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(54) **Title**: METHOD FOR IDENTIFYING SUBSTANCES WHICH PRIME CELLS FOR A STRESS RESPONSE AND CELLS FOR USE IN THIS METHOD

(57) **Abstract**: The present invention relates to a transgenic eukaryotic cell or non-human organism comprising one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery and an expression cassette which comprises a nucleic acid sequence coding for a reporter protein under the control of a promoter the methylation of which increases upon priming for a stress response. The present invention also relates to a method for identifying substances which prime eukaryotic cells for a stress response by using this transgenic cell or organism.

Method for identifying substances which prime cells for a stress response and cells for use in this method

FIELD OF THE INVENTION

The present invention relates to a transgenic eukaryotic cell or non-human organism comprising one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of the transcriptional machinery and an expression cassette which comprises a nucleic acid sequence coding for a reporter protein under the control of a promoter the methylation of which increases upon priming for a stress response. The present invention also relates to a method for identifying substances which prime eukaryotic cells for a stress response by using this transgenic cell or organism.

BACKGROUND OF THE INVENTION

Plant diseases which are caused by various pathogens such as viruses, bacteria, oomycetes and fungi or abiotic stress such as drought, cold, freeze and salt may lead to significant crop losses of cultivated plants, resulting in economic detriments and in threatening food and feed supply.

Since the last century, chemical pesticides have been used for controlling plant diseases. Nevertheless, at present at least 40% of the possible plant yield is lost due to diseases and abiotic stress such as drought. Hence, there is still a need for improved methods for controlling plant diseases and abiotic stresses. Due to the low acceptance of genetically modified plants in Europe and because of the increasing demand for substances which are uncritical for the environment, there is a huge interest in using natural or near-natural substances for an effective plant protection.

Natural or near-natural substances are particularly effective, if they do not only harm or kill pathogens, but also stimulate certain parts of the plant's endogenous immune system. This is particularly true for agents which act via the so-called "defence priming" (reviewed in Conrath (2011) Trends in Plant Science 16(10): 524-531; Conrath *et al.* (2006) Mol. Plant. Microbe Interact. 19(10): 1062-1071). Defence priming prepares the plant's endogenous immune system for a future challenge such as pathogen infection or abiotic stress, but does not directly activate immunity. It has been shown that treatment of plant cells with priming substances induce chromatin modifications on endogenous defence gene promoters that are normally found on active genes, although the genes remain inactive (Jaskiewicz *et al.* (2011) EMBO Reports 12(1): 50-55). However, primed plants respond to very low levels of a stimulus, such as biotic or abiotic stress, in a more rapid and robust manner than non-primed plants. Hence, pesticides which induce priming confer an increased stress resistance to plants without reducing the yield. Consequently, there is a strong demand for substances which induce defence priming in plants.

It has been shown that priming is not restricted to plant cells, but also occurs in animal cells with a similar mechanism (see, e.g., Hayes *et al.* (1991) J. Leukocyte Biol. 50: 176-181). For example, interferon-γ or GM-CSF can

prime the induction of the expression of the cytokines interferon-α, interferon-β, tumour necrosis factor α and IL-12 which cytokines are involved in the overall defence response (Hayes *et al.* (1995) Blood 86: 646-650). The induction of cytokine expression is mediated by signal transduction through p38 and ERK1/2 kinases.

However, since the priming process does not lead to a direct activation of the defence mechanisms, the search for substances which act via priming is rather difficult. So far, no system for the direct screening for priming compounds is known which is easy to handle and based on the use of plants. In the methods of the prior art, the cells have to be stimulated twice to be able to determine whether they were in a primed state after treatment with a specific substance. The first stimulus by a substance to be tested primes the cell, for example for gene expression and the second stimulus, e.g. an infection with a pathogen, induces the actual defence reaction which is used as a read-out system in this method to determine whether the substance induces priming or not. Both the first and the second stimulus are necessary to determine whether the cells were in a primed state after treatment with the substance to be tested. Such a system is described in Noutoshi *et al.* (2012) Plant Cell 24(9): 3795-3804 where Arabidopsis MM1 cell suspension cultures were first treated with potential immune-priming substances and then with bacteria Pst-avrRpm1, before immune-related cell death was measured by staining the dead plant cells with Evans blue dye. Hence, this system necessarily involves the death of plant cells.

Consequently, there is still a need for a method which enables the direct identification of substances which prime cells or organisms for a stress response in one treatment step and with a convenient read-out system.

OBJECT AND SUMMARY OF THE INVENTION

It is thus an object of the present invention to provide a method for identifying substances which prime cells or organisms for a stress response and to provide transgenic cells or organisms for use in this method.

These and further objects of the invention, as will become apparent from the description, are attained by the subject-matter of the independent claims.

Some of the preferred embodiments of the present invention form the subject-matter of the dependent claims.

The present inventors have found that in the induction of an enhanced stress-activated gene response after priming of plants two molecular events are involved:

(1) an epigenetic alteration, i.e. an increase in the methylation of histone H3 in promoters regulating the expression of genes which are involved in the defence response, and

(2) the activation of one or more signal transduction events involved in stress-induced gene expression or the pre-activation of the transcriptional machinery.

In the method of the present invention the activation of one or more signal transduction pathways involved in stress-induced gene expression or the pre-activation of the transcriptional machinery mimics the stress stimulus which induces the defence reaction, i.e. the expression of genes involved in the defence response. Hence, in the method of the present invention only one stimulus, i.e. the treatment with a substance which is supposed to induce the priming is required to determine whether this substance induces priming or

not. Consequently, it was possible to develop a simple method for directly identifying substances which induce the priming of cells.

Accordingly, the present invention provides a transgenic eukaryotic cell or non-human organism comprising:

a) one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery; and

b) an expression cassette which comprises:

(i) a promoter the methylation of which increases upon priming for a stress response or a functional fragment or functional derivative of such a promoter;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, further regulatory elements.

Preferably, the one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene and/or the pre-activation of one or more members of the transcriptional machinery lead to an altered expression or activity of a protein selected from the group consisting of RNA polymerase II, EDR1, MKP1, MKP2, MPK4, EDS1, PAD4, WRKY70, WRKY18, MYB44, SPT4, SPT5 and SNI1.

More preferably, the one or more genetic modifications lead to an increased expression or activity of RNA polymerase II. Even more preferably, the RNA polymerase II is constitutively phosphorylated and most preferably the RNA polymerase II is constitutively phosphorylated by reducing the activity of a phosphatase capable of dephosphorylating RNA polymerase II.

The promoter the methylation of which increases upon priming for a stress response may be the promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, PR5, ICS1, WRKY31, HEL, PDF1-2, p35, p40, tumour necrosis factor α, interferon-α and interferon-β or a functional fragment or functional derivative of any of these promoters.

The reporter protein may be selected from the group consisting of: fluorescent proteins, luciferase proteins, β-galactosidase, alkaline phosphatase, β-glucuronidase and chloramphenicol acetyltransferase.

The transgenic eukaryotic cell or non-human organism may be a plant cell or plant organism, preferably the plant cell is from an *Arabidopsis thaliana* plant or the plant organism is an *Arabidopsis thaliana* plant.

If the transgenic eukaryotic cell or non-human organism is a plant cell or plant organism, preferably an *Arabidopsis thaliana* cell or an *Arabidopsis thaliana* plant, the promoter the methylation of which increases upon priming for a stress response may be the promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, PR5, ICS1, WRKY31; HEL, PDF1-2 or a functional fragment or a functional derivative of any of these promoters.

In an alternative embodiment the transgenic eukaryotic cell or non-human organism may be an animal cell or a non-human animal organism, preferably a mammalian cell and more preferably a rodent or human cell.

If the transgenic eukaryotic cell or non-human organism is an animal cell or a non-human animal organism, preferably a mammalian cell and more preferably a rodent or human cell, the promoter the methylation of which increases upon priming for a stress response may be the promoter of a gene selected from the group consisting of: p35, p40, tumour necrosis factor α, interferon-α and interferon-β or a functional fragment or functional derivative of any of these promoters.

The present invention also relates to a transgenic plant cell or plant organism comprising:

(a) one or more genetic modifications leading to an altered expression or activity of a protein selected from the group consisting of RNA polymerase II, EDR1, MKP1, MKP2, MPK4, EDS1, PAD4, WRKY70, WRKY18, MYB44, SPT4, SPT5 and SNI1; and

(b) an expression cassette which comprises the following elements in 5' to 3' direction:

(i) a promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, WRKY31, PR5, ICS1, HEL, PDF1-2 or a functional fragment or functional derivative of any of these promoters;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, a transcription termination sequence.

In another embodiment, the present invention relates to a transgenic plant cell or plant organism comprising:

a) a genetic modification resulting in the constitutive phosphorylation of RNA polymerase II; and

b) an expression cassette which comprises the following elements in 5' to 3' direction:

(i) a promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, PR5, ICS1, WRKY31, HEL, PDF1-2 or a functional fragment or functional derivative of any of these promoters;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, a transcription termination sequence.

Preferably, the modification resulting in the constitutive phosphorylation of RNA polymerase II is a reduction of activity of a phosphatase capable of dephosphorylating RNA polymerase II.

Preferably, the phosphatase is CPL1 or CPL3 and more preferably the phosphatase has the nucleic acid sequence according to SEQ ID No. 1 or 2.

In a preferred embodiment the activity of the phosphatase is reduced by a T-DNA insertion or chemically induced mutation which leads to a reduction of phosphatase expression, by RNA interference, by miRNA, by an aptamer, by an antibody specifically binding to the phosphatase, by an antisense sequence, by TILLING, by TALENs or by post-transciptional gene silencing, more preferably it is reduced by a T-DNA insertion which leads to a reduction of phosphatase expression and most preferably the T-DNA insertion is the one present in the plants obtainable from The European Arabidopsis Stock Centre under a catalogue number selected from the group consisting of N6541, N415837, N481418, N861773, N865879 and N6542.

Preferably, the promoter is the promoter of the WRKY6 gene and more preferably it has the sequence according to SEQ ID No. 6 or is a functional fragment or functional derivative thereof.

Hence, the present invention in particular relates to a transgenic plant cell or plant organism comprising:

(a) a T-DNA insertion which leads to a reduction of expression of a phosphatase which is capable of dephosphorylating RNA polymerase II; and

(b) an expression cassette which comprises the following elements in 5' to 3' direction:

(i) a promoter of the WRKY6 gene or a functional fragment or functional derivative of this promoter;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, a transcription termination sequence.

In still another embodiment, the present invention relates to a method for identifying substances which prime eukaryotic cells for a stress response, comprising the steps of:

a) treating the transgenic eukaryotic cell or non-human organism or the transgenic plant cell or plant organism of the present invention with one or more candidate substances; and

b) determining expression of the reporter gene.

Hence, in particular the present invention relates to a method for identifying substances which prime eukaryotic cells for a stress response, comprising the steps of:

(a) treating a transgenic plant cell or plant organism comprising:

(i) a T-DNA insertion which leads to a reduction of expression of a phosphatase which is capable of dephosphorylating RNA polymerase II; and

(ii) an expression cassette which comprises the following elements in 5' to 3' direction:

- a promoter of the WRKY6 gene or a functional fragment or a functional derivative of this promoter;
- operatively linked thereto a nucleic acid sequence coding for a reporter protein; and
- optionally, a transcription termination sequence, with one or more candidate substances; and

(b) determining expression of the reporter gene.

The transgenic cell or non-human organism may be treated with the one or more candidate substances for a period between 6 hours and 5 days, preferably 10 hours to 4 days, more preferably 12 hours to 3 days and most preferably 48 to 72 hours.

Finally, the present invention relates to the use of the transgenic eukaryotic cell or non-human organism or the transgenic plant cell or plant organism of the present invention for the identification of substances which prime cells for a stress response.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Hypothetical model of priming and transcription-associated methylation on the WRKY6 promoter

a) After stress stimulus, without priming: During normal transcription lysine 4 of histone 3 (H3K4) becomes trimethylated (H3K4me3) by a methyltransferase complex that is recruited and activated by phosphorylated RNA polymerase II (RNAPII).

b) After priming: H3K4 becomes dimethylated and the WDR5 protein of the methyltransferase complex recruits H3K4me3 to chromatin.

c) Stress stimulus after priming: During primed transcription the combination of WDR5-dependent coupling of the H3K4me3 methyltransferase and the phosphorylation of RNA polymerase II both lead to a high trimethylation of the promoter and enhanced transcription.

DETAILED DESCRIPTION OF THE INVENTION

The present invention as illustratively described in the following may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein.

The present invention will be described with respect to particular embodiments, but the invention is not limited thereto, but only by the claims.

Where the term “comprising” is used in the present description and claims, it does not exclude other elements. For the purposes of the present invention, the term “consisting of” is considered to be a preferred embodiment of the term “comprising". If hereinafter a group is defined to comprise at least a certain number of embodiments, this is also to be understood to disclose a group which preferably consists only of these embodiments.

For the purposes of the present invention, the term “obtained” is considered to be a preferred embodiment of the term “obtainable”. If hereinafter e.g. a cell or organism is defined to be obtainable by a specific method, this is also to be understood to disclose a cell or organism which is obtained by this method.

Where an indefinite or definite article is used when referring to a singular noun, e.g. “a”, “an” or “the”, this includes a plural of that noun unless something else is specifically stated.

The term "transgenic" means that a cell or organism has been altered using recombinant DNA technology to contain a nucleic acid sequence which would otherwise not be present in said cell or organism. Within the present invention, the transgenic cell or organism contains a nucleic acid sequence coding for a reporter protein which is operably linked to a promoter to which the nucleic acid sequence is not linked in the genome of a non-transgenic cell or organism. The transgenic eukaryotic cell or non-human organism also contains a further modification by recombinant DNA technology which leads to the activation of one or more members of a signal transduction pathway leading to stress-induced gene expression or the pre-activation of one or more members of the transcriptional machinery, such as RNA polymerase II. Preferably, the genetic modification leads to the constitutive phosphorylation of RNA polymerase II.

Eukaryotic cells are characterized by a nucleus which is surrounded by a membrane and which distinguishes the eukaryotic cells from prokaryotic cells. In particular, eukaryotic cells include animal cells, plant cells and fungal cells. Preferably, the cells of the present invention are plant cells.

The term "cell" as used herein refers to a single cell and also includes a population of cells. The population may be a pure population comprising one cell type. Likewise, the population may comprise more than one cell type. A cell within the meaning of the invention may be isolated (e.g., in suspension culture) or comprised in a tissue, organ or organism at any developmental stage, such as a plant tissue, plant organ or plant organism.

The term “organism” as used herein refers to a living system which is capable of responding to stimuli, of reproduction, of growth and of development. An organism usually consists of many cells which are grouped into specialized tissues and organs. Preferably, the eukaryotic organism of the present invention is a plant organism.

The term “genetic modification” refers to the alteration of the genetic material of a cell or organism, which genetic modification is preferably stable so that it is inherited to future generations. By this genetic modification the genome of a transgenic cell can be distinguished from the genome of a non-transgenic cell.

The term “signal transduction pathway which is involved in stress-induced gene expression” is intended to include all proteins which are involved in the transduction of a stress stimulus from the extracellular membrane to the cell nucleus via a signaling cascade, thereby leading to the induction of gene expression. In particular, the signal transduction pathway includes signal

transduction molecules such as adaptor proteins, GTPases, kinases and phosphatases. The skilled person knows the members of the signal transduction pathways leading to stress-induced gene expression.

The signal transduction pathway which is involved in stress-induced gene expression is activated, if the stress-induced gene expression occurs earlier and/or to a higher extent after an additional stress stimulus than the stress-induced gene expression without activated signal transduction pathway leading to stress-induced gene expression. Usually, the members of the signal transduction pathway will be in a state which is the same as after stimulation of the cell with a stimulus which leads to the activation of the signal transduction pathway. For example, if a stress leads to the activation of a kinase present in the signal transduction pathway, the signal transduction pathway is activated in the cells of the present invention if the kinase is active without the stimulus. Alternatively, if a stress stimulus leads to the inactivation of a repressor or negative regulator, the signal transduction pathway is activated, if the function of this repressor or negative regulator is reduced or eliminated.

The term “members of the transcriptional machinery” is intended to include regulators of transcription, such as specific transcription factors, basal transcription factors, repressors and enhancers as well as RNA polymerase II which regulators are necessary to obtain gene expression and which regulators are differentially modified or bound to the DNA during the transcription cycle. Preferably, the members of the transcriptional machinery are transcriptional regulators which are able to bind to multiple promoters or at least to several promoters which are involved in stress responses.

The members of the transcriptional machinery are in a “pre-activated state”, if the transcription occurs earlier and/or to a higher extent after an additional

stimulus than the transcription without pre-activated members of the transcriptional machinery. For example, in the pre-activated state one or more transcription factors or other transcription-stimulating factors may already be bound to regulatory regions of a gene without activating transcription. Preferably, the member of the transcriptional machinery is RNA polymerase II and more preferably the RNA polymerase II is constitutively phosphorylated.

Preferably, the one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of the transcriptional machinery leads to an altered expression or activity of one or more proteins selected from the group consisting of RNA polymerase II, EDR1, MKP1, MKP2, MPK4, EDS1, PAD4, WRKY70, WRKY18, MYB44, SPT4, SPT5 and SNI1.

SPT4 and SPT5 are subunits of the negative elongation factor which cause the RNA polymerase to stay on the promoter without starting transcription (Wada et al. (1998) Genes Dev. 12: 343-356). Hence, the inhibition of the expression or activity of SPT4 and/or SPT5 will result in an activation of transcription.

SNI1 is a transcription factor which acts as negative regulator of stress responses in *Arabidopsis thaliana* (Mosher *et al.* (2006) The Plant Cell 18: 1750-1765). Hence, a reduced expression or activity of SNI1 will result in an activation of stress-induced transcription.

EDS1, PAD4, WRKY and MYB transcription factors are positive regulators of a stress response so that their expression leads to an activation of stress-induced gene expression (Li *et al.* (2004) The Plant Cell 16: 319-331; Chen

and Chen (2002) Plant Physiol. 129: 706-716; Shim *et al.* (2013) The Plant J. 73: 483-495; Thesis of Enrico Gobbato at the University of Cologne (2007)).

More preferably, the member of a signal transduction pathway which is involved in stress-induced gene expression or the member of the transcriptional machinery is RNA polymerase II and/or EDR1, and most preferably it is RNA polymerase II.

EDR1 is a protein kinase which negatively regulates defense signaling pathways (Christiansen *et al.* (2011) Mol. Plant. Pathol. 12(8): 746-758). Hence, inhibition of EDR1 expression or activity will result in an activation of stress-induced transcription.

RNA polymerase II is a core component of the transcription complex which catalyses mRNA synthesis and is also involved in the regulation of various mRNA maturation processes, such as capping, splicing and poly-adenylation. The largest subunit of RNA polymerase II contains a C-terminal domain which is composed of up to 52 repeats of the heptapeptide sequence YSPTSPS which repeats are essential for polymerase activity. It has been shown that the function of RNA polymerase II is determined by the phosphorylation status of the C-terminal domain. During the transcription cycle the repeats of the C-terminal domain are differentially phosphorylated, predominantly at serine 2 or serine 5.

In the transgenic cells and the method of the present invention, the RNA polymerase II is preferably constitutively phosphorylated. The term “constitutively phosphorylated” is intended to mean that the phosphorylation is constant and not significantly influenced by external stimuli. The phosphorylation status can be detected by immuno-precipitating the RNA polymerase II with a suitable antibody such as an antibody directed to RNA polymerase II and

detecting the phosphorylation of the protein with a phospho-specific antibody, such as an antibody directed to phospho-serine.

The skilled person is aware of methods for obtaining a constitutively phosphorylated RNA polymerase II. These methods include the overexpression of a kinase which is capable of phosphorylating RNA polymerase II, for example mitogen-activated protein kinase 3. It has been shown that RNA polymerase II is phosphorylated by mitogen-activated protein kinase 3 (Ueda *et al.* (2008) Plant Mol. Biol. 67:683-697). Further, this kinase was shown to the required for full priming in *Arabidopsis thaliana* (Beckers *et al.* (2009) Plant Cell 21:944-953). Another kinase which may be involved in the phoshorylation of RNA polymerase II may be mitogen-activated protein kinase 6 (MPK6).

Methods for the overexpression of proteins are known to the person skilled in the art. For example, the coding region of the protein may be operably linked to a promoter which shows strong and constitutive activity in the cells to be transformed and the resulting expression construct may be transformed into the host cells.

Another alternative for obtaining constitutively phosphorylated RNA polymerase II is to reduce or inhibit the activity of a phosphatase which is capable of dephosphorylating RNA polymerase II. Such phosphatases include, but are not limited to C-terminal domain phosphatase-like (CPL) 1 and 3 (see Koiwa *et al.* (2002) Proc. Natl. Acad. Sci. USA 99 (16): 10893-10898; Koiwa *et al.* (2004) Proc. Natl. Acad. Sci. USA 101 (40): 14539-14544). The nucleic acid sequences coding for CPL1 and CPL3 are depicted in SEQ ID Nos. 1 and 2.

The skilled person knows methods to reduce the expression of a certain protein, including, but not being limited to, T-DNA insertion which leads to a reduction of phosphatase expression, by RNA interference, by miRNA, by an

aptamer, by an antibody specifically binding to the phosphatase, by an anti-sense sequence or by post-transciptional gene silencing. Further methods include the expression of a ribozyme which specifically recognizes the nucleic acid sequence coding for the protein or the expression of ribonuclease P (RNAseP) together with a leading sequence which directs RNAseP to the mRNA of the protein. Further, the skilled person knows many techniques for suppressing or inhibiting the expression of an endogenous gene by a small double-stranded RNA molecule, so called small interfering RNAs or siRNAs.

Another possibility would be to integrate a nucleic acid sequence for reducing the expression of the phosphatase into the natural locus of the sequence by targeted homologous recombination. The activity may also be reduced by the targeted induced local lesions in genomes (TILLING) method or by transcription activator-like effector nucleases (TALENs).

Preferably, the activity of the phosphatase is reduced by a T-DNA insertion into the coding sequence of the phosphatase which insertion leads to an abrogation of expression of said phosphatase. Plants carrying such an insertion are known to the skilled person (Koiwa *et al.* (2002) Proc. Natl. Acad. Sci. USA 99 (16): 10893-10898; Koiwa *et al.* (2004) Proc. Natl. Acad. Sci. USA 101 (40): 14539-14544). Such plants can be obtained, for example, from The European Arabidopsis Stock Center (http://arabidopsis.info/; Nottingham Arabidopsis Stock Centre, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD United Kingdom) under any of the catalogue numbers N6541, N415837, N481418, N861773, N865879, N6542. If such plants are used, they are transformed with a vector carrying the expression cassette comprising the promoter the methylation of which increases upon priming for a stress response

operatively linked to a nucleic acid sequence coding for the reporter protein to obtain the transgenic plants or plant cells of the present invention.

Within the scope of the present invention, the term "expression cassette" means a nucleic acid molecule which contains all elements which are necessary for the expression of a nucleic acid sequence, i.e. the nucleic acid sequence to be expressed under the control of a suitable promoter and optionally further regulatory sequences such as termination sequences. An expression cassette of the present invention may be part of an expression vector which is transferred into a cell or may be integrated into the chromosome of a transgenic cell or organism after transformation.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and may be used herein interchangeably with the term "recombinant nucleic acid molecule". One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. For the transformation of plants, the vector can be a binary vector or a T-DNA that comprises a left and a right border and may include a gene of interest in between. The term "expression vector" means a vector capable of directing expression of a particular nucleotide sequence in an appropriate host cell. An expression vector comprises a regulatory nucleic acid element operably linked to a nucleic acid of interest, which is - optionally - operably linked to a termination signal and/or other regulatory element.

The term "promoter" as used herein refers to a DNA sequence which, when ligated to a nucleotide sequence of interest, is capable of controlling the transcription of the nucleotide sequence of interest into mRNA. A promoter is

typically, though not necessarily, located 5' (e.g., upstream) of a nucleotide sequence of interest (e.g., proximal to the transcriptional start site of a structural gene) whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and other transcription factors for initiation of transcription.

The methylation of histones on the promoter used in the present invention increases upon priming for a stress response, i.e. when the cell is treated with a substance which primes the cell for a stress response, the methylation of the histones present in the chromatin of the promoter used in the expression cassette of the present invention increases compared to the methylation of the histones present in the chromatin of the promoter of an untreated cell. The methylation of a promoter can for example be detected by chromatin immunoprecipitation (ChIP) which involves the cross-linking of proteins bound to chromatin with chromatin, the shearing of chromatin to produce smaller fragments, the precipitation of the DNA/protein complex with an antibody which is directed to the methylated histone protein, the de-crosslinking of proteins and chromatin and determination of the DNA sequence of the DNA-fragment that was bound to the protein when the protein was immunoprecipitated by amplifying the DNA sequence with suitable DNA primers. A suitable method for chromatin immunoprecipitation from plant cells is described in Haring *et al.* (2007) Plant Methods 3: 11-27.

Within the meaning of the present invention the term “methylation” is intended to mean the attachment of one or more methyl residues to lysine 4 of histone H3 and includes mono-, di- and trimethylation. The promoter is preferably trimethylated after priming for a stress response, i.e. three methyl residues are attached to lysine 4 of histone H3 proteins within the chromatin of the promoter sequence. Accordingly, the terms “monomethylated” and “dimethylated” are intended to refer to the attachment of one or two methyl

residues, respectively, to lysine 4 of histone H3 proteins within the chromatin of the promoter sequence

The antibody which is used to detect the trimethylation of a promoter is directed to H3K4me3, i.e. histone H3 which is trimethylated on lysine 4. Such an antibody is available from Abcam (Catalogue No. ab8580) and Diagenode (Catalogue No. pAB-003-50). The antibody which is used to detect the monomethylation of a promoter is directed to H3K4me1, i.e. histone H3 which is monomethylated on lysine 4. Such an antibody is available from Abcam (Catalogue No. ab8895). The antibody which is used to detect the dimethylation of a promoter is directed to H3K4me2, i.e. histone H3 which is dimethylated on lysine 4. Such an antibody is available from Abcam (Catalogue No. ab7766) and Upstate (Catalogue No. 07-030).

To identify promoters which are suitable for use in the present invention, i.e. promoters the methylation of which increases upon priming for a stress response, cells are treated with a substance which is known to induce histone H3 methylation and the priming for a stress response, such as benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH), salicylic acid (SA), beta-aminobutyric acid (BABA) or acetyl salicylic acid (aspirin) and the methylation of the promoter is detected as described above using chromatin immunoprecipitation and compared to the methylation of the promoter in an untreated cell. If the methylation of the promoters increases upon treatment with any of these substances, the promoter can be used in the present invention. Candidate promoters which can be tested for an increase in methylation after priming for a stress response are promoters which are known to be induced by exposure to biotic or abiotic stress and/or which are known to have a stronger activity or an earlier onset of activity after repeated exposure to stress.

Plant promoters for which it has been shown that their methylation increases after treatment with substances which are known to induce priming are the promoters of the WRKY29, WRKY6, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22 genes (Jaskiewicz *et al.* (2011) EMBO Reports 12(1): 50-55; Ding *et al.* (2012) Nature Communications 3: 740). Hence, the promoters of the WRKY29, WRKY6, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22 genes are preferred for use in plant cells of the present invention. The promoters of the WRKY29, WRKY6, WRKY70, WRKY53, PR1, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22 genes have been shown to be trimethylated after treatment with substances which are known to induce priming and are therefore particularly preferred for use in plant cells of the present invention. Further promoters which are suitable for use in plant cells of the present invention include the promoters of the WRKY38, PR5, ICS1, WRKY31, HEL and PDF1-2 genes.

The sequences of these promoters from *Arabidopsis thaliana* are depicted in SEQ ID No. 3 (WRKY29), SEQ ID No. 6 (WRKY6), SEQ ID No. 7 (WRKY70), SEQ ID No. 8 (WRKY53), SEQ ID No. 9 (PR1), SEQ ID No. 10 (PAL1), SEQ ID No. 11 (PAL2), SEQ ID No. 12 (4CL), SEQ ID No. 13 (C4H), SEQ ID No. 14 (RD29B), SEQ ID No. 15 (RAB18), SEQ ID No. 16 (P5CS1), SEQ ID No. 17 (LPT3), SEQ ID no. 18 (LPT4), SEQ ID No. 19 (HSFA6A), SEQ ID No. 20 (HIPP22), SEQ ID No. 21 (WRKY38), SEQ ID No. 22 (PR5), SEQ ID No. 23 (ICS1), SEQ ID No. 24 (WRKY31), SEQ ID No. 63 (PDF1-2) and SEQ ID No. 64 (HEL).

Promoters from animal cells which are known to be induced in the priming process include, but are not limited to, the promoters of the p35, p40, tumour

necrosis factor α (TNF-α), interferon-α (IFN-α) and interferon-β (IFN-β) genes.

The sequences of these promoters are depicted in SEQ ID No. 25 (p35), SEQ ID No. 26 (p40), SEQ ID No. 27 (TNF-α), SEQ ID No. 28 (IFN-α) and SEQ ID No. 29 (IFN-β).

In one embodiment, the promoter used in the expression cassette may be operably linked to a part or all of the coding sequence of the gene to which it is naturally linked, i.e. the nucleic acid sequence the expression of which it controls in a wild-type cell, in addition to the nucleic acid sequence coding for the reporter protein. For example, the promoter of the WRKY29 gene may be linked to the 5' untranslated region (5' UTR) and a part of the first exon of the WRKY29 gene or it may be linked to the 5' UTR, the first exon, the first intron, the second exon, the second intron and part of the third exon of the WRKY29 gene. Such sequences are shown in SEQ ID Nos. 4 and 5. The promoter of the WRKY6 gene may be linked to the first to third exon of the WRKY6 gene. The sequence of the first to third exon of the WRKY6 gene is shown in SEQ ID No. 50. The nucleic acid sequence coding for the reporter protein is located downstream of the part or all of the coding sequence of the gene to which the promoter is naturally linked. Additionally or alternatively, the promoter used in the expression cassette may be operably linked to the 5' UTR of a gene to which it is not naturally linked, such as the 5' UTR of chalcone synthase to provide a suitable ribosome binding site.

In an alternative embodiment, the promoter is not linked to a part or all of the coding sequence of the gene to which it is naturally linked, i.e. the nucleic acid sequence the expression of which it controls in a wild-type cell. Nevertheless, it may be operably linked to the 5' UTR of a gene to which it is

not naturally linked, such as the 5' UTR of chalcone synthase to provide a suitable ribosome binding site.

A "functional fragment" of a promoter refers to a smaller part of the promoter with a contiguous nucleotide sequence found in the full-length promoter. The fragment is functional, if it is able to bind the transcription complex and cause expression of the reporter gene to which it is linked and if it its methylation increases upon priming for a stress response.

A "functional derivative" of a promoter refers to a sequence which differs from the wild-type promoter, for example the promoter shown in any of SEQ ID Nos. 3 and 6 to 29, in one or more nucleotides, but to which the transcription complex can still bind and cause expression of the reporter gene and the methylation of which increases upon priming for a stress response. In particular, the term "functional derivative" is intended to include promoters from species other than *Arabidopsis thaliana* which promoters are homologous to the promoters shown in any of SEQ ID Nos. 3 and 6 to 24, i.e. they direct the expression of homologous genes. For example, a promoter homologous to the WRKY6 promoter directs the expression of the WRKY6 gene in another species.

The term "priming" refers to the induction of a physiological state that enables the primed cells to respond to very low levels of a stimulus, preferably an abiotic or biotic stress, in a faster and/or stronger manner than non-primed cells. Thus, primed plants show faster and/or stronger activation of stress responses when challenged by biotic or abiotic stress after priming.

Stress responses are the reaction of a cell or organism to biotic or abiotic stress and include, but are not limited to, enhanced transcription of defence genes such as PAL1, PAL2, 4CL, C4H, WRKY29, WRKY6 and WRKY53.

With respect to nucleic acid sequences or DNA sections in expression cassettes or vectors the terms "operatively linked" and "operably linked" mean that nucleic acid sequences are linked to each other such that the function of one nucleic acid sequence is influenced by the other nucleic acid sequence. For example, if a nucleic acid sequence is operably linked to a promoter, its expression is influenced by said promoter.

Reporter proteins which are encoded by reporter genes are proteins the expression of which can be easily detected and which are attached to a regulatory sequence of another gene to investigate the expression pattern of this regulatory sequence. Hence, the regulatory sequence, in particular the promoter, and the reporter gene are not operably linked in wild-type cells. In a preferred embodiment the expression of the reporter protein is not detected by measuring the expression level of the reporter protein by detecting the amount of mRNA or protein expressed, but by measuring an activity of the reporter protein.

Suitable reporter proteins include, for example the β-glucuronidase (GUS)-encoding *uidA* gene from *E.coli,* fluorescent proteins, luciferase proteins such as firefly luciferase from *Photinus pyralis*, renilla luciferase from *Renilla reniformis* or the NanoLuc™ luciferase, alkaline phosphatase and the β-galactosidase (lacZ) gene from *E. coli.* If expressed, the GUS, luciferase and β-galactosidase proteins catalyse a reaction which leads to a detectable product, whereas the expression of the fluorescent proteins can be directly determined by detecting the fluorescence.

Further reporter proteins are proteins which confer resistance to certain substances such as the proteins chloramphenicol transferase or neomycin

phosphotransferase which confer resistance to chloramphenicol and kanamycin, respectively.

Particularly preferred in the method of the present invention are fluorescent proteins such as AcGFP1, AmCyan1, AsRed2, mBanana, mCherry, Dendra2, DsRed2, E2-Crimson, GFP, HcRed1, mOrange, PAm cherry, mPlum, mRaspberry, mStrawberry, tandem-d-Tomato, Timer, Zsgreen1, Zsyellow1, mNeonGreen, mVenus and superfolder GFP and most preferred is mNeonGreen. The nucleic acid sequences coding for mOrange, tandem-d-tomato, superfolder GFP, mVenus and mNeonGreen are depicted in SEQ ID Nos. 30-34.

The skilled person is aware of methods for determining the expression of the reporter protein. In case of fluorescent proteins, the fluorescent protein is excited with a suitable wavelength and the emitted fluorescent is detected at a suitable wavelength. Suitable wavelengths for exciting and detecting fluorescence of fluorescent proteins are known to the person skilled in the art. For example, for detecting expression of the mNeonGreen protein the cells or organisms are irradiated with light of a wavelength of 480 nm and fluorescence is detected at a wavelength of 530 nm.

The vectors which are used in the method of the present invention may further comprise regulatory elements in addition to the nucleic acid sequence to be transferred. Which specific regulatory elements must be included in said vectors depends on the procedure which is to be used for said vectors. Those skilled in the art, who are familiar with the various methods for producing transgenic cells and organisms know which regulatory elements and also other elements said vectors must include.

Typically, the regulatory elements which are contained in the vectors ensure the transcription and, if desired, the translation in the plant cell.

The term "transcription regulatory element" as used herein refers to a polynucleotide that is capable of regulating the transcription of an operably linked polynucleotide. It includes, but is not limited to, promoters, enhancers, introns, 5' UTRs, and 3' UTRs.

For enhancing the fluorescence of the fluorescent protein, the fluorescent protein may be fused to a localization sequence which targets the protein to the endoplasmic reticulum and/or a retention sequence which retains the fluorescent protein in the endoplasmic reticulum of the cell. Suitable sequences for targeting and retaining proteins to the endoplasmic reticulum are known to the person skilled in the art and shown in SEQ ID Nos. 35 to 37.

So-called termination sequences are sequences which ensure that the transcription or the translation is terminated properly. If the introduced nucleic acids are to be translated, said nucleic acids are typically stop codons and corresponding regulatory sequences; if the introduced nucleic acids are only to be transcribed, said nucleic acids are normally poly-A sequences.

The transgenic eukaryotic cell may be a plant or an animal cell, such as a mammalian cell. Preferably it is a plant cell. The plant cell may be derived from a monocotyledonous or dicotyledonous plant and the plant organism may also be a dicotyledonous or monocotyledonous organism.

Examples of monocotyledonous plants are plants belonging to the genera Avena (oat), Triticum (wheat), Secale (rye), Hordeum (barley), Oryza (rice), Panicum, Pennisetum, Setaria, Sorghum (millet), Zea (maize), and the like.

Dicotyledonous useful plants comprise, *inter alia*, Arabidopsis, cotton, legumes, like leguminous plants and in particular alfalfa, soybean, rape, canola, tomato, sugar beet, potato, ornamental plants, and trees. Further useful plants can comprise fruit (in particular apples, pears, cherries, grapes, citrus, pineapple, and bananas), pumpkin, cucumber, wine, oil palms, tea shrubs, cacao trees, and coffee shrubs, tobacco, sisal, as well as, with medicinal plants, rauwolfia and digitalis.

Particularly preferred are plants or plant cells which are easy to manipulate and to cultivate such as *Arabidopsis thaliana* plants or plant cells or *Nicotiana tabacum* plants or plant cells. Most preferably, the plant cells are from *Arabidopsis thaliana* or the plant is *Arabidopsis thaliana.*

Suitable mammalian cells include, but are not limited to, human, mouse, rat, hamster, bovine and porcine cells. Preferably the cells are rodent or human cells. Examples of suitable mammalian cells include HeLa, NIH3T3, CHO and 293 cells.

The expression cassette can be introduced into the cells by any method known in the art.

For introducing DNA into a plant cell, a number of well-known techniques are available and those skilled in the art may easily determine the suitable technique for each case. Said techniques comprise the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation means, viral infection by using viral vectors (EP 0 067 553; US 4,407,956, WO 95/34668; WO 93/03161), the fusion of protoplasts, polyethylene glycol-induced DNA uptake, liposome-mediated transformation (US 4,536,475), incubation of dry embryos in DNA-comprising

solution, microinjection, the direct gene transfer of isolated DNA in protoplasts, the electroporation of DNA, the introduction of DNA by the biolistic procedure, as well as other possibilities. Thereby, stable as well as transient transformants may be produced.

Methods for introducing the expression cassette into mammalian cells include, but are not limited to, liposome mediated transfection, dendrimer based transfection, electroporation, microinjection, virus-mediated gene delivery, calcium phosphate precipitation, DEAE-dextran-mediated transfection and lipofection. Commercially available kits for transfection, such as SuperFect, PolyFect, Effectene (Qiagen), TransFast™, ProFection®, Transfectam® (Promega) and TransPass™ (NEB) may also be used. Preferably, the cells are transfected while in suspension and under serum-free conditions.

The transgenic cells of the present invention are usually stably transfected, which means that the transgene is stably integrated into the genome of the cell and that successfully transformed cells are selected after transfection by means of a selection agent which kills the non-transfected cells, whereas the transfected cells containing the resistance gene continue growing. For this purpose, the vector used for the transfection which comprises the expression cassette usually also comprises a nucleic acid sequence encoding a selection marker, such as a protein conferring resistance to an agent which is toxic for a wild-type cell.

The transgenic eukaryotic cell or non-human organism may be used in a method for identifying substances which prime eukaryotic cells for a stress response. Substances which prime eukaryotic cells, in particular plant cells, for a stress response prepare the immune system of the cells for future stress. In plants which have been primed for a stress response, the stress

response occurs earlier and is stronger, leading to a more effective resistance.

Substances which are known to prime plant cells for a stress response and which also increase the methylation of histones on promoters of genes involved in the stress response include benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH), salicylic acid (SA) or acetyl salicylic acid (aspirin).

The method of the present invention involves the treatment of the transgenic cells or the transgenic organism, preferably transgenic plant cells or a transgenic plant, with one or more candidate substances, alone or in combination, wherein the cells or organism is contacted with the substance for a certain time period, such as 6 hours and 5 days, preferably 10 hours to 4 days, more preferably 12 hours to 3 days and most preferably 48 to 72 hours. The person skilled in the art will appreciate that the actual incubation time may be different for different reporter genes.

Candidate substances are substances which should be tested for their ability to prime cells for a stress response and include natural and synthetic substances. The term “candidate substances” is also intended to include bacteria and other microorganisms which may secrete substances which prime cells for a stress response. It has been shown that the colonization of Arabidopsis roots with *Pseudomonas fluorescens* bacteria primes the plant for a response to pathogens (Hase *et al.* (2003) Physiological and Molecular Plant Pathology 62: 219-226).

After incubation of the substance with a cell or organism the expression of the reporter protein is determined by a method suitable for determining expression of this reporter protein. For example, if the reporter protein is a fluorescent protein, the expression is detected by exciting the fluorescent

protein with light of a suitable wavelength and measuring the emitted light at a suitable wavelength. Suitable incubation times for a particular reporter protein are known or can be easily determined.

As a positive control, the cell or organism may also be treated with one or more substances, from which it is known that they prime cells for a stress response, such as benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH), salicylic acid (SA) or acetyl salicylic acid (aspirin).

Further, the expression of the reporter protein is usually compared with the expression of this protein in cells or organisms which act as a negative control. Suitable negative control cells or organisms include, but are not limited to:

(i) cells or organisms of the present invention which have not been treated with the one or more candidate substances and

(ii) cells or organisms which carry the expression cassette comprising the promoter and the nucleic acid sequence encoding a reporter protein, but which do not comprise the one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery and which have been treated with the one or more candidate substances.

The negative control cells or organisms are from the same organisms or are the same organisms as the cells of the present invention which are incubated with candidate substances.

Those substances which lead to a significantly increased expression of the reporter protein in the treated cells of the present invention in comparison to

the negative control cells are substances which prime the cells for a stress response.

Instead of using the whole organism such as a whole plant or a cell from this organism in the method of the present invention, also parts of the organism can be used in the method of the present invention, such as stems, roots, ovules, stamens, leaves, embryos, meristematic regions, callus tissue, cell suspensions, seedlings, gametophytes, sporophytes, pollen, microspores, seeds and the like.

After identifying substances which are capable of priming a stress response by the method of the present invention, it can be confirmed that these substances prime the cells for a stress response by treatment of wild-type plants with this substance and then contacting the plants with pathogen or abiotic stress and measuring the stress response. It is confirmed that a substance primes cells for a stress response, if after treatment with the substance and contacting the plants with pathogen or abiotic stress the stress response in the plant treated with the substance occurs earlier and/or is stronger than in a plant not treated with the substance.

The production of a transgenic plant cell of the present invention as well as its use in a method of identifying substances which prime for a stress response is described in the following examples:

EXAMPLES

1. Preparation of a reporter gene construct

The expression cassette comprising the promoter the methylation of which increases after priming for a stress response operably linked to a reporter

protein was cloned into the pGreenII-0229 plasmid which carries a kanamycin and a phosphinotricin resistance gene (SEQ ID No. 65). The plasmid was cut with *Cla*I to remove the lacZ expression cassette and then self-ligated.

The WRKY6 promoter was amplified via PCR with the DNA primers according to SEQ ID Nos. 51 and 52 from *Arabidopsis thaliana* genomic DNA and ligated in the pGreenII-0229 plasmid which had been cut with *Eco*RV and *Eco*RI.

The 5' UTR from CHS was amplified from *Petroselinum crispum* genomic DNA using the primers according to SEQ ID Nos. 53 and 54 and ligated 3' of the WRKY6 promoter via the *Eco*RI and *Sma*I restriction sites.

The WAK1 ER localisation sequence was amplified from *Arabidopsis thaliana* genomic DNA using the primers according to SEQ ID Nos. 55 and 56 and ligated 3' of the 5' UTR from CHS via the *Sma*I and NotI restriction sites.

The WRKY coding sequence from the first to the third exon was amplified from *Arabidopsis thaliana* genomic DNA using the primers according to SEQ ID Nos. 57 and 58 and ligated 3' of the WAK1 ER localisation sequence via the *Not*I and *Ale*I restriction sites.

The nucleic acid sequence coding for the reporter protein monomeric NeonGreen was amplified from a plasmid obtained from Allele Biotechnology, 6404 Nancy Ridge Drive San Diego, CA 92121 (http://www.allelebiotech.com) using the primers according to SEQ ID Nos. 59 and 60. In this PCR reaction the nucleic acid sequence coding for the ER retention signal was fused to the nucleic acid sequence coding for

monomeric NeonGreen. The resulting nucleic acid molecule was ligated 3' of the WRKY6 coding sequence via the *Ale*I and *Sap*I restriction sites.

Finally, the terminator sequence from octopin synthase was amplified from plasmid pEarleyGate 100 (ABRC stock number CD3-724) using the primers according to SEQ ID Nos. 61 and 62 and ligated 3' of the nucleic acid sequence coding for monomeric NeonGreen via the *Sap*I restriction site.

The plasmid was transformed into *E. coli* cells by heat shock and positive colonies were selected with kanamycin.

2. Transformation of the reporter gene construct

The plasmid carrying the complete expression cassette of example 1 was transformed into *Agrobacterium tumefaciens* strain GV3101 using heat shock. Agrobacteria containing the plasmid were selected and used to transform *Arabidopsis thaliana* plants in which the activity of CPL1 was abrogated by a T-DNA insertion. These plants were obtained from The European Arabidopsis Stock Centre (http://arabidopsis.info/; Nottingham Arabidopsis Stock Centre, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD United Kingdom) under catalogue number N6541. For the transformation the floral dip method (Clough and Bent (1998) Plant J. 16: 735-743) was used. Seeds were obtained from the transformed plants and transferred to soil containing phosphinotricin to select for plants carrying the transformed plasmid. From positive seeds complete plants were recovered.

3. Identification of substances which induce priming

Plant seeds obtained from the plants prepared in Example 2 were transferred into soil and plants were grown for five weeks at a light intensity of 80 to 180 µmol/m^2/s. A solution or suspension of the substances to be tested was sprayed on the plants. Three days after the spraying the plants were irradiated with light with a wavelength of 480 nm and the emitted light was detected at a wavelength of 530 nm to determine the expression of the reporter protein. The intensity of the emitted light indicated whether the substance has induced the priming or not. If the intensity of the light emitted by the treated plants was stronger than the intensity of the light emitted by treated plants which carry only the reporter gene construct, but not the CPL1 mutation, the substance had an influence on the promoter of the reporter gene construct and on the priming of the cells.

Some embodiments of the present invention include:

1. Transgenic eukaryotic cell or non-human organism comprising:

a) one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery; and

b) an expression cassette which comprises:

(i) a promoter the methylation of which increases upon priming for a stress response or a functional fragment or functional derivative of such a promoter;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, further regulatory elements.

2. Transgenic eukaryotic cell or non-human organism of 1, wherein the one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery lead to an altered expression or activity of a protein selected from the group consisting of RNA polymerase II, EDR1, MKP1, MKP2, MPK4, EDS1, PAD4, WRKY70, WRKY18, MYB44, SPT4, SPT5 and SNI1.

3. Transgenic eukaryotic cell or non-human organism of 1 or 2, wherein the one or more genetic modifications lead to an increased expression or activity of a protein selected from the group consisting of RNA polymerase II, EDS1, PAD4, WRKY70, WRKY18 and MYB44.

4. Transgenic eukaryotic cell or non-human organism of 1 or 2, wherein the one or more genetic modifications lead to a decreased expression or activity of a protein selected from the group consisting of EDR1, MKP1, MKP2, MPK4, SPT4, SPT5 and SNI1.

5. Transgenic eukaryotic cell or non-human organism of 1 or 2, wherein the genetic modification providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery leads to an altered expression or activity of RNA polymerase II.

6. Transgenic eukaryotic cell or non-human organism of 5, wherein the RNA polymerase II is constitutively phosphorylated.

7. Transgenic eukaryotic cell or non-human organism of 6, wherein the RNA polymerase II is constitutively phosphorylated by reducing the activity of a phosphatase capable of dephosphorylating RNA polymerase II.

8. Transgenic eukaryotic cell or non-human organism of any of 1 to 7, wherein the methylation is the attachment of one, two or three methyl residues to lysine 4 of histone H3.

9. Transgenic eukaryotic cell or non-human organism of any of 1 to 7, wherein the methylation is the attachment of three methyl residues to lysine 4 of histone H3.

10. Transgenic eukaryotic cell or non-human organism of any of 9 to 7, wherein the promoter is the promoter of a gene selected from the group

consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, WRKY31, PR5, ICS1, HEL, PDF1-2, p35, p40, tumour necrosis factor α, interferon-α and interferon-β or a functional fragment or functional derivative of any of these promoters.

11. Transgenic eukaryotic cell or non-human organism of any of 1 to 10, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3, 6 to 29, 63 and 64 or is a functional fragment or functional derivative of any of these promoters.

12. Transgenic eukaryotic cell or non-human organism of any of 1 to 10, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY29, WRKY6, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22.

13. Transgenic eukaryotic cell or non-human organism of 12, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3 and 6 to 20 or is a functional fragment or functional derivative of any of these promoters.

14. Transgenic eukaryotic cell or non-human organism of any of 1 to 10, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY29, WRKY6, WRKY70, WRKY53, PR1, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22.

15. Transgenic eukaryotic cell or non-human organism of 14, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3, 6 to 9 and 14 to 20 or is a functional fragment or functional derivative of any of these promoters.

16. Transgenic eukaryotic cell or non-human organism of any of 1 to 15, wherein the reporter protein is selected from the group consisting of: fluorescent proteins, luciferase proteins, β-galactosidase, alkaline phosphatase, β-glucuronidase and chloramphenicol acetyltransferase.

17. Transgenic eukaryotic cell or non-human organism of 16, wherein the fluorescent protein is selected from the group consisting of AcGFP1, AmCyan1, AsRed2, mBanana, mCherry, Dendra2, DsRed2, E2-Crimson, GFP, HcRed1, mOrange, PAm cherry, mPlum, mRaspberry, mStrawberry, tandem-d-Tomato, Timer, Zsgreen1, Zsyellow1, mNeonGreen, mVenus and superfolder GFP.

18. Transgenic eukaryotic cell or non-human organism of 17, wherein the fluorescent protein is mNeonGreen.

19. Transgenic eukaryotic cell or non-human organism of 18, wherein the fluorescent protein is encoded by the nucleic acid sequence according to SEQ ID No. 34.

20. Transgenic eukaryotic cell or non-human organism of any of 1 to 19, being a plant cell or plant organism.

21. Transgenic eukaryotic cell or non-human organism of 20, wherein the plant cell is from an *Arabidopsis thaliana* plant or the plant organism is an *Arabidopsis thaliana* plant.

22. Transgenic eukaryotic cell or non-human organism of 20 or 21, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22,

WRKY38, WRKY31, PR5, ICS1, HEL, PDF1-2 or a functional fragment or functional derivative of any of these promoters.

23. Transgenic eukaryotic cell or non-human organism of 20 to 22, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3, 6 to 24, 63 and 64 or is a functional fragment or functional derivative of any of these promoters.

24. Transgenic eukaryotic cell or non-human organism of 20 to 22, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY29, WRKY6, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22.

25. Transgenic eukaryotic cell or non-human organism of 23, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3 and 6 to 20 or is a functional fragment or functional derivative of any of these promoters.

26. Transgenic eukaryotic cell or non-human organism of 20 to 22, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY29, WRKY6, WRKY70, WRKY53, PR1, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22.

27. Transgenic eukaryotic cell or non-human organism of 26, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3, 6 to 9 and 14 to 20 or is a functional fragment or functional derivative of any of these promoters.

28. Transgenic eukaryotic cell or non-human organism of any of 1 to 19, being an animal cell or a non-human animal organism.

29. Transgenic eukaryotic cell or non-human organism of 28, being a mammalian cell.

30. Transgenic eukaryotic cell or non-human organism of 28 or 29, wherein the promoter is the promoter of a gene selected from the group consisting of: p35, p40, tumour necrosis factor α, interferon-α and interferon-β or a functional fragment or functional derivative of any of these promoters.

31. Transgenic eukaryotic cell or non-human organism of 28 to 30, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 25 to 29 or is a functional fragment or functional derivative of any of these promoters.

32. Transgenic plant cell or plant organism comprising:

(a) one or more genetic modifications leading to an altered expression or activity of one or more proteins selected from the group consisting of RNA polymerase II, EDR1, MKP1, MKP2, MPK4, EDS1, PAD4, WRKY70, WRKY18, MYB44, SPT4, SPT5 and SNI1; and

(b) an expression cassette which comprises the following elements in 5' to 3' direction:

(i) a promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, WRKY31, PR5, ICS1, HEL, PDF1-2 or a functional fragment or functional derivative of any of these promoters;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, a transcription termination sequence.

33. Transgenic plant cell or plant organism of 32, wherein the one or more genetic modifications lead to an increased expression or activity of a protein selected from the group consisting of RNA polymerase II, EDS1, PAD4, WRKY70, WRKY18 and MYB44.

34. Transgenic plant cell or plant organism of 32, wherein the one or more genetic modifications lead to a decreased expression or activity of a protein selected from the group consisting of EDR1, MKP1, MKP2, MPK4, SPT4, SPT5 and SNI1.

35. Transgenic plant cell or plant organism comprising:

(a) a genetic modification resulting in the constitutive phosphorylation of RNA polymerase II ; and

(b) an expression cassette which comprises the following elements in 5' to 3' direction:

(i) a promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, WRKY31, PR5, ICS1, HEL, PDF1-2 or a functional fragment or functional derivative of any of these promoters;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, a transcription termination sequence.

36. Transgenic plant cell or plant organism of 35, wherein the modification results in the reduction of activity of a phosphatase capable of dephosphorylating RNA polymerase II.

37. Transgenic plant cell or plant organism of 36, wherein the phosphatase is CPL1 or CPL3.

38. Transgenic plant cell or plant organism of 37, wherein the nucleic acid sequence encoding CPL1 is the sequence according to SEQ ID No. 1.

39. Transgenic plant cell or plant organism of 37, wherein the nucleic acid sequence encoding CPL3 is the sequence according to SEQ ID No. 2.

40. Transgenic plant cell or plant organism of any of 35 to 39, wherein the activity of the phosphatase is reduced by a T-DNA insertion which leads to a reduction of phosphatase expression, by chemical mutagenesis, by RNA interference, by miRNA, by an aptamer, by an antibody specifically binding to the phosphatase, by an antisense sequence, by TILLING, by TALENS or by post-transcriptional gene silencing.

41. Transgenic plant cell or plant organism of 40, wherein the activity of the phosphatase is reduced by a T-DNA insertion which leads to a reduction of phosphatase expression.

42. Transgenic plant cell or plant organism of 41, wherein the T-DNA insertion is the one present in the plants obtainable from The European Arabidopsis Stock Centre under a catalogue number selected from the group consisting of N6541, N415837, N481418, N861773, N865879 and N6542.

43. Transgenic plant cell or plant organism of any of 32 to 42, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY29, WRKY6, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22.

44. Transgenic plant cell or plant organism of 43, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3 and 6 to 20 or is a functional fragment or functional derivative of any of these promoters.

45. Transgenic plant cell or plant organism of any of 32 to 42, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY29, WRKY6, WRKY70, WRKY53, PR1, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A and HIPP22.

46. Transgenic plant cell or plant organism of 45, wherein the promoter has a sequence selected from the group consisting of SEQ ID Nos. 3, 6 to 9 and 14 to 20 or is a functional fragment or functional derivative of any of these promoters.

47. Transgenic plant cell or plant organism of any of 32 to 46, wherein the promoter is the promoter of the WRKY6 gene.

48. Transgenic plant cell or plant organism of 47, wherein the promoter of the WRKY6 gene has the sequence according to SEQ ID No. 6 or is a functional derivative or functional fragment thereof.

49. Transgenic plant cell or plant organism of any of 32 to 48, wherein the reporter protein is selected from the group consisting of: fluorescent proteins, luciferase protein, β-galactosidase, alkaline phosphatase, β-glucuronidase and chloramphenicol acetyltransferase.

50. Transgenic plant cell or plant organism of 49, wherein the fluorescent protein is selected from the group consisting of: AcGFP1, AmCyan1, AsRed2, mBanana, mCherry, Dendra2, DsRed2, E2-Crimson, GFP, HcRed1, mOrange, PAm cherry, mPlum, mRaspberry, mStrawberry,

tandem-d-Tomato, Timer, Zsgreen1, Zsyellow1, mNeonGreen, mVenus and superfolder GFP.

51. Transgenic plant cell or plant organism of 50, wherein the fluorescent protein is mNeonGreen.

52. Transgenic plant cell or plant organism of 51, wherein the fluorescent protein is encoded by the nucleic acid sequence according to SEQ ID No. 34.

53. Transgenic plant cell or plant organism of any of 32 to 52, wherein the cell is from an *Arabidopsis thaliana* plant or the plant is an *Arabidopsis thaliana* plant.

54. Transgenic plant cell or plant organism comprising:

(a) a T-DNA insertion which leads to a reduction of the expression of a phosphatase capable of dephosphorylating RNA polymerase II;

(b) an expression cassette which comprises the following elements in 5' to 3' direction:

(i) a promoter of the WRKY6 gene or a functional fragment or functional derivative of this promoter;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, a transcription termination sequence.

55. Transgenic plant cell or plant organism of 54, wherein the T-DNA insertion is the one present in the plants obtainable from The European Arabidopsis Stock Centre under a catalogue number selected from the group consisting of N6541, N415837, N481418, N861773, N865879 and N6542.

56. Transgenic plant cell or plant organism of 54 or 55, wherein the promoter of the WRKY6 gene has the sequence according to SEQ ID No. 6 or is a functional derivative or functional fragment thereof.

57. Transgenic plant cell or plant organism of any of 54 to 56, wherein the reporter protein is selected from the group consisting of: proteins, luciferase protein, β-galactosidase, alkaline phosphatase, β-glucuronidase and chloramphenicol acetyltransferase.

58. Transgenic plant cell or plant organism of 57, wherein the fluorescent protein is selected from the group consisting of: AcGFP1, AmCyan1, AsRed2, mBanana, mCherry, Dendra2, DsRed2, E2-Crimson, GFP, HcRed1, mOrange, PAm cherry, mPlum, mRaspberry, mStrawberry, tandem-d-Tomato, Timer, Zsgreen1, Zsyellow1, mNeonGreen, mVenus and superfolder GFP.

59. Transgenic plant cell or plant organism of 58, wherein the fluorescent protein is mNeonGreen.

60. Transgenic plant cell or plant organism of 59, wherein the fluorescent protein is encoded by the nucleic acid sequence according to SEQ ID No. 34.

61. Transgenic plant cell or plant organism of any of 54 to 60, wherein the cell is from an *Arabidopsis thaliana* plant or the plant is an *Arabidopsis thaliana* plant.

62. Method for identifying substances which prime eukaryotic cells for a stress response, comprising the steps of:

(a) treating the transgenic eukaryotic cell or non-human of any of 1 to 31 or the transgenic plant cell or plant organism of any of 32 to 61 with one or more candidate substances; and

(b) determining expression of the reporter protein.

63. Method of 62, wherein the transgenic plant cell or plant organism of any of 32 to 61 is treated with the one or more candidate substances.

62. Method of 62 or 63, wherein the transgenic eukaryotic cell or non-human organism or the transgenic plant cell or plant organism is treated with the one or more candidate substances for a period of 6 hours to 5 days.

63. Method of 62 to 64, wherein increased expression of the reporter protein compared to the expression in the negative control indicates that the candidate substance primes the cells for the stress response.

64. Method of 65, wherein the negative control is selected from the group consisting of:

(i) cells of any of claims 1 to 61 which have not been treated with the one or more candidate substances; and

(ii) cells which carry the expression cassette of (b) of claims 1 to 61, but which do not comprise the one or more genetic modifications of (a) of claims 1 to 59 and which have been treated with the one or more candidate substances.

65. Use of the transgenic eukaryotic cell or non-human organism of any of 1 to 31 or the transgenic plant cell or plant organism of any of 32 to 61 for the identification of substances which prime cells for a stress response.

CLAIMS

1. Transgenic eukaryotic cell or non-human organism comprising:

a) one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery; and

b) an expression cassette which comprises:

(i) a promoter the methylation of which increases upon priming for a stress response or a functional fragment or functional derivative of such a promoter;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, further regulatory elements.

2. Transgenic eukaryotic cell or non-human organism of claim 1, wherein the one or more genetic modifications providing the activation of one or more signal transduction pathways which are involved in stress-induced gene expression and/or the pre-activation of one or more members of the transcriptional machinery lead to an altered expression or activity of one or more proteins selected from the group consisting of RNA polymerase II, EDR1, MKP1, MKP2, MPK4, EDS1, PAD4, WRKY70, WRKY18, MYB44, SPT4, SPT5 and SNI1.

3. Transgenic eukaryotic cell or non-human organism of claim 1 or 2, wherein the one or more members of the transcriptional machinery is RNA polymerase II which is constitutively phosphorylated.

4. Transgenic eukaryotic cell or non-human organism of claim 3,

wherein the RNA polymerase II is constitutively phosphorylated by reducing the activity of a phosphatase capable of dephosphorylating RNA polymerase II.

5. Transgenic eukaryotic cell or non-human organism of any of the preceding claims, being a plant cell or plant organism.

6. Transgenic eukaryotic cell or non-human organism of claim 5, wherein the plant cell is from an *Arabidopsis thaliana* plant or the plant organism is an *Arabidopsis thaliana* plant.

7. Transgenic eukaryotic cell or non-human organism of claim 5 or 6, wherein the promoter is the promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, WRKY31, PR5, ICS1, HEL; PDF1-2 or a functional fragment or a functional derivative of any of these promoters.

8. Transgenic eukaryotic cell or non-human organism of any of claims 1 to 4, being an animal cell or a non-human animal organism.

9. Transgenic eukaryotic cell or non-human organism of claim 8, wherein the promoter is the promoter of a gene selected from the group consisting of: p35, p40, tumour necrosis factor α, interferon-α and interferon-β or a functional fragment or functional derivative of any of these promoters.

10. Transgenic plant cell or plant organism comprising:

(a) a genetic modification resulting in the constitutive phosphorylation of RNA polymerase II ; and

(b) an expression cassette which comprises the following elements in 5' to 3' direction:

(i) a promoter of a gene selected from the group consisting of: WRKY6, WRKY29, WRKY70, WRKY53, PR1, PAL1, PAL2, 4CL, C4H, RD29B, RAB18, P5CS1, LPT3, LPT4, HSFA6A, HIPP22, WRKY38, WRKY31, PR5, ICS1, HEL, PDF1-2 or a functional fragment of any of these promoters;

(ii) operatively linked thereto a nucleic acid sequence coding for a reporter protein; and

(iii) optionally, a transcription termination sequence.

11. Transgenic plant cell or plant organism of claim 10, wherein the genetic modification results in the reduction of activity of a phosphatase capable of dephosphorylating RNA polymerase II

12. Transgenic plant cell or plant organism of claim 10 or 11, wherein the activity of the phosphatase is reduced by a T-DNA insertion which leads to a reduction of phosphatase expression, by RNA interference, by miRNA, by an aptamer, by an antibody specifically binding to the phosphatase, by an antisense sequence, by TILLING, by TALENs or by post-transcriptional gene silencing.

13. Transgenic plant cell or plant organism of any of claims 10 to 12, wherein the promoter is the promoter of the WRKY6 gene according to SEQ ID No. 6 or a functional fragment or a functional derivative thereof.

14. Method for identifying substances which prime eukaryotic cells for a stress response, comprising the steps of:

(a) treating the transgenic eukaryotic cell or non-human organism or the transgenic plant cell or plant organism of any of the preceding claims with one or more candidate substances; and

(b) determining expression of the reporter gene.

15. Use of the transgenic eukaryotic cell or non-human organism of any of claims 1 to 9 or the transgenic plant cell or plant organism of any of claims 10 to 13 for the identification of substances which prime eukaryotic cells for a stress response.

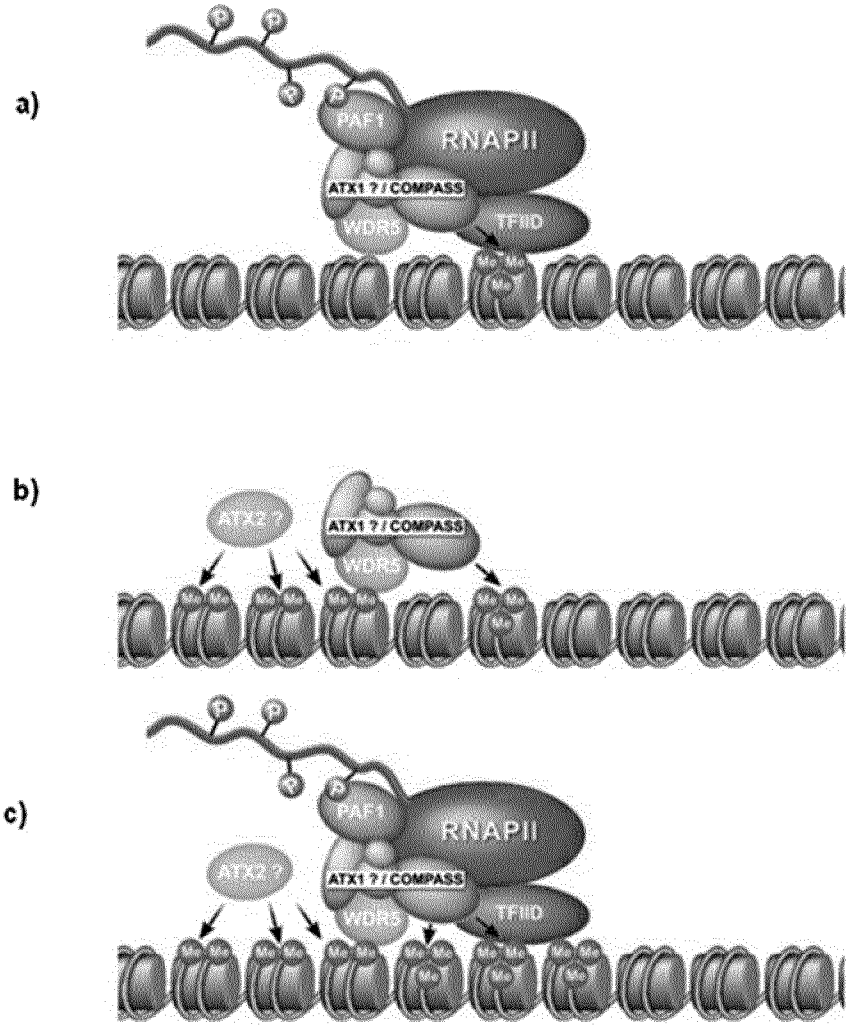
a)
PAF1
RNAPII
ATX1 ? / COMPASS
WDR5
TFIID
b)
ATX2 ?
ATX1 ? / COMPASS
WDR5
c)
PAF1
RNAPII
ATX2 ?
ATX1 ? / COMPASS
WDR5
TFIID

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2014/062266

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   - [ ] on paper
   - [X] in electronic form

   b. (time)

   - [X] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/062266

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H1/00 A01H1/06 C12N15/82 G01N33/00 G01N33/50
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JASKIEWICZ MICHAL ET AL: "Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response", EMBO REPORTS, vol. 12, no. 1, January 2011 (2011-01), pages 50-55, XP002715478, cited in the application	1
A	page 52 - page 54	2-15
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 September 2014	16/09/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Brero, Alessandro

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NOUTOSHI YOSHITERU ET AL: "Novel Plant Immune-Priming Compounds Identified via High-Throughput Chemical Screening Target Salicylic Acid Glucosyltransferases in Arabidopsis", PLANT CELL, vol. 24, no. 9, September 2012 (2012-09), pages 3795-3804, XP002715479, ISSN: 1040-4651 the whole document	1-15
A	Jaskiewicz, Michal Rafal: "Mechanismen der Genregulation bei der geprimten Stressantwort in Arabidopsis thaliana", RWTH Aachen Hoschschulbibliothek , 7 June 2013 (2013-06-07), XP002715480, Retrieved from the Internet: URL:http://darwin.bth.rwth-aachen.de/opus3/volltexte/2013/4500/ [retrieved on 2013-10-25] the whole document	1-15
A	UWE CONRATH: "Molecular aspects of defence priming", TRENDS IN PLANT SCIENCE, vol. 16, no. 10, 1 October 2011 (2011-10-01), pages 524-531, XP028304088, ISSN: 1360-1385, DOI: 10.1016/J.TPLANTS.2011.06.004 [retrieved on 2011-06-18] cited in the application abstract page 526 ff. figure 2	1-15
A	CHEN PO-WEN ET AL: "Priming of the Arabidopsis pattern-triggered immunity response upon infection by necrotrophic Pectobacterium carotovorum bacteria", MOLECULAR PLANT PATHOLOGY, vol. 14, no. 1, 4 January 2013 (2013-01-04), pages 58-70, XP055065250, ISSN: 1464-6722, DOI: 10.1111/j.1364-3703.2012.00827.x the whole document	1-15

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CONRATH UWE: "Priming of Induced Plant Defense Responses", ADVANCES IN BOTANICAL RESEARCH, LONDON, GB, 1 January 2009 (2009-01-01), pages 361-395, XP009173759, ISSN: 0065-2296, DOI: 10.1016/S0065-2296(09)51009-9 the whole document -----	1-15