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(54) Title: RESISTANCE TO POWDERY MILDEW AND ABSENCE OF NECROSIS IN CUCUMIS SATIVUS

(57) Abrégé/Abstract:
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(57) Abstract: The present invention relates to a powdery mildew-resistant Cucumis sativus plant, comprising in its genome a necrosis-suppressing genetic factor, which plant is both resistant to powdery mildew and is necrosis-free. The invention further relates to a method for obtaining a powdery mildew-resistant and necrosis-free Cucumis sativus plant, comprising of introducing a necrosis-suppressing genetic factor into the genome of a powdery mildew-resistant.

RESISTANCE TO POWDERY MILDEW AND ABSENCE OF NECROSIS IN CUCUMIS SATIVUS

The present invention relates to powdery mildew-resistant Cucumis sativus plants which are necrosis-free. In addition, the invention relates to a method for obtaining powdery mildew-resistant cucumber plants which are necrosis-free.

The cucumber plant (i.e. a plant of the botanical species Cucumis sativus) belongs to the gourd family of Cucurbitaceae, like melons and squash. The cucumbers are the edible fruits of the plant, which are cylindrical, green-skinned fruits, consisting of about 96% water. The cucumber plant, which has been cultivated since long, is an important horticultural crop worldwide. Cucumbers are commonly harvested in an unripe stadium and may be used for the pickling industry or the fresh market.

Powdery mildew is one of the main fungal diseases known in cucumber plants, both in the field and greenhouse. Powdery mildew can be caused by Sphaerotheca fuliginea (Schlecht. ex Fr.)(recently renamed: Podosphaera xanthii) and/or Erysiphe cichoracearum DC (ex Mérat emend. Salm)(recently renamed: Golovinomyces cichoracearum). In greenhouse cultivation powdery mildew is predominantly caused by the first species. The fungus occurs mainly on leaves, which are most susceptible 2 to 3 weeks after unfolding. However, in severely affected plants the fungus may also occur on the stem and even the fruits. Severely affected leaves can become dry and brittle, or can wither and die. Because of the infection, the fruits can be smaller in size, fewer in number, less able to be successfully stored, sun scalded, incompletely ripe, and have a poor flavour. It may also predispose plants to be more vulnerable to other pathogens. Eventually, the plant can die.

Until now, fungicide application and the use of varieties with some resistance to the fungus have been the major methods of disease control. Thus, a resistance against both fungi has been demonstrated in various commercial cultivars. It has been demonstrated that hypocotyl resistance is based on a recessive gene (s), while leaf resistance is controlled by the dominant leaf gene (R). Both genes are necessary for a high-level resistance at the whole plant level (Shanmugasundaram, et al., Phytopathology 61: 1218-1221, 1971).

Powdery mildew (PM)-resistant cultivars, however, generally suffer from necrosis under low-light conditions (i.e. conditions wherein the light exposure of the plants is such that less than 2000 J/cm^2 of energy is received by the plant = less than 286 J/cm^2 per day), in particular in combination with a high fruit load, i.e. at least one fully developed fruit in a harvestable stage per node. Such conditions often occur during autumn, winter and early spring, in particular in production areas in Northern European countries and Canada. The fact that resistance against powdery mildew is associated with necrosis of the plants severely limits the practical use of these powdery mildew resistant plants.

The symptoms of necrosis related to powdery mildew resistance in cucumber begin with a yellowing between the main veins of the leaves (chlorosis), eventually resulting in necrosis (i.e. death of the leaves). A positive correlation between mildew resistance and necrosis sensitivity has been demonstrated, which has led to the suggestion that both traits are genetically tightly linked or that necrosis is a pleiotropic effect of one or more of the resistance genes.

In EP 1 433 378 a breaking of the genetic linkage between powdery mildew resistance and leaf necrosis in one

Cucumis sativus line (DC-1) has been described. However, the genetic control of the powdery mildew resistance related necrosis phenomenon has not yet been elucidated, and many cucumber producers still suffer from the occurrence of necrosis in powdery mildew resistant cucumber cultivars. As a consequence, cucumber production still involves the use of fungicides for crop protection to control the infection with powdery mildew, which not only increases the costs involved but also is undesirable in view of a healthy environment.

In order to reduce the use of fungicides it thus is essential to provide plants, or to find new methods for providing plants, that are both resistant to powdery mildew and are necrosis-free.

The object of the invention is to provide _Cucumis sativus_ plants which both are resistant to powdery mildew infection and are necrosis-free.

This is achieved by the present invention by providing a powdery mildew-resistant _Cucumis sativus_ plant, comprising in its genome a necrosis-suppressing genetic factor, which plant is resistant to powdery mildew and is necrosis-free. The plant of the invention thus is resistant to powdery mildew and shows no symptoms of leaf necrosis under low-light conditions (i.e. conditions wherein the light exposure of the plants is such that less than 2000 J/cm^2 of energy is received by the plant = less than 286 J/cm^2 per day), in particular in combination with a high fruit load.

According to the present invention, a novel necrosis-suppressing genetic factor has been identified. In addition, suitable molecular markers have been developed which can be used to identify and provide _Cucumis sativus_ plants which both are resistant to powdery mildew and are necrosis-free. This novel genetic factor has been found to suppress the powdery mildew-related necrosis. This necrotic suppressing

genetic factor is a semi-dominant genetic factor, i.e. both when present in heterozygous and homozygous form, the phenotype will be "necrosis-free".

As demonstrated according to the invention (shown below), the cucumber plant described in EP 1 433 378 does not comprise the necrosis suppressing genetic factor.

In a preferred embodiment of the invention, the plant comprises the known hypocotyl resistance gene (s) and the leaf resistance gene (R) conferring a high level of resistance to the powdery mildew pathogen.

The presence of the powdery mildew resistance genes can be determined using specific molecular markers that are specifically linked to these resistance genes. Suitable markers are known in the art and have for example been described in WO 2007/053015. Thus, as disclosed in WO 2007/053015, the presence of the hypocotyl resistance gene (i.e. the genomic region responsible for the powdery mildew resistance referred to as *pm-h* in WO 2007/053015) is indicated by the presence of specific single nucleotide polymorphism (SNP) markers associated with said powdery mildew resistance gene in said plant. The presence of the leaf resistance gene (i.e. the genomic region responsible for the powdery mildew resistance referred to as *pm-l* in WO 2007/053015) is indicated by the presence of a specific single nucleotide polymorphism (SNP) marker, or a specific insertion mutation marker, indicated as the 5-bp insert 5-AATTT-3". Further markers that may be used to detect the presence of the powdery mildew resistance genes are the AFLP markers E16/M50-F-194, E11/M48-F-251, E23/M38-M001, E23/M40-M003, E24/M46-M002, E24/M46-M003, E12/M48-M003, E26/M43-M003, E14/M59-F-134 and E14/M59-F-200, as described in more detail in WO 2007/053015, to which express reference is made in this context.

According to the invention it has been demonstrated that the necrosis suppressing genetic factor is located on another chromosome as compared to the powdery mildew resistance genes: s and R. This was accomplished by mapping the specific markers for the powdery mildew resistance genes and the necrosis suppressing genetic factor, respectively, at the cucumber chromosomal map.

In a preferred embodiment of the invention the necrosis-suppressing genetic factor in the genome of said plant is also linked to one or more DNA markers, and can be determined using one or more of said DNA markers. By using DNA markers, plants with the desired combination of powdery mildew resistance and the necrosis-suppressing genetic factor can easily be identified, without the need for performing space and time-consuming necrosis tests. DNA markers may reveal genetic differences that can be visualized by gel electrophoresis and staining with chemicals (e.g. ethidium bromide) or detection with radio-active probes, which are well-known to the person skilled in the art.

According to a preferred embodiment of the present invention, the necrosis-suppressing genetic factor is linked to and can be identified by one or more of the DNA markers selected from the group consisting of a first DNA-marker of approximately 65 bp, identified by SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and a second DNA-marker of approximately 123 bp, identified by SEQ ID NO: 3 (GACTGCGTACCAATTCAC) and SEQ ID NO: 4 (GATGAGTCCTGAGTAATCG).

According to a preferred embodiment of the present invention, the homozygous presence of the necrosis-suppressing genetic factor in the genome of said plant is identified by the absence of at least one of said DNA markers. Preferably, the homozygous presence of said

necrosis-suppressing genetic factor in the genome of said plant is identified by the absence of both the first DNA-marker and the second DNA-marker.

In the research that led to the invention, it has been demonstrated that the absence of said specific molecular marker(s)of the invention in resistant plants is indicative for the necrosis-free fenotype. The molecular markers of the invention thus are a so-called "trans" markers. Homozygous presence of the DNA-fragment (allele) is correlated with the absence of the necrosis suppressing genetic factor and therefore indicative for the non-desired necrotic phenotype. Absence of this DNA-marker thus is indicative for the homozygous presence of the necrosis-suppressing genetic factor, i.e. when the DNA-marker(s) is/are absent, this means that the necrosis-suppressing genetic factor is homozygously present in the genome of the plant.

According to another preferred embodiment of the invention, the heterozygous presence of the necrosis-suppressing genetic factor is identified by the heterozygous presence of the DNA-marker(s). It has been found that the necrotic suppressing genetic factor is a semi-dominant genetic factor, i.e. both when present in homozygous and heterozygous form, the phenotype will be "necrosis-free". Accordingly, the heterozygous presence of the DNA marker(s) according to the invention is indicative for the heterozygous presence of the necrosis-suppressing genetic factor in the plant. Heterozygous presence of the DNA-marker(s) can e.g. be determined using suitable software, such as the AFLP-Quantar®*Pro* developed by Keygene (Wageningen, The Netherlands).

In a particularly preferred embodiment, the plant comprises a necrosis suppressing genetic factor derived from the Cucumis sativus plant, seeds of which have been deposited

on 14 February 2006 at the American type culture collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, United States of America under deposit number PTA-7394.

The present invention further relates to the seeds and/or other plant parts of the plants as described above. Plant parts according to the invention are for instance plant cells, pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, stems, seeds, protoplasts and calli derived from the plant.

In a preferred embodiment, the invention relates to cucumber fruits derived from the plant as described above.

The present invention furthermore relates to a method for obtaining a powdery-mildew resistant Cucumis sativus plant, which is necrosis-free, comprising of introducing a necrosis-suppressing genetic factor into the genome of a powdery mildew-resistant plant.

According to the invention, the powdery mildew resistance genes and the necrosis suppressing genetic factor can be introduced in the genome of the plant using well-known techniques, like classical breeding techniques and/or molecular biological techniques.

According to a preferred embodiment of said method, the powdery mildew resistance genes comprise the known hypocotyl resistance gene (s) and the leaf resistance gene (R). As indicated above, the presence of the powdery mildew resistance genes can be determined using specific markers that are specifically linked to these resistance genes. Suitable markers are known in the art and have for example been described above. In a preferred embodiment, the presence of the necrosis suppressing genetic factor is determined using one or more specific DNA markers. The present invention thus provides a simple and reliable method which ensures that

the plants of interest can be identified without the need to perform any disease resistance and/or necrosis tests.

Preferably, the DNA markers for identifying the necrosis-suppressing genetic factor are selected from the group consisting of a first DNA-marker of approximately 65 bp, identified by SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and a second DNA-marker of approximately 123 bp, identified by SEQ ID NO: 3 (GACTGCGTACCAATTCAC) and SEQ ID NO: 4 (GATGAGTCCTGAGTAATCG).

In a preferred embodiment, the homozygous presence of the necrosis-suppressing genetic factor is identified by the absence of at least one of said DNA markers. Preferably, the homozygous presence of the necrosis-suppressing genetic factor is identified by the absence of both the first and second DNA markers.

According to another preferred embodiment of the invention, the heterozygous presence of the necrosis-suppressing genetic factor is identified by the heterozygous presence of the DNA-marker(s).

In a particular preferred embodiment, the necrosis suppressing genetic factor is derived from the Cucumis sativus plant of which seeds have been deposited with the ATCC under no. PTA-7394.

The invention further relates to a powdery mildew-resistant Cucumis sativus plant, obtainable by the method as described above, which plant is necrosis-free, as well as to the seeds, and/or other plant parts and fruits of said plant.

In addition, the present invention relates to a method for the identification of necrosis tolerance in a Cucumis sativus plant, comprising detecting the presence of a necrosis-suppressing genetic factor in the genome of said plant using one or more DNA markers, wherein the DNA markers are selected from the group consisting of a first DNA-marker

of approximately 65 bp, identified by SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and a second DNA-marker of approximately 123 bp, identified by SEQ ID NO: 3 (GACTGCGTACCAATTCAC) and SEQ ID NO: 4
5 (GATGAGTCCTGAGTAATCG). Using the method of the invention, necrosis-tolerance can easily be detected in Cucumis sativus plants, already in seedlings and/or young plants.

In a preferred embodiment, the homozygous presence of the necrosis-suppressing genetic factor is identified by the
10 absence of at least one of said DNA markers. Preferably, the presence of the necrosis-suppressing genetic factor is identified by the absence of both the first and second DNA markers.

According to another preferred embodiment of the
15 invention, the heterozygous presence of the necrosis-suppressing genetic factor is identified by the heterozygous presence of the DNA-marker(s).

In another aspect, the invention provides a cell of a powdery mildew-resistant *Cucumis sativus* plant, wherein: (a) said plant is resistant to powdery mildew and is necrosis-free under conditions wherein the light exposure of the plant is
5 less than 2000 J/cm^2; (b) said plant homozygously comprises in its genome a necrosis-suppressing genetic factor of a *Cucumis sativus* plant of which seeds have been deposited with the American Type Culture Collection (ATCC) on 14 February 2006 under deposit number PTA-7394; and, (c) the presence of said
10 necrosis-suppressing genetic factor is determinable by the absence of a first DNA-marker of approximately 65 bp, amplifiable by polymerase chain reaction with a first primer comprising SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and a second primer comprising SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and the
15 absence of a second DNA-marker of approximately 123 bp, amplifiable by polymerase chain reaction with a third primer comprising SEQ ID NO: 3 (GACTGCGTACCAATTCAC) and a fourth primer comprising SEQ ID NO: 4 (GATGAGTCCTGAGTAATCG), said first and second DNA markers being present in *Cucumis sativus*
20 plants lacking said necrosis-suppressing genetic factor.

In another aspect, the invention provides a cell of a powdery mildew-resistant *Cucumis sativus* plant, wherein a representative sample of seed of said plant was deposited with the ATCC on February 14, 2006 under deposit number PTA-7394.

25 In another aspect, the invention provides use of the powdery mildew-resistant *Cucumis sativus* plant as defined herein for homozygously introducing a necrosis-suppressing genetic factor into the genome of a second powdery mildew resistant plant, wherein the presence of said necrosis-
30 suppressing genetic factor is determinable by the absence of a

first DNA-marker of approximately 65 bp, amplifiable by polymerase chain reaction with a first primer comprising SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and a second primer comprising SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and the absence
5 of a second DNA-marker of approximately 123 bp, amplifiable by polymerase chain reaction with a third primer comprising SEQ ID NO: 3 (GACTGCGTACCAATTCAC) and a fourth primer comprising SEQ ID NO: 4 (GATGAGTCCTGAGTAATCG), said first and second DNA markers being present in *Cucumis sativus* plants
10 lacking said necrosis-suppressing genetic factor.

In another aspect, the invention provides a method for identification of necrosis tolerance in a *Cucumis sativus* plant, said method comprising detecting the presence of a necrosis-suppressing genetic factor in the genome of said plant
15 using the absence of a first DNA-marker of approximately 65 bp, amplifiable by polymerase chain reaction with a first primer comprising SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and a second primer comprising SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and the absence of a second DNA-marker of approximately 123 bp,
20 amplifiable by a third primer comprising SEQ ID NO: 3 (GACTGCGTACCAATTCAC) and a fourth primer comprising SEQ ID NO: 4 (GATGAGTCCTGAGTAATCG), said first and second DNA markers being present in *Cucumis sativus* plants lacking said necrosis-suppressing genetic factor.

25 Explanation of definitions:

The symptoms of powdery mildew resistance can be classified as follows:

According to the present invention, the level of powdery mildew (PM) resistance can be classified as follows:

level 1 = less than 10% of the surface of first true leaf affected by PM after artificial inoculation, no
5 sporulation, classification: R/resistant;

level 2 = between 10-50% of surface of first true leave affected by PM after artificial inoculation, some sporulation, classification: IR/intermediate resistant;

level 3 = more than 50% of the surface of first true leaf affected by PM after artificial inoculation, sporulation, classification: S/susceptible.

5 According to the present invention, necrosis can be classified as followed:

Level 1: the leaves are green, and the plant is functioning and developing well (classification: necrosis-free).

10 Level 2: yellow spots appear on the leaves, and there is some growth reduction of the leaves (classification: intermediate level of necrosis)

Level 3: yellow green leaves with many yellow spots, very serious growth problems, ultimately resulting in 15 partially or complete dying leaves (necrosis) and sometimes even death of the plant (classification: necrosis).

The wording "low light conditions" relate to i.e. 20 conditions wherein the light exposure of the plants is such that less than 2000 J/cm^2 of energy is received by the plant = less than 286 J/cm^2 per day. Under these conditions, symptoms of necrosis will occur in plants that do not comprise the necrosis-suppressing genetic factor of the 25 invention.

A high fruit load according to the invention relates to a fruit load of at least one fully developed (i.e. in a harvestable stage) fruit per node.

30

The term "necrosis-suppressing genetic factor" as used according to the present invention relates to a DNA fragment determining and transmitting the necrosis-

suppressing property from parent to offspring. It has been found according to the invention that the necrosis-free genetic factor is semi-dominant, i.e. both when present homozygously and heterozygously, the necrosis-free phenotype
5 is observed.

A DNA marker according to the invention refers to a DNA sequence that can be identified by a simple assay, e.g. PCR followed by electrophoresis, allowing the presence or
10 absence of neighbouring stretches of the genome to be inferred. The marker may e.g. be an AFLP marker.

The present invention is further illustrated by the following Example.
15

EXAMPLES

In the research that led to the present invention a novel necrosis-reducing factor has been identified in Cucumis
20 sativus plants.

A segregating population of a powdery mildew hypocotyl and leaf resistant, necrotic Cucumis sativus (Code B, see table 1) X a powdery mildew hypocotyl and leaf resistant, necrosis-free Cucumis sativus (Code A, deposited
25 at 14 February 2006 with the ATCC under number PTA-7394) was produced. AFLP-markers linked to the necrosis-suppressing genetic factor were identified using a Bulked Segregant Analysis (BSA) approach (Michelmore et al., PNAS 88:9828-98232, 1991). Markers linked to the necrosis-suppressing
30 factor could be mapped on a linkage group which is distinct from the linkage group which is harboring the powdery mildew resistance genes.

Validation of the markers linked to the necrosis-suppressing genetic factor was performed by screening these markers on plants of the segregating population and a specific panel of breeding lines, according to well-known molecular biological methods.

The molecular markers described in WO 2007/053015 and identified in table 3, were used to determine the presence/absence of the powdery mildew resistance genes.

Table 1. Necrosis suppressing genetic factor

Marker results with different genotypes

Genotype	PM-resistance level	Necrosis level	Marker 65 bp	Marker 123 bp
Code A	1	1	-	-
Code B	1	3	+ homozygous	+ homozygous
cv Flamingo F1 *	2	2	+ homozygous	+ homozygous

+ = marker is present

- = marker is absent

* = plant according to EP 1 433 378

the scores 1-3 are explained above.

It thus becomes clear that in the plant according to the invention (Code A), both of the DNA markers, that have been identified as being linked to the novel necrosis suppressing genetic factor of the invention, are absent, indicating the presence of the necrosis suppressing genetic factor and thus of the necrosis-free fenotype. In contrast, in the plant indicated by Code B (resistant, necrotic) and in the plant described in EP 1 433 378, referred to above, both of these DNA markers are present, indicating that these

plants do not comprise the necrosis suppressing genetic factor of the present invention.

The presence of the powdery mildew resistance genes of these plants has also been tested using the molecular markers (listed in table 3). The results of both markers analyses have been summarized in table 2.

The results clearly show that the plants identified by Code A and Code B (genotypes A en B) score homozygous for all powdery mildew markers and show the highest level of powdery mildew resistance, whereas cv. Flamingo (i.e. the plant of EP 1 433 378) scores heterozygous for the PM markers identified by SEQ ID NO: 7 and 8 and shows a lower level of powdery mildew resistance.

These marker data thus show the independent segregation behaviour of the powdery mildew resistance markers relative to the necrosis markers. This clearly demonstrates that the powdery mildew resistance and the necrosis suppressing genetic factor are unlinked.

Combined PM/necrosis seedling test protocol:

Plant material

The time to perform the experiment in the Netherlands is from 1 November until 1 February (low light conditions, < 2000 J/cm^2 of energy per week = 286 J/cm^2 per day). Seedlings (test plants and controls) are grown at 24° C in vermiculite covered with sand. The seedlings are transplanted in a ground table after 4 to 5 days (cotyledons just spread). Controls are necrosis-susceptible, PM-resistant plants.

Pathogen

Sphaerotheca fuliginea (Podosphaera xanthii) race 2 multiplied on Kamaron, a commercially available F1 hybrid.

Table 2 Powdery mildew resistance

Marker results

5

Genotype	PM resistance level	Necrosis level	Marker SEQ ID NO: 5	Marker SEQ ID NO: 6	Marker SEQ ID NO: 7	Marker SEQ ID NO: 8	Marker 65 bp	Marker 123 bp
Code A	1	1	+ homozygous	+ homozygous	+ homozygous	+ homozygous	-	-
Code B	1	3	+ homozygous	+ homozygous	+ homozygous	+ homozygous	+ homozygous	+ homozygous
cv. Flamingo	2	2	+ homozygous	+ homozygous	+ *heterozygous*	+ *heterozygous*	+ homozygous	+ homozygous

10

Preparation of inoculum

Well sporulating leaves are taken and the spores rubbed off into water; the inoculum is sieved by pouring the inoculum through a funnel covered with thoroughly wetted cheesecloth.

The viability of the spores is checked by using an UV-microscope after staining with FDA (fluorescein diacetate) and, after counting, the concentration of viable spores is adjusted to approximately 1×10^5 viable spores/ml for the first inoculation on hypocotyls, and approximately 5×10^4 viable spores for the second inoculation on the first leaf.

Inoculation

The seedlings are inoculated (with a sprayer) 1-2 days after transplanting. A second infection is made when the first leaf has just spread (4 to 6 days a.t.).

The humidity can be increased by wetting the soil directly after inoculation to stimulate infection. Temperature at night: 18-20° C, in the daytime: 22-25° C.

Growth measurement

In case of low humidity after 5 days (after infection), the sporulation can be stimulated by wetting the soil once or twice every day.

Development of symptoms

The necrosis in young plants is scored approximately 14 days after the last inoculation. The scores of necrosis are determined as identified above. The powdery mildew infection on hypocotyl and leaves is also scored approximately 14 days after the last inoculation. The scores of mildew infection are determined as identified above.

Table 3 Marker sequences powdery mildew

>PMHypocotyl1- SEQ ID NO: 5

TCATAATGACACGTAATGATTGTCAGAG**R**AAATTTATAGAAACCTTTTGTTCAA
CTATCCAACAAATTACAATCAAGGCACTTCTGGAATGAGATAGTCA

>PMHypocotyl2- SEQ ID NO: 6

GTCGTCTTCGCCTATGCaAGACAAAATAAATGCTTGTTT**K**AGTCTAGCCAAAAA
TGGTGTAGAACAGTTGATCACAGTTCCTACGGACTA

>PM-Leaf1- SEQ ID NO: 7

TGGATAAGAGAGGTYCTTGTAAAATRTTATTTTTCATTTAGACCTTGATtttaaTTT
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>PM-Leaf 2- SEQ ID NO: 8

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SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 30582-48 Seq 11-FEB-09 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> Enza Zaden Beheer B.V.

<120> Resistance to powdery mildew and absence of necrosis in cucumis sativus

<130> 2M/2ET29

<140> PCT/EP2007/056911
<141> 2007-07-06

<160> 8

<170> PatentIn version 3.3

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ctcttaggtg ccacaatcga gagggtttat cttcatcttt c 101

CLAIMS:

1. A cell of a powdery mildew-resistant *Cucumis sativus* plant, wherein a representative sample of seed of said plant was deposited with the ATCC on February 14, 2006 under deposit
5 number PTA-7394.

2. The cell according to claim 1, wherein the cell is a cell of a seed.

3. Use of the powdery mildew-resistant *Cucumis sativus* plant defined in claim 1 for homozygously introducing a
10 necrosis-suppressing genetic factor into the genome of a second powdery mildew resistant plant,

wherein the presence of said necrosis-suppressing genetic factor is determinable by the absence of a first DNA-marker of approximately 65 bp, amplifiable by polymerase
15 chain reaction with a first primer comprising SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and a second primer comprising SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and the absence of a second DNA-marker of approximately 123 bp, amplifiable by polymerase chain reaction with a third primer comprising SEQ ID NO: 3
20 (GACTGCGTACCAATTCAC) and a fourth primer comprising SEQ ID NO: 4 (GATGAGTCCTGAGTAATCG), said first and second DNA markers being present in *Cucumis sativus* plants lacking said necrosis-suppressing genetic factor.

4. A method for identification of necrosis tolerance in
25 a *Cucumis sativus* plant, said method comprising detecting the presence of a necrosis-suppressing genetic factor in the genome of said plant using the absence of a first DNA-marker of

approximately 65 bp, amplifiable by polymerase chain reaction with a first primer comprising SEQ ID NO: 1 (GACTGCGTACCAATTCAA) and a second primer comprising SEQ ID NO: 2 (GATGAGTCCTGAGTAACCC), and the absence of a second
5 DNA-marker of approximately 123 bp, amplifiable by a third primer comprising SEQ ID NO: 3 (GACTGCGTACCAATTCAC) and a fourth primer comprising SEQ ID NO: 4 (GATGAGTCCTGAGTAATCG), said first and second DNA markers being present in *Cucumis sativus* plants lacking said necrosis-suppressing genetic
10 factor.