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(72) **Inventeurs/Inventors:**
RIKKERINK, HENDRIKUS ANTONIUS, NZ;
HILARIO-ANDRADE, ELENA MARIA, NZ;
DARE, ANDREW PATRICK, NZ;
GARDINER, SUSAN ELIZABETH, NZ;
YOON, MINSOO, NZ;
BUS, VINCENT GERARDUS MARIA, NZ

(73) **Propriétaire/Owner:**

(54) **Titre : GENE DE RESISTANCE ET SES UTILISATIONS**
(54) **Title: RESISTANCE GENE AND USES THEREOF**

(57) **Abrégé/Abstract:**
The invention provides methods for producing a plant with altered resistance to powdery mildew, the methods comprising transformation of a plant with a genetic construct including a polynucleotide encoding of a polypeptide with the amino acid sequence of SEQ ID NO- 1 or a variant of fragment thereof The invention also provides isolated polypeptides, polynucleotides, contructs and vectors useful for producing a plant cell and plants tranformed to contain and express the polypeptides, polynucleotides and constructs The invention also provides plants produced by methods of the invention.

(73) **Propriétaires(suite)/Owners(continued):**
THE NEW ZEALAND INSTITUTE FOR PLANT AND FOOD RESEARCH LIMITED, NZ

(74) **Agent:** AIRD & MCBURNEY LP

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(71) Applicant (*for all designated States except US*): THE HORTICULTURAL AND FOOD RESEARCH INSTITUTE OF NEW ZEALAND [NZ/NZ]; Mt Albert Research Centre, 120 Mt Albert Road, Mt Albert, Auckland (NZ).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): RIKKERINK, Hendrikus Antonius [NL/NZ]; 48 Rosewarne Crescent, Glendene, 0602 Waitakere (NZ). HILARIO-ANDRADE, Elena Maria [MX/NZ]; 5 Mountfield Terrace, Owairaka, 1025 Auckland (NZ). DARE, Andrew Patrick [NZ/NZ]; 26 Wyoming Avenue, Murrays Bay, 0630 North Shore City (NZ). GARDINER, Susan Elizabeth [NZ/NZ]; 115 Staces Road, RD 1, 4471 Palmerston North (NZ). YOON, Minsoo [NZ/NZ]; 467 Dominion Road, Mount Eden, 1024 Auckland (NZ). BUS, Vincent Gerardus Maria [NL/NZ]; 295 Meeanee Road, 4112 Napier (NZ).

(74) Agents: ADAMS, Matthew, D. et al.; A J Park, 6th Floor Huddart Parker Building, Post Office Square, P O Box 949, 6015 Wellington (NZ).

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(57) Abstract: The invention provides methods for producing a plant with altered resistance to powdery mildew, the methods comprising transformation of a plant with a genetic construct including a polynucleotide encoding of a polypeptide with the amino acid sequence of SEQ ID NO: 1 or a variant of fragment thereof. The invention also provides isolated polypeptides, polynucleotides, contructs and vectors useful for producing a plant cell and plants tranformed to contain and express the polypeptides, polynucleotides and constructs. The invention also provides plants produced by methods of the invention.

WO 2009/058030 A3

RESISTANCE GENE AND USES THEREOF

TECHNICAL FIELD

The present invention is in the field of disease resistance genes from plants.

BACKGROUND ART

The development of apple (*Malus x domestica)* cultivars carrying natural resistance against major pathogens and pests is a crucial component of any successful breeding strategy. The two most significant fungal diseases of apple are apple scab and powdery mildew. Powdery mildew (caused by *Podosphaera leucotricha)* is a particularly serious problem in relatively dry apple growing areas but is prevalent in all growing regions.

A number of sources of resistance to powdery mildew have been identified in non-commercial apples and breeding to incorporate these resistances into new commercial varieties is in progress using various strategies [11, 13, 26, 27].

Two sources of resistance in particular have been used in a number of different breeding programmes. These sources of powdery mildew resistance are: 1. An open-pollinated seedling of *Malus zumi* (MAL68/5) carrying the *Pl2* resistance locus, and 2. An open-pollinated seedling of *Malus robusta* (MAL59/9) carrying the *Pl1* resistance locus. Genetic analysis of these sources has indicated that in some genetic backgrounds at least these loci appear to segregate as a single major dominant locus for resistance [10, 24, 29]. Other powdery mildew resistance loci that have been genetically characterized include *PlMIS* [8], *Pld* [40], *Pl8* [25] and *Plw* [15]. None of the genes responsible for these resistances have been isolated.

To date, about 70 resistance genes have been cloned from at least 14 different plant species conferring resistance to various diseases [28]. The encoded proteins have been grouped into classes based on a number of characteristic domains.

The first class consists of genes encoding proteins with characteristics of serine/threonine (S/T) kinases. This class includes the first cloned plant disease resistance gene, *Pto* from tomato [29]. This class also includes two close relatives of the *Pto* gene, the *LhirPto* [33] and *Fen* [30, 34] genes, and the *Rpg1* gene [4].

The second class of resistance genes consists of those encoding proteins containing a central nucleotide binding site (NBS) and a carboxy terminal leucine-rich repeat (LRR). The first of these genes to be cloned were the *Arabidopsis thaliana RPS2* [2] and *Nicotiana tabacum N* [41] genes. The *N* gene represents the first member of a subclass with Toll-Interleukin-1 like receptor domains at the amino terminus. The *RPS2* gene represents the first member of the CC-NBS-LLR subclass with leucine zippers or coiled coil (CC) motifs at the amino terminus. This subclass is sometimes also referred to as non-TIR.

A third major class of resistance genes, the xLRR class, consists of those encoding proteins composed almost entirely of leucine rich repeats (LRRs) that are predicted to reside in an extracellular environment based on their amino acid sequence [21]. The *Cf-9* gene [22] was the first gene cloned in this class. Most of the genes in this class have been cloned from tomato (*Cf* genes) and confer resistance against the leaf mold *Cladosporium fulvum*. The V_f gene from apple confers resistance to apple scab and belongs to the xLRR class of resistance genes [1].

A fourth class of resistance genes consists of those encoding proteins with an amino terminal serine/threonine protein kinase domain with homology to the *Pto* gene, a carboxy terminal LRR domain with homology to the *Cf* genes and a central putative transmembrane region [38]. These genes have all the hallmarks of a transmembrane receptor kinase. Receptor kinases are often involved in mammalian ligand mediated signalling (e.g. hormone receptors) with the protein kinase acting as the signalling domain inside the cell and the LRR conferring specificity in the extracellular environment [3]. The *Xa21* gene from rice is a member of this class.

A small number of other disease resistance genes that do not fit neatly into one of these four classes have recently been cloned. These include the *mlo* gene [5] from barley, the $Hs1^{Pro\text{-}1}$ gene [6] from sugar beet and the *Ve* genes [23] from tomato. The

mlo gene has a putative 7 transmembrane structure and shares no domains with other cloned resistance genes whereas the $Hs1^{Pro\text{-}1}$ gene contains LRRs and the *Ve* gene contains LRRs, PEST sequences, leucine zippers and potential signals for receptor mediated endocytosis.

Powdery mildew resistance in apple is subject to heterogeneity at the phenotypic, and possibly also genetic, levels. Typically resistant progeny are not reliably identifiable based on nursery phenotypes [20] or using (macroscopic) symptom development in the field. Because of this, resistance is sometimes not scored until the plants have matured in the orchard over several years [10]. This makes the screening for resistance against this important pathogen of apple by traditional means especially difficult and time consuming.

The cloning of a gene for resistance against apple powdery mildew would constitute a significant advance and would have a number of advantages over the traditional breeding routes for resistance.

It is therefore an object of the invention to provide compositions and methods useful for conferring powdery mildew resistance in plants and/or at least to provide the public with a useful choice to this end.

SUMMARY OF THE INVENTION

In the first aspect the invention provides an isolated polynucleotide encoding a polypeptide that comprises the sequence of SEQ ID NO: 1 or a fragment or variant thereof, wherein the fragment or variant confers resistance to powdery mildew in a plant.

Preferably the polypeptide or variant has a sequence characteristic of a non-TIR class disease resistance protein. More preferably the polypeptide or variant has a sequence characteristic of a CC-NBS-LRR class disease resistance protein.

Preferably the fragment comprises sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a non-TIR class disease

resistance protein. More preferably the fragment comprises sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a CC-NBS-LRR class disease resistance protein.

Preferably the CC domain is at the N-terminal end of the polypeptide relative to the NBS domain.

In one embodiment the fragment comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 6.

In a further embodiment the fragment comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

In a further embodiment the fragment comprises a sequence the sequence of SEQ ID NO: 6.

In a further embodiment the fragment comprises a sequence the sequence of SEQ ID NO: 5.

In a further embodiment the variant comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 1.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 1.

In a further aspect the invention provides an isolated polynucleotide comprising the sequence of SEQ ID NO: 3 or a fragment or variant thereof, wherein the fragment or variant encodes a polypeptide that confers resistance to powdery mildew in a plant.

Preferably the polynucleotide or variant encodes a polypeptide with a sequence characteristic of a non-TIR class disease resistance protein. More preferably the polypeptide has a sequence characteristic of a CC-NBS-LRR class disease resistance protein.

Preferably the fragment encodes a polypeptide comprising sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a non-TIR class disease resistance protein. More preferably the fragment encodes a polypeptide comprising sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a CC-NBS-LRR class disease resistance protein.

Preferably the CC domain is at the N-terminal end of the polypeptide relative to the NBS domain.

In one embodiment the fragment comprises a sequence with at least 70% sequence identity to SEQ ID NO: 8.

In one embodiment the fragment comprises a sequence with at least 70% sequence identity to SEQ ID NO: 7.

In a further embodiment the fragment comprises the sequence of SEQ ID NO: 8.

In a further embodiment the fragment comprises the sequence of SEQ ID NO: 7.

In one embodiment the variant comprises a sequence with at least 70% sequence identity to sequence of SEQ ID NO: 3.

In a further embodiment the polynucleotide comprises the sequence of SEQ ID NO: 3.

In a further embodiment the variant comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 2.

In a further embodiment the polynucleotide comprises the sequence of SEQ ID NO: 2.

In a further aspect the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 1, or a fragment or variant thereof, wherein the fragment or variant confers resistance to powdery mildew in a plant.

Preferably the polypeptide or variant has a sequence characteristic of a non-TIR class disease resistance protein. More preferably the polypeptide or variant has a sequence characteristic of a CC-NBS-LRR class disease resistance protein.

Preferably the fragment comprises sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a non-TIR class disease resistance protein. More preferably the fragment comprises sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a CC-NBS-LRR class disease resistance protein.

Preferably the CC domain is at the N-terminal end relative to the NBS domain.

In one embodiment the fragment comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 6.

In a further embodiment the fragment comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

In a further embodiment the fragment comprises the sequence of SEQ ID NO: 6.

In a further embodiment the fragment comprises the sequence of SEQ ID NO: 5.

In a further embodiment the variant comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 1.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 1.

In a further aspect the invention provides an isolated polynucleotide encoding a polypeptide that comprises the sequence of SEQ ID NO: 6 or a variant thereof, wherein the variant confers resistance to powdery mildew in a plant.

Preferably the polypeptide or variant comprises sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a non-TIR class disease resistance protein. More preferably the fragment comprises sequences

characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a CC-NBS-LRR class disease resistance protein.

In one embodiment the variant comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 6.

In a further embodiment the variant comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 6.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 5.

In a further embodiment the polypeptide comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 1.

Preferably the polypeptide encodes a polypeptide with sequences characteristic of a CC-NBS-LRR class disease resistance protein.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 1.

In a further aspect the invention provides an isolated polynucleotide comprising the sequence of SEQ ID NO: 8 or a variant thereof, wherein the variant encodes a polypeptide that confers resistance to powdery mildew in a plant.

Preferably the polypeptide comprises sequences characteristic of a coiled coil (CC) and nucleotide binding site (NBS) domain of a non-TIR class disease resistance protein. More preferably the polypeptide comprises sequences characteristic of a coiled coil (CC) and a nucleotide binding site domain of a CC-NBS-LRR class disease resistance protein.

Preferably the CC domain is at the N-terminal end relative to the NBS domain.

In one embodiment the variant comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 8.

In one embodiment the polynucleotide comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 7.

In one embodiment the polynucleotide comprises the sequence of SEQ ID NO: 8.

In one embodiment the polynucleotide comprises the sequence of SEQ ID NO: 7.

In one embodiment the polynucleotide comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 1.

In one embodiment the polynucleotide comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 2.

In one embodiment the polynucleotide comprises the sequence of SEQ ID NO: 1.

In one embodiment the polynucleotide comprises the sequence of SEQ ID NO: 2.

In a further aspect the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 6, or a variant thereof, wherein the variant confers resistance to powdery mildew in a plant.

Preferably the variant comprises sequences characteristic of a coiled coil (CC) and a nucleotide binding site (NBS) domain of a non-TIR class disease resistance protein. More preferably the polypeptide comprises sequences characteristic of a coiled coil (CC) and a nucleotide binding site domain of a CC-NBS-LRR class disease resistance protein.

Preferably the CC domain is at the N-terminal end relative to the NBS domain.

In one embodiment the variant comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 6.

In a further embodiment the polypeptide comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

In a further embodiment the polypeptide comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 1.

Preferably the polypeptide comprises sequences characteristic of a CC-NBS-LRR class disease resistance protein.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 6.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 5.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 1.

In a further aspect the invention provides an isolated polynucleotide encoding a polypeptide of the invention.

In a further aspect the invention provides an isolated polynucleotide comprising:

a) a polynucleotide comprising a fragment, of at least 15 nucleotides in length, of a polynucleotide of the invention;

b) a polynucleotide comprising a complement, of at least 15 nucleotides in length, of the polynucleotide of the invention; or

c) a polynucleotide comprising a sequence, of at least 15 nucleotides in length, capable of hybridising to the polynucleotide of the invention under stringent hybridisation conditions.

In a further aspect the invention provides a genetic construct which comprises a polynucleotide of the invention.

In a further aspect the invention provides an expression construct which comprises a polynucleotide of the invention.

In a further aspect the invention provides an RNAi construct which comprises a polynucleotide of the invention.

In a further aspect the invention provides a vector comprising an expression construct, genetic construct or RNAi construct of the invention.

In a further aspect the invention provides a host cell comprising an expression construct or genetic construct of the invention.

In a further aspect the invention provides a host cell genetically modified to express a polynucleotide or polypeptide of the invention.

Preferably the host cell is genetically modified to express a polynucleotide encoding a polypeptide that confers powdery mildew resistance on a plant.

In a further aspect the invention provides a plant cell which comprises an expression construct, genetic construct or RNAi construct of the invention.

In a further aspect the invention provides a plant cell genetically modified to express a polynucleotide of the invention, or a polypeptide of the invention.

Preferably the plant cell is genetically modified to express a polynucleotide encoding a polypeptide that confers powdery mildew resistance on a plant.

In a further aspect the invention provides a plant which comprises a plant cell of the invention.

Preferably the plant has increased resistance to powdery mildew.

In a further aspect the invention provides a method for producing a plant cell or plant with increased resistance to powdery mildew, the method comprising transformation of a plant cell or plant with a polynucleotide encoding polypeptide with the amino acid sequence of SEQ ID NO: 1 or a fragment or variant thereof, wherein the fragment or variant confers resistance to powdery mildew in a plant.

Preferably the polypeptide has a sequence characteristic of a CC-NBS-LRR class disease resistance protein.

Preferably the fragment comprises sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a non-TIR class disease resistance protein. More preferably the polypeptide comprises sequences characteristic of a coiled coil (CC) and a nucleotide binding site domain of a CC-NBS-LRR class disease resistance protein.

In one embodiment the fragment comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 6.

In a further embodiment the fragment comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

In a further embodiment the fragment comprises the sequence of SEQ ID NO: 6.

In a further embodiment the fragment comprises a sequence the sequence of SEQ ID NO: 5.

In a further embodiment the variant comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 1.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 1.

In a further aspect the invention provides a method for producing a plant cell or plant with increased resistance to powdery mildew, the method comprising transformation of a plant cell or plant with a polynucleotide comprising the sequence of SEQ ID NO: 3 or a fragment or variant thereof, wherein the fragment or variant encodes a protein that confers powdery mildew resistance in a plant.

Preferably the polypeptide has a sequence characteristic of a CC-NBS-LRR class disease resistance protein.

In one embodiment the fragment comprises a sequence with at least 70% sequence identity to SEQ ID NO: 8.

In one embodiment the fragment comprises a sequence with at least 70% sequence identity to SEQ ID NO: 7.

In a further embodiment the fragment comprises the sequence of SEQ ID NO: 8.

In a further embodiment the fragment comprises the sequence of SEQ ID NO: 7.

In one embodiment the variant comprises a sequence with at least 70% sequence identity to sequence of SEQ ID NO: 3.

In a further embodiment the polynucleotide comprises the sequence of SEQ ID NO: 3.

In a further embodiment the variant comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 2.

In a further embodiment the polynucleotide comprises the sequence of SEQ ID NO: 2.

In a further aspect the invention provides a method for producing a plant cell or plant with increased resistance to powdery mildew, the method comprising transformation of a plant cell or plant with a polynucleotide encoding polypeptide with the amino acid sequence of SEQ ID NO: 6 or a variant thereof, wherein the variant confers resistance to powdery mildew in a plant.

Preferably the variant comprises sequence characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a CC-NBS-LRR class disease resistance protein.

In one embodiment the variant comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 6.

In a further embodiment the polypeptide comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 6.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 5.

In a further embodiment the polypeptide comprises a sequence with at least 70% identity to the sequence of SEQ ID NO: 1.

Preferably the variant encodes a polypeptide with sequences characteristic of a CC-NBS-LRR class disease resistance protein.

In a further embodiment the polypeptide comprises the sequence of SEQ ID NO: 1.

In a further aspect the invention provides a method for producing a plant cell or plant with increased resistance to powdery mildew, the method comprising transformation of a plant cell or plant with a polynucleotide comprising the sequence of SEQ ID NO: 8 or a variant thereof, wherein the variant encodes a polypeptide that confers powdery mildew resistance in a plant.

Preferably the polypeptide comprises sequences characteristic of a coiled coil (CC) domain and a nucleotide binding site (NBS) domain of a CC-NBS-LRR class disease resistance protein.

Preferably the CC domain is at the N-terminal end of the protein relative to the NBS domain.

In one embodiment the variant comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 8.

In one embodiment the polynucleotide comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 7.

In one embodiment the polynucleotide comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 1.

In one embodiment the polynucleotide comprises a sequence with at least 70% sequence identity to the sequence of SEQ ID NO: 2.

In one embodiment the variant comprises the sequence of SEQ ID NO: 8.

In one embodiment the polynucleotide comprises the sequence of SEQ ID NO: 7.

In one embodiment the polynucleotide comprises the sequence of SEQ ID NO: 1.

In one embodiment the polynucleotide comprises the sequence of SEQ ID NO: 2.

In a further aspect the invention provides a method for selecting a plant with increased resistance to powdery mildew, the method comprising testing of a plant for altered expression of a polynucleotide of the invention.

In a further aspect the invention provides a method for selecting a plant with increased resistance to powdery mildew, the method comprising testing of a plant for altered expression of a polypeptide of the invention.

In a further aspect the invention provides a plant cell or plant produced by the method of the invention. Preferably the plant is genetically modified to include a polynucleotide or polypeptide of the invention.

In a further aspect the invention provides a group of plants selected by the method of the invention.

In a further aspect the invention provides an antibody raised against a polypeptide of the invention.

The polynucleotides and variants of polynucleotides, of the invention may be derived from any species. The polynucleotides and variants may also be recombinantly produced and also may be the products of "gene shuffling' approaches.

In one embodiment the polynucleotide or variant, is derived from a plant species.

In a further embodiment the polynucleotide or variant, is derived from a gymnosperm plant species.

In a further embodiment the polynucleotide or variant, is derived from an angiosperm plant species.

In a further embodiment the polynucleotide or variant, is derived from a monocotyledonous plant species.

In a further embodiment the polynucleotide or variant, is derived from a dicotyledonuous plant species.

The polypeptides and variants of polypeptides of the invention may be derived from any species. The polypeptides and variants may also be recombinantly produced and may also be expressed from the products of "gene shuffling' approaches.

In one embodiment the polypeptides or variants of the invention are derived from plant species.

In a further embodiment the polypeptides or variants of the invention are derived from gymnosperm plant species.

In a further embodiment the polypeptides or variants of the invention are derived from angiosperm plant species.

In a further embodiment the polypeptides or variants of the invention are derived from monocotyledonous plant species.

In a further embodiment the polypeptides or variants of the invention are derived from dicotyledonous plant species.

The plant cells and plants of the invention, including those from which the polynucleotides, variant polynucleotides, polypeptide and variant polypeptides may be derived, and including plant cells and plants to be transformed or selected, may be from any species.

In one embodiment the plant cells and plants are from gymnosperm species.

In a further embodiment the plant cells and plants are from an angiosperm species.

In a further embodiment the plant cells and plants are from a dicotyledonuous species.

In a further embodiment the plant cells and plants are from a fruit species selected from a group comprising but not limited to the following genera: *Actinidia, Malus, Citrus, Fragaria, Vaccinium, Pyrus, Prunus, Rosa, Fragaria, Rubus, Cydonia Eriobotya, Mespilus, Photinia, Pyracantha, Sorbus, Humus, Ficus, Morus, Ulmus, Cucumus, Cucurbita, Arachis, Cicer, Lupinus, Cyamopsis, Lotus, Glycine, Phaseolus, Vigna, Medicago, Trifolium, Pisum, Vicia, Betula, Fagus, Juglans, Ricinus, Manihot, Hevea, Euphorbia, Saliceae.*

Particularly preferred fruit plant species are: *Actidinia deliciosa, A. chinensis, A. eriantha, A. arguta, hybrids of the four Actinidia species, Malus domestica, Malus zumi, Malus sylvestris, Malus sieversii and Malus sieboldii.*

The most preferred plant family is the Rosaceae.

The most preferred genus is *Malus*.

The most preferred *Malus* species are *Malus zumi* and *Malus domestica*.

In a further embodiment the plant cells and plants are from a vegetable species selected from a group comprising but not limited to the following genera: *Brassica, Lycopersicon and Solanum.*

5 Particularly preferred vegetable plant species are: *Lycopersicon esculentum* and *Solanum tuberosum.*

In a further embodiment the plant cells and plants of the invention are from monocotyledonous species.

10

In a further embodiment the plant cells and plants are from a crop species selected from a group comprising but not limited to the following genera: *Glycine, Zea, Hordeum and Oryza.*

15 Particularly preferred crop plant species are: *Oryza sativa, Glycine max* and *Zea mays.*

In accordance with another aspect, there is provided an isolated polynucleotide encoding a polypeptide that comprises the sequence of SEQ ID NO: 1 or a fragment
20 or variant thereof, wherein the fragment or variant confers resistance to powdery mildew in a plant, and wherein the fragment comprises:

a) a sequence with at least 70% identity to the entire length of sequence of SEQ ID NO: 6,
b) a sequence with at least 70% identity to the entire length of sequence of
25 SEQ ID NO: 5,
c) the sequence of SEQ ID NO: 6, or
d) the sequence of SEQ ID NO: 5, and wherein the variant comprises:
e) a sequence with at least 70% identity to the entire length of sequence of SEQ ID NO: 1.

30

In accordance with another aspect, there is provided anisolated polynucleotide comprising the sequence of SEQ ID NO: 3 or a fragment or variant thereof, wherein the fragment or variant encodes a polypeptide that confers resistance to powdery mildew in a plant, and wherein the fragment comprises:

a) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 8,
b) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 7,
5 c) the sequence of SEQ ID NO: 8, or
d) the sequence of SEQ ID NO: 7, and wherein the variant comprises:
e) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 3, or
f) a sequence with at least 70% sequence identity to the entire length of the
10 sequence of SEQ ID NO: 2.

In accordance with another aspect, there is provided anisolated polypeptide comprising the amino acid sequence of SEQ ID NO: 1, or a fragment or variant thereof, wherein the fragment or variant confers resistance to powdery mildew in a
15 plant, and wherein the fragment comprises:
a) a sequence with at least 70% identity to the entire length of the sequence of SEQ ID NO: 6,
b) a sequence with at least 70% identity to the entire length of the sequence of SEQ ID NO: 5,
20 c) the sequence of SEQ ID NO: 6, or
d) the sequence of SEQ ID NO: 5, and the variant comprises:
e) a sequence with at least 70% identity to the entire length of the sequence of SEQ ID NO: 1.

25 In accordance with another aspect, there is provided anisolated polynucleotide encoding a polypeptide that comprises the sequence of SEQ ID NO: 6 or a variant thereof, wherein the variant confers resistance to powdery mildew in a plant, and wherein the variant comprises at least one of:
a) a sequence with at least 70% identity to the sequence of SEQ ID NO: 6, and
30 b) a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

In accordance with another aspect, there is provided an isolated polynucleotide comprising the sequence of SEQ ID NO: 8 or a variant thereof, wherein the variant

encodes a polypeptide that confers resistance to powdery mildew in a plant, and wherein the variant comprises at least one of:

a) a sequence with at least 70% sequence identity to entire length of the the sequence of SEQ ID NO: 8, and

5 b) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 7.

In accordance with another aspect, there is provided an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 6, or a variant thereof, wherein

10 the variant confers resistance to powdery mildew in a plant, and wherein the variant comprises at least one of:

a) a sequence with at least 70% identity to the entire length of the sequence of SEQ ID NO: 6, and

b) a sequence with at least 70% identity to the entire length of the sequence of

15 SEQ ID NO: 5.

DETAILED DESCRIPTION

In this specification where reference has been made to patent specifications, other

20 external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

25

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in

30 the same manner.

Polynucleotides and fragments

The term “polynucleotide(s),” as used herein, means a single or double-stranded deoxyribonucleotide or ribonucleotide polymer of any length but preferably at least 15 nucleotides, and include as non-limiting examples, coding and non-coding sequences of a gene, sense and antisense sequences complements, exons, introns, genomic DNA, cDNA, pre-mRNA, mRNA, rRNA, siRNA, miRNA, tRNA, ribozymes, recombinant polypeptides, isolated and purified naturally occurring DNA or RNA sequences, synthetic RNA and DNA sequences, nucleic acid probes, primers and fragments.

A “fragment” of a polynucleotide sequence provided herein is a subsequence of the polynucleotide sequence consisting of a contiguous stretch of nucleotides of the polynucleotide sequence, e.g., a sequence that is at least 15 nucleotides in length. The fragments of the invention preferably comprise at least 15 nucleotides, preferably at least 20 nucleotides, more preferably at least 30 nucleotides, more preferably at least 50 nucleotides, more preferably at least 50 nucleotides and most preferably at least 60 nucleotides of contiguous nucleotides of a polynucleotide of the invention. A fragment of a polynucleotide sequence can be used in antisense, gene silencing, triple helix or ribozyme technology, or as a primer, a probe, included in a microarray, or used in polynucleotide-based selection methods of the invention.

The term “primer” refers to a short polynucleotide, usually having a free 3’OH group, that is hybridized to a template and used for priming polymerization of a polynucleotide complementary to the target.

The term “probe” refers to a short polynucleotide that is used to detect a polynucleotide sequence, that is complementary to the probe, in a hybridization-based assay. The probe may consist of a “fragment” of a polynucleotide as defined herein.

Polypeptides and fragments

The term “polypeptide”, as used herein, encompasses amino acid chains of any length but preferably at least 5 amino acids, including full-length proteins, in which amino acid residues are linked by covalent peptide bonds. Polypeptides of the present invention may be purified natural products, or may be produced partially or wholly using recombinant or synthetic techniques. The term may refer to a polypeptide, an aggregate of a polypeptide such as a dimer or other multimer, a fusion polypeptide, a polypeptide fragment, a polypeptide variant, or derivative thereof.

A “fragment” of a polypeptide is a subsequence of the longer polypeptide, consisting of a contiguous stretch of amino acids of the longer polypeptide, that performs a function that is required for the biological activity and/or provides three dimensional structure of the longer polypeptide. The term may refer to a polypeptide, an aggregate of a polypeptide such as a dimer or other multimer, a fusion polypeptide, a polypeptide fragment, a polypeptide variant, or derivative thereof capable of performing the above enzymatic activity.

The term “isolated” as applied to the polynucleotide or polypeptide sequences disclosed herein is used to refer to sequences that are removed from their natural cellular environment. An isolated molecule may be obtained by any method or combination of methods including biochemical, recombinant, and synthetic techniques.

The term “recombinant” refers to a polynucleotide sequence that is removed from sequences that surround it in its natural context and/or is recombined with sequences that are not present in its natural context.

A “recombinant” polypeptide sequence is produced by translation from a “recombinant” polynucleotide sequence.

The term “derived from” with respect to polynucleotides or polypeptides of the invention being derived from a particular genus or species, means that the polynucleotide or polypeptide has the same sequence as a polynucleotide or

polypeptide found naturally in that genus or species. The polynucleotide or polypeptide, derived from a particular genus or species, may therefore be produced synthetically or recombinantly.

Variants

As used herein, the term "variant" refers to polynucleotide or polypeptide sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variants may be from the same or from other species and may encompass homologues, paralogues and orthologues. Variants may also be naturally occurring or non-naturally occurring recombinants between alleles of these homologues, paralogues and orthologues. In certain embodiments, variants of the inventive polypeptides and polypeptides possess biological activities that are the same or similar to those of the inventive polypeptides or polypeptides. The term "variant" with reference to polypeptides and polypeptides encompasses all forms of polypeptides and polypeptides as defined herein.

Polynucleotide variants

Variant polynucleotide sequences preferably exhibit at least 50%, more preferably at least 51%, more preferably at least 52%, more preferably at least 53%, more preferably at least 54%, more preferably at least 55%, more preferably at least 56%, more preferably at least 57%, more preferably at least 58%, more preferably at least 59%, more preferably at least 60%, more preferably at least 61%, more preferably at least 62%, more preferably at least 63%, more preferably at least 64%, more preferably at least 65%, more preferably at least 66%, more preferably at least 67%, more preferably at least 68%, more preferably at least 69%, more preferably at least 70%, more preferably at least 71%, more preferably at least 72%, more preferably at least 73%, more preferably at least 74%, more preferably at least 75%, more preferably at least 76%, more preferably at least 77%, more preferably at least 78%, more preferably at least 79%, more preferably at least 80%, more preferably at least 81%, more preferably at least 82%, more preferably at least 83%, more preferably at least 84%, more preferably at least 85%, more preferably at least 86%, more

preferably at least 87%, more preferably at least 88%, more preferably at least 89%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, and most preferably at least 99% identity to a sequence of the present invention. Identity is found over a comparison window of at least 20 nucleotide positions, preferably at least 50 nucleotide positions, more preferably at least 100 nucleotide positions, and most preferably over the entire length of a polynucleotide of the invention.

Polynucleotide sequence identity can be determined in the following manner. The subject polynucleotide sequence is compared to a candidate polynucleotide sequence using BLASTN (from the BLAST suite of programs, version 2.2.5 [Nov 2002]) in bl2seq (Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250), which is publicly available from NCBI (ftp://ftp.ncbi.nih.gov/blast/). The default parameters of bl2seq are utilized except that filtering of low complexity parts should be turned off.

The identity of polynucleotide sequences may be examined using the following unix command line parameters:

bl2seq –i nucleotideseq1 –j nucleotideseq2 –F F –p blastn

The parameter –F F turns off filtering of low complexity sections. The parameter –p selects the appropriate algorithm for the pair of sequences. The bl2seq program reports sequence identity as both the number and percentage of identical nucleotides in a line “Identities = “.

Polynucleotide sequence identity may also be calculated over the entire length of the overlap between a candidate and subject polynucleotide sequences using global sequence alignment programs (e.g. Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453). A full implementation of the Needleman-Wunsch global alignment algorithm is found in the needle program in the EMBOSS package (Rice,P.

Longden,I. and Bleasby,A. EMBOSS: The European Molecular Biology Open Software Suite, Trends in Genetics June 2000, vol 16, No 6. pp.276-277) which can be obtained from http://www.hgmp.mrc.ac.uk/Software/EMBOSS/. The European Bioinformatics Institute server also provides the facility to perform EMBOSS-needle global alignments between two sequences on line at http:/www.ebi.ac.uk/emboss/align/.

Alternatively the GAP program may be used which computes an optimal global alignment of two sequences without penalizing terminal gaps. GAP is described in the following paper: Huang, X. (1994) On Global Sequence Alignment. Computer Applications in the Biosciences 10, 227-235.

A preferred method for calculating polynucleotide % sequence identity is based on aligning sequences to be compared using Clustal X (Jeanmougin *et al.*, 1998, *Trends Biochem. Sci.* **23,** 403-5.)

Polynucleotide variants of the present invention also encompass those which exhibit a similarity to one or more of the specifically identified sequences that is likely to preserve the functional equivalence of those sequences and which could not reasonably be expected to have occurred by random chance. Such sequence similarity with respect to polypeptides may be determined using the publicly available bl2seq program from the BLAST suite of programs (version 2.2.5 [Nov 2002]) from NCBI (ftp://ftp.ncbi.nih.gov/blast/).

The similarity of polynucleotide sequences may be examined using the following unix command line parameters:

bl2seq –i nucleotideseq1 –j nucleotideseq2 –F F –p tblastx

The parameter –F F turns off filtering of low complexity sections. The parameter –p selects the appropriate algorithm for the pair of sequences. This program finds regions of similarity between the sequences and for each such region reports an "E value" which is the expected number of times one could expect to see such a match by chance in a database of a fixed reference size containing random sequences. The size

of this database is set by default in the bl2seq program. For small E values, much less than one, the E value is approximately the probability of such a random match.

Variant polynucleotide sequences preferably exhibit an E value of less than 1×10^{-6} more preferably less than 1×10^{-9}, more preferably less than 1×10^{-12}, more preferably less than 1×10^{-15}, more preferably less than 1×10^{-18}, more preferably less than 1×10^{-21}, more preferably less than 1×10^{-30}, more preferably less than 1×10^{-40}, more preferably less than 1×10^{-50}, more preferably less than 1×10^{-60}, more preferably less than 1×10^{-70}, more preferably less than 1×10^{-80}, more preferably less than 1×10^{-90} and most preferably less than 1×10^{-100} when compared with any one of the specifically identified sequences.

Alternatively, variant polynucleotides of the present invention hybridize to the specified polynucleotide sequences, or complements thereof under stringent conditions.

The term "hybridize under stringent conditions", and grammatical equivalents thereof, refers to the ability of a polynucleotide molecule to hybridize to a target polynucleotide molecule (such as a target polynucleotide molecule immobilized on a DNA or RNA blot, such as a Southern blot or Northern blot) under defined conditions of temperature and salt concentration. The ability to hybridize under stringent hybridization conditions can be determined by initially hybridizing under less stringent conditions then increasing the stringency to the desired stringency.

With respect to polynucleotide molecules greater than about 100 bases in length, typical stringent hybridization conditions are no more than 25 to 30° C (for example, 10° C) below the melting temperature (Tm) of the native duplex (see generally, Sambrook *et al.*, Eds, 1987, Molecular Cloning, A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press; Ausubel *et al.*, 1987, Current Protocols in Molecular Biology, Greene Publishing,). Tm for polynucleotide molecules greater than about 100 bases can be calculated by the formula Tm = 81. 5 + 0. 41% (G + C-log (Na+). (Sambrook *et al.*, Eds, 1987, Molecular Cloning, A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press; Bolton and McCarthy, 1962, PNAS 84:1390). Typical stringent conditions for polynucleotide of greater than 100 bases in length would be

hybridization conditions such as prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65° C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

With respect to polynucleotide molecules having a length less than 100 bases, exemplary stringent hybridization conditions are 5 to 10° C below Tm. On average, the Tm of a polynucleotide molecule of length less than 100 bp is reduced by approximately (500/oligonucleotide length)° C.

With respect to the DNA mimics known as peptide nucleic acids (PNAs) (Nielsen *et al.*, Science. 1991 Dec 6;254(5037):1497-500) Tm values are higher than those for DNA-DNA or DNA-RNA hybrids, and can be calculated using the formula described in Giesen *et al.*, Nucleic Acids Res. 1998 Nov 1;26(21):5004-6. Exemplary stringent hybridization conditions for a DNA-PNA hybrid having a length less than 100 bases are 5 to 10° C below the Tm.

Variant polynucleotides of the present invention also encompasses polynucleotides that differ from the sequences of the invention but that, as a consequence of the degeneracy of the genetic code, encode a polypeptide having similar activity to a polypeptide encoded by a polynucleotide of the present invention. A sequence alteration that does not change the amino acid sequence of the polypeptide is a "silent variation". Except for ATG (methionine) and TGG (tryptophan), other codons for the same amino acid may be changed by art recognized techniques, e.g., to optimize codon expression in a particular host organism.

Polynucleotide sequence alterations resulting in conservative substitutions of one or several amino acids in the encoded polypeptide sequence without significantly altering its biological activity are also included in the invention. A skilled artisan will be aware of methods for making phenotypically silent amino acid substitutions (see, e.g., Bowie *et al.*, 1990, Science 247, 1306).

Variant polynucleotides due to silent variations and conservative substitutions in the encoded polypeptide sequence may be determined using the publicly available bl2seq

program from the BLAST suite of programs (version 2.2.5 [Nov 2002]) from NCBI (ftp://ftp.ncbi.nih.gov/blast/) via the tblastx algorithm as previously described.

Polypeptide variants

The term "variant" with reference to polypeptides encompasses naturally occurring, recombinantly and synthetically produced polypeptides. Variant polypeptide sequences preferably exhibit at least 50%, more preferably at least 51%, more preferably at least 52%, more preferably at least 53%, more preferably at least 54%, more preferably at least 55%, more preferably at least 56%, more preferably at least 57%, more preferably at least 58%, more preferably at least 59%, more preferably at least 60%, more preferably at least 61%, more preferably at least 62%, more preferably at least 63%, more preferably at least 64%, more preferably at least 65%, more preferably at least 66%, more preferably at least 67%, more preferably at least 68%, more preferably at least 69%, more preferably at least 70%, more preferably at least 71%, more preferably at least 72%, more preferably at least 73%, more preferably at least 74%, more preferably at least 75%, more preferably at least 76%, more preferably at least 77%, more preferably at least 78%, more preferably at least 79%, more preferably at least 80%, more preferably at least 81%, more preferably at least 82%, more preferably at least 83%, more preferably at least 84%, more preferably at least 85%, more preferably at least 86%, more preferably at least 87%, more preferably at least 88%, more preferably at least 89%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, and most preferably at least 99% identity to a sequences of the present invention. Identity is found over a comparison window of at least 20 amino acid positions, preferably at least 50 amino acid positions, more preferably at least 100 amino acid positions, and most preferably over the entire length of a polypeptide of the invention.

Polypeptide sequence identity can be determined in the following manner. The subject polypeptide sequence is compared to a candidate polypeptide sequence using BLASTP (from the BLAST suite of programs, version 2.2.5 [Nov 2002]) in bl2seq, which is publicly available from NCBI (ftp://ftp.ncbi.nih.gov/blast/). The default

parameters of bl2seq are utilized except that filtering of low complexity regions should be turned off.

Polypeptide sequence identity may also be calculated over the entire length of the overlap between a candidate and subject polynucleotide sequences using global sequence alignment programs. EMBOSS-needle (available at http:/www.ebi.ac.uk/emboss/align/) and GAP (Huang, X. (1994) On Global Sequence Alignment. Computer Applications in the Biosciences 10, 227-235.) as discussed above are also suitable global sequence alignment programs for calculating polypeptide sequence identity.

A preferred method for calculating polypeptide % sequence identity is based on aligning sequences to be compared using Clustal X (Jeanmougin *et al.*, 1998, *Trends Biochem. Sci.* **23**, 403-5.)

Polypeptide variants of the present invention also encompass those which exhibit a similarity to one or more of the specifically identified sequences that is likely to preserve the functional equivalence of those sequences and which could not reasonably be expected to have occurred by random chance. Such sequence similarity with respect to polypeptides may be determined using the publicly available bl2seq program from the BLAST suite of programs (version 2.2.5 [Nov 2002]) from NCBI (ftp://ftp.ncbi.nih.gov/blast/). The similarity of polypeptide sequences may be examined using the following unix command line parameters:

```
bl2seq –i peptideseq1 –j peptideseq2 -F F –p blastp
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Variant polypeptide sequences preferably exhibit an E value of less than 1×10^{-6} more preferably less than 1×10^{-9}, more preferably less than 1×10^{-12}, more preferably less than 1×10^{-15}, more preferably less than 1×10^{-18}, more preferably less than 1×10^{-21}, more preferably less than 1×10^{-30}, more preferably less than 1×10^{-40}, more preferably less than 1×10^{-50}, more preferably less than 1×10^{-60}, more preferably less than 1×10^{-70}, more preferably less than 1×10^{-80}, more preferably less than 1×10^{-90} and most preferably 1×10^{-100} when compared with any one of the specifically identified sequences.

The parameter –F F turns off filtering of low complexity sections. The parameter –p selects the appropriate algorithm for the pair of sequences. This program finds regions of similarity between the sequences and for each such region reports an "E value" which is the expected number of times one could expect to see such a match by chance in a database of a fixed reference size containing random sequences. For small E values, much less than one, this is approximately the probability of such a random match.

Conservative substitutions of one or several amino acids of a described polypeptide sequence without significantly altering its biological activity are also included in the invention. A skilled artisan will be aware of methods for making phenotypically silent amino acid substitutions (see, e.g., Bowie *et al.*, 1990, Science 247, 1306).

The function of a polypeptide variant in conferring resistance to powdery mildew may be assessed by the methods described in the Example section herein.

Constructs, vectors and components thereof

The term "genetic construct" refers to a polynucleotide molecule, usually double-stranded DNA, which may have inserted into it another polynucleotide molecule (the insert polynucleotide molecule) such as, but not limited to, a cDNA molecule or a molecule derived from genomic DNA region covering, but not restricted to, the open reading frame and any introns and exons within that region. A genetic construct may contain the necessary elements that permit transcribing the insert polynucleotide molecule, and, optionally, translating the transcript into a polypeptide. The insert polynucleotide molecule may be derived from the host cell, or may be derived from a different cell or organism and/or may be a recombinant polynucleotide. Once inside the host cell the genetic construct may become integrated in the host chromosomal DNA or be expressed transiently. The genetic construct may be linked to a vector.

The term "vector" refers to a polynucleotide molecule, usually double stranded DNA, which is used to transport the genetic construct into a host cell. The vector may be capable of replication in at least one additional host system, such as *E. coli*.

The term "expression construct" refers to a genetic construct that includes the necessary elements that permit transcribing the insert polynucleotide molecule, and, optionally, translating the transcript into a polypeptide. An expression construct typically comprises in a 5' to 3' direction:

a) a promoter functional in the host cell into which the construct will be transformed,
b) the polynucleotide to be expressed, and
c) a terminator functional in the host cell into which the construct will be transformed.

The term "coding region" or "open reading frame" (ORF) refers to the sense strand of a genomic DNA sequence or a cDNA sequence that is capable of producing a transcription product and/or a polypeptide under the control of appropriate regulatory sequences. The coding sequence is identified by the presence of a 5' translation start codon and a 3' translation stop codon. When inserted into a genetic construct, a "coding sequence" is capable of being expressed when it is operably linked to promoter and terminator sequences.

"Operably-linked" means that the sequenced to be expressed is placed under the control of regulatory elements that include promoters, tissue-specific regulatory elements, temporal regulatory elements, enhancers, repressors and terminators.

The term "noncoding region" refers to untranslated sequences that are upstream of the translational start site and downstream of the translational stop site. These sequences are also referred to respectively as the 5' UTR and the 3' UTR. These regions include elements required for transcription initiation and termination and for regulation of translation efficiency.

Terminators are sequences, which terminate transcription, and are found in the 3' untranslated ends of genes downstream of the translated sequence. Terminators are important determinants of mRNA stability and in some cases have been found to have spatial regulatory functions.

The term “promoter” refers to nontranscribed cis-regulatory elements upstream of the coding region that regulate gene transcription. Promoters comprise cis-initiator elements which specify the transcription initiation site and may include conserved boxes such as the TATA box, and motifs that are bound by transcription factors.

A “transgene” is a polynucleotide that is taken from one organism and introduced into a different organism by transformation. The transgene may be derived from the same species or from a different species as the species of the organism into which the transgene is introduced.

An “inverted repeat” is a sequence that is repeated, where the second half of the repeat is in the complementary strand, e.g.,

(5')GATCTA.......TAGATC(3')
(3')CTAGAT.......ATCTAG(5')

Read-through transcription will produce a transcript that undergoes complementary base-pairing to form a hairpin structure provided that there is a 3-5 bp spacer between the repeated regions.

Host cells

Host cells may be derived from, for example, bacterial, fungal, insect, mammalian or plant organisms.

A “transgenic plant” refers to a plant which contains new genetic material as a result of genetic manipulation or transformation. The new genetic material may be derived from a plant of the same species as the resulting transgenic plant or from a different species.

The applicants have identified a novel gene (SEQ ID NO: 2) that encodes a novel polypeptide (SEQ ID NO: 1) that confers powdery mildew resistance to a plant. SEQ ID NO: 3 shows the cDNA/open reading frame encoding the novel polypeptide of SEQ ID NO: 1.

The applicants have also shown that a polynucleotide sequence encoding a truncated polypeptide comprising just the colied coil (CC) domain and the nucleotide binding site (NBS) domain is sufficient to confer powdery mildew resistance to a plant.

The invention provides genetic constructs, vectors comprising the polynucleotides, including polynucleotides encoded the truncated polypeptide. The invention provides genetically modified host cells, plant cells and plants containing the novel polynucleotide sequences, genetic constructs and vectors. The invention also provides plants comprising the plant cells of the invention.

The invention provides plants altered in resistance to powdery mildew, relative to suitable control plants. The invention provides plants with increased resistance to powdery mildew.

The invention also provides methods for the production of such plants, and methods of selection of such plants.

The term "powdery mildew" as used herein refers to the commonly known disease of several plant species caused by organisms selected from but not limited to the following genera *Podosphaera, Blumeria, Arthrocladiella, Brasiliomyces, Caespitotheca, Cystotheca, Erysiphe, Golovinomyces, Leveillula, Microsphaera, Neoerysiphe, Oidiopsis, Oidium, Ovulariopsis, Parauncinula, Phyllactinia, Pleochaeta, Reticuloidium, Sawadaea, Sphaerotheca, Typhulochaeta and Uncinula.*

Preferably the causative pathogen is from the genus *Podosphaera*.

Preferably the causative pathogen is from the species *Podosphaera leucotricha*.

The term "increased resistance to powdery mildew" means that the plants of the invention, or plants produced or selected by the methods of the invention show reduced symptoms of powdery mildew infection, when challenged with causative pathogens, than do control plants under the same conditions.

Suitable control plants include non-transformed plants of the same species or variety or plants transformed with control constructs, such as, for example, empty vector constructs.

With respect to the selection methods of the invention, suitable control plants include non-selected members of the population from which selected plants are selected.

Methods for isolating or producing polynucleotides

The polynucleotide molecules of the invention can be isolated by using a variety of techniques known to those of ordinary skill in the art. By way of example, such polypeptides can be isolated through use of the polymerase chain reaction (PCR) described in Mullis *et al.*, Eds. 1994 The Polymerase Chain Reaction, Birkhauser, incorporated herein by reference. The polypeptides of the invention can be amplified using primers, as defined herein, derived from the polynucleotide sequences of the invention.

Further methods for isolating polynucleotides of the invention include use of all, or portions of, the polypeptides having the sequence set forth herein as hybridization probes. The technique of hybridizing labelled polynucleotide probes to polynucleotides immobilized on solid supports such as nitrocellulose filters or nylon membranes, can be used to screen a genomic or cDNA libraries. Exemplary hybridization and wash conditions are: hybridization for 20 hours at 65°C in 5. 0 X SSC, 0. 5% sodium dodecyl sulfate, 1 X Denhardt's solution; washing (three washes of twenty minutes each at 55°C) in 1. 0 X SSC, 1% (w/v) sodium dodecyl sulfate, and optionally one wash (for twenty minutes) in 0. 5 X SSC, 1% (w/v) sodium dodecyl sulfate, at 60°C. An optional further wash (for twenty minutes) can be conducted under conditions of 0. 1 X SSC, 1% (w/v) sodium dodecyl sulfate, at 60°C.

The polynucleotide fragments of the invention may be produced by techniques well-known in the art such as restriction endonuclease digestion, oligonucleotide synthesis and PCR amplification.

A partial polynucleotide sequence may be used, in methods well-known in the art to identify the corresponding full length polynucleotide sequence. Such methods include PCR-based methods, 5'RACE (Frohman MA, 1993, Methods Enzymol. 218: 340-56) and hybridization- based method, computer/database –based methods. Further, by way of example, inverse PCR permits acquisition of unknown sequences, flanking the polynucleotide sequences disclosed herein, starting with primers based on a known region (Triglia *et al.*, 1998, *Nucleic Acids Res* 16, 8186, incorporated herein by reference). The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template. Divergent primers are designed from the known region. In order to physically assemble full-length clones, standard molecular biology approaches can be utilized (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987).

It may be beneficial, when producing a transgenic plant from a particular species, to transform such a plant with a sequence or sequences derived from that species. The benefit may be to alleviate public concerns regarding cross-species transformation in generating transgenic organisms. Additionally when down-regulation of a gene is the desired result, it may be necessary to utilise a sequence identical (or at least highly similar) to that in the plant, for which reduced expression is desired. For these reasons among others, it is desirable to be able to identify and isolate orthologues of a particular gene in several different plant species.

Variants (including orthologues) may be identified by the methods described herein.

Methods for identifying variants

Physical methods

Variant polypeptides may be identified using PCR-based methods (Mullis *et al.*, Eds. 1994 The Polymerase Chain Reaction, Birkhauser). Typically, the polynucleotide sequence of a primer, useful to amplify variants of polynucleotide molecules of the invention by PCR, may be based on a sequence encoding a conserved region of the corresponding amino acid sequence.

Alternatively library screening methods, well known to those skilled in the art, may be employed (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987). When identifying variants of the probe sequence, hybridization and/or wash stringency will typically be reduced relatively to when exact sequence matches are sought.

Polypeptide variants may also be identified by physical methods, for example by screening expression libraries using antibodies raised against polypeptides of the invention (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987) or by identifying polypeptides from natural sources with the aid of such antibodies.

Computer based methods

The variant sequences of the invention, including both polynucleotide and polypeptide variants, may also be identified by computer-based methods well-known to those skilled in the art, using public domain sequence alignment algorithms and sequence similarity search tools to search sequence databases (public domain databases include Genbank, EMBL, Swiss-Prot, PIR and others). See, e.g., Nucleic Acids Res. 29: 1-10 and 11-16, 2001 for examples of online resources. Similarity searches retrieve and align target sequences for comparison with a sequence to be analyzed (i.e., a query sequence). Sequence comparison algorithms use scoring matrices to assign an overall score to each of the alignments.

An exemplary family of programs useful for identifying variants in sequence databases is the BLAST suite of programs (version 2.2.5 [Nov 2002]) including BLASTN, BLASTP, BLASTX, tBLASTN and tBLASTX, which are publicly available from (ftp://ftp.ncbi.nih.gov/blast/) or from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894 USA. The NCBI server also provides the facility to use the programs to screen a number of publicly available sequence databases. BLASTN compares a nucleotide query sequence against a nucleotide sequence database. BLASTP compares an amino acid query sequence against a

protein sequence database. BLASTX compares a nucleotide query sequence translated in all reading frames against a protein sequence database. tBLASTN compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames. tBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. The BLAST programs may be used with default parameters or the parameters may be altered as required to refine the screen.

The use of the BLAST family of algorithms, including BLASTN, BLASTP, and BLASTX, is described in the publication of Altschul *et al.*, Nucleic Acids Res. 25: 3389-3402, 1997.

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, BLASTX, tBLASTN, tBLASTX, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The BLASTN, BLASTP, BLASTX, tBLASTN and tBLASTX algorithms also produce "Expect" values for alignments. The Expect value (E) indicates the number of hits one can "expect" to see by chance when searching a database of the same size containing random contiguous sequences. The Expect value is used as a significance threshold for determining whether the hit to a database indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the database screened, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in that database is 1% or less using the BLASTN, BLASTP, BLASTX, tBLASTN or tBLASTX algorithm.

Multiple sequence alignments of a group of related sequences can be carried out with CLUSTALW (Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through

sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680, http://www-igbmc.u-strasbg.fr/BioInfo/ClustalW/Top.html) or T-COFFEE (Cedric Notredame, Desmond G. Higgins, Jaap Heringa, T-Coffee: A novel method for fast and accurate multiple sequence alignment, J. Mol. Biol. (2000) 302: 205-217)) or PILEUP, which uses progressive, pairwise alignments (Feng and Doolittle, 1987, J. Mol. Evol. 25, 351).

Pattern recognition software applications are available for finding motifs or signature sequences. For example, MEME (Multiple Em for Motif Elicitation) finds motifs and signature sequences in a set of sequences, and MAST (Motif Alignment and Search Tool) uses these motifs to identify similar or the same motifs in query sequences. The MAST results are provided as a series of alignments with appropriate statistical data and a visual overview of the motifs found. MEME and MAST were developed at the University of California, San Diego.

Coiled-coil (CC) regions within proteins can be detected by utilising the programme Pepcoil (Lupas A, van Dyke M & Stock J 1991. Science 252:1162-1164) which calculates probablilities that particular windows of 28 amino acid residues will form a coiled-coil structure.

PROSITE (Bairoch and Bucher, 1994, Nucleic Acids Res. 22, 3583; Hofmann *et al.*, 1999, Nucleic Acids Res. 27, 215) is a method of identifying the functions of uncharacterized proteins translated from genomic or cDNA sequences. The PROSITE database (www.expasy.org/prosite) contains biologically significant patterns and profiles and is designed so that it can be used with appropriate computational tools to assign a new sequence to a known family of proteins or to determine which known domain(s) are present in the sequence (Falquet *et al.*, 2002, Nucleic Acids Res. 30, 235). Prosearch is a tool that can search SWISS-PROT and EMBL databases with a given sequence pattern or signature.

The function of a variant polynucleotide of the invention as encoding a polypeptide conferring resistance to powdery mildew can be analysed by methods disclosed herein and well known to those skilled in the art. Such methods may involve transforming susceptible plants with polynucleotides of the invention and testing the resistance of

transformed plants to challenge with powdery mildew pathogens. Such methods are described in the Examples section of this specification.

Methods for isolating polypeptides

The polypeptides of the invention, including variant polypeptides, may be prepared using peptide synthesis methods well known in the art such as direct peptide synthesis using solid phase techniques (e.g. Stewart *et al.*, 1969, in Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco California, or automated synthesis, for example using an Applied Biosystems 431A Peptide Synthesizer (Foster City, California). Mutated forms of the polypeptides may also be produced during such syntheses.

The polypeptides and variant polypeptides of the invention may also be purified from natural sources using a variety of techniques that are well known in the art (e.g. Deutscher, 1990, Ed, Methods in Enzymology, Vol. 182, *Guide to Protein Purification,*).

Alternatively the polypeptides and variant polypeptides of the invention may be expressed recombinantly in suitable host cells and separated from the cells as discussed below.

Methods for producing constructs and vectors

The genetic constructs of the present invention comprise one or more polynucleotide sequences of the invention and/or polynucleotides encoding polypeptides of the invention, and may be useful for transforming, for example, bacterial, fungal, insect, mammalian or plant organisms. The genetic constructs of the invention are intended to include expression constructs as herein defined.

Methods for producing and using genetic constructs and vectors are well known in the art and are described generally in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987 ; Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing, 1987).

Methods for producing host cells comprising polynucleotides, constructs or vectors

The invention provides a host cell which comprises a genetic construct or vector of the invention.

Host cells comprising genetic constructs, such as expression constructs, of the invention are useful in methods well known in the art (e.g. Sambrook *et al.*, Molecular Cloning : A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987 ; Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing, 1987) for recombinant production of polypeptides of the invention. Such methods may involve the culture of host cells in an appropriate medium in conditions suitable for, or conducive to, expression of a polypeptide of the invention. The expressed recombinant polypeptide, which may optionally be secreted into the culture, may then be separated from the medium, host cells or culture medium by methods well known in the art (e.g. Deutscher, Ed, 1990, Methods in Enzymology, Vol 182, Guide to Protein Purification).

Methods for producing plant cells and plants comprising constructs and vectors

The invention further provides plant cells which comprise a genetic construct of the invention, and plant cells modified to alter expression of a polynucleotide or polypeptide of the invention. Plants comprising such cells also form an aspect of the invention.

Methods for transforming plant cells, plants and portions thereof with polypeptides are described in Draper *et al.*, 1988, Plant Genetic Transformation and Gene Expression. A Laboratory Manual. Blackwell Sci. Pub. Oxford, p. 365; Potrykus and Spangenburg, 1995, Gene Transfer to Plants. Springer-Verlag, Berlin.; and Gelvin *et al.*, 1993, Plant Molecular Biol. Manual. Kluwer Acad. Pub. Dordrecht. A review of transgenic plants, including transformation techniques, is provided in Galun and Breiman, 1997, Transgenic Plants. Imperial College Press, London.

Methods for genetic manipulation of plants

A number of plant transformation strategies are available (e.g. Birch, 1997, Ann Rev Plant Phys Plant Mol Biol, 48, 297, Hellens RP, et al (2000) Plant Mol Biol 42: 819-32, Hellens R et al Plant Meth 1: 13). For example, strategies may be designed to increase expression of a polynucleotide/polypeptide in a plant cell, organ and/or at a particular developmental stage where/when it is normally expressed or to ectopically express a polynucleotide/polypeptide in a cell, tissue, organ and/or at a particular developmental stage which/when it is not normally expressed. The expressed polynucleotide/polypeptide may be derived from the plant species to be transformed or may be derived from a different plant species.

Transformation strategies may be designed to reduce expression of a polynucleotide/polypeptide in a plant cell, tissue, organ or at a particular developmental stage which/when it is normally expressed. Such strategies are known as gene silencing strategies.

Genetic constructs for expression of genes in transgenic plants typically include promoters for driving the expression of one or more cloned polynucleotide, terminators and selectable marker sequences to detect the presence of the genetic construct in the transformed plant.

The promoters suitable for use in the constructs of this invention are functional in a cell, tissue or organ of a monocot or dicot plant and include cell-, tissue- and organ-specific promoters, cell cycle specific promoters, temporal promoters, inducible promoters, constitutive promoters that are active in most plant tissues, and recombinant promoters. Choice of promoter will depend upon the temporal and spatial expression of the cloned polynucleotide, so desired. The promoters may be those normally associated with a transgene of interest, or promoters which are derived from genes of other plants, viruses, and plant pathogenic bacteria and fungi. Those skilled in the art will, without undue experimentation, be able to select promoters that are suitable for use in modifying and modulating plant traits using genetic constructs comprising the polynucleotide sequences of the invention. Examples of constitutive plant promoters include the CaMV 35S promoter, the nopaline synthase promoter and

the octopine synthase promoter, and the Ubi 1 promoter from maize. Plant promoters which are active in specific tissues, respond to internal developmental signals or external abiotic or biotic stresses are described in the scientific literature. Exemplary promoters are described, e.g., in WO 02/00894.

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Exemplary terminators that are commonly used in plant transformation genetic construct include, e.g., the cauliflower mosaic virus (CaMV) 35S terminator, the *Agrobacterium tumefaciens* nopaline synthase or octopine synthase terminators, the *Zea mays* zein gene terminator, the *Oryza sativa* ADP-glucose pyrophosphorylase

10 terminator and the *Solanum tuberosum* PI-II terminator.

Selectable markers commonly used in plant transformation include the neomycin phophotransferase II gene (NPT II) which confers kanamycin resistance, the aadA gene, which confers spectinomycin and streptomycin resistance, the phosphinothricin

15 acetyl transferase (*bar* gene) for Ignite (AgrEvo) and Basta (Hoechst) resistance, and the hygromycin phosphotransferase gene (hpt) for hygromycin resistance.

Use of genetic constructs comprising reporter genes (coding sequences which express an activity that is foreign to the host, usually an enzymatic activity and/or a visible

20 signal (e.g., luciferase, GUS, GFP) which may be used for promoter expression analysis in plants and plant tissues are also contemplated. The reporter gene literature is reviewed in Herrera-Estrella *et al.*, 1993, Nature 303, 209, and Schrott, 1995, In: Gene Transfer to Plants (Potrykus, T., Spangenberg. Eds) Springer Verlag. Berline, pp. 325-336.

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Gene silencing strategies may be focused on the gene itself or regulatory elements which effect expression of the encoded polypeptide. "Regulatory elements" is used here in the widest possible sense and includes other genes which interact with the gene of interest.

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Genetic constructs designed to decrease or silence the expression of a polynucleotide/polypeptide of the invention may include an antisense copy of a

polynucleotide of the invention. In such constructs the polynucleotide is placed in an antisense orientation with respect to the promoter and terminator.

An "antisense" polynucleotide is obtained by inverting a polynucleotide or a segment of the polynucleotide so that the transcript produced will be complementary to the mRNA transcript of the gene, e.g.,

5'GATCTA 3' (coding strand)	3'CTAGAT 5' (antisense strand)
3'CUAGAU 5' mRNA	5'GAUCUCG 3' antisense RNA

Genetic constructs designed for gene silencing may also include an inverted repeat. An 'inverted repeat' is a sequence that is repeated where the second half of the repeat is in the complementary strand, e.g.,

5'-GATCTA.........TAGATC-3'
3'-CTAGAT.........ATCTAG-5'

The transcript formed may undergo complementary base pairing to form a hairpin structure. Usually a spacer of at least 3-5 bp between the repeated region is required to allow hairpin formation.

Another silencing approach involves the use of a small antisense RNA targeted to the transcript equivalent to an miRNA (Llave *et al.*, 2002, Science 297, 2053). Use of such small antisense RNA corresponding to polynucleotide of the invention is expressly contemplated.

The term genetic construct as used herein also includes small antisense RNAs and other such polypeptides effecting gene silencing.

Transformation with an expression construct, as herein defined, may also result in gene silencing through a process known as sense suppression (e.g. Napoli *et al.*, 1990, Plant Cell 2, 279; de Carvalho Niebel *et al.*, 1995, Plant Cell, 7, 347). In some cases sense suppression may involve over-expression of the whole or a partial coding sequence but may also involve expression of non-coding regions of the gene, such as an intron or a 5' or 3' untranslated region (UTR). Chimeric partial sense constructs

can be used to coordinately silence multiple genes (Abbott *et al.*, 2002, Plant Physiol. 128(3): 844-53; Jones *et al.*, 1998, Planta 204: 499-505). The use of such sense suppression strategies to silence the expression of a polynucleotide of the invention is also contemplated.

The polynucleotide inserts in genetic constructs designed for gene silencing may correspond to coding sequence and/or non-coding sequence, such as promoter and/or intron and/or 5' or 3' UTR sequence, or the corresponding gene.

Other gene silencing strategies include dominant negative approaches and the use of ribozyme constructs (McIntyre, 1996, Transgenic Res, 5, 257)

Pre-transcriptional silencing may be brought about through mutation of the gene itself or its regulatory elements. Such mutations may include point mutations, frameshifts, insertions, deletions and substitutions.

The following are representative publications disclosing genetic transformation protocols that can be used to genetically transform the following plant species: Rice (Alam *et al.*, 1999, Plant Cell Rep. 18, 572); apple (Yao *et al.*, 1995, Plant Cell Reports 14, 407-412); maize (US Patent Serial Nos. 5, 177, 010 and 5, 981, 840); wheat (Ortiz *et al.*, 1996, Plant Cell Rep. 15, 1996, 877); tomato (US Patent Serial No. 5, 159, 135); potato (Kumar *et al.*, 1996 Plant J. 9, : 821); cassava (Li *et al.*, 1996 Nat. Biotechnology 14, 736); lettuce (Michelmore *et al.*, 1987, Plant Cell Rep. 6, 439); tobacco (Horsch *et al.*, 1985, Science 227, 1229); cotton (US Patent Serial Nos. 5, 846, 797 and 5, 004, 863); grasses (US Patent Nos. 5, 187, 073 and 6. 020, 539); peppermint (Niu *et al.*, 1998, Plant Cell Rep. 17, 165); citrus plants (Pena *et al.*, 1995, Plant Sci.104, 183); caraway (Krens *et al.*, 1997, Plant Cell Rep, 17, 39); banana (US Patent Serial No. 5, 792, 935); soybean (US Patent Nos. 5, 416, 011 ; 5, 569, 834 ; 5, 824, 877 ; 5, 563, 04455 and 5, 968, 830); pineapple (US Patent Serial No. 5, 952, 543); poplar (US Patent No. 4, 795, 855); monocots in general (US Patent Nos. 5, 591, 616 and 6, 037, 522); brassica (US Patent Nos. 5, 188, 958 ; 5, 463, 174 and 5, 750, 871); cereals (US Patent No. 6, 074, 877); pear (Matsuda et al., 2005, Plant Cell Rep. 24(1):45-51); Prunus (Ramesh et al., 2006 Plant Cell Rep. 25(8):821-8; Song and Sink 2005 Plant Cell Rep. 2006 ;25(2):117-23; Gonzalez Padilla et al., 2003 Plant

Cell Rep.22(1):38-45); strawberry (Oosumi et al., 2006 Planta. 223(6):1219-30; Folta et al., 2006 Planta Apr 14; PMID: 16614818), rose (Li et al., 2003), Rubus (Graham et al., 1995 Methods Mol Biol. 1995;44:129-33), tomato (Dan et al., 2006, Plant Cell Reports V25:432-441), and *Actinidia eriantha* (Wang et al., 2006, Plant Cell Rep. 25,5: 425-31). Transformation of other species is also contemplated by the invention. Suitable methods and protocols are available in the scientific literature.

Several further methods known in the art may be employed to alter expression of a nucleotide and/or polypeptide of the invention. Such methods include but are not limited to Tilling (Till *et al.,* 2003, Methods Mol Biol, 2%, 205), so called "Deletagene" technology (Li *et al.,* 2001, Plant Journal 27(3), 235) and the use of artificial transcription factors such as synthetic zinc finger transcription factors. (e.g. Jouvenot *et al.,* 2003, Gene Therapy 10, 513). Additionally antibodies or fragments thereof, targeted to a particular polypeptide may also be expressed in plants to modulate the activity of that polypeptide (Jobling *et al.,* 2003, Nat. Biotechnol., 21(1), 35). Transposon tagging approaches may also be applied. Additionally peptides interacting with a polypeptide of the invention may be identified through technologies such as phase-display (Dyax Corporation). Such interacting peptides may be expressed in or applied to a plant to affect activity of a polypeptide of the invention. Use of each of the above approaches in alteration of expression of a nucleotide and/or polypeptide of the invention is specifically contemplated.

The terms "to alter expression of" and "altered expression" of a polynucleotide or polypeptide of the invention, are intended to encompass the situation where genomic DNA corresponding to a polynucleotide of the invention is modified thus leading to altered expression of a polynucleotide or polypeptide of the invention. Modification of the genomic DNA may be through genetic transformation or other methods known in the art for inducing mutations. The "altered expression" can be related to an increase or decrease in the amount of messenger RNA and/or polypeptide produced and may also result in altered activity of a polypeptide due to alterations in the sequence of a polynucleotide and polypeptide produced.

Methods of selecting plants

Methods are also provided for selecting plants with altered resistance to powdery mildew. Such methods involve testing of plants for altered expression of a polynucleotide or polypeptide of the invention. Such methods may be applied at a young age or early developmental stage when the altered resistance to powdery mildew may not necessarily be easily measurable.

The expression of a polynucleotide, such as a messenger RNA, is often used as an indicator of expression of a corresponding polypeptide. Exemplary methods for measuring the expression of a polynucleotide include but are not limited to Northern analysis, RT-PCR and dot-blot analysis (Sambrook *et al.*, Molecular Cloning : A Laboratory Manual, 2nd Ed. Cold Spring Harbor Press, 1987). Polynucleotides or portions of the polynucleotides of the invention are thus useful as probes or primers, as herein defined, in methods for the identification of plants with altered powdery mildew resistance. The polynucleotides of the invention may be used as probes in hybridization experiments, or as primers in PCR based experiments, designed to identify such plants.

Alternatively antibodies may be raised against polypeptides of the invention. Methods for raising and using antibodies are standard in the art (see for example: Antibodies, A Laboratory Manual, Harlow A Lane, Eds, Cold Spring Harbour Laboratory, 1998). Such antibodies may be used in methods to detect altered expression of polypeptides which modulate powdery mildew resistance in plants. Such methods may include ELISA (Kemeny, 1991, A Practical Guide to ELISA, NY Pergamon Press) and Western analysis (Towbin & Gordon, 1994, J Immunol Methods, 72, 313).

These approaches for analysis of polynucleotide or polypeptide expression and the selection of plants with altered resistance to powdery mildew are useful in conventional breeding programs designed to produce resistant varieties.

Plants

The term "plant" is intended to include a whole plant, any part of a plant, propagules and progeny of a plant.

The term 'propagule' means any part of a plant that may be used in reproduction or propagation, either sexual or asexual, including seeds and cuttings.

The plants of the invention may be grown and either selfed or crossed with a different plant strain and the resulting hybrids, with the desired phenotypic characteristics, may be identified. Two or more generations may be grown to ensure that the subject phenotypic characteristics are stably maintained and inherited. Plants resulting from such standard breeding approaches also form an aspect of the present invention.

This invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be better understood with reference to the accompanying drawings in which:

Figure 1 shows a Southern blot analysis of genomic DNA from a test population of powdery mildew resistant and susceptible parents and progeny of the cross 'Royal Gala' X A689-24. DNA samples were digested with *Dra*I, and the Southern blot hybridized with the mflc9 (NBS) probe. Lane 1; Powdery mildew (PM) resistant parent A689-24. lane 2; PM susceptible parent 'Royal Gala'. lanes 3-5; PM resistant progeny. lanes 6-8; PM susceptible progeny. Two polymorphic restriction fragments

(approximately 5.7 kb and 5.5 kb in size) putatively segregating with the resistance phenotype are shown by arrows.

Figure 2A shows a diagram of a Southern blot analysis of restriction fragment sizes in the cosmid clone pk4-051-2N. DNA of the cosmid clone was cut with the restriction endonucleases indicated, separated by gel electrophoresis, transferred to N+ nylon membrane and probed with the NBS clone mf1c9. Lane 1; *Apa*I, lane 2; *Bam*HI, lane 3; *Dra*I lane 4; *Eco*RV, lane 5; *Kpn*I, lane 6; *Sal*I, lane 7; *Spe*I, lane 8; *Xho*I, lane 9; *Not*I and *Spe*I, lane 10; *Not*I and *Xho*I, lane 11; *Hind*III endonuclease digest of Lambda marker. Three fragments that were subcloned are indicated with white arrows.

Figure 2B shows a schematic diagram of the restriction sites, coding region and surrounding sequenced region of the *Pl2.1* candidate gene. The 4.1 kb open reading frame is shown as a grey box and the sequenced 8.5 kb *Avr*II genomic DNA fragment containing a small 95 bp intron (light grey), the 4.4 kb cDNA fragments and a scale bar of 2 kb in dark grey are indicated below the figure. Restriction endonuclease sites; P; *Spe*I, A; *Avr*II, D; *Dra*I, X; *Xho*I, V; *Eco*RV, S; *Sac*I, B; *Bam*HI. The dotted line indicates the position of the 2NA fragment used as a probe in subsequent analysis.

Figure 3 shows DNA sequence and translation of the *Pl2.1* candidate gene region. Based on comparisons between cDNA and genomic sequence, an intron in the 5' UTR is shown in grey and the deduced translation of an uninterupted 1367 residue open reading frame is shown from base 2788 to base 6891 with the amino acid translation shown in bold as an additional line below the corresponding DNA sequence. Underlined regions mark the position of the Pl2.1 primers used in the analysis. As named in the text they are given in the following order; 5'UTR, Pl2.1 5' utr F1, Pl2.1rt 5'F1, Pl2.1 5' CCF1 and Pl2.1 3' CCR1 (overlapping in sequence and indicated within the same region), F2 P2N, R2 P2N, R1-2NA, F1-2NA, Pl2.1 3' utr R1 and 3'UTR respectively. Two *Xmn*I restriction sites used to develop the deletion construct are italicized at positions 4796-4805 and 6156-6165.

Figure 4 shows an RFLP linkage analysis with an internal fragment of the cosmid pk051-2N resistance gene candidate *Pl2.1* A. Southern blot of *Eco*RV digested

genomic DNA probed with the 2NA internal fragment of the cosmid R gene candidate. lane 1 - 1 kb marker lane 2; the susceptible parent 'Royal Gala'; lane 3 - the resistant parent A689-24; lanes 4 to 9- susceptible progeny; lanes 10 to 19- resistant progeny; note that the progeny in lane 6 was subsequently identified as a probable rogue based on microsatellite analysis. The two arrows on the right mark two polymorphic restriction fragment (approximately 4.9 kb and 4.7 kb in size) segregating with the resistance phenotype. B. Segregation analysis in the 'Pinkie' X 'Braeburn' population. Southern blot of genomic DNA cut with the restriction endonuclease *Dra*I and probed with the 2NA fragment produced by PCR amplification of the corresponding region from the cosmid clone with primers F1-2NA and R1 2NA. Lane 1; 1 kb marker, lane 2; Lambda *Hind*III marker, lane 3; 'Pinkie', lane 4; 'Braeburn', lane 5; A689-24, lane 6 to lane 16; progeny of the 'Pinkie' X 'Braeburn' population. Three black arrows on the left indicate RFLPs segregating as a closely linked set and including two RFLPs of identical size segregating with resistance in the 'Royal Gala' X A689-24 population, the top fragment indicated by the arrows does not segregate in the 'Royal Gala' X A689-24 population, but segregates in a 3:1 ratio in the 'Pinkie' X 'Braeburn' population.

Figure 5A shows an analysis of meiotic cross-over events in the region of the *Pl2* resistance gene. DNA samples of progeny were analysed with the genetic markers indicated below. Key; AC, the SCAR marker NZscOPAC15/AZ16; *Pl2* the powdery mildew (cumulative) resistance phenotype; N18, U2, and S5, the SCAR markers OPN18, NZscOPU02 and S5 respectively; XO the actual number of cross-overs characterized in the region indicated by the filled in box. An estimated distance based on the % of deduced cross-overs is given in italics below the diagram and the black bar indicates the region expanded in Figure 5B. Note that the distance was calculated based on the proportion of cross-overs in the total number of progeny fully characterized for the flanking markers concerned - in the case of region **A** that was 217 progeny, for regions **B** and **C** that was the full dataset of 443 progeny, and for region **D** that was 190 progeny.

Figure 5B shows a more detailed analysis of meiotic cross-over events in the region between N18 and the *Pl2* resistance gene. Key; as for Figure5A; RV, the 4.9 kb and 4.7 kb *Eco*RV restriction fragment markers, <u>SNP</u>, the SNP marker derived from the

Pl2.1 candidate gene (note although this marker is shown to the right of the *Pl2* gene it could be on either side of this gene or in the gene itself). An estimated distance based on the % of deduced cross-overs is given in italics below the diagram, the distance between the SNP marker and the *Pl2* gene is underlined. Note that the distance was calculated based on the proportion of cross-overs in the following way - in the case of region **B1** cross-overs in the full dataset of 443 progeny, for region **B2** the proportion of cross-overs in a subset of 13 progeny with cross-overs between OPN18 and the resistance gene which were also analysed for the presence of the *Eco*RV RFLP markers (note that 7 progeny with possible gene conversion events involving this region were not counted), and for region **C1** based on finding no progeny with cross-overs between the SNP marker and the powdery mildew phenotype out of 411 that were analysed for the SNP marker, the flanking NZscOPU02 and OPN18 markers and the powdery mildew phenotype (cumulative) score.

Figure 6A shows alignments of the *MxdPl2.1* protein with known resistance genes and candidate genes from other plants. The N terminal CC region and the central NBS region sequences were aligned using the Gonnet scoring matrix and the complete alignment option in ClustalX [39] which compares all sequences by pairwise alignments, constructs a dendrogram and then performs the final multiple alignment using the dendrogram as a guide. Alignments are displayed using Genedoc [31]. The consensus protein sequence is shown under the alignment with similarity groups used (1= DN 2= EQ 3= ST 4= KR 5= FYW 6= ILVM). Characteristic motifs in the CC and NBS regions are indicated in bold below the consensus sequence, dashes being used to indicate the approximate length of the motifs. Genbank sequence identifications for abbreviations in the alignment; MxdPl21 the apple *Pl2.1* candidate gene, AthaQ9LRR4 (Arabidopsis, Q9LRR4), Lescl2 (tomato, AAD27815), TaesPM3b (wheat, AAQ96158), OsatXA1 (rice, BAA25068), OsatAAO379 (rice, AAO37954), OsatP0514H (rice, BAD52970).

Figure 6B shows alignment of the 32 putative leucine rich repeats (LRR) in *Pl2.1* to each other, alignments were performed by matching the repeats to the LRR core LXXLXLXXC/N consensus sequence shown above the first deduced LRR. The short remaining C terminal sequence is also shown after the deduced LRR 32. C) adjusted

alignments were converted into phylogenetic trees using ClustalX [39], bootstrapped 1000 times and drawn by NJ plot [32], trees display bootstrap values and the bar in the right hand corner is a scale for branch lengths. Abbreviations as for A) with an additional motif identifier at the end of the name; CC signifies the coiled coil region, NB signifies the nucleotide binding site region and LRR signifies the leucine rich repeat region. The beginning and end of the region deleted in the Pl2 deletion construct are underlined in LRR 5 and LRR 23 respectively.

Figure 7 shows macroscopic and microscopic reactions on untransformed 'Royal Gala' and *Pl2.1* transgenic 'Royal Gala' plants infected with powdery mildew. A)-B) Macroscopic symptoms on control (c) untransformed and transformed (t) 'Royal Gala' C)-E) microscopic symptoms and reactions with a scale bar showing size. C) Abundant hyphal growth on 'Royal Gala' untransformed youngest leaves (leaf 1-20). D) Line A24 Youngest leaves (leaf 1-20) almost no spores visible. E) Line A24 middle of the plant (leaves 20-40) with spores visible but mostly not germinated. F) Line A24, 12 X magnified view of un-germinated powdery mildew spore marked with arrow from view E).

Figure 8 microscopic reactions on transformed *Pl2.1* transgenic 'Royal Gala' plants infected with powdery mildew (scale bar shown). A) Line 24 field of view with no visible hypersensitive response (HR) reactions on older leaves (leaf 40-80). B)–F) fields of view with HR reactions on older leaves (leaf 40-80). B) Line A24, showing a chain of HR reactions associated with hyphae more easily visible in another plane of view. C) Line A7, showing a hypha closely associated with a single HR reaction. D) Line A7, the same view taken at different exposure and plane of view to highlight the presence of a hypha protruding from the main hyphal branch down to the HR reaction. E) Line A5, showing a series of HR reactions occurring directly below powdery mildew hyphae. F) Line A25, showing a chain of HR reactions directly below a hypha which was more visible in a different plane of view.

Figure 9 shows representative microscopic images of leaf tissue innoculated with powdery mildew for the plants described in Example 4: A) *Pl2.1* A25 line, youngest leaves (leaf 1-20) with no spores visible. B) Royal Gala control micrografted line showing abundant hyphal growth on 'Royal Gala' untransformed youngest leaves

(leaf 1-20). C) *Pl2.1* DA4 deletion line, youngest leaves (leaf 1-20) with no spores visible.

EXAMPLES

The invention will now be illustrated with reference to the following non-limiting example.

Example 1: Isolation of the powdery mildew resistance gene of the invention

Plant material and population assessments

Three segregating families were used for the mapping analysis presented below. Two of these families (S2 and S9) have the same parents (the susceptible variety 'Royal Gala' crossed with the powdery mildew resistant clone A689-24) but were derived from seed generated in subsequent years (1993 and 1994).

The powdery mildew phenotype of each individual of the populations was assessed after the seedlings had been transplanted into the orchard for 2 to 6 years. Each year powdery mildew growth on the progeny was scored using a 5 point scale where 0 represents no visible symptoms of powdery mildew growth (resistant or escape plant) and 5 represents abundant powdery mildew growth (susceptible plant). An average score over the years was used to test segregation of the gene for goodness of fit with the model of a single major gene.

It is difficult to assign progeny with intermediate scores (between 1 and 2) to their appropriate resistance class since the field based assessment of mildew resistance suffers from some problems [10, 20]. Therefore we modified the suggestion of Dunemann et al. [10] that scores of 0 or 1 indicate resistance by taking an average score of less than or equal to 1 as resistant and an average score greater than 2 as susceptible, scores in between were treated as inconclusive.

According to the method of Gardiner et al. [36], a small subset of three resistant and three susceptible progeny from these 2 populations were used to generate a mini-population that could be rapidly screened by Southern hybridisation with multiple probes.

A third population ('Pinkie' X 'Braeburn') was used to confirm co-segregation of restriction fragments from 'Pinkie' since this cultivar contains the *Pl2* gene and was the genomic DNA source for the large DNA insert library used below.

DNA isolation, primers and PCR

Genomic DNA was extracted from the parents and progeny of the above apple crosses using the Nucleon Phytopure Plant DNA extraction kit (Amersham Biosciences) and following the manufacturers instructions. Primers used for PCR were purchased from Invitrogen Corporation (Carlsbad, CA USA). The name and sequence of the primers used was as follows; *Pl2.1* specific SNP primers; R2 P2N (the mismatched site is underlined) 5' TCATAATTTACCGGCTTTCC<u>T</u>G 3', F2 P2N 5' TCTGATGACTTC GATGTTGAA 3', 2NA probe primers; F1-2NA 5' CACCACAAAAGAGGCAGT G 3' R1-2NA 5' CATTGCTGGTCGATTTGATG 3'. The reaction conditions and sequence of the SCAR primers were based on published protocols as follows; S5 [19] and NZscOPU02, OPN18, NZscOPAC16/OPAZ16 [16]. The specific SNP marker used the same conditions specified for marker NZscOPU02 with the PCR reaction containing 1% formamide. The PCR programme used consisted of an initial denaturing step of 94°C for 4 min followed by 30 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 30 s and a final extension step of 72°C for 5 min. Two sets of *Pl2.1* specific primers that together span the entire *Pl2.1* open reading frame were used in reverse transcriptase (RT)-PCR reactions to assess if the entire *Pl2.1* transcript (and therefore the entire *Pl2.1* gene) is present in the transformants. Set 1 amplifies the region from just outside the initiating methionine to the 3' end of the coiled coil domain and set 2 amplifies from the 3' end of the coiled coil region to the beginning of 3' UTR. The name and sequence of these primers pairs is: set 1- Pl2.1 5' utr F1 (GCGATTCGGTCTTTCTTTGA) and Pl2.1 3' CCR1 (CTACACCAAAATTGACGGCATCTGT); set 2- Pl2.1 5' CCF1 (CAAAAAAATACGAGCATACAGATGCC) and Pl2.1 3' utr R1

(AAAACATTCCTCGACAGATGA). The RT-PCR reactions consisted of 2 uL of diluted cDNAs in a 20 uL reaction volume using Platinum Taq polymerase (Invitrogen, Carlsbad). The following PCR programme was used: an initial denaturation cycle at 94°C for 4 min; followed by 20 touchdown cycles of 94°C for 30s, 65°C to 55°C (0.5°C decrease at each cycle) for 30s, 72°C for 30s; followed by 10 cycles at 94°C for 30s, 55°C for 30s and 72°C for 30s, and a final extension at 72°C for 5 min. Transcript expression analysis was performed with the transformants and control in the following way. Quantitative real time (qRT)-PCR reactions were set up and consisted of 5 uL of diluted cDNAs used in 20 uL reaction volume in an ABI7700 Real time PCR machine following the manufacturer's instructions (ABI, Foster City, USA). A Pl2.1-specific primer pair was used to amplify a 135 bp Pl2.1-specific PCR product. The PCR programme used was as follows; 10 min at 95°C, followed by a 40 cycles of a two step PCR consisting of 95°C for 15s and 60°C for 1 min. The name of the primers used is Pl2.1rt 5' F1 (sequence AGGAATCGCGAAGT CTACCA) and Pl2.1 3' CCR1 (sequence given above); A pair of primers that amplify an apple actin gene were used as the internal control. The same PCR conditions and reaction set up was used for this internal control. The name and sequence of the primers used are ACT2F (GCAGAGCGTGAAATTGTGAG) and ACT2R (ATGACCTGCCCATCTGGTAA).

Southern Blot analysis, RFLP mapping PCR mapping and linkage analysis:

Restriction digests were performed according to the manufacturers instructions and Southern blotting was performed according to the method of Sambrook et al. [35]. The same technique was used to generate blots of mini-populations for rapid screening and blots containing the entire progeny set from populations S2, S9 and ‘Pinkie’ X ‘Braeburn’. The size of RFLPs was estimated by scanning lumigrams with the geldoc (BioRad) system and using labeled marker lanes to estimate the size of the hybridising bands. A number of existing SCAR markers that are known to be closely linked to the *Pl2* resistance gene based on previous analysis [16] were also included in the mapping analysis and 456 progeny were scored for the closest known flanking markers (N18 and NZscOPU2 SCAR). In one case sequence data from the *Pl2.1* candidate gene and an NBS gene sequence database developed previously by the applicants (data not shown) was used to develop a PCR-based single nucleotide

polymorphism test that was used to accurately place a candidate gene onto the *Pl2* genetic map. This test used the primers R2 P2N and F2 P2N given above and the R2 P2N primer contained a deliberate mismatch following the method of Drenkard et al. [9]. As gene conversion events can interfere with the ordering of markers that are close together, fine scale ordering of markers was based on progeny containing recombination events that were diagnostic for marker order and using that information to develop the most parsimonious marker order.

Screening of a cosmid library from 'Pinkie'

A 7n haploid equivalent cosmid library (SuperCos I 168,960 clones) generated by *Sau*3AI partial digestion of genomic DNA from the resistant cv 'Pinkie' was screened with the NBS clone that revealed RFLPs linked to powdery mildew resistance. One duplicate copy of the cosmid library was used to generate 6 copies of a high density (3X3) array of 384 well plates on Hybond N+ membranes using the 384 pin HDR tool of the Biomek 2000 by the following method. Hybond N+ membranes were overlayed on LB agar omnitray plates containing 75 ml of solidified LB agar medium. The culture medium from four 384 well microtitre plates was subsequently inoculated in duplicate spots onto a single Hybond membrane (with one of the nine available positions being left empty). These plates were then grown at 37°C overnight and the membranes were removed and processed by a colony hybridisation method [35]. Hybridisation was carried out by using ECL labeled insert DNA and following the manufacturers instructions (MD Biosciences, Zurich) for probing and stringent washing conditions.

Screening of NBS clones to identify RFLPs segregating with powdery mildew resistance

The applicants previously constructed a phylogenetic tree from the NBS regions of putative apple resistance gene analogue clones that were isolated by PCR (data not shown). Representatives from the main branches of this phylogenetic tree were screened across mini-population blots by Southern hybridisation in order to search for restriction fragment length polymorphisms (RFLPs) that putatively segregate with the powdery mildew resistance phenotype. Clones that generated RFLPs putatively

linked to the powdery mildew resistance gene *Pl2* were then screened across an enlarged population to test if the co-segregation with phenotype remained consistent.

RNA extraction and cDNA cloning

RNA was extracted from 'Pinkie' by the method of Chang et al. [7]. DNA sequence data from the putative 3' un-translated region of a homologous ESTs with a high degree of sequence identity to *Pl2.1* was used to identify a GC clamp next to the poly A region that was used to reverse transcribe RNA of 'Pinkie'. DNA sequence data generated from the resistance gene candidate *Pl2.1* was used to design 2 specific cDNA primers; 3'UTR (sequence 5' CTTGACCCAAACCAAAATATG 3') and 5'UTR (5' TTGACTGTTGATCTTCCCTTC 3'). These primers were used to amplify cDNA copies of the gene from reverse transcribed RNA of 'Pinkie'. The following PCR programme was used to amplify candidate cDNAs that match the DNA sequence of *Pl2.1*; 4 min at 95°C, followed by a 30 cycles of a three step PCR consisting of 95°C for 30s, 55°C for 30s and 68°C for 90s. The reaction concentrations were 0.2 uM for dNTPs and 2 uM of each primer and amplification was driven by 2 units of the Expand DNA Polymerase (Roche) system in a final reaction volume of 50 ul.

RNA was extracted from transformants using 100 mg of leaf tissues with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Then 500 ng of total RNAs were used to make first-strand cDNA using SuperScriptII (Invitrogen, Carlsbad, USA). The synthesised cDNA was diluted 10-fold in TE buffer and stored at -20°C until further use for RT-PCR and qRT-PCR analysis of the transformants.

DNA sequencing and sequence analysis

DNA sequencing reactions (using universal forward, reverse or custom designed primers for primer walking reactions) were performed by a cycle sequencing method according to manufacturers instructions using ABI Big dye terminator sequencing mix (Applied Biosystems, Foster City, CA) and analysed on an ABI PRISM™ 377-XL or ABI3100 genetic analyser sequencers. The results from the DNA sequencer were analysed using ABI PRISM™ DNA sequencing analysis software version 3.0

(Applied Biosystems, Foster City, CA). Base calling was performed by the semiadaptive base calling method and the resulting files were imported into Sequencher^{TM} 3.0 (Genecodes Corp., Ann Arbor, MI) where the sequences from forward, reverse and primer walking reactions for the same clone were aligned and manually checked and edited. In the case of the resistance gene analogue *Pl2.1* all of the sequences (including 3 independently cloned cDNA copies, 5' and 3' sequences) were joined together into a single project to identify putative start and stop sites, the open reading frame and any putative introns/exons.

Progeny and orchard based powdery mildew phenotype analysis

Progeny were screened with 6 microsatellite markers and 12 progeny were identified as having a marker profile that was inconsistent with being derived from a cross between 'Royal Gala' and A689-24A689-24. These progeny were eliminated from further consideration. The remaining progeny of the S2 and S9 crosses were analysed for mildew resistance in the field over several growing seasons from 1998 to 2002 and classified as resistant (scoring an average of 1 or less), susceptible (scoring an average of 2 or greater) or inconclusive. The phenotype score of the majority of the 443 progeny putatively placed them into either the resistant or susceptible classes (198 resistant versus 221 susceptible). Taking marker data into consideration, the resistant and susceptible classes showed 97% and 96% concordance respectively between the anticipated phenotype based on marker profile and the observed phenotype in the orchard. By deduction there is a high probability that the linked *Pl2* allele is identical by descent (IBD) amongst (but not between) each of these two classes. Based on this interpretation, powdery mildew resistance segregated in a simple 1:1 ratio (Probability Chi squared =0.26) in this population suggesting that the gene might be amenable to being cloned by map-based techniques. The remaining 24 progeny were treated as inconclusive scoring an average between 1 and 2. Genetic marker data analysis of these progeny confirmed that these scores generated the greatest degree of discordance between marker profile and observed phenotype. It indicated that 10 of these progeny showed alleles IBD from the chromosome carrying the resistance allele of *Pl2* and 14 showed alleles IBD from the chromosome carrying the susceptible allele of *Pl2*.

Mapping strategy and identification of an RGA that co-segregates with resistance

The 443 progeny of the S2 and S9 populations were genotyped and scored for the presence or absence of the flanking OPN18 and NZscOPU02 SCAR markers. Progeny from these populations were also screened with a number of nucleotide binding site domain fragments isolated previously by PCR (data not shown). Given that a large database of NBS sequences had been developed a process needed to be developed to prioritise the clones to be mapped. We based this process on the position of NBS sequences on a phylogenetic tree. Fourteen NBS clones were chosen from main branches of a phylogenetic tree constructed (data not shown). These were screened by hybridisation across Southern blots of genomic DNA digested with *Eco*RV and *DraI* containing a small exploratory set of resistant and susceptible progeny and the two parents of crosses S2 and S9 (mini-population).

One of these original 14 clones, mf1c9, showed a possible co-segregation pattern of polymorphic restriction fragments with powdery mildew resistance in the mini-population (Figure 1). Two RFLP fragments in each of two different restriction endonuclease digests (4.9 kb and 4.7 kb *Eco*RV fragments and 5.7 kb and 5.5 kb *Dra*I fragments) were present in the resistant progeny and absent from the susceptible progeny. A series of 11 to 13 restriction fragments were revealed with this probe, suggesting the presence of a large gene family of homologous resistance gene candidates. A high rate of co-segregation of the *Eco*RV RFLPs with the resistance phenotype and their absence in susceptible progeny (93% concordance) was confirmed by the analysis of an enlarged dataset of 150 phenotyped individuals using Southern blots of genomic DNA. Almost complete concordance between the presence or absence of both of the OPN18 and NZscOPU02 SCAR markers and the presence or absence of these two RFLPs was found. Just one individual exhibited the RFLP fragments but lacked both the OPN18 and NZscOPU02 SCAR markers.

Screening the Pinkie cosmid library and sequencing of a Pl_2 candidate gene region

The mfl c9 clone was used as a hybridisation probe to screen the cosmid library for candidate genes homologous to the probe in order to be able to correlate the segregation patterns of specific RFLPs with a specific gene candidate. The screen with mfl c9 identified two positive clones Pk4-051-2N and Pk4-051-4F. Restriction mapping and hybridisation analysis of these clones identified that both contained a 5.7 kb *Dra*I restriction fragment that is the same size as one of the RFLPs above that putatively co-segregates with the powdery mildew resistance phenotype and is therefore a candidate for the *Pl2* resistance gene. The two cosmids showed identical restriction endonuclease profiles and the results for Pk4-051-2N are shown in Figure 2A. Analysis of Pk4-051-2N identified restriction fragments of convenient size for sub-cloning that hybridised to the probe mfl c9 which were selected as initial sub-cloning targets (9 kb *ApaI* - lane 1, 5.7 kb *Dra*I - lane 3 and 3.6 kb *SpeI*-lane 7). Initial blast searches with sequence information from these clones suggested the presence of at least one open reading frame with homology to the known tomato wilt (*Fusarium oxysporum*) disease resistance gene I2 [37]. Further restriction enzyme analyses of the cosmid clones (not presented) were used to identify sub-cloning strategies for regions near the gene not contained in these initial clones (*Avr*II, *Sac*I). These fragment were cloned and sequenced and a restriction map derived from this sequence information is shown in Figure 2B. The complete DNA sequence of the *Avr*II fragment and its deduced amino acid translation is shown in Figure 3.

PCR based mapping and RFLP mapping with the 2NA fragment from candidate gene $Pl_{2.1}$

A fragment from the middle of the candidate gene clone (2NA, position shown in Figure 2B.) was used as a probe for further RFLP mapping of this candidate gene and other candidate genes able to hybridise to this fragment. This region of the gene showed 43 % identity at the protein level over 133 residues to a region identified by Simons et al. [37] as a leucine rich region containing a potential leucine zipper domain within the tomato I2 gene. Genomic DNA of parents and progeny of the S2 and S9 populations was digested with restriction endonuclease *Eco*RV and polymorphic RFLPs segregating in these populations were analysed by Southern

blotting and hybridization with the 2NA probe (Figure 4A). This data was added to the data derived from the powdery mildew phenotype scores and SCAR mapping data. The two polymorphic RFLPs scored (4.9kb and 4.7 kb fragments) showed almost perfect co-segregation with the set of flanking markers surrounding the resistance gene. A similar RFLP analysis to investigate co-segregation of *Dra*I RFLPs was also carried out in the population 'Pinkie' X 'Braeburn' (Figure 4B.). This indicated that three RFLPs including the 5.7 kb and 5.5 kb *Dra*I fragments and a third 6.2 kb *Dra*I fragment co-segregate, we named the gene represented by the 5.7 kb RFLP *Pl2.1* whereas the 5.5 kb and 6.2 kb fragments represent additional candidate genes for the *Pl2* mediated resistance.

A PCR based SNP marker likely to be specific to the *Pl2.1* candidate gene (5.7 kb *Dra*I fragment) was designed based on the sequences of the NBS region of the gene. The polymorphic site was identified by comparing this sequence with our large database of other apple NBS sequences. This marker was screened over the entire S2 and S9 populations and this marker data together with RFLP marker data (where the markers could be confidently scored) were integrated into a genetic map. Flanking markers were previously ordered with respect to each other and the resistance phenotype by JoinMap [16] while the most parsimonious order of the closer (resistance candidate gene based) markers to the resistance phenotype was determined by choosing the order that required invoking the smallest number of double cross-over or gene conversion events possible. This allowed us to identify likely gene conversion events. The resulting gene order and the deduced number and location of meiotic cross-over events are shown in Figure 5. Based on this analysis only 3 putative cross-over events were found between the *Pl2* candidate gene represented by the *EcoRV* fragments and the resistance phenotype and no cross-over events were detected between the 5.7 kb *DraI* fragment of *Pl2.1*, the *Pl2.1* SNP marker and the resistance phenotype.

Among the progeny showing no recombination events near the *Pl2* locus there were ten progeny (five resistant and 5 susceptible) where the phenotype of the progeny did not match the anticipated phenotype based on IBD. Progeny like this have been labeled Genotype-Phenotype Incongruence (GPI) progeny and sometimes excluded from particular analyses to determine the exact position of resistance genes [12, 18].

These progeny can be interpreted in several possible ways as either gene conversion events (in which case changes in any candidate gene would be expected but not necessarily always detectable by the marker display methods used), a double cross-over event between the closest flanking markers on either side and the resistance gene, or a progeny where the resistance phenotype has not fully penetrated. Lack of penetration of the resistance phenotype could be because of other factors segregating in the background of the progeny, due to the biology of the pathogen, or a combination of quantitative factors coming together to give resistance in the absence of a major resistance gene. Evidence for gene conversion at one of the markers around the gene was found amongst four of the five resistant progeny. These GPI progeny could give misleading evidence against associations between candidate genes and their phenotypic effects, as it is not possible to distinguish between many of these possibilities and only some of them would eliminate candidate genes from contention for being responsible for the *Pl2* mediated resistance.

Only the recombinations which are likely to be due to meiotic cross-over events were therefore considered useful to eliminate candidate genes from contention. Such cross-over events should have evidence of recombination in the flanking markers as well as possibly some of the closer gene markers not derived from the gene(s) primarily responsible for the *Pl2* powdery mildew resistance phenotype. Considering only such recombination events, we could not detect any cross-over events between the *Pl2.1* candidate gene and the resistance phenotype. Therefore the analysis was consistent with the *Pl2.1* candidate gene being responsible for the powdery mildew resistance phenotype of the *Pl2* locus. The sequence of the full-length *Pl2.1* protein is shown in SEQ ID NO: 1. The sequence of the *Pl2.1* gene is shown in SEQ ID NO: 2. The sequence of the open reading frame/cDNA encoding *Pl2.1* is shown in SEQ ID NO: 3.

Gene constructs containing the *Pl2.1* coding region and up and downstream regulatory regions were introduced into a plant transformation vector in order to assay gene function by transformation.

Example 2: Characterisation of the *Pl2.1* protein and comparison with other CC-NBS-LRR known resistance genes

Similarity with other known resistance genes can be used to indicate the presence of a series of protein domains required to impart an ability to confer resistance. BlastP searches with the deduced protein sequences of the $Pl_{2.1}$ candidate gene identified a number of genes from other species, including three known resistance genes, *I2* from tomato [37], *Pm3b* from wheat [42] and *Xa1* from rice [44], as the closest matches. The Q9LRR4 gene from Arabidopsis, with similarity to the known resistance gene *RPP13,* is the closest match in Arabidopsis (Figure 6A).

The highest degree of identity was in the NBS region where Q9LRR4 was the closest match at 46% identity followed by *I2* (42% identity), *PM3b* and *Xa1* (34% and 33% identity respectively).

Lower levels of identity were found between the proteins encoded by these genes in the CC region (32%, 25% and 18% for Q9LRR4, *I2* and *PM3b* respectively), whereas *Xa1* contained a much longer N terminal region which was difficult to align with the other 3 proteins.

In the LRR region the *I2* protein was the most similar to $Pl_{2.1}$ at 28% identity.

Phylogenetic trees drawn from these alignments show a similar relationship between the proteins regardless of the region of the alignment used to draw the tree (Figure 6C). The trees and alignments also contain sequences from two rice proteins (identified by their accession numbers AAO37954 and P0514H03.24) that have previously been shown [42] to fall into a common clade with the wheat *PM3b* protein.

Example 3: Use of polynucleotides of the invention encoding the full length *Pl2.1* protein to confer powdery mildew resistance

Plant transformation vector constructs, transformation and transcription analysis

An 8.5 kb *Avr*II fragment containing the entire putative open reading frame of the resistance gene candidate *Pl2.1* and 4.4 kb of adjacent sequence (2.8 kb at the 5' end and 1.6 kb at the 3' end) was cloned into the plant transformation binary vector pART27 [17] to generate the vector p*Pl2.1*-clone10. This p*Pl2.1*-clone10 was introduced into *Agrobacterium tumefaciens* strain LBA4404 and kanamycin resistant transformants of apple were selected using plant transformation protocols previously described by Yao et al. [43]. Putative transformants were analysed by PCR to determine whether the entire *Pl2.1* gene was introduced and transcription of the gene was analysed by qRT PCR according to the method of Zhang et al. [45].

Functional analysis of Pl2.1 transformants

Twenty-five independent transformed lines of 'Royal Gala' were generated by transformation with the p*Pl2.1*-clone10 construct. Single shoots regenerated from each callus were separated and multiplied by subculturing to derive multiple copies of 25 independent lines. Shoots of each copy of each independent line were rooted and transferred into the glass house for growing under controlled conditions.

To test for the presence of an intact *Pl2.1* transgene a small leaf was removed from each of the putative transformed plants at an early stage of growth and both DNA and RNA isolated from this leaf for subsequent PCR based analysis. RT-PCR analysis using two sets of *Pl2.1*-specific primer pairs suggested all transformants tested (a total of 8 lines) had full-length *Pl2.1* transcripts. qRT-PCR analysis showed that there was a varying degree of *Pl2.1* transcript level in these lines. Table 1 shows a difference of up to 9-fold among the 11 independently transformed lines that were tested. The level of *Pl2.1* transcripts detected in Royal Gala was so low that it is not statistically significant above background.

Table 1.

qPCR of transformants		
Transformant line	Average Expression	Standard Deviation
A2	0.32	0.068
A4	0.35	0.023
A6	0.65	0.036
A7	0.30	0.034
A8	0.39	0.037
A10	0.34	0.036
A14	1.97	0.111
A15	0.22	0.032
A18	0.45	0.009
A19	1.06	0.024
A25	0.64	0.063
Control	0.01	0.005

Table 1. Quantitative PCR (qPCR) of 11 independently transformed lines carrying the Pl2.1 gene. The relative expression level of 11 independent lines was assessed using the primer pair Pl2.1rt 5' F1 and Pl2.1 3' CCR1 and compared with an Actin internal control (using primers ACT2F and ACT2R) and an untransformed Royal Gala control. Values are given as ratios relative to expression of the Actin gene in each sample which was set arbitrarily at 1.0. Standard deviations were calculated based on the analysis which was done in triplicate.

Representatives of the transformed plants and control plants were introduced into a glasshouse in order to test the effect of the candidate gene on the powdery mildew phenotype of the normally susceptible 'Royal Gala' host.

A minimum of three copies of 9 independent lines were generated and introduced into the glasshouse together with control untransformed 'Royal Gala'. The challenge was initiated by introducing 'Royal Gala' plants heavily infected with powdery mildew and the infection process on the uninfected control and transgenic plants was followed over a period of several months at the macroscopic symptom level.

In the first season in the glasshouse macroscopic symptoms were recorded by visual inspection and estimating the proportion of the top 20 leaves heavily infected with

mildew. At the end of the first season plants were pruned down to a 50 cm height and treated with Hicane to induce uniform budbreak [14].

The results of monitoring the macroscopic symptoms of these plants during the first spring-summer season are presented in Table 2 .

Table 2.

Macroscopic phenotypic scoring of transformants				
		Months after challenge		
Line	**Description**	**1**	**2**	**6**
'Royal Gala' A2	*Pl2.1*Transformant	0*[1]	0	0
'Royal Gala' A3	*Pl2.1*Transformant	0	0	0
'Royal Gala' A4	*Pl2.1*Transformant	0	0	0
'Royal Gala' A5	*Pl2.1*Transformant	0	0	0*[2]
'Royal Gala' A7	*Pl2.1*Transformant	0	0	0
'Royal Gala' A8	*Pl2.1*Transformant	0	0	0
'Royal Gala' A10	*Pl2.1*Transformant	0	0	0
'Royal Gala' A24	*Pl2.1*Transformant	0	0	0*[2]
'Royal Gala' A25	*Pl2.1*Transformant	0	0	0
'Royal Gala'	Untransformed	53±15%*[3]	82%±8%*[3]	93%±3%*[3]

Table 2. Powdery mildew phenotypes of 9 independent transformed lines of 'Royal Gala' with the Pl2.1 gene in the first year in the glasshouse. Plants were maintained in triplicate in a glasshouse into which heavily infected mildew plants were introduced and the infection of transgenic and susceptible control plants was followed over 6 months. 0[1] clear, no visible symptoms on any leaves, *[2] some fungal growth just visible to the naked eye *[3] proportion (percentage) of the top 20 leaves infected with visible symptoms over an average of 3 plants scored (standard deviation).*

Examples of the appearance of these plants are illustrated in Figure 7 (panels A and B). Macroscopic powdery mildew infection symptoms appeared consistently on the untransformed control plants and appeared on most of their leaves over time. In contrast no macroscopic symptoms could be detected on any of the lines transformed with the *Pl2.1* gene in this first year of infection.

Microscopic symptoms of infection on *Pl2.1* transgenic plants were too difficult to detect on a regular basis in order to be able to assess the response of the host when it carries the candidate gene.

The plants were maintained in the glasshouse over winter and treated with Hicane in the spring to induce budbreak. A natural infection cycle was allowed to initiate infection in the second season in the glasshouse. The infections were followed by regular macroscopic observations of the plants. Susceptible control plants again rapidly became infected with powdery mildew. Three months into the second season a detailed microscopic analysis of the response of the plants was carried out by inspecting several leaves from each plant proceeding from the youngest to the oldest leaf to determine if there were any spores or hyphae present, whether these spores germinated or the hyphae attempted to penetrate the host and the range of responses of the control and transgenic plants to powdery mildew. Example of the response of the control and *Pl2.1* transgenic plants to powdery mildew spores detected by monitoring the microscopic symptoms during this second period of infection are presented in Figure 7 (panels C to E) and Figure 8.

On the control plants the leaves were usually covered with dense growth of powdery mildew hyphae over a period of a few weeks (Figure 7C). In contrast the transgenic plants containing the *Pl2.1* gene contained few or no spores on the youngest leaves (Figure 7D). A few spores could be detected on middle aged leaves (Figure 7E), but in most cases these spores were not germinating (Figure 7F).

This initially made it difficult to detect whether the plants responded with a hypersensitive reaction to attempts by the spores to penetrate the host. A more detailed examination some of the older leaves from the transgenic plants (Figure 8) did however reveal increasing numbers of germinating powdery mildew spores and hyphae, albeit that this growth was always much slower and at a much lower level than found on the susceptible controls.

Even on the older leaves there were still many un-germinated spores visible and no obvious response from the host was visible in most fields of view (Figure 8A). However some of the hyphae on these plants were closely associated with a response

that has the hallmarks of a hypersensitive response (HR), as illustrated in Fig 8B to 8F.

Two of the nine lines eventually showed some signs of fungal growth that were just visible to the naked eye (lines A5 and A24), but even these were clearly distinguishable from the control plants which were heavily infected by this stage.

These observations were consistent between the replicates of the individual transformed and control lines. At the microscopic level all of the lines examined which contained the *Pl2.1* gene showed HR-like reactions. In many cases several HR reactions could be found in a single field of view and usually some signs of hyphal growth could be found adjacent or right above these reactions when focusing the microscope on different planes of view. The range of HR reactions is displayed in Figure 8. This data suggests that the *Pl2.1* gene is responsible for at least a major part of the resistance response (if not the entire response) conferred by the *Pl2* powdery mildew resistance locus and operates, at least in part, by the mechanism of a hypersensitive response.

Summary

A targeted map-based cloning strategy has been used in apple to identify a gene for the Pl_2 locus that co-segregates with the Pl_2 resistance phenotype. This candidate gene, named $Pl_{2.1}$, has been fully sequenced, consists of a continuous open reading frame of over 1300 amino acids and has all of the hallmark domains of a plant disease resistance gene in the coiled-coil, nucleotide binding site domain, leucine-rich repeat (CC-NBS-LRR) class. When this gene was transformed into susceptible apple plants they showed an enhanced resistance to mildew in glasshouse trials associated with hypersensitive response reactions. This allele of the $Pl_{2.1}$ locus therefore confers powdery mildew resistance in apple. This finding constitutes the first plant resistance gene in the NBS class cloned from the Rosaceae for which a function has been confirmed and the first powdery mildew resistance gene cloned within the Rosaceae.

Example 4: Expression of a truncated *Pl2.1* protein, including only a coiled-coil and a nucleotide binding site domain, confers powdery mildew resistance in transgenic plants

The applicants also prepared a *Pl2.1* deletion construct that would express a truncated *Pl2.1* protein. Two *Xmn*I restriction sites identified in Figure 3 were used to excise a 1.4 kb fragment from the *Pl2.1* gene while retaining its original promoter and terminator sequences. In addition to excising the 1.4 kb fragment, the excision results in most of the remaining leucine rich repeat region being out of frame with the N terminal portion of the *Pl2.1* gene. Figure 6B shows where these deletion events occur within the leucine rich repeat region. Plants carrying this modified construct would thus express a protein with only the first two domains (the coiled-coil and nucleotide binding site domains) intact. This allowed the applicants to test if the first two domain are sufficient to provide resistance. The sequence of the *Pl2.1* deletion construct is shown in SEQ ID NO: 4. The sequence of the truncated *Pl2.1* protein, expressed by the construct of SEQ ID NO: 4, is shown in SEQ ID NO: 5. The cDNA sequence encoding the truncated *Pl2.1* protein is shown in SEQ ID NO: 7. The sequence of a truncated *Pl2.1* protein extending from the full-length N-terminus to the end of the NBS domain is shown in SEQ ID NO: 6. The cDNA encoding the polypeptide of SEQ ID NO: 6 is shown in SEQ ID NO: 8.

The *Pl2.1* deletion construct was cloned into pART27, introduced into *A. tumefaciens* strain LBA4404 and used to transform apple as described in Example 3.

Functional analysis of Pl2.1 deletion and full-length Pl2.1 transformants

Further testing was carried out in the glasshouse in the third season to assess the effect of grafting and to compare symptom development on transgenic plants carrying a complete open reading frame of *Pl2.1* with plants carrying a deletion allele of *Pl2.1* and missing more than half of the protein in the correct reading frame. Five of the *Pl2.1* plant lines tested in Example 3 (lines A3, A5 A7 A8 and A25) were used to prepare plants for testing using the simple test of bench-grafting onto Royal Gala stock plants, and five plant lines carrying a *Pl2.1* deletion allele (lines DA2, DA3, DA4 DA5 and DA6) were used to prepare plants for testing using the simple test of

micro-grafting onto Royal Gala stock plants to create a suitable comparison between these two types of lines and the bench-grafted (line B) and micro-grafted (line M) control lines. The results of monitoring the macroscopic symptoms of these plants during the third spring-summer season are presented in Table 3. Examples of the microscopic appearance of these plants are illustrated in Figure 9 (panels A, B and C). Microscopic powdery mildew infection symptoms appeared consistently on the grafted untransformed control lines and appeared on most of their leaves over time. In contrast no microscopic symptoms could be detected on any of the grafted transformant lines carrying the complete *Pl2.1* gene. This illustrates that grafting is a simple test that can speed up the analysis of testing for the function of genes of this nature. In addition no microscopic symptoms could be detected on any of the grafted transformant lines carrying the *Pl2.1* deletion allele. This illustrates that the *Pl2.1* protein sustaining large deletions of more than half of the *Pl2.1* gene can still provide functional resistance. This also provides strong evidence that proteins including only the first two domains are sufficient to provide the resistance function.

Table 3.

Phenotypic comparison of transformants with *PL2.1* full length or deletion allele			
		Days after challenge	
Line (clones)	**Description**	**40**	**60**
Royal Gala A3 (2)	*Pl2.1* Transformant	0	0
Royal Gala A5 (2)	*Pl2.1* Transformant	0	0
Royal Gala A7 (1)	*Pl2.1* Transformant	0	0
Royal Gala A8 (1)	*Pl2.1* Transformant	0	0
Royal Gala A25 (2)	*Pl2.1* Transformant	0	0
Royal Gala B (3)	Bench-graft controls	60	90
Royal Gala DA2 (5)	*Pl2.1* deletion allele	0	0
Royal Gala DA3 (2)	*Pl2.1* deletion allele	0	0
Royal Gala DA4 (5)	*Pl2.1* deletion allele	0	0
Royal Gala DA5 (3)	*Pl2.1* deletion allele	0	0
Royal Gala DA6 (1)	*Pl2.1* deletion allele	0	0
Royal Gala M (5)	Micro-graft controls	60	90

Table 3. Powdery mildew phenotypes of 5 independent transformed lines of 'Royal Gala' with the full length Pl2.1 gene and 5 independent transformed lines with the Pl2.1 deletion allele in the third year in the glasshouse. Transformed shoots were used as micrografts onto M9 rootstocks. When the shoots were approximately 30 cm

in length, the shoots were cut back and single node cuttings were grafted on to Royal Gala stock plants. Full length Pl2.1 transformants were bench grafted whereas transformants carrying the Pl2.1 deletion allele were micro-grafted. Royal Gala control plants were bench-grafted and micro-grafted at the same time. These plants were grown for a further three months, then exposed to powdery mildew spores and the infection of transgenic and untransformed plants was followed over 2 months. The resulting phenotypes were scored as for Table 2.

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Summary of Sequences

SEQ ID NO:	Sequence type	Information	Species
1	polypeptide	full-length *Pl2.1* protein	*Malus zumi*
2	polynucleotide	*Pl2.1* gene	*Malus zumi*
3	polynucleotide	open reading frame/cDNA encoding full-length *Pl2.1* protein	*Malus zumi*
4	polynucleotide	*Pl2.1* deletion construct for expessing truncated *Pl2.1* protein of SEQ ID NO: 5	*Malus zumi*
5	polypeptide	*truncated Pl2.1* protein	*Malus zumi*
6	polypeptide	fragment containing CC domain and NBS domain only of *Pl2.1* protein	*Artificial*
7	polynucleotide	cDNA encoding truncated *Pl2.1* protein of SEQ ID NO: 5	*Artificial*
8	polynucleotide	cDNA encoding truncated *Pl2.1* protein of SEQ ID NO: 5	*Artificial*
9	polynucleotide	R2 P2N primer	*Artificial*
10	polynucleotide	F2 P2N primer	*Artificial*
11	polynucleotide	F1 2NA probe primer	*Artificial*
12	polynucleotide	R1 2NA probe primer	*Artificial*
13	polynucleotide	1 - Pl2.1 5' utr F1 primer	*Artificial*
14	polynucleotide	Pl2.1 3' CCR1 primer	*Artificial*
15	polynucleotide	Pl2.1 5' CCF1 primer	*Artificial*
16	polynucleotide	Pl2.1 3' utr R1 primer	*Artificial*
17	polynucleotide	Pl2.1rt 5' F1 primer	*Artificial*
18	polynucleotide	ACT2F primer	*Artificial*
19	polynucleotide	ACT2R primer	*Artificial*
20	polynucleotide	cDNA primer 3'UTR	*Artificial*
21	polynucleotide	cDNA primer 5'UTR	*Artificial*

CLAIMS:

1. An isolated polynucleotide encoding a polypeptide that comprises the sequence of SEQ ID NO: 1 or a fragment or variant thereof, wherein the fragment or variant confers resistance to the powdery mildew fungus *Podosphaera leucotricha* in a Rosaceae plant, and wherein the fragment comprises:

a) a sequence with at least 70% identity to the entire length of sequence of SEQ ID NO: 6,

b) a sequence with at least 70% identity to the entire length of sequence of SEQ ID NO: 5,

c) the sequence of SEQ ID NO: 6, or

d) the sequence of SEQ ID NO: 5, and wherein the variant comprises:

e) a sequence with at least 70% identity to the entire length of sequence of SEQ ID NO: 1.

2. The isolated polynucleotide of claim 1, wherein the polypeptide comprises the sequence of SEQ ID NO: 1.

3. An isolated polynucleotide comprising the sequence of SEQ ID NO: 3 or a fragment or variant thereof, wherein the fragment or variant encodes a polypeptide that confers resistance to powdery mildew fungus *Podosphaera leucotricha* in a Rosaceae plant, and wherein the fragment comprises:

a) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 8,

b) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 7,

c) the sequence of SEQ ID NO: 8, or

d) the sequence of SEQ ID NO: 7, and wherein the variant comprises:

e) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 3, or

f) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 2.

4. The isolated polynucleotide of claim 3, wherein the polynucleotide comprises the sequence of SEQ ID NO: 3.

5. The isolated polynucleotide of claim 3, wherein the polynucleotide comprises the
5 sequence of SEQ ID NO: 2.

6. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 1, or a fragment or variant thereof, wherein the fragment or variant confers resistance to the powdery mildew fungus *Podosphaera leucotricha* in a Rosaceae plant, and wherein
10 the fragment comprises:

a) a sequence with at least 70% identity to the entire length of the sequence of SEQ ID NO: 6,
b) a sequence with at least 70% identity to the entire length of the sequence of SEQ ID NO: 5,
15 c) the sequence of SEQ ID NO: 6, or
d) the sequence of SEQ ID NO: 5, and the variant comprises:
e) a sequence with at least 70% identity to the entire length of the sequence of SEQ ID NO: 1.

20 7. The isolated polypeptide of claim 6, wherein the polypeptide comprises the sequence of SEQ ID NO: 1.

8. An isolated polynucleotide encoding a polypeptide that comprises the sequence of SEQ ID NO: 6 or a variant thereof, wherein the variant confers resistance to powdery
25 mildew fungus *Podosphaera leucotricha* in a Rosaceae plant, and wherein the variant comprises at least one of:

a) a sequence with at least 70% identity to the sequence of SEQ ID NO: 6, and
b) a sequence with at least 70% identity to the sequence of SEQ ID NO: 5.

30 9. The isolated polynucleotide of claim 8, wherein the polypeptide comprises the sequence of SEQ ID NO: 6.

10. The isolated polynucleotide of claim 8, wherein the polypeptide comprises the sequence of SEQ ID NO: 5.

11. An isolated polynucleotide comprising the sequence of SEQ ID NO: 8 or a variant thereof, wherein the variant encodes a polypeptide that confers resistance to powdery mildew fungus *Podosphaera leucotricha* in a Rosaceae plant, and wherein
5 the variant comprises at least one of:
   a) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 8,
   b) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 7.

10

12. The isolated polynucleotide of claim 11, wherein the polynucleotide comprises the sequence of SEQ ID NO: 8.

13. The isolated polynucleotide of claim 11, wherein the polynucleotide comprises a
15 sequence of SEQ ID NO: 7.

14. An isolated polypeptide comprising the amino acid of SEQ ID NO: 6 or a variant thereof, wherein the variant confers resistance to powdery mildew fungus *Podosphaera leucotricha* in a Rosaceae plant, and wherein the variant comprises at
20 least one of:
   a) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 6, and
   b) a sequence with at least 70% sequence identity to the entire length of the sequence of SEQ ID NO: 5.

25

15. The isolated polypeptide of claim 14, wherein the polypeptide comprises the sequence of SEQ ID NO: 6.

16. The isolated polypeptide of claim 14, wherein the polypeptide comprises the
30 sequence of SEQ ID NO: 5.

17. A genetic construct which comprises a polynucleotide of any one of claims 1 to 5 and 8 to 13.

18. An expression construct which comprises a polynucleotide of any one of claims 1 to 5 and 8 to 13.

19. A host cell comprising the construct any one of claims 17 to 18.

5

20. A plant cell which comprises the construct of any one of claims 17 to 18.

21. A plant cell genetically modified to express a polynucleotide of any one of claims 1 to 5 and 8 to 13, or a polypeptide of any one of claims 6 or 7 and 15 to 16.

10

22. The plant cell of claim 20 or 21 which has increased resistance to powdery mildew.

23. A method for producing a plant cell or plant with increased resistance to powdery

15 mildew fungus *Podosphaera leucotricha*, the method comprising transformation of a Rosaceae plant cell or plant with the polynucleotide of any one of claims 1 to 5 and 8 to 13.

24. A method for selecting a Rosaceae plant with increased resistance to powdery

20 mildew fungus *Podosphaera leucotricha*, the method comprising testing of a plant for presence of the polynucleotide of any one of claims 1 to 5 and 8 to 13, or the polypeptide of claim 6, 7 and 14 to 16.

25. An antibody raised against a polypeptide of any one of claims 6, 7 and 14 to 16.

25

Figure 1

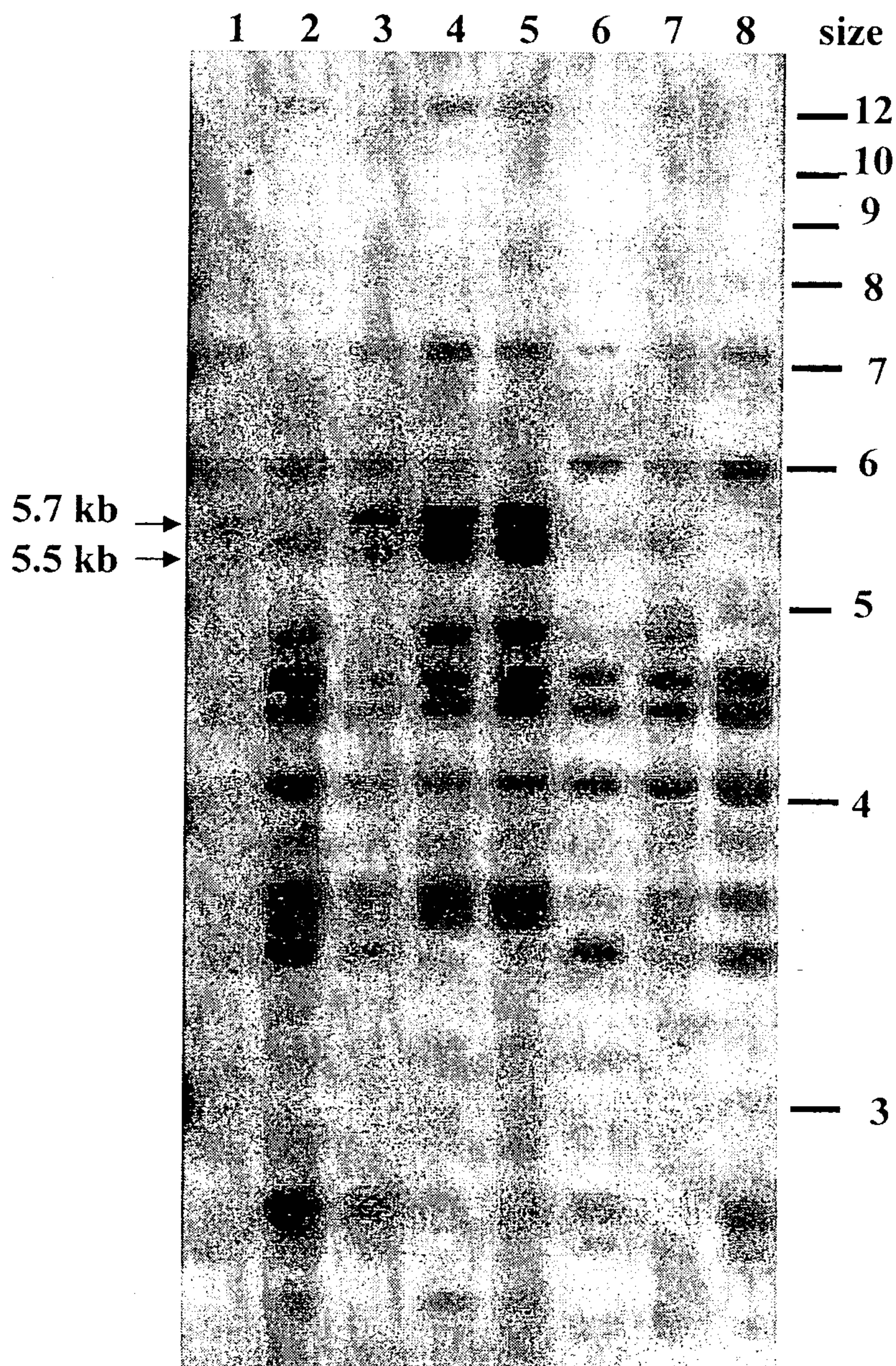

Figure 2A

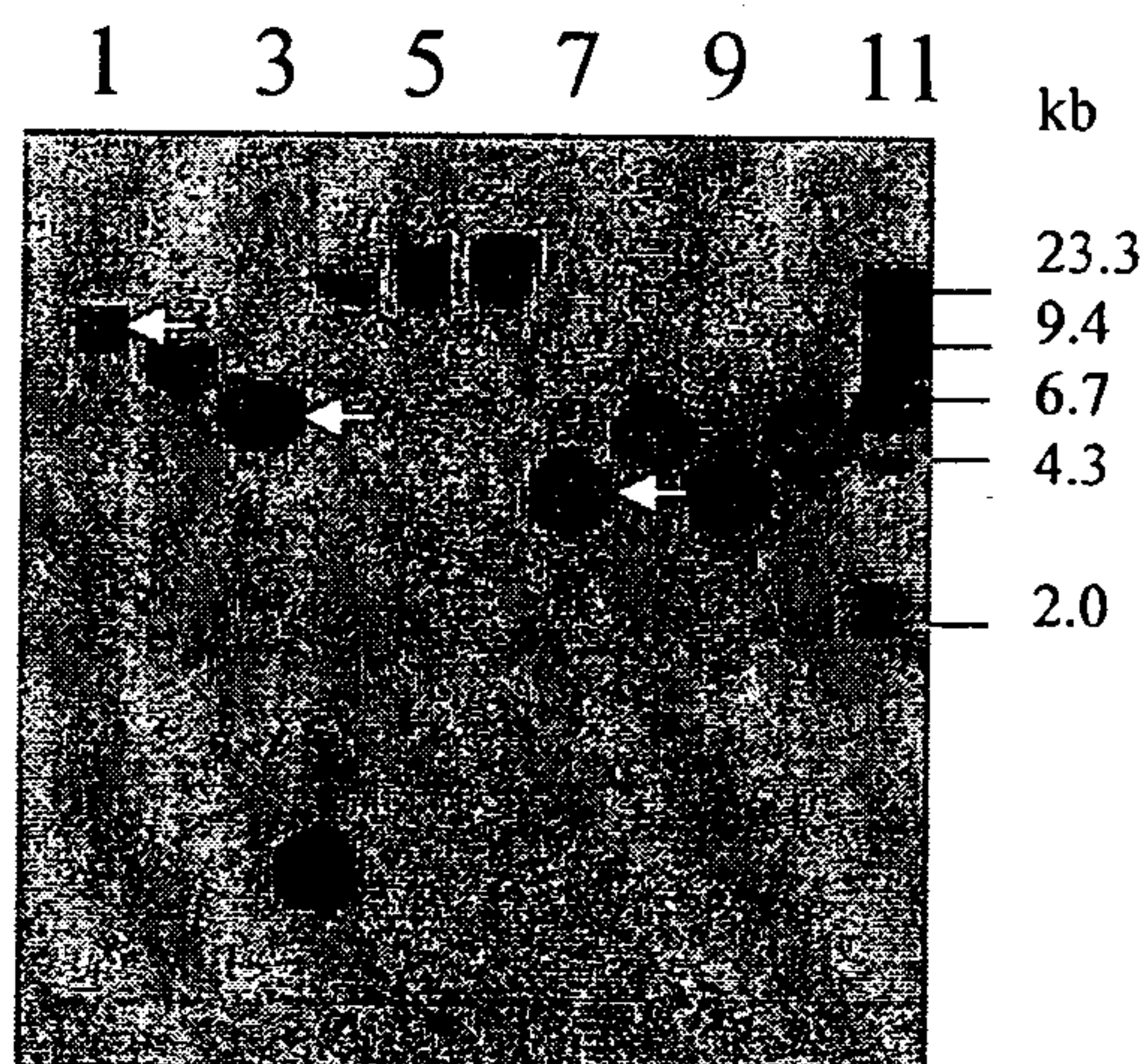

Figure 2B

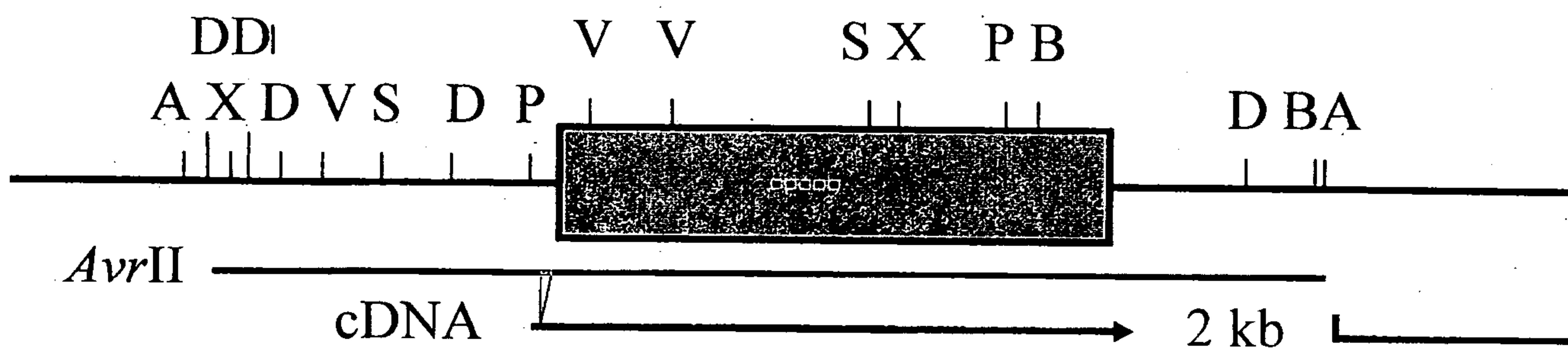

Figure 3

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   1  CTAGTTCTAGGATGATGTTAGAGAGACTATATATATATATATATATTTTTTTTTTTTAAG  60
  61  AAATGATATTATCTATACTAAAGAAAATTGATGAGTTCAGCGTCACAAAGTTATTAATAA  120
 121  TGTTGTTCAAATTTATATTTGGTAAAAAATCAAACTTAAAGAGTCTACTTTTAAAAAGGT  180
 181  ATGCTTATAAAATATATGAACTGACAGAAAGTTTGTAACATCTCATATCGTCTAGGGGAG  240
 241  TGGATCATCTATGTCTTATATCTATATTTCATCTCTACCTAGCACGAGACATTTTGGGA  300
 301  GCTTACTGGTTTCGGGTTCCATCGAAACTCAGCGAGTTCGCTCGAGAGCAATCTCATGAT  360
 361  ACGTAGCACGATATTGCCTAAAACGAATAATGTCATGCTATGACGAAATCGAGACTATAT  420
 421  GTGATGGGTCCGATCCAAAATGTGACAAAATTCATGAAAAACTCTTTTAAAAGAATCTTA  480
 481  TTAGCATTTTTCTTTTTCTAAACAGCATAAATATTTGGGGATTGATATTTACGAGCGTGT  540
 541  CGGAAGCAGCCGAATCGAAGTCACGAGATACGAAAAGAGTAGGCGTGGGCCGGAATGAAC  600
 601  GGATATTATGCATTTGCTGAGTCGATTCCACGACATGCAGAGTCAGATACACGTAGAAGC  660
 661  CGTCCGTTGTGGTCCCACATCTTAATTAATTAATTTCCATTTGTAGTTGGGTAGGTTTAA  720
 721  AGGATAAATTAGGATTATCACGCTCACAAAATAACATGATCAGCATTGCAAGGGAATGTT  780
 781  GCTCAGTTGTTTACGAGTCACCTTTGCACCCGAAGTTTCATGTTTGGTTCGCCATCTCCA  840
 841  AAATCGATCGCATAAAGGAAAAAAGAAATTGTAGCAAGTCTCTCAATTAAAAATTCATGA  900
 901  TCATTTGTCTCTTAATTTCCCAAAACGTGCGGCTATAGTTATTTTTGTCAACTTTATCAT  960
 961  AACTTTTGTCAAAACAAGTTAAGTTGAAAAAAACATTGCTATAATTGTATTAAAGTTAAG  1020
1021  ATATCATTGTTTCAATTAGATTAAAATTGAGAAACTATTTTTACAATTTTCTCATTTAGT  1080
1081  TTTCCATATTCGCTTCTTGATTCGGCCTCATGTGCCTAGCACGTGACTCATTTTGACGAA  1140
1141  AATTTTAGCGGTGTTGACGAAATGGACTTTAGCTGCACATTTTAATGAGTTAAGAGACCA  1200
1201  ATTATCATGAATTTTTAATTAAGAGATCATTATTCTAATTTGATTAAAATTGAGAGATCA  1260
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1321  AGAAAGCAATCAAATGATAAAACAATATGACCCCACGAAAAATAAAAAGAGAGGAAAAAG  1380
1381  AGGAGTCTAACTCTCCTTTTGGGCAGAAGGAAGAAGACTTCGTTATGGTCTTTCAACACC  1440
1441  TAATTATCTCAGCAAATTGAAGAGCTCTAAAGTAGTCTAGATCTGTTGATCTTAATGATC  1500
1501  TATGGAAACAAGTCCCTTCATCTTCATCATCATCTGAAAACTCCAACGCAGTAAGTTCCT  1560
1561  CTTGCAACTATACATGCCAAATCCATTATTTATTTATTTCATTTTTCTTCAAGTTTGTAT  1620
1621  GCTTAATTAATTATTTTATCAATAACCATCTATATAATCTGTGCACTTTTCAGGCTTAGT  1680
1681  TATTCCATCGTTCAGTCTGGATTTGTTGATCATAAGTGCAAACAAGTGCCCTCATGACCT  1740
1741  GATTGACTCCCATGAAACTGCTTCTTGCTACAGATCATGTCCAATATTCGTGCAAGTTCG  1800
1801  AGTCTTGAAACGTAGGATTGCAAGGTAATCCCAAAGCTGCCCATTTGTTTGTTCAACATG  1860
1861  TTAAAATTTAAGAAAAATAACCATTTATTTGTTTAACATGTTAAAATTTAAGAAAATAAT  1920
1921  GTATACTAATACATGATTTTTGCAATCATATATGATATGTTTTATTATGCTTTATTATTT  1980
1981  AAAGAGTAATGCTAGAAAAACTACATATTTATACTATATTAGTGTATCATCTCATATGGC  2040
2041  AAGTGGTGGACACAATCAATATCCTGTGAGATAAATTCATAAATTGACGTTACGTGTATA  2100
2101  CCTCACTTGCCACATTAGATGGTACATAAATGTGACACAAAAATATGTGATCTTCCCAAC  2160
2161  ATTTTCCTATTAAGTAATATTATGTGGTGTGTTTTATTGTGCTTTATACATACAATATCA  2220
2221  AGTTTTTTTTATTTTTTATTGAAACATATAATATCAAGTTATTAGATTATTTGGTAGTAC  2280
2281  TATATTAGGGATTGTATACCTGGCGATACGGTTATGATGTTGGCAACCTTGATCTGAATG  2340
2341  CGTCCTGTTGCCTGCCATCTCTAGTCCCAAATTGTCACCTCTAGAAATGTTTACTAGGAG  2400
2401  TCCATTAATTGATTATTTCACTTTTACTATAAATTTGTATATGATTGCGCATGCATCAAT  2460
2461  AATTAGTTCTCATCATTTTAATTGGTGCAGTTGTGACTATTTCTGAGTCTCGACAGATCA  2520
2521  AGCTTCCAAATTTCCAAGGAACTAGTATTTGACTGTTGATCTTCCCTTCGTTGAGTCTGT  2580
2581  ATTTGTTGATCACAAGCAGAGTTAGATTTGAACTTCCATGTAAGTTCTTATTATTTGTTT  2640
2641  GAATAATACATTGGTTGTTTTGCATTTTATTTGAACCACTTTTTATTCATTTGTTTCTTC  2700
2701  ACTTAATTTGTACAGTTCTGCACCTCAATATTGCTCAAGAGCGATTCGGTCTTTCTTTGA  2760

2761  CTGCTTGGAGTAATCCTCGCTCTTAATATGGCACTGGGAGAGGTTTTTCTTGCGGCGTTT  2820
   1                             M  A  L  G  E  V  F  L  A  A  F   11
2821  CTACAGTTGCTGCTCGACAGGTTGACCCCTCGCGAGATTCTTGAGTACTTGGGAAATTTC  2880
  12  L  Q  L  L  L  D  R  L  T  P  R  E  I  L  E  Y  L  G  N  F   31
2881  CGGGGCGTCGGACAGAAGCTGGAGAAGTGGAGGACCACGTTGTCTACAATCGGAGCGGTG  2940
  32  R  G  V  G  Q  K  L  E  K  W  R  T  T  L  S  T  I  G  A  V   51
2941  CTGAGCGACGCTGAGGAAAGGCAACTGACTGAAGGTGGTGTGAAACTGTGGCTGGATGAT  3000
  52  L  S  D  A  E  E  R  Q  L  T  E  G  G  V  K  L  W  L  D  D   71
3001  CTCAGAGACCTGGCCTATGATATCGAAGACATGTTGGACAAATTTGCCGTTAAAATGTTG  3060
  72  L  R  D  L  A  Y  D  I  E  D  M  L  D  K  F  A  V  K  M  L   91
3061  AAGCGCATGATAGAGGGATGTGATCAAGCCAGCACAAGCAGGAAGGTACGGAGATCATTT  3120
  92  K  R  M  I  E  G  C  D  Q  A  S  T  S  R  K  V  R  R  S  F   111
3121  TATAAAGTTAAATTGAGTTTTGATATGAACTCCGAAATGAAGAAGATTACGAAGCGGTTG  3180
 112  Y  K  V  K  L  S  F  D  M  N  S  E  M  K  K  I  T  K  R  L   131
3181  CAAGACATATCTGAACGGAAAGATAAGTTTGGCTTGAAAGATATTGGGACGTCTGCTAAG  3240
 132  Q  D  I  S  E  R  K  D  K  F  G  L  K  D  I  G  T  S  A  K   151
3241  GAATCGCGAAGTCTACCAAGTTCAGACGTGTTAGATGAAAAGCTTGTTGTTGGAAGAGAT  3300
 152  E  S  R  S  L  P  S  S  D  V  L  D  E  K  L  V  V  G  R  D   171
3301  GGTGACAAATGGGAGATTATTGAATTGTTGTCAAAAAAATACGAGCATACAGATGCCGTC  3360
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172 G D K W E I I E L L S K K Y E H T D A V 191
3361 AATTTGGTGTAGTTGCTATAGTTGGCATGCCCGGAGTCGGGAAGACAACACTTGCTCAA 3420
192 N F G V V A I V G M P G V G K T T L A Q 211
3421 CTTGTATTCAACCGCAAAGATGATGCCATGAAGGAGTTTGAGCTAAAGGTGTGGGTATGT 3480
212 L V F N R K D D A M K E F E L K V W V C 231
3481 GTGTCTGATGACTTCGATGTTGAACGAGTGACGAAGGCAATTCTTGAATCAATCACATCC 3540
232 V S D D F D V E R V T K A I L E S I T S 251
3541 CGACCCGTTCAAGTGCAGGAGTTTAGTCAAATTCAGCATGATTTGAGTGAGCAATTAAGA 3600
252 R P V Q V Q E F S Q I Q H D L S E Q L R 271
3601 GGAAAAAAGTTTTTAATCGTTTTAGATGATATCTGGAACAAAGATGACTCTGATCTATAC 3660
272 G K K F L I V L D D I W N K D D S D L Y 291
3661 GATCTCTGGACAAGACTTCAATCCCCTTTTGGCATCGGAGCAGGAGGAAGTAAGATTATT 3720
292 D L W T R L Q S P F G I G A G G S K I I 311
3721 GTGACAACCCGTGATGTGAATGTTGCAAAGATTATGGGAGCCACTGGAGTTCATAATTTG 3780
312 V T T R D V N V A K I M G A T G V H N L 331
3781 GAGTGTATGGCAGATGATGATTGTTTGGAAATATTTGAGCGACATGCGTTCAGGGGAATT 3840
332 E C M A D D D C L E I F E R H A F R G I 351
3841 AATACTGGAAAGCCGGTAAATTATGATTTAATTAAGACAAGAATTGTTGAAAAATGTCGT 3900
352 N T G K P V N Y D L I K T R I V E K C R 371
3901 GGCTTACCATTAGCTGCAAGGACTCTCGGTGGTCTTTTACGTTGCAAAGAAAAAGATGAG 3960
372 G L P L A A R T L G G L L R C K E K D E 391
3961 TGGGGAGAAATATTGAACAACAAGTTATGGAATCTAGCAGACAAGAGTGGCATTCTCCCC 4020
392 W G E I L N N K L W N L A D K S G I L P 411
4021 GTACTAAAGTTGAGCTATCACTATCTTCCATCAAATTTGAAGAGGTGTTTTGCATATTGC 4080
412 V L K L S Y H Y L P S N L K R C F A Y C 431
4081 TCAATACTTCCAAATGACTATGAATTTGGGGAGAAGCAGCTCATTCTTTTGTGGATGGCA 4140
432 S I L P N D Y E F G E K Q L I L L W M A 451
4141 GAGGGTTTGATTCAACAAAATCCTGACGACAATAAACAAATAGAGGATTTGGGCCGCGAC 4200
452 E G L I Q Q N P D D N K Q I E D L G R D 471
4201 TACTTTCGAGAGCTATTAGCAAGGTCGCTGTTTCAAGAATCAAGCAAAAACAATTCACGA 4260
472 Y F R E L L A R S L F Q E S S K N N S R 491
4261 TATGTAATGCATGACCTCGTTAATGATTTAGCACAATGGGCAGCAGGTGAAATATGTTTT 4320
492 Y V M H D L V N D L A Q W A A G E I C F 511
4321 AGATTGGAAGATAAGCAAGGTAATAACTTGCAAAGCAATTGCTTTCGAAGGGCTCGCCAT 4380
512 R L E D K Q G N N L Q S N C F R R A R H 531
4381 TCGTCTTTCATTGCTGGTCGATTTGATGGAGTTATGAGATTTGAGGACTTTCCAAAAGTT 4440
532 S S F I A G R F D G V M R F E D F P K V 551
4441 GAACGTTTGCGAACATTCCTGCCACTTTCACTTTCAGATTCCAGGGGATGGGCCAAATAT 4500
552 E R L R T F L P L S L S D S R G W A K Y 571
4501 TTGTCTCGTAAGGTTACTTTTGAGCTATTACCACAGTTGCAATACTTACGAGTGCTCTCT 4560
572 L S R K V T F E L L P Q L Q Y L R V L S 591
4561 TTCAATGACTACACAATAACTGAGCTGCCAGACTCAATCGGTGATTTGAGGTTGTTACAG 4620
592 F N D Y T I T E L P D S I G D L R L L Q 611
4621 TATCTTGACCTTTCCTATACACATATAGCCAGTTTGCCTAAATCAACAAGCACTCTTTAC 4680
612 Y L D L S Y T H I A S L P K S T S T L Y 631
4681 CACTTGCAAACATTGATATTGGAAGGTTGTTCTCAATTGAAGTCATTGCCCGCGAACATG 4740
632 H L Q T L I L E G C S Q L K S L P A N M 651
4741 AGTAATCTAATTAATTTGCGCCATCTCAACAACTCAGATGCATCTTCGTTGAAAG*GAATG* 4800
652 S N L I N L R H L N N S D A S S L K G M 671
4801 *CCTTC*GCAACTAGGTCGATTGACAAATCTACAATCACTGCCTCTTTTTGTGGTGAGCGAA 4860
672 P S Q L G R L T N L Q S L P L F V V S E 691
4861 GGAAGTGATCATTCAGGGATAAGAGAGATAGGGCCCCTATTGCATCTCCGAGGGACATTG 4920
692 G S D H S G I R E I G P L L H L R G T L 711
4921 TGCCTCTTAGGATTGGAGAATGTGACTGATGTCGAGGATGCCAGGAGGGCCAACTTGAAA 4980
712 C L L G L E N V T D V E D A R R A N L K 731
4981 TGCAAGGAGAGGCTTGATTCACTGGTCCTAAAATGGTATCATTCAAGCGACACGAGAGAA 5040
732 C K E R L D S L V L K W Y H S S D T R E 751
5041 ACAGAATCCGCTGTGCTTGACATGTTACAGCCTCATACAAAGCTCAAGGAGCTCACCATC 5100
752 T E S A V L D M L Q P H T K L K E L T I 771
5101 AAGGGTTATGCCAGAGAGGAATTTTCATCATGGGTTGGAGGTCCCTTGTTCTCTAATATG 5160
772 K G Y A R E E F S S W V G G P L F S N M 791
5161 GTGCTTGTGCGCTTAGAGGAATGTAACAATTGTTTATCGTTGCCACCTCTCGGACAATTG 5220
792 V L V R L E E C N N C L S L P P L G Q L 811
5221 CCTCGTCTCAAAGAGCTTTATATTGGAGGAATGAATGCAGTCGAAAGTGTTGGTGCTGAG 5280
812 P R L K E L Y I G G M N A V E S V G A E 831
5281 TTTTATGGTGAGTGTGTCATGCCTTTTCCGCTGTTAGAGATTCTCGAGTTTGTGGATATG 5340
832 F Y G E C V M P F P L L E I L E F V D M 851

5341 CGGCATTGGAAGGTGTGGCTTCCTTTCCAACTGGATCACGGAAGTGGTGTTTTCCCTTTC 5400
852 R H W K V W L P F Q L D H G S G V F P F 871
5401 CTAAAAAGGCTTTCAATCCAGGAATGTTCTAAGTTGGAAGGTAAACTGCCAGAGAAGCTT 5460
872 L K R L S I Q E C S K L E G K L P E K L 891
5461 GATTTGTTAGCCGAACTTGAAATTGTTAAATGTGAGGAATTGACGGTTTCGATTGCCAAC 5520
892 D L L A E L E I V K C E E L T V S I A N 911
5521 TACAAACAGCTTCGTCAGCTAAACATTGACGGTTGTAAAGTGTTGGAACATACAGCTGCT 5580
912 Y K Q L R Q L N I D G C K V L E H T A A 931
5581 AAGGTTGAGTTTGAGTTATTAGAGTCCTTGTGCATTTCAAACATTTCAGAGGTGATGTCT 5640
932 K V E F E L L E S L C I S N I S E V M S 951
5641 CGGCCAACAGGGGAATTGTTCAGGAAGGGACTAAGCAAGGTTAGAGATTTGAAGATCAAT 5700
952 R P T G E L F R K G L S K V R D L K I N 971
5701 GGATGTGAGAAGCTGACGTCTTCACTGAAGAATGAGGCTAGATTATTGCAGCGGTTGACT 5760
972 G C E K L T S S L K N E A R L L Q R L T 991
5761 TCTCTTGGCCGTTTGGAAATTAAAGACAACTCTCGTCTAGTTGAAGAATTGGGAGAAGAA 5820
992 S L G R L E I K D N S R L V E E L G E E 1011
5821 GCAGAGGAGTTGCTGCAATTGCAAATATTGGATTGCAAGCTTGAACTTCTAAAGTTAAGA 5880
1012 A E E L L Q L Q I L D C K L E L L K L R 1031
5881 AAGTGCGAAAATCTTTTGAAGCTACCAAAAGGGTTAAATCAGCTGTCGTCTCTTCAAAAG 5940
1032 K C E N L L K L P K G L N Q L S S L Q K 1051
5941 CTTCGCATAGTAGGATGTTCAAGTCTAGTTTCTTTTCCAGATGTTGGTCTGCCACCTTCT 6000
1052 L R I V G C S S L V S F P D V G L P P S 1071
6001 CTTAAAGACATCTGGATTGCAGAGTGCAATTCGTTGATATATTTTGCAAAATTCCAGATT 6060
1072 L K D I W I A E C N S L I Y F A K F Q I 1091
6061 CCCCAAAATCTCAGAATAATACAGATAAGAGGGTGCAAAAGTTTGAAATCACTAGTAGAT 6120
1092 P Q N L R I I Q I R G C K S L K S L V D 1111
6121 GAGGAGGAATGTGAACGACTGGGTTTAATAGCACC*GAACGGGTTC*TTCAGCGACAACACC 6180
1112 E E E C E R L G L I A P N G F F S D N T 1131
6181 AATCACTGCCTTGAATCTATTTTGATCTGGAAGTGCCAAAATCTGAAATCCTTACCGGAT 6240
1132 N H C L E S I L I W K C Q N L K S L P D 1151
6241 GGCTTATGCCACCTCAGCAATCTTCAAACTCTAAGAATCGAATACTGTGGAAGTCTTGTT 6300
1152 G L C H L S N L Q T L R I E Y C G S L V 1171
6301 TCCATCCCGAGACTGAGTGGGGGAGAAGACCCTCCAACCTGAGAGAGATCTGGATCCGA 6360
1172 S I P R L S G G R R P S N L R E I W I R 1191
6361 GATTGCGAGAAATTGGAGGCGTTGCCCGAAGACATGCACAATCTCAACTCTCTTGAGGAA 6420
1192 D C E K L E A L P E D M H N L N S L E E 1211
6421 TTGAGGATCGACTACCGGGAAGGTTTGACTTTTCCTCCCAACCTAAAATCACTTGGAATT 6480
1212 L R I D Y R E G L T F P P N L K S L G I 1231
6481 AGGAAGGTCAAGAGCTGTAAGTCATTGTGGGAGTTGGAGTGGGGGTTGCACAGACTCACC 6540
1232 R K V K S C K S L W E L E W G L H R L T 1251
6541 TCTCTTAAAATCGGTGGTGAAGACCCGGATACGGTGTCGTTTCCACCCGACATGGTACGG 6600
1252 S L K I G G E D P D T V S F P P D M V R 1271
6601 ATGGAGACGCTCTTCCCCAAATCTCTCACTAGCCTCTCAATAGATGGCTTCCCGAATTTG 6660
1272 M E T L F P K S L T S L S I D G F P N L 1291
6661 AAGAAACTGAGCAGCAAGGGCTTTCAATTCCTCACCTCCCTTCAATCTCTTACACTCTTG 6720
1292 K K L S S K G F Q F L T S L Q S L T L L 1311
6721 GATTGTCCAAAGCTAGCATCCATTCCAGAGGAGGGTCTGCCTCCTTCACTAGAGGAATTA 6780
1312 D C P K L A S I P E E G L P P S L E E L 1331
6781 ATCATCGATGGGTGTCCAGTGCTAAAAGAGAGATGCCAACCAGGAAAAGGACGCTACTGG 6840
1332 I I D G C P V L K E R C Q P G K G R Y W 1351
6841 CACAAAATATCCCACATCCCTTTCATAGAGATAGATTGGCACATAATTTGATGCAGATGG 6900
1352 H K I S H I P F I E I D W H I I * 1367
6901 TCCAGGTATCATCTGTCGAGGAATGTTTTAATATTAATATTATTATTATTATTATCTTTA 6960
6961 TTCTTTTTTTTTTGGGTAATTAATCATGTTTATTCTTGTAAAGTAAAGTAATCCACATAT 7020
7021 TTTGGTTTGGGTCAAGGTTTTGGGGGTTGAGGAGTGGCTGCTTTGGGAGGTCTTCAATTG 7080
7081 GCGACAACCTGGATATTTCTCGGCCAACGTTTCTTCTCCCGGATTCCTATATTTTCTTTT 7140
7141 TTTATTTTTTTGGTAAACGATTGCTATGTTTGCTTGGCCCCTTTTCACTGATTTGTATTA 7200
7201 TAACTTTGTAAATCAGGCCACTTTCACCTAATGTTTTATTTTATTTTAGCAAATTTTGAC 7260
7261 TTATGAGCTAGTTATCTTAATGCATCGAGAATAAGCAATCAACAAAGAAATGGAAATGCA 7320
7321 AATGATGACCACTAAATGACTTCCTTTACCATAACTTGTCTTCCTGAAAGTAGATAATAT 7380
7381 GTGCCAACAAATTTTGGTCAATCAAATCCTTATACCAAGCCTTCGTCACTTATTCAAAAA 7440
7441 GTGCATTATTCATCTAATTAATATGTAATAAATTGGTGACGATTATACATTTTAACTGAG 7500
7501 TTCTAAGGAAAATTGAGGGTAGTAATACATCAGTACTAAAACGTGAATTTGTGAAAAACT 7560
7561 GGTTTGATTTTATTCGTGGATTTTATGTGGGATAGTGATTGTGCAATTTTCCATTTTTTA 7620
7621 TTTGACCCTCAATTTTATTCCAAACGCACAAACCACTTCAGGGCTGACGCCGACTTAACG 7680
7681 GCGATCCGAATCATCTTTCCGTTCCGTCATTTTAAAATTATTTAGTAATCCTAAATTTCT 7740
7741 TCATCTAAACATAGTGTAACTCGTTAAGAAGACTCTCGAAGTCGAGAGAAAAAGATGAGC 7800
7801 GACGCGTGGAAAATAGGGACACAAACGGAAAGAAAACAGAGAAGCGAGGGCAGTTCCGTC 7860

```
7861  CGGACACTGGTGATCAACAGTACCGGGGGAAACTCACGTTTAGTATATATGATTTACAAG 7920
7921  CTTTATTTGGGACTGCCCGAATTTGATTCTTAAGCAATGTTTATTAAAGTGAGGTGATCT 7980
7981  GATAATGACCTGATTCATTAACAAGTTATGTTGTTTTATGTTGGGTTAATGGGTCCTGCA 8040
8041  AGACATTGCTTAATGTCTAAATTTGGCAAACAATATTTTGTCGAGTTCTTGATTCAACAA 8100
8101  TGACTCAACTTGTTAAAGGGTTCTTAATGAGTCCTAATAACGACTCGACTTGTTAACAGG 8160
8161  TTGACTAAAAACCTATTACTTTCATGTCGTTTTGTATCAAATGAACGAGTCGTGCAATAA 8220
8221  ATTGTCAGACCTAATGGGACCGTGCCAAACAATTGATCGTAGCACGATATTATCTGCTTT 8280
8281  AGGTCCTGACCACACCCTCACGGTTTTGTTTCTGATAACTCACACGAAAACTTCCCAGTG 8340
8341  GGTCATCCATCATGGGATTGCTCTCGCGCAATCTCGCTTAACTTCGAAGTTCCTATGAAA 8400
8401  TCTGAAGCCAGTGAGTTCCAAAAAGACCTTGTGCTAATTGGAGGTAGGAATGTACATATA 8460
8461  AGGCATAGAGGATCCACTCCCCTAG 8485
```

Figure 4A

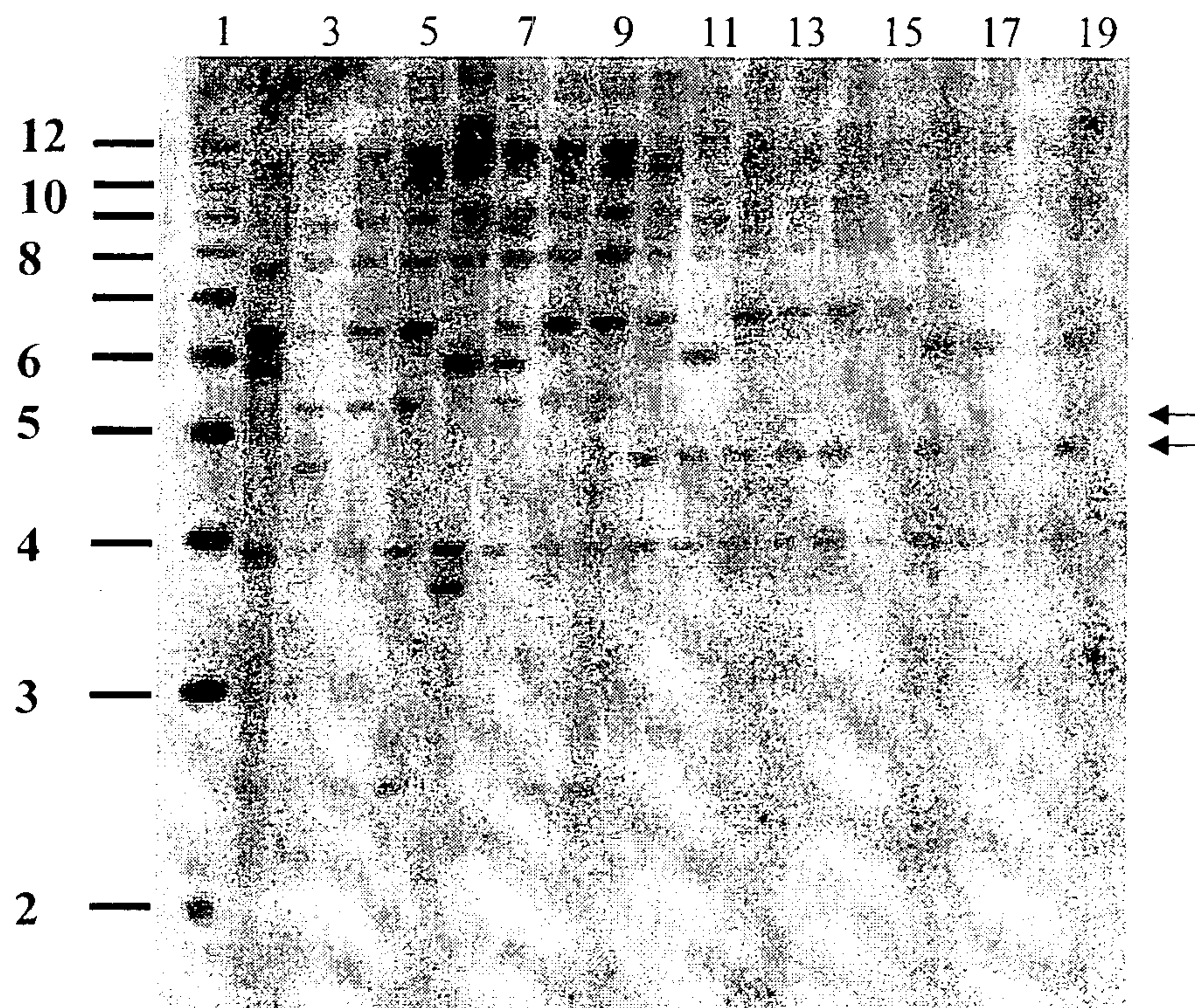

Figure 4B

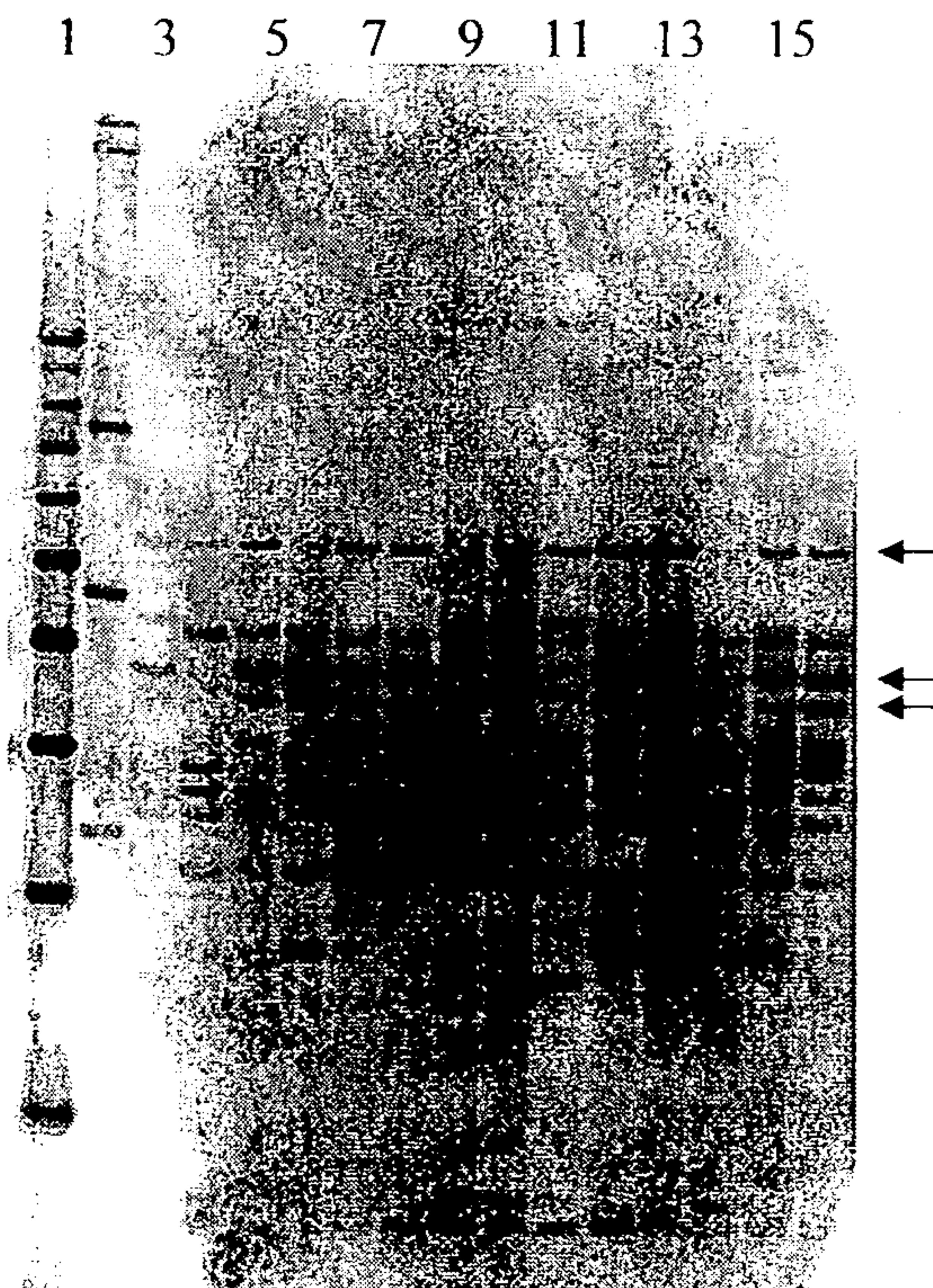

Figure 5A

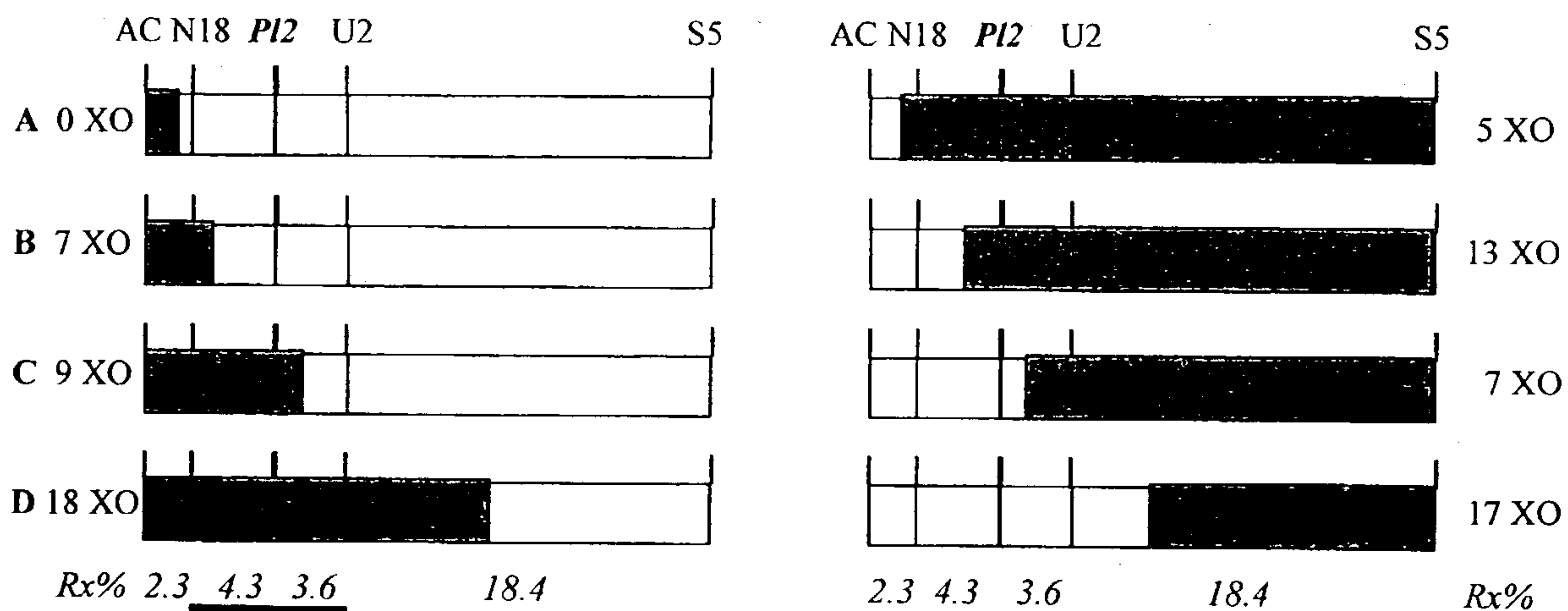

Figure 5B

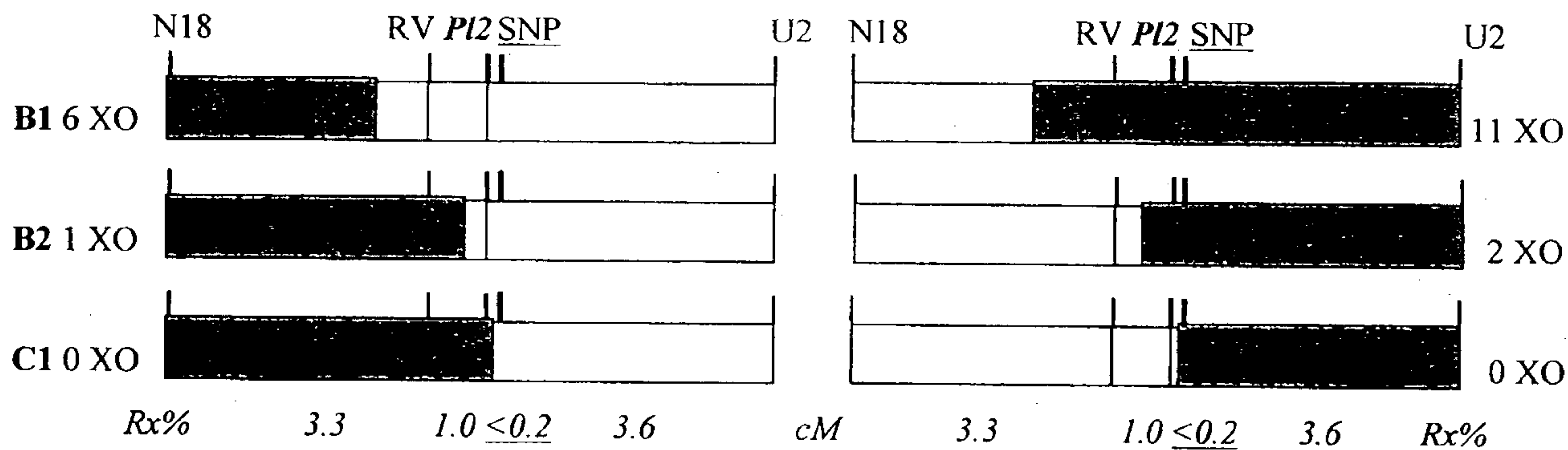

Figure 6A

```
CC region               *       20         *       40         *       60
MxdPl2.1CC : ----MAIGEVEILAAFLQELLLDRLTPR---EILEYLGNFREVGQKLEKWRTTLSTIGAVLS :  53
AthaQ9LRR4 : ---MTGIGEMELAAFLQALFQTLVSE---PFRSFFKRRELNENLLERLSTALLTITAVLI :  54
LescI2CC   : MEIGLAVGGAFLSSALNVLFDRLAPNG--DLLNMFRKHKDHVKLLKKLKMTLRGIQIVLS :  58
OsatXA1CC  : ---MEEVEAGWLEGGIRWLAETILDNLDADKLDEWIRQIRLAADTEKLRAEIEKVDGVVA :  57
TaesPM3bCC : --MAERVVTMAIGPLVSMLKDKASS----YLLDQYKVMEGMEEQHKILKRKLPAILDVIT :  54
OsatAAO379 : --MAELMATMVVGPLVSMVKEKASS----YLMEQYKVMEGMEEQHKILKRKLPAILDVIA :  54
OsatP0514H : -----MATSMLLGPLIALVNRQVSN----YELQQYQELDGMEEQETILERKLPAILDVII :  51
                       6    6  6                              1   6   6  V6
                        *       80         *      100         *      120
MxdPl2.1CC : DAEERQ-LTEGGVKLWLDDLRDLAYDIEDMLDKEAVKMLKRMIEGCDQASTSRKVRR--- : 109
AthaQ9LRR4 : DAEEKQ-ITNPVVEKWVNELRDVVYHAEDALDDIATEALRLNIGAESSSSNRLRQLR--- : 110
LescI2CC   : DAENKQ-ASNPSVRDWLNELRDAVDSAENLIEEVNYEALRLKVEG--QHQNFSETSN--- : 112
OsatXA1CC  : AVKGRA-IGNRSLARSLGRLRGLLYDADDAVDELDYFRLQQQVEGGVTTRFEAEETVGDG : 116
TaesPM3bCC : DVEEQAMAQREGAKAWLQELRTVAYVANEVFDEFKYEALRREAKKNGHYIKLGFDVI--- : 111
OsatAAO379 : DAEEQAAKHREGAKAWLEELRKVAYQANDVFDEFKYEALRRKAKANWQYKMLGMDVI--- : 111
OsatP0514H : DAEEQG-THRPGVSAWLKALKAVAYKANDIFDEFKYEALRREAKRRGNHGNLSTSLV--- : 107
             d e             w6  L4   y a    d       L
                          ---Coiled coil---
                        *      140         *      160         *      180
MxdPl2.1CC : --------------------------------------------------------SFYK : 113
AthaQ9LRR4 : --------------------------------------------------------GRMS : 114
LescI2CC   : --------------------------------------------------------QQVS : 116
OsatXA1CC  : AEDEDDIPMDNTDVPEAVAAGSSKKRSKAWEHFTTVEFTADGKDSKARCKYCHKDLCCTS : 176
TaesPM3bCC : --------------------------------------------------------KLFP : 115
OsatAAO379 : --------------------------------------------------------KLFP : 115
OsatP0514H : --------------------------------------------------------LAN- : 110
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```
                      *        200          *        220          *        240
MxdP12.1CC  : VKLSFDMNS-----EMKKITKRLQDISERKDKEGLK---DIGTSAKESRSLPSSDVLDEK : 165
AthaQ9LRR4  : LGDFLDGNSEHLETRLEKVTIRLERLASQRNILGLK---ELTAMIPKQR-LPTTSLVDES : 170
LescI2CC    : DDFFLNIKD-----KIEDTEETLKDLQEQIGLLGLK---EYFDSTKLETRRPSTSVDDES : 168
OsatXA1CC   : KNGTSALRNHENVCKRKRVTSTDQPVNPSSAGEGASNATGNSVGRKRMRMDGTSTHHEAV : 236
TaesPM3bCC  : THNRVAFRYKMG-RKICLIEQAVEVLIAEMQVEGFKYQPQPPVS-KEWRHTD-YVSEDPQ : 172
OsatAAO379  : THNRIVFRYRMG-NKLRMILNAIEVLITEMNAFREKFRPEPPMGSMKWRKTDSKISEHSM : 174
OsatP0514H  : --NPLVFRYRMS-KKLRKIVSSIEDLVADMNAEGFRYRPQMPTS-KQWRQTD-SIEIDSE : 165
                                        6     g              r

                      *        260          *        280
MxdP12.1CC  : ---------------LVVGR--DGDKWEIIEELSKKYEHTDAVNFG : 194
AthaQ9LRR4  : ---------------EVFGR--DDDKDEIMRFLIPENGKDNGIT-- : 197
LescI2CC    : ---------------DIFGR--QSEIEDLIDRLLSEGASGKKET-- : 195
OsatXA1CC   : STHPWNKAELSNRIQCMTHQLEEAVNEVMREC RSSSSNQSRQGTP : 281
TaesPM3bCC  : ---------------EIASRSRHEDKKNIIGILVDEASNADLT--- : 200
OsatAAO379  : ---------------DIANRSREEDRQKIVKSLISQASNGDLT--- : 202
OsatP0514H  : ---------------NIVSR--EKEKQHIVNLLLTDASNRNLM--- : 191
                                   r          66 l
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```
NBS region           *        20         *        40         *        60
MxdP12.1NB : VVAIVGMPGVGKTTLAQLVFNRKDDAMKEFELKVWVCVSDDFDVERVTKAILESITSRPV :  60
AthaQ9LRR4 : VVAIVGIGGVGKTTLSQLLYN-DQHVRSYFGTKVWAHVSEEFDVFKITKKVYESVTSRPC :  59
LescI2NB   : VVPIVGMGGQGKTTLAKAVYN-DERVKNHFDLKAWYCVSEGFDALRITKELLQEIGKFDS :  59
OsatXA1NB  : VLPIVGNGGIGKTTLAQLVCK-DLVIKSQFNVKIWVYVSDKFDVVKITRQILDHVSNQSH :  59
TaesPM3bNB : VVPVVAMGGLGKTTLAQLIYN-DPEIQKHFQLLLWVCVSDTFDVNSLAKSIVEAS-PNKN :  58
OsatAAO379 : VIPIVGMGGMGKTTLAQLIYN-DPQIQKHFQLLLWVCVSDNFDVDSLAKSIVEAARKQKN :  59
OsatP0514H : VLPIIGMGGLGKTTFAQLIYN-DPEIQKHFQLRKWVCVLDDFDVTSIANKISMSHEKECE :  59
             V6 66g gG GKTTlaq 6 n d      F    W  Vs  FDv   6    6
             ----P loop----               RNBS-A

                     *        80         *       100         *       120
MxdP12.1NB : Q---VQEFSQIQHDLSEQLRGK--KFLIVLDDIWNKDDSDLYDLWTRLQSPFGIG----- : 110
AthaQ9LRR4 : E---FTDLDVLQVKLKERLTGTGLPFLLVLDDLWNEN----FADWDLLRQPFIHA----- : 107
LescI2NB   : KDV-HNNLNQLQVKLKESLKGK--KFLIVLDDVWNEN----YNEWNDLRNIFAQG----- : 107
OsatXA1NB  : EG--ISNIDTLQQDLEEQMKSK--KFLIVLDDVWEIR----TDDWKKLLAPLRPNDQVNS : 111
TaesPM3bNB : VD----TDKPPLARLQKLVSGQ--RYLLVLDDVWDNKE---LRKWERLKVCLQH------ : 103
OsatAAO379 : C-----NER---AEFKEVVNGQ--RFLLVLDDVWN-RE---ASKWEALKSYVQH------ :  99
OsatP0514H : N-----ADE----KLQQEVRGK--RYLLILDDVWN-CD---ADKWAKLKYCLQQY----- :  99
                           l    6 g   5L66LDD6W            W  L
                                  ---Kinase-2---

                     *       140         *       160         *       180
MxdP12.1NB : ----AGGSKIIVTTRDVNVAKIMGATG-VHNLECMADDDCLEIFERHAFRGINTGKPVNY : 165
AthaQ9LRR4 : ----AQGSQILVTTRSQRVASIMCAVH-VHNLQPLSDGDCWSLFMKTVFGNQEPCLNREI : 162
LescI2NB   : ----DIGSKIIVTTRKDSVALMMGNEQ-IR-MGNLSTEASWSLFQRHAFENMDPMGHPEL : 161
OsatXA1NB  : SQEEATGNMIILTTRIQSIAKSLGTVQ-SIKLEALKDDDIWSLFKVHAFGNDKHDSSPGL : 170
TaesPM3bNB : ---GGMGSAVLTTTRDKRVAEIMGADRAAYNLNALEDHFIKEIIVDRAFSSENGK-IPEL : 159
OsatAAO379 : ---GGSGSSVLTTTRDKTVAEIMAPPKEVHHLKDLNENFIKEIIERSAFNSEEEKRQSEL : 156
OsatP0514H : ---GGVGSAILMTTRDQGVAQLMGTTK-AHQLVRMEKEDDLAIFEKRAFRFDEQK-PDEL : 154
                  Gs 66 TTR   6A  6       6  6      6    aF
                  --Kinase-3--                      -RNBS-C

                     *       200         *       220         *       240
MxdP12.1NB : DLIKTRIVEKCRGLPLAARTLGGLLRCKEKD-EWGEILNNKLWNL-ADKSGILPVLKLSY : 223
AthaQ9LRR4 : GDLAERIVHKCRGLPLAVKTLGGVLRFEGKVIEWERVLSSRIWDLPADKSNLLPVLRVSY : 222
LescI2NB   : EEVGRQIAAKCKGLPLALKTLAGMLRSKSEVEEWKRILRSEIWEL--PHNDILPALMLSY : 219
OsatXA1NB  : QVLGKQIASELKGNPLAAKTVGSLLGTNLTIDHWDSIIKSEEWKSLQQAYGIMQALKLSY : 230
TaesPM3bNB : LEMVGEIVKRCCGSPLAASALGSVLRTKTTVKEWNAIASRSSICT--EETGILPILKLSY : 217
OsatAAO379 : LEMVGDIAKKCSGSPLAATALGSTLRTKTTKKEWEAILRRSTICD--EENGILPILKLSY : 214
OsatP0514H : VQIGWEIMDRCHGSPLAAKALGSMLSTRKAVEEWRAVLTKSSICD--DENGILPILKLSY : 212
              6    I   c G PLA   6g   L       eW 6            66p L 6SY
                   ---TM---
```

```
                         *          260            *            280            *          300
MxdP12.1NB : HYLPSNLKRCFAYCSILPNDYEFGEKQLILLWMAEGLIQQNPDDNKQIEDLGRDYFRELL : 283
AthaQ9LRR4 : YYLPAHLKRCFAYCSIFPKGHAFEKDKVVLLWMAEGFLQQTRS-SKNLEELGNEYFSELE : 281
LescI2NB   : NDLPAHLKRCFSFCAIFPKDYPFRKEQVIHLWIANGLVEVKDE---INQDLGNQYFLELR : 276
OsatXA1NB  : DHLSNPLQQCVSYCSLFPKGYSFSKAQLIQIWIAQGFVEESSE---KLEQKGWKYLAELV : 287
TaesPM3bNB : NDLPSHMKQCFAFCAVFPKDYKIDVAKLIQLWIANGFIPEHKE--DSLETIGQLIFDELA : 275
OsatAAO379 : NCLPSYMRQCFAFCAIFPKDHVIDVEMLIQLWMANCFIPEQQG--ECPEISGKRIFSELV : 272
OsatP0514H : DDLPSYMKQCFAFCAIFPKNYVIDVEMLILLWMANDFIPSEEA--IRPETKGKQIFNELA : 270
               Lp  6   Cf 5C 6fPk       66 6W6A   6           2  G   f EL
                       --RNBS-D--         ----WMx-G

                         *          320            *            340            *          360
MxdP12.1NB : ARSLEQESSKNNSR-----------YVMHDLVNDLAQWAAGEIGFRLEDKQGNNIQSNCF : 332
AthaQ9LRR4 : SRSLLQ---KTKTR-----------YIMHDFINELAQFASGEFSSKFED--GCKLQVS-- : 323
LescI2NB   : SRSLFEKVPNPSKRNIEE------LFLMHDLVNDLAQLASSKLCIRLEESQGSHMLEQ-- : 328
OsatXA1NB  : NSGFLQQVESTRFSS--E------YFVMHDLMHDLAQKVSQTEYATIDGSECTELAPS-- : 337
TaesPM3bNB : SRSFFLDIEKSKEDWEYY---SRTTCKIHDLMHDIAMSVMEKECVVATMEPSEIEWLP-- : 330
OsatAAO379 : SRSFFQDVKGIPFEFHDIKD-SKITAKIHDLMHDVAQSSMGKECAAIDSESIGSEDFP-- : 329
OsatP0514H : SRSFFQDVKEVPLHKDESGHSYRTICSIHDLMHDVAVSVIGKECFTIAEGHNYIEFLP-- : 328
              rs                           6HDl6 d6A
                                           MHD------

                    *
MxdP12.1NB : RRARHSSFIAG : 343
AthaQ9LRR4 : ERTRYLSYLRD : 334
LescI2NB   : --CRHLSYSIG : 337
OsatXA1NB  : --IRHLSIVT- : 345
TaesPM3bNB : DTARHLFLSC- : 340
OsatAAO379 : YSARHLFLSG- : 339
OsatP0514H : NTVRHLFLCS- : 338
                Rhl
```

Figure 6B

L	XX**LXL**XX	C/N Consensus	LRR
KVERLR	TF**LPL**SL	SDSRGWAKYLSRKVT	LRR 1
FELLPQL	QY**LRV**LS	FNDYTITELPDS	LRR 2
IGDLRLL	QY**LDL**SY	THIASLPKS	LRR 3
TSTLYHL	QT**LIL**EG	CSQLKS	LRR 4
LPANMS	NL**INL**RH	LNNSDASSLKGMPSQ	LRR 5
LGRLTNL	QS**LPL**FV	VSEGSDHSGIREIGP	LRR 6
LLHLR	GT**LCL**LG	LENVTDVEDARRAN	LRR 7
LKCKERL	DS**LVL**KW	YHSSDTRETESAVLDM	LRR 8
LQPHTKL	KE**LTI**KG	YAREEFSSWVGGP	LRR 9
LFSNM	VL**VRL**EE	CNNCLSLPP	LRR 10
LGQLPRL	KE**LYI**GG	MNAVESVGAEFYG	LRR 11
ECVMPF	PL**LEI**LE	FVDMRHWKVWLPFQLDH	LRR 12
GSGVFPFL	KR**LSI**QE	CSKLEGKLP	LRR 13
EKLDLL	AE**LEI**VK	CEELTVS	LRR 14
IANYKQL	RQ**LNI**DG	CKVLEHTAAK	LRR 15
VEFELL	ES**LCI**SN	ISEVMSRPGELF	LRR 16
RKGLSKV	RD**LKI**NG	CEKLTSSLKNEAR	LRR 17
LLQRLTSL	GR**LEI**KD	NSRLVEELGEEAEELLQ	LRR 18
LQILDCKL	EL**LKL**RK	CENLLKLPK	LRR 19
GLNQLSSL	QK**LRI**VG	CSSLVSFP	LRR 20
DVGLPPSL	KD**IWI**AE	CNSLIYFAKF	LRR 21
QIPQNL	RI**IQI**RG	CKSLKS	LRR 22
LVDEEEC	ER**LGL**IA	PNGFF	LRR 23
SDNTNHCL	ES**ILI**WK	CQNLKSLPDG	LRR 24
LCHLSNL	QT**LRI**EY	CGSLVSIPRLSG	LRR 25
GRRPSNL	RE**IWI**RD	CEKLEALPED	LRR 26
MHNLNSL	EE**LRI**DY	REGLTFP	LRR 27
PNL	KS**LGI**RK	VKSCKSLWEL	LRR 28
EWGLHRL	TS**LKI**GG	EDPDTVSFPPDMVRME	LRR 29
TLFPKSL	TS**LSI**DG	FPNLKKLSSKG	LRR 30
FQFLTSL	QS**LTL**LD	CPKLASIP	LRR 31
EEGLPPSL	EE**LII**DG	CPVLKER	LRR 32

CQPGKGRYWHKISHIPFIEIDWHII

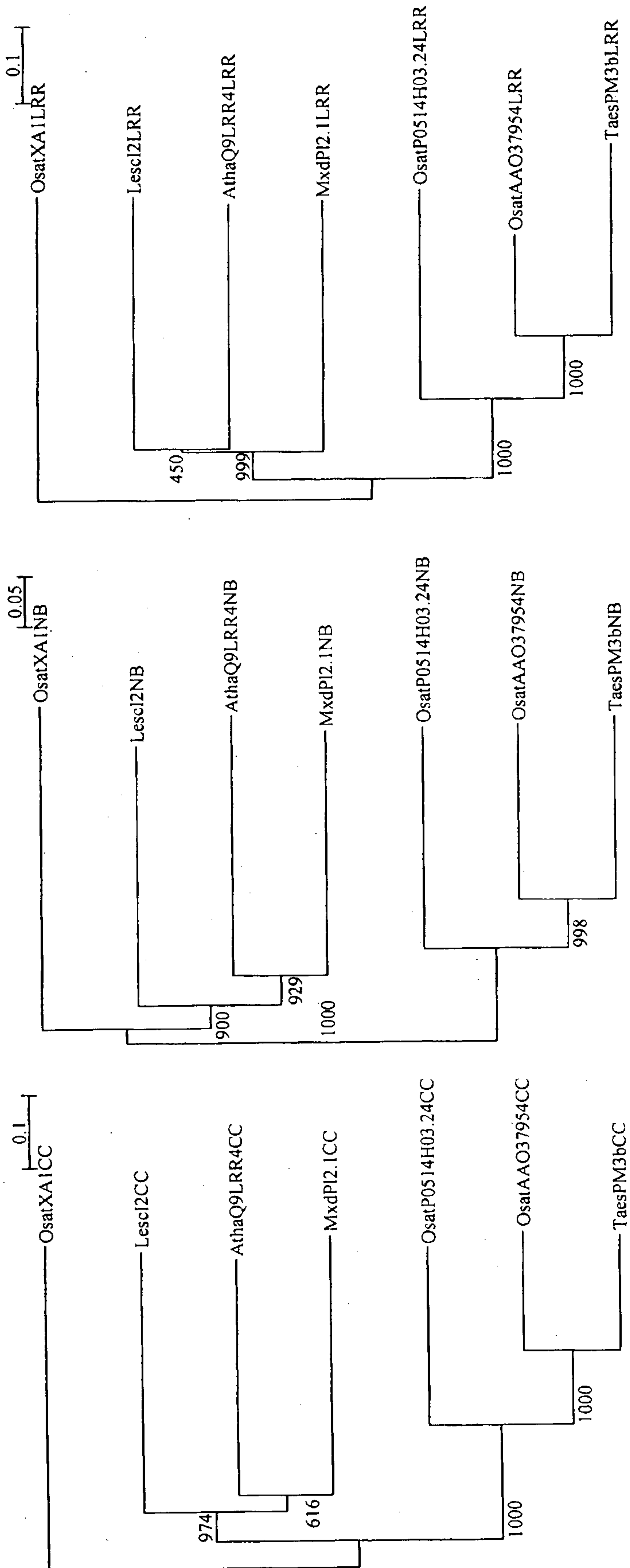


Figure 6C

Figure 7

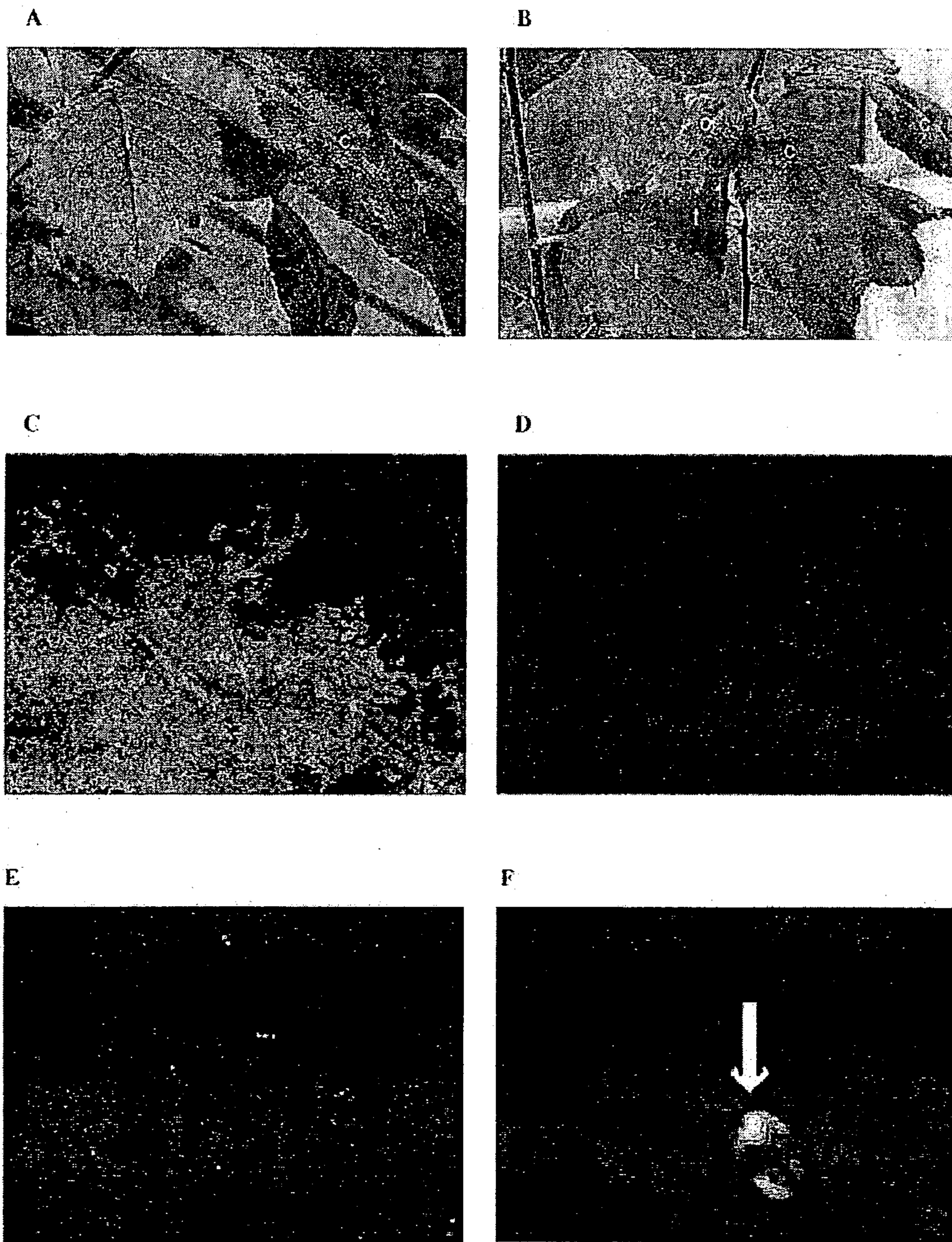


Figure 8

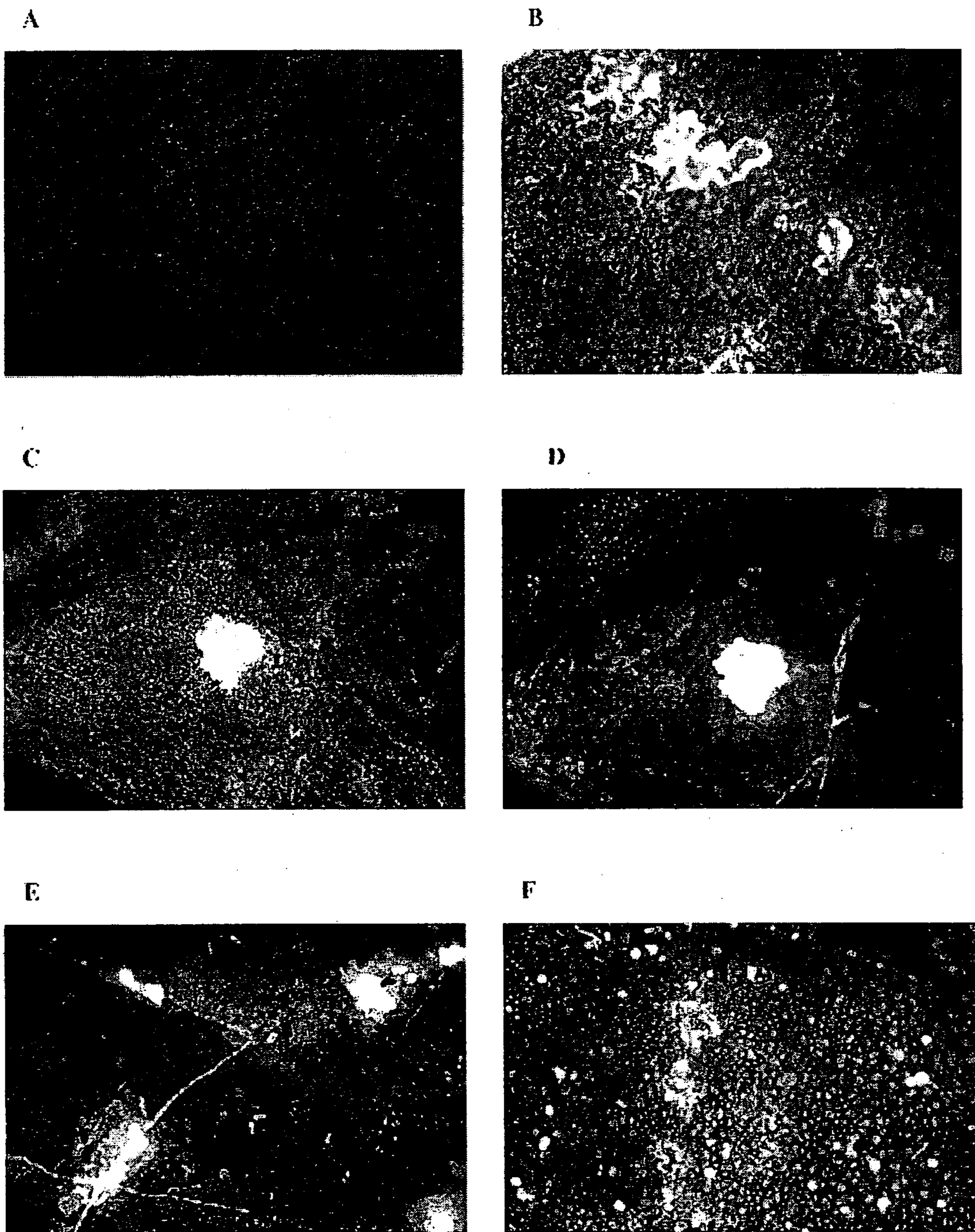
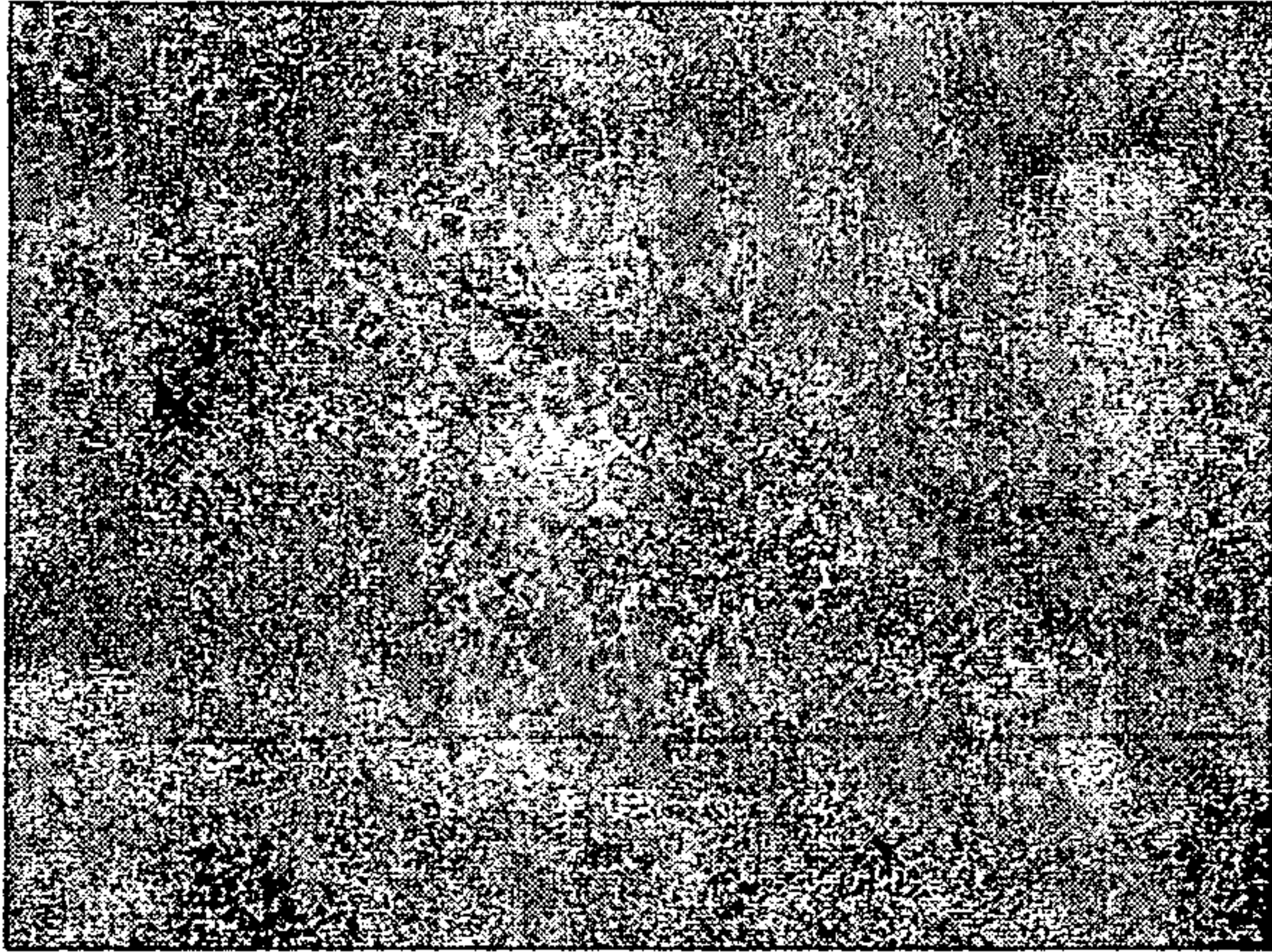
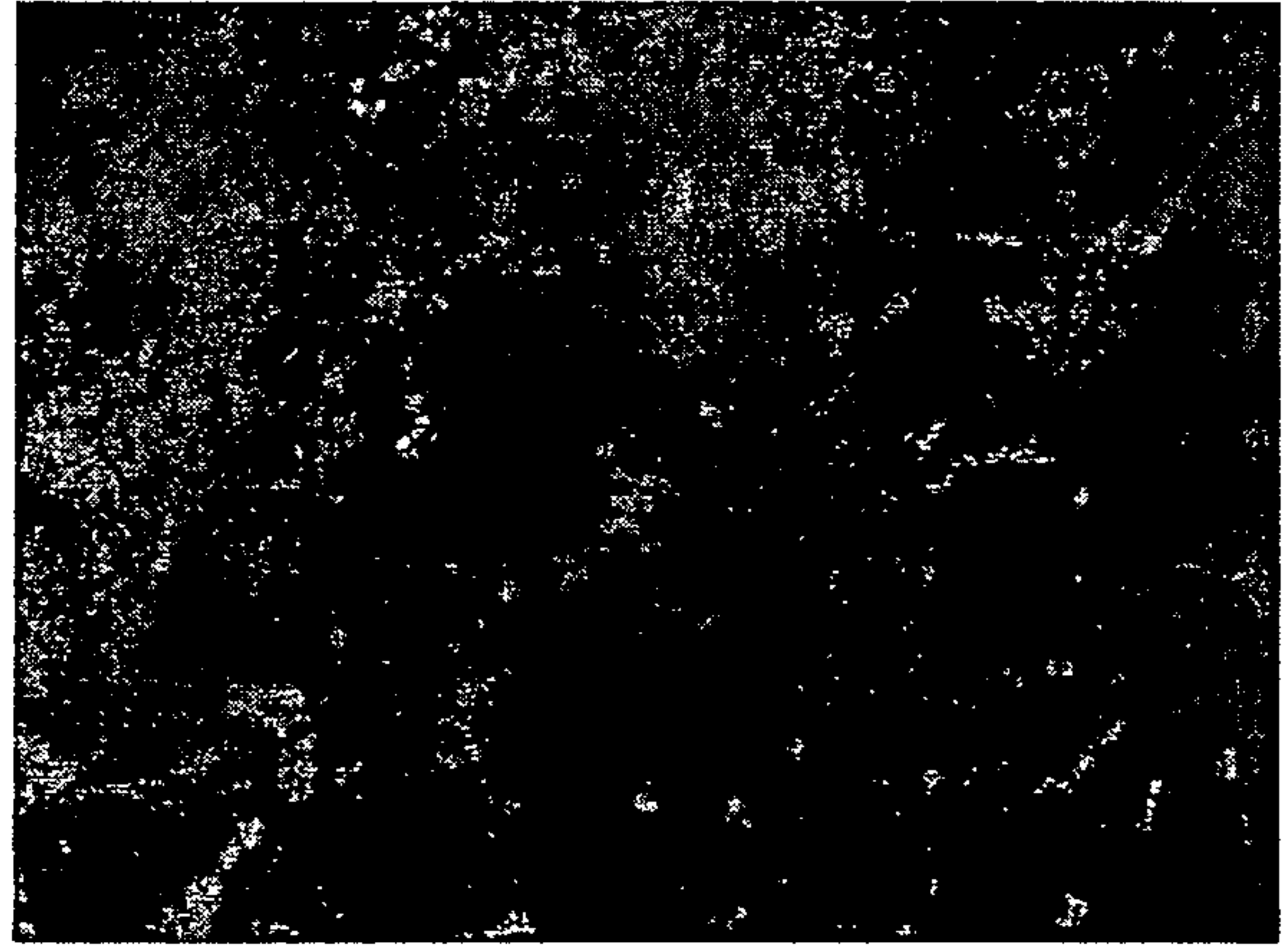


Figure 9

A



B



C