

Review

Gene-edited protein kinases and phosphatases in molecular plant breeding

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Protein phosphorylation, the most common and essential post-translational modification, belongs to crucial regulatory mechanisms in plants, affecting their metabolism, intracellular transport, cytoarchitecture, cell division, growth, development, and interactions with the environment. Protein kinases and phosphatases, two important families of enzymes optimally regulating phosphorylation, have now become important targets for gene editing in crops. We review progress on gene-edited protein kinases and phosphatases in crops using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9). We also provide guidance for computational prediction of alterations and/or changes in function, activity, and binding of protein kinases and phosphatases as consequences of CRISPR/Cas9-based gene editing with its possible application in modern crop molecular breeding towards sustainable agriculture.

Protein kinases and phosphatases as targets for gene editing

Plants respond to various environmental stresses and maintain their homeostasis via regulatory and signaling mechanisms that developed throughout the process of evolution. Protein phosphorylation, the most common and basic post-translational modification, belongs to crucial regulatory mechanisms of plant stress resistance, closely linked to plant growth and development. It is a very efficient way of signal transduction because only one ATP molecule is needed for the introduction of a negative charge during the phosphorylation of an amino acid residue. Phosphorylation may directly affect proteins, phospholipids, and other biomolecules by changing their structure, function, activity, binding specificity, and subcellular localization. This leads to alterations in metabolism, intracellular transport, cytoarchitecture, cell division, growth, and development. Due to the reversible addition or elimination of the phosphate group, proteins can switch between phosphorylated and dephosphorylated states, resulting in various functional consequences. Protein kinases and phosphatases are two important families of cellular enzymes that optimally regulate the phosphorylation status of proteins and maintain the balance between phosphorylation and dephosphorylation [1,2] (Box 1 and Box 2).

Thanks to their irreplaceable role and importance, protein kinases and phosphatases have come to the attention of researchers as well as breeders and are promising targets for plant gene editing. Overexpressions and knockouts of protein kinases and phosphatases play an important role in elucidating their biological functions in plants. While transfer DNA (T-DNA) insertion is a suitable approach to knock out a single gene in a diploid organism such as the model plant *Arabidopsis thaliana* (*arabidopsis*), preparation of gene knockout for polyploid crops is challenging. The invention of gene editing technologies has overcome methodological and technical problems of the classical mutagenesis methods, which were inexact and time-consuming. These modern methods are evolving quickly and allow precise knockout of the genes and/or editing their sequences. Among others, three techniques widely used for editing protein kinase and

Highlights

Recently, significant advances have been achieved in gene editing of plant protein kinases and phosphatases using clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology.

Progress in computer science and programming could significantly improve CRISPR/Cas9 gene-editing predictions and subsequent wet-laboratory experiments on protein kinases and phosphatases.

Gene-edited protein kinases and phosphatases could considerably improve desirable crop traits and stress tolerance.

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