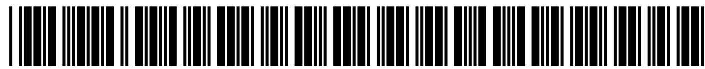

US 20100115668A1

(19) **United States**
(12) **Patent Application Publication**
Coego González et al.
(10) **Pub. No.: US 2010/0115668 A1**
(43) **Pub. Date: May 6, 2010**

(54) **OCP3 GENE OF ARABIDOPSIS THALIANA AND THE OCP3 RECESSIVE MUTATION THEREOF, AND THE USE OF SAME AS A RESISTANCE REGULATOR IN PLANTS WITH DISEASE CAUSED BY NECROTROPHIC FUNGAL PATHOGENS**

(75) Inventors: **Alberto Coego González**, Valencia (ES); **Pablo Vera Vera**, Valencia (ES); **Vicente Ramirez García**, Valencia (ES); **María José Gil Morrio**, Valencia (ES)

Correspondence Address:
SUGHRUE MION, PLLC
2100 PENNSYLVANIA AVENUE, N.W., SUITE 800
WASHINGTON, DC 20037 (US)

(73) Assignee: **CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS**, Madrid (ES)

(21) Appl. No.: **11/919,007**

(22) PCT Filed: **Apr. 20, 2006**

(86) PCT No.: **PCT/ES2006/070050**

§ 371 (c)(1),
(2), (4) Date: **Dec. 28, 2009**

(30) **Foreign Application Priority Data**

Apr. 22, 2005 (ES) P200501035

Publication Classification

(51) **Int. Cl.**
A01H 11/00 (2006.01)
C07H 21/04 (2006.01)
C07H 21/00 (2006.01)

(52) **U.S. Cl.** **800/295**; 536/23.2

(57) **ABSTRACT**

The invention relates to the technical field of plant biotechnology and, more specifically, to the OCP3 gene of *Arabidopsis* and the ocp3 mutation of same, as well as to the use thereof in the regulation of resistance to diseases caused by necrotrophic pathogens and to the applications of same in the generation of transgenic plants that are resistant to said type of pathogens.

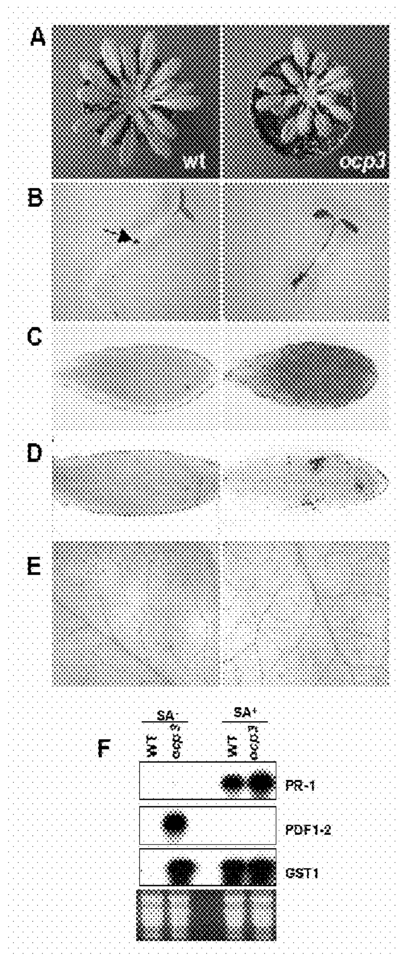
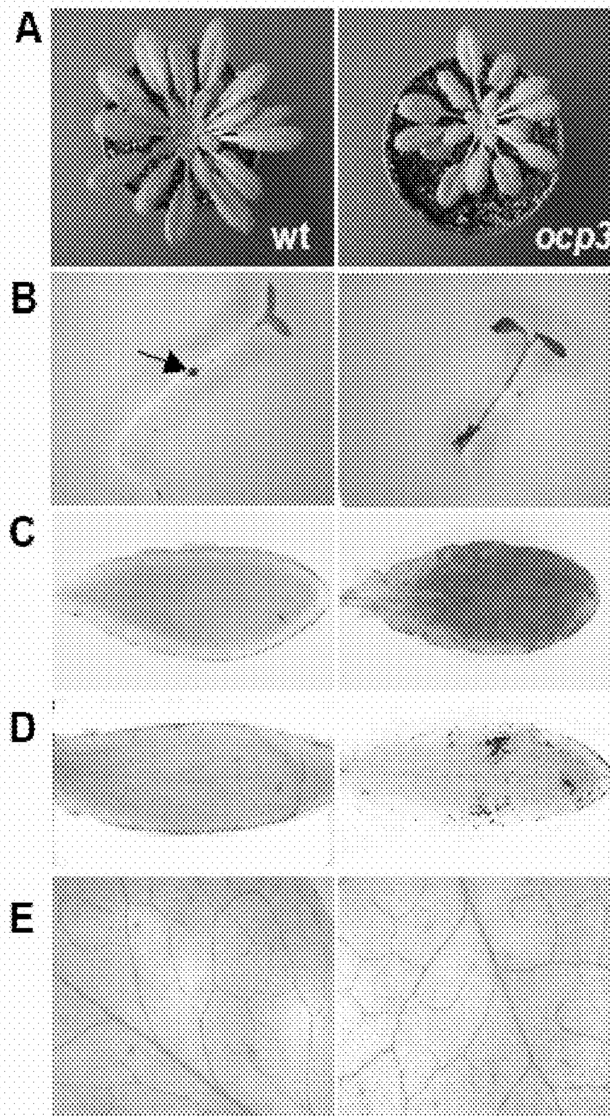


FIG. 1



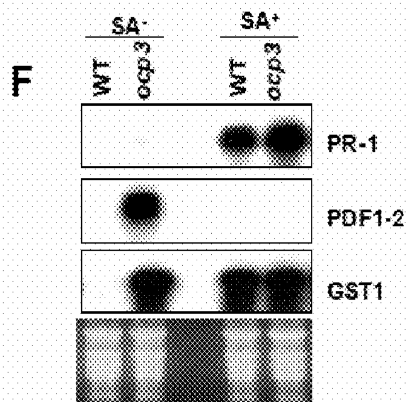


FIG. 2

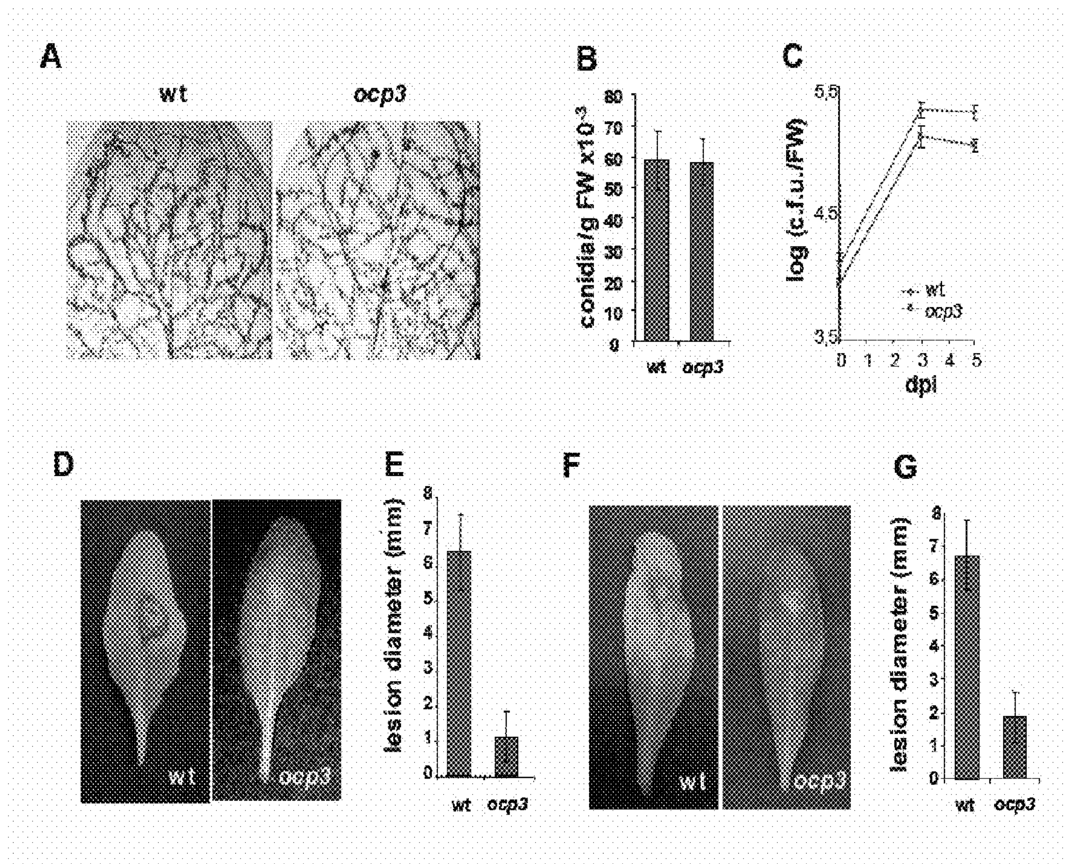


FIG. 3

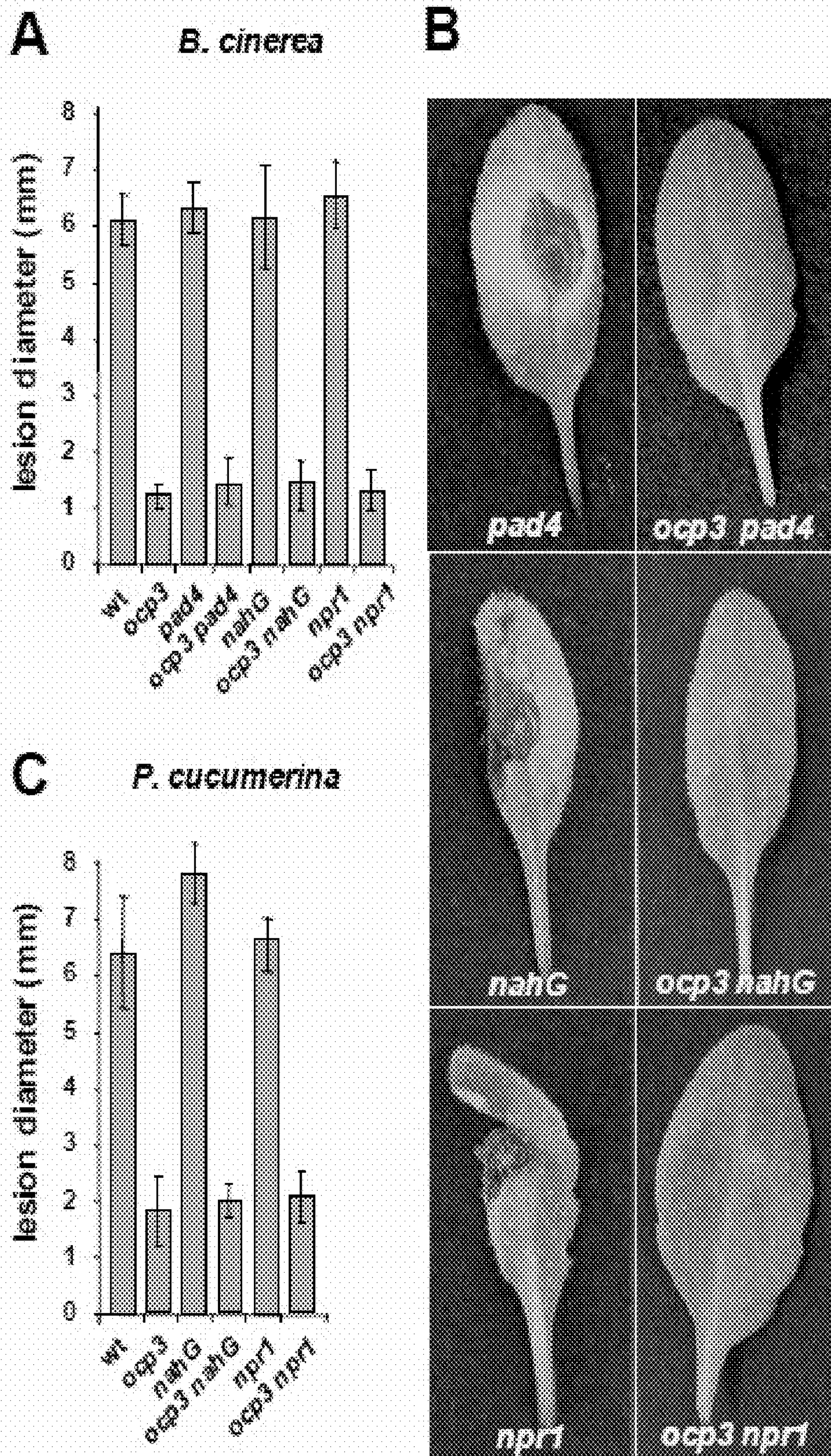


FIG. 4

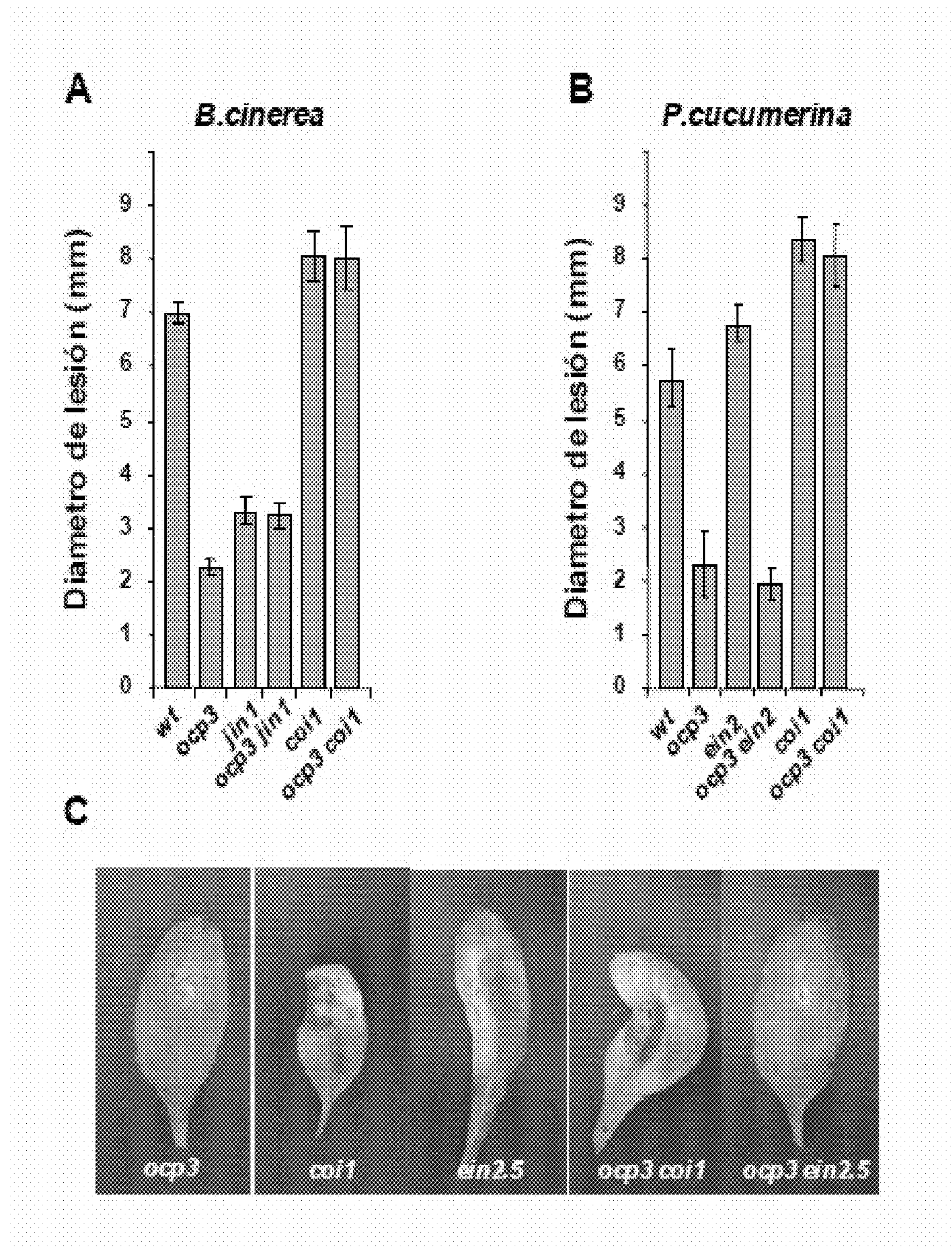


FIG. 5

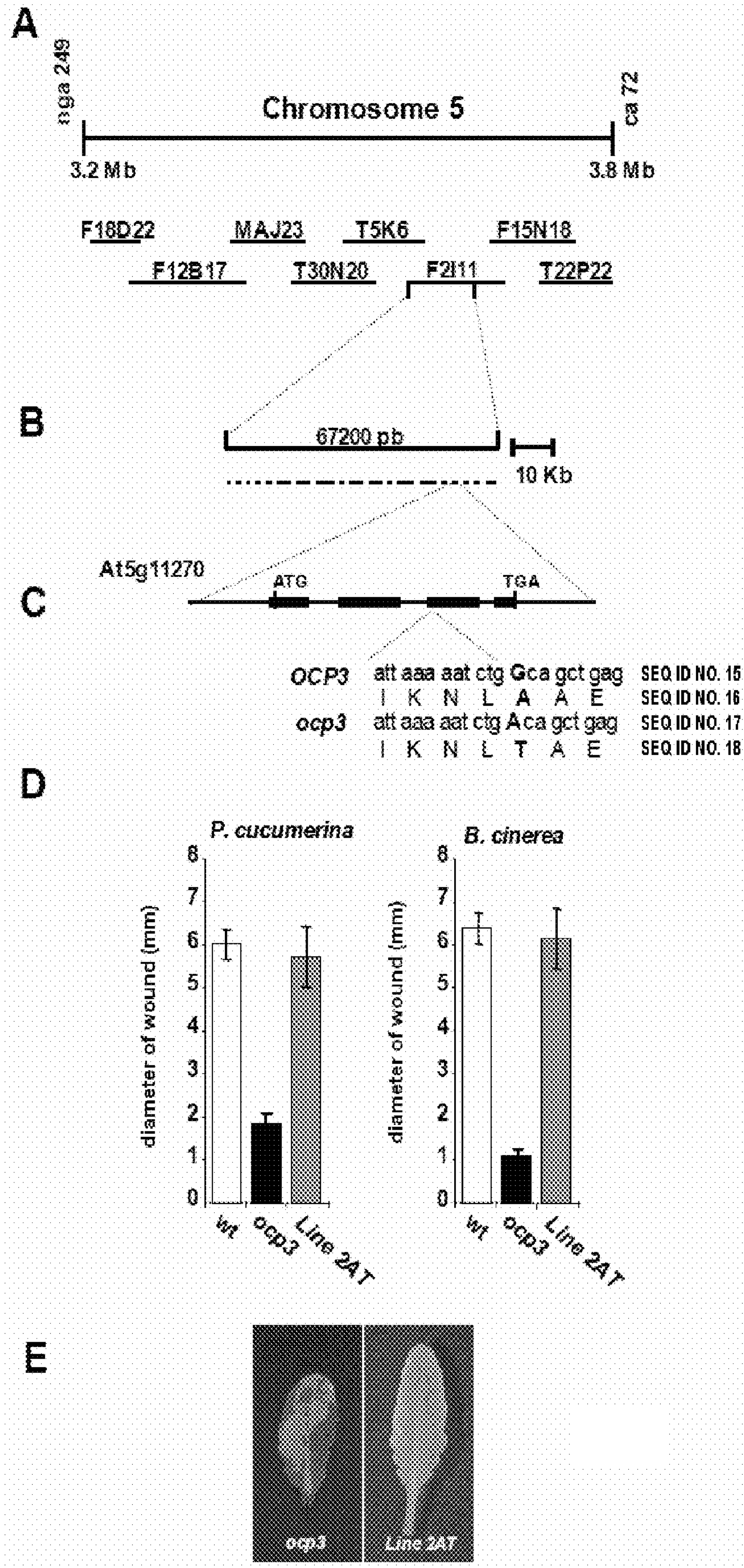


FIG. 6

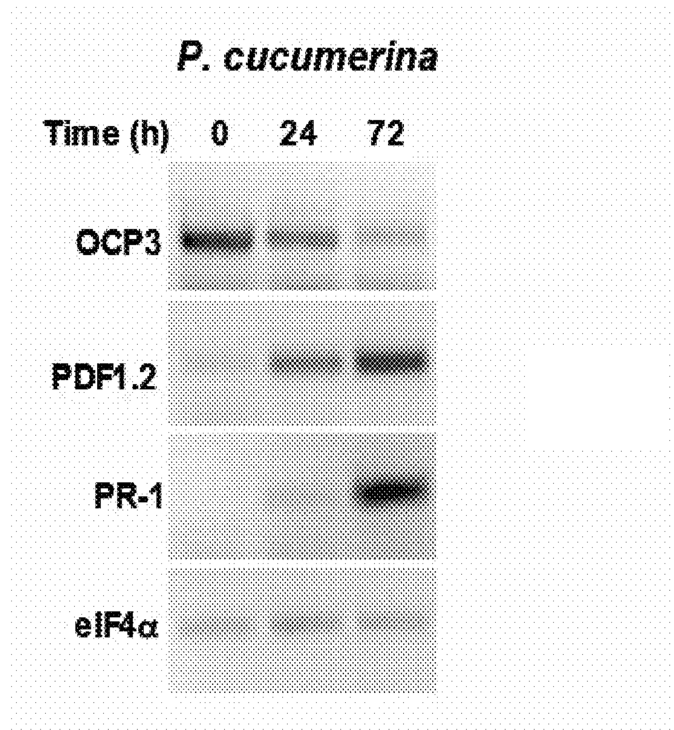


FIG. 7

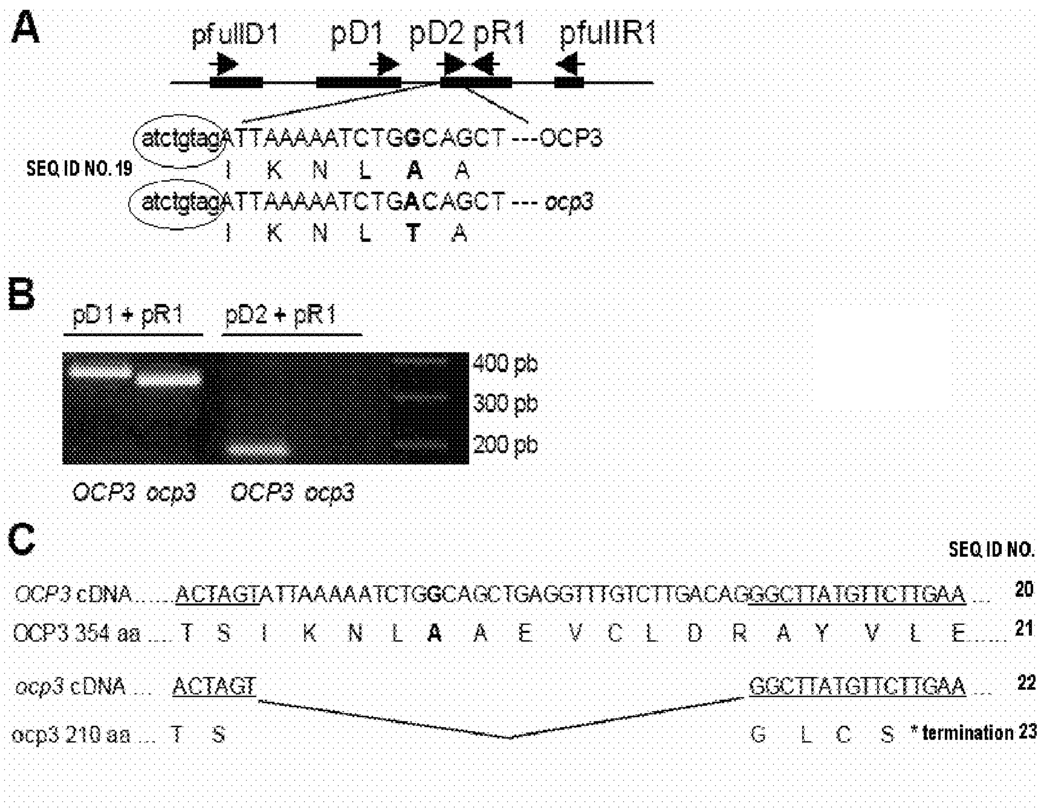
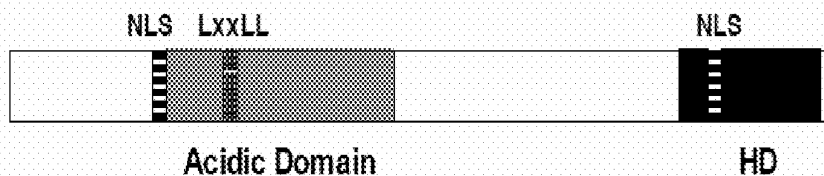


FIG. 8

A

```
1    MIKAMALSSAGVVSHLHPPSFSSSSGLSVNRVLFRNRNASPCGLSLPILNPSRSVLVFAR
61   GKNRKGFVSSSSSSPKKNKKKSLDGADNGGEEEEDPFEALFNLLEEDLKNDNSDDEEIS
121  EEELEALADELARALGVGDDVDDIDLFGSVTGDVDVDVDNDDDDNDDDINDDDDDDSEED
181  ERPTKLKNWQLKRLAYALKAGRRKTSIKNLAAEVCLDRAYVLELLRDPPPKLLMLSATLP
241  DEKPPVAAPENSSPDPSPVESLSAEDVVVEPKEKVKDEAVHVNQQRWSAQKRVKKAHIET
301  LEKVYRRSKRPTNAVVSSIVQVTNLPRKRVLKWFEDKRAEDGVPDKRAPYQAPV SEQ ID NO 24
```

B



C

HELIX 1 HELIX 2 HELIX 3/4

Name	SEQ ID
OCP3	SEQ ID NO. 25
ATKNAT3	SEQ ID NO. 26
ATKNAT4	SEQ ID NO. 27
ATKNAT2	SEQ ID NO. 28
ATBEL1	SEQ ID NO. 29
ATHB-1	SEQ ID NO. 30
ATHB-10	SEQ ID NO. 31
ATANTHOC	SEQ ID NO. 32
ATHB-2	SEQ ID NO. 33
ATHAT1	SEQ ID NO. 34
ATHB-5	SEQ ID NO. 35
ATHB-6	SEQ ID NO. 36
ATHB-3	SEQ ID NO. 37
ATHB-7	SEQ ID NO. 38
ATHB12	SEQ ID NO. 39
ATHAT3	SEQ ID NO. 40
ATHAT4	SEQ ID NO. 41
ATPRH	SEQ ID NO. 42
ATLUMININ	SEQ ID NO. 43
ATWUSHEL	SEQ ID NO. 44

OCP3 GENE OF ARABIDOPSIS THALIANA AND THE OCP3 RECESSIVE MUTATION THEREOF, AND THE USE OF SAME AS A RESISTANCE REGULATOR IN PLANTS WITH DISEASE CAUSED BY NECROTROPHIC FUNGAL PATHOGENS

[0001] The present invention relates to the technical field of plant biotechnology and specifically to the OCP3 gene of *Arabidopsis* and ocp3 mutation thereof, as well as to the use thereof in the regulation of resistance to diseases caused by necrotrophic pathogens, and to the applications of same in the generation of transgenic plants resistant to this type of pathogens.

STATE OF THE ART

[0002] Plants react to phytopathogenic microorganism attacks with a series of inducible responses leading to the local and systemic expression of a broad spectrum of antimicrobial defenses. These defenses include the strengthening of mechanical barriers, oxidative burst, de novo production of antimicrobial compounds and the induction of the hypersensitive response (HR) mechanism in which the tissue surrounding the site of infection dies and in turn limits the growth of the pathogen, preventing it from spreading (Hammond-Kosack and Parker, (2003) Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. Curr. Op. Biotechnol. 14, 177-193)

[0003] Our understanding of how plants activate defense responses has substantially been developed, and this has been aided in part by cloning and characterization of factors of resistance to plant diseases recognizing the corresponding avirulent factors of the pathogen to induce the hypersensitive response or HR (Dangl and Jones, (2001) Plant pathogens and integrated defense responses to infection. Nature 411, 826-33)

[0004] The induction of the hypersensitive response is often associated with the development of systemic acquired resistance (SAR), another well-studied defense response providing long-lasting protection in the entire plant against a broad spectrum of pathogens (Durrant and Dong, (2004) Systemic acquired resistance. Ann. Rev. Phytopathol. 42, 185-209)

[0005] The isolation and analysis of mutants with altered defense responses (Durrant and Dong, 2004; Kunkel and Brooks, (2002) Cross talk between signaling pathways in pathogen defense. Curr. Op. Plant Biol. 5, 325-331) are aiding in the characterization of cellular components involved in signal transduction and in understanding the role of plant defense signal molecules. These studies are of vital importance for understanding the coupling of pathogen recognition to the activation of defense responses in the plant. Salicylic acid (SA), a benzoic acid derivative, is a central signal molecule central mediating different aspects of HR and SAR responses. Some time ago it was demonstrated that the synthesis and accumulation of salicylic acid is essential for creating an effective defense response against bacterial pathogens and oomycetes (Gaffney, T, Friedrich, L., Vernooij, B., Negretto, D., Nye, G, Uknes, S., Ward, E., Kessmann, H., and Ryals, J. (1993). Requirement of salicylic acid for induction of systemic acquired resistance. Science 261, 754-756). And the signaling thereof is mediated for the most part by an ankyrin repeat protein, NPR1/NIM1 (Cao, H., Glazebrook, J., Clarke, J. D., Volko, S., and Dong, X. (1997). The *Arabidopsis* NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88, 57-63) however, independent NPR1 pathways have been proposed and genetically identified for channeling salicylic acid signaling (Clarke, J. D., Liu, Y., Klessig, D. F., and Dong, X. (1998). Uncoupling PR gene expression from NPR1 and bacterial resistance: Characterization of the dominant *Arabidopsis* cpr6-1 mutant. Plant Cell 10, 557-569; Clarke, J. D., Volko, S. M., Ledford, H., Ausubel, F. M., and Dong, X (2000). Roles of Salicylic Acid, Jasmonic Acid, and Ethylene in cpr-Induced Resistance in *Arabidopsis*. Plant Cell 12, 2175-2190; Mayda, E., Mauch-Mani, B., and Vera, P. (2000). The *Arabidopsis* dth9 mutant is compromised in systemic acquired resistance without affecting SA-dependent responses. Plant Cell 12, 2119-2128; Shah, J., Kachroo, P., and Klessig, D. F. (1999). The *Arabidopsis* ss1 mutation restores pathogenesis-related gene expression in npr1 plants and renders defensin gene expression salicylic acid dependent. Plant Cell 11, 191-206)

[0006] In addition to salicylic acid, it has been demonstrated that other signaling molecules such as jasmonic acid (JA) and ethylene (ET), alone or in coordinated combination, regulate other aspects of the defense responses of plants (Kunkel and Brooks, 2002; Turner, J. G., Ellis, C, and Devoto, A. (2002). The jasmonate signal pathway. Plant Cell (suppl), S153-S164), and genetic indicia of this involvement in the response to fungal pathogens have also been provided. For example, certain *Arabidopsis* mutants that cannot produce jasmonic acid (for example, a triple fad3 fad7 fad8 mutant), or which cannot perceive this hormone (for example, coil, jin1 or jar1/jin4) had altered susceptibility to different necrotrophic pathogens {Kunkel and Brooks, 2002; Lorenzo O., Chico, J. M., Sánchez-Serrano, J. J., Solano, R. (2004). JASMONATE-INSENSITIVE1 Encodes a MYC Transcription Factor Essential to Discriminate between Different Jasmonate-Regulated Defense Responses in *Arabidopsis*. Plant Cell 16, 1938-1950; Staswick, P. E., Yuen, G. Y., and Lehman, C. C. (1998). Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. Plant J. 15, 747-54; Thomma, B. P. H. J., Eggermont, K. Penninckx, I. A. M. A., Mauch-Mani, B., Vogelsang, R., Cammue, B. P. A. and Broekaert, W. F. (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. USA. 95, 15107-11; Thomma, B. P., Penninckx, I. A., Broekaert, W. F., and Cammue, B. P. (2001). The complexity of disease signaling in *Arabidopsis*. Curr Opin Immunol. 13, 63-68; Vijayan P, Shockey J, Levesque C A, Cook R J., Browse J. (1998). A role for jasmonate in pathogen defense of *Arabidopsis*. Proc. Natl. Acad. Sci. USA. 95, 7209-7214)

[0007] Furthermore, a mutual antagonistic relationship between the signaling pathways by salicylic acid and jasmonic acid during the resistance response to diseases has been described (Kunkel and Brooks, 2002). To that respect, *Arabidopsis* mutants deficient in the accumulation of salicylic acid (SA) (for example, pad4 and eds1) or with an altered response to salicylic acid (for example, npr1) present a better induction of genes responding to jasmonic acid (JA) (Penninckx, I. A., Eggermont, K., Terras, F. R., Thomma, B. P., De Samblanx, G. W., Buchala, A., Metraux, J. P., Manners, J. M., Broekaert, W. F. (1996). Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a

salicylic acid-independent pathway. Plant Cell 8, 2309-23; Clarke et al., 1998; Gupta, V., Willits, M. G., and Glazebrook, J. (2000). *Arabidopsis thaliana* EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: evidence for inhibition of jasmonic acid signaling by SA. Mol. Plant. Microbe Interact. 13, 503-511)

[0008] It has been considered that the normal suppression of the response genes to JA by SA is regulated by the different cellular location of the NPR1 protein (Spoel, S. H., Koornneef, A., Claessens. S. M. C., Korzelius, J. P., van Pelt, J. A., Mueller, M. J., Buchala, A. J., Metraux, J., Brown, R., Kazan, K., Van Loon, L. C., Dong, X., and Pieterse, C. M. J. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel use in the cytosol. Plant Cell 15; 760-770)

[0009] Similarly, certain genetic studies provide indications that signaling with JA can also negatively control the expression of genes responding to SA in *Arabidopsis* (Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H. B., Lacy, M., Austin, M. J., Parker, J. E., Sharma, S. B., Klessig, D. F., Martienssen, R., Mattsson, O., Jensen, A. B., and Mundy J. (2000). *Arabidopsis* MAP Kinase 4 Negatively Regulates Systemic Acquired Resistance. Cell 103, 1111-1120; Kloek et al, 2001; Li, J, Brader, G., and Palva, E. T. (2004). The WRKY70 Transcription Factor: A Node of Convergence for Jasmonate-Mediated and Salicylate-Mediated Signals in Plant Defense. Plant Cell 16, 319-333)

[0010] The molecular mechanism explaining such pathway replica is not yet well understood. Therefore, the characterization of molecular components finally coordinating the signaling pathways by SA and JA is essential for understanding, and finally for creating by means of genetic engineering, very regulated resistance mechanisms providing effective protection to specific pathogen sub-series. In addition to the previously mentioned signal molecules, the production and accumulation of reactive oxygen species (ROS), mainly superoxide (O_2) and hydrogen peroxide (H_2O_2), during the course of a plant-pathogen interaction has been recognized for quite some time (Apostol, L, Heinstein, F. H., Low. P. S. (1989). Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Role in defense and signal transduction. Plant Physiol. 90, 109-116; Baker, CJ, and Orlandi, E. W. (1995). Active oxygen species in plant pathogenesis. Annu. Rev. Phytopathol. 33, 299-321)

[0011] Indicia suggest that the oxidative burst and the subsequently associated related redox signaling may have an important central role in the integration of a diverse series of defense responses of plants (Alvarez M E, Pennell R I, Meijer P J, Ishikawa A, Dixon R A, Lamb C (1998). Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92, 773-784; Grant, J. J., and Loake, G. J. (2000). Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol, 124, 21-29). Furthermore, a replica between ROS- and SA-dependent defense responses has also been documented in plants (Kauss, H., Jeblick, W. (1995). Pretreatment of Parsley Suspension Cultures with Salicylic Acid Enhances Spontaneous and Elicited Production of H_2O_2. Plant Physiol. 108. 1171-1178; Mur, L. A., Brown, I. R., Darby, R. M., Bestwick, C. S., Bi, Y. M., Mansfield, J. W., Draper, J. (2000). A loss of resistance to avirulent bacterial pathogens in tobacco is associated with the attenuation of a salicylic acid-potentiated oxidative burst. Plant J. 23, 609-621; Shirasu, K., Nakajima, K, Rajasekhar, V. K., Dixon, R. A., and Lamb, C. (1997). Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. Plant Cell. 9, 261-70; Tierens, K. F., Thomma, B. P., Bari, R. P., Garmier, M., Eggermont, K., Brouwer, M., Penninckx, I. A., Broekaert, W. F., and Cammue, B. P. (2002). Esa1, an *Arabidopsis* mutant with enhanced susceptibility to a range of necrotrophic fungal pathogens, shows a distorted induction of defense responses by reactive oxygen generating compounds. Plant J. 29, 131-40), but the exact mechanism and the components associating redox signaling with the induced defense response is still not well understood.

[0012] The Ep5C gene of tomato plants, which encodes a cationic peroxidase, has recently been identified and has been used as a marker for early transcription-dependent responses controlled by H_2O after the perception of a pathogen, and with a conserved gene activation mode both in tomato plants and in *Arabidopsis* plants (Coego, A., Ramirez, V., Ellul, P., Mayda, E., and Vera, P. (2005). The H_2O_2-regulated Ep5C gene encodes a peroxidase required for bacterial speck susceptibility in tomato. Plant J. "in press"). Since the Ep5C pathogen-induced expression is based on the production and accumulation of H_2O_2 by the affected plant cell, Ep5C is signaled as a marker for finding new defense components finally participating in the defense-related pathways in plants. For this purpose, the present invention describes the isolation and characterization of the ocp3 mutant of *Arabidopsis thaliana* desregulated in the expression of the previously identified H_2O_2-inducible Ep5C gene. It is demonstrated that OCP3 encodes a homeobox-type transcription factor regulating different aspects of the defense response. By means of the analysis of ocp3 mutant plants and the analysis of epistasis with other defense-related mutants, it is proposed that OCP3 controls critical aspects of the JA-mediated pathway in necrotrophic pathogens.

DESCRIPTION OF THE INVENTION

Isolation and Characterization of the ocp3 Mutant of *Arabidopsis*

[0013] As discussed above, the Ep5C gene encodes an extracellular cationic peroxidase and is transcriptionally activated by the H_2O_2 generated during the course of plant-pathogen interactions (Coego et al., 2005). To identify signals and mechanisms involved in the induction of the Ep5C gene and testing the effect that this pathway can have on the resistance to diseases, a search was conducted for mutants using transgenic plants of *Arabidopsis* having an Ep5C-GUS gene construct (the GUS gene encodes the β-Glucuronidase enzyme). The logical basis of this research was that searching for mutants which showed constitutive expression of the indicator gene in plants cultured in non-inductive conditions, mutations which affect the regulation of this signaling pathway would be identified. Therefore, one of the transgenic lines of *Arabidopsis* Ep5C-GUS, previously characterized with ethyl methanesulfonate (EMS) was mutated and M2 plants (second generation of a mutant induced in this case by chemical or physical agents) were used to determine the existence of any constitutive expresser of GUS in the absence of any pathogenic threat. Out of approximately 10,000 M2 plants investigated, 18 constitutive expressers of GUS were identified and were left to produce seeds. The GUS activity was assayed again in the progeny of all these supposed

mutants to confirm if the phenotype was inheritable. Eight lines corresponding to six complementation groups maintained the GUS constitutive activity in subsequent generations. These were called ocp (overexpression of cationic peroxidase gene promoter) mutants and the mutant selected for additional analysis was ocp3 (FIG. **1**). Macroscopically, the ocp3 plants were not very different from the wild-type plants in terms of the architecture of the plant and in terms of the growth habitat (FIG. **1**A). However, in early stages of plant development, the ocp3 plants showed a delayed growth rate compared to wild-type plants. This delayed growth rate is also accompanied by the presence of a less intense green color in young leaves. Histochemical staining was carried out to investigate the expression model of the constitutive indicator gene in the ocp3 mutants compared to non-mutated wild-type parent plants. As shown in FIG. **1**B, GUS activity was not detected in the parent seedlings except in a discrete area in the connection between the root and the stem (see the arrow of the left panel of FIG. **1**B). In contrast, GUS activity was detected in the ocp3 seedlings in the expanding leaves as well as in the cotyledons and in the stem, but very little activity was detected in the roots. In the same way, in rosette leaves of the ocp3 plants, GUS activity was distributed throughout the entire upper side of the leaf, whereas the leaves of the parent plants did not show detectable GUS expression detectable (FIG. **1**C).

[0014] As it was proposed that H_2O_2 was the signal molecule that triggered the activation of Ep5C transcription after perception of the pathogen (Coego et al., 2005), it was hypothesized that the accumulation of H_2O_2 increases in ocp3 plants or, as an alternative, the ocp3 mutant must be hypersensitive to ROS. To analyze if ocp3 plants showed any phenotype related thereto, the sensitivity to H_2O_2 or to reagents directly or indirectly generating H_2O_2 was studied. ocp3 seeds and seeds of the parent line were left to germinate in MS (Murashige and Skoog) medium which contained different amounts of H_2O_2 and the growth was recorded at different time intervals. No significant differences were found in the inhibition of growth for ocp3 with regard to the parent seedlings. Similarly, the inhibition of growth was similar in ocp3 and wild-type seedlings when it was assayed with light in the presence of ROS generating molecules, with Bengal rose (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein) or paraquat (methyl viologen). According to these assays, the ocp3 mutation therefore does not confer increased sensitivity or greater resistance to oxidative stress. However, Northern blot analysis with mRNA of wild-type plants and ocp3 plants showed (FIG. **1**F; lanes on the right-hand side) that the mutant seedlings constitutively expressed GST6, a gene which, as previously demonstrated, was controlled by H_2O_2 (Alvarez et al., 1998; Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79, 583-93). This reflects that ocp3 plants can be producing and/or accumulating higher levels of H_2O_2 than those that are normally found in the wild-type plants. To assay this, leaves were stained in situ with 3,3'-diaminobenzidine (DAB), a histochemical reagent which polymerizes in the presence of H_2O_2 to produce a visible reddish-brown precipitate (Thordal-Christensen, H., Zhang, Z., Wei, Y., and Collinge, D. B. (1997). Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. Plant J. 11, 1187-1194). Very little staining with DAB could be observed in the wild-type plant leaves (FIG. **1**D, left-hand side). In contrast, the ocp3 plant leaves showed different staining foci with DAB spread out along the entire upper side of the leaf (FIG. **1**D, right-hand side). Furthermore, the ocp3 plants did not show any sign of cell death or cellular collapse, as was later shown with Trypan blue staining (FIG. **1**E) and showed no differences compared to the wild-type when the production of superoxide anions (O_2^-) was assayed by nitro blue tetrazolium staining.

[0015] The greatest observed accumulation of H_2O_2 and the induction of GST6 in ocp3 plants further suggest that the mutation produces an oxidative stress-related signal, but it does not induce a cell death response. This is consistent with the prior observation in which when H_2O_2 is generated during plant-pathogen interaction or when it is generated in situ by infiltration with different systems of generating H_2O_2, it is the signal that triggers the activation of Ep5C-GUS transcription, typical in transgenic *Arabidopsis* plants (Coego et al., 2005). Therefore, both H_2O_2 generation and the activation of the signaling mechanism leading to the activation of Ep5C transcription occur in the ocp3 mutant.

The ocp3 Mutant has Greater Resistance to Necrotrophic Pathogens, but not to Biotrophic Pathogens.

[0016] To study a causal relationship between the signaling pathway mediating the activation of Ep5C-GUS in ocp3, and the one mediating susceptibility to diseases, the response of this mutant to different pathogens generating diseases in *Arabidopsis* was assayed. FIG. **2** shows the response of ocp3 plants to the obliged virulent biotrophic oomycete *Peronospora parasitica* and its comparison to the response of wild-type parent plants. The growth of the pathogen was assayed by direct observation by stained hyphae in infected leaves (FIG. **2**A) and by the count of spores produced in the infected leaves (FIG. **2**B). Using these two measurements, there was no significant difference in the growth of pathogens between the wild-type plants and the ocp3 plants. Sporulation occurred in 50% of the leaves of both wild-type plants and ocp3 plants. Therefore, the ocp3 mutation does not affect the susceptibility of the plant to colonization by *P. parasitica*.

[0017] Changes in the susceptibility of ocp3 plants to pathogens were additionally studied using the virulent bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 (Pst DC3000) and controlling the growth rate of this bacteria in the inoculated leaves. The resulting growth curves are shown in FIG. **2**C. As occurs with *P. parasitica*, the growth rate of Pst DC3000 in ocp3 plants was not significantly different from the growth rate observed in wild-type plants. Therefore, the susceptibility of the wild-type plant and of the ocp3 mutant also continues virtually intact after local inoculation with this pathogen.

[0018] To determine if the ocp3 mutation could cause changes in susceptibility to necrotrophic pathogens, plants were inoculated with *Botrytis cinerea*. The disease as evaluated between 5 and 10 days after the inoculation by tracking the degree of necrosis and death that occurred in the inoculated leaves. As was to be expected, the wild-type plants were very susceptible to *Botrytis*, and all the inoculated plants showed necrosis accompanied by extensive proliferation of the fungal mycelium (FIG. **2**F-G). However, and in considerable contrast, none of the ocp3 plants which were inoculated with the same fungi showed extended necrosis in the inoculated leaves (FIG. **2**F-G). Furthermore, the proliferation of the fungal mycelium in ocp3 plants was drastically inhibited. This indicates that the resistance to this necrotrophic

pathogen in the ocp3 mutant was spectacularly enhanced or the susceptibility was blocked.

[0019] To assay if the altered susceptibility to ocp3 diseases is specific for *Botrytis*, the plants were exposed to *Plectosphaerella cucumerina*, another necrotroph. The infection of wild-type plants with *P. cucumerina* also led to a strong degradation of the tissue of the leaf, manifested as extended wounds and chlorosis which increases in diameter as the infection progresses throughout the inoculated leaf (FIG. **2**D). In contrast, the ocp3 plants showed a high degree of resistance to this fungal pathogen, since the visible and measurable necrosis of the inoculated leaves was drastically reduced (FIG. **2**D-E), and the proliferation of the fungal mycelium was also drastically inhibited.

[0020] Based on these results, it can be concluded that the susceptibility to necrotrophic fungi is a typical feature associated with the OCP3 locus, and the mutation identified in this locus causes greater resistance to the same pathogens. This consideration is also consistent with the observation that PDF1.2, a marker gene the expression of which is inducible for the response to ET/JA defense pathway against necrotrophic fungal pathogens (Turner et al, 2002), is constitutively expressed in ocp3 plants (FIG. **1**F).

The Greater Resistance of ocp3 Plants to Necrotrophic Fungi Requires Jasmonic Acid (JA) but not Salicylic Acid (SA) or Ethylene (ET)

[0021] The constitutive expression in ocp3 plants of the GST6 gene inducible by H_2O_2 and of the PDF1.2 gene by JA, but not of the PR-1 gene inducible by SA, (FIG. **1**F), suggests an association between oxidative stress and signaling with JA, which is apparently independent of SA. In the complex scheme of interactions taking place during the resistance responses of plants, an antagonistic relationship between the SA and JA/ET pathway has been documented for some time (Kunkel and Brooks, 2002) and indicates that the constitutive activation of the pathway leading to the expression of the PDF1.2 gene in ocp3 plants could be the negation of the expression of SA-dependent genes. However, the exogenous application of SA promotes the expression of the PR-1 marker gene both in ocp3 plants and in wild-type plants (FIG. **1**F), indicating that the ocp3 plants are not affected in SA perception, and coincides with the observation that resistance response to biotrophic pathogens is also intact in this mutant (FIG. **2** A-C). The exogenous application of SA furthermore annuls the constitutive expression of PDF1.2 taking place in ocp3 plants (FIG. **1**F). This antagonistic effect of SA was specific for the expression of PDF1.2, since the expression of GST6 was not repressed in ocp3 after the treatment with SA. Instead, SA promoted the activation of GST1 in wild-type plants (FIG. **1**F). This last observation reinforces the association existing between SA and ROS as previously documented by other authors (Mur et al, 2000; Shirasu et al, 1997; Tierens et al, 2002), but it also indicates that the oxidative stress mediating the expression of GST1 in ocp3 plants and the joint expression of PDF1.2 could be independent of SA.

[0022] To more directly evaluate if SA could contribute to the ocp3 plant phenotype in relation to the observed resistance to necrotrophic pathogens, the nahG transgene is crossed in ocp3. nahG encodes a salicylate hydroxylase blocking the SA pathway by means of SA degradation (Delaney, T. P., Uknes, S., Vernooij, S., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gutrella, M., Kessmann, H., Ward, E., and Ryals, J. (1994). A central role of salicylic acid in plant disease resistance. Science, 266, 1247-1250).

[0023] The ocp3 nahG plants retained the resistance to infections by *B. cinerea* (FIG. **3**A-B) and *P. cucumerina* (FIG. **3**C) at levels similar to those of the ocp3 plants. Similarly, when pad4 plants, which are also affected in the accumulation of SA after the attack by pathogens (Zhou, N, Tootle, T. L., Tsui, F., Klessig, D. F., and Glazebrook, J. (1998). PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. Plant Cell 10, 1021-1030), were subjected to introgression in ocp3 plants, the resulting ocp3 pad4 plants remained as resistant to *B. cinerea* (FIG. **3**A-B) or to *P. cucumerina* (FIG. **3**C) as the ocp3 plants.

[0024] A double ocp3 npr1-1 mutant was created to additionally broaden these studies. The npr1-1 mutant was originally identified by its insensitivity to SA and is currently considered the main regulator of SA-mediated responses (Durrant and Dong, 2004). As is observed for ocp3 nahG and ocp3 pad4 plants, the resistance of ocp3 npr1-1 plants to necrotrophic fungi also remained unchanged with regard to that observed in ocp3 plants (FIG. **3**A-C). All these results therefore indicate that it seems that SA is not required to improve the resistance to necrotrophic pathogens that can be attributed to the ocp3 mutation.

[0025] The importance of JA in the contribution to the ocp3 plant phenotype was evaluated as an alternative to SA. It was assayed if a defect in the perception of this hormone may affect the better observed resistance of ocp3 plants to necrotrophic fungi. The coil mutant of *Arabidopsis* is completely insensitive to JA and the COI1 protein is required for all JA-dependent responses identified up until now. COI1 encodes an F-box protein involved in the ubiquitin-mediated degradation of the signaling by JA by means of the formation of functional E3-type ubiquitin ligase complexes (Xie, D. X., Feys, B. F., James, S., Nieto-Rostro, M., and Turner, J. G. (1998). COI1: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science 280, 1091-1094; Devoto, A., Nieto-Rostro, M., Xie, D., Ellis, C, Harmston, R., Patrick, E., Davis, J., Sharratt, L., Coleman, M., and Turner, J. G. (2002). COI1 links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in *Arabidopsis*. Plant J. 32, 457-466). Furthermore, the coil plants cannot express PDF1.2 and show greater sensitivity to necrotrophic fungi (Thomma et al., 1998; Turner et al. 2002). All this indicates the importance of JA in the resistance of plants to this type of pathogens and justifies the introgression of coil in ocp3 to generate double ocp3 coil mutant plants (FIG. **4**). The greater resistance observed in ocp3 plants to *B. cinerea* and *P. cucumerina* is significantly annulled when the coil mutation is present (FIG. **4** A-C). The ocp3 coil plants behave like coil plants which are very affected after infection with any fungus, the necrotic wounds extending throughout the inoculated leaves as shown in FIG. **4**C for the response to *P. cucumerina*. The study of jin1, another mutant insensitive to JA was considered in addition to coil (Berger, S., Bell, E., and Mullet, J. E. (1996). Two Methyl Jasmonate-Insensitive Mutants Show Altered Expression of AtVsp in Response to Methyl Jasmonate and Wounding. Plant Physiol. 111, 525-531) in relation to the ocp3 mutant. JIN1 is a bHLHzip-type MYC-type transcription factor which functions dependently on COI1 (Lorenzo et al., 2004). Unlike that which occurs with coil and despite the defect in signaling with JA, the jin1 plants show greater resistance to necrotrophic pathogens, indicating that JIN1 can function as a repressor of the resistance to this type of pathogens. Curiously enough, double ocp3 jin1 mutant plants remained very resistant when they were assayed

against infection by *B. cinerea* (FIG. **4**A) and at levels comparable to those obtained by jin1 or ocp3 plants. It must be mentioned that the ocp3 mutation does not confer sensitivity to JA (according to the assay for inhibiting the growth of roots in the presence of JA) nor is it allelic to jin1. This indicates that there may be certain redundancy or overlapping of functions for the two mutants under consideration to improve the resistance to *B. cinerea* that is finally induced by JA and controlled by COI1.

[0026] It has also been demonstrated that ethylene (ET) mediates certain aspects of the responses of the plants to pathogens (Berrocal-Lobo, M., Molina, A., and Solano, R. (2002). Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant J. 29, 23-32; Thomma et al., 2001). However, signaling with ET can also function independently of JA, or it can even inhibit JA-dependent responses (Ellis, C. and Turner, J. G. (2001). The *Arabidopsis* mutant cev1 has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell, 13, 1025-1033; Thomma et al, 2001). ocp3 plants were crossed with the ein2 mutant insensitive to ET to assay the importance of ET in the ocp3 mutation-mediated resistance response (Alonso, J. M., Hirayama, T., Roman, G., Nourizadeh, S. and Ecker, J. R. (1999) EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science, 284, 2148-2152) to generate the double ocp3 ein2 mutant. As can be seen in FIG. **2**B-C, the resistance of ocp3 ein2 plants to *P. cucumerina* remained unchanged compared to the resistance observed in ocp3 plants (FIG. **4**A-C), thus indicating that the ET plant hormone is essential for the observed ocp3-mediated resistance.

Isolation of OCP3

[0027] Wild-type ocp3/ocp3 plants and OCP3/OCP3 plants that contained the Ep5C-GUS transgene were backcrossed and the progeny was analyzed to determine the nature of the mutation. The constitutive expression of GUS activity in the 21 assayed seedlings was absent in the F1 plants resulting from this crossing, and the expression was present in 31 of 118 seedlings in the F2 plants. The F2 segregation ratio of the phenotype conferred by ocp3 was 1:3 (constitutive expressers:non-expressers, α^2=1.48 (0.1>P>0.5)), indicating a single recessive mutation. The ocp3 mutant was subjected to backcrossing with wild-type *Landsberg erecta* (Ler) to generate an F2 mapping population and the recombinant seedlings were identified by means of the use of single sequence length polymorphism (SSLP) markers (Bell, C J., and Ecker, J. R. (1994). Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19, 137-144). DNA was initially isolated from 38 homozygotic ocp3 plants and the segregation of the SSLP markers indicated that ocp3 showed an association to the Nga249 marker in chromosome 5, in which all of the 76 alleles analyzed were Col-0 alleles (0 erecta: 76 Col-0). Another analysis of the ocp3 plants screened with additional markers available for chromosome 5 identified the SSLP, Nga249 and ca72 markers as the closest markers that flanked the ocp3 mutation on each side (FIG. **5**A). The screening of 1100 plants randomly chosen from an F2 Ler×ocp3 mapping population with the SSLP, Nga249 and ca72 markers identified 29 plants which had a recombination in the interval. By using these 29 recombinant plants it could be seen that OCP3 was located at 4 cM from Nga249 and at 1.9 cM from ca72. Other polymorphic markers were designed for the region comprised between Nga249 and ca72 and the position of OCP3 narrowed to a genome region which included the end of the bacterial artificial clone (BAC) T5 KB and the start of the BAC clone F2I11. 19 genes are present in the mentioned sequence comprised within these two BAC clones (FIG. **5**B). The entire coding region of each of these genes in ocp3 plants was amplified and the PCR product sequences were determined. The sequence corresponding to the At5g11270 gene was identified as the only sequence that showed a single nucleotide substitution (G-to-A in the coding chain (exon 3)) that caused a single amino acid substitution (from Ala to Thr) (FIG. **5**C). No mutation was found in the remaining eighteen genes. At5g11270 contains two introns and encodes a protein with 553 amino acids.

[0028] A 3.2 Kb fragment containing At5g11270 in ocp3 was introduced by the *Agrobacterium*-mediated transformation to unequivocally assign At5g11270 as OCP3. Three transgenic lines were assayed with regard to the constitutive expression of GUS and with regard to the resistance to *B. cinerea* and *P. cucumerina*. In all these lines, the constitutive expression of GUS was annulled and normal susceptibility to the fungal pathogens had been recovered, demonstrating that At5g11270 is OCP3 (FIG. **5**D-E summarizes the result of this complementation for one of the transgenic lines generated, line 2AT).

The Expression of OCP3 is Partially Repressed by Fungal Infection

[0029] The expression of OCP3 in response to infection with a necrotrophic fungal pathogen in wild-type plants at different time intervals after the infection was analyzed. Levels of OCP3 mRNA could not be detected by Northern blot analysis in any analyzed tissue, indicating that the OCP3 gene is transcribed at a very low rate. To solve this problem, the presence of OCP3 mRNA was studied by RT-PCR. These analyses showed that OCP3 is constitutively expressed in healthy plant leaf tissue. FIG. **6** demonstrates that after infection with *P. cucumerina* there is a reduction in the level of accumulation of OCP3 mRNA, being more evident 72 hours after infection. Together with this reduction, and inversely correlated, the marker gene inducible by JA and by PDF1.2 fungi is positively regulated after infection with *P. cucumerina*. An induced expression of the defense-related PR1 gene takes place in post-infection steps and is indicative of the deterioration of tissues which occurs as a result of the growth habitat of the fungus.

[0030] The negative regulation of OCP3 after fungal infection, its inverse correlation with the induced expression of PDF1.2 and the recessive nature of the ocp3 mutation favors the interpretation that OCP3 functions as a repressor of the resistance response to fungal pathogens in wild-type plants.

Aberrant Processing of At5g11270 mRNA in the ocp3 Mutant

[0031] A 1.2 Kb fragment was amplified by reverse transcription-mediated polymerase chain reaction (RT-PCR) from wild-type and ocp3 mutant plants to identify the structure of the OCP3 gene and its ocp3 mutant allele, using primers designed according to the mentioned sequence of the At5g11270 gene. Direct sequencing and comparison of the RT-PCR products showed that the cDNA derived from ocp3 has an internal deletion of 36 nucleotides instead of the expected substitution of a single nucleotide, identified in the genome sequences (FIG. **7**). This deletion corresponds to the first 36 nucleotides of exon III. The G-to-A transition identi-

fied at the genome level in the encoding chain of the ocp3 allele (FIG. **5**C) thus causes an alteration in the normal processing for the mRNA derived from ocp3. This short deletion causes a phase shift in the open reading frame of ocp3 resulting in the generation of an in-phase termination codon causing a truncated protein with 210 amino acid residues instead of the 354 residues of the wild-type protein (see below, FIG. **8**). This deletion was additionally confirmed in different ocp3 plants by RT-PCR using a series of internal primers designed from the genome sequence (FIG. **7**A). Products having expected lengths were obtained in all the reactions except when the internal primer in the deleted sequence was used, which did not result in any RT-PCR product in samples derived from ocp3 plants (FIG. **7**B). The lack of use genetically attributed to the ocp3 recessive mutation therefore is not due to a change of amino acid due to the single substitution of nucleotides observed in the genome sequence; instead it is based on a abnormal processing of the mRNA transcribed from the mutated ocp3 version which, after translation, produces a truncated protein lacking 144 amino acid residues of the C-terminal part (FIGS. **7** and **8**).

OCP3 Encodes a Homeobox Transcription Factor

[0032] DNA sequencing demonstrated that OCP3 cDNA encodes a protein having 354 amino acid residues (FIG. **8**A) of 39111 D and a pI of 4.53. OCP3 contains different detectable characteristics. A 60 amino acid domain (position 284 to 344) resembling that of a homeodomain (HD) encoded by homeobox genes of different organisms is identified close to the C-terminal end (Gehring, W. J., Affolter, M. and Bürglin, T. (1994). Homeodomain proteins. Annu Rev Biochem, 63, 487-526). The homeodomain of OCP3 shares the majority of the very conserved amino acids forming the typical signature of the 60 amino acid HD module. The conservation of these critical residues (for example, L-16, Y-20 instead of F-20, I/L-34, I/L/M-40, W-48, F-49 and R-53) is easily identified in comparison with different proteins containing homeodomains of *Arabidopsis* which belong to different subgroups of proteins (FIG. **8**C). The inspection of amino acid sequence of OCP3 also showed the presence of two canonic bipartite nuclear localization signals (NLS) (Dingwall, C, and Laskey, R. A. (1991). Nuclear targeting sequences-a consensus? Trends Biochem. Sci. 16, 478-481; Nigg, E. A. (1997). Nucleocytoplasmic transport: signals, mechanism and regulation. Nature 386: 779-787), RK-$(X)_{10}$-KKNKKK in positions 64-81, and KK-$(X)_{10}$-RRSKR in positions 294-310, the latter being hidden within the homeodomain (FIG. **8**A). These characteristics could be mediating a direction of the protein to the nucleus. Another detectable characteristic of OCP3 is the presence of an extended region rich in acid residues (positions 84-181), a common characteristic of several transcription activators (Cress, W. D., and S. J. Triezenberg. 1991. Critical structural elements of the VP16 transcriptional activation domain. Science 251: 87-90). The last identifiable characteristic within OCP3 is the presence of the canonic LxxLL motif in positions 101-105 (FIG. **8**A). This motif is a typical sequence aiding the interaction of different transcription co-activators with nuclear receptors, and it is thus a defining characteristic identified in several nuclear proteins (Heery, D. M., E, Kalkhoven, S. Hoare, and M. G. Parker. 1997. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387: 733-736). All these structural motifs strongly indicate that OCP3 is a nuclear protein involved in the regulation of transcription in *Arabidopsis*. According to a general classification scheme for homeobox genes (http://www.homeobox.cjb.net/) OCP3 is unique since it is different from the main classes of proteins containing homeodomains found in plants, including KNOX or HD-ZIP. OCP3 is furthermore present as a gene of a single copy in the genome of *Arabidopsis*. The searches for sequences in databases showed an extensive identity of OCP3 with six other proteins—tomato protein (GenBank accession number AW223899, 48.9% identity), potato protein (GenBank accession number BQ112211, 48.3% identity), grape protein (GenBank accession number CD003732, 51.5% identity), rice protein (GenBank accession number AY224485, 49.5% identity), wheat protein (GenBAnk accession number CK205563, 49.4% identity) and corn protein (GenBAnk accession number BG840814, 51.3% identity)—which, as was seen, had a high degree of sequence similarity with OCP3 and with the conservation of the main structural motifs previously shown. This indicates that the use of this type of transcription regulator has been well conserved in plants throughout evolution.

[0033] The data set forth in the present invention provides evidence of a role of OCP3 in the regulation of resistance to necrotrophic pathogen microorganisms. A recessive mutation in the OCP3 gene resulted in a greater resistance of ocp3 plants to the fungal pathogens *Botrytis cinerea* and *Plectosphaerella cucumerina*, whereas the resistance to the infection by the oomycete *Peronospora* parasitica or the bacterium *Pseudomonas syringae* DC3000 remained invariable in the same plants. Interestingly enough, the OCP3 gene was expressed in very low levels in healthy plants and this constitutive expression is partially repressed during the infection with a fungal necrotroph. The resistance phenotype conferred by the ocp3 mutation is furthermore blocked when the assay is carried out in the coil mutant as a base organism, the double ocp3 coil mutant plants retaining the greater sensitivity to the necrotrophs that can be attributed to coil. This means that OCP3 participates in the defense response regulated by JA. In fact, the ocp3 recessive mutation confers constitutive expression of the PDF1.2 gene, encoding a defense protein with a defined role in the JA-mediated defense response of the plants (Thomma et al., 1998). Since the expression of PDF1.2 is completely dependent on COI1 (Turner et al., 2002) and is in healthy mutant ocp3 plants, the consideration that OCP3 is functioning in a COI1-dependant manner, acting as a negative regulator of the JA-mediated defense response to necrotrophic pathogens, is reinforced. Another important feature of ocp3 is the greater accumulation of H_2O_2 which is observed to occur in rest conditions in the leaves of ocp3 plants, followed by H_2O_2-inducible marker gene GST1 (Levine et al., 1994; Alvarez et al., 1998) but without symptoms indicating cell death. H_2O_2 and other ROI molecules are normally produced in high levels during the infection by both biotrophic pathogens and necrotrophic pathogens and have been involved as regulating signals for the baseline resistance response to these pathogens (Mengiste, T, Chen, X., Salmerón, J. M., and Dietrich, R. A. (2003). The BOS1 gene encodes an R2R3MYB transcription factor protein that is required for biotic and abiotic stress responses in *Arabidopsis*. Plant Cell 15, 2551-2565; Tiedemann, A. V. (1997). Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. Physiol. Mol. Plant. Pathol. 50, 151-166) However, out of the pathogens assayed in ocp3 plants, an increase of the resistance was only observed towards necrotrophic patho-

gens, whereas the resistance to biotrophic pathogens remained intact. This significant difference indicates that the ocp3 mutation can affect specific functions related to ROI by means of the regulation of certain effector molecules aimed at the sensitization and identification of a necrotroph. Interestingly enough, it has been shown that SA and H_2O_2 form a circuit which is a feedback loop during the course of a plant-pathogen interaction (Shirasu et al., 1997; Draper, J. (1997). Salicylate, superoxide synthesis and cell suicide in plant defense. Trends Plant Sci. 2, 162-165), and there are indications suggesting that SA can be necessary for a local response local to a necrotroph such as *Botrytis* at the infection point (Govrin, E. M., and Levine, A. (2000). The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Curr. Biol. 10, 751-757; Ferrari, S., Plotnikova, J. M., De Lorenzo, G., and Ausubel, F M. (2003). *Arabidopsis* local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. Plant J. 35, 193-205).

[0034] However, the synthesis and accumulation of SA is neither increased nor repressed in ocp3 plants. Furthermore, the analysis of double mutant plants for ocp3 and key regulators of the accumulation and perception of SA, such as the double mutants ocp3 pad4, ocp3 nahG or ocp3 npr1 generated in the present invention, indicate that SA is not required for ocp3-mediated resistance to necrotrophs. Furthermore, the plant hormone ethylene (ET) does not seem to be necessary for achieving the greatest resistance of ocp3. The lack of perception of this hormone, as studied with the double mutant ocp3 ein2 plants, does not here repress or reduce the characteristics resistance of the ocp3 plants to *P. cucumerina*. Since JA and ET can function in association or independently for the activation of specific signaling pathways (Thomma et al., 2001, Ellis and Turner, 2001), the independent resistance of ET of ocp3 indicates that OCP3 regulates a specific branch of the JA pathway. This branch further seems to be independent of another branch which has been proposed to be controlled by the transcription factor JIN1 regulated by JA (Lorenzo et al., 2004), because epistasis between ocp3 and jin1 was not found at least when the resistance to *B. cinerea* was assayed. All these observations thus confirm the important role of OCP3 in the specific regulation of a COI1-dependant resistance to necrotrophic pathogens. OCP3 is a member of the homeobox gene family. Homeobox proteins are ubiquitous in higher organisms and represent master control changes involved in development processes and in cell adaptation to changes in the medium. They function as transcription regulators which are characterized by the presence of an evolutionally conserved homeodomain (HD) responsible for the specific binding to DNA (Gehring et al., 1994). Two main classes of genes encoding HD have been identified in plants: the HD class represented by KNOTTED1 (Vollbrecht, E., Veit, S., Sinha, N. and Hake, S. (1991). The developmental gene Knotted-1 is a member of a maize homeobox gene family. Nature 350, 241-243) and the HD-Zip protein family (Schena, M., and Davis, R. W. (1992). HD-Zip proteins: members of an *Arabidopsis* homeodomain superfamily. Proc Natl Acad Sd USA, 89, 3894-3898) The latter is characterized by an additional leucine zip motif, adjacent to the HD facilitating the homo- and heterodimerization of transcription regulators. The functional characterization of some members of the homeobox family confirms a role, for some of them, as key regulators of the signaling with hormones (Himmelbach, A., Hoffmann, T., Laube, M., Hohener. B., and Grill, E. (2002). Homeodomain protein ATHB6 is a target of the ABI1 protein phosphatase and regulates hormone responses in *Arabidopsis*. EMBO J 21: 3029-3038), in adaptation responses to environmental parameters (Steindler, C., Matteucci, A., Sessa, G., Weimar, T., Ohgishi, M., Aoyama, T., Morelli, G. and Ruberti I. (1999). Shade avoidance responses are mediated by the ATHB-2 HD-Zip protein, a negative regulator of gene expression. Development, 126, 4235-4245; Zhu, J., Shi, H., Lee, B-h., Damsz, B., Cheng, S., Stirm, V., Zhu J-K., Hasegawa, P., Bressan, R. A. (2004). An *Arabidopsis* homeodomain transcription factor gene, HOS9, mediates cold tolerance through a CBF-independent pathway. Proc. Natl. Acad. Sci. USA 101, 9873-9878) and in pathogen-derived signaling processes (Mayda, E., Tornero, P., Conejero, V., and Vera, P. (1999). A tomato homeobox gene (HD-Zip) is involved in limiting the spread of programmed cell death. Plant J. 20, 591-600).

[0035] The isolated mutation identified in ocp3 results in an abnormal processing of the corresponding transcript causing an internal deletion where the first 36 nucleotides of exon III are no longer present in mature ocp3 mRNA. This short deletion causes a phase shift in the ORF of ocp3 resulting in the generation of a premature termination codon. This mutation thus produces a truncated ocp3 protein consisting of 210 amino acid residues instead of the 354 amino acid residues characteristic of OCP3. The expected 10 amino acid domain corresponding to the homeodomain (HD) is located within the 144 amino acid C-terminal domain missing in ocp3. It is conceivable that the mutated ocp3 protein no longer functions as a transcription regulator since this domain is a determining factor for the homeobox proteins to function as transcription regulators, because this is the site where contact with DNA is established, mainly through helix 3 of the HD domain, which is directed to the main groove of the DNA helix present in the downstream gene promoting region (Gehring et al. 1994). Therefore, OCP3 functions as a specific transcription factor of the COI1-dependant, JA-mediated plant cell signal translation pathway and modulates the transcription of important genes for the defense response or responses to necrotrophic pathogens.

[0036] The identification of target OCP3 genes and the interaction with molecules of associated proteins is the challenge for the future. Furthermore, the possible interaction of OCP3 with other transcription regulators involved in the defense response to necrotrophic pathogens such as the JIN1 protein related to MYC {Lorenzo et al., 2004), the AP2-type ERF1 protein (Lorenzo, O., Piqueras, R., Sánchez-Serrano, J. J., and Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15, 165-178), the BOS1 protein related to MYB (Mengiste et al., 2003) or the transcription factor WRKY70 (Li et al., 2004), and how they function in a coordinated manner is another interesting challenge for the future. All these strategies must aid in understanding the regulating mechanism of the use of OCP3 and how this use can be exploited to generate plants that are more resistant to fungal pathogens without affecting the defense responses against other types of pathogens. The genetic manipulation of the OCP3 gene by means mechanisms of reverse genetics in transgenic plants repressing or altering the gene expression of OCP3, or altering its function as a transcription factor, will allow the agronomic exploitation of the

use of OCP3 in the regulation of the defensive response of the plants against said pathogenic aggressions.

DESCRIPTION OF THE DRAWINGS

[0037] FIG. **1**. Characterization of ocp3 plants and comparison with wild-type plants.

[0038] (A) A comparison of the external appearance of ocp3 plants (at the right-hand side) and the wild-type parent plants (wt) (at the left-hand side). The plants were photographed when they were 3.5 weeks old.

[0039] (B) Histochemical staining of GUS activity directed by the Ep5C promoter in a 10-day-old wild-type transgenic seedling (at the left-hand side) and seedlings ocp3 (at the right-hand side) cultivated in MS agar medium. The arrow indicates a discrete tissue area in the connection of the hypocotyl and the root where GUS activity is observed in the wild-type seedlings.

[0040] (C) Completely expanded rosette leaves of transgenic wild-type plants (at the left-hand side) and of ocp3 plants (at the right-hand side), stained to determine the GUS activity.

[0041] (D) H_2O_2 production in wild-type plants (at the left-hand side) and ocp3 plants (at the right-hand side). H_2O_2 production was assayed using 3,3'-diaminobenzidine. The reddish-brown color indicates the polymerization of 3,3'-diaminobenzidine in the H_2O_2 production site.

[0042] (E) Staining of leaf tissue of wild-type plants (at the left-hand side) and ocp3 plants (at the right-hand side) with Trypan blue in the search for signs of cell death. The absence of cell collapse is revealed by the lack of intense blue spots after staining with Trypan blue.

[0043] (F) Expression of genes markers PR-1, PDF1.2 and GST1 in wild-type and ocp3 plants 36 hours after the plants have been sprayed with (+SA) or without (−SA) a buffer solution containing 0.3 mM salicylic acid (SA).

[0044] FIG. **2**. Resistance of ocp3 plants to necrotrophic pathogens but not to biotrophic pathogens.

[0045] (A) Resistance response of wild-type *Arabidopsis* plants and ocp3 mutants to virulent *Peronospora* parasitica. Seven days after the inoculation by spraying 2-week-old plants with 10^5 conidiospores per milliliter of *Peronospora*, the leaves were stained with lactophenol-Trypan blue and were viewed under a microscope to reveal the extensive growth characteristics of the hyphae and the conidiospores.

[0046] (B) To quantify the resistance to *P. parasitica*, conidiospore production was quantified seven days after the inoculation with the aid of a hemocytometer. The plants carrying the ocp3 mutation were as resistant to this pathogen as the wild-type plants.

[0047] (C) Growth of *Pseudomonas syringae* pv. tomato DC3000 in ocp3 and wild-type plants. A bacterial suspension was infiltrated in 4-week-old plants, and the bacterial titer, measured as c.f.u. by fresh weight, was determined 0, 3 and 5 days after the infection in wild-type plants (solid lines) and ocp3 (dotted lines) The error bars show the 95% confidence limits of the data transformed into logarithms. 8 samples were taken for each genotype at each point in time. The experiment was repeated 3 times, similar results being obtained.

[0048] (D) Representative leaves of ocp3 and wild-type plants 4 days after the inoculation with a drop of 6 μl of a spore suspension (5×10^6 spores/ml) of *P. cucumerina.*

[0049] (E) The symptoms of the disease measured as the size of the wound were evaluated 6 days after the inoculation with *P. cucumerina*, determining the mean diameters of the wound in 3 leaves of 8 plants. The data points show the mean size of the wound±SE of the measurements.

[0050] (F) Representative leaves of ocp3 and wild-type plants 4 days after the inoculation with a drop of 6 μl of spores of *Botrytis cinerea* (2.5×10^4 conidia/ml).

[0051] (G) The size of the wound generated by *Botrytis cinerea* was measured 6 days after the inoculation. The data points show the mean size of the wound±SE of the measurements for at least 30 wounds.

[0052] All the experiments were repeated at least 3 times with similar results, wt, wild-type.

[0053] FIG. **3**. Effect of mutations related to SA on the resistance response to diseases of ocp3 plants

[0054] (A) Resistance response of ocp3 nahG, ocp3 npr1 and ocp3 pad4 double mutants to *Botrytis cinerea* compared to that of simple mutant genotype and wild-type plants. The plants were inoculated and the symptoms of the disease were evaluated as described in FIG. **2**, determining the mean diameters of the in 3 leaves of 8 plants.

[0055] (B) Representative leaves of each genotype, showing symptoms of the disease observed 5 days after the inoculation with a drop of 6 μl of *Botrytis cinerea* spores (2.5×10^4 conidia/ml).

[0056] (C) Resistance response of ocp3 nahG, ocp3 npr1 and ocp3 pad4 double mutants to *Plectosphaerella cucumerina* compared to that of simple mutant genotypes and wild-type plants. The wound was measured by determining the mean diameters of the wound in 3 leaves of 8 plants. The data points show the mean size of the wound±SE of the measurements.

[0057] FIG. **4**. Effect of mutations related to JA and ET on the resistance response to diseases of ocp3 plants.

[0058] (A) Resistance response of ocp3 coil and ocp3 jin1 double mutants to *Botrytis cinerea* compared to that of simple mutant genotypes and wild-type plants. The plants were inoculated and the symptoms of the disease were evaluated as described in FIG. **2**, determining the mean diameters of the wound in 3 leaves of 8 plants.

[0059] (B) Resistance response of ocp3 coil and ocp3 jin1 double mutants to *Plectosphaerella cucumerina* compared to that of simple mutant genotypes and wild-type plants. The symptoms of the disease were evaluated by determining the mean diameters of the wound in 3 leaves of 8 plants.

[0060] (C) Representative leaves of each genotype, showing symptoms of the disease observed 7 days after the inoculation with a drop of 6 μl of *P. cucumerina* spores (5×10^6 spores/ml). The data points show the mean size of the wound±SE of the measurements.

[0061] FIG. **5**. Positional cloning of ocp3 and reverse complementation

[0062] (A) 0.6 Mb region at the upper part of chromosome 5 with overlapping BACs flanked by the SSLP markers Nga249 and ca72 used for screening recombinations in 2200 chromosomes.

[0063] (B) Location of OCP3 in the sequenced BAC clone F2111. OCP3 was placed between two SSLP markers comprising a 67.2 Kb region of BAC F2111. The mentioned 19 genes included in this region are indicated.

[0064] (C) Structure of OCP3 exons/introns. The encoding regions are indicated with thick lines. The insert shows the nucleotide change and its influence on the protein sequence. The allele mutant is indicated under the wild-type sequence. The lower case letters mark nucleotide sequences at the start of exon 3 (SEQ ID NO:17). The G-to-A transition due to the

mutagen is indicated in upper case letters in bold print (SEQ ID NO; 15). The deduced amino acid sequences are indicated in the single-letter code with upper case letters under each nucleotide triplet and the bold letters mark the amino acid changes (from Ala to Thr) in the protein sequences (SEQ ID NO:16 and 18).

[0065] (D) Resistance response of transgenic ocp3 plants (line 2AT) stably transformed with a 3.2 Kb genomic DNA sequence including the entire At5g11270 gene and comparison with the resistance response observed in the wild-type plants and in the ocp3 mutant. The plants were inoculated as described in FIG. **2** with *B. cinerea* (at the right-hand side) and *P. cucumerina* (at the left-hand side) and the symptoms of the disease were evaluated by determining the mean diameters of the wound in 3 leaves of 8 plants. The data points show the mean size of the wound±SE of the measurements.

[0066] (E) Histochemical staining of the GUS activity directed by the Ep5C promoter in completely expanded rosette leaves obtained from ocp3 plants (at the left-hand side) and from transgenic ocp3 plants transformed with the At5g11270 gene (line 2AT) (at the right-hand side).

[0067] FIG. **6**. Expression of OCP3 and marker genes of defense response after the infection with *P. cucumerina*

[0068] RT-PCR analysis of leaf tissue infected with *P. cucumerina*. Wild-type plants were inoculated by spraying with a suspension of 10^5 spores/ml, and the tissue was frozen for RNA extraction. The numbers indicate the hours after the inoculation. The gels of the lower part show the RT-PCR for the internal elF4α gene used as a load control. The experiment was repeated twice, similar results being obtained.

[0069] FIG. **7**. Analysis of OCP3 and ocp3 cDNA

[0070] (A) Diagram of the structure of OCP3 exons/introns. The exons are indicated with thick lines. The insert indicates the nucleotide sequence in the splice junction exon 3. The lower case letters mark intron sequences (SEQ ID NO:19), the upper case letters mark exon sequences (from nucleotide residues 1 to 18 of SEQ ID NO:15 and from nucleotide residues 1 to 18 of SEQ ID NO:17), the bold upper case letters indicate amino acids and the arrows indicate the corresponding substitution of nucleotides and of amino acids (from amino acid residues 1 to 6 of SEQ ID NO:16 and from amino acid residues 1 to 6 of SEQ ID NO:18). The arrows at the upper part of the schematized gene indicate the different position of the primers used in the RT-PCR experiments.

[0071] PfullD1 (SEQ ID NO:2) is located at the start of exon 1; pfullR1 (SEQ ID NO:3) is located at the end of exon 4; pD1 (SEQ ID NO:4) is located at the end of exon 2; pD2 (SEQ ID NO:6) is located at the start of exon 3 and pR1 (SEQ ID NO:5) is located in the middle of exon 3. D indicates the direct orientation (from 5' to 3') whereas R indicates the reverse orientation (from 3' to 5').

[0072] (B) Electrophoresis in agarose gel of RT-PCR products obtained when mRNA from wild-type plants (wt) and ocp3 and a different combination of primers is used. The reduction of the molecular weight and thus, the faster migration of the amplified band from ocp3 plants with pD1+pR1 primers compared to that obtained from wild-type plants should be observed. The absence of the amplified DNA product when pD2+pR1 primers and mRNA transcribed with reverse transcriptase from ocp3 plants, but no from wild-type plants, should also be observed. The absence of amplified product indicates a lack of recognition by one of the two primers in the cDNA template generated from ocp3 plants. The experiment was repeated several times with mRNA derived from 4 different ocp3 and wild-type plants.

[0073] (C) Nucleotides sequence of and amino acid sequence derived from cDNA clones derived from mRNA isolated from wild-type (OCP3) (SEQ ID NO:20 and 21) and mutant (ocp3) (SEQ ID NO:22 and 23) plants. The reversely transcribed products were amplified with the pfullD1 and pfullR1 primers and the two chains were completely sequenced. The internal deletion of 36 nucleotides in all the sequenced ocp3 cDNAs should be observed. The nucleotide sequence common to all the cDNAs derived from ocp3 and OCP3 is underlined. The internal deletion in the ocp3 cDNA affects the derived amino acid sequence and causes a phase shift generating a premature termination codon in the ocp3 protein. The bold upper case letters mark amino acids and the asterisks indicate a termination codon. The arrow indicates the presence and position of the nucleotide (G) in OCP3 cDNA, producing the ocp3 phenotype if it is mutated. The results were reproduced several times with mRNA derived from different wild-type and ocp3 plants and in different stages of growth.

[0074] FIG. **8**. Protein OCP3 and comparison with other proteins containing *Arabidopsis* homeodomains.

[0075] (A) Expected amino acid sequence of OCP3 (SEQ ID NO:24). The homeodomain is shown in bold. The two identification signals conserved for the nuclear localization are underlined. The acid domain is shown in italics and the domain interacting with the nuclear protein (LxxLL), included within this acid region, is underlined.

[0076] (B) Expected protein structure of OCP3. The relative position of the nuclear localization signals (NLS), of the domain of interaction with the nuclear protein (LxxLL), of the acid domain and of the homeodomain (HD) is indicated.

[0077] (C) Alignment of sequences showing the OCP3 C-terminal amino acid sequence with the homeodomain of different homeobox *Arabidopsis* genes including the members of the KN and HD-Zip family (SEQ ID NO:25 to 44). The asterisk above the alignments corresponds to amino acid positions in the HD which are very conserved in all organisms and define the identification signal of the homeodomain. The black shading indicates amino acids conserved in all the entries, and the gray shading indicates amino acids with very similar physical and chemical characteristics.

DETAILED EXPLANATION OF AN EMBODIMENT

Plants, Growth Conditions and Treatments

[0078] *Arabidopsis thaliana* plants were cultured in substrate or in plates containing Murashige and Skoog (MS) medium, as previously described (Mayda et al., 2000). The ocp3 mutant was isolated in an investigation of constitutive expressers of the Ep5C-GUS indicator gene in Columbia transgenic plants (Col-0) mutated with ethyl methanesulfonate (EMS), as previously described for another mutant (Mayda et al., 2000). The ocp3 mutant line used in these experiments was subjected three times to backcrossing with the wild-type parent line. The plants were cultured in a growth chamber at 20-22° C., with a relative moisture of 85%, and 100 $\mu Em^{-2} sec^{-1}$ of fluorescent illumination, in a 14 hour light and 10 hour dark cycle.

[0079] Completely expanded leaves of four week-old plants were used for all the experiments (unless otherwise indicated). Staining was performed to determine the presence

of H_2O_2 through the 3,3'-diaminobenzidine (DAB) uptake method, as previously described (Thordal-Christensen et al, 1997). Staining was performed to determine the presence of GUS activity as previously described (Mayda et al, 2000).

Infection with Pathogens

[0080] *Pseudomonas syringae* pv. tomato DC3000 (P.s. tomato DC3000) was cultured and prepared for the inoculation as previously described (Mayda et al. 2000). The density of the bacteria populations was determined by culturing serial dilutions in King's B agar medium supplemented with rifampicin (50 μg/ml) at 28° C. and by counting the colony forming units. The data was presented as means and standard deviations of the logarithm (cfu/crm^2) of at least six replicas. Three week-old plants were sprayed with a *P. parasitica* conidial suspension (10^5 conidiospores ml^{-1} of running water) as previously described (Mayda et al, 2000) for the resistance to *Peronospora parasitica* experiments. The density of the spores in the seedlings (seven pots per treatment, each pot treated separately) was evaluated on the seventh day using a hemocytometer. As an alternative, the leaf samples were stained with lactophenol-Trypan blue on different days after the inoculation and were examined under the microscope as previously described (Mayda et al, 2000). Three week-old seedlings were transplanted in individual pots and were cultured at 22° C. during the day/18° C. at night, with twelve hours of light every 24 hours for the resistance to *Plectosporium* and *Botrytis*. When the plants were six weeks old they were inoculated by applying drops of 6 ml drops of *Plectosphaerella cucumerina* (5×10^6 spores ml^{-1}) or *Botrytis cinerea* (2.5×10^4 conidium ml^{-1}) spore suspension to 3 completely expanded leaves per plant. *P. cucumerina* was isolated from naturally infected *Arabidopsis* (*Landsberg erecta* access) and was cultured in 19.5 g/l of potato-dextrose agar (Difco, Detroit) at room temperature for 2 weeks before collecting the spores and suspending them in 10 mM $MgSO_4$. *B. cinerea* (BMM1 strain isolated from Pelargonium zonale) was cultured in 19.5 g/l of potato-dextrose agar (Difco, Detroit) at 20° C. for 10 days. The conidia were collected and resuspended in sterile PDS (12 g l^{-1}, Difco). The plants were maintained with a relative moisture of 100% and the symptoms of the disease were evaluated from 4 to 10 days after the inoculation, determining the mean diameter of the wound in 3 leaves from 5 plants.

Genetic Analysis

[0081] Crosses were performed by emasculating unopened buds and using the pistils as pollen receptors. Backcrossings were performed with the parent transgenic line using Ep5C-GUS plants as pollen donors. Reciprocal crosses were also performed. F1 and F2 plants were cultured in MS plates and assayed with regard to GUS activity. The segregation of the phenotype in the F2 generation was analyzed with a chi-square test to check the goodness of fit.

PCR-Based Mapping

[0082] An ocp3 plant (in Columbia as a basis) was crossed with *Landsberg erecta* and used for mapping the progeny which segregated ocp3 homozygotic mutants after self-pollination. Seedlings of the F2 population where selected for DNA extraction, and the recombinant seedlings were identified using single sequence length polymorphism (SSLP) markers according to the protocol described by Bell and Ecker (1994) and with new markers as indicated on the *Arabidopsis* database webpage (http://genome-www.stanford.edu).

Generation of Double Mutants

[0083] The mutant alleles used throughout this invention were npr1-7 (Cao et al., 1997), pad4-1 (Zhou et al., 1998), coil-1 (Xie et al., 1998), ein2-5 (Alonso et al., 1999) and jin1-1 (Lorenzo et al., 2004). Transgenic plants (in the Columbia ecotype) which expressed the bacterial nahG gene have been described (Reuber et al. 1998). The double mutants ocp3 npr1, ocp3 pad4, ocp3 coil, ocp3 ein2, ocp3 jin1 and ocp3 nahG double mutants were generated using ocp3 as a pollen receptor. The homozygosity of the loci was confirmed using molecular markers for each of the alleles in segregation populations. All the double mutants were confirmed in the F3 generation, except the ocp3 coil plants which were sterile and could not be propagated in heterozygosity for coil. For the double mutant that contained ein2-5, the F2 seeds were cultured in plates with MS that contained 20 μM 1-aminocyclopropane-1-carboxylic (ACC) acid and were placed in a growth chamber. After three days in the dark, the seedlings were evaluated with regard to the presence or absence of the ethylene (ET)-induced triple response (Guzman and Ecker 1990). The ein2 mutant, which was insensitive to ET, did not have the triple response. F2 plants lacking the triple response were collected and transferred to the substrate to evaluate the homozygosity for ocp3.

Genomic DNA and cDNA Cloning

[0084] The genome sequence was used as a basis for the cDNA cloning and genomic clones. Poly(A*) RNA was isolated from different wild-type and ocp3 plants and were transcribed with reverse transcriptase using oligo-primers (dT) as described (Mayda et al., 1999). They were used as templates for amplifying OCP3 and ocp3 cDNA using different combinations of sense and anti-sense primers with gene specificity:

```
pfuIId1
(5'-GAATTCATGATAAAAGCCATGG-5'),      SEQ ID NO; 2

pfuIIR1
5'-GTTAACTCTAGATCTTTCCGGAG-5'),      SEQ ID NO: 3

pD1
(5'-GGTGATGTTGATGTTGATGTTG-3'),      SEQ ID NO: 4

pR1
(5'-CTTAGGTTCGACCACAACATCTTCAG-5')   SEQ ID NO: 5
and

pD2
(5'-ATCTGGCAGCTGAGGTTTGTCTTG-5').    SEQ ID NO: 6
```

Reverse Complementation

[0085] The OCP3 genomic region was amplified by PCR using primers with gene specificity designed to include the 1.5 Kb upstream region of the initiation codon and a part of region 3' after the termination codon. The sequences of the advance and reverse genomic primers of OCP3 used were:

[0086] (5'-GAGATTGGAACGTGGGTCGACTTTAG-3) SEQ ID NO:7 and

[0087] (5'-TTCCTGAATTCATACTTTATCATAG-3') SEQ ID NO:8, respectively.

[0088] A 3.2 Kb genomic fragment which contained the wild-type At5g11270 gene was obtained by PCR using primers and was cloned into pCAMBIA1300 to produce the clone pCAMBIAOCP3 which was transferred to *Agrobacterium* and used to transform ocp3 plants by the floral immersion method (Bechtold, N, Ellis, J. and Pelletier, G. (1993). In plant *Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. C. R. Acad. Sd. Paris Life Sci. 316, 1194-1199)

Expression Analysis

[0089] To analyze the level of gene expression by reverse transcriptase-mediated PCR, total RNA samples were prepared from leaf tissue using the Totally RNA kit of Ambion (Austin, Tex.). Reverse transcription was performed using the RT kit for PCR of Clontech (Palo Alto, Calif.).

[0090] The series of oligonucleotide primers (50 pmol each) used for amplifying OCP3 were: OCP3PCR1 (5'-GCTTAAAAGACTGGCTTATGCATTG-3') SEQ ID NO:9/OCP3PCR2 (5'-GCTTTGGAGCGGGTCACGAAG-3') SEQ ID NO:10.

[0091] The primers used for amplifying PDF1.2 were PDF1.2PCR1 (5'-ATGGCTAAGTTTGCTTCCAT-3') SEQ ID NO:11/PDF1.2PCR2 (5'-ACATGGGACGTAACAGATAC-3') SEQ ID NO:12.

[0092] The primers used for amplifying PR1 were PR1PCR1 (5'-ATGAATTTTACTGGCTATTC-3') SEQ ID NO:13/PR1PCR2 (5'-AACCCACATGTTCACGGCGGA-3') SEQ ID NO:14.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 44

<210> SEQ ID NO 1
<211> LENGTH: 354
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 1

Met Ile Lys Ala Met Ala Leu Ser Ser Ala Gly Val Val Ser His Leu
1   5   10   15

His Pro Pro Ser Phe Ser Ser Ser Ser Gly Leu Ser Val Asn Arg Val
            20   25   30

Leu Phe Arg Asn Arg Asn Ala Ser Pro Cys Gly Leu Ser Leu Pro Ile
        35   40   45

Leu Asn Pro Ser Arg Ser Val Leu Val Phe Ala Arg Gly Lys Asn Arg
    50   55   60

Lys Gly Phe Val Ser Ser Ser Ser Ser Ser Pro Lys Lys Asn Lys Lys
65   70   75   80

Lys Ser Leu Asp Gly Ala Asp Asn Gly Gly Gly Glu Glu Glu Glu Asp
                85   90   95

Pro Phe Glu Ala Leu Phe Asn Leu Leu Glu Glu Asp Leu Lys Asn Asp
            100   105   110

Asn Ser Asp Asp Glu Glu Ile Ser Glu Glu Glu Leu Glu Ala Leu Ala
        115   120   125

Asp Glu Leu Ala Arg Ala Leu Gly Val Gly Asp Asp Val Asp Asp Ile
    130   135   140

Asp Leu Phe Gly Ser Val Thr Gly Asp Val Asp Val Asp Val Asp Asn
145   150   155   160

Asp Asp Asp Asp Asn Asp Asp Asp Asp Asn Asp Asp Asp Asp Asp Asp
                165   170   175

Ser Glu Glu Asp Glu Arg Pro Thr Lys Leu Lys Asn Trp Gln Leu Lys
            180   185   190

Arg Leu Ala Tyr Ala Leu Lys Ala Gly Arg Arg Lys Thr Ser Ile Lys
        195   200   205

Asn Leu Ala Ala Glu Val Cys Leu Asp Arg Ala Tyr Val Leu Glu Leu
    210   215   220

Leu Arg Asp Pro Pro Pro Lys Leu Leu Met Leu Ser Ala Thr Leu Pro
225   230   235   240

-continued

```
Asp Glu Lys Pro Pro Val Ala Ala Pro Glu Asn Ser Ser Pro Asp Pro
                245                 250                 255

Ser Pro Val Glu Ser Leu Ser Ala Glu Asp Val Val Val Glu Pro Lys
            260                 265                 270

Glu Lys Val Lys Asp Glu Ala Val His Val Met Gln Gln Arg Trp Ser
        275                 280                 285

Ala Gln Lys Arg Val Lys Lys Ala His Ile Glu Thr Leu Glu Lys Val
    290                 295                 300

Tyr Arg Arg Ser Lys Arg Pro Thr Asn Ala Val Val Ser Ser Ile Val
305                 310                 315                 320

Gln Val Thr Asn Leu Pro Arg Lys Arg Val Leu Lys Trp Phe Glu Asp
                325                 330                 335

Lys Arg Ala Glu Asp Gly Val Pro Asp Lys Arg Ala Pro Tyr Gln Ala
            340                 345                 350

Pro Val


<210> SEQ ID NO 2
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer with gene Specificity pFuIId1

<400> SEQUENCE: 2

gaattcatga taaaagccat gg                                              22


<210> SEQ ID NO 3
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer with gene specificity pfuIIR1

<400> SEQUENCE: 3

gttaactcta gatctttccg gag                                             23


<210> SEQ ID NO 4
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer with gene specificity pD1

<400> SEQUENCE: 4

ggtgatgttg atgttgatgt tg                                              22


<210> SEQ ID NO 5
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primers with gene specificity pR1

<400> SEQUENCE: 5

cttaggttcg accacaacat cttcag                                          26


<210> SEQ ID NO 6
<211> LENGTH: 24
<212> TYPE: DNA
```

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer with gene specificity pD2

<400> SEQUENCE: 6

atctggcagc tgaggtttgt cttg 24

<210> SEQ ID NO 7
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: advance genomic primer of OCP3

<400> SEQUENCE: 7

gagattggaa cgtgggtcga ctttag 26

<210> SEQ ID NO 8
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: reverse genomic primer of OCP3

<400> SEQUENCE: 8

ttcctgaatt catactttat catag 25

<210> SEQ ID NO 9
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide primer OCP3PCR1

<400> SEQUENCE: 9

gcttaaaaga ctggcttatg cattg 25

<210> SEQ ID NO 10
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide primer OCP3PCR2

<400> SEQUENCE: 10

gctttggagc gggtcacgaa g 21

<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primers used for amplifying PDF1.2: PDF1.2PCR1

<400> SEQUENCE: 11

atggctaagt ttgcttccat 20

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primers used for amplifying PDF1.2: PDF1.2PCR2

-continued

<400> SEQUENCE: 12

acatgggacg taacagatac 20

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer used for amplifying PR1: PR1PCR1

<400> SEQUENCE: 13

atgaatttta ctggctattc 20

<210> SEQ ID NO 14
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer used for amplifying PR1: PR1PCR2

<400> SEQUENCE: 14

aacccacatg ttcacggcgg a 21

<210> SEQ ID NO 15
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Part of wild type sequence of OCP3 at the start
of exon 3

<400> SEQUENCE: 15

attaaaaatc tggcagctga g 21

<210> SEQ ID NO 16
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Part of wild type OCP3 protein at the start of
exon 3

<400> SEQUENCE: 16

Ile Lys Asn Leu Ala Ala Glu
1 5

<210> SEQ ID NO 17
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Part of the allele mutant sequence of OCP3 at
the start of exon 3

<400> SEQUENCE: 17

attaaaaatc tgacagctga g 21

<210> SEQ ID NO 18
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Part of Mutant OCP3 protein at the start of
exon 3

-continued

<400> SEQUENCE: 18

Ile Lys Asn Leu Thr Ala Glu
1 5

<210> SEQ ID NO 19
<211> LENGTH: 8
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sequence in the splice junction of exon 3 of
OCP3

<400> SEQUENCE: 19

atctgtag 8

<210> SEQ ID NO 20
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nucleotide sequence derived from cDNA clones
derived from mRNA isolated from wild-type OCP3

<400> SEQUENCE: 20

actagtatta aaaatctggc agctgaggtt tgtcttgaca gggcttatgt tcttgaa 57

<210> SEQ ID NO 21
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequence derived from cDNA clones
derived from mRNA isolated from wild-type OCP3

<400> SEQUENCE: 21

Thr Ser Ile Lys Asn Leu Ala Ala Glu Val Cys Leu Asp Arg Ala Tyr
1 5 10 15

Val Leu Glu

<210> SEQ ID NO 22
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nucleotide sequence derived from cDNA clones
derived from mRNA isolated from mutant ocp3

<400> SEQUENCE: 22

actagtggct tatgttcttg aa 22

<210> SEQ ID NO 23
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amino acid sequence derived from cDNA clones
derived from mRNA isolated from mutant ocp3

<400> SEQUENCE: 23

Thr Ser Gly Leu Cys Ser
1 5

-continued

```
<210> SEQ ID NO 24
<211> LENGTH: 354
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Expected amino acid sequence of OCP3

<400> SEQUENCE: 24

Met Ile Lys Ala Met Ala Leu Ser Ser Ala Gly Val Val Ser His Leu
1               5                   10                  15

His Pro Pro Ser Phe Ser Ser Ser Ser Gly Leu Ser Val Asn Arg Val
            20                  25                  30

Leu Phe Arg Asn Arg Asn Ala Ser Pro Cys Gly Leu Ser Leu Pro Ile
        35                  40                  45

Leu Asn Pro Ser Arg Ser Val Leu Val Phe Ala Arg Gly Lys Asn Arg
    50                  55                  60

Lys Gly Phe Val Ser Ser Ser Ser Ser Ser Pro Lys Lys Asn Lys Lys
65                  70                  75                  80

Lys Ser Leu Asp Gly Ala Asp Asn Gly Gly Gly Glu Glu Glu Glu Asp
                85                  90                  95

Pro Phe Glu Ala Leu Phe Asn Leu Leu Glu Glu Asp Leu Lys Asn Asp
            100                 105                 110

Asn Ser Asp Asp Glu Glu Ile Ser Glu Glu Glu Leu Glu Ala Leu Ala
        115                 120                 125

Asp Glu Leu Ala Arg Ala Leu Gly Val Gly Asp Asp Val Asp Asp Ile
    130                 135                 140

Asp Leu Phe Gly Ser Val Thr Gly Asp Val Asp Val Asp Val Asp Asn
145                 150                 155                 160

Asp Asp Asp Asp Asn Asp Asp Asp Asp Asn Asp Asp Asp Asp Asp Asp
                165                 170                 175

Ser Glu Glu Asp Glu Arg Pro Thr Lys Leu Lys Asn Trp Gln Leu Lys
            180                 185                 190

Arg Leu Ala Tyr Ala Leu Lys Ala Gly Arg Arg Lys Thr Ser Ile Lys
        195                 200                 205

Asn Leu Ala Ala Glu Val Cys Leu Asp Arg Ala Tyr Val Leu Glu Leu
    210                 215                 220

Leu Arg Asp Pro Pro Pro Lys Leu Leu Met Leu Ser Ala Thr Leu Pro
225                 230                 235                 240

Asp Glu Lys Pro Pro Val Ala Ala Pro Glu Asn Ser Ser Pro Asp Pro
                245                 250                 255

Ser Pro Val Glu Ser Leu Ser Ala Glu Asp Val Val Val Glu Pro Lys
            260                 265                 270

Glu Lys Val Lys Asp Glu Ala Val His Val Met Gln Gln Arg Trp Ser
        275                 280                 285

Ala Gln Lys Arg Val Lys Lys Ala His Ile Glu Thr Leu Glu Lys Val
    290                 295                 300

Tyr Arg Arg Ser Lys Arg Pro Thr Asn Ala Val Val Ser Ser Ile Val
305                 310                 315                 320

Gln Val Thr Asn Leu Pro Arg Lys Arg Val Leu Lys Trp Phe Glu Asp
                325                 330                 335

Lys Arg Ala Glu Asp Gly Val Pro Asp Lys Arg Ala Pro Tyr Gln Ala
            340                 345                 350

Pro Val
```

-continued

<210> SEQ ID NO 25
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: OCP3 C-terminal sequence

<400> SEQUENCE: 25

```
Arg Trp Ser Ala Gln Lys Arg Val Lys Lys Ala His Ile Glu Thr Leu
1               5                   10                  15

Glu Lys Val Tyr Arg Arg Ser Lys Arg Pro Thr Asn Ala Val Val Ser
            20                  25                  30

Ser Ile Val Gln Val Thr Asn Leu Pro Arg Lys Arg Val Leu Lys Trp
        35                  40                  45

Phe Glu Asp Lys Arg Ala Glu Asp Gly Val Pro Asp
    50                  55                  60
```

<210> SEQ ID NO 26
<211> LENGTH: 63
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: homeodomain of ATKNAT3

<400> SEQUENCE: 26

```
Arg Lys Arg Arg Ala Gly Lys Leu Pro Gly Asp Thr Thr Ser Val Leu
1               5                   10                  15

Lys Ala Trp Trp Gln Ser His Ser Lys Trp Pro Tyr Pro Thr Glu Glu
            20                  25                  30

Asp Lys Ala Arg Leu Val Gln Glu Thr Gly Leu Gln Leu Lys Gln Ile
        35                  40                  45

Asn Asn Trp Phe Ile Asn Gln Arg Lys Arg Asn Trp His Ser Asn
    50                  55                  60
```

<210> SEQ ID NO 27
<211> LENGTH: 63
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: homeodomain of ATKNAT4

<400> SEQUENCE: 27

```
Arg Lys Arg Arg Ala Gly Lys Leu Pro Gly Asp Thr Thr Ser Val Leu
1               5                   10                  15

Lys Ser Trp Trp Gln Ser His Ser Lys Trp Pro Tyr Pro Thr Glu Glu
            20                  25                  30

Asp Lys Ala Arg Leu Val Gln Glu Thr Gly Leu Gln Leu Lys Gln Ile
        35                  40                  45

Asn Asn Trp Phe Ile Asn Gln Arg Lys Arg Asn Trp His Ser Asn
    50                  55                  60
```

<210> SEQ ID NO 28
<211> LENGTH: 63
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ATKNAT2

<400> SEQUENCE: 28

-continued

```
Lys Lys Lys Lys Lys Gly Lys Leu Pro Arg Glu Ala Arg Gln Ala Leu
1               5                   10                  15

Leu Asp Trp Trp Asn Val His Asn Lys Trp Pro Tyr Pro Thr Glu Gly
            20                  25                  30

Asp Lys Ile Ala Leu Ala Glu Glu Thr Gly Leu Asp Gln Lys Gln Ile
        35                  40                  45

Asn Asn Trp Phe Ile Asn Gln Arg Lys Arg His Trp Lys Pro Ser
    50                  55                  60


<210> SEQ ID NO 29
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATBEL1

<400> SEQUENCE: 29

Arg Pro Gln Arg Gly Leu Pro Glu Arg Ala Val Thr Thr Leu Arg Ala
1               5                   10                  15

Trp Leu Phe Glu His Phe Leu His Pro Tyr Pro Ser Asp Val Asp Lys
            20                  25                  30

His Ile Leu Ala Arg Gln Thr Gly Leu Ser Arg Ser Gln Val Ser Asn
        35                  40                  45

Trp Phe Ile Asn Ala Arg Val Arg Leu Trp Lys Pro Met
    50                  55                  60


<210> SEQ ID NO 30
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHB-1

<400> SEQUENCE: 30

Lys Arg Lys Lys Tyr His Arg His Thr Thr Asp Gln Ile Arg His Met
1               5                   10                  15

Glu Ala Leu Phe Lys Glu Thr Pro His Pro Asp Glu Lys Gln Arg Gln
            20                  25                  30

Gln Leu Ser Lys Gln Leu Gly Leu Ala Pro Arg Gln Val Lys Phe Trp
        35                  40                  45

Phe Gln Asn Arg Arg Thr Gln Ile Lys Ala Ile Gln
    50                  55                  60


<210> SEQ ID NO 31
<211> LENGTH: 57
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHB-10

<400> SEQUENCE: 31

Lys Arg Lys Lys Tyr His Arg His Thr Thr Asp Gln Ile Arg His Met
1               5                   10                  15

Glu Ala Leu Phe Lys Glu Thr Pro His Pro Asp Glu Lys Gln Arg Gln
            20                  25                  30

Gln Leu Ser Lys Gln Leu Gly Leu Ala Pro Arg Gln Val Lys Phe Trp
        35                  40                  45
```

-continued

```
Phe Gln Asn Arg Arg Thr Gln Ile Lys
    50                  55


<210> SEQ ID NO 32
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATANTHOC

<400> SEQUENCE: 32

Arg Lys Lys Arg Tyr His Arg His Thr Pro Gln Gln Ile Gln Glu Leu
1               5                   10                  15

Glu Ser Met Phe Lys Glu Cys Pro His Pro Asp Glu Lys Gln Arg Leu
            20                  25                  30

Glu Leu Ser Lys Arg Leu Cys Leu Glu Thr Arg Gln Val Lys Phe Trp
        35                  40                  45

Phe Gln Asn Arg Arg Thr Gln Met Lys Thr Gln Leu
    50                  55                  60


<210> SEQ ID NO 33
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain ATHB-2

<400> SEQUENCE: 33

Asn Ser Arg Lys Lys Leu Arg Leu Ser Lys Asp Gln Ser Ala Ile Leu
1               5                   10                  15

Glu Glu Thr Phe Lys Asp His Ser Thr Leu Asn Pro Lys Gln Lys Gln
            20                  25                  30

Ala Leu Ala Lys Gln Leu Gly Leu Arg Ala Arg Gln Val Glu Val Trp
        35                  40                  45

Phe Gln Asn Arg Arg Ala Arg Thr Lys Leu Lys Gln
    50                  55                  60


<210> SEQ ID NO 34
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHAT1

<400> SEQUENCE: 34

Thr Cys Arg Lys Lys Leu Arg Leu Ser Lys Asp Gln Ser Ala Val Leu
1               5                   10                  15

Glu Asp Thr Phe Lys Glu His Asn Thr Leu Asn Pro Lys Gln Lys Leu
            20                  25                  30

Ala Leu Ala Lys Lys Leu Gly Leu Thr Ala Arg Gln Val Glu Val Trp
        35                  40                  45

Phe Gln Asn Arg Arg Ala Arg Thr Lys Leu Lys Gln
    50                  55                  60


<210> SEQ ID NO 35
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHB-5
```

-continued

```
<400> SEQUENCE: 35

Ala Ala Glu Lys Lys Arg Arg Leu Gly Val Glu Gln Val Lys Ala Leu
1               5                   10                  15

Glu Lys Asn Phe Glu Ile Asp Asn Lys Leu Glu Pro Glu Arg Lys Val
            20                  25                  30

Lys Leu Ala Gln Glu Leu Gly Leu Gln Pro Arg Gln Val Ala Ile Trp
        35                  40                  45

Phe Gln Asn Arg Arg Ala Arg Trp Lys Thr Lys Gln
    50                  55                  60


<210> SEQ ID NO 36
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHB-6

<400> SEQUENCE: 36

Leu Ser Glu Lys Lys Arg Arg Leu Ser Ile Asn Gln Val Lys Ala Leu
1               5                   10                  15

Glu Lys Asn Phe Glu Leu Glu Asn Lys Leu Glu Pro Glu Arg Lys Val
            20                  25                  30

Lys Leu Ala Gln Glu Leu Gly Leu Gln Pro Arg Gln Val Ala Val Trp
        35                  40                  45

Phe Gln Asn Arg Arg Ala Arg Trp Lys Thr Lys Gln
    50                  55                  60


<210> SEQ ID NO 37
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHB-3

<400> SEQUENCE: 37

Leu Gly Glu Lys Lys Lys Arg Leu Asn Leu Glu Gln Val Arg Ala Leu
1               5                   10                  15

Glu Lys Ser Phe Glu Leu Gly Asn Lys Leu Glu Pro Glu Arg Lys Met
            20                  25                  30

Gln Leu Ala Lys Ala Leu Gly Leu Gln Pro Arg Gln Ile Ala Ile Trp
        35                  40                  45

Phe Gln Asn Arg Arg Ala Arg Trp Lys Thr Lys Gln
    50                  55                  60


<210> SEQ ID NO 38
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHB-7

<400> SEQUENCE: 38

Asn Lys Asn Asn Gln Arg Arg Phe Ser Asp Glu Gln Ile Lys Ser Leu
1               5                   10                  15

Glu Met Met Phe Glu Ser Glu Thr Arg Leu Glu Pro Arg Lys Lys Val
            20                  25                  30

Gln Leu Ala Arg Glu Leu Gly Leu Gln Pro Arg Gln Val Ala Ile Trp
```

-continued

```
        35                  40                  45

Phe Gln Asn Lys Arg Ala Arg Trp Lys Ser Lys Gln
    50                  55                  60


<210> SEQ ID NO 39
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHB12

<400> SEQUENCE: 39

Lys Ser Asn Asn Gln Lys Arg Phe Asn Glu Glu Gln Ile Lys Ser Leu
1               5                   10                  15

Glu Leu Ile Phe Glu Ser Glu Thr Arg Leu Glu Pro Arg Lys Lys Val
            20                  25                  30

Gln Val Ala Arg Glu Leu Gly Leu Gln Pro Arg Gln Met Thr Ile Trp
        35                  40                  45

Phe Gln Asn Lys Arg Ala Arg Trp Lys Thr Lys Gln
    50                  55                  60


<210> SEQ ID NO 40
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHAT3

<400> SEQUENCE: 40

Ser Ser Ser Ser Ala Cys Lys Gln Thr Asp Pro Lys Thr Gln Arg Leu
1               5                   10                  15

Tyr Ile Ser Phe Gln Glu Asn Gln Tyr Pro Asp Lys Ala Thr Lys Glu
            20                  25                  30

Ser Leu Ala Lys Glu Leu Gln Met Thr Val Lys Gln Val Asn Asn Trp
        35                  40                  45

Phe Lys His Arg Arg Trp Ser Ile Asn Ser Lys Pro
    50                  55                  60


<210> SEQ ID NO 41
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATHAT4

<400> SEQUENCE: 41

Ser Ser Ser Ser Ala Cys Lys Gln Thr Asp Pro Lys Thr Gln Arg Leu
1               5                   10                  15

Tyr Ile Ser Phe Gln Glu Asn Gln Tyr Pro Asp Lys Ala Thr Lys Glu
            20                  25                  30

Ser Leu Ala Lys Glu Leu Gln Met Thr Val Lys Gln Val Asn Asn Trp
        35                  40                  45

Phe Lys His Arg Arg Trp Ser Ile Asn Ser Lys Pro
    50                  55                  60
```

-continued

```
<210> SEQ ID NO 42
<211> LENGTH: 59
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATPRH

<400> SEQUENCE: 42

Gly Arg Arg Arg Met Phe Arg Leu Pro Arg Asn Ala Val Glu Lys Leu
1               5                   10                  15

Arg Gln Val Phe Ala Glu Thr Glu Leu Pro Ser Lys Ala Val Arg Asp
            20                  25                  30

Arg Leu Ala Lys Glu Leu Ser Leu Asp Pro Glu Lys Val Asn Lys Trp
        35                  40                  45

Phe Lys Asn Thr Arg Tyr Met Ala Leu Arg Asn
    50                  55


<210> SEQ ID NO 43
<211> LENGTH: 59
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATLUMININ

<400> SEQUENCE: 43

Gly Lys Arg Pro Arg Asp Leu Leu Asn Pro Lys Ala Val Lys Tyr Leu
1               5                   10                  15

Gln Ala Val Phe Ala Ile Lys Asp Ala Ile Ser Lys Arg Glu Ser Arg
            20                  25                  30

Glu Ile Ser Ala Leu Phe Gly Ile Thr Val Ala Gln Val Arg Glu Phe
        35                  40                  45

Phe Val Thr Gln Lys Thr Arg Val Arg Lys Gln
    50                  55


<210> SEQ ID NO 44
<211> LENGTH: 66
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Homeodomain of ATWUSHEL

<400> SEQUENCE: 44

Gln Thr Ser Thr Arg Trp Thr Pro Thr Thr Glu Gln Ile Lys Ile Leu
1               5                   10                  15

Lys Glu Leu Tyr Tyr Asn Asn Ala Ile Arg Ser Pro Thr Ala Asp Gln
            20                  25                  30

Ile Gln Lys Ile Thr Ala Arg Leu Arg Gln Phe Gly Lys Ile Glu Gly
        35                  40                  45

Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln
    50                  55                  60

Lys Lys
65
```

1. An isolated polynucleotide encoding OCP3 having a recessive mutation, wherein said recessive mutation confers, to a mutant plant comprising said polynucleotide, resistance to necrotrophic fungal pathogens.

2. The isolated polynucleotide according to claim **1**, wherein said mutation is an ocp3 mutation.

3. The isolated polynucleotide according to claim **2**, wherein said mutation gives rise to constitutive expression of a polynucleotide encoding PDF1.2 encoding a defense protein in jasmonic acid-mediated defense response of plants.

4. The isolated polynucleotide according to claim **3**, wherein the polynucleotide encoding PDF1.2 is a marker of jasmonic acid-mediated physiological/molecular response of plants.

5. The isolated polynucleotide according to claim **2** wherein said polynucleotide encoding OCP3 is cDNA which has an internal deletion corresponding to the first 36 nucleotides of exon III.

6. The isolated polynucleotide according to claim **5**, wherein said polynucleotide encoding OCP3 produces a truncated protein of 210 amino acid residues.

7. (canceled)

8. A transgenic plant comprising a polynucleotide encoding OCP3 having a recessive mutation, wherein said recessive mutation confers, to said transgenic plant, resistance to necrotrophic fungal pathogens.

9. The isolated polynucleotide according to claim **3**, wherein said polynucleotide encoding OCP3 is cDNA which has an internal deletion corresponding to the first 36 nucleotides of exon III.

10. The isolated polynucleotide according to claim **4**, wherein said polynucleotide encoding OCP3 is cDNA which has an internal deletion corresponding to the first 36 nucleotides of exon III.

11. The isolated polynucleotide according to claim **9**, wherein said polynucleotide encoding OCP3 produces a truncated protein of 210 amino acid residues.

11. The isolated polynucleotide according to claim **9**, wherein said polynucleotide encoding OCP3 produces a truncated protein of 210 amino acid residues.

12. The isolated polynucleotide according to claim **10**, wherein said polynucleotide encoding OCP3 produces a truncated protein of 210 amino acid residues.

13. The transgenic plant according to claim **8**, wherein said polynucleotide encoding OCP3 is cDNA which has an internal deletion corresponding to the first 36 nucleotides of exon III.

14. The transgenic plant according to claim **13**, wherein said polynucleotide encoding OCP3 produces a truncated protein of 210 amino acid residues.

* * * * *