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Geeta Prakash
Department of Botany, Ramjas
College, University of Delhi,
Delhi, India-110007

Suresh Kumar
Department of Botany, Ramjas
College, University of Delhi,
Delhi, India-110007

Khaling Mikawlawng
Department of Botany, Ramjas
College, University of Delhi,
Delhi, India-110007

Metabolic Pathways Engineering: an emerging approach in abiotic stress tolerance in plants

Geeta Prakash, Suresh Kumar, Khaling Mikawlawng

ABSTRACT

Abiotic stress factors, such as drought, salinity, temperature extremes (high and low) and submergence are major limitations to crop yield. Different approaches have been proposed for the management of different kind of stresses. Biotechnology has emerged as an important tool for molecular understanding of plant response to various abiotic stresses. Currently, numerous stress-responsive genes have been identified and functionally analyzed in transgenic plants. However, very inadequate success has been achieved in developing abiotic stress-tolerant cultivars. Therefore, there are immense needs to identify stress responsive genes/pathways for effectively overcoming several abiotic stresses in plants. Recently, metabolic pathways involved in abiotic stress responses have developed wide attention of researchers, a better understanding of which can help achieve this target. Metabolic engineering in plants can be used to increase the plenty of specific valuable metabolites, but single-gene introduction or single interventions generally do not improve the yields of target metabolites and sometimes it also causes the increase in undesirable metabolic products. Targeting multiple genes in a pathway by regulating the expression of key elements, such as a transcription factors is another means, but this approach is only possible if such core regulators are worked out exist and identified. A more potential approach is the simultaneous expression of various multiple genes in the pathway, if possible representing every critical step of enzymatic reactions, therefore removing all hindrances and surely completely unobstructed metabolic flux. This approach needs to the transformation of multiple enzyme-encoding genes to the recipient plant, which is attained most efficiently if at the same time all genes are transferred. The present review discusses the recent findings on the significance of metabolic pathways or metabolic engineering during abiotic stresses in plants.

Keywords: Abiotic Stress, Multigene Transformation, Osmoprotectants, Metabolic Pathways.

1. Introduction

Abiotic stresses are one of the major causes of crop losses throughout the world. Abiotic stresses such as drought, salinity, extreme temperature and ion imbalance are key limitations for growth and productivity of crop plants. Organisms that live in habitats where these factors predominate have evolved various adaptations to these stresses. Certain plants, marine algae, bacteria and other organism have developed a number of adaptations in responses to such abiotic stresses. Different approaches have been proposed to overcome from these adverse conditions. As abiotic stress is influenced by many factors, conventional breeding have resulted less success in getting stress tolerant plant. It is well distinguished that metabolic changes are the important part of any response to stress in microbes ^[1, 2]. Current gene expression analysis has also proved that genes involved in various metabolic pathways are also influenced by abiotic stresses in plants ^[3-6]. In a detailed study in model plant *Arabidopsis thaliana*, the strong effect of abiotic stress was identified on several metabolic pathways such as the tricarboxylic acid cycle, glycolysis and oxidative pentose phosphate pathway ^[4]. Further, a coordinated response of the gene transcription involved in different metabolism was found to redesign various metabolic reactions to overcome with several metabolic changes. Notably, the overall metabolic reaction of *Arabidopsis* cells was found similar to the microbes ^[4], which showed that awareness of microbial system can also be replicated in plants to analyze the important core regulators of responses during stress conditions.

2. Metabolic Pathway

Most of the important agronomic traits in plants are regulated by multiple genes, also the synthesis of complex organic compounds from primary and secondary metabolisms are most often regulated by long and complex metabolic pathways. Therefore, genetic engineering has seen a progressive alteration from single-gene involvement to multigene transformation to attempt increasingly determined goals ^[7].

Correspondence:
KHALING MIKAWLRAWNG
Department of Botany, Ramjas
College, University of Delhi,
Delhi, India-110007.
Email: khaling55@gmail.com
Tel: +919953228994

This principle was applied in the examples of metabolic engineering, which engage the modification of endogenous metabolic pathways to increase change towards exacting advantageous molecules or even new molecules for example osmoprotectants [8]. Multigene transformation (MGT) is now being conventional move to generate plants with more determined phenotypes, including more complex examples of metabolic engineering [9]. Examples of metabolic engineering in plants include primary metabolic pathways (carbohydrates, lipids and amino acids,) and secondary metabolic pathways (e.g. alkaloids, terpenoids, flavonoids, lignins, quinones, and other benzoic acid derivatives) [10]. These metabolic pathways produce a large number of compounds that are valuable to plants during various stress conditions to adapt themselves, and also for improved food quality. In this review, we focus on the metabolic engineering of osmoprotectants, because they provide illustrative examples of applied MGT.

3. Manipulating the Osmoprotectants

Genetic transformation technology enables us to achieve gene transfer in a specific and, to some extent, expected manner. Metabolic traits, especially the pathways with few enzymes, are better characterized genetically and more acquiescent to such manipulations than structural and developmental traits. Thus, the metabolic engineering of pathways that resulted in the synthesis of osmoprotectants in plants. Some osmoprotectants also protect the metabolic machinery against various abiotic stress damage. Many major crops are not able to synthesize the unusual osmoprotectants that are naturally accumulated by stress-tolerant organisms. Therefore, it was hypothesized that establishing osmoprotectant synthesis pathways is a potential approach to produce stress-tolerant crops. Proving this, new engineering efforts in model species led to important but significant enhancement in stress tolerance of transgenic plants. Future research avenues include the identification and exploitation of diverse osmoprotectants in naturally stress-tolerant organisms, and the use of multiple genetic engineering to increase osmoprotectant change in response to stress. High-throughput genomic technologies offer a number of tools to refine this by rapidly identifying genes, pathways, and regulatory controls.

Osmoprotectants are produced by the many of metabolic pathways, such as sugars (trehalose, sucrose and fructan), amino acids (tryptophan and proline) and ammonium compounds (polyamines and glycinebetaine) biosynthesis pathways. These osmoprotectant molecules accumulate in plants in high concentration under stress conditions as an adaptive mechanism, which can provide stress tolerance. The introduction of genes responsible for the production of such osmoprotectants has been used to improve stress tolerance in crop plants [11-13]. And most significant metabolic pathways are those regulated various reactions involved in detoxification of reactive oxygen species (ROS) produced during in response to abiotic stress conditions. ROS are toxic compounds, which cause damage to normal cellular metabolic pathways. The modification of genes responsible for the production of enzymes (peroxidase and catalase) involved in scavenging of ROS can enhance tolerance to abiotic stresses. Metabolic pathways which are responsible for hormone production has also become known as an important factor in regulating plant stress response. Even though accurate mechanism is still unknown, genes involved in metabolism pathways and enzymes of different plant hormone like auxin, cytokinin, ethylene and abscisic acid have been concerned in different stresses [14,15]. In fact, detailed knowledge on levels of some of these plant hormones involved in the production of many

secondary metabolites and osmoprotectants are well known [16,17]. The metabolic pathways that regulate the biosynthesis of cell wall components, such as cellulose and suberin, also play a significant role in adaptation during various stress conditions [18,19]. Lipid and carbohydrate metabolic pathways are also significant objective of research as some of their components have been found to be regulated by abiotic stresses [20, 21]. A few studies have already proved that modification of levels of soluble sugars and plant sterols can develop stress tolerance in plants [21, 22]. It has been verified that enhanced carbohydrate metabolic reactions can act as preventing strategy for plants under submergence stress conditions in an effort to grow above water level and resume photosynthesis [23].

Recently, metabolomics has been projected as a secondary and significant approach to the recombinant DNA technology-assisted selection for crop improvement [24, 25]. A small number of mQTLs have already been recognized in *Arabidopsis*, tomato and *Populus* and have been revealed to have intermediate heritability [26, 27]. The incorporation of gene expression and QTL mapping with metabolite profiling illustrated a composite relation among them [28].

4. Synthetic biology as the next step for metabolic pathway engineering

Synthetic biology describes the *de novo* assembly of genetic organization using confirmed components [29]. In relation to metabolic engineering in plants, a synthetic biology approach would employ specific promoters, genes, and other regulatory components to generate perfect genetic path that make possible the accumulation of certain important metabolites. The idea of synthetic biology creates engineering and mathematical modelling to expect and check the performance of the resulting system, which can be taken into account as the next move in multigene metabolic engineering because it eliminate any dependence on naturally occurring sequences and permit the design of ideal functional genetic path from first principles. Thus far, most work on synthetic biology has been proficient with microorganisms, in spite of a number of limiting feature, such as the ability of currently used methods to accumulate complex DNA molecules encoding multiple genetic elements in predefined arrangements [30]. Simple synthetic biology approaches have been described in plants, mostly in the perspective of signaling pathways and development, but also in the development of phytodetectors [31] and biofortified crops [9]. The use of synthetic biology in metabolism as well as development is important because it not only regulates the metabolic activity of a cell, but also one step ahead in terms of organization and use of particular promoters and genes that control developmental and metabolic processes during various stress conditions to generate novel tissues, in which the cells have specialized biosynthetic or storage organs which accumulate target products. This approach will facilitate the attainment of goals that are not possible through conventional genetic engineering, such as the development of novel organisms with medical functions, the production of biofuels, and the removal of hazardous waste [32].

5. Multiple gene transformation for pathway engineering

Genetic engineering for metabolic pathway involves the handling of specific enzymatic reactions for enhancement of cellular characteristics during stress conditions. Recent studies have already established the important role of metabolomics for improving stress tolerance in plants. So far, the core of such genetic engineering research has been on the manipulation of a one gene i.e. responsible for a specific metabolic pathway for improvement of

stress tolerance in plants. However, taking into account the involvement of multiple genes in abiotic stress, the engineering of single a gene may not be very successful. Therefore, it would be further significant to modify multiple complex enzymatic reactions involved in same or different metabolic pathways to enhance abiotic stress tolerance in plants. A few reported examples already listed, which used this technology to develop plants with improved stress tolerance [13]. These studies reveal the high potential of simultaneous manipulation of many steps of single enzymatic reactions or multiple enzymatic reactions. Further, the identification of core regulators of significant metabolic pathways, such as transcription factors, can also provide a valuable approach for improving of stress-tolerance in plants through manipulating a single gene only.

6. Role of Computational Biology

The various involvement of metabolic pathways build a challenge in recognizing the core regulatory components of metabolic pathways involved in abiotic stress responses. A few databases of identified metabolic pathways in different organisms are available and few models have also been predicted [33-36], which can provide the knowledge about the key metabolic pathways involved in a particular biological and physiological response. Although better and efficient databases and tools are mandatory, the existing databases and models can also be used to identify and reveals the key metabolic pathways and responsive functional components for abiotic stress conditions. The accessibility of such tools can pave the way for genetic annotations and elucidate of role of unique genes in the whole metabolic network of an organism.

7. Conclusion

The significant importance of metabolic pathways in abiotic stress response is apparent. Modification of metabolic pathways is now being increasingly used to improve plant stress responses. Therefore, there is an immense necessity of methodical thorough investigations to describe their precise role and identify the involvement of significant enzymes/pathways. Further detailed studies in model plants can lay down the system to unravel the core regulators as an objective for manipulation of stress responsive genes for improved tolerance in crop plants.

7. References

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