

(SD2019-269) Use of M3K-delta Protein for Improvement of Plant Drought and Salinity Stress Resistance

Tech ID: 31836 / UC Case 2019-269-0

BACKGROUND

The response of plants to reduced water availability is controlled by a complex osmotic stress and abscisic acid (ABA)-dependent signal transduction network. The core ABA signaling components are snf1-related protein kinase2s (SnRK2s) which are activated by ABA-dependent inhibition of type 2C protein phosphatases and by an unknown ABA-independent osmotic stress signaling pathway.

Limited water availability is one of the key factors that negatively impacts crop yields. The plant hormone abscisic acid (ABA) and the signal transduction network it activates, enhance plant drought tolerance through stomatal closure, and inhibition of seed germination and growth. As plants are constantly exposed to changing water conditions, reversibility and robustness of the ABA signal transduction cascade is important for plants to balance growth and drought stress resistance. Core ABA signaling components have been established the ABA receptors PYRABACTIN RESISTANCE (PYR/PYL) or REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) inhibit type 2C protein phosphatases (PP2Cs) resulting in the activation of the SnRK2 protein kinases SnRK2.2, 2.3 and OST1/SnRK2.6 . However, it has remained unclear whether direct autophosphorylation or trans-phosphorylation by unknown protein kinases re-activates these SnRK2 protein kinases in response to stress. The osmotic stress sensing mechanism and upstream signal transduction mechanisms leading to SnRK2 activation remain largely unknown in plants.

TECHNOLOGY DESCRIPTION

Biologists from UC San Diego have identified functionally-redundant Raf-like MAPKK kinases (M3Ks) that activate OST1/SnRK2 kinases. These M3Ks are essential for the re-activation of SnRK2 kinases after deactivation by the PP2C phosphatases through a specific M3K-targeted phosphorylation site in the SnRK2 kinase activation-loop (OST1-Ser-171) which is essential for SnRK2 kinase function and ABA signal transduction. CRISPR/Cas9 deletion, T-DNA insertion and amiRNA knock-down plants independently reveal that these M3Ks play an important role in ABA responses. The identified M3Ks provide an opportune mechanism for tuning the ABA and abiotic stress response of plants. Reactivation of dephosphorylated SnRK2 requires these M3Ks, and ABA-induced OST1/SnRK2.6 activation and S-type anion channel activation requires the presence of M3Ks. M3K knock-out plants show not only reduced sensitivity to ABA but also strongly impaired osmotic stress-induced SnRK2 activation. These results demonstrate that these Raf-like M3Ks are required for ABA- and osmotic stress-activation of SnRK2 kinases, ensuring robust ABA and osmotic stress signal transduction. Our advance additionally provides an important mechanism for modulating the osmotic stress sensing machinery.

APPLICATIONS

M3Ks provide a new genetic engineering target to improve plant abiotic stress resistance when global fresh water supply is limiting and the effects of abiotic stress or climate-linked stress on plants are limiting to crop yields and robust tree growth. Interestingly, the findings further demonstrate that these M3Ks are required for the rapid osmotic stress activation of SnRK2 protein kinases through an ABA-independent pathway. Genome editing can be accomplished using unrestricted Thalen technology

This invention can be used to enhance:

- drought tolerance of crop plants
- salinity of tolerance of crop plants
- early monitoring of drought, salinity and cold stress by crop plants and trees
- early mounting of stress resistance in crop plants and trees.

ADVANTAGES

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OTHER INFORMATION

KEYWORDS

plant hormones, drought tolerance,

salinity, cold stress, plant regulation,

plant signaling, SnRK2 protein

kinases, functionally-redundant Raf-

like MAPKK kinases, M3K

CATEGORIZED AS

- **Agriculture & Animal Science**
 - Plant Traits
- **Biotechnology**
 - Food
- **Engineering**
 - Engineering

RELATED CASES

2019-269-0

Based on the ubiquitous important role of SnRK2 protein kinases in plant drought and abiotic stress resistance, M3K-mediated modulation of SnRK2s could be an effective approach for increasing plant stress resistance, which could be broadly applicable to agricultural crops. Prior to this advance, no methodology has been established to selectively activate SnRK2 kinases in plants without applications of ABA or osmotic stress.

STATE OF DEVELOPMENT

The underlying mechanisms and genes have been discovered. The present stage suggests that enhanced osmotic stress sensing and drought and salinity resistance can be engineered.

INTELLECTUAL PROPERTY INFO

The invention is patent-pending and is available for licensing and collaborations.

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(12)

**United States
Patent Application Publication**
TAKAHASHI et al.

(10)
(43)

Pub. No.: US 2022/0154204 A1
Pub. Date: May 19, 2022

(54)	ENHANCING DROUGHT, SALINITY AND COLD TOLERANCE IN PLANTS AND TREES	Publication Classification
(71)	Applicant: The Regents of the University of California , Oakland, CA (US)	(51) Int. Cl. <i>C12N 15/82</i> (2006.01) <i>C12N 9/12</i> (2006.01)
(72)	Inventors: Yohei TAKAHASHI , San Diego, CA (US); Felix HAUSER , San Diego, CA (US); Julian I. SCHROEDER , La Jolla, CA (US)	(52) U.S. Cl. CPC <i>C12N 15/8273</i> (2013.01); <i>C12N 9/12</i> (2013.01); <i>C12Y 207/12002</i> (2013.01)
(21)	Appl. No.: 17/437,586	(57) ABSTRACT
(22)	PCT Filed: Mar. 11, 2020	In alternative embodiments, provided are methods for: enhancing drought tolerance of crop plants and trees, enhancing salinity of tolerance of plants such as crop plants, enhancing early monitoring of drought, salinity and cold stress by plants such as crop plants and trees, enhancing stress resistance in plants such as crop plants and trees, by increasing the expression of or the activity of a Raf-like mitogen-activated protein (MAP) kinase kinase (MAPKK) kinase delta B3 family enzyme (or a Raf-like MAPKK kinase delta B3 family enzyme) (a M3K B3 family enzyme) in a plant or a tree cell or a plant or a tree.
(86)	PCT No.: PCT/US2020/022013 § 371 (c)(1), (2) Date: Sep. 9, 2021	
	Related U.S. Application Data	
(60)	Provisional application No. 62/816,492, filed on Mar. 11, 2019.	

RELATED MATERIALS

► [Takahashi Y, Zhang J, Hsu PK, Ceciliato PHO, Zhang L, Dubeaux G, Munemasa S, Ge C, Zhao Y, Hauser F, Schroeder JI. MAP3Kinase-dependent SnRK2-kinase activation is required for abscisic acid signal transduction and rapid osmotic stress response. Nat Commun. 2020 Jan 2;11\(1\):12. - 01/02/2020](#)

PATENT STATUS

Patent Pending