Improving plant stress-tolerance: potential applications of engineered MAPK cascades

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Abstract

There will be a paramount need to develop solutions to the problem of worldwide food crop scarcity under exacerbated climate change. This motivates an effort to leverage agricultural biotechnology to improve plant adaptation to severe and multiple, simultaneous environmental stresses. Consequently, engineering synthetic signaling pathways, particularly mitogen activated protein kinase (MAPK) cascades utilizing components already involved in plant stress tolerance, is a worthy focus for research to breed new plant varieties with enhanced stress-tolerance traits.

Keyword: MAP kinase, mitogen activated protein kinase, MAPK transgenic circuit, environmental stress, plant adaptation.

Rationale for engineering plant MAPKs to enhance stress tolerance

MAPKs are involved in multiple environmental biotic and abiotic stress signaling pathways, reviewed in ([Pitzschke et al., 2009](#)) ([Rodriguez et al., 2010](#)) ([Moustafa et al., 2014](#)). Overexpression of single MAPKs genes in plants yields positive effects on stress adaptation *in vitro* and under laboratory conditions, but results of single genes approach under field conditions are still below expectations ([Castiglioni et al., 2008](#), [Mittler, 2006](#)). This suggests that a single gene approach is not sufficient to produce inherently stress-adapted varieties. Overexpression of a single MAPK gene may result in a mismatch in stoichiometry between the gene product and its target, leading to inefficient signal transduction and poor cellular function. Overexpression of all components of a MAPK cascade (receptor, adaptors, kinases, effectors), would ensure correct stoichiometry. Augmenting the concentrations of MAPK components can also increase the sensitivity and decrease the activation threshold of a cascade ([O'Shaughnessy et al., 2011](#)). Additionally, given the similarity and conservation of components from different MAPK pathways ([Widmann et al., 1999](#)), components from one pathway or plant species could be used in another, or multiple pathways could be connected. These “rewired” components could comprise one or more synthetic MAPK circuits to generate new plant genotypes with improved adaptability to multiple environmental stresses. The output of such circuits can be assessed by measuring the amount of osmolytes and reactive oxygen species under both biotic and abiotic stress conditions.

How would a synthetic MAPK cascade improve plant stress tolerance?

Gene candidates for engineered MAPK cascades

The ABA receptor ([Kim et al., 2014](#)) ([Okamoto et al., 2013](#)), the kinases MKKK20 ([Kim et al., 2012](#)), ZmMKK1/ZmMKK3 ([Cai et al., 2014](#)) ([Zhang et al., 2012](#)), and ZmMPK5/ZmSIMK1 ([Zhang et al., 2014](#)) ([Wang et al., 2014](#)), and the transcription factors WRKY ([Okay et al., 2014](#), [Pan and Jiang, 2014](#)) were all implicated in plant responses to environmental stress. This provides a diverse repertoire of candidate genes for assembling into synthetic circuits (Figure 1) and testing for improved plant stress tolerance. To improve plant resistance to biotic stresses, a complete MAPK cascade comprising the flagellin receptor (FLS2), the kinases MEKK1, MKK4/MKK5, and MPK3/MPK6, and the transcription factor WRKY ([Asai et al., 2002](#)) would be a worthy choice for engineering. In an abiotic stress context, a good candidate is an incomplete cascade comprising the kinases MEKK1, MKK2, and MPK4/MPK6) ([Teige et al., 2004](#)). Although this cascade lacks a receptor and effector, the core components could still be used.

Potential crosstalk with native pathways

A major concern of a synthetic MAPK circuit is the potential crosstalk between the synthetic circuit and the native signal pathways, which may produce unexpected or deleterious effects. One possible strategy to circumvent any potential undesirable crosstalk is to design synthetic circuits as orthogonal or heterologous modules, utilizing components from other organisms, which would not interact with native signaling components. However, many multiplex transgenic corn lines were created, expressing at least five genes involved in vitamin metabolic pathways, with no undesirable effects observed ([Naqvi et al., 2009](#)). An entire metabolic pathway was engineered in Arabidopsis with only minor inadvertent effects on the metabolome and transcriptome ([Kristensen et al., 2005](#)). In bacteria, the rewiring of promoters with different transcription or sigma factor genes in about 600 combinations was also tolerated by the organism with only minor effects on growth ([Isalan et al., 2008](#)).

Alternative deployment of synthetic MAPK circuits

In addition to responding to natural stimuli, synthetic MAPK circuits can be engineered to respond to non-natural stimuli by linking MAPK component gene expression to inducible promoters, driven by molecules such as estradiol ([O'Shaughnessy et al., 2011](#)). Estradiol-induced gene expression of the heat-shock factor HSFA4A confers enhanced tolerance to salt and oxidative stresses in Arabidopsis ([Pérez-Salamó et al., 2014](#)). This demonstrates the viability of exogenous applications of appropriate inducers to drive gene expression in whole plants. In the field, the circuits could be activated by spraying plants with a solution containing the appropriate inducers (e.g. ligand, abscissic acid or others) at the concentrations determined to give the best output, similar to how we currently deliver some pesticides or herbicides. Another option is to dissolve the inducers in the crop irrigation water.

Figure 1. A synthetic *MAPK transgenic circuit* could be engineered from genes involved in stress responses, from receptor to targeted transcription factor. ABA receptor (Kim et al., 2014) (Okamoto et al., 2013), MKKK20, ZmMKK1/3, ZmSIMK1/ZmPK5 and WRKY transcription factors are all involved in environmental stress-responses; they could be assembled from different plant species into a synthetic MAPK transgenic circuit (or synthetic signaling modules) to transform plant and assess plant-stress tolerance :

ABA receptor-MPKKK20-ZmKK1/ZmKK3-ZmSIMK1/ZmPK5-WRKY

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