Strategies Applied in Phytohormone-Targeted Genetic Engineering

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As abiotic and biotic stresses cause severe losses in agriculture, it is also crucial to obtain varieties that are more tolerant to these factors. In the past, traditional breeding methods were used to obtain new varieties displaying demanded traits. Nowadays, genetic engineering is another available tool. An important direction of the research on genetically modified plants concerns the modification of phytohormone metabolism.

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1. Introduction

Due to the constantly growing human population, ensuring high crop productivity is an important challenge for 21st century agriculture. The research aimed at obtaining high-yielding varieties is being carried out [1]. Another important issue is to obtain varieties displaying enhanced tolerance to biotic and abiotic stresses that cause significant loss of yield. Among the abiotic stresses, the most important are drought, thermal stress (too high or too low temperature), light stress, salt stress, and stress caused by environmental pollution, e.g., by heavy metal ions. Due to anthropogenic climate change, an increase in abiotic-stress-evoked losses of crop yield is expected in the near future [2]. Apart from abiotic factors, the biotic ones, such as pathogens, competing plants, parasites, and herbivores, also limit plant growth and productivity.

The application of mineral fertilizers, herbicides, and pesticides, as well as growing high-yielding varieties obtained via traditional breeding methods, enabled a significant increase in crop productivity during the second half of the 20th century [3]. For example, the average cereal yield in 1951 was 1.2 t/ha, while in 1993 it was 2.3 t/ha [4]. However, this yield increase has slowed down in the 21st century. It is currently believed that for the most important crop species, further increases in their productivity obtained by traditional breeding methods are possible only to a small extent. For this reason, research based on genetic engineering became crucial for the future of agriculture [1]. In addition to experiments on transgenic organisms, extensive genome analyses of major crop species are also being carried out. Their goal is to identify quantitative trait loci (QTLs), which are genes determining quantitative traits [5].

Research on transgenic plants conducted over the past decades resulted in the development of various strategies of genetic modification aimed at obtaining lines with increased yield or improved tolerance to stress [6][7]. One of the promising research directions is associated with the modulation of phytohormone levels [2]. Phytohormones participate in the regulation of plant growth and development. They also play a role in response to environmental factors. These compounds include auxins, cytokinins, gibberellins, abscisic acid (ABA), ethylene, jasmonic acid (JA) and its derivatives, and brassinosteroids. Auxins, cytokinins, gibberellins, and brassinosteroids are considered particularly important for the regulation of plant growth and development, while JA, ABA, and ethylene play crucial roles in stress response. However, one needs to remember that growth-stimulating hormones participate in stress responses, while those primarily associated with the stress response are also involved in the regulation of various plant developmental processes, such as dormancy, fruit maturation, or senescence [8]. Plant hormones have pleiotropic effects. Furthermore, the result of their action often depends on cross-talk between various phytohormones and signaling molecules [9]. Phytohormones occur in plants at very low concentrations; their biosynthesis and degradation are strictly regulated. In some cases, reversible inactivation by conjugate formation is also possible [10].

2. Strategies Applied in Phytohormone-Targeted Genetic Engineering

The research aimed at improving crop performance by modification of phytohormone metabolism and signaling starts with identification of the crucial genes. This is possible mainly due to the studies carried out on mutants or by comparing crop varieties displaying desirable traits with the other ones [10]. Gene and genome sequencing enables the identification of loci crucial for the observed effects. Analyses of phenotypes and detailed analyses at the biochemical level, i.e., determination of phytohormone content, enable scientists to discover gene functions. When the sequence and function of its product are known, bioinformatics provides tools to find homologues in other species. At this point, the plant transformation can be carried out to increase or decrease the level of a certain phytohormone. The increase in hormone level can be achieved by the overexpression of the gene encoding enzyme participating in the phytohormone biosynthetic pathway or silencing of the gene whose product catalyzes hormone degradation. The decrease can be achieved by silencing of the gene crucial for phytohormone biosynthesis or by overexpression of the gene whose product is involved in hormone degradation. The manipulation of the genes encoding enzymes carrying out phytohormone conjugation was also carried out [10]. Sometimes, the increase in phytohormone level may be achieved by the enhanced production of an enzyme catalyzing the formation of a metabolite that serves as a phytohormone precursor (for example, xanthophyll precursors of ABA biosynthesis) or a cofactor needed by the hormone-synthetizing enzyme (for example, molybdenum cofactor required for abscisic aldehyde oxidase activity). The research on the engineering of phytohormone transport was also carried out. The significant progress in our understanding of phytohormone signaling opens a wide range of possibilities, as various elements of signaling cascades, transcription factors, and miRNAs are emerging targets for potential modifications. In the early research, scientists used strong, constitutive promoters to provide the overexpression of desired genes. The discovery of tissue-specific, developmental-stage-specific, and stress-responsive promoters enabled the improved control of the time and site of transgene expression [10]. Furthermore, artificial promoters have been developed. Considering gene silencing, various constructs may be applied, including antisense sequences, 3′-untranslated regions, and hairpin constructs. The recent development of the CRISPR/Cas9 system paved the way for extensive genome editing.

The progress in this field is possible due to broadening of the knowledge concerning the regulation of plant growth, development, and stress response, as well as due to the improvement of the methodology used. Many genes that can be targets of genetic engineering have been identified up to date [7]. The extensive research aiming at deciphering phytohormone signaling pathways is being carried out. The modification of this signaling at various levels, from elements of signaling cascades, through transcription factors to miRNAs, is a very promising direction of genetic engineering of crop plants.

Considering the methods of genetic engineering, the most promising innovation is genome editing using the so-called CRISPR/Cas9 system [11]. The system is based on nucleases that can be relatively easily programmed to search for specific DNA sequences. Available variants of effector nucleases allow various modifications of the target region. This makes CRISPR/Cas9 a fast, effective, and precise genome editing tool [11]. It is used both to discover functions of certain genes and to obtain plants of potential application in agriculture. CRISPR/Cas9 genome editing seems especially promising in research aimed at modulation of cytokinin levels [12].

Intensive research on the regulation of gene expression led to the discovery of many promoters specific to certain tissues, organs, or stage of plant development. The application of these promoters allows better control of the time and site of transgene expression. Scientists also designed artificial promoters [13]. There are systems enabling us to combine and introduce multiple genes at once (such as the Golden Gate modular cloning box), as well as methods for the introduction of large DNA fragments into plant cells. New successful protocols of crop species transformation are being developed [7].

An important obstacle in obtaining transgenic plants with improved yield is the well-known trade-off between stress defense and plant growth. One of its reasons is the energetic cost of the development and maintenance of various protective mechanisms, both biochemical and morphological. However, the negative effect of defense induction on growth often results from antagonistic crosstalk between phytohormones rather than from an identified metabolic expenditure. Sometimes, it is caused by pleiotropic effects of certain resistance traits or is a consequence of genetic linkage [14]. Therefore, it is possible to reduce the costs of plant defense. The strategies aimed at such a reduction were summarized by Karasov et al. [14].

To date, the majority of studies on transgenic lines with altered phytohormone content or signaling have been conducted under laboratory conditions. To obtain improved varieties suitable for regular cultivation, it is necessary to carry out large-scale field tests to determine whether the modifications introduced allow us to obtain the desired phenotype under natural conditions. At the same time, care should be taken to minimize the risk of transgene leak, so that genetically modified varieties would not pose the threat of contamination to the genomes of closely related wild species.

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