



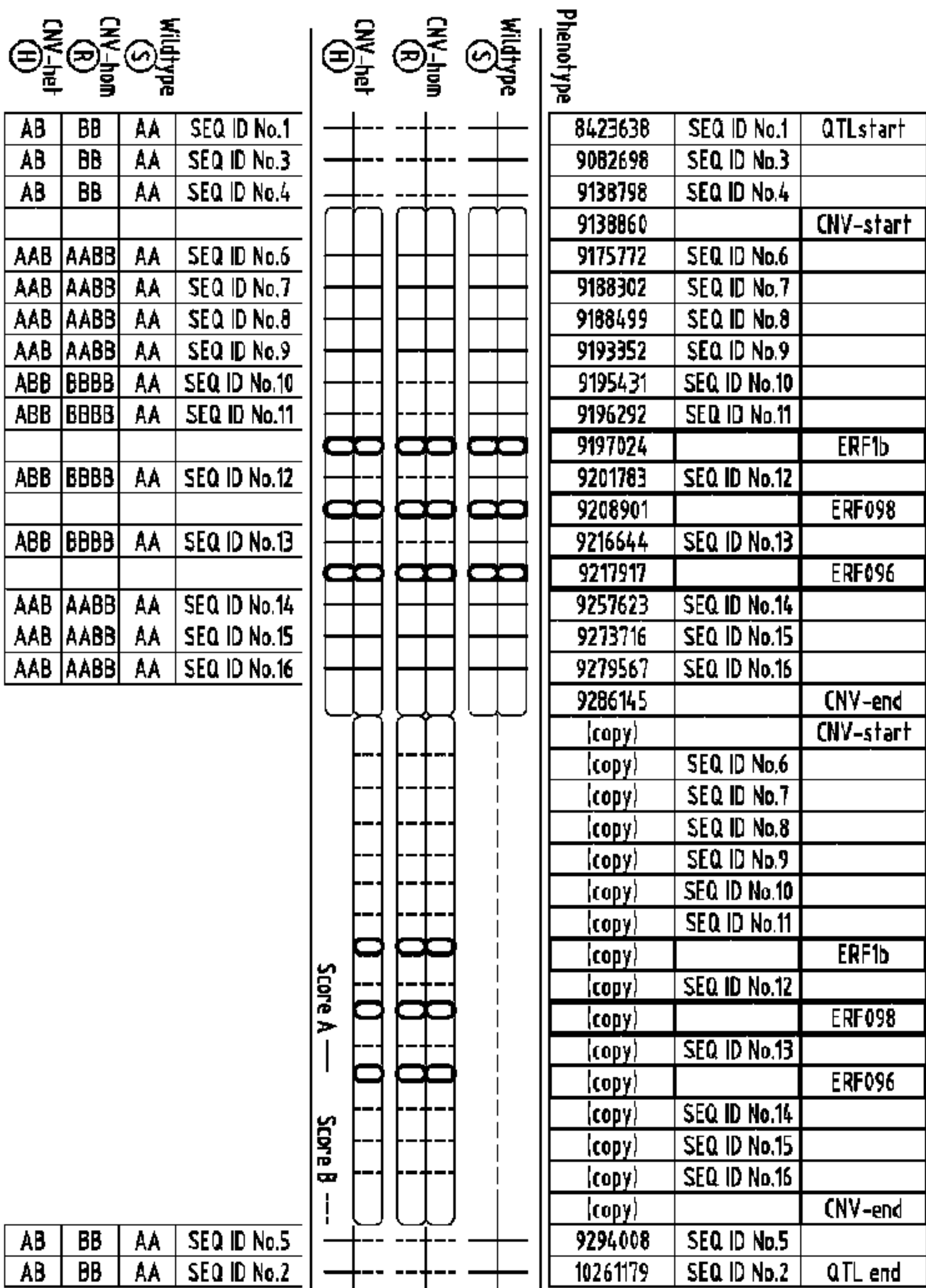
(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION
(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2018/07/19
(87) Date publication PCT/PCT Publication Date: 2019/01/24
(85) Entrée phase nationale/National Entry: 2020/01/15
(86) N° demande PCT/PCT Application No.: EP 2018/069649
(87) N° publication PCT/PCT Publication No.: 2019/016323
(30) Priorité/Priority: 2017/07/20 (EP PCT/EP2017/068398)

(51) Cl.Int./Int.Cl. *A01H 5/08* (2018.01),
C12N 15/82 (2006.01)
(71) Demandeur/Applicant:
RIJK ZWAAN ZAADTEELT EN ZAADHANDEL B.V., NL
(72) Inventeurs/Inventors:
HAARING, CORNELIS, NL;
KOEKEN, ADRIANUS CORNELIS, NL;
HUIJBREGTS-DOORDUIN, LENA JOHANNA, NL
(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : BASE GENETIQUE DE RESISTANCE AU PYTHIUM
(54) Title: GENETIC BASIS FOR PYTHIUM RESISTANCE

FIG. 2



(57) **Abrégé/Abstract:**
The present invention relates to a Cucumis sativus plant comprising a QTL, a copy number variant region, at least two copies of an ERF gene, or a mutation leading to increased expression of an ERF gene, which leads to Pythium resistance. The invention further relates to propagation material suitable for producing such Cucumis sativus plant. The invention also relates to a method for producing such Cucumis sativus plant and to methods for identification and selection of such a plant. In addition, the invention relates to a marker for identification of the QTL or copy number variant region, or for identification of the presence of at least two copies of an ERF gene resulting in Pythium resistance in Cucumis sativus, and to use of said marker. The invention also relates to seed comprising the QTL, copy number variant region, at least two copies of an ERF gene, or a mutation leading to increased expression of an ERF gene, which leads to Pythium resistance in the plant grown from such seed.

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
24 January 2019 (24.01.2019)



(10) International Publication Number
WO 2019/016323 A1

(51) International Patent Classification:

A01H 5/08 (2018.01) C12N 15/82 (2006.01)

(21) International Application Number:

PCT/EP2018/069649

(22) International Filing Date:

19 July 2018 (19.07.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PCT/EP2017/068398

20 July 2017 (20.07.2017)

EP

(71) Applicant: RIJK ZWAAN ZAADTEELT EN ZAAD-
HANDEL B.V. [NL/NL]; Burgemeester Crezeelaan 40,
2678 KX De Lier (NL).

(72) Inventors: HAARING, Cornelis; Burgemeester Crezée-
laan 40, 2678 KX De Lier (NL). KOEKEN, Adrianus
Cornelis; Burgemeester Crezéelaan 40, 2678 KX De Li-
er (NL). HUIJBREGTS-DOORDUIN, Lena Johanna;
Burgemeester Crezéelaan 40, 2678 KX De Lier (NL).

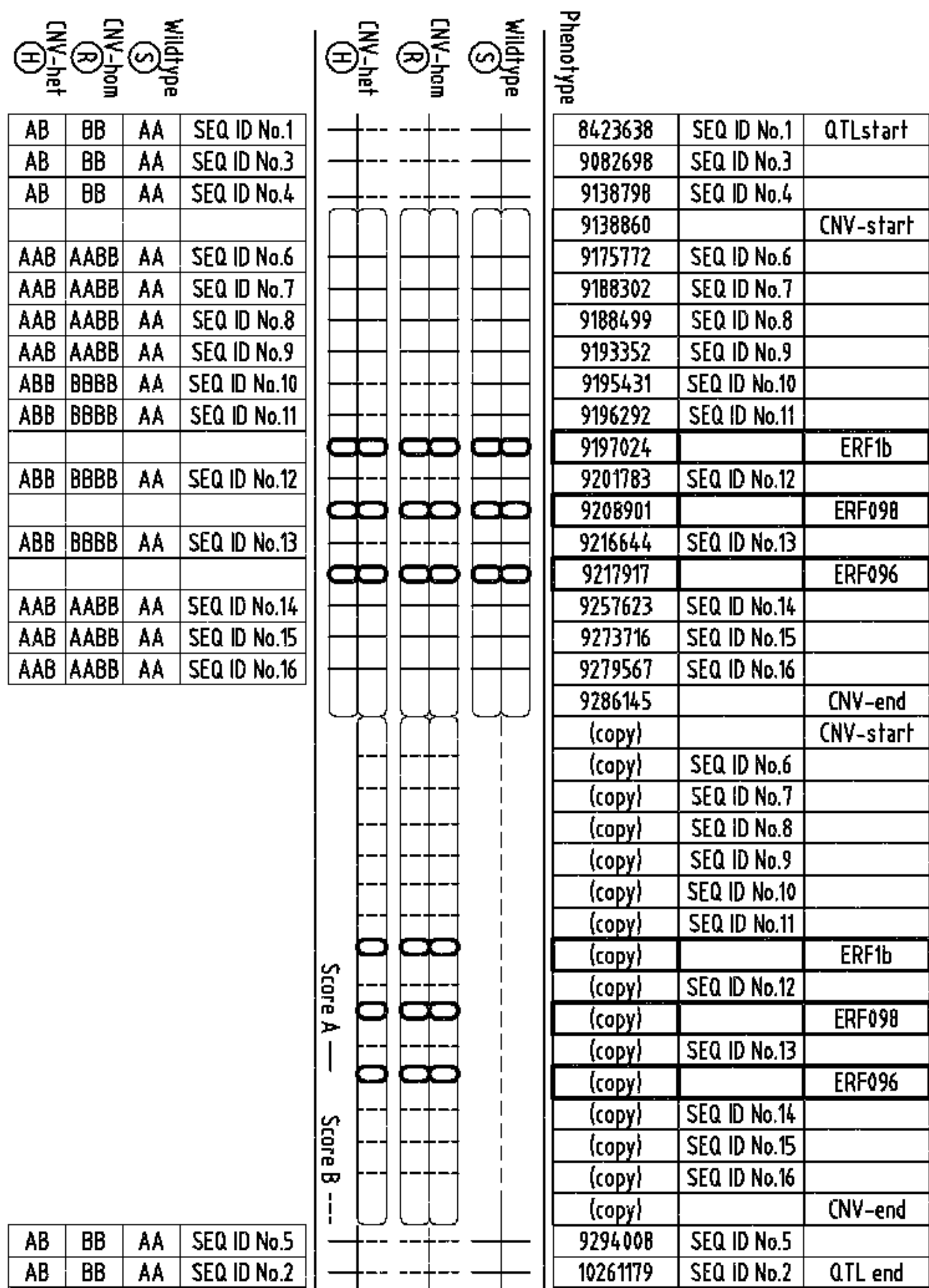
(74) Agent: VAN SOMEREN, Petronella Francisca Hendri-
ka Maria et al.; Arnold & Siedsma, Bezuidenhoutseweg 57,
2594 AC Den Haag (NL).

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

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

(54) Title: GENETIC BASIS FOR PYTHIUM RESISTANCE

FIG. 2



(57) Abstract: The present invention relates to a *Cucumis sativus* plant comprising a QTL, a copy number variant region, at least two copies of an ERF gene, or a mutation leading to increased expression of an ERF gene, which leads to *Pythium* resistance. The invention further relates to propagation material suitable for producing such *Cucumis sativus* plant. The invention also relates to a method for producing such *Cucumis sativus* plant and to methods for identification and selection of such a plant. In addition, the invention relates to a marker for identification of the QTL or copy number variant region, or for identification of the presence of at least two copies of an ERF gene resulting in *Pythium* resistance in *Cucumis sativus*, and to use of said marker. The invention also relates to seed comprising the QTL, copy number variant region, at least two copies of an ERF gene, or a mutation leading to increased expression of an ERF gene, which leads to *Pythium* resistance in the plant grown from such seed.

WO 2019/016323 A1

Published:

- *with international search report (Art. 21(3))*
- *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

GENETIC BASIS FOR PYTHIUM RESISTANCE

The present invention relates to a *Pythium* resistant *Cucumis sativus* plant comprising a QTL, or a copy number variant region, or at least two copies of an *ERF* gene, or a mutant *ERF* gene. The invention further relates to a method for producing such *Cucumis sativus* plant and methods for identification and selection of such a plant. The invention also relates to progeny, seed and fruit of the *Pythium* resistant *Cucumis sativus* plant, to propagation material suitable for producing the *Cucumis sativus* plant, and to a food product comprising such cucumber fruit or part thereof. The invention also relates to a marker for identification of the QTL or copy number variant region or at least two copies of an *ERF* gene resulting in *Pythium* resistance in *Cucumis sativus*, and to use of said marker.

In many crops, the raising of seedlings or the initial growth stage of a plant is hampered by a phenomenon known as ‘damping off’. Damping off is a soil-borne problem that can be caused by a number of pathogens. The most common of these pathogens are various *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Fusarium* species. Damping off is also known as root rot, since the symptoms are usually visible as rotting of the stem and root tissues above or below the soil surface. Damping off can occur pre-emergence, whereby it can initially be confused with a poor seed viability. Often, however, the tissue of just germinated plants becomes water-soaked near the soil surface, after which the seedlings topple over and die.

Very often the cause of damping off turns out to be one of a rather large number of *Pythium* species. *Pythium*, like *Phytophthora*, is a genus of the Oomycetes; the *Pythium* species are usually very generalistic and have a large number of hosts. The differences between the various *Pythium* species lie therefore not in their host-range, but in the different environmental conditions under which they can optimally affect the plants. Although *Pythium* is mostly infecting seedlings, it is also possible that older plants are affected. Because of their non-host specificity, a cultivation method such as crop rotation is not very effective in controlling the disease. In addition, *Pythium* can easily survive in soil and on plant debris for several years, making it difficult to eradicate the pathogen.

Genetic resistance against *Pythium* is not known, and *Pythium* is therefore one of the diseases against which biochemical control is extensively used. *Pythium* can occur in many different environments, also depending on the species; it is often found in protected cultivation, and can be present in soil as well as in various substrates that are used in high-end cultivation systems. Generally wet soil, large temperature changes, and high levels of fertilizer favor the development of the disease. Various fungicides and biological control agents can be used to prevent the occurrence or the spread of the disease. Once plants are infected, treatment to cure them is not effective. Application can be done for example as a seed treatment, soil drenching, or

foliar spray. Most effective however is to maintain a strict hygiene system and high level crop maintenance in order to prevent the pathogen from entering the growing system.

It is an object of the present invention to provide a *Cucumis sativus* plant that is resistant to *Pythium*.

5 Cucumber and gherkin, both belonging to the species *Cucumis sativus*, are among the crops that can be severely affected by various *Pythium* species, among which *Pythium aphanidermatum*, *Pythium dissotocum*, and *Pythium ultimum*. Since no varieties exist that are resistant to *Pythium*, a research program was started to develop *C. sativus* plants that are resistant to this pathogen.

10 The research program identified a population of plants that showed a remarkably good resistance to *Pythium*. This population of plants, however, had many agronomic characteristics such as pronounced dark warts and spines that needed to be overcome before it could be used in a breeding program to develop commercially suitable *Pythium* resistant cucumber varieties. A lot of effort had to be put in, whereby the plants were combined with various internal
15 breeding lines to develop cucumber material with different backgrounds that could be further used in different combinations for the development of *C. sativus* varieties of different types.

To confirm the resistance and to follow the resistance in populations during the breeding process a bio-assay for *Pythium* resistance was regularly carried out on relevant material (Example 1). However, because bio-assays are commonly time consuming, and logistically
20 challenging since for example a suitable area, sufficient inoculum, and good timing of the evaluation is required, it is more efficient to develop a marker screen. For this purpose a QTL mapping study was performed and a QTL region was identified on chromosome 3 between SEQ ID No. 1 and SEQ ID No. 2 (Example 2). A marker within this region that is linked to the QTL is represented by SEQ ID No. 3. The presence of the QTL that leads to *Pythium* resistance can be
25 identified by one or both of SEQ ID No. 1 and SEQ ID No. 3; SEQ ID No. 1 and SEQ ID No. 3 are linked to the resistance; SEQ ID No. 1 and SEQ ID No. 2 indicate the position of the QTL.

The present invention relates to a QTL that is present on chromosome 3 between SEQ ID No. 1 and SEQ ID No. 2 of the *C. sativus* genome, and preferably is present between SEQ ID No. 4 and SEQ ID No. 2. This QTL leads to resistance against *Pythium* when present in a
30 cucumber plant, and is further referred to herein as the “QTL of the invention”. The “QTL of the invention” also encompasses that the presence of this QTL can be identified by at least one of the markers selected from the group consisting of SEQ ID No. 1, SEQ ID No. 3, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16. The “QTL of the
35 invention” further encompasses that this QTL comprises and therefore is linked to at least one of the markers selected from the group consisting of SEQ ID No. 1, SEQ ID No. 3, SEQ ID No. 5,

SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16.

The present invention provides a *C. sativus* plant comprising the QTL of the invention.

5 Through further research it was determined that when a certain region of approximately 30 genes in the QTL on chromosome 3 is present in duplicate in a *C. sativus* plant, this duplication leads to resistance against *Pythium* (Example 3). When a duplication, or other multiplication, of a gene or a region of genes is present on a chromosome in a genome, this is called a copy number variant. The presence of multiple copies of a certain gene can lead to an
10 increased expression of said gene, and/or an increase of the product produced by said gene. This increase can subsequently lead to resistance. In the present invention, the duplication of the genes, i.e. a copy number variant, present in the region between SEQ ID No. 4 and SEQ ID No. 5 was determined to be related to resistance to *Pythium*.

The present invention relates to a copy number variant region (CNV) that is
15 present on chromosome 3 between SEQ ID No. 4 and SEQ ID No. 5 of the *C. sativus* genome. This CNV leads to resistance against *Pythium* when present in a cucumber plant, and is further referred to herein as the “copy number variant region of the invention” or the “CNV of the invention”. The “CNV of the invention” also encompasses that the presence of this CNV can be identified by at least one of the markers selected from the group consisting of SEQ ID No. 5, SEQ
20 ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16. The “CNV of the invention” further encompasses that this CNV comprises and therefore is linked to at least one of the markers selected from the group consisting of SEQ ID No. 1, SEQ ID No. 3, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11,
25 SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16.

The present invention provides a *C. sativus* plant comprising the copy number variant region of the invention.

It was further determined that the sequences of the two copies of the duplicated region on chromosome 3 were not identical. Several SNPs were identified which are either present
30 in both copies or only in one of the two copies. The SNPs present in only one of the two copies of the CNV region can be identified by using a polymorphic marker. Examples of markers that are polymorphic between the CNV copies are represented by SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16. These markers are indicated herein as ‘heterozygous CNV markers’.

35 The SNPs present in both copies can be identified by using markers that are polymorphic between both copies of the CNV region on the one hand and only a single, wildtype,

copy on the other hand. Examples of markers that are polymorphic between both copies of the CNV region on the one hand and only a single, wildtype, copy on the other hand are represented by SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, and SEQ ID No. 13. These markers are indicated herein as ‘homozygous CNV markers’.

Table 2 shows the marker score for the presence of one copy, and the marker score to identify the presence of the copy number variant region and therefore a resistant plant. When the sequences of the markers are positioned on version 2 of the publicly available genome reference sequence for *C. sativus*, that is based on Cs9930, the physical position to which the SNP polymorphism in said marker sequence corresponds is also indicated in **Table 2**. The public *C. sativus* genome reference sequence based on Cs9930 can for example be accessed at: http://www.icugi.org/cgi-bin/gb2/gbrowse/cucumber_v2/, and is the reference for ‘the public cucumber genome’ as used herein. The positions of the QTL or CNV region and the markers of the invention are therefore also derivable from this public map and these positions are relative to said physical positions.

As used herein a marker is genetically “linked”, and can be used for the identification of the QTL or CNV region of the invention, when the sequence of said marker is present in the QTL or CNV of the invention.

Figure 2 shows the location of the various markers within the QTL and the CNV region, and a representation of the difference in scoring between the so-called heterozygous and homozygous CNV markers.

Within the CNV region of the invention three *ERF* genes are present. An *ERF* gene within the CNV region can be identified by the presence of an AP-2 domain. The AP-2 domain is a conserved DNA-binding domain found in transcription regulators in plants, and the skilled person is aware of how to identify the presence of an AP-2 domain in a gene. An AP-2 domain can for example be identified by using the EMBL-EBI database through <http://pfam.xfam.org/family/AP2>. A search for the relevant sequences can subsequently be performed with for example the use of the hmmsearch from HMMER 3.1b2, using an e-value of 1e-4.

The present invention relates to an *ERF* gene of which at least two copies are present within the CNV of the invention, on chromosome 3 between SEQ ID No. 4 and SEQ ID No. 5 of the *C. sativus* genome. The presence of at least two copies of said *ERF* gene leads to resistance against *Pythium* when present in a cucumber plant, and is further referred to herein as the “at least two copies of an *ERF* gene of the invention”. The “at least two copies of an *ERF* gene of the invention” encompasses that the presence of said at least two copies of an *ERF* gene can be identified by at least one of the markers selected from the group consisting of SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID

No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16. The “*ERF* gene of the invention” encompasses an *ERF* gene that can be identified by determining the presence of an AP-2 domain represented by SEQ ID No. 20 or SEQ ID No. 21 or SEQ ID No. 22, or by a sequence having a sequence identity of at least 70% to any of those sequences. In order of increased preference, the AP-2 domain sequence of an *ERF* gene of the invention has a sequence identity of 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, 99%, or 100% to any of the sequences represented by SEQ ID No. 20 or SEQ ID No. 21 or SEQ ID No. 22 (**Figure 3**). The “*ERF* gene of the invention” also encompasses an *ERF* gene comprising a coding sequence represented by SEQ ID No. 17 or by a sequence having at least 95% sequence identity thereto, designated herein as an *ERF1B* gene; an *ERF* gene comprising a coding sequence represented by SEQ ID No. 18 or by a sequence having at least 95% sequence identity thereto, designated herein as an *ERF098* gene; or an *ERF* gene comprising a coding sequence represented by SEQ ID No. 19 or by a sequence having at least 95% sequence identity thereto, designated herein as an *ERF096* gene. Preferably, the presence of the at least two copies of an *ERF* gene of the invention leads to increased expression of said *ERF* gene.

The present invention also provides a mutant *ERF* gene of the invention, the presence of which mutant *ERF* gene leads to *Pythium* resistance when present in a *C. sativus* plant. The mutant *ERF* gene has a higher expression than the wild-type *ERF* gene, and is further referred to herein as the “mutant *ERF* gene of the invention”. A “mutant *ERF* gene of the invention” encompasses an *ERF* gene of the invention having a mutation in the promoter region, a mutation in the 5'-UTR, a mutation in the coding sequence, and/or a mutation in the 3'UTR.

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.

Increased expression is expression as compared to a plant comprising a single, wild-type, copy of the *ERF* gene, which plant is not resistant to *Pythium*. Increased expression is optionally determined in the presence of *Pythium* infection. An increased expression is an at least 1.5 fold increased expression, in order of increased preference an at least 1.9 fold, 2 fold, 2.5 fold, 2.8 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 4.8 fold, 5 fold, 7 fold, 9 fold, 10 fold, 12 fold, 15 fold, 16 fold, 18 fold, 20 fold, 22 fold, 22.9 fold, 25 fold, 30 fold, 35 fold, or higher, up to an at least 100 fold increased expression.

The present invention provides a plant that is resistant to *Pythium*, which plant comprises at least two copies of an *ERF* gene of the invention, or comprises a mutant *ERF* gene of the invention.

A plant of the invention preferably comprises two copies of an *ERF* gene comprising a coding sequence represented by SEQ ID No. 17 or by a sequence having at least 95% sequence identity thereto, designated herein as an *ERF1B* gene, two copies of an *ERF* gene comprising a coding sequence represented by SEQ ID No. 18 or by a sequence having at least 95% sequence identity thereto, designated herein as an *ERF098* gene, and two copies of an *ERF* gene comprising a coding sequence represented by SEQ ID No. 19 or by a sequence having at least 95% sequence identity thereto, designated herein as an *ERF096* gene.

As used herein, a copy of an *ERF* gene is a gene comprising at least 95% sequence identity, preferably in order of increased preference at least 96%, 97%, 98%, 99%, or 100% sequence identity, to another gene that is present in the CNV of the invention.

As used herein, a gene comprises the promoter, the 5'-UTR, the coding sequence (CDS) or gDNA sequence, and the 3'UTR of that gene. The promoter suitably comprises a sequence of up to 2kb upstream of the ATG start codon of the CDS of that gene.

The present invention provides a *C. sativus* plant that is resistant to *Pythium*, which *C. sativus* plant comprises at least two copies of at least one *ERF* gene of the invention, or comprises a mutant *ERF* gene of the invention, wherein the presence of the at least two copies or the mutant *ERF* gene of the invention leads to increased expression of said *ERF* gene as compared to a *C. sativus* plant comprising a single, wild-type, copy of said *ERF* gene.

As used herein, *Pythium* resistance is resistance to one or more *Pythium* species, in particular to one or more of the species of the group comprising *Pythium aphanidermatum*, *Pythium dissotocum*, and *Pythium ultimum*. As used herein, *Pythium* resistance comprises resistance to at least the species *Pythium aphanidermatum*.

The *Pythium* resistance of the present invention inherits in a monogenic, incompletely dominant, manner. As used herein, incompletely dominant means that when the QTL, the copy number variant region, the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention is homozygously present, it gives a higher level of *Pythium* resistance than when the QTL, the copy number variant region, the at least two copies of an *ERF* gene, the mutant *ERF* gene of the invention is heterozygously present. The heterozygous presence of the QTL, the copy number variant region, the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention however still confers improved *Pythium* resistance. The improved *Pythium* resistance of both homozygous and heterozygous plants makes the plants more suitable for cultivation under conditions where *Pythium* is present. Therefore both levels of resistance are considered to be improved agronomic characteristics.

The presence of *Pythium* resistance can be determined through a bioassay under conditions that are suitable for *Pythium* infection. For example the cucumber seedling bioassay as described in Chen et al, 1987 can be used. (Factors affecting suppression of *Pythium* damping-off

in container media amended with composts. Chen et al, Phytopathology 77:755-760, 1987). As container medium a suitable growth medium for the plants to be tested can be used. Regular potting soil is an example of a container medium that can be used in a *Pythium* bioassay for cucumber.

5 As used herein, *Pythium* resistance is determined by comparison to a control variety known to be *Pythium* susceptible. Resistance is suitably scored on 10-12 plants of a certain line or other plant population to be tested. The use of replicates is advisable, especially when conditions cannot be optimally controlled. Since *Pythium* shows very severe symptoms, and no adequate cure is possible once a plant has been affected, scoring can be done in just two categories:
10 either resistant or dead/wilted. Scoring is suitably done 10 – 14 days after inoculation. A genotype is considered to be resistant when in a bioassay significantly more plants score resistant than the susceptible control variety in that same bioassay. Depending on the number of plants that is used in the assay, statistical methods known to the skilled person can optionally be used to determine a significant difference.

15 *C. sativus* varieties that are susceptible to *Pythium* and do not have the QTL or the copy number variant region of the invention, and have only one copy of a wild-type *ERF* gene of the present invention, are for example the hybrid variety Ventura and the hybrid variety Roxanna. These varieties can be used as a susceptible control variety. The *ERF* gene expression of these varieties can also be used as a control to compare with the increased *ERF* expression of a plant of
20 the invention. Other publicly available and commonly grown *C. sativus* varieties of various types can also be used as susceptible controls, such as Marketmore 70 or Poinsett 76.

A *C. sativus* genotype that has the *Pythium* resistance of the invention is deposited as NCIMB 42776. A plant grown from NCIMB 42776, or a progeny thereof, can be used as a resistant control variety in a *Pythium* bio-assay. When a plant, line, or population to be assessed
25 shows the same level of resistance as NCIMB 42776 in a bio-assay, this plant, line, or population is considered to be *Pythium* resistant and is therefore a plant of the invention.

A plant of the present invention is preferably a cultivated plant having improved agronomic characteristics that make it suitable for commercial cultivation. The invention also relates to a cucumber fruit harvested from a plant of the invention, wherein the cucumber fruit
30 comprises the QTL, the copy number variant region, the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention in its genome which leads to *Pythium* resistance in the plant. This cucumber fruit is also referred to herein as ‘the fruit of the invention’ or ‘the cucumber fruit of the invention’. As used herein, ‘cucumber fruit’ comprises a fruit produced by a plant of the species *Cucumis sativus*.

The present invention relates to a method for producing a *Pythium* resistant *C. sativus* plant comprising introducing the QTL of the invention in a *C. sativus* plant, or introducing the copy number variant region of the invention a *C. sativus* plant.

The present invention also relates to a method for producing a *Pythium* resistant plant comprising increasing the expression of an *ERF* gene of the invention in a plant, whereby increasing the expression is achieved by introduction of an extra copy of an *ERF* gene of the invention in a plant or through mutation of an *ERF* gene of the invention. Mutation of an *ERF* gene comprises mutation of the promoter region, mutation of the 5'-UTR, mutation of the coding sequence, and/or mutation of the 3'UTR.

In a preferred embodiment the *Pythium* resistant plant is a *C. sativus* plant, in particular a cultivated *C. sativus* plant.

The QTL of the invention, the copy number variant region of the invention, the at least two copies of an *ERF* gene of the invention, or the mutant *ERF* gene of the invention can be introduced from another plant which comprises the QTL, or the copy number variant region, or at least two copies of an *ERF* gene, or the mutant *ERF* gene, through commonly used breeding techniques, such as crossing and selection, when the plants are sexually compatible. Introduction of an extra copy of the *ERF* gene can likewise be done from another plant that comprises more than one *ERF* gene. 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 are used to follow the incorporation of the QTL, or the copy number variant region, or the at least two copies, or the mutant *ERF* gene into another plant.

The above method can in particular be used to introduce the QTL, or the copy number variant region, or the two copies of the *ERF* gene, or the mutant *ERF* gene of the invention into a plant species that is suitable for incorporation of such genetic information. Said QTL, copy number variant region, two copies of an *ERF* gene, or the mutant *ERF* gene can be introduced from a *Cucumis sativus* plant comprising the QTL, copy number variant region, two copies of an *ERF* gene, or the mutant *ERF* gene into a *Cucumis sativus* plant lacking said genetic information using standard breeding methods.

The QTL, copy number variant region, or two copies of an *ERF* gene of the invention can be introduced from a *Cucumis sativus* plant representative seed of which was deposited with the NCIMB under deposit number NCIMB 42776, or from the deposited seeds NCIMB 42776, or from sexual or vegetative descendants thereof. Introduction of the QTL, or the copy number variant region, or the copy of an *ERF* gene in *Cucumis sativus* leads to *Pythium* resistance.

A plant grown from NCIMB 42776 has the *Pythium* resistance of the invention, in which the QTL of the invention is present. A plant grown from NCIMB 42776 also has the copy number variant region of the invention.

In a plant grown from NCIMB 42776, or a progeny thereof, the presence of the QTL can be identified by determining the presence of one or both of SEQ ID No. 1 and SEQ ID No. 3, in particular by determining the presence of the SNP in SEQ ID No. 1 or SEQ ID No. 3 as compared to the wildtype sequence. SEQ ID Nos. 5-16 can also be used to determine the presence of the QTL of the invention. The locations and scores of the SNPs which show the sequences of the markers that identify resistance are indicated in **Table 2**.

In a plant grown from NCIMB 42776, or a progeny thereof, the presence of the copy number variant region can be identified by determining the presence of at least one of the markers selected from the group consisting of SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16, in particular by determining the presence of the SNP in one or more of SEQ ID Nos. 5-16 as compared to the wildtype sequence. The locations and scores of the SNPs which show the sequences of the markers that identify resistance are indicated in **Table 2**.

A plant grown from NCIMB 42776, or a progeny thereof, has two copies of an *ERF* gene comprising a coding sequence represented by SEQ ID No. 17, two copies of an *ERF* gene comprising a coding sequence represented by SEQ ID No. 18, and two copies of an *ERF* gene comprising a coding sequence represented by SEQ ID No. 19 on chromosome 3.

Alternatively the QTL, or the copy number variant region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention can be transferred from another, sexually incompatible, plant, for example by using a transgenic approach. Techniques that can suitably be used comprise general plant transformation techniques known to the skilled person, such as the use of an *Agrobacterium*-mediated transformation method. Genome editing methods such as the use of a CRISPR/Cas system might also be employed to obtain a plant of the invention. Genome editing can be used to develop a *Pythium* resistant plant through duplication of the region comprising the approximately 30 genes, or duplication of an *ERF* gene, or by increasing the expression of an *ERF* gene through modification of the gene. Modification of an *ERF* gene comprises modification of the promoter region, modification of the 5'-UTR, modification of the coding sequence, and/or modification of the 3'UTR.

The plant of the invention may comprise the QTL of the invention, or the copy number variant region of the invention, or the at least two copies of an *ERF* gene of the invention, or the mutant *ERF* gene of the invention either homozygously or heterozygously.

The plant of the invention may be a plant of an inbred line, a hybrid, a doubled haploid, or a plant of a segregating population. Preferably, the plant of the invention is a non-transgenic plant.

The invention also relates to a *Cucumis sativus* seed comprising the QTL of the invention, or the copy number variant region of the invention, or the at least two copies of an *ERF* gene of the invention, or the mutant *ERF* gene of the invention, wherein the plant grown from the seed is a plant that is resistant to *Pythium*. The invention also relates to seeds produced by a plant of the invention. These seeds harbor the QTL of the invention, or the copy number variant region of the invention, or the at least two copies of an *ERF* gene of the invention, or the mutant *ERF* gene of the invention, and as such, a plant grown from said seed is a plant of the invention.

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

The invention also relates to propagation material suitable for producing a *Cucumis sativus* plant of the invention, wherein the propagation material is suitable for sexual reproduction, and is in particular selected from a microspore, pollen, an ovary, an ovule, an embryo sac, or an egg cell, or is suitable for vegetative reproduction, and is in particular selected from a cutting, a root, a stem cell, or a protoplast, or is suitable for tissue culture of regenerable cells, and is in particular selected from a leaf, pollen, an embryo, a cotyledon, a hypocotyl, a meristematic cell, a root, a root tip, an anther, a flower, a seed and a stem, and wherein the propagation material comprises the QTL of the invention, or the copy number variant region of the invention, or the at least two copies of an *ERF* gene of the invention, or the mutant *ERF* gene of the invention that confers *Pythium* resistance. A plant of the invention may be used as a source of the propagation material.

The invention further relates to a cell of a plant of the invention. Such a cell may either be in isolated form or a part of the complete plant or parts thereof and still constitutes a cell of the invention because such a cell harbours the genetic information that leads to the *Pythium* resistance of a cultivated *C. sativus* plant. Each cell of a plant of the invention carries the genetic information that leads to *Pythium* resistance. A cell of the invention may also be a regenerable cell that can regenerate into a new plant of the invention. The presence of the genetic information in a cell of the invention in this context is the presence of the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene as defined herein.

The invention further relates to plant tissue of a plant of the invention, which comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene, of the invention. The tissue can be undifferentiated tissue or already differentiated tissue. Undifferentiated tissue is for example a stem tip, an anther, a petal, or pollen, and can be used in micropropagation to obtain new plantlets that are grown into new plants of the invention. The tissue can also be grown from a cell of the invention.

The invention moreover relates to progeny of a plant, a cell, a tissue, or a seed of the invention, which progeny comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene, of the invention that leads to *Pythium* resistance. Such progeny can in itself be a plant, a cell, a tissue, or a seed.

As used herein “progeny” is intended to mean the first and all further descendants from a cross with a plant of the invention.

“Progeny” also encompasses a *C. sativus* plant that carries the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention, which plant has the *Pythium* resistance of the invention, and is obtained from another plant, or progeny of a plant, of the invention by vegetative propagation or multiplication.

The invention further relates to a part of a *C. sativus* plant of the invention that is suitable for sexual reproduction and which comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention. Such a part is for example selected from the group consisting of a microspore, pollen, an ovary, an ovule, an embryo sac, and an egg cell. Additionally, the invention relates to a part of a *C. sativus* plant of the invention that is suitable for vegetative reproduction, which is in particular a cutting, a root, a stem, a cell, or a protoplast that comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention. The part of a plant as previously mentioned is considered propagation material. The plant that is produced from the propagation material comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention that leads to *Pythium* resistance.

The invention further relates to tissue culture of a plant of the invention, which is also propagation material and which comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene or the mutant *ERF* gene of the invention. The tissue culture comprises regenerable cells. Such tissue culture can be selected or derived from any part of the plant, in particular from a leaf, pollen, an embryo, a cotyledon, a hypocotyl, a meristematic cell, a root, a root tip, an anther, a flower, a seed, and a stem. The tissue culture can be regenerated into a *C. sativus* plant comprising the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention, wherein the regenerated *C. sativus* plant expresses the trait of the invention and is also part of the invention.

The invention additionally relates to the use of a plant of the invention in plant breeding. The invention thus also relates to a breeding method for the development of cultivated *C. sativus* plants that are resistant against *Pythium* wherein germplasm comprising the QTL, or the CNV region, or the at least two copies of an *ERF* gene for conferring said resistance is used. Seed
5 being representative for the germplasm was deposited with the NCIMB under accession number NCIMB 42776.

The invention also concerns the use of the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or a mutant *ERF* gene of the invention for the development of *Cucumis sativus* plants that have resistance to *Pythium*.

10 The invention also relates to a marker for the identification of *Pythium* resistance in a *Cucumis sativus* plant, which marker is selected from the group consisting of SEQ ID Nos. 1-16. The presence of at least one of the markers selected from the group consisting of SEQ ID Nos. 1-16, preferably the presence of at least one of the markers selected from the group consisting of SEQ ID Nos. 5-16, is indicative of the presence of resistance to *Pythium*. The use of any of the
15 markers represented by SEQ ID Nos. 1-16, preferably the use of any of the markers represented by SEQ ID Nos. 5-16, for identification of *Pythium* resistance in a *Cucumis sativus* plant is also part of the invention. All these markers can also be used to develop other markers for the identification of the QTL, or the CNV region, or for the identification of the presence of at least two copies of an *ERF* gene, or of a mutation in an *ERF* gene of the invention leading to *Pythium* resistance, which
20 use is also part of the present invention.

The present invention also relates to a method for selecting a *Pythium* resistant *Cucumis sativus* plant, comprising determining the presence of the QTL, or the copy number variant region of the invention, or the presence of at least two copies of an *ERF* gene, or of the mutant *ERF* gene of the invention, and selecting a plant that comprises the QTL, or the copy
25 number variant region of the invention, or at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention as a *Pythium* resistant plant.

The present invention also relates to a method for selecting a *Pythium* resistant plant, comprising determining increased expression of at least one *ERF* gene of the invention, and selecting a plant with increased expression as a *Pythium* resistant plant. Increased expression can
30 be determined by performing a qPCR assay as described in **Example 4**. A plant with increased expression of at least one *ERF* gene of the invention is suitably a plant comprising at least two copies of said *ERF* gene, or a plant comprising a mutant *ERF* gene of the invention.

The invention also relates to a method of testing a *Cucumis sativus* plant for the presence of the QTL, or of the copy number variant region of the invention conferring *Pythium*
35 resistance in its genome, comprising detecting a marker sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID

No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16, or detecting any combination thereof, in the genome of the *Cucumis sativus* plant.

The invention also relates to a method of testing a *Cucumis sativus* plant for the presence of at least two copies of an *ERF* gene of the invention, comprising detecting a marker sequence selected from the group consisting of SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16, or detecting any combination thereof, in the genome of the *Cucumis sativus* plant. The presence of at least one of the markers selected from SEQ ID Nos. 6-16, i.e. of the B allele of the SNP, is indicative for the presence of at least two copies of an *ERF* gene of the invention.

In one embodiment of the invention, the method of testing a *Cucumis sativus* plant for the presence of the QTL, or the copy number variant region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention conferring *Pythium* resistance in its genome further comprises selecting a *Cucumis sativus* plant that comprises the QTL, or the copy number variant region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention in its genome as a *Pythium* resistant plant.

The invention also relates to a method for the production of a *Cucumis sativus* plant which is resistant against *Pythium*, said method comprising:

- a) crossing a plant of the invention with a plant not comprising the QTL of the invention, or the CNV region of the invention, or the at least two copies of an *ERF* gene of the invention, or the mutant *ERF* gene of the invention, to obtain an F1 population;
- b) optionally performing one or more rounds of selfing and/or crossing a plant from the F1 to obtain a further generation population;
- c) selecting from the population a plant that comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or a mutation leading to an increased expression of an *ERF* gene and is resistant against *Pythium*, suitably by using a molecular marker linked to the QTL, or the CNV region, or the presence of at least two copies of an *ERF* gene, or a mutation leading to an increased expression of an *ERF* gene of the invention.

The marker of step c) of the method can be a marker represented by any of SEQ ID Nos. 1-16, preferably by any of SEQ ID Nos. 5-16. The plant can also be phenotypically selected for having resistance to *Pythium*.

The plant of the invention used in the method for the production of a *Cucumis sativus* plant which is resistant against *Pythium* is optionally a plant grown from seed deposited under NCIMB accession number 42776, or progeny thereof.

The invention additionally provides for a method of introducing another desired trait into a *Cucumis sativus* plant comprising *Pythium* resistance, comprising:

- a) crossing a *Cucumis sativus* plant of the invention with a second *Cucumis sativus* plant that comprises the other desired trait to produce F1 progeny;
- 5 b) selecting an F1 progeny that comprises *Pythium* resistance and the other desired trait;
- c) crossing the selected F1 progeny with either parent, to produce backcross progeny;
- d) selecting backcross progeny comprising *Pythium* resistance and the other desired trait; and
- e) optionally repeating steps c) and d) one or more times in succession to produce
- 10 selected fourth or higher backcross progeny that comprises the other desired trait and has resistance *Pythium*.

The plant of the invention used in the method of introducing another desired trait into a *Cucumis sativus* plant comprising resistance to *Pythium* is optionally a plant grown from seed deposited under NCIMB accession number 42776, or progeny thereof.

- 15 Optionally, selfing steps are performed after any of the crossing or backcrossing steps in the described method. Selection of a plant comprising *Pythium* resistance and the other desired trait can alternatively be done following any crossing or selfing step of the method. The desired trait can be selected from, but is not limited to, the following group: resistance to bacterial, fungal or viral diseases, insect or pest resistance, improved germination, plant size, plant type,
- 20 improved shelf-life, water stress and heat stress tolerance, and male sterility. The invention includes a *Cucumis sativus* plant produced by this method and the *Cucumis sativus* fruit obtained therefrom.

- 25 The invention further relates to a method for the production of a *Cucumis sativus* plant comprising the QTL of the invention, or the CNV region of the invention, or the at least two copies of an *ERF* gene of the invention, or the mutant *ERF* gene of the invention that leads to resistance to *Pythium*, by using tissue culture of plant material that comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention in its genome.

- 30 The invention further relates to a method for the production of a *Cucumis sativus* plant comprising the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention that leads to resistance to *Pythium*, by using vegetative reproduction of plant material that comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention in its genome.

- 35 The invention further provides a method for the production of a *Cucumis sativus* plant having resistance to *Pythium* as defined herein by using a doubled haploid generation technique to generate a doubled haploid line that homozygously comprises the QTL, or the CNV

region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention and is resistant against *Pythium*.

The invention further relates to a method for the production of a *Cucumis sativus* plant comprising the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention wherein said QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention leads to *Pythium* resistance, which method comprises growing a seed comprising the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or a mutant *ERF* gene of the invention into the said *Cucumis sativus* plant. The seed used in the method is optionally seed deposited with the NCIMB under deposit number 42776, or progeny seed thereof.

The invention further relates to a method for seed production comprising growing a *Cucumis sativus* plant from seed of the invention, allowing the plant to produce fruits with seed, and harvesting those seed. Production of the seed is suitably done by crossing or selfing. Preferably, the seed that is so produced has the capability to grow into plants that are resistant to *Pythium*.

The invention further relates to hybrid seed and to a method for producing said hybrid seed, comprising crossing a first parent plant with a second parent plant and harvesting the resultant hybrid seed, wherein the first parent plant and/or the second parent plant is a plant of the invention. The resultant hybrid plant comprising the QTL, or the CNV region, or at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention and which exhibits resistance to *Pythium* is also a plant of the invention.

It is clear that the parent that provides the trait of the invention is not necessarily a plant grown directly from the deposited seed. The parent can also be a progeny plant from the deposited seed, or a progeny plant from seed that is identified to have obtained the trait of the invention by other means.

Introgression of the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention as used herein means introduction of the QTL, or the CNV, or the at least two copies of an *ERF* gene, or the mutant *ERF* of the invention from a donor plant comprising said QTL, or CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene into a recipient plant not carrying said QTL, or CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene by standard breeding techniques wherein selection for plants comprising the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention can be performed phenotypically by means of observation of the resistance to *Pythium*, or selection can be performed with the use of markers through marker assisted breeding, or combinations of these. Selection is started in the F1 or any further generation from a cross between the recipient plant and the donor plant, suitably by using markers as

identified herein by SEQ ID Nos 1-16. The skilled person is familiar with creating and using new molecular markers that can be used to identify or are linked to the trait of the invention. Development and use of such markers for identification and selection of plants of the invention is also part of the invention.

5 The phrase “trait” in the context of this application refers to the phenotype of the cultivated *Cucumis sativus* plant. In particular, the word “trait” refers to the trait of the invention, more in particular to the resistance to *Pythium*. When a cultivated *C. sativus* plant exhibits the trait of the invention, its genome comprises the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention causing the trait of the invention. Hence, the
10 “trait of the invention” as used herein is intended to refer to the trait of resistance to *Pythium* caused by the QTL, or the CNV region, or the at least two copies of an *ERF* gene, or the mutant *ERF* gene of the invention.

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

DEPOSIT

 Seed of cucumber *Cucumis sativus* EX 5.014 was deposited with NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, UK on 11/07/2017 under
20 deposit accession number NCIMB 42776. The seed of the deposit comprises the QTL, the copy number variant region, and at least two copies of an *ERF* gene of the invention homozygously. Plants grown from this seed are thus resistant against *Pythium*.

FIGURES

25 **Figure 1** – Genomic coding sequences of **SEQ ID No. 17**, **SEQ ID No. 18**, and **SEQ ID No. 19**.

Figure 2 – Representation of the presence of the markers, the location of the *ERF* genes including the start position of the gene, and the difference between the score of a heterozygous CNV marker and a homozygous CNV marker. CNV-hom shows the marker scores when the QTL
30 or CNV region is homozygously present in a plant. CNV-het shows the marker score when the QTL or CNV region is heterozygously present in a plant.

Figure 3 – Sequences of the AP-2 domains **SEQ ID No. 20**, **SEQ ID No. 21**, and **SEQ ID No. 22** of the 3 *ERF* genes that are present in the CNV region.

Figure 4 – Relative expression of *ERF* genes that are present in the CNV region of a plant
35 of the invention. *ERF1B* is the gene represented by **SEQ ID No. 17**; *ERF098* is the gene represented by **SEQ ID No 18**; *ERF096* is the gene represented by **SEQ ID No. 19**.

EXAMPLES

EXAMPLE 1

Bio-assay for Pythium resistance and deposit development in C. sativus

5 A *Pythium* resistant *Cucumis sativus* source that was identified in a germplasm screen was first crossed with various internal breeding lines to create a number of backgrounds in which the resistance would be present. These crosses were put through several cycles of backcrossing and inbreeding to develop cultivated lines that have commercially acceptable standards. During this process, continuous selection for *Pythium* resistance was done, since the
10 resistance was not uniformly present in the source, and to make sure the resistance was not lost in the process.

 Selection for *Pythium* resistance was done with a bio-assay. Plants were sown in trays filled with potting soil in a greenhouse under normal cucumber growing conditions, at a day temperature of around 23⁰ C. Seven days after sowing, multiplication of the *Pythium* pathogen was
15 started on standard agar plates containing oatmeal medium. At 14 days after sowing the inoculum was prepared by blending around 2 agar plates of the *Pythium* with one liter of water. The seedlings were taken out of the soil and dipped in the inoculum for around 5 minutes. After inoculation plants were replanted in pots with regular potting soil. Assessment for resistance was done 14 days after inoculation, whereby each plant was scored in 2 categories: 0 (resistant) and 1
20 (dead/wilted). At least 2 replicates of 10 plants were done for each assay. Because the scores can vary somewhat depending on the conditions, it is essential that sufficient susceptible control plants are included to verify the intensity of the test. When the average number of plants that is resistant in a certain population was statistically higher than the number of resistant plants in the susceptible control in the same experiment, this population was considered to be resistant. However, if the
25 difference was not very convincing the experiment would be repeated to confirm the presence of the resistance.

 After several uniform lines were obtained in which the disease resistance did not segregate anymore, these lines were crossed again with internally developed *Pythium* susceptible breeding lines. Again, backcrossing and some inbreeding was done to develop improved material
30 with *Pythium* resistance. From two segregating populations, one based on a cross with susceptible internal line 021, and one based on a cross with susceptible internal line 029, DH lines were created to obtain completely homozygous lines in which the resistance could be optimally assessed. DH line 002 from the combination with line 021 was selected, and DH lines 027 and 053 from the combination with line 029 were selected. All three lines showed a good level of *Pythium*
35 resistance (**Table 1**).

Table 1 – *Pythium* bio-assay in *C. sativus* lines.

	Number		Line	Pythium plant score R/S	Pythium line score
5	15175	DH	1138	8/4	R
	15224	R source	1137 - R control	10/2	R
	15223	S line	021	0/12	S
	15226	DH	002 - F1BC3 with line 21	4/3	R
	15320	DH	002 - F1BC3 with line 21	6/0	R
	11099	F1	VENTURA - S control	0/12	S
10	11100	DH	1138	6/0	R
	11245	R source	1137 - R control	12/0	R
	11401	R source	1137 - R control	10/2	R
	11400	DH	021	2/10	S
	11244	DH	021	6/6	S
	11246	DH	002 - F1BC3 with line 21	11/1	R
	11402	DH	002 - F1BC3 with line 21	12/0	R
15	13007	F1	VENTURA - S control	2/10	S
	13008	R source	1137 - R control	12/0	R
	13010	R source	1137 - R control	12/0	R
	13097	R source	1137 - R control	11/1	R
	13028	S line	029	0/12	S
	13115	S line	029	0/12	S
20	13048	DH	027 - F2BC1 with line 29	8/4	R
	13135	DH	027 - F2BC1 with line 29	8/4	R
	13064	DH	053 - F2BC1 with line 29	10/2	R
	13151	DH	053 - F2BC1 with line 29	10/2	R

25 Crosses were made between line 002 and line 027, and between line 002 and line 053. All resulting seeds were homozygous for *Pythium* resistance. Seeds of the crosses were deposited under accession number NCIMB 42776.

EXAMPLE 2

30 *QTL mapping and marker development*

In order to map the *Pythium* resistance conferring QTL of the invention, two populations from the 1137 source in combination with susceptible internal breeding lines were developed through backcrossing and selfing. Both populations, having the same source but different backgrounds, were subsequently crossed with susceptible line 021 to develop further backcross populations that represent cultivated cucumber plants. From one population, a final BC4F1 was taken, and from the other population a BC3F1.

From the BC4F1 population and the BC3F1 population DH lines were generated using standard DH generation techniques for *C. sativus*. In this way homozygous lines could be obtained that are most suitable for mapping purposes.

From the BC4F1 population 42 DH lines were genotyped and phenotyped for *Pythium* resistance. From the BC3F1 population 43 DH lines were genotyped and phenotyped. A total of 9 highly resistant *Pythium* lines were selected for further breeding. Phenotyping was done as described in **Example 1**. Susceptible and resistant parents were also genotyped and phenotyped in the same way. Phenotypic scores were used as input for the mapping.

Genotype data for the 85 DH lines and their parents were obtained starting with an internal set of 66 SNP markers. A good linkage map was obtained covering all 7 *C. sativus* chromosomes wherein the markers were relatively equally represented. Because all material was homozygous, only A and B scores were given, indicating the presence of either the allele from the resistant source or the allele from the susceptible cultivated background.

QTL analysis was performed, and mapping of the data resulted in the identification of a QTL on chromosome 3 that still covered a relatively large area between 8,3 and 46,1 cM. To zoom in within this region a larger number of SNP markers known to be located in this stretch were used for further genotyping, resulting in finemapping of the QTL. The markers that resulted from the QTL analysis after finemapping as flanking the QTL on chromosome 3 are indicated with SEQ ID No. 1 and SEQ ID No. 2. SEQ ID No. 1 flanks the region but also co-segregates with the QTL and allele B is therefore linked to the QTL conferring resistance. SEQ ID No. 2 flanks the region and is indicative for the position of the QTL, but depending on the background allele B will be present in the resistant background, but the susceptible background can score either the A or the B allele.

The mapping of this population also resulted in the identification of a number of polymorphic SNP markers that can be used to identify the presence of the QTL on chromosome 3. The SNP markers resulting from mapping of this population that are linked to the resistance conferring QTL are indicated as SEQ ID Nos. 3, and SEQ ID Nos. 5 to 16. The sequence of these markers, as well as their genetic position on the genetic map and corresponding physical positions on the publicly available *C. sativus* genome reference sequence based on Cs9930 are listed in **Table 2**. Thus these markers may be used to identify individuals of other populations that comprise the resistance conferring QTL on chromosome 3 in their genome.

In the deposit NCIMB 42776, the presence of the QTL and the *Pythium* resistant genotype is linked to SNP markers with SEQ ID No. 1, SEQ ID No. 3, and SEQ ID Nos. 5 – 16. These SNP sequences can be used as molecular markers for identifying *Pythium* resistant plants grown from said deposit. Furthermore, since the markers were also positioned on the *C. sativus* public genome map and the actual physical positions determined (**Table 2**), these markers may be

used to identify the presence of the QTL on chromosome 3 in any other population that comprises said QTL.

Table 2 – SNP marker sequences and locations.

H>B indicates a heterozygous CNV marker, which are the markers represented by SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16. One copy of the CNV region has the SNP score of the susceptible background for these markers, the other copy of the CNV region has a SNP unique to the resistant background and therefore the presence of the CNV region. When running the marker assay for a H>B marker, a score AA means there is a homozygous presence of the A allele – the CNV region is not present, and the plant is susceptible. When the score is AABB, it means the CNV region is present and the plant is resistant. In this situation, the AABB score therefore indicates the homozygous presence of the B allele. Because normally an AABB score would be viewed as heterozygous, the marker is called herein a ‘heterozygous CNV marker’, and the score is indicated below as ‘H>B’.

A plant heterozygous for the presence of the CNV region scores AAB for heterozygous CNV markers – an A score for the chromosome without the CNV region, i.e. the A allele, and an AB score for the chromosome with the CNV region, i.e. the B allele. For homozygous CNV markers, which are markers represented by SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, and SEQ ID No. 13, the heterozygous presence of the CNV region gives an ABB score. This AAB or ABB score represents the H (heterozygous) presence of both an A and a B allele, and is present in a plant that is heterozygous for *Pythium* resistance.

The B allele of the SNPs in the sequences below, which is the allele as presented in column 2 of the Table, is present in the *Pythium* resistant plant.

Marker name	Sequence; indicating the SNP	Position of the SNP in the sequence and type of SNP (alleles A/B)	Position of the SNP in the public <i>C. sativus</i> genome 9930 V2
SEQ ID No. 1	TGGCTCCTGATCGTGGTGCCACAGTCCCAA AATTTGGTGACTGGGACGAAAGCGATCCA TCGTCATCTGAGAACTGCACTAATATTTTCA CCAGAGTGCGTGTGGAGAGACAAACGGAA GACGGAAGTTTGCCAGCTGGGACCAATGT TTCTAGTATTCGTAGTCGTTCTAGTGCCGA AAACTCAAAGAGATGTTGCTGTT	101 C/T	8423638

5	SEQ ID No. 2	ATTAAACTTTATGAAGGTTTCCCTTTTATC TTTCCTTTATCCAACAAATAAATTCTTATTT ACAACTCCACAACTTAATTATATCCCAATT TGGTATC <u>TTT</u> CAATCTTCTTCTCTTAAACA GCAACTTGGGCTGCAGCCAATCTTGCTACC GGA ACTCTGAAAGGAGAACAAGAAACATA GTCAAGTCCAGCCTCAGC	101 C/T (flanking the QTL region; score B can also be present in a susceptible plant)	10261179
10	SEQ ID No. 3	TAATAAAACATTATATATATATTTTCATCAA ATATATATAAATTAATAAATAAATAGAGA CTCGTAAGAAAGGTGTAAATCAAATAACA AAAAAATTTT <u>G</u> AAATAAATTTAACTCACTTC TTATCTTATTCAATTATTTTATCTTGATGA AATTTTGTTAAGAATAATAGTTTCTATATTT AGAGATCKRTTAAKATTT	101 A/G	9082698
20	SEQ ID No. 4	GATACGAAACTGTCTTTAATTATTTAACTT GAGTGTGGATCAAAAACACATTTACAATAG TCGTTAAAGATTAGAGAAAGCTTGGATTTT AGGAATTAAAGATTTCAAACCATTATTTGG TCACTTTGGTAGGATTGCAAGCTTCAAGAG GACTTGACAGATGAAATGGTTGGTTTAGC AAAGCAGTCGAAGAGAGCAGTCTGATAAC GAG <u>G</u> CCAATCCTTAGAGAGCATTGAGAAAG TATATTTTCTCTACTAATCGTTCATTGCTGA CGTAGTAGATATAGAAATTTTATACCTTTG GTTTCTTCAAAATATAAACAAACGCTATAG AAATTAAATAGGAAAGTCTTCGATTCGTC CCATATTCAAAGCATAGTTTGAGCAATTCT GTTTTTCAGTG	212 A/G (flanking the CNV region; score B can also be present in a susceptible plant)	9138798
30	SEQ ID No. 5	ATTTAATCTAATAGAGAGTGATTA ACTCA TGCTAGGCACATTTTAATAATCATATTA ACT GCATTCTAATTTAACAATGTAAAATGACTTT TATTATAC <u>C</u> TATTTGGAGAATTTTGTTATTT TATTATTATTGTTTTTTTTTACTACACAATTT TCATTTAAATAACCAGAAAAATGATTGACT TCCACTATTTTAAAAA	101 T/C	9294008
35				

5	SEQ ID No. 6	AAATATTTCCATATACGTGTAGGCAATGGT GGTAAATACCTAGAGGCGTCAAGATCCAA TCATAGGAGCACAGCGATCTCAACTATTTT GTGGTTGCRCCGAAAATGGGNNNNNNN NNNN <u>I</u> GAGGATGAGGAAGAGGAGAGGG AGGGGCTATGGAGAGAAATGGGGGAGGA GGTGATGGGGATATTTAAGAA	122 G/T H>B	9175772
10	SEQ ID No. 7	ATATCAGAAGAAAAAATAGACTGAATT AATCATGGATTTTGAAAA <u>AC</u> TTTTTTTTTTT TCAAGTAATATATTCTTAAGAAACCTTTAG AATCCATTTTGCATAAAATGACATTCTTTTT TATTTACATTTGGAAAAATACCATTTTTTTA TTCAAATGAAAATTATGAAAAATAGCTAAA TAAATAAAATATT	48 C/AC H>B	9188302
15	SEQ ID No. 8	GTCCTTTATAAAAAATTTGAAACGTAAATA GTTGGATTTTTCTNAAAAAA <u>A</u> AAATAGAGA CTATGTGTCATTTATATCATGTCAATAAGAT GGTCTAGAA	51 C/A H>B	9188499
20	SEQ ID No. 9	AAGCAACCTATTAATTATTCTATTGAGGGT AGCAGATTCCATCTTAGAGTTCCTGCTTCA ATCCAAGTGAAAAAAGAAAAAGAAAAAA GAAAAWAAN <u>A</u> TCATTCAGATCAAACAAAA TAGCAGTAGTAAAGTAAAAAGAAAAAA AAAAAAAAAGCAGAATAAAGGCAGCAAC GTACTTGGATGTTGAGGGG	99 T/A H>B	9193352
25	SEQ ID No. 10	NACACACACACACATTATCAAGCAAAC ATCACTAATTCCAAATGATAATTGTGTAAC ATTAATTGAAAATTCCACATGAAAAAACTG AAAGAATGAA <u>I</u> TGACATCACACTCTATTAT AATATATATGAACTGCTTTCTTAATTCTCAA TTAGTTTTGAGATAAAAAATAAATATATTAA	101 C/T	9195431
30	SEQ ID No. 11	TATTATTGGAAATGATTTTGATTTTGAATA NAAAAAAGGTGAGATCCATGTTATGTATA CATTACAATCAAATGATAAAYAATGAATTA ATGTGTGGAAT <u>G</u> ATGCAATCTAAAATTTTG GACATGCACAAAATAGAAAACATCATGCAT CNNNNNNNNNNNNNNNTACATATACTT TTAGAACAACCTTCCATCAAAT	101 C/G	9196292
35				

5

10

15

20

25

30

35

SEQ ID No. 12	TAAAAAATGTTAGATAACAAATGACAGCTA GAAAATAAATTAGTATTTTATCTTATATGA GTTTTTTTTTTTTTTTTTAAAAATATCTTCTTC TAAGAAATGAAATTATTTTCCTTTTATAGAAA TTTCTAAAATTTAGGAAATCAAATTATTCTC CCTTTTCAAATCTTTAAAATTTAGGACGTAA ATAATTTGATATTTGG	101 C/G	9201783
SEQ ID No. 13	TTATGTAAAATATCAAATACAAAAGTGAAA ATATAGAAATTATTCGTGTGATAACTTACT AATTTCAATACATTTAAGGTTAAAATTTTAA TTATTATTTCAAAGAAGTATTGTTTACCTCA AGGAAGAATCTTACATGAATCAATAATCAT TGGTCTTATGATTAATCTTTTTTTCTTTCGA AACATTT	101 G/C	9216644
SEQ ID No. 14	GGGCACTGATATAGCCAAAGCTATCCCTCC AATTGGTRATGGCTGCAGAGTACAGATCG AAATTACTATTGTAAATCAAAGGTTTGGTG AGGTCGCGAGTTTAATATTGTTTCTTGGAT TCGGTTTCTTGCTCGTGAAATCTTCGAACT GCATCTTTCATCTCCTCAAGAACGTTCTCCG GAATCCCATGGTTAATCAGTT	101 G/T H>B	9257623
SEQ ID No. 15	TCACAATGAATTCTTTTTTTATTTGAARAAC ATCTATTAGAAAGACTGTTTATCATGATCCC TTCCCTTCAGGTACCACCCATGGGTTTATTC TTCCCAACGCTTTTGGTTCCAGATGTGTATC CACCACCACCCCGTGCATGGTCAGTTGCTA ATAATCATATTCAACTAGTTTTTCATGTCAAA ATATACCTGTATGATG	101 T/C H>B	9273716
SEQ ID No. 16	TAAAMAGAGCCATAAAGTAATCAGGTAGA GTAAACCCAGATCCAACCTCCCGCATGTAC AGGACCCCCCGATCGCTCCTCATGATCAAA TAGTCATGGCTGAGTGCCAATGTAAAGCC AGCGGCAGCAGCGTGTCTGTAAATGGCAG CAATGGTAGGCATAGGAAGGGAAATGART TCGGCAACGACGGACTTGAAGATCT	101 C/G H>B	9279567

Figure 2 shows a representation of the position of the markers and the scoring for the homozygous and heterozygous CNV markers.

EXAMPLE 3

5 Identification of the copy number variant region within the QTL

Whole genome sequencing data of various *Cucumis sativus* lines, including material with *Pythium* resistance were mapped and subsequently analyzed using WGS read alignment visualization tools. After this, the sequence read data were aligned and compared against an internally generated reference genome sequence of *Cucumis sativus*. It was then determined that
10 within the QTL region on chromosome 3 that was indicative for *Pythium* resistance, a stretch could be found wherein the read depth indicated the presence of multiple copies. This meant a copy number variant (CNV) region was present in the QTL.

Marker assays combined with the CNV information resulted in markers that were flanking the CNV, and could therefore be used to indicate the position of the CNV region. These
15 flanking CNV markers are represented by SEQ ID No. 4 and SEQ ID No. 5. Because they are not within the CNV region, they score with just two alleles – A or B. Within the CNV region, around 30 annotated genes are present.

To determine if this CNV region was related to the *Pythium* resistance, markers were designed to identify SNPs that were present within this region. Since within the CNV region
20 each sequence is present twice, also a double marker score would be observed. It was found that several SNPs were present in one copy of the CNV region, but the other copy would have the same sequence as the reference or susceptible, i.e. the wildtype, genome. The scoring of these markers was rather difficult, since the sequence of the wildtype, which is indicated herein as allele A, is present twice, while the sequence of the SNP that is present in one copy of the CNV in the resistant
25 material is also present twice. The SNP sequence relating to the resistant material is indicated as allele B. This results in an ‘AABB’ marker score to indicate the homozygous presence of the CNV region. These markers are the ‘heterozygous CNV markers’.

The markers that scored in this way are represented by SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16.

30 In addition to these markers, however, also SNPs could be identified that were present in both copies of the CNV region. These markers, again scored double because of the presence of the two copies, would have a BBBB score when the CNV region is homozygously present in a plant. These markers are called herein the ‘homozygous CNV markers’. The markers that score in this way are represented by SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, and
35 SEQ ID No. 13.

Figure 2 shows the position and the scoring of these and other markers for *Pythium* resistance.

Next it was determined if the marker scores that indicated the presence of the CNV region were co-segregating with, and therefore linked to and indicative of, *Pythium* resistance. It was indeed found that the presence of the CNV region in a plant was indicative of *Pythium* resistance of that plant. It was therefore concluded that the presence of the CNV region leads to *Pythium* resistance in *Cucumis sativus*.

EXAMPLE 4

ERF gene expression of *Pythium* infected *C. sativus* plants

The CNV region that was identified to be linked to *Pythium* resistance comprises 30 genes, 3 of which were designated to be *ERF* genes. To determine the expression levels of these 3 *ERF* genes within the CNV region on chromosome 3 in cucumber, an expression analysis was performed. For this experiment the expression of these genes in plants of the invention, represented by plants of deposit NCIMB 42776, was compared with the expression in plants of the *Pythium* susceptible hybrid cucumber variety Roxanna as a control.

For both genotypes of plants of the invention and the control variety 3 plants were treated with *Pythium* infection according to the protocol described in **Example 1**. To facilitate *Pythium* infection, the roots were wounded before dipping them in the inoculum. As a control for the treatment, of each genotype also 3 plants were used as control, undergoing the same treatment including wounding of the roots, but without actual *Pythium* infection.

Subsequently, for each plant 3 stem- samples and 1 root-sample were taken and RNA was extracted. qPCR primers were designed for all 3 *ERF* genes to detect the expression. All samples were tested by performing the qPCR using SYBR-green and a relative expression analysis was performed. Results of the averages of the stem samples are presented in **Table 3**.

Table 3 – *ERF* relative gene expression comparison between resistant and susceptible plants

	Pythium	ERF1B	ERF098	ERF096
ROX	-	0,0399	0,2926	0,0584
GBN	-	0,1129	1,4026	1,3386
ROX	+	1,0505	0,3470	4,7459
GBN	+	2,0048	5,5484	7,4518

The treatment without *Pythium* infection is indicated with a '-'; when *Pythium* infection was present it is indicated with a '+'. 'GBN' are *Pythium* resistant plants of the invention; 'ROX' are plants of susceptible control variety Roxanna. *ERF1B* is the gene represented by **SEQ ID No. 17**;

ERF098 is the gene represented by **SEQ ID No 18**; *ERF096* is the gene represented by **SEQ ID No. 19**.

5 The experiment showed that the expression of each *ERF* gene was clearly higher in the resistant plants than in the susceptible control variety. The results are also graphically presented in **Figure 4**. Expression was increased when *Pythium* infection was present, but also non-infected GBN plants showed a higher expression for all genes than the susceptible control variety. The analysis to determine increased expression can therefore be performed both with and without disease pressure.

CLAIMS

1. A *Cucumis sativus* plant comprising a QTL on chromosome 3 between SEQ ID No. 1 and SEQ ID No. 2, the presence of which QTL leads to resistance against *Pythium*.

5 2. A *Cucumis sativus* plant as claimed in claim 1, wherein the *Pythium* resistance is due to the presence of a copy number variant region within the QTL, which copy number variant region is flanked by SEQ ID No. 4 and SEQ ID No. 5.

3. A *Cucumis sativus* plant as claimed in claim 2, wherein the presence of the copy number variant region can be identified by determining the presence of at least one of the markers
10 selected from the group consisting of SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, and SEQ ID No. 16.

4. A *Cucumis sativus* plant as claimed in any of the claims 1-3, wherein the *Pythium* resistance is due to the presence of at least two copies of an *ERF* gene within the copy
15 number variant region, and/or a mutant *ERF* gene within the copy number variant region.

5. A *Cucumis sativus* plant as claimed in claim 4, wherein the presence of the at least two copies of an *ERF* gene and/or the mutant *ERF* gene leads to increased expression of said *ERF* gene.

6. A *Cucumis sativus* plant as claimed in any of the claims 1-5, wherein the QTL, or the copy number variant region, or the at least two copies of an *ERF* gene is as comprised in the
20 genome of a *Cucumis sativus* plant representative seed of which was deposited with the NCIMB under deposit number NCIMB 42776.

7. Propagation material suitable for producing a *Cucumis sativus* plant as claimed in any one of the claims 1-6, wherein the propagation material is suitable for sexual reproduction, and is in particular selected from a microspore, pollen, an ovary, an ovule, an embryo sac, or an
25 egg cell; or is suitable for vegetative reproduction, and is in particular selected from a cutting, a root, a stem, a cell, a protoplast; or is suitable for tissue culture of regenerable cells, and is in particular selected from a leaf, pollen, an embryo, a cotyledon, a hypocotyl, a meristematic cell, a root, a root tip, an anther, a flower, a seed, or a stem, and wherein the propagation material
30 comprises the QTL as defined in claim 1, or the copy number variant region as defined in claim 2 or 3, or the two copies of an *ERF* gene within the copy number variant region as defined in claim 4, or the mutant *ERF* gene as defined in claim 4 or 5.

8. Marker for the identification of *Pythium* resistance in a *Cucumis sativus* plant, which marker is selected from the group consisting of SEQ ID Nos. 1-16.

9. Use of a marker as claimed in claim 8 for identification of *Pythium* resistance in a *Cucumis sativus* plant.

10. Method for producing a *Pythium* resistant *Cucumis sativus* plant comprising introducing a QTL as defined in claim 1, or introducing a copy number variant region as defined in claim 2 or 3, or introducing at least one extra copy of an *ERF* gene as defined in claim 4, or introducing a mutant *ERF* gene as defined in claim 4 or 5.

11. Method for selecting a *Pythium* resistant *Cucumis sativus* plant, comprising determining the presence of the QTL as defined in claim 1, or the copy number variant region as defined in claim 2 or 3, or determining the presence of at least two copies of an *ERF* gene or of a mutant *ERF* gene as defined in claim 4 or 5, and selecting a plant that comprises the QTL, or the copy number variant region, or the at least two copies of an *ERF* gene or the mutant *ERF* gene as a *Pythium* resistant plant.

12. Seed, wherein the seed comprises the QTL as defined in claim 1, or the copy number variant region as defined in claim 2 or 3, or the at least two copies of an *ERF* gene or the mutant *ERF* gene as defined in claim 4 or 5.

13. A method for producing a *Cucumis sativus* plant which is resistant against *Pythium*, said method comprising:

a) crossing a plant as claimed in any one of the claims 1-6 with another plant to obtain an F1 population;

b) optionally performing one or more rounds of selfing and/or crossing a plant from the F1 to obtain a further generation population;

c) selecting from the population a plant that comprises the QTL as defined in claim 1, or the copy number variant region as defined in claim 2 or 3, or the at least two copies of an *ERF* gene a mutant *ERF* gene as defined in claim 4 or 5, which plant is resistant against *Pythium*.

14. The method as claimed in claim 13, wherein the plant as claimed in any one of the claims 1-6 is a plant grown from seed deposited under NCIMB accession number 42776, or from progeny thereof.

FIG. 1***Ethylene responsive transcription factor (ERF) gene sequences***

SEQ ID No. 17 – Cucumis sativus

> class=Sequence position=cs_9930 V2 Chr3:9197024..9197710 (- strand)

ATGGATTATT CTGCATTCAT CTCCCCGCTT TCTGATTTCT CATCCGAATC ATCTTTCGGT TCACCCGAAT CCTCCTTCAC
CAATTTGGAC CATAATTTTC TCCCTTTCAA TGAAAATGAC TCAGAGGAAA TGCTTCTTTA CGGCCTAATC
TCCGAGGGCA CATACTGAATC ATTCGATACA AGTATCGGAA CCGTGCAAGT GAAGGAAGAG GAAGTCGATT
CCATCGGAGA AGAAAGCCCG AAGAAAGAGA GGGCTTATAG AGGAGTTCGC CGCCGTCCAT GGGGGAAATT
TGCGGCGGAA ATTAGAGATT CCACTAGACA TGGTACAAGG GTATGGTTGG GAACTTTCGA TAGTGCTGAA
GCCGCCGCTT TGGCTTACGA TCAAGCTGCC TTTTCGATGA GGGGCGCTGC CGCAATTCTC AATTTTCCTG
TCGACAGAGT TAGAGAGTCT CTCAAAGAGA TGAACGCCGG CAGTGGGGGC AGCGGTGATA GTTTAGCCGA
AGACGGCGGC TCTCCGGTAG TGGCGTTAAA AAGAAAACAC TCGATTAGAA GGAAAGCCAT AGGTAAAAAG
AGCAAAGAGA GAGATGTGAG GATTCAAAC GTGGTGGTTT TGGAAGATTT AGGGACAGAG TATTTGGAAG
AACTTTTGGG GTCTTCTCAA AGTGATAGCC CTTCTTGTTT TTTCTAA

SEQ ID No. 18 – Cucumis sativus

> class=Sequence position=cs_9930 V2 Chr3:9208901..9209353 (+ strand)

ATGGAGGATC ATCGTAAGGG TAAAGAACAA CAAAAGCATG GTGACGATGG GATCAAGTAC CGGGGTGTGC
GACGTCGCCC ATGGGGGAAA TATGCAGCGG AGATACGTGA TCCGTCGAAG AATGGGGCTA GACAATGGCT
TGGGACCTAC GAAACGGCGG AGGATGCAGC TAGGGCTTAC GATCAGAGGG CATTTCAGTT GAAAGGTCAT
CTTGCTAGTT TGAATTTTCC TAGTGAATAT TATGCTCGTG TCATGGGTTT ACCTCCTCAT CCTCCTAACT TGTTTTCTTC
GACTTCGATC AATTCGGGTT TTAGACAGCGG TGGTGTGGT GGTGGATCGT CGACTTCTAA CATCGATCCT
CACAAAGTTA TTGTGTTTGA GTATGTGGAT GGTAGGGTTT TGGAAGACCT TCTGGCTCAA GAGGATAAAA
AGAAGAAGAA GAATAGTAAA TAA

SEQ ID No. 19 – Cucumis sativus

> class=Sequence position=cs_9930 V2 Chr3:9217516..9217917 (- strand)

ATGGACGAGA GTGGTGGTCG TGGAAGAGGT TATGGGGACG ACTCCACAGG CAGCAGAGAG ATTCGTTACC
GGGGAGTACG ACGTCGGCCA TGGGGAAAAT TCGCTGCTGA AATACGAGAC TCTAGAAGGC AAGGAGTACG
GATATGGCTA GGGACTTTCA AACTGCAGA AGAAGCAGCA CGAGCTTACG ATCGAGCGGC CTACAACATG
AGGGGTCATT TGGCCATTTT GAATTTTCCT AATGAATATC CGCTTACCAG GGGTGGGGCT TATTCGAGTG
GGTCATCTTC TTCTTCTCA ATGTCAATGC GGCAAATGA AGTGATTGAA TTTGAGTATT TGGATGATAA
AGTGCTGGAA GATCTTCTTG ACTATGGAGA AGAAAGTGAT AAGAGAAGCT AA

FIG. 2

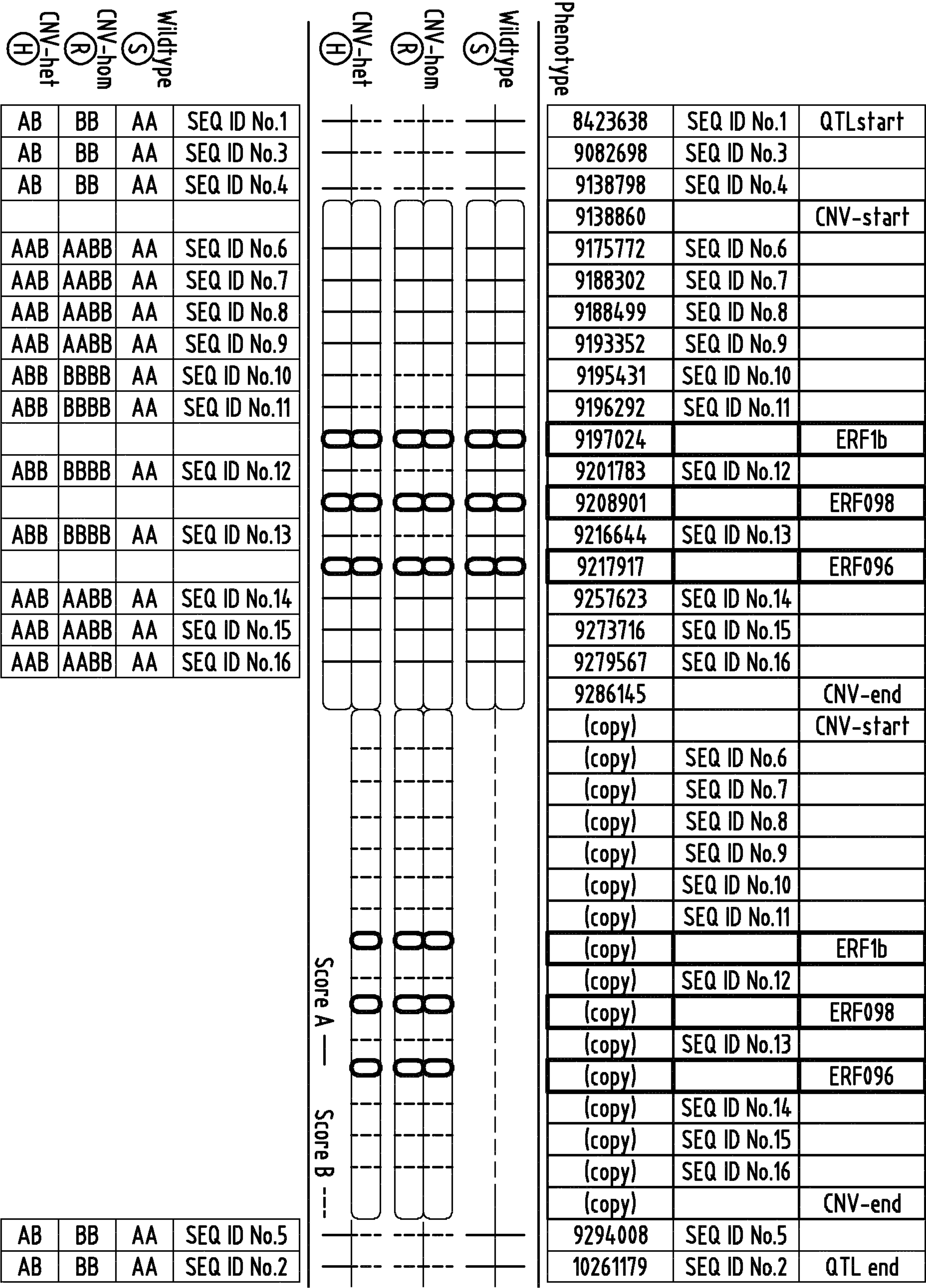


FIG. 3

AP-2 domain sequences of the *ERF* genes in the CNV

SEQ ID No. 20 – AP-2 domain of the *ERF* gene represented by SEQ ID No. 17

YRGVRRRPWKGFAAEIRDSTRHGTRVWLGTFDSEAAAALAYDQAAFSMRGAAAILNFPVD

SEQ ID No. 21 – AP-2 domain of the *ERF* gene represented by SEQ ID No. 18

YRGVRRRPWKGKYAAEIRDPSKNGARQWLGTYETAEDAARAYDQRAFQLKGHLASLNFPSE

SEQ ID No. 22 – AP-2 domain of the *ERF* gene represented by SEQ ID No. 19

YRGVRRRPWKGFAAEIRDSRRQGVRIWLGTFTAEAAARAYDRAAYNMRGHLAILNFPNE

FIG. 4

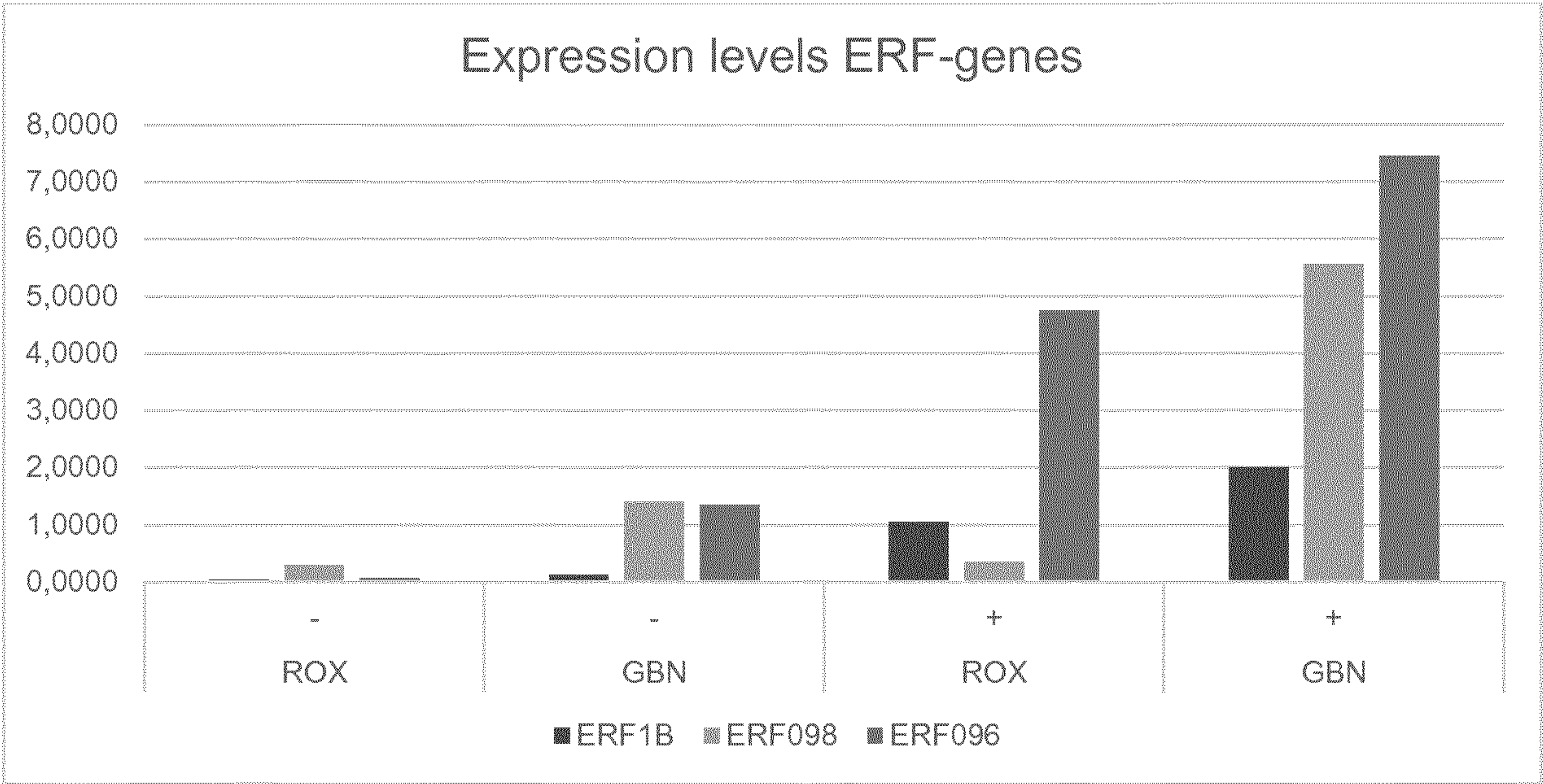
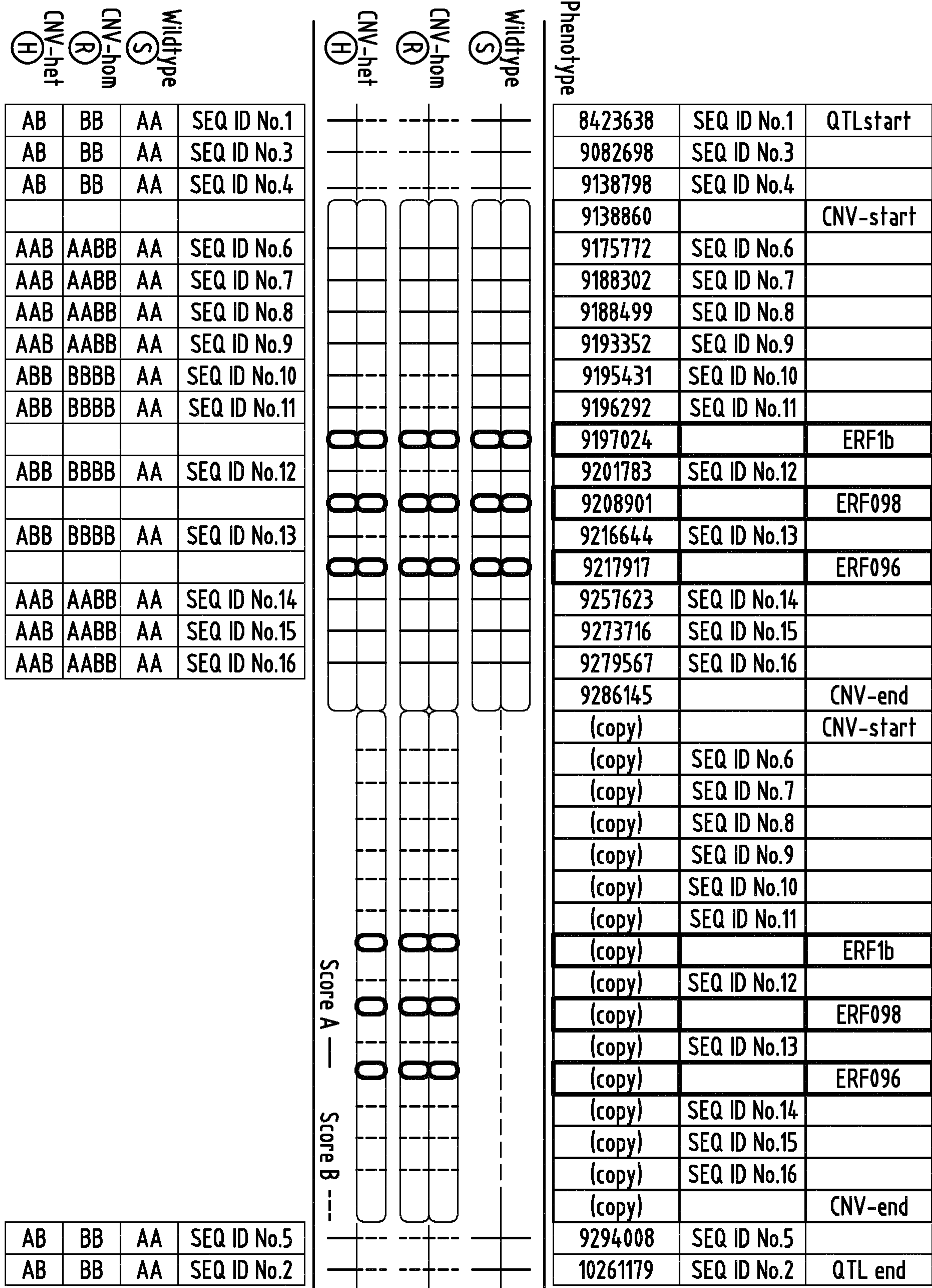


FIG. 2



AB	BB	AA	SEQ ID No.5
AB	BB	AA	SEQ ID No.2