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REVIEW

Implications of Plants Genetic Transformation Assessed by Geneticist, Biochemist and Physiologist

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Transgenic plants creation methodology developed for several decades has gained significant advances. However, problems of unanticipated effects of transgenosis, stability of GMO characteristics and establishing criteria of their safety evaluation remain unresolved. The analysis of different approaches to assessing the impact of plant genetic transformation is presented. It is concluded that the profound studies on the physiology of plant-agrobacterial symbiosis as a methodological basis of plants genetic engineering can answer many unresolved issues of genetic engineering.

Key words: transgenosis, assessment of implications, different approaches

Creating of transgenic plants with new properties is a complex, multi-step process involving experts of various biological branches: geneticists and molecular biologists, biochemists, physiologists. Each stage addresses a specific range of issues from target gene transfer to the recipient plant to a comprehensive assessment of new biological systems. The need to deal with a wide range of tasks entails the use of different research strategies, different approaches and criteria for evaluation of results. However, ultimately the process of producing a transgenic plant is reduced to two main tasks. The first one is the transfer of the target gene in the genome of the recipient plant, together the stable expression of the transgene, which provides the necessary level of biosynthesis of encoded proteins. The second one is a minimization of the potential pleiotropic effects and, ultimately, elimination of risks of GMO.

Currently, significant success was achieved in solving the first of the above problems. The modern pool of methods allows for the transformation of a very wide range of plants. The transformation process, to a great extent, became routine (Transgenic plants: methods and protocols, 2005; Genetic Engineering: Principles and Methods, 2007). The elaboration of antisense strategy as a tool for the regulation of gene expression has opened additional prospects for the development of genetic engineering (Bourque, 1995). The technologies for creating of transplastomic plants have been developed (Shchelkunov *et al.*, 2011). The second task is still far from a final fulfillment. The existing body of knowledge doesn't provide a definite answer about the properties and characteristics of transgenic plants, the causes and mechanisms of unforeseen transgenic effects (Sorochinskii *et al.*, 2011). Finally, the status of genetically modified plants is not determined: whether they are new varieties, new artificially created species or a new kind of biological system. The consequence is the lack of unified criteria for the safety assessment of GMOs, identifying of the causes and ways to minimize the risks of their use (Meyer, 2011).

The prospects for the practical use of transgenic plants in human economic activities largely depend on the clear interaction between experts in various disciplines. However, significant differences in the rate of molecular biological, biochemical and physiological research areas not only didn't contribute to the development of common criteria, but, on the contrary, led to considerable discrepancies in the assessment of the transgenic effects. The purpose of this article is to give a brief analysis of the problems that are being tackeled today in each approach as well as to assess the prospects for their rapprochement.

The approaches of a geneticist and a molecular biologist

Selection of criteria to measure the impact of genetic transformation of plants is largely determined by the nature of the problem that are being solved in the framework of a particular biological discipline. According to most molecular biologists working in the field of genetic engineering of plants, place of incorporation of exogenous DNA is accidental for each fact of transformation (Tinland, 1996). The effect of regulations i.e. the effect of environment on transgene expression (Matzke, Matzke, 1998; Baudo *et al.*, 2006), the insertion of exogenous DNA in intergenic regions and plant genes encoding portions - insertional mutagenesis (Alonso *et al.*, 2003; Feldmann *et al.*, 1989; Day *et al.*, 2000; Krysan *et al.*, 1999, 2002), are regarded as the main causes of multiple genetic transformation effects and GMO risks (Bartlett *et al.*, 2014). At the same time, now it has accumulated a large amount of data questioning the randomness of the incorporation place. Many authors reported about the presence of "hot spots" where insertion of T-DNA occurred much more often than in other parts of the host genome (Filipenko *et al.*, 2009; Paepe *et al.*, 2013). The plant transformation is often accompanied by transfer and stable integration of vector DNA fragments, that also can cause a whole range of negative consequences, such as breach of a transgene expression and the ability to embed a vector DNA under the plant promoter followed by plasmid gene expression (Permyakova *et al.*, 2009). The step of tissue culture, as part of the creating transgenic plants process, may provoke additional changes of the transformant properties (Fonseca *et al.*, 2015; Kawakatsu *et al.*, 2013; Zhou *et al.*, 2012). In our opinion, the impact of this phase of transgenic plants on the transformants' properties is exaggerated. Somaclones getting through tissue culture has been used in biotechnology for a long time (Reshetnikov *et al.,* 2014), and the variability of symptoms in this case is not comparable with the effects of transgenesis (Sorochinskii *et al.*, 2011).

The problem of instability of the transgenic plants properties has stimulated considerable interest in the study of regulation mechanisms of expression of the transgene at the epigenetic level (Magori, Citovsky, 2011). Silencing of transferred genes, despite significant advances in the study of molecular mechanisms of this process (Marenkova-Novoselia *et al.*, 2007; Finn *et al.*, 2011; Yamasaki *et al.*, 2011), remains a "headache" of specialists in biotechnology (Dietz-Pfeilstetter, 2010). A separate item of this research is the analysis of transgene copy number and influence of this factor on its expression (Reddy *et al.*, 2003). It is found that the maximum expression level is provided by inserting a single copy of T-DNA into the chromosome. Inserting two or more copies entails a transgene silencing (Tang *et al.*, 2007). However, there is conflicting data that the copy number of bets doesn't affect on transgene expression (Joyce *et al.*, 2014; Kohli *et al.*, 1999).

Specificity of problems of molecular genetic research areas limits the criteria for evaluating the effects of transgenesis, analysis of transgene expression and structural genome rearrangements induced by T-DNA insertion. Outside the field of research is the nature of the transgene (the degree of phylogenetic distance of the donor and recipient gene) and the nature of its interaction with the genome of the transformed plant, though this fact may be a key factor in determining the nature of the effects of transgenesis (Nielsen, 2003). Ultimately, the copy number of the transgene and the success of its expression is the only solution of a specific problem of biotechnology. Physiological effects of transgenesis largely determined by the fact of insertion of T-DNA into plant genome of the plant, irrespective of its subsequent expression (Enikeev *et al.*, 2010, 2015).

To date, there is a significant gap between the study of molecular-genetic mechanisms of transformation and the research of the transgenic plant in the physiology, which makes interpretation of the consequences of the transformation of the above-described one-sided and incomplete positions.

Assessment of the transgenesis effects at the biochemical level

GMP significantly differ from varieties established with the help of methods of classical breeding by ways of the making. There aren't standard methods for the safety assessment of GMOs, and their development requires a large amount of scientific research (Magaña-Gómez, Calderón de la Barca, 2009). Changes in the metabolism of transgenic plants unrelated to the expression of the target gene have been described in the literature and have been called "unintended consequences of transformation" (Holdrege, 2008; Sorochinskii *et al.*, 2011). Nowadays, one of the main strategy for risk assessment of GMOs is to identify the differences between transgenic organisms and original forms (Fonseca *et al.*, 2015).

As a possible way of solving the problem the technology of "molecular profiling" ("omics") - a parallel analysis of the compounds of the transgenic and non-transgenic plants and original form - was examined. These technologies, including metabolomics (the analysis of a number of primary and secondary metabolites), proteomics (analysis of polypeptides) and transcriptomics (comparative analysis of gene expression), have proliferated in recent decades due to significant advances in the development of mass spectroscopy methods, NMR spectroscopy, and the development of bioinformatics technologies (Cellini *et al.*, 2004; Li *et al.*, 2005; Rischer, Oksman-Caldentey, 2006; Simó *et al.*, 2014; Zolla *et al.*, 2008). Comparative analysis of the metabolic profiles of genetically modified plants and non-transgenic forms was carried out by many authors, and in most cases, differences in the content of the individual compounds, enzyme activity and other characteristics were revealed (Chang *et al.*, 2012; Kim *et al.*, 2013; Xu *et al.*, 2013). However, they were accidental, excluding the possibility of any generalizations. A more detailed analysis of the proposed approaches has revealed a number of disadvantages of these technologies, it casts doubt on the possibility of their use for the safety assessment of GMOs. Identified differences generally tend to be associated with the biosynthesis of secure compounds. Similar and even greater effect could be caused by a change in environmental conditions (Plischke *et al.*, 2012; Zhao *et al.*, 2013). Moreover, the comparative analysis of two classic varieties could reveal large differences than between transgenic and non-transgenic original plants (Defernez *et al.*, 2004).

Despite the difficulties the use of omics-technologies remains one of the promising areas of possible risks evaluation of using GMOs. However, the possibilities of molecular profiling methods can not be fully realized without detection units of metabolism most exposed to transgenesis (Kim *et al.*, 2011).

Otherwise, these technologies can turn into a "finding a needle in a haystack."

The transgenic plant in the eyes of physiologist

Notwithstanding that more than 30 years passed since the creation of the first transgenic plants, gene engineers did not manage to answer the question about the physiological nature of the new biotechnology facility. Meanwhile, the solution to this problem is the key to the development of a methodology for assessing the consequences of the transformation, including forecasts of the possible risks of GMP using. Several transgenic properties can not be explained by expression of the target gene or by the result of insertional mutagenesis. For example, a significant increase of stability of the transformants to heavy metals and other toxic compounds is showed in a large number of independent studies (Brichkova *et al.*, 2007; Enikeev *et al.*, 2010; Kolodyazhnaya *et al.*, 2006). Chemical analysis of Bt-corn plants of different origin revealed a significantly higher content of lignin as compared to non-transgenic isolines (Flores *et al.*, 2005; Saxena, Stotzky, 2001). Analysis of the causes and mechanisms of these changes in the GMP properties is impossible without solving the above problem.

Two groups of factors determine the parameters of transgenic plants: the nature of the inserted gene and the method of transfer of the genetic construct into the plant genome. In 2003 K. Nielsen proposed the concept of different transgenic levels, according to it, the stability of the transgenic organism is reduced, and the probability of pleiotropic effects increases with the genetic distance between gene donor and gene recipient (Nielsen, 2003). The article of Nielsen was not unanimously accepted (Cayford, 2003), but, of course, it has great importance for further development of the study methodology of the physiological features of a transgenic plant.

Proposals to use for creating of new varieties using genetic engineering technologies, only intragenic (transformation of their own genes) or cisgenic (closely related species, between which normal mating is possible) plants are being actively discussed in the scientific literature (Espinoza *et al.*, 2013; Holme *et al.*, 2013; Jacobsen, Schouten, 2009). According to the authors, it would eliminate the risks associated with the insertion of foreign DNA into genome of the recipient plant. Some authors consider that it is necessary to withdraw such organisms from standards regulation of GMOs regulation, a priori considering them safe (Hou *et al.*, 2014; Rommens *et al.*, 2007). These proposals, however, are disputed by other researchers (Russell , Sparrow, 2008).

The most common mistake of GMOs' creators is identifying of a gene with a certain physiological characteristics of the organism. However, the concept of transgenesis as a method of "gene transfer" is based on this approach. Most of the economically valuable and important adaptive traits are polygenic and controlled by complex mechanisms of coadaptation (Zuchenko, 2003). The basis for their formation are gene networks - groups of coordinated functioning genes (Kolchanov *et al.*, 2013). The insertion of a new gene (an additional link in the chain) or damage of the recipient plant genes (using direct transformation methods), will unavoidably caused changes in the functioning of the entire chain, which is likely to be one of the causes of pleiotropic effects.

Despite the presence of a rather wide arsenal of methods of gene transfer, agrobacterium transformation is the main method of the GMP creating, and it is rightly called the "workhorse" of biotechnology (Choi *et al.*, 2015). Mainly specialists in the field of plant hormones biochemistry have studied tumorigenic process induced by agrobacterium for a long time (Gohlke, Deeken, 2014; Mano, Nemoto, 2012). With the birth of genetic engineering of plants, the focus of researchers was aimed at studying of molecular transformation mechanisms (Gelvin, 2000; Tinland, 1996), while the physiology of plant-agrobacterial symbiosis almost fell out of the field of researchers' view.

The evolution of parasitic systems is on the path of mutual adaptation of the partners, which is manifested in reduction of pathogenicity of the parasite and increase the stability of the host (Roitman, Be'er, 2008). *Status quo* of a symbiotic system is provided by complicated interaction mechanisms at the level of the genome, including the exchange of genes between the partners (Antia *et al.*, 1994; Provorov, Tikhonovich, 2014 a,b). Long-term co-evolution of plant-agrobacterial symbiosis casts doubt on the thesis of the accident of insert place of T-DNA and insertional mutagenesis as the primary cause of pleiotropic effects.

Mechanisms of partners' interaction in the symbiosis are very complex and mobile (Kier, van der Heijden, 2006; Smith, Read, 2008; Thrall *et al.*, 2006), even minor changes in the properties of one of the partners (a mutation in a single gene) may lead to fundamental changes in the nature of the interaction (Johnson, Oelmüller, 2009; Redman *et al.*, 2001). T-DNA insert genes from phylogenetically distant plant species, entails a violation of the existing balance. In other words, agrobacterium with a built-in T-DNA target gene may be perceived as a new unknown pathogen species, causing a stress response (Enikeev *et al.*, 2015) and significant restructuring metabolism aimed at the activation of defense mechanisms, which may be another reason for the pleiotropic effects.

CONCLUSIONS

Constant increase of the GMOs use requires the developing of comprehensive evaluation criteria of their safety. The solution to this problem is impossible without studying the characteristics of transgenic plants as a new artificial biological system. Achieving this goal is possible only by combining the efforts of specialists in different branches of biological science. The starting point of this research should be the study of the physiological parameters of the GMP taking into consideration that changes on the biochemical and molecular genetic levels are only the realization of physiological response mechanisms to the insertion of phylogenetically unfamiliar for host plant genes and the cascade of defense reaction. To study the mechanisms of plant-agrobacterial symbiosis may become a key to understand the features of the transgenic plant physiology.

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