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(54) Titre : PROCEDE D'AUGMENTATION DE LA RESISTANCE AUX CHAMPIGNONS DANS LES PLANTES
(54) Title: METHODS FOR INCREASING THE RESISTANCE IN PLANTS TO BIOTROPIC FUNGI

(57) Abrégé/Abstract:
The present invention relates to methods for generating or increasing resistance in a plant or a part of a plant to at least one biotrophic pathogen by increasing the amount of protein or function of at least one Bax inhibitor 1 (BI-1) protein in at least one part of the plant. The invention additionally relates to polypeptide and nucleic acid sequences which code for a BI-1 protein, and expression cassettes, vectors and organisms which include such sequences or such a protein.

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(54) **Title:** METHODS FOR INCREASING THE RESISTANCE IN PLANTS TO BIOTROPIC FUNGI

(54) **Bezeichnung:** VERFAHREN ZUR ERHÖHUNG DER RESISTENZ GEGEN BIOTROPHE PILZE IN PFLANZEN

(57) **Abstract:** The present invention relates to methods for generating or increasing resistance in a plant or a part of a plant to at least one biotrophic pathogen by increasing the amount of protein or function of at least one Bax inhibitor 1 (BI-1) protein in at least one part of the plant. The invention additionally relates to polypeptide and nucleic acid sequences which code for a BI-1 protein, and expression cassettes, vectors and organisms which include such sequences or such a protein.

(57) **Zusammenfassung:** Die vorliegende Erfindung betrifft Verfahren zur Erzeugung oder Erhöhung von Resistenz gegen mindestens ein biotrophes Pathogen in einer Pflanze oder einem Teil einer Pflanze durch Erhöhung der Proteinmenge oder Funktion mindestens eines Bax Inhibitor-1 (BI-1) Proteins in mindestens einem Teil der Pflanze. Die Erfindung betrifft zudem Polypeptid- und Nukleinsäuresequenzen, die für ein BI-1 Protein codieren, und Expressionskassetten, Vektoren und Organismen, die solche Sequenzen oder ein solches Protein umfassen.

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METHODS FOR INCREASING THE RESISTANCE IN PLANTS TO BIOTROPIC FUNGI

The present invention relates to methods for generating or increasing resistance to at least one biotrophic pathogen in a plant or a part of a plant by increasing the protein quantity or function of at least one Bax inhibitor-1 (BI-1) protein in at least one part of the plant. Moreover, the invention relates to polypeptide sequences and nucleic acid sequences which code for a BI-1 protein, and to expression cassettes, vectors and organisms which comprise such sequences or such a protein, in particular to recombinant plants, to cultures, parts or recombinant propagation material derived there from, and to the use of same for the production of foodstuffs, feeding stuffs, seed, pharmaceuticals or fine chemicals.

The cultivation of agricultural crop plants serves mainly for the production of foodstuffs for humans and feedingstuffs for animals. The last 25 years have seen pronounced yield increases in crop production. This was the result of a good combination of altered production techniques, newly developed varieties, fertilization and, last but not least, increased crop protection. In the light of an ever increasing world population, safeguarding food production gains increasingly in importance. It has been estimated that 7 billion people will inhabit Earth in 2010. To feed all these people, without the proportion of malnourished people increasing, food production would have to be increased by 60% (Entrup N.L. et al., Lehrbuch des Pflanzenbaues [Textbook of crop production], Thomas Mann Verlag, Gelsenkirchen, 2000). Efficient crop protection is a decisive factor in this context. Monocultures in particular, which are the rule nowadays, are highly susceptible to an epidemic-like spreading of diseases. The result are markedly reduced yields. To date, the pathogenic organisms have been controlled mainly by using pesticides. Nowadays, in contrast, the possibility of directly modifying the genetic disposition of a plant or pathogen is open to man.

Fungi are distributed worldwide so they may form a heterogeneous group with a range of species. They are eukaryotes, do not contain chlorophyll and are therefore heterotrophic. Hence, they rely on external carbon sources which they tap as parasites, saprophytes or symbionts. Saprophytes live exclusively on dead plant material. Parasitic fungi feed on live tissue and must have concluded their development before the plant has died. Facultative parasites can feed both on live and on dead tissue. Symbionts, such as mycorrhiza, live in close association with the plants. Fungi have one or more nuclei per cell and are homokaryotic or heterokaryotic. Fungi have a firm cell wall during at least one stage in their life history. This cell wall usually consists of chitin or, in some cases such as the Oomycota, of cellulose. The vegetative part of the fungus (thallus) is usually haploid, in rare cases diploid. The thallus of

lower fungi (Myxomycota, inter alia) consists of ameboidal cells or plasmodia (naked, polynuclear protoplasma). Eumycota have budding cells, as in the case of yeasts (for example Saccharomyces cerevisiae), or form a mycelium which consists of threadlike hyphae. As the result of hyphal aggregation, specific organs for propagation (fruiting bodies) or for surviving unfavorable environmental conditions (sclerotia) may be formed. Propagation and multiplication is usually by way of spores; asexually by means of conidia, uredospores, sporangiospores, chlamydospores and zoospores, and sexually with oospores, ascospores, zygospores and basidiospores.

Approximately 100 000 different fungal species are known to date. Among these, however, only 5% are plant pathogens. The Basidiomycota are a division of the true fungi which are characterized by the development of a particular structure, the basidium, on which the basidiospores mature. The Basidiomycota also include the generally known mushrooms. The Basidiomycota are predominantly heterothallic and self-sterile. Mating occurs by somatogamy. During somatogamy, two compatible, haploid, mononuclear mycelia or sporidia coalesce. The resulting dikaryotic mycelium constitutes the dominant phase of the life cycle over a prolonged period. The only two phytopathogenic genera of the Basidiomycota are the smuts (Ustilages) and the rusts (Uredinales). Smuts only attack angiosperms and use to be of great economical importance. Nowadays they are controlled successfully by suitable active substances and tight seed control. The rusts are still somewhat more important nowadays. They are biotrophic and can have a complicated development cycle with up to five different spores stages (spermatium, aecidiospore, uredospore, teleutospore and basidiospore). Rusts which develop all spore stages are referred to as macrocyclic rusts. If some stages are absent, these rusts are referred to as being macrocyclic. "Imperfect rusts" lack the basidiospores. Some rusts change their hosts during their development. These are referred to as heteroecious. Host alternation can be linked to nuclear-phase alternation. In contrast, autoecious rusts complete all of their development on one host. A traditional example of a macrocyclic heteroecious rust is black rust of cereals, Puccinia graminis. P. graminis, in its dikaryotic stage, attacks predominantly wheat. The haplont is pathogenic to barberry (Börner H., Pflanzenkrankheiten und Pflanzenschutz [Plant disease and plant protection], Ulmer Verlag Stuttgart, 1997; Sitte P. et al., Strasburger - Lehrbuch der Botanik [Textbood of Botany], Gustav Fischer Verlag, Stuttgart, 1998; Entrup N.L. et al., Lehrbuch des Pflanzenbaues [Textbook of crop production], Thomas Mann Verlag, Gelsenkirchen, 2000).

During the infection of plants by pathogenic fungi, different phases are usually observed. The first phases of the interaction between phytopathogenic fungi and their potential host plants are

decisive for the colonization of the plant by the fungus. During the first stage of the infection, the spores become attached to the surface of the plants, germinate, and the fungus penetrates the plant. The attachment of the spores requires either an active metabolism, which is the case in Colletotrichum graminicola, or it is passive, as is the case with Magnaporthe grisea. In the latter case, moisture leads to the secretion of a "mucilage adhesive", by means of which the spore attaches (Howard R.J. et al., Annu. Rev. Microbiol. 50, 491 (1996)). Spore germination is induced either by unspecific inductors such as water, nutrients, ethylene or, more rarely, as is the case in Phyllosticta ampelicida, by hydrophobic surfaces (Kuo K. et al., Fungal Genet. Biol. 20, 18 (1996)). Some fungi develop two germ tubes, as is the case in powdery mildew cereals, Blumeria graminis, while other fungi develop only one germ tube (Green J.R. et al., The powdery mildews, a comprehensive treatise; The formation and function of infection and feeding structures, APS Press, 2002). Fungi may penetrate the plant via existing ports such as stomata, lenticels, hydatodes and wounds, or else they penetrate the plant epidermis directly as the result of the mechanical force and with the aid of cell-wall-digesting enzymes. Specific infection structures are developed for penetration of the plant. These pressure organs are referred to as appressoria and allow the fungus to build up a high pressure above a discrete point. It is estimated that appressorium of M. grisea reaches a pressure of 80 bar (Howard R.J. et al., Annu. Rev. Microbiol. 50, 491 (1996)). Most rusts, in contrast, penetrate the plant via the stomata. The soya rust Phakopsora pachyrhizi directly penetrates the plant epidermis and therefore resembles powdery mildew of cereals, B. graminis, in its penetration behavior (Koch E. et al., Phytopath. p. 106, 302 (1983); Tucker S.L. et al., Annu. Rev. Phytopathol. 39, 385 (2001); Green J.R. et al., The powdery mildews, a comprehensive treatise; The formation and function of infection and feeding structures, APS Press, 2002).

Phytopathogenic fungi do not always colonize all of the plant; in contrast, sometimes it is only specific areas or tissues which are colonized. Following the successful invasion of the plant, phytopathogenic fungi follow different nutritional strategies. Pertotrophic or necrotrophic pathogens kill the host cells by means of extracellular enzymes or toxins and feed by degrading the dead cells. Some genera with pertotrophic nutrition are the Fusaria sp., Alternaria sp. and Cochliobolus. Most fungi use this feeding strategy. The biotrophic phytopathogenic fungi, such as mildew and many rusts, depend, for their nutrition, on the metabolism of live cells. An intermediate position is occupied by the hemibiotrophic pathogenic fungi, which include the genera Phythophtora and Peronospora. Most of these are biotrophs at the beginning of their development and only change over to a pertotrophic lifestyle during the later stages of their development (Prell H.H., Interaktionen von Pflanzen und phytopathogenen Pilzen [Interactions between plants and phytopathogenic fungi], Gustav Fischer Verlag, Jena, 1996). The plants

have developed defense mechanisms to avoid infection. Another intermediate position is occupied for example by soybean rust, which penetrates the epidermis directly, whereupon the penetrated cell becomes necrotic; after the penetration, the fungus changes over to an obligatory-biotrophic lifestyle. The subgroup of the biotrophic fungal pathogens which follows essentially such an infection strategy will, for the purposes of the present description, be referred to as being "heminecrotrophic".

It must be emphasized that plants, during their development, are exposed to constant attack by a large number of phytopathogenic organisms. Nevertheless, colonization of the plant by phytopathogenic organisms is the exception rather than the rule. Before a pathogen can attack the plant, it has to overcome a series of barriers. Frequently, the pathogen has developed specific pathogenicity factors which are adapted to the plant, known as virulence factors, in order to overcome these barriers. In such a case, the plant becomes a host plant for the pathogen, i.e. the latter is virulent on the plant. There is a basic compatibility between the plant and the pathogen. This means that the physiological and biochemical prerequisites which are required for the colonization are already in existence or have been produced (Prell H.H., Interaktionen von Pflanzen und phytopathogenen Pilzen [Interactions between plants and phytopathogenic fungi], Gustav Fischer Verlag, Jena, 1996). In the case of compatible interaction, the pathogen develops at the expense of the plant, thus causing the formation of disease symptoms such as wilting, necroses and chloroses. However, if basic compatibility exists, the plant can still defend itself against the pathogen when a resistance mutation has taken place in the plant. The resistance of the plant, thus acquired, is referred to as host resistance. It is only directed against a certain individual pathogen and can be overcome readily by the latter. The pathogens in question are mostly, but not always, biotrophic pathogens. Host resistance can be subdivided into non-race-specific horizontal resistances which, in most cases, involves several genes, and race-specific vertical resistance. The latter is only effective against certain, individual races of a pathogen, while the plant defends itself *a priori* against most pathogens. This phenomenon is referred to as basic incompatibility or non-host resistance. In contrast to host resistance, non-host resistance is based on a series of causes and not on individual genes. Firstly, the pathogen may lack the necessary pathogenicity factors, or else the plant is capable of recognizing, and successfully defending itself, against the pathogen. Another term which is important in particular for agriculture is tolerance. A plant is tolerant to a pathogen when it can be attacked, but the attack does not lead to the development of disease systems and yield reduction (Prell H.H., Interaktionen von Pflanzen und phytopathogenen Pilzen [Interactions between plants and phytopathogenic fungi], Gustav Fischer Verlag, Jena, 1996).

For the purposes of the description of the present invention, generating, or increasing, a

resistance is to comprise an increase or generation of any type of resistance, but also of tolerance, i.e. in particular all cases of an increased tolerance or resistance which lead to reduced yield losses as caused by the pathogen.

In connection with resistance responses of plants, the term resistance factors includes structures, substances and processes which prevent or inhibit attack of the plant by potential pathogens. If the resistance factors are already constitutively present in the plant, they are referred to as pre-formed resistance factors. Induced resistance factors are only formed when a recognition response between the plant and the potential pathogen has taken place. Recognition can be described as a signal/sensor response (elicitor/receptor model, Keen N.T. et al., Phytopathology 62, 768 (1972)). The signal are plant substances or substances produced by the pathogen, known as elicitors, which bind to a sensor or receptor which is specific for the elicitor in question. This binding triggers one or more effectors, which may be, for example, signal transduction chains and induce the resistance response. A whole series of substances act as elicitors. These include proteins, glycoproteins, glucans and lipids (Garcia Brugger et al., MPMI 19, 711 (2006)). Plant cell wall degradation products which are released by enzymes of the pathogen or else by wounding of the plant may also induce a resistance response. In this context, an avirulence factor of the pathogen and the corresponding resistance gene of the plant is frequently mentioned ("Gene-for-gene hypothesis", Flor, J. Agric. Res. 74, 241 (1947)). The pathogen can prevent recognition by the plant by means of structural modifications of the elicitor, masking of the recognition sequence or by competition with another substance for the binding sites on the elicitor or receptor.

Preformed resistance factors form the first defense against colonization by pathogenic organisms. These factors can be morphological factors or else substances of the secondary plant metabolism (phytoanticipins). Morphological factors which prevent colonization are hairy leaves, stomatal density and shape, and the nature of the cuticle and of the cell wall.

Recognition, of the pathogen, by the plant may also lead to the induction of resistance factors, i.e. morphological and physiological resistance responses. Many of these responses are the result of a signal cascade. Signal molecules such as Ca^{2+}, NO, reactive oxygen compounds and phytohormones such as ethylene and jasmonate are involved in the cascades and contribute to the crosslinking of the signal pathways. Resistance responses in which morphological structures in the plant cell are modified are the formation of cell wall appositions (papillae), cork and abscission layers, thyllae and the impregnation of the cell wall. The beginning of penetration by a pathogenic fungus can trigger the formation of papillae. Lignin, callose, suberin

and hydroxyproline-rich proteins are deposited at the inner cell wall opposite the potential penetration site and are crosslinked with one another. Callose can be stained by the intercalation of aniline blue. In addition, the papilla formed accumulates phenols, reactive oxygen species and hydrolases (Hückelhoven R. et al., Plant Physiol. 119, 1251 (1999); Assaad F.F. et al., Mol. Biol. of the Cell, 15, 5118 (2004)). The development of papillae leads to a substantially thickened cell wall and may prevent penetration of the pathogenic fungus. Physiological processes which contribute to induced resistance are depolarization of the cell membrane, the oxidative burst, the hypersensitive reaction, the formation of phytoalexins and the expression of pathogenesis-related proteins (PR proteins). One of the first responses to contact with an elicitor is the depolarization of the cell membrane. This results in a pronounced efflux of Cl^- and K^+ ions, linked with pronounced water loss. It is assumed that depolarization triggers an increased Ca^{2+} concentration, which is an important signal molecule (Ward J.M. et al., Plant Cell 7, 833 (1995)) and plays a role in the hypersensitive reaction (HR) (Wendehenne D. et al., Plant Cell 14, 1937 (2002)). HR in plants is a form of programmed cell death. It allows the plant to stop the fungus even after penetration of the latter by denying it a source of nutrients. The course of HR appears to depend on the combination of plant and pathogen. However, protein biosynthesis, an intact cytoskeleton and salicylic acid appear to be necessary for inducing HR (Heath M., Plant Mol. Biol. 44, 321 (2000)).

A very rapid response to the pathogen is the oxidative burst, the formation of reactive oxygen species, such as the superoxide anion O_2^-, the hydroxyl radical OH and hydrogen peroxide. These compounds are formed by various oxidases. The hydroxyl radical acts locally, while H_2O_2 can diffuse via the membranes. Both oxidize polyunsaturated fatty acids and can thus destroy membranes (Grant J.J. et al., Plant Physiol. 124, 21 (2000)). H_2O_2 is also suspected of performing a function in gene regulation. In addition, the compounds support the defense responses by crosslinking the cell wall components, by increasing lignification and by exerting a toxic effect on pathogens (Garcia–Brugger A. et al., MPMI 19, 711 (2006)). Last but not least, the pathogen attack leads to the expression of genes which code for PR proteins and for phytoalexins. PR proteins are a heterogeneous group of proteins which have a toxic effect on penetrating fungi. The term phytoalexins refers to low-molecular-weight antimicrobially active substances whose synthesis is triggered by biotic or abiotic stress (Prell H.H., Interaktionen von Pflanzen und phytopathogenen Pilzen [Interactions between plants and phytopathogenic fungi], Gustav Fischer Verlag, Jena, 1996; van Loon L.C. et al., Physiol. Mol. Plant Physiol. 55, 85 (1999)). The responses described proceed partly not only when the pathogen interact with a host plant, but also when it reacts with a non-host-plant. Decisive for pathogen defense is the

quality of the recognition and the quantity and speed of the resistance response (Thordal-Christensen H., Current Opinion in Plant Biology 6, 351 (2003)).

A plant disease which has become increasingly important in recent times is soybean rust. The disease is caused by the pathogenic rusts Phakopsora pachyrhizi (Sydow) and Phakopsora meibomiae (Arthur). They belong to the class Basidiomycota, order Uredinales, family Phakopsoraceae. The two species are very closely related with one another. The intergenic sequences of their rRNA genes show 80% similarity (Frederick R.D. et al., Phytopathology 92, 217 (2002)). The species are distinguished by morphological characteristics of the teliospores (Ono Y. et al., Mycol. Res. 96, 825 (1992)). Both rusts infect a wide spectrum of host plants. P. pachyrhizi, also referred to as Asian soybean rust, is the more aggressive pathogen on soybeans (Glycine max), and is therefore, at least currently, of great importance for agriculture. P. pachyrhizi is capable of infecting 31 species from 17 families of the Leguminosae under natural conditions and is capable of growing on further 60 species under controlled conditions (Sinclair et al. (eds.), Proceedings of the soybean rust workshop (1995), National Soybean Research Laboratory, Publication No. 1 (1996); Rytter J.L. et al., Plant Dis. 87, 818 (1984)). P. meibomiae has been found in the Caribbean Basin and in Puerto Rico, and has not caused substantial damage as yet.

P. pachyrhizi was originally discovered in Japan in 1902. From there, P. pachyrhizi spread over large parts of Asia and over India and Australia and, finally, reached Africa in 1996. In 2001, the fungus arrived in South America and reached America for the first time in 2004 (Sconyers E.L. et al., www.ers.usda.gov/Features/SoyBeanRust/ (2005)). In South America in particular, P. pachyrhizi caused big yield losses of up to 80%. It is estimated that 1.5 million tons of the Brazilian soybean harvest 2005/2006 alone have succumbed to infection with soybean rust. P. pachyrhizi is a hemicyclic rust which forms three types of spores. The formation of teliospores was observed in Asia towards the end of the vegetation period (Yeh C.C. et al., Phytopathology 71, 1111 (1981)). The formation of basidiospores, in contrast, is only known under laboratory conditions. The most important spore form are the uredospores, which are formed over the entire vegetation period and which serve to spread the disease. These spores are formed in large amounts and are capable of spreading over wide distances with the aid of wind and rain. P. pachyrhizi is an obligate biotroph. If a uredospore arrives on a suitable host, it germinates with a single germ tube. At the end of this germ tube, an appressorium develops and rapidly reaches the size of the spore. With the appressorium, the fungus is capable of building up a large pressure and of penetrating the epidermal cells directly with the aid of a penetration hypha. The penetration hypha of P. pachyrhizi grows through the epidermal cell

and, once it reaches the intercellular space in the leaf, forms the first septum. It now continues to grow in the leaf as a primary hypha. As early as 24-48 h after the infection, the first haustorial mother cell is divided by a septum, and a sacciform haustorium is formed in a mesophyll cell of the leaf. The cell wall of the mesophyll cell is penetrated, but the plasmalemma is only folded, so that the cell remains alive and can act as a nutrient source. The nutrients travel from the membrane of the live host cell via the extrahaustorial matrix to the haustorium. The epidermal cell which has been penetrated at the beginning turns necrotic shortly after penetration. This manner of infection of a biotrophic pathogen, as is used by P. pachyrhizi, will therefore be referred to as "heminecrotrophic" for the purposes of the description of the present invention. The first uredospores are found only 11-12 days after the infection, and the cycle can start afresh (Koch E. et al., Phytopath, p. 106, 302 (1983)).

The crop plant soybean Glycine max (L.) Merr. belongs to the family Leguminosae, subfamily Papilionoideae, tribe Phaseoleae, genus Glycine Willd. and subgenus soja (Moench). Soybean is planted in more than 35 countries. Some of the most important production areas are located in the United States, China, Korea, Argentina and Brazil. It is considered to be one of the oldest crop plants and was domesticated for the first time in China between the 11th and 17th century (Hymowitz T., Econ. Bot. 24, 408 (1970)). It was introduced into the United States in 1765; the United States are currently one of the largest soya production areas. Wild soybean species can be found in China, Korea, Japan, Taiwan and the former USSR. Morphological, cytological and molecular evidence suggests that G. soja is the ancestor of the cultivated form G. max. Being a subtropical plant, soybeans prefer a mean annual temperature of 5.9-27°C; they are not frost resistant (OECD, Consensus document on the biology of Gycine max (L.) Merr. (Soybean); Series on harmonization of regulatory oversight in biotechnology No. 15, ENV/JM/MONO(2000)9). Soybeans are currently an important oil and protein source. This extensive use of soya in food production underlines the importance of efficient control of soybean rust.

Soybean plants are infected by P. pachyrhizi by windborne uredospores. The first discernible symptoms are small yellow to reddish-brown lesions on the upper surface of the leaf, which later spread further until all of the leaf finally turns chlorotic and dies. Upon advanced infection, the lesions are found on all of the plant. The first uredia have a diameter of 100-200 µm and are found on the underside of the leaf 10-14 days after the infection; they can produce spores for three to six weeks. Telia are formed subepidermally and mostly occur on the periphery of the lesions. The spores are first yellow to brown and later turn black. The first symptoms are frequently first observed on the older leaves. The rapid development of the disease correlates

with the beginning of flowering (R1+) and finally destroys all of the foliage. The fact that most of the photosynthetically active area is destroyed and that water and nutrients are extracted by the fungus leads to reduced productivity of the plant (Sconyers E.L. et al., www.ers.usda.gov/Features/SoyBeanRust/ (2005)).

In order to germinate, P. pachyrhizi requires moisture in the form of dew or the like on the upper surface of the leaf. The fungus is encouraged in particular by frequent rain and temperatures of between 15 and 29°C (Sconyers E.L. et al., www.ers.usda.gov/Features/SoyBeanRust/ (2005)). Frequently, the disease starts at discrete locations and subsequently spreads rapidly over the entire field. The fungus is autoecious, i.e. it requires no host alternation for its development, and it can persist readily on its numerous alternative host plants. In the United States, kudzu vine (Pueraria lobata), which originates in Japan, is considered to be a potential host plant on which P. pachyrhizi can overwinter and provide fresh inoculum in the next spring.

P. pachyrhizi can currently be controlled in the field only by means of fungicides. Soybean plants with resistance to the entire spectrum of the isolates are not available. When searching for resistant plants, four dominant genes Rpp1-4, which mediate resistance of soya to P. pachyrhizi, were discovered; however, this resistance is only isolate-specific (Hartwig E.E. et al., Crop Science, 23, 237 (1983); Hartwig E.E., Crop Science 26, 1135 (1986)). Since the resistance was only based on individual genes, it was lost rapidly. Only the Rpp4-mediated resistance has as yet only been broken down under greenhouse conditions (Posada-Buitrago M.L. et al., Fungal Genetics and Biology 42, 949 (2005). The utilization of potential resistance sources from representatives of the perennial subgenus soja is limited (Hartman G.I. et al., Plant Disease 76, 396 (1992)). So far, all crosses have only led to sterile progeny (Singh R. et al., Wendl. Theor. Appl. Genet. 74, 391 (1987).

The efficient control of soybean rust with fungicides requires low application into the foliage of the plants, since infection occurs first on the lower leaves. A double treatment has proved to be effective. A disadvantage of the fungicides used is that, as the result of their specific mechanism of action, resistances may develop readily. A potential alternative to the use of fungicides is the use of glyphosate-resistant soybean plants. In a greenhouse experiment, soybean plants were treated with the herbicide three days before inoculation, and a reduction of rust-caused lesions by 46-70% was observed (Feng C.C.P. et al., PNAS 102, 17290 (2005)). Whether this effect will also be retained in field trials remains to be seen.

In recent years, P. pachyrhizi has gained in importance as pest in soybean production. There was therefore a demand in the prior art for developing methods of controlling the fungus. Right now, plant breeding cannot be expected to contribute since the available resistance sources are not accessible. Treatment with fungicides has a limited efficiency and is only effective when the disease is yet to break out. This is why in particular the pathosystem soya/P. pachyrhizi is the method of choice for a recombinant approach. For an approach to be successful, it is initially important to have detailed knowledge of the course of infection and the response of the plant, in particular during the first stages of the infection. Potential candidate genes which confer resistance must be identified, and their effect in the interaction between plant and pathogen must be characterized. The stable transformation of plants is very time-consuming and costly, which is why transient transformation, which makes possible a characterization of the plant/pathogen interaction at the cell level, is preferred. The method of choice here is the transient transformation of leaves with the gene gun. In plants, non-host-resistance is particularly effective and durable and based on the fact that quantity and speed of the resistance response is increased in comparison with the compatibility response. Thus, the characterization of decisive genes in non-host-resistance can serve to identify potential candidate genes for the generation of resistant plants. Thus, to also study the response of a non-host-plant to soya rust, the system barley (Hordeum vulgare)/P. pachyrhizi was chosen since barley is a model plant which has been described in detail. Moreover, the pathosystem barley/ with Blumeria graminis f.sp. hordei (Bgh) and with Blumeria graminis f.sp. tritici (Bgt), which is incompatible, has been studied in great detail. Although Bgh and P. pachyrhizi differ in some respects, they share at least phenotypically some steps at the beginning of the infection process. Thus, the analysis in barley can provide information on the mechanisms of the non-host-resistance to P. pachyrhizi in comparison with the host resistance of barley. Thus, candidate genes were tested in transiently transformed barley leaves for their effect in the resistance to P. pachyrhizi. The transient transformation with the gene gun provides a method of studying, in the pathosystem barley/P. pachyrhizi, a series of genes which may be involved in the resistance response.

Programmed cell death (PCD) is an important process in the development and stress response of plants and animals. Some morphological and biochemical changes in the cell, such as chromatin condensation, shrinking of the cytoplasm and DNA fragmentation, appear to be shared by plants and animals (Lam E. et al., Nature 411, 848 (2001)). A clear distinction between PCD and HR, which is caused by biotic or abiotic stress, is not possible (Heath M., Plant Mol. Biol. 44, 321 (2000)). One activator of PCD in animals is BAX. BAX develops channels in the outer mitochondrial membrane and causes the release of cytochrome c. This

triggers a caspase cascade, and thus the proteolysis of proteins which the cell needs to survive (Green D.R. et al., Science 281, 1309 (1998). The overexpression of BAX in tobacco (N. tabacum, Lacomme C. et al., Proc. Nat. Acad. Sci. USA 96, 7956 (1999)) and Arabidopsis thaliana (Kawai-Yamada M. et al., Plant Cell 16, 21 (2004)) causes PCD and thus suggests similar mechanisms in plants and animals. However, no BAX homologs have been identified in plants. However, a BAX-antagonistic regulator of PCD, the Bax inhibitor-1 (BI-1), is conserved in plants, animals and other organisms such as yeast. Similar proteins have been identified since in A. thaliana, H. vulgare, Brassica napus, Brassica oleracea, Oryza sativa and N. tabacum (Hückelhoven R., Apoptosis 9, 299 (2004)). In experiments with BI-1/GFP fusion proteins, a localization of BI-1 in the membrane of the endoplasmic reticulum (ER) and the nuclear membrane has been observed (Eichmann R. et al., Mol. Plant Microbe Interact. 17, 484 (2004)). The protein has a size of 25-27 kDa and has 6-7 transmembrane domains. The C-terminal end, which is probably located in the cytoplasm (Bolduc N. et al., Planta 216, 377 (2003)), is essential for the function of BI-1 (Kawai-Yamada et al., Plant Cell 16, 21 (2004)). It is possible that the transmembrane domains form an ion channel (Bolduc N. et al., Planta 216, 377 (2003)). Thus, BI-1 might have a function in regulating the cytosolic Ca^{2+} level and/or the redox state of the cell as the result of the ER's storage function for Ca^{2+} (Xu Q. et al., Mol. Cell 18, 1084 (1998); Balduc N. et al., FEBS Lett. 532, 111 (2003); Hückelhoven R. et al., Proc. Natl. Acad Sci. USA 29, 5555 (2003); Matsumura H. et al., Plant J. 33, 425 (2003)). In animal cells, there is no direct physical interaction between BI-1 and Bax. However, BI00-1 interacts with other PCD regulators (Xu Q. et al., Mol. Cell 18, 1084 (1998)). It is probable that BI-1, in plants, also interacts with other PCD regulators, thus influencing the resistance responses. In Arabidopsis, BI-1 is capable of suppressing BAX and the H_2O_2 have induced PCD (Baek et al., Plant Mol. Biol. 56, 15 (2004); Kawai-Yamada M. et al., Plant Cell 16, 21 (2004)). Therefore, it probably regulates the processes at a level lower than the oxidative stress response (Kawai-Yamada M. et al., Plant Cell 16, 21 (2004)).

The expression of BI-1 is induced by biotic and abiotic stress such as attack by pathogens or wounding, but also in aging tissues (Balduc N. et al., FEBS Lett. 532, 111 (2003); Hückelhoven R., Apoptosis 9, 299 (2004)). In Arabidopsis, the mRNA levels of BI-1 are increased after heat shock (Watanabe N. et al., Plant J. 45, 884 (2006)). BI-1 expression is induced in tomato (Lycopersicum esculentum) by H_2O_2, and in Arabidopsis by H_2O_2 and salicylic acid (Hückelhoven R., Apoptosis 9, 299 (2004); Kawai-Yamada M. et al., Plant Cell 16, 21 (2004)). This suggests that BI-1 has a function in pathogen defense, because this is where both substances play an important role (Prell H.H., Interaktionen von Pflanzen und phytopathogenen Pilzen [Interactions between plants and phytopathogenic fungi], Gustav Fischer Verlag, Jena, 1996).

Accordingly, the infection of barley with Bgh or Bgt triggers an increased expression of BI-1 (Hückelhoven R. et al., Plant Mol. Biol. 47, 739 (2001); Eichmann R. et al., Mol. Plant Microbe Interact. 17, 484 (2004)). In rice, the expression is biphasic after infection with M. grisea. It is first slightly increased, but is reduced 12 hours after the infection only to rise again (Matsumura H. et al., Plant J. 33, 425 (2003)). The increased expression of BI-1 in Arabidopsis cells after treatment with fumonisin B1 (Watanabe N. et al., Plant J. 45, 884 (2006)), and the reduction of the BI-1 expression after the treatment of rice cells with M. grisea elicitor extract, demonstrates that the expression patterns can differ greatly, depending on the inducing factor and on the plant. The expression patterns suggest a role of BI-1 in the regulation of the stress-induced PCD or HR and in the resistance response to pathogens. The influence on the HR can increase the resistance of a plant, especially if the pathogens are pertotrophic or hemibiotrophic fungi. Overexpression of BI-1 in carrots (Daucus carota ssp. sativa) leads to resistance of the plants to Botrytis cinerea (Imani J. et al., Mol. Plant Physiol. in press). In tomatoes, the expression of the PCD inhibitor p35 protects against Alternaria alternata, Colletotrichum coccodes and Pseudomonas syringae (Lincoln J.E. et al., Proc. Nat. Acad. Sci. USA 99, 15217 (2002)).

Against this background, there was a continuous demand in the prior art for crop plants with an increased resistance to pathogens. Only few approaches exist which confer, to plants, a resistance to a broader spectrum of pathogens, especially fungal pathogens. Systemic acquired resistance (SAR) – a defense mechanism in various plant/pathogen interactions – can be conferred by application of endogenous messenger substances such as jasmonate (JA) or salicylic acid (SA) (Ward J.M. et al., Plant Cell 3, 1085 (1991); Uknes et al., Plant Cell 4(6), 645 (1992)). Similar effects can also be brought about by synthetic compounds such as 2,6-dichloroisonicotinic acid (DCINA) or benzo(1,2,3)thiadiazole-7-thiocarboxylic acid S-methyl ester (BTH; Bion®) (Friedrich et al., Plant J. 10(1), 61 (1996); Lawton et al., Plant J. 10, 71 (1996)). Also, expression of "pathogenesis-related" (PR) proteins, which has been upregulated in the context of SAR, may partly bring about resistance to pathogens.

In barley, the Mlo locus has been described as a negative regulator of pathogen defense. The loss, or loss of function, of the Mlo gene brings about an increased, race-unspecific resistance to a large number of mildew isolates (Büschges R. et al., Cell 88, 695 1997); Jorgensen J.H., Euphytica 26, 55 (1997); Lyngkjaer M.F. et al., Plant Pathol 44, 786 (1995)).

The Mio gene has been described (Büschges R. et al., Cell 88, 695 (1997); WO 98/04586; Schulze-Lefert P. et al., Trends Plant Sci. 5, 343 (2000)). Various Mlo homologs from other

cereal species have been isolated. Methods using these genes for obtaining pathogen resistance have been described (WO 98/04586; WO 00/01722; WO 99/47552). The disadvantage is that Mlo-deficient plants also initiate the abovementioned defense mechanism in the absence of a pathogen, which manifests itself in the spontaneous dying of plant cells (Wolter M. et al., Mol. Gen. Genet. 239, 122 (1993)). As the result, mlo-resistant plants suffer a yield loss of up to 5% (Jörgensen J.H. Euphytica 63, 141 (1992)). The spontaneous dying of the leaf cells furthermore brings about a disadvantageous hypersusceptibility to necrotrophic and hemibiotrophic pathogens such as Magnaporthe grisea (M. grisea) or Cochliobolus sativus (Bipolaris sorokiniana) (Jarosch B. et al., Mol Plant Microbe Interact. 12, 508 (1999); Kumar J. et al., Phytopathology 91, 127 (2001)).

Apoptosis, also referred to as programmed cell death, is an essential mechanism for maintaining tissue homoeostasis, and, as such, counteracts cell division as a negatively-regulating mechanism. In the multi-celled organism, apoptosis is a natural component of ontogenesis, and involved, inter alia, in organ development and the removal of senescent, infected or mutated cells. As the result of apoptosis, undesired cells are eliminated in an efficient manner. Interference with, or inhibition of, apoptosis contributes to the pathogenesis of a variety of diseases, among which carcinogenesis. The main effectors of apoptosis are aspartate-specific cysteine proteases, which are known by the name of caspases. They can generally be activated by at least two apoptotic signal pathways: firstly by the activation of the TNF (tumor necrosis factor) receptor family; secondly, the mitochondria play a central role. Activation of the mitochondrial apoptosis signal pathway is regulated by proteins of the Bcl-2 family. This protein family consists of antiapoptotic and proapoptotic proteins such as, for example, Bax. In the case of an apoptotic stimulus, the Bax protein undergoes an allosteric conformation change, which leads to the anchoring of the protein in the external mitochondrial membrane, and to its oligomerization. As the result of these oligomers, proapoptotic molecules are released from the mitochondria into the cytosol and bring about an apoptotic signal cascade and, ultimately, the degradation of specific cellular substrates, resulting in cell death. The Bax inhibitor-1 (BI1) was isolated via its property of inhibiting the proapoptotic effect of BAX (Xu Q. et al., Mol Cell 1(3), 337 (1998)). BI1 is a highly conserved protein. It is found predominantly as an integral constituent of intracellular membranes. BI1 interacts with bcl-2 and bcl-xl. The overexpression of BI1 in mammalian cells suppresses the proapoptotic effect of BAX, etoposid and staurosporin, but not of Fas antigen (Roth W. et al., Nat. Med. 8, 216 (2002)). The inhibition of BI1 by antisense RNA, in contrast, induces apoptosis (Xu Q. et al., Mol Cell 1(3), 337 (1998)). The first plant homologs of BI1 have been isolated from rice and Arabidopsis (Kawai et al., FEBS Lett 464, 143 (1999); Sanchez et al., Plant J. 21, 393 (2000)). These plant proteins

suppress the BAX-induced cell death in yeast. The amino acid sequence homology with human BI1 is approximately 45%. In recombinant plants, the Arabidopsis homolog AtBI1 is capable of suppressing the proapoptotic effect of murine BAX (Kawai-Yamada M. et al., Proc. Natl. Acad. Sci. USA 98(21), 12295 (2001)). The rice (Oryza sativa) BI1 homolog OsBI1 is expressed in all plant tissues (Kawai et al., FEBS Lett 464, 143(1999)). Furthermore described are BI1 genes from barley (Hordeum vulgare; GenBank Acc.-No.: AJ290421), rice (GenBank Acc.-No.: AB025926), Arabidopsis (GenBank Acc.-No.: AB025927), tobacco (GenBank Acc.-No.: AF390556) and oilseed rape (GenBank Acc.-No.: AF390555, Bolduc N. et al., Planta 216, 377-386 (2003)). The expression of BI1 in barley is upregulated as the result of infection with mildew (Hückelhoven R. et al., Plant Mol. Biol. 47(6), 739 (2001)).

WO 00/26391 describes the overexpression, in plants, of the antiapoptotic genes Ced-9 from C. elegans, sfIAP from Spodoptera frugiperda, bcl-2 from humans and bcl-xl from chicken for increasing the resistance to necrotrophic or hemibiotrophic fungi. Plant BI1 homologs are not disclosed. Expression is under the control of constitutive promoters. Furthermore described is the expression of a BI1 protein from Arabidopsis under the strong constitutive 35S CaMV promoter in rice cells and a hereby–induced resistance to cell-death-inducing substances from Magnaporthe grisea (Matsumura H. et al., Plant J. 33, 425 (2003)).

Originally, the prior art described that constitutive expression of an inhibitor of the programmed cell death in plants can bring about resistance to necrotrophic fungi.

However, the person skilled in the art was faced in particular with the problem of providing methods for the pathogen defense in plants, in particular against biotrophic pathogens.

Surprisingly, the problem is solved by the inventive methods, peptide sequences, nucleic acid sequences, expression cassettes, vectors and organisms defined in the main claims, using a BI1 protein. The dependent claims define specific, especially preferred use forms of the present invention.

The roll of BI-1 has been tested in three independent experiments in the transient transformation system. In the control, 53% (averaged over the experiments) of the transformed cells which interacted with P. pachyrhizi were penetrated, while only 37% of the BI-1 transformed cells were penetrated (FIG. 8; Table FIG. 10). The data are based on three independent experiments. Barley leaves were transformed with the reporter gene construct pGY1-GFP and the blank vector in order to act as controls. The penetration rate in the BI-1

transformed cells differs significantly from the WT ($P<0.05$). After the evaluation, the cells which have been transiently transformed with BI-1 therefore surprisingly show a significantly increased penetration resistance to *P. pachyrhizi* ($P< 0.05$; Fig. 8; Table FIG. 10). A noteworthy aspect of the observation under the microscope was that the BI-1-forming cells had a markedly more vital appearance than cells, which expressed GFP or ADF3.

Thus, even the transient overexpression of the cell death inhibitor BI-1 in barley revealed, surprisingly, the trend to an increased resistance of the plant cells to penetration by soybean rust. To confirm this, the interaction between transgenic barley plants cv. "Golden Promise" (GP) which contain a GFP-BI-1 overexpression construct was studied under the microscope in comparison with the wild type (WT) of this variety. To generate the transgenic plants, a GFP-BI-1 fusion under the control of the constitutive CaMV 35S promoter was used, thus ensuring sufficient expression of the protein.

In preliminary experiments, leaves of the WT cv. "Golden Promise" and of the transgenic barley line (cv. "Golden Promise") #6(1)E8L1(T1)' were inoculated with *P. pachyrhizi* and, 24 hours after the inoculation, fixed in destaining solution. After destaining was complete, the leaves were stained with aniline blue. Aniline blue intercalates into the structure of callose and thus preferentially stains papillae where callose undergoes accumulation and crosslinking with other polymeric substances. Cells which, as the result of a hypersensitive response (HR), have undergone a similar process as apoptosis in mammalian cells, will, after staining with aniline blue, also show a light fluorescence. The number of spores, the germinated spores with germ tubes which had already formed an appressorium and, as cell response, the appressoria with underlying papillae and the HR were counted on the inoculated barley leaves. Larger spore agglomerations where an assignation of the appressoria to the spores was no longer possible were not included. As far as possible, at least 100 spores with appressoria were counted per leaf. Even in this experiment, a significantly increased formation of papillae and a significantly decreased HR of the cells was observed in the transgenic line ($P<0.01$; Table FIG. 11 A/B, FIG. 12).

Both Bgh (Hückelhoven R., FEMS Microbiol. Letters 245, 9(2005)) and *P. pachyrhizi* (Koch E. et al., Phytopath, p. 106, 302 (1983)) are biotrophic pathogens. An HR of the infected cells can therefore stop the development of the fungi since it deprives them from their food source. If the HR is prevented, the cells may become more sensitive to infection by biotrophic pathogens. Despite this, barley cells which have been transiently transformed with a BI-1 overexpression construct surprisingly show an increased resistance to penetration by *P. pachyrhizi*. This is

surprising because an increased sensitivity to the biotrophic fungus would have been expected. However, the HR is not the only resistance reaction in the resistance of barley and wheat to the incompatible pathogen P. pachyrhizi, and perhaps not the decisive one. Thus, the defense of wheat against P. pachyrhizi is papille formation (Hoppe H.H. et al., Pro. Intern. Congress of SABRAO (Bangkok 1985) 1986). Barley responds with papilla formation and with HR of the infected cells, depending on the variety. Moreover, barley with the mlo5 allele responds with papilla increased papilla formation. The defense of barley to Bgt is also affected by papilla formation, but mostly by an HR of infected cells (Hückelhoven R. et al., Mol. Plant Pathol. 2, 199 (2001). In contrast to P. pachyrhizi, the transient overexpression of BI-1 in barley leads to an increased penetration rate of the cells by Bgt (Eichmann R. et al., Mol. Plant Microbe Interact. 17, 484 (2004)). Likewise, barley plants with the mlo5 allele, which confers broad resistance to Bgh demonstrate, in the case of transient overexpression of BI-1, greater sensitivity of the cells to penetration by Bgh (Hückelhoven R. et al., Proc. Natl. Acad. Sci. USA, 29, 5555 (2003). Although the resistance of barley with the mlo5 allele is based not on an HR of the infected cells, but on a more efficient accumulation of antimicrobial compounds, H_2O_2 and an increased formation of papillae, the inhibition of the HR also appears to affect the other resistance responses (Hückelhoven R. et al., Plant Physiol. 119, 1251 (1999)). Without causing limitation by theory, these observations of a reduced penetration resistance as the result of inhibition of HR suggests that crosslinked regulation of the resistance responses. The suspicion that the resistance responses are subject to crosslinked regulation is supported by the microscopic analysis of transgenic barley plants which have been inoculated with P. pachyrhizi. These barley plants contain a BI-1 overexpression construct and respond to infection with increased papilla formation. While only 16-26% of the infected cells show HR on the leaves of the transgenic plants, HR was observed in approximately 50% of the infected cells on the WT leaves. Consequently, in the barley variety "Golden Promise", the HR of the cells also appears to be an important resistance mechanism against P. pachyrhizi, which is a biotroph, although P. pachyrhizi does not utilize the epidermal cells as a food source, at least not in soybean, but only forms haustoria in the mesophyll (Koch E. et al., Phytopath, p. 106, 302 (1983)). In general, however, the fungus did not reach this stage on the non-host plant barley. Inhibition of the HR makes it possible for Bgt to penetrate the cells, and the fungus can establish itself successfully (Eichmann R. et al., Mol. Plant Microbe Interact. 17, 484 (2004)). Further fungus-specific factors appear to be necessary for this process. It appears that these specific factors of Bgt are capable of suppressing either the alternative resistance response of the plant cell or the recognition by the latter. These factors are probably absent in P. pachyrhizi. Thus, the plant cell is perhaps capable of recognizing P. pachyrhizi and, since BI-1 suppresses the HR, capable of inducing an alternative resistance response, papilla formation. Recognition of P. pachyrhizi by the plant is

supported by the observation of increased papilla formation in barley with the mlo5 allele. However, the reason why the plant is capable of recognizing the pathogen P. pachyrhizi, which is specialized to host plants of a completely different order, remains unexplained (Sinclair J. B. et al. (eds.), Proceedings of the soybean rust workshop (1995), National Soybean Research Laboratory, Publication No. 1 (1996)).The "recognition feature" of P. pachyrhizi might be an unspecific elicitor ('pathogenesis-associated molecular patterns' PAMP) such as the INF1 from P. infestans, which triggers an HR in Nicotiana sp. (Kamoun S. et al., Plant Cell 10, 1413 (1998)). PAMPs are recognized by the plant as foreign molecules and trigger a resistance response. Not all leaves of the transgenic plant show increased papilla formation, although the BI-1 gene construct has been identified in them by means of PCR. This might be the consequence of an unfavorable insertion type of the construct, which prevents an effective expression of BI-1 and/or leads to antagonistic effects by other genes. Since the identification was performed at the DNA level, no comments can be made on the expression of BI-1. It must also be borne in mind that even minor damage to the seeds or the leaves can have a decisive effect, or prevent, the development of the plant. Thus, some seeds of the line #6(2)E15L7P2 (T2) did not germinate.

Consequently, a first subject matter of the invention relates to a method of generating or increasing a resistance in the plant, or a part of a plant, to a pathogen which is preferably a biotroph. The method comprises a step in which the amount of a Bax inhibitor-1 (BI-1) protein or its function in the plant or at least a part of a plant is increased. A part of a plant is understood as meaning the entire plant, one or more of its organs, a tissue or at least a cell. The method furthermore comprises the step of selecting a plant or a part of a plant in which the BI1 protein or its function has been increased in such a way that it shows increased resistance in comparison with a starting plant, in which the increase in the amount of function of the BI1 protein in comparison with the starting plant or its part is increased.

In a preferred embodiment, the resistance is a resistance to at least one biotrophic pathogen, preferably a biotrophic fungus, and especially preferably a heminecrotrophic fungus as defined herein. In preferred embodiments of the present invention, the biotrophic fungus is selected from the group Basidiomycota, preferably the Uredinales (rusts), especially preferably the Melompsoraceae, and in particular the genus Phacopsora. In especially preferred embodiments, the pathogen is Phacopsora pachyrhizi and/or P. meibomiae (together also referred to as "soybean rust" or "soya rust"). Preference being given to the former. When the pathogen is selected from the group of the biotrophic pathogens or fungi, it is preferred in some embodiments that the pathogen is other than powdery mildew or downy mildew.

In preferred embodiments, the step of increasing the amount or function of the BI1 protein is accomplished by biotechnological methods. The term "biotechnological" methods comprises, inter alia, the recombinant expression of the protein in a cell, preferably in operable linkage with, and driven by, a heterologous promoter. The abovementioned increase, however, can also be accomplished for example by substituting only the endogenous promoter or, for example, the silencing of factors which generally inhibit the expression of the endogenous BI1 protein. Accordingly, the term "biological method" comprises all methods which are open to modern molecular biology, in particular the methods of recombinant gene technology, which are known to the skilled worker and which will be discussed in greater detail in the course of the description.

The plant in which the resistance is generated is preferably selected from the group of the monocotyledonous plants, in particular comprising wheat, oats, millet, barley, rye, maize, rice, sorghum, triticale, spelt or sugar cane; or from the group of the dicotyledonous plants, in particular comprising Arabidopsis, cotton, buckwheat, potato, cabbages, cress, linseed, oil seed rape, tomato, aubergine, bell peppers, sunflower, tobacco, Tagetes, lettuce, Calendula, melon, pumpkin/squash, courgette, sugarbeet, ornamentals, trees and legumes. Since soybean rust attacks more than 70 leguminous species, the plant is especially preferably selected, among the legumes, at least in cases where the pathogen is a heminecrotrophic pathogen or a rust or soybean rust pathogen.

Consequently, the plant is selected especially preferably among the legumes, comprising plants of the genus Phaseolus (comprising French bean, dwarf bean, climbing bean (Phaseolus vulgaris), Lima bean (Phaseolus lunatus L.), Tepary bean (Phaseolus acutifolius A. Gray), runner bean (Phaseolus coccineus)); the genus Glycine (comprising Glycine soja, soybeans (Glycine max (L.) Merill)); pea (Pisum) (comprising shelling peas (Pisum sativum L. convar. sativum), also called smooth or round-seeded peas; marrowfat pea (Pisum sativum L. convar. medullare Alef. emend. C.O. Lehm), sugar pea (Pisum sativum L. convar. axiphium Alef emend. C.O. Lehm), also called snow pea, edible-podded pea or mangetout, (Pisum granda sneida L. convar. sneidulo p. shneiderium)); peanut (Arachis hypogaea), clover (Trifolium spec.), medick (Medicago), kudzu vine (Pueraria lobata), common lucerne, alfalfa (M. sativa L.), chickpea (Cicer), lentils (Lens) (Lens culinaris Medik.), lupins (Lupinus); vetches (Vicia), field bean, broad bean (Vicia faba), vetchling (Lathyrus) (comprising chickling pea (Lathyrus sativus), heath pea (Lathyrus tuberosus)); genus Vigna (comprising moth bean (Vigna aconitifolia (Jacq.) Maréchal), adzuki bean (Vigna angularis (Willd.) Ohwi & H. Ohashi), urd bean (Vigna mungo

(L.) Hepper), mung bean (Vigna radiata (L.) R. Wilczek), bambara groundnut (Vigna subterrane (L.) Verdc.), rice bean (Vigna umbellata (Thunb.) Ohwi & H. Ohashi), Vigna vexillata (L.) A. Rich., Vigna unguiculata (L.) Walp., in the three subspecies asparagus bean, cowpea, catjang bean)); pigeonpea (Cajanus cajan (L.) Millsp.), the genus Macrotyloma (comprising geocarpa groundnut (Macrotyloma geocarpum (Harms) Maréchal & Baudet), horse bean (Macrotyloma uniflorum (Lam.) Verdc.)); goa bean (Psophocarpus tetragonolobus (L.) DC.), African yam bean (Sphenostylis stenocarpa (Hochst. ex A. Rich.) Harms), Egyptian black bean, dolichos bean, lablab bean (Lablab purpureus (L.) Sweet), yam bean (Pachyrhizus), guar bean (Cyamopsis tetragonolobus (L.) Taub.); and the genus Canavalia (comprising jack bean (Canavalia ensiformis (L.) DC.), sword bean (Canavalia gladiata (Jacq.) DC.)).

In any of the embodiments of the present invention which are disclosed herein, it is especially preferred that the amount or function of the BI1 protein is increased at least in the epidermis, preferably essentially in a tissue-specific manner in the epidermis; in particular, it is preferred that the amount or function of the BI1 protein is specifically increased in the epidermis and/or essentially not increased in the mesophyll.

By epidermis, the skilled worker means the predominant epidermal tissue of primary aerial plant parts, for example of the shoot, the leaves, flowers, fruits and seeds. The epidermal cells secrete outwardly a water-repellent layer, the cuticle. The roots are surrounded by the rhizodermis, which, in many ways, resembles the epidermis, but also shows pronounced differences. While the outermost layer of the apical meristem gives rise to the epidermis, the formation of the rhizodermis is much less clear. Depending on the species, it can be considered, in phylogenetic terms, either as part of the calyptra or as part of the primary cortex. The epidermis has a number of functions: it protects the plant against desiccation and regulates the transpiration rate. It protects the plant against a wide range of chemical and physical external influences, against being fed upon by animals and against attack by parasites. It is involved in gas exchange, in the secretion of certain metabolites and in the absorption of water. It comprises receptors for light and mechanical stimuli. It thus acts as a signal transformer between the environment and the plant. In accordance with its various functions, the epidermis comprises a number of differently differentiated cells. To this must be added species-specific variants and different organizations of the epidermides in the individual parts of a plant. Essentially, it consists of three categories of cells: the "actual" epidermal cells, the cells of the stomata and of the trichomes (Greek: trichoma, hair), epidermal appendages of varying shape, structure and function.

The "actual", i.e. the least specialized, epidermal cells account for the bulk of the cells of the epidermal tissue. In topview, they appear either polygonal (slab or plate shaped) or elongated. The walls between them are often wavy or sinuate. It is not known what induces this shape during development; existing hypotheses only offer unsatisfactory explanations herefor.

Elongated epidermal cells can be found in organs or parts of organs that are elongated themselves, thus, for example, in stems, petioles, leaf veins and on the leaves of most monocots. The upper surface and undersurface of laminae can be covered in epidermides with different structures, it being possible for the shape of the cells, the wall thickness and the distribution and number of specialized cells (stomata and/or trichomes) per unit area to vary. A high degree of variation is also found within individual families, for example in the Crassulaceae. In most cases, the epidermis consists of a single layer, though multi-layered water-storing epidermides have been found among species from a plurality of families (Moraceae: most Ficus species; Piperaceae: Peperonia, Begoniaceae, Malvaceae and the like). Epidermal cells however secrete a cuticle on the outside which covers all epidermal surfaces as an uninterrupted film. It may either be smooth or structured by bulges, rods, folds and furrows. However, the folding of the cuticle, which can be observed when viewing the surface, is not always caused by cuticular rods. Indeed, there are cases where cuticular folding is merely the expression of the underlying bulges of the cell wall. Epidermal appendages of various form, structure and function are referred to as trichomes and, in the present context, likewise come under the term "epidermis". They occur in the form of protective hairs, supportive hairs and gland hairs in the form of scales, different papillae and, in the case of roots, as absorbent hairs. They are formed exclusively by epidermal cells. Frequently, a trichome is formed by only one such a cell, however, occasionally, more than one cell is involved in its formation.

The term "epidermis" likewise comprises papillae. Papillae are bulges of the epidermal surface. The textbook example are the papillae on flower surfaces of pansy (Viola tricolor) and the upper surfaces of the leaves of many species from tropical rain forests. They impart a velvet-like consistency to the surface. Some epidermal cells can form water stores. A typical example are the water vesicles at the surfaces of many Mesembryanthemum species and other succulents.

In some plants, for example in the case of campanula (Campanula persicifolia), the outer walls of the epidermis are thickened like a lens.

The bulk of all tissues is the parenchyma. The parenchymatic tissues include the mesophyll which, in leaves, can be differentiated into palisade parenchyma and spongy parenchyma.

Accordingly the skilled worker understands, by mesophyll, a parenchymatic tissue. Parenchymatic cells are always alive, in most cases isodiametric, rarely elongated. The pith of the shoots, the storage tissues of the fruits, seeds, the root and other underground organs are also parenchymas, as is the mesophyll.

In the leaves of most ferms and phanerogams, especially in the case of the dicots and many monocots, the mesophyll is subdivided into palisade parenchyma and spongy parenchyma. A "typical" leaf is of dorsiventral organization. In most cases, the palisade parenchyma is at the upper surface of the leaf immediately underneath the epidermis. The sponge parenchyma fills the underlying space. It is interspersed by a voluminous intercellular system whose gas space is in direct contact with the external space via the stomata.

The palisade parenchyma consists of elongated cylindrical cells. In some species, the cells are irregular, occasionally bifurcate (Y-shaped: arm palisade parenchyma). Such variants are found in ferns, conifers and a few angiosperms (for example in Ranunculaceae and Caprifoliaceae species [example: elder]). Besides the widest-spread organization form which has just been described, the following variants have been found:

- palisade parenchyma on the abaxial leaf surface. Particularly noticeable in scaly leaves. Example: arbor vitae (Thuja), and on the leaves of wild garlic (Allium ursinum).
- palisade parenchyma on both leaf surfaces (adaxial and abaxial). Frequently in plants which grow in dry habitats (xerophytes). Example: prickly lettuce (Lactuca serriola);
- ring-shaped closed palisade parenchyma: In cylindrically organized leaves and in conifers' needles.

The variability of the cells of the spongy parenchyma, and the organization of the spongy parenchyma itself, are even more varied than that of the palisade parenchyma. It is most frequently referred to as aerenchyma since it comprises a multiplicity of interconnected intercellular spaces.

The mesophyll may comprise what is known as the assimilation tissue, but the terms mesophyll and assimilation tissue are not to be used synonymously. There are chloroplast-free leaves whose organization differs only to a minor extent from comparable green leaves. As a consequence, they comprise mesophyll, but assimilation does not take place; conversely, assimilation also takes place in, for example, sections of the shoot.

In the present description, the epidermis is characterized in biochemical terms. In a preferred embodiment, the epidermis can be characterized by the activity of one or more of the following promoters:

- WIR5 (=GstA1); acc. X56012; Dudler & Schweizer,
- GLP4, acc. AJ310534; Wei Y., Zhang Z., Andersen C.H., Schmelzer E., Gregersen P.L., Collinge D.B., Smedegaard-Petersen V. and Thordal-Christensen H., Plant Molecular Biology 36, 101 (1998),
- GLP2a, acc. AJ237942, Schweizer P., Christoffel A. and Dudler R., Plant J. 20, 541 (1999);
- Prx7, acc. AJ003141, Kristensen B.K., Ammitzböll H., Rasmussen S.K. and Nielsen K.A., Molecular Plant Pathology, 2(6), 311 (2001);
- GerA, acc. AF250933; Wu S., Druka A., Horvath H., Kleinhofs A., Kannangara G. and von Wettstein D., Plant Phys Biochem 38, 685 (2000);
- OsROC1, acc. AP004656
- RTBV, acc. AAV62708, AAV62707; Klöti A., Henrich C., Bieri S., He X., Chen G., Burkhardt P.K., Wünn J., Lucca P., Hohn T., Potrykus I. and Fütterer J., PMB 40, 249 (1999);
- Chitinase ChtC2-Promotor from potato (Ancillo et al., Planta. 217(4), 566, (2003));
- AtProT3 Promotor (Grallath et al., Plant Physiology. 137(1), 117 (2005))
- SHN-Promotors from Arabidopsis (AP2/EREBP transcription factors involved in cutin and wax production) (Aarón et al., Plant Cell. 16(9), 2463 (2004));
- GSTA1 from wheat (Dudler et al., WP2005306368 and Altpeter et al., Plant Molecular Biology. 57(2), 271 (2005)).

In preferred embodiments, the epidermis is characterized by the fact that all the abovementioned promoters are active in the tissue or the cell. In other preferred embodiments, the epidermis is characterized by the fact that only some of the promoters are active, for example preferably 2, 3, 5 or most preferably 7 or more, but at least from only one of those detailed above.

In a preferred embodiment, the mesophyll is characterized in biochemical terms. The mesophyll can be characterized by the activity of one or more of the following promoters:

- PPCZm1 (=PEPC); Kausch A.P., Owen T.P., Zachwieja S.J., Flynn A.R. and Sheen J., Plant Mol. Biol. 45, 1 (2001);
- OsrbcS, Kyozuka et al., PlaNT Phys 102, 991 (1993); Kyozuka J., McElroy D., Hayakawa T., Xie Y., Wu R. and Shimamoto K., Plant Phys. 102, 991 (1993);
- OsPPDK, acc. AC099041;

- TaGF-2.8, acc. M63223; Schweizer P., Christoffel A. and Dudler R., Plant J. 20, 541 (1999);
- TaFBPase, acc. X53957;
- TaWIS1, acc. AF467542; US 200220115849;
- HvBIS1, acc. AF467539; US 200220115849;
- ZmMIS1, acc. AF467514; US 200220115849;
- HvPR1a, acc. X74939; Bryngelsson et al., Mol. Plant Microbe Interacti. 7 (2), 267 (1994);
- HvPR1b, acc. X74940; Bryngelsson et al., Mol. Plant Microbe Interact. 7(2), 267 (1994);
- HvB1,3gluc; acc. AF479647;
- HvPrx8, acc. AJ276227; Kristensen et al., Molecular Plant Pathology, 2(6), 311 (2001);
- HvPAL, acc. X97313; Wei Y., Zhang Z., Andersen C.H., Schmelzer E., Gregersen P.L., Collinge D.B., Smedegaard-Petersen V. and Thordal-Christensen H. Plant Molecular Biology 36, 101 (1998).

In preferred embodiments, the mesophyll is characterized by the fact that all the abovementioned promoters are active in the tissue or the cell. In another embodiment, the mesophyll comprises the fact that only some of the promoters are active, for example preferably 2, 3, 5 or especially preferably 7 or more, but at least from only one of those detailed above.

In preferred embodiments, all of the abovementioned promoters are active in a plant used or produced in accordance with the invention or in the epidermis and in the mesophyll in a plant according to the invention. In one embodiment, only some of the abovementioned promoters are active, for example preferably 2, 5, or especially preferably 7 or more; however, at least one of the promoters detailed above is active in each case.

In preferred embodiments, the increase in the protein quantity or function of the BI1 protein takes place in a constitutive or tissue-specific manner. In especially preferred embodiments, an essentially tissue-specific increase in the protein quantity or protein function takes place in an essentially epidermis-specific manner, for example by recombinant expression of a nucleic acid sequence coding for said BI1 protein under the control of an epidermis-specific promoter. In particular, the increase in the expression or function of the BI1 protein takes place in the epidermis, where, however, the expression of the BI-1 protein in the mesophyll remains essentially unchanged, or it is reduced, and where other tissues are unaffected.

As described in the present text, in one embodiment, the expression or function of the protein according to the invention or of the BI-1 characterized in the present text is increased at least in

the epidermis of a plant. An increase in expression can be achieved as described hereinbelow. By increased expression or function, the present text means both the activation or enhancement of the expression or function of the endogenous protein including a *de novo* expression, but also an increase in or enhancement as the result of the expression of a transgenic protein or factor.

In an especially preferred embodiment, the increase in the protein quantity or function of at least one plant BI1 protein can be combined with an mlo-resistant phenotype or with the inhibition or reduction, in comparison with a control plant, of the expression of MLO, RacB and/or NaOx in the plant or a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a considerable number of the epidermal cells, and/or with the increase in the expression or function of PEN2 and/or PEN1 in the plant, for example constitutively, or a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or in a considerable number of epidermal cells, with the proviso that the expression of a plant BI1 protein in the leaf epidermis remains essentially unchanged or is reduced.

The Mlo locus has been described in barley as negative regulator of pathogen defense. The loss, or loss of function, of the Mlo gene brings about an increased, race-unspecific resistance to a number of mildew isolates (Büschges R. et al., Cell 88, 695 (1997); Jorgensen J.H., Euphytica 26, 55 (1977); Lyngkjaer M.F. et al., Plant Pathol. 44, 786 (1995)). An mlo-resistant phenotype can be obtained as described in the prior art. Methods using these genes for obtaining a pathogen resistance are described, inter alia, in WO 98/04586; WO 00/01722; WO 99/47552.

In one embodiment of the present invention, the activity, expression or function of MLO, RacB and/or NaOx in the plant or a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a substantial number of epidermal cells, can advantageously be inhibited or reduced in comparison with a control plant or a part thereof. By reducing the activity or function of MLO, RacB and/or NaOx in the plant or a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a substantial number of epidermal cells, it is preferred to increase the resistance, or withstanding power, to biotrophic pathogens in plants produced in accordance with the invention. The activity or function of MLO, RacB and/or NaOx can be reduced or inhibited analogously to what has been described for MLO in WO 98/04586; WO 00/01722; WO 99/47552 and the other publications mentioned hereinbelow, whose content is herewith expressly incorporated into the present description, in particular for describing the activity and inhibition of MLO. The description of the

abovementioned publications describes processes, methods and especially preferred embodiments for reducing or inhibiting the activity or function of MLO; the examples detail specifically how this can be performed.

The reduction of the activity or function, if appropriate the expression, of RacB is described in detail in WO 2003/20939, which is herewith expressly incorporated into the present description.

The description of the abovementioned publication describes processes and methods for reducing or inhibiting the activity or function of proteins; the examples detail specifically how this can be performed. It is especially preferred to carry out the reduction or inhibition of the activity or function of RacB as described in the embodiments and the examples which are especially preferred in WO 2003/20939 and in the organisms specified therein as being especially preferred, in particular in a plant or a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a substantial number of epidermal cells. The reduction of the activity or function, if appropriate the expression, of RacB is described in detail in WO 2003/20939. In WO 2003/20939, the skilled worker can find the sequences which code for RacB proteins and can also identify RacB by means of the method provided in WO 2003/20939.

The reduction of the activity or function, if appropriate of the expression, of NaOX is described in detail in WO 2004/09820 (= PCT/EP/03/07589) which is herewith expressly incorporated into the present description. The description of the abovementioned publication describes processes and methods for reducing or inhibiting the activity or function of NaOx; the examples detail specifically how this can be performed. It is especially preferred to carry out the reduction or inhibition of the activity or function of NaOx as described in the embodiments and the examples which are especially preferred in WO 2004/09820 (= PCT/EP/03/07589) and in the organisms specified therein as being especially preferred, in particular in a plant or a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a substantial number of epidermal cells. In WO 2004/09820 (= PCT/EP/03/07589), the skilled worker can find the sequences which code for NaOx proteins and can also identify NaOx by means of the method provided in WO 2004/09820 (= PCT/EP/03/07589).

In one embodiment of the present invention, the activity, expression or function of PEN1, PEN2 and/or SNAP34 can advantageously be increased in the plant, for example constitutively, or in a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a substantial number of epidermal cells. The increase in activity, which also comprises a *de novo*

expression, of PEN1, PEN2 and/or SNAP34 in the plant, for example constitutively, or in a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a substantial number of epidermal cells will preferably increase the resistance or withstanding power to biotrophic pathogens in the plants produced in accordance with the invention. The increase in the activity or function, if appropriate the expression, of PEN2 is described in detail in WO 03/074688, which is herewith expressly incorporated into the present description. The description of the abovementioned publication describes processes and methods for reducing or inhibiting the activity or function of PEN2; the examples detail specifically how this can be performed. The reduction or inhibition of the activity or function of PEN2 is especially preferably carried out in accordance with the embodiments and examples which are especially preferred in WO 03/074688 and in the organisms detailed therein as being especially preferred, in particular in plants, for example constitutively, or in a part thereof, for example in a tissue, but especially advantageously at least in the epidermis or a considerable part of the epidermal cells. In WO 03074688, the skilled worker will find the sequences which code for PEN2 proteins and can also identify PEN2 by means of the method provided in WO 03/074688.

The expression of PEN1 and SNAP34 can be increased analogously to the methods described in WO 03/074688. Owing to his general expert knowledge and the prior art with which he is familiar, the skilled worker can isolate and overexpress PEN1 and SNAP34 nucleic acid sequences and protein sequences. SEQ ID No: 39 describes the nucleic acid sequence which codes for PEN1 from barley; the protein sequence is described in SEQ ID No: 40. SEQ ID No: 41 describes the nucleic acid sequences which codes for PEN1 from Arabidopsis thaliana; the protein sequence is described in SEQ ID No: 42. PEN1 from Arabidopsis thaliana is published under the accession numbers NM 202559 and NM 112015. The homolog from barley is disclosed in accession numbers AY246907 and AY246906 as ROR2. They are members of the fairly large family of the syntaxin proteins. Thus, the skilled worker can use simple homology comparisons for identifying further syntaxin proteins which are expressed as potential resistance genes in the method according to the invention.

SEQ ID No: 43 describes the nucleic acid sequence which codes for SNAP34 from barley; the protein sequence is described in SEQ ID No: 44. The SNAP-34 homolog from barley is also published as AY 247208 (SNAP-34). Homologs whose function is unknown and which might play a role in the resistance are published as AY 247209 (SNAP-28) and AY 247210 (SNAP-25). The following Arabidopsis genes show a higher degree of homology with barley SNAP34 than barley SNAP-28 or SNAP-25 to SNAP-34 and can thus advantageously be co-overexpressed as potential resistance-mediating genes:

AAM 62553 - Arabidopsis SNAP25a

NP 200929 - Arabidopsis SNAP33b

NP 172842 - Arabidopsis SNAP30

NP 196405 - Arabidopsis SNAP29

Accordingly, the invention also relates to a plant in which a polypeptide whch is encoded by a nucleic acid molecule comprising the sequences shown in SEQ ID No: 39, 41 or 43 or one of the sequences shown in the abovementioned database publications or which comprises one of the amino acid sequences shown in the abovementioned database publications or in SEQ ID No: 40, 42 or 44, or which is a functional equivalent thereof or which has at least 50%, preferably 70%, more preferably 80%, even more preferably 90%, 95% or more homology with the abovementioned sequences at the coding nucleic acid molecule level or, preferably, at the amino acid level is overexpressed at least furthermore in the epidermis, or relates to a plant in which the above-characterized polypeptide is activated, or its activity or function increased, constitutively or in a part, for example in a tissue, but especially advantageously at least in the epidermis or a substantial number of epidermal cells.

A reduction of the expression or activity of a protein can be brought about by the methods with which the skilled worker is familiar, for example mutagenesis, for example EMS, if appropriate TILLING, iRNA; ribozyme, silencing, knockout, and the like. Reduction methods are described in particular in WO 2003/20939, whose methods can readily be adapted to the sequences described herein, which is why the content of WO 2003/20939 is explicitly incorporated herein.

The lowering or reduction of the expression of a protein, the activity or the function can be performed in many ways.

"Lowering", "to lower", "reduction" or "to reduce" is to be understood in the broad sense in connection with the present invention and comprises the partial or essentially complete prevention or blocking of the functionality or a protein, as the result of different cell-biological mechanisms.

A reduction for the purposes of the invention also comprise a quantitative reduction of a protein down to an essentially complete absence of the protein (i.e. lacking detectability of activity or function or lacking immunological detectability of the protein). In this context, the expression of a certain protein or the activity, or 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%.

The methods of dsRNAi, cosuppression by means of sense RNA and "VIGS" ("virus induced gene silencing") are also referred to as "post-transcriptional gene silencing" (PTGS). PTGS methods, like the reduction of the function or activity with dominant-negative variants, are especially advantageous because the requirements to homology between the endogenous gene to be suppressed and the recombinantly expressed sense or dsRNA nucleic acid sequence (or between the endogenous gene and its dominant-negative variant, respectively) are lower than, for example in the case of a traditional antisense approach. Such homology criteria are mentioned in the description of the dsRNAi method and can generally be applied to PTGS methods or dominant-negative approaches.

"Introduction" comprises, within the context of the present invention, all methods which are capable of introducing a compound, directly or indirectly, into the epidermis or a substantial part of the epidermal cells, compartment or tissues of same, or which are suitable for generating it therein. This comprises direct and indirect methods. The introduction can lead to a transient presence of a compound (for example a dsRNA) or else to a stable presence. Introducing, comprises, for example, methods such as transfection, transduction or transformation.

In expression constructs of the present invention, a nucleic acid molecule disclosed herein, whose expression (transcription and, if appropriate, translation) generates a corresponding amino acid molecule, is preferably in operable linkage with at least one genetic control element (for example a promoter) which ensures expression in an organism, preferably in plants, preferably an epidermis-specific expression. If the expression construct is to be introduced directly into the plant, plant-specific genetic control elements (for example promoters) are preferred, where, as can be seen from what has been said above, the epidermis-specific activity of the promoter is mandatory in most use forms, as described herein above.

Operable linkage is understood as meaning, for example, the sequential arrangement of a promoter and the nucleic acid sequence to be expressed 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 in the recombinant expression of the nucleic acid sequence, depending on the arrangement of the nucleic acid sequences to make sense RNA or antisense RNA. This does not necessarily require direct linkage in the chemical sense. Genetic control sequences such as, for example, enhancer sequences, can also exert their function on the target sequence

from positions which are somewhat distant, or indeed from other DNA molecules (cis or trans localization). Preferred arrangements are those in which the nucleic acid sequence to be expressed recombinantly is positioned downstream of the sequence which acts as promoter, so that the two sequences are covalently bonded with one another. 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.

The generation of an operable linkage can be accomplished by means of current recombination and cloning techniques, as is the generation of an expression cassette. Such techniques are described, for example, in Maniatis T., Fritsch E.F. and Sambrook J., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY) (1989), in Silhavy T.J., Berman M.L. and Enquist L.W., Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY) (1984), in Ausubel F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience (1987) and in Gelvin et al. in Plant Molecular Biology Manual (1990). However, it is also possible to position, between the two sequences, further sequences which have, for example, the function of a linker with certain restriction enzyme cleavage sites, or of a signal peptide. 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 as integrated into a vector and can be inserted into a plant genome by, for example, transformation. The control elements preferably mediate an epidermis-specific expression.

For the purposes of the present invention, "approximately" in connection with numbers or sizes means a range of numbers or sizes around the numerical value or the size. In general, the term "approximately" means a range of in each case 10% above and below the value detailed.

For the purposes of the present invention, "plant" means all genera and species of higher and lower plants of the plant kingdom. The term includes the mature plants, seed, fruits and seedlings, and parts, propagation material, plant organs, tissue, protoplasts, callus and other cultures, for example cell cultures, derived therefrom, and any other types of associations of plants cells to the functional or structural units. Mature plants means plants at any developmental stage beyond the seedling stage. Seedling means a young, immature plant in an early developmental stage.

"Plant" comprises all annual and perennial, 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, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solarium, Petunia, Digitalis, Majorana, Ciahorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Picea and Populus.

The term "plant" preferably comprises the monocotyledonous and dicotyledonous crop plants. Preferred within the scope of the invention are plants which are employed as foodstuffs or feedingstuffs, very especially preferred are agriculturally important monocotyledonous and dicotyledonous genera and species, as detailed in the claims.

"Pathogen resistance" means the reduction or diminishing of disease symptoms of a plant as the result of attack by at least one pathogen. The symptoms can be manifold in nature, but preferably comprise those which directly or indirectly lead to a negative effect on plant quality, yield quantity, the suitability for use as foodstuff or feedingstuff, or else which make sowing, planting, harvesting or processing of the crop more difficult. For the purposes of the present invention, "pathogen tolerance" is, in particular, to be considered as being comprised by "pathogen resistance".

"Confering", "existing", "generating" or "increasing" (of) a resistance means that the defense mechanisms of a particular plant species or variety displays increased resistance to one or more pathogens, as the result of the application of the method according to the invention, in comparison with the wild type of the plant ("starting plant") to which this method according to the invention has not been applied, under otherwise essentially identical conditions (such as, for example, climatic conditions, culture conditions, type of stress, pathogen species and the like). In this context, the increased resistance preferably manifests itself in a reduced manifestation of the disease symptoms, where disease symptoms – in addition to the abovementioned adverse effects – also comprises for example the penetration efficiency of a pathogen into the plant or plant cells, or the proliferation efficiency in or on same. In this context, the disease symptoms are preferably reduced by at least 5%, 10% or at least 20%, especially preferably by at least 40% or 60%, very especially preferably by at least 70% or 80%, most preferably by at least 90% or 95%.

"Selection" means, with regard to plants where – as opposed to, or in comparison with, the starting plant – resistance to at least one pathogen exists or is increased, all those methods which are suitable for recognizing an existing or increasing pathogen resistance. This can be for example symptoms of the pathogen infection (for example development of necroses in the case of fungal infection), but may also comprise the above-described symptoms, which affect the quality of the plant, the quantity of the yield, the suitability for use as feedstuff or foodstuff, and the like.

For the purposes of the invention, "pathogen" means by way of example, but not by limitation, viruses or viroids, bacteria, fungi, animal pests such as, for example, insects or nematodes. Fungi, in particular biotrophic or heminecrotrophic fungi as defined herein, are especially preferred. However, it can be assumed that the mesophyll-specific expression of a BI1 protein also brings about a resistance to other pathogens since a resistance to stress factors in total is being generated.

Pathogens which may be mentioned by way of example, but not by limitation, are the following:

1. Fungal pathogens of fungus-like pathogens:

Fungal pathogens or fungus-like pathogens (such as, for example, Chromista) preferably belong to the group comprising Plasmodiophoramycota, Oomycota, Ascomycota, Chytridiomycetes, Zygomycetes, Basidiomycota and Deuteromycetes (Fungi imperfecti). Pathogens which may be mentioned by way of example, but not by limitation, are those detailed in Tables 1 to 4, and the diseases which are associated with them.

Table 1: Diseases caused by biotrophic phytopathogenic fungi

Disease	Pathogen
Leaf rust	Puccinia recondita
Yellow rust	P. striiformis
Powdery mildew	Erysiphe graminis / Blumeria graminis
Rust (common corn)	Puccinia sorghi
Rust (Southern corn)	Puccinia polysora
Tobacco leaf spot	Cercospora nicotianae

Rust (soybean)	Phakopsora pachyrhizi, P. meibomiae
Rust (tropical corn)	Physopella pallescens, P. zeae = Angiopsora zeae

Table 2: Diseases caused by necrotrophic and/or hemibiotrophic fungi and Oomycetes

Disease	Pathogen
Plume blotch	Septoria (Stagonospora) nodorum
Leaf blotch	Septoria tritici
Ear fusarioses	Fusarium spp.
Eyespot	Pseudocercosporella herpotrichoides
Smut	Ustilago spp.
Late blight	Phytophthora infestans
Bunt	Tilletia caries
Take-all	Gaeumannomyces graminis
Anthrocnose leaf blight Anthracnose stalk rot	Colletotrichum graminicola (teleomorph: Glomerella graminicola Politis); Glomerella tucumanensis (anamorph: Glomerella falcatum Went)
Aspergillus ear and kernel rot	Aspergillus flavus
Banded leaf and sheath spot ("Wurzeltöter")	Rhizoctonia solani Kuhn = Rhizoctonia microsclerotia J. Matz (telomorph: Thanatephorus cucumeris)
Black bundle disease	Acremonium strictum W. Gams = alosporium acremonium Auct. non Corda
Black kernel rot	Lasiodiplodia theobromae = Botryodiplodia theobromae
Borde blanco	Marasmiellus sp.
Brown spot (black spot, stalk rot)	Physoderma maydis
Cephalosporium kernel rot	Acremonium strictum = Cephalosporium acremonium
Charcoal rot	Macrophomina phaseolina
Corticium ear rot	Thanatephorus cucumeris = Corticium sasakii

Disease	Pathogen
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)
Didymella leaf spot	Didymella exitalis
Diplodia ear and stalk rot	Diplodia frumenti (teleomorph: Botryosphaeria festucae)
Diplodia ear and stalk rot, seed rot and seedling blight	Diplodia maydis = Stenocarpella maydis
Diplodia leaf spot or streak	Stenocarpella macrospora = Diplodialeaf macrospora
Brown stripe downy mildew	Sclerophthora rayssiae var. zeae
Crazy top downy mildew	Sclerophthora macrospora = Sclerospora macrospora
Green ear downy mildew (graminicola downy mildew)	Sclerospora graminicola
Dry ear rot (cob, kernel and stalk rot)	Nigrospora oryzae (teleomorph: Khuskia oryzae)
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 maydicus, Rhizopus microsporus Tiegh., R. stolonifer = R. nigricans, Scopulariopsis brumptii

Disease	Pathogen
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)
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,

Disease	Pathogen
	S. zeina
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)
Penicillium ear rot (blue eye, blue mold)	Penicillium spp., P. chrysogenum, P. expansum, P. oxalicum
Phaeocytostroma stalk and root rot	Phaeocytostroma ambiguum, = Phaeocytosporella zeae
Phaeosphaeria leaf spot	Phaeosphaeria maydis = Sphaerulina maydis
Physalospora ear rot (Botryosphaeria ear rot)	Botryosphaeria festucae = Physalospora zeicola (anamorph: Diplodia frumenti)
Purple leaf sheath	Hemiparasitic bacteria and fungi
Pyrenochaeta stalk and root rot	Phoma terrestris = Pyrenochaeta terrestris
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
Rhizoctonia ear rot (sclerotial rot)	Rhizoctonia zeae (teleomorph: Waitea circinata)
Rhizoctonia root and stalk rot	Rhizoctonia solani, Rhizoctonia zeae
Root rots (minor)	Alternaria alternata, Cercospora sorghi, Dictochaeta fertilis, Fusarium acuminatum (teleomorph: Gibberella acuminata), F. equiseti (teleomorph: G. intricans), F. oxysporum, F. pallidoroseum, F. poae, F. roseum, G. cyanogena, (anamorph: F. sulphureum), Microdochium bolleyi, Mucor sp., Periconia circinata, Phytophthora cactorum, P. drechsleri, P. nicotianae var. parasitica, Rhizopus arrhizus
Rostratum leaf spot (Helminthosporium leaf disease, ear and stalk rot)	Setosphaeria rostrata, (anamorph: xserohilum rostratum = Helminthosporium

Disease	Pathogen
	rostratum)
Java downy mildew	Peronosclerospora maydis = Sclerospora maydis
Philippine downy mildew	Peronosclerospora philippinensis = Sclerospora philippinensis
Sorghum downy mildew	Peronosclerospora sorghi = Sclerospora sorghi
Spontaneum downy mildew	Peronosclerospora spontanea = Sclerospora spontanea
Sugarcane downy mildew	Peronosclerospora sacchari = Sclerospora sacchari
Sclerotium ear rot (southern blight)	Sclerotium rolfsii Sacc. (teleomorph: Athelia rolfsii)
Seed rot-seedling blight	Bipolaris sorokiniana, B. zeicola = Helminthosporium carbonum, Diplodia maydis, Exserohilum pedicillatum, Exserohilum turcicum = Helminthosporium turcicum, Fusarium avenaceum, F. culmorum, F. moniliforme, Gibberella zeae (anamorph: F. graminearum), Macrophomina phaseolina, Penicillium spp., Phomopsis sp., Pythium spp., Rhizoctonia solani, R. zeae, Sclerotium rolfsii, Spicaria sp.
Selenophoma leaf spot	Selenophoma sp.
Sheath rot	Gaeumannomyces graminis
Shuck rot	Myrothecium gramineum
Silage mold	Monascus purpureus, M ruber
Smut, common	Ustilago zeae = U. maydis
Smut, false	Ustilaginoidea virens
Smut, head	Sphacelotheca reiliana = Sporisorium holcisorghi
Southern corn leaf blight and stalk rot	Cochliobolus heterostrophus (anamorph: Bipolaris maydis = Helminthosporium maydis)
Southern leaf spot	Stenocarpella macrospora = Diplodia macrospora
Stalk rots (minor)	Cercospora sorghi, Fusarium episphaeria, F. merismoides, F. oxysporum Schlechtend, F. poae, F. roseum, F. solani (teleomorph: Nectria

Disease	Pathogen
	haematococca), F. tricinctum, Mariannaea elegans, Mucor sp., Rhopographus zeae, Spicaria sp.
Storage rots	Aspergillus spp., Penicillium spp. und weitere Pilze
Tar spot	Phyllachora maydis
Trichoderma ear rot and root rot	Trichoderma viride = T. lignorum teleomorph: Hypocrea sp.
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

Table 4: Diseases caused by fungi and Oomycetes with unclear classification regarding biotrophic, hemibiotrophic or necrotrophic behavior

Disease	Pathogen
Hyalothyridium leaf spot	Hyalothyridium maydis
Late wilt	Cephalosporium maydis

The following are especially preferred:

- Plasmodiophoromycota such as Plasmodiophora brassicae (clubroot of crucifers), Spongospora subterranea, Polymyxa graminis,
- 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 (for example damping-off of Beta beet caused by P. debaryanum), Phytophthora infestans (late blight in potato and in tomato and the like), Albugo spec.
- 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), Uncinula 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, 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).

- 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).
- Deuteromycetes (Fungi imperfecti) such as Septoria (Stagonospora) nodorum (glume blotch) of wheat (Septoria tritici), Pseudocercosporella herpotrichoides (eyespot of wheat, barley, rye), Rynchosporium 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).

Especially preferred are biotrophic pathogens, among which in particular heminecrotrophic pathogens, i.e. Phakopsora pachyrhizi and/or those pathogens which have essentially a similar infection mechanism as Phakopsora pachyrhizi, as described herein. Particularly preferred are pathogens from the group Uredinales (rusts), among which in particular the Melompsoraceae. Especially preferred are Phakopsora pachyrhizi and/or Phakopsora meibomiae.

2. Bacterial pathogens:

The pathogens and the diseases associated with them which are mentioned in Table 5 may be mentioned by way of example but not by limitation.

Table 5: Bacterial diseases

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

3. Viral pathogens:

"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.

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

Table 6: Viral diseases

Disease	Pathogen
American wheat striate (wheat striate mosaic)	American wheat striate mosaic virus (AWSMV)
Barley stripe mosaic	Barley stripe mosaic virus (BSMV)
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)
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)
Cynodon chlorotic streak	Cynodon chlorotic streak virus (CCSV)
Johnsongrass mosaic	Johnsongrass mosaic virus (JGMV)
Maize bushy stunt	Mycoplasma-like organism (MLO) associated
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)
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
Maize pellucid ringspot	Maize pellucid ringspot virus (MPRV)
Maize raya gruesa	Maize raya gruesa virus (MRGV)
Maize rayado fino (fine striping disease)	Maize rayado fino virus (MRFV)
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)
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)
Maize stripe (maize chlorotic stripe, maize hoja blanca)	Maize stripe virus
Maize stunting	Maize stunting virus
Maize tassel abortion	Maize tassel abortion virus (MTAV)
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)
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)
Rice black-streaked dwarf	Rice black-streaked dwarf virus (RBSDV)
Rice stripe	Rice stripe virus (RSV)
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)
Wheat spot mosaic	Wheat spot mosaic virus (WSMV)

4. Animal pests

4.1 Pathogenic insects:

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, Diabrotica undecimpunctata, Diabrotica virgifera, Agrotis ipsilon, Crymodes devastator, Feltia ducens, Agrotis gladiaria, Melanotus spp., Aeolus mellillus, Aeolus mancus, Horistonotus uhlerii, Sphenophorus maidis, Sphenophorus zeae, Sphenophorus parvulus, Sphenophorus callosus, Phyllogphaga spp., Anuraphis maidiradicis, Delia platura, Colaspis brunnea, Stenolophus lecontei and Clivinia impressifrons.

Other examples are: barley leaf beetle (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 7 may be mentioned by way of example, but not by limitation.

Table 7: Parasitic nematodes

Damage	Pathogenic nematode
Awl	Dolichodorus spp., D. heterocephalus
Bulb and stem nematode disease; bulb eelworm	Ditylenchus dipsaci
Burrowing	Radopholus similis
Cyst nematode disease	Heterodera avenae, H. zeae, Punctodera chalcoensis
Dagger	Xiphinema spp., X. americanum, X. mediterraneum
False root-knot	Nacobbus dorsalis
Lance, Columbia	Hoplolaimus columbus

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

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).

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

1. Barley:

Fungal, bacterial and viral pathogens: Puccinia graminis f.sp. hordei, barley yellow dwarf virus (BYDV),

Pathogenic insects/nematodes: Ostrinia nubilalis (European corn borer); Agrotis ipsilon; Schizaphis graminum; Blissus leucopterus leucopterus; Acrosternum hilare; Euschistus servus; Deliaplatura; Mayetiola destructor; Petrobia latens.

2. Soybean:

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 sojina, Peronospora manshurica, Colletotrichum dematium (Colletotrichum truncatum), Corynespora cassiicola, Septoria glycines, Phyllosticta sojicola, Alternaria alternata, Pseudomonas syringae p.v. glycinea, Xanthomonas campestris p.v. phaseoli, Microsphaera diffussa, Fusarium semitectum, Phialophora gregata, soybean mosaic virus, soybean rust, Glomerella glycines, tobacco ring spot virus, tobacco streak virus, Phakopsorapachyrhizi, Pythium aphanidermatum, Pythium ultimum, Pythium debaryanum, tomato spotted wilt virus, Heterodera glycines Fusarium solani; particularly soybean rust.

Pathogenic insects/nematodes: Pseudoplusia includens; Anticarsia gemmatalis; Plathypena scabra; Ostrinia nubilalis; Agrotis ipsilon; Spodoptera exigua; Heliothis virescens; Helicoverpa zea; Epilachna varivestis; Myzus persicae; Empoasca fabae; Acrosternum hilare; Melanoplus femurrubrum; Melanoplus differentialis; Hylemya platura; Sericothrips variabilis; Thrips tabaci; Tetranychus turkestani; Tetranychus urticae;

3. Oil seed rape:

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

4. Alfalfa:

Fungal, bacterial or viral pathogens: Clavibater michiganese 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:

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, Collotetrichum graminicola, Erysiphe graminis f.sp. tritici, Puccinia graminis f.sp. tritici, Puccinia

recondita f.sp. tritici, Puccinia striiformis, Pyrenophora tritici-repentis, Septoria (Stagonospora) nodorum, Septoria tritici, Septoria avenae, Pseudocercosporella herpotrichoides, Rhizoctonia solani, Rhizoctonia cerealis, Gaeumannomyces graminis var. tritici, Pythium aphanidermatum, Pythium arrhenomanes, Pythium ultimum, Bipolaris sorokiniana, barley tellow dwarf virus, brome mosaic virus, soil borne wheat mosaic virus, wheat streak mosaic virus, wheat spindle streak virus, American wheat striate virus, Claviceps purpurea, Tilletia tritici, Tilletia laevis, Ustilago tritici, Tilletia indica, Rhizoctonia solani, Pythium arrhenomannes, Pythium gramicola, Pythium aphanidermatum, high plains virus, European wheat striate virus, Puccinia graminis f.sp. tritici (wheat stem rust), Blumeria (Erysiphe) graminis f.sp. tritici (wheat powdery mildew).

Pathogenic insects/nematodes: Pseudaletia unipunctata; Spodoptera, frugiperda; Elasmopalpus lignosellus; Agrotis orthogonia; Elasmopalpus Zignosellus; Oulema melanopus; Hypera punctata; Diabrotica undecimpunctata howardi; Russian wheat aphid; Schizaphis graminum; Macrosiphum avenae; Melanoplus femurrubrum; Melanoplus differentialis; Melanoplus sanguinipes; Mayetiola destructor; Sitodiplosis mosellana; Meromyza americana; Hylemya coarctata; Frankliniella fusca; Cephus cinctus; Aceria tulipae;

6. Sunflower:

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.

Pathogenic insects/nematodes: Suleima helianthana; Homoeosoma electellum; zygogramma exclamationis; Bothyrus gibbosus; Neolasioptera murtfeldtiana;

7. Maize:

Fungal, bacterial or viral pathogens: Fusarium moniliforme var. subglutinans, Erwinia stewartii, Fusarium moniliforme, Gibberella zeae (Fusarium graminearum), Stenocarpella maydi (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 corotovora, Cornstunt spiroplasma, Diplodia macrospora, Sclerophthora macrospora, Peronosclerospora sorghi, Peronosclerospora philippinesis, Peronosclerospora maydis, Peronosclerospora sacchari, Spacelotheca reiliana, Physopella zeae, Cephalosporium maydis, Caphalosporium acremonium, maize chlorotic mottle virus, high plains virus, maize mosaic virus, maize rayado fino virus, maize streak virus (MSV), maize stripe virus, maize rough dwarf virus.

Pathogenic insects/nematodes: Ostrinia nubilalis; Agrotis ipsilon; Helicoverpa zea; Spodoptera frugiperda; Diatraea grandiosella; Elasmopalpus lignosellus; Diatraea saccharalis; Diabrotica virgifera; Diabrotica longicornis barberi; Diabrotica undecimpunctata howardi; Melanotus spp.; Cyclocephala borealis; Cyclocephala immaculata; Popillia japonica; Chaetocnema pulicaria; Sphenophorus maidis; Rhopalosiphum maidis; Anuraphis maidiradicis; Blissus leucopterus leucopterus; Melanoplus femurrubrum; Melanoplus sanguinipes; Hylemva platura; Agromyza parvicornis; Anaphothrips obscrurus; Solenopsis milesta; Tetranychus urticae.

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 monilifonne, 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, Sclerophthona macrospora, Peronosclerospora sorghi, Peronosclerospora philippinensis, Sclerospora graminicola, Fusarium graminearum, Fusarium oxysporum, Pythium arrhenomanes, Pythium graminicola.

Pathogenic insects/nematodes: Chilo partellus; Spodoptera frugiperda; Helicoverpa zea; Elasmopalpus lignosellus; Feltia subterranea; Phvllophaga crinita ; Eleodes, Conoderus und Aeolus spp.; Oulema melanopus; Chaetocnema pulicaria; Sphenophorus maidis;

Rhopalosiphum maidis; Siphaflava; Blissus leucopterus leucopterus; Contarinia sorghicola; Tetranychus cinnabarinus; Tetranychus urticae.

9. Cotton:

Pathogenic insects/nematodes: Heliothis virescens; Helicoverpa zea; Spodoptera exigua; Pectinophora gossypiella; Anthonomus grandis grandis; Aphis gossypii; Pseudatomoscelis seriatus; Trialeurodes abutilonea; Lygus lineolaris; Melanoplus femurrubrum; Melanoplus differentialis; Thrips tabaci (onion thrips); Franklinkiella fusca; Tetranychus cinnabarinus; Tetranychus urticae.

10. Rice:

Pathogenic insects/nematodes: Diatraea saccharalis; Spodoptera frugiperda; Helicoverpa zea; Colaspis brunnea; Lissorhoptrus oryzophilus; Sitophilus oryzae; Nephotettix nigropictus; Blissus leucopterus leucopterus; Acrosternum hilare.

11. Oilseed rape:

Pathogenic insects/nematodes: Brevicoryne brassicae; Phyilotreta cruciferae; Mamestra conjgurata; Plutella xylostella; Delia ssp..

The processes and methods according to the invention firstly relate by preference to soya, plant parts, cells and/or seed thereof. Thereof. Equally, the processes and methods according to the invention relate by preference to soybean rust.

For the purposes of the invention, "BI1 protein" means polypeptides which have at least one sequence with at least 50%, preferably at least 80%, especially preferably at least 90%, very especially preferably at least 95% and especially preferably 100% homology with a BI1 consensus motif selected from the group consisting of

a) H(L/I)KXVY
b) AXGA(Y/F)XH
c) NIGG
d) P(V/P)(Y/F)E(E/Q)(R/Q)KR
e) (E/Q)G(A/S)S(V/I)GPL
f) DP(S/G)(L/I)(I/L)
g) V(G/A)T(A/S)(L/I)AF(A/G)CF(S/T)
h) YL(Y/F)LGG, preferably EYLYLGG

i) L(L/V)SS(G/W)L(S/T)(I/M)L(L/M)W

j) DTGX(I/V)(I/V)E.

Especially preferred in this context is the BI consensus motif f) YL(Y/F)LGG, very especially preferred is (EYLYLGG). This motif is characteristic for plant BI1 proteins. Sequences with homology to at least 2 or 3 of these motifs (a to j) are especially preferably found in a BI1 protein, very especially preferably at least 4 or 5, most preferably all motifs a to j. Further BI1-typical sequence motifs can be derived by the skilled worker without difficulty from the sequence alignment of BI1 proteins as shown in FIG. 1 or 6.

Especially preferred for the use in the methods disclosed herein are BI1 proteins which are encoded by a polypeptide which comprises at least one sequence selected from the group consisting of:

a) the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46;

b) sequences with at least 50%, more preferably 60%, 70%, 80%, 85% or 90%, especially preferably 95, 97 or 99% or more identity with one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46; and

c) sequences which comprise at least one part-sequence of at least 10 contiguous amino acid residues of one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46,
and/or which comprise at least a part-sequence of at least 20 contiguous amino acid residues, where the part-sequence has at 80%, preferably 85% or 90%, especially preferably 95, 97 or 99% or more identity with the corresponding part-sequence from one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46.

Comprised in accordance with the invention by the term BI protein are in particular natural or artificial mutations of the BI1 polypeptides as shown in SEQ ID NO: 2, 4, 6, 8, 10, 38 and in particular 46, and homologous or similar polypeptides from other organisms, preferably plants, which furthermore have essentially identical properties. Mutations comprise substitutions, additions, deletions, inversions or insertions of one or more amino acid residues.

Also comprised are thus use forms utilizing BI1 proteins from nonplant organisms such as for example humans (GenBank Acc.-No.: P55061), rat (GenBank Acc.-No.: P55062) or Drosophila

(GenBank Acc.-No.: Q9VSH3). Motifs which are conserved between plant and nonplant BI1 proteins can be identified readily by sequence alignments (cf. alignment in Bolduc N. et al., Planta 216, 377 (2003); Fig. 1 and 6).

Thus, the present invention also comprises for example those polypeptides which are obtained by modification of a polypeptide as shown in SEQ ID NO: 2, 4, 6, 8, 10, 38 and 46.

The sequences from other plants which are homologous to the BI1 sequences disclosed within the scope of the present invention can be found for example by

a) database search in libraries of organisms whose genomic or cDNA sequence is known in full or in part, using the BI1 sequences provided as search sequence, or
b) screening gene libraries or cDNA libraries using the BI1 sequences provided as probe.

Screening cDNA libraries or genomic libraries (for example using one of the nucleic acid sequences described under SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 37 and 45 or parts of these as probe) is a method of identifying homologous or similar or identical sequences, which method is known to the skilled worker. In this context, the probes derived from the nucleic acid sequences as shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 37 and 45 have a length of at least 20 bp, preferably at least 50 bp, especially preferably at least 100 bp, very especially preferably at least 200 bp, most preferably at least 400 bp. A DNA strand which is complementary to the sequences described under SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 37 and 45 may also be employed for screening the libraries.

Homology between two nucleic acid sequences is understood as meaning, in the present context, the identity of the nucleic acid sequence over in each case the entire sequence length which, in turn, is calculated by alignment 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., Nucleic Acids Res. 25, 3389 (1997)) 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 alignment with the

sequence SEQ ID NO: 1 by the above program algorithm with the above parameter set, has at least 80% homology.

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 alignment with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap weight: 8 Length weight: 2

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

BI1 proteins also comprise those polypeptides which are encoded by nucleic acid sequences which hybridize under standard conditions with one of the BI1 nucleic acid sequences described by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 37 and 45, the nucleic acid sequence which is complementary thereto or parts of the above, and which have essentially identical properties as the proteins described under SEQ ID NO: 2, 4, 6, 8, 10, 38 and 46.

"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 E.F., 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.

"Essential properties" means, with regard to a BI protein, one or more of the following properties:

a) Conferring or increasing the pathogen resistance to at least one pathogen while increasing the protein quantity or function of said BI protein in at least one tissue of the plant, preferably at least in the epidermis of the plant.

b) Nonappearance of a spontaneously induced cell death when increasing the protein quantity or the function of said BI protein.

c) The property of significantly inhibiting the BAX-induced apoptosis in the case of transient cotransfection of Bax with said BI1 protein, for example in HEK293 cells. Suitable methods have been described (Bolduc N. et al., Planta 216, 377 (2003)).

d) The presence of five to seven putative transmembrane domains within said BI1 protein.

e) Preferential localization in cell membranes, in particular the nuclear membrane, the ER membrane and/or the thylakoid membrane.

In this context, the quantitative manifestation of said properties of a BI1 protein may deviate up or down in comparison with the value obtained for the BI1 protein as shown in SEQ ID NO: 2, 4, 6, 8, 10, 38 or 46.

For the purposes of the present invention, the term "increasing the BI1 protein quantity or function" is to be understood in the broad sense and may be based on different cell-biological mechanisms.

"Protein quantity" means the amount of a BI1 protein in the organism, tissue, cell or cell compartment detailed.

"Increasing the protein quantity" means the quantitative increase of the amount of a BI1 protein in the organism, tissue, cell or cell compartment detailed, for example by means of one of the methods described hereinbelow, in comparison with the wild type of the same genus and species, to which this method has not been applied, but on the otherwise identical conditions (such as, for example, culture conditions, age of the plants and the like). In this context, the increase amounts to at least 10%, preferably at least 30% or at least 50%, especially preferably at least 70% or 100%, very especially preferably at least 200% or 500%, most preferably at

least 1000%. The protein quantity can be determined by means of a variety of methods with which the skilled worker is familiar. Examples which may be mentioned, but not by limitation, are: the micro-biuret method (Goa J., Scand J. Clin. Lab. Invest. 5, 218 (1953)), the Folin-Ciocalteu method (Lowry O.H. et al., J Biol Chem 193, 265 (1951)) or the measurement of the adsorption of CBB G-250 (Bradford M.M., Analyt. Biochem. 72, 248 (1976)). Furthermore, a quantification can be accomplished via immunological methods such as, for example, Western blot. The generation of suitable BI1 antibodies and the procedure of BI1 Western blots is described (Bolduc N. et al., FEBS Lett 532, 111 (2002)). An indirect quantification can be accomplished via Northern blots, where, as a rule, the mRNA quantity correlates well with the resulting protein quantity. Suitable methods have been described (Bolduc N. et al., Planta 216, 377 (2003); Matsumura H. et al., Plant J. 33, 425 (2003)).

"Function" preferably means the property of a BI1 protein of reducing the spontaneously induced cell death and/or the property of inhibiting the apoptosis-inducing effect of Bax. Such functions are among the essential properties of a BI1 protein.

Within the scope of the present invention, "increasing" the function means, for example, the quantitative increase of the inhibitor effect on the Bax-induced apoptotic cell death, which can be determined quantitatively by methods with which the skilled worker is familiar (see herein above). In this context, the increase amounts to at least 10%, preferably at least 30% or at least 50%, especially preferably at least 70% or 100%, very especially preferably at least 200% or 500%, most preferably at least 1000%. Methods of increasing the function comprise, beside the above-described method of increasing the protein quantity (which, as a rule, also increases the function) furthermore – by way of example, but not by limitation – in particular the introduction of mutations into a BI1 protein or the inhibition of a putative BI1 inhibitor, and the like.

The BI1 protein quantity can be increased for example, but not by limitation, by one of the following methods:

a) recombinant expression or overexpression of a BI1 protein by introducing a recombinant expression cassette comprising a nucleic acid sequence coding for a BI1 protein under the control of a tissue-specific promoter, where said promoter has activity preferably essentially specifically in the leaf epidermis and/or no activity in the mesophyll.

b) modification (for example substitution) of the regulatory regions (for example the promoter region) of an endogenous BI1 gene, for example substitution for a tissue-specific promoter

by means of homologous recombination, where said promoter has activity preferably essentially specifically in the leaf epidermis and/or no activity in the mesophyll.

c) insertion of a nucleic acid sequence, coding for a BI1 protein, into the plant genome downstream of a tissue-specific promoter by means of homologous recombination, where said promoter has activity preferably essentially specifically in the leaf epidermis and/or no activity in the mesophyll.

d) increasing the expression of an endogenous BI1 protein by introducing a transcription factor (for example artificial transcription factor from the class of the zinc finger proteins) which is suitable for inducing the expression of said BI1 protein. Preferred is the introduction of a recombinant expression cassette comprising a nucleic acid sequence coding for said transcription factor under the control of a tissue-specific promoter, where said promoter has activity preferably essentially specifically in the leaf epidermis and/or no activity in the mesophyll.

For the purposes of the present invention, the term "to introduce/introduction" generally comprises all methods which are suitable for transferring the compound to be introduced, either directly or indirectly, into a plant or into a cell, compartment, tissue, organ or seed thereof, or generating it therein. This comprises direct and indirect methods. The introduction can lead to a transient presence of said compound or else to a stable or inducible presence. "Introducing" comprises for example methods such as transfection, transduction or transformation.

In the recombinant expression cassettes which are employed within the scope of the present invention, a nucleic acid molecule (for example coding for a BI1 protein) is in operable linkage with at least one tissue-specific promoter, where said promoter has activity preferably essentially specifically in the leaf epidermis and/or no activity in the mesophyll, and where the promoter is heterologous with regard to the nucleic acid sequence to be expressed, i.e. does not naturally occur in combination with same. The recombinant expression cassettes according to the invention may optionally comprise further genetic control elements.

Operable linkage is to be understood as meaning, for example, the sequential arrangement of said promoter with the nucleic acid sequence to be expressed 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. 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 downstream of 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 a recombinant expression cassette, can be generated by means of customary recombination and cloning techniques as are described above. 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, a recombinant expression cassette also denotes those constructions in which the promoter is positioned upstream of an endogenous BI1 gene, for example by means of homologous recombination, thus controlling the expression of the BI1 protein. Analogously, the nucleic acid sequence to be expressed (for example coding for a BI1 protein) can be placed downstream of an endogenous promoter in such a way that the same effect is manifested. Both approaches lead to inventive recombinant expression cassettes.

By "tissue-specific promoter with activity essentially specifically in the leaf epidermis" there are generally to be understood, for the purposes of the present invention, those promoters which are suitable of ensuring or increasing recombinant expression of a nucleic acid sequence in at least one plant tissue with the proviso that

a) the expression is manifested at least in the epidermis and preferably not in the mesophyll, or remains essentially unchanged in the mesophyll, where tissues other than the two tissues mentioned are not been taken into consideration, and

b) the recombinant expression under the control of said promoter in said plant tissue amounts to at least five times, preferably at least ten times, especially preferably at least hundred times, the expression of a comparative plant.

Genetic control sequences furthermore also encompass 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 been demonstrated that they may play a significant role 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., Nucl. Acids Res. 15, 8693 (1987)) and the like. Furthermore, they may promote tissue specificity (Rouster J. et al., Plant J 15, 435 (1998)).

The recombinant expression cassette may advantageously comprise one or more 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 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 are plant polyadenylation signals, preferably those which essentially correspond to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*, in particular the OCS (octopin synthase) terminator and the NOS (nopalin synthase) terminator.

Control sequences are furthermore to be understood as those which 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 BI1 gene may be exchanged for one of the preferred tissue-specific promoters. Methods such as the cre/lox technology permit a tissue-specific, if appropriate inducible, removal of the recombinant expression cassette from the genome of the host organism (Sauer B., Methods. 14(4), 381 (1998)). In this method, specific flanking sequences (lox sequences), which later allow removal by means of cre recombinase, are attached to the target gene.

A recombinant expression cassette 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 elements which have an effect on the generation, amplification or function of the recombinant expression cassettes, vectors or recombinant organisms according to the invention. The following may be mentioned by way of example, but not by limitation:

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 markers are those which confer resistance to herbicides. Examples which may be mentioned are: DNA sequences which code for phosphinothricin acetyltransferases (PAT) and which inactivate glutamin synthase inhibitors (bar and pat genes), 5-enolpyruvylshikimate-3-phosphate synthase genes (EPSP synthase genes), which confer resistance to Glyphosat® (N-(phosphonomethyl)glycine), the gox gene, which encodes Glyphosat®-degrading enzymes (Glyphosate oxidoreductase), the deh gene (encoding a dehalogenase which inactivates Dalapon®), 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 (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), and the acetolactate synthase gene (ALS), which confers resistance to imidazolinone herbicides.

b) Reporter genes which code for 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 coding for reporter proteins (Schenborn E., Groskreutz D., Mol. Biotechnol. 13(1), 29 (1999)) such as the green fluorescent protein (GFP) (Sheen et al., Plant Journal 8(5), 777 (1995); Haseloff et al., Proc. Natl. Acad. Sci. USA 94(6), 2122 (1997); Reichel et al., Proc. Natl. Acad. Sci. USA 93(12), 5888 (1996); Tian et al., Plant Cell Rep. 16, 267 (1997); WO 97/41228; Chui W.L. et al., Curr. Biol. 6, 325 (1996); Leffel S.M. et al., Biotechniques 23(5), 912 (1997)), chloramphenicol transferase, a luciferase (Ow et al., Science 234, 856 (1986); Millar et al., Plant Mol. Biol. Rep. 10, 324 (1992)), the aequorin gene (Prasher et al., Biochem. Biophys. Res. Commun. 126(3), 1259 (1985)), 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 (1988)), with β–glucuronidase being very especially preferred (Jefferson et al., EMBO J. 6, 3901 (1987)).

c) Origins of replication, which ensure amplification of the recombinant 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., Plant Cell Reports 5, 81 (1986)).

The introduction of a recombinant 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 recombinant expression cassettes. The recombinant 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 recombinant 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 plurality of methods are available for this procedure, which is referred to as transformation (or transduction or transfection) (Keown et al., Methods Enzymol. 185, 527 (1990); Jenes B. et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited

by Kung S.D. and Wu R., Academic Press, p. 128-143 (1993), and in Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42, 205 (1991)).

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., Gene 100, 247 (1991); Scheid et al., Mol. Gen. Genet. 228, 104 (1991); Guerche et al., Plant Science 52, 111 (1987); Neuhause et al., Theor. Appl. Genet. 75, 30 (1987); Klein et al., Nature 327, 70 (1987); Howell et al., Science 208, 1265 (1980); Horsch et al., Science 227, 1229 (1985); DeBlock et al., Plant Physiol 91, 694 (1989)).

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 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, 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 R.B. et al., Science 225, 1229 (1985).

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 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 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., Mol. Gen. Genet. 163, 181 (1978)). The selection marker gene permits a 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 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 Kanters B.V., Alblasserdam, Chapter V; An et al., EMBO J. 4, 277 (1985)). Various binary vectors are known, some of which are commercially available such as, for example, pBI101.2 or pBIN19 (Bevan et al., Nucl. Acids Res. 12, 8711 (1984); Clontech Laboratories, Inc. USA). Further promoters which are suitable for expression in plants have been described (Rogers et al., Methods Enzymol. 153, 253 (1987); Schardl et al., Gene 61, 1(1987); Berger et al., Proc. Natl. Acad. Sci. USA 86, 8402 (1989)).

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 may 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 comprise 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 of suitable selection markers are mentioned above. As soon as a transformed plant cell has been generated, a complete plant can be obtained by using methods known to the skilled worker. Starting material in this context is, for example, callus cultures. The development of shoot and root and can be induced in the known manner in these as yet undifferentiated cell biomasses. The plantlets obtained can be grown on and bred. The skilled worker is familiar with methods of regenerating plant parts of entire plants starting from plant cells. For example, methods described by Fennell et al., Plant Cell Rep. 11, 567 (1992); Stoeger et al., Plant Cell Rep. 14, 273 (1995); Jahne et al., Theor. Appl. Genet. 89, 525 (1994) are used in this context. The plants obtained can be bred and/or hybridized in the customary manner. Two or more generations should be grown in order to ensure that the genomic integration is stable and hereditary.

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 and the like), stress resistance or another improvement of the plant properties. Examples are mentioned, inter alia, by Dunwell J.M., J. Exp. Bot. 51 (Spec No), 487 (2000).

With regard to, for example 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, the term "recombinant" means all those constructs which are the result of recombinant methods and in which either

a) the BI1 nucleic acid sequence or
b) a genetic control sequence, for example promoter, which is operably linked with the BI1 nucleic acid sequence, or
c) (a) and (b)

are not located in their natural genetic environment or have been modified by recombinant methods, an example of the modification being a substitution, addition, deletion, inversion or insertion 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 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 occurring expression cassette - for example the naturally occurring combination of the BI1 promoter with the corresponding BI1 gene - becomes a recombinant expression cassette when it is modified by non-natural, synthetic “artificial” methods such as, for example, mutagenization. Such methods have been described (US 5,565,350; WO 00/15815; also see above).

The invention also relates to recombinant 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. Host or starting organisms which are preferred as recombinant organisms are mainly plants in accordance with the above definition.

The invention furthermore relates to the use of the recombinant 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 recombinant plant organisms - derived from them, and to recombinant propagation material such as seeds or fruits, for the production of foodstuffs or feedingstuffs, pharmaceuticals or fine chemicals.

Furthermore is a nucleic acid molecule which is antisense to the nucleic acid according to the invention, a monoclonal antibody which binds specifically to the polypeptide according to the invention, and a fungicide which comprises the nucleic acid according to the invention, the vector according to the invention, in particular an infectious, for example viral, vector according to the invention, the polypeptide according to the invention in a form which is suitable for application to plants, for example in encapsulated form or in an infectious organism which is preferably suitable for transferring nucleic acids or for expressing genes in a cell, such as an agrobacterium or a virus.

In one embodiment, the invention relates to the use of a BI-1 encoding nucleic acid molecule or of a BI-1 protein for the generation of a pathogen-resistant plant, preferably for the generation of a fungus-resistant plant or for the generation of a fungicide which brings this about, or for controlling or treating plants which are attacked, or liable to be attacked, by pathogens.

Sequences

1. SEQ ID NO: 1 : Nucleic acid sequence coding for a BI1 protein from barley (Hordeum vulgare).
2. SEQ ID NO: 2 : Amino acid sequence coding for a BI1 protein from barley (Hordeum vulgare).
3. SEQ ID NO: 3 : Nucleic acid sequence coding for a BI1 protein from Arabidopsis thaliana.
4. SEQ ID NO: 4 : Amino acid sequence coding for a BI1 protein from Arabidopsis thaliana.
5. SEQ ID NO: 5 : Nucleic acid sequence coding for a BI1 protein from tobacco.
6. SEQ ID NO: 6 : Amino acid sequence coding for a BI1 protein from tobacco.

7. SEQ ID NO: 7 : Nucleic acid sequence coding for a BI1 protein from rice.

8. SEQ ID NO: 8: Amino acid sequence coding for a BI1 protein from rice.

9. SEQ ID NO: 9 : Nucleic acid sequence coding for a BI1 protein from oil seed rape.

10. SEQ ID NO: 10 : Amino acid sequence coding for a BI1 protein from oil seed rape.

11. SEQ ID NO: 11 : Nucleic acid sequence coding for part of a BI1 protein from soybean.

12. SEQ ID NO: 12: Amino acid sequence coding for part of a BI1 protein from soybean.

13. SEQ ID NO: 13 : Nucleic acid sequence coding for part of a BI1 protein from soybean.

14. SEQ ID NO: 14: Amino acid sequence coding for part of a BI1 protein from soybean.

15. SEQ ID NO: 15 : Nucleic acid sequence coding for part of a BI1 protein from wheat.

16. SEQ ID NO: 16 : Amino acid sequence coding for part of a BI1 protein from wheat.

17. SEQ ID NO: 17 : Nucleic acid sequence coding for part of a BI1 protein from maize.

18. SEQ ID NO: 18 : Amino acid sequence coding for part of a BI1 protein from maize.

19. SEQ ID NO: 19 : Nucleic acid sequence coding for part of a BI1 protein from wheat.

20. SEQ ID NO: 20 : Amino acid sequence coding for part of a BI1 protein from wheat.

21. SEQ ID NO: 21 : Nucleic acid sequence coding for part of a BI1 protein from maize.

22. SEQ ID NO: 22 : Amino acid sequence coding for part of a BI1 protein from maize.

23. SEQ ID NO: 23 : Nucleic acid sequence coding for part of a BI1 protein from maize.

24. SEQ ID NO: 24 : Amino acid sequence coding for part of a BI1 protein from maize.

25. SEQ ID NO: 25 : Nucleic acid sequence coding for part of a BI1 protein from wheat.

26. SEQ ID NO: 26 : Amino acid sequence coding for part of a BI1 protein from wheat.

27. SEQ ID NO: 27 : Nucleic acid sequence coding for part of a BI1 protein from maize.

28. SEQ ID NO: 28 : Amino acid sequence coding for part of a BI1 protein from maize.

29. SEQ ID NO: 29 : Nucleic acid sequence coding for the patatin promote from potato.

30. SEQ ID NO: 30 : Nucleic acid sequence coding for the Germin 9f-3.8 promoter from wheat.

31. SEQ ID NO: 31 : Nucleic acid sequence coding for the Arabidopsis CAB-2 promoter

32. SEQ ID NO: 32 : Nucleic acid sequence coding for the PPCZm1 promoter from maize.

33. SEQ ID NO: 33 : Nucleic acid sequence coding for the recombinant expression vector pUbiBI-1

34. SEQ ID NO: 34 : Nucleic acid sequence coding for the recombinant expression vector pLo114UbiBI-1

35. SEQ ID NO: 35 : Nucleic acid sequence coding for the recombinant expression vector pOXoBI-1

36. SEQ ID NO: 36 : Nucleic acid sequence coding for the recombinant expression vector pLo114OXoBI-1

37. SEQ ID NO: 37: Nucleic acid sequence coding for BI-1 protein from wheat

38. SEQ ID NO: 38: Amino acid sequence coding for BI-1 protein from wheat

39. SEQ ID NO: 39: Nucleic acid sequence for PEN1 (= ROR2) from barley

40. SEQ ID NO: 40: Amino acid sequence coding for PEN1 (= ROR2) from barley

41. SEQ ID NO: 41: Nucleic acid sequence for PEN1 (= ROR2) from Arabidopsis thaliana

42. SEQ ID NO: 42: Amino acid sequence coding for PEN1 (= ROR2) from Arabidopsis thaliana

43. SEQ ID NO: 43: Nucleic acid sequence coding for SNAP34 from barley

44. SEQ ID NO: 44: Amino acid sequence coding for SNAP34 from barley

45. SEQ ID NO: 45: Nucleic acid sequence coding for BI-1 from soya

46. SEQ ID NO: 46: Amino acid sequence coding for BI-1 from soya

47. SEQ ID NO: 47: GFP primer 1 (see herein below)

48. SEQ ID NO: 48: GFP primer 2 (see herein below)

Figures

1. Fig. 1a-d: Alignment of protein sequences of various BI-1 proteins from plants. AtBI-1: Arabidopsis; BnBI-1: Brassica napus (oil seed rape); GmBI2: Glycine max (soybean; variant 1); GmBI3: Glycine max (soybean; variant 2); HVBI-1: Hordeum vulgare (barley); NtBI-1: Nicotiana tabacum (tobacco); OsBI-1: Oryza sativa (rice); TaBI11: Triticum aestivum (wheat, variant 1); TaBI18: Triticum aestivum (wheat, variant 2); TaBI5 neu: Triticum aestivum (wheat, variant 3); ZmBI14: Zea mays (maize; variant 1); ZmBI16: Zea mays (maize; variant 2); ZmBI33: Zea mays (maize; variant 3); ZmBI8: Zea mays (maize; variant 4); Consensus: consensus sequence derived from the alignment.

2. Fig. 2: Vector map for the vector pUbiBI-1 (Ubi: ubiquitin promoter; BI-1 nucleic acid sequence coding for barley BI1 protein; ter: transcription terminator). Also indicated is the localization of the cleavage sites of various restriction enzymes.

3. Fig. 3: Vector map for the vector pLO114UbiBI-1 (Ubi: ubiquitin promoter; BI-1 nucleic acid sequence coding for barley BI1 protein; ter: transcription terminator). Also indicated is the localization of the cleavage sites of various restriction enzymes.

4. Fig. 4: Vector map for the vector pOxoBI-1 (Oxo: TaGermin 9f-2.8 promoter; BI-1 nucleic acid sequence coding for barley BI1 protein; ter: transcription terminator). Also indicated is the localization of the cleavage sites of various restriction enzymes.

5. Fig. 5: Vector map for the vector pLO114OxoBI-1 (Oxo: TaGermin 9f-2.8 promoter; BI-1 nucleic acid sequence coding for barley BI1 protein; ter: transcription terminator). Also indicated is the localization of the cleavage sites of various restriction enzymes.

6. Fig. 6: Alignment of the protein sequences of BI-1 proteins from barley (Hordeum vulgare, GenBank Acc.-No.: CAC37797), rice (Oryza sativa, GenBank Acc.-No.: Q9MBD8), Arabidopsis thaliana (GenBank Acc.-No.: Q9LD45) and humans (Homo sapiens, GenBank Acc.-No.: AAB87479). Amino acids shown against the black background are identical in all species. Amino acids shown against a gray background are

only identical in plants. Bars indicate the predicted seven transmembrane domains in HvBI-1.

7. Fig. 7: Diagram of the transformation rate as a function of the conditions during the transformation of soybean leaves (A) and barley leaves (B) with the gene gun.
For the transformation, in each case 1.6 µg of DNA were used per bombardment; particles of diameter 0.6 µm were used for barley leaves and with 1 µm diameter for soybean. The number of transformed cells was determined 24 hours after the transformation.

8. Fig. 8: Diagram of the penetration rate of P. pachyrhizi in barley cells which have been transformed transiently with a BI-1 overexpression construct in comparison with the control. The data are based on three independent experiments. By way of control, barley leaves were transformed with the reporter gene construct pGY1-GFP and with the blank vector. In the BI-1 transformed cells, the penetration rate differs significantly from the WT ($P<0.05$).

9. Fig. 9: PCR for detecting the GFP-BI-1 construct in the transgenic barley lines #6(1)E4L3P5 (T2), 1#6(2)E15L7P1 (T2), #6(2)E15L7P2 (T2) and #6(1)E8L1(T1) ('Golden Promise'). The PCR was carried out with genomic DNA of the plants with the GFP-specific primers. In positive transgenic plants, the entire GFP, which is 740 bp in size is amplified. The negative control used was genomic DNA from the WT 'Golden Promise' (WT) or water (NTC). The GFP of plasmid pGY1-GFP was amplified as the positive control (PC).

10. Fig. 10: Data obtained for the penetration rate of P. pachyrhizi in barley cells which have been transformed transiently with a BI-1 overexpression construct. The data shown are those of Fig. 8 (see also examples hereinbelow).

11. Fig. 11 A/B: Counted spores and cell responses in the interaction between soybean rust and transgenic barley lines with the GFP-BI-1 overexpression construct #6(1)E4L3P5(T2), 1#6(2)E15L7P1(T2), #6(2)E15L7P2(T2) and #6(1)E8L1(T1) CV. 'Golden Promise'. The WT acted as the control. Leaves of 7-day old plants were inoculated with soybean rust, fixed after 24 hours and stained with aniline blue for counting.

12. Fig. 12: Relative fraction of papillae formation and HR following inoculation with P. pachyrhizi in barley leaves of the transgenic lines (CV. 'Golden Promise') #6(1)E4L3P5

(T2), 1#6(2)E15L7P1 (T2), #6(2)E15L7P2 (T2) and #6(1)E8L1(T1) in comparison with the WT. The primary leaves were removed seven days after sowing the barley seeds inoculated with P. pachyrhizi and evaluated 24 hours after the inoculation. The data shown are the mean values of the WT and of the individual lines. The error bars represent the standard deviation. A Student's t-test reveals that the relative papillae formation and HR in the transgenic lines are significantly different from the WT, $P < 0.001$.

Examples

General methods:

The chemical synthesis of oligonucleotides can be effected, for example, in the known fashion using the phosphoamidite 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 gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking DNA fragments, transformation of E. coli cells, bacterial cultures, phage multiplication and sequence analysis of recombinant DNA, are carried out as described by Sambrook et al. Cold Spring Harbor Laboratory Press (1989), 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., Proc. Natl. Acad. Sci. USA 74, 5463 (1977)).

Example 1 : Inoculation with P. pachyrhizi

Suitable spore material (Uredospores) of the pathogen Phakopsora pachyrhizi was obtained from BASF Aktiengesellschaft. To this end, spores of soybean plants which had been inoculated 2-3 weeks earlier were used directly or shaken onto aluminum foil and stored in the dark at room temperature on a desiccant (TROPAGel, Tropack, Lahnau).

To prepare a spore suspension, the spores were washed from the leaves with Tween/H_2O (0.1%). The spores were counted using a Thoma hematocytometer under the light microscope. To carry out the inoculation, the leaves were fixed on 1% H_2O agar using a metal ring. Various methods were finally used for the inoculation. For the spray inoculation, the spore suspension was placed into a spray bottle operated with pressurized air and were distributed uniformly on the plants until the upper surface of the leaf was thoroughly moistened. To obtain a higher spore density, leaves which had previously been sprayed with Tween/H_2O were additionally dry-inoculated in a 'precipitation column'. To this end, the column is placed over the plates with the moistened leaves. The young spores of infected soybean plants are blown into the column via an inlet flap. As the result of the draft, the spores are fluidized, finely distributed, and fall down onto the leaves.

To obtain higher inoculation densities without spore aggregation, the leaves were, as an alternative, covered with a layer of a spore suspension and, after 15 minutes, removed from the

suspension (layering method). After this time, sufficient adhesion of the precipitated spores was already ensured. The inoculated leaves were incubated in a chamber with on average 25°C and an atmospheric humidity of 71.2%.

To assess the course of the infection under the microscope, leaf samples were taken 0 hours post inoculation (hpi), 6 hpi, 24 hpi and 48 hpi, and stained by different methods.

a) Coomassie staining

To carry out the Coomassie staining, the infected leaf material was harvested and destained overnight at room temperature. For the microscopy, the leaf material was covered in Coomassie solution, and the solution was rinsed off after 5 minutes using a little water. The sample was viewed under the microscope immediately thereafter.

b) Calcofluor staining

To prepare the Calcofluor staining solution, 0.03% of Calcofluor White (Sigma-Aldrich) was dissolved in 50 mM Tris/HCl pH 8.0 nd 0.01% Tween 20. To stain the extracellular fungal structures, the infected leaf was immersed in the staining solution for 30 seconds and then washed with water for 10 seconds. The stained fungal structures fluoresce pale blue under UV excitation.

c) Aniline blue staining

The leaf material was transferred into Falcon tubes or dishes with destaining solution and incubated overnight at RT. Thereafter, the destaining solution was removed and the leaves were washed 2× with water. For the staining, the leaves were covered for 1.5-2 h in aniline blue staining solution and subsequently viewed directly under the microscope.

d) Wheat germ agglutinin Alexa Fluor 488 staining (WGA staining)

For the WGA staining of P. pachyrhizi, inoculated barley leaves were placed into 10% (w/v) KOH for 30-45 minutes at RT. Inoculated soybean leaves were dissected into 1 cm^2 sections and boiled for 5 minutes in 10% (w/v) KOH. Thereafter, the barley and the soybean leaves were washed 5 times for 3-5 minutes with 1 x PBS buffer. For the staining, the leaf material was placed into WGA staining solution and allowed to infiltrate for 10 minutes under a residual pressure of 100 mbar. Thereafter, the material was viewed directly under the microscope or stored in the staining solution at 4°C in the dark.

Example 2 : Transient biolistic transformation of plant cells

For the transient overexpresssion of the Bax inhibitor (for constructs and other methods, see patent WO 2004/081217) by biolistic transformation, 30-100 mg of gold particles were weighed, resuspended in 1 ml of 70% strength EtOH and shaken for 3-5 minutes. After incubation for 1 hour at room temperature, pelletization was effected by centrifugation (1 min, 10 000 rpm, Eppendorf microcentrifuge). Thereafter, the particles were washed 3× with sterile water and subsequently taken up in sterile 50% (v/v) glycerol and stored at 4°C. When 30 mg of gold particles were weighed in, the solution contained approximately 25 mg/ml gold. Prior to use, the particles were again shaken in order to distribute them thoroughly and sonicated for 15 seconds in a sonicator.

In general, enough gold particles for at least three bombardments were prepared. Up to the precipitation step, the mixture was shaken vigorously for a few seconds on a vortex mixer after each step. For the DNA precipitation into gold particles for 3 bombardments, one 12.5 µl of the thoroughly shaken gold particle glycerol solution were removed and treated with the desired DNA (pGY1-BI-1, pGY1-GFP, pGY1, see WO 2004/081217). 1.6 µg/µl DNA were employed per bombardment on the plasmid. Thereafter, 12.5 µl of 2.5 M $CaCl_2$ solution and 5 µl of 0.1 M spermidine solution were added. The mixture was shaken for 3 minutes, treated with 70 µl of 70% (v/v) ethanol and carefully inverted. After addition of 70 µl of 100% ethanol, the mixture was again mixed thoroughly by inverting and incubated at -20°C for up to 1 hour. The particles were pelleted by centrifugation (1 min, 11000 rpm, Eppendorf microcentrifuge, Wesseling-Berzdorf), resuspended in 18 µl of 100% ethanol and distributed as uniformly as possible on the macrocarriers (Bio-Rad, Munich), which had previously been washed with 100% ethanol and dried, within a radius of approx. 1 cm. The macrocarriers were then incubated at room temperature until all of the ethanol had evaporated.

The transient biolistic transformation of barley and soya leaves was performed using the Biolistic Particle Delivery System PDS-1000/He system (Bio-Rad, Munich). The material used for the transformation was preferably barley leaves of 7-day old barley plants (cv. 'Hanna') or the first two leaves (two-leaf stage) of soybean plants (cv. 'Oxford'). The leaves to be transformed were placed on 1% (w/v) water agar and fixed using a metal ring.

In order to determine the optimum ratio between pressure applied and residual pressure in the vacuum chamber for the transformation, various pressure/residual-pressure combinations were tested. Residual pressures of 15-27 inches Hg and rupture disks rated at 650-1800 psi were used. After the bombardment, the chamber was ventilated, the carrier together with the leaves

was removed, and the leaves were incubated for at least 24 hours at room temperature before being viewed under the microscope or stained.

Gold particles were used for the ballistic transformation of the leaves. It emerged that bombardment with particles 0.6 µm in diameter was best suited to barley since they caused less tissue injury. In the case of the transient transformation of soybeans, the highest transformation rate was obtained with the 1 µm particles.

For the transient transformation to be successful, the ratios between the pressure for accelerating the particles, the residual pressure in the vacuum chamber and the distance between sample and particles must be adapted to each other. To achieve this, various conditions were tested for barley leaves and soybean leaves. In the case of soybean, a pressure of 900 psi with a residual pressure in the vacuum chamber of 25 inches Hg and a sample distance of 9 cm has proven successful (FIG. 7A). In the case of barley leaves, the highest cell numbers were obtained with a pressure of 1100 psi, a residual pressure in the vacuum chamber of 25 inches Hg and a distance between leaves and particles of 9 cm (FIG. 7B).

To study the effects of BI-1 on the interaction between P. pachyrhizi and barley, use was made of the transient biolistic transformation. The primary leaves of seven-day old barley plants (cv. 'Hanna') were bombarded with overexpression constructs of BI-1 (pGY1-BI-1, Hückelhoven R. et al., 2001). These plasmids contain a Camv 35S promoter, which ensures constitutive expression of the genes. A CaMV 35S/GFP construct as reporter gene was bombarded into the cells together with the expression plasmids (Schweizer P. et al., MPMI 12, 647 (1999)). The blank vector pGY-1 together with the GFP/reporter gene construct was used as the control. The inoculation with P. pachyrhizi was effected 24 hours after the transformation by precipitation from a spore suspension (layering method, 2-3 × 10^4 spores/ml). After incubation for 18 hours, the interactions were evaluated under the microscope following Calcofluor staining of the extracellular fungal structures. The structures counted were GFP-forming cells which had been penetrated by the fungus and GFP-forming cells which had been attacked by the fungus, but could either successfully defend themselves against it (or where the fungus has died prior to penetration). In addition, the total number of GFP-forming cells was counted. To carry out the evaluation, the relative penetration rates were calculated on the basis of the total number of transformed cells which interacted with soybean rust.

In the control, 53% of the transformed cells which interacted with P. pachyrhizi were penetrated (averaged over the experiments), while only 37% of the BI-1-transformed cells were penetrated (see Table FIG. 10 and FIG. 8). According to the evaluation, the penetration resistance of the

cells which had been transformed transiently with BI-1 is significantly increased over P. pachyrhizi. Viewing under the microscope revealed that the BI-1-forming cells had a markedly more vital appearance than cells which only expressed GFP.

Example 3 : Stable transformation of barley, and detection of the GFP:BI-1 transgene

To study the effect of BI-1 on interaction of barley with Phakopsora pachyrhizi in greater detail, stably transformed barley plants which overexpressed a GFP:BI-1 fusion protein were generated. The agrobacterium-mediated stable transformation used for this purpose is a technique which has been established for years and whose methods have already been published in a number of review articles (Cheng Z. et al., Plant Mol. Biol. 60, 583 (2004); Taylor et al., DNA & Cell Biology. 21(12), 963 (2002); Rakoczy-Trojanowska, Cellular & Molecular Biology Letters. 7(3), 849 (2002); Grabowska A., Acta Physiologiae Plantarum. 26(4), 451 (2004); Chesnokov V., Sel'skokhozyaistvennaya Biologiya. 1, 26 (2004). Even the transformation of monocotyledonous species such as, for example, barley, which was still difficult in the 1990s, is now a standard technique (Travella S. et al., Plant Cell Reports 23(12), 780 (2005); Murray F. et al., Plant Cell Reports 22(6), 397 (2004)).

Since it was not known of the resulting transgenic plants whether they were homozygous lines, the presence of the overexpression construct had first to be detected. To this end, the genomic DNA was isolated from one leaf of all of the plants. This was done using the DNeasy 96 Plant Kit (Quiagen, Hilden; following the manufacturer's instructions).
The overexpression construct GFP-BI-1 was detected with GFP-specific primers by means of PCR for the presence of the construct. The Phusion Hot Start (Finnzymes, Espoo) was used for the *in-vitro* amplification of DNA by PCR (Mullis & Faloona, 1987).

Primer 1: 5'-ATGGTGAGCAAGGGCGAGGA-3' (SEQ ID NO: 47)
Primer 2: 5'-TTGAACAACGATGTGCAAGACTCCTTGTACAGCTCGTCCATGC-3') (SEQ ID NO: 48)

PCR approach for detecting the GFP:BI-1 transgene:

DMSO	0.6 µl
Buffer (5x) (F-519 GC)	4 µl
DNA	3 µl
dNTP mix (10 mM each)	0.4 µl

Primer 1 (20 pmol)	1 µl
Primer 2 (20 pmol)	1 µl
Hot Start Phusion F 540L	0.2 µl
Water	9.8 µl

The PCR was carried out a thermocycler with heatable lid (Tgradient; Biometra, Göttingen).

PCR Programme:

1.	Initial denaturation	98°C	30sec	
2.	Denaturation	98°C	10sec	35 cycles of steps 2-4
3.	Annealing	58°C	30sec	
4.	Elongation	72°C	30sec	
5.	Elongation	72°C	5 min	
6.	Storage	4°C		

Using standard methods, the DNA was separated and analyzed by gel electrophoresis in a 1% agarose gel ([w/v], INVITROGEN, Karlsruhe; in 1 x TAE buffer) (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY) 1989).

In a total of six plants out of the transgenic lines #6(2)E15L7P2 (T2) and #6(1)E8L1(T1), it was not possible to detect the 740 bp GFP fragment, and thus the construct. The data of these plants [plants 5, 10 and 19 of line #6(1)E8L1(T1) and plant 3, 11 and 12 of line #6(2)E15L7P2 (T2)] were therefore not taken into consideration in the calculations which follow (see FIG. 9).

Example 4 : Interaction of P. pachyrhizi with transgenic Bax inhibitor-1 overexpressing barley

To verify the resistance-increasing effect as the result of transient overexpression of BI-1, the interaction between P. pachyrhizi and transgenic barley plants cv. 'Golden Promise' (GP), which contain a GFP-BI-1 overexpression construct was studied under the microscope in comparison with the wild type (WT) of this variety. To generate the transgenic plants, a GFP-BI-1 fusion under the control of the constitutive CaMV 35S promoter was used, thus ensuring a sufficient expression of the protein.

In each case 20 plants of the wild type and of four transgenic lines #6(1)E4L3P5 (T2), 1#6(2)E15L7P1 (T2), #6(2)E15L7P2 (T2) and #6(1)E8L1(T1) were grown. Only 14 plants

germinated from the seeds of line #6(2)E15L7P2 (T2). Since there was only a limited amount of seed available, the experiments were carried out with a smaller number of plants of this line. Seven days after sowing, the primary leaf was removed, inoculated with the P. pachyrhizi with the aid of the precipitation column and fixed in destaining solution 24 hours after the inoculation. After destaining of the leaves was complete, they were stained with aniline blue. Aniline blue intercollates into the structure of callose and thus preferentially stains papillae where callose accumulates and crosslinks with other polymeric substances. Cells which have, as the result of a hypersensitivity response (HR), undergone a process which resembles apoptosis in mammalian cells, also show a light fluorescence after staining with aniline blue. The number of spores, the germinated spores with germ tube which had already formed an appressorium and, as cell response, the appressoria with the papillae which lie underneath, and the HR, were counted on the inoculated barley leaves. Larger spore accumulations where an allocation of the appressoria to the spores was no longer possible, were not included in the count. As far as possible, at least 100 spores with appressoria were counted per leaf (see Table in FIG. 11).

The analysis of the leaves under the microscope revealed the formation of papillae, which was frequently markedly increased in the transgenic lines. In contrast, an appearance of HR in the infected cells was observed less frequently in the transgenic plants. In the wild type, an average of 36% of the infected cells developed papillae as defence response, 45% of the cells responded with an HR. In contrast, the formation of papillae in the transgenic lines amounted to between 50 and 60%. The HR, which amounted to 16-26%, was also markedly less pronounced in comparison with the wild type (FIG. 12). According to examination with the so-called “Student test” (t-Test), the relative formation of papillae in the transgenic lines is significantly increased over the WT and the HR is significantly reduced ($P < 0.001$). According to the observations in these experiments, BI-1 prevents the programmed cell death and promotes papillae formation, by the cells, as alternative defence.

Example 5: Interaction of P. pachyrhizi with transgenic Bax-inhibitor-1-overexpressing soya

Soya plants which overexpress NtBI-1 were generated by methods known per se and were inoculated with P. pachyrhizi as described above. The NtBI-1-transformed soya plants showed a markedly reduced soya rust infection in comparison with the wild-type soya plants - the reduction amounted on average to in the region of over 30%.

Likewise, soya plants transformed with NTBI-1 and with SELDA were generated and inoculated with P. pachyrhizi as described above. The NtBI-1 + SELDA-transformed soya plants likewise

showed a markedly reduced soya rust infection in comparison with the wild-type soya plants - the reduction here amounted on average to in the region of over 15%.

DEMANDE OU BREVET VOLUMINEUX

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CECI EST LE TOME 1 DE 2
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We claim:

1. A method of generating or increasing a resistance to at least one biotrophic fungus in a plant or a part of a plant, where the part of a plant comprises a tissue or a cell, comprising the following steps:

   a) increasing the protein quantity or function of at least one Bax inhibitor-1 (BI1) protein in the plant or at least a part of a plant over the protein quantity or function in the starting plant or part thereof, and, if appropriate

   b) selecting the plant or a part of a plant where a resistance to at least one biotrophic fungus has been generated or increased in comparison with the starting plant or part thereof.

2. The method according to claim 1, where the at least one biotrophic fungus is selected from the group of the heminecrotrophic fungi.

3. The method according to claim 1 or 2, where the at least one biotrophic fungus is selected from the group consisting of the group of the Basidiomycota, preferably the Uredinales (rusts), especially preferably the Melompsoraceae, and in particular from the genus Phakopsora.

4. The method according to claim 3, where the at least one biotrophic fungus is selected from the group consisting of Phakopsora pachyrhizi and Phakopsora meibomiae.

5. The method according to claim 1 or 2, where the at least one biotrophic fungus is not a powdery or downy mildew.

6. The method according to any of the preceding claims, where the BI1 protein comprises at least one sequence which has at least 50% homology with at least one BI1 consensus motif selected from the group consisting of

   a) H(L/I)KXVY,
   b) AXGA(Y/F)XH,
   c) NIGG,
   d) P(V/P)(Y/F)E(E/Q)(R/Q)KR,
   e) (E/Q)G(A/S)S(V/I)GPL,
   f) DP(S/G)(L/I)(I/L),
   g) V(G/A)T(A/S)(L/I)AF(A/G)CF(S/T),
   h) YL(Y/F)LGG,
   i) L(L/V)SS(G/W)L(S/T)(I/M)L(L/M)W, and
   j) DTGX(I/V)(I/V)E.

7. The method according to any of the preceding claims, where the BI1 protein is encoded by a polypeptide which comprises at least one sequence selected from the group consisting of:

a) the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46;

b) sequences with at least 50% identity with one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46; and

c) sequences which comprise at least one part-sequence of at least 10 contiguous amino acid residues of one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46,
and/or which comprise at least a part-sequence of at least 20 contiguous amino acid residues, where the part-sequence has at least 80% identity with the corresponding part-sequence from one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46.

8. The process according to any of the preceding claims, where the step of increasing the protein quantity or function of at least one BI1 protein is achieved by a biotechnological method.

9. The process according to any of the preceding claims, where the step of increasing the protein quantity or function of at least one BI1 protein is achieved by recombinant expression of the BI1 protein.

10. The process according to any of the preceding claims, where the step of increasing the protein quantity or function of at least one BI1 protein is achieved by increasing, by means of biotechnology, the endogenous BI1 protein.

11. The method according to any of the preceding claims, comprising

i) stable transformation of a plant cell with a recombinant expression cassette comprising a nucleic acid sequence, which codes for a BI1 protein, in operable linkage with a tissue-specific promoter, the promoter being heterologous with regard to the nucleic acid sequence which codes for the BI1 protein;
ii) regeneration of the plant from the plant cell; and
iii) expression of the nucleic acid sequence which codes for the BI1 protein in an amount, in at least one tissue and over a period sufficient for generating or increasing a fungal resistance in the plant.

12. The method according to any of the preceding claims, where the plant is selected among monocotyledonous and dicotyledonous plants.

13. The method according to any of the preceding claims, where the plant is selected among the group of the monocotyledonous plants, in particular comprising wheat, oats, millet, barley, rye, maize, rice, sorghum, triticale, spelt or sugar cane.

14. The method according to any of the preceding claims, where the plant is selected among the group of the dicotyledonous plants, in particular comprising Arabidopsis, cotton, buckwheat, potato, cabbages, cress, linseed, oil seed rape, tomato, aubergine, bell pepper, sunflower, tobacco, Tagetes, lettuce, Calendula, melon, pumpkin/squash, courgette, sugarbeet, ornamentals, trees and legumes.

15. The method according to claim 14, where the plant is selected among the genus Phaseolus (comprising French bean, dwarf bean, climbing bean (Phaseolus vulgaris), Lima bean (Phaseolus lunatus L.), Tepary bean (Phaseolus acutifolius A. Gray), runner bean (Phaseolus coccineus)); the genus Glycine (comprising Glycine soja, soybean (Glycine max (L.) Merill)); pea (Pisum) (comprising shelling peas (Pisum sativum L. convar. sativum), also called smooth or round-seeded peas; marrowfat pea (Pisum sativum L. convar. medullare Alef. emend. C.O. Lehm), sugar pea (Pisum sativum L. convar. axiphium Alef emend. C.O. Lehm), also called snow pea, edible-podded pea or mangetout, (Pisum granda sneida L. convar. sneidulo p. shneiderium)); peanut (Arachis hypogaea), clover (Trifolium spec.), medick (Medicago), kudzu vine (Pueraria lobata), common lucern, alfalfa (M. sativa L.), chickpea (Cicer), lentils (Lens) (Lens culinaris Medik.), lupins (Lupinus); vetches (Vicia), field bean, broad bean (Vicia faba), vetchling (Lathyrus) (comprising chickling pea (Lathyrus sativus), heath pea (Lathyrus tuberosus)); genus Vigna (comprising moth bean (Vigna aconitifolia (Jacq.) Maréchal), adzuki bean (Vigna angularis (Willd.) Ohwi & H. Ohashi), urd bean (Vigna mungo (L.) Hepper), mung bean (Vigna radiata (L.) R. Wilczek), bambara groundnut (Vigna subterrane (L.) Verdc.), rice bean (Vigna umbellata (Thunb.) Ohwi & H. Ohashi), Vigna vexillata (L.) A. Rich., Vigna unguiculata (L.) Walp., in the three subspecies asparagus bean, cowpea, catjang bean)); pigeonpea (Cajanus cajan (L.) Millsp.), the genus Macrotyloma (comprising geocarpa groundnut (Macrotyloma geocarpum (Harms) Maréchal & Baudet), horse bean (Macrotyloma uniflorum (Lam.) Verdc.)); goa bean (Psophocarpus tetragonolobus (L.) DC.), African yam bean (Sphenostylis stenocarpa (Hochst. ex A. Rich.) Harms), Egyptian black bean, dolichos bean, lablab bean (Lablab purpureus (L.) Sweet), yam bean (Pachyrhizus), guar bean (Cyamopsis tetragonolobus (L.) Taub.); and the genus Canavalia (comprising jack bean (Canavalia ensiformis (L.) DC.), sword bean (Canavalia gladiata (Jacq.) DC.)).

16. The method according to claim 14, where the plant is selected among the legumes, preferably the tribe *Phaseolae*, especially preferably the genus *Glycine*, and in particular soybean (*Glycine max*).

17. The method according to any of the preceding claims, wherein the expression of the BI1 protein in a plant is increased at least in the epidermis, preferably essentially tissue-specifically in the epidermis, and/or essentially not increased in the mesophyll.

18. The method according to any of the preceding claims, where the plant additionally has an mlo-resistent phenotype, or the expression or function of MLO, RacB and/or NaOx is inhibited or reduced, in comparison with a control plant, at least in the epidermis and/or the expression or function of PEN2, SNAP34 and/or PEN1 is increased at least in the epidermis in comparison with a control plant.

19. A polypeptide sequence coding for BI1 protein comprising at least one sequence selected from the group consisting of

a) the sequences as shown in SEQ ID NO: 12, 14, 16, 18, 20, 22, 24, 28, 30, 32, 38 or 46;

b) sequences with at least 50% identity with one of the sequences as shown in SEQ ID NO: 12, 14, 16, 18, 20, 22, 24, 28, 30, 32, 38 or 46; and

c) sequences which comprise at least one part-sequence of at least 10 contiguous amino acid residues of one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46,
and/or which comprise at least a part-sequence of at least 20 contiguous amino acid residues, where the part-sequence has at least 80% identity with the corresponding part-sequence from one of the sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 38 or 46.

20. A nucleic acid sequence coding for a polypeptide sequence according to claim 19.

21. A recombinant expression cassette comprising a nucleic acid sequence, which codes for a BI1 protein, in operable linkage with a promoter, the promoter being heterologous with regard to said nucleic acid sequence which codes for the BI1 protein.

22. The recombinant expression cassette according to claim 21, where

a) the BI1 protein is characterized by a polypeptide sequence as defined in claim 19, and/or

b) the promoter is a tissue-specific promoter selected from the group of the epidermis-specific promoters.

23. A recombinant vector comprising an expression cassette according to one of claims 21 or 22.

24. A recombinant organism comprising at least one expression cassette according to one of claims 19 or 20 and/or at least one vector according to claim 23.

25. The recombinant organism according to claim 24 selected from the group consisting of bacteria, fungi, in particular yeasts, nonhuman animals and plants.

26. The recombinant organism according to claim 24 or 25, selected from the group of the plants as defined in any of claims 12 to 16.

27. The recombinant organism according to one of claims 24 or 25, where the organism is a plant which additionally has an mlo-resistant phenotype.

Application number / numéro de demande: ep20070061436

Figures: 6, 7

Pages: 8, 9

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Figure 1a (Page 1 of 3)

```
1                                                                    50
    AtBI-1     (1) ----------MDAFSSFFDSQPGS---RSWSYDSLKNFRQISPAVQNHLKR
    BnBI-1     (1) ----------MDSFSSFFDSQPGS---RSWSYDSLKNLRQISPSVQNHLKR
     GmBI2     (1) -----RLQAMDAFNSFFDS------RNRWNYDTLKNFRQISPVVQNHLKQ
     GmBI3     (1) ITKTIRFDSLFSMDTFFKSPSSSSSRSRWSYDTLKNFREISPLVQNHIKL
    HVBI-1     (1) ------------MDAFYSTS---SAAASGWGHDSLKNFRQISPAVQSHLKL
    NtBI-1     (1) ----------MESCTSFFNSQSASS-RNRWSYDSLKNFRQISPFVQTHLKK
    OsBI-1     (1) ------------MDAFYSTSSAYGAAASGWGYDSLKNFRQISPAVQSHLKL
    TaBI11     (1) ------------------------------------------------
    TaBI18     (1) --------------------FSGTFRNSRSDDFVLCELQRELPRCRDATLTV
 TaBI5 new     (1) ------------------------------------------------VAMPGR
    ZmBI14     (1) ------------------------------------------------
    ZmBI16     (1) ------------------------------------------------
    ZmBI33     (1) ------------------------------------------------
     ZmBI8     (1) ------------------------------------------------
 Consensus     (1)                   F  S          W YDSLKN R ISP VQ HLK

                   51                                                100
    AtBI-1    (39) VYLTLCCALVASAFGAYLHVLWNIGGILTTIGCIGTMIWLLSCPPYEHQK
    BnBI-1    (39) VYLTLCCALVASAFGAYLHVLWNIGGILTTIGCFGSMIWLLSCPPYEQQK
     GmBI2    (40) VYFTLCFAVVAAAVGAYLHVLLNIGGFLTTVACMGSSFWLLSTPPFEERK
     GmBI3    (51) VYFTLCCAVVAAAVGAFLHVLWNIGGFLTTLASIGSMFWLLSTPPFEEQK
    HVBI-1    (37) VYLTLCFALASSAVGAYLHIALNIGGMLTMLACVGTIAWMFSVPVYEERK
    NtBI-1    (41) VYLSLCCALVASAAGAYLHILWNIGGLLTTLGCVGSIVWLMATPLYEEQK
    OsBI-1    (40) VYLTLCVALAASAVGAYLHVALNIGGMLTMLGCVGSIAWLFSVPVFEERK
    TaBI11     (1) ------------------------------------------------
    TaBI18    (33) VYVIPIVGRIKSAAGAYLHIALNIGGMLTMLACIGTIAWMFSVPVYEERK
 TaBI5 new     (7) RFRLTYALPGLICRGCLPAHCPEHWRDADNARVYRNHRLDVLGASLRGEE
    ZmBI14     (1) ----------------------------------GSIAWLFSVPVYEERK
    ZmBI16     (1) --------------------WNIGVRLTMLGCIGSIDWLFSVPVYEERK
    ZmBI33     (1) --------------------WNIGGTLTMLGCVGSIAWLFSVPVYEERK
     ZmBI8     (1) ------------------------------------------------
 Consensus    (51) VY TLC AL ASA GAYLHV  NIGG LT LGCIGSI WL S PVYEERK

                   101                                               150
    AtBI-1    (89) RLSLLFVSAVLEGASVGPLIKVAIDVDPSILITAFVGTAIAFVCFSAAAM
    BnBI-1    (89) RLSLLFLSAVLEGASVGPLIKVAVDFDPSILITAFVGTAIAFICFSGAAM
     GmBI2    (90) RVTLLMAASLFQGSSIGPLIDLAIHIDPSLIFSAFVGTALAFACFSGAAL
     GmBI3   (101) RLSLLMASALFQGASIGPLIDLAFAIDPGLIIGAFVATSLAFACFSAVAL
    HVBI-1    (87) RFGLLMGAALLEGASVGPLIELAIDFDPSILVTGFVGTAIAFGCFSGAAI
    NtBI-1    (91) RIALLMAAALFKGASIGPLIELAIDFDPSIVIGAFVGCAVAFGCFSAAAM
    OsBI-1    (90) RFGILLAAALLEGASVGPLIKLAVDFDSSILVTAFVGTAIAFGCFTCAAI
    TaBI11     (1) ----------------------------------------------AAI
    TaBI18    (83) RFGLLMGAALLEGASVGPLIELAIDFDPSILVTGFVGTAIAFGCFSGAAI
 TaBI5 new    (57) EVWAADGCSLLEGASVGPLIELAIDFDPSILVTGFVGTAIAFGCFSGAAI
    ZmBI14    (17) RYWLLMAAALLEGASVGPLIKLAVEFDPSILVTAFVGTAIAFACFSCAAM
    ZmBI16    (30) RYGLLMAAALLEGASVGPLVKLAVEFDPSILVTAFVGTAIAFACFSGAAM
    ZmBI33    (30) RYGLLMAAALLEGASVGPLVKLAVEFDPSILVTAFVGTAIAFACFSGAPW
     ZmBI8     (1) ----------------------VIDLDSRILVTAFVGTAVAFACFSGAAI
 Consensus   (101) R  LLMAAALLEGASVGPLI LAIDFDPSILVTAFVGTAIAFACFSGAAI
```

Figure 1b (Page 2 of 3)

```
                    151                                                 200
AtBI-1      (139)   LARRREYLYLGGLLSSGLSMLMWLQFASSIFG-GSASIFKFELYFGLLIF
BnBI-1      (139)   LARRREYLYLGGLLSSGLSMLMWLQFASSIFG-GSASIFKFELYFGLLIF
 GmBI2      (140)   VARRREYLYLGGLVSSGLSILLWLHFASSIFG-GSTALFKFELYFGLLVF
 GmBI3      (151)   VARRREYLYLGGLLSSWLSILMWLHSDSSLFG-GSIALFKFELYFGLLVF
HVBI-1      (137)   IAKRREYLYLGGLLSSGLSILLWLQFVTSIFGHSS-GSFMFEVYFGLLIF
NtBI-1      (141)   VARRREYLYLGGLLSSGLSILFWLHFASSIFG-GSMALFKFEVYFGLLVF
OsBI-1      (140)   VAKRREYLYLGGLLSSGLSILLWLQFAASIFGHST-GSFMFEVYFGLLIF
TaBI11        (4)   IAKRREYLYLGGLLSSGLSILLWLQFATSIFGHSS-GSFMFEVYFGLLIF
TaBI18      (133)   IAKRREYLYLGGLLSSG----------------------------LTIL
TaBI5 new   (107)   IAKRREYLYLGGLLSSGLSILLWLQFATSIFGHSS-GSFMFEVYFGLLIF
ZmBI14       (67)   VAKRREYLYLGGLLSSGLSILLWLQFAASIFGHQSTSSFMFEVYFGLLIF
ZmBI16       (80)   VARRREYLYLGGLLSSGLSILLWLQLAASIF-GHSATSFMFEVYFGLLIF
ZmBI33       (80)   WQAR-EYLYLGGCSRRGSPSCSGCSSPPPSS--ALRNSFMFEVYFGLLIL
 ZmBI8       (29)   IAKRREYLYLGGLLSSGLSILLWLQFATSIFGHTS-ATFMFELYFGLLVF
Consensus   (151)   VAKRREYLYLGGLLSSGLSILLWLQFASSIFG  S ASFMFEVYFGLLIF

                    201                                                 250
AtBI-1      (188)   VGYMVVDTQEIIEKAHLGDMDYVKHSLTLFTDFVAVFVRILIIMLKNSAD
BnBI-1      (188)   VGYMVVDTQDIIEKAHLGDMDYVKHSLTLFTDFVAVFVRVLIIMLKNSAD
 GmBI2      (189)   VGYIVVDTQEIVERAHLGDLDYVKHALTLFTDLVAVFVRILVIMLKNSTE
 GmBI3      (200)   VGYVIVDTQEIIERAHFGDLDYVKHALTLFTDLAAIFVRILIIMLKNSSE
HVBI-1      (186)   LGYMVYDTQEIIERAHHGDMDYIKHALTLFTDFVAVLVRVLIIMLKNAGD
NtBI-1      (190)   VGYIIFDTQDIIEKAHLGDLDYVKHALTLFTDFVAVFVRILIIMLKNASD
OsBI-1      (189)   LGYMVYDTQEIIERAHHGDMDYIKHALTLFTDFVAVLVRILVIMLKNASD
TaBI11       (53)   LGYMVYDTQEIIERAHHGDMDYIKHALTLFTDFVAVLVRILIIMLKNAGD
TaBI18      (154)   L-------------------------------------------------
TaBI5 new   (156)   LGYMVYDTQEIIERAHHGDMDYIKHALTLFTDFVAVLVRVLIILLKNAAD
ZmBI14      (117)   LGYMVYDTQEVIERAHHG-----------------------------------
ZmBI16      (129)   LGYVVYDT---------------------------------------------
ZmBI33      (127)   LG---------------------------------------------------
 ZmBI8       (78)   LGYMVFDTQEIIERAHRGDMDYIKHALTLFTDFVAVLVRILVIMMKNAQE
Consensus   (201)   LGYMVYDTQEIIERAH GDMDYIKHALTLFTDFVAV VRILIIMLKNA D

                    251                                                 300
AtBI-1      (238)   KEEKKKKRRN-------GDVK-I-LYGCYRVWPL-RYYLLALSIGDQTCF
BnBI-1      (238)   KEDKKKRRRN-----D-KVRKKAK-SGCYVCFKK-----------KRVG
 GmBI2      (239)   RNEKKKKRRD-------------------------------------------
 GmBI3      (250)   RNEKKKKRRD--ADRPTRAQASLQ-FSLWRIHN-------LFR-CWSLV-
HVBI-1      (236)   KSEDKKKRKRG-------------------S---------------------
NtBI-1      (240)   KEEKKKKRRN----CISGYSKTL-L-NLAFSCS---TSVDLRQVCC--FG
OsBI-1      (239)   KSEEKKRKKRS-ELLFPLCT-EKTTAAIASTYYDRAALQLGFMVNTSSFA
TaBI11      (103)   KSEDKKKRKRRS-----------------------------------------
TaBI18      (155)   -----------------------------------------------------
TaBI5 new   (206)   KVGGQEEEEEKS-----------------------------------------
ZmBI14      (135)   -----------------------------------------------------
ZmBI16      (137)   -----------------------------------------------------
ZmBI33      (129)   -----------------------------------------------------
 ZmBI8      (128)   KSQDEKKRK-------------------------------------------
Consensus   (251)   K E KKKRR
```

Figure 1c (Page 3 of 3)

```
                    301                                                 350
AtBI-1      (278)   H-KG-SACFTSAQVPSSDCK--------------LECCSSFHKLLFFKSL
BnBI-1      (269)   VISTDMIALVFFTCLEQFW-------------QHTLRICVFLLVTPDCEWI
 GmBI2      (249)   --------------------------------------------------
 GmBI3      (288)   LVSYVFAVMVNVRISFKHLHMYLPIS-CVV-HHTLV-KKKKKKKKKKKKK
HVBI-1      (248)   --------------------------------------------------
NtBI-1      (279)   NASD-AARLCYAACQCGYGGT-MVLF----PKHTIK-HACLHYIDNLRVY
OsBI-1      (287)   FC-YGVNLLRFVVVVALQILACYMTRIFL-WWSR-SKRENTSSFATNLFA
Consensus   (301)

                    351                                                 400
AtBI-1      (312)   VLLIASYQAKNNVGK---------SCLNFLKCVHFRKKKKKKKKK-----
BnBI-1      (307)   SILKLC-KLSVGS-----------------------------------
 GmBI2      (249)   --------------------------------------------------
 GmBI3      (335)   KKKKKXXXXXXXXXXXX--XXXXXGVCGLRYSRHSSNH-EGSLW-PGLC-
HVBI-1      (248)   --------------------------------------------------
NtBI-1      (322)   YLFLLPFAVLGCS-LYS-FSVMLDHLLS-RLISHIDGRNENSHRRPNLFK
OsBI-1      (334)   FW-LMMILSPKKKK-----------------------------------
Consensus   (351)

                    401                                                 450
AtBI-1      (348)   --------------------------------------------------
BnBI-1      (319)   --------------------------------------------------
 GmBI2      (249)   --------------------------------------------------
 GmBI3      (380)   ACIDTVH-FGCNLCANS-YNVE-FI-EK-EEEEERLIG-PIAMCRVIWFV
HVBI-1      (248)   --------------------------------------------------
NtBI-1      (369)   TEAQL-------------------------------------------
Consensus   (401)

                    451                                                 500
 GmBI3      (424)   ENT-LAV-KLLVPLCS--LAMCLL-W-MSGFLLNIFICIC--S-YIV-TS
Consensus   (451)

                    501      512
 GmBI3      (464)   FLGLKKEKKKKK
Consensus   (501)
```

Figure 2

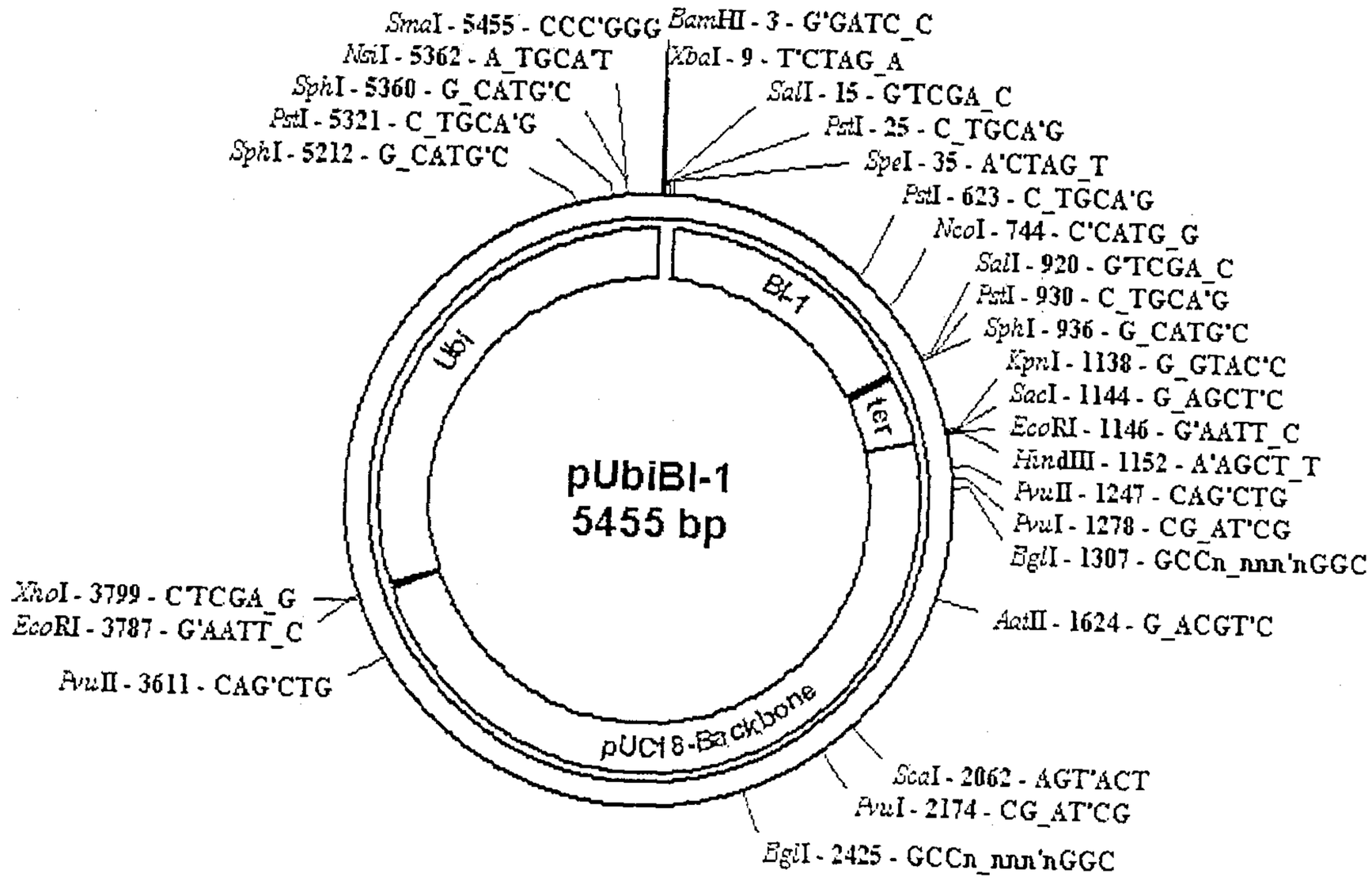

SmaI - 5455 - CCC'GGG
NciI - 5362 - A_TGCA'T
SphI - 5360 - G_CATG'C
PstI - 5321 - C_TGCA'G
SphI - 5212 - G_CATG'C
BamHI - 3 - G'GATC_C
XbaI - 9 - T'CTAG_A
SalI - 15 - G'TCGA_C
PstI - 25 - C_TGCA'G
SpeI - 35 - A'CTAG_T
PstI - 623 - C_TGCA'G
NcoI - 744 - C'CATG_G
SalI - 920 - G'TCGA_C
PstI - 930 - C_TGCA'G
SphI - 936 - G_CATG'C
KpnI - 1138 - G_GTAC'C
SacI - 1144 - G_AGCT'C
EcoRI - 1146 - G'AATT_C
HindIII - 1152 - A'AGCT_T
PvuII - 1247 - CAG'CTG
PvuI - 1278 - CG_AT'CG
BglI - 1307 - GCCn_nnn'nGGC
AatII - 1624 - G_ACGT'C
ScaI - 2062 - AGT'ACT
PvuI - 2174 - CG_AT'CG
BglI - 2425 - GCCn_nnn'nGGC
XhoI - 3799 - C'TCGA_G
EcoRI - 3787 - G'AATT_C
PvuII - 3611 - CAG'CTG
BL1
ter
Ubi
pUbiBI-1
5455 bp
pUC18-Backbone

Figure 3

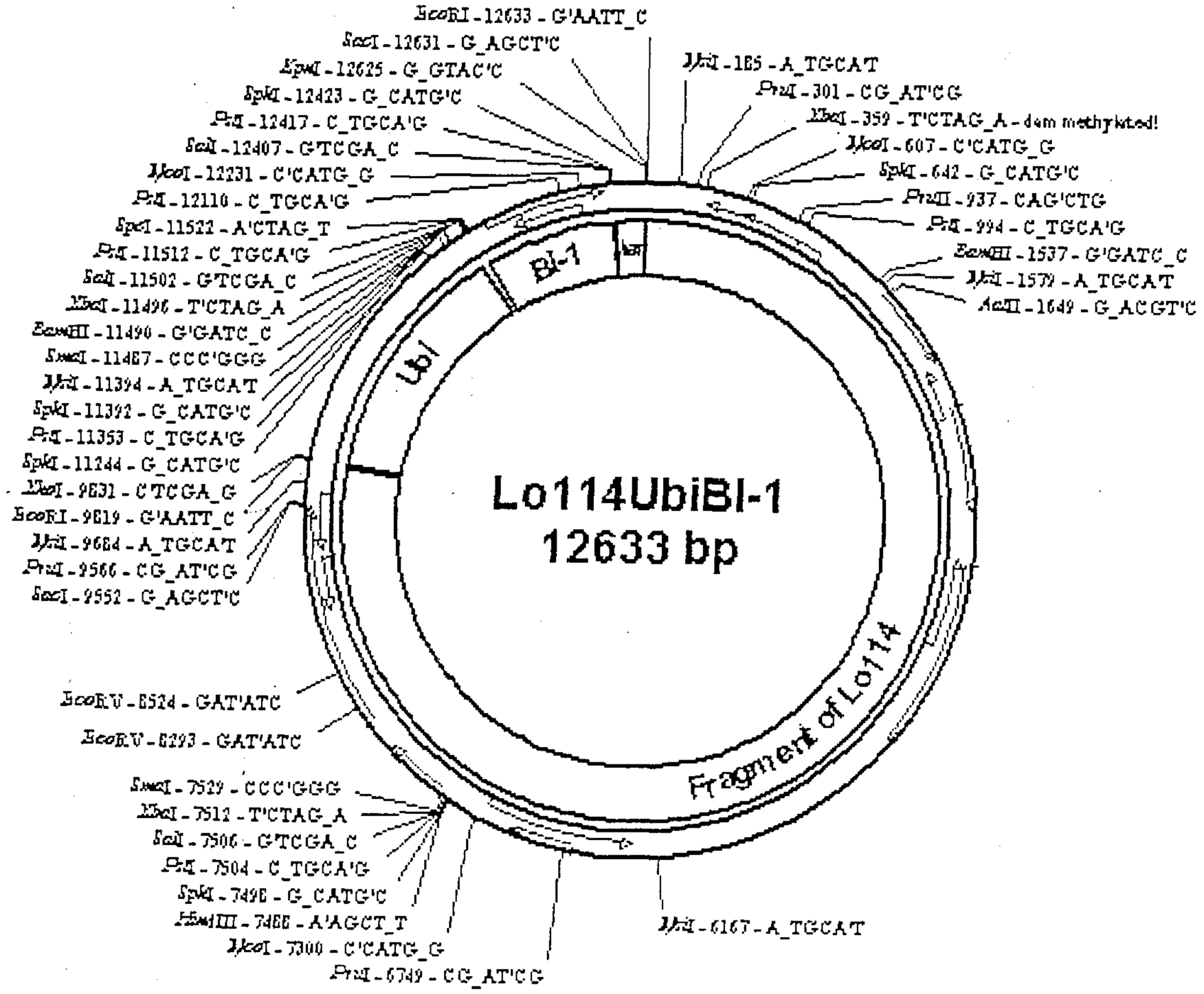

EcoRI - 12633 - G'AATT_C
SacI - 12631 - G_AGCT'C
KpnI - 12625 - G_GTAC'C
SphI - 12623 - G_CATG'C
PstI - 12617 - C_TGCA'G
SalI - 12607 - G'TCGA_C
NcoI - 12231 - C'CATG_G
PstI - 12110 - C_TGCA'G
SpeI - 11522 - A'CTAG_T
PstI - 11512 - C_TGCA'G
SalI - 11502 - G'TCGA_C
XbaI - 11496 - T'CTAG_A
BamHI - 11490 - G'GATC_C
SmaI - 11487 - CCC'GGG
NheI - 11394 - A_TGCAT
SphI - 11392 - G_CATG'C
PstI - 11353 - C_TGCA'G
SphI - 11244 - G_CATG'C
XbaI - 9631 - C'TCGA_G
EcoRI - 9619 - G'AATT_C
NheI - 9584 - A_TGCAT
PvuI - 9566 - CG_AT'CG
SacI - 9552 - G_AGCT'C
EcoRV - 8524 - GAT'ATC
EcoRV - 8293 - GAT'ATC
SmaI - 7529 - CCC'GGG
XbaI - 7512 - T'CTAG_A
SalI - 7506 - G'TCGA_C
PstI - 7504 - C_TGCA'G
SphI - 7498 - G_CATG'C
HindIII - 7488 - A'AGCT_T
NcoI - 7300 - C'CATG_G
PvuI - 6749 - CG_AT'CG
NheI - 6167 - A_TGCAT
NheI - 1579 - A_TGCAT
PvuI - 301 - CG_AT'CG
XbaI - 359 - T'CTAG_A - dam methylated!
NcoI - 607 - C'CATG_G
SphI - 642 - G_CATG'C
PvuII - 937 - CAG'CTG
PstI - 994 - C_TGCA'G
BamHI - 1537 - G'GATC_C
NheI - 1579 - A_TGCAT
AccII - 1649 - G_ACGT'C
Lo114UbiBI-1
12633 bp
BI-1
Ubi
Fragment of Lo114

Figure 4

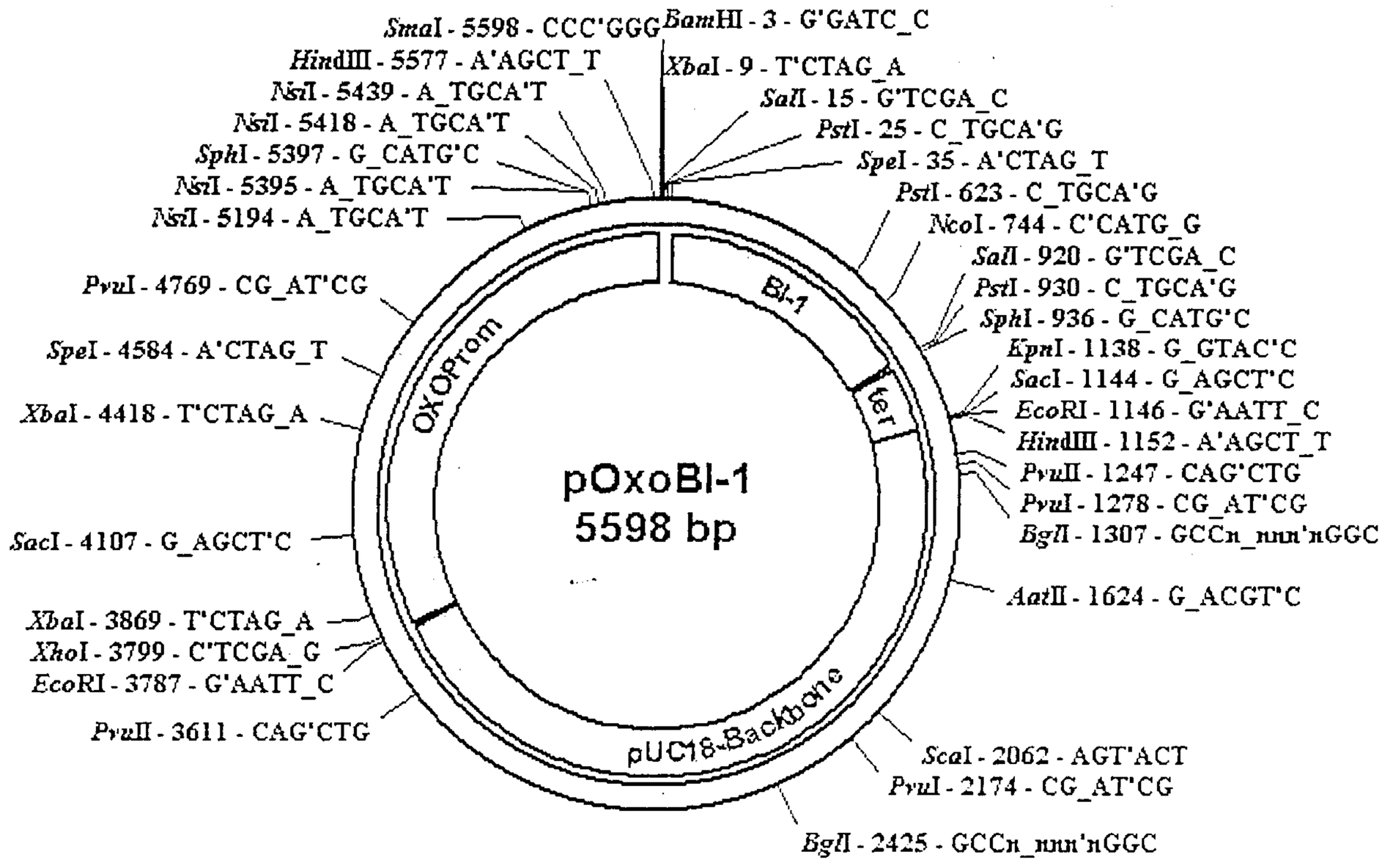

SmaI - 5598 - CCC'GGG
BamHI - 3 - G'GATC_C
HindIII - 5577 - A'AGCT_T
XbaI - 9 - T'CTAG_A
NsiI - 5439 - A_TGCA'T
SalI - 15 - G'TCGA_C
NsiI - 5418 - A_TGCA'T
PstI - 25 - C_TGCA'G
SphI - 5397 - G_CATG'C
SpeI - 35 - A'CTAG_T
NsiI - 5395 - A_TGCA'T
PstI - 623 - C_TGCA'G
NsiI - 5194 - A_TGCA'T
NcoI - 744 - C'CATG_G
SalI - 920 - G'TCGA_C
PvuI - 4769 - CG_AT'CG
PstI - 930 - C_TGCA'G
SphI - 936 - G_CATG'C
SpeI - 4584 - A'CTAG_T
EpnI - 1138 - G_GTAC'C
SacI - 1144 - G_AGCT'C
XbaI - 4418 - T'CTAG_A
EcoRI - 1146 - G'AATT_C
HindIII - 1152 - A'AGCT_T
PvuII - 1247 - CAG'CTG
PvuI - 1278 - CG_AT'CG
BglI - 1307 - GCCn_nnn'nGGC
SacI - 4107 - G_AGCT'C
AatII - 1624 - G_ACGT'C
XbaI - 3869 - T'CTAG_A
XhoI - 3799 - C'TCGA_G
EcoRI - 3787 - G'AATT_C
PvuII - 3611 - CAG'CTG
ScaI - 2062 - AGT'ACT
PvuI - 2174 - CG_AT'CG
BglI - 2425 - GCCn_nnn'nGGC
Bl-1
tetR
OXOPtet
pUC18-Backbone
pOxoBl-1
5598 bp

Figure 5

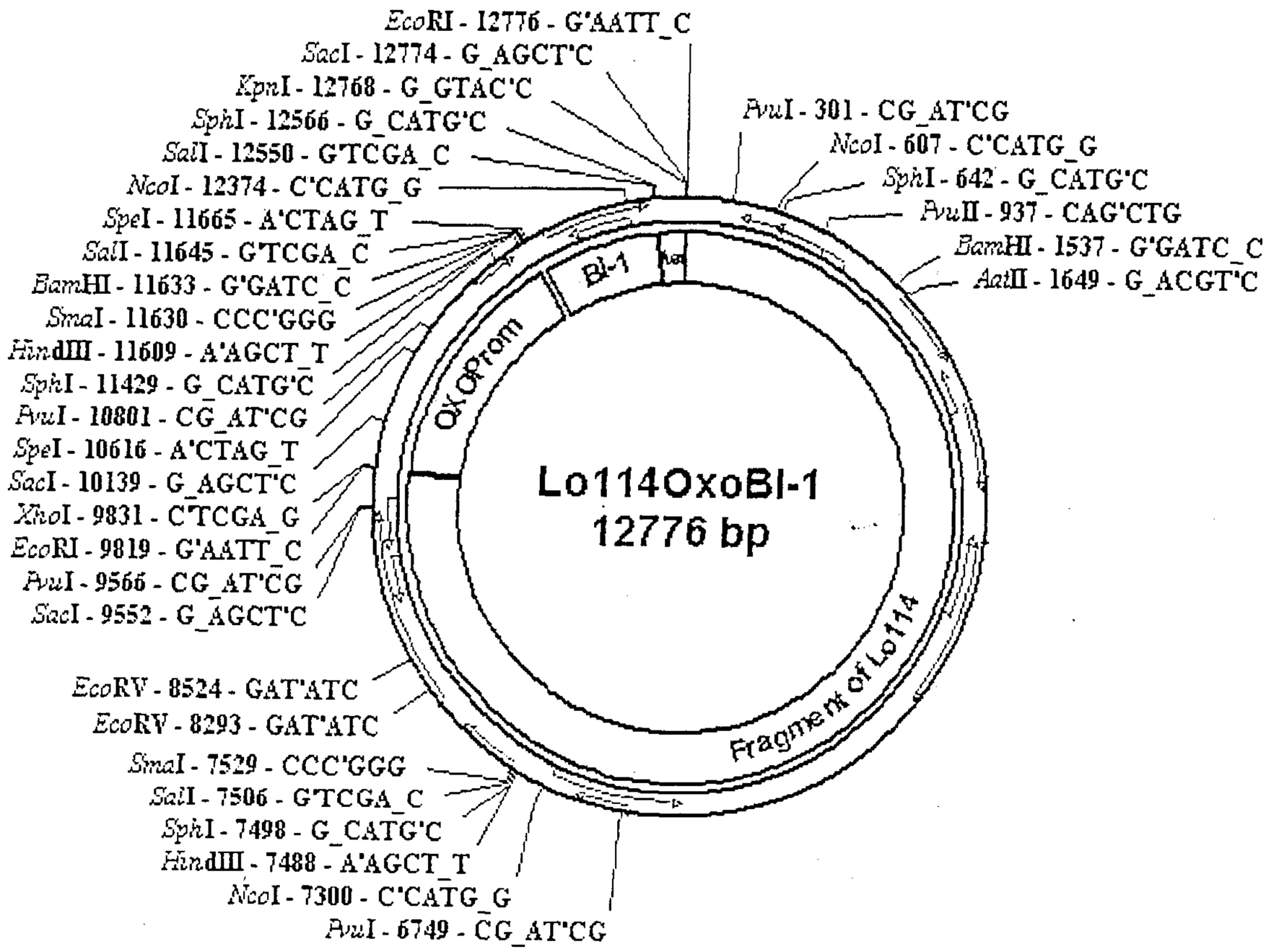

EcoRI - 12776 - G'AATT_C
SacI - 12774 - G_AGCT'C
KpnI - 12768 - G_GTAC'C
SphI - 12566 - G_CATG'C
SalI - 12550 - G'TCGA_C
NcoI - 12374 - C'CATG_G
SpeI - 11665 - A'CTAG_T
SalI - 11645 - G'TCGA_C
BamHI - 11633 - G'GATC_C
SmaI - 11630 - CCC'GGG
HindIII - 11609 - A'AGCT_T
SphI - 11429 - G_CATG'C
PvuI - 10801 - CG_AT'CG
SpeI - 10616 - A'CTAG_T
SacI - 10139 - G_AGCT'C
XhoI - 9831 - C'TCGA_G
EcoRI - 9819 - G'AATT_C
PvuI - 9566 - CG_AT'CG
SacI - 9552 - G_AGCT'C
EcoRV - 8524 - GAT'ATC
EcoRV - 8293 - GAT'ATC
SmaI - 7529 - CCC'GGG
SalI - 7506 - G'TCGA_C
SphI - 7498 - G_CATG'C
HindIII - 7488 - A'AGCT_T
NcoI - 7300 - C'CATG_G
PvuI - 6749 - CG_AT'CG
PvuI - 301 - CG_AT'CG
NcoI - 607 - C'CATG_G
SphI - 642 - G_CATG'C
PvuII - 937 - CAG'CTG
BamHI - 1537 - G'GATC_C
AatII - 1649 - G_ACGT'C
Bl-1
OxOProm
Lo114OxoBI-1
12776 bp
Fragment of Lo114

Figure 8

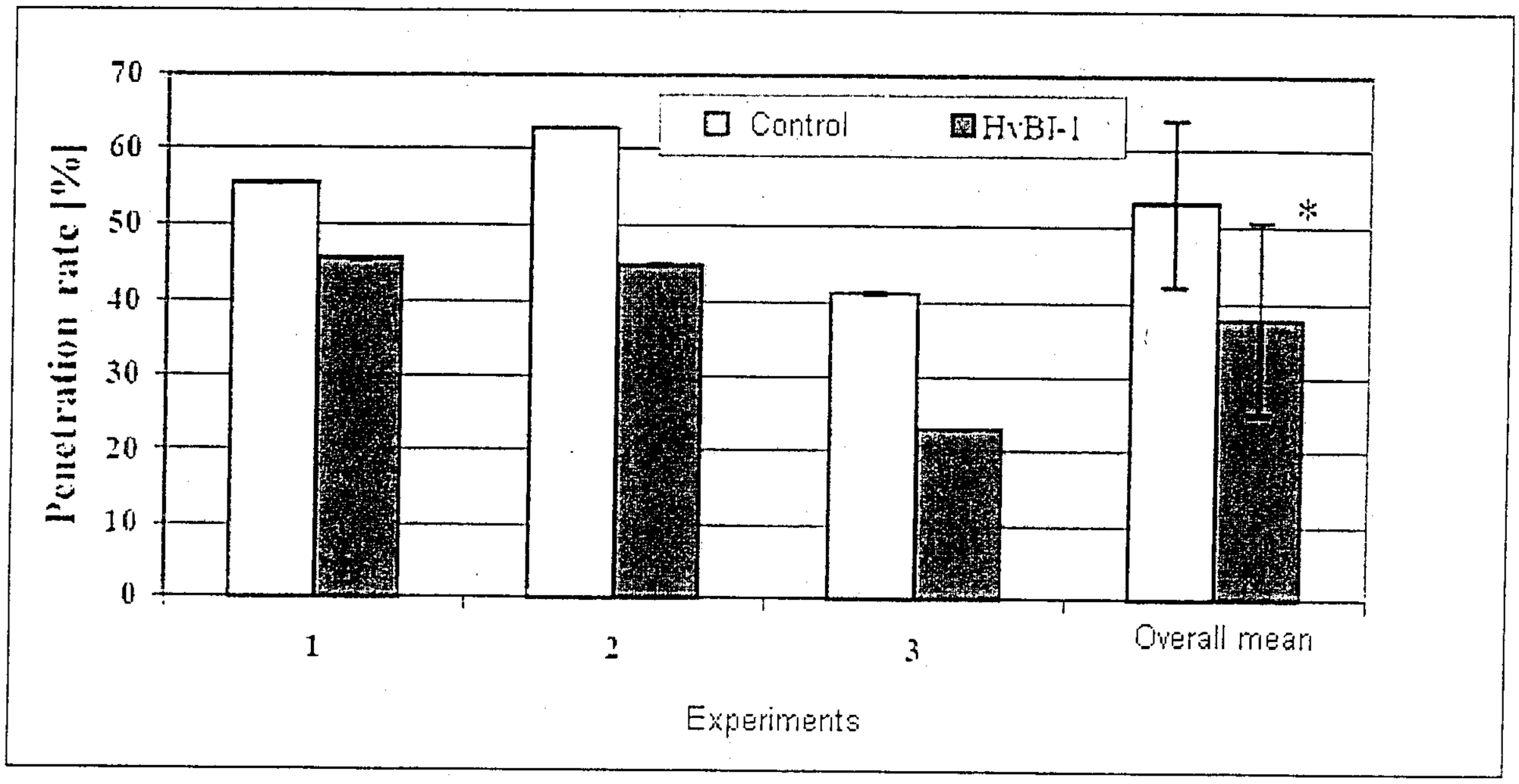

70
60
50
40
30
20
10
0
Penetration rate [%]
Control
HvBI-1
*
1
2
3
Overall mean
Experiments

Figure 9

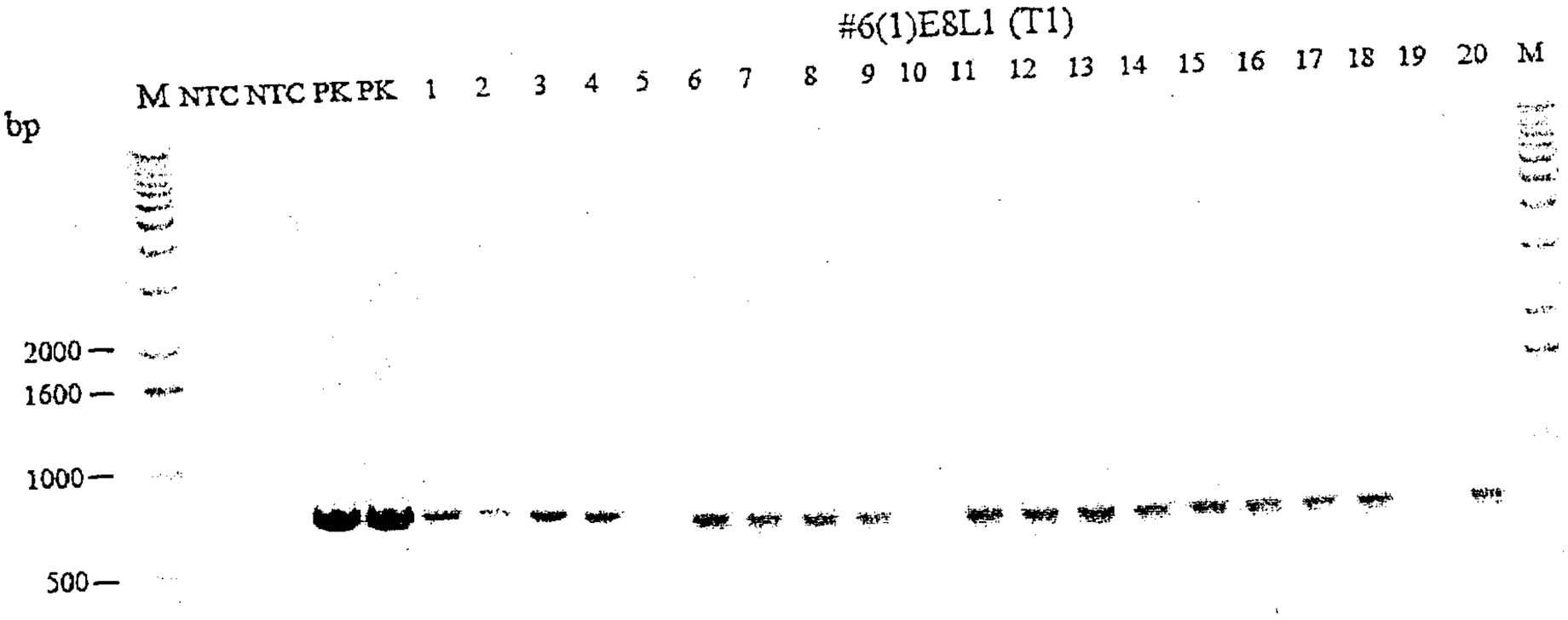

#6(1)E8L1 (T1)
bp
M NTC NTC PK PK 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 M
2000
1600
1000
500

Figure 10

Experiment	Constructs used	Transformed cells which interact with soybean rust	Transformed cells which are penetrated by soybean rust	Penetration rate of the transformed cells [%]
1	pGY1-GFP+pGY1	46	19	41.30
	pGY1-GFP+ pGY1-HvBI	13	3	23.08
2	pGY1-GFP+pGY1	65	36	55.38
	pGY1-GFP+ pGY1-HvBI	136	62	45.59
3	pGY1-GFP+pGY1	126	79	62.70
	pGY1-GFP+ pGY1-HvBI	203	91	44.83

	Control	HvBI-1
Overall mean averaged over the experiments	53.13	37.83
standard deviation	10.88	12.78
t-test	0.0309	

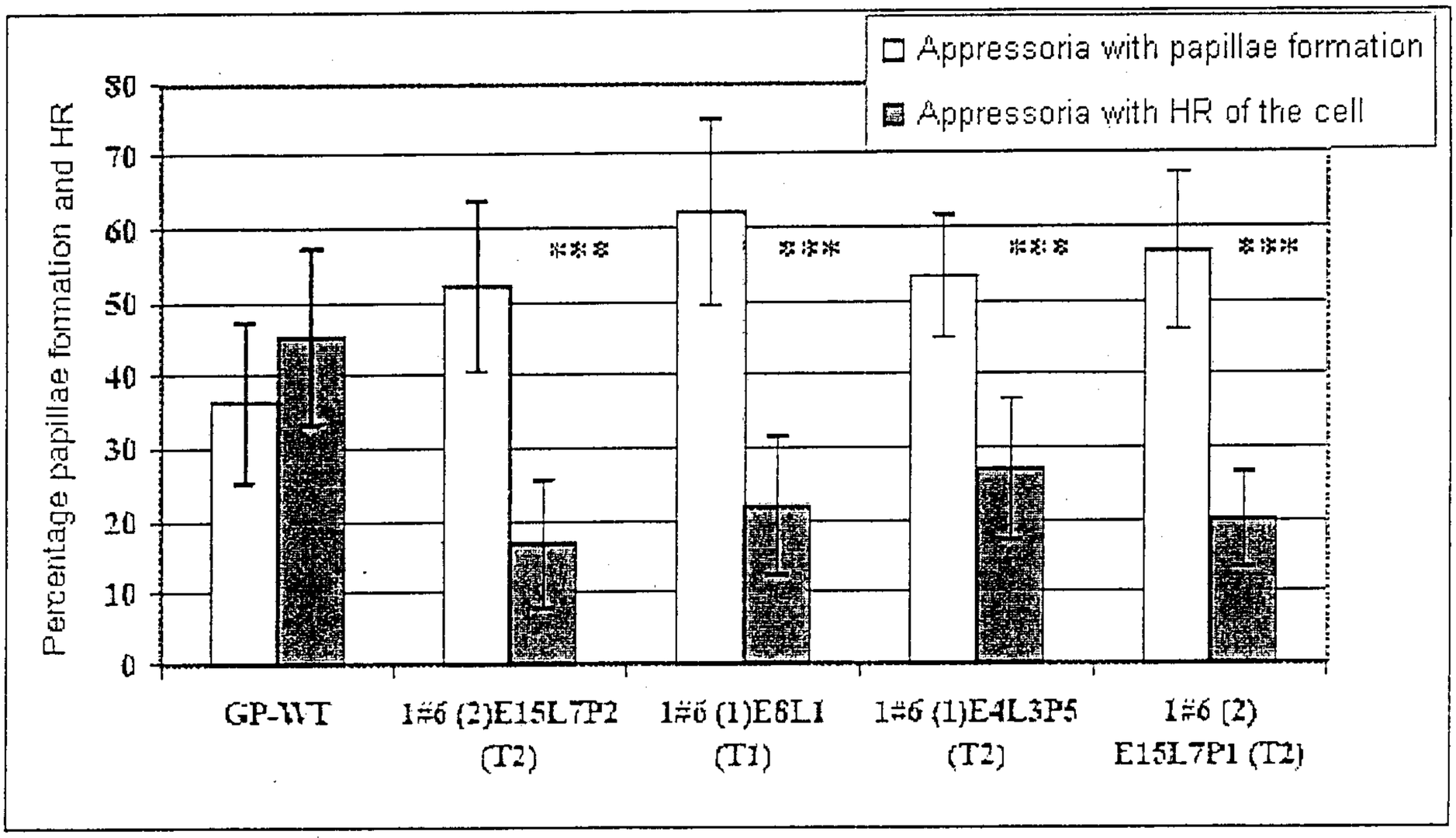
Figure 11a (Page 1 of 2)

Barley	Plant No.	Spores	Spores with appressoria	Appressoria with papillae	HR of infected cells
1#6 (1)E8L1 (T1)	1	139	122	33	55
1#6 (1)E8L1 (T1)	2	167	136	76	23
1#6 (1)E8L1 (T1)	3	167	131	87	21
1#6 (1)E8L1 (T1)	4	194	124	81	26
1#6 (1)E8L1 (T1)	6	169	121	71	34
1#6 (1)E8L1 (T1)	7	188	123	73	28
1#6 (1)E8L1 (T1)	8	143	123	84	33
1#6 (1)E8L1 (T1)	9	156	122	82	16
1#6 (1)E8L1 (T1)	20	147	105	61	24
1#6 (1)E8L1 (T1)	18	175	138	97	25
1#6 (1)E8L1 (T1)	17	134	129	94	16
1#6 (1)E8L1 (T1)	16	176	122	50	51
1#6 (1)E8L1 (T1)	15	168	123	100	23
1#6 (1)E8L1 (T1)	14	168	124	77	11
1#6 (1)E8L1 (T1)	13	143	125	86	20
1#6 (1)E8L1 (T1)	12	153	122	83	25
1#6 (1)E8L1 (T1)	11	141	122	82	31
1#6 (2)E15L7P2 (T2)	1	156	136	72	12
1#6 (2)E15L7P2 (T2)	2	151	125	78	13
1#6 (2)E15L7P2 (T2)	4	173	127	77	21
1#6 (2)E15L7P2 (T2)	14	131	111	40	35
1#6 (2)E15L7P2 (T2)	13	177	122	58	44
1#6 (2)E15L7P2 (T2)	10	175	127	60	22
1#6 (2)E15L7P2 (T2)	9	164	114	60	16
1#6 (2)E15L7P2 (T2)	8	175	126	96	17
1#6 (2)E15L7P2 (T2)	7	152	131	52	18
1#6 (2)E15L7P2 (T2)	6	181	128	53	10
1#6 (2)E15L7P2 (T2)	5	207	129	74	19
GP-WT	1	159	115	54	42
GP-WT	2	150	127	54	58
GP-WT	3	169	123	21	80
GP-WT	4	175	120	68	35
GP-WT	5	162	125	52	66
GP-WT	6	160	121	53	61
GP-WT	7	137	124	45	59
GP-WT	8	160	120	43	63
GP-WT	9	161	126	30	90
GP-WT	10	125	119	46	64
GP-WT	11	165	138	43	53
GP-WT	12	146	126	55	33
GP-WT	13	139	113	34	51
GP-WT	14	147	123	47	50
GP-WT	15	151	130	25	65
GP-WT	16	209	124	33	75
GP-WT	17	210	147	29	46
GP-WT	18	151	132	72	42
GP-WT	19	207	122	50	50
GP-WT	20	199	133	52	52

Figure 11b (Page 2 of 2)

Barley	Plant No.	Spores	Spores with appressoria	Appressoria with papillae	HR of infected cells
1#6 (2) E15L7P1 (T2)	20	149	100	46	26
1#6 (2) E15L7P1 (T2)	19	176	120	61	15
1#6 (2) E15L7P1 (T2)	18	181	129	94	12
1#6 (2) E15L7P1 (T2)	17	200	122	70	21
1#6 (2) E15L7P1 (T2)	16	170	121	76	20
1#6 (2) E15L7P1 (T2)	15	170	128	62	22
1#6 (2) E15L7P1 (T2)	14	156	126	44	43
1#6 (2) E15L7P1 (T2)	13	116	94	34	32
1#6 (2) E15L7P1 (T2)	12	204	130	75	30
1#6 (2) E15L7P1 (T2)	11	194	122	69	30
1#6 (2) E15L7P1 (T2)	10	145	121	60	20
1#6 (2) E15L7P1 (T2)	9	165	121	64	30
1#6 (2) E15L7P1 (T2)	8	192	122	80	23
1#6 (2) E15L7P1 (T2)	7	184	127	74	28
1#6 (2) E15L7P1 (T2)	6	167	121	85	13
1#6 (2) E15L7P1 (T2)	5	169	129	67	20
1#6 (2) E15L7P1 (T2)	4	182	124	80	22
1#6 (2) E15L7P1 (T2)	3	147	117	77	24
1#6 (2) E15L7P1 (T2)	2	151	121	81	25
1#6 (2) E15L7P1 (T2)	1	174	119	59	23
1#6 (1)E4L3P5 (T2)	20	153	121	74	36
1#6 (1)E4L3P5 (T2)	19	150	143	82	39
1#6 (1)E4L3P5 (T2)	18	152	122	82	19
1#6 (1)E4L3P5 (T2)	17	143	131	81	12
1#6 (1)E4L3P5 (T2)	16	179	149	76	39
1#6 (1)E4L3P5 (T2)	15	153	121	46	43
1#6 (1)E4L3P5 (T2)	14	155	124	58	41
1#6 (1)E4L3P5 (T2)	13	145	126	67	33
1#6 (1)E4L3P5 (T2)	12	160	125	67	45
1#6 (1)E4L3P5 (T2)	11	169	123	65	46
1#6 (1)E4L3P5 (T2)	10	157	124	57	46
1#6 (1)E4L3P5 (T2)	9	180	124	68	47
1#6 (1)E4L3P5 (T2)	8	168	120	78	25
1#6 (1)E4L3P5 (T2)	7	153	128	76	15
1#6 (1)E4L3P5 (T2)	6	165	127	78	37
1#6 (1)E4L3P5 (T2)	5	144	136	68	37
1#6 (1)E4L3P5 (T2)	4	134	132	53	55
1#6 (1)E4L3P5 (T2)	3	168	156	75	34
1#6 (1)E4L3P5 (T2)	2	158	130	77	14
1#6 (1)E4L3P5 (T2)	1	162	123	51	30

Figure 12


Appressoria with papillae formation
Appressoria with HR of the cell
Percentage papillae formation and HR
80
70
60
50
40
30
20
10
0
***
***
***
***
GP-WT
1#6 (2)E15L7P2 (T2)
1#6 (1)E6L1 (T1)
1#6 (1)E4L3P5 (T2)
1#6 (2) E15L7P1 (T2)