraan, waarbij de aanwezigheid van de genetische determinant in een *Cucumis sativus* plant leidt tot virusresistentie tegen Cucumber Green Mottle Mosaic Virus en Cucumber Vein Yellowing Virus.

11. Werkwijze voor het produceren van een virusresistente plant omvattende het introduceren van de genetische determinant zoals geclaimd in een der conclusies 1-10 in een plant.

12. Werkwijze voor het selecteren van een virusresistente plant, omvattende het bepalen van het kopie-aantal van een combinatie van twee nauw gekoppelde *RDR1* genen die omgekeerd aanwezig zijn, en het selecteren van een plant dat ten minste twee kopieën, bij voorkeur tenminste drie kopieën, van de combinatie omvat als een virusresistente plant omvattende de genetische determinant zoals geclaimd in een der conclusies 1-10.

13. Werkwijze zoals geclaimd in conclusie 11 of 12 waarbij de plant behoort tot een soort gekozen uit de groep bestaande uit *Phaseolus vulgaris*, *Beta vulgaris*, *Brassica oleracea*, *Daucus carota*, *Lactuca sativa*, *Cucumis melo*, *Cucumis sativus*, *Cucumis pepo*, *Spinacia oleracea*, *Solanum lycopersicum*, *Capsicum annuum*, en *Citrullus lanatus*.

5

14. Werkwijze zoals geclaimd in conclusie 11, 12, of 13, waarbij het virus van de familie *Potyviridae*, *Bromoviridae*, en/of van de familie *Virgaviridae* is.

10

15. Werkwijze zoals geclaimd in een der conclusies 11-14, waarbij de combinatie van twee nauw gekoppelde RDR1 genen tenminste een RDR1 gen omvat dat wordt voorgesteld door **SEQ ID No. 1** of een sequentie-identiteit heeft van ten minste 70% daaraan, en/of tenminste een RDR1 gen omvat dat wordt voorgesteld door **SEQ ID No. 3** of een sequentie-identiteit heeft van ten minste 70% daaraan.

15

16. Werkwijze zoals geclaimd in een der conclusies 11-14, waarbij de combinatie van twee nauw gekoppelde RDR1 genen tenminste een RDR1 gen omvat dat wordt voorgesteld door **SEQ ID No. 3** of een sequentie-identiteit heeft van ten minste 70% daaraan, en/of tenminste een RDR1 gen omvat dat wordt voorgesteld door **SEQ ID No. 5** of een sequentie-identiteit heeft van ten minste 70% daaraan.

20

17. Werkwijze zoals geclaimd in een der conclusies 11-14, waarbij de virusresistente plant een *Cucumis sativus* plant is welke resistent is tegen Cucumber Green Mottle Mosaic Virus, en optioneel resistent tegen Cucumber Vein Yellowing Virus.

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18. Werkwijze zoals geclaimd in conclusie 15 of 17, waarbij de combinatie van twee nauw gekoppelde RDR1 genen *CsRDR1\_II* omvat, welke wordt voorgesteld door **SEQ ID No. 1** of een gen dat een sequentie-identiteit heeft van ten minste 90% daaraan, en *CsRDR1\_I* welke wordt voorgesteld door **SEQ ID No. 3** of gen dat een sequentie-identiteit heeft van ten minste 90% daaraan, en waarin de gekozen virus-resistente plant een *Cucumis sativus* plant is die twee kopieën, bij voorkeur drie of meer kopieën, van de combinatie bevat, waarvan de aanwezigheid leidt tot resistentie tegen Cucumber Green Mottle Mosaic Virus.

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19. Werkwijze zoals geclaimd in conclusie 16 of 17, waarbij de combinatie van twee nauw gekoppelde RDR1 genen *CsRDR1\_II* omvat, welke wordt voorgesteld door **SEQ ID No. 5** of een gen dat een sequentie-identiteit heeft van ten minste 90% daaraan, en *CsRDR1\_I* welke wordt voorgesteld door **SEQ ID No. 3** of gen dat een sequentie-identiteit

heeft van ten minste 90% daaraan, en waarin de gekozen virus-resistente plant een *Cucumis sativus* plant is die twee kopieën, bij voorkeur drie of meer kopieën, van de combinatie bevat, waarvan de aanwezigheid leidt tot resistentie tegen Cucumber Green Mottle Mosaic Virus en tegen Cucumber Vein Yellowing Virus.

5

20. Plant, welke resistent is tegen een of meerdere virussen door de aanwezigheid in zijn genoom van de genetische determinant zoals geclaimd in een der conclusies 1-10.

10

21. Zaad, waarbij een plant gegroeid uit het zaad resistent is tegen een of meerdere virussen door de aanwezigheid in zijn genoom van de genetische determinant zoals geclaimd in een der conclusies 1-10.

15

22. Plant zoals geclaimd in conclusie 20, of zaad zoals geclaimd in conclusie 21, waarbij het virus van de familie *Potyviridae*, *Bromoviridae*, en/of van de familie *Virgaviridae* is.

20

23. Plant zoals geclaimd in conclusie 20 of 22, of zaad zoals geclaimd in conclusie 21 of 22, welke een plant of een zaad is van een soort gekozen uit de groep bestaande uit *Phaseolus vulgaris*, *Beta vulgaris*, *Brassica oleracea*, *Daucus carota*, *Lactuca sativa*, *Cucumis melo*, *Cucumis sativus*, *Cucumis pepo*, *Spinacia oleracea*, *Solanum lycopersicum*, *Capsicum annuum*, en *Citrullus lanatus*.

25

24. Plant zoals geclaimd in conclusie 20, 22 of 23, welke een *Cucumis sativus* plant is omvattende de genetische determinant zoals geclaimd in conclusie 9 of 10, waarin de genetische determinant ten minste twee kopieën, bij voorkeur tenminste drie kopieën van de combinatie bevat, welke *Cucumis sativus* plant resistent is tegen Cucumber Green Mottle Mosaic Virus, en optioneel resistent is tegen Cucumber Vein Yellowing Virus.

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25. *Cucumis sativus* plant zoals geclaimd in conclusie 24 omvattende de genetische determinant zoals geclaimd in conclusie 10, welke plant resistent is tegen Cucumber Green Mottle Mosaic Virus en tegen Cucumber Vein Yellowing Virus door de aanwezigheid van de genetische determinant.

Fig. 1-1

Genomic sequences SEQ ID Nos. 1, 3, and 5

SEQ ID No. 1 - *CsRDR1\_II*

>*Cucumis sativus*\_cs9930v2\_emv\_14138\_genomic\_sequence

```
AATACTACAACAATAATTCTTCTCCCAAACACATACTATCATAATCCTTCTCCCAAACAC
ATACAATCATAACACTACCATTTCATATTCCTTCCCCCAAATAACACATATTACCATAACA
CTACCAATAATAACCCAAACCTTAAACACATATTATCATAACACCAAGATTATTATAACA
CTAGGATTGCCATAATCTTTCCCTCCCAAATGCACCCCTAAGAATTTTGCCATATTTGCA
AAATTATAAATCAATGTGCTATATTTGTGATAACATGTTCTCAAAATGCTACCTACTACA
ACTTTTCAATAAATAAGTAGAGACTAACTAGAGCAAGGTCAGGACAGGGAGTGTCTTCAT
CTTGGTTTAGCTCACAGTGAGTTTAAATTTTTTTTTTTTNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNCTTCCACTCCCTCTCCATTCTCCACGTGGTTTCAGTGCAGGT
CTCGGGCACCCGTCTCACTGGAAAAATGGACATGTCTAGAAATATTTAAAGCATATCTC
AAAGTTTACGGTCATTGGTATTCTCTCTATGAAGACCTTCAAAATATTATTTAACACGGT
CACAATTAATATTTGAGAGAGAAACAACGTAAGTATTTCAAAATATGTATCAATAAATT
TTGTAGGTATTTCCATATTTATGTAGATTATTGTGAATCAACCTTTGTATCATATGATTA
AAAATATATATATGAACAACAAAAATGTACTAATATGTAAATCTAATATAATATAAACAA
TATGGTATATTTTCTATTGATTCCTTTAATAAGAAAAATGTTTTCTATAATTTTTTTTAAA
AAAATATCAATCCACATAGAAAATTCATATCCATTGGCGGCTCATTCAATAATTTAATAT
ATTCTTTTCGAAAACCTAGAAGCCAAAATTAAGAAAAAGAAATTAATTCAATAGAGA
ATATTTGGTGTATGGCCATGGAAAGCTCAAAAAGAAAGACCTGTCAATGAAAGTCTTTC
TTTACTCTTAAGCTAAGGCCCCCAATTATGGAAATATATCTCTTCATTCTCCATTTTC
GTTTCTCCATTCCCAACTCTCCTATTTTGGCACTACACTGTTCTCTACTGCCCTCTGCAT
CCTCTTTTCATGAATCAATCTGCTTGGTATTCACCTAACTTTTCTTCCATTGTTGAGAA
TAGATGGACTATTGATGTGTTTTCTTTTATATTGTAAAGCTATTCTTCTTTCTTTGTG
TTTCTTCATCTGGGTTTCATTTTTTATCATGTTTTTCCCATTTCTTTTTGTTCCCTGTG
TTTTCTTTGTATTTAGCAACGTATCCTCTCTGCTCTCTCTGTAGATTCTTACTGCTTCT
GGGGCTGTTTATGATCTGGGGTTGTTTCTGTCTTCAAATTTTAGTTTTCACTATGTGGG
TGTCGGTTTGATTATGAAAACGTTTATTCGTATGTTCCACACATTTTCTTGATCATGT
ATGAGTTACCATTAGTATGCATTCGTCTCTTTACCAATGAGTATAATGTGATCTAGCTT
TCTCTATTAATGTCGGTGAGATCCTCTATATCTTGAATGTGTACATACCTTTTCAATTTG
TCAAGATGATAATGTTTTTGCATTTGGAATGAAGTTATATATAGAACTTATGGAAAAG
GGTTAAATAAATATTAATCTTTCCTCGATGGAATGTAAGAAACACTTTTTAATCTATCTG
CTCACCTCTTTATTTTGGAGACTTGGTTTTTGGGTTGAATAATATATGGGGTGAGGTATT
TGAACAGTTGATCTTTTGGTCAAGGCTACATATATTATGCTAGTTGAACTTGGCTTTCTT
TTTAGGCTTCATCATATGCATTTGTAATCAATTTGTTTATATGACAGAAAGAAGTTCCGA
GTTGATTTTTCTTGGATTGGATGGGTAAACAATTCAGCTTTTGGATTCCCTTCTCGTG
TATTGCAAGAAFCAGTTAAGACGTTTGTAGAGGGAATTACAGGCACAGGAAGTATTGATG
CCATAAATACGAAACGTTTGAAGGAGGAGGAAGACGAGTGTATGCTATCATCCAGTTTA
CTGATGAAGAAGGTGCTAAGTCAATTATATCTAAGGCTACTGAACGCCTTTGTTATGGTA
CTTCTTATCTGAAGGCAAGGAGATGAAACATGATATTCTACCAGATCCGCTTGTCTTTG
ATTACAACCTTCAAAGCTCTAAGACTACATCTTGGCTGTGAGATATCAAAGGAAAGTTTTT
CCGTGTTATGGACAGAGTCGAATGTTTCTGTAGATTTCGGGTTTGGCTGCGCAAGCTTT
ATTTCTTCATATCCTATCCTCGTGTGACTACATGCTCGTATTGCGCTACGAGAACATTT
GGCAGGTTGAGTTACACAAGCCACATGGTCAATCTGTAGATTATCTTCTGATTACAGGTT
ATCCATTAACCTTTGAACAATGTCATGTATTAGTGTACTGTTGTATTTTCTCCTCACTAT
TGAGAAATATCATTGATTATCCCAAGCAAGTTTCACTAAATTTTTCACTTTATTCATG
GTATTGTTCTTAATTACGGGGATTCAACTACTGACTCATGTACGTGCTCATAGGCCTGA
TTTCCATCACAGAACAGTGGACGGATATAAATGATACTGAAAATAAAAATTTAGTGAA
CCACTAAAATCATCATTATACCTAAGTTCTGAGAGAAATATATAGACTGAACACTTTA
TGGGACAAAGGAATTAAGTGAATTTATTGATAACTTCGATGCAAAAAGAACTGAGAAAC
GATCAAGGTTTTATCAAAAGATTGTAAGGGGATAGTGGAAGATAGCTGTAGATAAATTC
CAGTGCTTCAAATGGGTGAAAGAAGCTATAATTTTATTAAAAGGTGTCTTAGTTGATAA
```

Fig. 1-2

TTTTATCATACTTTTTCTCCAACCTTGATAACTTCAAGACTATGGGTAGGATTTGGATA  
TAATGAGATTTTGAAGCCATATAAGGTTAATGTTGTTTAGTAATTGTAATCTGGCAGGATA  
TGTTTTCTTTGAACAGAGCTAAACATGTCCCTAGATATGAATTTTAAACAAGCTAAGTAT  
AAACAGAACTAAGCTTGCAACTTTTCTATATTTCTATACTTCAGGATAAGCTTATAAACG  
CAGGTAATCCGTGCAAGTGAACATATGTTTCATAAAAAACAAATATGCTGTCTTCATACT  
GATGTTGAAATAAGCAAGTCAAAGTTCATGGGCAAGAAATTTGAGAATAGCTTAGGTTCT  
TGGCCCATGCACATTTTATGTTGTATATATTCTAACTATGACATGTTTGTACTGTTAGTT  
ATTTGGTGCTCCACGGATTTATGAAAGAGATGCAAGGTCTTTTGGACTCATTACTGAAGA  
CCCTTTCTTAAACTTTTCCACGGAAATGACACCCCAATGGTTTGGAGCAACTGATTTTAC  
TCCATCATGTAGTATTTGGACAATCTGCTGCTTATGCTTGGAGATTCCCTACGGTCGCCA  
GCTCCCTAATTTTCATGATAAATTTGCTTACTTCAAAGAAATCAAGGGTAAATTTACATT  
GGTCAGTGGTTCTACTTATTCTCTCAATGTAAACTTTGGTACCTGTAGTTACACCTCCTCG  
AACCATCAACTTGCCATATACAATTTTGTTTAAGATAAAATTTGTTGGTACAACAAGGATG  
TCTTCCAGGCCCCAGCTCTTGATATTAGTTTCTATCAGATGGTAGATTCTCAGATATACAA  
TACTGCCGTCATAGATCATGCGTTAAAGAACTTCTCCACTTGAAGAGAGTGTTCGTATATA  
CCCTTCAAAATGGTTAGATGAGGAATACAGAAAGTACTTCAAATTAAGAATCCCCCACA  
GCCACCTATTTTGACCTTGAATGAAGGGTTACTCTATGTACACAGGGTTCAAGTGACACC  
TTGTAAGTTTACTTTTGTGGTCCAGAAGTTAACATTTCAAATCGTGTATTACGCCGGTA  
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GTATTCAACTGAGTTGTCTCCACGTGCATCTTCTCTTGGAGGATGGAAAGACAAAAAT  
TTTTAAACGGATTCTTTCAGTTCTAAGAGATGGCATCACTATTGGTGATAAGAGTTTGA  
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AAATGAACCTTACTGCAGCTAAATAAGGCAATGGATGGGAGATTTTCATAATATACGAAA  
TGAGCCCAAGTATGCTGCTAGACTAGGCCAATCCTTTGGTTCAACACAGAACTTTAAG  
TGTCAGTAGACGTGAAGTTAAAGTTATCTCTGATATTGAAGTTGAATCAGGTAGTGGTGT  
CAATTATGCTCTCTGATGGTATTGGGAAATAGCAGCTAGTTTGTAGAAAAGTGGC  
TAAAAATGTGGATCAGGCATACACCATCTGCTTTTCAGATTGCTTATGCTGGTTTTAA  
AGGTGTTATTTCTGTTGATCCTACCTCATCAGTAAATATTCGCTAAGCAACAGCATGCT  
CAAGTATGAATCAACAGACACGAAGCTTGATGTTTTATCATGAGTAATATCATCCTTG  
CTTTCTAAATCGTCAGTTGATTACTCTTTTGTCTACACTTGGAGTTCAGGATCATGTTTT  
TGAGAGTAACAACAGGAGTTGATTGATGAATGGACACCATTTTATGATCCATTGAA  
GGCTCAGCAGGCTCTTGAGCTAATGTCTCCAGGAGAGAATACCAAGATACTTAAGGAAAT  
GATGTTGTGCGGTTACAAACCTGATTCTGAACCTTTCTTAAGAATGATGTTGCACACATT  
CAGAGAATCAAAGTTGATGGAATTCGAATGAAGTCAAGGATCTTCAATCCAAATGGAAG  
AGCAATGATGGGATGCTCTGACGAAACAAGAACTTGGAAATATGGGAGGTATTTGTGCA  
GTGTTCTGCACATCAGCAGCTGCATGACCATCGCGTAATCTTTAAGAGAATAAAATCGAA  
CCGCAATTCATTGTAACCTGGAACAGTTGTAGTGGCCAAAACCCCTGCTTGCACCCAGG  
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CAGTTCAAGTAAAATAAAATATATTTCTCCTTTTCAGTCTTAGATATATGTTTATACTCG  
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AAGTATTTTAGAGGAAAAGGTTGTTCTATAGTGTAAATGTTGTAATGCAGGAGGTCCAG  
GAGTATTTTGCAAATTTATGTTCAATGACAGTTTAGGAATCATTGCCAATGCTCATACA  
GCTTTTGCAGATAAAGAGCCAAAGAAAGCAATGAGCAATCCTTGATACAGCTCGCAAAA  
CTATTCTCAATTCAGTCCGACTTTCCGAAAACCTGCAGTCCCTGCTTTAATACCTGCTAAT  
CTAAGAGTAAAAGAAATATCCGGATTTTCATGGATAAAGCCGACAAAGTGACATACGAGTCG

Fig. 1-3

GAGAATGTTACTGGGGAACTATTTAGAATGTTGGATAGCATTGGTCCAAACATTAAGAAT  
ATCAGGTCTTCAACTATACGCCGAGATGGCTCGGCAAGATTATGACCCTGACATGGAA  
GTTGAAGGTTTCGAGGAGTACCTCGACGATGCAATATATCACAAGAACAACATGACATG  
AGGTTGGGAAATTTGATGCACTATCATAAGATCAAACTGAGGCGGAATTGATCAGTGGT  
GGTAGTTTGACGTCATCATTATCTTTACCATGAAAAATGAAGCGGAATCGATTATCTTG  
GCTGTGAAGTCTGCTGCGAAAGGAGGCGAGGGGCTGGTTCAATGAGAAAGCAGACTTACAT  
TATGGACATCATACTAATGTGTATGCAAGAGCTTCAGCATGGTATTTTGTACATATCAT  
CACACCTACTGGGGGTGGTCTGATGGCAGAAAGAATCATGGCCATTTTCTTAGCTTTCCA  
TGGTGTGTTTATGATAAACTCATCCGTATCAAGCACCGCAAAATTAATTGTAGAGCTCGC  
TATTGA

SEQ ID No. 3 - *CsRDR1\_I*

>Cucumis sativus\_cs9930v2\_emv\_14137\_genomic\_sequence

TTAAATCACGTTTTTAAAAATGAAAACTACCATATCAAGCATTAGTATGGTCAATAAGTGGGTGTTTTGTTGAA  
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CAATATCGAACTTATTTTGTATGCAAATTTAGTTTAAATATGTTTGAATAGTTGTAAATATAACAAATA  
AATTTAAATAAATTAAGAATATAACAACATTTTAAAAAATTGCAAATATAACAAATCTGTAAAGTCTATC  
AATAATAGATTATGTTGCAAATATTGGTCTATCATAATAAATCATAAGAGTCTAGTGTAGACTTTGCAATAT  
TTACAATGTTTTTAAATGCTGTTATATACTTAATTATTATTTCTAAACTGTTATCCATTATAATTACTCAT  
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CACTCCTCTTGTTACCAATAATCTATTTTGGCTTTCCAAACAACCGATAAGGATCAATGTAAATAGTTAAAAAG  
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CAACGAGTAAAAAGAATAAAAGTAACCTAAATGGAGGAAAAATTTAAATGTTTCGTAAAAACATGGTTGAAGGA  
AAGTTTGAAGAAGATAAAATGTTACCACTAACTAATGTGTTAGGAAAGAAAGTAAGAATTTGAAAGATA  
ATGAAGCAAATTAATTATAAAATAATGTAATTAATAAATTCCTTTACAAAAGTCTACTTAGTTATTTACTTTT  
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TTAATGTGAATTGAATAATATTATTGAAAGTGAGAAATCATATAATTTGGTTTGTGTTTTTATTAAGAATA  
GAAAAAGAGAAATAATTGTACTAGAAAGGTTAACTTAGGTAGCAAGTTTGTGTTGATTTTTCCCATCTGG  
CGTCAAGTCAAGGCTTTTGGGAAATGAAGTCTATTATTAAAGCTTTCAAGTTCTTCTCATGCCCCACAAAAAC  
ATTTTTAAGAATATTACTTTACTTGAAATTAATTATTTTACTTATTCTTACTTTTCAGTACGCTTTATCTTT  
AATGTAATCATATAATAGAAACACACTAAAATTTAATTAGCATCAATAAGTAAATTTGAAATCAAGGAACA  
TAAACCTAAAATAAAGGGAACCCCATGTTGAAATTTGTGCATTAAATAGCAAAATTTGACTTTTGATCCA  
CAGCCTTATTTGGTGAATTACTCCATGATGTTTGGATTTGATTTAGACCATATTGGTAAACATATTCTAAG  
TCCTTCTTTTAGCTCTCCCAACCGTCCCTTATTTATGGATGTTTCAATATTTCAGTCATAGTGTGCAACTT  
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ACAATTTGACTGGTTACACCTCACTTGATCAGTCTCTACTTCACGATCCCTCGTCTCGTATGGGCTCT  
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AGTTTTTGACATCCATTAACCTCTTCTGTAAATTACCACTAACTGGGGAAGTGGGCTTGGCTGCTTGGCTC  
TTGACTCTTCGCAATCTCTGTTTTTACTCTGTTTTTACACTGTTTTTGGTTTTGATTGCTCTACTGGGTT  
ATATGGAAACTTCAAAATCCTAAAGTTTTCATTTCCGTTTATCGATTGTGCCACTTGGAGGGGATTTTTCAT

Fig. 1-4

GTTTTTTTTTTTTTAACTGTGGGTTTCTCTGTGTTTCTTCTGCTCATATCTTTTGTGCCTTTAATTGTCTT  
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ACCTTATTTTCTTCACATTGTCTATGGAATGCTCCATTCAAATTGGAACCCCAATACGCATAGGAGCATAGAAG  
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GAGTTGATCATTTCTTTAGCTAATCAACGTCTATGGTACGGATCTTCTTATCTTAAGGCTCGGGCAACCGAGG  
TTGATATTGTACAAAACCTAGGACATACATGTATACCTTGGAAAGAGTTGCTGCTATGCTTTGCTGTCAAGT  
CTCAACTGAAAAGTTTCTGTCTATGGGAAGGAAATGTTGATTGTTGACTTTTGGAAATGGAATCGGAAA  
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TCTTCTTCTTTATGTTTGAAGCTACCTAATGGCCCTCAACTTCCACCTTTTAAACAAAATTTTGCTTATTATG  
AAGAATTTGAACATGAATTCGGCTTGATAGATGAAGATGCCAATTTTTCTTTTTGTAGAGATCTTGCTCCCAT  
TGTTGATTCTCGTTCTCATGTTCTGCCGTATAAAAATTTGTTTAAAATAAATGCATTAGTTCAATATGGTTGC  
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AGGGTTCAAATAACACCTTGTAAGGTGTTCTTCTGTGGTCTGAAAGTCAATGTCTCAAATCGGGTGTGCGCC  
ATTTTTCTCAATATATTGATAATTTCTTCTGTGTCTTTTGTGATGAGGAGTGGGATAAAATGCGTTCAAC  
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TATTTGTGAACATACATGTTAATGATAGTCTTGAATCAATGCCAATGCTCATACTGCCTTTGCAGATAAAG  
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Fig. 1-5

AACTGGAGTACCAGCTATAATACCTTCTCATTATATGTCAAAGAGTTTCCTGACTTTATGGAGAAGCCTGAC  
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GGTATCAAGTCTGAGGCAGAAGTACTTAGTGGGAGTATCATGAGGATGTCCAAGTCTTTCACCAGGAGAAGAG  
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TGGGGCTGCTATAATGAGGGAATGAAACGCGACCATTATTTGAGCTTCCCCTGGTGTGTTTACGACAACTGA  
TGCAATCAAGGAGAATAATTTGAGAAGAAGAGAGAGAGCTGCAAGACTGGCAAGTTTCGACAGATTCCGACA  
TGTGTTAAATCTTGGTGGGAGTTGAAGAATGATCAATATGGTTGGTTTGGCTGTCAGATTGAACATAAATTTTC  
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SEQ ID No. 5 - *CsRDR1\_II* modified with indel (CAGGT - bold)  
>*Cucumis sativus*\_14138 modified\_genomic\_sequence

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AAGAATTTTGCCATATTTGCAAAATTATAAATCAATGTGCTATATTTGTGATAACATGTTCTCAAAATGCTAC  
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TAATTTAATATATTTCTTTTCGAAAACCTAGAAAGCCAAATTAAGGACCTTATGGAATTATATCTCTTCATTCTCCAT  
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GAGAACATTTGGCAGGTTGAGTTACACAAGCCACATGGTCAATCTGTAGATTATCTTCTGATTCAAGTTTCATC

Fig. 1-6

CATTAACTTTGAACAATGTCATGTCATTAGTGTACTGTTGTTATTTCTCCTCACTATTGAGAAATATCATTGA  
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GACATGTTTGTACTGTTAGTTATTTGGTGTCTCCACGGATTTATGAAAGAGATGCCAAGCTCTTTGGACTCATT  
ACTGAAGACCTTTCTTAACTTTTCCACGGAAATGACACCCAATGGTTTCGAGCAACTGATTTTACTCCAT  
CATGTAGTATTGGACAATCTGCTGCTTTATGCTTGGAGATTCCCTACGGTCGCCAGCTCCCTAATTTTTCATGA  
TAAATTTGCTTACTTCAAAGAAATCAAGGGTAAATTTACATTGGTCAGTGGTTCTACTTATTCCTCAATGTA  
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CAATACTGCCGTATAGATCATGCGTTAAAGAACTTCTCCACTTGAAAGAGTGTGCTATAACCTTCAAAA  
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CAATGGATGGGAGATTTTCATAATATACGAAATGTAGCCAAGTATGCTGCTAGACTAGGCCAATCTTTGGTT  
CATCAACAGAACTTTAAGTGTCACTAGACGTGAAGTTAAAGTTATTCCTGATATTGAAGTTGAATCAGGTAG  
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CGCCGGAGATGGCTCGCAAGATTATGACCTGACATGGAAGTTGAAGGTTTCGAGGAGTACCTCGACGATGC  
AATATATCAAGAACAACCTATGACATGAGGTTGGGAAATTTGATGCACTATCATAAGATCAAACCTGAGGCG  
GAATTGATCAGTGGTGGTAGTTTGACGTATCATTATCTTTCAACATGAAAAATGAAGCGGAATCGATTATCT

Fig. 1-7

TGGCTGTGAAGTCGCTGCGAAAGGAGGCGAGGGGCTGGTTCAATGAGAAAGCAGACTTACATTATGGACATCA  
TACTAATGTGTATGCAAGAGCTTCAGCATGGTATTTTGTACATATCATCACACCTACTGGGGCTGGTCTGAT  
GGCAGAAAGAATCATGGCCATTTTCTTAGCTTTCATGGTGTGTTTATGATAAACTCATCCGTATCAAGCACC  
GCAAAATTAATTGTAGAGCTCGCTATTGA

Fig. 2-1

Protein sequences SEQ ID Nos. 2 and 4

SEQ ID No. 2 - CsRDR1\_II

>Cucumis sativus\_cs9930v2\_emv\_14138\_protein sequence

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VLRZENIWQVELHKKPHGQSVDYLLIQLFGAPRIYERDARSFGLITEDPFLNFSTEIDTQWFRATDFTPSCSIG  
QSAALCLEIPYGRQLPNFHDKEAYFKEIKGKFTLVSGSTYSSNVNLVPVVTTPRTINLPYTLFKINLLVQQG  
CLPGPALDISFYQMVDSDIYNTAVIDHALKLLHLKECCYNPSKWLDDEYRKYFKLKNPPQPPILTLNEGLVY  
VHRVQVTPCKVYFCGPEVNISNRVLRYPDYIDNFLRVSFVDEELGKMYSTELSPRASSSLEDCKTKIFKRIL  
SVLRDGITIGDKKFEFLAYSSSQLRENAAMWFAPKNELTAAKIRQWMDPHNIRNVAKYAARLGQSFGSSTET  
LSVSRREVKVIPDIEVESGSGVNVVFSGIGKIAASFARKVAKKCGIRHTPSAFQIRYAGFKGVISVDFTSSV  
KLSLRNSMLKYESTDTKLDVLSWSKYHPCFLNRQLITLLSTLGVCQHVFSKQQLIDELDTIFSDPLKAQQA  
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PNECSGSDLDGDIYFVCWDPDLIPQQVEPMDYTPVPSQVLDHVDVMEEVQSYFANYMVNDLSGIIANAHTAF  
ADKEPKKAMSNCIQLAKLFSIAVDFPKTVGPALIPANLRVKEYPDFMDKADKVTESENVLGKLFRLDSIG  
FNIKIRSFNYTPEMARQDYDPDMEVEGFEEYLLDAIYHKNYDMRLGNLMHYHKIKTEAELISGGSLSLSSLS  
FTMKNEAESIIILAVKSLRKEARGWPFNEKADLHYGHHTNVYARASAWYFVITYHHTYWGWSGDRKNHGHFLSFPW  
CVYDKLIRIKHRKINCRARY

SEQ ID No. 4 - CsRDR1\_I

>Cucumis sativus\_cs9930v2\_emv\_14137\_protein sequence

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GQSSSLCLKLPNGRQLPFFKQNFAYYEEFEHEFLIDEDANFSFCRDLAPIVDSRSHVLPYKILFKINALVQY  
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VRRVQITPCKVFFCGPEVNVSNRVLRFHSQYIDNFLRVSFVDEEWDKMRSTDLLPRMSSKSEDEGKTDIYRIL  
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3A

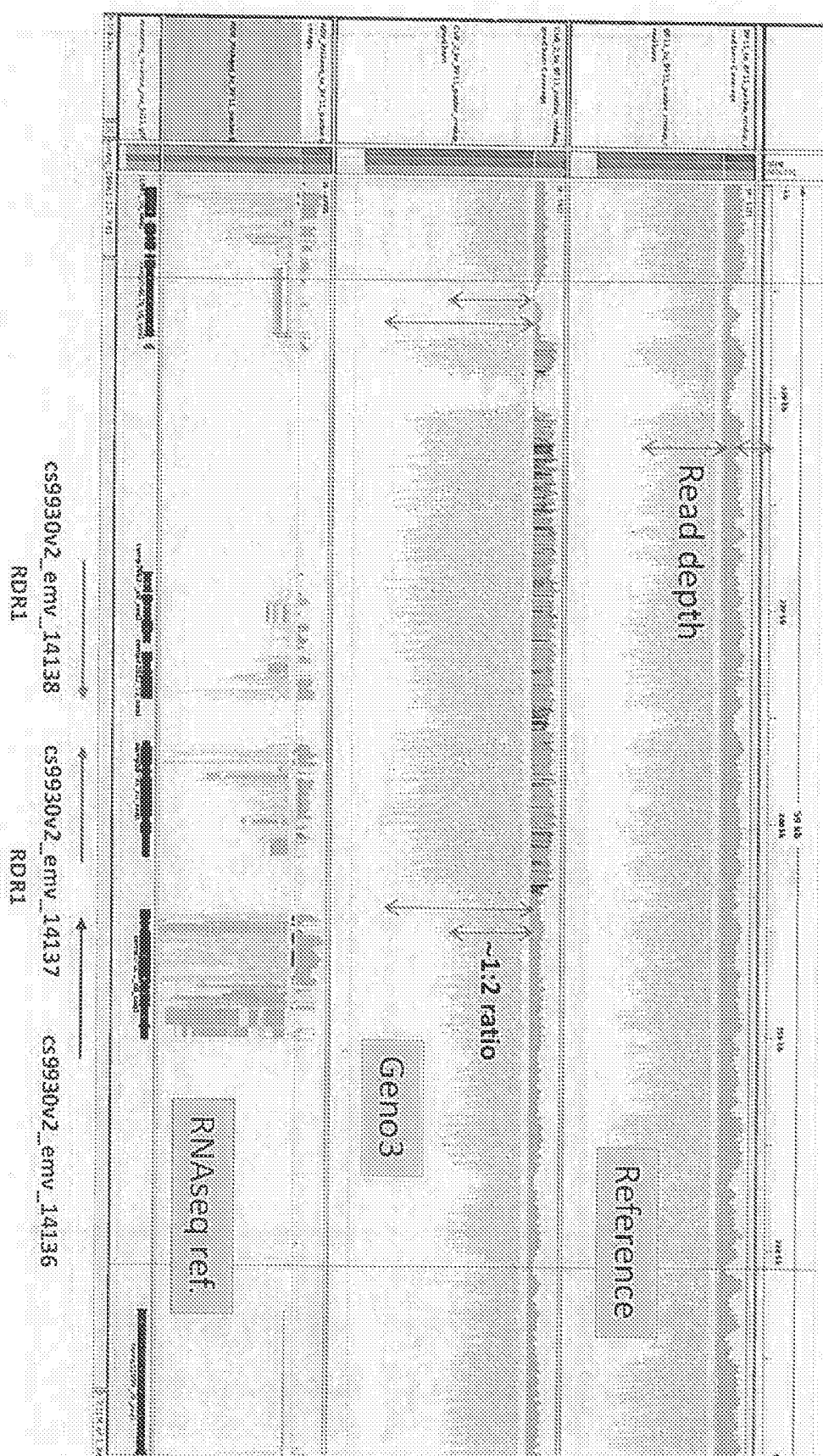
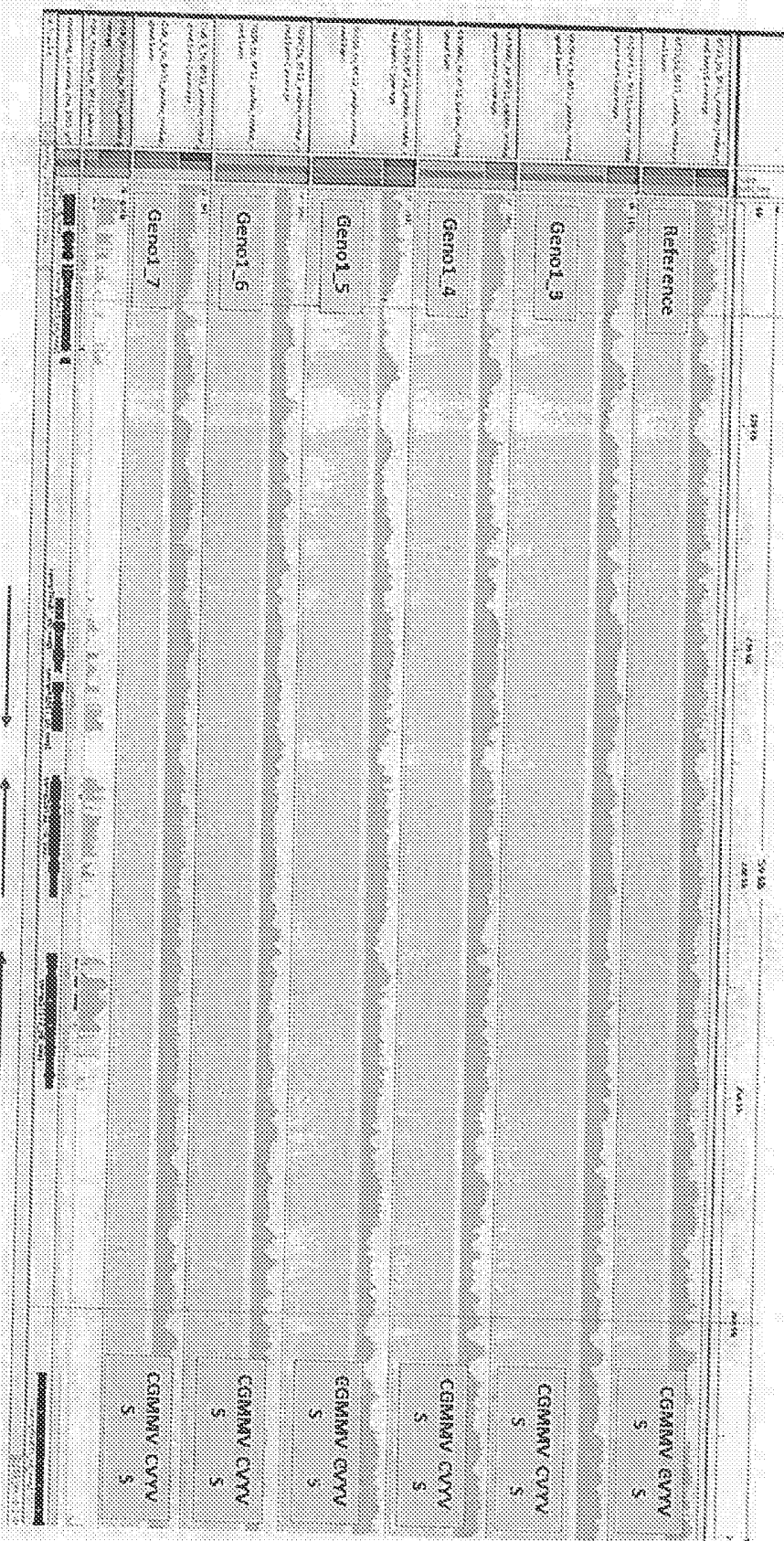


Fig. 3B



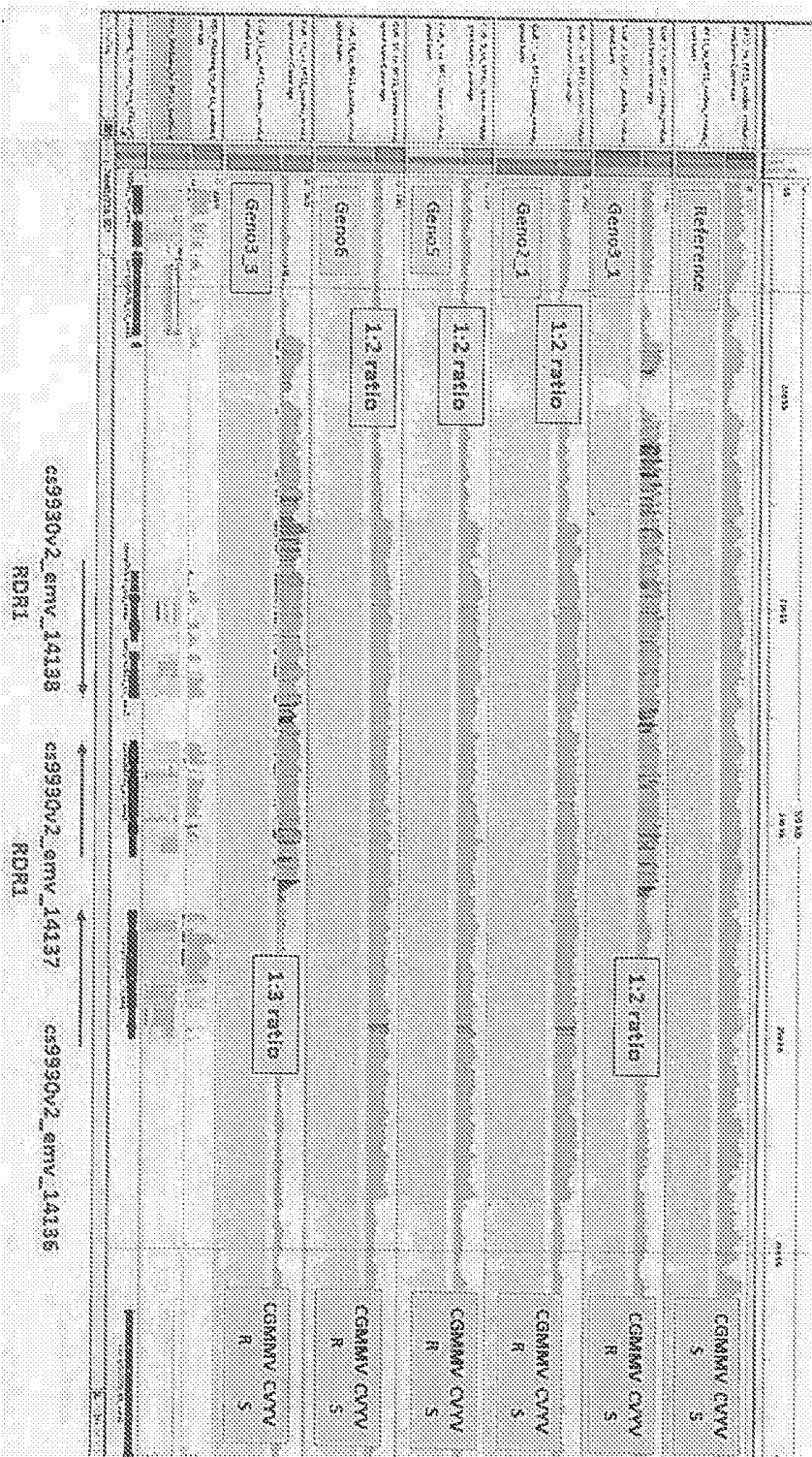


Fig. 3D

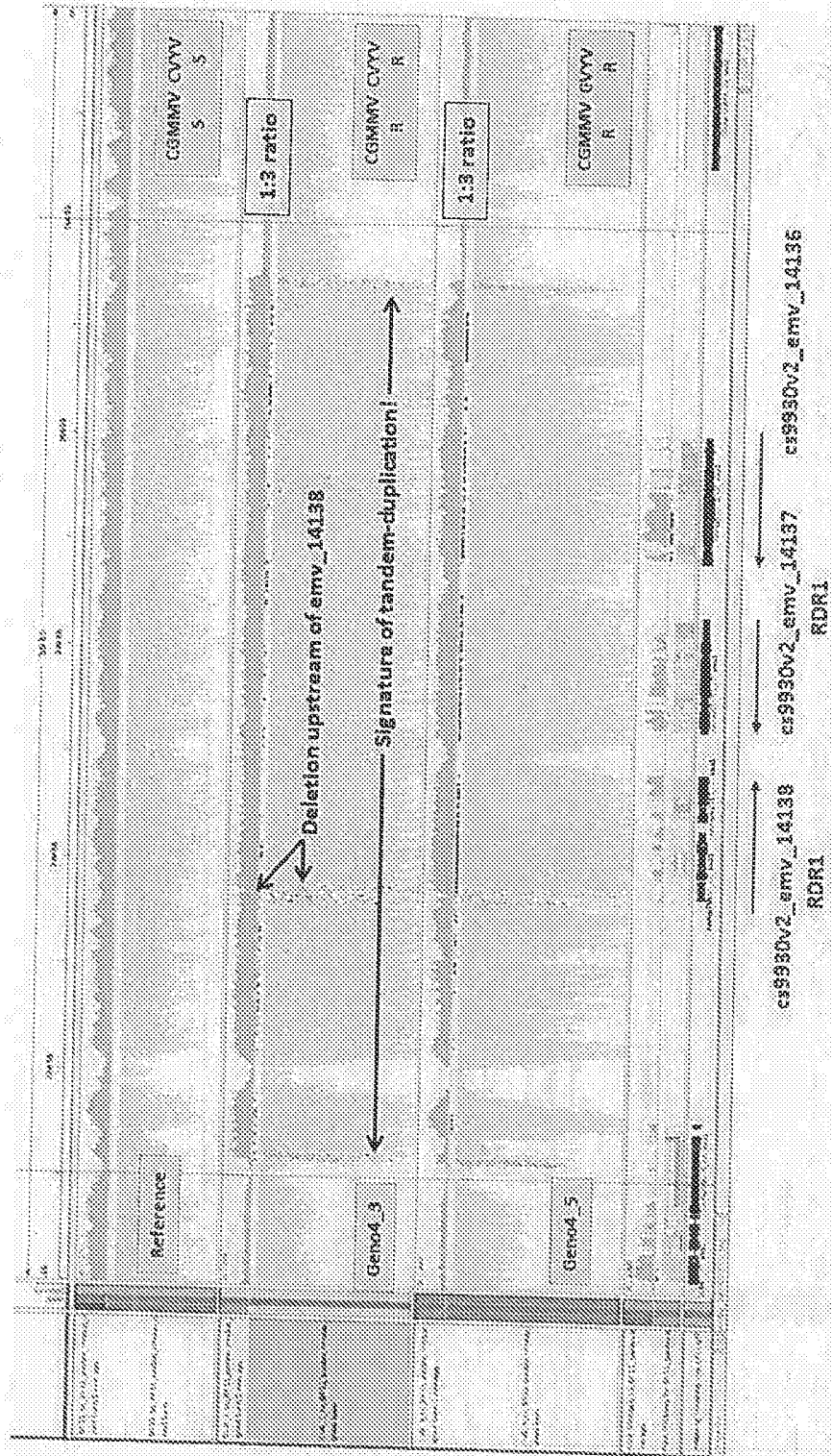
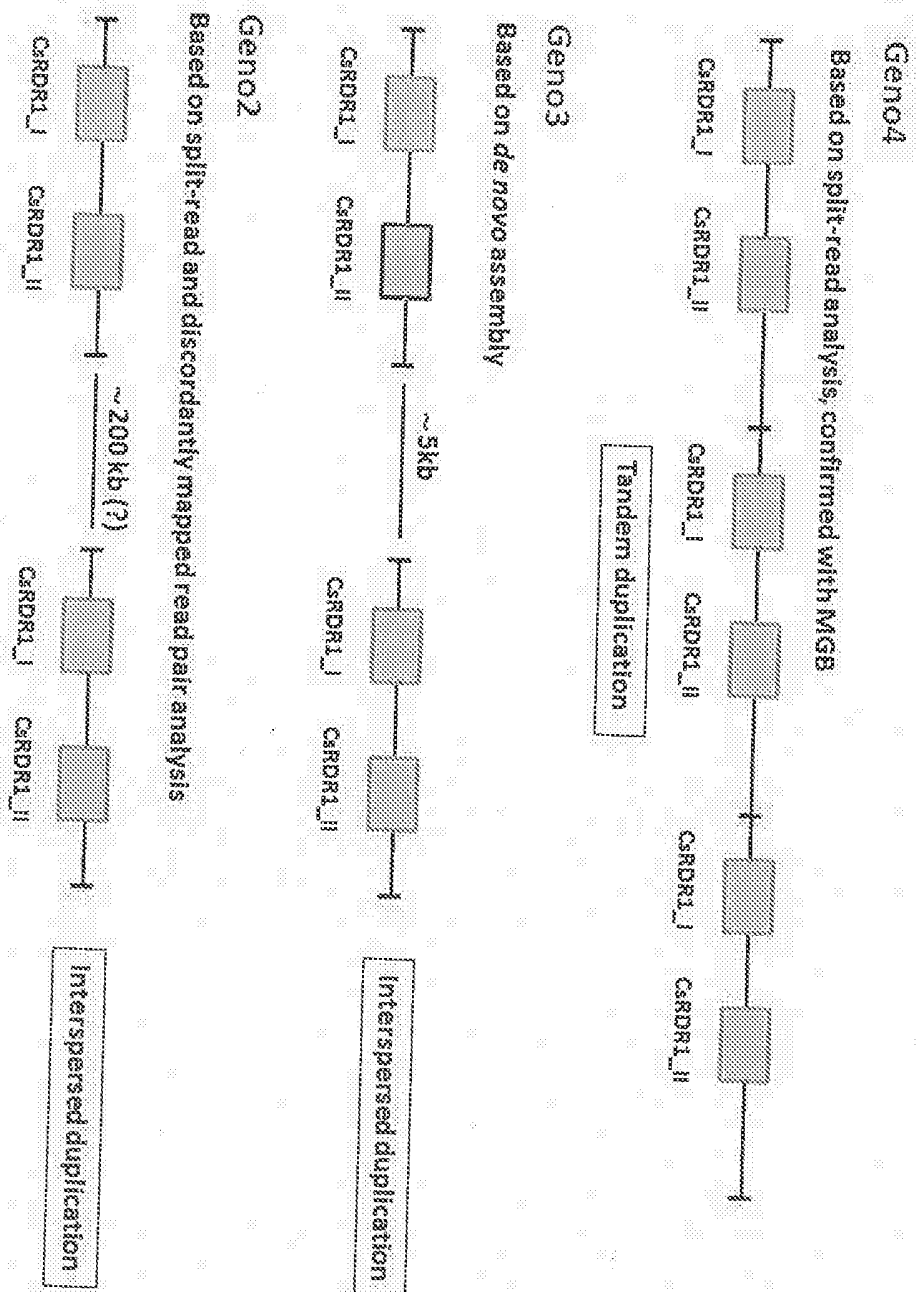


Fig. 4



**ABSTRACT**

The present invention relates to a genetic determinant comprising at least two copies of a combination of two closely linked *RDR1* genes, which two closely linked *RDR1* genes are inversely oriented, and which genetic determinant leads to virus resistance when present in a plant. In one embodiment, of the *RDR1* genes in the combination is represented by **SEQ ID No. 1** or has a sequence identity of at least 70% thereto, and one of the *RDR1* genes in the combination is represented by **SEQ ID No. 3** or has a sequence identity of at least 70% thereto; or one of the *RDR1* genes in the combination encodes a protein represented by **SEQ ID No. 2** or a protein that has a sequence identity of at least 70% thereto, and one of the *RDR1* genes encodes a protein represented by **SEQ ID No. 4** or a protein that has a sequence identity of at least 70% thereto.

## SAMENWERKINGSVERDRAG (PCT)

### RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

|                                                                                                                                                                                                                    |                                                                                                                                                |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| IDENTIFICATIE VAN DE NATIONALE AANVRAGE                                                                                                                                                                            | KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE<br><br><b>L/2SF16/TG/377</b>                                                                    |
| Nederlands aanvraag nr.<br><br><b>2015547</b>                                                                                                                                                                      | Indieningsdatum<br><br><b>02-10-2015</b>                                                                                                       |
|                                                                                                                                                                                                                    | Ingeroepen voorrangsdatum                                                                                                                      |
| Aanvrager (Naam)<br><br><b>Rijk Zwaan Zaadteelt en Zaadhandel B.V.</b>                                                                                                                                             |                                                                                                                                                |
| Datum van het verzoek voor een onderzoek van internationaal type<br><br><b>23-04-2016</b>                                                                                                                          | Door de instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr.<br><br><b>SN66249</b> |
| I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)<br>Volgens de internationale classificatie (IPC)<br><br><b>A01H5/08;C12N9/12;C12Q1/68</b> |                                                                                                                                                |
| II. ONDERZOChte GEBIEDEN VAN DE TECHNIEK<br>Onderzochte minimumdocumentatie                                                                                                                                        |                                                                                                                                                |
| Classificatiesysteem                                                                                                                                                                                               | Classificatiesymbolen                                                                                                                          |
| <b>IPC</b>                                                                                                                                                                                                         | <b>A01H;C12N;C12Q</b>                                                                                                                          |
| Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen<br><br><div style="height: 40px;"></div>                                   |                                                                                                                                                |
| III.                                                                                                                                                                                                               | <input type="checkbox"/> GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES (opmerkingen op aanvullingsblad)                                     |
| IV.                                                                                                                                                                                                                | <input type="checkbox"/> GEBREK AAN EENHEID VAN UITVINDING (opmerkingen op aanvullingsblad)                                                    |

**ONDERZOEKSRAPPORT BETREFFENDE HET  
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND  
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar  
de stand van de techniek

NL 2015547

**A. CLASSIFICATIE VAN HET ONDERWERP**

INV. A01H5/08 C12N9/12 C12Q1/68  
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

**B. ONDERZOCHE GEBIEDEN VAN DE TECHNIEK**

Onderzochte minimum documentatie (classificatie gevolgd door classificatiesymbolen)

A01H C12N C12Q

Onderzochte andere documentatie dan de minimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)

EPO-Internal, WPI Data, Sequence Search, BIOSIS

**C. VAN BELANG GEACHTE DOCUMENTEN**

| Categorie * | Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages                                                                                                                                                                                                                                                                   | Van belang voor<br>conclusie nr. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| X           | WO 2011/003440 A1 (ENZA ZADEN BEHEER BV<br>[NL]; MAZEREEUW JAAP [NL]; VAN KAMPEN<br>BRIGIT [NL]) 13 januari 2011 (2011-01-13)<br>* voorbeelden 1,3 *                                                                                                                                                                                                      | 16-21                            |
| X           | PICO B ET AL: "Screening Cucumis sativus<br>landraces for resistance to cucumber vein<br>yellowing virus",<br>PLANT BREEDING, PAUL PAREY SCIENTIFIC<br>PUBL., BERLIN, DE,<br>deel 122, nr. 5,<br>1 oktober 2003 (2003-10-01), bladzijden<br>426-430, XP002572460,<br>ISSN: 0179-9541, DOI:<br>10.1046/J.1439-0523.2003.00882.X<br>* het gehele document * | 16-21                            |



Verdere documenten worden vermeld in het vervolg van vak C.



Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

**\* Speciale categorieën van aangehaalde documenten**

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvraag vermeld

"E" eerdere octrooi(aanvraag), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwaarlijk is voor de octrooiaanvraag, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"Z" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid

26 juli 2016

Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type

Naam en adres van de instantie

European Patent Office, P.B. 5818 Patentlaan 2  
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Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

De bevoegde ambtenaar

Blanco Urgoiti, B

**ONDERZOEKSRAPPORT BETREFFENDE HET  
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND  
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar  
de stand van de techniek

NL 2015547

**C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN**

| Categorie * | Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Van belang voor<br>conclusie nr. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| X           | <p>D. Leibman, D. Wolf, S. Haviv, M. Brumin,<br/>V. Gaba, A. Gal-On: "The role of Cucumis<br/>spp RNA-dependent RNA polymerase genes in<br/>antiviral defense",<br/>XVI international conference on<br/>plant-microbe interactions 2014. Book of<br/>abstracts.</p> <p>6 juli 2014 (2014-07-06), XP002760250,<br/>Gevonden op het Internet:<br/>URL:<a href="http://www.mpmi2014rhodes-hellas.gr/bo&lt;br/&gt;okOfAbstracts/Posters/P414.pdf">http://www.mpmi2014rhodes-hellas.gr/bo<br/>okOfAbstracts/Posters/P414.pdf</a><br/>[gevonden op 2016-07-25]<br/>* samenvatting *</p> | 1-21                             |
| A           | <p>-----</p> <p>DATABASE EMBL [Online]</p> <p>31 januari 2011 (2011-01-31),<br/>"Cucumis sativus RNA-dependent RNA<br/>polymerase 1a mRNA, complete cds.",<br/>XP002760251,<br/>gevonden in EBI accession no.<br/>EM STD:HQ738485<br/>Database accession no. HQ738485<br/>* sequentie *</p>                                                                                                                                                                                                                                                                                       | 1-21                             |
| A           | <p>-----</p> <p>DATABASE EMBL [Online]</p> <p>31 januari 2011 (2011-01-31),<br/>"Cucumis sativus RNA-dependent RNA<br/>polymerase 1b mRNA, complete cds.",<br/>XP002760252,<br/>gevonden in EBI accession no.<br/>EM STD:HQ738486<br/>Database accession no. HQ738486<br/>* sequentie *</p> <p>-----</p>                                                                                                                                                                                                                                                                          | 1-21                             |

**ONDERZOEKSRAPPORT BETREFFENDE HET  
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND  
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar  
de stand van de techniek

NL 2015547

| In het rapport<br>genoemd octrooigescrift | Datum van<br>publicatie | Overeenkomend(e)<br>geschrift(en) | Datum van<br>publicatie |
|-------------------------------------------|-------------------------|-----------------------------------|-------------------------|
| WO 2011003440                             | A1                      | 13-01-2011                        |                         |
|                                           |                         | AU 2009349358 A1                  | 09-02-2012              |
|                                           |                         | CA 2766190 A1                     | 13-01-2011              |
|                                           |                         | CN 102770018 A                    | 07-11-2012              |
|                                           |                         | EP 2453730 A1                     | 23-05-2012              |
|                                           |                         | JP 2013505002 A                   | 14-02-2013              |
|                                           |                         | US 2012137388 A1                  | 31-05-2012              |
|                                           |                         | WO 2011003440 A1                  | 13-01-2011              |
| -----                                     |                         |                                   |                         |

## WRITTEN OPINION

|                                                                              |                                            |                                |                              |
|------------------------------------------------------------------------------|--------------------------------------------|--------------------------------|------------------------------|
| File No.<br>SN66249                                                          | Filing date (day/month/year)<br>02.10.2015 | Priority date (day/month/year) | Application No.<br>NL2015547 |
| International Patent Classification (IPC)<br>INV. A01H5/08 C12N9/12 C12Q1/68 |                                            |                                |                              |
| Applicant<br>Rijk Zwaan Zaadteelt en Zaadhandel B.V.                         |                                            |                                |                              |

This opinion contains indications relating to the following items:

- ☒ Box No. I      Basis of the opinion
- ☐ Box No. II      Priority
- ☐ Box No. III      Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV      Lack of unity of invention
- ☒ Box No. V      Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI      Certain documents cited
- ☐ Box No. VII      Certain defects in the application
- ☒ Box No. VIII      Certain observations on the application

|  |                               |
|--|-------------------------------|
|  | Examiner<br>Blanco Urgoiti, B |
|--|-------------------------------|

## WRITTEN OPINION

Application number  
NL2015547

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### Box No. I Basis of this opinion

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1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:
  - a. type of material:
    - ☒ a sequence listing
    - ☐ table(s) related to the sequence listing
  - b. format of material:
    - ☐ on paper
    - ☒ in electronic form
  - c. time of filing/furnishing:
    - ☒ contained in the application as filed.
    - ☐ filed together with the application in electronic form.
    - ☐ furnished subsequently for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

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### Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

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#### 1. Statement

|                          |             |       |
|--------------------------|-------------|-------|
| Novelty                  | Yes: Claims | 1-15  |
|                          | No: Claims  | 16-21 |
| Inventive step           | Yes: Claims |       |
|                          | No: Claims  | 1-21  |
| Industrial applicability | Yes: Claims | 1-21  |
|                          | No: Claims  |       |

#### 2. Citations and explanations

**see separate sheet**

## WRITTEN OPINION

Application number  
NL2015547

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**Box No. VIII    Certain observations on the application**

---

**see separate sheet**

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1 Reference is made to the following documents:

- D1 WO 2011/003440 A1 (ENZA ZADEN BEHEER BV [NL]; MAZEREEUW JAAP [NL]; VAN KAMPEN BRIGIT [NL]) 13 January 2011 (2011-01-13)
- D2 PICO B ET AL: "Screening Cucumis sativus landraces for resistance to cucumber vein yellowing virus",  
PLANT BREEDING, PAUL PAREY SCIENTIFIC PUBL., BERLIN, DE,  
vol. 122, no. 5, 1 October 2003 (2003-10-01), pages 426-430,  
XP002572460,  
ISSN: 0179-9541, DOI: 10.1046/J.1439-0523.2003.00882.X
- D3 D. Leibman, D. Wolf, S. Haviv, M. Brumin, V. Gaba, A. Gal-On: "The role of Cucumis spp RNA-dependent RNA polymerase genes in antiviral defense",  
XVI international conference on plant-microbe interactions 2014. Book of abstracts.  
, 6 July 2014 (2014-07-06),  
Retrieved from the Internet:  
URL:<http://www.mpmi2014rhodes-hellas.gr/bookOfAbstracts/Posters/P414.pdf>  
[retrieved on 2016-07-25]
- D4 DATABASE EMBL [Online] 31 January 2011 (2011-01-31),  
"Cucumis sativus RNA-dependent RNA polymerase 1a mRNA, complete cds.",  
retrieved from EBI accession no. EM\_STD:HQ738485  
Database accession no. HQ738485
- D5 DATABASE EMBL [Online] 31 January 2011 (2011-01-31),  
"Cucumis sativus RNA-dependent RNA polymerase 1b mRNA, complete cds.",  
retrieved from EBI accession no. EM\_STD:HQ738486  
Database accession no. HQ738486

2 NOVELTY.

- 2.1 D1 and D2 disclose cucumber plants resistant to CVYV or to both CVYV and CGMMV. The plants of the present application relate to known CVYV and/or CGMMV virus resistant cucumber lines found to comprise RDR1I and II located closely linked, inversely oriented. Novelty of claims 16-21 cannot be therefore acknowledged in view of any of D1 or D2.
- 2.2 D3 teaches that RDR1a, RDR1b and RDR1c are identified in cucumber. Virus susceptibility/resistance can be associated with RDR1 level. Transgenic plants resistant to virus show an increased expression of RDR1b. Infected plants show an increased expression of RDR1c. Ectopic application of salicylic acid induced an increased expression of all three.
- 2.3 D4 discloses the polypeptide sequence of RDR1a from cucumber being identical over the whole length to SEQ ID NO: 4 of the present application. The cDNA sequence of RDR1a is identical over a stretch of 1890 nts to SEQ ID NO:3 of the present application.
- 2.4 D5 discloses the polypeptide sequence of RDR1b from cucumber being identical over the whole length to SEQ ID NO: 2 of the present application. The cDNA sequence of RDR1a is identical over a stretch of 1888 nts to SEQ ID NO:1 and 5 of the present application.
- 2.5 In view of the prior art the subject-matter of claims 1 to 15 is novel.
- 3 INVENTIVE STEP.
- 3.1 D3 can be considered the closest prior art for the subject-matter of claim 1. The difference lies in the combination of RDR1 variants and genomic organization associated to virus resistant cucumber plants.
- 3.2 The problem to be solved can be seen as the provision of a further combination of RDR1 variants associated to virus resistance in cucumber.
- 3.3 The solution as claimed is the provision of a genetic determinant comprising at least two copies of the combination of two closely linked RDR1 genes, inversely oriented.
- 3.4 However, the problem is not solved over the whole scope claimed contrary to the requirements of inventive step. Only resistance to cucumber green mottle mosaic virus (CGMMV) seems to be correlated to having at least two copies of of two closely linked RDR1 genes as claimed. The resistance to cucumber vein yellowing virus (CVYV) seems to be linked to the presence of the modified RDR1\_II of SEQ ID NO:5 independently of the copy number (see table 1, p. 12 and I.16 -19). Moreover, when the copies are located far from

each other in the genome, the plants are not resistant to CGMMV (see line "Geno2\_1" in table 1 and p.11, l.35-p.12, l.2). The same is true for dependent claims 9 to 10, and for the methods of claims 11 to 19.

**4 INDUSTRIAL APPLICABILITY**

- 4.1 The subject-matter of claims 1-25 seems to be susceptible of Industrial Applicability.

**Re Item VIII**

**5 DISCLOSURE, SUPPORT AND CLARITY**

- 5.1 The subject-matter of claim 11 lacks support in the description and sufficiency of disclosure. The application concerns the correlation of existing resistant or susceptible plants to CGMMV and/or CVYV and the location of genomic variants of RDR1 gene. The application does not disclose that a susceptible plant can result in resistance upon the introduction of the genetic determinant as defined in the claims, and it would be considered an undue burden and a new line of research to do so.