

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2008202603 B2**

(54) Title
Novel Nucleic Acid Sequences and their Use

(51) International Patent Classification(s)
C12N 15/82 (2006.01) **C07K 14/415** (2006.01)
A01H 5/00 (2006.01) **C12N 15/29** (2006.01)

(21) Application No: **2008202603** (22) Date of Filing: **2008.06.12**

(43) Publication Date: **2008.07.17**

(43) Publication Journal Date: **2008.07.17**

(44) Accepted Journal Date: **2011.07.21**

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(56) Related Art
Emble online Accession no. AF230327, 2000
US 6555732, 29 April 2003
WO 2000/015815, 23 March 2000

ABSTRACT

The invention relates to novel RacB cDNA sequences from barley and to expression cassettes and vectors comprising said sequences. The invention also relates to transgenic plants transformed with said expression cassettes or vectors, cultures derived there from, fragments or transgenic propagating material and to the use thereof in the production of foodstuffs, animal foods, seeds, pharmaceuticals or fine chemicals. The invention further relates to the production or enhancement of pathogen resistance in plants by reducing the expression of a RacB protein or a functional equivalent.

2008202603 12 Jun 2008

P001
Section 29
Regulation 3.2(2)

AUSTRALIA
Patents Act 1990

COMPLETE SPECIFICATION STANDARD PATENT

Application Number:

Lodged:

Invention Title: **Novel Nucleic Acid Sequences and their Use**

The following statement is a full description of this invention, including the best method of performing it known to us:

2008202603 12 Jun 2008

Novel nucleic acid sequences and their use in methods of obtaining a pathogen resistance in plants

- 5 The invention relates to novel RacB cDNA sequences from barley and to expression cassettes and vectors comprising these sequences. The invention furthermore relates to transgenic plants transformed with these expression cassettes or vectors, to cultures, parts or transgenic propagation material derived from
- 10 them, and to their use for the production of foodstuffs, feeding stuffs, seed, pharmaceuticals or fine chemicals. The invention furthermore relates to methods of generating or increasing a pathogen resistance in plants by reducing the expression of an RacB protein or of a functional equivalent thereof.

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- The aim of plant biotechnology work is the generation of plants with advantageous novel properties, for example for increasing agricultural productivity, increasing the quality in the case of foodstuffs, or for producing specific chemicals or
- 20 pharmaceuticals (Dunwell JM (2000) J Exp Bot 51 Spec No:487-96). The plant's natural defense mechanisms against pathogens are frequently insufficient. Fungal diseases alone result in annual yield losses of many billions of US\$. The introduction of foreign genes from plants, animals or microbial sources can increase the
- 25 defenses. Examples are the protection of tobacco against feeding damage by insects by expressing *Bacillus thuringiensis* endotoxins under the control of the 35S CaMV promoter (Vaeck et al. (1987) Nature 328:33-37) or the protection of tobacco against fungal infection by expressing a bean chitinase under the control of the
- 30 CaMV promoter (Broglie et al. (1991) Science 254:1194-1197). However, most of the approaches described only offer resistance to a single pathogen or a narrow spectrum of pathogens.

- Only a few approaches exist which impart a resistance to a
- 35 broader spectrum of pathogens, in particular fungal pathogens, to plants. Systemic acquired resistance (SAR) - a defense mechanism in a variety of plant/pathogen interactions - can be mediated by the application of endogenous messenger substances such as jasmonate (JA) or salicylic acid (SA) (Ward, et al. (1991) Plant
- 40 Cell 3:1085-1094; Uknes, et al. (1992) Plant Cell 4(6):645-656). Similar effects can also be achieved by synthetic compounds such as 2,6-dichloroisonicotinic acid (INA) or S-methyl benzo(1,2,3)thiadiazole-7-thiocarboxylate (BTH; Bion®) (Friedrich et al. (1996) Plant J 10(1):61-70; Lawton et al. (1996) Plant J.
- 45 10:71-82). The expression of pathogenesis-related (PR) proteins,

which are highly regulated in the case of an SAR, may also cause pathogen resistance in some cases.

- In barley, the Mlo locus has been described for some time as a negative regulator of plant defense. The loss, or loss of function, of the Mlo gene causes an increased and, above all, race-unspecific resistance for example against a large number of mildews (Büschges R et al. (1997) Cell 88:695-705; Jorgensen JH (1977) Euphytica 26:55-62; Lyngkjaer MF et al. (1995) Plant Pathol 44:786-790). The Mlo phenotype is inherited recessively, which also suggests a function as a susceptibility gene. Mlo-deficient barley varieties obtained by traditional breeding are already being widely used in agriculture. Although these varieties are being grown intensively, this resistance has proved to be extraordinarily durable, probably owing to the recessivity. Resistance breakdown has not been observed as yet. Mlo-like resistances in other plants, especially in cereal species, have not been described even though wheat, rye and other cereals are also attacked by comparable mildew pathogens. The reason in the case of wheat may be, for example, the existence of a hexaploid genome, which makes the identification of mutants in which each of the six copies of the gene has been inactivated extremely difficult.
- The Mlo gene has only recently been cloned (Büschges R et al. (1997) Cell 88:695-705; WO 98/04586; Schulze-Lefert P, Vogel J (2000) Trends Plant Sci. 5:343-348). As a consequence, various homologs have been isolated from other cereal species. Various methods for obtaining pathogen resistance using these genes have been described (WO 98/04586; WO 00/01722; WO 99/47552).

- Mlo resistance of a plant to mildew pathogens manifests itself in two important events, both of which bring about resistance to penetration: cell wall apposition (CWA) underneath the penetration site of the pathogen in the epidermal cell wall. Spreading of this fungal pathogen is almost exclusively restricted to this subcellular structure (Jorgensen JH and Mortensen K (1977) Phytopathology 67:678-685; Freialdenhoven A et al. (1996) Plant Cell 8:5-14). This reaction is caused by the genes Ror1 and Ror2, which are required for the effect of Mlo (Peterhänsel C et al. (1997) 9:1397-1409).

- The disadvantage in Mlo pathogen resistance is that Mlo-deficient plants - even in the absence of a pathogen - initiate a defense mechanism which manifests itself for example in the spontaneous death of leaf cells (Wolter M et al. (1993) Mol Gen Genet 239:122-128). A further disadvantage is that the Mlo-deficient

genotypes are hypersusceptible to hemibiotrophic pathogens such as *Magnaporthe grisea* (*M. grisea*) and *Cochliobolus sativus* (*Bipolaris sorokiniana*) (Jarosch B et al. (1999) *Mol Plant Microbe Interact* 12:508-514; Kumar J et al. (2001) *Phytopathology* 91:127-133). The Mlo gene therefore appears to be a negative regulator of cell death. Again, the cause is probably the induction of cell death in the absence of the Mlo gene, which increases the susceptibility to these fairly necrotrophic pathogens. This ambivalent effect, which limits the biotechnological use of Mlo, is probably due to the fact that necrotrophic fungi are capable of exploiting the more pronounced HR of the Mlo-deficient host plant for their infection process. A resistance comparable to Mlo deficiency, but without the characteristic of inducing cell death, would be desirable.

The proteins Rho, Rac and Cdc42 are members of the small GTP (guanosine triphosphate) binding protein family and regulate a large number of intracellular processes as "molecular switches", both in plant and animal organisms. As elements of signal transduction, they play an important role in the conversion of extracellular stimuli. For example, they regulate NADPH oxidase and thus the release of reactive oxygen molecules ("oxidative burst"). Animal or human Rac1 is essential for the formation of the active NADPH oxidase complex which, in turn, is important for the formation of superoxide, thus contributing to plant defense (Irani K and Goldschmidt-Clermont PJ (1998) *Biochem Pharmacol* 55: 1339-1346). The function in plant defense in plants and animals is largely analogous (Kwong et al. (1995) *J Biol Chem* 270(34): 19868-19872; Dusi et al. (1996) *Biochem J* 314:409-412; Diekmann et al. (1994) *Science* 265:531-533; Purgin et al. (1997) *The Plant Cell* 9:2077-2091; Kleinberg et al. (1994) *Biochemistry* 33:2490-2495; Prigmore et al. (1995) *Journal of Biol Chem* 27(18): 10717-10722; Irani et al. (1997) *Science* 275:1649-1652; Low et al. (1994) *Advances in Molecular Genetics of Plant-Microbe Interactions* 3:361-369 (1994) eds. MJ Daniels, Kluwer Academic Publishers, Netherlands; Mehdy et al. (1994) *Plant Physiol* 105: 467-472; Sundaresan et al. (1996) *Biochem J* 318:379-382). Moreover, GTP binding proteins function in restructuring the cytoskeleton and in cell transformation (Symon M. (1996) *TIBS* 21: 178-181), and also in the activation of transcription (Hill et al. (1995) *Cell* 81:1159-1170; Chandra et al. (1996) *Proc Natl Acad Sci USA* 93:13393-13397).

In plants, there exists a substantial family of Rac-like proteins (Winge et al. (1997) *Plant Mol Biol* 35:483-495), which is also termed Rop family (Lin et al. (1997) *The Plant Cell* 9:1647-1659). In plants, the Rac proteins appear to have a function in the

release of reactive oxygen molecules as the consequence of pathogen infection (Groom QJ et al. (1996) Plant J 10: 515-522; Hassanain HH et al. (2000) Biochem Biophys Res Commun 272(3):783-788; Ono E et al. (2001) Proc Natl Acad Sci USA 98: 759-764). Rac modulates, inter alia, cell wall architecture, signal transduction in the meristem and the defense against pathogens (Valster AH et al. (2000) Trends Cell Biol 10(4):141-146). When the constitutively active form is overexpressed, Rac1 from rice is capable of inducing a hypersensitive response (HR) at the sites of *M. grisea* attack, thus causing pathogen resistance. Analogously, the expression of a negative dominant form of Rac1 brings about an increased susceptibility to *M. grisea* (Kawasaki T et al. (1999) Proc Natl Acad Sci USA 96:10922-10926; Ono E et al. (2001) Proc Natl Acad Sci USA 98: 759-764). These findings suggest that an overexpression of Rac proteins in the plant can bring about advantageous effects with regard to plant defense.

WO 00/15815 describes five Rac genes from maize. Although methods for both an up regulation and a down regulation of Rac proteins are described and speculatively discussed in connection with obtaining a resistance to pathogens (p. 55/line 25 et seq.), the only technical teaching, which describes this use in real terms, concerns merely an overexpression of the claimed Rac genes for obtaining pathogen resistance (p. 60/line 21 et seq.). The author postulates quite unambiguously and in agreement with the situation described in the prior art (p. 60/line 31 et seq.): "Thus the present invention is useful in protecting plants from pathogens. Once a plant is transformed with a polynucleotide sequence encoding an Rac polypeptide, expression of the polypeptide in the plant confers resistance to infection by plant pathogens." The rationale behind this hypothesis (plants defense via reactive oxygen molecules) is explained hereinbelow and supported by a large number of references. Beyond this, no differentiation is being made between the five claimed Rac genes.

It is an object of the present invention to provide novel methods of plant defense against pathogens which bring about an effective defense against as broad a spectrum of pathogens as possible in as large a number of plant species as possible, preferably the crop plants used in agriculture. We have found that this object is achieved by the method according to the invention.

The invention firstly comprises a method of generating or increasing the resistance to at least one pathogen in plants, which comprises the following steps

- a) reducing the amount, activity or function of an RacB protein in a plant or a tissue, organ, part or cell thereof, and
- b) selecting those plants in which, as opposed or as compared to the original plant, the resistance to at least one pathogen exists or is increased.

According to one aspect of the invention, there is provided a method of generating or increasing a resistance to at least one pathogen in a plant, which comprises:

reducing an amount, activity or function of an RacB protein in a plant or a tissue, organ, part or cell thereof,

wherein the RacB protein is a polypeptide comprising the sequence of SEQ ID NO: 2 or a functional equivalent thereof

having at least 64% homology to the sequence of SEQ ID NO: 2;

wherein the reducing comprises introducing to the plant, tissue, organ, part or cell thereof:

a double-stranded RacB RNA or an expression cassette comprising said double-stranded RacB RNA; and

wherein the pathogen is selected from the group consisting of Plasmodiophoramyxota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota, Deuteromycetes, and combinations thereof.

According to another aspect of the invention there is provided an isolated nucleic acid comprising a nucleic acid sequence encoding a barley RacB protein, wherein the RacB protein is a polypeptide comprising the sequence of SEQ ID NO: 2.

According to yet another aspect of the invention there is provided an isolated nucleic acid comprising the sequence of SEQ ID NO: 1, a sequence complementary thereto, or a sequence derived from said sequence or said complementary sequence by degeneracy of the genetic code.

According to yet another aspect of the invention there is provided a recombinant double-stranded RNA molecule that is capable of reducing expression of an RacB protein, wherein:

one of the two RNA strands of said molecule is essentially identical to at least part of a nucleic acid that encodes a RacB protein; and

the other RNA strand of said molecule is essentially identical to at least part of the complementary strand of said nucleic acid; and

5 wherein one of the two RNA strands comprises the sequence of SEQ ID No: 1 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 1.

According to yet another aspect of the invention there is provided a recombinant double-stranded RNA molecule that is capable of reducing expression of an RacB protein, comprising:

10 a sense strand comprising a sequence which is essentially identical to at least part of the sense RNA transcript of a sequence encoding an RacB protein; and

15 an antisense strand, which is essentially complementary to the sense strand;

wherein one of the strands comprises the sequence of SEQ ID NO: 1 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 1.

20 According to yet another aspect of the invention there is provided a method of selecting a plant cell with an increased resistance to a pathogen comprising:

25 introducing a nucleic acid or an expression cassette comprising or encoding the nucleic acid to a plant cell that reduces an amount, activity or function of an RacB protein of the plant cell; and

selecting at least one plant cell wherein resistance to a pathogen is increased as compared to an untransformed cell;

30 wherein the nucleic acid comprises a double-stranded RacB RNA;

wherein the RacB protein is a polypeptide comprising the sequence of SEQ ID NO: 2 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 2; and

35 wherein the pathogen is selected from the group consisting of Plasmodiophoromycota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota, Deuteromycetes, and combinations thereof.

40 According to yet another aspect of the invention there is provided a recombinant plant cell wherein an expressed amount,

activity or function of an endogenous RacB protein is reduced by stable transformation with a nucleic acid or an expression cassette comprising or encoding the nucleic acid as compared to an untransformed plant cell, wherein the nucleic acid comprises a double-stranded RacB RNA; and wherein the nucleic acid comprises the sequence of SEQ ID NO: 1 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 1.

Surprisingly, the Rac homolog RacB from barley (*Hordeum vulgare*) (SEQ ID NO: 1) (hereinbelow: hvRacB), despite a great similarity with rice Rac1, has a negative control function upon attack by powdery mildew of barley *Blumeria* (*syn. Erysiphe*) *graminis* f.sp. *hordei* (Bgh), as opposed to the former: reducing the hvRacB expression in the epidermal cell by a sequence-specific RNA interference approach using double-stranded hvRacB dsRNA ("gene silencing") significantly prevented the development of haustoria owing to Bgh infection. Further experiments demonstrated (cf. Example 7) that this phenotype cannot be observed in an *mlo5-ror1*-mutant genotype, namely barley A89. This suggests that RacB is linked operably to Mlo or Ror1 or both, that is to say they probably act within a signal cascade.

Similarly to the loss of function of Mlo, that of HvRacB confers broad resistance to various *Blumeria graminis* f.sp. *hordei* isolates. In transient gene silencing experiments, HvRacB reduced the penetration efficiency (development of haustoria) of Bgh by 44% (cf. Example 7), an effect whose magnitude corresponds to the effect achieved by Mlo dsRNA (Schweizer P et al. (2000) Plant J 24:895-903). In the wild-type barley variety Ingrid, approximately 60% of the fungal penetrations resulted in the development of haustoria, while the penetration rate in BCIngrid-mlo5 is virtually 0%. The barley variety A89 (*mlo-ror1* dual mutant) shows a penetration efficiency of approximately 20 to 35%. An altered RacB expression owing to Bgh inoculation was observed in none of these variants (cf. Example 7; Fig. 3). The fact that only a penetration of approximately 50% can be observed even in pathogen-sensitive wild-type varieties, such as Pallas or Ingrid, can be attributed to the basal resistance which is always present.

Interestingly, the gene silencing of hvRacB only enhances cell wall apposition, but apparently not the spontaneous cell death of the plant, which is in contrast to Mlo. Thus, HvRacB differs from OsRac1, a rice homolog of Rac1 (Ono E et al. (2001) Proc Natl Acad Sci USA 98: 759-764). HvRacB acts predominantly as negative

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regulator of cell wall apposition. This difference is of outstanding importance for its use for obtaining pathogen resistance in plants. As already described above, Mlo resistance

to biotrophic fungi (for example mildew fungi) is indeed caused, inter alia, by increased cell wall apposition, but the trade-off is a higher susceptibility to necrotrophic fungi (Jarosch B et al. (1999) Mol Plant Microbe Interact 12:508-514; Kumar J et al. 5 (2001) Phytopathology 91:127-133). Since HvRacB only affects cell wall apposition, this problem of ambivalence can be circumvented.

Owing to the above findings, RacB must be considered as a key element for the successful penetration of a pathogen such as Bgh 10 into the plant cell. Accordingly, the method according to the invention has all the advantages of Mlo deficiency without simultaneously showing its biggest shortcoming, namely increased spontaneous cell death.

15 Moreover, the method outperforms all those methods in which a pathogen-resistant phenotype is realized by overexpressing a resistance-conferring protein. Switching off a gene can be realized without expressing a (foreign) protein. In an ideal case, all that needs doing is to deactivate the endogenous gene.

20 This has not inconsiderable advantages for approval and acceptance by the consumer, who is frequently apprehensive toward plants with foreign proteins. Very especially advantageous in this context is the use of inducible promoters for reducing the amount, activity or function of RacB protein, which, for example

25 when using pathogen-inducible promoters, allows expression only when required (i.e. pathogen infection).

A partial sequence of the barley RacB cDNA (HvRacB-cDNA) (GenBank Acc. No.: AJ290240), which is highly conserved relative to rice 30 RacB (GenBank Acc. No.: AF250327) and maize RacB (GenBank Acc. No.: AF126053) and very similar to rice Rac1 has been described. Maize RacB is also one of the five Rac genes in the abovementioned application WO 00/15815 (Sequence No. 3). The complete coding sequence of the HvRacB protein has not been

35 described as yet (see Example 1). Barley RacB has a homology of 95% identity with rice RacB and maize RacB and is over 55% identical to human RAC1 or RAC2 (Hassanain et al. 2000, Fig. 1). HvRacB is expressed constitutively in primary leaves of barley (epidermis-specifically) and its expression level is not affected

40 substantially by Bgh infection. Expression thus takes place in the tissue which interacts directly with the Bgh pathogen.

In principle, the method according to the invention can be applied to all plant species, preferably to those in which an 45 RacB protein or a functional equivalent thereof is expressed naturally. Since the function of RacB is closely connected functionally to the Mlo gene and the latter has been identified

in a large number of plants, including dicots (Devoto A et al. (1999) J Biol Chem 274(49):34993-5004), it can be assumed that RacB and its homologs are similarly widely distributed. The sequences from other plants (for example *Arabidopsis thaliana*) which are homologous to the RacB sequences disclosed within the scope of the present invention can be found readily for example by database searches or by screening genetic libraries using the RacB sequences as search sequence or probe.

- 10 The term "plant" as used herein refers to all genera and species of higher and lower plants of the Plant Kingdom. The term includes the mature plants, seed, shoots and seedlings and their derived parts, propagation material, plant organs, tissue, protoplasts, callus and other cultures, for example cell
- 15 cultures, and any other type of plant cell grouping to give functional or structural units. Mature plants refers to plants at any desired developmental stage beyond that of the seedling. Seedling refers to a young immature plant at an early developmental stage. "Plant" comprises all annual and perennial
- 20 monocotyledonous and dicotyledonous plants and includes by way of example but not by limitation those of the genera *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*,
- 25 *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solarium*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesis*, *Pelargonium*, *Panieum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Pisum*, *Phaseolus*,
- 30 *Lolium*, *Oryza*, *Zea*, *Avena*, *Hordeum*, *Secale*, *Triticum*, *Sorghum*, *Picea* and *Populus*.

The term "plant" preferably comprises monocotyledonous crop plants such as, for example, cereal species such as wheat,

- 35 barley, millet, rye, triticale, maize, rice, sorghum or oats and also sugar cane.

The term furthermore comprises dicotyledonous crop plants such as, for example,

- 40 - Brassicaceae such as oilseed rape, canola, cress, *Arabidopsis*, cabbages or canola, Leguminosae such as soybean, alfalfa, pea, beans or peanut

- Solanaceae such as potato, tobacco, tomato, egg plant or pepper, Asteraceae such as sunflower, Tagetes, lettuce or Calendula,
- 5 - Cucurbitaceae such as melon, pumpkin/squash or zucchini, and also linseed, cotton, hemp, clover, spinach, flax, red pepper, carrot, beet, radish, sugar beet, sweet potato, cucumber, chicory, cauliflower, broccoli, asparagus, onion, garlic,
- 10 celery/celeriac, strawberry, raspberry, blackberry, pineapple, avocado and the various tree, bush, nut and vine species. Tree species preferably comprise plum, cherry, peach, nectarine, apricot, banana, papaya, mango, apple, pear, quince.
- 15 Also comprised are ornamental plants, useful trees, ornamental trees, flowers, cut flowers, shrubs or lawns such as by way of example but not by limitation the families of the Rosaceae such as rose, Ericaceae such as rhododendrons and azaleas, Euphorbiaceae such as poinsettias and croton, Caryophyllaceae
- 20 such as carnations, Solanaceae such as petunias, Gesneriaceae such as African violet, Balsaminaceae such as touch-me-not, Orchidaceae such as orchids, Iridaceae such as gladioli, iris, freesia and crocus, Compositae such as calendula, Geraniaceae such as geraniums, Liliaceae such as dracaena, Moraceae such as
- 25 ficus, Araceae such as philodendron and many others.

Preferred within the scope of the invention are those plants which are employed as foodstuffs or feeding stuffs, very especially preferably monocotyledonous genera and species like the

30 above-described cereal species.

The method is applied very especially preferably to monocotyledonous plants, most preferably to agriculturally important plants such as wheat, oats, millet, barley, rye, maize,

35 rice, buckwheat, sorghum, triticale, spelt, linseed or sugar cane.

"Pathogen resistance" denotes the reduction or weakening of disease symptoms of a plant following infection by a pathogen.

40 The symptoms can be manifold, but preferably comprise those which directly or indirectly have an adverse effect on the quality of the plant, the quantity of the yield, the suitability for use as feeding stuff or foodstuff, or else which make sowing, planting, harvesting or processing of the crop difficult.

"Conferring", "existing", "generating" or "increasing" a pathogen resistance means that the defense mechanisms of a specific plant species or variety is increasingly resistant to one or more pathogens due to the use of the method according to the invention in comparison with the wild type of the plant ("original plant"), to which the method according to the invention has not been applied, under otherwise identical conditions (such as, for example, climatic conditions, growing conditions, pathogen species and the like). The increased resistance manifests itself preferably in a reduced manifestation of the disease symptoms, disease symptoms comprising - in addition to the abovementioned adverse effects - for example also the penetration efficiency of a pathogen into the plant or plant cells or the proliferation efficiency in or on the same. In this context, the disease symptoms are preferably reduced by at least 10% or at least 20%, especially preferably by at least 40% or 60%, very especially preferably by at least 70% or 80% and most preferably by at least 90% or 95%.

"Selection" with regard to plants in which - as opposed or as compared to the original plant - resistance to at least one pathogen exists or is increased means all those methods which are suitable for recognizing an existing or increased resistance to pathogens. These may be symptoms of pathogen infection (for example the development of haustoria in the case of fungal infection), but may also comprise the above-described symptoms which relate to the quality of the plant, the quantity of the yield, the suitability for use as feeding stuff or foodstuff and the like.

"Pathogen" within the scope of the invention means by way of example but not by limitation viruses or viroids, bacteria, fungi, animal pests such as, for example, insects or nematodes. Especially preferred are fungi such as, for example, mildew. However, it can be assumed that a reduced expression of an RacB protein, its activity or function also brings about resistance to other pathogens. Changes in the cell wall structure can constitute a prime mechanism of pathogen resistance.

The following pathogens may be mentioned by way of example but not by limitation:

1. Fungal pathogens or fungus-like pathogens:

Fungal pathogens or fungus-like pathogens (e.g. Chromista) are preferably from the group comprising the Plasmodiophoromycota, Oomycota, Ascomycota, Chytridiomycetes,

Zygomycetes, Basidiomycota and Deuteromycetes (Fungi imperfecti). The pathogens mentioned in Tables 1 and 2 and the diseases with which they are associated may be mentioned by way of example but not by limitation.

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Table 1: Fungal plant diseases

	Disease	Pathogen
10	Leag rust	Puccinia recondita
	Yellow rust	P. striiformis
	Powdery mildew	Erysiphe graminis/Blumeria graminis
	Glume blotch	Septoria nodorum
	Leaf blotch	Septoria tritici
15	Ear fusarioses	Fusarium spp.
	Eyespot	Pseudocercospora herpotrichoides
	Smut	Ustilago spp.
	Bunt	Tilletia caries
20	Take-all	Gaeumannomyces graminis
	Anthracnose leaf blight Anthracnose stalk rot	Colletotrichum graminicola (teleomorph: Glomerella graminicola Politis); Glomerella tucumanensis (anamorph: Glomerella falcatum Went)
25	Aspergillus ear and kernel rot	Aspergillus flavus
	Banded leaf and sheath spot	Rhizoctonia solani Kuhn = Rhizoctonia microsclerotia J. Matz (telomorph: Thanatephorus cucumeris)
30	Black bundle disease	Acremonium strictum W. Gams = Cephalosporium acremonium Auct. non Corda
	Black kernel rot	Lasioidiplodia theobromae = Botryodiplodia theobromae
	Borde blanco	Marasmiellus sp.
35	Brown spot (black spot, stalk rot)	Physoderma maydis
	Cephalosporium kernel rot	Acremonium strictum = Cephalosporium acremonium
	Charcoal rot	Macrophomina phaseolina
40	Corticium ear rot	Thanatephorus cucumeris = Corticium sasakii

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	Disease	Pathogen
5	Curvularia leaf spot	Curvularia clavata, C. eragrostidis, = C. maculans (teleomorph: Cochliobolus eragrostidis), Curvularia inaequalis, C. intermedia (teleomorph: Cochliobolus intermedius), Curvularia lunata (teleomorph: Cochliobolus lunatus), Curvularia pallescens (teleomorph: Cochliobolus pallescens), Curvularia senegalensis, C. tuberculata (teleomorph: Cochliobolus tuberculatus)
10	Didymella leaf spot	Didymella exitalis
	Diplodia ear rot and stalk rot	Diplodia frumenti (teleomorph: Botryosphaeria festucae)
15	Diplodia ear rot, stalk rot, seed rot and seedling blight	Diplodia maydis = Stenocarpella maydis
	Diplodia leaf spot or streak	Stenocarpella macrospora = Diplodia leaf macrospora

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Table 2: Downy mildew

	Disease	Pathogen
25	Brown stripe downy mildew	Sclerophthora rayssiae var. zeae
	Crazy top downy mildew	Sclerophthora macrospora = Sclerospora macrospora
30	Green ear downy mildew (graminicola downy mildew)	Sclerospora graminicola
	Java downy mildew	Peronosclerospora maydis = Sclerospora maydis
	Philippine downy mildew	Peronosclerospora philippinensis = Sclerospora philippinensis
35	Sorghum downy mildew	Peronosclerospora sorghi = Sclerospora sorghi
	Spontaneum downy mildew	Peronosclerospora spontanea = Sclerospora spontanea
40	Sugarcane downy mildew	Peronosclerospora sacchari = Sclerospora sacchari
	Dry ear rot (cob, kernel and stalk rot)	Nigrospora oryzae (teleomorph: Khuskia oryzae)

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Disease	Pathogen
Ear rots, minor	Alternaria alternata = A. tenuis, Aspergillus glaucus, A. niger, Aspergillus spp., Botrytis cinerea (teleomorph: Botryotinia fuckeliana), Cunninghamella sp., Curvularia pallescens, Doratomyces stemonitis = Cephalotrichum stemonitis, Fusarium culmorum, Gonatobotrys simplex, Pithomyces maydis, Rhizopus microsporus Tiegh., R. stolonifer = R. nigricans, Scopulariopsis brumptii
Ergot (horse's tooth)	Claviceps gigantea (anamorph: Sphacelia sp.)
Eyespot	Aureobasidium zeae = Kabatiella zeae
Fusarium ear and stalk rot	Fusarium subglutinans = F. moniliforme var. subglutinans
Fusarium kernel, root and stalk rot, seed rot and seedling blight	Fusarium moniliforme (teleomorph: Gibberella fujikuroi)
Fusarium stalk rot, seedling root rot	Fusarium avenaceum (teleomorph: Gibberella avenacea)
Gibberella ear and stalk rot	Gibberella zeae (anamorph: Fusarium graminearum)
Gray ear rot	Botryosphaeria zeae = Physalospora zeae (anamorph: Macrophoma zeae)
Gray leaf spot (Cercospora leaf spot)	Cercospora sorghi = C. sorghi var. maydis, C. zeae-maydis
Helminthosporium root rot	Exserohilum pedicellatum = Helminthosporium pedicellatum (teleomorph: Setosphaeria pedicellata)
Hormodendrum ear rot (Cladosporium rot)	Cladosporium cladosporioides = Hormodendrum cladosporioides, C. herbarum (teleomorph: Mycosphaerella tassiana)
Hyalothyridium leaf spot	Hyalothyridium maydis
Late wilt	Cephalosporium maydis

	Disease	Pathogen
5	Leaf spots, minor	Alternaria alternata, Ascochyta maydis, A. tritici, A. zeicola, Bipolaris victoriae = Helminthosporium victoriae (teleomorph: Cochliobolus victoriae), C. sativus (anamorph: Bipolaris sorokiniana = H. sorokinianum = H. sativum), Epicoccum nigrum, Exserohilum prolatum = Drechslera prolata (teleomorph: Setosphaeria prolata) Graphium penicillioides, Leptosphaeria maydis, Leptothyrium zeae, Ophiosphaerella herpotricha, (anamorph: Scolecosporiella sp.), Paraphaeosphaeria michotii, Phoma sp., Septoria zeae, S. zeicola, S. zeina
10		
15		
20	Northern corn leaf blight (white blast, crown stalk rot, stripe)	Setosphaeria turcica (anamorph: Exserohilum turcicum = Helminthosporium turcicum)
	Northern corn leaf spot Helminthosporium ear rot (race 1)	Cochliobolus carbonum (anamorph: Bipolaris zeicola = Helminthosporium carbonum)
25	Penicillium ear rot (blue eye, blue mold)	Penicillium spp., P. chrysogenum, P. expansum, P. oxalicum
	Phaeocytostroma stalk rot and root rot	Phaeocytostroma ambiguum, = Phaeocytosporella zeae
	Phaeosphaeria leaf spot	Phaeosphaeria maydis = Sphaerulina maydis
30	Physalospora ear rot (Botryosphaeria ear rot)	Botryosphaeria festucae = Physalospora zeicola (anamorph: Diplodia frumenti)
	Purple leaf sheath	Hemiparasitic bacteria and fungi
	Pyrenochaeta stalk rot and root rot	Phoma terrestris = Pyrenochaeta terrestris
35	Pythium root rot	Pythium spp., P. arrhenomanes, P. graminicola
	Pythium stalk rot	Pythium aphanidermatum = P. butleri L.
	Red kernel disease (ear mold, leaf and seed rot)	Epicoccum nigrum
40	Rhizoctonia ear rot (sclerotial rot)	Rhizoctonia zeae (teleomorph: Waitea circinata)
	Rhizoctonia root rot and stalk rot	Rhizoctonia solani, Rhizoctonia zeae

	Disease	Pathogen
5	Root rots, minor	<i>Alternaria alternata</i> , <i>Cercospora sorghi</i> , <i>Dictochaeta fertilis</i> , <i>Fusarium acuminatum</i> (teleomorph: <i>Gibberella acuminata</i>), <i>F. equiseti</i> (teleomorph: <i>G. intricans</i>), <i>F. oxysporum</i> , <i>F. pallidoroseum</i> , <i>F. poae</i> , <i>F. roseum</i> , <i>G. cyanogena</i> , (anamorph: <i>F. sulphureum</i>), <i>Microdochium bolleyi</i> , <i>Mucor</i> sp., <i>Periconia circinata</i> , <i>Phytophthora cactorum</i> , <i>P. drechsleri</i> , <i>P. nicotianae</i> var. <i>parasitica</i> , <i>Rhizopus arrhizus</i>
10		
15	Rostratum leaf spot (Helminthosporium leaf disease, ear and stalk rot)	<i>Setosphaeria rostrata</i> , (anamorph: <i>Exserohilum rostratum</i> = <i>Helminthosporium rostratum</i>)
	Rust, common corn	<i>Puccinia sorghi</i>
	Rust, southern corn	<i>Puccinia polysora</i>
20	Rust, tropical corn	<i>Physopella pallescens</i> , <i>P. zeae</i> = <i>Angiopsora zeae</i>
	Sclerotium ear rot (southern blight)	<i>Sclerotium rolfsii</i> Sacc. (teleomorph: <i>Athelia rolfsii</i>)
25	Seed rot-seedling blight	<i>Bipolaris sorokiniana</i> , <i>B. zeicola</i> = <i>Helminthosporium carbonum</i> , <i>Diplodia maydis</i> , <i>Exserohilum pedicellatum</i> , <i>Exserohilum turcicum</i> = <i>Helminthosporium turcicum</i> , <i>Fusarium avenaceum</i> , <i>F. culmorum</i> , <i>F. moniliforme</i> , <i>Gibberella zeae</i> (anamorph: <i>F. graminearum</i>), <i>Macrophoma phaseolina</i> , <i>Penicillium</i> spp., <i>Phomopsis</i> sp., <i>Pythium</i> spp., <i>Rhizoctonia solani</i> , <i>R. zeae</i> , <i>Sclerotium rolfsii</i> , <i>Spicaria</i> sp.
30		
	Selenophoma leaf spot	<i>Selenophoma</i> sp.
	Sheath rot	<i>Gaeumannomyces graminis</i>
35	Shuck rot	<i>Myrothecium gramineum</i>
	Silage mold	<i>Monascus purpureus</i> , <i>M. ruber</i>
	Smut, common	<i>Ustilago zeae</i> = <i>U. maydis</i>
	Smut, false	<i>Ustilagoidea virens</i>
40	Smut, head	<i>Sphacelotheca reiliana</i> = <i>Sporisorium holcisorghi</i>
	Southern corn leaf blight and stalk rot	<i>Cochliobolus heterostrophus</i> (anamorph: <i>Bipolaris maydis</i> = <i>Helminthosporium maydis</i>)
45	Southern leaf spot	<i>Stenocarpella macrospora</i> = <i>Diplodia macrospora</i>

	Disease	Pathogen
5	Stalk rots, minor	Cercospora sorghi, Fusarium episphaeria, F. merismoides, F. oxysporum Schlechtend, F. poae, F. roseum, F. solani (teleomorph: Nectria haematococca), F. tricinctum, Mariannaea elegans, Mucor sp., Rhopoglyphus zeae, Spicaria sp.
10	Storage rots	Aspergillus spp., Penicillium spp. and other fungi
	Tar spot	Phyllachora maydis
	Trichoderma ear rot and root rot	Trichoderma viride = T. lignorum teleomorph: Hypocrea sp.
15	White ear rot, root and stalk rot	Stenocarpella maydis = Diplodia zeae
	Yellow leaf blight	Ascochyta ischaemi, Phyllosticta maydis (teleomorph: Mycosphaerella zeae-maydis)
	Zonate leaf spot	Gloeocercospora sorghi

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The following are especially preferred:

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- Plasmodiophoromycota such as Plasmodiophora brassicae (clubroot of crucifers), Spongospora subterranea (powdery scab of potato tubers), Polymyxa graminis (root disease of cereals and grasses),

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- Oomycota such as Bremia lactucae (downy mildew of lettuce), Peronospora (downy mildew) in snapdragon (P. antirrhini), onion (P. destructor), spinach (P. effusa), soybean (P. manchurica), tobacco ("blue mold"; P. tabacina) alfalfa and clover (P. trifolium), Pseudoperonospora humuli (downy mildew of hops), Plasmopara (downy mildew in grapevines) (P. viticola) and sunflower (P. halstedii), Sclerophthora macrospora (downy mildew in cereals and grasses), Pythium (seed rot, seedling damping-off, and root rot and all types of plants, for example damping-off of Beta beet caused by P. debaryanum), Phytophthora infestans (blight in potato, brown rot in tomato and the like), Albugo spec. (white rust on cruciferous plants).

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- Ascomycota such as Microdochium nivale (snow mold of rye and wheat), Fusarium graminearum, Fusarium culmorum (partial ear sterility mainly in wheat), Fusarium oxysporum (Fusarium wilt of tomato), Blumeria graminis (powdery mildew of barley (f.sp. hordei) and wheat (f.sp. tritici)), Erysiphe pisi (powdery mildew of pea), Nectria galligena (Nectria canker of

- fruit trees), *Unicnula necator* (powdery mildew of grapevine), *Pseudopeziza tracheiphila* (red fire disease of grapevine), *Claviceps purpurea* (ergot on, for example, rye and grasses), *Gaeumannomyces graminis* (take-all on wheat, rye and other grasses), *Magnaporthe grisea* (rice blast disease), *Pyrenophora graminea* (leaf stripe of barley), *Pyrenophora teres* (net blotch of barley), *Pyrenophora tritici-repentis* (leaf blight of wheat), *Venturia inaequalis* (apple scab), *Sclerotinia sclerotium* (stalk break, stem rot), *Pseudopeziza medicaginis* (leaf spot of alfalfa, white and red clover).
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- Basidiomycetes such as *Typhula incarnata* (typhula blight on barley, rye, wheat), *Ustilago maydis* (blister smut on maize), *Ustilago nuda* (loose smut on barley), *Ustilago tritici* (loose smut on wheat, spelt), *Ustilago avenae* (loose smut on oats), *Rhizoctonia solani* (rhizoctonia root rot of potato), *Sphacelotheca* spp. (head smut of sorghum), *Melampsora lini* (rust of flax), *Puccinia graminis* (stem rust of wheat, barley, rye, oats), *Puccinia recondita* (leaf rust on wheat), *Puccinia dispersa* (brown rust on rye), *Puccinia hordei* (leaf rust of barley), *Puccinia coronata* (crown rust of oats), *Puccinia striiformis* (yellow rust of wheat, barley, rye and a large number of grasses), *Uromyces appendiculatus* (brown rust of bean), *Sclerotium rolfsii* (root and stem rots of many plants).
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- Deuteromycetes (Fungi imperfecti) such as *Septoria nodorum* (glume blotch) of wheat (*Septoria tritici*), *Pseudocercospora herpotrichoides* (eyespot of wheat, barley, rye), *Rhynchosporium secalis* (leaf spot on rye and barley), *Alternaria solani* (early blight of potato, tomato), *Phoma betae* (blackleg on Beta beet), *Cercospora beticola* (leaf spot on Beta beet), *Alternaria brassicae* (black spot on oilseed rape, cabbage and other crucifers), *Verticillium dahliae* (verticillium wilt), *Colletotrichum lindemuthianum* (bean anthracnose), *Phoma lingam* (blackleg of cabbage and oilseed rape), *Botrytis cinerea* (grey mold of grapevine, strawberry, tomato, hops and the like).
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- 40 Most preferred are *Phytophthora infestans* (potato blight, brown rot in tomato and the like), *Microdochium nivale* (previously *Fusarium nivale*; snow mold of rye and wheat), *Fusarium graminearum*, *Fusarium culmorum* (partial ear sterility of wheat), *Fusarium oxysporum* (*Fusarium* wilt of tomato), *Blumeria graminis*
- 45 (powdery mildew of barley (f. sp. *hordei*) and wheat (f. sp. *tritici*)), *Magnaporthe grisea* (rice blast disease), *Sclerotinia sclerotium* (stalk break, stem rot), *Septoria nodorum* and *Septoria*

tritici (glume blotch of wheat), *Alternaria brassicae* (black spot of oilseed rape, cabbage and other crucifers), *Phoma lingam* (blackleg of cabbage and oilseed rape).

5 2. Bacterial pathogens:

The pathogens and the diseases associated with them which are mentioned in Table 3 may be mentioned by way of example but not by limitation.

Table 3: Bacterial diseases

	Disease	Pathogen
15	Bacterial leaf blight and stalk rot	<i>Pseudomonas avenae</i> subsp. <i>avenae</i>
	Bacterial leaf spot	<i>Xanthomonas campestris</i> pv. <i>holcicola</i>
	Bacterial stalk rot	<i>Enterobacter dissolvens</i> = <i>Erwinia dissolvens</i>
20	Bacterial stalk and top rot	<i>Erwinia carotovora</i> subsp. <i>carotovora</i> , <i>Erwinia chrysanthemi</i> pv. <i>zeae</i>
	Bacterial stripe	<i>Pseudomonas andropogonis</i>
	Chocolate spot	<i>Pseudomonas syringae</i> pv. <i>coronafaciens</i>
25	Goss's bacterial wilt and blight (leaf freckles and wilt)	<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> = <i>Corynebacterium michiganense</i> pv. <i>andnebraskense</i>
	Holcus spot	<i>Pseudomonas syringae</i> pv. <i>syringae</i>
	Purple leaf sheath	Hemiparasitic bacteria
30	Seed rot-seedling blight	<i>Bacillus subtilis</i>
	Stewart's disease (bacterial wilt)	<i>Pantoea stewartii</i> = <i>Erwinia stewartii</i>
35	Corn stunt (achapparramiento, maize stunt, Mesa Central or Rio Grande maize stunt)	<i>Spiroplasma kunkelii</i>

The following pathogenic bacteria are very especially preferred:

40 *Corynebacterium sepedonicum* (bacterial ring rot of potato), *Erwinia carotovora* (black leg of potato), *Erwinia amylovora* (fire blight of pear, apple, quince), *Streptomyces scabies* (potato scab), *Pseudomonas syringae* pv. *tabaci* (wildfire of tobacco), *Pseudomonas syringae* pv. *phaseolicola* (grease spot of dwarf bean), *Pseudomonas syringae* pv. *tomato* (bacterial speck of

45 tomato), *Xanthomonas campestris* pv. *malvacearum* (bacterial blight of cotton) and *Xanthomonas campestris* pv. *oryzae* (bacterial leaf

blight of rice and other grasses).

3. Viral pathogens:

- 5 "Viral pathogens" includes all plant viruses such as, for example, tobacco or cucumber mosaic virus, ringspot virus, necrosis virus, maize dwarf mosaic virus and the like.

- 10 The pathogens and diseases associated with them which are mentioned in Table 4 may be mentioned by way of example, but not by limitation.

Table 4: Viral diseases

15	Disease	Pathogen
	American wheat striate (wheat striate mosaic)	American wheat striate mosaic virus (AWSMV)
	Barley stripe mosaic	Barley stripe mosaic virus (BSMV)
20	Barley yellow dwarf	Barley yellow dwarf virus (BYDV)
	Brome mosaic	Brome mosaic virus (BMV)
	Cereal chlorotic mottle	Cereal chlorotic mottle virus (CCMV)
	Corn chlorotic vein banding (Brazilian maize mosaic)	Corn chlorotic vein banding virus (CCVBV)
25	Corn lethal necrosis	Virus complex of Maize chlorotic mottle virus (MCMV) and Maize dwarf mosaic virus (MDMV) A or B or Wheat streak mosaic virus (WSMV)
	Cucumber mosaic	Cucumber mosaic virus (CMV)
30	Cynodon chlorotic streak	Cynodon chlorotic streak virus (CCSV)
	Johnsongrass mosaic	Johnsongrass mosaic virus (JGMV)
	Maize bushy stunt	Mycoplasma-like organism (MLO) associated
35	Maize chlorotic dwarf	Maize chlorotic dwarf virus (MCDV)
	Maize chlorotic mottle	Maize chlorotic mottle virus (MCMV)
	Maize dwarf mosaic	Maize dwarf mosaic virus (MDMV) strains A, D, E and F
	Maize leaf fleck	Maize leaf fleck virus (MLFV)
40	Maize line	Maize line virus (MLV)
	Maize mosaic (corn leaf stripe, enanismo rayado)	Maize mosaic virus (MMV)
	Maize mottle and chlorotic stunt	Maize mottle and chlorotic stunt virus
45	Maize pellucid ringspot	Maize pellucid ringspot virus (MPRV)
	Maize raya gruesa	Maize raya gruesa virus (MRGV)

	Disease	Pathogen
	Maize rayado fino (fine striping disease)	Maize rayado fino virus (MRFV)
5	Maize red leaf and red stripe	Mollicute
	Maize red stripe	Maize red stripe virus (MRSV)
	Maize ring mottle	Maize ring mottle virus (MRMV)
	Maize rio IV	Maize rio cuarto virus (MRCV)
10	Maize rough dwarf (nanismo ruvido)	Maize rough dwarf virus (MRDV) (Cereal tillering disease virus)
	Maize sterile stunt	Maize sterile stunt virus (strains of barley yellow striate virus)
	Maize streak	Maize streak virus (MSV)
15	Maize stripe (maize chlorotic stripe, maize hoja blanca)	Maize stripe virus
	Maize stunting	Maize stunting virus
	Maize tassel abortion	Maize tassel abortion virus (MTAV)
20	Maize vein enation	Maize vein enation virus (MVEV)
	Maize wallaby ear	Maize wallaby ear virus (MWEV)
	Maize white leaf	Maize white leaf virus
	Maize white line mosaic	Maize white line mosaic virus (MWLMV)
25	Millet red leaf	Millet red leaf virus (MRLV)
	Northern cereal mosaic	Northern cereal mosaic virus (NCMV)
	Oat pseudorosette (zakuklivanie)	Oat pseudorosette virus
	Oat sterile dwarf	Oat sterile dwarf virus (OSDV)
30	Rice black-streaked dwarf	Rice black-streaked dwarf virus (RBSDV)
	Rice stripe	Rice stripe virus (RSV)
35	Sorghum mosaic	Sorghum mosaic virus (SrMV) (auch: sugarcane mosaic virus (SCMV) Stämme H, I and M)
	Sugarcane Fiji disease	Sugarcane Fiji disease virus (FDV)
	Sugarcane mosaic	Sugarcane mosaic virus (SCMV) strains A, B, D, E, SC, BC, Sabi and MB (formerly MDMV-B)
40	Wheat spot mosaic	Wheat spot mosaic virus (WSMV)

4. Animal pests

45 4.1 Insect pathogens:

The following may be mentioned by way of example, but not by limitation: insects such as, for example, beetles, caterpillars, lice or mites. Preferred insects are those of the genera Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc. Especially preferred are coleopteran and lepidopteran insects such as, for example, the European corn borer (ECB), *Diabrotica barberi* (northern corn rootworm), *Diabrotica undecimpunctata* (southern corn rootworm), *Diabrotica virgifera* (Western corn rootworm), *Agrotis ipsilon* (black cutworm), *Crymodes devastator* (glassy cutworm), *Feltia ducens* (dingy cutworm), *Agrotis gladiaria* (claybacked cutworm), *Melanotus* spp., *Aeolus mellillus* (wireworm), *Aeolus mancus* (wheat wireworm), *Horistonotus uhlerii* (sand wireworm), *Sphenophorus maidis* (maize billbug), *Sphenophorus zeae* (timothy billbug), *Sphenophorus parvulus* (bluegrass billbug), *Sphenophorus callosus* (southern corn billbug), *Phyllogphaga* spp. (white grubs), *Anuraphis maidiradicis* (corn root aphid), *Delia platura* (seedcorn maggot), *Colaspis brunnea* (grape colaspis), *Stenolophus lecontei* (seedcorn beetle) and *Clivinia impressifrons* (lender seedcorn beetle).

Other examples are: lema (*Oulema melanopus*), frit fly (*Oscinella frit*), wireworms (*Agrotis lineatus*) and aphids (such as, for example, the oat grain aphid *Rhopalosiphum padi*, the blackberry aphid *Sitobion avenae*).

4.2 Nematodes:

The pathogens and the diseases associated with them mentioned in Table 6 may be mentioned by way of example, but not by limitation.

Table 6: Parasitic nematodes

Damage	Pathogenic nematode
Awl	<i>Dolichodorus</i> spp., <i>D. heterocephalus</i>
Bulb and stem nematode disease; Europe	<i>Ditylenchus dipsaci</i>
Burrowing	<i>Radopholus similis</i>
Cyst nematode disease	<i>Heterodera avenae</i> , <i>H. zeae</i> , <i>Punctodera chalconensis</i>
Dagger	<i>Xiphinema</i> spp., <i>X. americanum</i> , <i>X. mediterraneum</i>
False root-knot	<i>Nacobbus dorsalis</i>

	Damage	Pathogenic nematode
	Lance, Columbia	Hoplolaimus columbus
	Lance	Hoplolaimus spp., H. galeatus
5	Lesion	Pratylenchus spp., P. brachyurus, P. crenatus, P. hexincisus, P. neglectus, P. penetrans, P. scribneri, P. thornei, P. zaeae
	Needle	Longidorus spp., L. breviannulatus
10	Ring	Criconemella spp., C. ornata
	Root-knot disease	Meloidogyne spp., M. chitwoodi, M. incognita, M. javanica
	Spiral	Helicotylenchus spp.
	Sting	Belonolaimus spp., B. longicaudatus
15	Stubby-root	Paratrachodorus spp., P. christiei, P. minor, Quinisulcius acutus, Trichodorus spp.
	Stunt	Tylenchorhynchus dubius

- 20 Very especially preferred are Globodera rostochiensis and G. pallida (cyst eelworm on potato, tomato and other Solanaceae), Heterodera schachtii (beet eelworm on sugar and fodder beet, oilseed rape, cabbage and the like), Heterodera avenae (oat cyst nematode on oat and other cereal species), Ditylenchus dipsaci (stem or bulb eelworm, stem eelworm of rye, oats, maize, clover, tobacco, beet), Anguina tritici (grain nematode, cockle disease of wheat (spelt, rye), Meloidogyne hapla (root-knot nematode of carrot, cucumber, lettuce, tomato, potato, sugar beet, lucerne).

- 30 Examples of preferred fungal or viral pathogens for the individual varieties are:

1. Barley:

- 35 Fungal, bacterial and viral pathogens: Puccinia graminis f.sp. hordei (barley stem rust), Blumeria (Erysiphe) graminis f.sp. hordei (barley powdery mildew), barley yellow dwarf virus (BYDV),
- 40 Pathogenic insects/nematodes: Ostrinia nubilalis (European corn borer); Agrotis ipsilon (black cutworm); Schizaphis graminum (greenbug); Blissus leucopterus leucopterus (chinch bug); Acrosternum hilare (green stink bug); Euschistus servus (brown stink bug); Delia platura (seedcorn maggot); Mayetiola destructor (Hessian fly); Petrobia latens (brown wheat mite).
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2. Soybean:

- 5 Fungal, bacterial or viral pathogens: *Phytophthora megasperma* fsp.glycinea, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Diaporthe phaseolorum* var. *sojae* (*Phomopsis sojae*), *Diaporthe phaseolorum* var. *caulivora*, *Sclerotium rolfsii*, *Cercospora kikuchii*, *Cercospora sojae*, *Peronospora manshurica*, *Colletotrichum dematium* (*Colletotrichum truncatum*),
- 10 *Corynespora cassiicola*, *Septoria glycines*, *Phyllosticta sojicola*, *Alternaria alternata*, *Pseudomonas syringae* p.v. *glycinea*, *Xanthomonas campestris* p.v. *phaseoli*, *Microsphaera diffusa*, *Fusarium semitectum*, *Phialophora gregata*, soybean mosaic virus, *Glomerella glycines*, Tobacco Ring spot virus,
- 15 Tobacco Streak virus, *Phakopsora pachyrhizi*, *Pythium aphanidermatum*, *Pythium ultimum*, *Pythium debaryanum*, Tomato spotted wilt virus, *Heterodera glycines* *Fusarium solani*.
- 20 Pathogenic insects/nematodes: *Pseudoplusia includens* (soybean looper); *Anticarsia gemmatilis* (velvetbean caterpillar); *Plathypena scabra* (green cloverworm); *Ostrinia nubilalis* (European corn borer); *Agrotis ipsilon* (black cutworm); *Spodoptera exigua* (beet armyworm); *Heliothis virescens* (cotton budworm); *Helicoverpa zea* (cotton bollworm);
- 25 *Epilachna varivestis* (Mexican bean beetle); *Myzus persicae* (green peach aphid); *Empoasca fabae* (potato leaf hopper); *Acrosternum hilare* (green stink bug); *Melanoplus femurrubrum* (redlegged grasshopper); *Melanoplus differentialis* (differential grasshopper); *Hylemya platura* (seedcorn maggot);
- 30 *Sericothrips variabilis* (soybean thrips); *Thrips tabaci* (onion thrips); *Tetranychus turkestanii* (strawberry spider mite); *Tetranychus urticae* (two-spotted spider mite).

3. Canola:

- 35 Fungal, bacterial or viral pathogens: *Albugo candida*, *Alternaria brassicae*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Mycosphaerella brassicicola*, *Pythium ultimum*, *Peronospora parasitica*, *Fusarium roseum*,
- 40 *Alternaria alternata*.

4. Alfalfa:

- 45 Fungal, bacterial or viral pathogens: *Clavibacter michiganense* subsp. *insidiosum*, *Pythium ultimum*, *Pythium irregulare*, *Pythium splendens*, *Pythium debaryanum*, *Pythium aphanidermatum*, *Phytophthora megasperma*, *Peronospora*

trifoliorum, *Phoma medicaginis* var. *medicaginis*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Leptotrochila medicaginis*, *Fusarium*, *Xanthomonas campestris* p.v. *alfalfae*, *Aphanomyces euteiches*, *Stemphylium herbarum*, *Stemphylium alfalfae*.

5. Wheat:

10 Fungal, bacterial or viral pathogens: *Pseudomonas syringae* p.v. *atrofaciens*, *Urocystis agropyri*, *Xanthomonas campestris* p.v. *translucens*, *Pseudomonas syringae* p.v. *syringae*, *Alternaria alternata*, *Cladosporium herbarum*, *Fusarium graminearum*, *Fusarium avenaceum*, *Fusarium culmorum*, *Ustilago tritici*, *Ascochyta tritici*, *Cephalosporium gramineum*,
15 *Collotetrichum graminicola*, *Erysiphe graminis* f.sp. *tritici*, *Puccinia graminis* f.sp. *tritici*, *Puccinia recondita* f.sp. *tritici*, *Puccinia striiformis*, *Pyrenophora tritici-repentis*, *Septoria nodorum*, *Septoria tritici*, *Septoria avenae*, *Pseudocercospora herpotrichoides*, *Rhizoctonia solani*,
20 *Rhizoctonia cerealis*, *Gaeumannomyces graminis* var. *tritici*, *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium ultimum*, *Bipolaris sorokiniana*, Barley Yellow Dwarf Virus, Brome Mosaic Virus, soil borne wheat mosaic virus, wheat streak mosaic virus, wheat spindle streak virus, American
25 wheat striate virus, *Claviceps purpurea*, *Tilletia tritici*, *Tilletia laevis*, *Ustilago tritici*, *Tilletia indica*, *Rhizoctonia solani*, *Pythium arrhenomanes*, *Pythium graminicola*, *Pythium aphanidermatum*, high plains virus, European wheat striate virus, *Puccinia graminis* f.sp. *tritici* (wheat stem
30 rust), *Blumeria* (*Erysiphe*) *graminis* f.sp. *tritici* (wheat powdery mildew).

Pathogenic insects/nematodes: *Pseudaletia unipunctata* (armyworm); *Spodoptera frugiperda* (fall armyworm);
35 *Elasmopalpus lignosellus* (lesser cornstalk borer); *Agrotis orthogonia* (western cutworm); *Elasmopalpus lignosellus* (lesser cornstalk borer); *Oulema melanopus* (cereal leaf beetle); *Hypera punctata* (clover leaf weevil); *Diabrotica undecimpunctata howardi* (southern corn rootworm); Russian
40 wheat aphid; *Schizaphis graminum* (greenbug); *Macrosiphum avenae* (English grain aphid); *Melanoplus femurrubrum* (redlegged grasshopper); *Melanoplus differentialis* (differential grasshopper); *Melanoplus sanguinipes* (migratory grasshopper); *Mayetiola destructor* (Hessian fly);
45 *Sitodiplosis mosellana* (wheat midge); *Meromyza americana* (wheat stem maggot); *Hylemya coarctata* (wheat bulb fly);

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Frankliniella fusca (tobacco thrips); Cephus cinctus (wheat stem sawfly); Aceria tulipae (wheat curl mite).

6. Sunflower:

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Fungal, bacterial or viral pathogens: Plasmophora halstedii, Sclerotinia sclerotiorum, aster yellows, Septoria helianthi, Phomopsis helianthi, Alternaria helianthi, Alternaria zinniae, Botrytis cinerea, Phoma macdonaldii, Macrophomina phaseolina, Erysiphe cichoracearum, Rhizopus oryzae, Rhizopus arrhizus, Rhizopus stolonifer, Puccinia helianthi, Verticillium dahliae, Erwinia carotovorum p.v. Carotovora, Cephalosporium acremonium, Phytophthora cryptogea, Albugo tragopogonis.

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Pathogenic insects/nematodes: Suleima helianthana (sunflower bud moth); Homoeosoma electellum (sunflower moth); zygogramma exclamationis (sunflower beetle); Bothyrus gibbosus (carrot beetle); Neolasioptera murtfeldtiana (sunflower seed midge).

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7. Maize:

Fungal, bacterial or viral pathogens: Fusarium moniliforme var. subglutinans, Erwinia stewartii, Fusarium moniliforme, Gibberella zeae (Fusarium graminearum), Stenocarpella maydis (Diplodia maydis), Pythium irregulare, Pythium debaryanum, Pythium graminicola, Pythium splendens, Pythium ultimum, Pythium aphanidermatum, Aspergillus flavus, Bipolaris maydis 0, T (Cochliobolus heterostrophus), Helminthosporium carbonum I, II & III (Cochliobolus carbonum), Exserohilum turcicum I, II & III, Helminthosporium pedicellatum, Physoderma maydis, Phyllosticta maydis, Kabatiella maydis, Cercospora sorghi, Ustilago maydis, Puccinia sorghi, Puccinia polysora, Macrophomina phaseolina, Penicillium oxalicum, Nigrospora oryzae, Cladosporium herbarum, Curvularia lunata, Curvularia inaequalis, Curvularia pallescens, Clavibacter michiganese subsp. nebraskense, Trichoderma viride, maize dwarf mosaic virus A & B, wheat streak mosaic virus, maize chlorotic dwarf virus, Claviceps sorghi, Pseudonomas avenae, Erwinia chrysanthemi p.v. Zea, Erwinia carotovora, cornstunt spiroplasma, Diplodia macrospora, Sclerophthora macrospora, Peronosclerospora sorghi, Peronosclerospora philippinesis, Peronosclerospora maydis, Peronosclerospora sacchari, Spacelotheca reiliana, Physopella zeae, Cephalosporium maydis, Cephalosporium acremonium, maize chlorotic mottle virus, high plains virus, maize mosaic virus, maize rayado

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finio virus, maize streak virus (MSV), maize stripe virus, maize rough dwarf virus.

- Pathogenic insects/nematodes: *Ostrinia nubilalis* (European corn borer); *Agrotis ipsilon* (black cutworm); *Helicoverpa zea* (corn earworm); *Spodoptera frugiperda* (fall armyworm); *Diatraea grandiosella* (Southwestern corn borer); *Elasmopalpus lignosellus* (lesser cornstalk borer); *Diatraea saccharalis* (surgarcane borer); *Diabrotica virgifera* (Western corn rootworm); *Diabrotica longicornis barberi* (Northern corn rootworm); *Diabrotica undecimpunctata howardi* (Southern corn rootworm); *Melanotus* spp. (wireworms); *Cyclocephala borealis* (Northern masked chafer; white grub); *Cyclocephala immaculata* (Southern masked chafer; white grub); *Popillia japonica* (Japanese beetle); *Chaetocnema pulicaria* (corn flea beetle); *Sphenophorus maidis* (maize billbug); *Rhopalosiphum maidis* (corn leaf aphid); *Anuraphis maidiradicis* (corn root aphid); *Blissus leucopterus leucopterus* (chinch bug); *Melanoplus femur-rubrum* (red-legged grasshopper); *Melanoplus sanguinipes* (migratory grasshopper); *Hylemya platura* (seedcorn maggot); *Agromyza parvicornis* (corn blot leafminer); *Anaphothrips obscurus* (grass thrips); *Solenopsis milesta* (thief ant); *Tetranychus urticae* (two-spotted spider mite).

25 8. Sorghum:

- Fungal, bacterial or viral pathogens: *Exserohilum turcicum*, *Colletotrichum graminicola* (*Glomerella graminicola*), *Cercospora sorghi*, *Gloeocercospora sorghi*, *Ascochyta sorghina*, *Pseudomonas syringae* p.v. *syringae*, *Xanthomonas campestris* p.v. *holcicola*, *Pseudomonas andropogonis*, *Puccinia purpurea*, *Macrophomina phaseolina*, *Perconia circinata*, *Fusarium moniliforme*, *Alternaria alternate*, *Bipolaris sorghicola*, *Helminthosporium sorghicola*, *Curvularia lunata*, *Phoma insidiosa*, *Pseudomonas avenae* (*Pseudomonas alboprecipitans*), *Ramulispora sorghi*, *Ramulispora sorghicola*, *Phyllachara sacchari*, *Sporisorium reilianum* (*Sphacelotheca reiliana*), *Sphacelotheca cruenta*, *Sporisorium sorghi*, sugarcane mosaic H, maize dwarf mosaic virus A & B, *Claviceps sorghi*, *Rhizoctonia solani*, *Acremonium strictum*, *Sclerophthora macrospora*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Sclerospora graminicola*, *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium arrhenomanes*, *Pythium graminicola*.

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Pathogenic insects/nematodes: *Chilo partellus* (sorghum borer); *Spodoptera frugiperda* (fall armyworm); *Helicoverpa zea* (corn ear-worm); *Elasmopalpus lignosellus* (lesser cornstalk borer); *Feltia subterranea* (granulate cutworm);
 5 *Phyllophaga crinita* (white grub); *Eleodes*, *Conoderus* and *Aeolus* spp. (wireworm); *Oulema melanopus* (cereal leaf beetle); *Chaetocnema pulicaria* (corn flea beetle); *Sphenophorus maidis* (maize billbug); *Rhopalosiphum maidis* (corn leaf aphid); *Siphaflava* (yellow sugarcane aphid);
 10 *Blissus leucopterus leucopterus* (chinch bug); *Contarinia sorghicola* (sorghum midge); *Tetranychus cinnabarinus* (carmine spider mite); *Tetranychus urticae* (two-spotted spider mite).

9. Cotton:

15 Pathogenic insects/nematodes: *Heliothis virescens* (cotton budworm); *Helicoverpa zea* (cotton bollworm); *Spodoptera exigua* (beet armyworm); *Pectinophora gossypiella* (pink bollworm); *Anthonomus grandis grandis* (boll weevil); *Aphis*
 20 *gossypii* (cotton aphid); *Pseudatomoscelis seriatus* (cotton fleahopper); *Trialeurodes abutilonea* (banded-winged whitefly); *Lygus lineolaris* (tarnished plant bug); *Melanoplus femurrubrum* (red-legged grasshopper); *Melanoplus differentialis* (differential grasshopper); *Thrips tabaci*
 25 (onion thrips); *Frankliniella fusca* (tobacco thrips); *Tetranychus cinnabarinus* (carmine spider mite); *Tetranychus urticae* (two-spotted spider mite).

10. Rice:

30 Pathogenic insects/nematodes: *Diatraea saccharalis* (sugarcane borer); *Spodoptera frugiperda* (fall armyworm); *Helicoverpa zea* (corn earworm); *Colaspis brunnea* (grape colaspis); *Lissorhoptrus oryzophilus* (rice water weevil); *Sitophilus*
 35 *oryzae* (rice weevil); *Nephotettix nigropictus* (rice leafhopper); *Blissus leucopterus leucopterus* (chinch bug); *Acrosternum hilare* (green stink bug).

11. Oilseed rape:

40 Pathogenic insects/nematodes: *Brevicoryne brassicae* (cabbage aphid); *Phyllotreta cruciferae* (flea beetle); *Mamestra conjugata* (bertha armyworm); *Plutella xylostella* (diamond-back moth); *Delia* ssp. (root maggots).
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For the purposes of the invention, "RacB protein" is understood as meaning the RacB protein from barley as shown in SEQ ID NO: 2, and its homologs from rice (*Oryza sativa*) as shown in SEQ ID NO: 4 and maize (*Zea mays*) as shown in SEQ ID NO: 6, and 5 functional equivalents of the abovementioned.

"Amount of protein" is understood as meaning the amount of an RacB polypeptide in an organism, a tissue, a cell or a cell compartment. "Reduction" of the amount of protein means the 10 quantitative reduction of the amount of an RacB protein in an organism, a tissue, a cell or a cell compartment - for example by one of the methods described hereinbelow - in comparison with the wild type of the same genus and species, to which this method had not been applied, under otherwise identical conditions (such as, 15 for example, culture conditions, plant age and the like). The reduction amounts to at least 10%, preferably at least 10% or at least 20%, especially preferably at least 40% or 60%, very especially preferably at least 70% or 80%, most preferably at least 90% or 95%.

20 "Activity" is preferably understood as meaning the GTPase activity of an RacB polypeptide in an organism, a tissue, a cell or a cell compartment. "Reduction" of the activity is understood as meaning the reduction of the total activity of an RacB protein 25 in an organism, a tissue, a cell or a cell compartment - for example by one of the methods described hereinbelow - in comparison with the wild type of the same genus and species, to which this method had not been applied, under otherwise identical conditions (such as, for example, culture conditions, plant age 30 and the like). The reduction amounts to at least 10%, preferably at least 10% or at least 20%, especially preferably at least 40% or 60%, very especially preferably at least 70% or 80%, most preferably at least 90% or 95%.

35 "Function" is preferably understood as meaning the substrate-binding capacity of an RacB polypeptide in an organism, a tissue, a cell or a cell compartment. Suitable substrates are low-molecular-weight compounds such as GTP, but also the protein interaction partners of an RacB protein. "Reduction" of the 40 function is understood as meaning, for example, the quantitative reduction of the binding capacity or binding strength of an RacB protein to at least one substrate in an organism, a tissue, a cell or a cell compartment - for example by one of the methods described hereinbelow - in comparison with the wild type of the 45 same genus and species, to which this method had not been applied, under otherwise identical conditions (such as, for example, culture conditions, plant age and the like). Reduction

is also understood as meaning the change in substrate specificity as can be expressed for example by the k_{cat}/K_m value. The reduction amounts to at least 10%, preferably at least 10% or at least 20%, especially preferably at least 40% or 60%, very especially preferably at least 70% or 80%, most preferably at least 90% or 95%. Binding partners for RacB can be identified in a manner with which the skilled worker is familiar, for example by the yeast-2-hybrid system.

- 10 Methods of determining the amount of protein, the activity of GTPases or the substrate binding capacity are known to the skilled worker and have been described on a number of occasions for GTPases and for Rac proteins from a variety of genera and species (see, inter alia, Benard V et al. (1999) J Biol Chem 15 274(19):13198-204; Burstein ES (1998) Oncogene. 17(12):1617-23).

"Functional equivalents" of an RacB protein is preferably understood as meaning those sequences which are derived from, or are homologous to, an RacB protein described by SEQ ID NO: 2, 4 20 or 6 and which have essentially the same properties.

"Essentially the same properties" of a functional equivalent is above all understood as meaning conferring a pathogen-resistant phenotype or conferring or increasing the resistance to at least 25 one pathogen while reducing the amount of protein, activity or function of said functional RacB equivalent in a plant or in a tissue, part or cells of the same. The absence of a spontaneously induced cell death in combination with said reduction of the amount of protein, activity or function of the functional 30 equivalent is furthermore understood as an essential property.

In this context, the efficacy of the pathogen resistance can deviate both downward or upward in comparison with a value obtained when reducing one of the RacB proteins as shown in SEQ 35 ID NO: 2, 4 or 6. Preferred functional equivalents are those in which the efficacy of the pathogen resistance - measured, for example, by the penetration efficacy of a pathogen (formation of haustoria) - differs by not more than 50%, preferably 25%, especially preferably 10% from a comparative value obtained by 40 reducing an RacB protein as shown in NO: 2, 4 or 6. Especially preferred are those sequences where the reduction increases the efficacy of pathogen resistance quantitatively by more than 50%, preferably 100%, especially preferably 500%, very especially preferably 1000% based on a comparative value obtained by 45 reducing one of the RacB protein as shown in SEQ ID NO: 2, 4 or 6.

The comparison is preferably carried out under analogous conditions. "Analogous conditions" means that all conditions such as, for example, culture or growing conditions, assay conditions (such as buffer, temperature, substrates, pathogen concentration and the like) are kept identical between the experiments to be compared and that the set-ups differ only by the sequence of the RacB polypeptides to be compared, their organism of origin and, if appropriate, the pathogen. When choosing the pathogen, each comparison requires that the pathogen be chosen which is most similar to the other equivalent, taking into consideration the species specificity.

"Functional equivalents" is understood as meaning, in particular, natural or artificial mutations of the RacB polypeptides as shown in SEQ ID NO: 2, 4 or 6 and homologous polypeptides from other plants which continue to have essentially the same properties. Homologous polypeptides from the above-described preferred plants are preferred. The sequences from other plants (for example *Arabidopsis thaliana*) which are homologous to the RacB sequences disclosed within the scope of the present invention can be found readily for example by database search or by screening genetic libraries using the RacB sequences as search sequence or probe.

Mutations comprise substitutions, additions, deletions, inversion or insertions of one or more amino acid residues. Thus, the present invention also comprises those polypeptides which are obtained by modification of a polypeptide as shown in SEQ ID NO: 2, 4 or 6.

Homology between two nucleic acid sequences is understood as meaning the identity of the nucleic acid sequence over in each case the entire sequence length which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA; Altschul et al. (1997) *Nucleic Acids Res.* 25:3389 et seq.), setting the following parameters:

Gap weight: 50

Length weight: 3

Average match: 10

Average mismatch: 0

For example a sequence which has at least 80% homology with sequence SEQ ID NO: 1 at the nucleic acid level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 1 by the above program algorithm with the above parameter set, has at least 80% homology.

30

Homology between two polypeptides is understood as meaning the identity of the amino acid sequence over in each case the entire sequence length which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0,

- 5 University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap weight: 8

Length weight: 2

10 Average match: 2,912

Average mismatch:-2,003

For example a sequence which has at least 80% homology with sequence SEQ ID NO: 2 at the protein level is understood as meaning a sequence which, upon comparison with the sequence SEQ

- 15 ID NO: 2 by the above program algorithm with the above parameter set, has at least 80% homology.

Functional equivalents derived from one of the polypeptide as shown in SEQ ID NO: 2, 4 or 6 according to the invention by

- 20 substitution, insertion or deletion have at least 60%, preferably at least 80%, by preference at least 90%, especially preferably at least 95%, very especially preferably at least 98%, homology with one of the polypeptide as shown in SEQ ID NO: 2, 4 or 6 according to the invention and are distinguished by essentially
- 25 the same properties as the polypeptide as shown in SEQ ID NO: 2, 4 or 6.

Functional equivalents derived from the nucleic acid sequence as shown in SEQ ID NO: 1, 3 or 5 according to the invention by

- 30 substitution, insertion or deletion have at least 60%, preferably at least 80%, by preference at least 90%, especially preferably at least 95%, very especially preferably at least 98%, homology with one of the polypeptides as shown in SEQ ID NO: 1, 3 or 5 according to the invention and encode polypeptides having
- 35 essentially the same properties as the polypeptide as shown in SEQ ID NO: 2, 4 or 6.

The RacB proteins comprised as functional equivalents preferably have at least one of the following sequence motifs:

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- a) A G1 element GXXXXGKS/T preferably in the N-terminal region, very especially preferably an element with the sequence GDGAVGKT, most preferably an element with the sequence KCVTVGDGAVGKTC.

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- b) A G2 effector region comprising a sequence motif with PTVFDN, especially preferably NTFPTDYVPTVFDNFSANVV.
- c) A G3 element comprising LWDTAGQ, especially preferably
5 NLGLWDTAGQEDYN.
- d) A G4 element TKXD, especially preferably TKLD, very especially preferably LVGTKLRLRDDKQ.
- 10 e) A G5 element EXS, preferably ECSS, very especially preferably ECSSKTG.
- f) A C-terminal isoprenylation motif (CXXX, Hassanain HH et al. (2000) Biochem Biophys Res Commun. 272(3):783-8.), especially
15 preferably CSIL.

Especially preferably, at least 2 or 3 of these motifs (a to f) occur in a functionally equivalent RacB protein, very especially preferably at least 4 or 5 of these motifs, most preferably all
20 motifs a to f. Further sequence motifs which are typical for RacB, in particular also motifs which constitute a delimitation against Rac1 proteins, can be deduced readily by the skilled worker from the sequence alignment of the known RacB (or Rac1) proteins, as shown in Fig. 1.

25 Examples of the functional equivalents to the RacB proteins as shown in SEQ ID NO: 2, 4 or 6, which equivalents are to be reduced in the method according to the invention, can be identified for example from a variety of organisms whose genomic
30 sequence is known, such as, for example, from Arabidopsis thaliana, Brassica napus, Nicotiana tabacum, Solanum tuberosum, or Helianthum from databases of homology comparisons.

The screening of cDNA libraries or genomic libraries of other
35 organisms, preferably of the plant species which are mentioned further below as hosts for the transformation, using the nucleic acid sequences described under SEQ ID NO: 1, 3 or 5 or parts of these as probe is also a method of identifying homologs in other species with which the skilled worker is familiar. In this
40 context, the probes derived from the nucleic acid sequences as shown in SEQ ID NO: 1, 3 or 5 have a length of at least 20 bp, preferably 50 bp, particularly preferably 100 bp, very especially preferably 200 bp, and most preferably 400 bp. A DNA strand which is complementary to the sequences described under SEQ ID NO: 1, 3
45 or 5 may also be employed for screening the libraries.

Functional equivalents, accordingly, comprise DNA sequences which hybridize under standard conditions with the RacB nucleic acid sequence described by SEQ ID NO: 1, 3 or 5, with the sequence complementary thereto or parts of the abovementioned and which, as complete sequences, encode proteins which have the same properties as the proteins described under SEQ ID NO: 2, 4 or 6.

"Standard hybridization conditions" is to be understood in the broad sense and means stringent or else less stringent hybridization conditions. Such hybridization conditions are described, inter alia, by Sambrook J, Fritsch EF, Maniatis T et al., in Molecular Cloning (A Laboratory Manual), 2nd Edition, Cold Spring Harbor Laboratory Press, 1989, pages 9.31-9.57) or in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

For example, the conditions during the wash step can be selected from the range of conditions delimited by low-stringency conditions (approximately 2X SSC at 50°C) and high-stringency conditions (approximately 0.2X SSC at 50°C, preferably at 65°C) (20X SSC: 0.3M sodium citrate, 3M NaCl, pH 7.0). In addition, the temperature during the wash step can be raised from low-stringency conditions at room temperature, approximately 22°C, to higher-stringency conditions at approximately 65°C. Both of the parameters salt concentration and temperature can be varied simultaneously, or else one of the two parameters can be kept constant while only the other is varied. Denaturants, for example formamide or SDS, may also be employed during the hybridization. In the presence of 50% formamide, hybridization is preferably effected at 42°C. Some examples of conditions for hybridization and wash step are shown hereinbelow:

(1) Hybridization conditions be selected, for example, from the following conditions:

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a) 4X SSC at 65°C,

b) 6X SSC at 45°C,

40

c) 6X SSC, 100 µg/ml denatured fragmented fish sperm DNA at 68°C,

d) 6X SSC, 0.5% SDS, 100 µg/ml denatured salmon sperm DNA at 68°C,

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e) 6X SSC, 0.5% SDS, 100 µg/ml denatured fragmented salmon sperm DNA, 50% formamide at 42°C,

f) 50% formamide, 4XSSC at 42°C, or

g) 50% (vol/vol) formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer pH 6.5, 750 mM NaCl, 75 mM sodium citrates at 42°C, or

h) 2X or 4X SSC at 50°C (low-stringency condition),

i) 30 to 40% formamide, 2X or 4X SSC at 42°C (low-stringency condition).

(2) Wash steps can be selected, for example, from the following conditions:

a) 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.

b) 0.1X SSC at 65°C.

c) 0.1X SSC, 0.5 % SDS at 68°C.

d) 0.1X SSC, 0.5% SDS, 50% formamide at 42°C.

e) 0.2X SSC, 0.1% SDS at 42°C.

f) 2X SSC at 65°C (low-stringency condition).

Functional equivalents derived from a polypeptide as shown in SEQ ID NO: 2, 4 or 6 comprises in particular also the proteins having the SEQ ID NO: 35, 37, 39, 41, 43, 45, 47, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68 or 70. "Functional equivalents" is to be understood as meaning, in particular, proteins encoded by a nucleic acid sequence having the SEQ ID NO: 34, 36, 38, 40, 42, 44, 46, 48, 49, 51, 53, 55, 57, 61, 63, 65, 67 or 69.

The reduction of the expression of an RacB protein, the RacB activity or the RacB function can be realized in many ways.

"Reduction" or "reducing" in connection with an RacB protein, an RacB activity or an RacB function is to be interpreted in the wide sense and comprises the partial or essentially complete

inhibition or blocking of the functionality of an RacB protein in a plant or a part, tissue, organ, cells or seeds thereof, which inhibition or blocking is based on a variety of cytobiological

mechanisms.

For the purposes of the invention, a reduction also comprises a quantitative reduction of an RacB protein down to the essential complete absence of the RacB protein (i.e. lacking detectability of RacB activity or RacB function, or lacking immunological detectability of the RacB protein). In this context, the expression of a particular RacB protein, or the RacB activity or RacB function, in a cell or an organism is preferably reduced by more than 50%, especially preferably by more than 80%, very especially preferably by more than 90%.

A variety of strategies for reducing the expression of an RacB protein, the RacB activity or RacB function are comprised in accordance with the invention. The skilled worker is aware of a series of different methods being available for influencing the expression of an RacB protein, the RacB activity or the RacB function in the desired manner.

A reduction of the RacB activity or the RacB function is preferably achieved by reduced expression of an endogenous RacB protein.

A reduction of the amount of RacB protein, the RacB activity or the RacB function can be effected using the following methods:

- a) Introduction of a double-stranded RacB RNA nucleic acid sequence (RacB dsRNA) or an expression cassette(s) ensuring the expression thereof
- b) Introduction of an RacB antisense nucleic acid sequences or an expression cassette ensuring expression thereof. comprised are those methods in which the antisense nucleic acid sequence is directed against an RacB gene (i.e. genomic DNA sequences) or an RacB gene transcript (i.e. RNA sequences). α -Anomeric nucleic acid sequences are also comprised
- c) Introduction of an RacB antisense nucleic acid sequences in combination with a ribozyme or an expression cassette ensuring expression thereof
- d) Introduction of RacB sense nucleic acid sequences for inducing cosuppression or an expression cassette ensuring expression thereof

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- e) Introduction of a nucleic acid sequence encoding dominant-negative RacB protein or an expression cassette ensuring expression thereof
 - 5 f) Introduction of DNA- or protein-binding factors against RacB genes, RacB RNAs or RacB proteins or an expression cassette ensuring expression thereof
 - 10 g) Introduction of viral nucleic acid sequences and expression constructs causing RacB RNA degradation or an expression cassette ensuring expression thereof
 - 15 h) Introduction of constructs for inducing a homologous recombination on endogenous RacB genes, for example for generating knock-out mutants
 - i) Introduction of mutations into endogenous RacB genes for generating a loss of function (for example generation of stop codons, reading-frame shifts and the like)

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In this context, each and every of these methods may bring about a reduction of the RacB expression, RacB activity or RacB function for the purposes of the invention. A combined use is also feasible. Further methods are known to the skilled worker and can comprise the hindering or prevention of RacB protein processing, of the RacB protein or RacB mRNA transport, inhibition of ribosome attachment, inhibition of RNA splicing, induction of an RacB-RNA-degrading enzyme and/or inhibition of translational elongation or termination.

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The individual processes which are preferred may be described in greater detail hereinbelow:

- a) Introduction of a double-stranded RacB RNA nucleic acid sequence (RacB dsRNA)

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The method of regulating genes by means of double-stranded RNA ("double-stranded RNA interference"; dsRNAi) has been described repeatedly for animal and plant organisms (for example Matzke MA et al. (2000) Plant Mol Biol 43:401-415; Fire A. et al (1998) Nature 391:806-811; WO 99/32619; WO 99/53050; WO 00/68374; WO 00/44914; WO 00/44895; WO 00/49035; WO 00/63364). Express reference is made to the processes and methods described in the above references.

Effective gene suppression can also be demonstrated upon transient expression or following transient transformation for example as the consequence of biolistic transformation

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(Schweizer P et al. (2000) Plant J 2000 24: 895-903). dsRNAi methods are based on the phenomenon that the simultaneous introduction of complementary strand and counterstrand of a gene transcript causes the expression of the gene in question to be suppressed in a highly efficient manner. The phenotype caused greatly resembles a corresponding knock-out mutant (Waterhouse PM et al. (1998) Proc Natl Acad Sci USA 95:13959-64).

- 5 10 15 20 25 30 35 40
- The dsRNAi method has proved to be particularly effective and advantageous for reducing the RacB expression. As described, inter alia, in WO 99/32619, dsRNAi approaches are markedly superior to traditional antisense approaches.
- The invention therefore furthermore relates to double-stranded RNA molecules (dsRNA molecules) which, upon introduction into a plant (or a cell, tissue, organ or seed derived therefrom), bring about the reduction of an RacB.
- In the double-stranded RNA molecule for reducing the expression of an RacB protein,
- a) one of the two RNA strands is essentially identical to at least a portion of an RacB nucleic acid sequence, and
- b) the corresponding other RNA strand is essentially identical to at least a portion of the complementary strand of an RacB nucleic acid sequence.
- In a further preferred embodiment, the double-stranded RNA molecule for reducing the expression of an RacB protein comprises
- a) a sense RNA strand comprising at least one ribonucleotide sequence which is essentially identical to at least part of the sense RNA transcript of a nucleic acid sequence encoding an RacB protein, and
- b) an antisense RNA strand which is essentially - preferably completely - complementary to the RNA sense strand under a).

With respect to the double-stranded RNA molecules, RacB nucleic acid sequence is to be understood as meaning, preferably, a sequence as shown in SEQ ID NO: 1, 3 or 5 or a functional equivalent thereof as shown in SEQ ID NO: 34, 36,

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38, 40, 42, 44, 46, 48, 49, 51, 53, 55, 57, 61, 63, 65, 67 or 69

5 "Essentially identical" means that the dsRNA sequence can also show insertions, deletions or individual point mutations compared with the RacB target sequence or a functionally equivalent target sequence while still bringing about an effective reduction of the expression. The homology in accordance with the above definition preferably amounts to at least 75%, preferably at least 80%, very especially preferably at least 90%, most preferably 100%, between the sense strand of an inhibitory dsRNA and at least part of the sense RNA transcript of a nucleic acid sequence encoding an RacB protein or a functional equivalent thereof (or between the antisense strand of the complementary strand of a nucleic acid sequence encoding an RacB protein or a functional equivalent thereof).

20 The length of the part-segment amounts to at least 10 bases, preferably at least 25 bases, especially preferably at least 50 bases, very especially preferably at least 100 bases, most preferably at least 200 bases or at least 300 bases.

25 As an alternative, an "essentially identical" dsRNA can also be defined as a nucleic acid sequence which is capable of hybridizing with part of a storage protein gene transcript (for example in 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA at 50°C or 70°C for 12 to 16 h).

30 "Essentially complementary" is to be understood as meaning that the antisense RNA strand may also contain insertions, deletions and individual point mutations, compared to the complement of the sense RNA strand. Preferably, the homology between the antisense RNA strand and the complement of the sense RNA strand is at least 80%, preferably at least 90%, very particularly preferably at least 95%, most preferably 100% .

40 "Part of the sense RNA transcript" of a nucleic acid sequence encoding an RacB protein or a functional equivalent thereof is to be understood as meaning fragments of an RNA or mRNA transcribed from a nucleic acid sequence, preferably an RacB gene, encoding an RacB protein or a functional equivalent thereof. Here, the fragments preferably have a sequence length of at least 20 bases, preferably at least 50 bases, particularly preferably at least 100 bases, very particularly preferably at least 200 bases, most preferably at least 500

bases. Also comprised is the complete transcribed RNA or mRNA.

5 Also comprised is the use of the dsRNA molecules according to the invention in the methods according to the invention for generating a pathogen resistance in plants.

10 The dsRNA can be composed of one or more strands of polymerized ribonucleotides. Modifications both of the sugar-phosphate backbone and of the nucleosides may be present. For example, the phosphodiester bonds of the natural RNA can be modified in such a way that they comprise at least one nitrogen or sulfur hetero atom. Bases can be modified in such a way that the activity of, for example, adenosine
15 deaminase is restricted. These and other modifications are described hereinbelow in the methods of stabilizing antisense RNA.

20 To achieve the same purpose, it is, of course, also possible to introduce a plurality of individual dsRNA molecules each comprising one of the ribonucleotide sequence sections defined above into the cell or the organism.

25 The dsRNA can be generated enzymatically or fully or partially synthesized chemically.

30 The double-stranded dsRNA structure can be formed starting from two complementary, separate RNA strands or, preferably, strands starting from an individual self-complementary RNA strand.

35 In a single self-complementary strand, sense and antisense sequence may be linked by a linking sequence ("linker") and can form for example a hairpin structure. The linking sequence can preferably be an intron which is spliced out after the dsRNA has been synthesized.

40 The nucleic acid sequence encoding a dsRNA can comprise further elements such as, for example, transcription termination signals or polyadenylation signals.

If the two dsRNA strands are to be combined in a cell or plant, this can be effected in various ways:

45 a) transformation of the cell or plant with a vector comprising both expression cassettes,

- b) cotransformation of the cell or plant with two vectors, one of them comprising the expression cassettes with the sense strand and the other comprising the expression cassettes with the antisense strand,
- c) hybridizing two plants, each of which has been transformed with one vector, one of the vectors comprising the expression cassettes with the sense strand and the other comprising the expression cassettes with the antisense strand.

The formation of the RNA duplex can be initiated either outside or within the cell. Like in WO 99/53050, the dsRNA can also comprise a hairpin structure by linking sense and antisense strand by means of a linker (for example an intron). The self-complementary dsRNA structures are preferred since they only require the expression of one construct and always comprise the complementary strands in an equimolar ratio.

The expression cassettes encoding the antisense or sense strand of a dsRNA or the self-complementary strand of the dsRNA are preferably inserted into a vector and, using the methods described hereinbelow, stably inserted into the genome of a plant in order to ensure permanent expression of the dsRNA, using selection markers for example.

The dsRNA can be introduced using a quantity which allows at least one copy per cell. Greater quantities (for example at least 5, 10, 100, 500 or 1000 copies per cell) may bring about a more effective reduction.

As already described, 100% sequence identity between dsRNA and an RacB gene transcript or the gene transcript of a functionally equivalent gene is not necessarily required in order to bring about an effective reduction of the RacB expression. Accordingly, there is the advantage that the method is tolerant with regard to sequence deviations as may exist as the consequence of genetic mutations, polymorphisms or evolutionary divergence. Thus, for example, it is possible to use the dsRNA generated on the basis of the RacB sequence of one organism to suppress the RacB expression in another organism. The high sequence homology between the RacB sequences from rice, maize and barley allows the conclusion that this protein is conserved to a high degree within plants, so that the expression of a dsRNA derived from one of the disclosed RacB sequences as shown in SEQ ID NO: 1, 3 or 5

appears to have an advantageous effect in other plant species as well.

Furthermore, owing to the high homology between the individual RacB proteins and their functional equivalents, it is possible using a single dsRNA generated from a certain RacB sequence of an organism to suppress the expression of further homologous RacB proteins and/or their functional equivalents of the same organism or else the expression of RacB proteins in other related species. For this purpose, the dsRNA preferably comprises sequence regions of RacB gene transcripts which correspond to conserved regions. Said conserved regions can easily be found by comparing sequences.

The dsRNA can be synthesized either in vivo or in vitro. To this end, a DNA sequence encoding a dsRNA can be brought into an expression cassette under the control of at least one genetic control element (such as, for example, promoter, enhancer, silencer, splice donor or splice acceptor or polyadenylation signal). Suitable advantageous constructions are described hereinbelow. Polyadenylation is not required, nor do elements for initiating translation have to be present.

A dsRNA can be synthesized chemically or enzymatically. Cellular RNA polymerases or bacteriophage RNA polymerases (such as, for example, T3, T7 or SP6 RNA polymerase) can be used for this purpose. Suitable methods for expression of RNA in vitro are described (WO 97/32016; US 5,593,874; US 5,698,425, US 5,712,135, US 5,789,214, US 5,804,693). A dsRNA which has been synthesized in vitro chemically or enzymatically can be isolated completely or to some degree from the reaction mixture, for example by extraction, precipitation, electrophoresis, chromatography or combinations of these methods, before being introduced into a cell, tissue or organism. The dsRNA can be introduced directly into the cell or else be applied extracellularly (for example into the interstitial space).

However, it is preferred to transform the plant stably with an expression construct which brings about the expression of the dsRNA. Suitable methods are described hereinbelow.

b) Introduction of an RacB antisense nucleic acid sequence

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Methods for suppressing a specific protein by preventing its mRNA from accumulating by means of antisense technology have been described in many instances, including in the case of plants (Sheehy et al. (1988) Proc Natl Acad Sci USA 85: 8805-8809; US 4,801,340; Mol JN et al. (1990) FEBS Lett 268(2):427-430). The antisense nucleic acid molecule hybridizes, or binds, with the cellular mRNA and/or genomic DNA encoding the RacB target protein to be suppressed. This suppresses the transcription and/or translation of the target protein. Hybridization can originate conventionally by the formation of a stable duplex or - in the case of genomic DNA - by the antisense nucleic acid molecule binding to the duplex of the genomic DNA by specific interaction in the major groove of the DNA helix.

An antisense nucleic acid sequence suitable for reducing an RacB protein can be deduced using the nucleic acid sequence encoding this protein, for example the nucleic acid sequence as shown in SEQ ID NO: 1, 3 or 5, or the nucleic acid sequence encoding a functional equivalent thereof, for example a sequence as shown in SEQ ID NO: 34, 36, 38, 40, 42, 44, 46, 48, 49, 51, 53, 55, 57, 61, 63, 65, 67 or 69, following Watson and Crick's base pairing rules. The antisense nucleic acid sequence can be complementary to all of the transcribed mRNA of said protein, be limited to the coding region, or else only be composed of a nucleotide, which is partially complementary to the coding or noncoding sequence of the mRNA. Thus, for example, the oligonucleotide can be complementary to the region comprising the translation start for said protein. Antisense nucleic acid sequences can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length, but may also be longer and comprise at least 100, 200, 500, 1000, 2000 or 5000 nucleotides. Antisense nucleic acid sequences can be expressed recombinantly or synthesized chemically or enzymatically using methods known to the skilled worker. In the case of chemical synthesis, natural or modified nucleotides may be used. Modified nucleotides can impart an increased biochemical stability to the antisense nucleic acid sequence and lead to an increased physical stability of the duplex formed of antisense nucleic acid sequence and sense target sequence. The following can be used: for example phosphorothioate derivatives and acridine-substituted nucleotides such as 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthin, xanthin, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine,

- 5-carboxymethylaminomethyluracil, dihydrouracil, β -D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, β -D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl)uracil and 2,6-diaminopurine.
- 15 In a further preferred embodiment, the expression of an RacB protein can be inhibited by nucleotide sequences which are complementary to the regulatory region of an RacB gene (for example an RacB promoter and/or enhancer) and which form triple-helical structures with that DNA double helix so that the transcription of the RacB gene is reduced. Such methods have been described (Helene C (1991) *Anticancer Drug Res* 6(6):569-84; Helene C et al. (1992) *Ann NY Acad Sci* 660:27-36; Maher LJ (1992) *Bioassays* 14(12):807-815).
- 20 In a further embodiment, the antisense nucleic acid molecule can be an α -anomeric nucleic acid. Such α -anomeric nucleic acid molecules form specific double-stranded hybrids with complementary RNA in which - as opposed to the conventional β -nucleic acids - the two strands run parallel to one another (Gautier C et al. (1987) *Nucleic Acids Res* 15:6625-6641). The antisense nucleic acid molecule can furthermore also comprise 2'-O-methylribonucleotides (Inoue et al. (1987) *Nucleic Acids Res* 15:6131-6148) or chimeric RNA/DNA analogs (Inoue et al. (1987) *FEBS Lett* 215:327-330).
- 30 c) Introduction of an RacB antisense nucleic acid sequence in combination with a ribozyme
- The above-described antisense strategy can be combined advantageously with a ribozyme method. Catalytic RNA molecules or ribozymes can be adapted to suit any target RNA and cleave the phosphodiester backbone at specific positions, functionally deactivating the target RNA (Tanner NK (1999) *FEMS Microbiol Rev* 23(3):257-275). The ribozyme itself is not modified thereby, but is capable of cleaving further target RNA molecules analogously, thereby assuming the qualities of an enzyme. The incorporation of ribozyme sequences into antisense RNAs confers this enzyme-like RNA-cleaving quality

to precisely these antisense RNAs, thus increasing their efficacy in inactivating the target RNA. The generation and the use of such ribozyme antisense RNA molecules is described, for example, in Haseloff et al. (1988) Nature 334: 585-591.

In this manner, ribozymes (for example "hammerhead" ribozymes; Haselhoff and Gerlach (1988) Nature 334:585-591) can be used catalytically to cleave the mRNA of an enzyme to be suppressed, for example RacB, and to prevent translation. The ribozyme technique can increase the efficacy of an antisense strategy. Methods of expressing ribozymes for reducing specific proteins are described in (EP 0 291 533, EP 0 321 201, EP 0 360 257). The expression of ribozyme in plant cells has also been described (Steinecke P et al. (1992) EMBO J 11(4):1525-1530; de Feyter R et al. (1996) Mol Gen Genet. 250(3):329-338). Suitable target sequences and ribozymes can be determined as described for example by "Steinecke P, Ribozymes, Methods in Cell Biology 50, Galbraith et al. eds, Academic Press, Inc. (1995), pp. 449-460" by calculating the secondary structure of ribozyme RNA and target RNA as well as by their interaction (Bayley CC et al. (1992) Plant Mol Biol. 18(2):353-361; Lloyd AM and Davis RW et al. (1994) Mol Gen Genet. 242(6):653-657). For example, derivatives of the Tetrahymena L-19 IVS RNA with regions which are complementary to the mRNA of the RacB protein to be suppressed can be constructed (see also US 4,987,071 and US 5,116,742). As an alternative, such ribozymes can also be identified from a library of diverse ribozymes via a selection process (Bartel D and Szostak JW (1993) Science 261:1411-1418).

- d) Introduction of an RacB sense nucleic acid sequence for inducing cosuppression

The expression of an RacB nucleic acid sequence in sense orientation can lead to cosuppression of the corresponding homologous endogenous gene. The expression of sense RNA with homology with an endogenous gene can reduce or switch off the expression of the former, similarly to what has been described for antisense approaches (Jorgensen et al. (1996) Plant Mol Biol 31(5):957-973; Goring et al. (1991) Proc Natl Acad Sci USA 88:1770-1774; Smith et al. (1990) Mol Gen Genet 224:447-481; Napoli et al. (1990) Plant Cell 2:279-289; Van der Krol et al. (1990) Plant Cell 2:291-99). In this context, the homologous gene to be reduced can be represented either fully or only in part by the construct introduced. The

possibility of translation is not required. The application of this technique to plants is described, for example, by Napoli et al. (1990) *The Plant Cell* 2: 279-289 and in US 5,034,323.

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The cosuppression is preferably realized by using a sequence essentially identical to at least part of the nucleic acid sequence encoding an RacB protein or a functional equivalent thereof, for example the nucleic acid sequence as shown in SEQ ID NO: 1, 3 or 5, or the nucleic acid sequence encoding a functional equivalent thereof, for example a sequence as shown in SEQ ID NO: 34, 36, 38, 40, 42, 44, 46, 48, 49, 51, 53, 55, 57, 61, 63, 65, 67 or 69.

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- 15 e) Introduction of nucleic acid sequences encoding a dominant-negative RacB protein

The function or activity of an RacB protein can also be brought about efficiently by expressing a dominant-negative variant of this RacB protein. Methods of reducing the function or activity of a protein by coexpressing its dominant-negative form are known to the skilled worker (Lagna G and Hemmati-Brivanlou A (1998) *Current Topics in Developmental Biology* 36:75-98; Perlmutter RM and Alberola-Ila J (1996) *Current Opinion in Immunology* 8(2):285-90; Sheppard D (1994) *American Journal of Respiratory Cell & Molecular Biology*. 11(1):1-6; Herskowitz I (1987) *Nature* 329(6136):219-22).

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For example, a dominant-negative RacB variant can be brought about by modifying the amino acid threonine at position 20 in the RacB proteins from maize, rice or barley to, preferably, aspartic acid. The threonine which is preferably to be mutated, or, if appropriate, also serine (similarly to threonine at position 20 in RacB from maize, rice or barley) in RacB homologs from other species can be determined for example by computer-aided comparison ("alignment"). These mutations for achieving a dominant-negative RacB variant are preferably carried out at the level of the nucleic acid sequence encoding RacB proteins. A corresponding mutation can be brought about for example by PCR-mediated in-vitro mutagenesis using suitable oligonucleotide primers, by which the desired mutation is introduced. This is done using methods known to the skilled worker; for example, the "LA PCR in vitro Mutagenesis Kit" (Takara Shuzo, Kyoto) may be used for this purpose. A method of generating a dominant-negative

variant of the maize RacB protein is also described in WO 00/15815 (Example 4, p. 69).

5 Especially preferred are the dominant-negative variants of the RacB proteins from barley, rice or maize described under SEQ ID NO: 7, 8 and 9.

f) Introduction of DNA- or protein-binding factors against RacB genes, RacB RNAs or RacB proteins

10 RacB gene expression may also be reduced using specific DNA-binding factors, for example factors of the zinc finger transcription factor type. These factors attach to the genomic sequence of the endogenous target gene, preferably in
15 the regulatory regions, and bring about repression of the endogenous gene. The use of such a method makes possible the reduction of the expression of an endogenous RacB gene without it being necessary to recombinantly manipulate its sequence. Suitable methods for the preparation of suitable
20 factors have been described (Dreier B et al. (2001) J Biol Chem 276(31):29466-78; Dreier B et al. (2000) J Mol Biol 303(4):489-502; Beerli RR et al. (2000) Proc Natl Acad Sci USA 97 (4):1495-1500; Beerli RR et al. (2000) J Biol Chem 275(42):32617-32627; Segal DJ and Barbas CF 3rd. (2000) Curr Opin Chem Biol 4(1):34-39; Kang JS and Kim JS (2000) J Biol
25 Chem 275(12):8742-8748; Beerli RR et al. (1998) Proc Natl Acad Sci USA 95(25):14628- 14633; Kim JS et al. (1997) Proc Natl Acad Sci USA 94(8):3616 -3620; Klug A (1999) J Mol Biol 293(2):215-218; Tsai SY et al. (1998) Adv Drug Deliv Rev 30(1-3):23-31; Mapp AK et al. (2000) Proc Natl Acad Sci USA
30 97(8):3930-3935; Sharrocks AD et al. (1997) Int J Biochem Cell Biol 29(12):1371-1387; Zhang L et al. (2000) J Biol Chem 275(43):33850-33860).

35 These factors can be selected using any desired portion of an RacB gene. This segment is preferably located in the promoter region. For gene suppression, however, it may also be in the region of the coding exons or introns. The segments in question can be obtained by the skilled worker from Genbank
40 by database search or, starting from an RacB cDNA whose gene is not present in Genbank, by screening a genomic library for corresponding genomic clones. The skilled worker is familiar with the methods required therefor.

45 Furthermore, it is possible to introduce, into cells, factors which inhibit the RacB target protein itself. The protein-binding factors can be, for example, aptamers

(Famulok M and Mayer G (1999) Curr Top Microbiol Immunol 243:123-36) or antibodies or antibody fragments or single-chain antibodies. Methods for obtaining these factors have been described and are known to the skilled worker. For example, a cytoplasmic scFv antibody was employed to modulate the activity of the phytochrome A protein in genetically modified tobacco plants (Owen M et al. (1992) Biotechnology (N Y) 10(7):790-794; Franken E et al. (1997) Curr Opin Biotechnol 8(4):411-416; Whitelam (1996) Trend Plant Sci 1:286-272).

Gene expression may also be suppressed by tailor-made low-molecular-weight synthetic compounds, for example of the polyamide type (Dervan PB and Bürli RW (1999) Current Opinion in Chemical Biology 3:688-693; Gottesfeld JM et al. (2000) Gene Expr 9(1-2):77-91). These oligomers are composed of the units 3-(dimethylamino)propylamine, N-methyl-3-hydroxypyrrole, N-methylimidazole and N-methylpyrrole and can be adapted to any piece of double-stranded DNA in such a way that they bind into the major groove in a sequence-specific manner and block the expression of these gene sequences. Suitable methods have been described (see, inter alia, Bremer RE et al. (2001) Bioorg Med Chem. 9(8):2093-103; Ansari AZ et al. (2001) Chem Biol. 8(6):583-92; Gottesfeld JM et al. (2001) J Mol Biol. 309(3):615-29; Wurtz NR et al. (2001) Org Lett 3(8):1201-3; Wang CC et al. (2001) Bioorg Med Chem 9(3):653-7; Urbach AR and Dervan PB (2001) Proc Natl Acad Sci USA 98(8):4343-8; Chiang SY et al. (2000) J Biol Chem. 275(32):24246-54).

- g) Introduction of viral nucleic acid sequences and expression constructs which cause RacB RNA degradation

RacB expression can also be brought about efficiently by inducing the specific RacB RNA degradation by the plant with the aid of a viral expression system (amplicon) (Angell, SM et al. (1999) Plant J. 20(3):357-362). These systems - also termed "VIGS" (viral induced gene silencing) - introduce, into the plant, nucleic acid sequences with homology to the transcripts to be suppressed, with the aid of viral vectors. Then, transcription is switched off, probably mediated by plant defense mechanisms against viruses. Suitable techniques and methods have been described (Ratcliff F et al. (2001) Plant J 25(2):237-45; Fagard M and Vaucheret H (2000) Plant Mol Biol 43(2-3):285-93; Anandalakshmi R et al. (1998) Proc

Natl Acad Sci USA 95(22):13079-84; Ruiz MT (1998) Plant Cell 10(6): 937-46).

- h) Introduction of constructs for inducing a homologous recombination on endogenous RacB genes, for example for generating knock-out mutants

An example of what is used for generating a homologously recombinant organism with reduced RacB activity is a nucleic acid construct comprising at least part of an endogenous RacB gene which is modified by a deletion, addition or substitution of at least one nucleotide in such a way that its functionality is reduced or fully destroyed. The modification may also relate to the regulatory elements (for example the promoter) of the gene, so that the coding sequence remains unmodified, but expression (transcription and/or translation) does not take place and is reduced.

In the case of conventional homologous recombination, the modified region is flanked at its 5' and 3' end by further nucleic acid sequences which must be sufficient in length for making possible recombination. They are, as a rule, in the range of several hundred bases to several kilobases in length (Thomas KR and Capecchi MR (1987) Cell 51:503; Strepp et al. (1998) Proc Natl Acad Sci USA 95(8):4368-4373). For homologous recombination, the host organism - for example a plant - is transformed with the recombination construct using the methods described hereinbelow, and clones which have successfully undergone recombination are selected, for example using a resistance to antibiotics or herbicides.

Homologous recombination is a relatively rare event in higher eukaryotes, especially in plants. Random integrations into the host genome predominate. One possibility of eliminating the randomly integrated sequences and thus increasing the number of cell clones with a correct homologous recombination is the use of a sequence-specific recombination system as described in US 6,110,736, by which unspecifically integrated sequences can be deleted again, which simplifies the selection of events which have integrated successfully via homologous recombination. A large number of sequence-specific recombination systems can be used, examples being the Cre/lox system of bacteriophage P1, the FLP/FRT system of yeast, the Gin recombinase of phage Mu, the Pin recombinase from E. coli, and the R/RS system of the pSR1 plasmid. The bacteriophage P1 Cre/lox and the yeast FLP/FRT system are preferred. The FLP/FRT and cre/lox recombinase system has

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already been applied to plant systems (Odell et al. (1990) Mol Gen Genet 223: 369-378).

- i) Introduction of mutations into endogenous RacB genes for generating a loss of function (for example generation of stop codons, reading-frame shifts and the like)

- Further suitable methods for reducing the RacB activity are the introduction of nonsense mutations into endogenous RacB genes, for example by introducing RNA/DNA oligonucleotides into the plant (Zhu et al. (2000) Nat Biotechnol 18(5):555-558) and the generation of knock-out mutants with the aid of, for example, T-DNA mutagenesis (Koncz et al. (1992) Plant Mol Biol 20(5):963-976), ENU (N-ethyl-N-nitrosourea) mutagenesis or homologous recombination (Hohn B and Puchta (1999) H Proc Natl Acad Sci USA 96:8321-8323.). Point mutations can also be generated by means of DNA-RNA hybrids also known under the name "chimeraplasty" (Cole-Strauss et al. (1999) Nucl Acids Res 27(5):1323-1330; Kmiec (1999) Gene therapy American Scientist 87(3):240-247).

- The methods of dsRNAi, cosuppression by means of sense RNA and "VIGS" ("virus induced gene silencing") are also termed
- "post-transcriptional gene silencing" (PTGS). PTGS methods, like the reduction of the RacB function or activity with dominant-negative RacB variants, are especially advantageous because the demands regarding the homology between the endogenous gene to be suppressed and the sense or dsRNA nucleic acid sequence expressed recombinantly (or between the endogenous gene and its dominant-negative variant) are lower than, for example, in the case of a traditional antisense approach. Such criteria with regard to homology are mentioned in the description of the dsRNAi method and can generally be applied to PTGS methods or dominant-negative approaches. Owing to the high degree of homology between the RacB proteins from maize, rice and barley, a high degree of conservation of this protein in plants can be assumed. Thus, using the RacB nucleic acid sequences from barley, maize or rice, it is presumably also possible efficiently to suppress the expression of homologous RacB proteins in other species without the isolation and structure elucidation of the RacB homologs occurring therein being required. Considerably less labor is therefore required. Similarly, the use of dominant-negative variants of an RacB protein from rice, maize or barley can presumably also efficiently reduce or suppress the function/activity of its homolog in other plant species.

All of the substances and compounds which directly or indirectly bring about a reduction in protein quantity, RNA quantity, gene activity or protein activity of an RacB protein shall subsequently be combined in the term "anti-RacB" compounds. The
5 term "anti-RacB" compound explicitly includes the nucleic acid sequences, peptides, proteins or other factors employed in the above-described methods.

For the purposes of the invention, "introduction" comprises all
10 of the methods which are capable of directly or indirectly introducing an "anti-RacB" compound into a plant or a cell, compartment, tissue, organ or seed thereof, or of generating such a compound there. Direct and indirect methods are comprised. The introduction can lead to a transient presence of an "anti-RacB"
15 compound (for example a dsRNA) or else to its stable presence.

Owing to the different nature of the above-described approaches, the "anti-RacB" compound can exert its function directly (for example by insertion into an endogenous RacB gene). However, its
20 function can also be exerted indirectly following transcription into an RNA (for example in the case of antisense approaches) or following transcription and translation into a protein (for example in the case of binding factors). The invention comprises both directly and indirectly acting "anti-RacB" compounds.

25 The term "introducing" comprises for example methods such as transfection, transduction or transformation.

"Anti-RacB" compounds therefore also comprises recombinant
30 expression constructs which bring about expression (i.e. transcription and, if appropriate, translation), for example of an RacB dsRNA or an RacB "antisense" RNA, preferably in a plant or a part, tissue, organ or seed thereof.

35 In said expression constructs, a nucleic acid molecule whose expression (transcription and, if appropriate, translation) generates an "anti-RacB" compound is preferably operably linked to at least one genetic control element (for example a promoter) which ensures expression in an organism, preferably in plants. If
40 the expression construct is to be introduced directly into a plant and the "anti-RacB" compound (for example the RacB dsRNA) is to be generated therein in plantae, plant-specific genetic control elements (for example promoters) are preferred. However, the "anti-RacB" compound may also be generated in other organisms
45 or in vitro and then be introduced into the plant (as described in Examples 6 and 7). Preferred in this context are all of the prokaryotic or eukaryotic genetic control elements (for example

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promoters) which permit the expression in the organism chosen in each case for the preparation.

Operable linkage is to be understood as meaning, for example, the sequential arrangement of a promoter with the nucleic acid sequence to be expressed (for example an "anti-RacB" compound) and, if appropriate, further regulatory elements such as, for example, a terminator in such a way that each of the regulatory elements can fulfil its function when the nucleic acid sequence is expressed recombinantly, depending on the arrangement of the nucleic acid sequences in relation to sense or antisense RNA. To this end, direct linkage in the chemical sense is not necessarily required. Genetic control sequences such as, for example, enhancer sequences, can also exert their function on the target sequence from positions which are further away, or indeed from other DNA molecules. Preferred arrangements are those in which the nucleic acid sequence to be expressed recombinantly is positioned behind the sequence acting as promoter, so that the two sequences are linked covalently to each other. The distance between the promoter sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 200 base pairs, especially preferably less than 100 base pairs, very especially preferably less than 50 base pairs.

Operable linkage, and an expression cassette, can be generated by means of customary recombination and cloning techniques as are described, for example, in Maniatis T, Fritsch EF and Sambrook J (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Silhavy TJ, Berman ML and Enquist LW (1984) *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Ausubel FM et al. (1987) *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley Interscience and in Gelvin et al. (1990) *In: Plant Molecular Biology Manual*. However, further sequences which, for example, act as a linker with specific cleavage sites for restriction enzymes, or as a signal peptide, may also be positioned between the two sequences. The insertion of sequences may also lead to the expression of fusion proteins. Preferably, the expression cassette, consisting of a linkage of promoter and nucleic acid sequence to be expressed, can exist in a vector-integrated form and be inserted into a plant genome, for example by transformation.

However, expression cassette also denotes those constructions in which a promoter is positioned behind an endogenous RacB gene, for example by means of homologous recombination, and the reduction according to the invention of an RacB protein is

brought about by the expression of an antisense RacB RNA. Analogously, an "anti-RacB" compound (for example a nucleic acid sequence encoding an RacB dsRNA or an RacB antisense RNA) can be positioned behind an endogenous promoter in such a way that the same effect is manifested. Both approaches lead to inventive expression cassettes.

The term plant-specific promoters is understood as meaning, in principle, any promoter which is capable of governing the expression of genes, in particular foreign genes, in plants or plant parts, plant cells, plant tissues or plant cultures. In this context, expression can be, for example, constitutive, inducible or development-dependent.

The following are preferred:

a) Constitutive promoters

Preferred vectors are those which make possible constitutive expression in plants (Benfey et al. (1989) EMBO J 8:2195-2202). "Constitutive" promoter is understood as meaning those promoters which ensure expression in a large number of, preferably all, tissues over a substantial period of plant development, preferably at all stages of plant development. In particular a plant promoter or a promoter derived from a plant virus are preferably used. Particularly preferred is the promoter of the CaMV cauliflower mosaic virus 35S transcript (Franck et al. (1980) Cell 21:285-294; Odell et al. (1985) Nature 313:810-812; Shewmaker et al. (1985) Virology 140:281-288; Gardner et al. (1986) Plant Mol Biol 6:221-228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J 8:2195-2202). Another suitable constitutive promoter is the "Rubisco small subunit (SSU)" promoter (US 4,962,028), the leguminB promoter (GenBank Acc. No. X03677), the Agrobacterium nopaline synthase promoter, the TR dual promoter, the Agrobacterium OCS (octopine synthase) promoter, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29:637-649), the ubiquitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18:675-689; Bruce et al. (1989) Proc Natl Acad Sci USA 86:9692-9696), the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits or the promoter of a proline-rich protein from wheat (WO 91/13991), and further promoters of genes whose constitutive expression in plants is known to the skilled worker. A particularly preferred constitutive

promoter is the promoter of the nitrilase-1 (nit1) gene from *A. thaliana* (GenBank Acc.-No.: Y07648.2, nucleotides 2456-4340, Hillebrand et al. (1996) Gene 170:197-200).

5 b) Tissue-specific promoters

Preferred are furthermore promoters with specificity for the anthers, ovaries, flowers, leaves, stems, roots and seeds.

10 Seed-specific promoters

such as, for example, the phaseolin promoter (US 5,504,200; Bustos MM et al. (1989) Plant Cell 1(9):839-53), the 2S albumin gene promoter (Joseffson LG et al. (1987) J Biol Chem 262:12196-12201), the legumin promoter (Shirsat A et al. (1989) Mol Gen Genet 215(2): 326-331), the USP (unknown seed

15 protein) promoter (Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67), the napin gene promoter (US 5,608,152; Stalberg K et al. (1996) L Planta 199:515-519), the sucrose binding protein promoter (WO 00/26388) or the legumin B4 promoter (LeB4; Bäumlein H et al. (1991) Mol Gen Genet 225: 20 121-128; Baeumlein et al. (1992) Plant Journal 2(2):233-9; Fiedler U et al. (1995) Biotechnology (NY) 13(10):1090f), the Arabidopsis oleosin promoter (WO 98/45461), the Brassica Bce4 promoter (WO 91/13980). Further suitable seed-specific

25 promoters are those of the genes encoding the high-molecular-weight glutenin (HMWG), gliadin, branching enzyme, ADP glucose pyrophosphatase (AGPase) or starch synthase. Furthermore preferred are promoters which permit seed-specific expression in monocots such as maize, barley, 30 wheat, rye, rice and the like. The following can be employed advantageously: the promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) or the promoters described in WO 99/16890 (promoters of the hordein gene, the glutelin gene, the oryzin gene, the prolamin gene, the gliadin gene, 35 the glutelin gene, the zein gene, the kasirin gene or the secalin gene).

Tuber-, storage-root- or root-specific promoters such as, for example, the patatin promoter class I (B33), the potato 40 cathepsin D inhibitor promoter.

Leaf-specific promoters

such as the potato cytosolic FBPase promoter (WO 97/05900), the Rubisco (ribulose-1,5-bisphosphate carboxylase) SSU 45 (small subunit) promoter or the ST-LSI promoter from potato (Stockhaus et al. (1989) EMBO J 8:2445-2451). Very especially preferred are epidermis-specific promoters such as, for

example, the OXLP gene (oxalate-oxidase-like protein) promoter (Wei et al. (1998) Plant Mol. Biol. 36:101-112).

Flower-specific promoters

- 5 such as, for example, the phytoene synthase promoter (WO 92/16635) or the promoter of the P-rr gene (WO 98/22593).

Anther-specific promoters

- 10 such as the 5126 promoter (US 5,689,049, US 5,689,051), the glob-1 promoter and the γ -zein promoter.

c) Chemically inducible promoters

- 15 The expression cassettes can also comprise a chemically inducible promoter (review article: Gatz et al. (1997) Annu Rev Plant Physiol Plant Mol Biol 48:89-108), by which the expression of the exogenous gene in the plant at a particular point in time can be controlled. Such promoters such as, for example, the PRP1 promoter (Ward et al. (1993) Plant Mol Biol 22:361-366), a salicylic-acid-inducible promoter 20 (WO 95/19443), a benzenesulfonamide-inducible promoter (EP 0 388 186), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J 2:397-404), an abscisic-acid-inducible promoter (EP 0 335 528) or an ethanol- or 25 cyclohexanone-inducible promoter (WO 93/21334) can likewise be used.

d) Stress- or pathogen-inducible promoters

- 30 Further preferred promoters are those which are induced by biotic or abiotic stress such as, for example, the pathogen-inducible promoter of the PRP1 gene (or *gst1* promoter) e.g. from potato (WO 96/28561; Ward et al. (1993) Plant Mol Biol 22:361-366), the tomato 35 high-temperature-inducible hsp70 or hsp80 promoter (US 5,187,267), the potato low-temperature-inducible alpha-amylase promoter (WO 96/12814), the light-inducible PPDK promoter, or the wounding-induced pinII promoter (EP-A 0 375 091).

- 40 Further pathogen-inducible promoters comprise the *Fis1* promoter from flax (WO 96/34949), the *Vst1* promoter (Schubert et al. (1997) Plant Mol Biol 34:417-426) and the EAS4 sesquiterpene cyclase promoter from tobacco (US 6,100,451).

45

Pathogen-inducible promoters comprise those of genes which are induced as a consequence of infection by pathogens, such as, for example, genes of PR proteins, SAR proteins, β -1,3-glucanase, chitinase and the like (for example Redolfi et al. (1983) *Neth J Plant Pathol* 89:245-254; Uknes, et al. (1992) *The Plant Cell* 4:645-656; Van Loon (1985) *Plant Mol Biol* 4:111-116; Marineau et al. (1987) *Plant Mol Biol* 9:335-342; Matton et al. (1987) *Molecular Plant-Microbe Interactions* 2:325-342; Somssich et al. (1986) *Proc Natl Acad Sci USA* 83:2427-2430; Somssich et al. (1988) *Mol Gen Genetics* 2:93-98; Chen et al. (1996) *Plant J* 10:955-966; Zhang and Sing (1994) *Proc Natl Acad Sci USA* 91:2507-2511; Warner, et al. (1993) *Plant J* 3:191-201; Siebertz et al. (1989) *Plant Cell* 1:961-968(1989).

Also comprised are wounding-inducible promoters such as that of the pinII gene (Ryan (1990) *Ann Rev Phytopath* 28:425-449; Duan et al. (1996) *Nat Biotech* 14:494-498), of the wun1 and wun2 gene (US 5,428,148), of the win1 and win2 gene (Stanford et al. (1989) *Mol Gen Genet* 215:200-208), of systemin (McGurl et al. (1992) *Science* 225:1570-1573), of the WIP1 gene (Rohmeier et al. (1993) *Plant Mol Biol* 22:783-792; Eckelkamp et al. (1993) *FEBS Letters* 323:73-76), of the MPI gene (Corderok et al. (1994) *Plant J* 6(2):141-150) and the like.

A source of further pathogen-inducible promoters is the PR gene family. A number of elements in these promoters have been found to be advantageous. Thus, the region -364 to -288 in the promoter of PR-2d provides salicylate specificity (Buchel et al. (1996) *Plant Mol Biol* 30, 493-504). The sequence 5'-TCATCTTCTT-3' is encountered repeatedly in the promoter of barley β -1,3-glucanase and more than 30 further stress-induced genes. In tobacco, this region binds a nuclear protein whose abundance is increased by salicylate. The PR-1 promoters from tobacco and Arabidopsis (EP-A 0 332 104, WO 98/03536) are likewise suitable for use as pathogen-inducible promoters. "Acidic PR-5"-(aPR5)-promoters from barley (Schweizer et al. (1997) *Plant Physiol* 114:79-88) and wheat (Rebmann et al. (1991) *Plant Mol Biol* 16:329-331) are preferred, since they are particularly specifically pathogen-induced. aPR5 proteins accumulate within about 4 to 6 hours after pathogen attack and have only very limited background expression (WO 99/66057). One approach to achieve higher pathogen-induced specificity is the preparation of synthetic promoters from combinations of known pathogen-responsive elements (Rushton et al. (2002) *Plant Cell* 14, 749-762; WO 00/01830; WO 99/66057). Further pathogen-inducible promoters

from different species are known to the person skilled in the art (EP-A 1 165 794; EP-A 1 062 356; EP-A 1 041 148; EP-A 1 032 684;

5 e) Development-dependent promoters

Further suitable promoters are, for example, fruit-maturation-specific promoters such as, for example, the tomato fruit-maturation-specific promoter (WO 94/21794, EP 409 625). Development-dependent promoters comprise partly the tissue-specific promoters, since individual tissues develop by nature in a development-dependent fashion.

Especially preferred are constitutive promoters and leaf- and/or stem-specific, pathogen-inducible and epidermis-specific promoters, with pathogen-inducible and epidermis-specific promoters being most preferred.

Furthermore, further promoters may be linked operably to the nucleic acid sequence to be expressed, which promoters make possible the expression in further plant tissues or in other organisms, such as, for example, *E. coli* bacteria. Suitable plant promoters are, in principle, all of the above-described promoters.

The nucleic acid sequences present in the expression cassettes or vectors according to the invention can be linked operably to further genetic control sequences in addition to a promoter. The term "genetic control sequences" is to be understood in the broad sense and refers to all those sequences which have an effect on the materialization or the function of the expression cassette according to the invention. For example, genetic control sequences modify the transcription and translation in prokaryotic or eukaryotic organisms. Preferably, the expression cassettes according to the invention comprise the promoter with specificity for the embryonal epidermis and/or the flower 5'-upstream of the nucleic acid sequence in question to be expressed recombinantly, and 3'-downstream a terminator sequence as additional genetic control sequence and, if appropriate, further customary regulatory elements, in each case linked operably to the nucleic acid sequence to be expressed recombinantly.

Genetic control sequences also comprise further promoters, promoter elements or minimal promoters, all of which can modify the expression-governing properties. Thus, for example, the tissue-specific expression may additionally depend on certain stressors, owing to genetic control sequences. Such elements have

been described, for example, for water stress, abscisic acid (Lam E and Chua NH, J Biol Chem 1991; 266(26): 17131 -17135) and heat stress (Schoffl F et al., Molecular & General Genetics 217(2-3):246-53, 1989).

5

Further advantageous control sequences are, for example, in the Gram-positive promoters amy and SPO2, and in the yeast or fungal promoters ADC1, MFa, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH.

10 In principle, all natural promoters with their regulatory sequences like those mentioned above may be used for the method according to the invention. In addition, synthetic promoters may also be used advantageously.

15 Genetic control sequences furthermore also comprise the 5'-untranslated regions, introns or noncoding 3'-region of genes, such as, for example, the actin-1 intron, or the Adh1-S introns 1, 2 and 6 (general reference: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994)). It has

20 been demonstrated that they may play a significant roles in the regulation of gene expression. Thus, it has been demonstrated that 5'-untranslated sequences can enhance the transient expression of heterologous genes. Examples of translation enhancers which may be mentioned are the tobacco mosaic virus 5' leader sequence (Gallie et al. (1987) Nucl Acids Res 15:8693-8711) and the like. Furthermore, they may promote tissue specificity (Rouster J et al. (1998) Plant J 15:435-440).

The expression cassette may advantageously comprise one or more
30 of what are known as enhancer sequences, linked operably to the promoter, which make possible an increased recombinant expression of the nucleic acid sequence. Additional advantageous sequences, such as further regulatory elements or terminators, may also be inserted at the 3' end of the nucleic acid sequences to be
35 expressed recombinantly. One or more copies of the nucleic acid sequences to be expressed recombinantly may be present in the gene construct.

Polyadenylation signals which are suitable as control sequences
40 are plant polyadenylation signals, preferably those which essentially correspond to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*, in particular gene 3 of the T-DNA (octopin synthase) of the Ti plasmid pTiACHS (Gielen et al. (1984) EMBO J 3:835 et seq.) or functional equivalents thereof.
45 Examples of terminator sequences which are especially suitable

are the OCS (octopin synthase) terminator and the NOS (nopalin synthase) terminator.

Control sequences are furthermore to be understood as those which
 5 make possible homologous recombination or insertion into the
 genome of a host organism or which permit removal from the
 genome. In the case of homologous recombination, for example the
 natural promoter of a particular gene may be exchanged for a
 promoter with specificity for the embryonal epidermis and/or the
 10 flower. Methods such as the cre/lox technology permit a
 tissue-specific, if appropriate inducible, removal of the
 expression cassette from the genome of the host organism (Sauer B
 (1998) Methods. 14(4):381-92). In this method, specific flanking
 sequences (lox sequences), which later allow removal by means of
 15 cre recombinase, are attached to the target gene.

An expression cassettes and the vectors derived from it may
 comprise further functional elements. The term functional element
 is to be understood in the broad sense and refers to all those
 20 elements which have an effect on the generation, amplification or
 function of the expression cassettes, vectors or transgenic
 organisms according to the invention. The following may be
 mentioned by way of example, but not by limitation:

- 25 a) Selection markers which confer a resistance to a metabolism
 inhibitor such as 2-deoxyglucose-6-phosphate (WO 98/45456),
 antibiotics or biocides, preferably herbicides, such as, for
 example, kanamycin, G 418, bleomycin or hygromycin, or else
 phosphinothricin and the like. Especially preferred selection
 30 markers are those which confer resistance to herbicides.
 Examples which may be mentioned are: DNA sequences which
 encode phosphinothricin acetyl transferases (PAT) and which
 inactivate glutamin synthase inhibitors (bar and pat genes),
 5-enolpyruvylshikimate-3-phosphate synthase genes (EPSP
 35 synthase genes), which confer resistance to Glyphosate®
 (N-(phosphonomethyl)glycine), the gox gene, which encodes
 Glyphosate®-degrading enzymes (Glyphosate oxidoreductase),
 the deh gene (encoding a dehalogenase which inactivates
 dalapon), sulfonylurea- and imidazolinone-inactivating
 40 acetolactate synthases, and bxn genes, which encode
 bromoxynil-degrading nitrilase enzymes, the aasa gene, which
 confers resistance to the antibiotic apectinomycin, the
 streptomycin phosphotransferase (SPT) gene, which allows
 resistance to streptomycin, the neomycin phosphotransferase
 45 (NPTII) gene, which confers resistance to kanamycin or
 geneticidin, the hygromycin phosphotransferase (HPT) gene,
 which mediates resistance to hygromycin, the acetolactate

synthase gene (ALS), which confers resistance to sulfonylurea herbicides (for example mutated ALS variants with, for example, the S4 and/or Hra mutation).

- 5 b) Reporter genes which encode readily quantifiable proteins and, via their color or enzyme activity, make possible an assessment of the transformation efficacy, the site of expression or the time of expression. Very especially preferred in this context are genes encoding reporter proteins (Schenborn E, Groskreutz D. Mol Biotechnol. 1999; 13(1):29-44) such as the green fluorescent protein (GFP) (Sheen et al. (1995) Plant Journal 8(5):777-784; Haseloff et al. (1997) Proc Natl Acad Sci USA 94(6):2122-2127; Reichel et al. (1996) Proc Natl Acad Sci USA 93(12):5888-5893; Tian et al. (1997) Plant Cell Rep 16:267-271; WO 97/41228; Chui WL et al. (1996) Curr Biol 6:325-330; Leffel SM et al. (1997) Biotechniques. 23(5):912-8), chloramphenicol transferase, a luciferase (Ow et al. (1986) Science 234:856-859; Millar et al. (1992) Plant Mol Biol Rep 10:324-414), the aequorin gene (Prasher et al. (1985) Biochem Biophys Res Commun 126(3):1259-1268), β -galactosidase, R locus gene (encoding a protein which regulates the production of anthocyanin pigments (red coloring) in plant tissue and thus makes possible the direct analysis of the promoter activity without addition of further auxiliary substances or chromogenic substrates; Dellaporta et al., In: Chromosome Structure and Function: Impact of New Concepts, 18th Stadler Genetics Symposium, 11:263-282, 1988), with β -glucuronidase being very especially preferred (Jefferson et al., EMBO J. 1987, 6, 3901-3907).
- c) Origins of replication, which ensure amplification of the expression cassettes or vectors according to the invention in, for example, E. coli. Examples which may be mentioned are ORI (origin of DNA replication), the pBR322 ori or the P15A ori (Sambrook et al.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).
- d) Elements which are necessary for Agrobacterium-mediated plant transformation, such as, for example, the right or left border of the T-DNA or the vir region.

To select cells which have successfully undergone homologous recombination, or else to select transformed cells, it is, as a rule, necessary additionally to introduce a selectable marker, which confers resistance to a biocide (for example herbicide), a

metabolism inhibitor such as 2-deoxyglucose-6-phosphate (WO 98/45456) or an antibiotic to the cells which have successfully undergone recombination. The selection marker permits the selection of the transformed cells from untransformed ones (McCormick et al. (1986) Plant Cell Reports 5:81-84).

The introduction of an expression cassette according to the invention into an organism or cells, tissues, organs, parts or seeds thereof (preferably into plants or plant cells, tissue, organs, parts or seeds) can be effected advantageously using vectors which comprise the expression cassettes. The expression cassette can be introduced into the vector (for example a plasmid) via a suitable restriction cleavage site. The plasmid formed is first introduced into *E. coli*. Correctly transformed *E. coli* are selected, grown, and the recombinant plasmid is obtained by the methods familiar to the skilled worker. Restriction analysis and sequencing may serve to verify the cloning step.

Examples of vectors may be plasmids, cosmids, phages, viruses or else agrobacteria. In an advantageous embodiment, the expression cassette is introduced by means of plasmid vectors. Preferred vectors are those which make possible stable integration of the expression cassette into the host genome.

The generation of a transformed organism (or of a transformed cell or tissue) requires introducing the DNA, RNA or protein in question into the relevant host cell.

A multiplicity of methods are available for this procedure, which is termed transformation (or transduction or transfection) (Keown et al. (1990) Methods in Enzymology 185:527-537). For example, the DNA or RNA can be introduced directly by microinjection or by bombardment with DNA-coated microparticles. Also, the cell can be permeabilized chemically, for example using polyethylene glycol, so that DNA can enter the cell by diffusion. The DNA can also be introduced by protoplast fusion with other DNA-containing units such as minicells, cells, lysosomes or liposomes. Another suitable method of introducing DNA is electroporation, where the cells are permeabilized reversibly by an electrical pulse. Suitable methods have been described (for example by Bilang et al. (1991) Gene 100:247-250; Scheid et al. (1991) Mol Gen Genet 228:104-112; Guerche et al. (1987) Plant Science 52:111-116; Neuhauser et al. (1987) Theor Appl Genet 75:30-36; Klein et al. (1987) Nature 327:70-73; Howell et al. (1980) Science 208:1265; Horsch et al. (1985) Science 227:1229-1231; DeBlock et al. (1989) Plant Physiology 91:694-701; Methods for Plant Molecular Biology

(Weissbach and Weissbach, eds.) Academic Press Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press Inc. (1989)).

- 5 In plants, the above-described methods of transforming and regenerating plants from plant tissues or plant cells are exploited for transient or stable transformation. Suitable methods are especially protoplast transformation by polyethylene-glycol-induced DNA uptake, the biolistic method with
10 the gene gun, what is known as the particle bombardment method, electroporation, incubation of dry embryos in DNA-containing solution, and microinjection.

- In addition to these "direct" transformation techniques,
15 transformation can also be effected by bacterial infection by means of *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. The *Agrobacterium*-mediated transformation is best suited to dicotyledonous plant cells. The methods are described, for example, by Horsch RB et al. (1985) Science 225: 1229f.

- 20 When *agrobacteria* are used, the expression cassette must be integrated into specific plasmids, either into a shuttle or intermediate vector, or into a binary vector. If a Ti or Ri plasmid is to be used for the transformation, at least the right
25 border, but in most cases the right and left border, of the Ti or Ri plasmid T-DNA is linked to the expression cassette to be introduced in the form of a flanking region.

- Binary vectors are preferably used. Binary vectors are capable of
30 replication both in *E. coli* and in *Agrobacterium*. As a rule, they comprise a selection marker gene and a linker or polylinker flanked by the right and left T-DNA border sequence. They can be transferred directly into *Agrobacterium* (Holsters et al. (1978) Mol Gen Genet 163:181-187). The selection marker gene permits the
35 selection of transformed *agrobacteria* and is, for example, the *nptII* gene, which confers resistance to kanamycin. The *Agrobacterium* which acts as host organism in this case should already contain a plasmid with the *vir* region. The latter is required for transferring the T-DNA to the plant cell. An
40 *Agrobacterium* transformed in this way can be used for transforming plant cells. The use of T-DNA for transforming plant cells has been studied and described intensively (EP 120 516; Hoekema, In: The Binary Plant Vector System, Offsetdrukkerij Kanthers B.V., Alblasterdam, Chapter V; An et al. (1985) EMBO J
45 4:277-287). Various binary vectors are known, some of which are

commercially available such as, for example, pBI101.2 or pBIN19 (Clontech Laboratories, Inc. USA).

Further promoters which are suitable for expression in plants have been described (Rogers et al. (1987) Meth in Enzymol 153:253-277; Schardl et al. (1987) Gene 61:1-11; Berger et al. (1989) Proc Natl Acad Sci USA 86:8402-8406).

Direct transformation techniques are suitable for any organism and cell type.

The plasmid used need not meet any particular requirements in the case of the injection or electroporation of DNA or RNA into plant cells. Simple plasmids such as those of the pUC series can be used. If complete plants are to be regenerated from the transformed cells, it is necessary for an additional selectable marker gene to be located on the plasmid.

Stably transformed cells, i.e. those which contain the introduced DNA integrated into the DNA of the host cell, can be selected from untransformed cells when a selectable marker is part of the DNA introduced. Examples of genes which can act as markers are all those which are capable of conferring resistance to antibiotics or herbicides (such as kanamycin, G 418, bleomycin, hygromycin or phosphinothricin) (see above). Transformed cells which express such marker genes are capable of surviving in the presence of concentrations of a corresponding antibiotic or herbicide which kill an untransformed wild type. Examples are mentioned above and preferably comprise the bar gene, which confers resistance to the herbicide phosphinothricin (Rathore KS et al. (1993) Plant Mol Biol 21(5):871-884), the nptII gene, which confers resistance to kanamycin, the hpt gene, which confers resistance to hygromycin, or the EPSP gene, which confers resistance to the herbicide Glyphosate. The selection marker permits the selection of transformed cells from untransformed cells (McCormick et al. (1986) Plant Cell Reports 5:81-84). The resulting plants can be bred and hybridized in the customary fashion. Two or more generations should be grown in order to ensure that the genomic integration is stable and hereditary.

The abovementioned methods are described, for example, in Jenes B et al. (1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by SD Kung and R Wu, Academic Press, pp. 128-143 and in Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225). The construct to be expressed is preferably cloned into a vector which is suitable

for the transformation of *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al. (1984) Nucl Acids Res 12:8711f).

As soon as a transformed plant cell has been generated, a
 5 complete plant can be obtained using methods known to the skilled worker. For example, callus cultures are used as starting material. The development of shoot and root can be induced in this as yet undifferentiated cell biomass in a known fashion. The shoots obtained can be planted out and bred.

10 The skilled worker is familiar with such methods of regenerating plant parts and intact plants from plant cells. Methods to do so are described, for example, by Fennell et al. (1992) Plant Cell Rep. 11: 567-570; Stoeger et al (1995) Plant Cell
 15 Rep. 14:273-278; Jahne et al. (1994) Theor Appl Genet 89:525-533.

The method according to the invention can advantageously be combined with further methods which bring about pathogen resistance (for example to insects, fungi, bacteria, nematodes
 20 and the like), stress resistance or another improvement of the plant properties. Examples are mentioned, inter alia, by Dunwell JM, Transgenic approaches to crop improvement, J Exp Bot. 2000;51 Spec No; pages 487-96.

25 The invention furthermore relates to the barley RacB protein as shown in SEQ ID NO: 2, and to dominant-negative variant thereof, for example described by SEQ ID NO: 7.

The invention furthermore relates to nucleic acid sequences
 30 encoding the barley RacB protein, preferably the nucleic acid sequence as shown in SEQ ID NO: 1, the nucleic acid sequence complementary thereto, and the sequences derived owing to degeneracy of the genetic code.

35 The invention furthermore relates to the polypeptide encoding functional equivalents of the RacB protein from barley as shown in SEQ ID NO: 35, 37 or 39.

The invention furthermore relates to nucleic acid sequences
 40 encoding functional equivalents of the RacB protein from barley, preferably the nucleic acid sequence as shown in SEQ ID NO: 34, 36 or 38, the nucleic acid sequence complementary thereto and the sequences derived by degeneration of the genetic code.

45 The invention furthermore relates to transgenic expression cassettes comprising one of the nucleic acid sequences according to the invention. In the transgenic expression cassettes

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according to the invention, the nucleic acid sequence encoding the barley RacB protein is linked to at least one genetic control element as defined above in such a manner that it is capable of expression (transcription and, if appropriate, translation) in
5 any organism, preferably in plants. Suitable genetic control elements are described above. The transgenic expression cassettes may also comprise further functional elements in accordance with the above definition. The inserted nucleic acid sequence encoding a barley RacB protein can be inserted in the expression cassette
10 in sense or antisense orientation and thus lead to the expression of sense or antisense RNA. Transgenic vectors comprising the transgenic expression cassettes are also in accordance with the invention.

15 "Transgenic", for example regarding a nucleic acid sequence, an expression cassette or a vector comprising said nucleic acid sequence or an organism transformed with said nucleic acid sequence, expression cassette or vector, refers to all those constructs originating by recombinant methods in which either
20 a) the RacB nucleic acid sequence, or

b) a genetic control sequence linked operably to the RacB nucleic acid sequence, for example a promoter, or
25 c) (a) and (b)

are not located in their natural genetic environment or have been modified by recombinant methods, an example of a modification
30 being a substitutions, additions, deletions, inversion or insertions of one or more nucleotide residues. Natural genetic environment refers to the natural chromosomal locus in the organism of origin, or to the presence in a genomic library. In the case of a genomic library, the natural genetic environment of
35 the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least at one side and has a sequence of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, very especially preferably at least 5000 bp, in length. A naturally
40 occurring expression cassette - for example the naturally occurring combination of the RacB promoter with the corresponding RacB gene - becomes a transgenic expression cassette when it is modified by non-natural, synthetic "artificial" methods such as, for example, mutagenization. Such methods have been described
45 (US 5,565,350; WO 00/15815; also see above).

The invention also relates to transgenic organisms transformed with at least one of the nucleic acid sequences according to the invention, expression cassette according to the invention or vector according to the invention, and to cells, cell cultures, tissues, parts - such as, for example, leaves, roots and the like in the case of plant organisms - or propagation material derived from such organisms. The term organism is to be understood in the broad sense and refers to prokaryotic and eukaryotic organisms, preferably bacteria, yeasts, fungi, animal organisms and plant organisms.

The following are preferred:

- a) fungi such as *Aspergillus*, *Eremothecium*, *Trichoderma*, *Ashbya*, *Neurospora*, *Fusarium*, *Beauveria* or other fungi described in Indian Chem Eng. Section B. Vol 37, No. 1,2 (1995) on page 15, Table 6. Especially preferred is the filamentous hemiascomycete *Ashbya gossypii* or *Eremothecium ashbyii*,
 - b) yeasts such as *Candida*, *Saccharomyces*, *Hansenula* or *Pichia*, with *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178) being especially preferred,
 - c) plants in accordance with the above definition of "plants",
 - d) vertebrates and invertebrates. Especially preferred vertebrates are nonhuman mammals such as in dog, cat, sheep, goat, chicken, mouse, rat, cattle or horse. Preferred animal cells comprise CHO, COS and HEK293 cells. Preferred invertebrates comprise insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 or Sf21 cells,
 - e) prokaryotic organisms such as Gram-positive or Gram-negative bacteria such as *Acetobacter*, *Gluconobacter*, *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Clostridium*, *Cyanobacter*, *Escherichia* (mainly *Escherichia coli*), *Serratia*, *Staphylococcus*, *Aerobacter*, *Alcaligenes*, *Penicillium* or *Klebsiella*.
- Host or starting organisms which are preferred as transgenic organisms are mainly plants in accordance with the above definition. Included within the scope of the invention are all genera and species of higher and lower plants of the Plant Kingdom. Furthermore included are the mature plants, seed, shoots and seedlings, and parts, propagation material and cultures derived therefrom, for example cell cultures. Mature plants refers to plants at any developmental stage beyond that of the

seedling. The term seedling refers to a young immature plant in an early developmental stage. Plants preferred as host organisms are in particular plants which can be used for the process according to the invention to obtain a pathogen resistance according to the criteria mentioned above. Very particularly preferred are monocotyledonous plants, such as wheat, oats, millet, barley, rye, maize, rice, buckwheat, sorghum, triticale, spelt, linseed, sugar cane, as dicotyledonous crop plants, such as oil seed rape, canola, cress, Arabidopsis, cabbages, soya, alfalfa, pea, beans, peanut, potato, tobacco, tomato, eggplant, bell pepper, sunflower, Tagetes, lettuce, Calendula, melon, pumpkin/squash or zucchini.

The transgenic organisms can be generated with the above-described methods for the transformation or transfection of organisms.

The invention furthermore relates to the use of the transgenic organisms according to the invention and of the cells, cell cultures, parts - such as, for example, roots, leaves and the like in the case of transgenic plant organisms - derived from them, and to transgenic propagation material such as seeds or fruits, for the production of foodstuffs or feeding stuffs, pharmaceuticals or fine chemicals.

Furthermore preferred is a method for the recombinant production of pharmaceuticals or fine chemicals in host organisms, wherein a host organism is transformed with one of the above-described expression cassettes and this expression cassette comprises one or more structural genes which encode the desired fine chemical or catalyze the biosynthesis of the desired fine chemical, the transformed host organism is cultured, and the desired fine chemical is isolated from the culture medium. This method can be applied widely to fine chemicals such as enzymes, vitamins, amino acids, sugars, fatty acids, and natural and synthetic flavorings, aroma substances and colorants. Especially preferred is the production of tocopherols and tocotrienols and carotenoids. The transformed host organisms are cultured and the products are isolated from the host organisms or the culture medium by methods known to the skilled worker. The production of pharmaceuticals such as, for example, antibodies or vaccines, is described by Hood EE, Jilka JM. (1999) Curr Opin Biotechnol. 10(4):382-6; Ma JK, Vine ND. (1999) Curr Top Microbiol Immunol. 236:275-92.

Sequences

1. SEQ ID NO: 1 : Nucleic acid sequence encoding the barley
(Hordeum vulgare) RacB protein.
- 5
2. SEQ ID NO: 2 : Amino acid sequence encoding the barley
(Hordeum vulgare) RacB protein.
- 10
3. SEQ ID NO: 3 : Nucleic acid sequence encoding the rice
(Oryza sativa) RacB protein.
4. SEQ ID NO: 4 : Amino acid sequence encoding the rice
(Oryza sativa) RacB protein.
- 15
5. SEQ ID NO: 5 : Nucleic acid sequence encoding the maize
(Zea mays) RacB protein.
6. SEQ ID NO: 6 : Amino acid sequence encoding the maize
(Zea mays) RacB protein.
- 20
7. SEQ ID NO: 7 : Amino acid sequence encoding a
dominant-negative variant of the RacB protein
(Hordeum vulgare).
- 25
8. SEQ ID NO: 8 : Amino acid sequence encoding a
dominant-negative variant of the rice
(Oryza sativa) RacB protein.
9. SEQ ID NO: 9 : Amino acid sequence encoding a
dominant-negative variant of the maize
(Zea mays) RacB protein.
- 30
10. SEQ ID NO: 10 Oligonucleotide primer ONP-1
5'-GGATCCGATGAGCGCGTCCAGGTT-3'
- 35
11. SEQ ID NO: 11 Oligonucleotide primer ONP-2
5'-GTCGACCTTCGCCCTTGTTCTTTGTC-3'
12. SEQ ID NO: 12 RACE-RacB primer
5'-gtgggcacatagtcggtggggaaggt-3'
- 40
13. SEQ ID NO: 13 GeneRacer™ 5' primer:
5'-CGACTGGAGCACGAGGACACTGA-3
- 45
14. SEQ ID NO: 14 GeneRacer™ 5' nested primer:
5'-GGACACTGACATGGACTGAAGGAGTA-3

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15. SEQ ID NO: 15 RacB sense primer
5'-gttcatcaagtgcgtcaccgtg-3'
- 5 16. SEQ ID NO: 16 RacB antisense primer
5'-ttagcttcctcagttcttccctg-3'
17. SEQ ID NO: 17 BAS sense primer
5'-cgcgccgcagccgagtagc-3'
- 10 18. SEQ ID NO: 18 BAS antisense primer
5'-gtcacaaaaacacatgtaacc-3'
19. SEQ ID NO: 19 OXLP sense primer
5'-ggccgacatgcattcaccag-3'
- 15 20. SEQ ID NO: 20 OXLP antisense primer
5'-catctgatattgctgggtctg-3'
21. SEQ ID NO: 21 UBI sense primer
5'-ccaagatgcagatcttcgtga-3'
- 20 22. SEQ ID NO: 22 UBI antisense primer
5'-ttcgcgataggtaaaagagca-3'
23. SEQ ID NO: 23 M13 fwd primer
5'-GTAAACGACGGCCAGTG-3'
24. SEQ ID NO: 24 M13 rev primer
5'-GGAAACAGCTATGACCATG-3'
- 30 25. SEQ ID NO: 25 HvRop6 LEFT PRIMER
5'-GTGGAGGCGCGGCGAGA-3'
26. SEQ ID NO: 26 HvRop6 RIGHT PRIMER
5'-CCATGCTTCATCTCCATAGTCA-3'
- 35 27. SEQ ID NO: 27 HvRacD LEFT PRIMER
5'-ggatccCGATTCCATCAGGAAAGCAT-3'
28. SEQ ID NO: 28 HvRacD RIGHT PRIMER
5'-gtcgacGCGAGACACTGCAAAACAAA-3'
- 40 29. SEQ ID NO: 29 HvRop4 LEFT PRIMER
5'-GGATCCttctcgtccatttagccggc-3'
- 45

30. SEQ ID NO: 30 HvRop4 RIGHT PRIMER
5'-GTCGACTgatcacttgaagcatgccag-3'
31. SEQ ID NO: 31 RacB5'BamHI Primer
5'-GGATCCGATGAGCGCGTCCAGGTT-3'
32. SEQ ID NO: 32 RacB3'SalI Primer
5'-GTCGACCTTCGCCCTTGTTCTTTGTC-3'
33. SEQ ID NO: 33 V15 mutagenesis Primer
5'-ACCGTGGGGGACGTCGCCGTCGGCAAGAC-3'
34. SEQ ID NO: 34 : Nucleic acid sequence encoding the RacB
homolog HvRop6 from barley (*Hordeum vulgare*).
35. SEQ ID NO: 35 : Amino acid sequence encoding the RacB
homolog HvRop6 from barley (*Hordeum vulgare*).
36. SEQ ID NO: 36 : Nucleic acid sequence encoding the RacB
homolog HvRacD from barley (*Hordeum vulgare*).
37. SEQ ID NO: 37 : Amino acid sequence encoding the RacB
homolog HvRacD from barley (*Hordeum vulgare*).
38. SEQ ID NO: 38 : Nucleic acid sequence encoding the RacB
homolog HvRop4 from barley (*Hordeum vulgare*).
39. SEQ ID NO: 39 : Nucleic acid sequence encoding the RacB
homolog HvRop4 from barley (*Hordeum vulgare*).
40. SEQ ID NO: 40 : Nucleic acid sequence encoding the RacB
homolog Zea mays ROP6 (GenBank Acc.-No.:
AJ278665)
41. SEQ ID NO: 41 Amino acid sequence encoding the RacB
homolog Zea mays ROP6
42. SEQ ID NO: 42 Nucleic acid sequence encoding the RacB
homolog *Oryza sativa* subsp. *japonica* RACDP
(RACD) (GenBank Acc.-No.: AF218381)
43. SEQ ID NO: 43 Amino acid sequence encoding the RacB
homolog *Oryza sativa* subsp. *japonica* RACDP

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44. SEQ ID NO: 44 Nucleic acid sequence encoding the RacB
homolog *Oryza sativa* ROP4 (GenBank Acc.-No.:
AF380335)
- 5 45. SEQ ID NO: 45 Amino acid sequence encoding the RacB
homolog *Oryza sativa* ROP4
46. SEQ ID NO: 46 Nucleic acid sequence encoding the RacB
homolog *Zea mays* RACA (GenBank Acc.-No.:
AF126052)
- 10 47. SEQ ID NO: 47 Amino acid sequence encoding the RacB
homolog *Zea mays* RACA
- 15 48. SEQ ID NO: 48 Nucleic acid sequence encoding an RacB
homolog from *Hordeum vulgare* (GenBank Acc.-
No.: BM816965)
49. SEQ ID NO: 49 Nucleic acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At3g51300)
- 20 50. SEQ ID NO: 50 Amino acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At3g51300)
- 25 51. SEQ ID NO: 51 Nucleic acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At2g17800)
52. SEQ ID NO: 52 Amino acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At2g17800)
- 30 53. SEQ ID NO: 53 Nucleic acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At4g35950)
54. SEQ ID NO: 54 Amino acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At4g35950)
- 35 55. SEQ ID NO: 55 Nucleic acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At1g75840)
- 40 56. SEQ ID NO: 56 Amino acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At1g75840)
57. SEQ ID NO: 57 Nucleic acid sequence encoding an RacB
homolog from *Arabidopsis thaliana* (At4g35020)
- 45

70

58. SEQ ID NO: 58 Amino acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At4g35020)
59. SEQ ID NO: 59 Nucleic acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At1g20090)
60. SEQ ID NO: 60 Amino acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At1g20090)
61. SEQ ID NO: 61 Nucleic acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At5g45970)
62. SEQ ID NO: 62 Amino acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At5g45970)
63. SEQ ID NO: 63 Nucleic acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At3g48040)
64. SEQ ID NO: 64 Amino acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At3g48040)
65. SEQ ID NO: 65 Nucleic acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At5g62880)
66. SEQ ID NO: 66 Amino acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At5g62880)
67. SEQ ID NO: 67 Nucleic acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At4g28950)
68. SEQ ID NO: 68 Amino acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At4g28950)
69. SEQ ID NO: 79 Nucleic acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At2g44690)
70. SEQ ID NO: 70 Amino acid sequence encoding an RacB
homolog from Arabidopsis thaliana (At2g44690)
71. SEQ ID NO: 71 Oligonucleotide primer Fra 186
5'-ATGAGCGCGTCCAGGTTTCATA-3'
72. SEQ ID NO: 72 Oligonucleotide primer Fra 187
5'-ATCAAACACGCCCTTCACGTT-3'

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73. SEQ ID NO: 73 Transgenic expression vector
pSUN3NIT_AtRacB_s for expression of
Arbidopsis thaliana RacB in sense
orientation
74. SEQ ID NO: 74 Transgenic expression vector
pSUN3NIT_AtRacB_as for expression of
Arbidopsis thaliana RacB in antisense
orientation
75. SEQ ID NO: 75 Transgenic expression vector
pSUN3NIT_HvRacB_s for expression of a
barley RacB fragment in sense
orientation
76. SEQ ID NO: 76 Transgenic expression vector
pSUN3NIT_HvRacB_as for expression of a
barley RacB fragment in antisense
orientation

Figures

1. Fig. 1: Alignment of the amino acid sequences of barley
RacB, rice RacB, maize RacB, and human Rac1 and Rac2
proteins.

Regions shaded in gray show the position of the G1 element
(GXXXXGKS/T; amino acid 13 to 20), of the G2 effector region
(amino acid 29 to 45), of the G3 element (LWDTAGQ; amino acid
58 to 64), of the G4 element (TKXD; amino acid 118 to 121),
of the G5 element (EXS) and of the C-terminal isoprenylation
motif (CXXX, Hassanain HH et al. (2000) Biochem Biophys Res
Commun. 272(3):783-8.). Hyphens indicate sequence gaps.
Asterisks denote amino acids which are identical in all
homologs. Amino acids which differ between barley on the one
hand and maize and rice on the other hand are shown white
against black. The position which is advantageously modified
to obtain a dominant-negative RacB variant is marked by a
black triangle above the sequence.

2. Fig. 2: Expression of RacB in epidermal tissue

RT-PCR of RNA from the barley lines Pallas and BCPM1a12 (P10)
24 h post-inoculation ("hai" hours after inoculation) with
BgHA6. To extract the RNA, strips of the abaxial epidermis
(E, from inoculated locations of leaves) were removed from
the mesophyll and the adaxial epidermis (M). Ubiquitin 1

(Ubi) acted as marker for tissue-unspecific expression, OXLP as positive control for gene expression in the epidermis, and Bas as positive control for gene expression in mesophyll cells. The RT-PCR was carried out over 25 amplification cycles as described hereinbelow. RT-PCR products were denatured in the gel, blotted and detected by means of antisense RNA probes under stringent conditions.

3. Fig. 3: *RacB* is expressed constitutively in various resistant barley lines.

RNA was isolated from the variety Ingrid (*Mlo*, *Ror1*, *Bgh*-susceptible), BCIngrid-*mlo5* (*mlo5*, *Ror1*, *Bgh*-resistant) and A89 (*mlo5*, *ror1*, moderately susceptible to *BghA6*) immediately prior to inoculation (0 Ø) or 8, 15 or 24 h post-inoculation with *Bgh* and 24 h thereafter from noninoculated control plants (24 Ø). Ubiquitin 1 (*Ubi*) was used as marker for constitutive expression, OXLP as positive control for *Bgh*-induced gene expression in the epidermal layer. OXLP expression was detected via Northern blot. The RT-PCR for *RacB* and *Ubi* was carried out as described over 25 amplification cycles. The PCR products were denatured in the gel, blotted and detected by means of antisense RNA samples under stringent conditions.

4. Fig. 4: "RNA interference" with *RacB*-dsRNA reduces the penetration efficacy of barley powdery mildew *BghA6* in barley.

The relative penetration efficacy (RPE) was determined in six individual experiments with inoculation with *Bgh* from barley cv. Pallas. The RPE was calculated as the difference between the penetration efficacy of cells transformed with *RacB*-dsRNA and the penetration efficacy of cells transformed with control dsRNA (here: average penetration efficacy 57%). The RPE percentage (%-RPE) is calculated from the RPE minus 1, multiplied by 100.

$$\text{RPE} = \frac{[\text{PE of cells transformed with RacB-dsRNA}]}{[\text{PE of cells transformed with control dsRNA}]}$$

$$\text{\%-RPE} = 100 * (\text{RPE}-1)$$

The black columns represent the %-RPE upon evaluation of at least 100 interaction sites for in each case one independent experiment. The white column represents the average %-RPE of

the experiments with the RacB-dsRNA ("RACB-dsRNA"). The error bar indicates the standard error.

"control" represents the parallel experiments with a control dsRNA.

In cells which had been bombarded with RacB-dsRNA, the %RPE was markedly reduced in comparison with cells which had been bombarded with a control dsRNA (TR: human thyroid receptor dsRNA).

5. Fig 5: Effect of the genetic background on RacB function

The %RPE was studied in 5 independent experiments by inoculating barley cv. Pallas, Ingrid or A89, which had previously been transformed with RacB-dsRNA, with BghA6.

The %RPE is markedly reduced in Pallas (*Mlo Ror1*, black bars, experiments 1 and 2) or Ingrid (*Mlo Ror1*, black bars, experiments 3, 4 and 5). The %RPE of the susceptible mutant A89 (*mlo5 ror1*, black bars, experiments 1 to 5), however, was not reduced. White bars indicate the mean, error bars the standard error.

6. Fig.6: Overexpression of a constitutively active RacB mutant in barley cv. Pallas

A constitutively active mutant of barley RACB (exchange G->V in position 15; RacB-V15) was transiently overexpressed in the barley variety Pallas in 5 independent experiments using the expression construct pGY-RacBV15. For comparison, corresponding experiments were carried out using the vector alone, without RacB insert (pGY).

The expression of a constitutive RacB mutant results in significantly higher susceptibility to pathogen attack by mildew of barley, compared to the controls (Fig. 6-A). In all cases, the relative susceptibility to the fungal pathogen is increased (Fig. 6-B). These results, too, demonstrate the key function of racB in the defense of pathogens.

7. Fig. 7: Plasmid map for expression vector pGY-1 (Schweizer P et al. (1999) Mol Plant Microbe Interact 12: 647-54; Shinshi H et al. (1990) Plant Mol Biol 14:357-368).

Examples

General methods:

- 5 The chemical synthesis of oligonucleotides can be effected, for example, in the known fashion using the phosphoramidite method (Voet, Voet, 2nd Edition, Wiley Press New York, pages 896-897). The cloning steps carried out for the purposes of the present invention such as, for example, restriction cleavages, agarose
- 10 gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking DNA fragments, transformation of *E. coli* cells, growing bacteria, multiplying phages and sequence analysis of recombinant DNA, are carried out as described by Sambrook et al. (1989) Cold Spring
- 15 Harbor Laboratory Press; ISBN 0-87969-309-6. The sequencing of recombinant DNA molecules is carried out with an MWG-Licor laser fluorescence DNA sequencer following the method of Sanger (Sanger et al. (1977) Proc Natl Acad Sci USA 74:5463-5467).

20 Example 1: Plants, pathogens and inoculation

The barley variety Ingrid is from James McKey, University of Uppsala, Sweden. The variety Pallas and backcrossed line BCIngrid-mlo5 was donated by Lisa Munk, Department of Plant

- 25 Pathology, Royal Veterinary and Agricultural University, Copenhagen, Denmark. Its production has been described (Kølster P et al. (1986) Crop Sci 26: 903-907). Line A89 was provided by Paul Schulze-Lefert (Max-Plank-Institut für Züchtungsforschung, Cologne, Germany).

30

Unless otherwise specified, the seed which had been pregerminated on moist filter paper for 12 to 36 hours in the dark was sown along the edge of a square pot (8 x 8 cm; 5 kernels per pot) in Fruhstorfer soil, type P, covered with soil and watered regularly

- 35 with tap water. All of the plants were cultured in controlled-environment cabinets or chambers for 5 to 8 days at 16 to 18°C, 50 to 60% relative atmospheric humidity and a 16-hr-light/8-hr-dark rhythm at 3000 or 5000 lux (photon flow density 50 and 60 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, respectively) and used in the
- 40 experiments during the seedling stage. In experiments in which applications to the primary leaves were carried out, the latter were developed fully.

Before the transient transfection experiments were carried out,

- 45 the plants were grown in controlled-environment cabinets or chambers at a daytime temperature of 24°C, a nighttime temperature

of 20°C, 50 to 60% relative atmospheric humidity and a 16-hr light/8-hr-dark rhythm at 30 000 lux.

- Barley powdery mildew *Blumeria graminis* (DC) Speer f.sp. *hordei*
- 5 Em. Marchal race A6 (Wiberg A (1974) Hereditas 77: 89-148) (BghA6) was used for the inoculation of barley plants. The fungus was provided by the Department of Biometry, JLU Gießen. Inoculum was grown in controlled-environment cabinets under identical conditions to those described above for the plants by
- 10 transferring the conidia of infected plant material at a rate of 100 conidia/mm² to 7-day-old barley plants cv. Golden Promise, which were grown regularly.

- The inoculation with BghA6 was carried out using 7-day-old
- 15 seedlings by shaking off the conidia of already infected plants in an inoculation tower at a rate of approx. 100 conidia/mm² (unless otherwise specified).

Example 2: RNA extraction

20

Total RNA was extracted from 8 to 10 primary leaf segments (length 5 cm) by means of "RNA extraction buffer" (AGS, Heidelberg, Germany).

- 25 To this end, the central primary leaf segments 5 cm in length were harvested and homogenized in liquid nitrogen in mortars. The homogenate was stored at -70°C until the RNA was extracted.

- Total RNA was extracted from the deep-frozen leaf material with
- 30 the aid of an RNA extraction kit (AGS, Heidelberg). To this end, 200 mg of the deep-frozen leaf material were covered with 1.7 ml RNA extraction buffer (AGS) in a microcentrifuge tube (2 ml) and immediately mixed thoroughly. After addition of 200 µl of chloroform, the mixture was again mixed thoroughly and shaken for
- 35 45 minutes on a horizontal shaker at 200 rpm at room temperature. To separate the phases, the tubes were subsequently centrifuged for 15 minutes at 20 000 g and 4°C, and the upper, aqueous phase was transferred into a fresh microcentrifuge tube, while the bottom phase was discarded. The aqueous phase was repurified with
- 40 900 µl of chloroform by homogenizing for 10 seconds and recentrifuging (see above) and removing the aqueous phase (3 times). Then, 850 µl of 2-propanol were added and the mixture was homogenized and placed on ice for 30 to 60 minutes in order to precipitate the RNA. Thereafter, the mixture was centrifuged
- 45 for 20 minutes (see above), the supernatant was carefully decanted off, 2 ml of 70% strength ethanol (-20°C) were pipetted in, and the mixture was mixed and recentrifuged for 10 minutes.

Then, the supernatant was again decanted off, and the pellet was carefully freed from residual fluid, using a pipette, and then dried in a stream of clean air in a clean bench. Then, the RNA was dissolved in 50 μ l of DEPC water on ice, mixed and centrifuged for 5 minutes (see above). 40 μ l of the supernatant, constituting the RNA solution, were transferred into a fresh microcentrifuge tube and stored at -70°C .

The RNA concentration was determined photometrically. To this end, the RNA solution was diluted 1:99 (v/v) with distilled water, and the absorption was measured at 260 nm (Beckman Photometer DU 7400); ($E_{260\text{ nm}} = 1$ at 40 μg RNA/ml). The concentrations of the RNA solutions were subsequently adjusted to 1 $\mu\text{g}/\mu\text{l}$ with DEPC water to match the calculated RNA contents and verified in an agarose gel.

To verify the RNA concentrations in a horizontal agarose gel (1% agarose in 1 x MOPS buffer with 0.2 $\mu\text{g}/\text{ml}$ ethidium bromide), 1 μl of RNA solution was treated with 1 μl of 10 x MOPS, 1 μl of color marker and 7 μl of DEPC water, separated according to size in 1 x MOPS running buffer over 1.5 hours at a voltage of 120 V in the gel, and photographed under UV light. Any differences in concentration of the RNA extracts were adjusted with DEPC water, and the adjustment was rechecked in the gel.

25

Example 3: Cloning the barley RacB cDNA sequence

The cDNA fragments required for isolating the HvRacB cDNA, cloning it, sequencing it and generating probes were obtained by means of RT-PCR using the "One Step RT-PCR Kit" (Life Technologies, Karlsruhe, Germany or Qiagen, Hilden, Germany). To this end, total RNA from barley seedlings was used as template. The RNA was isolated from Pallas 3, 5 and 7 days after germination. Moreover, RNA was isolated from Pallas and the backcrossed lines with *mlo5*, *Mlg* or *Mla12* 1, 2 and 5 days after inoculation with *BghA6* on day 7 after germination. The following primers are used for the RT-PCR:

ONP-1 5'-GGATCCGATGAGCGCGTCCAGGTT-3' (SEQ ID NO: 10) and

ONP-2 5'-GTCGACCTTCGCCCTTGTTCTTTGTC-3' (SEQ ID NO: 11)

1000 ng of total RNA, 0.4 mM dNTPs, in each case 0.6 mM OPN-1 and OPN-2 primer, 10 μl of RNase inhibitor and 1 μl of enzyme mix in 1x RT buffer (One Step RT-PCR Kit, Qiagen, Hilden) were employed for each reaction (25 μl batch).

The following temperature program is used (PTC-100TM model 96V; MJ Research, Inc., Watertown, Massachusetts):

- 1 cycle of 30 min at 50°C
- 5 1 cycle of 150 sec at 94°C
- 30 cycles of 45 sec at 94°C, 1 min at 55°C and 2 min at 72°C
- 1 cycle of 7 min at 72°C

- 10 The PCR product was separated by 2% w/v agarose gel electrophoresis. This gave an RT-PCR product of in total 642 bp which was composed of the RacB sequence (SEQ ID NO: 1) and terminal sequences encoding restriction endonuclease restriction sites. The fragment encodes a 591 bp open reading frame encoding
- 15 a polypeptide of 197 amino acids. The corresponding cDNA was isolated from an agarose gel and cloned into vector pGEM-T (Promega, Mannheim, Germany) by means of T-overhang ligation. The cDNAs were sequenced starting from the plasmid DNA using the "Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing
- 20 Kit" (Amersham, Freiburg, Germany).

- Since a primer has been deduced from the rice RacB sequence as starting primer OPN-1 (GenBank Acc. No.: AF250327), this region (i.e. the 5'-end) of the barley RacB cDNA was reverified by means
- 25 of RACE technology using the "GeneRacer Kit" (INVITROGENE Life Technologies). To this end, 100 ng of poly-A mRNA, 1 µl of 10xCIP buffer, 10 units of RNase inhibitor, 10 units of CIP ("calf intestinal phosphatase") and DEPC-treated water were treated for 1 hour at 50°C in a total volume of 10 µl. To precipitate the RNA,
- 30 a further 90 µl of DEPC water and 100 µl of phenol:chloroform were added and the mixture was mixed thoroughly for approximately 30 seconds. After centrifugation for 5 minutes at 20 000 g, the top phase was treated with 2 µl of 10 mg/ml mussel glycogen, 10 µl of 3 M sodium acetate (pH 5.2) in a fresh micro reaction vessel.
- 35 220 µl of 95% ethanol were added and the mixture was incubated on ice. The RNA was subsequently precipitated by centrifugation for 20 minutes at 20 000 g and 4°C. The supernatant was discarded, 500 µl of 75% ethanol were added, and the mixture was vortexed briefly and recentrifuged for 2 minutes (20 000 g). Again, the
- 40 supernatant was discarded, and the precipitate was dried in the air for 2 minutes at room temperature and subsequently suspended in 6 µl of DEPC water. mRNA CAP structures were removed by adding 1 µl of 10x TAP buffer, 10 units of RNasin and 1 unit of TAP (tobacco acid pyrophosphatase). The mixture was incubated for 1 h
- 45 at 37°C and subsequently cooled on ice. Again, the RNA was precipitated as described above and transferred into a reaction vessel with 0.25 µg of GeneRacer oligonucleotide primer. The

oligonucleotide primer was resuspended in the RNA solution, and the mixture was incubated for 5 minutes at 70°C and then ice-cooled. 1 µl of 10x ligase buffer, 10 mM ATP, 1 unit of RNAsin and 5 units of T4 RNA ligase were added, and the batch was

5 incubated for 1 h at 37°C. Again, the RNA was precipitated as described above and resuspended in 13 µl of DEPC water. 10 pmol of oligo-dT primer were added to the RNA, and the mixture was immediately heated at 70°C and again cooled on ice. 1 µl of each dNTP solution (25 mM), 2 µl of 10x RT buffer, 5 u (1 µl) of AMV

10 reverse transcriptase and 20 units of RNAsin were added, and the reaction solution was incubated for 1 hour at 42°C and subsequently for 15 minutes at 85°C. The first-strand cDNA thus prepared was stored at -20°C.

15 The following primer was used to amplify the 5'-cDNA ends:

RACE RacB primer:

5'-gtgggcacatagtcggtggggaaggt-3' (SEQ ID NO: 12)

20 GeneRacer™ 5'-primer:

5'-CGACTGGAGCACGAGGACACTGA-3 (SEQ ID NO: 13)

GeneRacer™ 5'-nested primer:

5'-GGACACTGACATGGACTGAAGGAGTA-3 (SEQ ID NO: 14)

25

The batch (total volume 25 µl) was composed as follows:

1 µl primer RACE-RacB (5 pmol/µl),
0.5 µl GeneRacer 5'-primer (10 pmol/µl)

30 2.5 µl 10x buffer Qiagen,
2.5 µl dNTPs (2mM)
0.5 µl cDNA
0.2 µl QiagenTAG (5u/µl)
17.8 µl H2O

35

The PCR conditions were:

	94°C	denaturation for 5 minutes
40	5 cycles of	30 seconds at 70°C (annealing), 1 min at 72°C (extension), 30 seconds at 94°C (denaturation)
45	5 cycles of	30 seconds at 68°C (annealing), 1 min at 72°C (extension), 30 seconds at 94°C (denaturation)

79

- 28 cycles of 30 seconds at 66°C (annealing),
1 min at 72°C (extension),
30 seconds at 94°C (denaturation)
- 5 72°C final extension for 10 minutes
4°C cooling until further use

The PCR gave a product of approx. 400 bp product. Starting from this product, a nested PCR with the RacB-specific oligonucleotide
10 primer and the "GeneRacer nested 5'-primer" was carried out:

- 94°C denaturation for 5 minutes
- 15 30 cycles of 30 sec at 64°C (annealing),
1 min at 72°C (extension),
30 sec at 94°C (denaturation)
- 72°C final extension for 10 minutes
4°C cooling until further use

20

The PCR product obtained was isolated via a gel, extracted from the gel, cloned into pGEM-T by means of T-overhang ligation, and sequenced. The sequence in the region of the primer OPN-1 was absolutely identical to the sequence of rice racB, so that no
25 point mutations could be generated by means of primers. Thus, the sequence shown under SEQ ID NO: 1 is identical to the barley RacB sequence.

Example 4: Reverse transcription polymerase chain reaction
30 (RT-PCR)

The "One Step RT-PCR Kit" (Qiagen, Hilden, Germany) was used for the semi-quantitative RT-PCR. In doing so, RNA (prepared as above) was first translated into cDNA (reverse transcription) and
35 the sought cDNA was amplified in a subsequent PCR reaction using specific primers. To estimate the initial amount of template RNA, the amplification was interrupted during the exponential phase in order to reflect differences in the target RNA. The PCR products were separated by means of an agarose gel, denatured, blotted
40 onto nylon membranes, and detected with specific non-radiolabeled probes under stringent standard conditions. Hybridization, wash steps and immunodetection were carried out as described under "Northern blot".

45

The following components were combined for the individual reactions (25 µl batch) using the "One Step RT-PCR Kit" (Qiagen, Hilden, Germany):

- 5 1000 ng total RNA of a specific sample
- 0.4 mM dNTPs
- 0.6 µM of each *sense and antisense* primer
- 0.1 µl RNase inhibitor
- 1 µl enzyme mix in 1x RT buffer

10

cDNA synthesis (reverse transcription) was carried out for 30 minutes at 50°C. The reverse transcriptase was subsequently inactivated for 15 minutes at 95°C, which simultaneously causes activation of DNA polymerase and denaturation of cDNA. A PCR was

15 subsequently carried out with the following program:

- denaturation for 1 minute at 94°C
- 25 cycles of 1 minute at 54°C primer annealing
- 1 minute at 72°C primer extension
- 20 10 minutes at 72°C completion of the DNA
- duplexes
- then: termination of the reaction at 4°C

The PCR products were separated in a 1x TBE agarose gel using

25 ethidium bromide.

The following oligonucleotide primer pairs were used for the amplifications in the individual batches:

- 30 a) amplification of a 387 bp fragment of the barley RacB cDNA

RacB sense 5'-gttcatcaagtgcgtcaccgtg-3' (SEQ ID NO: 15)
 RacB antisense 5'-ttagcttcctcagttcttcctg-3' (SEQ ID NO: 16)

- 35 b) amplification of a 674 bp fragment of barley BAS cDNA
 (GenBank Acc. No. Z34917)

BAS sense 5'-cgcgccgcagccgagtagc-3' (SEQ ID NO: 17)
 BAS antisense 5'-gtcacaaaaacacatgtaacc-3' (SEQ ID NO: 18)

40

- c) amplification of a 506 bp OXLP cDNA fragment (GenBank Acc. No. X93171)

- OXLP sense 5'-ggccgacatgcattcaccag-3' (SEQ ID NO: 19)
- 45 OXLP antisense 5'-catctgatattgctgggtctg-3' (SEQ ID NO: 20)

- d) amplification of a 513 bp *Ubi* cDNA fragment (GenBank accession M60175)

UBI sense 5'-ccaagatgcagatcttcgtga-3' (SEQ ID NO: 21)
 5 UBI antisense 5'-ttcgcgataggtaaaagagca-3' (SEQ ID NO: 22)

All of the fragments obtained were additionally ligated into the vector pGEM-T by means of T-overhang ligation and were used as starting plasmids for the generation of probes (for example for
 10 Northern blots) or dsRNA. The individual constructs were named pGEMT-RAC1, pGEMT-BAS, pGEMT-OXLP, pGEMT-UBI.

Example 5: Northern blot analysis

- 15 To prepare the Northern blotting, the RNA was separated in an agarose gel under denaturing conditions. To this end, part of the RNA solution (corresponding to 5 µg of RNA) was mixed with an identical volume of sample buffer (with ethidium bromide), denatured for 5 minutes at 94°C, placed on ice for 5 minutes,
 20 centrifuged briefly and applied to the gel. The 1 x MOPS gel (1.5% agarose, ultra pure grade) contained 5 percent by volume of concentrated formaldehyde solution (36.5% [v/v]). The RNA was separated for 2 hours at 100 V and subsequently blotted.
- 25 Northern blotting was done as an upward capillary RNA transfer. To this end, the gel was first agitated gently for 30 minutes in 25 mM sodium hydrogen/dihydrogen phosphate buffer (pH 6.5) and cut to size. A piece of Whatman paper was prepared in such a way that it rested on a horizontal support and extended on 2 sides
 30 into a trough with 25 mM sodium hydrogen/dihydrogen phosphate buffer (pH 6.5). This piece of paper was covered with the gel, uncovered parts of the piece of Whatman paper being covered with a plastic film. The gel was then covered with a positively charged nylon membrane (Boehringer-Mannheim), avoiding air
 35 bubbles, whereupon the membrane was recovered to a height of approximately 5 cm with a stack of blotting paper. The blotting paper was additionally weighed down with a sheet of glass and with a 100 g weight. Blotting was carried out overnight at room temperature. The membrane was rinsed briefly in twice-distilled
 40 water and irradiated with UV light in a crosslinking apparatus (Biorad) with a light energy of 125 mJ in order to immobilize the RNA. The uniformity of the RNA transfer to the membrane was checked on a UV light bench.

To detect barley mRNA, 10 µg of total RNA from each sample were resolved in an agarose gel and blotted onto a positively charged nylon membrane by capillary transfer. Detection was effected using the DIG system.

5

Probe preparation: Digoxigenin - or fluorescein-labeled RNA probes were prepared for hybridization with the mRNAs to be detected. The probes were generated by *in-vitro* transcription of a PCR product by means of a T7 or SP6 RNA polymerase, using labeled UTPs. The template for the PCR-aided amplification was provided by the above-described plasmid vectors pGEMT-RAC1, pGEMT-BAS, pGEMT-OXLP, pGEMT-UBI.

Depending on the orientation of the insert, different RNA polymerases were used for generating the antisense strand. T7 RNA polymerase was used for pGEMT-BAS and pGEMT-OXLP, while SP6-RNA polymerase was used for pGEMT-RAC1 and pGEMT-UBI.

The insert of the individual vector was amplified via PCR using flanking standard primers (M13 fwd and rev). The reaction proceeded with the following end concentrations in a total volume of 50 µl of PCR buffer (Silverstar):

M13-fwd: 5'-GTAAACGACGGCCAGTG-3' (SEQ ID NO: 23)

25 M13-rev: 5'-GGAAACAGCTATGACCATG-3' (SEQ ID NO: 24)

10% dimethyl sulfoxide (v/v)

2 ng/µl of each primer (M13 forward and reversed)

1.5 mM MgCl₂,

30 0.2 mM dNTPs,

4 units Taq polymerase (Silverstar),

2 ng/µl plasmid DNA.

The amplification was carried out in a *Thermocycler* (Perkin-Elmer 35 2400) with the following temperature program:

94°C denaturing for 3 minutes

30 cycles of 30 seconds at 94°C (denaturing)
40 30 seconds at 58°C (annealing),
1.2 minutes at 72°C (extension),

72°C final extension for 5 minutes

4°C cooling until further use

45

The success of the reaction was verified in a 1% agarose gel. The products were subsequently purified using a "High Pure PCR-Product Purification Kit" (Boehringer-Mannheim). This gave approximately 40 µl of column eluate, which was again verified in the gel and stored at -20°C.

The RNA polymerization, the hybridization and the immunodetection were carried out largely following the kit manufacturer's instructions regarding the nonradioactive RNA detection (DIG

- 10 System User's Guide, DIG-Luminescence detection Kit, Boehringer-Mannheim, Kogel et al. (1994) Plant Physiol 106:1264-1277). 4 µl of purified PCR product were treated with 2 µl of transcription buffer, 2 µl of NTP labeling mix, 2 µl of NTP mix and 10 µl of DEPC water. Then, 2 µl of the T7 RNA
- 15 polymerase solution were pipetted in. The reaction was then carried out for 2 hours at 37°C and then made up to 100 µl with DEPC water. The RNA probe was detected in an ethidium bromide gel and stored at -20°C.
- 20 To prepare the hybridization, the membranes were first agitated gently for 1 hour at 68°C in 2 x SSC (salt, sodium citrate), 0.1% SDS buffer (sodium dodecyl sulfate), the buffer being renewed twice or 3 times. The membranes were subsequently applied to the internal wall of hybridization tubes preheated at 68°C and
- 25 incubated for 30 minutes with 10 ml of Dig-Easy hybridization buffer in a preheated hybridization oven. In the meantime, 10 µl of probe solution were denatured for 5 minutes at 94°C in 80 µl of hybridization buffer, and the mixture was subsequently placed on ice and centrifuged briefly. For the hybridization, the probe was
- 30 then transferred into 10 ml of hybridization buffer at a temperature of 68°C, and the buffer in the hybridization tube was replaced by this probe buffer. Hybridization was then carried out overnight, likewise at 68°C.
- 35 Prior to the immunodetection of RNA-RNA hybrids, the blots were washed twice under stringent conditions for in each case 20 minutes in 0.1% (w/v) SDS, 0.1 x SSC at 68°C.

- For the immunodetection, the blots were first agitated twice for
- 40 5 minutes in 2 x SSC, 0.1% SDS at RT. 2 stringent wash steps were subsequently carried out for in each case 15 minutes at 68°C in 0.1 x SSC, 0.1% SDS. The solution was then replaced by wash buffer without Tween. The reaction mix was shaken for 1 minute and the solution was exchanged for blocking reagent. After a
- 45 further 30 minutes' shaking, 10 µl of anti-fluorescein antibody solution were added, and shaking was continued for 60 minutes. This was followed by two 15-minute wash steps in Tween-containing

wash buffer. The membrane was subsequently equilibrated for 2 minutes in substrate buffer and, after being left to drain, transferred to a sheet of acetate paper. A mixture of 20 μ l CDP-StarTM and 2 ml of substrate buffer was then divided uniformly on the "RNA side" of the membrane. The membrane was subsequently covered with a second sheet of acetate paper and the edges were heat-sealed to provide a water-tight seal, avoiding air bubbles. In a dark room, the membrane was then covered for 10 minutes with an X-ray film and the film was subsequently developed. The exposure time was varied as a function of the luminescence reaction.

Unless otherwise specified, the solutions were part of the kit as delivered (*DIG-Luminescence detection Kit*, Boehringer-Mannheim). All the others were prepared from the following stock solutions by dilution with autoclaved distilled water. Unless otherwise specified, all the stock solutions were made with DEPC (like DEPC water) and subsequently autoclaved.

- 20 - DEPC water: distilled water is treated overnight at 37°C with diethyl pyrocarbonate (DEPC, 0.1%, w/v) and subsequently autoclaved.
- 10 x MOPS buffer: 0.2 M MOPS (morpholine-3-propanesulfonic acid), 0.05 M sodium acetate, 0.01 M EDTA, pH brought to 7.0 with 10 M NaOH.
- 25 - 20 x SSC (sodium chloride/sodium citrate, salt/sodium citrate): 3 M NaCl, 0.3 M trisodium citrate x 2 H₂O, pH brought to 7.0 with 4 M HCl.
- 30 - 1% SDS (sodium dodecyl sulfate) sodium dodecyl sulfate (w/v), without DEPC.
- 35 - RNA sample buffer: 760 μ l formamide, 260 μ l formaldehyde, 100 μ l ethidium bromide (10 mg/ml), 80 μ l glycerol, 80 μ l bromophenol blue (saturated), 160 μ l 10 x MOPS, 100 μ L water.
- 10 x wash buffer without Tween: 1.0 M maleic acid, 1.5 M NaCl; without DEPC, bring to pH 7.5 with NaOH (solid, approx. 77 g) and 10 M NaOH.
- 40 - Tween-containing wash buffer: made by adding Tween to wash buffer without Tween (0.3%, v/v).
- 45

- 10 x blocking reagent: suspend 50 g of blocking powder (Boehringer-Mannheim) in 500 ml of wash buffer without Tween.
- Substrate buffer: bring 100 mM Tris (trishydroxymethylaminomethane), 150 mM NaCl to pH 9.5 with 4 M HCl.
- 10 x color marker: 50% glycerol (v/v), 1.0 mM EDTA pH 8.0, 0.25% bromophenol blue (w/v), 0.25% xylene cyanole (w/v).

10

Example 6: In vitro synthesis of the RacB dsRNA

- All of the plasmids (pGEMT-RAC1, pGEMT-BAS, pGEMT-OXLP, pGEMT-UBI) which were employed for in-vitro transcription
- 15 comprise the T7 and SP6 promoters (pGEM-T, Promega) at the respective ends of the nucleic acid sequence inserted, which makes possible the synthesis of sense or antisense RNA. The plasmids can be linearized with suitable restriction enzymes in order to ensure correct transcription of the nucleic acid
- 20 sequence inserted and to prevent reading being continued into vectorial sequences.

- To this end, 10 µg of plasmid DNA were cleaved with in each case at the side of the insert which was located distally from the
- 25 promoter. The cleaved plasmids are extracted in 200 µl of water with an identical volume of phenol/chloroform/isoamyl alcohol, transferred into a new Eppendorf reaction vessel (RNase-free) and centrifuged for 5 minutes at 20 000 g. 180 µl of the plasmid solution were treated with 420 µl of ethanol, and the mixture was
- 30 placed on ice and subsequently precipitated by centrifugation for 30 minutes at 20 000 g and -4°C. The precipitate was taken up in 10 µl of TE buffer.

- To prepare the RacB dsRNA, the plasmid pGEMT-Rac1 was digested
- 35 with SpeI, and sense RNA was transcribed using T7 RNA polymerase. Furthermore, pGEMT-Rac1 was digested with NcoI, and antisense RNA was transcribed using SP6 RNA polymerase. RNA polymerases were obtained from Roche Molecular Biology, Mannheim, Germany.

- 40 Each transcription reaction contained the following in a volume of 40 µl:

- 2 µl of linearized plasmid DNA (1 µg)
- 2 µl of NTPs (25 mM) (1.25 mM of each NTP)
- 45 4 µl of 10x reaction buffer (Roche Molecular Biology),
- 1 µl of RNasin (27 units; Roche Molecular Biology),

2 µl of RNA polymerase (40 units)
29 µl of DEPC water

Following incubation for 2 hours at 37°C, part of the reactions
5 from the transcription of the sense and antisense strand,
respectively, were mixed, denatured for 5 minutes at 95°C and
subsequently hybridized (annealed) by cooling to a final
temperature of 37°C over 30 minutes. Alternatively, it is also
possible first to denature the mixture of sense and antisense
10 strand and then to cool it for 30 minutes at -20°C. The protein
precipitate which formed during denaturing and hybridization was
removed by briefly centrifuging the reaction at 20 800 g, and the
supernatant was used directly for coating tungsten particles (see
hereinbelow). For analysis, 1 µl of each RNA strand and of the
15 dsRNA were resolved on a nondenaturing agarose gel. Successful
hybridization manifested itself by a band shift toward higher
molecular weight in comparison with the single strands.

4 µl of the dsRNA were ethanol-precipitated (by adding 6 µl of
20 water, 1 µl of 3M sodium acetate solution and 25 µl of ethanol,
and centrifugation for at least 5 minutes at 20 000 g and 4°C) and
the pellet was resuspended in 500 µl of water. The absorption
spectrum between 230 and 300 nm was measured or the absorption at
280 and 260 nm was determined in order to determine the purity
25 and concentration of the dsRNA. As a rule, 80 to 100 µg of dsRNA
with an OD₂₆₀/OD₂₈₀ ratio of 1.80 to 1.95 were obtained. Digestion
with DNase I can optionally be carried out, but has no
significant effect on the results which follow.

30 The control dsRNA used was the human thyroid receptor dsRNA
(starting vector pT7betaSal (Norman C et al. (1988) Cell
55(6):989-1003), provided by Dr. Baniahmad, Department of
Genetics, Gießen, Germany; the sequence of the insert is
described under the GenBank Acc. No.: NM_000461). To generate the
35 sense RNA, the plasmid was digested with PvuII, to generate the
antisense RNA, it was digested with HindIII, and the RNA was then
transcribed with T7 and SP6 RNA polymerase, respectively. The
individual process steps for generating the control dsRNA are
carried out analogously to those described above for the RacB
40 dsRNA.

Example 7: Transient transformation, RNAi, and evaluation of the
development of the fungal pathogen

45 Barley cv. Pallas leaf segments were transformed with an RacB
dsRNA together with a GFP expression vector. The leaves were
subsequently inoculated with Bgh, and the result was analyzed

after 48 hours by light and fluorescence microscopy. The penetration into GFP-expressing cells was assessed by detecting haustoria in live cells and by assessing the fungal development on precisely these cells. In all six experiments, bombardment of
 5 barley cv. Pallas with *RacB* dsRNA resulted in a reduced number of successfully Bgh-penetrated cells in comparison with cells which had been bombarded with a foreign control dsRNA (human thyroid hormone receptor dsRNA, TR). The resistance-inducing effect of the *RacB* dsRNA caused an average reduction in Bgh penetration
 10 efficacy by 44% (Fig. 4).

A method was employed for the transient transformation which had already been described for the biolistic introduction of dsRNA into epidermal cells of barley leaves (Schweizer P et al. (1999)
 15 Mol Plant Microbe Interact 12:647-54; Schweizer P et al. (2000) Plant J 2000 24: 895-903). Tungsten particles with a diameter of 1.1 μm (particle density 25 mg/ml) were coated with dsRNA (preparation see above) together with plasmid DNA of the vector pGFP (GFP under the control of the CaMV 35S promoter) as
 20 transformation marker. For each bombardment, the following amounts of dsRNA and reporter plasmid were used for coating: 1 μg of pGFP and 2 μg of dsRNA. Double-stranded RNA was synthesized in vitro by annealing sense and antisense RNA (see above).

25 To prepare microcarriers, 55 mg of tungsten particles (M 17, diameter 1.1 μm ; Bio-Rad, Munich) were washed twice with 1 ml of autoclaved distilled water and once with 1 ml of absolute ethanol, dried and taken up in 1 ml of 50% strength glycerol (approx. 50 mg/ml stock solution). The solution was diluted to
 30 25 mg/ml with 50% strength glycerol, mixed thoroughly prior to use, and suspended in an ultrasonic bath. To coat the microcarriers for each bombardment, 1 μg of plasmid, 2 μg of dsRNA (1 μl), 12.5 μl of tungsten particle suspension (25 mg/ml), 12.5 μl of 1 M $\text{Ca}(\text{NO}_3)_2$ solution (pH 10) were combined dropwise
 35 with constant mixing, the mixture was left to stand for 10 minutes at RT and then briefly centrifuged, and 20 μl of the supernatant were drawn off. The remainder with the tungsten particles is resuspended (ultrasonic bath) and employed in the experiment.

40 Segments (approx. 4 cm in length) of barley primary leaves were used. The tissue was placed on 0.5% Phytagar (GibcoBRL™ Life Technologies™, Karlsruhe) supplemented with 20 $\mu\text{g}/\text{ml}$ benzimidazole in Petri dishes (diameter 6.5 cm), and the edges
 45 were covered directly prior to particle bombardment with a stencil provided with a rectangular opening of 2.2 cm x 2.3 cm. One after the other, the dishes were placed on the bottom of the

vacuum chamber (Schweizer P et al. (1999) Mol Plant Microbe Interact 12:647-54) over which a nylon mesh (mesh size 0.2 mm, Millipore, Eschborn) on an apertured plate had been inserted (5 cm above the bottom, 11 cm underneath the macrocarrier, see 5 hereinbelow) to act as diffuser in order to disperse particle aggregates and to slow down the particle stream. For each bombardment, the macrocarrier (plastic syringe filter holder, 13 mm, Gelman Sciences, Swinney, UK), which was attached at the top of the chamber, was loaded with 5.8 µl of DNA-coated tungsten 10 particles (microcarrier, see hereinbelow). The pressure in the chamber was reduced by 0.9 bar using a diaphragm vacuum pump (Vacuubrand, Wertheim), and the surface of the plant tissue was bombarded with the tungsten particles at a helium gas pressure of 9 bar. The chamber was aerated immediately thereafter. To label 15 transformed cells, the leaves were bombarded with the plasmid (pGFP; vector pUC18-based, CaMV 35S promoter/terminator cassette with inserted GFP gene; Schweizer P et al. (1999) Mol Plant Microbe Interact 12:647-54; provided by Dr. P. Schweizer Schweizer P, Institut für Pflanzengenetik [Department of Plant 20 Genetics] IPK, Gatersleben, Germany). Each time before another plasmid was used for the bombardment, the macrocarrier was cleaned thoroughly with water. Following incubation for four hours after the bombardment with slightly open Petri dishes, RT and daylight, the leaves were inoculated with 100 conidia/mm² of 25 the barley powdery mildew fungus (race A6) and incubated for a further 36 to 48 hours under identical conditions.

Leaf segments were bombarded with the coated particles using a particle inflow gun. 312 µg of tungsten particles were applied per 30 bombardment. 4 hours after bombardment, the leaf segments were inoculation inoculated with *Blumeria graminis* f.sp. *hordei* mildew (race A6) and, after a further 40 hours, evaluated with regard to infection symptoms. The result (for example the penetration efficacy, defined as percentage of attacked cells with a mature 35 haustorium and a secondary hypha (secondary elongating hyphae) was analyzed by fluorescence and light microscopy. Inoculation with 100 conidia/mm² results in an attack frequency of approximately 50% of the transformed cells. A minimum of 100 interaction sites were evaluated for each individual 40 experiment. Transformed (GFP- expressing) cells were identified under excitation with blue light. Three different categories of transformed cells were distinguished:

1. Penetrated cells comprising a readily recognizable 45 haustorium. A cell with more than one haustorium counted as one cell.

2. Cells which were attacked by a fungal appressorium, but comprise no haustorium. A cell which was attacked repeatedly by Bgh, but contains no haustorium, counted as one cell.

- 5 3. Cells which are not attacked by Bgh.

Stomatal cells and subsidiary cells were excluded from the evaluation. Surface structures of Bgh were analyzed by light microscopy or fluorescent staining of the fungus with 0.1%

- 10 Calcofluor (w/v in water) for 30 sec. The fungal development can be evaluated readily by staining with Calcofluor followed by fluorescence microscopy. While the fungus develops a primary germ tube and an appressorial germ tube in cells transformed with RacB dsRNA, it fails to develop a haustorium. The development of
15 haustoria is a precondition for the development of a secondary hyphae.

The relative penetration efficacies (RPEs) were calculated as the difference between the penetration efficacies in transformed

- 20 cells (transformation with RacB dsRNA or control dsRNA) and the penetration efficacies of untransformed cells (here: average penetration efficacy 57%). The percentage RPE (%-RPE) is calculated from the RPE minus 1, multiplied by 100.

$$25 \quad \text{RPE} = \frac{[\text{PE of cells transformed with RacB dsRNA}]}{[\text{PE of cells transformed with control dsRNA}]}$$

$$\% \text{-RPE} = 100 * (\text{RPE} - 1)$$

- 30 The %-RPE value (deviation from the average penetration efficacy of the control) serves to determine the susceptibility of cells transfected with RacB dsRNA (Fig. 4).

In the case of the control dsRNA, no difference between the

- 35 transfection with the control dsRNA and with water was found in five independent experiments with regard to the penetration efficacy of Bgh.

The deviation of the PE in various genotypes was also studied. To
40 demonstrate the operable linkage with the Mlo gene, an *mlo5* genotype (A89, *mlo5 ror1*, background: Ingrid), which owing to a mutation of the *Ror1* gene has only moderate susceptibility to Bgh attack (Freialdenhoven et al. (1996) Plant cell 8:5-14), was employed. In this doubly-mutant genotype, the efficacy of RacB

- 45 dsRNA was studied in comparison with a wild-type *Mlo* genotype. However, no prevention of the development of haustoria in A89 was observed in five independent experiments, while in parallel

experiments with Pallas and Ingrid the PE was markedly reduced (Fig. 5). Interestingly, the effect of the *RacB* dsRNA was more pronounced in Pallas than in Ingrid (Fig. 5, Experiments 1 and 2 in comparison with 3, 4 and 5).

5

To rule out an effect of the dsRNA on the transformation rate or survival rate of the attacked cells, the number of GFP-expressing cells was compared between control and *RacB* dsRNA experiments (Table 7). The *RacB* dsRNA had no effect on the total number, or
10 the number of attacked, GFP-expressing cells.

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Table 7: Transformation rates of barley leaves following bombardment with dsRNA

Line	Number of GFP-expressing cells per bombardment ^a				n ^c
	Total (Control dsRNA)	Attacked (Control dsRNA)	Total (<i>RacB</i> dsRNA)	Attacked (<i>RacB</i> dsRNA)	
10 Pallas (<i>Mlo Ror1</i>)	34.3±4.6	16.0±2.2	33.9±4.8	15.5±1.4	6 (21)
Ingrid (<i>Mlo Ror1</i>)	51.0±8.9	27.6±8.7	49.9±5.6	31.5±7.8	3 (11)
15 A89 (<i>mlo5 ror1</i>)	34.4±5.4	18.1±4.0	34.1±5.5	16.7±3.8	5 (22)

a: 4 leaves were bombarded per bombardment.

The data shown are means and standard error.

c: Number of independent experiments (bombardments n in each case for control and *RacB* dsRNA).

Example 8: Constitutively active RACB mutant

A putatively constitutively active barley RACB mutant

(substitution G→V at position 15; *RacB*-V15) was generated and overexpressed in the barley variety Pallas in order to positively identify RACB as susceptibility factor. First, full-length RACB was synthesized via RT-PCR. The following oligonucleotide primers were employed for this purpose:

RacB5'BamHI : 5'-GGATCCGATGAGCGCGTCCAGGTT-3' (SEQ ID NO: 31)

RacB3'SalI : 5'-GTCGACCTTCGCCCTTGTTCTTTGTC-3' (SEQ ID NO: 32)

The cDNA was cloned into pGEM-T and subsequently excised via the primer cleavage sites and cloned into pGY-1 (Schweizer P et al. (1999) Mol Plant Microbe Interact 12: 647-54; Fig. 7) via BamHI/SalI cleavage sites. The construct is referred to as pGY1-RacB.

The nucleic acid sequence encoding the constitutively active *RacB* mutant *RACB*-V15 was generated using the "Transformer® Site-Directed Mutagenesis Kit" (Clontech, Heidelberg), following the

manufacturer's instructions. The starting vector employed was pGY1-RacB. The following oligonucleotide was used as mutagenesis primer:

5 V15 : 5'-ACCGTGGGGGACGTCGCCGTCGGCAAGAC-3' (SEQ ID NO: 33)

Then, RACB-V15 was then overexpressed transiently in 5 independent experiments in the barley variety Pallas under the control of the 35S CamV promoter. The experiments were carried
 10 out as described by Schultheiss et al. (Schultheiss H et al. (2002) Plant Physiol 128:1447-1454), except that, after the particle bombardment, 24 hours instead of 4 hours elapsed prior to inoculation. The particles were coated as described in
 15 Schweizer et al. (Schweizer P et al. (1999) Mol Plant Microbe Interact 12:647-54).

The expression of a constitutive RacB mutant brings about a significantly increased susceptibility to pathogen attack by powdery mildew of barley in comparison with the controls. Again,
 20 these results confirm the key function of RacB in the defense of pathogens. The RACB-V15 effects (see fig. 6-A/B) are significant in the t-test when a two-tail paired test is carried out. The relative susceptibility to the fungal pathogen is increased in all cases (fig. 6-B).

25

Example 9: Further HvRac homologs

All full-length sequences were isolated from RNA using specific primers and cloned into pGEM-T and sequenced (Hückelhoven et al.
 30 (2001) Plant Mol Biol; Schultheiss et al. (2002) Plant Physiol 128:1447-1454). In some cases, the sequences are very similar to RacB.

a) HvRop6:

35

LEFT PRIMER 5'-GTGGAGGCGCGGCGAGA-3' (SEQ ID NO: 25)
 RIGHT PRIMER 5'-CCATGCTTCATCTCCATAGTCA-3' (SEQ ID NO: 26)

b) HvRacD:

40

LEFT PRIMER 5'-ggatcccGATTCCATCAGGAAAGCAT-3' (SEQ ID NO: 27)
 RIGHT PRIMER 5'-gtcgacGCGAGACACTGCAAAACAAA-3' (SEQ ID NO: 28)

c) HvRop4:

45

LEFT PRIMER 5'-GGATCCttctcgtccatttagccggc-3' (SEQ ID NO: 29)

RIGHT PRIMER 5'-GTCGACTgatcacttgaagcatgccag-3' (SEQ ID NO: 30)

Example 10: Generation of sense and antisense constructs with the gene *AtRacB* for expression in *Arabidopsis thaliana*

5

A fragment of the *Arabidopsis RacB* homolog (MIPS-Code: AT4g35950; SEQ ID NO: 53; hereinbelow *AtRacB*) is isolated via PCR from an *Arabidopsis thaliana* cDNA library. The primer sequences used are:

10 Fra 186: 5'-ATGAGCGCGTCCAGGTTTCATA-3' (SEQ ID NO: 71)

Fra 187: 5'-ATCAAACACGCCCTTCACGTT-3' (SEQ ID NO: 72)

The amplification proceeds in a T3 thermocycler from Biometra

15 with the following temperature profile:

35 cycles of 1 min at 95°C, 0.5 min at 59°C and 3 min at 72°C.
Subsequent extension for 5 min at 72°C.

20 The PCR products is cloned into the vector pCR2.1 (in accordance with the *PCR Script Cloning Kit*, Stratagene, Heidelberg) following the manufacturer's instructions. A fragment is excised from the vector construct via the restriction enzyme *EcoRI* (Roche, Mannheim). The fragment can be isolated via gel

25 electrophoresis and subsequent purification using anion exchanger columns (QIAex Purification Kit, Qiagen, Hilden). Accordingly, the binary vector pSUN3-Nit is opened up via the enzymes *XmaI* and *EcoRI* and subjected to purification by means of gel electrophoresis followed by elution over anion exchanger columns
30 (QIAex Purification Kit, Qiagen, Hilden).

Since blunt ends have to be generated for the cloning of insert and vector with 5'-overhangs, both the eluted *AtRacB* fragment and the eluted pSUN3-Nit fragment are treated with 2 µl of dNTP mix I
35 (10 mM each of dATP, dCTP, dGTP, dTTP; Pharmacia, Freiburg) and 1.6 µl of Klenow fragment (USB/Amersham, Braunschweig, 2 U/µl) for filling up the overhang and incubated for 30 min at 37°C. To prevent religation, the vectors are first purified via a QIAquick Spin Column (Qiagen, Hilden), treated with CIAP (Calf Intestinal
40 Alkaline Phosphatase, GibcoBRL, Eggenstein, 1 U/µl) and finally purified via a 0.8% strength agarose gel.

To prepare the following ligation mix in a total volume of 50 µl, 34 µl of H₂O, 5 µl of ligation buffer and 1 µl of T4 ligase
45 (Roche, Mannheim) are additionally added to the 10 µl of cut DNA. This mixture is incubated overnight at 16°C. The ligase is subsequently inactivated for 10 min at 65°C. The ligation is now

followed by precipitation with 0.1 volume of sodium acetate (pH 5.2) and 2.5 volumes of ethanol. After centrifugation (30 min, 15 000 g, 4°C), the pellet is dried in 70% ethanol and resuspended in 10 µl of H₂O. 2 µl of this pellet are transformed

5 by electroporation (*E. coli* Pulser, Bio-Rad) into *Escherichia coli* bacteria, strain DH5α. The DNA-treated bacteria are plated onto LB plates supplemented with the antibiotic ampicillin (50 mg/l). After incubation for 16 h at 37°C, bacteria are scraped from the colonies which have grown and transferred into tubes

10 containing 3 ml of LB-Amp liquid medium each. After incubation for 16 h at 37°C, the cultures, which have grown to great density, are centrifuged. Plasmid DNA is isolated from the bacterial pellets by means of the QIAprep DNA miniprep kit (Qiagen, Hilden) following the manufacturer's instructions and subjected to

15 analytic digests with various enzyme combinations. These control digests allow the isolation of constructs in which the AtRacB gene is cloned in sense or antisense orientation behind the of the *A. thaliana* nitrilase-1 (*nit1*) gene, which is constitutively active in plants (GenBank Acc.-No.: Y07648.2, nucleotides

20 2456-4340, Hillebrand et al. (1996) Gene 170:197-200). These constructs are named pSUN3NIT_AtRacB_s (SEQ ID NO: 73) and pSUN3NIT_atRacB_as (SEQ ID NO: 74) and used for the transformation of *Arabidopsis* plants. The constructs comprise the complete sequence of AtRacB, so that the expression vector

25 pSUN3NIT_HvRacB_s, which comprises the fragment in sense orientation, is capable of expressing a functional AtRacB protein. The vector acts primarily as negative control and leads in most cases to reduced pathogen resistance, but in some cases (see hereinbelow) also to an increase in pathogen resistance,

30 presumably via a cosuppression effect.

Example 11: Generation of sense and antisense constructs with the gene *HvRacB* for expression in *Arabidopsis thaliana*

35 Various nonfunctional fragments of the gene *HvRacB* are to be prepared for expression in *Arabidopsis* plants. To this end, the plasmid, which contains the *HvRacB* gene subcloned into the bacterial vector pGEM-T, is digested with the enzyme combinations BamHI / HindIII (Roche, Mannheim). Overhanging 5'-single strands

40 are filled up by treatment with Klenow polymerase in the presence of a mixture of nucleotides (see above). The resulting *HvRacB* fragment with these blunt ends is cloned directly into a pSUN3NIT vector, which is opened up with the enzymes BglII and SpeI (Roche, Mannheim) in its multiple cloning site and whose

45 5'-overhangs are filled up by means of treatment with Klenow polymerase (as described above). For the ligation batches, each of which has a total volume of 50 µl, 34 µl of H₂O, 5 µl of

ligation buffer and 1 µl of T4 ligase (Roche, Mannheim) are additionally added to the 10 µl of cut DNA. This mixture is incubated overnight at 16°C. The ligase is subsequently inactivated for 10 min at 65°C. The ligation is now followed by

5 precipitation with 0.1 volume of sodium acetate (pH 5.2) and 2.5 volumes of ethanol. After centrifugation (30 min, 15 000 g, 4°C), the pellet is dried in 70% ethanol and resuspended in 10 µl of H₂O. 2 µl of this pellet are transformed by electroporation (*E. coli* Pulser, Bio-Rad) into *Escherichia coli* bacteria, strain

10 DH5α. The DNA-treated bacteria are plated onto LB plates supplemented with the antibiotic ampicillin (50 mg/l). After incubation for 16 h at 37°C, bacteria are scraped from the colonies which have grown and transferred into tubes containing 3 ml of LB-Amp liquid medium each. After incubation for 16 h at

15 37°C, the cultures, which have grown to great density, are centrifuged. Plasmid DNA is isolated from the bacterial pellets by means of the QIAprep DNA miniprep kit (Qiagen, Hilden) following the manufacturer's instructions and subjected to analytic digests with various enzyme combinations. These control

20 digests allow the identification of constructs in which the appropriate gene construct is cloned in sense or antisense orientation behind the promoter of the *A. thaliana* nitrilase-1 gene, which is constitutively active in plants (see above). These constructs are named pSUN3NIT_HvRacB_s (SEQ ID NO: 75) and

25 pSUN3NIT_HvRacB_as (SEQ ID NO: 76) and used for the transformation of *Arabidopsis* plants. The constructs comprise a truncated fragment of hvRacB, so that not even the expression vector pSUN3NIT_HvRacB_s, which comprises the fragment in sense orientation, is capable of expressing a functional HvRacB

30 protein. The vector acts primarily as negative control but leads in some cases (see hereinbelow) also to an increase in pathogen resistance, presumably via a cosuppression effect.

35 Example 12: Transformation of *Arabidopsis thaliana*, and analysis of the fungal resistance

Wild-type *A. thaliana* plants (Columbia) are with the *Agrabacterium tumefaciens* strain (EHA105) based on a modified method (Steve Clough and Andrew Bent (1998) Plant J 16(6):735-743) of the

40 vacuum infiltration method of Bechtold et al. (Bechtold N et al. (1993) CR Acad Sci Paris, Life Sciences 316:1194-1199).

The *A. tumefaciens* cells used are previously transformed with the plasmids pSUN3NIT_AtRacB_s (SEQ ID NO: 73), pSUN3NIT_atRacB_as

45 (SEQ ID NO: 74), pSUN3NIT_HvRacB_s (SEQ ID NO: 75) and pSUN3NIT_HvRacB_as (SEQ ID NO: 76).

Seeds of the *Agrobacterium*-transformed primary transformants are selected on the basis of their kanamycin resistance. Antibiotic-resistant seedlings are planted in soil and, when grown into fully developed plants, used for biochemical analysis.

5

To analyze the resistance of the transgenic *Arabidopsis* plants to pathogenic fungi, inoculations with the biotrophic fungi *Peronospora parasitica* and *Erysiphe cichoracearum* are performed.

10 a) *Peronospora parasitica*

Plants aged 5 to 8 weeks are sprayed with a conidia spore suspension (approx. 10^6 spores/ml). The inoculated plants are kept overnight in a refrigerator at approximately 16°C under dark and damp conditions, being covered with a plastic bag. After one day, the plastic bag is opened slightly and later removed completely. Six days after inoculation, the plants are again covered with the plastic bag overnight, whereby sporulation is induced. On the next day, the leaves are examined for the appearance of conidiophores. Over the next days, the intercellular growth of the fungus leads to the induction of weak chloroses up to severe necroses in the leaves. These symptoms are quantified and tested for significance.

25 b) *Erysiphe cichoracearum*

The biotrophic mildew fungus is grown on *Arabidopsis* plants. To infect the 4-week-old transgenic *RacB*-expressing *Arabidopsis* plants, conidiophores are removed from the leaf surface with a fine brush and applied to the leaves of the transgenic plants. The plants are incubated for 7 days at 20°C. 7 days after the inoculation, the conidiophores appear on the leaves, and chloroses and necroses can be observed over the following days. These symptoms are quantified and tested for significance.

35

c) Results

The transgenic *Arabidopsis* plants which express antisense sequences for *AtRacB* or *HvRacB* are significantly more resistant to *Peronospora parasitica* and to *Erysiphe cichoracearum* than nontransgenic wild-type plants.

The transgenic *Arabidopsis* plants which express sense sequences for the complete *AtRacB* are in are in most cases significantly more susceptible to both *Peronospora parasitica* and *Erysiphe cichoracearum* than nontransgenic wild-type plants. In some cases,

however, an increased resistance can also be observed (presumably via a cosuppression effect).

5 Transgenic Arabidopsis plants which express sense sequences for the an HvRacB fragment are in are in some cases significantly more resistant to both *Peronospora parasitica* and *Erysiphe cichoracearum* than nontransgenic wild-type plants.

10 Comprises/comprising and grammatical variations thereof when used in this specification are to be taken to specify the presence of stated features, integers, steps or components or groups thereof, but do not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of generating or increasing a resistance to at least one pathogen in a plant, which comprises:
 - reducing an amount, activity or function of an RacB protein in a plant or a tissue, organ, part or cell thereof,
 - wherein the RacB protein is a polypeptide comprising the sequence of SEQ ID NO: 2 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 2;
 - wherein the reducing comprises introducing to the plant, tissue, organ, part or cell thereof:
 - a double-stranded RacB RNA or an expression cassette comprising said double-stranded RacB RNA; and
 - wherein the pathogen is selected from the group consisting of Plasmodiophoromycota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota, Deuteromycetes, and combinations thereof.
2. The method of claim 1, comprising:
 - stably transforming a plant cell with a recombinant expression cassette comprising a nucleic acid encoding;
 - a double-stranded RacB RNA;
 - regenerating a plant from the plant cell; and
 - expressing said nucleic acid in an amount and over a period of time sufficient to generate or increase said resistance.
3. The method of claim 1 or 2, wherein the plant or the tissue, organ, part or cell thereof is selected or derived from a monocot or a dicot.
4. The method of claim 3, wherein the monocot is selected from the group consisting of wheat, oats, millet, barley, rye, maize, rice, buckwheat, sorghum, triticale, spelt, linseed, sugar cane, and combinations thereof.
5. An isolated nucleic acid when used in the method according to any one of claims 1 to 4, said isolated nucleic acid comprising a nucleic acid sequence encoding a barley RacB protein, wherein the RacB protein is a polypeptide comprising the sequence of SEQ ID NO: 2.

6. An isolated nucleic acid when used in the method according to any one of claims 1 to 4, said isolated nucleic acid comprising the sequence of SEQ ID NO: 1, a sequence complementary thereto, or a sequence derived from

said sequence or said complementary sequence by degeneracy of the genetic code.

7. A recombinant double-stranded RNA molecule that is capable of reducing expression of an RacB protein, wherein:

one of the two RNA strands of said molecule is essentially identical to at least part of a nucleic acid that encodes a RacB protein; and

the other RNA strand of said molecule is essentially identical to at least part of the complementary strand of said nucleic acid; and

wherein one of the two RNA strands comprises the sequence of SEQ ID No: 1 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 1.

8. A recombinant double-stranded RNA molecule that is capable of reducing expression of an RacB protein, comprising:

a sense strand comprising a sequence which is essentially identical to at least part of the sense RNA transcript of a sequence encoding an RacB protein; and

an antisense strand, which is essentially complementary to the sense strand;

wherein one of the strands comprises the sequence of SEQ ID NO: 1 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 1.

9. A transgenic expression cassette comprising the nucleic acid of claim 4 in operable linkage with a promoter.

10. The transgenic expression cassette of claim 9, wherein the promoter is functional in a plant.

11. The transgenic expression cassette of claim 10, wherein the promoter is a pathogen-inducible promoter.

12. A transgenic vector comprising the expression cassette of claim 8.

13. A plant or a tissue, organ, part or cell thereof made by the method of any one of claims 1 to 4.

14. A method of selecting a plant cell with an increased resistance to a pathogen comprising:

introducing a nucleic acid or an expression cassette comprising or encoding the nucleic acid to a plant cell that reduces an amount, activity or function of an RacB protein of the plant cell; and

selecting at least one plant cell wherein resistance to a pathogen is increased as compared to an untransformed cell;

wherein the nucleic acid comprises a double-stranded RacB RNA;

wherein the RacB protein is a polypeptide comprising the sequence of SEQ ID NO: 2 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 2; and

wherein the pathogen is selected from the group consisting of Plasmodiophoromycota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota, Deuteromycetes, and combinations thereof.

15. A transgenic organism comprising the nucleic acid of claim 5, the expression cassette of claim 9, or the vector of claim 12, wherein the organism is selected from the group of organisms consisting of bacteria, yeasts, and plants.

16. The transgenic organism of claim 15, wherein the plants are selected from the group consisting of wheat, oats, millet, barley, rye, maize, rice, buckwheat, sorghum, triticale, spelt, linseed, sugar cane, oil seed rape, canola, cress, Arabidopsis, cabbages, soya, alfalfa, pea, beans, peanut, potato, tobacco, tomato, eggplant, bell pepper, sunflower, Tagetes, lettuce, Calendula, melon, pumpkin, squash and zucchini.

17. A recombinant plant cell wherein an expressed amount, activity or function of an endogenous RacB protein is reduced by stable transformation with a nucleic acid or an expression cassette comprising or encoding the nucleic acid as compared to an untransformed plant cell, wherein the nucleic acid comprises a double-stranded RacB RNA; and wherein the nucleic acid comprises the sequence of SEQ ID NO: 1 or a functional equivalent thereof having at least 64% homology to the sequence of SEQ ID NO: 1.

18. The method of any one of claims 1 to 4, or the cell of claim 17, wherein activity comprises GTPase activity of said RacB protein.

19. The method of any one of claims 1 to 4 or 18, or the cell of claim 17 or 18, wherein function comprises a substrate-binding capacity of said RacB protein.

20. The method of any one of claims 1 to 4, 18 or 19, or the cell of any one of claims 17 to 19, wherein the reduction is at least 60%.

21. The method of any one of claims 1 to 4, 18 or 19, or the cell of any one of claims 17 to 19, wherein the reduction is at least 90%.

22. The cell of any one of claims 17 to 21, wherein the double-stranded RNA molecule comprises a sense strand comprising a sequence which is essentially identical to at least part of the sense RNA transcript of a sequence encoding the RacB protein; and an antisense strand which is essentially complementary to the sense strand.

23. The cell of any one of claims 17 to 22, wherein the reduction provides the cell with an increased resistance to a pathogen as compared to an untransformed cell.

24. The cell of claim 23, wherein the pathogen is selected from the group consisting of Plasmodiophoromycota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota, Deuteromycetes, and combinations thereof.

25. The method of any one of claims 1 to 4 or 18 to 21, wherein the functional equivalent is a polypeptide that has a capability of conferring a pathogen-resistant phenotype or an increased resistance to at least one pathogen, to the plant, tissue, organ, part or cell thereof.

26. The dsRNA molecule of claim 7 or 8, wherein the two RNA strands are linked covalently to each other.

27. A transgenic expression cassette comprising a nucleic acid encoding the dsRNA molecule of claim 7 or 8 in operable linkage with a promoter.
28. A transgenic organism comprising the dsRNA of claim 7 or 8, wherein the organism is selected from the group of organisms consisting of bacteria, yeasts, and plants.
29. A transgenic organism comprising a nucleic acid encoding the dsRNA molecule of claim 7 or 8, an expression cassette comprising the nucleic acid, or a vector comprising the expression cassette, wherein the organism is selected from the group of organisms consisting of bacteria, yeasts, and plants.
30. A stably transformed plant cell made by the method of claim 2.
31. A plant comprising the transformed plant cell of claim 2.
32. A plant comprising the recombinant plant cell of any one of claims 17 to 21.
33. A transformed plant cell obtained by the method of claim 14.
34. A plant comprising the transformed plant cell of claim 33.
35. The method of any one of claims 1 to 4, 14, 18 to 21, or 25, the isolated nucleic acid of claim 5 or 6, the recombinant nucleic acid of claim 7 or 8, the transgenic expression cassette of any one of claims 9 to 11 or 27, the transgenic vector of claim 12, a plant or a tissue, organ, part of cell thereof according to claim 13, the transgenic organism according to any one of claims 15, 16, 28 or 29, a recombinant plant cell according to any one of claims 17 to 24, the double stranded RNA molecule of any one claims 7, 8 or 26, a plant according to claim 31, 32 or 34, a transformed plant cell according to claim 33, substantially as hereinbefore described.

BASF PLANT SCIENCE GMBH

WATERMARK PATENT AND TRADE MARKS ATTORNEYS

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RACB <i>H.vul.</i>	MSASRFIKCVTVCDGAVGKTCMLISYTSNFEEDYVPTFEDNFSANVVDGNTVNLGAND	60
RACB <i>Z.mays</i>	MSASRFIKCVTVCDGAVGKTCMLISYTSNFEEDYVPTFEDNFSANVVDGNTVNLGAND	60
RACB <i>O.sat.</i>	MSASRFIKCVTVCDGAVGKTCMLISYTSNFEEDYVPTFEDNFSANVVDGNTVNLGAND	60
RAC2 <i>H.sap.</i>	MQA---IKCVVVGDCAVGKTCMLISYTTNFEEDYVPTFEDNFSANVVDGSKPVNLGAND	57
RAC1 <i>H.sap.</i>	MQA---IKCVVVGDCAVGKTCMLISYTTNFEEDYVPTFEDNFSANVVDGSKPVNLGAND	57
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RACB <i>O.sat.</i>	YAGQEDYNRLRPLSYRGADVFLAFSLISKASYENVSKKWIPELKHYAPGVPIILVGKRL	120
RAC2 <i>H.sap.</i>	YAGQEDYDRLRPLSYRQTDVFLICFSLVSPASYENVRAKWFEVRHHCPTPIILVGKRL	117
RAC1 <i>H.sap.</i>	YAGQEDYDRLRPLSYRQTDVFLICFSLVSPASFENVRAKWFEVRHHCPTPIILVGKRL	117
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Fig. 1

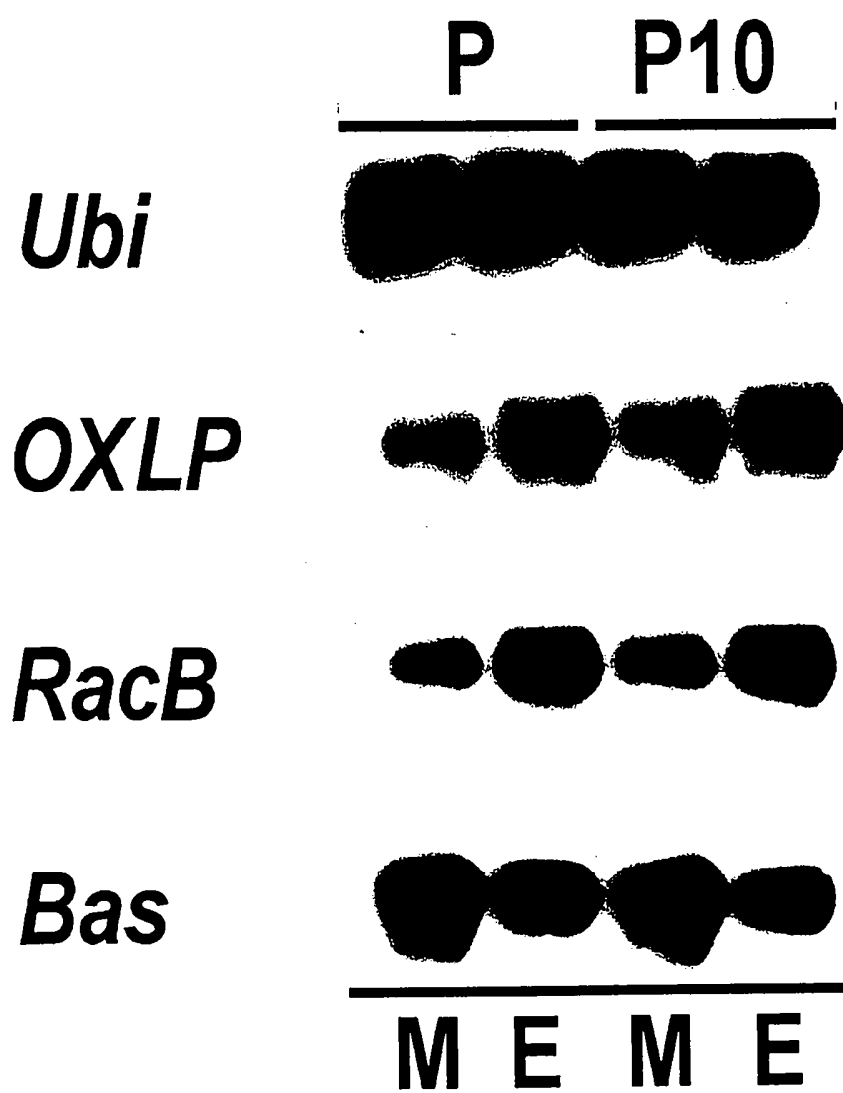
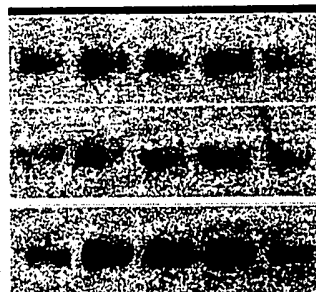
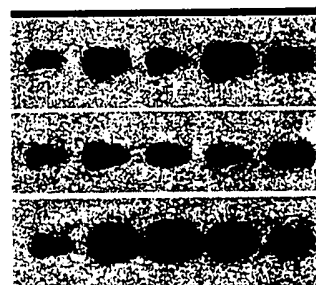
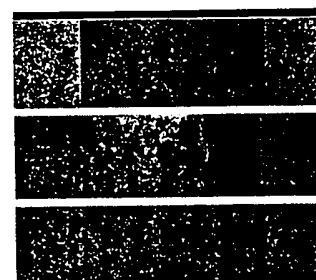


Fig. 2

2008202603 12 Jun 2008

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Fig. 3

2008202603 12 Jun 2008

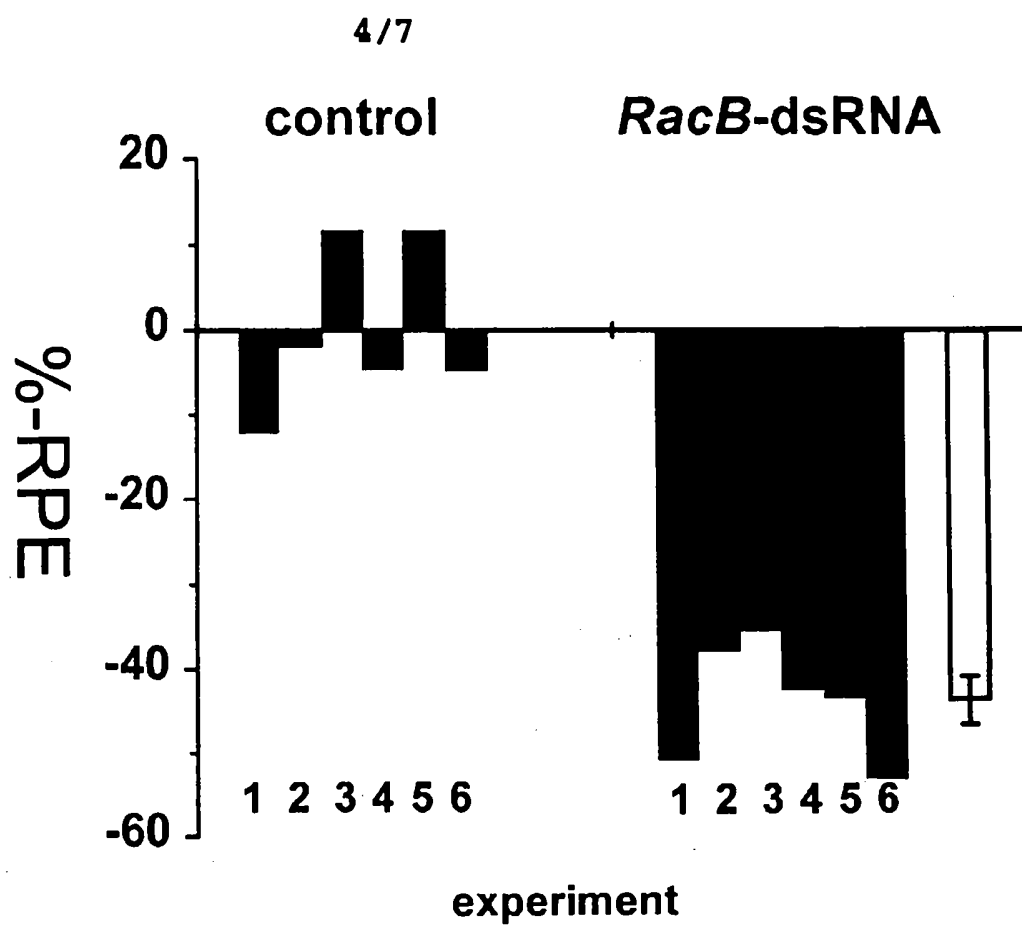


Fig. 4

2008202603 12 Jun 2008

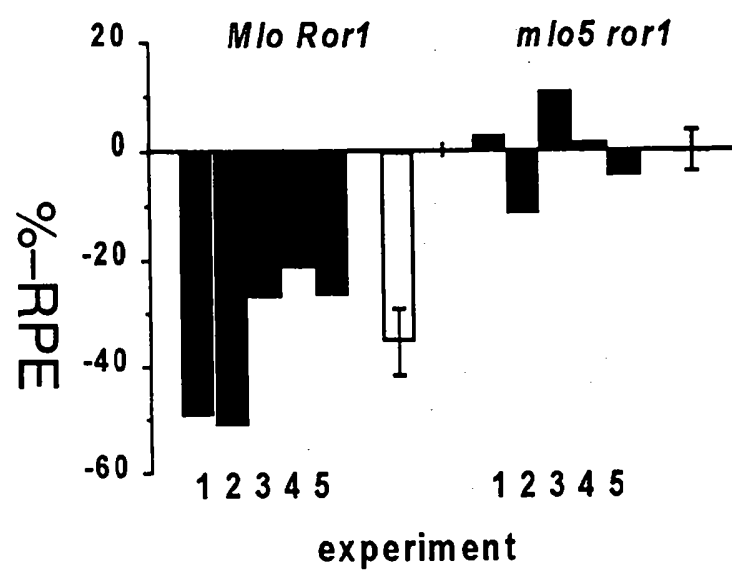


Fig. 5

2008202603 12 Jun 2008

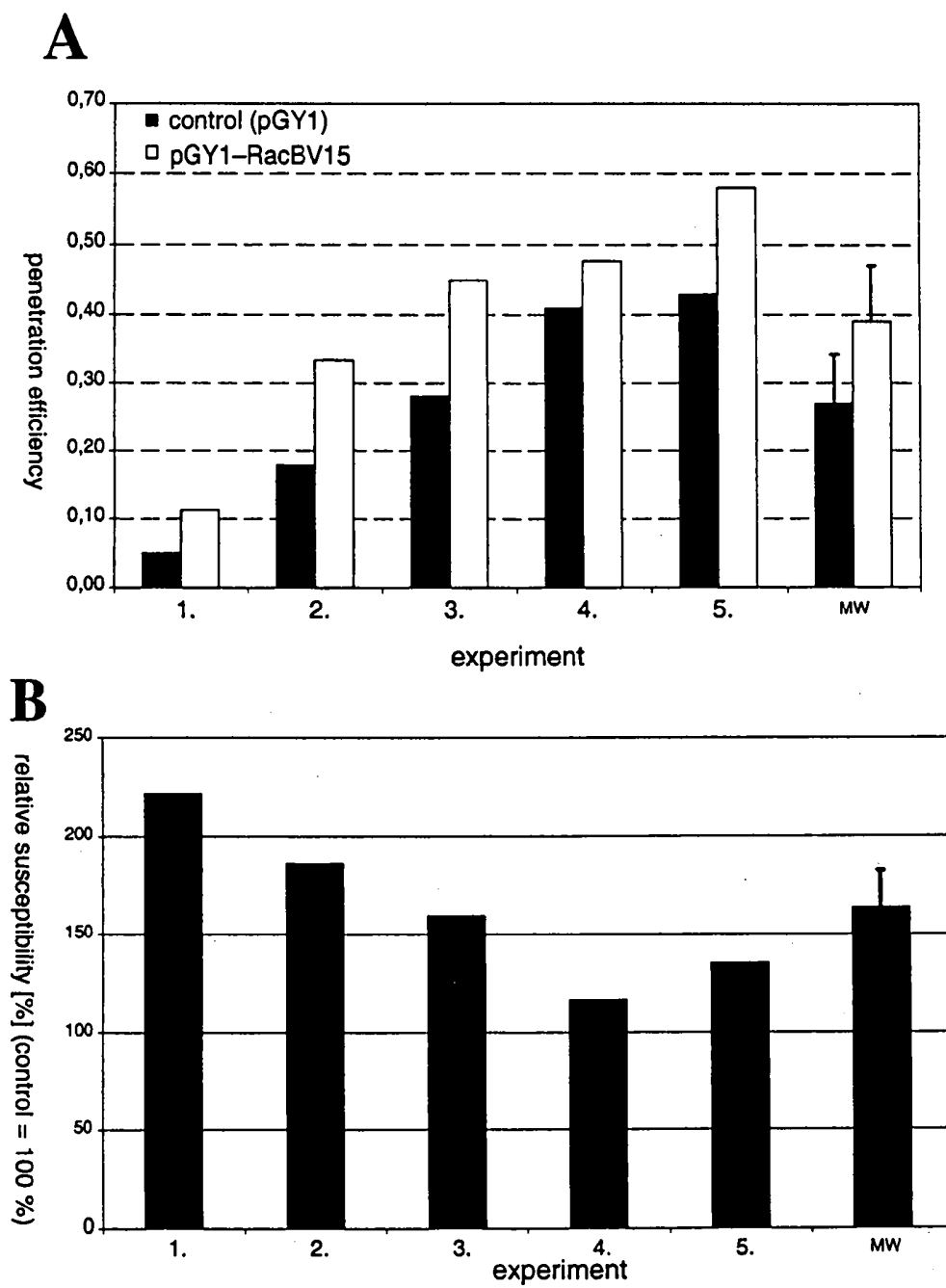


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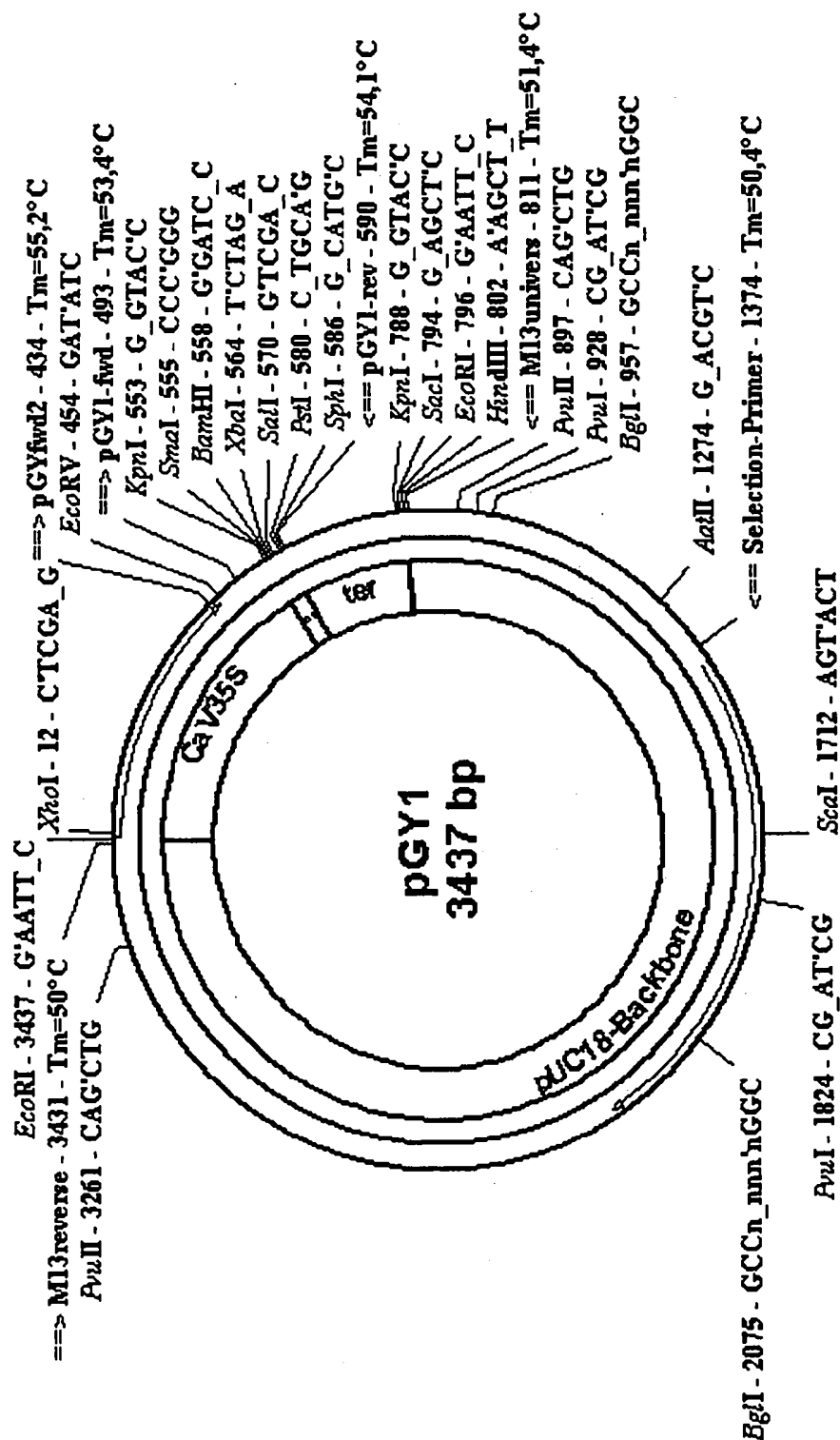


Fig. 7

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ata aag tgc gtc acc gtc ggg gac ggc gcc gtc ggc aag acc tgc atg 223
Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val Gly Lys Thr Cys Met
                10                15                20

ctc atc tcc tac acc tcc aac acc ttc ccc act gat tat gtt ccg acg 271
Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro Thr Asp Tyr Val Pro Thr
                25                30                35

gtg ttt gac aac ttc agt gcc aac gtc gtg gtt gat ggt aac acc gtc 319
Val Phe Asp Asn Phe Ser Ala Asn Val Val Val Asp Gly Asn Thr Val
                40                45                50

aac ctc ggg cta tgg gac act gca ggt cag gag gat tac aac aga ctg 367
Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu Asp Tyr Asn Arg Leu
                55                60                65                70

aga cca ctg agt tat cgt gga gct gat gtt ttc ctt ctg gcc ttc tcg 415
Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe Leu Leu Ala Phe Ser
                75                80                85

cta atc agc aag gcc agc tat gag aat gtt tca aag aag tgg ata cct 463
Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val Ser Lys Lys Trp Ile Pro
                90                95                100

gag ctg aag cat tat gca cct ggt gtt cct atc atc ctt gtg gga aca 511
Glu Leu Lys His Tyr Ala Pro Gly Val Pro Ile Ile Leu Val Gly Thr
                105                110                115

aag ctt gat ctt cga gat gac aag cag ttt ttt gtg gac cat cct ggt 559
Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe Phe Val Asp His Pro Gly
                120                125                130

gct gtt cct atc acc act gct cag gga gag gaa cta aga aag caa ata 607
Ala Val Pro Ile Thr Thr Ala Gln Gly Glu Glu Leu Arg Lys Gln Ile
                135                140                145                150

ggc gcc cca tac tac atc gaa tgc agc tca aag acc caa cta aac gtc 655
Gly Ala Pro Tyr Tyr Ile Glu Cys Ser Ser Lys Thr Gln Leu Asn Val
                155                160                165

aag ggc gtt ttc gat gcg gca ata aag gtg gtg ctg cag cca ccc aag 703
Lys Gly Val Phe Asp Ala Ala Ile Lys Val Val Leu Gln Pro Pro Lys
                170                175                180

gcg aag aag aag aaa aag gcg caa agg ggg gcg tgc tcc att ttg 748
Ala Lys Lys Lys Lys Lys Ala Gln Arg Gly Ala Cys Ser Ile Leu
                185                190                195

tgatctaadc atcagtagac gacgaagaag aagaacgatg aagttgccag gctttattat 808
tgttgcgtct tgcttcagcg aaacagcatt catggtccgg ggatcctagt ttactggcag 868
ctgcagcaag gcctctttgt cgaggcaatg agcgatccgt ttgtttcatt ttctcctttc 928
tgccttgatga ttatctcgtg tgactgacaa gtcgtggcaa ttaggtaact ttcctagatg 988

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gtatttcctg tgtttgagaa aaaaaattct tgttatccct gtttcataag tagacatgat 1048
 gtaatcgac tcagtttatt cttttccttc ttatttcact tcaatggaaa attatgtttc 1108
 ccttc 1113

<210> 4
 <211> 197
 <212> PRT
 <213> Oryza sativa

<400> 4
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45
 Val Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95
 Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110
 Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 Phe Val Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 Glu Leu Arg Lys Gln Ile Gly Ala Pro Tyr Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Leu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Lys Val
 165 170 175
 Val Leu Gln Pro Pro Lys Ala Lys Lys Lys Lys Lys Ala Gln Arg Gly
 180 185 190
 Ala Cys Ser Ile Leu
 195

<210> 5
 <211> 1393
 <212> DNA
 <213> Zea mays
 <220>
 <221> CDS
 <222> (398)..(988)

<400> 5
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 cgggccacca ccaccactc taccctcccc tccccaccac cactagcacc caccgtcccg 120
 gcgcggagac cgcttccttc cctccgcctc cgcaacctc tcccgctcgc cccgcgcctc 180
 cctccatttg tccgcggctc ccctccctcc cgatcttaac caccgcccac ccggttctct 240

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ctcccccttc ttcctccctc aaaccagacg ctgcaccccc tttctccac gcctatcttc 300
ttcagacgac cagcaggagg tacgaggaag accacctagg aggcctctct ctctctctcc 360
ccagccaccc ccgtagcgag agggagggcg gaagagg atg agc gcg tcc agg ttc 415
                               Met Ser Ala Ser Arg Phe
                               1           5
ata aag tgc gtc acg gtc ggg gac ggc gcc gtc ggc aag acc tgc atg 463
Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val Gly Lys Thr Cys Met
          10          15          20
ctc atc tcc tac acc tcc aac acc ttc ccc acc gac tat gtt ccg aca 511
Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro Thr Asp Tyr Val Pro Thr
          25          30          35
gtg ttt gat aac ttc agt gcc aac gtt gtg gtt gat ggt aat act gtc 559
Val Phe Asp Asn Phe Ser Ala Asn Val Val Val Asp Gly Asn Thr Val
          40          45          50
aac ctc ggc ctc tgg gac act gca ggt caa gag gat tac aac aga ctg 607
Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu Asp Tyr Asn Arg Leu
          55          60          65          70
aga cca ctg agc tat cgt gga gct gat gtt ttt ctt ctg gct ttc tca 655
Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe Leu Leu Ala Phe Ser
          75          80          85
ctg atc agt aag gcc agc tat gag aat gtt tcg aag aag tgg ata cct 703
Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val Ser Lys Lys Trp Ile Pro
          90          95          100
gaa ctg aag cat tat gca cct ggt gtg cca att att ctc gta ggg aca 751
Glu Leu Lys His Tyr Ala Pro Gly Val Pro Ile Ile Leu Val Gly Thr
          105          110          115
aag ctt gat ctt cga gac gac aag cag ttc ttt gtg gac cat cct ggt 799
Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe Phe Val Asp His Pro Gly
          120          125          130
gct gtc cct atc act act gct cag gga gag gag cta aga aag caa ata 847
Ala Val Pro Ile Thr Thr Ala Gln Gly Glu Glu Leu Arg Lys Gln Ile
          135          140          145          150
ggc gct cca tac tac atc gaa tgc agc tcg aag acc caa cta aac gtg 895
Gly Ala Pro Tyr Tyr Ile Glu Cys Ser Ser Lys Thr Gln Leu Asn Val
          155          160          165
aag ggc gtc ttc gat gcg gcg ata aag gtt gtg ctg cag ccg cct aag 943
Lys Gly Val Phe Asp Ala Ala Ile Lys Val Val Leu Gln Pro Pro Lys
          170          175          180
gcg aag aag aag aaa aag gtg cag agg ggg gcg tgc tcc att ttg 988
Ala Lys Lys Lys Lys Lys Val Gln Arg Gly Ala Cys Ser Ile Leu
          185          190          195
tgatctaadc atcggtagat gaagaaacaa gggcgaagggt gccatggctt tatcatcgtc 1048
gcgtcttgct tcagtggaac agcatgaatg gtccccaccc cctctagggt tactggcggc 1108
tcggctgcag cgagttctca tctctttgtc gaggcattga gcgatatgtt tgtttcattt 1168
tcctccttcc tgccttggtga ttatctggtg tgtgtgtgtg tgtgactgac gaagtcgcgg 1228
cgattaggta actcgcttag aaggtatttc ccgtgtttga gcaaaagaaa gtatccctgt 1288
tatctctgtt ccataagtta gacatgatgt aatcgacta agtttatttt tacttatttc 1348
acttgaatgg aaaagtatgc ttccatttta aaaaaaaaaa aaaaa 1393

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<210> 6
 <211> 197
 <212> PRT
 <213> Zea mays

<400> 6
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45
 Val Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95
 Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110
 Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 Phe Val Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 Glu Leu Arg Lys Gln Ile Gly Ala Pro Tyr Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Leu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Lys Val
 165 170 175
 Val Leu Gln Pro Pro Lys Ala Lys Lys Lys Lys Lys Val Gln Arg Gly
 180 185 190
 Ala Cys Ser Ile Leu
 195

<210> 7
 <211> 197
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Description of the artificial sequence:
 dominant-negative mutant of barley RacB protein

<220>
 <221> SITE
 <222> (20)
 <223> Thr/Asn mutation for dominant-negative phenotype

<400> 7
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 Val Gly Lys Asn Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45

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Val Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60

Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80

Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95

Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110

Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125

Phe Val Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
 130 135 140

Glu Leu Lys Lys Leu Ile Gly Ala Pro Tyr Tyr Ile Glu Cys Ser Ser
 145 150 155 160

Lys Thr Gln Leu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Lys Val
 165 170 175

Val Leu Gln Pro Pro Lys Ala Lys Lys Lys Lys Lys Ala Gln Arg Gly
 180 185 190

Ala Cys Ser Ile Leu
 195

<210> 8

<211> 197

<212> PRT

<213> Artificial sequence

<220>

<223> Description of the artificial sequence: :
 dominant-negative mutant of rice RacB protein

<220>

<221> SITE

<222> (20)

<223> Thr/Asn mutation for dominant-negative phenotype

<400> 8

Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15

Val Gly Lys Asn Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30

Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45

Val Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60

Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80

Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95

Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110

Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125

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Phe Val Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 Glu Leu Arg Lys Gln Ile Gly Ala Pro Tyr Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Leu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Lys Val
 165 170 175
 Val Leu Gln Pro Pro Lys Ala Lys Lys Lys Lys Lys Ala Gln Arg Gly
 180 185 190
 Ala Cys Ser Ile Leu
 195

<210> 9
 <211> 197
 <212> PRT
 <213> Artificial sequence
 <220>
 <221> SITE
 <222> (20)
 <223> Thr/Asn mutation for dominant-negative phenotype
 <220>
 <223> Description of the artificial sequence:
 dominant-negative mutant of maize RacB protein

<400> 9
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 Val Gly Lys Asn Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45
 Val Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95
 Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110
 Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 Phe Val Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 Glu Leu Arg Lys Gln Ile Gly Ala Pro Tyr Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Leu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Lys Val
 165 170 175
 Val Leu Gln Pro Pro Lys Ala Lys Lys Lys Lys Val Gln Arg Gly
 180 185 190
 Ala Cys Ser Ile Leu
 195

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<210> 10
<211> 24
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
oligonucleotide primer
<400> 10
ggatccgatg agcgcgtcca gggt 24
<210> 11
<211> 26
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
oligonucleotide primer
<400> 11
gtcgaccttc gcccttggtc ttgtc 26
<210> 12
<211> 26
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
oligonucleotide primer
<400> 12
gtgggcacat agtcggtggg gaaggt 26
<210> 13
<211> 23
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
oligonucleotide primer
<400> 13
cgactggagc acgaggacac tga 23
<210> 14
<211> 26
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
oligonucleotide primer
<400> 14
ggacactgac atggactgaa ggagta 26
<210> 15
<211> 22
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
oligonucleotide primer

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<400> 15	
gttcatcaag tgcgtcaccg tg	22
<210> 16	
<211> 23	
<212> DNA	
<213> Artificial sequence	
<220>	
<223> Description of the artificial sequence: oligonucleotide primer	
<400> 16	
ttagcttcct cagttcttcc ctg	23
<210> 17	
<211> 21	
<212> DNA	
<213> Artificial sequence	
<220>	
<223> Description of the artificial sequence: oligonucleotide primer	
<400> 17	
cgcgccgcag ccgagtacga c	21
<210> 18	
<211> 21	
<212> DNA	
<213> Artificial sequence	
<220>	
<223> Description of the artificial sequence: oligonucleotide primer	
<400> 18	
gtcacaaaaa cacatgtaac c	21
<210> 19	
<211> 20	
<212> DNA	
<213> Artificial sequence	
<220>	
<223> Description of the artificial sequence: oligonucleotide primer	
<400> 19	
ggccgacatg cattcaccag	20
<210> 20	
<211> 21	
<212> DNA	
<213> Artificial sequence	
<220>	
<223> Description of the artificial sequence: oligonucleotide primer	
<400> 20	
catctgatat tgctgggtct g	21
<210> 21	
<211> 21	
<212> DNA	
<213> Artificial sequence	

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<220>
 <223> Description of the artificial sequence:
 oligonucleotide primer

 <400> 21
 ccaagatgca gatcttcgtg a 21

 <210> 22
 <211> 21
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer

 <400> 22
 ttcgcatag gtaaaagagc a 21

 <210> 23
 <211> 18
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer

 <400> 23
 gtaaaacgac ggccagtg 18

 <210> 24
 <211> 19
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer

 <400> 24
 ggaaacagct atgaccatg 19

 <210> 25
 <211> 17
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer

 <400> 25
 gtggaggcgc ggcgaga 17

 <210> 26
 <211> 22
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer

 <400> 26
 ccatgcttca tctccatagt ca 22

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<210> 27
 <211> 26
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer
 <400> 27
 ggatcccgat tccatcagga aagcat 26
 <210> 28
 <211> 26
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer
 <400> 28
 gtcgacgcga gacactgcaa aacaaa 26
 <210> 29
 <211> 26
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer
 <400> 29
 ggatccttct cgtccattta gccggc 26
 <210> 30
 <211> 27
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer
 <400> 30
 gtcgactgat cacttgaagc atgccag 27
 <210> 31
 <211> 24
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer
 <400> 31
 ggatccgatg agcgcgtcca gggt 24
 <210> 32
 <211> 26
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Description of the artificial sequence:
 oligonucleotide primer

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<400> 32
gtcgcaccttc gcccttggtc tttgtc 26
<210> 33
<211> 29
<212> DNA
<213> Artificial sequence
<220>
<223> Description of the artificial sequence:
        oligonucleotide primer
<400> 33
accgtggggg acgtcgccgt cggcaagac 29
<210> 34
<211> 721
<212> DNA
<213> Hordeum vulgare
<220>
<221> CDS
<222> (38)..(673)
<223> coding for RacB homologue (Rop6)
<400> 34
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                               Met Ser Val Thr Lys Phe
                               1           5

atc aag tgc gtc acg gtg ggg gac ggc gcc gtc ggc aag acc tgc atg 103
Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val Gly Lys Thr Cys Met
           10           15           20

ctc atc tgc tac acc agc aac agg ttc ccc agt gat tac atc ccc acg 151
Leu Ile Cys Tyr Thr Ser Asn Arg Phe Pro Ser Asp Tyr Ile Pro Thr
           25           30           35

gtg ttc gac aac ttc agc gcc aac gtc tcc gtc gac ggc aac atc gtc 199
Val Phe Asp Asn Phe Ser Ala Asn Val Ser Val Asp Gly Asn Ile Val
           40           45           50

aac ctc ggc cta tgg gac acc gcc ggg caa gaa gac tac agc cgg ctg 247
Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu Asp Tyr Ser Arg Leu
           55           60           65           70

agg ccg ctg agc tac aga ggc gcc gac gtg ttc gtg ctc gcc ttc tcc 295
Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe Val Leu Ala Phe Ser
           75           80           85

ctc atc agc agc gcc agc tac gag aat gtt ctt aag aag tgg atg cca 343
Leu Ile Ser Ser Ala Ser Tyr Glu Asn Val Leu Lys Lys Trp Met Pro
           90           95           100

gag ctc cgc cgg ttc gcg ccg aat gtc ccc att gtt ctt gtt ggg acc 391
Glu Leu Arg Arg Phe Ala Pro Asn Val Pro Ile Val Leu Val Gly Thr
           105           110           115

aag cta gat ctg cgt gac cac aga gcc tac ctc gcc gac cac ccc ggt 439
Lys Leu Asp Leu Arg Asp His Arg Ala Tyr Leu Ala Asp His Pro Gly
           120           125           130

gct tca gca atc aca act gca cag ggt gaa gaa ctt agg aag cag atc 487
Ala Ser Ala Ile Thr Thr Ala Gln Gly Glu Glu Leu Arg Lys Gln Ile
           135           140           145           150

ggc gcc gcg gct tac atc gag tgc agc tcc aag aca cag cag aac gtc 535
Gly Ala Ala Ala Tyr Ile Glu Cys Ser Ser Lys Thr Gln Gln Asn Val
           155           160           165

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aag gct gtg ttt gac acc gcc ata aag gtg gtc ctc cag ccg ccg agg 583
 Lys Ala Val Phe Asp Thr Ala Ile Lys Val Val Leu Gln Pro Pro Arg
 170 175 180

aga agg gag gtg atg tcc gcc agg aag aaa acc agg cga agc tct gga 631
 Arg Arg Glu Val Met Ser Ala Arg Lys Lys Thr Arg Arg Ser Ser Gly
 185 190 195

tgc tcc atc aag cac ttg atc tgc ggg agt acg tgc gct gct 673
 Cys Ser Ile Lys His Leu Ile Cys Gly Ser Thr Cys Ala Ala
 200 205 210

tgaattagca ccatggaggc ctggactgac tatggagatg aagcatgg 721

<210> 35
 <211> 212
 <212> PRT
 <213> Hordeum vulgare

<400> 35
 Met Ser Val Thr Lys Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Arg Phe Pro
 20 25 30
 Ser Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Ser
 35 40 45
 Val Asp Gly Asn Ile Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 Glu Asp Tyr Ser Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 Phe Val Leu Ala Phe Ser Leu Ile Ser Ser Ala Ser Tyr Glu Asn Val
 85 90 95
 Leu Lys Lys Trp Met Pro Glu Leu Arg Arg Phe Ala Pro Asn Val Pro
 100 105 110
 Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp His Arg Ala Tyr
 115 120 125
 Leu Ala Asp His Pro Gly Ala Ser Ala Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 Glu Leu Arg Lys Gln Ile Gly Ala Ala Ala Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Thr Ala Ile Lys Val
 165 170 175
 Val Leu Gln Pro Pro Arg Arg Arg Glu Val Met Ser Ala Arg Lys Lys
 180 185 190
 Thr Arg Arg Ser Ser Gly Cys Ser Ile Lys His Leu Ile Cys Gly Ser
 195 200 205
 Thr Cys Ala Ala
 210

<210> 36
 <211> 699
 <212> DNA
 <213> Hordeum vulgare

<220>
 <221> CDS
 <222> (67)..(657)
 <223> coding for RacB homologue (RacD)
 <400> 36
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 tcggcg atg agc gca tct cgg ttc atc aag tgc gtg acg gtg ggg gac 108
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp
 1 5 10
 ggc gcc gtg gga aag aca tgc ctc ctc atc tca tac aca tcc aac acc 156
 Gly Ala Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr
 15 20 25 30
 ttc ccc aca gac tat gtc cca aca gtt ttc gac aac ttc agc gct aac 204
 Phe Pro Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn
 35 40 45
 gtc gtg gtt gac ggc agc acc gtc aac ctc gga tta tgg gat act gca 252
 Val Val Val Asp Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60
 gga caa gaa gac tat aat cga cta cgc cca cta agc tac cgt ggt gcc 300
 Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75
 gat gtc ttc ctg ctc gcc ttt tct ctc atc agc aaa gca agc tac gag 348
 Asp Val Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu
 80 85 90
 aat gtc act aag aag tgg att cca gag tta cgg cac tat gct cct ggc 396
 Asn Val Thr Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly
 95 100 105 110
 gtg ccc ata att ctt gtt gga aca aag ctt gat ctg cgg gat gac aag 444
 Val Pro Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys
 115 120 125
 cag ttt ttt gtg gat cac cct ggg gcg gtt cct att tcc act gct cag 492
 Gln Phe Phe Val Asp His Pro Gly Ala Val Pro Ile Ser Thr Ala Gln
 130 135 140
 ggt gaa gag ctg aag aag gtg att ggc gcg act gcc tac atc gag tgc 540
 Gly Glu Glu Leu Lys Lys Val Ile Gly Ala Thr Ala Tyr Ile Glu Cys
 145 150 155
 agc tca aaa aca cag cag aac atc aag gcg gtg ttt gat gcg gcg atc 588
 Ser Ser Lys Thr Gln Gln Asn Ile Lys Ala Val Phe Asp Ala Ala Ile
 160 165 170
 aag gtg gtc ctc cag cct ccg aag cag aag cgg aag aag agg aag tca 636
 Lys Val Val Leu Gln Pro Pro Lys Gln Lys Arg Lys Lys Arg Lys Ser
 175 180 185 190
 cag aaa gga tgc agc atc ttg taaagctaaa atcccttttg ttttgcagtg 687
 Gln Lys Gly Cys Ser Ile Leu
 195
 tctcgcgtcg ac 699
 <210> 37
 <211> 197
 <212> PRT
 <213> Hordeum vulgare

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<400> 37

Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
1 5 10 15

Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
20 25 30

Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
35 40 45

Val Asp Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
50 55 60

Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
65 70 75 80

Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
85 90 95

Thr Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro
100 105 110

Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
115 120 125

Phe Val Asp His Pro Gly Ala Val Pro Ile Ser Thr Ala Gln Gly Glu
130 135 140

Glu Leu Lys Lys Val Ile Gly Ala Thr Ala Tyr Ile Glu Cys Ser Ser
145 150 155 160

Lys Thr Gln Gln Asn Ile Lys Ala Val Phe Asp Ala Ala Ile Lys Val
165 170 175

Val Leu Gln Pro Pro Lys Gln Lys Arg Lys Lys Arg Lys Ser Gln Lys
180 185 190

Gly Cys Ser Ile Leu
195

<210> 38

<211> 677

<212> DNA

<213> Hordeum vulgare

<220>

<221> CDS

<222> (27)..(665)

<223> coding for RacB homologue (Rop4)

<400> 38

ggatccttct cgtccattta gccggc atg gcg tcc agc gcc tcc cgg ttc atc 53
Met Ala Ser Ser Ala Ser Arg Phe Ile
1 5

aag tgc gtc acc gtc ggg gac ggc gcc gtc ggc aag acc tgc atg ctc 101
Lys Cys Val Thr Val Gly Asp Gly Ala Val Gly Lys Thr Cys Met Leu
10 15 20 25

atc tgc tac acc agc aac aag ttc ccc acc gac tac gtg ccc acc gtg 149
Ile Cys Tyr Thr Ser Asn Lys Phe Pro Thr Asp Tyr Val Pro Thr Val
30 35 40

ttc gac aat ttc agc gcg aac gtg gtg gtg gac ggc acc acc gtg aac 197
Phe Asp Asn Phe Ser Ala Asn Val Val Val Asp Gly Thr Thr Val Asn
45 50 55

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ctg ggc ctc tgg gac act gca ggg cag gag gac tac aac aga ttg aga	245
Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu Asp Tyr Asn Arg Leu Arg	
60 65 70	
ccg ctg agc tac cgg gga gcc gac gtc ttc gtg ctc tcc ttc tcg ctc	293
Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe Val Leu Ser Phe Ser Leu	
75 80 85	
gtc agc cga gcc agc tac gag aat gtc atg aag aag tgg cta ccg gag	341
Val Ser Arg Ala Ser Tyr Glu Asn Val Met Lys Lys Trp Leu Pro Glu	
90 95 100 105	
ctt cag cac cat gca ccc ggc gtg cca aca gtg ctg gtt ggt aca aag	389
Leu Gln His His Ala Pro Gly Val Pro Thr Val Leu Val Gly Thr Lys	
110 115 120	
cta gat cta cgt gaa gac aag caa tac tta ctt gac cac ccc ggc gtg	437
Leu Asp Leu Arg Glu Asp Lys Gln Tyr Leu Leu Asp His Pro Gly Val	
125 130 135	
gtg cct gtt act aca gct cag ggg gag gaa ctc cgc aag cac atc ggt	485
Val Pro Val Thr Thr Ala Gln Gly Glu Glu Leu Arg Lys His Ile Gly	
140 145 150	
gca act tgt tat gtc gaa tgc agc tca aag aca cag cag aat gtc aaa	533
Ala Thr Cys Tyr Val Glu Cys Ser Ser Lys Thr Gln Gln Asn Val Lys	
155 160 165	
gct gtg ttt gat gct gcc atc aag gta gtg atc aaa cct cca aca aag	581
Ala Val Phe Asp Ala Ala Ile Lys Val Val Ile Lys Pro Pro Thr Lys	
170 175 180 185	
cag agg gaa agg agg aag aag aaa gca cgg caa gga tgt gca tca ttg	629
Gln Arg Glu Arg Arg Lys Lys Lys Ala Arg Gln Gly Cys Ala Ser Leu	
190 195 200	
ggt acc ctg tca aga agg aag ctg gca tgc ttc aag tgatcagtcg ac	677
Gly Thr Leu Ser Arg Arg Lys Leu Ala Cys Phe Lys	
205 210	
<210> 39	
<211> 213	
<212> PRT	
<213> Hordeum vulgare	
<400> 39	
Met Ala Ser Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp	
1 5 10 15	
Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys	
20 25 30	
Phe Pro Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn	
35 40 45	
Val Val Val Asp Gly Thr Thr Val Asn Leu Gly Leu Trp Asp Thr Ala	
50 55 60	
Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala	
65 70 75 80	
Asp Val Phe Val Leu Ser Phe Ser Leu Val Ser Arg Ala Ser Tyr Glu	
85 90 95	
Asn Val Met Lys Lys Trp Leu Pro Glu Leu Gln His His Ala Pro Gly	
100 105 110	
Val Pro Thr Val Leu Val Gly Thr Lys Leu Asp Leu Arg Glu Asp Lys	
115 120 125	

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Gln Tyr Leu Leu Asp His Pro Gly Val Val Pro Val Thr Thr Ala Gln
 130 135 140
 Gly Glu Glu Leu Arg Lys His Ile Gly Ala Thr Cys Tyr Val Glu Cys
 145 150 155 160
 Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile
 165 170 175
 Lys Val Val Ile Lys Pro Pro Thr Lys Gln Arg Glu Arg Arg Lys Lys
 180 185 190
 Lys Ala Arg Gln Gly Cys Ala Ser Leu Gly Thr Leu Ser Arg Arg Lys
 195 200 205
 Leu Ala Cys Phe Lys
 210

<210> 40

<211> 945

<212> DNA

<213> Zea mays

<220>

<221> CDS

<222> (37)..(672)

<223> coding for RacB homologue (Rop6)

<400> 40

gagaagaagg gggcctgccg gccggggctg ggagac atg agc gtg acc aag ttc 54
 Met Ser Val Thr Lys Phe
 1 5
 atc aag tgc gtc acg gtg ggc gac ggc gcg gtg ggc aag acc tgc atg 102
 Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val Gly Lys Thr Cys Met
 10 15 20
 ctc atc tgc tac acc agc aac aag ttc ccc acg gac tac atc ccc acg 150
 Leu Ile Cys Tyr Thr Ser Asn Lys Phe Pro Thr Asp Tyr Ile Pro Thr
 25 30 35
 gtg ttc gac aac ttc agc gcc aac gtc tcc gtg gac ggc agc atc gtc 198
 Val Phe Asp Asn Phe Ser Ala Asn Val Ser Val Asp Gly Ser Ile Val
 40 45 50
 aac ctg ggc ctc tgg gac acc gcg ggg caa gag gac tac agc agg ctg 246
 Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu Asp Tyr Ser Arg Leu
 55 60 65 70
 cgg ccg ctg agc tac agg ggc gcg gac gtg ttc gtg ctg gcc ttc tcc 294
 Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe Val Leu Ala Phe Ser
 75 80 85
 ctg atc agc agg gcg agc tac gag aac gtt ctt aag aag tgg gtg cca 342
 Leu Ile Ser Arg Ala Ser Tyr Glu Asn Val Leu Lys Lys Trp Val Pro
 90 95 100
 gag ctt cgc aga ttc gcg ccc aac gtc ccg gtc gtt ctt gtt ggg acc 390
 Glu Leu Arg Arg Phe Ala Pro Asn Val Pro Val Val Leu Val Gly Thr
 105 110 115
 aag tta gat ctc cgc gac cac aga gcc tac ctc gcc gac cat cct gga 438
 Lys Leu Asp Leu Arg Asp His Arg Ala Tyr Leu Ala Asp His Pro Gly
 120 125 130
 gct tca gca gtc acc acg gcg cag ggt gag gaa ctg agg aag cag atc 486
 Ala Ser Ala Val Thr Thr Ala Gln Gly Glu Glu Leu Arg Lys Gln Ile
 135 140 145 150

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ggc gct gcg gcc tac atc gag tgc agt tcc aaa acc cag cag aac gtc 534
Gly Ala Ala Ala Tyr Ile Glu Cys Ser Ser Lys Thr Gln Gln Asn Val
155 160 165

aag tct gtc ttc gat acg gcc atc aaa gtg gtc ctt cag ccc cca cgg 582
Lys Ser Val Phe Asp Thr Ala Ile Lys Val Val Leu Gln Pro Pro Arg
170 175 180

agg agg gag gca gtg cct gcc agg aag aag aac agg cgt ggc tcc gga 630
Arg Arg Glu Ala Val Pro Ala Arg Lys Lys Asn Arg Arg Gly Ser Gly
185 190 195

tgc tct ata atg aac ctt gtg tgt ggc agc aca tgt gct gct 672
Cys Ser Ile Met Asn Leu Val Cys Gly Ser Thr Cys Ala Ala
200 205 210

tagggagtct actagaacac tgaaccggaa gggaggtgaa ggcgtgattc atggtgtgta 732
atgtgctgtg gcaactggca agttagtttg ctatagatga ggatgactgc tgcttttgtt 792
ttccttgccc catctgctgt agttcgtcag gctcttcaag ggctgacttt ttaccagact 852
gcagtgttgt gtaagaagtt tgctagacgc tgtaactgta atgttctccg ctgatgtggt 912
ataactaaga tacgagtaag cttgagcgtg ttc 945

<210> 41
<211> 212
<212> PRT
<213> Zea mays

<400> 41
Met Ser Val Thr Lys Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
1 5 10 15
Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys Phe Pro
20 25 30
Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Ser
35 40 45
Val Asp Gly Ser Ile Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
50 55 60
Glu Asp Tyr Ser Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
65 70 75 80
Phe Val Leu Ala Phe Ser Leu Ile Ser Arg Ala Ser Tyr Glu Asn Val
85 90 95
Leu Lys Lys Trp Val Pro Glu Leu Arg Arg Phe Ala Pro Asn Val Pro
100 105 110
Val Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp His Arg Ala Tyr
115 120 125
Leu Ala Asp His Pro Gly Ala Ser Ala Val Thr Thr Ala Gln Gly Glu
130 135 140
Glu Leu Arg Lys Gln Ile Gly Ala Ala Ala Tyr Ile Glu Cys Ser Ser
145 150 155 160
Lys Thr Gln Gln Asn Val Lys Ser Val Phe Asp Thr Ala Ile Lys Val
165 170 175
Val Leu Gln Pro Pro Arg Arg Arg Glu Ala Val Pro Ala Arg Lys Lys
180 185 190
Asn Arg Arg Gly Ser Gly Cys Ser Ile Met Asn Leu Val Cys Gly Ser
195 200 205

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Thr Cys Ala Ala
210

<210> 42

<211> 850

<212> DNA

<213> Oryza sativa

<220>

<221> CDS

<222> (112)..(702)

<223> coding for RacB homologue (RACDP / RACD)

<400> 42

agcaagcagc agctgaggtg aggtccgtgg cggttgagtg aggactgagg aggaagaaga 60

gggcgggatac tagggtaccg gatgcgctgg ctgtgctgag tgagagtaga g atg agc 117
Met Ser
1

gcg tct cgg ttc atc aag tgc gtc acc gtg ggg gac ggc gcc gtg ggc 165
Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val Gly
5 10 15

aag acc tgc atg ctc atc tcc tac acc tcc aac acc ttc ccc acg gac 213
Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro Thr Asp
20 25 30

tat gtt cca act gtt ttt gat aac ttc agt gca aat gtt gtg gtc gat 261
Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val Val Asp
35 40 45 50

ggg agc act gtg aac ttg ggg ttg tgg gat aca gca gga caa gag gac 309
Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu Asp
55 60 65

tac aat agg cta cgc ccg ttg agc tat cgt ggc gct gat gtt ttc ctg 357
Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe Leu
70 75 80

ctg gcc ttt tct ctg atc agc aaa gca agc tat gag aat gtt tct aaa 405
Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val Ser Lys
85 90 95

aag tgg ata cct gaa tta agg cat tat gct cct ggt gtg cca ata att 453
Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro Ile Ile
100 105 110

ctc gtt gga aca aag ctt gat ctg cgg gat gat aag caa ttt ttc gta 501
Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe Phe Val
115 120 125 130

gat cac cct ggt gct gta cct att tcc act gct cag ggc gaa gag ctg 549
Asp His Pro Gly Ala Val Pro Ile Ser Thr Ala Gln Gly Glu Glu Leu
135 140 145

agg aaa ctc att ggt gca gcg gca tac att gaa tgc agt tca aaa aca 597
Arg Lys Leu Ile Gly Ala Ala Ala Tyr Ile Glu Cys Ser Ser Lys Thr
150 155 160

cag caa aac atc aag gca gtt ttc gat gct gcg att aag gtg gtt ctc 645
Gln Gln Asn Ile Lys Ala Val Phe Asp Ala Ala Ile Lys Val Val Leu
165 170 175

cag cct cca aag caa aag aag aag aag aaa aag gcg cag aaa gga tgt 693
Gln Pro Pro Lys Gln Lys Lys Lys Lys Lys Lys Ala Gln Lys Gly Cys
180 185 190

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gcc atc ttg taattaaatg gtagacagtg cagtgcagat cgatgtatcc      742
Ala Ile Leu
195

cttcatttgt agcctctggc ttcaatcgtc gcttgtttgt ataattacgc tagatgccac 802
cggcagaaga tataatatag tcctcctgcc tttgtggtgt tgggtctct      850
<210> 43
<211> 197
<212> PRT
<213> Oryza sativa
<400> 43
Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1          5          10          15
Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20          25          30
Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35          40          45
Val Asp Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50          55          60
Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65          70          75          80
Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85          90          95
Ser Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro
100          105          110
Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
115          120          125
Phe Val Asp His Pro Gly Ala Val Pro Ile Ser Thr Ala Gln Gly Glu
130          135          140
Glu Leu Arg Lys Leu Ile Gly Ala Ala Ala Tyr Ile Glu Cys Ser Ser
145          150          155          160
Lys Thr Gln Gln Asn Ile Lys Ala Val Phe Asp Ala Ala Ile Lys Val
165          170          175
Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Lys Lys Lys Ala Gln Lys
180          185          190
Gly Cys Ala Ile Leu
195

<210> 44
<211> 1169
<212> DNA
<213> Oryza sativa
<220>
<221> CDS
<222> (169)..(813)
<223> coding for RacB homologue (Rop 4)
<400> 44
aacagttcag agaggaagca tgtgacactt cctctgtccc ctctctctct ctctagcctc 60
caaccatcgc ttcaccaaga agccatcacc tcctcctctc tatcaagttc tctcccctct 120

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cttgctgtct ctgcttgctg ctgctgctgc tcgattcggc cggcggcc atg gcg tcc 177
                                   Met Ala Ser
                                   1

agc gcg tcg cgg ttc atc aag tgc gtc acg gtc ggg gac ggc gcc gtc 225
Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val
      5                      10                      15

ggc aag acc tgc atg ctc atc tgc tac acc agc aac aag ttc ccc act 273
Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys Phe Pro Thr
  20                      25                      30                      35

gat tac gta ccc act gtt ttt gac aat ttc agt gca aac gtg gtg gtc 321
Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val Val
                      40                      45                      50

gac ggc acc acg gtg aat ttg ggt ctc tgg gat act gca ggg cag gaa 369
Asp Gly Thr Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu
      55                      60                      65

gat tac aac aga ttg agg ccg cta agc tac cgt ggc gcc gat gtc ttt 417
Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe
      70                      75                      80

gtg ctt gcc ttc tcc cta gtg agc cga gct agc tat gag aat gtc atg 465
Val Leu Ala Phe Ser Leu Val Ser Arg Ala Ser Tyr Glu Asn Val Met
      85                      90                      95

aag aag tgg tta cca gag ctt cag cat tat gca cca ggg gtg cca att 513
Lys Lys Trp Leu Pro Glu Leu Gln His Tyr Ala Pro Gly Val Pro Ile
  100                      105                      110                      115

gtg ttg gtt ggg acc aaa ttg gat ctt cgt gaa gat aaa cac tac tta 561
Val Leu Val Gly Thr Lys Leu Asp Leu Arg Glu Asp Lys His Tyr Leu
                      120                      125                      130

ctt gac cat cct agc ttg gtg cct gtg act aca gca cag gga gag gaa 609
Leu Asp His Pro Ser Leu Val Pro Val Thr Thr Ala Gln Gly Glu Glu
                      135                      140                      145

ctc cgc aag cac att ggc gca acg tgt tac atc gaa tgc agc tca aag 657
Leu Arg Lys His Ile Gly Ala Thr Cys Tyr Ile Glu Cys Ser Ser Lys
      150                      155                      160

aca cag cag aat gta aaa gct gtg ttt gat gct gcc atc aag gta gta 705
Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile Lys Val Val
      165                      170                      175

atc aag cct cca aca aag cag agg gac agg aag aag aag aaa aca cgg 753
Ile Lys Pro Pro Thr Lys Gln Arg Asp Arg Lys Lys Lys Lys Thr Arg
  180                      185                      190                      195

cgc gga tgt tct ttc ttc tgc aag ggt gtc atg tcc aga aga agg cta 801
Arg Gly Cys Ser Phe Phe Cys Lys Gly Val Met Ser Arg Arg Arg Leu
                      200                      205                      210

gta tgc ttc aag tgaacaagag gggttctttg atgagcagag cagaggtctg 853
Val Cys Phe Lys
      215

tgagacaaaa tgatgtcttg tgtttgataa ttgctttatc tcaaaagttc cagtttgata 913

gttgcatcttc caacctatat atatcctgtt tggcaattaa ctactacctc cgtcccaaaa 973

tataacaact tttggctatg aatctgaacg cacagttatc cagattcata gctaaaaata 1033

cttatattttt gggacggagg gagtactagt agtagattac tatcctgtcc atgtaatgta 1093

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ttaggaaggt taatagcact ccctacatct cagaatggaa gttgttttgg ttttaaaaaa 1153
 aaaaaaaaaa aaaaaa 1169

<210> 45

<211> 215

<212> PRT

<213> Oryza sativa

<400> 45

Met Ala Ser Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp
 1 5 10 15

Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys
 20 25 30

Phe Pro Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn
 35 40 45

Val Val Val Asp Gly Thr Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60

Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75 80

Asp Val Phe Val Leu Ala Phe Ser Leu Val Ser Arg Ala Ser Tyr Glu
 85 90 95

Asn Val Met Lys Lys Trp Leu Pro Glu Leu Gln His Tyr Ala Pro Gly
 100 105 110

Val Pro Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Glu Asp Lys
 115 120 125

His Tyr Leu Leu Asp His Pro Ser Leu Val Pro Val Thr Thr Ala Gln
 130 135 140

Gly Glu Glu Leu Arg Lys His Ile Gly Ala Thr Cys Tyr Ile Glu Cys
 145 150 155 160

Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile
 165 170 175

Lys Val Val Ile Lys Pro Pro Thr Lys Gln Arg Asp Arg Lys Lys Lys
 180 185 190

Lys Thr Arg Arg Gly Cys Ser Phe Phe Cys Lys Gly Val Met Ser Arg
 195 200 205

Arg Arg Leu Val Cys Phe Lys
 210 215

<210> 46

<211> 1127

<212> DNA

<213> Zea mays

<220>

<221> CDS

<222> (190)..(831)

<223> coding for RacB homologue (RacA)

<400> 46

gtcgaccac gcgtccgccc agaagtcacg caccaaacac caccaccaa gaaggcgaga 60

acgtactcgg tccctcccct cccctcccct ccccttcccc tcgaggctcc aggaccgtct 120

cctcgctgc tcattccgccc ctgcttcct tctctgggct cggagaaccg gagagaagcg 180

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cgcgcgggcc atg gcg tcc agc gcc tct cgg ttc atc aag tgc gtc acg gtc 231
      Met Ala Ser Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val
            1              5              10

ggc gac ggt gcc gtg ggc aag aca tgt atg ctc atc tgc tac acc agc 279
Gly Asp Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser
      15              20              25              30

aac aag ttc ccc act gac tac ata cct acg gtg ttc gac aat ttc agt 327
Asn Lys Phe Pro Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser
            35              40              45

gca aat gta gtt gtg gat ggc acc act gtg aat ttg ggc ctt tgg gat 375
Ala Asn Val Val Val Asp Gly Thr Thr Val Asn Leu Gly Leu Trp Asp
            50              55              60

acc gct ggg cag gaa gat tac aac cgc ctg agg cct cta agc tac cga 423
Thr Ala Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg
            65              70              75

ggg gca gat gtt ttc gtg ctt gca ttc tca ctt gtg agc cga gct agc 471
Gly Ala Asp Val Phe Val Leu Ala Phe Ser Leu Val Ser Arg Ala Ser
            80              85              90

tat gag aat atc atg aag aag tgg ata cca gag ctt caa cat tat gca 519
Tyr Glu Asn Ile Met Lys Lys Trp Ile Pro Glu Leu Gln His Tyr Ala
            95              100              105              110

cct ggg gtg ccc gtt gtt ttg gca ggc aca aaa ttg gat ctt cgt gaa 567
Pro Gly Val Pro Val Val Leu Ala Gly Thr Lys Leu Asp Leu Arg Glu
            115              120              125

gac aag cac tac ttg atg gac cat cct gga ttg gtg cct gtt acc act 615
Asp Lys His Tyr Leu Met Asp His Pro Gly Leu Val Pro Val Thr Thr
            130              135              140

gca cag ggg gag gaa ctt cgt aga caa att ggt gct atg tat tac att 663
Ala Gln Gly Glu Glu Leu Arg Arg Gln Ile Gly Ala Met Tyr Tyr Ile
            145              150              155

gaa tgc agc tca aag aca cag cag aat gtc aaa gct gtg ttc gat gct 711
Glu Cys Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala
            160              165              170

gcc atc aag gta gta atc cag cct cca act aaa ata aga gaa aag aag 759
Ala Ile Lys Val Val Ile Gln Pro Pro Thr Lys Ile Arg Glu Lys Lys
            175              180              185              190

aag aaa aaa tca cgc aaa gga tgt tct atg atg aac atc ttc ggt gga 807
Lys Lys Lys Ser Arg Lys Gly Cys Ser Met Met Asn Ile Phe Gly Gly
            195              200              205

aga aaa atg cta tgc ttc aag tcc tgaatgggttc aaggggggtct tacatggact 861
Arg Lys Met Leu Cys Phe Lys Ser
            210

gataccacga gtgtgacccc gagtttgcca agcttgaaat cttgatgtgc tcgttgcgca 921
tgtgtatatatt tgcacctttg gttattaatg actagaggta ggtaattgaa actagtctgc 981
ttaagcgttc tgcactgctg gtgtgggttag ctctatgagt taagcagttc gacagaggcc 1041
aaaccgacag tgagattttg ttctttcatg gaaatgtgcc aatgtcacag ctttttcgtg 1101
aaaaaaaaa aaaaaaaaaa aaaaaa 1127

<210> 47
<211> 214

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<212> PRT

<213> Zea mays

<400> 47

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Met Ala Ser Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp
  1           5           10           15
Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys
          20           25           30
Phe Pro Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Ala Asn
          35           40           45
Val Val Val Asp Gly Thr Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
          50           55           60
Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
  65           70           75           80
Asp Val Phe Val Leu Ala Phe Ser Leu Val Ser Arg Ala Ser Tyr Glu
          85           90           95
Asn Ile Met Lys Lys Trp Ile Pro Glu Leu Gln His Tyr Ala Pro Gly
          100          105          110
Val Pro Val Val Leu Ala Gly Thr Lys Leu Asp Leu Arg Glu Asp Lys
          115          120          125
His Tyr Leu Met Asp His Pro Gly Leu Val Pro Val Thr Thr Ala Gln
          130          135          140
Gly Glu Glu Leu Arg Arg Gln Ile Gly Ala Met Tyr Tyr Ile Glu Cys
  145          150          155          160
Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile
          165          170          175
Lys Val Val Ile Gln Pro Pro Thr Lys Ile Arg Glu Lys Lys Lys Lys
          180          185          190
Lys Ser Arg Lys Gly Cys Ser Met Met Asn Ile Phe Gly Gly Arg Lys
          195          200          205
Met Leu Cys Phe Lys Ser
          210

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<210> 48

<211> 901

<212> DNA

<213> Hordeum vulgare

<220>

<221> misc_feature

<222> (1)..(901)

<223> coding for RacB homologue (RacA)

<400> 48

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ccggggtgc aggaattcgg caccaggcaa gaagtcacgc accaaacacc acccccatc 60
accgccgctc cgctccccag tccccaccc ctcttcgcc ccttcctcg agccgagctc 120
cgggggaagg aatcgggagag gccggcgcg gcgagccat ggcgtccagc gcctcccggt 180
tcatcaagtg cgtcacggtg ggcgacggcg ccgtcggcaa gacctgcatg ctcatctgct 240
acaccagcaa caagttcccc accgactaca taccacgggt gttcgacaat ttcagcgcca 300
acgtggtggc ggacggcacc acggtgaatt tgggcctttg ggacaccgcc gggcaggagg 360
attacaaccg gctgaggcct ctaagctacc gcggcgccga cgttttcgtg cttgccttct 420
cccttgtagg ccgagctagc tatgagaata tcatgaagaa gtggataccg gagcttcagc 480

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attacgcgcc cggcgtaacct gttgtgctgg taggcacaaa actggatcct cgtgaagata 540
agcactatatt gctggaccac cctgggatga taccggttac cacagcacag ggggaggaac 600
ttcgtaaagca agttgggtgct ttatattaca tagagtgcag ctcaaagaca caacagaatg 660
tcaaagctgt gtttgatgct gctatcaagg tagtaatcca gccccccact aaacaaagag 720
aaaagaagaa aaagaaacag cgtcgggggat gttctatgat gaacttcagc ggaaggaaat 780
gctatgcttc aaatcctgaa tgatgaaaga gaagggttcct tgcctngaac gattgtcacg 840
gttgcgctgc accaatttga caacacctcc aaaccggttg aatgtgctgg attgcaccgt 900
c 901

<210> 49
<211> 594
<212> DNA
<213> Arabidopsis thaliana
<220>
<221> CDS
<222> (1)..(591)
<223> coding for RacB homologue
<400> 49
atg agc gct tcg agg ttc gta aag tgc gtg acg gtt ggt gat gga gct 48
Met Ser Ala Ser Arg Phe Val Lys Cys Val Thr Val Gly Asp Gly Ala
  1          5          10          15
gtc gga aaa act tgt ttg ttg att tct tac aca agc aac act ttc cct 96
Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
          20          25          30
acg gat tat gtg cct acc gtt ttc gat aat ttc agt gcc aat gtt gtg 144
Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
          35          40          45
gtt aat gga agc act gtg aat ctt gga ttg tgg gac act gca ggg caa 192
Val Asn Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
          50          55          60
gag gat tac aat aga tta aga cca ctg agt tac cgt gga gca gat gtt 240
Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
          65          70          75          80
ttc att ttg gcc ttc tct ctt atc agt aaa gcc agt tat gaa aac gtc 288
Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
          85          90          95
tcc aaa aag tgg atc ccg gag ttg aaa cat tac gcg cct ggt gtc ccc 336
Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
          100          105          110
atc gtc ctt gtt gga aca aag ctt gat ctt cga gat gat aaa cag ttc 384
Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
          115          120          125
ttt atc gac cat cct ggt gct gtt ccg att act act gct cag gga gag 432
Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
          130          135          140
gag ctg agg aag caa ata gga gca cct act tac atc gaa tgc agt tcc 480
Glu Leu Arg Lys Gln Ile Gly Ala Pro Thr Tyr Ile Glu Cys Ser Ser
          145          150          155          160
aaa act caa gag aat gtg aag gcg gtg ttt gac gca gcc atc cga gtg 528
Lys Thr Gln Glu Asn Val Lys Ala Val Phe Asp Ala Ala Ile Arg Val
          165          170          175
gtg ttg caa ccg cca aag cag aag aag aag aag agc aaa gcg cag aag 576
Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Lys Ser Lys Ala Gln Lys
          180          185          190

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gca tgc tcc att cta tga
Ala Cys Ser Ile Leu
195

<210> 50
<211> 197
<212> PRT
<213> Arabidopsis thaliana

<400> 50
Met Ser Ala Ser Arg Phe Val Lys Cys Val Thr Val Gly Asp Gly Ala
1 5 10 15
Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
20 25 30
Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
35 40 45
Val Asn Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
50 55 60
Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
65 70 75 80
Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
85 90 95
Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
100 105 110
Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
115 120 125
Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
130 135 140
Glu Leu Arg Lys Gln Ile Gly Ala Pro Thr Tyr Ile Glu Cys Ser Ser
145 150 155 160
Lys Thr Gln Glu Asn Val Lys Ala Val Phe Asp Ala Ala Ile Arg Val
165 170 175
Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Ser Lys Ala Gln Lys
180 185 190
Ala Cys Ser Ile Leu
195

<210> 51
<211> 594
<212> DNA
<213> Arabidopsis thaliana
<220>
<221> CDS
<222> (1)..(591)
<223> coding for RacB homologue

<400> 51
atg agc gct tgc agg ttc ata aag tgt gtc acc gtt ggc gac gga gct 48
Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
1 5 10 15
gtt ggt aaa acc tgt ttg ctg att tct tac acc agc aac act ttt cct 96
Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
20 25 30

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acg gat tat gta ccg act gtt ttc gat aac ttt agc gca aat gtg gtt 144
Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
      35                      40                      45

gtt aat gga gcc act gtg aat ctt ggg cta tgg gat acc gca ggg cag 192
Val Asn Gly Ala Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
      50                      55                      60

gag gat tat aac aga tta aga cct ttg agt tac cgc ggt gct gat gtt 240
Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
      65                      70                      75                      80

ttc atc tta gca ttc tct ctt atc agt aag gct agt tat gag aat gtc 288
Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
      85                      90                      95

tcc aag aag tgg atc cca gag ctg aag cat tat gcc cct ggt gtc cct 336
Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
      100                     105                     110

ata gtt ctt gtt gga acc aaa cta gat ctt cgg gat gac aaa cag ttc 384
Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
      115                     120                     125

ttc att gac cac cct ggc gct gta cca att act act gct cag gga gag 432
Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
      130                     135                     140

gaa ctg aag aaa cta att gga gct ccc gca tac atc gag tgc agt tca 480
Glu Leu Lys Lys Leu Ile Gly Ala Pro Ala Tyr Ile Glu Cys Ser Ser
      145                     150                     155                     160

aaa aca caa gag aac gtg aaa gga gta ttt gat gca gcg atc cga gtg 528
Lys Thr Gln Glu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Arg Val
      165                     170                     175

gtt ctt caa cct cca aag cag aag aaa aag aaa agc aaa gca caa aaa 576
Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Lys Ser Lys Ala Gln Lys
      180                     185                     190

gcc tgc tcc att ttg taa 594
Ala Cys Ser Ile Leu
      195

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<210> 52

<211> 197

<212> PRT

<213> Arabidopsis thaliana

<400> 52

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Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
  1           5           10           15

Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
      20           25           30

Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
      35           40           45

Val Asn Gly Ala Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
      50           55           60

Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
      65           70           75           80

Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
      85           90           95

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Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110
 Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 Glu Leu Lys Lys Leu Ile Gly Ala Pro Ala Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Glu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Arg Val
 165 170 175
 Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Ser Lys Ala Gln Lys
 180 185 190
 Ala Cys Ser Ile Leu
 195

<210> 53

<211> 594

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(591)

<223> coding for RacB homologue

<400> 53

atg agc gca tca agg ttc ata aag tgc gtc acc gtt ggt gat gga gct 48
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 gtt ggt aaa acc tgt ttg ctg att tct tat acc agc aac acc ttt ccc 96
 Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 acg gat tat gtt ccg act gtt ttc gat aac ttt agt gca aat gtg gtt 144
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45
 gtt aat gga gcc acg gtg aat ctt gga ttg tgg gat act gca ggg caa 192
 Val Asn Gly Ala Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 gag gac tat aac aga tta aga cct ttg agt tac cgt ggt gct gat gtt 240
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 ttc att ctt gcc ttc tct ctc att agt aag gct agt tat gag aat gtt 288
 Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95
 tcc aag aag tgg att cct gag ttg aag cac tat gct cct ggt gtc cca 336
 Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110
 att gtc ctt gtt gga acc aaa cta gat ctt cga gat gac aaa cag ttt 384
 Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 ttc atc gac cat cct ggt gct gtc cct att acc act gtt cag gga gag 432
 Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Val Gln Gly Glu
 130 135 140

30

gag ctg aag aag cta att gga gcg cca gct tac atc gag tgc agt tca 480
 Glu Leu Lys Lys Leu Ile Gly Ala Pro Ala Tyr Ile Glu Cys Ser Ser
 145 150 155 160

aaa tca caa gag aac gtg aag ggc gtg ttt gat gca gcg atc aga gtg 528
 Lys Ser Gln Glu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Arg Val
 165 170 175

gtc ctt caa cct cca aag cag aag aaa aag aag aac aaa gca caa aag 576
 Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Asn Lys Ala Gln Lys
 180 185 190

gcc tgc tcc atc ttg taa 594
 Ala Cys Ser Ile Leu
 195

<210> 54
 <211> 197
 <212> PRT
 <213> Arabidopsis thaliana

<400> 54
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45
 Val Asn Gly Ala Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95
 Ser Lys Lys Trp Ile Pro Glu Leu Lys His Tyr Ala Pro Gly Val Pro
 100 105 110
 Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Val Gln Gly Glu
 130 135 140
 Glu Leu Lys Lys Leu Ile Gly Ala Pro Ala Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Ser Gln Glu Asn Val Lys Gly Val Phe Asp Ala Ala Ile Arg Val
 165 170 175
 Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Asn Lys Ala Gln Lys
 180 185 190
 Ala Cys Ser Ile Leu
 195

<210> 55
 <211> 591
 <212> DNA
 <213> Arabidopsis thaliana
 <220>
 <221> CDS

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<222> (1)..(588)

<223> coding for RacB homologue

<400> 55

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atg agt gct tcg agg ttt ata aag tgt gtc acc gtc ggc gat ggt gcc 48
Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
  1           5           10           15

gtc gga aaa act tgt atg ctg att tct tac aca agc aac act ttc cct 96
Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
          20           25           30

acg gac tat gtt cca act gtt ttc gac aac ttc agt gct aat gtg gtt 144
Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
          35           40           45

gta gat ggg aac acg gtg aat ctt gga ttg tgg gat aca gct ggt caa 192
Val Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
          50           55           60

gaa gac tat aac agg tta aga ccg ttg agt tac cgt ggt gcc gat gtc 240
Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
          65           70           75           80

ttc att ctt gca ttc tcg ctt att agc aaa gct agc tac gag aat gta 288
Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
          85           90           95

gcc aag aag tgg att cct gag ctt agg cat tat gcc cct ggt gtt cct 336
Ala Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro
          100          105          110

ata atc ctc gtt gga acg aaa ctc gat ctt cga gat gac aag caa ttc 384
Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
          115          120          125

ttc ata gac cat cct ggt gca gtg cct att act aca aac cag gga gag 432
Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Asn Gln Gly Glu
          130          135          140

gaa cta aag aaa ctg ata gga tca cca atc tac att gaa tgt agt tca 480
Glu Leu Lys Lys Leu Ile Gly Ser Pro Ile Tyr Ile Glu Cys Ser Ser
          145          150          155          160

aag act cag cag aat gtg aaa gca gtc ttt gac gca gcc ata aaa gtg 528
Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile Lys Val
          165          170          175

gtg ctt cag cca ccg aaa cag aag aag aag aaa aag aac aag aac cgc 576
Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Lys Lys Asn Lys Asn Arg
          180          185          190

tgc gtg ttc ttg tga 591
Cys Val Phe Leu
          195

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<210> 56

<211> 196

<212> PRT

<213> Arabidopsis thaliana

<400> 56

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Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
  1           5           10           15

Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
          20           25           30

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Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45
 Val Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 Phe Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95
 Ala Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro
 100 105 110
 Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 Phe Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Asn Gln Gly Glu
 130 135 140
 Glu Leu Lys Lys Leu Ile Gly Ser Pro Ile Tyr Ile Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile Lys Val
 165 170 175
 Val Leu Gln Pro Pro Lys Gln Lys Lys Lys Lys Asn Lys Asn Arg
 180 185 190
 Cys Val Phe Leu
 195

<210> 57

<211> 597

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(594)

<223> coding for RacB homologue

<400> 57

atg agt gct tca agg ttt atc aag tgt gtc act gtc ggc gac ggt gct 48
 Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 gtt gga aag act tgt ctt ctc atc tcc tac act agc aac act ttc ccc 96
 Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 acg gat tat gtg cca act gtg ttc gat aat ttc agt gcc aat gtg att 144
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Ile
 35 40 45
 gtt gat ggc aac act atc aac ttg gga ttg tgg gat act gca ggg caa 192
 Val Asp Gly Asn Thr Ile Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 gag gac tac aat aga cta aga cct ttg agc tat cgc ggt gca gat gtc 240
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 ttc tta ctt gca ttc tca ctt gtc agc aaa gct agc tat gaa aat gtt 288
 Phe Leu Leu Ala Phe Ser Leu Val Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95

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tct aaa aag tgg gtt cct gaa ctg aga cat tat gct cct ggt gtt ccc 336
 Ser Lys Lys Trp Val Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro
 100 105 110

atc atc ctc gtt gga aca aag ctt gat ctt cga gat gat aag caa ttc 384
 Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125

ttt gcc gag cac cct ggt gct gtg cct atc tct acc gct cag ggt gaa 432
 Phe Ala Glu His Pro Gly Ala Val Pro Ile Ser Thr Ala Gln Gly Glu
 130 135 140

gaa cta aag aag ctg att ggg gcg cct gct tat atc gaa tgc agt gca 480
 Glu Leu Lys Lys Leu Ile Gly Ala Pro Ala Tyr Ile Glu Cys Ser Ala
 145 150 155 160

aaa act caa cag aat gtg aaa gca gtg ttt gat gcg gct atc aag gtc 528
 Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile Lys Val
 165 170 175

gtt ctc cag cca cca aaa aac aag aag aag aag aga aaa tct cag 576
 Val Leu Gln Pro Pro Lys Asn Lys Lys Lys Lys Arg Lys Ser Gln
 180 185 190

aaa ggt tgt tct ata ctc tga 597
 Lys Gly Cys Ser Ile Leu
 195

<210> 58
 <211> 198
 <212> PRT
 <213> Arabidopsis thaliana
 <400> 58

Met Ser Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15

Val Gly Lys Thr Cys Leu Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30

Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Ile
 35 40 45

Val Asp Gly Asn Thr Ile Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60

Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80

Phe Leu Leu Ala Phe Ser Leu Val Ser Lys Ala Ser Tyr Glu Asn Val
 85 90 95

Ser Lys Lys Trp Val Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro
 100 105 110

Ile Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125

Phe Ala Glu His Pro Gly Ala Val Pro Ile Ser Thr Ala Gln Gly Glu
 130 135 140

Glu Leu Lys Lys Leu Ile Gly Ala Pro Ala Tyr Ile Glu Cys Ser Ala
 145 150 155 160

Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile Lys Val
 165 170 175

Val Leu Gln Pro Pro Lys Asn Lys Lys Lys Lys Arg Lys Ser Gln
 180 185 190

Lys Gly Cys Ser Ile Leu
195

<210> 59

<211> 588

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(585)

<223> coding for RacB homologue

<400> 59

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atg gcg tca agg ttt ata aag tgt gtg acc gtc gga gat ggt gcc gtc      48
Met Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val
  1             5             10             15

gga aaa act tgc atg ctc att tct tac act agc aat act ttt cct act      96
Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro Thr
             20             25             30

gat tat gtg cca act gtt ttc gac aac ttc agt gct aat gtg gtt gtt      144
Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val Val
             35             40             45

gat ggc aac act gtc aat ctt gga ttg tgg gat act gct ggt caa gag      192
Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu
             50             55             60

gac tac aac agg tta cga cct ttg agt tac cgt ggt gct gat gtt ttc      240
Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe
             65             70             75             80

att ctt gct ttc tct ctt att agc aag gct agc tat gag aat ata gcc      288
Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Ile Ala
             85             90             95

aag aag tgg att cct gag ctc agg cat tat gct cct ggt gtt ccc att      336
Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro Ile
             100            105            110

atc ctt gtt ggg aca aaa ctc gat ctt cga gat gac aag caa ttc ttt      384
Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe Phe
             115            120            125

ata gat cat cct ggt gct gtg cca att act aca aac cag gga gag gaa      432
Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Asn Gln Gly Glu Glu
             130            135            140

ctg aag aaa ctg att gga tct gct gtc tac att gaa tgt agt tca aag      480
Leu Lys Lys Leu Ile Gly Ser Ala Val Tyr Ile Glu Cys Ser Ser Lys
             145            150            155            160

aca cag cag aac gtg aag gca gtg ttt gat gca gct ata aaa gtg gtg      528
Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile Lys Val Val
             165            170            175

ctt cag cca cca aag cag aag aag aag aaa aag aat aag aac cgt tgc      576
Leu Gln Pro Pro Lys Gln Lys Lys Lys Lys Lys Asn Lys Asn Arg Cys
             180            185            190

gcg ttc ttg tga
Ala Phe Leu
             195

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<210> 60
 <211> 195
 <212> PRT
 <213> Arabidopsis thaliana
 <400> 60
 Met Ala Ser Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala Val
 1 5 10 15
 Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro Thr
 20 25 30
 Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val Val
 35 40 45
 Asp Gly Asn Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln Glu
 50 55 60
 Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val Phe
 65 70 75 80
 Ile Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Ile Ala
 85 90 95
 Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala Pro Gly Val Pro Ile
 100 105 110
 Ile Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe Phe
 115 120 125
 Ile Asp His Pro Gly Ala Val Pro Ile Thr Thr Asn Gln Gly Glu Glu
 130 135 140
 Leu Lys Lys Leu Ile Gly Ser Ala Val Tyr Ile Glu Cys Ser Ser Lys
 145 150 155 160
 Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile Lys Val Val
 165 170 175
 Leu Gln Pro Pro Lys Gln Lys Lys Lys Lys Lys Asn Lys Asn Arg Cys
 180 185 190
 Ala Phe Leu
 195

<210> 61
 <211> 606
 <212> DNA
 <213> Arabidopsis thaliana
 <220>
 <221> CDS
 <222> (1)..(603)
 <223> coding for RacB homologue

<400> 61
 atg agc aca gca aga ttc att aag tgt gtg act gtc gga gat gga gca 48
 Met Ser Thr Ala Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 gtg gga aag act tgt atg ctc att tca tat acc agc aat acg ttt cct 96
 Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 acg gat tat gtt cca aca gtt ttc gac aac ttc agc gca aat gtg gtg 144
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45

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gtc gac ggg agt acc gtg aac ctt ggc ctg tgg gat act gcc ggt cag 192
 Val Asp Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 gaa gat tat aat agg ctt agg cct ttg agt tac aga gga gca gat gtc 240
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 ttc tta tta gca ttt tcc ctt ata agc aag gcc agt tac gag aat att 288
 Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Ile
 85 90 95
 cac aaa aag tgg ctt ccg gag ctg aaa cat tat gct cct gcc atc ccc 336
 His Lys Lys Trp Leu Pro Glu Leu Lys His Tyr Ala Pro Gly Ile Pro
 100 105 110
 att gtg ctc gtc gga aca aaa tta gat ttg agg gat gac aag cag ttc 384
 Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125
 ttg aag gat cat cca gga gca gct tct ata aca act gct cag gga gaa 432
 Leu Lys Asp His Pro Gly Ala Ala Ser Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 gaa tta agg aaa atg att gga gct gtt agg tac tta gag tgc agc tcc 480
 Glu Leu Arg Lys Met Ile Gly Ala Val Arg Tyr Leu Glu Cys Ser Ser
 145 150 155 160
 aaa acc caa cag aat gtg aag gca gtg ttt gat aca gcg ata agg gta 528
 Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Thr Ala Ile Arg Val
 165 170 175
 gct ttg agg cca cca aag gca aag aaa aag ata aaa cca ttg aag act 576
 Ala Leu Arg Pro Pro Lys Ala Lys Lys Lys Ile Lys Pro Leu Lys Thr
 180 185 190
 aag aga tca aga ata tgc ttt ttc cta taa 606
 Lys Arg Ser Arg Ile Cys Phe Phe Leu
 195 200

<210> 62

<211> 201

<212> PRT

<213> Arabidopsis thaliana

<400> 62

Met Ser Thr Ala Arg Phe Ile Lys Cys Val Thr Val Gly Asp Gly Ala
 1 5 10 15
 Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr Phe Pro
 20 25 30
 Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Val
 35 40 45
 Val Asp Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Val
 65 70 75 80
 Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Ile
 85 90 95
 His Lys Lys Trp Leu Pro Glu Leu Lys His Tyr Ala Pro Gly Ile Pro
 100 105 110
 Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gln Phe
 115 120 125

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Leu Lys Asp His Pro Gly Ala Ala Ser Ile Thr Thr Ala Gln Gly Glu
 130 135 140
 Glu Leu Arg Lys Met Ile Gly Ala Val Arg Tyr Leu Glu Cys Ser Ser
 145 150 155 160
 Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Thr Ala Ile Arg Val
 165 170 175
 Ala Leu Arg Pro Pro Lys Ala Lys Lys Lys Ile Lys Pro Leu Lys Thr
 180 185 190
 Lys Arg Ser Arg Ile Cys Phe Phe Leu
 195 200

<210> 63

<211> 606

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(603)

<223> coding for RacB homologue

<400> 63

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 Met Ala Ser Ser Ala Ser Lys Phe Ile Lys Cys Val Thr Val Gly Asp
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 ggt gcc gtt gga aaa act tgt atg ctc atc tgc tac act agc aac aaa 96
 Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys
 20 25 30
 ttc cct act gac tac ata cca aca gtt ttt gac aac ttt agt gtt aat 144
 Phe Pro Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Val Asn
 35 40 45
 gtt gtg gtt gaa ggc atc act gtg aac tta ggc ctt tgg gac act gcc 192
 Val Val Val Glu Gly Ile Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60
 ggg caa gaa gac tat aac aga cta agg cct tta agt tac aga gga gca 240
 Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75 80
 gat gtt ttt gtg ttg gct ttc tca ttg atc agc cga gct agc tat gag 288
 Asp Val Phe Val Leu Ala Phe Ser Leu Ile Ser Arg Ala Ser Tyr Glu
 85 90 95
 aat gtg ttt aaa aag tgg atc cct gaa ctc caa cac ttt gca cca gga 336
 Asn Val Phe Lys Lys Trp Ile Pro Glu Leu Gln His Phe Ala Pro Gly
 100 105 110
 gtc ccc att gtg ctt gtt ggt acc aaa atg gat ctt cgt gaa gat aga 384
 Val Pro Ile Val Leu Val Gly Thr Lys Met Asp Leu Arg Glu Asp Arg
 115 120 125
 cat tac ttg tct gat cat cct gga ctg tcc ccg gta act aca tca cag 432
 His Tyr Leu Ser Asp His Pro Gly Leu Ser Pro Val Thr Thr Ser Gln
 130 135 140
 gga gag gaa ctc cgc aag cat atc gga gcg act tat tac att gaa tgt 480
 Gly Glu Glu Leu Arg Lys His Ile Gly Ala Thr Tyr Tyr Ile Glu Cys
 145 150 155 160

agc tca aaa act caa cag aat gtg aaa gcc gta ttt gat gct gct att 528
 Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile
 165 170 175

aaa gta gta att aaa cca gca gtg aaa caa aag gag aag aag aag aag 576
 Lys Val Val Ile Lys Pro Ala Val Lys Gln Lys Glu Lys Lys Lys Lys
 180 185 190

cag aag cct cgc agc gga tgt ctc tcg taa 606
 Gln Lys Pro Arg Ser Gly Cys Leu Ser
 195 200

<210> 64

<211> 201

<212> PRT

<213> Arabidopsis thaliana

<400> 64

Met Ala Ser Ser Ala Ser Lys Phe Ile Lys Cys Val Thr Val Gly Asp
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Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys
 20 25 30

Phe Pro Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Val Asn
 35 40 45

Val Val Val Glu Gly Ile Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60

Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75 80

Asp Val Phe Val Leu Ala Phe Ser Leu Ile Ser Arg Ala Ser Tyr Glu
 85 90 95

Asn Val Phe Lys Lys Trp Ile Pro Glu Leu Gln His Phe Ala Pro Gly
 100 105 110

Val Pro Ile Val Leu Val Gly Thr Lys Met Asp Leu Arg Glu Asp Arg
 115 120 125

His Tyr Leu Ser Asp His Pro Gly Leu Ser Pro Val Thr Thr Ser Gln
 130 135 140

Gly Glu Glu Leu Arg Lys His Ile Gly Ala Thr Tyr Tyr Ile Glu Cys
 145 150 155 160

Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile
 165 170 175

Lys Val Val Ile Lys Pro Ala Val Lys Gln Lys Glu Lys Lys Lys Lys
 180 185 190

Gln Lys Pro Arg Ser Gly Cys Leu Ser
 195 200

<210> 65

<211> 648

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(645)

<223> coding for RacB homologue

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 1 5 10 15
 ggt gct gtt ggt aaa acc tgt atg ctc atc tgc tac acc agc aat aaa 96
 Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys
 20 25 30
 ttc ccc act gac tac ata cca aca gtt ttt gac aac ttt agt gca aat 144
 Phe Pro Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Ala Asn
 35 40 45
 gtt gtt gtt gaa ggc acc act gtc aat ttg ggg ctt tgg gac act gct 192
 Val Val Val Glu Gly Thr Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60
 ggg caa gaa gac tat aac aga tta agg cct tta agt tac agg gga gca 240
 Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75 80
 gat gtt ttc gtc ttg tct ttc tca tta gtc agc cga gct agc tac gag 288
 Asp Val Phe Val Leu Ser Phe Ser Leu Val Ser Arg Ala Ser Tyr Glu
 85 90 95
 aat gtt ttt aaa aag tgg atc cct gaa ctc caa cac ttt gct cca gga 336
 Asn Val Phe Lys Lys Trp Ile Pro Glu Leu Gln His Phe Ala Pro Gly
 100 105 110
 gtt ccc ctt gtc ctt gtt ggt acc aaa tta gat ctt cgt gaa gat aag 384
 Val Pro Leu Val Leu Val Gly Thr Lys Leu Asp Leu Arg Glu Asp Lys
 115 120 125
 cat tat ttg gct gat cat cct gga cta tcc cct gta act act gca cag 432
 His Tyr Leu Ala Asp His Pro Gly Leu Ser Pro Val Thr Thr Ala Gln
 130 135 140
 gga gag gag ttg cgt aag cta att ggt gcg acg tat tac att gag tgt 480
 Gly Glu Glu Leu Arg Lys Leu Ile Gly Ala Thr Tyr Tyr Ile Glu Cys
 145 150 155 160
 agt tca aaa act caa cag aat gtg aaa gca gtt ttt gat tct gcg ata 528
 Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ser Ala Ile
 165 170 175
 aag gaa gtg atc aaa cct ctg gtt aaa caa aag gag aag act aag aag 576
 Lys Glu Val Ile Lys Pro Leu Val Lys Gln Lys Glu Lys Thr Lys Lys
 180 185 190
 aag aag aag caa aag tcg aat cac ggc tgt tta tca aat gtt ctg tgt 624
 Lys Lys Lys Gln Lys Ser Asn His Gly Cys Leu Ser Asn Val Leu Cys
 195 200 205
 ggg agg ata gtg act cgg cat tga 648
 Gly Arg Ile Val Thr Arg His
 210 215
 <210> 66
 <211> 215
 <212> PRT
 <213> Arabidopsis thaliana
 <400> 66
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 1 5 10 15
 Gly Ala Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys
 20 25 30

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Phe Pro Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Ala Asn
 35 40 45
 Val Val Val Glu Gly Thr Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60
 Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75 80
 Asp Val Phe Val Leu Ser Phe Ser Leu Val Ser Arg Ala Ser Tyr Glu
 85 90 95
 Asn Val Phe Lys Lys Trp Ile Pro Glu Leu Gln His Phe Ala Pro Gly
 100 105 110
 Val Pro Leu Val Leu Val Gly Thr Lys Leu Asp Leu Arg Glu Asp Lys
 115 120 125
 His Tyr Leu Ala Asp His Pro Gly Leu Ser Pro Val Thr Thr Ala Gln
 130 135 140
 Gly Glu Glu Leu Arg Lys Leu Ile Gly Ala Thr Tyr Tyr Ile Glu Cys
 145 150 155 160
 Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ser Ala Ile
 165 170 175
 Lys Glu Val Ile Lys Pro Leu Val Lys Gln Lys Glu Lys Thr Lys Lys
 180 185 190
 Lys Lys Lys Gln Lys Ser Asn His Gly Cys Leu Ser Asn Val Leu Cys
 195 200 205
 Gly Arg Ile Val Thr Arg His
 210 215

<210> 67

<211> 630

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(627)

<223> coding for RacB homologue

<400> 67

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 1 5 10 15
 gtt ggg aag aca tgt atg ctt atc tgt tac act agc aac aag ttt cct 96
 Val Gly Lys Thr Cys Met Leu Ile Cys Tyr Thr Ser Asn Lys Phe Pro
 20 25 30
 act gat tat ata ccg act gtg ttc gac aat ttc agt gcc aat gta gct 144
 Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Ala
 35 40 45
 gtg gat gga caa atc gtt aat tta ggg cta tgg gac act gcc ggt caa 192
 Val Asp Gly Gln Ile Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln
 50 55 60
 gaa gat tac agt agg tta aga cca ttg agt tat aga gga gct gat atc 240
 Glu Asp Tyr Ser Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Ile
 65 70 75 80

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ttc gtc tta gcc ttt tcg ctt att agc aag gcg agt tac gaa aat gta	288
Phe Val Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val	
85 90 95	
ctc aag aag tgg atg cct gaa ctt cgt cgg ttt gcg cca aat gtt ccc	336
Leu Lys Lys Trp Met Pro Glu Leu Arg Arg Phe Ala Pro Asn Val Pro	
100 105 110	
ata gtt ctt gtt ggt aca aag cta gat ctc cgg gat gac aag gga tac	384
Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gly Tyr	
115 120 125	
ctc gcg gat cac acc aat gtc att acc tct act cag gga gag gaa ttg	432
Leu Ala Asp His Thr Asn Val Ile Thr Ser Thr Gln Gly Glu Glu Leu	
130 135 140	
agg aag caa att ggt gca gct gct tat att gag tgt agt tcc aag act	480
Arg Lys Gln Ile Gly Ala Ala Tyr Ile Glu Cys Ser Ser Lys Thr	
145 150 155 160	
caa caa aat gtg aaa gca gtg ttt gat aca gcg atc aag gtg gtt ctt	528
Gln Gln Asn Val Lys Ala Val Phe Asp Thr Ala Ile Lys Val Val Leu	
165 170 175	
cag cct cca agg agg aaa gag gtc ccg agg agg agg aag aat cat aga	576
Gln Pro Pro Arg Arg Lys Glu Val Pro Arg Arg Arg Lys Asn His Arg	
180 185 190	
aga tcc ggt tgc tcc att gcg agt att gtc tgt gga ggt tgc acc gct	624
Arg Ser Gly Cys Ser Ile Ala Ser Ile Val Cys Gly Gly Cys Thr Ala	
195 200 205	
gct taa	630
Ala	
<210> 68	
<211> 209	
<212> PRT	
<213> Arabidopsis thaliana	
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20 25 30	
Thr Asp Tyr Ile Pro Thr Val Phe Asp Asn Phe Ser Ala Asn Val Ala	
35 40 45	
Val Asp Gly Gln Ile Val Asn Leu Gly Leu Trp Asp Thr Ala Gly Gln	
50 55 60	
Glu Asp Tyr Ser Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala Asp Ile	
65 70 75 80	
Phe Val Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu Asn Val	
85 90 95	
Leu Lys Lys Trp Met Pro Glu Leu Arg Arg Phe Ala Pro Asn Val Pro	
100 105 110	
Ile Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys Gly Tyr	
115 120 125	
Leu Ala Asp His Thr Asn Val Ile Thr Ser Thr Gln Gly Glu Glu Leu	
130 135 140	

Arg Lys Gln Ile Gly Ala Ala Ala Tyr Ile Glu Cys Ser Ser Lys Thr
 145 150 155 160
 Gln Gln Asn Val Lys Ala Val Phe Asp Thr Ala Ile Lys Val Val Leu
 165 170 175
 Gln Pro Pro Arg Arg Lys Glu Val Pro Arg Arg Arg Lys Asn His Arg
 180 185 190
 Arg Ser Gly Cys Ser Ile Ala Ser Ile Val Cys Gly Gly Cys Thr Ala
 195 200 205

Ala

<210> 69

<211> 600

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(597)

<223> coding for RacB homologue

<400> 69

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 1 5 10 15
 tgt gtc act gtt ggc gat gga gct ctt ttg gtg act gtt gag atc ttg 96
 Cys Val Thr Val Gly Asp Gly Ala Leu Leu Val Thr Val Glu Ile Leu
 20 25 30
 tta tta cag gat tat gtt cca aca gtg ttc gac aat ttc aat gct aat 144
 Leu Leu Gln Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Asn Ala Asn
 35 40 45
 gtt tta gtc gat ggt aaa act gtc aat ctg ggt ctc tgg gat act gct 192
 Val Leu Val Asp Gly Lys Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60
 ggt caa gaa gac tac aat agg gtt aga cca ttg agt tac aga gga gca 240
 Gly Gln Glu Asp Tyr Asn Arg Val Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75 80
 gat gtt ttc att ctt gcc ttc tca ctt att agc agg cct agc ttt gag 288
 Asp Val Phe Ile Leu Ala Phe Ser Leu Ile Ser Arg Pro Ser Phe Glu
 85 90 95
 aac att gct aaa aag tgg gta ccc gag ctg aga cat tat gcc ccg act 336
 Asn Ile Ala Lys Lys Trp Val Pro Glu Leu Arg His Tyr Ala Pro Thr
 100 105 110
 gtg cct att gtt ctt gtg gga acc aaa tca gat cta aga gac aac atg 384
 Val Pro Ile Val Leu Val Gly Thr Lys Ser Asp Leu Arg Asp Asn Met
 115 120 125
 cag ttc cca aag aat tat cca ggt gct tgc aca atc ttc cca gaa cag 432
 Gln Phe Pro Lys Asn Tyr Pro Gly Ala Cys Thr Ile Phe Pro Glu Gln
 130 135 140
 ggt caa gaa cta aga aag gaa ata gga gca tta gca tac ata gag tgc 480
 Gly Gln Glu Leu Arg Lys Glu Ile Gly Ala Leu Ala Tyr Ile Glu Cys
 145 150 155 160

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agc tca aaa gca caa atg aac gta aaa gcc gtg ttt gat gaa gcg atc 528
 Ser Ser Lys Ala Gln Met Asn Val Lys Ala Val Phe Asp Glu Ala Ile
 165 170 175

aaa gta gtt tta cat cct cct tca aag act aag aag cga aag aga aag 576
 Lys Val Val Leu His Pro Pro Ser Lys Thr Lys Lys Arg Lys Arg Lys
 180 185 190

atc ggt tta tgc cat gtt ctt tga 600
 Ile Gly Leu Cys His Val Leu
 195

<210> 70

<211> 199

<212> PRT

<213> Arabidopsis thaliana

<400> 70

Met Ala Ala Thr Ser Thr Ser Ser Ala Thr Ala Thr Thr Phe Ile Lys
 1 5 10 15

Cys Val Thr Val Gly Asp Gly Ala Leu Leu Val Thr Val Glu Ile Leu
 20 25 30

Leu Leu Gln Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Asn Ala Asn
 35 40 45

Val Leu Val Asp Gly Lys Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
 50 55 60

Gly Gln Glu Asp Tyr Asn Arg Val Arg Pro Leu Ser Tyr Arg Gly Ala
 65 70 75 80

Asp Val Phe Ile Leu Ala Phe Ser Leu Ile Ser Arg Pro Ser Phe Glu
 85 90 95

Asn Ile Ala Lys Lys Trp Val Pro Glu Leu Arg His Tyr Ala Pro Thr
 100 105 110

Val Pro Ile Val Leu Val Gly Thr Lys Ser Asp Leu Arg Asp Asn Met
 115 120 125

Gln Phe Pro Lys Asn Tyr Pro Gly Ala Cys Thr Ile Phe Pro Glu Gln
 130 135 140

Gly Gln Glu Leu Arg Lys Glu Ile Gly Ala Leu Ala Tyr Ile Glu Cys
 145 150 155 160

Ser Ser Lys Ala Gln Met Asn Val Lys Ala Val Phe Asp Glu Ala Ile
 165 170 175

Lys Val Val Leu His Pro Pro Ser Lys Thr Lys Lys Arg Lys Arg Lys
 180 185 190

Ile Gly Leu Cys His Val Leu
 195

<210> 71

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence:
 oligonucleotide primer

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<210> 72

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence:
oligonucleotide primer

<400> 72

atcaaacacg cccttcacgt t

21

<210> 73

<211> 10828

<212> DNA

<213> Artificial sequence

<220>

<223> Description of the artificial sequence: transgenic
expression vector pSUN3NIT_AtRacB_s for expression
of A-thalianan RacB sense RNA

<400> 73

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