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(54) Title: DROUGHT TOLERANT PLANTS

(57) Abstract: The invention provides methods and compositions for enhancing drought tolerance in plants. Nucleic acid constructs therefore are also described. Transgenic plants are also provided that exhibit enhanced agronomic properties. The inventors have demonstrated increased drought tolerance in connection with increased expression of the Xa21 gene.



## **DROUGHT TOLERANT PLANTS**

### **CROSS REFERENCE TO RELATED APPLICATIONS**

[001] This application claims the priority of U.S. Provisional Application No. 62/335,241, filed on May 12, 2016, the entire disclosure of which is incorporated herein by  
5 reference.

### **STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT**

[002] The U.S. government has certain rights in this invention, pursuant to USDA-NIFA Grant No. 2015-67013-22910, awarded by the U.S. Department of Agriculture.

### **INCORPORATION OF SEQUENCE LISTINGS**

10 [003] The sequence listing that is contained in the file named "UFFL080WO\_ST25.txt", which is 18.5 kilobytes (as measured in Microsoft Windows®) and was created on May 11, 2017, is filed herewith by electronic submission and is incorporated by reference herein.

### **FIELD OF THE INVENTION**

15 [004] The present invention relates generally to the field of molecular biology. More specifically, the invention relates to plant genes involved in drought tolerance and methods of use thereof.

### **BACKGROUND OF THE INVENTION**

20 [005] Drought is a major constraint to crop production worldwide. The greenhouse effect is predicted to raise temperatures and to prolong droughts. Human-induced climate change is predicted to put pressure on the supply of water for agriculture. At the same time the world population is estimated to exceed 9.5 billion by the year 2050. Therefore, central to long-term agricultural security is implementing a sustainable system that is more resilient and productive, while at the same time requires less of the increasingly costly inputs such as water.

### **SUMMARY OF THE INVENTION**

25 [006] The present invention provides a method of increasing drought tolerance in a plant, comprising expressing in the plant a heterologous receptor kinase XA21 coding region, wherein the drought tolerance of the plant is increased when compared to a control plant that

lacks the expressing of the heterologous XA21 coding region. In certain embodiments the expressing comprises introducing into the plant a DNA construct comprising the heterologous receptor kinase XA21 coding region operably linked to a native receptor kinase XA21 promoter. In other embodiments the expressing comprises introducing into the plant a DNA construct comprising the heterologous receptor kinase XA21 coding region operably linked to a heterologous promoter functional in the plant. In some aspects the promoter is a constitutive or inducible promoter.

[007] In certain aspects of the present invention, the heterologous receptor kinase XA21 coding region comprises a polynucleotide sequence at least 85%, 90%, 95%, 97%, 98% 99%, or 100% identical to the rice receptor kinase XA21 coding region (SEQ ID NO:1), or a homolog thereof. In particular aspects the heterologous receptor kinase XA21 coding region comprises a polynucleotide sequence encoding an XA21 protein at least 90%, 95%, 97%, 98%, 99% or 100% identical to the rice receptor kinase XA21 protein (SEQ ID NO:2), or a homolog thereof.

[008] In some embodiments of the present invention the plant is a monocotyledonous plant, such as a monocotyledonous plant selected from the group consisting of maize, wheat, rice, sorghum (*Sorghum bicolor*), oats, barley, sugar cane, African oil palm (*Elaeis guineensis*), or switchgrass. In other embodiments of the present invention the plant is a dicotyledonous plant, such as a dicotyledonous plant selected from the group consisting of *Arabidopsis*, peanut (*Arachis hypogaea*), barrel medic (*Medicago truncatula*), carrot, soybean (*Glycine max*), cotton, Brassica, canola, tomato, potato, alfalfa, grape, clover, poplar, willow, eucalyptus, hemp, a *Lotus* sp., a *Vinca* sp., a *Nicotiana* sp., a *Vitis* sp., or a *Ricinus* sp.

[009] The present invention further provides a plant, or part thereof, expressing a heterologous receptor kinase XA21 coding region, wherein drought tolerance of the plant or part thereof is increased when compared to a control plant or part thereof that lacks the expressing of the heterologous XA21 coding region. In certain aspects the expressing comprises introducing into the plant or part thereof a DNA construct comprising the heterologous receptor kinase XA21 coding region operably linked to a native receptor kinase XA21 promoter. In other aspects the overexpressing comprises introducing into the plant or part thereof a DNA construct comprising the heterologous receptor kinase XA21 coding region operably linked to a heterologous promoter

functional in the plant or part thereof. In particular embodiments the part thereof is a cell, meristem, root, leaf, node, pistil, anther, flower, seed, embryo, stalk or petiole.

[0010] The present invention additionally provides a method of producing food for human or animal consumption, comprising obtaining a plant, or part thereof, expressing a heterologous receptor kinase XA21 coding region, wherein drought tolerance of the plant or part thereof is increased when compared to a control plant or part thereof that lacks the expressing, and preparing food for human or animal consumption from the plant or part thereof. In some aspects, the food is starch, protein, meal, flour or grain.

[0011] The present invention also provides a method of increasing drought tolerance in a rice plant, comprising introducing into the rice plant a DNA construct comprising the rice receptor kinase XA21 coding region or a heterologous receptor kinase XA21 coding region operably linked to a heterologous promoter functional in the rice plant, wherein the drought tolerance of the rice plant is increased when compared to a control rice plant that lacks the XA21 coding region or heterologous XA21 coding region.

[0012] The present invention further provides a method of producing a drought tolerant plant, comprising crossing a plant expressing a heterologous receptor kinase XA21 coding region, wherein drought tolerance of the plant is increased when compared to a control plant that lacks the expressing of the XA21 coding region, with a second plant to produce at least a first progeny plant comprising drought tolerance. In certain aspects the drought tolerant plant is a drought tolerant rice plant.

[0013] The present invention additionally provides a method of increasing drought tolerance in a plant, comprising introducing into the plant a DNA construct comprising a heterologous receptor kinase XA21 coding region operably linked to a promoter, and selecting a progeny plant that has increased drought tolerance when compared to a control plant that lacks the DNA construct. In some embodiments the promoter is a drought-inducible promoter.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

[0014] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better

understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

**[0015]** FIG. 1. A double-tagged XA21 confers resistance to *Xoo* PXO99A. FIG. 1A. Schematic representation of a double-tagged XA21. An 8,739 bp genomic fragment containing the *Xa21* coding region, its own intron (not indicated in FIG. 1A) and the native 5' and 3' regulatory sequences was used to express the gene. Domains of XA21 were as described previously (Song, *et al.*, *Science* 270:1804-1806, 1995): LRRs, leucine-rich repeats; TM, transmembrane domain; JM, juxtamembrane domain. A triple FLAG tag was inserted into the *Dra* III site, whereas a c-Myc tag was fused in frame to the C-terminus of XA21. FIG. 1B. Lesion length data of inoculated plants (n = 11 each line). The asterisk signs indicate statistically significant difference from the control A36 as calculated by Student's t test (\*\*: p<0.01).

**[0016]** FIG. 2. XA21-expressing plants display milder drought-related injuries than the control after *Xoo* infection. Growth of PXO99A in 4021-3 and A36 plants. Each data point represents mean  $\pm$  SD (n = 3 each). CFU, colony-forming unit. Blue, A36; pink, 4021-3. The asterisk signs indicate statistically significant difference from the control A36 as calculated by Student's t test (\*\*: p<0.01).

**[0017]** FIG. 3. XA21 confers tolerance to drought in rice. FIG. 3A. RWC of A36 and B7-12 plants during the course of drought treatment. # indicates the day at which A36 leaves were rolled. FIG. 3B. Survival rates of the drought-treated seedlings. FIG. 3C. Survival rates of the drought-treated seedlings. Results in FIG. 3A, FIG. 3B and FIG. 3C are means  $\pm$  s.e.m. (n = 3). Data sets with asterisks indicate statistically significant difference from A36 (\*: p<0.05; \*\*: p<0.01).

**[0018]** FIG. 4. FIG. 4A. The XA21 line B7-11 displays tolerance to drought. Survival rates of the drought-treated seedlings. Results are means  $\pm$  s.e.m. (n = 3). The asterisk signs indicate statistically significant difference from A36 as calculated using Student's t test (\*\*: p<0.01). FIG. 4B. The XA21 line 4021-3 is tolerant to drought. Survival rates of the stress-treated seedlings. FIG. 4C. The XA21 line IRBB21 is tolerant to drought. Survival rates of the drought-treated seedlings. Results in FIG. 4B and FIG. 4C are means  $\pm$  s.e.m. (n = 3). The asterisk signs indicate statistically significant difference from A36 or IR24 as calculated by Student's t test (\*: p<0.05; \*\*: p<0.01). Of note, transgenic lines containing *Xa21* are often more

resistant to *Xoo* PXO99A than the introgression line IRBB21, likely due to higher levels of transgene expression (Song, *et al.*, *Science* 270:1804-1806, 1995).

**[0019]** FIG. 5. IRBB13 seedlings display no tolerance to drought. Survival rates of the drought-treated seedlings (n = 20). Results are means  $\pm$  s.e.m. (n = 3).

5 **[0020]** FIG. 6. XA21 potentiates expression of drought-related genes in response to water stress. Four DEGs identified by RNA-seq, *OsLEA1* (FIG. 6A), *OsLEA3* (FIG. 6B), *OsLEA33* (FIG. 6C), and *OsNAC10* (FIG. 6D) were validated in A36 (blue) and B7-12 (pink) seedlings using q-PCR. FIG. 6E. Comparisons of ABA levels in A36 and the XA21-expressing line B7-12 after drought treatment. ABA contents of leaves were determined from two-week-old  
10 seedlings. Each data point represents mean of three independent biological replicates. White, A36; gray, B7-12.

**[0021]** FIG. 7. Severe drought-stress induces XA21-dependent defense against the compatible *Xoo* strain DY87031. Two-week-old A36 and B7-12 seedlings were inoculated with *Xoo* strain DY87031 and incubated with or without 13% PEG to initiate disease development.  
15 FIG. 7A. Lesion length measurements of inoculated plants (n = 15 each line) at 7 dpi. FIG. 7B. Growth of *Xoo* in inoculated plants. Data points represent mean  $\pm$  SD (n = 3 each). CFU, colony-forming unit. The asterisk signs indicate statistically significant difference from the control A36 (treated with 13% PEG) as calculated using Student's t test (\*: p<0.05; \*\*: p<0.01).

**[0022]** FIG. 8. Suppression of XA21-mediated resistance to the incompatible *Xoo* strain  
20 PXO99A by PEG or ABA. Two-week-old A36 and B7-12 seedlings were inoculated with *Xoo* PXO99A and incubated with or without 13% PEG (FIG. 8A and FIG. 8B) or ABA (FIG. 8C and FIG. 8D). Lesion lengths of diseased leaves (n=15 each line) at 10 dpi (FIG. 8A and FIG. 8C). Growth of PXO99A in PEG- (FIG. 8B) or ABA- (FIG. 8D) treated plants. The asterisk signs indicate statistically significant difference from the control B7-12 (water, FIG. 8A and FIG. 8B)  
25 or B7-12 (0  $\mu$ M ABA, FIG. 8C and FIG. 8D) as calculated using Student's t test (\*: p<0.05; \*\*: p<0.01).

**[0023]** FIG. 9. The rice *Xb3* gene acts as a drought regulator. FIG. 9A. Survival rates of the drought-treated lines. FIG. 9B. Survival rates of the indicated lines after drought stress. FIG. 9C. RWC of A36 and OXB3-70 plants during the course of drought treatment. Results in

are means  $\pm$  s.e.m. (n = 3). Data sets with asterisks indicate statistically significant difference from A36 (\*: p<0.05; \*\*: p<0.01).

[0024] FIG. 10. XA21 regulates XB3 nuclear accumulation. Structure and domain organization of XB3. The predicted XB3 domains include an N-terminal putative myristoylation motif in which the myristoylation residue G<sup>2</sup> (Gly-2) is indicated, an ankyrin domain, a RING finger (RF) motif and the C-terminal region possessing a newly identified NLS (the basic residues underlined, the number indicates position of the Lys residue in the full-length XB3). The insertion sites for eGFP and mCherry are shown.

[0025] FIG. 11. Working model for XA21-mediated immunity. At the early stage of incompatible *Xoo* infection, progressive bacterial growth in rice leaves triggers XA21-mediated defense signaling (green dashed lines), which leads to the restriction of bacterial over-proliferation and disease development. With the *Xoo* levels reaching a plateau in the xylem vessels, drought stress becomes evident, which in turn activates XA21-mediated drought signaling (red lines) that involves a release of XB3 from the XA21 complex into the nucleus, where the E3 ubiquitin ligase might degrade a substrate(s) for tolerance to drought.

#### DETAILED DESCRIPTION OF THE INVENTION

[0026] The following detailed description is provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0027] It has surprisingly been shown that transgenic plants expressing an *Xa21* gene displayed strong drought tolerance, as compared to control plants that lack the *Xa21* gene. The invention thus represents a significant advance in the art by providing methods and compositions that permit engineering of plants for drought tolerance. In this manner, agronomic performance of crop plants may be increased, particularly when plants are subject to osmotic stress at any given stage of growth. This is particularly important in avoiding crop loss and also in increasing water use efficiency. The invention thus provides methods and compositions for obtaining improvements in osmotic stress tolerance. In specific embodiments, expression cassettes comprising an *Xa21* nucleotide sequence are described operably linked to a promoter that directs expression or overexpression of the *Xa21* nucleotide sequence in the plant cell. In additional

embodiments, a plurality of XA21 transgenic plants are generated, and plants having improved drought tolerance compared to a control plant are selected.

[0028] Innate immunity plays an important role in protecting evolutionarily diverse species from pathogen infection. To perceive pathogenic invaders, hosts have evolved pattern-recognition receptors (PRRs) for detecting pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) and receptors for recognizing virulence effectors produced by pathogens for manipulating PAMP-triggered immunity and/or host cell physiology (Chisholm, *et al.*, *Cell* 124:803-814, 2006; Jones and Dangl, *Nature* 444:323-329, 2006). Plant PRRs are cell-surface proteins belonging to receptor kinase and receptor-like protein superfamilies, whereas the majority of effector-recognizing receptors are intracellular proteins possessing nucleotide-binding (NB) and leucine-rich repeat (LRR) domains (Couto and Zipfel, *Nat. Rev. Immunol.* 16:537-552, 2016; Dangl and Jones, *Nature* 411:826-833, 2001). Well-studied PRRs include Arabidopsis flagellin sensitive 2 (FLS2), elongation factor receptor (EFR) and chitin elicitor receptor kinase 1 (CREK1) that recognize bacterial flagellin, elongation factor Tu (EF-Tu) and the fungal cell wall component chitin, respectively (Gómez-Gómez and Boller, *Mol. Cell* 5:1003-1011, 2000; Zipfel, *et al.*, *Cell* 125:749-760, 2006; Miya, *et al.*, *Proc. Natl. Acad. Sci. USA* 104:19613-19618, 2007). Many NB-LRR proteins are encoded by classic disease resistance genes and NB-LRR-encoding sequences represent one of the largest gene families in plants (Meyers, *et al.*, *Plant Cell* 15:809-834, 2003; Sanseverino, *et al.*, *Nucleic Acids Res.* 38(Database issue):D814-821, 2010). Upon activation, immune receptors mobilize a defense response leading to restriction of pathogen proliferation.

[0029] The Gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the causal agent of bacterial leaf blight disease of rice (*Oryza sativa* L.). After entering leaves, *Xoo* exclusively accumulates and spreads in xylem vessels, causing phenotypes (*i.e.*, leaf rolling and wilting) similar to those seen in plants stressed by drought (Niño-Liu, *et al.*, *Mol. Plant Pathol.* 7:303-324, 2006). The product of the rice gene *Xa21* confers resistance to *Xoo* and is among the first cell-surface receptors identified in the innate immune system of plants and animals (Song, *et al.*, *Science* 270:1804-1806, 1995; Chen, *et al.*, *Mol. Plant.* 3:917-926, 2010; Park, *et al.*, *PLoS One* 5:e9262, 2010). Like FLS2 and EFR, XA21 is a LRR-receptor kinase whose intracellular domain belongs to the non-RD subclass of the receptor-like kinase/Pelle family (Song, *et al.*, *Science* 270:1804-1806, 1995; Dardick and Ronald, *PLoS Pathog.* 2:e2,

2006). Evidence has been shown to support XA21 as a PRR recognizing the *Xoo* protein 'required for activation of XA21' (RaxX) (Pruitt *et al.*, *Sci. Adv.* 1:e1500245, 2015). *Xa21*-mediated resistance is only fully expressed in adult plants (Century, *et al.*, *Plant J.* 20:231-236, 1999), however the inventors have shown that the developmentally-regulated resistance can be restored at the seedling stage by a low temperature (23-27°C) treatment. In highly resistant plants expressing XA21, incompatible *Xoo* strains (*e.g.*, PXO99A) still grow and propagate to a significant level ( $\sim 10^7$  to  $10^8$  bacterial cells/infected leaf), but they induce only shorter disease lesions and weaker water stress phenotypes than observed in susceptible individuals (FIG. 2).

**[0030]** Under normal growth conditions devoid of *Xoo*, XA21 is constitutively expressed and likely forms stable protein complexes with XA21 binding proteins (XBs) in multiple subcellular compartments. Aside from the plasma membrane, XA21 is also localized to the endoplasmic reticulum (ER) (Park, *et al.*, *PLoS One* 5:e9262, 2010). Co-immunoprecipitation experiments have detected five XBs in XA21 precipitates prepared from fully mature leaves. They are XB3, the ATPase XB24, the ER chaperone luminal-binding protein 3 (OsBiP3), XB25, and rice somatic embryogenesis receptor kinase 2 (OsSERK2) (Park, *et al.*, 2010, *supra*; Wang, *et al.*, *Plant Cell* 18:3635-3646, 2006; Chen, *et al.*, *Proc. Natl. Acad. Sci. USA* 107:8029-8034, 2010; Jiang, *et al.*, *Plant J.* 73:814-823, 2013; Chen, *et al.*, *Mol. Plant* 7:874-892, 2014).

**[0031]** The first reported XA21 binding partner XB3 possesses an N-terminal myristoylation site, eight imperfect copies of ankyrin repeats, a RING finger (RF) domain, and a C-terminal region (XB3-C) (Wang, *et al.*, 2006, *supra*). XB3 binds to the intracellular domain of XA21 through its ankyrin repeats, while the RF motif of XB3 is responsible for ubiquitin ligase activity. The *Xb3* gene is required for full XA21 accumulation and resistance. When over-expressed in *Nicotiana benthamiana* (*N. benthamiana*), XB3 and its orthologs from diverse plant species are capable of triggering rapid cell death (Huang, *et al.*, *PLoS One* 8: e63868, 2013). Despite these informative findings, the function and subcellular localization of XB3 are not fully understood.

**[0032]** The inventors have previously shown in rice that an N-terminal c-Myc epitope-tagged XA21 (Myc-XA21, ~140 kDa) is sensitive to proteolytic cleavage by an unidentified

protease(s) resulting in an N-terminal cleavage product (XA21<sup>ncp</sup>) of ~100 kDa (Xu, *et al.*, *Plant J.* 45:740-751, 2006). XA21<sup>ncp</sup> can also be detected in microsomal fractions and XA21 immunoprecipitates (Park, *et al.*, 2010, *supra*; Wang, *et al.*, 2006, *supra*; Chen, *et al.*, 2010, *supra*; Jiang, *et al.*, 2013, *supra*; Xu, *et al.*, 2006, *supra*; Park and Ronald, *Nat. Commun.* 3:920, 2012). The C-terminal portion of cleaved XA21 (XA21<sup>ccp</sup>, ~37 kDa) is detectable in the nucleus (Park and Ronald, 2012, *supra*). Kinase inactive (Myc-XA21<sup>K736E</sup>) and autophosphorylation (Myc-XA21<sup>S686A/T688A/S689A</sup>) mutants both appear to be more sensitive to cleavage, suggesting that autophosphorylation protects XA21 from degradation (Xu, *et al.*, 2006, *supra*). In addition to XA21, proteolysis has been observed from other PRRs/receptor-like kinases including the Arabidopsis CERK1 and brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1); and the symbiotic receptor kinase (SYMRK) from *Lotus japonicas* (Petutschnig, *et al.*, *New Phytol.* 204:955-967, 2014; Domínguez-Ferreras, *et al.*, *Plant Physiol.* 168:1106-1121, 2015; Antolín-Llovera, *et al.*, *Curr. Biol.* 24:422-427, 2014).

[0033] The inventors have shown that XA21 signaling has a significant role in counteracting drought, which is surprising and unexpected because an immune sensor has never before been assigned a similar function under physiological conditions.

#### NUCLEIC ACIDS, POLYPEPTIDES AND PLANT TRANSFORMATION CONSTRUCTS

[0034] Certain embodiments of the current invention concern the use of recombinant nucleic acid sequences comprising an *Xa21* coding sequence. Complements to any nucleic acid sequences described herein can also be used in certain embodiments. In addition, *Xa21* homologs (Song, *et al.*, *Plant Cell* 9:1279-1287, 1997) can also be used in some aspects of the present invention.

[0035] In further embodiments, nucleic acids and polypeptides are used that have at least about 80% (percent) sequence identity, about 85% sequence identity, about 90% sequence identity, about 91% sequence identity, about 92% sequence identity, about 93% sequence identity, about 94% sequence identity, about 95% sequence identity, about 96% sequence identity, about 97% sequence identity, about 98% sequence identity, and about 99% sequence identity to any of the nucleic acid or protein sequences described herein. As used herein, the term "percent sequence identity" or "% sequence identity" refers to the percentage of identical nucleotides or amino acids in a linear polynucleotide or polypeptide sequence of a reference

("query") sequence (or its complementary strand) as compared to a test ("subject") sequence (or its complementary strand) when the two sequences are optimally aligned (with appropriate nucleotide or amino acid insertions, deletions, or gaps totaling less than 20 percent of the reference sequence over the window of comparison). Methods to determine "percent sequence identity" are codified in numerous publicly available programs including, but are not limited to, GCG (also known as The Wisconsin Package™), and the BLAST programs that are publicly available from NCBI. Optimal alignment of sequences for aligning a comparison window are well known to those skilled in the art and may be conducted by tools including, but not limited to, the local homology algorithm of Smith and Waterman (*Adv. Appl. Math.* 2:482-489, 1981), the homology alignment algorithm of Needleman and Wunsch (*J. Mol. Biol.* 48:443-453, 1970), and the search for similarity method of Lipman and Pearson (*Science* 227:1435-1441, 1985).

**[0036]** The nucleic acids for use in the present invention may be from any source, *e.g.*, identified as naturally occurring in a plant, or synthesized, *e.g.*, by mutagenesis. In certain embodiments, the naturally occurring sequence may be from any plant. In some embodiments, the plant may be a dicotyledonous plant, for example, *Arabidopsis*, peanut (*Arachis hypogaea*), barrel medic (*Medicago truncatula*), carrot, soybean (*Glycine max*), cotton, *Brassica*, canola, tomato, potato, alfalfa, grape, clover, poplar, willow, eucalyptus, hemp, a *Lotus* sp., a *Vinca* sp., a *Nicotiana* sp., a *Vitis* sp., or a *Ricinus* sp.. In other embodiments, a plant useful for the present invention may be a monocotyledonous plant, for example maize, wheat, rice, sorghum (*Sorghum bicolor*), oats, barley, sugar cane, African oil palm (*Elaeis guineensis*), or switchgrass.

**[0037]** Coding sequences used in the present invention may be provided in a recombinant vector operably linked to a homologous or heterologous promoter functional in plants. Expression constructs may also be used comprising these sequences. In other embodiments, plants and plant cells transformed with the sequences may be provided. The construction of vectors which may be employed in conjunction with plant transformation techniques using these or other sequences according to the invention will be known to those of skill of the art in light of the present disclosure (see, for example, Sambrook, *et al.*, *Molecular Cloning: a Laboratory Manual*, Volume 3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). The techniques of the current invention are thus not limited to the use of any particular nucleic acid sequences.

[0038] The choice of any additional elements used in conjunction with the Xa21 coding sequences may depend on the purpose of the transformation. One of the major purposes of transformation of crop plants is to add commercially desirable, agronomically important traits to the plant, as described herein.

5 [0039] Vectors used for plant transformation may include, for example, plasmids, cosmids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes) or any other suitable cloning system, as well as fragments of DNA therefrom. Thus when the term "vector" or "expression vector" is used, all of the foregoing types of vectors, as well as nucleic acid sequences obtained therefrom and otherwise, are included. It is contemplated that  
10 utilization of cloning systems with large insert capacities will allow introduction of large DNA sequences comprising more than one selected gene. In accordance with the invention, this could be used to introduce genes corresponding to, *e.g.*, an entire biosynthetic pathway, into a plant.

[0040] Particularly useful for transformation are expression cassettes which have been derived from such vectors. DNA segments used for transforming plant cells will generally  
15 comprise the cDNA, gene or genes which one desires to introduce into and have expressed in the host cells. These DNA segments can further include structures such as promoters, enhancers, polylinkers, or even regulatory genes as desired. The DNA segment or gene chosen for cellular introduction will often encode a protein which will be expressed in the resultant recombinant cells resulting in a screenable or selectable trait and/or which will impart an improved phenotype  
20 to the resulting transgenic plant. Preferred components likely to be included with vectors used in the current invention are as follows.

#### A. REGULATORY ELEMENTS

[0041] In certain embodiments, exemplary promoters for expression of a nucleic acid sequence include plant promoters such as the CaMV 35S (Odell, *et al.*, *Nature* 313:810-812,  
25 1985), CaMV 19S (Lawton, *et al.*, *Plant Mol. Biol.* 9:315-324, 1987), nos (Ebert, *et al.*, *Proc. Natl. Acad. Sci. USA* 84:5745-5749, 1987), actin (Wang, *et al.*, *Mol. Cell. Biol.* 12:3399-3406, 1992), UDP glucose flavonoid glycosyl-transferase gene promoter (Ralston, *et al.*, *Genet.* 119:185-197, 1988), MPI proteinase inhibitor (Cordero, *et al.*, *Plant J.* 6141-150, 1994), and the glyceraldehyde-3-phosphate dehydrogenase (Kohler, *et al.*, *Plant Mol. Biol.* 29:1293-1298,  
30 1995; Quigley, *et al.*, *J. Mol. Evol.* 29:412-421, 1989; Martinez, *et al.*, *J. Mol. Biol.* 208:551-565,

1989) promoter, and the ubiquitin promoters from maize or rice, or ubiquitin promoters for use in various monocotyledonous plants (Christensen and Quail, *Transgenic Res.* 5:213-218, 1996).

**[0042]** Tissue-specific promoters, such as Adh (Walker, *et al.*, *Proc. Natl. Acad. Sci. USA* 84:6624-6628, 1987), sucrose synthase (Yang and Russell, *Proc. Natl. Acad. Sci. USA* 87:4144-4148, 1990),  $\alpha$ -tubulin (Kim and An, *Transgenic Research* 1:188-194, 1992), cab (Sullivan, *et al.*, *Mol. Gen. Genet.* 215:431-440, 1989), PEPCase (Hudspeth and Grula, *Plant Mol. Biol.* 12:579-589, 1989), lectin (Vodkin, *et al.*, *Cell* 34:1023, 1983; Lindstrom, *et al.*, *Dev. Genet.* 11:160, 1990), corn alcohol dehydrogenase 1 (Vogel, *et al.*, *J. Cell. Biochem.* 13:Part D, 1989; Dennis, *et al.*, *Nucl. Acids Res.* 12:3983-4000, 1984); corn light harvesting complex (Simpson, *Science* 233:34, 1986; Bansal, *et al.*, *Proc. Natl. Acad. Sci. USA* 89:3654-3658, 1992), corn heat shock protein (Rochester, *et al.*, *EMBO J.* 5:451-458, 1986), pea small subunit RuBP carboxylase (Poulsen, *et al.*, *Mol. Gen. Genet.* 205:193-200, 1986; Cashmore, *et al.*, *Gen. Eng. of Plants*, Plenum Press, New York, 29-38, 1983), Ti plasmid mannopine synthase or nopaline synthase (Langridge, *et al.*, *Proc. Natl. Acad. Sci. USA* 86:3219-3223, 1989), petunia chalcone isomerase (Van Tunen, *et al.*, *EMBO J.* 7:1257, 1988), bean glycine rich protein 1 (Keller, *et al.*, *EMBO J.* 8:1309-1314, 1989), potato patatin promoters (Wenzler, *et al.*, *Plant Mol. Biol.* 12:41-50, 1989), root cell promoters (Conkling, *et al.*, *Plant Physiol.* 93:1203-1211, 1990), maize zein (Reina, *et al.*, *Nucl. Acids Res.* 18:6426, 1990; Kriz, *et al.*, *Mol. Gen. Genet.* 207:90-98, 1987; Wandelt and Feix, *Nucl. Acids Res.* 17:2354, 1989; Langridge and Feix, *Cell* 34:1015-1022, 1983; Reina, *et al.*, *Nucl. Acids Res.* 18:6426, 1990), globulin-1 (Belanger and Kriz, *Genet.* 129:863-872, 1991), R gene complex-associated promoters (Chandler, *et al.*, *The Plant Cell* 1:1175-1183, 1989), and chalcone synthase (Franken, *et al.*, *EMBO J.* 10:2605-2612, 1991), or tissue selective promoters and tissue-specific enhancers (Fromm, *et al.*, *Nature* 312:791-793, 1986; Fromm, *et al.*, *The Plant Cell* 1:977-984, 1989) are also contemplated to be useful in certain embodiments, as are inducible promoters such as ABA- and turgor-inducible promoters, as well as drought-inducible promoters. Any suitable promoters known in the art may be used to express XA21 coding sequences in a plant. In certain embodiments of the invention a drought-inducible or osmotic stress-inducible promoter may be used to express XA21 coding sequences in a plant.

**[0043]** The DNA sequence between the transcription initiation site and the start of the coding sequence, *i.e.*, the untranslated leader sequence, can also influence gene expression. One

may thus wish to employ a particular leader sequence with a transformation construct of the invention. In certain embodiments, leader sequences are contemplated to include those which comprise sequences predicted to direct optimum expression of the attached gene, *i.e.*, to include a consensus leader sequence which may increase or maintain mRNA stability and prevent inappropriate initiation of translation. The choice of such sequences will be known to those of skill in the art in light of the present disclosure. In some embodiments, sequences that are derived from genes that are highly expressed in plants may be used for expression of XA21 coding sequences.

## B. TERMINATORS

[0044] Transformation constructs prepared in accordance with the invention may include a 3' end DNA sequence that acts as a signal to terminate transcription and allow for the polyadenylation of the mRNA produced by coding sequences operably linked to a promoter. In one embodiment of the invention, the native terminator of a XA21 coding sequence may be used. Alternatively, a heterologous 3' end may enhance the expression of XA21 coding sequences. Non-limiting examples of terminators that may be used in this context include those from the nopaline synthase gene of *Agrobacterium tumefaciens* (nos 3' end) (Bevan, *et al.*, *Nucl. Acids Res.* 11:369-385, 1983), the terminator for the T7 transcript from the octopine synthase gene of *Agrobacterium tumefaciens*, and the 3' end of the protease inhibitor I or II gene from potato or tomato. Regulatory elements such as an Adh intron (Callis, *et al.*, *Genes Dev.* 1:1183-1200, 1987), sucrose synthase intron (Vasil, *et al.*, *Plant Physiol.* 91:1575-1579, 1989) or TMV omega element (Gallie, *et al.*, *The Plant Cell* 1:301-311, 1989), may further be included in certain embodiments where desired.

## C. TRANSIT OR SIGNAL PEPTIDES

[0045] Sequences that are joined to the coding sequence of an expressed gene, which are removed post-translationally from the initial translation product and which facilitate the transport of the protein into or through intracellular or extracellular membranes, are termed transit (usually into vacuoles, vesicles, plastids and other intracellular organelles) and signal sequences (usually to the endoplasmic reticulum, Golgi apparatus and outside of the cellular membrane). By facilitating the transport of the protein into compartments inside and outside the cell, these sequences may increase the accumulation of gene products by protecting them from proteolytic

degradation. These sequences also allow for additional mRNA sequences from highly expressed genes to be attached to the coding sequence of the genes. Since mRNA being translated by ribosomes is more stable than naked mRNA, the presence of translatable mRNA in front of the gene may increase the overall stability of the mRNA transcript from the gene and thereby increase synthesis of the gene product. Since transit and signal sequences are usually post-translationally removed from the initial translation product, the use of these sequences allows for the addition of extra translated sequences that may not appear on the final polypeptide. It further is contemplated that targeting of certain proteins may be desirable in order to enhance the stability of the protein (U.S. Patent No. 5,545,818, incorporated herein by reference in its entirety).

[0046] Additionally, vectors may be constructed and employed in the intracellular targeting of a specific gene product within the cells of a transgenic plant or in directing a protein to the extracellular environment. This generally will be achieved by joining a DNA sequence encoding a transit or signal peptide sequence to the coding sequence of a particular gene. The resultant transit or signal peptide will transport the protein to a particular intracellular or extracellular destination, respectively, and will then be post-translationally removed.

#### D. MARKER GENES

[0047] By employing a selectable or screenable marker, one can provide or enhance the ability to identify transformants. "Marker genes" are genes that impart a distinct phenotype to cells expressing the marker protein and thus allow such transformed cells to be distinguished from cells that do not have the marker. Such genes may encode either a selectable or screenable marker, depending on whether the marker confers a trait which one can "select" for by chemical means, *i.e.*, through the use of a selective agent (*e.g.*, a herbicide, antibiotic, or the like), or whether it is simply a trait that one can identify through observation or testing, *i.e.*, by "screening" (*e.g.*, the green fluorescent protein). Of course, many examples of suitable marker proteins are known to the art and can be employed in the practice of the invention.

[0048] Many selectable marker coding regions are known and could be used with the present invention including, but not limited to, neo (Potrykus, *et al.*, *Mol. Gen. Genet.* 199:183-188, 1985), which provides kanamycin resistance and can be selected for using kanamycin, G418, paromomycin, *etc.*; bar, which confers bialaphos or phosphinothricin

resistance (Rathore, *et al.*, *Plant Mol. Biol.* 21:871-884, 1993); a mutant EPSP synthase protein (Hinchee, *et al.*, *Bio/Technol.* 6:915-922, 1988) conferring glyphosate resistance; a nitrilase such as bxn from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker, *et al.*, *Science* 242:419-423, 1988); a mutant acetolactate synthase (ALS) which confers resistance to imidazolinone, sulfonylurea or other ALS inhibiting chemicals (European Patent Application 154204, 1985); a methotrexate resistant DHFR (Thillet, *et al.*, *J. Biol. Chem.* 263:12500-12508, 1988), a dalapon dehalogenase that confers resistance to the herbicide dalapon (Buchanan-Wollaston, *et al.*, *Plant Cell Reports* 11:627-631, 1992); or a mutated anthranilate synthase that confers resistance to 5-methyl tryptophan (Li and Last, *Plant Physiol.* 110:51-59, 1996).

[0049] An illustrative embodiment of selectable marker capable of being used in systems to select transformants are those that encode the enzyme phosphinothricin acetyltransferase, such as the bar gene from *Streptomyces hygroscopicus* or the pat gene from *Streptomyces viridochromogenes*. The enzyme phosphinothricin acetyl transferase (PAT) inactivates the active ingredient in the herbicide bialaphos, phosphinothricin (PPT). PPT inhibits glutamine synthetase (Murakami, *et al.*, *Mol. Gen. Genet.* 205:42-50, 1986; Twell, *et al.*, *Plant Physiol.* 91:1270-1274, 1989), causing rapid accumulation of ammonia and cell death.

## GENETIC TRANSFORMATION

[0050] Additionally provided herein are transgenic plants transformed with the above-identified recombinant vectors encoding XA21, or a sequence modulating up-regulation thereof, and exhibiting tolerance to drought.

[0051] Suitable methods for transformation of plant or other cells for use with the current invention are believed to include virtually any method by which DNA can be introduced into a cell, such as by direct delivery of DNA such as by PEG-mediated transformation of protoplasts (Omirulleh, *et al.*, *Plant Mol. Biol.* 21:415-428, 1993), by desiccation/inhibition-mediated DNA uptake (Potrykus, *et al.*, *Mol. Gen. Genet.* 199:183-188, 1985), by electroporation (U.S. Patent No. 5,384,253, specifically incorporated herein by reference in its entirety), by agitation with silicon carbide fibers (Kaeppeler, *et al.*, *Plant Cell Reports* 9:415-418, 1990; U.S. Patent No. 5,302,523 and U.S. Patent No. 5,464,765, both specifically incorporated herein by reference in its entirety), by *Agrobacterium*-mediated transformation (U.S. Patent No. 5,591,616 and U.S.

Patent No. 5,563,055; both specifically incorporated herein by reference) and by acceleration of DNA coated particles (U.S. Patent No. 5,550,318; U.S. Patent No. 5,538,877; and U.S. Patent No. 5,538,880; each specifically incorporated herein by reference in its entirety). Through the application of techniques such as these, the cells of virtually any plant species may be stably transformed, and these cells developed into transgenic plants.

**[0052]** Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of Agrobacterium-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example, the methods described by Horsch, *et al.* (Science 227:1229-1231, 1985), Rogers and Klee (*Plant DNA Infectious Agents*, Chapter 7, Springer-Verlag/Wein, 1987) and U.S. Patent No. 5,563,055, specifically incorporated herein by reference in its entirety.

**[0053]** Agrobacterium-mediated transformation is most efficient in dicotyledonous plants and is the preferable method for transformation of dicots, including Arabidopsis, tobacco, tomato, alfalfa and potato. Indeed, while Agrobacterium-mediated transformation has been routinely used with dicotyledonous plants for a number of years, including alfalfa (Thomas, *et al.*, *Plant Sci.* 69:189-198, 1990), it has only more recently become applicable to monocotyledonous plants. Advances in Agrobacterium-mediated transformation techniques have now made the technique applicable to nearly all monocotyledonous plants. For example, Agrobacterium-mediated transformation techniques have now been applied to rice (Hiei, *et al.*, *Plant Mol. Biol.* 35:205-218, 1997; U.S. Patent No. 5,591,616, specifically incorporated herein by reference in its entirety), wheat (McCormac, *et al.*, *Euphytica* 99:17-25, 1998), barley (Tingay, *et al.*, *The Plant Journal* 11:1369-1376, 1997) and maize (Ishidia, *et al.*, *Nature Biotechnology* 14:745-750, 1996).

**[0054]** One also may employ protoplasts for electroporation transformation of plants (Bates, *Mol. Biotechnol.* 2:135-145, 1994; Lazzeri, *Methods Mol. Biol.* 49:95-106, 1995). Another method for delivering transforming DNA segments to plant cells in accordance with the invention is microprojectile bombardment (U.S. Patent No. 5,550,318; U.S. Patent No. 5,538,880; U.S. Patent No. 5,610,042; and PCT Application WO 94/09699; each of which is

specifically incorporated herein by reference in its entirety). In this method, particles may be coated with nucleic acids and delivered into cells by a propelling force.

[0055] The transgenic plants of the present invention expressing heterologous XA21 and exhibiting drought tolerance can be of any species. The plants can be an R0 transgenic plant (i.e., a plant derived from the original transformed tissue). The plants can also be a progeny plant of any generation of an R0 transgenic plant, wherein the transgenic plant comprises the nucleic acid sequence from the R0 transgenic plant.

[0056] Seeds of the above-described transgenic plants may also be provided, particularly where the seed comprises the nucleic acid sequence. Additionally contemplated are host cells transformed with the above-identified recombinant vector. In some embodiments, the host cell is a plant cell.

[0057] The plants of these embodiments having increased or enhanced expression of XA21 and drought tolerance may be of any species. The species may be any monocotyledonous or dicotyledonous plant, such as those described herein. One of skill in the art will recognize that the present invention may be applied to plants of other species by employing methods described herein and others known in the art.

[0058] Tissue cultures may be used in certain transformation techniques for the preparation of cells for transformation and for the regeneration of plants therefrom. Maintenance of tissue cultures requires use of media and controlled environments. "Media" refers to the numerous nutrient mixtures that are used to grow cells in vitro, that is, outside of the intact living organism. A medium usually is a suspension of various categories of ingredients (salts, amino acids, growth regulators, sugars, buffers) that are required for growth of most cell types. However, each specific cell type requires a specific range of ingredient proportions for growth, and an even more specific range of formulas for optimum growth. The rate of cell growth also will vary among cultures initiated with the array of media that permit growth of that cell type.

#### **PRODUCTION AND CHARACTERIZATION OF STABLY TRANSFORMED PLANTS**

[0059] After effecting delivery of exogenous DNA to recipient cells, the next steps generally concern identifying the transformed cells for further culturing and plant regeneration. In order to improve the ability to identify transformants, one may desire to employ a selectable

or screenable marker gene with a transformation vector prepared in accordance with the invention. In this case, one would then generally assay the potentially transformed cell population by exposing the cells to a selective agent or agents, or one would screen the cells for the desired marker gene trait. Potentially transformed cells then are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene has been integrated and expressed at sufficient levels to permit cell survival. Cells may be tested further to confirm stable integration of the exogenous DNA.

[0060] One herbicide which constitutes a desirable selection agent is the broad-spectrum herbicide bialaphos. Another example of a herbicide which is useful for selection of transformed cell lines in the practice of the invention is the broad-spectrum herbicide glyphosate. Glyphosate inhibits the action of the enzyme EPSPS which is active in the aromatic amino acid biosynthetic pathway. Inhibition of this enzyme leads to starvation for the amino acids phenylalanine, tyrosine, and tryptophan and secondary metabolites derived therefrom. U.S. Patent No. 4,535,060 describes the isolation of EPSPS mutations which confer glyphosate resistance on the EPSPS of *Salmonella typhimurium*, encoded by the gene *aroA*. The EPSPS gene from *Zea mays* was cloned and mutations similar to those found in a glyphosate resistant *aroA* gene were introduced in vitro. Mutant genes encoding glyphosate resistant EPSPS enzymes are described in, for example, International Patent Application Publication Number WO 97/4103.

[0061] The transformed cells, identified by selection or screening and cultured in an appropriate medium that supports regeneration, will then be allowed to mature into plants. Developing plantlets can be transferred to soilless plant growth mix, and hardened, *e.g.*, in an environmentally controlled chamber, for example, at about 85% relative humidity, 600 ppm CO<sub>2</sub>, and 25-250 microeinsteins m<sup>-2</sup> s<sup>-1</sup> of light. Plants may be matured in a growth chamber or greenhouse. Plants can be regenerated after a transformant is identified, depending on the initial tissue. During regeneration, cells are grown on solid media in tissue culture vessels. Illustrative embodiments of such vessels are petri dishes and Plant Cons. Regenerating plants can be grown at about 19 to 28°C, for example. After the regenerating plants have reached the stage of shoot and root development, they may be transferred to a greenhouse for further growth and testing.

[0062] To confirm the presence of the exogenous DNA or "transgene(s)" in the regenerating plants, a variety of assays may be performed. Such assays include, for example,

"molecular biological" assays, such as Southern and Northern blotting and PCR™; "biochemical" assays, such as detecting the presence of a protein product, *e.g.*, by immunological means (ELISAs and Western blots) or by enzymatic function; plant part assays, such as leaf or root assays; and also, by analyzing the phenotype of the whole regenerated plant.

5       **[0063]**     The expression of a gene product is often determined by evaluating the phenotypic results of its expression. These assays also may take many forms including but not limited to analyzing changes in the chemical composition, morphology, or physiological properties of the plant. Chemical composition may be altered by expression of genes encoding enzymes or storage proteins which change amino acid composition and may be detected by  
10    amino acid analysis, or by enzymes that change starch quantity which may be analyzed by near infrared reflectance spectrometry. Morphological changes may include greater stature or thicker stalks. Most often changes in response of plants or plant parts to imposed treatments are evaluated under carefully controlled conditions termed bioassays. Such assays for determining drought tolerance are well-described herein

## 15    **BREEDING PLANTS OF THE INVENTION**

**[0064]**     In addition to direct transformation of a particular plant genotype with a construct prepared according to the current invention, transgenic plants may be made by crossing a plant having a selected DNA of the invention to a second plant lacking the construct. For example, a selected XA21 coding sequence can be introduced into a particular plant variety by crossing,  
20    without the need for ever directly transforming a plant of that given variety. Therefore, the current invention not only encompasses a plant directly transformed or regenerated from cells which have been transformed in accordance with the current invention, but also the progeny of such plants. As used herein, the term "progeny" denotes the offspring of any generation of a parent plant prepared in accordance with the instant invention, wherein the progeny comprises a  
25    selected DNA construct prepared in accordance with the invention. "Crossing" a plant to provide a plant line having one or more added transgenes relative to a starting plant line, as disclosed herein, is defined as the techniques that result in a transgene of the invention being introduced into a plant line by crossing a plant of a starting line with a plant of a donor plant line that comprises a transgene of the invention. To achieve this one could, for example, perform the  
30    following steps: (a) plant seeds of the first (starting line) and second (donor plant line that

comprises a transgene of the invention) parent plants; (b) grow the seeds of the first and second parent plants into plants that bear flowers; (c) pollinate a flower from the first parent plant with pollen from the second parent plant; and (d) harvest seeds produced on the parent plant bearing the fertilized flower. Backcrossing is herein defined as the process including the steps of:

- 5 (a) crossing a plant of a first genotype containing a desired gene, DNA sequence or element to a plant of a second genotype lacking the desired gene, DNA sequence or element; (b) selecting one or more progeny plant containing the desired gene, DNA sequence or element; (c) crossing the progeny plant to a plant of the second genotype; and (d) repeating steps (b) and (c) for the purpose of transferring a desired DNA sequence from a plant of a first genotype to a plant of a second genotype. In any one or more generations of crossing, selection may be made for drought tolerance, yielding drought tolerant progeny.

- [0065] Introgression of a DNA element into a plant genotype is defined as the result of the process of backcross conversion. A plant genotype into which a DNA sequence has been introgressed may be referred to as a backcross converted genotype, line, inbred, or hybrid.
- 15 Similarly a plant genotype lacking the desired DNA sequence may be referred to as an unconverted genotype, line, inbred, or hybrid.

## DEFINITIONS

- [0066] Expression: The combination of intracellular processes, including transcription and translation, undergone by a coding DNA molecule such as a structural gene to produce a polypeptide.
- 20

[0067] Genetic Transformation: A process of introducing a DNA sequence or construct (*e.g.*, a vector or expression cassette) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

- [0068] Heterologous: A sequence which is not normally present in a given host genome
- 25 in the genetic context in which the sequence is currently found. In this respect, the sequence may be native to the host genome, but be rearranged with respect to other genetic sequences within the host sequence. For example, a regulatory sequence may be heterologous in that it is linked to a different coding sequence relative to the native regulatory sequence.

**[0069]** Obtaining: When used in conjunction with a transgenic plant cell or transgenic plant, obtaining means either transforming a non-transgenic plant cell or plant to create the transgenic plant cell or plant, or planting transgenic plant seed to produce the transgenic plant cell or plant. Such a transgenic plant seed may be from an R0 transgenic plant or may be from a progeny of any generation thereof that inherits a given transgenic sequence from a starting transgenic parent plant.

**[0070]** Overexpression: The increase in the expression of a DNA or RNA transcript and/or the function or activity of a protein relative to a control or naturally-occurring counterpart.

**[0071]** Promoter: A recognition site on a DNA sequence or group of DNA sequences that provides an expression control element for a structural gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

**[0072]** R0 transgenic plant: A plant that has been genetically transformed or has been regenerated from a plant cell or cells that have been genetically transformed.

**[0073]** Regeneration: The process of growing a plant from a plant cell (*e.g.*, plant protoplast, callus or explant).

**[0074]** Selected DNA: A DNA segment which one desires to introduce or has introduced into a plant genome by genetic transformation.

**[0075]** Transformation construct: A chimeric DNA molecule which is designed for introduction into a host genome by genetic transformation. Preferred transformation constructs will comprise all of the genetic elements necessary to direct the expression of one or more exogenous genes. In particular embodiments of the instant invention, it may be desirable to introduce a transformation construct into a host cell in the form of an expression cassette.

**[0076]** Transformed cell: A cell in which the DNA complement has been altered by the introduction of an exogenous DNA molecule into that cell.

**[0077]** Transgene: A segment of DNA which has been incorporated into a host genome or is capable of autonomous replication in a host cell and is capable of causing the expression of one or more coding sequences. Exemplary transgenes will provide the host cell, or plants regenerated therefrom, with a novel phenotype relative to the corresponding non-transformed cell or plant. Transgenes may be directly introduced into a plant by genetic transformation, or

may be inherited from a plant of any previous generation which was transformed with the DNA segment.

[0078] Transgenic plant: A plant or progeny plant of any subsequent generation derived therefrom, wherein the DNA of the plant or progeny thereof contains an introduced exogenous DNA segment not naturally present in a non-transgenic plant of the same strain. The transgenic plant may additionally contain sequences which are native to the plant being transformed, but wherein the "exogenous" gene has been altered in order to alter the level or pattern of expression of the gene, for example, by use of one or more heterologous regulatory or other elements.

[0079] Up-regulation: The increase in the expression of a DNA or RNA transcript and/or the function or activity of a protein relative to a control or naturally-occurring counterpart.

[0080] Vector: A DNA molecule designed for transformation into a host cell. Some vectors may be capable of replication in a host cell. A plasmid is an exemplary vector, as are expression cassettes obtained therefrom.

### EXAMPLES

[0081] The following examples are included to demonstrate illustrative embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

#### **Example 1 - Plasmid construction and plant transformation.**

[0082] The 3xFLAG-XA21-Myc construct was made using an 8.7 kb genomic fragment containing the c-Myc-tagged *Xa21* coding region, intron and the native 5' and 3' regulatory

sequences (FIG. 1A). To delete the extra 3' from the 9.9 kb *Xa21*-containing fragment previously used for rice transformation (Wang, *et al.*, *Plant Cell* 18:3635-3646, 2006), a *KpnI-SpeI* fragment with *Myc-Xa21* was mobilized from the plasmid pBEK822-Bm into the vector pKBluescript to generate pKBXA21KS-M. An additional 1.8 kb 3' sequence, PCR  
5 amplified from the 9.9 kb *Xa21* fragment with primers XA21-Tail-F (CTTTCCGAAGACGAGT ATATCTAACG; SEQ ID NO:3)/XA21-Tail-R (ACTAGTGGTACCCGTCTTATATC GCCTCA; SEQ ID NO:4), was added to the 3' end of the *KpnI-SpeI* fragment of pBXA21KS-M using the *SpeI* site. The resultant construct, namely pKB-Myc-XA21-S, contains a c-Myc tag (EQKLISEEDLLEK; SEQ ID NO:15) in the N-terminal region (domain B) of XA21. To  
10 introduce a c-Myc tag to the C-terminus of XA21, the *EcoRI* fragment of pKB-Myc-XA21-S was replaced by one with the tag fused to the C-terminus of XA21. The N-terminal c-Myc tag in the construct was replaced with 3xFLAG (DYKDDDDKGGDYKDDDDKGGDYKDDDDK; SEQ ID NO:16) using the *DraIII* site. The 8.7 kb *KpnI* fragment containing *Myc-Xa21-3xFLAG* was then subcloned into the binary vector pCAMBIA1300. *Agrobacterium*-mediated rice  
15 transformation was performed using cultivar TP309 as recipient as described previously (Wang, *et al.*, 2006, *supra*).

**[0083]** For protoplast transformation, enhanced green fluorescent protein (eGFP) with its own start codon removed was in-frame fused with XB3 between residues Thr-10 and Gly-11 to make pCR8GW-eGFP-XB3New-3xFLAG using primers SEQ ID NO:22 (GTGTGGA  
20 TCCATGGGTCACGGTGTCTAGCTGCGCCCGCACCCCTAGGGTGAGCAAGGGCGAGGA G; GFP-F) and SEQ ID NO:23 (GAATAGGGAATTCTCCCAGCCGAA; XB3seq-2). The XB3-mCherry fusion plasmid was constructed by replacing the C-terminal tag of XB3-3xFLAG in pCR8GW-XB3New-3xFLAG (Huang, *et al.*, *PLoS One* 8: e63868, 2013) with mCherry fluorescent protein. The *mCherry* open reading frame was PCR amplified from pmCherry-C1  
25 (Clontech) using primers mCherry-F (GTGCGGCCGCACTAGTGGCGGAATGGTGAGC AAGGGCGAGGAGGA; SEQ ID NO:30)/mCherry-R (GTAGATCTTTACTTGTACAGCT CGTCCATGCCGC; SEQ ID NO:31). To generate the eGFP-XB3<sup>G2A</sup> mutant, PCR was carried out using primers XB3New-2 (GTTCTAGAAGATCTTCATAGATCGTGCTCAG  
GCTTGTCCA; SEQ ID NO:25)/ XB3New-3 (GTTCTAGAGGATCCATGGCTCACGGTGT  
30 CAGCTGCGCCCG; SEQ ID NO:24) (carrying a mutation leading to substitution of Gly-2 in XB3 to Ala) and the mutated *Xb3* gene was cloned into the vector pCR8GW (ThermoFisher

Scientific). To construct the eGFP-XB3<sup>NLS</sup> mutant, site-directed mutagenesis was performed using primers XB3NLS-3 (TGACAAGCCGTCATCCCTGCAACTCACCCGGGAGGAGT CGGAACGATCTCACAACCTCAGTGAGG; SEQ ID NO:26)/XB3NLS-4 (CCTCA CTGAGGTTGTGAGATCGTTCCGACTCCTCCCGGGTGAGTTGCAGGGATGACGGCTTG TCA; SEQ ID NO:27) and the template plasmid pCR8GW-XB3New-3xFLAG. *eGFP-Xb3* and its mutants were then cloned into the binary vector pCAMBIA1300S containing a rice gene expression cassette with a double cauliflower mosaic virus (CaMV) 35S promoter. To fuse a functional NLS (PKKKRKVG; SEQ ID NO:17 from SV40 T antigen) to the C-terminus of *Discosoma* sp. red fluorescent protein (DsRed), PCR was carried out using primers DsRed-F (GTGTTCTAGAACTAGTATGGCCTCCTCCGAGGACGTCA; SEQ ID NO:28)/DsRed-R (GTGTTCTAGACTATCCCACCTTACGCTTTTTCTTAGGTCCCAGGAACAGGTGGTGGC GGCC; SEQ ID NO:29) to amplify *DsRed-NLS*. The resultant product was cloned into pCAMBIA1300S. The XA21-eGFP fusion was made by using NEBuilder® HiFi DNA Assembly Kit (New England Biolabs). The coding sequences for the XA21 kinase domain and eGFP were PCR amplified using primer pairs XA21eGFP-1 (CTGGATCATTTGGC TCAGTATACA; SEQ ID NO:18)/XA21eGFP-2 (AAATTCAAGGCTCCCACCTTCA; SEQ ID NO:19) and XA21eGFP-3 (GGTGGGAGCCTTGAATTTGTGCGACATGGTGAGCAAG GCGAGGA; SEQ ID NO:20)/XA21eGFP-4 (TGATCGTGTGGTAGATAACCACTGCAGT CAGTCGACCTTGTACAGCTCG TCCATGCCGA; SEQ ID NO:21), respectively. Full-length *Xa21-eGFP* was assembled using the PCR products and a restriction fragment coding for the N-terminal half of XA21. The resultant gene was inserted into pCAMBIA1300S for protein expression in rice protoplasts.

**[0084]** For *agrobacterium*-mediated transient gene expression, Myc-XA21 was generated by cloning a c-Myc-tagged *Xa21* cDNA into pCAMBIA1300S. The plasmid pCAMBIA1303 was used to express the GUS-GFP-6xHis fusion. pCAMBIA1300S-XB3-3xFLAG for expressing XB3-3xFLAG was described previously (Huang, *et al.*, 2013, *supra*). All constructs were introduced into *Agrobacterium tumefaciens* strain EHA105. Infiltration of *N. benthamiana* was performed as described previously (Huang, *et al.*, 2013, *supra*), except for tissue collection at 42 hours post infiltration. All constructs were verified by DNA sequencing.

**Example 2 - Transient expression of fluorescent proteins in rice protoplasts.**

[0085] Rice protoplasts were isolated from cultivar TP309 as described (Zhang, *et al.*, *Plant Methods* 7:30, 2011) except for the use of eight-day-old, dark-grown seedlings. Sixteen hours after transfection with the constructs described above, the protoplasts were visualized using a 40 x objective with a Zeiss LSM800 confocal laser scanning microscope. N-(3 triethylammoniumpropyl)-4-(6-(4(diethylamino) phenyl) hexatrienyl) pyridinium dibromide (FM4-64) staining was performed by incubating the dye [final concentration 1% (v/v)] with transfected protoplasts for 10 min at room temperature. eGFP, DsRed, mCherry and FM4-64 were excited with 488, 561, 561 and 488 nm laser lines, respectively. Fluorescence emissions were captured at 410-535 nm for eGFP, at 410-585 nm for DsRed, at 600-617 nm for mCherry and at 650-700 nm for FM4-64. Images were analyzed using ZEN 2.0 software packages.

**Example 3 - Plant growth and treatments.**

[0086] Rice seeds were surface sterilized and germinated on half-strength Murashige-Skoog (MS) medium supplemented with 30 g/L sucrose (for wild-type) or the same medium with 30 g/L sucrose and 50 µg/ml hygromycin (for transgenic lines) for nine days at 25°C under fluorescent light with a 16-hour photoperiod. Germinated seedlings of both XA21-expressing lines and the vector control A36 were transferred into soil and grown in shared soil-holding trays prepared with evenly distributed holes on the bottom for absorbing water. The trays were maintained in large tanks filled with water in a greenhouse under nature light conditions in Gainesville, Florida. For drought treatments, the plant trays were transferred to a bench and kept under natural light conditions without watering for approximately 20-40 days depending on the season. To recover drought-stressed plants, the trays were returned to water tanks for 12 days before survivors were scored. RWC of drought-stressed leaves was determined using the equation:  $RWC = (FW - DW)/(TW - DW)$ , where FW is the fresh weight of the leaf discs collected. Turgid weight (TW) was measured after floating the leaf discs on water for 24 hours at room temperature in dark. Dry weight (DW) was determined by weighing the leaves after drying at 65°C for three days, which was adequate to assure complete drying of the biomass.

[0087] For seedling air-drying assays, germinated individuals were cultured in water for an additional two (for *indica* lines) and five (for transgenic *japonica* lines) days, respectively. Two-week-old *japonica* seedlings were air-dried in a growth chamber (23°C) for three and half hours followed by a recovery in half-strength MS medium for three days. Survivors were defined as individuals possessing at least one true leaf flattened after recovery. A similar method, except that a five-hour-drought treatment and 11-day-old seedlings, was used to dehydrate the *indica* lines.

#### Example 4 - Total RNA extraction, RNA-seq, q-PCR and RNA blot analyses.

[0088] Transgenic A36 and B7-12 plants were subjected to drought stress treatments for 15 days at which point most of the treated A36 leaves, but not the B7-12 leaves, were rolled. Leaf tissues from five plants were harvested and pooled for each sample in order to minimize individual variations. Total RNA was extracted using the TRIzol Reagent (Ambion) according to the manufacturer's instruction. After treatment with RNase-free DNase (Qiagen) to eliminate genomic DNA contamination followed by further purification using RNeasy MinElute Cleanup Kit (Qiagen), the purified RNA was used for RNA-seq library construction and sequencing using the HiSeq 2000 platform (Illumina).

[0089] The obtained reads were aligned to the *O. sativa* Nipponbare reference genome using TopHat version 2.013 (Kawahara, *et al.*, *Rice* 6:4, 2013; Trapnell, *et al.*, *Bioinformatics* 25:1105-1111, 2009). Ambiguous reads that mapped to more than one region in the genome or those with a MAPQ score of less than 10 were removed. Transcript quantification was carried out by the Partek Genomics Suite (version 6.4, Partek, Inc.) to obtain raw read counts and normalized read counts (RPKM: Reads per kilobase per million mapped reads) (Mortazavi, *et al.*, *Nat. Methods* 5:621-628, 2008). Differential gene expression was analyzed using generalized linear model approaches (GLM) implemented in the BioConductor edgeR package. Significant differential expression genes (DEGs) were selected based on the following criteria: fold change over 2, p-value less than 0.05 and RPKM greater than 1 for B7-12 in up-regulation or RPKM greater 1 for A36 in down-regulation.

[0090] For q-PCR analysis, two-week-old seedlings were subjected to dehydration followed by RNA isolation as described above. cDNA was synthesized with 1µg of total RNA using RT<sup>2</sup> First Strand Kit (Qiagen). Q-PCR was performed under the following conditions:

95°C, 2 min; (95°C, 5 s; 60°C, 5 s) × 40 cycles, 72°C, 5 min using the CFX 96 Real-Time PCR Detection System (Bio-Rad) according to the manufacturer's instruction. Results were normalized to the expression of the rice reference gene Os06g11170.1 (Narsai, *et al.*, *BMC Plant Biol.* 10:56, 2010). Primer sequences SEQ ID NO:7 (GTACATCTAGATTTGGGGTAGA; forward) and SEQ ID NO:8 (GTACGAACACAAGCTAACACGA; reverse) were used for *OsLEA1*, SEQ ID NO:9 (CCAAGCAGAAGACCGCCGA; forward) and SEQ ID NO:10 (GTCATCCCCAGCGTGCTCA; reverse) were used for *OsLEA3*, SEQ ID NO:11 (CGATGACGACGCTGAGTGAA; forward) and SEQ ID NO:12 (CAGGTGACATCACACGCTTGA; reverse) were used for *OsLEA33*, SEQ ID NO:13 (TAACAGCACACCACCACAA; forward) and SEQ ID NO:14 (GTCTTCAAGCTGTTCGACGG; reverse) were used for *OsNAC10*, and SEQ ID NO:5 (GGAATGTGGACGGTGACACT; forward) and SEQ ID NO:6 (TCAAAATAGAGTCCAGTAGATTTGTCA; reverse) were used for Os06g11170.1.

**[0091]** RNA blot analysis was performed using a radiolabeled *Xb3*-specific probe as described previously (Wang, *et al.*, 2006, *supra*).

#### **Example 5 - Immunodetection.**

**[0092]** To generate monoclonal anti-XA21K antibody, the intracellular kinase domain of XA21 was expressed in *E. coli* and the purified fusion protein was used as immunogen in mice. Antibody production was performed as described (Rong, *et al.*, *J. Integrative Agricultural* 15:726-734, 2016).

**[0093]** Nuclear fraction was isolated by homogenization of leaf tissues in 1x nuclei isolation buffer (2.5% Ficoll 400, 0.4 M sucrose, 25% glycerol, 25 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM DTT, 1 mM PMSF, and 1x complete protease inhibitor cocktail) using a mortar and pestle. The homogenate was sequentially filtered through one-layer of 75 μm nylon mesh, two-layers of miracloth (Millipore) and four-layers of miracloth. After addition of Triton X-100 to a final concentration of 0.5%, the homogenate was incubated on ice for 15 min and centrifuged at 1,500 g for 5 min. The supernatant was saved as a nuclei-depleted fraction and the pellet was washed with washing buffer (1x nuclei isolation buffer containing 0.1% Triton X-100) and centrifuged at 100 x g for 1 min to remove starch and cell debris. The pellet was further washed three times using washing buffer, and then resuspended in 1 ml of washing buffer. After centrifuging at 1,800 x g for 5 min, the nuclei-enriched pellet was collected.

[0094] Microsomal fraction was isolated by homogenization of leaf tissue harvested from two-month-old plants in 1x extraction buffer (50 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1 mM EDTA; 10% glycerol; 1 mM PMSF, and 1x complete protease inhibitor cocktail), filtrated through Miracloth, and centrifuged at 1,000 x g for 10 min at 4°C. The supernatant was re-centrifuged at 15,000 x g for 5 min at 4°C. The resultant supernatant was centrifuged at 150,000 x g for 60 min at 4°C. The pellet was re-suspended in solubilization buffer (extraction buffer containing 0.1% Triton X-100, but lacking glycerol) and stored at -70°C until used.

[0095] Protein extraction and protein blot analysis was performed as previously described (Xu, *et al.*, *Plant J.* 45:740-751, 2006).

#### Example 6 - Inoculation of plants with *Xoo*.

[0096] Six-week-old plants were inoculated with *Xoo* strains using the leaf-clipping method (Kauffman, *et al.*, *Plant Disease Rep.* 57:537-541, 1993). After inoculation, disease lesion development and bacterial population were determined as described previously (Song, *et al.*, *Science* 270:1804-1806, 1995).

#### Example 7 - XA21 confers drought tolerance in rice.

[0097] After infection, incompatible *Xoo* strains (*e.g.*, PXO99A) can propagate to a significant level in rice; but they cause short disease lesions and weak water stress injuries (FIG. 2). The inventors reasoned that XA21 might play a role in counteracting drought, although an immune sensor has never before been assigned a similar function under physiological conditions. The ability of XA21 to confer drought tolerance was tested in the absence of *Xoo*. Newly generated XA21 lines (B7-12 and B7-11) and the empty-vector control A36 [both using cultivar *O. sativa* ssp. *japonica* cv. TaiPei309 (TP309) as recipient] were stressed by withholding water. The XA21 lines, expressing 3xFLAG-XA21-Myc under the control of its native promoter, conferred resistance to PXO99A (FIG. 1B). Remarkably, XA21 plants displayed delayed drought-related symptoms and were recovered from the water stress with significantly higher survival rates than A36. The tolerance appeared to be influenced by the seasons and developmental stages with greater degree in winter and at the older stages. The leaf water status of stressed plants was measured and it was confirmed that the B7-12 line was able to maintain higher levels of relative water content (RWC) than did A36 during drought conditions (FIG. 3A).

[0098] The novel function of XA21 was confirmed in temperature-controlled laboratory settings. Air-drying of two-week-old seedlings for three and half hours at 23°C induced more than 54% mortality in A36, but caused less than 25% death in B7-12, B7-11 and the previously characterized line 4021-3 with a higher level of Myc-XA21 (FIG. 3B; FIG. 4A; FIG. 4B; Wang, *et al.*, 2006, *supra*). Relative to the near-isogenic recurrent parent IR24 (*O. sativa* ssp. *indica*), the *Xa21* introgression line IRBB21 (the original source for isolating the *Xa21* gene, Khush, *et al.*, *Rice Genetics Newsletter* 7:121-122, 1990) as well as IRBB14 seedlings, which carry the genetically-characterized BLB resistance gene *Xa14* (Taura, *et al.*, *Rice Genetics Newsletter* 4:101-102, 1987), exhibit better performance after five hour of drought treatment (FIG. 4C; FIG. 3C). By contrast, IRBB13 seedlings containing the recessive resistance gene *xa13* coding for a sugar transporter (Chu, *et al.*, *Genes Dev.* 20:1250-1255, 2006; Yang, *et al.*, *Proc. Natl. Acad. Sci. USA* 103:10503-10508, 2006; Chen, *et al.*, *Annu. Rev. Biochem.* 84:865-894, 2015) are sensitive to water stress (FIG. 5).

#### Example 8 - Water deficit induces heightened expression of drought stress-related genes in an XA21 dependent manner.

[0099] To determine the molecular mechanisms underlying XA21-mediated drought response, RNA-seq analysis was performed. Adult plants were drought-stressed under greenhouse conditions for 15 days at which point most of the treated A36 leaves, but not the B7-12 leaves, were rolled, a phenotypic sign of early stage water deficit. Total RNA was isolated and subjected to library construction and sequencing. More than 61 million reads were generated from each sample and the obtained reads were aligned to the *O. sativa* Nipponbare reference genome using TopHat version 2.013 (Kawahara, *et al.*, 2013, *supra*; Trapnell, *et al.*, 2009, *supra*). A total of 430 differentially expressed genes (DEGs) were identified between B7-12 and A36 after drought treatment, with 17 of them previously known to be water stress regulated (Table 1). In Table 1, known drought/dehydration-responsive genes are indicated in bold, genes whose differential expression was validated by q-PCR are indicated in bold and underlining, and known drought/dehydration-responsive genes whose differential expression was validated by q-PCR are underlined (no bold). Fold change and p-value were generated by edgeR, and RPKM value is average value for each line. Real-time quantitative reverse transcription-PCR (q-PCR) validated the drought induction and differential expression of four DEGs (*OsLEA1*, *OsLEA3*, *OsLEA33* and *OsNAC10*) (FIG. 6A, FIG. 6B, FIG. 6C and FIG. 6D,

respectively). These data indicate that in response to water stress XA21 triggers heightened expression of a battery of drought-responsive genes.

Table 1

| Gene model          | Annotation                                                                               | Fold change drought (B7-12/A36) | p-value         | B7-12 (RPKM)   | A36 (RPKM)       |
|---------------------|------------------------------------------------------------------------------------------|---------------------------------|-----------------|----------------|------------------|
| Os08g36740.1        | bHelix-loop-helix transcription factor, putative, expressed                              | 65.60                           | 0.014347791     | 1.05888        | 0.0101797        |
| Os02g20940.2        | CHCH domain containing protein, expressed                                                | 64.92                           | 0.015035143     | 1.31103        | 0.0123909        |
| <b>Os07g48450.2</b> | <b>NAC domain transcription factor</b>                                                   | <b>57.63</b>                    | <b>2.91E-07</b> | <b>3.48369</b> | <b>0.0360582</b> |
| Os07g40460.2        | prefoldin, putative, expressed                                                           | 57.40                           | 0.025212223     | 1.39779        | 0.0133264        |
| Os05g47860.1        | expressed protein                                                                        | 56.03                           | 0.027707752     | 1.30644        | 0.014704         |
| Os06g42430.2        | expressed protein                                                                        | 46.81                           | 0.004351535     | 1.62423        | 0.0252887        |
| Os08g18974.2        | expressed protein                                                                        | 36.56                           | 0.015416347     | 1.47982        | 0.0254192        |
| Os01g65090.1        | aminotransferase, classes I and II, domain containing protein, expressed                 | 32.25                           | 4.74E-06        | 2.51309        | 0.0474212        |
| Os02g58730.2        | ras-related protein, putative, expressed                                                 | 27.11                           | 0.014580597     | 1.13705        | 0.0277986        |
| Os03g17870.1        | metallothionein, putative, expressed (related to OsMT1a)                                 | 25.45                           | 0.006840116     | 5.56079        | 0.137775         |
| <b>Os05g47730.1</b> | <b>LTPL153 - Protease inhibitor/seed storage/LTP family protein precursor, expressed</b> | <b>19.93</b>                    | <b>7.64E-07</b> | <b>15.8955</b> | <b>0.502877</b>  |
| Os11g25040.1        | plant-specific domain TIGR01615 family protein, expressed                                | 17.47                           | 0.006340921     | 1.35904        | 0.0490498        |
| <b>Os08g31860.1</b> | <b>expressed protein</b>                                                                 | <b>15.54</b>                    | <b>3.00E-09</b> | <b>23.074</b>  | <b>0.936311</b>  |
| Os03g08360.1        | 3-ketoacyl-CoA synthase 10, putative, expressed                                          | 15.35                           | 4.78E-05        | 2.42981        | 0.100974         |
| Os04g33920.1        | LTPL102 - Protease inhibitor/seed storage/LTP family protein precursor, expressed        | 14.33                           | 1.17E-08        | 35.8224        | 1.57634          |
| Os01g62420.3        | triosephosphate isomerase, cytosolic, putative, expressed                                | 12.90                           | 0.01944993      | 1.73492        | 0.0852586        |
| Os05g31140.2        | glycosyl hydrolases family 17, putative, expressed                                       | 12.43                           | 2.42E-07        | 12.3571        | 0.626934         |
| Os05g11320.1        | metallothionein-like protein                                                             | 12.30                           | 1.57E-09        | 691.652        | 35.4509          |

|                     |                                                                                    |              |                    |                |                 |
|---------------------|------------------------------------------------------------------------------------|--------------|--------------------|----------------|-----------------|
|                     | 3B, putative, expressed                                                            |              |                    |                |                 |
| Os10g38470.1        | glutathione S-transferase, putative, expressed                                     | 11.44        | 2.51E-07           | 21.5953        | 1.19037         |
| Os08g44270.1        | vignain precursor, putative, expressed                                             | 11.28        | 0.018962743        | 1.03103        | 0.0576694       |
| Os07g23640.1        | retrotransposon protein, putative, Ty3-gypsy subclass, expressed                   | 11.10        | 0.004396684        | 2.18513        | 0.124103        |
| <b>Os01g45640.1</b> | <b>tat pathway signal sequence family protein, expressed</b>                       | <b>10.95</b> | <b>4.59E-08</b>    | <b>73.6573</b> | <b>4.24383</b>  |
| Os11g18570.1        | cytochrome P450, putative, expressed                                               | 10.78        | 4.03E-06           | 6.69536        | 0.391673        |
| Os12g09300.1        | amino acid transporter, putative, expressed                                        | 10.78        | 0.000118804        | 3.17876        | 0.185964        |
| Os04g33510.1        | expressed protein                                                                  | 10.75        | 0.01806747         | 1.66628        | 0.101577        |
| Os01g46120.1        | GDSL-like lipase/acylhydrolase, putative, expressed                                | 10.61        | 0.004705021        | 1.64294        | 0.0968729       |
| Os01g62420.1        | triosephosphate isomerase, cytosolic, putative, expressed                          | 10.50        | 0.020205805        | 1.26389        | 0.0724757       |
| Os01g43740.1        | cytochrome P450 72A1, putative, expressed                                          | 9.72         | 0.006632195        | 1.15791        | 0.0751394       |
| <b>Os09g29660.1</b> | <b>white-brown complex homolog protein 11, putative, expressed</b>                 | <b>9.69</b>  | <b>0.000123738</b> | <b>2.50208</b> | <b>0.162849</b> |
| Os10g35070.4        | alpha-galactosidase precursor, putative, expressed                                 | 9.40         | 0.004817214        | 2.52422        | 0.167932        |
| <b>Os12g41680.1</b> | <b>NAC domain transcription factor</b>                                             | <b>8.82</b>  | <b>8.61E-06</b>    | <b>10.5794</b> | <b>0.756102</b> |
| Os01g03730.1        | nuclease PA3, putative, expressed                                                  | 8.72         | 5.59E-06           | 14.1191        | 1.02108         |
| Os01g43372.1        | expressed protein                                                                  | 8.64         | 0.000127875        | 3.45949        | 0.251629        |
| Os05g48510.1        | phenylalanyl-tRNA synthetase beta chain, putative, expressed                       | 8.60         | 0.005416772        | 1.08485        | 0.0800341       |
| <u>Os05g46480.2</u> | <u>late embryogenesis abundant protein, group 3, putative, expressed (OsLEA3)</u>  | <u>8.42</u>  | <u>1.08E-05</u>    | <u>18.4447</u> | <u>1.38329</u>  |
| Os07g37690.1        | UDP-glucuronosyl and UDP-glucosyl transferase domain containing protein, expressed | 8.42         | 0.012705122        | 1.21277        | 0.0908814       |
| Os02g16000.1        | E1-BTB1 - Bric-a-Brac, Tramtrack, and Broad Complex                                | 8.41         | 0.001700045        | 1.86881        | 0.138122        |

|                     |                                                                                   |             |                    |                |                 |
|---------------------|-----------------------------------------------------------------------------------|-------------|--------------------|----------------|-----------------|
|                     | domain with E1 subfamily conserved sequence, expressed                            |             |                    |                |                 |
| Os01g73830.1        | expressed protein                                                                 | 8.11        | 0.028925119        | 1.32138        | 0.102768        |
| Os01g68290.1        | expressed protein                                                                 | 8.07        | 2.86E-07           | 408.154        | 31.9028         |
| Os01g06310.1        | glycine-rich cell wall structural protein precursor, putative, expressed          | 8.06        | 1.41E-06           | 66.2272        | 5.18048         |
| Os12g33130.1        | expressed protein                                                                 | 7.81        | 1.44E-06           | 119.968        | 9.68975         |
| Os03g18850.1        | pathogenesis-related Bet v I family protein, putative, expressed                  | 7.80        | 0.001346482        | 6.01608        | 0.486655        |
| Os08g19420.1        | O-methyltransferase, putative, expressed                                          | 7.62        | 1.94E-05           | 11.9352        | 0.987687        |
| Os06g49660.1        | transferase family protein, putative, expressed                                   | 7.43        | 0.004939444        | 1.95157        | 0.165563        |
| Os06g46270.1        | NAC domain transcription factor                                                   | 7.40        | 0.0320483          | 1.26111        | 0.107533        |
| Os01g55940.1        | OsGH3.2 - Probable indole-3-acetic acid-amido synthetase, expressed               | 7.14        | 9.95E-05           | 4.82056        | 0.425924        |
| Os06g36390.2        | expressed protein                                                                 | 7.04        | 0.000893281        | 4.66685        | 0.418074        |
| Os12g07970.2        | transporter, major facilitator family, putative, expressed                        | 6.86        | 0.019455049        | 1.20617        | 0.110105        |
| <u>Os04g49980.1</u> | <u>late embryogenesis abundant group 1, putative, expressed (OsLEA1)</u>          | <u>6.66</u> | <u>0.000841869</u> | <u>10.3023</u> | <u>0.975863</u> |
| Os02g28170.1        | transferase family protein, putative, expressed                                   | 6.49        | 0.01744985         | 1.28606        | 0.124938        |
| Os01g42860.1        | inhibitor I family protein, putative, expressed                                   | 6.45        | 0.049838642        | 3.9417         | 0.385182        |
| Os11g40530.1        | LTPL162 - Protease inhibitor/seed storage/LTP family protein precursor, expressed | 6.30        | 7.07E-06           | 179.129        | 17.9183         |
| Os01g09220.1        | transposon protein, putative, CACTA, En/Spm sub-class, expressed                  | 5.88        | 0.019435644        | 1.45257        | 0.155772        |
| Os04g54330.1        | acetyltransferase, GNAT family, putative, expressed                               | 5.82        | 0.045569669        | 1.40486        | 0.153768        |
| Os03g08550.1        | STRUBBELIG-RECEPTOR FAMILY 6 precursor, putative, expressed                       | 5.80        | 0.002096038        | 2.17117        | 0.234815        |

|                            |                                                                                       |                    |                           |                       |                       |
|----------------------------|---------------------------------------------------------------------------------------|--------------------|---------------------------|-----------------------|-----------------------|
| Os03g20870.1               | zinc finger, C3HC4 type domain containing protein, expressed                          | 5.79               | 0.000419056               | 7.89185               | 0.859687              |
| Os05g43300.1               | expressed protein                                                                     | 5.78               | 0.00324686                | 2.50565               | 0.27477               |
| Os08g31340.1               | heavy metal-associated domain containing protein, expressed                           | 5.76               | 0.010627881               | 2.69415               | 0.29489               |
| Os04g54330.3               | acetyltransferase, GNAT family, putative, expressed                                   | 5.74               | 0.04074359                | 1.50025               | 0.1642                |
| Os07g10420.1               | expressed protein                                                                     | 5.66               | 4.95E-05                  | 39.7652               | 4.43137               |
| Os03g43720.1               | transporter family protein, putative, expressed                                       | 5.61               | 0.006395436               | 2.00027               | 0.226837              |
| Os12g38270.1               | metallothionein, putative, expressed (related to OsMT1a)                              | 5.53               | 0.000403064               | 22.7044               | 2.58901               |
| Os10g34614.1               | csAtPR5, putative, expressed                                                          | 5.49               | 0.039014666               | 1.34781               | 0.152235              |
| Os05g07940.1               | glyoxalase family protein, putative, expressed                                        | 5.46               | 0.000258157               | 13.8468               | 1.60099               |
| Os02g08440.1               | WRKY71, expressed                                                                     | 5.38               | 0.03227178                | 1.41166               | 0.164446              |
| Os03g04500.1               | tetratricopeptide repeat domain containing protein, expressed                         | 5.33               | 0.021962974               | 1.04358               | 0.124314              |
| Os03g55590.1               | MYB family transcription factor, putative, expressed                                  | 5.28               | 9.62E-05                  | 18.0639               | 2.15755               |
| Os12g42910.1               | sodium/calcium exchanger protein, putative, expressed                                 | 5.26               | 0.013891386               | 1.61749               | 0.194023              |
| Os08g35620.1               | HD domain containing protein, putative, expressed                                     | 5.23               | 0.01181755                | 1.29624               | 0.156381              |
| Os04g38680.1               | transmembrane amino acid transporter protein, putative, expressed                     | 5.23               | 0.000612057               | 7.00345               | 0.844909              |
| Os04g58750.3               | protein kinase family protein, putative, expressed                                    | 5.23               | 0.010002973               | 2.09933               | 0.251298              |
| Os05g01280.2               | expressed protein                                                                     | 5.08               | 0.041787996               | 4.1354                | 0.508973              |
| Os02g36974.2               | 14-3-3 protein, putative, expressed                                                   | 5.07               | 0.002758265               | 5.64025               | 0.70212               |
| Os01g53240.1               | BURP domain containing protein, expressed                                             | 5.07               | 0.000123555               | 22.1057               | 2.7515                |
| Os01g66100.1               | gibberellin 20 oxidase 2 (semi-dwarfing gene SD1)                                     | 4.91               | 0.0045523                 | 4.05657               | 0.521365              |
| <b><u>Os06g23350.1</u></b> | <b><u>late embryogenesis abundant protein D-34, putative, expressed (OsLEA33)</u></b> | <b><u>4.90</u></b> | <b><u>0.013580481</u></b> | <b><u>3.53686</u></b> | <b><u>0.45529</u></b> |
| Os02g16630.1               | tryptophan biosynthesis                                                               | 4.87               | 0.034101964               | 1.78049               | 0.228005              |

|              |                                                                          |      |             |         |          |
|--------------|--------------------------------------------------------------------------|------|-------------|---------|----------|
|              | protein trpCF, putative, expressed                                       |      |             |         |          |
| Os04g42250.3 | transferase family protein, putative, expressed                          | 4.86 | 0.0027841   | 4.33123 | 0.561044 |
| Os01g27020.3 | transposon protein, putative, unclassified, expressed                    | 4.76 | 0.046556802 | 1.02605 | 0.134374 |
| Os05g23440.2 | solute carrier family 35 member F1, putative, expressed                  | 4.74 | 0.027841494 | 2.05889 | 0.273971 |
| Os04g02900.1 | dehydrogenase E1 component domain containing protein, expressed          | 4.70 | 0.000341814 | 14.179  | 1.90125  |
| Os02g42290.3 | OsClp3 - Putative Clp protease homologue, expressed                      | 4.66 | 0.000410464 | 19.8523 | 2.68277  |
| Os03g07130.1 | RING finger protein 13, putative, expressed                              | 4.65 | 0.015820352 | 1.75596 | 0.23755  |
| Os07g47450.1 | flowering promoting factor-like 1, putative, expressed                   | 4.41 | 0.006286889 | 15.6035 | 2.23356  |
| Os05g45090.1 | anthocyanidin 5,3-O-glucosyltransferase, putative, expressed             | 4.38 | 0.044069989 | 1.4007  | 0.201701 |
| Os05g15770.1 | glycosyl hydrolase, putative, expressed                                  | 4.35 | 0.000540829 | 25.293  | 3.66743  |
| Os10g25130.1 | aminotransferase, classes I and II, domain containing protein, expressed | 4.32 | 0.001426589 | 7.73144 | 1.12752  |
| Os05g09600.3 | GA11916-PA, putative, expressed                                          | 4.32 | 0.030862698 | 3.39147 | 0.498372 |
| Os05g49940.1 | expressed protein                                                        | 4.14 | 0.001070071 | 35.3562 | 5.37991  |
| Os05g07940.5 | glyoxalase family protein, putative, expressed                           | 4.13 | 0.00368885  | 10.1542 | 1.548    |
| Os08g08960.1 | Cupin domain containing protein, expressed                               | 4.12 | 0.027926452 | 3.87949 | 0.593737 |
| Os02g55590.1 | expressed protein                                                        | 4.07 | 0.011803933 | 2.68421 | 0.414516 |
| Os01g46600.1 | seed maturation protein PM41, putative, expressed                        | 4.06 | 0.003422302 | 41.6122 | 6.46556  |
| Os02g07230.2 | porphobilinogen deaminase, chloroplast precursor, putative, expressed    | 4.05 | 0.028910079 | 2.56107 | 0.401008 |
| Os01g72890.2 | transposon protein, putative, CACTA, En/Spm sub-class, expressed         | 4.03 | 0.019251511 | 2.75861 | 0.42939  |
| Os10g18150.1 | crooked neck, putative,                                                  | 4.02 | 0.018603025 | 1.86837 | 0.292938 |

|                     |                                                                                    |             |                    |                |                 |
|---------------------|------------------------------------------------------------------------------------|-------------|--------------------|----------------|-----------------|
|                     | expressed                                                                          |             |                    |                |                 |
| Os09g29710.1        | beta-expansin precursor, putative, expressed                                       | 4.02        | 0.041299195        | 4.5672         | 0.717197        |
| Os05g08980.3        | expressed protein                                                                  | 4.00        | 0.007464156        | 5.90306        | 0.929681        |
| Os09g21120.1        | armadillo/beta-catenin repeat family protein, putative, expressed                  | 4.00        | 0.017785079        | 1.85889        | 0.293372        |
| Os07g04220.1        | wound and phytochrome signaling involved receptor like kinase, putative, expressed | 3.93        | 0.043253877        | 1.08147        | 0.17371         |
| Os09g33810.2        | ankyrin repeat domain containing protein, putative, expressed                      | 3.92        | 0.009507488        | 4.72312        | 0.76076         |
| Os04g33500.1        | protein kinase, putative, expressed                                                | 3.92        | 0.035154191        | 2.07574        | 0.334254        |
| Os01g59600.2        | peptidase, T1 family, putative, expressed                                          | 3.92        | 0.026888423        | 3.62538        | 0.583029        |
| Os04g55850.1        | nuclease PA3, putative, expressed                                                  | 3.89        | 0.003666708        | 11.3396        | 1.84064         |
| Os09g19940.2        | cwfJ-like family protein, putative, expressed                                      | 3.87        | 0.048843213        | 1.31393        | 0.214269        |
| Os10g39920.1        | expressed protein                                                                  | 3.85        | 0.006408314        | 12.0164        | 1.9664          |
| <b>Os02g32520.1</b> | <b>early-responsive dehydration 1 (ERD1) protein</b>                               | <b>3.85</b> | <b>0.004053087</b> | <b>3.76972</b> | <b>0.618829</b> |
| Os10g38610.1        | glutathione S-transferase, putative, expressed                                     | 3.83        | 0.007759607        | 8.05908        | 1.32678         |
| Os03g08580.1        | expressed protein                                                                  | 3.80        | 0.008598883        | 7.34964        | 1.21944         |
| Os01g08440.1        | UDP-glucuronosyl and UDP-glucosyl transferase domain containing protein, expressed | 3.79        | 0.009586004        | 4.33448        | 0.721899        |
| Os04g10680.3        | zinc finger, C3HC4 type domain containing protein, expressed                       | 3.70        | 0.041016557        | 1.24967        | 0.212153        |
| Os01g51010.1        | DUF292 domain containing protein, expressed                                        | 3.70        | 0.032642746        | 1.00933        | 0.172579        |
| Os05g10940.1        | metal cation transporter, putative, expressed                                      | 3.68        | 0.013041639        | 4.15535        | 0.712355        |
| Os03g03810.1        | DEF8 - Defensin and Defensin-like DEFL family, expressed                           | 3.67        | 0.002356129        | 56.5489        | 9.72839         |
| Os07g36465.1        | vacuolar ATP synthase subunit H, putative, expressed                               | 3.65        | 0.045822573        | 6.42183        | 1.10929         |
| <b>Os11g47809.1</b> | <b>metallothionein, putative, expressed (OsMT1a)</b>                               | <b>3.63</b> | <b>0.001210069</b> | <b>156.213</b> | <b>27.1128</b>  |

|                     |                                                                                            |             |                    |                |                 |
|---------------------|--------------------------------------------------------------------------------------------|-------------|--------------------|----------------|-----------------|
| Os01g70920.2        | cullin-1, putative, expressed                                                              | 3.62        | 0.040235358        | 2.53091        | 0.442728        |
| <b>Os05g34830.3</b> | <b>NAC domain transcription factor</b>                                                     | <b>3.59</b> | <b>0.010772328</b> | <b>6.60158</b> | <b>1.16018</b>  |
| Os01g65480.1        | dnaJ domain containing protein, expressed                                                  | 3.57        | 0.016264368        | 4.51583        | 0.797834        |
| Os09g26620.4        | auxin-repressed protein, putative, expressed                                               | 3.57        | 0.039860726        | 5.27219        | 0.926879        |
| Os01g19150.5        | CGMC_GSK.3 - CGMC includes CDA, MAPK, GSK3, and CLKC kinases, expressed                    | 3.55        | 0.032144494        | 2.3096         | 0.410895        |
| Os06g37010.2        | metal cation transporter, putative, expressed                                              | 3.53        | 0.035643776        | 2.62741        | 0.46961         |
| Os10g33960.2        | START domain containing protein, expressed                                                 | 3.52        | 0.024508117        | 1.76838        | 0.317967        |
| Os04g52450.1        | aminotransferase, putative, expressed                                                      | 3.46        | 0.005097202        | 8.77895        | 1.59814         |
| Os06g03660.1        | peroxisomal biogenesis factor 11, putative, expressed                                      | 3.44        | 0.010123999        | 6.15175        | 1.12762         |
| <u>Os11g03300.2</u> | <u>NAC domain transcription factor (OsNAC10)</u>                                           | <u>3.42</u> | <u>0.043369255</u> | <u>3.19666</u> | <u>0.590187</u> |
| Os07g48830.2        | glycosyl transferase 8 domain containing protein, putative, expressed                      | 3.36        | 0.003255207        | 29.1802        | 5.47601         |
| Os02g12650.1        | puromycin-sensitive aminopeptidase, putative, expressed                                    | 3.33        | 0.005200384        | 7.0731         | 1.33811         |
| Os01g09300.3        | oxidoreductase, putative, expressed                                                        | 3.31        | 0.040225522        | 3.25537        | 0.622002        |
| Os03g61920.1        | electron transfer flavoprotein subunit alpha, mitochondrial precursor, putative, expressed | 3.30        | 0.007785135        | 10.1092        | 1.93468         |
| Os04g56110.1        | protein kinase, putative, expressed                                                        | 3.29        | 0.009049751        | 8.07154        | 1.54822         |
| Os04g53690.1        | expressed protein                                                                          | 3.29        | 0.036186551        | 4.03281        | 0.77431         |
| Os09g34280.1        | ankyrin repeat-containing protein, putative, expressed                                     | 3.25        | 0.010233398        | 5.23619        | 1.01564         |
| Os07g48160.1        | alpha-galactosidase precursor, putative, expressed                                         | 3.23        | 0.020628362        | 4.7077         | 0.918123        |
| Os04g54830.1        | expressed protein                                                                          | 3.19        | 0.033398369        | 2.32475        | 0.459516        |
| Os01g37750.1        | glutathione S-transferase, putative, expressed                                             | 3.18        | 0.0105181          | 14.6467        | 2.90378         |
| Os10g11810.1        | dehydrogenase, putative, expressed                                                         | 3.11        | 0.022703831        | 5.79326        | 1.17309         |

|                     |                                                                                       |             |                    |                |                |
|---------------------|---------------------------------------------------------------------------------------|-------------|--------------------|----------------|----------------|
| Os08g05570.3        | monodehydroascorbate reductase, putative, expressed                                   | 3.11        | 0.019060015        | 4.55583        | 0.926648       |
| Os03g50290.1        | 14-3-3 protein, putative, expressed                                                   | 3.09        | 0.005406408        | 31.7782        | 6.47917        |
| Os04g58360.1        | expressed protein                                                                     | 3.09        | 0.025484871        | 18.1069        | 3.69421        |
| Os03g17930.2        | alpha-taxilin, putative, expressed                                                    | 3.06        | 0.047655945        | 3.01106        | 0.621429       |
| Os02g57840.2        | remorin C-terminal domain containing protein, putative, expressed                     | 3.05        | 0.021179187        | 11.2032        | 2.31616        |
| Os08g38880.4        | WD-40 repeat family protein, putative, expressed                                      | 3.04        | 0.012817107        | 9.44055        | 1.95841        |
| <b>Os01g53880.5</b> | <b>OsIAA6 - Auxin-responsive Aux/IAA gene family member, expressed</b>                | <b>3.02</b> | <b>0.017470191</b> | <b>6.78062</b> | <b>1.41427</b> |
| <b>Os05g34830.1</b> | <b>NAC domain transcription factor</b>                                                | <b>3.02</b> | <b>0.012973598</b> | <b>11.6291</b> | <b>2.426</b>   |
| Os05g03920.1        | TKL_IRAK_DUF26-lf.3 - DUF26 kinases have homology to DUF26 containing loci, expressed | 3.02        | 0.014560547        | 5.98364        | 1.25081        |
| Os12g03470.4        | alpha-N-arabinofuranosidase A, putative, expressed                                    | 3.02        | 0.028865128        | 2.99124        | 0.626884       |
| Os09g28050.1        | aspartate aminotransferase, putative, expressed                                       | 3.01        | 0.00959124         | 13.2161        | 2.7631         |
| Os11g03780.2        | alpha-N-arabinofuranosidase, putative, expressed                                      | 3.00        | 0.011278122        | 8.13877        | 1.71027        |
| Os04g33590.1        | hydrolase, alpha/beta fold family protein, putative, expressed                        | 2.99        | 0.044269298        | 3.8648         | 0.816558       |
| Os11g40090.2        | A49-like RNA polymerase I associated factor family protein, expressed                 | 2.98        | 0.024667002        | 5.07588        | 1.07563        |
| Os06g10750.1        | integral membrane protein DUF6 containing protein, expressed                          | 2.96        | 0.01881857         | 7.51249        | 1.59882        |
| Os08g35740.1        | 12-oxophytodienoate reductase, putative, expressed                                    | 2.95        | 0.016204039        | 9.46148        | 2.02497        |
| Os09g31130.1        | citrate transporter, putative, expressed                                              | 2.94        | 0.041760309        | 2.64974        | 0.568961       |
| Os03g44900.1        | CCR4-NOT transcription factor, putative, expressed                                    | 2.93        | 0.043888981        | 2.01017        | 0.432212       |

|                     |                                                                                   |             |                    |                |                |
|---------------------|-----------------------------------------------------------------------------------|-------------|--------------------|----------------|----------------|
| Os09g21230.2        | AMP-binding enzyme, putative, expressed                                           | 2.87        | 0.011871401        | 11.3363        | 2.49409        |
| <u>Os05g46480.1</u> | <u>late embryogenesis abundant protein, group 3, putative, expressed (OsLEA3)</u> | <u>2.84</u> | <u>0.008633078</u> | <u>70.9919</u> | <u>15.7612</u> |
| Os01g63990.2        | hydrolase, alpha/beta fold family protein, putative, expressed                    | 2.84        | 0.011984955        | 20.3489        | 4.51509        |
| Os01g59000.1        | cytochrome P450, putative, expressed                                              | 2.84        | 0.026288698        | 6.02527        | 1.33845        |
| Os02g52560.1        | xyloglucan fucosyltransferase, putative, expressed                                | 2.83        | 0.019421299        | 7.09289        | 1.57996        |
| Os10g41060.1        | expressed protein                                                                 | 2.81        | 0.034573323        | 4.15975        | 0.932969       |
| <b>Os12g03040.1</b> | <b>NAC domain transcription factor</b>                                            | <b>2.80</b> | <b>0.022774518</b> | <b>7.96675</b> | <b>1.79473</b> |
| Os04g35060.1        | nicotinate phosphoribosyltransferase family domain containing protein, expressed  | 2.80        | 0.026759488        | 5.27013        | 1.18742        |
| Os08g03290.1        | glyceraldehyde-3-phosphate dehydrogenase, putative, expressed                     | 2.79        | 0.007669592        | 174.12         | 39.3703        |
| Os07g12150.1        | acyl carrier protein, putative, expressed                                         | 2.76        | 0.040950598        | 11.6442        | 2.65945        |
| Os05g42210.1        | serine/threonine-protein kinase receptor precursor, putative, expressed           | 2.75        | 0.018907713        | 6.76181        | 1.54937        |
| <b>Os09g11460.2</b> | <b>AP2 domain containing protein, expressed</b>                                   | <b>2.75</b> | <b>0.024284631</b> | <b>9.94016</b> | <b>2.27893</b> |
| Os06g24730.1        | hydrolase, alpha/beta fold family domain containing protein, expressed            | 2.75        | 0.009674252        | 47.9783        | 11.0139        |
| Os06g41930.3        | zinc-binding protein, putative, expressed                                         | 2.73        | 0.036007727        | 6.44591        | 1.48721        |
| Os06g47200.1        | LTPL85 - Protease inhibitor/seed storage/LTP family protein precursor, expressed  | 2.70        | 0.027390758        | 20.0421        | 4.6762         |
| Os02g56850.1        | glutathione reductase, putative, expressed                                        | 2.70        | 0.019441752        | 11.4648        | 2.68255        |
| Os01g50450.1        | expressed protein                                                                 | 2.68        | 0.023521504        | 14.7349        | 3.46828        |
| Os07g13270.1        | SNF7 domain containing protein, putative, expressed                               | 2.64        | 0.025151051        | 18.5537        | 4.43033        |

|                     |                                                                                |             |                    |                |                |
|---------------------|--------------------------------------------------------------------------------|-------------|--------------------|----------------|----------------|
| Os04g49757.1        | purine permease, putative, expressed                                           | 2.63        | 0.037495399        | 7.75265        | 1.86432        |
| Os02g15860.1        | expressed protein                                                              | 2.62        | 0.015275712        | 187.296        | 45.0737        |
| Os01g62060.1        | plant-specific domain TIGR01589 family protein, expressed                      | 2.58        | 0.040598341        | 17.8803        | 4.36682        |
| Os03g56460.1        | glucose-6-phosphate isomerase, putative, expressed                             | 2.57        | 0.039210869        | 5.37327        | 1.32074        |
| Os04g55720.2        | D-3-phosphoglycerate dehydrogenase, chloroplast precursor, putative, expressed | 2.55        | 0.021651388        | 16.1942        | 4.00543        |
| Os01g17190.1        | OsCam3 - Calmodulin, expressed                                                 | 2.54        | 0.02590334         | 33.2291        | 8.24689        |
| Os05g46560.2        | RAN GTPase-activating protein 1, putative, expressed                           | 2.53        | 0.033625822        | 8.10648        | 2.0235         |
| Os01g07950.1        | OsGrx_S15.2 - glutaredoxin subgroup II, expressed                              | 2.53        | 0.03846351         | 15.8821        | 3.96206        |
| Os04g56160.1        | plasma membrane ATPase, putative, expressed                                    | 2.53        | 0.031998692        | 5.10151        | 1.27298        |
| Os01g58380.1        | 3-hydroxybutyryl-CoA dehydrogenase, putative, expressed                        | 2.51        | 0.028660723        | 18.6086        | 4.66751        |
| Os06g48500.1        | expressed protein                                                              | 2.51        | 0.031334088        | 13.4713        | 3.38703        |
| Os05g24550.4        | Papain family cysteine protease domain containing protein, expressed           | 2.50        | 0.017546457        | 117.503        | 29.6923        |
| Os03g05310.1        | pheophorbide a oxygenase, chloroplast precursor, putative, expressed           | 2.48        | 0.021235417        | 27.755         | 7.05028        |
| Os10g35070.1        | alpha-galactosidase precursor, putative, expressed                             | 2.46        | 0.019744005        | 103.457        | 26.5642        |
| Os01g73170.1        | peroxidase precursor, putative, expressed                                      | 2.45        | 0.041846292        | 10.9312        | 2.81781        |
| Os04g55720.1        | D-3-phosphoglycerate dehydrogenase, chloroplast precursor, putative, expressed | 2.44        | 0.024351298        | 25.7436        | 6.64834        |
| <b>Os10g31330.1</b> | <b>retrotransposon protein, putative, unclassified, expressed</b>              | <b>2.39</b> | <b>0.025256249</b> | <b>86.8535</b> | <b>22.9056</b> |
| Os02g07260.1        | phosphoglycerate kinase protein, putative, expressed                           | 2.37        | 0.026635993        | 53.5015        | 14.2099        |
| Os04g38870.4        | 14-3-3 protein, putative, expressed                                            | 2.34        | 0.040467828        | 23.7275        | 6.38233        |

|              |                                                                                               |       |             |         |         |
|--------------|-----------------------------------------------------------------------------------------------|-------|-------------|---------|---------|
| Os08g33710.1 | ribonuclease T2 family domain containing protein, expressed                                   | 2.34  | 0.035044134 | 33.4444 | 9.00889 |
| Os02g32520.2 | ERD1 protein, chloroplast precursor, putative, expressed                                      | 2.33  | 0.03683252  | 14.9923 | 4.05293 |
| Os06g07760.1 | sulfiredoxin-1, putative, expressed                                                           | 2.33  | 0.046904416 | 29.9865 | 8.1148  |
| Os06g03800.1 | pollen ankyrin, putative, expressed                                                           | 2.32  | 0.045719504 | 12.2605 | 3.33104 |
| Os01g10890.1 | CAMK_KIN1/SNF1/Nim1_like.8 - CAMK includes calcium/calmodulin dep. protein kinases, expressed | 2.31  | 0.039219237 | 18.5848 | 5.07691 |
| Os05g47700.1 | LTPL152 - Protease inhibitor/seed storage/LTP family protein precursor, expressed             | 2.30  | 0.042697449 | 52.4999 | 14.3715 |
| Os08g25720.1 | pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha, putative, expressed   | 2.30  | 0.048255047 | 8.9484  | 2.45378 |
| Os06g29180.1 | erythronate-4-phosphate dehydrogenase domain containing protein, expressed                    | 2.30  | 0.033926849 | 44.5829 | 12.2327 |
| Os01g68300.1 | expressed protein                                                                             | 2.22  | 0.035781973 | 1111.02 | 315.67  |
| Os02g52390.1 | M16 domain containing zinc peptidase, putative, expressed                                     | 2.20  | 0.039703736 | 44.7604 | 12.8433 |
| Os07g05360.1 | photosystem II 10 kDa polypeptide, chloroplast precursor, putative, expressed                 | 2.18  | 0.045788927 | 59.2679 | 17.1484 |
| Os12g33120.1 | expressed protein                                                                             | 2.17  | 0.045013224 | 237.343 | 68.9371 |
| Os01g37910.1 | vacuolar-processing enzyme precursor, putative, expressed                                     | 2.15  | 0.04915593  | 48.0585 | 14.1222 |
| Os03g57220.2 | hydroxyacid oxidase 1, putative, expressed                                                    | -2.11 | 0.04973388  | 120.76  | 160.463 |
| Os08g44680.1 | photosystem I reaction center subunit II, chloroplast precursor, putative, expressed          | -2.11 | 0.048774331 | 646.373 | 859.146 |
| Os09g00999.1 | expressed protein                                                                             | -2.16 | 0.042040381 | 624.184 | 849.324 |
| Os07g09800.1 | expressed protein                                                                             | -2.19 | 0.043369594 | 41.2813 | 57.066  |
| Os04g21350.1 | flowering promoting factor-like 1, putative, expressed                                        | -2.22 | 0.039080205 | 81.6275 | 114.133 |

|              |                                                                                           |       |             |         |         |
|--------------|-------------------------------------------------------------------------------------------|-------|-------------|---------|---------|
| Os08g01380.1 | 2Fe-2S iron-sulfur cluster binding domain containing protein, expressed                   | -2.23 | 0.036387607 | 104.838 | 147.563 |
| Os04g16872.1 | photosystem II D2 protein, putative, expressed                                            | -2.29 | 0.039753954 | 14.3788 | 20.7646 |
| Os03g18770.1 | wound-induced protein WI12, putative, expressed                                           | -2.32 | 0.037385003 | 17.4302 | 25.5421 |
| Os08g01170.1 | acetyltransferase, GNAT family, putative, expressed                                       | -2.34 | 0.047340648 | 9.44318 | 13.9644 |
| Os01g58049.1 | photosystem I assembly protein ycf4, putative, expressed                                  | -2.36 | 0.038341618 | 15.4432 | 23.0173 |
| Os01g43980.1 | retrotransposon protein, putative, unclassified, expressed                                | -2.36 | 0.042151575 | 1.88686 | 2.81276 |
| Os12g35465.1 | expressed protein                                                                         | -2.36 | 0.040741528 | 6.94725 | 10.3569 |
| Os06g15360.3 | RAD23 DNA repair protein, putative, expressed                                             | -2.38 | 0.044872431 | 5.14721 | 7.72242 |
| Os12g02340.2 | LTPL14 - Protease inhibitor/seed storage/LTP family protein precursor, expressed          | -2.38 | 0.049365188 | 12.9394 | 19.4327 |
| Os11g35710.1 | cycloartenol synthase, putative, expressed                                                | -2.39 | 0.033210561 | 4.83313 | 7.29655 |
| Os02g32250.1 | retrotransposon protein, putative, unclassified, expressed                                | -2.40 | 0.029254255 | 5.09024 | 7.69633 |
| Os06g16330.1 | BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed | -2.41 | 0.037727183 | 3.61924 | 5.49781 |
| Os01g01120.1 | enolase-phosphatase E1, putative, expressed                                               | -2.42 | 0.028980149 | 13.3159 | 20.327  |
| Os08g11470.1 | transposon protein, putative, CACTA, En/Spm sub-class, expressed                          | -2.42 | 0.040771816 | 2.54775 | 3.89277 |
| Os02g01590.2 | glycosyl hydrolases, putative, expressed                                                  | -2.42 | 0.038144132 | 3.89457 | 5.9538  |
| Os07g01480.2 | oxygen evolving enhancer protein 3 domain containing protein, expressed                   | -2.43 | 0.021063133 | 65.8037 | 100.78  |
| Os12g43380.1 | thaumatin, putative, expressed                                                            | -2.45 | 0.025703885 | 23.6051 | 36.423  |

|              |                                                                                                                         |       |             |         |         |
|--------------|-------------------------------------------------------------------------------------------------------------------------|-------|-------------|---------|---------|
| Os04g16722.1 | uncharacterized protein ycf68, putative, expressed                                                                      | -2.45 | 0.018254508 | 9550.19 | 14744.2 |
| Os09g08910.1 | ATP synthase, putative, expressed                                                                                       | -2.46 | 0.024377738 | 5.18384 | 8.03774 |
| Os06g01210.1 | plastocyanin, chloroplast precursor, putative, expressed                                                                | -2.47 | 0.017359677 | 505.916 | 788.873 |
| Os04g40630.3 | BTBZ4 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with TAZ zinc finger and Calmodulin-binding domains, expressed | -2.48 | 0.040222601 | 3.98385 | 6.23537 |
| Os08g23410.1 | rubredoxin family protein, putative, expressed                                                                          | -2.49 | 0.021188627 | 22.0919 | 34.7154 |
| Os02g56940.2 | expressed protein                                                                                                       | -2.50 | 0.048028799 | 4.29532 | 6.76306 |
| Os11g02424.2 | LTPL9 - Protease inhibitor/seed storage/LTP family protein precursor, expressed                                         | -2.50 | 0.025681419 | 17.3126 | 27.3406 |
| Os01g09620.1 | zinc finger/CCCH transcription factor, putative, expressed                                                              | -2.51 | 0.026677194 | 6.04229 | 9.57034 |
| Os09g26420.4 | AP2 domain containing protein, expressed                                                                                | -2.51 | 0.020683786 | 13.4701 | 21.3636 |
| Os04g40100.1 | BTBN11 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with non-phototropic hypocotyl 3 NPH3 domain, expressed       | -2.52 | 0.028632449 | 3.80953 | 6.06242 |
| Os02g45750.1 | protein kinase domain containing protein, expressed                                                                     | -2.53 | 0.045403651 | 2.33871 | 3.73764 |
| Os10g24004.1 | expressed protein                                                                                                       | -2.54 | 0.02111726  | 27.7315 | 44.3977 |
| Os11g13890.5 | chlorophyll A-B binding protein, putative, expressed                                                                    | -2.56 | 0.018394166 | 25.1011 | 40.4963 |
| Os03g28960.1 | DNA-directed RNA polymerase subunit, putative, expressed                                                                | -2.56 | 0.03528912  | 2.1012  | 3.39128 |
| Os03g54150.1 | expressed protein                                                                                                       | -2.57 | 0.032852054 | 1.98813 | 3.21744 |
| Os06g39708.1 | photosystem II P680 chlorophyll A apoprotein, putative, expressed                                                       | -2.58 | 0.023484887 | 5.94125 | 9.65741 |
| Os03g17174.1 | PsbP, putative, expressed                                                                                               | -2.58 | 0.034992189 | 5.56692 | 9.05769 |

|              |                                                                                                              |       |             |         |         |
|--------------|--------------------------------------------------------------------------------------------------------------|-------|-------------|---------|---------|
| Os04g33660.1 | bifunctional monodehydroascorbate reductase and carbonic anhydrase nectarin-3 precursor, putative, expressed | -2.62 | 0.037315729 | 3.86508 | 6.38636 |
| Os08g17390.1 | expressed protein                                                                                            | -2.63 | 0.024950826 | 3.38554 | 5.6187  |
| Os02g40240.1 | receptor kinase, putative, expressed                                                                         | -2.63 | 0.03901775  | 1.04241 | 1.73003 |
| Os07g22650.1 | expressed protein                                                                                            | -2.63 | 0.031528124 | 4.61748 | 7.66277 |
| Os02g45520.1 | uncharacterized membrane protein, putative, expressed                                                        | -2.64 | 0.020549594 | 3.45497 | 5.74754 |
| Os01g01280.1 | expressed protein                                                                                            | -2.67 | 0.015628319 | 13.0445 | 21.9808 |
| Os04g33830.1 | membrane protein, putative, expressed                                                                        | -2.68 | 0.010216745 | 211.314 | 356.58  |
| Os12g27370.1 | expressed protein                                                                                            | -2.71 | 0.041699717 | 1.6132  | 2.75822 |
| Os07g13969.1 | expressed protein                                                                                            | -2.71 | 0.0181913   | 10.7634 | 18.4216 |
| Os12g34054.1 | mitochondrial ribosomal protein S3, putative, expressed                                                      | -2.73 | 0.049477102 | 1.26015 | 2.1663  |
| Os07g35260.1 | TKL_IRAK_DUF26-lc.9 - DUF26 kinases have homology to DUF26 containing loci, expressed                        | -2.76 | 0.019314796 | 3.40647 | 5.92903 |
| Os08g15296.1 | photosystem II reaction center protein H, putative, expressed                                                | -2.77 | 0.009029597 | 65.7876 | 114.745 |
| Os01g57968.1 | expressed protein                                                                                            | -2.77 | 0.007427768 | 1261.8  | 2207.7  |
| Os02g42810.1 | oxidoreductase, short chain dehydrogenase/reductase family domain containing protein, expressed              | -2.78 | 0.013632634 | 8.61573 | 15.1268 |
| Os05g48630.1 | expressed protein                                                                                            | -2.79 | 0.007437456 | 246.127 | 432.689 |
| Os12g41560.1 | expressed protein                                                                                            | -2.80 | 0.02902244  | 3.1284  | 5.52978 |
| Os10g21310.1 | photosystem II P680 chlorophyll A apoprotein, putative, expressed                                            | -2.80 | 0.015449955 | 4.52616 | 8.0044  |
| Os01g41710.1 | chlorophyll A-B binding protein, putative, expressed                                                         | -2.81 | 0.006905218 | 323.987 | 573.608 |
| Os08g06090.1 | zinc finger, C3HC4 type domain containing protein, expressed                                                 | -2.82 | 0.036649395 | 2.8734  | 5.11857 |
| Os03g52239.2 | homeobox domain containing protein, expressed                                                                | -2.85 | 0.020717277 | 3.22534 | 5.788   |

|               |                                                                                           |       |             |          |         |
|---------------|-------------------------------------------------------------------------------------------|-------|-------------|----------|---------|
| Os02g24598.1  | chloroplast envelope membrane protein, putative, expressed                                | -2.85 | 0.007994176 | 36.1604  | 64.9277 |
| Os04g12080.1  | TKL_IRAK_DUF26-lc.7 - DUF26 kinases have homology to DUF26 containing loci, expressed     | -2.85 | 0.041070403 | 1.31646  | 2.36718 |
| Os04g44924.1  | short-chain dehydrogenase/reductase, putative, expressed                                  | -2.86 | 0.017612658 | 4.64346  | 8.3898  |
| Os02g01140.1  | GDSL-like lipase/acylhydrolase, putative, expressed                                       | -2.87 | 0.01280911  | 6.24649  | 11.3211 |
| Os12g33946.1  | cytochrome c oxidase subunit 1, putative, expressed                                       | -2.89 | 0.014298673 | 3.79342  | 6.92534 |
| Os10g21230.1  | ATP synthase C chain, putative, expressed                                                 | -2.91 | 0.044044755 | 4.26926  | 7.82835 |
| Os09g37710.1  | NIN, putative, expressed                                                                  | -2.93 | 0.046112809 | 0.58783  | 1.08828 |
| Os05g11064.1  | expressed protein                                                                         | -2.94 | 0.028587135 | 2.32589  | 4.31324 |
| Os09g17850.1  | acetyltransferase type B catalytic subunit, putative, expressed                           | -2.95 | 0.040313841 | 1.18819  | 2.21185 |
| Os06g40640.2  | fructose-bisphosphate aldolase isozyme, putative, expressed                               | -2.97 | 0.005871929 | 18.5791  | 34.7809 |
| Os03g11250.1  | expressed protein                                                                         | -2.97 | 0.037124169 | 1.90148  | 3.56756 |
| Os02g42310.2  | OsSCP8 - Putative Serine Carboxypeptidase homologue, expressed                            | -2.98 | 0.007515762 | 8.86358  | 16.6299 |
| Os07g12800.2  | expressed protein                                                                         | -3.00 | 0.040948201 | 0.964209 | 1.82098 |
| Os12g19470.1  | ribulose bisphosphate carboxylase small chain, chloroplast precursor, putative, expressed | -3.00 | 0.006825462 | 9.71274  | 18.4053 |
| Os12g34108.1  | ATP synthase protein 9, mitochondrial, putative, expressed                                | -3.01 | 0.01931871  | 3.70372  | 7.04199 |
| Os12g16350.12 | enoyl-CoA hydratase/isomerase family protein, putative, expressed                         | -3.03 | 0.015357674 | 2.53417  | 4.84772 |
| Os01g57960.1  | retrotransposon protein, putative, unclassified, expressed                                | -3.05 | 0.003686299 | 113.058  | 217.275 |

|              |                                                                            |       |             |          |         |
|--------------|----------------------------------------------------------------------------|-------|-------------|----------|---------|
| Os04g02920.1 | leucine-rich repeat family protein, putative, expressed                    | -3.05 | 0.040133619 | 0.894626 | 1.71945 |
| Os06g15730.1 | expressed protein                                                          | -3.06 | 0.01207292  | 2.04551  | 3.94106 |
| Os01g51410.2 | glycine dehydrogenase, putative, expressed                                 | -3.06 | 0.004114518 | 12.9951  | 25.0591 |
| Os02g39570.6 | ACT domain containing protein, expressed                                   | -3.09 | 0.041130025 | 1.10089  | 2.13905 |
| Os08g02070.1 | OsMADS26 - MADS-box family gene with MIKCC type-box, expressed             | -3.09 | 0.008546826 | 6.93434  | 13.5106 |
| Os12g42250.1 | ZOS12-10 - C2H2 zinc finger protein, expressed                             | -3.11 | 0.043405939 | 1.68039  | 3.29125 |
| Os09g01000.1 | expressed protein                                                          | -3.13 | 0.002835193 | 28626.9  | 56678.2 |
| Os01g48950.1 | expressed protein                                                          | -3.14 | 0.009488916 | 9.21536  | 18.2327 |
| Os01g53330.1 | anthocyanidin 5,3-O-glucosyltransferase, putative, expressed               | -3.15 | 0.03594689  | 0.977994 | 1.94335 |
| Os02g36210.1 | ent-kaurene synthase, chloroplast precursor, putative, expressed           | -3.16 | 0.013488185 | 1.26204  | 2.51531 |
| Os11g41034.1 | expressed protein                                                          | -3.17 | 0.026471925 | 1.06521  | 2.13003 |
| Os02g24614.1 | DNA-directed RNA polymerase subunit beta, putative, expressed              | -3.18 | 0.020292773 | 1.16077  | 2.32663 |
| Os06g43350.1 | cytochrome P450, putative, expressed                                       | -3.19 | 0.021363349 | 1.41985  | 2.86078 |
| Os02g42330.2 | nitrilase, putative, expressed                                             | -3.20 | 0.031210755 | 1.40234  | 2.82859 |
| Os03g04060.1 | CHIT16 - Chitinase family protein precursor, expressed                     | -3.20 | 0.004653141 | 11.2584  | 22.7463 |
| Os04g52479.3 | peptidase, trypsin-like serine and cysteine proteases, putative, expressed | -3.22 | 0.01634472  | 1.2232   | 2.48759 |
| Os05g26070.1 | cyclin, putative, expressed                                                | -3.22 | 0.024496667 | 1.48547  | 3.01958 |
| Os11g13890.4 | chlorophyll A-B binding protein, putative, expressed                       | -3.23 | 0.002404666 | 108.795  | 221.402 |
| Os06g03580.1 | zinc RING finger protein, putative, expressed                              | -3.24 | 0.044828258 | 1.12059  | 2.29091 |
| Os01g45274.1 | carbonic anhydrase, chloroplast precursor, putative, expressed             | -3.26 | 0.002517656 | 38.5148  | 79.1304 |
| Os03g12660.1 | cytochrome P450, putative, expressed                                       | -3.32 | 0.013360285 | 1.53999  | 3.22067 |
| Os02g24100.1 | expressed protein                                                          | -3.32 | 0.044769085 | 1.86548  | 3.90163 |

|              |                                                                                                 |       |             |          |         |
|--------------|-------------------------------------------------------------------------------------------------|-------|-------------|----------|---------|
| Os04g24300.1 | OsWAK35a - OsWAK short gene, expressed                                                          | -3.32 | 0.027020786 | 0.623301 | 1.30685 |
| Os10g21190.1 | expressed protein                                                                               | -3.33 | 0.001711351 | 2445.89  | 5139.54 |
| Os11g06800.1 | retrotransposon protein, putative, unclassified, expressed                                      | -3.39 | 0.049559639 | 1.39094  | 2.97058 |
| Os11g46810.1 | retrotransposon protein, putative, unclassified, expressed                                      | -3.42 | 0.002118456 | 13.8543  | 29.8772 |
| Os01g57020.1 | expressed protein                                                                               | -3.44 | 0.003842246 | 4.58919  | 9.94446 |
| Os01g48990.2 | uncharacterized kinase mug58, putative, expressed                                               | -3.44 | 0.003696533 | 5.13813  | 11.1609 |
| Os12g34018.1 | ATP synthase protein YMF19, putative, expressed                                                 | -3.45 | 0.044235735 | 1.23426  | 2.68572 |
| Os01g44020.1 | expressed protein                                                                               | -3.45 | 0.041502973 | 2.27925  | 4.96304 |
| Os10g37180.1 | glycine cleavage system H protein, putative, expressed                                          | -3.46 | 0.001738309 | 32.6701  | 71.3354 |
| Os01g12710.2 | oxidoreductase, short chain dehydrogenase/reductase family domain containing protein, expressed | -3.47 | 0.008159118 | 1.98177  | 4.34252 |
| Os03g22200.2 | nodulin MtN3 family protein, putative, expressed                                                | -3.48 | 0.049214892 | 1.06068  | 2.32982 |
| Os12g44030.1 | purple acid phosphatase precursor, putative, expressed                                          | -3.49 | 0.011989244 | 1.94701  | 4.28544 |
| Os03g14040.2 | expressed protein                                                                               | -3.50 | 0.028420934 | 1.76265  | 3.89431 |
| Os02g22100.3 | OsRhmbd6 - Putative Rhomboid homologue, expressed                                               | -3.54 | 0.042450158 | 0.656258 | 1.47098 |
| Os12g24320.1 | ATPase 3, putative, expressed                                                                   | -3.55 | 0.018458942 | 0.924916 | 2.06871 |
| Os05g45890.2 | tRNAHis guanylyltransferase family protein, expressed                                           | -3.60 | 0.039618106 | 0.528332 | 1.20289 |
| Os10g21396.1 | NADPH-dependent oxidoreductase, putative, expressed                                             | -3.60 | 0.008362727 | 2.31162  | 5.25042 |
| Os07g01480.1 | oxygen evolving enhancer protein 3 domain containing protein, expressed                         | -3.60 | 0.03269576  | 0.946308 | 2.14243 |
| Os10g42020.1 | RALFL29 - Rapid ALkalinization Factor RALF family protein precursor, expressed                  | -3.64 | 0.002649312 | 11.3037  | 25.9462 |

|              |                                                                                          |       |             |          |         |
|--------------|------------------------------------------------------------------------------------------|-------|-------------|----------|---------|
| Os07g05480.2 | photosystem I reaction center subunit, chloroplast precursor, putative, expressed        | -3.66 | 0.003326406 | 8.08208  | 18.6791 |
| Os06g38450.1 | vignain precursor, putative, expressed                                                   | -3.66 | 0.002890345 | 4.15974  | 9.60965 |
| Os08g15322.1 | cytochrome b559 subunit alpha, putative, expressed                                       | -3.66 | 0.008488865 | 2.77526  | 6.41236 |
| Os03g34310.2 | expressed protein                                                                        | -3.67 | 0.038910641 | 0.830196 | 1.91576 |
| Os03g15460.1 | expressed protein                                                                        | -3.68 | 0.019181151 | 1.94767  | 4.51519 |
| Os11g13890.6 | chlorophyll A-B binding protein, putative, expressed                                     | -3.70 | 0.005435782 | 2.43461  | 5.68905 |
| Os01g69840.1 | expressed protein                                                                        | -3.71 | 0.002170427 | 14.9403  | 34.9671 |
| Os07g48040.1 | peroxidase precursor, putative, expressed                                                | -3.71 | 0.002173163 | 5.36435  | 12.5589 |
| Os11g40090.1 | A49-like RNA polymerase I associated factor family protein, expressed                    | -3.73 | 0.017651288 | 0.842065 | 1.98187 |
| Os12g34094.1 | NADH-ubiquinone oxidoreductase chain 4, putative, expressed                              | -3.75 | 0.028523155 | 0.860706 | 2.03713 |
| Os04g16820.1 | DNA-directed RNA polymerase subunit beta, putative, expressed                            | -3.77 | 0.009887744 | 0.555736 | 1.32178 |
| Os06g46434.1 | cytochrome c biogenesis protein ccsA, putative, expressed                                | -3.78 | 0.046199711 | 0.769943 | 1.83503 |
| Os04g10750.4 | inorganic phosphate transporter, putative, expressed                                     | -3.80 | 0.007369382 | 1.16562  | 2.7978  |
| Os08g35740.2 | 12-oxophytodienoate reductase, putative, expressed                                       | -3.80 | 0.006247429 | 1.87677  | 4.50112 |
| Os01g63990.5 | hydrolase, alpha/beta fold family protein, putative, expressed                           | -3.81 | 0.029529997 | 0.763068 | 1.82868 |
| Os03g45779.1 | expressed protein                                                                        | -3.83 | 0.012357599 | 1.6604   | 4.01537 |
| Os06g41010.3 | zinc finger A20 and AN1 domain-containing stress-associated protein, putative, expressed | -3.86 | 0.019926181 | 1.28767  | 3.1428  |
| Os10g42040.1 | expressed protein                                                                        | -3.88 | 0.030731625 | 2.21964  | 5.4372  |
| Os10g01080.1 | SOR/SNZ family protein, putative, expressed                                              | -3.89 | 0.005069858 | 2.20006  | 5.39425 |

|              |                                                                         |       |             |          |         |
|--------------|-------------------------------------------------------------------------|-------|-------------|----------|---------|
| Os06g40640.1 | fructose-bisphosphate aldolase isozyme, putative, expressed             | -3.91 | 0.001400842 | 4.08166  | 10.0541 |
| Os07g11110.1 | NAD dependent epimerase/dehydratase family protein, putative, expressed | -3.93 | 0.000675528 | 11.7843  | 29.1984 |
| Os02g45520.2 | uncharacterized membrane protein, putative, expressed                   | -3.94 | 0.00421714  | 1.15253  | 2.87185 |
| Os01g16920.1 | embryonic protein DC-8, putative, expressed                             | -3.98 | 0.006093606 | 2.35729  | 5.91148 |
| Os06g23870.2 | acyl-CoA dehydrogenase domain protein, putative, expressed              | -3.98 | 0.001086021 | 12.4808  | 31.332  |
| Os08g31410.5 | sulfate transporter, putative, expressed                                | -3.98 | 0.018120375 | 0.481531 | 1.21264 |
| Os10g38940.1 | fatty acid hydroxylase, putative, expressed                             | -3.98 | 0.012011389 | 1.32302  | 3.32473 |
| Os01g45274.4 | carbonic anhydrase, chloroplast precursor, putative, expressed          | -4.00 | 0.000390136 | 71.9813  | 181.803 |
| Os09g24200.1 | RAD23 DNA repair protein, putative, expressed                           | -4.06 | 0.011957671 | 0.943316 | 2.41856 |
| Os01g61880.2 | respiratory burst oxidase, putative, expressed                          | -4.11 | 0.001153124 | 2.15394  | 5.57829 |
| Os11g01240.1 | IBR domain containing protein, expressed                                | -4.11 | 0.021607225 | 0.542908 | 1.40751 |
| Os03g39610.2 | chlorophyll A-B binding protein, putative, expressed                    | -4.11 | 0.000332278 | 30.8309  | 79.9466 |
| Os01g13120.1 | aquaporin protein, putative, expressed                                  | -4.12 | 0.026464665 | 0.811939 | 2.1104  |
| Os04g29550.1 | RIPER2 - Ripening-related family protein precursor, expressed           | -4.12 | 0.007320102 | 2.32311  | 6.04122 |
| Os04g01780.1 | uncharacterized ACR, COG1399 family protein, expressed                  | -4.13 | 0.003012322 | 2.22556  | 5.79825 |
| Os05g37170.8 | transcription factor, putative, expressed                               | -4.14 | 0.0137674   | 0.798359 | 2.07846 |
| Os09g26210.1 | ZOS9-12 - C2H2 zinc finger protein, expressed                           | -4.16 | 0.017146048 | 1.47155  | 3.85929 |
| Os04g38600.1 | glyceraldehyde-3-phosphate dehydrogenase, putative, expressed           | -4.23 | 0.002482698 | 1.73177  | 4.6172  |

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|--------------|--------------------------------------------------------------|-------|-------------|----------|---------|
| Os01g28500.1 | SCP-like extracellular protein, expressed                    | -4.25 | 0.006643665 | 2.32969  | 6.24835 |
| Os02g46380.2 | purine permease, putative, expressed                         | -4.33 | 0.033108641 | 0.449198 | 1.21927 |
| Os03g31026.2 | retrotransposon protein, putative, unclassified, expressed   | -4.33 | 0.004295454 | 0.867985 | 2.37493 |
| Os12g33962.1 | uncharacterized tatC-like protein ymf16, putative, expressed | -4.34 | 0.048989573 | 0.531805 | 1.45633 |
| Os01g32460.1 | expressed protein                                            | -4.39 | 0.03102075  | 0.964544 | 2.67056 |
| Os12g39620.5 | disease resistance protein, putative, expressed              | -4.41 | 0.012012142 | 0.436545 | 1.21301 |
| Os01g25484.3 | ferredoxin--nitrite reductase, putative, expressed           | -4.43 | 0.000827728 | 2.18109  | 6.1005  |
| Os04g16770.1 | photosynthetic reaction center protein, putative, expressed  | -4.46 | 0.000193741 | 17.4533  | 49.0407 |
| Os06g40640.3 | fructose-bisphosphate aldolase isozyme, putative, expressed  | -4.46 | 0.000239742 | 9.6605   | 27.1746 |
| Os12g12580.2 | NADP-dependent oxidoreductase, putative, expressed           | -4.46 | 0.008349851 | 1.02209  | 2.88737 |
| Os01g51570.1 | glycosyl hydrolases family 17, putative, expressed           | -4.51 | 0.040226868 | 0.445291 | 1.26603 |
| Os08g01690.1 | retrotransposon protein, putative, unclassified, expressed   | -4.52 | 0.007619224 | 0.811031 | 2.30538 |
| Os10g21418.1 | NADPH-dependent oxidoreductase, putative, expressed          | -4.54 | 0.000351456 | 3.03207  | 8.68233 |
| Os01g62060.3 | plant-specific domain TIGR01589 family protein, expressed    | -4.57 | 0.042980678 | 0.944953 | 2.72623 |
| Os04g40530.1 | methyltransferase domain containing protein, expressed       | -4.60 | 0.001394639 | 2.6646   | 7.7336  |
| Os08g01240.1 | expressed protein                                            | -4.65 | 0.007485999 | 1.61447  | 4.73775 |
| Os01g64660.4 | fructose-1,6-bisphosphatase, putative, expressed             | -4.79 | 0.007454393 | 0.760764 | 2.29732 |
| Os08g35420.1 | photosynthetic reaction center protein, putative, expressed  | -4.81 | 7.82E-05    | 27.3698  | 83.0373 |
| Os02g01380.1 | expressed protein                                            | -4.85 | 0.005170394 | 0.670185 | 2.05088 |
| Os10g42030.1 | expressed protein                                            | -4.87 | 0.01204109  | 0.834753 | 2.56518 |
| Os12g38170.1 | osmotin, putative, expressed                                 | -4.88 | 5.60E-05    | 80.4968  | 247.673 |

|              |                                                                                               |       |             |          |         |
|--------------|-----------------------------------------------------------------------------------------------|-------|-------------|----------|---------|
| Os10g04730.1 | TKL_IRAK_DUF26-la.6 - DUF26 kinases have homology to DUF26 containing loci, expressed         | -4.96 | 0.000585907 | 1.3146   | 4.1092  |
| Os04g52260.1 | LTPL124 - Protease inhibitor/seed storage/LTP family protein precursor, expressed             | -4.96 | 0.001867307 | 2.12163  | 6.63514 |
| Os02g57180.2 | NADH dehydrogenase 1 alpha subcomplex subunit 9, mitochondrial precursor, putative, expressed | -5.12 | 0.006046562 | 0.649766 | 2.10466 |
| Os04g58050.1 | expressed protein                                                                             | -5.13 | 0.002431313 | 1.06758  | 3.45488 |
| Os01g03310.1 | BBT11 - Bowman-Birk type bran trypsin inhibitor precursor, expressed                          | -5.14 | 5.53E-05    | 19.5107  | 63.2453 |
| Os07g40690.1 | NAD dependent epimerase/dehydratase family protein, putative, expressed                       | -5.18 | 0.009711085 | 0.587754 | 1.92089 |
| Os01g55570.4 | expressed protein                                                                             | -5.20 | 0.002342732 | 1.82155  | 5.97436 |
| Os06g21980.2 | pantothenate kinase 4, putative, expressed                                                    | -5.23 | 0.044238551 | 0.307777 | 1.00544 |
| Os01g55974.3 | deoxycytidylate deaminase, putative, expressed                                                | -5.29 | 0.014567188 | 0.470402 | 1.57056 |
| Os05g10310.1 | acid phosphatase, putative, expressed                                                         | -5.37 | 0.016168211 | 0.382125 | 1.29311 |
| Os01g74160.1 | carboxyl-terminal peptidase, putative, expressed                                              | -5.37 | 0.000165502 | 3.05855  | 10.3628 |
| Os02g14170.2 | peroxidase precursor, putative, expressed                                                     | -5.38 | 0.006864916 | 0.652723 | 2.21656 |
| Os11g05190.1 | phytosulfokines precursor, putative, expressed                                                | -5.48 | 0.01678246  | 0.678707 | 2.34629 |
| Os10g41999.1 | RALFL27 - Rapid ALkalinization Factor RALF family protein precursor, expressed                | -5.52 | 0.040682104 | 0.495348 | 1.72315 |
| Os06g39120.1 | expressed protein                                                                             | -5.53 | 0.000226805 | 3.33059  | 11.6259 |
| Os12g39620.4 | disease resistance protein, putative, expressed                                               | -5.55 | 0.003196162 | 0.39917  | 1.40199 |
| Os12g24050.1 | retrotransposon protein, putative, unclassified, expressed                                    | -5.56 | 0.000491875 | 0.388465 | 1.36269 |

|              |                                                                                            |       |             |          |         |
|--------------|--------------------------------------------------------------------------------------------|-------|-------------|----------|---------|
| Os07g13770.1 | UDP-glucuronosyl and UDP-glucosyl transferase domain containing protein, expressed         | -5.73 | 0.002184869 | 0.618458 | 2.23392 |
| Os02g24642.1 | photosystem II reaction center protein K precursor, putative, expressed                    | -5.76 | 0.020955552 | 0.944201 | 3.43118 |
| Os03g14669.2 | core histone H2A/H2B/H3/H4, putative, expressed                                            | -5.81 | 2.24E-05    | 5.57868  | 20.4331 |
| Os11g13890.2 | chlorophyll A-B binding protein, putative, expressed                                       | -6.10 | 0.016302812 | 0.309686 | 1.19768 |
| Os01g42210.1 | LTPL47 - Protease inhibitor/seed storage/LTP family protein precursor, putative, expressed | -6.14 | 0.016649766 | 0.564518 | 2.18593 |
| Os02g42310.4 | OsSCP8 - Putative Serine Carboxypeptidase homologue, expressed                             | -6.16 | 0.007460782 | 0.402748 | 1.56816 |
| Os07g03750.1 | SCP-like extracellular protein, expressed                                                  | -6.26 | 0.021306065 | 0.629922 | 2.48532 |
| Os09g16950.1 | cysteine-rich receptor-like protein kinase 25 precursor, putative, expressed               | -6.47 | 0.001787905 | 0.393259 | 1.60501 |
| Os06g06980.3 | caffeoyl-CoA O-methyltransferase, putative, expressed                                      | -6.62 | 0.000382154 | 1.29041  | 5.38687 |
| Os01g65090.2 | aminotransferase, classes I and II, domain containing protein, expressed                   | -7.08 | 0.006093234 | 0.291077 | 1.31301 |
| Os01g03320.1 | BBTI2 - Bowman-Birk type bran trypsin inhibitor precursor, expressed                       | -7.11 | 2.37E-05    | 3.12949  | 14.0342 |
| Os02g51770.2 | TLD family protein, putative, expressed                                                    | -7.13 | 0.002653059 | 0.434182 | 1.96715 |
| Os04g31804.1 | OsMADS64 - MADS-box family gene with M-alpha type-box, expressed                           | -7.26 | 0.00051423  | 0.810366 | 3.70925 |
| Os04g32240.1 | retrotransposon, putative, centromere-specific, expressed                                  | -7.68 | 0.018735285 | 0.231279 | 1.12061 |
| Os10g40210.1 | retrotransposon protein, putative, Ty3-gypsy subclass                                      | -7.80 | 0.035737399 | 0.342591 | 1.68629 |
| Os03g14334.1 | expressed protein                                                                          | -7.94 | 0.015788103 | 0.239218 | 1.21521 |

|                     |                                                                                |               |                    |                  |                |
|---------------------|--------------------------------------------------------------------------------|---------------|--------------------|------------------|----------------|
| Os02g47744.3        | MYB family transcription factor, putative, expressed                           | -8.32         | 0.002155136        | 0.206919         | 1.09648        |
| Os02g21530.1        | expressed protein                                                              | -9.07         | 0.020448368        | 0.268415         | 1.53588        |
| Os10g42020.2        | RALFL29 - Rapid ALkalinization Factor RALF family protein precursor, expressed | -9.24         | 0.021519728        | 0.381216         | 2.18003        |
| Os11g26010.1        | retrotransposon protein, putative, unclassified, expressed                     | -9.89         | 0.000658558        | 0.218587         | 1.36391        |
| Os07g36080.3        | oxygen evolving enhancer protein 3 domain containing protein, expressed        | -10.04        | 1.73E-07           | 5.56036          | 35.2314        |
| Os03g13976.1        | expressed protein                                                              | -10.81        | 0.029242121        | 0.197642         | 1.38591        |
| Os01g47200.1        | retrotransposon protein, putative, unclassified, expressed                     | -12.25        | 5.76E-05           | 0.290781         | 2.2469         |
| Os08g09240.2        | autophagy-related protein, putative, expressed                                 | -12.93        | 0.016103366        | 0.1591           | 1.3233         |
| Os06g25010.1        | glycosyl hydrolase, putative, expressed                                        | -13.59        | 0.007430365        | 0.122906         | 1.05329        |
| Os01g01307.2        | translocon-associated protein beta domain containing protein, expressed        | -14.63        | 0.000460409        | 0.294688         | 2.771          |
| Os01g02790.1        | protein kinase domain containing protein, expressed                            | -18.02        | 0.000612146        | 0.089325         | 1.01503        |
| Os01g45914.1        | expressed protein                                                              | -23.65        | 1.07E-12           | 8.78029          | 130.938        |
| Os03g51530.1        | expressed protein                                                              | -27.22        | 0.000248169        | 0.162814         | 2.79487        |
| Os07g04930.1        | retrotransposon protein, putative, unclassified, expressed                     | -27.51        | 0.000226137        | 0.103328         | 1.79281        |
| Os03g26210.3        | helix-loop-helix DNA-binding domain containing protein, expressed              | -28.64        | 0.001162253        | 0.0557487        | 1.03806        |
| Os02g06215.1        | RNA-directed DNA polymerase, putative, expressed                               | -33.24        | 3.25E-06           | 0.0862936        | 1.80899        |
| <b>Os03g02670.3</b> | <b>transporter family protein, putative, expressed</b>                         | <b>-42.44</b> | <b>0.000115651</b> | <b>0.0417516</b> | <b>1.17481</b> |
| Os12g04870.1        | expressed protein                                                              | -43.17        | 9.89E-05           | 0.0913211        | 2.48629        |
| Os01g52240.1        | chlorophyll A-B binding protein, putative, expressed                           | -50.81        | 9.01E-12           | 0.255551         | 8.18971        |
| Os07g06834.1        | expressed protein                                                              | -72.95        | 1.14E-17           | 0.722018         | 33.2201        |

|              |                                                            |          |          |            |         |
|--------------|------------------------------------------------------------|----------|----------|------------|---------|
| Os12g02210.1 | RING finger protein, putative, expressed                   | -110.89  | 3.36E-12 | 0.130078   | 9.09714 |
| Os08g14195.1 | expressed protein                                          | -154.67  | 5.22E-17 | 0.0767163  | 7.48325 |
| Os07g15460.2 | metal transporter Nramp6, putative, expressed              | -185.36  | 8.47E-10 | 0.0156898  | 1.63297 |
| Os08g28010.1 | expressed protein                                          | -224.48  | 1.76E-14 | 0.0183586  | 2.5991  |
| Os03g18779.1 | expressed protein                                          | -334.14  | 1.98E-14 | 0.0244952  | 5.1619  |
| Os07g26100.1 | expressed protein                                          | -427.31  | 4.85E-09 | 0.00865809 | 2.33326 |
| Os08g29980.1 | retrotransposon protein, putative, unclassified, expressed | -818.52  | 1.24E-22 | 0.0052153  | 2.69221 |
| Os11g35300.1 | expressed protein                                          | -1027.30 | 6.27E-16 | 0.0092041  | 5.96317 |

**[00100]** ABA levels increase when plants sense drought, and ABA-dependent signaling plays a predominant role in the plant response to water deficit (Nambara and Marion-Poll, *Annu. Rev. Plant Biol.* 56:165-185, 2005; Zhu, *Annu. Rev. Plant Biol.* 53:247-273, 2002). ABA contents were compared between A36 and B7-12 seedlings after drought treatment. Two-week-old seedlings were air-dried for 0, 1, 3 or 4 hours in a growth chamber (23°C). Fully-expanded leaves (~100 mg FW) were harvested from the treated seedlings and immediately frozen in liquid nitrogen. After lyophilization, the dried materials were weighed and then ground in liquid nitrogen. ABA extraction was performed in darkness for 16 hours at 4°C with extraction buffer (80% methanol, 100 mg/L butylated hydroxytoluene and 500 mg/L citric acid monohydrate). Quantification of ABA was carried out using a Phytodetek ABA ELISA kit (Agdia Inc., Elkhart, IN) following the manufacturer's instructions. No significant difference was observed despite dramatically elevated levels of ABA observed in the treated seedlings of both genotypes (FIG. 6E). The results suggest that the heightened expression of drought/ABA-responsive genes described above is likely due to an increased ABA sensitivity in XA21 plants.

**[00101]** Since the infection of rice by any *Xoo* strains causes water stress, activation of XA21 by drought raised the possibility of some degree of non-race-specific defense against this bacterial pathogen. Plants were inoculated with *Xoo* strains at the seedling or adult stages using the leaf-clipping method (Kauffman, *et al.*, *Plant Disease Rep.* 57:537-541, 1973). For the adult inoculation, plants were grown in the greenhouse for 6 weeks, transferred to a controlled facility and clipped with scissors dipped in the *Xoo* inoculum. Seedling inoculation was performed at the 2-week-old stage. After inoculation, seedlings were cultured in water in growth chambers

(27°C, under florescent light with light/dark photoperiod of 16/8) for the indicated time period. For hormone and water stress treatment assays, inoculated seedlings were grown in water supplemented with ABA and PEG, respectively. Disease lesion development and bacterial population were determined as described previously (Xu, *et al.*, *Plant J.* 45:740-751, 2006).

5       **[00102]** Strain DY87031 possesses mutations in the sulfenylation system required by *Xoo* to trigger *Xa21*-mediated resistance (Burdman, *et al.*, *Mol. Plant Microbe Interact.* 17:602-612, 2004) and induced similar lesion developments between A36 and B7-12 plants. Water stress was further enhanced by incubating challenged seedlings with polyethylene glycol (PEG), a nonionic water-soluble polymer widely used to simulate drought in plants (Lagerwerff, *et al.*,  
10   *Science* 133:1486-1487, 1961). Beginning at 5 days post-inoculation (dpi), B7-12 seedlings repeatedly showed a reduction in disease progression relative to A36 (FIG. 7A for the data at 7 dpi). Growth of *Xoo* in B7-12 was also at lower levels than that seen in A36 (FIG. 7B). However, at 10 dpi, lesion had spread to the entire length of infected B7-12 leaves. Therefore, cumulative drought stress does elicit an XA21-dependent defense response capable of providing  
15   partial protection against *Xoo*. Notably, this defense mechanism might help explain the puzzling phenomenon that, under high temperatures in greenhouses (presumably elevating the water stress of infected leaves), adult IRBB21 plants (containing *Xa21*) exhibited shorter lesions than IR24 controls after challenge with compatible *Xoo* strains of different origins (Gu, *et al.*, *Theor. Appl. Genet.* 108:800-807, 2003).

20       **[00103]** The interplay between drought and XA21 resistance in the context of incompatible interactions was also examined. Treatment of B7-12 seedlings with PEG suppressed XA21 resistance to incompatible PXO99A as evidenced by the reduction in lesion lengths and bacterial growth (FIG. 8A and FIG. 8B). Likewise, high temperatures appear to have a negative impact on XA21 resistance at the adult stage (Gu, *et al.*, *Theor. Appl. Genet.*  
25   108:800-807, 2003). The apparent paradoxical effects of PEG/drought on XA21 defense and resistance responses would suggest that distinct defense signaling pathways are initiated by XA21 after *Xoo* infection (FIG. 9).

**[00104]** Treatment of rice seedlings with PEG rapidly and significantly increases endogenous ABA accumulation (Ye, *et al.*, *Plant Cell Physiol.* 52:689-698, 2011). The ability of  
30   ABA to suppress XA21 resistance was tested by introduction of this hormone into the root zone.

ABA can be taken up by roots from soil or cultural medium and transported via xylem to leaf blades (Schraut, *et al.*, *J. Exp. Bot.* 55:1635-1641, 2004; Sauter, *et al.*, *J. Exp. Bot.* 52:1991-1997, 2001). Application of ABA significantly compromised XA21 resistance in a dosage dependent manner (FIG. 8C and FIG. 8D). The suppression effect can be attributed to the positive role of ABA in enhancing *Xoo* virulence (Xu, *et al.*, *BMC Plant Biol.* 15:10, 2015; Xu, *et al.*, *PLoS One* 8:e67413, 2013) and the potentially negative impacts of the hormone on XA21 signaling. Support for the later possibility comes from the well-established suppression effects of ABA on plant defense events (Mang, *et al.*, *Plant Cell* 24:1271-1284, 2012).

#### Example 9 - XB3 functions as a drought regulator.

[00105] The inventors previously showed that XB3 interacts with XA21 *in planta* (Wang, *et al.*, 2006, *supra*). Interestingly, *Xb3* transcripts were markedly induced by drought stress in wild-type cultivar TP309 (carrying no *Xa21*). Stress treatment of previously characterized RNA interference (RNAi) lines, A13 and 37-2 (Wang, *et al.*, 2006, *supra*), revealed that down-regulation of *Xb3* increased drought sensitivity (FIG. 7A). The inventors predicted that over-expression of *Xb3* might enhance rice tolerance to drought. To test this, a 3xFLAG tagged *Xb3* was placed in an expression cassette under the control of the maize ubiquitin promoter. More than 50 independent transgenic rice lines were generated, and found to represent a range of transgene expression based on RNA blot analysis. Six transgenic lines with moderate and high levels of *Xb3* transcripts were randomly chosen for further characterization. Importantly, all plants were similar in size when grown under normal conditions. By contrast, drastically enhanced abilities to survive severe drought stress and to maintain water status were observed in *Xb3* over-expression transgenic plants compared with the control lines A36 and TP309 (FIG. 7B, FIG. 7C). Notably, the extent of damage caused by the stress was inversely correlated with levels of *Xb3* expression. These results indicate that accumulation of *Xb3* transcript levels improves drought tolerance in rice.

#### Example 10 - XB3 is localized to both the plasma membrane and the nucleus.

[00106] In this Example, a monopartite nuclear localization signal (NLS) was identified in XB3-C that is in addition to other previously reported domains (FIG. 8; Wang, *et al.*, 2006, *supra*). Based on this and the presence of the putative N-myristoylation site (a membrane targeting signal, FIG. 8), the inventors predicted that XB3 is localized in both the plasma

membrane and the nucleus. This hypothesis was tested by performing confocal microscopic analysis using rice protoplasts expressing fluorescently tagged XB3 fusion proteins under the control of a double cauliflower mosaic virus (CaMV) 35S promoter. To avoid disturbance of the putative membrane localization, enhanced green fluorescent protein (eGFP) was placed in-frame with XB3 between Thr10 and Gly11 (downstream of the N-myristoylation site). Transient co-expression of eGFP-XB3 with *Discosoma* sp. red fluorescent protein (DsRed)-nuclear localization signal fusion (DsRed-NLS) resulted in rice protoplasts with yellow nuclei. *N*-(3-Triethylammoniumpropyl)-4-(6-(4-(Diethylamino) Phenyl) Hexatrienyl) Pyridinium Dibromide (FM4-64) dye has been widely used to stain plasma membrane (red color) and visualize endocytosis (Vida and Emr, *J. Cell Biol.* 128:779-792, 1995). FM4-64 staining of rice protoplasts expressing eGFP-XB3 confirmed the plasma membrane localization of the fusion protein. To demonstrate co-localization of XB3 and XA21, the fluorescent tags mCherry and eGFP were fused to the C-termini of these two proteins, respectively. Consistent with the observations made for eGFP-XB3, red XB3-mCherry signals were clearly seen in the plasma membrane and the nucleus. Green fluorescence was detected in the plasma membrane and an ER-like compartment as previously reported (Park, *et al.*, *PLoS One* 5:e9262, 2010). Co-localization of XB3-mCherry and XA21-eGFP in the plasma membrane was evidenced by the yellow color that resulted from superimposed single color images of the two fluorescent proteins. By contrast, mutations of the predicted NLS and the putative N-myristoylation residue Gly-2 eliminated the fluorescent signals from the nucleus and the plasma membrane, respectively. As a control, GFP was present in both the cytoplasm and the nucleus.

**[00107]** To confirm the subcellular localizations of functional XB3 expressed by its native promoter *in planta*, the nuclear and membrane fractions were purified from leaf tissues of TP309 and the Myc-XA21 line 4021-3, respectively. 4021-3, rather than double-tagged B7-12, plants were selected for protein-related experiments because of the relatively easy detection of XA21 by anti-c-Myc in this line coupled with the successful development of a monoclonal antibody that recognizes the C-terminal kinase domain of XA21 in the later stage of this study. As expected, XB3 was detected in both the nuclei-enriched and nuclei-depleted fractions. Fractionations were validated by the nuclear marker histone H3 and the chloroplast protein ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco, nuclei-depleted). XB3 was also found in the microsomal pool of 4021-3 plants, in which membrane-localized XA21, but not the

cytoplasmic marker UGPase, was present. Purification of the microsomal fraction led to XA21 degradation as evidenced by the accumulation of XA21<sup>nep</sup> observed previously (Xu, *et al.*, 2006, *supra*). The presence of XB3 in the cytosolic fraction (S) might be due to a leak from the nuclei and a release from the XA21 complex through cleavage during purification. Taken together, these results indicate that XB3 is co-localized with XA21 to the plasma membrane and also accumulates in the nucleus.

#### **Example 11 - XA21 mediates a higher steady-state level of XB3.**

[00108] Because *Xb3* is required for full accumulation of XA21 in rice (Wang, *et al.*, 2006, *supra*), it was examined whether the opposite scenario also holds true. It was found that abundance is increased in the Myc-XA21 line 4021-3 relative to the empty-vector control line A36. Furthermore, epitope-tagged XB3-3xFLAG was co-expressed with Myc-XA21 in *N. benthamiana* using a well-established transient system mediated by *Agrobacterium* transformation (Huang, *et al.*, 2013, *supra*). Consistently, XB3 accumulated to a markedly higher level when co-expressed with XA21 in the infiltrated leaves than when expressed with the empty vector.

#### **Example 12 - XA21 increases the nuclear accumulation of XB3 in rice.**

[00109] It was next determined whether XA21 regulates subcellular distributions of XB3. It was decided to focus on the nuclear abundance of XB3 because it is unlikely that nuclear trafficking of proteins is influenced by the process of protein purification. By contrast, XB3 in the membrane pool could be released by cleavage of XA21 during sample preparation (see above). In addition, a monoclonal antibody was developed, anti-XA21K, against the C-terminal intracellular domain of XA21. This antibody specifically recognized the 140 kDa Myc-XA21 in 4021-3 plants. It was determined that XB3 is readily detectable in the nuclear fraction prepared from two-week-old untreated seedlings of 4021-3 but not in that of A36 control. In the XA21 nuclear pool, the cleaved XA21<sup>ccp</sup> product of 37 kDa was also detected using anti-XA21K. XA21<sup>ccp</sup> did not react with anti-c-Myc. Thus, XA21 is cleaved constitutively to some extent *in vivo* at the seedling stage, coinciding with the accumulation of XB3 in the same subcellular compartment.

[00110] In response to water stress, a marked increase in XB3 protein levels followed by a decline was observed in the A36 control. By contrast, XB3 accumulation in the nucleus of

XA21 seedlings reached higher levels and was sustained at five hours post drought stress (hpd). These changes in protein abundance likely result from a redistribution of pre-existing XB3 since no apparent difference in *Xb3* mRNA was observed between the two lines during the time period of stress treatment, and the induction of *Xb3* transcripts by drought in seedlings occurred at seven  
5 hpd. XA21<sup>ccp</sup> was detectable after drought, but appeared to be decreased at five hpd.

**Example 13 - Drought tolerance mediated by XA21 is associated with nuclear accumulation of XB3.**

[00111] Plants, unlike mammals, lack the advanced adaptive immunity required to eliminate most infectious pathogens. This results in significant burden of invaders remaining  
10 inside the host for an extended period of time or even a lifetime. An ability to cope with pathogen-induced stresses would therefore be beneficial for infected plants. In the case of rice BLB, cumulative growth of xylem-limited *Xoo* can induce water deficit in diseased leaves. However, injury is largely reduced in plants expressing the immune receptor XA21. The simplest interpretation of this phenomenon is the lower level of *Xoo* in the resistant plants than  
15 that in susceptible individuals. The present disclosure, however, reveals a novel function of XA21, namely drought tolerance. Without being bound by any one theory, the inventors believe that XA21 carries an integrated ability to suppress bacterial over-accumulation during early stage infection and then contributes to the control of drought effects. Both of these functions serve to limit water loss injury (FIG. 11).

[00112] The present disclosure demonstrates that XB3, an E3 ubiquitin ligase associating with XA21, also acts as a drought regulator. Unlike *Xa21*, which is natural only in the wild species *O. longistaminata* (Khush, *et al.*, 1990, *supra*), *Xb3* is a member of an evolutionarily conserved plant gene family (Huang, *et al.*, 2013, *supra*). In addition to *Xb3* another member, *AdZFP1* from the drought-tolerant species *Artemisia desertorum* Spreng, has been shown to be  
25 water-stress-responsive and capable of enhancing drought tolerance when over-expressing in tobacco (Yang, *et al.*, *J. Biosci.* 33:103-112, 2008). Thus, XB3 likely represents a regulator of a conserved plant drought signaling network and the immune receptor XA21 is linked to this network through binding to XB3. Furthermore, the present results show that these two proteins are co-localized in the membrane system and that the expression of XA21 leads to a higher  
30 abundance of XB3. Therefore, XA21 appears to promote storage of XB3 under normal growth

conditions, potentially enhancing the ability of rice plants to survive drought stress. Of note, increased XB3 was not only observed in the total protein extracts, but also in the nuclei-enriched fraction from 23°C treated two-week-old seedlings that accumulate the cleaved XA21<sup>ccp</sup>. Independent studies have confirmed the proteolytic cleavage of XA21 by an unidentified protease at a site (designated XA21CS-1) near the transmembrane domain (Park, *et al.*, 2010, *supra*; Wang, *et al.*, 2006, *supra*; Jiang, *et al.*, *Plant J.* 73:814-823, 2013; Chen, *et al.*, *Mol. Plant* 7:874-892, 2014; Xu, *et al.*, 2006, *supra*; Park and Ronald, *Nat. Commun.* 3:920, 2012). In contrast to the observations made using adult plants (Park and Ronald, *Nat. Commun.* 3:920, 2012, *supra*), the present data indicate that XA21 is constitutively cleaved to some extent at the seedling stage, which provides an explanation for the XA21-dependent nuclear accumulation of XB3.

**[00113]** Without being bound to any one theory, there are a number of ways that drought conditions can be perceived by the receptor kinase XA21 in a pathogen ligand-independent manner. One possibility is that water stress induces the production of a rice protein/peptide that can be recognized by the LRR domain of XA21. Since there is no homologs of RaxX identified in the rice genome, this would imply that XA21 is capable of recognizing two distinct ligands for pathogen defense and drought response, respectively. It has been shown that the Arabidopsis damage-associated molecule AtPep1 binds to the LRR-receptor kinase AtPEPR1 and activates immune responses (Huffaker, *et al.*, *Proc. Natl. Acad. Sci. USA* 103:10098-10103, 2006; Yamaguchi, *et al.*, *Proc. Natl. Acad. Sci. USA* 103:10104-10109, 2006). An alternative scenario is that drought might induce proteolysis of XA21, which in turn leads to a release and translocation of XA21-associated drought regulators (*e.g.*, XB3) into the nucleus. However, no significant increase in XA21<sup>ccp</sup> levels was observed in the nuclei-enriched pool after drought stress treatment. By contrast, the nuclear abundance of XB3 was markedly increased after drought in an XA21-dependent manner. The distinct kinetics of XB3 and XA21<sup>ccp</sup> accumulation might reflect a difference in their stabilities in drought environments. Alternatively, XB3 might be released by a second, drought-induced cleavage of XA21 that results in short-lived intermediates. It has been well-documented in the animal system that cell-surface receptors can be activated via complex proteolysis (Kopan and Ilagan, *Cell* 137:216-33, 2009; Rawson, *Biochim. Biophys. Acta* 1828:2801-2807, 2013). Regardless of the explanation,

drought-triggered, XA21-dependent accumulation of XB3 might allow the E3 ubiquitin ligase to exceed a threshold in the nucleus leading to the degradation of its substrate(s) (FIG. 11).

[00114] XA21-mediated drought tolerance and defense likely utilize different signaling mechanisms. In response to water stress, the present studies identified 17 DEGs between XA21 and control plants known to be drought-responsive. Of these, four were subjected to q-PCR analysis to validate drought induction and differential expression. They include three *later embryogenesis abundant* (LEAs) genes (*OsLEA1*, *OsLEA3* and *OsLEA33*) and *OsNAC10*. LEAs encode hydrophilic proteins that potentially function in cellular protection during water deficit, whereas *OsNAC10* codes for a transcription factor of the NAM ATAF CUC2 (NAC) family (Xiao, *et al.*, *Theor. Appl. Genet.* 115:35-46, 2007; Jeong, *et al.*, *Plant Physiol.* 153:185-197, 2010; Battaglia, *et al.*, *Plant Physiol.* 148:6-24, 2008). Over-expression of either *OsLEA3* or *OsNAC10* in transgenic rice plants enhances drought tolerance. In contrast, no defense marker genes, including *PR10b* (*Os12g36850*), *Os04g10010* and *Os12g36830*, previously shown to be induced by RaxX treatments in an XA21-dependent manner (Pruitt, *et al.*, *Sci. Adv.* 1:e1500245, 2015), were detected as DEGs following drought. These results strongly suggest that in XA21 plants water stress triggers a heightened drought response signaling, but does not activate pathogen defense.

[00115] In conclusion, the immune sensor XA21 was surprisingly demonstrated to confer tolerance to drought. This novel function may act directly or indirectly through sensing water stress and subsequently activating of drought regulators (*e.g.*, *OsNAC10*). Based on these results, an integrated ability for XA21 to suppress *Xoo* over-accumulation during early stage infection and then to control the water deficit caused by remaining bacteria may be achieved (FIG. 9). These findings therefore unify biotic and abiotic signaling under the control of a single stress sensor, XA21.

[00116] Rice is the staple food of more than half of the population in the world. Demonstration of the drought tolerance function of XA21 allows development of rice varieties with a broad-spectrum resistance/tolerance to environmental stresses using a single gene/pathway.

\* \* \* \* \*

[00117] All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

**What is claimed is:**

Claim 1. A method of increasing drought tolerance in a plant, comprising expressing in the plant a heterologous receptor kinase XA21 coding region, wherein the drought tolerance of the plant is increased when compared to a control plant that lacks said expressing.

Claim 2. The method of claim 1, wherein said expressing comprises introducing into the plant a DNA construct comprising said heterologous receptor kinase XA21 coding region operably linked to a native receptor kinase XA21 promoter.

Claim 3. The method of claim 1, wherein said expressing comprises introducing into the plant a DNA construct comprising said heterologous receptor kinase XA21 coding region operably linked to a heterologous promoter functional in the plant.

Claim 4. The method of claim 3, wherein the promoter is a constitutive or inducible promoter.

Claim 5. The method of claim 1, wherein said heterologous receptor kinase XA21 coding region comprises a polynucleotide sequence at least 85% identical to SEQ ID NO:1.

Claim 6. The method of claim 5, wherein said heterologous receptor kinase XA21 coding region comprises a polynucleotide sequence at least 90% identical to SEQ ID NO:1.

Claim 7. The method of claim 5, wherein said heterologous receptor kinase XA21 coding region comprises a polynucleotide sequence at least 95% identical to SEQ ID NO:1.

Claim 8. The method of claim 1, wherein the plant is a monocotyledonous plant.

Claim 9. The method of claim 8, wherein the monocotyledonous plant is selected from the group consisting of maize, wheat, rice, sorghum (*Sorghum bicolor*), oats, barley, sugar cane, African oil palm (*Elaeis guineensis*), or switchgrass.

Claim 10. The method of claim 1, wherein the plant is a dicotyledonous plant.

Claim 11. The method of claim 10, wherein the dicotyledonous plant is selected from the group consisting of *Arabidopsis*, peanut (*Arachis hypogaea*), barrel medic (*Medicago truncatula*), carrot, soybean (*Glycine max*), cotton, Brassica, canola, tomato, potato, alfalfa, grape, clover, poplar, willow, eucalyptus, hemp, a *Lotus* sp., a *Vinca* sp., a *Nicotiana* sp., a *Vitis* sp., or a *Ricinus* sp.

Claim 12. A plant, or part thereof, expressing a heterologous receptor kinase XA21 coding region, wherein drought tolerance of the plant or part thereof is increased when compared to a control plant or part thereof that lacks said expressing.

Claim 13. The plant, or part thereof, of claim 12, wherein said expressing comprises introducing into the plant or part thereof a DNA construct comprising said heterologous receptor kinase XA21 coding region operably linked to a native receptor kinase XA21 promoter.

Claim 14. The plant, or part thereof, of claim 12, wherein said expressing comprises introducing into the plant or part thereof a DNA construct comprising said heterologous receptor kinase XA21 coding region operably linked to a heterologous promoter functional in the plant or part thereof.

Claim 15. The plant, or part thereof, of claim 12, wherein the part thereof is a cell, meristem, root, leaf, node, pistil, anther, flower, seed, embryo, stalk or petiole.

Claim 16. A method of producing food for human or animal consumption, comprising:

- a) obtaining the plant, or part thereof, of claim 12; and
- b) preparing food for human or animal consumption from the plant or part thereof.

Claim 17. The method of claim 16, wherein the food is starch, protein, meal, flour or grain.

Claim 18. A method of increasing drought tolerance in a rice plant, comprising introducing into the rice plant a DNA construct comprising a rice receptor kinase XA21 coding region operably linked to a heterologous promoter functional in the rice plant, wherein the drought tolerance of the rice plant is increased when compared to a control rice plant that lacks said DNA construct.

Claim 19. A method of producing a drought tolerant plant, comprising crossing the plant of claim 12 with a second plant to produce at least a first progeny plant comprising drought tolerance.

Claim 20. The method of claim 19, wherein said drought tolerant plant is a drought tolerant rice plant.

Claim 21. A method of increasing drought tolerance in a plant, comprising introducing into the plant a DNA construct comprising a heterologous receptor kinase XA21 coding region operably linked to a promoter, and selecting a progeny plant that has increased drought tolerance when compared to a control plant that lacks said DNA construct.

Claim 22. The method of claim 21, wherein said promoter is a drought-inducible promoter.

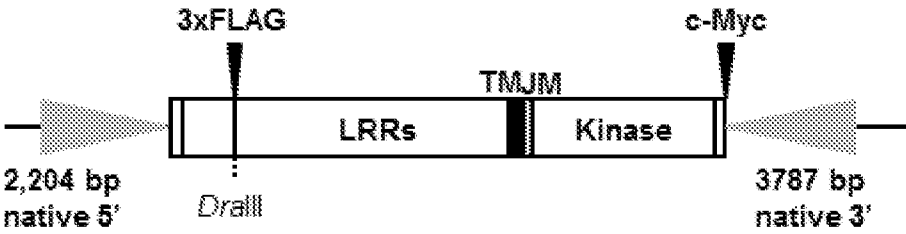


FIG. 1A

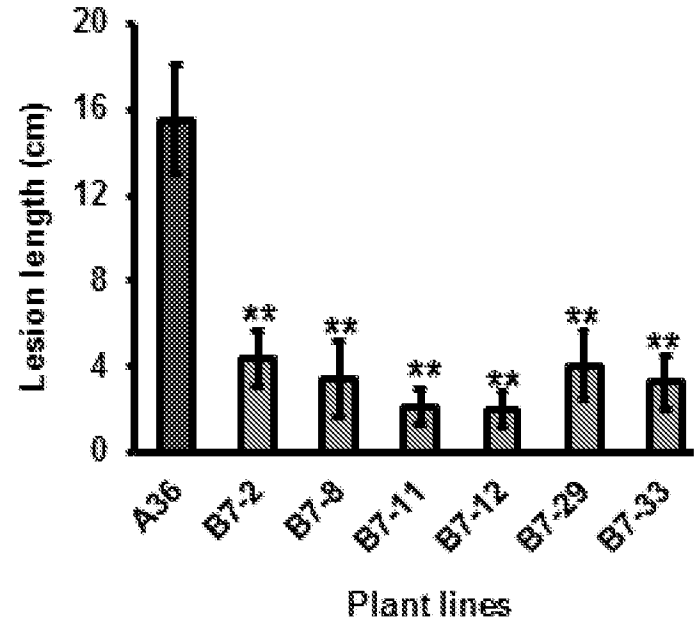


FIG. 1B

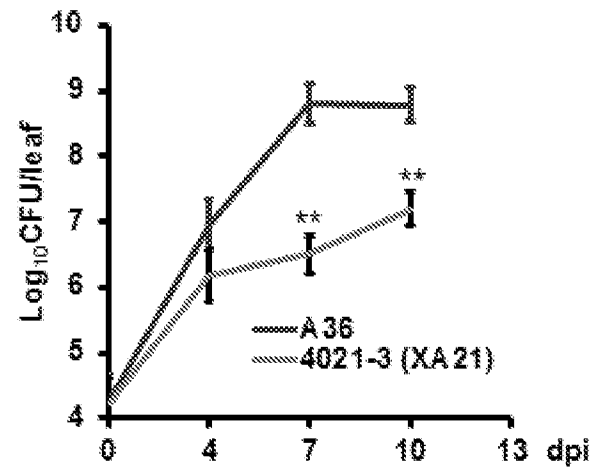


FIG. 2

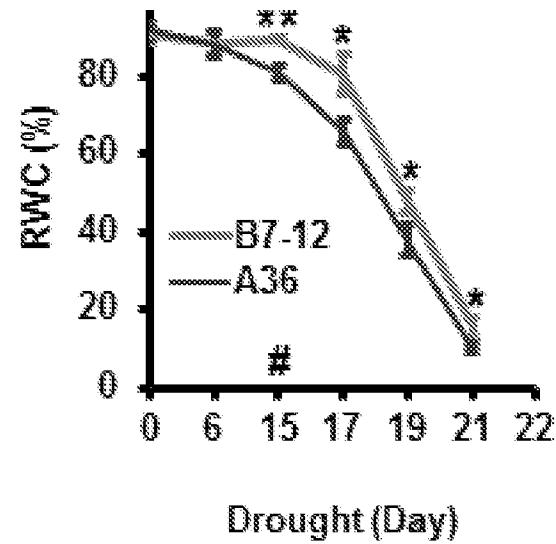


FIG. 3A

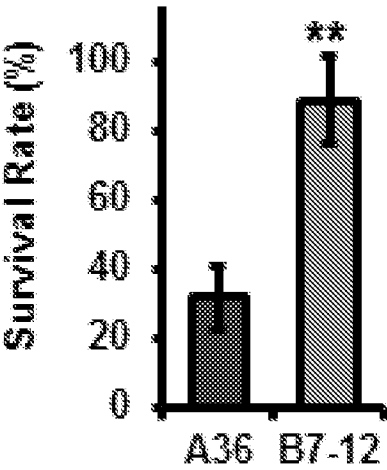


FIG. 3B

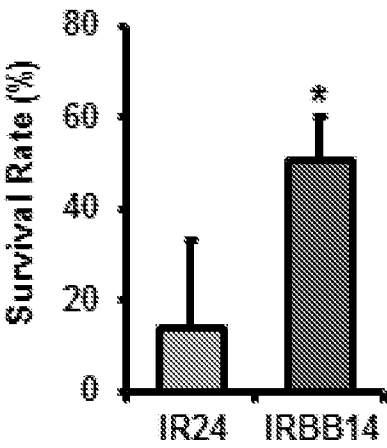


FIG. 3C

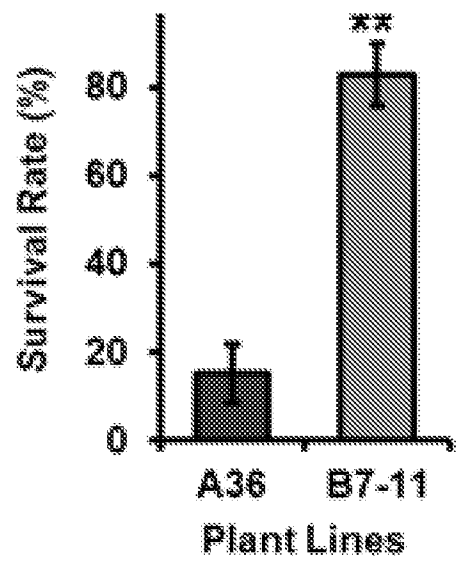


FIG. 4A

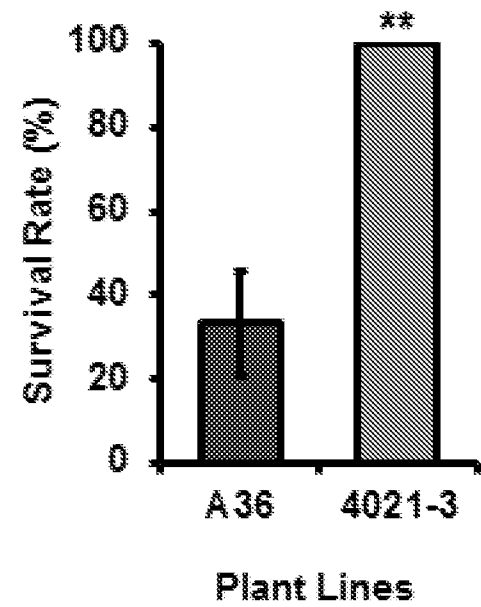


FIG. 4B

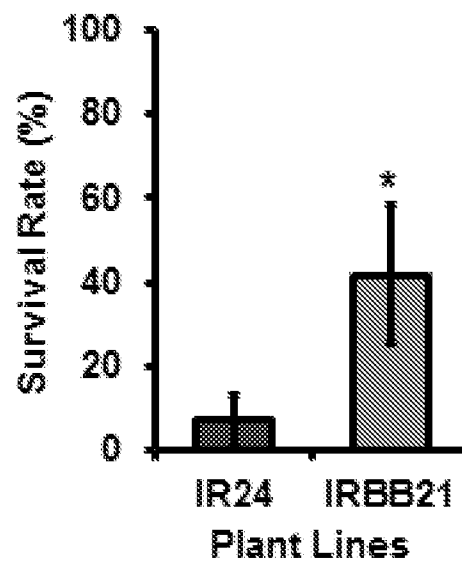


FIG. 4C

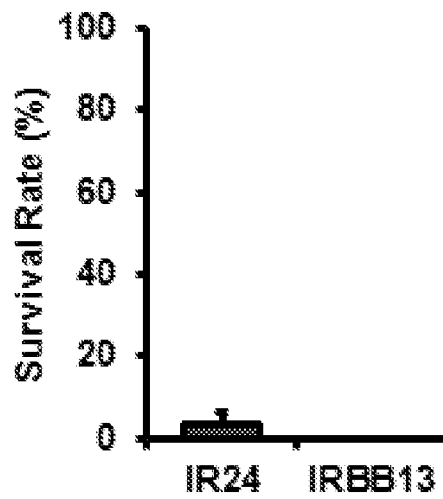


FIG. 5

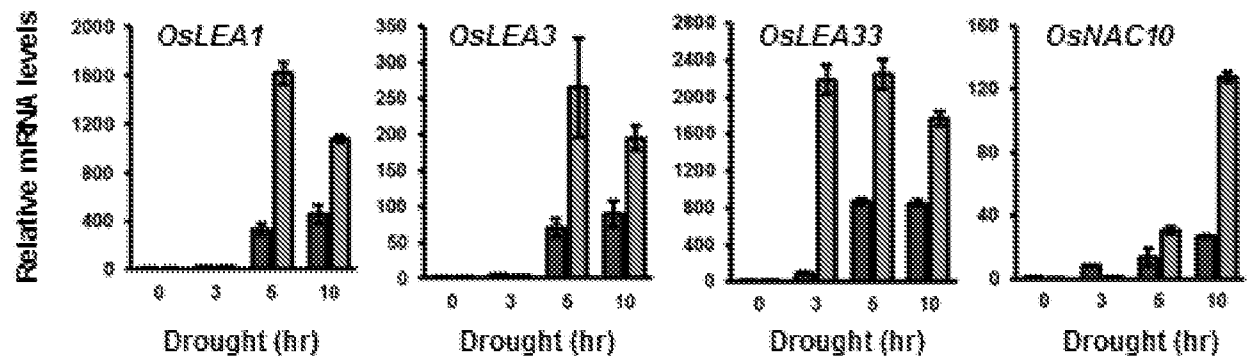


FIG. 6A

FIG. 6B

FIG. 6C

FIG. 6D

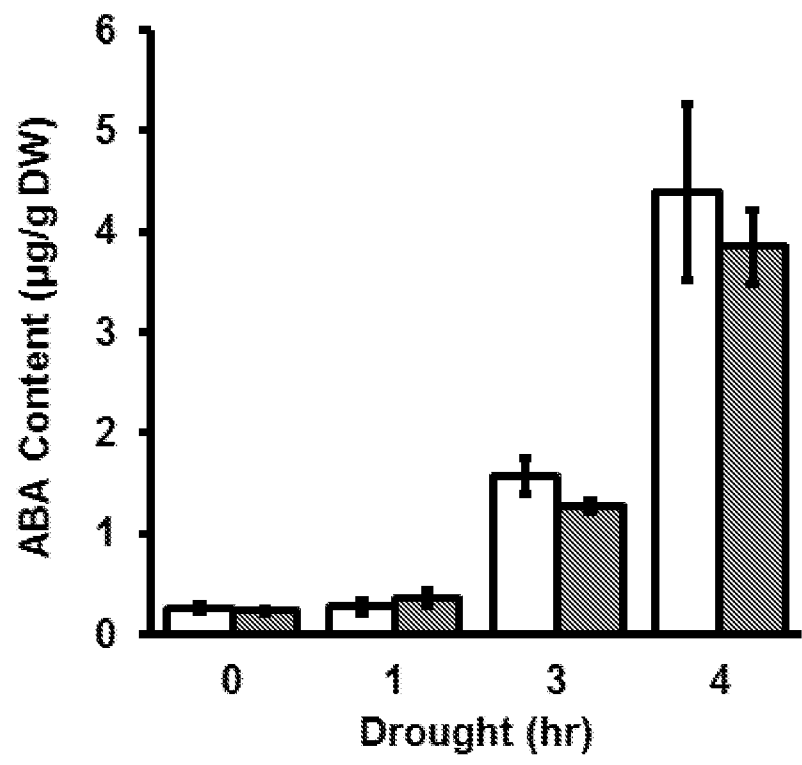


FIG. 6E

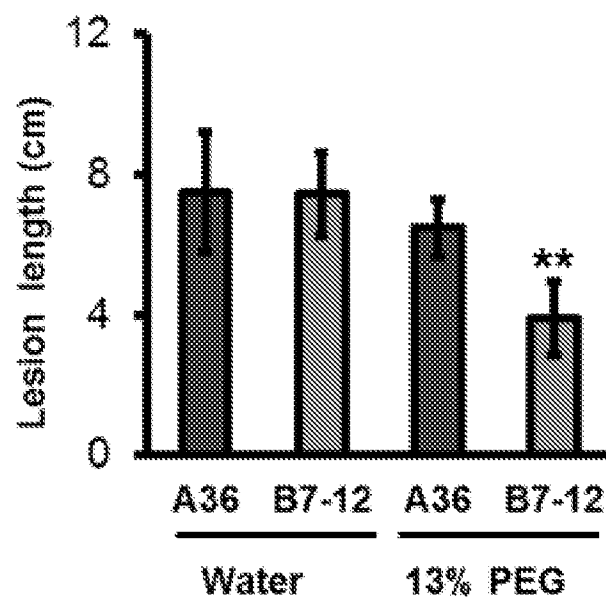


FIG. 7A

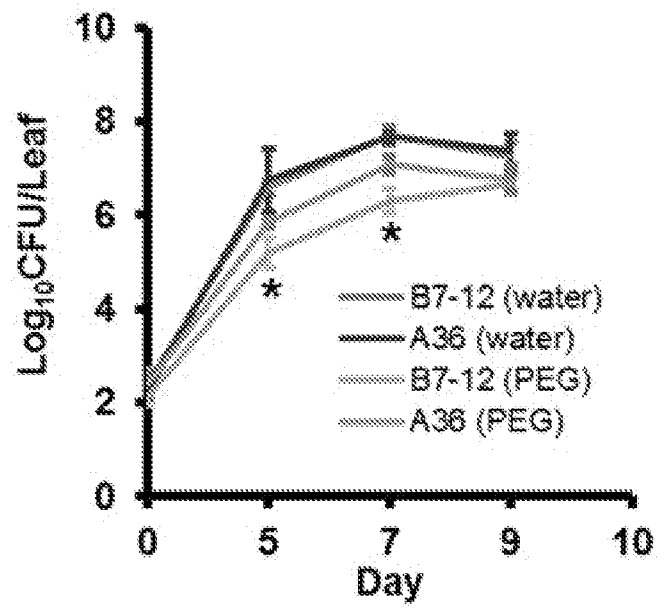


FIG. 7B

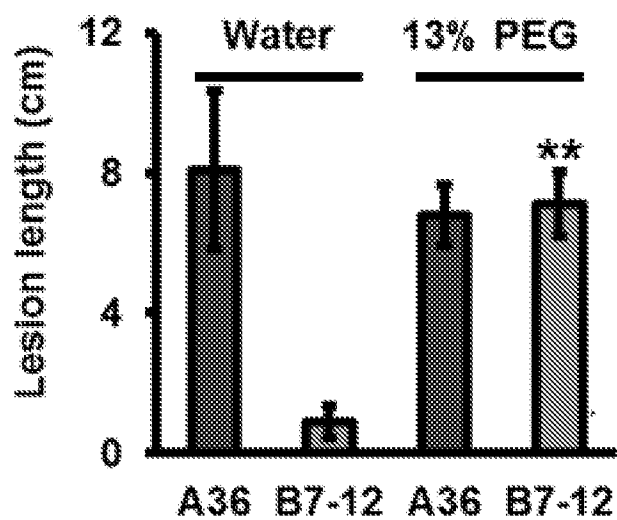


FIG. 8A

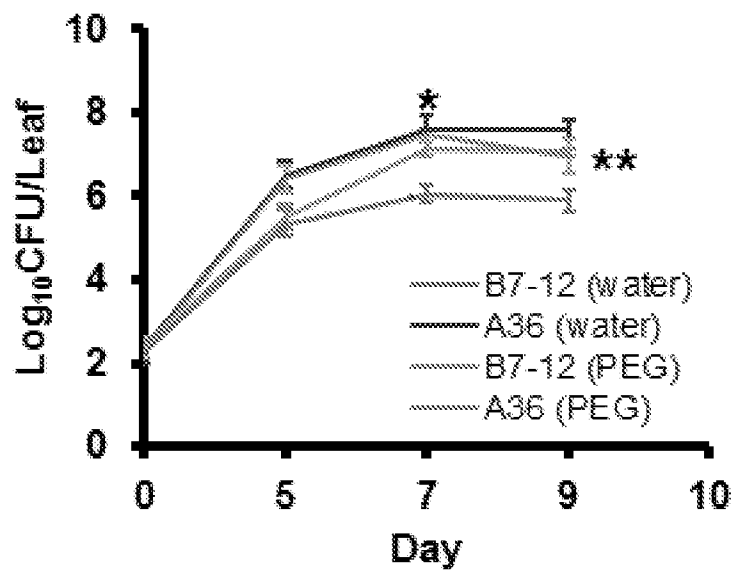


FIG. 8B

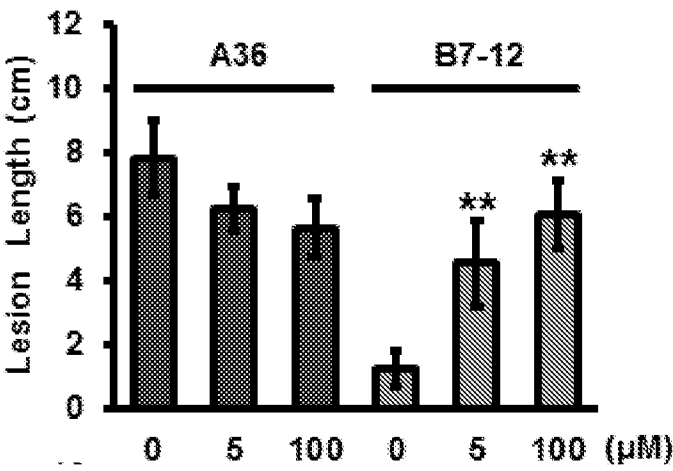


FIG. 8C

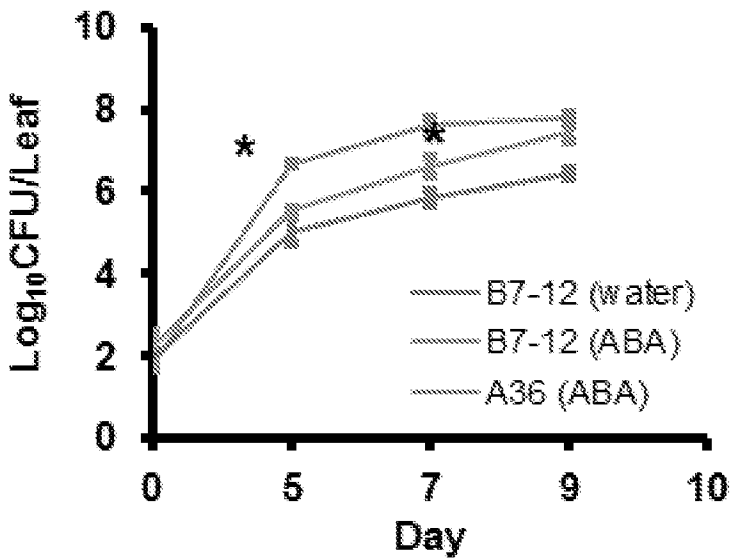


FIG. 8D

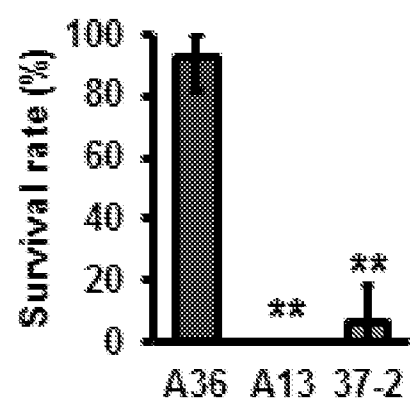


FIG. 9A

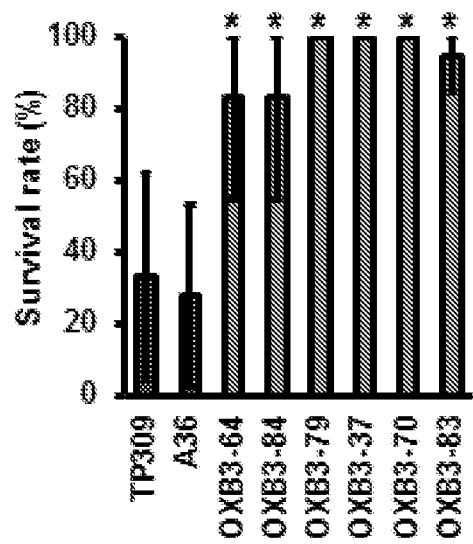


FIG. 9B

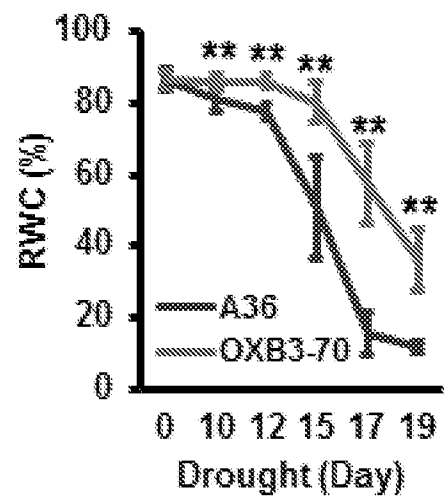


FIG. 9C

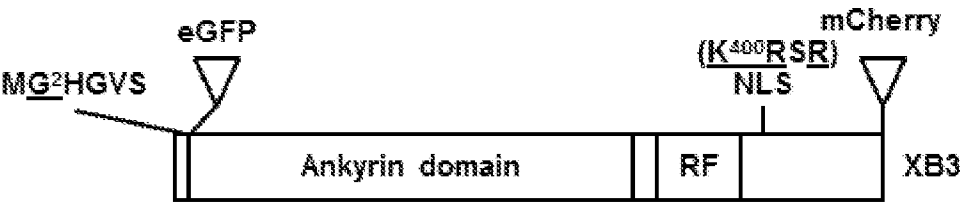


FIG. 10

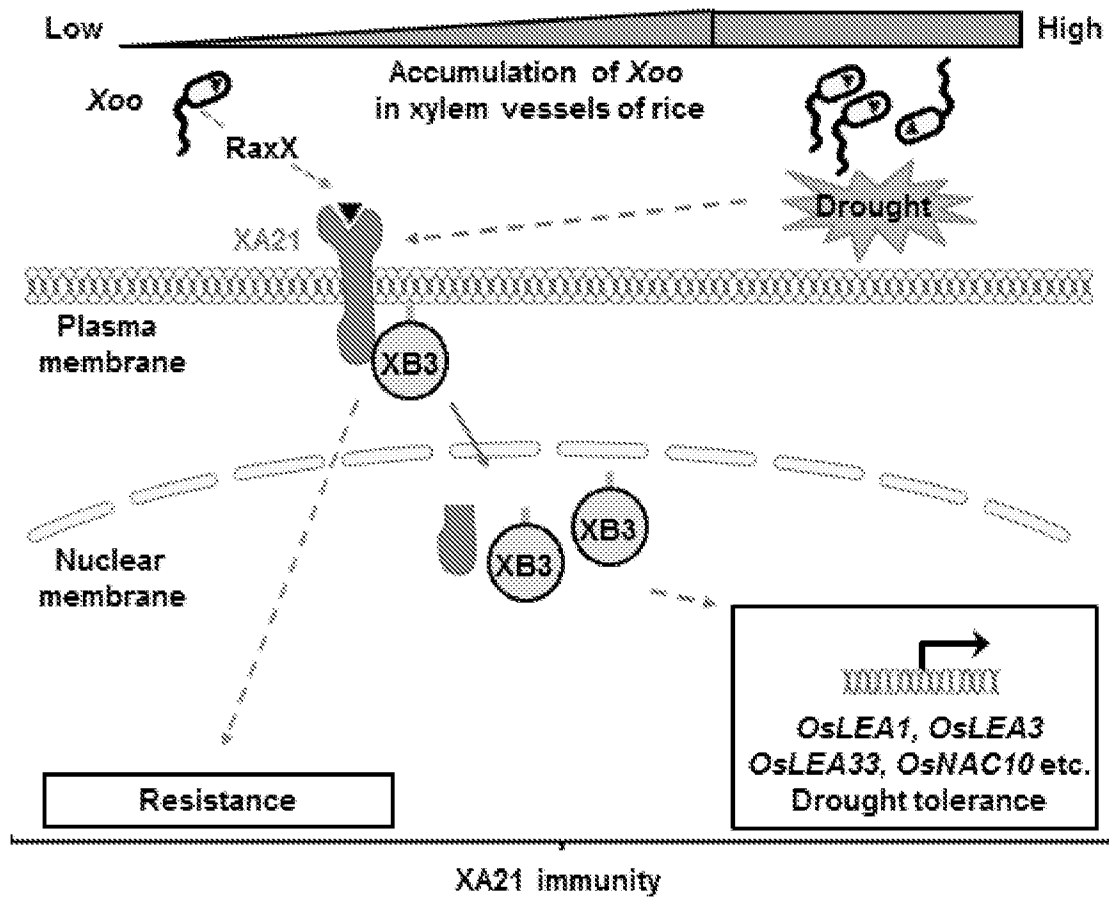


FIG. 11

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/32502

A. CLASSIFICATION OF SUBJECT MATTER  
IPC(8) - A01H 1/00, A01H 1/06, A01H 5/00, A01N 25/00 (2017.01)  
CPC - C12N 9/12, C12N 15/8282

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                              | Relevant to claim No. |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y         | WO 2015/081061 A2 (UNIVERSITY OF FLORIDA RESEARCH FOUNDATION INC.) 4 June 2015 (04.06.2015) Claim 1; Claim 8; Claim 11; Claim 16-18; p2, ln18-21; p12, ln 16-26; p12, ln26-p13, ln2; p16, ln 2-13; p23, ln 19-p24, ln 3; p23, ln 21-p 24, ln 10 | 1-22                  |
| Y         | WANG et al., Rice XA21 Binding Protein 3 Is a Ubiquitin Ligase Required for Full Xa21-Mediated Disease Resistance. Plant Cell. Dec. 2006, Vol 18, No.12, pp 3635-3646. entire document, especially Abstract.                                    | 1-22                  |
| Y         | WO 1999/045129 A1 (MOGEN INTERNATIONAL N.V.) 10 September 1999 (10.09.1999) Claim 16; SEQ ID NO: 28.                                                                                                                                            | 5-7                   |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

27 July 2017

Date of mailing of the international search report

22 AUG 2017

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