ABSTRACT

A METHOD OF PRODUCING STRESS TOLERANT PLANTS

The present disclosure relates to a method of producing transgenic plants that over-express the chickpea protein, CaSUN1, expression of which enhances the stress tolerance of the transgenic plants. The disclosure further provides recombinant DNA constructs, recombinant DNA vectors, and recombinant host cells comprising the cDNA encoding CaSUN1.

I/We claim:

1. A recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene , wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.
2. The recombinant DNA construct as claimed in claim 1, wherein the stress tolerance gene has a polynucleotide sequence as set forth in SEQ ID NO: 2.
3. The recombinant DNA construct as claimed in claims 1,wherein the promoter is selected from the group consisting of pUbi, CamV 35S, Act-1, Adh-1, and opine promoters.
4. The recombinant DNA construct as claimed in claim 3, wherein the promoter is CamV 35S.
5. A recombinant DNA vector comprising the recombinant DNA construct as claimed in claim 1.
6. A recombinant host cell comprising the recombinant DNA construct as claimed in claim 1.
7. The recombinant host cell as claimed in claim 6, wherein the host cell is selected from the group consisting of a plant cell, an animal cell, a fungal cell, and a bacterial cell.
8. The recombinant host cell as claimed in claim 7, wherein the host cell is *Agrobacterium.*
9. The recombinant host cell as claimed in claim 7, wherein the host cell is a plant cell.
10. A cDNA encoding a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.
11. The cDNA as claimed in claim 10, wherein the cDNA has a polynucleotide sequence as set forth in SEQ ID NO: 2.
12. The cDNA as claimed in claim 10, wherein expression of a polypeptide encoded by the cDNA in cells enhances tolerance against stress, wherein the stress is selected from the group consisting of, salt stress, thermal stress, dehydration stress, and salicylic acid stress.
13. A method of producing a transgenic plant with enhanced tolerance to stress, said method comprising:
    a. transforming plant cells with host cells as claimed in claim 7;
    b. selecting a transgenic plant cell comprising the recombinant DNA construct as claimed in claim 1; and

c. developing a transgenic plant, which shows enhanced tolerance to stress.

14. The method as claimed in claim 13, wherein the stress is selected from the group consisting of, salt stress, thermal stress, dehydration stress, and salicylic acid stress.

15. The method as claimed in claim 13, wherein the transgenic plant is a moncot or a dicot.

16. The method as claimed in claim 13, wherein the transgenic plant is selected from the group consisting of corn, rice, wheat, rye, millet, banana, beans, peas, potato, eggplant, peppers, squash, melons, coffee, citrus, broccoli, turnips, legumes, yams, arabidopsis, and apples.

17. A transgenic plant or parts thereof including seeds, and progeny that exhibit enhanced stress tolerance, wherein the transgenic plant or part thereof including seeds, and progeny encode in its nuclear genome the recombinant DNA construct as claimed in claim 1.

18. The transgenic plant or part thereof including seeds, and progeny as claimed in claim 17, wherein the stress is selected from the group consisting of, salt stress, thermal stress, dehydration stress, and salicylic acid stress.

19. The transgenic plant or parts thereof including seeds, and progeny as claimed in claim 17, wherein the transgenic plant or parts thereof including seeds, and progeny is a monocot or a dicot.

20. The transgenic plant or parts thereof including seeds, and progeny as claimed in claim 17, wherein the transgenic plant or parts thereof including seeds, and progeny is selected from the group consisting of corn, rice, wheat, rye, millet, banana, beans, peas, potato, eggplant, peppers, squash, melons, coffee, citrus, broccoli, turnips, legumes, yams, arabidopsis, and apples.

Dated this 02 January, 2014

Malathi Lakshmikumaran

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Agent for the Applicant

To,

The Controller of Patents,

The Patent Office, at New Delhi

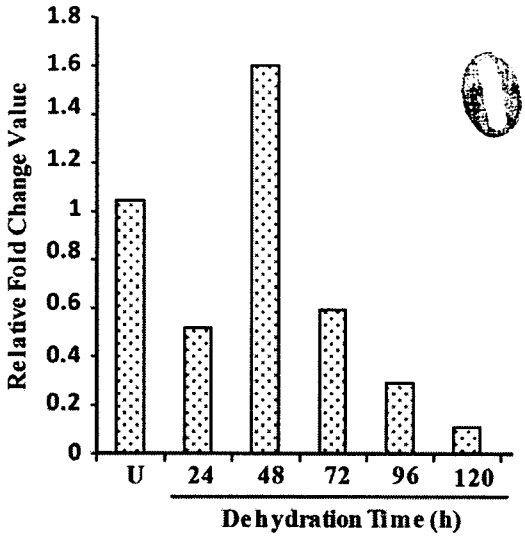

Figure 1

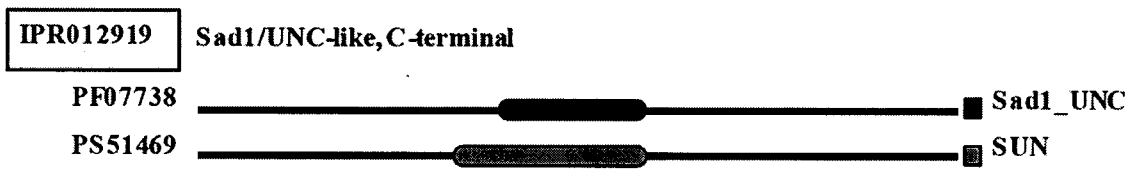

Figure 2

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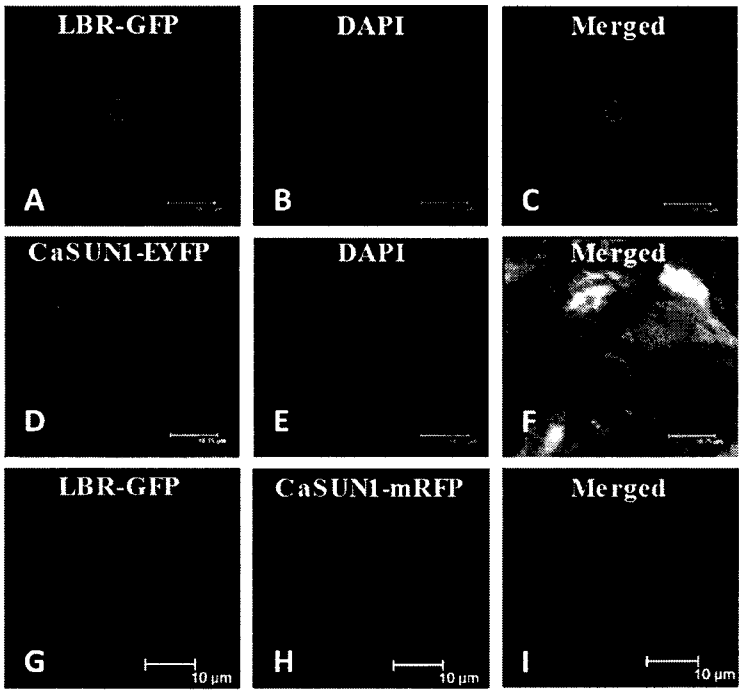

Figure 3

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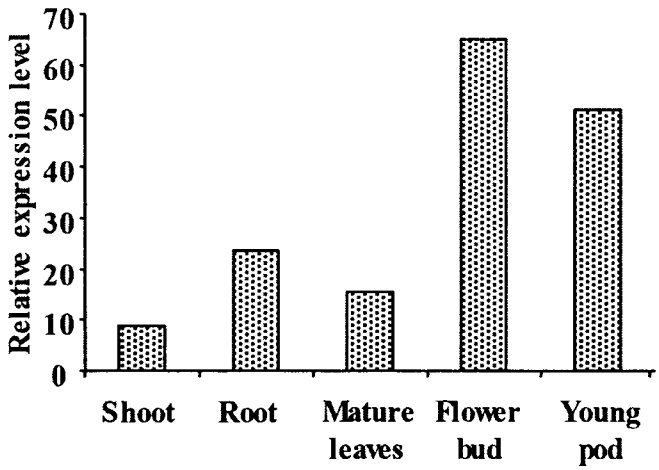

Figure 4

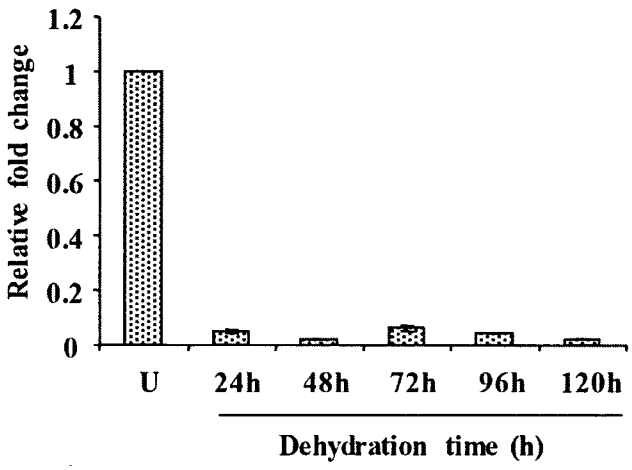

Figure 5A

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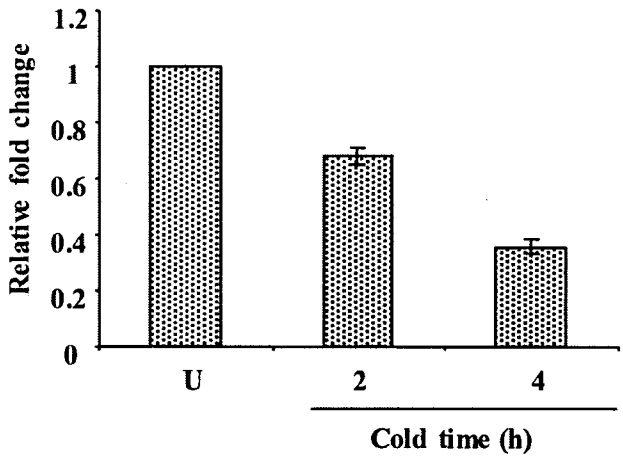

Figure 5B

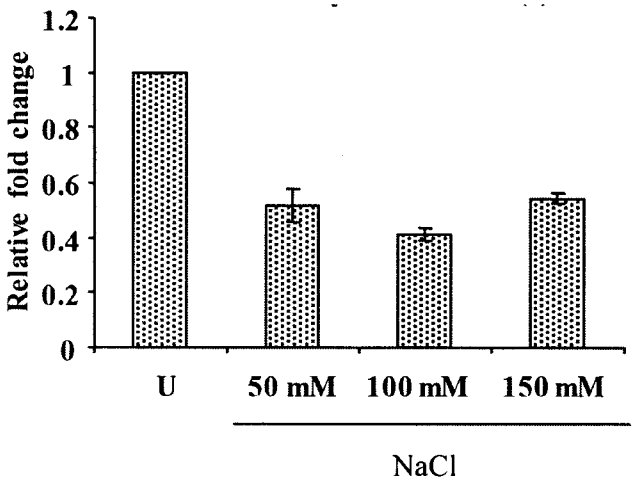

Figure 5C

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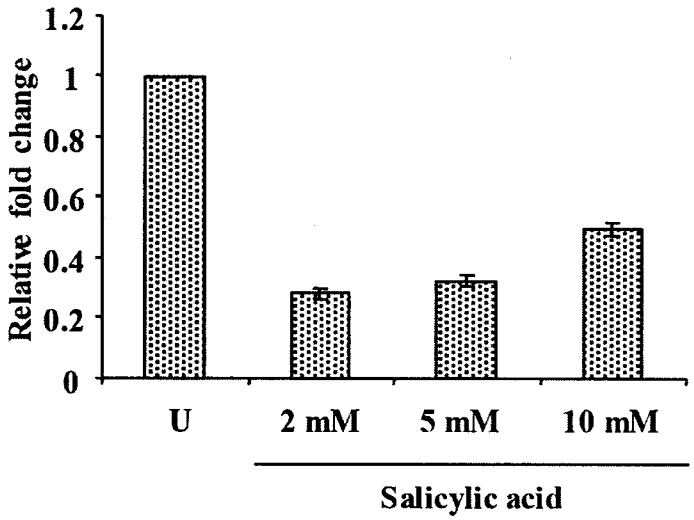

Figure 5D

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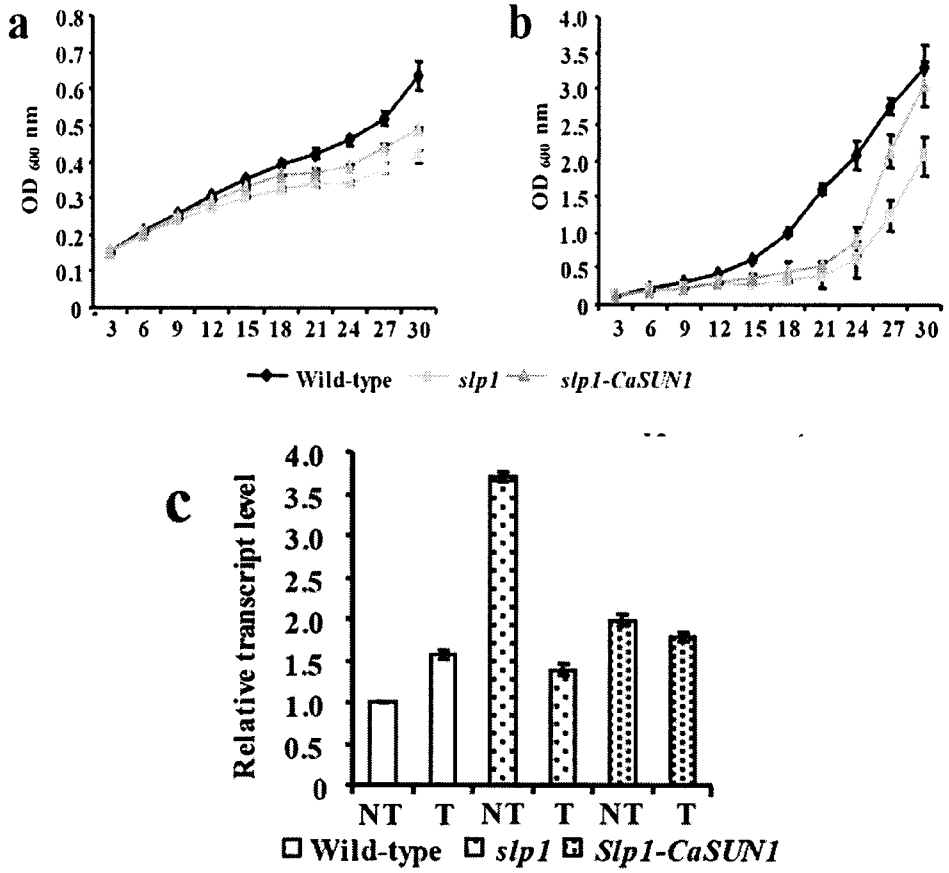

Figure 6

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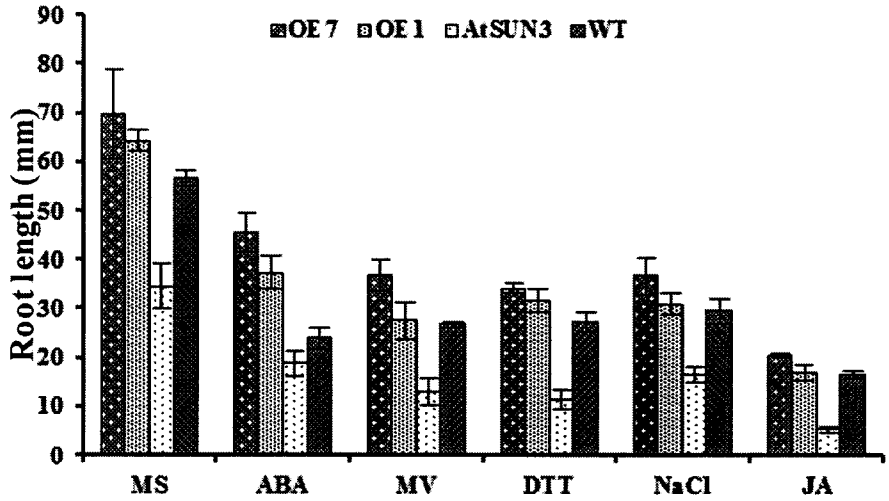

Figure 7

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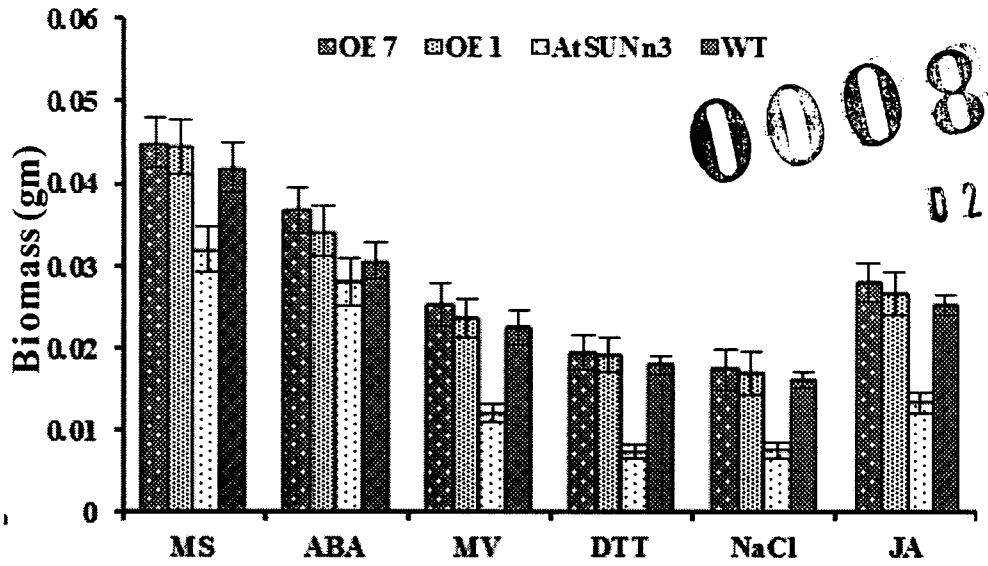

Figure 8

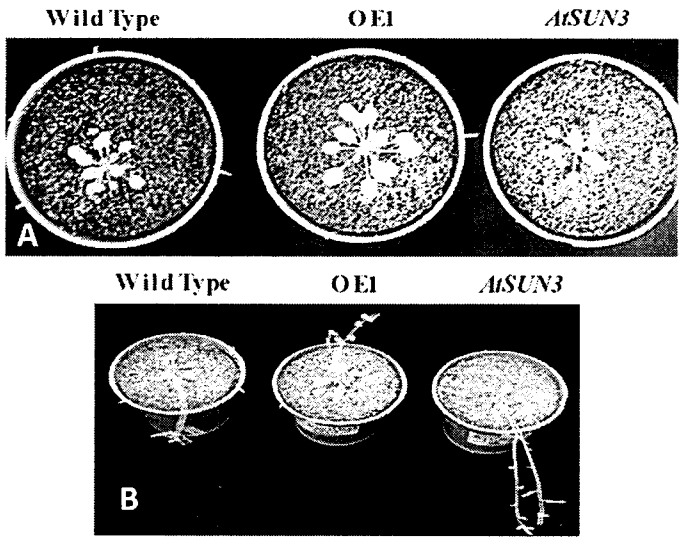

Figure 9

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FIELD OF INVENTION

[0001] The present disclosure relates to a method of producing transgenic plants over-expressing *CaSUN1* gene. The transgenic plants have enhanced abiotic and biotic stress tolerance. The disclosure also provides cDNA, recombinant DNA constructs, recombinant DNA vectors, and recombinant host cells comprising the *CaSUN1* cDNA.

BACKGROUND OF THE INVENTION

[0002] Water-deficit or dehydration is considered to be a predominant environmental stress and is often associated with other stresses viz., salinity, high temperature, and nutritional deficiencies. Plants, being sessile, have evolved distinct mechanisms to sense such adverse conditions and initiate defense responses. During the past decade, the physiological and molecular basis for plant responses to dehydration tolerance has been the subject of priority research (Krasensky *et al.*, *J. Exp. Bot.*, 2012, *63*, 1593-1608; Lawlor *et al.*, *J. Exp. Bot.*, 2013, *64*, 83-108). Most of the earlier understanding of cellular responses to dehydration came from gene expression studies (Matsui *et al.*, *Plant Cell Physiol*, 2008, *49*, 1135-1149; Shinozaki *et al.*, *J. Exp. Bot.*, 2007, *58*, 221-227)

[0003] Although such strategies allow identification of stress-responsive genes, they do not necessarily reflect the actual dynamics of final gene products, the proteins (Dumas-Gaudot *et al.*, *Proteomics*, 2004, *4*, 451-453). Proteomic analysis offers an opportunity to catalog temporal patterns of protein accumulation during stress perception, adaptation and cell defense (Abdalla *et al.*, *J. Proteomics*, 2012, *75*, 2361-2374; Bhushan *et al*, *J. Proteomics*, 2011, *10*, 2027-2046). Further, the level of proteins integrates post-transcriptional and post-translational processing that modulates the quantity, localization and efficiency of the final cell products. This information can hence be included with the annotation of the corresponding gene (Baginsky *et al.*, *J. Exp. Bot.*, 2006, *57*, 1485-1491). Thus, identifying novel proteins, determining their expression patterns in dehydration response and understanding their functions would provide the basis for effective engineering strategies to improve crop stress tolerance.

[0004] Increasing world-wide demand for staple food products such as rice has put an ever increasing pressure on both agricultural practices and scientific innovation on increasing yield of plants. Concurrently, there has been a demand for sustainable agriculture in the face of

increased use of growth stimulants, and vagaries of nature such as water availability or temperature conditions. There is a current pressing need to develop methods, and new varieties of food crops that are better equipped to handle both abiotic and biotic stress factors.

SUMMARY OF INVENTION

[0001] In an aspect of the present disclosure, there is provided a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene , wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[0002] In an aspect of the present disclosure, there is provided a recombinant DNA vector comprising a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene , wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[0003] In an aspect of the present disclosure, there is provided a recombinant host cell comprising of a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene , wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[0004] In an aspect of the present disclosure, there is provided a recombinant host cell comprising of **a** recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene , wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[0005] In an aspect of the present disclosure, there is provided a cDNA encoding a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[0006] In an aspect of the present disclosure, there is provided a method of producing a transgenic plant with enhanced tolerance to stress, said method comprising: (a) transforming plant cells with recombinant host cells comprising a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene , wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1, (b) selecting a transgenic plant cell comprising the recombinant DNA construct from (a), and (c) developing a transgenic plant which shows enhanced tolerance to stress.

[0007] In an aspect of the present disclosure, there is provided a transgenic plant or parts thereof including seeds, and progeny that exhibit enhanced stress tolerance, wherein the transgenic plant

or part thereof including seeds, and progeny encode in its nuclear genome a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene , wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[0008] These and other features, aspects and advantages of the present subject matter will be better understood with reference to the following description and appended claims. This summary is provided to introduce a selection of concepts. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used for to limiting the scope of the claimed subject matter.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS

[0009] The following drawings form part of the present specification and are included to further illustrate aspects of the present disclosure. The disclosure may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

[00010] Figure 1 shows the graphical depiction of down-regulation of *CaSUN1* transcript levels in chickpea seedlings, in accordance with an embodiment of the present disclosure.

[00011] Figure 2 shows the depiction of genomic organization of CaSUN1, in accordance with an embodiment of the present disclosure.

[00012] Figure 3A-I shows the localization of CaSUN1 in *Nicotiana* epidermal cells, in accordance with an embodiment of the present disclosure.

[00013] Figure 4 shows the graphical representation of tissue specific distribution of CaSUN1, in accordance with an embodiment of the present disclosure.

[00014] Figure 5A-D shows the graphical representation of *CaSUN1* transcript levels in chickpea seedlings exposed to stress, in accordance with an embodiment of the present disclosure.

[00015] Figure 6A-C shows the effect of *CaSUN1* on growth of *Δslp1* yeast strain, in accordance with an embodiment of the present disclosure.

[00016] Figure 7 shows the effect of CaSUN1 over-expression in arabidopsis on root length in response to stress, in accordance with an embodiment of the present disclosure.

[00017] Figure 8 shows the effect of CaSUN1 over-expression in arabidopsis on biomass in response to stress, in accordance with an embodiment of the present disclosure.

[00018] Figure 9A-B shows the effect of CaSUN1 over-expression in arabidopsis in response to dehydration, in accordance with an embodiment of the present disclosure.

DETAILED DESCRPTION OF THE INVENTION

[00019] Those skilled in the art will be aware that the invention described herein is subject to variations and modifications other than those specifically described. It is to be understood that the invention described herein includes all such variations and modifications. The invention also includes all such steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any or more of such steps or features.

Definitions

[00020] For convenience, before further description of the present invention, certain terms employed in the specification, example and appended claims are collected here. These definitions should be read in the light of the remainder of the disclosure and understood as by a person of skill in the art. The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

[00021] The articles "a", "an" and "the" are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

[00022] The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included. It is not intended to be construed as "consists of only.

[00023] Throughout this specification, unless the context requires otherwise the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated element or step or group of element or steps but not the exclusion of any other element or step or group of element or steps. The term "including" is used to mean "including but not limited to". "Including" and "including but not limited to" are used interchangeably.

[00024] "Primers" are synthesized nucleic acids that anneal to a complementary target DNA strand by hybridization to form a hybrid between the primer and the target DNA strand, and then

extended along the target DNA strand by polymerase activity, e.g., a DNA polymerase. Primer pairs described in the present invention refer to their use for amplification of a target nucleic acid sequence, e.g., by polymerase chain reaction or other conventional nucleic-acid amplification methods.

[00025] The term "genetic transformation" refers to a process of introducing a DNA sequence or construct (e.g., a vector or expression cassette) into a cell in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

[00026] The term "transgenic" refers to a cell contains a transgene, or whose genome has been altered by the introduction of a transgene. The term "transgenic" when used in reference to a tissue or to a plant refers to a tissue or plant, respectively, which comprises one or more cells that contain a transgene, or whose genome has been altered by the introduction of a transgene.

[00027] The term "transgene" refers to any nucleic acid sequence which is introduced into the genome of a cell by experimental manipulations. A transgene may be an "endogenous DNA sequence," or a "heterologous DNA sequence" (i.e., "foreign DNA"). A transgene is capable of causing the expression of one or more cellular products. 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.

[00028] The term "vector" refers to a DNA molecule capable of replication in a host cell and/or to which another DNA segment can be operably linked so as to bring about replication of the attached segment. A plasmid is an exemplary vector.

[00029] The term "expression vector" refers to a vector comprising an expression cassette.

[00030] The term "polypeptide" and "peptide are used interchangeably for the purposes of the present disclosure.

[00031] The term "transformed cell" refers to a cell, the DNA complement of which has been altered by the introduction of an exogenous DNA molecule into that cell.

[00032] The term "transgenic plant" refers to 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 originally 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.

[00033] The term "polynucleotide" used in the present invention refers to a DNA polymer composed of multiple nucleotides chemically bonded by a series of ester linkages between the phosphoryl group of one nucleotide and the hydroxyl group of the sugar in the adjacent nucleotide.

[00034] SEQ ID NO: 1 shows the amino acid sequence of CaSUN1.

[00035] SEQ ID NO: 2 shows the polynucleotide sequence of *CaSUN1*.

[00036] SEQ ID NO: 3 shows the forward primer for cloning *CaSUN1*.

[00037] SEQ ID NO: 4 shows the reverse primer for cloning *CaSUN1*.

[00038] In an embodiment of the present disclosure, there is provided a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene, wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1..

[00039] In an embodiment of the present disclosure, there is provided a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene, wherein the stress tolerance gene has a polynucleotide sequence as set forth in SEQ ID NO: 2.

[00040] In an embodiment of the present disclosure, the promoter is selected from the group consisting of pUbi, CamV 35S, opine promoters, Adh-1, and Act-1.

[00041] In a preferred embodiment of the present disclosure, the promoter is CamV 35S.

[00042] In an embodiment of the present disclosure, there is provided a a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene, wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[00043] In an embodiment of the present disclosure, there is provided a recombinant DNA vector comprising a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene, wherein the stress tolerance gene has a polynucleotide sequence as set forth in SEQ ID NO: 2.

[00044] In an embodiment of the present disclosure, there is provided a recombinant host cell comprising of a recombinant DNA vector comprising of a recombinant DNA construct

comprising of a promoter operably linked to a stress tolerance gene, wherein the stress tolerance gene encodes a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[00045] In an embodiment of the present disclosure, there is provided a recombinant host cell comprising of a recombinant DNA vector comprising of a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene, wherein the stress tolerance gene has a polynucleotide sequence as set forth in SEQ ID NO: 2.

[00046] In an embodiment of the present disclosure, the recombinant host cell is a plant cell.

[00047] In an embodiment of the present disclosure, the recombinant host cell is an animal cell.

[00048] In an embodiment of the present disclosure, the recombinant host cell is a fungal cell.

[00049] In an embodiment of the present disclosure, the recombinant host cell is a bacterial cell.

[00050] In an embodiment of the present disclosure, the recombinant host cell is *Agrobacterium tumefaciens*.

[00051] In an embodiment of the present disclosure, there is provided a cDNA encoding a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[00052] In an embodiment of the present disclosure, there is provided a cDNA having polynucleotide sequence as set forth in SEQ ID NO: 2.

[00053] In an embodiment of the present disclosure, there is provided a cDNA, wherein expression of the cDNA in cells enhances tolerance against stress, wherein the stress is selected from the group consisting of, salt stress, thermal stress, dehydration stress, and salicylic acid stress.

[00054] In an embodiment of the present disclosure, there is provided a method of producing a transgenic plant with enhanced tolerance to stress, said method comprising of (a) transforming plant cells with host cells comprising of a recombinant DNA vector comprising of a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene encoding a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1, (b) selecting a transgenic plant comprising the recombinant DNA construct from (a), and (c) developing a transgenic plant which shows enhanced tolerance to stress.

[00055] In an embodiment of the present disclosure, the stress is selected from the group consisting of dehydration stress, salt stress, salicylic acid stress, and thermal stress.

[00056] In an embodiment of the present disclosure, the method of transformation is selected from the group consisting of *Agrobacterium* mediated transformation, biolistics, electroporation, protoplast fusion, and liposome mediated transformation.

[00057] In a preferred embodiment of the present disclosure, the method of transformation is *Agrobacterium* mediated transformation.

[00058] In an embodiment of the present disclosure, there is provided a transgenic plant or parts thereof, including seeds, and progeny that express a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1.

[00059] In an embodiment of the present disclosure, there is provided a transgenic plant or parts thereof, including seeds, and progeny that express a polypeptide encoded by a polynucleotide sequence as set forth in SEQ ID NO: 2.

[00060] In an embodiment of the present disclosure, there is provided a transgenic plant or parts thereof, including seeds, and progeny that express a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1, wherein the transgenic plant or parts thereof, including seeds, and progeny is a monocot.

[00061] In an embodiment of the present disclosure, the monocot is selected from the group consisting of corn, rice, wheat, rye, millet, and banana.

[00062] In an embodiment of the present disclosure, there is provided a transgenic plant or parts thereof, including seeds, and progeny that express a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1, wherein the transgenic plant or parts thereof, including seeds, and progeny is a dicot.

[00063] In an embodiment of the present disclosure, the dicot is selected from the group consisting of beans, peas, potato, eggplant, peppers, squash, melons, coffee, citrus, broccoli, turnips, legumes, yams, arabidopsis, and apples.

[00064] In an embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to salt stress.

[00065] In an embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to thermal stress.

[00066] In a preferred embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to cold stress.

[00067] In an embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to dehydration induced stress.

[00068] In an embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to salicylic acid induced stress.

[00069] In an embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to salt stress, thermal stress, dehydration induced stress, and salicylic acid induced stress.

[00070] In a preferred embodiment of the present disclosure, the dicot transgenic plant or part thereof including seeds that shows enhanced tolerance to stress is *Arabidopsis*.

[00071] In an embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to stress, wherein a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene encoding a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 is nuclear genome encoded.

[00072] In embodiment of the present disclosure, there is provided a transgenic plant or part thereof including seeds, and progeny that show enhanced tolerance to stress, wherein a recombinant DNA construct comprising of a promoter operably linked to a stress tolerance gene having a polynucleotide sequence as set forth in SEQ ID NO: 2 is nuclear genome encoded.

[00073] In an embodiment of the present disclosure, the polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 is localized to the inner nuclear membrane.

[00074] In an embodiment of the present disclosure, the transcript levels of the polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in a plant cell are downregulated in response to dehydration induced stress.

[00075] In an embodiment of the present disclosure, the transcript levels of the polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in a plant cell are downregulated in response to cold induced stress.

[00076] In an embodiment of the present disclosure, the transcript levels of the polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in a plant cell are downregulated in response to salt induced stress.

[00077] In an embodiment of the present disclosure, the transcript levels of the polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in a plant cell are downregulated in response to salicylic acid induced stress.

[00078] In an embodiment of the present disclosure, a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 is expressed in shoot, root, mature leaves, flower bud, and young pod tissue.

[00079] In an embodiment of the present disclosure, a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 is maximally expressed in flower buds.

[00080] In an embodiment of the present disclosure, expression of a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in *Δslp1* yeast strain rescues the growth defect.

[00081] In an embodiment of the present disclosure, over-expression of a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in *AtSUN3* mutants rescues the root length phenotype of *AtSUN3* mutants.

[00082] In an embodiment of the present disclosure, over-expression of a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in *AtSUN3* mutants rescues the biomass phenotype of *AtSUN3* mutants.

[00083] In an embodiment of the present disclosure, over-expression of a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in *AtSUN3* mutants rescues the dehydration phenotype of *AtSUN3* mutants.

[00084] In an embodiment of the present disclosure, over-expression of a polypeptide having amino acid sequence as set forth in SEQ ID NO: 1 in plants results in enhanced stress tolerance.

EXAMPLES

[00085] The disclosure will now be illustrated with working examples, which is intended to illustrate the working of disclosure and not intended to take restrictively to imply any limitations on the scope of the present disclosure. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skills in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein. It is to be understood that this disclosure is not limited to particular methods, and experimental conditions described, as such

methods and conditions may vary. The example is provided just to illustrate the invention and therefore, should not be construed to limit the scope of the invention.

Example 1: Identification and down-regulation of dehydration responsive gene, *CaSUN1*

[00086] Three-week old chickpea seedlings were subjected to progressive dehydration over a period of 120 hours. The membrane associated proteins were extracted from unstressed and stressed seedlings, resolved on to 2D gels, followed by detection by mass spectrometry (MS) and analysis.

[00087] The MS/MS analysis identified 95 dehydration-responsive proteins (DRPs), the ion scores of which were statistically significant ($p<0.05$). A candidate protein that was downregulated in response to dehydration showed homology to membrane protein CH1-like, and was putatively given the name CaSUN1.

[00088] Figure 1 shows the transcript levels of *CaSUN1* in dehydrated chickpea seedlings. It can be seen that transcript levels of *CaSUN1* are decreased by five-fold over a period of 120 hours.

[00089] Figure 2 shows the genomic organization of CaSUN1. There is a putative SUN domain at mid position. The C-terminal shows homology to Sad1/UNC like.

[00090] *In-silico* analysis of CaSUN1 shows the presence of three transmembrane domains, a coiled-coil region and a secretory signal.

[00091] Isolation of *CaSUN1*, and sequence analysis: The full length cDNA of *CaSUN1* was cloned by RACE, using the SMART™ RACE cDNA amplification kit (Clonetech Laboratories).

Example 2: Multi-organelle localization of CaSUN1

[00092] *In-vivo* targeting and expression of CaSUN1 was examined in *Nicotiana* epidermal cells using CaSUN1-EYFP and CaSUN1-mRFP fusion proteins. The transient expression was detected by confocal microscopy. The plant nuclear envelope marker, LBR-GFP was used a positive control.

[00093] Primers used for the PCR for cloning *CaSUN1* are as set forth in SEQ ID NO: 3 and SEQ ID NO: 4. The PCR condition used are outlined in Table 1. Primers were obtained from Sigma. A 50 µl reaction mixture contained 10-50 ng DNA template, 1 µl of 10 µM of each primer, 1 µl of 10 mM dNTPs, 5 µl of 10X Taq buffer and 2.5 units of Taq polymerase.

Table 1

Step	Number of cycles	Temperature (°C)	Time (sec)
Initial denaturation	1	95	240
Denaturation	30	94	30
Annealing		60	60
Extension		72	120

[00094] Figure 3 shows the localization of CaSUN1-EYFP to the nuclear envelope. Figure 3A0C shows the nuclear envelope localization of the positive control, LBR-GFP. CaSUN1-EYFP expression can also be seen in the endoplasmic reticulum and in small vacuolar membranes suggesting trafficking of synthesized protein to the nuclear envelope (3D-F). Co-localization of CaSUN1-mRFP with LBR-GFP suggests that CaSUN1 is localized to the inner nuclear envelope (3G-I).

[00095] The open reading frame of *CaSUN1* that lacked the stop codon was PCR amplified and cloned in to the pENTR-D/TOPO vector (Invitrogen). The resultant plasmid pENTR-CaSUN1 was subjected to the LR reaction using destination vector pGWB411, 441, and 454 to produce a binary vector containing the C-terminal FLAG, EYFP, and mRFP tag respectively, under the control of the CaMV 35S promoter. Further, pENTR-CaSUN1 was subjected to the LR reaction using the yeast destination vector pAG426GPD-ccdB-EGFP and pAG426GPD-ccdB-EYFP (Addgene) to produce C-terminal tags under the control of *GAP* promoter.

[00096] Transient assay of CaSUN1 in *Nicotiana* leaves: *A.tumefaciens* strain GV3101 was transformed with the candidate gene construct by freeze-thaw method for transient expression of CaSUN1. The transformed *Agrobacterium* cells were grown at 28°C in YEP medium supplemented with appropriate antibiotics to an OD_{600} of 0.6-1. The cells were recovered by centrifugation at 500g for 15 minutes and resuspended in media containing 10% sucrose, 10mM $MgCl_2$, 2.6mM MES (pH 5.8), and 150mM acetosyringone. Cells were incubated in this medium for at least three hours and infiltrated in to four-week old tobacco leaves. The images were taken 2-3 days after infiltration.

Example 3: Tissue specific expression of CaSUN1

[00097] Figure 4 shows the graphical representation of tissue specific expression of *CaSUN1* in chickpea seedlings. While *CaSUN1*transcripts could be detected in all tissue types such as shoot,

root, mature leaves, flower bud, and young bud, maximal transcript level was detected in flower buds, which was over six-fold more than in shoots which had the least expression levels.

Example 4: Stress-responsive transcriptional regulation of *CaSUN1*

[00098] Transcript levels of *CaSUN1* in chickpea seedlings were quantified under dehydration conditions using quantitative real time-polymerase chain reaction (q RT-PCR). Primers used for detection of *CaSUN1* transcript is as set forth in SEQ ID NO: 3 (forward) and SEQ ID NO: 4 (reverse). Figure 5A shows that transcript levels of *CaSUN1* at various time intervals post-dehydration. It can be seen that there is a severe and significant decrease in *CaSUN1* transcript levels as early as 24 hours post dehydration. There is approximately more than a nine-fold decrease by 24 hours compared to controls.

[00099] Figure 5B shows the transcript levels of *CaSUN1* at various time intervals in response to cold stress. It can be seen that, in response to cold stress, transcript levels of *CaSUN1* are reduced by approximately 50% by four hours post cold stress with respect to controls.

[000100] Figure 5C shows the transcript levels of *CaSUN1* in response to varying concentration of salt in a test for salt stress. It can be seen that at salt concentrations ranging from 50mM-150mM, transcript levels of *CaSUN1* are reduced by approximately 50% compared to controls.

[000101] To examine whether CaSUN1 is responsive to pathogen infection/biotic stress, chickpea seedlings were treated with salicylic acid as such compounds are reported to mimic pathostress response (Clarke *et al.*, *Plant Cell*, 2000, *12,* 2175-2190). Figure 5D shows the transcript levels of *CaSUN1* in response to salicylic acid. It can be seen that *CaSUN1* transcript levels are suppressed by 50%-70% upon salicylic acid treatment ranging from 2mM-10mM. A general trend of salicylic acid mediated *CaSUN1* suppression can be seen in chickpea seedlings.

[000102] qRT-PCR: Total RNA was isolated either using the RNeasy Plant Mini kit (Qiagen) or the TriPure reagent (Invitrogen). cDNA was prepared using SuperScript® VILO™ eDNA Synthesis Kit (Invitrogen) according to manufacturer's instructions. The qRT-PCR assays were performed with the ABI PRISM 7700 sequence detection system (Applied Biosystems) using SYBR Green PCR Master mix in a final volume of 20µL including cDNA template and appropriate primer pairs. The internal standards *EFla* and *ACTJ* were used for normalizing the qRT-PCR data.

[000103] Growth conditions and stress treatment: The seedlings were grown in pots (10-12 seedlings/1.5L capacity pots with 18cm diameter) containing a mixture of soil and soilrite (2:1

w/w ratio) in an environmentally controlled growth room and maintained at 25±2°C, 50±5% relative humidity under 16 hours photoperiod (270μmol/m^2/second light intensity). A gradual dehydration condition was applied on three-week old seedlings by withdrawing water for a period of 120 hours. In a separate experiment, seedlings were also subjected to other stresses such as varying NaCl concentrations (100, 250, and 500mM), and low temperature (4°C). Different concentrations of ABA (25, 50, and 100μM), methyl viologen (50, 100μM), and salicylic acid (2, 5, and 10mM) were applied by spraying the respective solutions on the seedlings. The tissues were harvested, flash frozen in liquid nitrogen, and stored at -80°C.

Example 5: Functional complementation of yeast *slp1* mutant by *CaSUN1*

[000104] *slp1*mutants are hypersensitive to stress inducers that activate the unfolded protein response pathway (UPR). To ascertain the functional role of CaSUN1 in ER stress response, the *slp1* mutant was transformed with the yeast expression vector, pYES2, in which *CaSUN1* was cloned under the transcriptional control of *GAL* promoter.

[000105] Figure 6A, B show that expression of CaSUN1 in *slp1* mutant yeast background significantly restored the growth defect of *slp1* mutant yeast strain. This suggests that CaSUN1 may have a functional regulatory role in mediating the activation of the intracellular stress response pathway.

[000106] KAR2 is a molecular chaperone and indicator of UPR stress in yeast. Figure 6C shows the transcript levels of *CaSUN1* in *slp1* yeast mutant background in response to DTT treatment, which is a known inducer of UPR stress. It can be seen that expression of CaSUN1 ameliorates *KAR2* expression suggesting that *CaSUN1* may be functional *in-vivo* in protecting yeast cells from UPR stress, and that the phenotypic complementation is due to *CaSUN1*.

[000107] Yeast transformation and complementation assay: The pYES2 vector (Invitrogen) was constructed with *CaSUN1* cDNA via BamHI/XhoI to generate plasmid pYES2-CaSUN1 for complementation assay. The *slp1* mutant (FY; Mat α; ura3-52; HIS3; leu2Δ1; LYS2; TRP1; YOR154w(4,1762)::kanMX4) and wild-type strains (Mat α; ura3-52; HIS3; leu2Δ1; TRP1; GAL2) were obtained from EUROSCARF. Wild-type or mutant yeast cells were transformed with plasmids pYES2-CaSUN1 or the empty vector pYES2. Yeast transformation was performed using the lithium acetate method (Geno Technology Inc.) and selected on SD-Ura (Invitrogen) by growing the cells at 30°C for 3-4 days. The respective strains were grown in SD-Ura (non-inducible) and SD-Gal/Raf-URA (inducible) media. The overnight grown cultures were diluted

to OD_{600} ~0.1 in the respective medium containing 2mM DTT. The growth was monitored every three hours by measuring the OD_{600} of the cultures.

Example 6: Over-expression of CaSUN1 in arabidopsis enhances stress tolerance

[000108] Transgenic *Arabidopsis* were developed in which CaSUN1 is constitutively over-expressed under the control of CaMV 35S promoter. No phenotypic abnormalities were observed in unstressed conditions in at least two transgenic lines, designated OE-1, and OE-2.

[000109] OE-1 and OE-2 were subjected to various stress conditions such as salt exposure, DTT treatment, oxidative stress, and JA.

[000110] Figure 7 shows the effect of various stressors in OE-1 and OE-2 transgenic plants compared to AtSUN1 mutant and control plants with respect to root length. It can be seen that over-expression of *CaSUN1* rescues the defects seen in both *AtSUN1* mutants and wild-type arabidopsis exposed to various stressors, and in many cases enhances the protective effect of CaSUN1 over-expression, suggesting that CaSUN1 may have a role in regulating plant growth and health in response to stress.

[000111] Figure 8 shows the effect of various stressors in OE-1 and OE-2 transgenic plants compared to AtSUN1 mutant and control plants with respect to biomass. It can be seen that over-expression of *CaSUN1* rescues the defect seen in *AtSUN1* mutants with regard to biomass, suggesting that CaSUN1 may have a role in regulating plant growth and yield in response to stress.

[000112] The sensitivity of wild type and *AtSUN1* plants and OE-1 was also compared in response to dehydration treatment (18 days) of four-week old plants. Figure 9 shows that while wild type and mutants show wilting, OE-1 transgenic plant shows better adaptation.

[000113] Overall, OE-1 plants are more tolerant to multivariate stresses than their wild-type counterparts. Additionally, the transgenic seedlings showed similar phenotypes as that of wild-type seedlings when assayed for germination rate in the presence or absence of exogenously applied ABA.

[000114] Genetic transformation of *Arabidopsis*: The *CaSUN1* constructs were transformed in to *Agrobacterium tumefaciens* GV3101 cells, which were used to transform *Arabidopsis* by floral dip method (Clough *et al.*, *Plant J.*, 1998, *16*, 735-743). Putative transgenic seedlings were selected on MS medium containing 50mg/L kanamycin. For stress treatment, seeds of wild-type and transgenic plants were sterilized, stratified at 4 °C for 72 hours and then grown on MS

plates. Seeds were also kept onto identical plates supplemented with DTT (1mM or 2.5mM), MV (2μM or 8μM), ABA (0.5μM or 2μM), NaCl (50mM or 100mM), and JA (2μM or 8μM).