Screening for Gene Regulation Mutants by Bioluminescence Imaging

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Abstract

Because plants cannot move, they have evolved complex sensing and response systems to cope with the physical environment. Adverse environmental conditions, such as those causing abiotic stress, often cause significant losses in crop productivity and quality. Because of a paucity of well-defined visible phenotypes, conventional genetic screens have not been very successful in isolating abiotic stress signal transduction mutants of plants. Here, we describe a reporter gene-based strategy to screen for mutants affected in abiotic stress-regulated gene transcription. Our genetic screen uses the firefly luciferase reporter gene driven by the cold, drought, salt, and abscisic acid (ABA)-responsive *RD29A* promoter (*RD29A::LUC*). *Arabidopsis* plants transformed with the *RD29A::LUC* reporter emit bioluminescence in response to cold, drought, salt, or ABA treatment. After mutagenesis of these plants with ethyl methanesulfonate (EMS), mutants can be screened from the M2 population by monitoring the level of stress-inducible bioluminescence with a high-throughput, low-light imaging system. This protocol describes in detail the procedures for this luciferase reporter-based genetic screen for *Arabidopsis* mutants defective in abiotic stress signaling.

Introduction

Because plants are sessile, their success depends on their ability to respond to environmental stresses. In addition, crop productivity and quality are significantly affected by abiotic stresses. Knowledge of cell signaling under abiotic stresses is a prerequisite for rational breeding or engineering of stress-resistant crops. Environmental stresses, such as salinity or drought, regulate the expression of hundreds of plant genes that encode stress tolerance effectors (*1*). How plants perceive environmental stimuli and transduce the resulting signals to the nucleus to activate or repress gene transcription is not well understood. Functional analysis of mutants continues to contribute significantly to a better understanding of cellular signal transduction in plants under stress (*2*).

The genome of the model plant *Arabidopsis* contains more than 3000 genes that encode transcription factors, of which only 1709 proteins show some similarity to transcription factors from other eukaryotic systems. This suggests that there are significant differences in gene regulation between plants and other eukaryotes. Many of the signaling pathways conserved among *Caenorhabditis elegans*, *Drosophila*, and human are not found in *Arabidopsis* (*3*). Spatial and temporal separation of signaling pathways, cross-talk in signaling networks, redundancy in signaling pathways, and their interaction with tissue-specific transcriptional activators make abiotic stress signal transduction a very complex process. The intensity, duration, fluctuation, and occurrence of stress in relation to plant ontogeny further complicate stress signaling. Because of this complexity, the effect of a single mutation on visible stress tolerance phenotypes is often obscured. Consequently, only a small number of mutants have been recovered from genetic screens based on visible tolerance phenotypes.

In higher plants, only the salt overly sensitive (SOS) pathway of salt stress signaling has been elucidated by a conventional genetic screen, with well-defined signal transducers and signal output. SOS3, a calcineurin B-like protein, senses the rise in Ca^{2+} concentration caused by salt stress and interacts with SOS2, a protein kinase, which in turn activates SOS1, a plasma membrane Na^{+}-H^{+} antiporter. This system restores ion homeostasis of the cells under salt stress (*2*). The initial salt stress sensor of this pathway has yet to be identified. This type of conventional genetic screen has provided only limited understanding of osmotic-stress and cold-stress signaling. Hence, to unravel the complexity of abiotic stress signaling networks, new screens must be devised that can detect normally invisible plant responses.

One such approach is genetic screening based on tissue- or process specific-promoter driven reporter gene expression. In this system, the preferred reporter genes are firefly luciferase (*LUC*) and β-glucuronidase (*GUS*). The use of transgenic *Arabidopsis* expressing β-1,3-glucanase promoter-driven β-glucuronidase (*BGL2::GUS*) to isolate the nonexpressor of pathogenesis related genes (*npr1*) mutant and chlorophyll a/b binding protein gene promoter-driven firefly luciferase (*CAB2::LUC*) to isolate timing of chlorophyll a/b-binding protein expression (*toc1*) mutants in the circadian clock demonstrated the power of genetic screens using inducible-promoter driven reporter genes (*4-6*). The disadvantages of *GUS* reporter-based genetic screens are that (i) detection of GUS activity requires relatively time-consuming fluorometric determination or tissue staining; (ii) detection of real-time signaling events are difficult because of the high stability of the GUS enzyme; and (iii) many compounds produced under stress can interfere with the fluorometric GUS assay by producing unacceptable levels of background fluorescence. The luciferase method avoids these drawbacks. The *LUC* reporter-based genetic screen is more advantageous, because LUC-imaging is noninvasive, and the short half-life of LUC protein [3 hours in mammalian cell cultures (*7*)] makes it suitable for monitoring real-time signaling events. Because no luminescence-emitting compounds are produced in response to abiotic stresses, there is no background interference in a LUC reporter screen. In addition, the *LUC* reporter system is much less time-consuming and is easier to use than the GUS system.

When transgenic plant-expressing firefly *LUC* are sprayed with luciferin, luciferase catalyzes the adenylation of luciferin to produce luciferyl adenylate [which uses adenosine triphosphate (ATP) produced within the cell], which then reacts with molecular oxygen to form photon-emitting oxyluciferin. This bioluminescence can be monitored by a low light imaging camera. Millar and co-workers showed that *CAB2::LUC* mRNA abundance was closely correlated with temporal and spatial regulation of luciferase activity measured in vitro from tissue extracts and in vivo by bioluminescence *(4)*. Thus, gene activity can be measured with extreme precision by an imaging camera.

The success of a reporter-based genetic screen depends on the promoter used to drive the reporter. To dissect environmental stress signaling, Ishitani *et al*. chose the responsive to dehydration (*RD29A*) promoter and constructed the *RD29A* promoter-driven *LUC* reporter gene (*RD29A::LUC*) *Arabidopsis* plants (*8*). The *RD29A* promoter contains both the dehydration-responsive elements (DREs) and abscisic acid (ABA)-responsive element (ABRE), cis-elements that confer responsiveness to drought, salinity, cold, and ABA (*9*).

In this protocol, we present an overview of the details of the *RD29A::LUC* genetic screening method. The firefly LUC coding sequence (*4*) under the control of the *RD29A* promoter was placed in a binary vector, which is a vehicle plasmid for *Agrobacterium*-mediated plant transformation (*8*). The *RD29A* promoter, −650 to −1 base pair (bp), was obtained by polymerase chain reaction (PCR) using the following primer pairs: 5′-TCGGGATCCGGTGAATTAAGAGGAGAGAGGAGG-3′ and 5′-GACAAGCTTTGAGTAAAACAGAGGAGGGTCTCAC-3′. *Arabidopsis thaliana* ecotype C24 was transformed with this *RD29A::LUC* construct by the *Agrobacterium tumefaciens* root infection method (*10*). Plants homozygous for the *RD29A::LUC* gene were selected from the second generation after transformation (T1 plants). One such plant, with a single copy *RD29A::LUC* transgene, was selected for subsequent experiments (hereafter referred to as wild type). The *RD29A::LUC* plant seeds were mutagenized with ethyl methanesulfonate (EMS). These first-generation mutagenized seeds (M1) were grown in soil to produce M2 seeds (naturally self-pollinated), which were then used for mutant screening. Because the *RD29A* promoter is induced by drought, salinity, cold, or ABA, the *RD29A::LUC* genetic screen is useful in isolating mutants that are either specifically defective in a particular abiotic stress signaling pathway, or defective in a combination of the pathways. Here, we describe a protocol to use the *RD29A::LUC* genetic screen to isolate constitutive expression of osmotically responsive genes (*cos*), low expression of osmotically responsive genes (*los*), and high expression of osmotically responsive genes (*hos*) mutants, which are altered in abiotic stress signaling.

Materials

M2 seeds from EMS-mutagenized *Arabidopsis RD29A::LUC* plants (seeds of *Arabidopsis RD29A::LUC* wild-type plants can be obtained from our lab)

Reagents and Supplies

(±)-cis-trans-abscisic acid (Sigma-Aldrich #A1049)

Agar (Sigma-Aldrich #A1296)

Bleach (commercial, ~5.25% sodium hypochlorite)

Disposable petri plates (150 mm × 15 mm; Falcon #1058)

Ethanol (absolute)

Fertilizer (water-soluble, with NPK ratio of 20-20-20) (Scotts-Sierra Horticultural Products, Marysville, OH)

KOH

Luciferin (Promega #E1603)

Murashige and Skoog (MS) salt (JRH Biosciences #56740-50L, Lenexa, KS)

NaCl

Parafilm

Plastic pots, 2.25 × 2.25 inch (Hummert Intl. #16-1052, Earth City, MO)

Plastic transfer pipettes

Polyethylene glycol (PEG) with average molecular mass of 6000 (Sigma-Aldrich #P2139)

Potting medium, Metro Mix 350 (Scotts-Sierra Horticultural Products)

Sucrose

Triton X-100 (Fisher Scientific #BP151-500)

Whatman No. 1 filter paper

Equipment

Luciferase Imaging System (Fig. 1)

Camera controller (Model #ST-138S, 12-16 bits, serial, Princeton Instruments, Trenton, NJ)

Casina TV lens (F 0.95)

Charge coupled device (CCD) (Princeton Instruments)

Computer (Windows 95 or newer operating system, 32 megabytes of RAM and VGA video card with at least 256 colors and 512 kilobytes of memory; two-button Microsoft-compatible mouse; hard drive with sufficient memory to store the images or CD writer, or both)

Cryogenic cooler (Cryotiger, IGC APD Cryogenic, Allentown, PA)

WinView32 software (Princeton Instruments)

Luciferase Sprayer

Fine misting sprayer attached to a pump spray bottle of 30-ml volume

Note: We cut the tip of a fine-misting sprayer from a nasal decongestant bottle to fit into the screw-on cap of another, larger-volume pump spray bottle (such as a hair spray pump bottle). We also curve the solution guide tube of the sprayer so that its inlet end is at the top of the bottle to facilitate the entry of solution when the sprayer is held upside down while spraying.

General Equipment

Cold room or refrigerator (4°C)

Plant growth chamber [22°C, light intensity at 100 μmol per m^2s photosynthetically active radiation (PAR), 16 hours light and 8 hours dark photoperiod, and 70% relative humidity (RH)]

Refrigerator (0 ± 0.1°C)

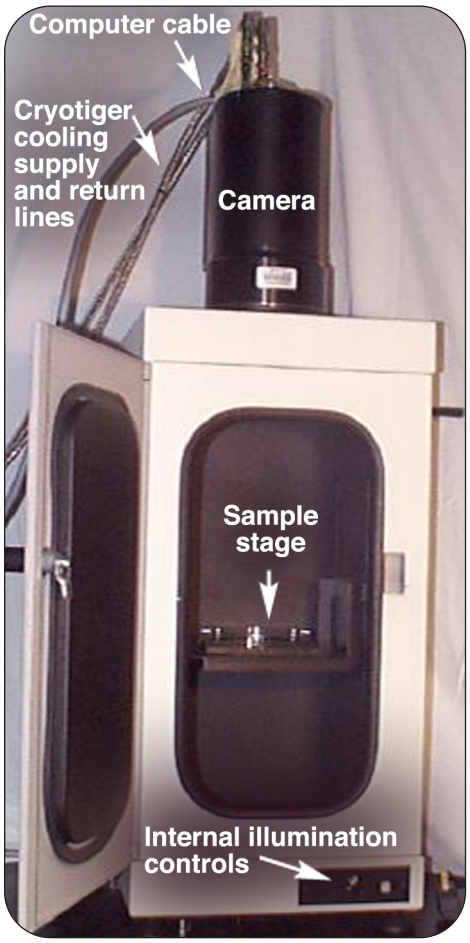

Fig. 1. Luminescence imaging camera and sample chamber.

Recipes

Recipe 1: MS Agar Plates

MS salt	4.31 g
Sucrose	30 g

Dissolve in 800 ml of distilled water. Adjust the pH to 5.7 with 0.1 N KOH.

Agar	6 g

Add the agar to the MS salt-sucrose mixture. Adjust the volume to 1000 ml with distilled water. Autoclave at 121°C for 20 min on slow exhaust. Pour about 75 ml of the MS agar into each 150 mm × 15 mm petri plate in a laminar flow hood. Wrap and store the MS agar plates at 4°C once allowed to solidify for 2 hours.

Recipe 2: Seed Sterilization Bleach

Triton X-100	100 mg
Commercial bleach	1000 ml

Add Triton X-100 into bleach. Store at room temperature.

Recipe 3: 100 mM ABA Stock

(±)-*cis-trans*-abscisic acid	2.643 g

Dissolve in 100 ml of absolute ethanol. Store at −20°C in aliquots of 100 μl in 0.5-ml microcentrifuge tubes.

Recipe 4: 100 μM ABA Solution

Mix 100 μl of 100 mM ABA Stock (Recipe 3) in sterile distilled water to a final volume of 100 ml. Store at 4°C.

Note: A fine-misting sprayer similar to that of the luciferin sprayer can be used to spray ABA.

Recipe 5: 300 mM NaCl

NaCl	17.53 g
MS salt	4.31 g
Sucrose	30 g

Dissolve in 800 ml of sterile distilled water. Adjust the pH to 5.7 with 0.1 Normal (N) KOH and then adjust the volume to 1000 ml with sterile distilled water. Autoclave at 121°C for 20 min. Store at 4°C.

Recipe 6: 500 mM PEG

PEG	300 g
MS salt	4.31 g
Sucrose	30 g

Dissolve in 800 ml of sterile distilled water. Adjust the pH to 5.7 with 0.1 N KOH and then adjust the volume to 1000 ml with sterile distilled water. Store at 4°C.

Recipe 7: 100 mM Luciferin Stock

Luciferin	2.803 g

Dissolve in 100 ml of sterile distilled water. Store at −80°C in aliquots of 100 μl in 0.5 ml microcentrifuge tubes. Cover the tubes with aluminum foil to exclude light.

Note: Luciferin is sensitive to photo-oxidation and should be stored in the dark.

Recipe 8: 0.01% Triton X-100

Triton X-100	100 mg

Dissolve in 1000 ml of sterile distilled water. Store at 4°C.

Recipe 9: 1 mM Luciferin

100 mM Luciferin Stock (Recipe 7)	100 µl
0.01% Triton X-100 (Recipe 8)	9.9 ml

Mix well. Store in dark at 4°C.

Note: This solution (Recipe 9) can be stored in dark at 4°C for up to a week. Keep luciferin sprayer containing this solution also at 4°C.

Instructions

Seedling Culture

1. Prepare MS Agar Plates (Recipe 1).
2. Place an appropriate amount (~5000 seeds) of M2 seeds of the *RD29A::LUC Arabidopsis* plants in a microcentrifuge tube.
3. Add 1.5 ml of Seed Sterilization Bleach (Recipe 2).
4. Incubate for 5 to 10 min at room temperature with intermittent mixing by inversion.
5. Remove the sterilization solution with a disposable plastic transfer pipette.
6. Wash the seeds with 1.5 ml of sterile distilled water five times.
7. Resuspend the seeds in 0.5 ml of sterile distilled water.
8. Plate about 500 M2 *Arabidopsis RD29A::LUC* seeds on each MS Agar Plate (150 mm × 15 mm) (Recipe 1) with a disposable plastic transfer pipette.
9. Seal the plate with parafilm.
10. Incubate the seeds on the plates at 4°C for 2 days to break seed dormancy and to achieve uniform germination.
11. Transfer the plates to room temperature (22 + 2°C) and keep them in the dark or dim light for 1 to 2 days to facilitate proper germination.
12. Incubate the plates at 22°C, 100 µmol per m^2s PAR, with a 16 hours light and 8 hours dark photoperiod and 70% RH, on a raised wire mesh or rack in a growth chamber to facilitate air circulation underneath the plates. This helps prevent condensation of water inside the plates.
13. Grow the seedlings for 5 to 7 days.

Stress Treatments

Seedlings can be sequentially screened for mutants that have constitutive *RD29A::LUC* expression, or increased or decreased *RD29A::LUC* induction under cold stress, ABA, osmotic stress, or a combination of these conditions. To identify mutants that constitutively express stress responsive *RD29A::LUC*, 5- to 7-day-old seedlings are directly imaged for luciferase expression. To identify mutants with increased or decreased induction of *RD29A::LUC*, the seedlings are treated as described below. The LUC activity is assayed in control, unstressed plants and in plants after the first stress (cold) treatment. Then the plants are subjected to sequential, additional stress conditions.

By comparing the luminescence images of mutants with that of the wild type, we grouped the mutants into *cos*, *los*, or *hos* categories (Fig. 2). Because imposed osmotic stress is severe, putative *cos* mutants and mutants with altered response to cold or ABA, or both, may die during osmotic treatment. To avoid loss of these mutants, they are transferred to soil to grow without being subjected to osmotic stress. The osmotic stress response of these putative mutants can be tested using aliquots of their progenies.

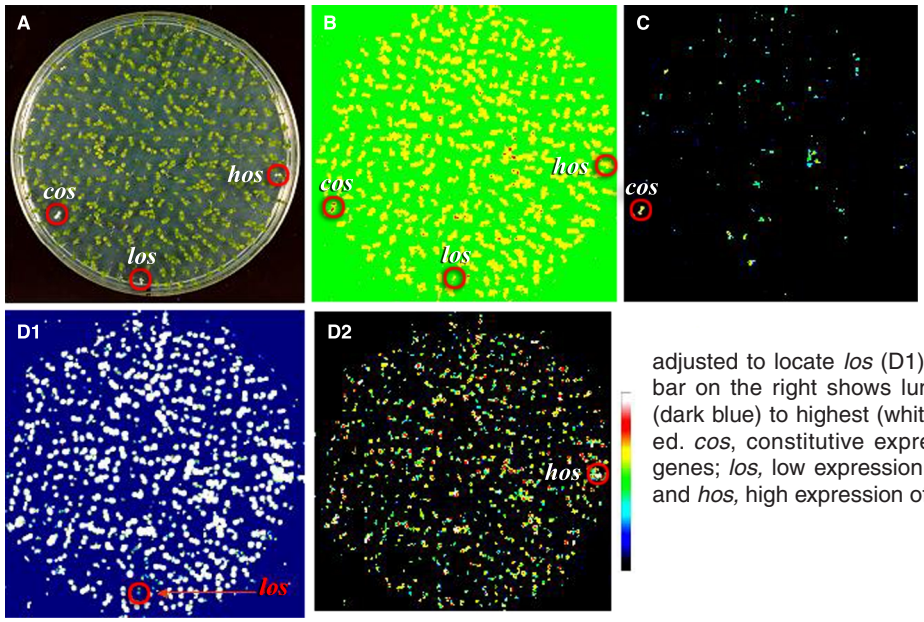

Fig. 2. Screening for stress signaling mutants. (**A**) Six-day-old *RD29A::LUC Arabidopsis* M2 plants in an agar plate, (**B**) chlorophyll fluorescence image of the seedlings, (**C**) luminescence image of the seedlings before stress treatment, and (**D**) luminescence image of the seedlings after cold stress treatment (0°C for 48 hours). The contrast of this image is adjusted to locate *los* (D1) and *hos* (D2) mutants. The color bar on the right shows luminescence intensity from lowest (dark blue) to highest (white). Putative mutants are highlighted. *cos*, constitutive expression of osmotically responsive genes; *los,* low expression of osmotically responsive genes; and *hos,* high expression of osmotically responsive genes.

Cold stress treatment

1. Place 5- to 7-day-old seedlings growing in an MS agar plate at 0°C in the dark for 48 hours.

   Note: Do not stack the plates in the refrigerator, because this may prevent uniform cold stress to seedlings of all plates.

2. Remove the plates from the refrigerator and incubate at room temperature for 15 to 20 min before LUC imaging.

   Note: Incubation of cold-treated plants at room temperature (22±1°C) for 20 min before imaging is essential to achieve high luciferase activity and thus luminescence.

3. Perform LUC imaging (see below).

ABA treatment

1. After LUC imaging of the cold stress-treated plates, incubate the plates at 22°C, 100 µmol per m^2s PAR, with a 16 hours light and 8 hours dark photoperiod and 70% RH, in a plant growth chamber for 24 hours.

   Note: This allows the luciferase synthesized in response previous stress treatments to be eliminated.

2. Spray 100 µM ABA Solution (Recipe 4) onto the leaves of the seedlings until the leaves are wet.
3. Incubate the plates at 22°C, 100 µmol per m^2s PAR and 70% RH, in a plant growth chamber for 3 hours.
4. Perform LUC imaging (see below).

Osmotic stress treatment

1. After LUC imaging of the ABA-treated plates, incubate the plates at 22°C, 100 µmol per m^2s PAR, with a 16 hours light and 8 hours dark photoperiod and 70% RH, in a plant growth chamber for 24 hours.
2. Gently pull the seedlings out of the MS agar plate and transfer them to a petri plate containing one layer of Whatman No. 1 filter paper soaked with 300 mM NaCl (Recipe 5) or 500 mM PEG (Recipe 6).

   Note: PEG is used to impose osmotic stress. NaCl is used to impose osmotic and ionic stress.

3. Incubate the plates at room temperature under light for 3 hours.
4. Perform LUC imaging (see below).

Imaging System

The imaging system consists of a cooled CCD, a camera controller, and a computer with Win View/32 software. The camera has 1300 × 1340 pixel resolution, which allows an image to contain a large number of small plants and also enables one to identify the part of the plant that emits luminescence. The camera uses a Casina TV lens (F 0.95). The CCD chip inside the camera is cooled to −100°C by a compressed gas cryogenic cooler system. The cooling of the CCD chip is essential to reduce the noise-to-luciferase signal ratio. The camera is mounted on the top of a dark chamber with a variable height sample holding platform, so that the distance between the sample and lens can be adjusted. This whole setup is placed inside a dark room and connected by a cable to the camera controller. The controller controls the shutter and temperature of the camera and converts the analog output of the camera to digital input required by the computer. The controller is connected to the computer by a PCI serial computer interface card.

Mutant Screening

Background subtraction

Background images must be used because of internal noise of the imaging system, which is usually around 200 counts per pixel. When the background exceeds 1000 counts per pixel, the detector should be sent to the manufacturer to restore the vacuum in the CCD chip chamber.

1. Turn the luciferase imaging system on and allow it to cool until the CCD chip temperature reaches −100°C.
2. Place an empty petri plate on the sample stage inside the camera chamber.
3. Close the camera chamber door.
4. Run the WinView32 software; use “acquire background” menu for automatic background subtraction.

   Note: The exposure time for luciferase imaging of the samples should be the same as that used for background image. Usually, a 5-min exposure time is sufficient.

Luminescence imaging

Chlorophyll fluorescence occurs in green seedlings growing under light and can interfere with imaging the luciferin fluorescence. We recommend dark incubation of seedlings for 5 min before luminescence imaging for this chlorophyll fluorescence to decay, thus preventing interference. In addition, a full 5-min dark incubation allows full penetration of luciferin into cells.

1. After each treatment, spray 1 mM Luciferin (Recipe 9) on the seedlings in a petri plate.
2. Place the plate (without lid) immediately onto the sample holder inside the camera chamber and close the camera chamber door.
3. Wait 5 min, then acquire the bioluminescence image.
4. Spray the next plate of seedlings with 1 mM Luciferin (Recipe 9) and keep inside a dark box in the dark room.
5. After the imaging of the first plate is completed, place the next luciferin-treated plate inside the camera chamber (without exposing it to light) and image directly without further dark incubation.

Imaging fluorescence

The fluorescence image provides a digital position of all green seedlings in the plate, which may help in matching luciferase signal to a particular seedling in the plate.

1. After obtaining luminescence image, open the camera door briefly (30 s) to expose the seedling to light.

   Note: Don’t move the plate!
2. Close the camera door.
3. Acquire fluorescence emitted by the seedling immediately by running WinView32 software set at 30-s exposure time.

Identification of mutants

In a plate, most of the seedlings will show similar luminescence intensity. These are wild-type plants. Putative mutant seedlings will be those with luminescence intensities that are higher or lower than most of the rest of seedlings in the same plate (Fig. 3).

1. Subtract the background image.

2. Adjust the image contrast.
3. Select the putative mutant seedlings, which show high, low, or no luminescence signal when compared to the wild-type seedlings.

4a. To locate the putative mutant seedlings on the plate, superimpose the plate on the luminescence image of that plate on the computer monitor, and, with a glass marker pen, mark mutant seedlings on the plate.

4b. Alternatively, compare the fluorescence image with luminescence image to locate the mutant seedlings.

5. For the selected mutants, estimate the luminescence intensity using WinView32 software and compare it with the lumines cence intensity emitted from the same number of pixels by a wild-type seedling.
6. Transfer each of the putative mutant seedlings separately to soil medium in plastic pots.

Note: After identifying the mutants in the NaCl- or PEG-treated plants, remove the selected putative mutants immediately and briefly rinse them in water before transferring them to soil to grow to maturity.

7. Place these pots at 22°C, with a 16 hours light and 8 hours dark photoperiod and 70% RH, in a plant growth chamber.
8. Irrigate the plants with water once every 5 days and fertilizer solution once every 15 days.
9. Harvest the seeds of each putative mutant (naturally self-pollinated) separately.
10. Use 15 to 20 progeny seedlings from a putative mutant for the second screening by the luciferase imaging, as described above.
11. If all the progenies of a putative mutant retain the mutant luciferase phenotype in the second screening, this putative mu tant then becomes a confirmed mutant.

Note: If the mutation is dominant and the mutant is heterozygous, it will show segregation for luminescence in second screening.

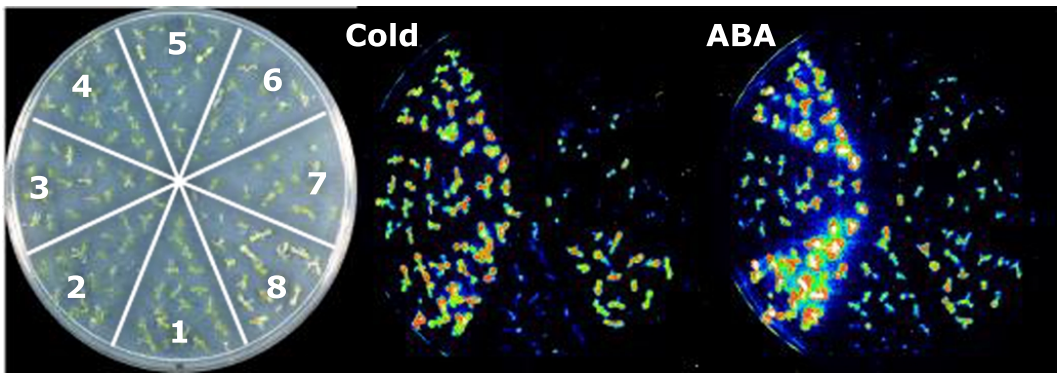

Fig. 3. Examples of mutants with altered *RD29A::LUC* expression in response to stress treatments. (**left**) Photograph of wild type (WT; 1) and mutants of *Arabidopsis* grown in an agar plate; (**middle**) luminescence image after a low-temperature treatment at 0°C for 24 hours; (**right**) luminescence image after treatment with 100 μM ABA for 3 hours. (7), a *los* mutant with a reduced response to cold; (5, 6), a *los* mutant with a reduced response to cold and ABA; (3), a *hos* mutant with an enhanced response to cold; and (2, 4, 8), a *hos* mutant with an enhanced response to cold and ABA.

Notes and Remarks

Our *RD29A::LUC* reporter-based genetic screen is a high-throughput method for selection of plant mutants altered in cold, osmotic stress or ABA signal transduction, or a combination of these pathways. Luciferase imaging of up to 1000 seedlings from mutagenized seeds in a single petri plate takes 5 min or less, and hence as many as 30,000 plants can be screened in one day. Comparison of the luminescence intensities of plants in the same petri plate allows easy identification of mutants that have altered stress sensing or signaling, as indicated by altered luciferase expression. Because luciferase has a very short half-life, the same plants can be subjected to successive and different stress treatments. Thus, the same plants can be successively screened for responses to the stresses. This is comparable to the advantages of replica plating in prokaryotic genetics.

The success of the LUC reporter genetic screen depends on the promoter used. The *RD29A* promoter is activated by cold, drought, salt, or ABA. This makes it possible to select mutants that either are defective in response to a particular stress or have defects in signaling in response to combination of stresses. The *RD29A* gene encodes a stress-responsive protein that might play a vital role in cellular stress protection. Expression of *RD29A* is regulated by the transcription factors DREB1A (also known as CBF3), DREB1B (also known as CBF1), and DREB1C (also known as CBF2). A *DREB1::LUC* construct can be used to isolate mutants that have defects in the regulation of DREB1 transcription factors. Hence, the same approach can be used to specifically dissect the upstream parts of the signaling pathways. The luminescence intensity of the LUC reporter depends on the promoter strength, as well as the plant ecotype. For example, *Arabidopsis thaliana* ecotype C24 with *RD29A::LUC* shows higher luminescence intensities than does the Columbia ecotype. The luminescence intensities of *DREB1A::LUC*, *DREB1B::LUC*, or *DREB1C::LUC* plants are lower than that of *RD29A::LUC* plants.

Because luciferase imaging is quantitative, it is possible to isolate mutants with qualitative and quantitative differences in signaling. Temporal analysis of signaling events at high spatial resolution in a single plant is also possible with this genetic screen. Isolation of mutants that emit luminescence from only a particular part of the plant may elucidate tissue specific signaling. The *RD29A::LUC* reporter imparts a visible phenotype to abiotic stress signaling. This genetic screen was used successfully to isolate many *cos*, *los*, and *hos* mutants of the abiotic stress signaling network (*8*, *11-15*).

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