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A Method for Screening Sensors of Calcium Signaling in Rice

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Abstract

Although rice is an important staple food crop of nearly half of the world population, the discoveries in calcium ion signal were mainly focused on the model plant *Arabidopsis* and researches in rice are very superficial and no calcium ion sensors have been found yet. Recently, the recombinant aequorin was introduced into rice, and the Ca^{2+} responses to NaCl and H_2O_2 in rice roots were characterized. This system can also be used to identify novel Ca^{2+}-coupled stress sensors or pathways after the establishment of an EMS-mutagenized population. In this paper, we present the screening design of the specific program in details.

Keywords: Rice; EMS; Screening; Calcium signaling sensor

Engineering Modelling and Learning

Rice (*Oryza sativa*) is the most widely consumed staple food over the world, especially in Asia. According to the data of FAOSTAT 2012 (http://faostat.fao.org/site/339/default.aspx), it is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize. Based on archaeological evidence, it was commonly accepted that rice was first domesticated in the region of the Yangtze River valley in China [1]. However, the current view is different from the previous. Genetic evidence showed that all forms of Asian rice, both *indica* and *japonica*, spring from a single domestication that occurred 8,200–13,500 years ago in China of the wild rice *Oryza rufipogon* [2]. Through a map of rice genome variation, a study indicated that the domestication of rice occurred in the Pearl River valley region of China based on the genetic evidence. From East Asia, rice was spread to South and Southeast Asia [3]. During the whole growth period of rice, the environmental impacts and pests and diseases threat to rice production. Calcium is one of the most important signaling molecules, which makes the plants overcome the stress and make appropriate adjustment under the outside of the stress.

Aequorin-based platform is the most effective indicator of calcium ion signaling in whole plants [4]. Based on this system, two sensors of calcium signaling have been characterized in *Arabidopsis* [5,6]. However, due to the lack of similar system, no calcium sensors have been reported in rice. Recently, Zhang et al. introduced recombinant aequorin into rice (*Oryza sativa*) and examined the change of Ca^{2+} concentration in response to salt and H_2O_2 stresses [7]. Because typical forward genetic screening is always useful to find genes involved in Ca^{2+} signaling, this newly established system can be used to identify novel Ca^{2+}-coupled stress sensors or pathways in rice after the establishment of an EMS-mutagenized population. Here we present a protocol for screening of NaCl insensitive mutants and isolation of the corresponding genes.

- First of all, we shall establish an EMS-mutagenized population of rice expressing apoaequorin.
- Before luminescence imaging, the M2 seedlings are sprayed with coelenterazine solution (final concentration 10 μM) for reconstitution. Reconstitution was allowed to proceed for 5 hours at 21 °C in the dark.
- For screening for the mutant insensitive to NaCl, the rice seedlings are treated with 0.25 M NaCl.
- Aequorin bioluminescence imaging is performed using a ChemiPro HT system (Roper Scientific) equipped with a light-tight box, a cryogenically cooled and back-illuminated CCD camera and a liquid nitrogen autofiller. The camera is controlled by WinView/32 (Roper) and bioluminescence images are analysed using MetaMorph 6.3 (Molecular Devices).
- All M2 seedlings that show weaker luminescence in roots are selected. These candidates are collected for M3 seeds individually. The M3 individual lines are confirmed by second round screens and lines with stable phenotypes are NaCl insensitive mutants.
- The mutants are backcrossed (BC) with the aequorin expressing wild-type. The BCF2 population of mutant lines that showed a 1:3 (mutant: wild-type) ratio are mapping populations described by Abe et al. [8].
- If we use a traditional map-based cloning strategy to isolate the corresponding gene, the mutant should be crossed to wild-type of different ecotypes and followed by self-fertilizing to yield an F2 population.
- To identify a mapping population from the F2 population, F2 seedlings are treated with NaCl, and seedlings with weaker luminescence are selected. Note that seedlings without any luminescence should be discarded, because these seedlings may have lost the apoaequorin after crossing and self-fertilizing (about 25% of F2 would lost apoaequorin). These populations are candidates of mapping populations.

The seedlings from candidates of mapping population are self-fertilized and collected for F3 seeds individually. The F3 individual lines are confirmed by NaCl treatments again. The F2 candidates with stable phenotypes in F3 generation are the final mapping population. This step is necessary, because the mapping lines should be homozygous at both the aequorin and mutant loci.

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