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Genetic improvement of fruit crops using biotechnological tools

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Abstract: -

The duration of their juvenile period, auto-incompatibility, and high degree of heterozygosity are the key reasons for the constraints of improving woody fruit species through traditional plant breeding methods. More accurate and quicker genetic changes of plants are now possible thanks to the advent of new biotechnology techniques (NBTs) like RNA interference (RNAi), trans-grafting, cisgenesis/intragenesis, and genome editing tools like zinc-finger and CRISPR/Cas9. This feature is especially crucial when introducing or changing individual qualities in woody fruit species while keeping the general traits of a chosen cultivar unaltered. Additionally, several of these new technologies make it possible to obtain changed fruit tree genomes without transgenes, which should boost consumer acceptance. Biotechnological instruments have advanced quickly throughout the years, and plant breeders are always learning new and useful methods. In order to satisfy the demand for sustained agricultural output, this enables the quicker and more effective creation of desired woody fruit types. Despite sharing the objective of accurate, quick, and efficient crop improvement, NBTs differ greatly from one another in terms of methodology and traits. This paper examines the relationship between these biotechnological tools and the EU biosafety rules applied to the plants and products obtained through these procedures, as well as their mechanisms and uses for improving fruit trees.

Introduction

The sluggish and challenging nature of conventional breeding for the genetic enhancement of woody fruit crops is exacerbated by autoincompatibility, prolonged juvenile periods, and high heterozygosity.

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Furthermore, because woody fruit species have a long generation time, improving them through traditional breeding techniques takes time. The rapid insertion of crucial genes into the genome of commercial woody fruit cultivars can be facilitated by new biotechnological tools (NBTs), such as genetic engineering techniques. This will increase the efficiency and dependability of genetic improvement of clonal propagated plants while preserving the high stability of the clone's primary traits. The topic of plant biotechnology has enormous promise thanks to the development of recombinant DNA technology. NBTs for producing genetically modified (GM) plants with beneficial agronomic and quality traits are already of significant significance for many crops in order to achieve food security and to ensure nutritional quality.

For over thirty years, genetic engineering has been used in plants. The main techniques for introducing heterologous DNA into plants have been direct transformation techniques (Biolistic) and indirect techniques (Agrobacterium tumefaciens-mediated transformation), both of which were created decades ago. One of these techniques was used to create all commercially growing genetically modified crops, including woody fruit species. Depending on the genotype and the type of starting plant tissue used, a well-established in vitro regeneration protocol is frequently required in order to produce fruit tree plants with novel traits or mutations through genetic engineering or NBTs. Furthermore, because most of these species have a high degree of heterozygosity, it is more prudent from an agronomic perspective to in vitro regenerate a new fruit tree plant from mature tissues. For some hard-to-transform woody species, like peach or grapevine genotypes, significant advancements have been made in the past 20 years. Effective protocols for the regeneration of adventitious shoots have been developed, beginning with adult tissues.

By directly modifying an organism's genome to express or mute particular features, genetic engineering techniques can introduce one or more additional genes or regulatory elements. Globally significant transgenic approaches focus primarily on producing crops with new genes that resist pests and diseases or herbicides, like Monsanto's roundup-ready crops as well as plants with improved nutritional levels and desirable traits, like the golden rice with higher vitamin A content.

Cisgenesis and Intragenesis

The genetic modification of plants using genes that come solely from the species or from a species that may be crossed conventionally with this species is known as cisgenesis. A natural variant, the added gene is an additional copy of the current genome that

has its introns bordering the native promoter and terminator in a normal sense orientation. The inserted genetic element (intragene) in intragenesis comes from either the same species or a species from a gene pool that is sexually compatible. Since the intragenes can be driven by distinct promoter or terminator regions of distinct genes and loci, they are regarded as hybrid genes. In comparison to the initial genome, the inserted DNA sequence will create a novel configuration of genetic components, resulting in a modified functional version. Additionally, in intragenic plants, plant-derived transfer DNA (P-DNA) boundaries sequences from the sexually compatible DNA pool are utilized to prevent unintentional insertion of vector sequences when employing Agrobacterium-mediated transformation as a technique to install the new trait. As a result, it is feasible to produce altered plants devoid of any foreign DNA. According to Jacobsen and Schouten (2007), these methods circumvent the possibility of "linkage drag," which is the transmission of additional unwanted genes together with the gene of interest and is linked to classical introgression in traditional breeding. Although whole genome sequencing research is revealing which cisgenes can be utilized to enhance the genetic makeup of particular crops, there are frequently few effective marker genes and cisgenic promoters available. Apples are among the woody fruit species that have used cisgenesis and intragenesis. Apple diseases, such as fire blight, which is brought on by Erwinia amylovora, are being addressed by fruit breeders. Using the cisgene FB_MR5 from the wild apple Malus × robusta 5 (Mr5), which gives resistance to fire blight, Kost et al. (2015) recently created a cisgenic apple line C44.4.146 from a cultivar "Gala Galaxy" that is vulnerable to fire blight. Following the heat-induced recombinase removal of the selectable markers, no transgenes were found by PCR or Southern blot analysis. Only the cisgene FB_MR5 and its native regulatory sequences were present in the converted line C44.4.146. Additionally, cisgenesis and intragenesis have been effectively used to make apples and other woody fruit trees and vines resistant to other diseases.

RNA INTERFERENCE (RNAi)

In 1990, researchers who were attempting to intensify the purple hue of petunias by overexpressing the Chalcone synthase gene discovered the initial discovery of the silencing phenomena in plants. They were surprised to see that the blossoms turned white, a sign that the gene had been disabled. In petunia, the process of introducing a homologous region into the genome to inhibit indigenous gene expression was known as "co-suppression". This phenomena was later linked

to post-transcriptional gene silencing (PTGS). RNA interference (RNAi) is a naturally occurring biological process that controls gene expression prior to translation or "turns off" undesirable or hazardous particular nucleic sequences. Numerous creatures, including ciliates, fungi, and animals, have been found to have RNAi. More recently, plants have been the subject of studies on RNAi. The term "RNAi" describes a group of biological processes that are primarily responsible for suppressing or inhibiting gene expression and are triggered by the presence of double-stranded RNA molecules. The ability to create unique "knock-downs" of gene activity was made possible by the discovery of this technique. In both animals and plants RNA interference (RNAi) has been demonstrated to use dsRNAs as trigger molecules to identify homologous mRNAs with negatively regulated. Thus, in fungi, insects, bacteria, viruses, and plants, RNA silencing has become the preferred technique for gene targeting. PTGS, transcriptional gene silencing, and microRNA silencing are some of the current methods of gene silencing found in plants. These pathways all depend on the presence of dsRNA molecules of various sizes, which are processed into the plant cell by particular protein families, such as RNA-dependent RNA polymerases, Argonaute (AGO), and Dicer or Dicer-like (DCL).

Trans-Grafting Technique

Grafting, a horticultural method that has been used for centuries to increase the output and quality of fruit crops, is the primary emphasis of this technique. The technique combines two independent genotypes that were chosen separately based on their capacity for roots and fruiting. To combine their outstanding qualities in the scion and the rootstock, they are grafted together. It has been widely utilized to spread woody perennial crops including fruits and ornamental plants, as well as to increase crop quality and productivity. Although the rootstock and scion maintain their genetic integrity—that is, their grafted tissues are linked but their genetic materials do not mix—the rootstock can change the scion's phenotype, for instance by decreasing its vigor and promoting more fruit set. Applications for further tissue grafting techniques range from animal organ transplantation to plant breeding. Grafting has historically been employed to enhance growth traits like rooting ability, nutrient and water uptake, and disease resistance, particularly against soil-borne fungus and bacteria. Trans grafting is a technique that blends genetically modifying plants with conventional grafting techniques. Grafting a nongenetically altered scion onto a genetically changed rootstock is the method used. Transgenes in the rootstock give the scion advantages and characteristics,

but the final products—like fruits—do not include the transgene and are therefore not genetically modified.

Biosafety Considerations for the Application of Nbts in Fruit Trees

Technological developments in agriculture provide new goods and ways to achieve a sustainable future. But these also bring with them new worries and problems to deal with. The principles, practices, and regulations of biosafety risk assessment have been implemented to guarantee the environmental and individual safety of genetically modified organisms (GMOs). There are many biosafety concerns with genetically modified crops, and before GM crops are released into the environment for commercial use, plant breeders must prove their product's safety. Compared to traditional and some early genetic modification techniques, NBTs have been created to allow for more accurate genetic changes of plants. It is still unclear, nevertheless, if crops produced by some of these methods belong in the same category as products of conventional breeding or mutagenesis or should be categorized and regulated as GMOs. While the global scientific community recommends that the assessment of plants obtained by NBTs should concentrate on the modifications made to the plant itself and the final products obtained, there is a lack of clear regulation regarding the use of these new techniques, particularly at the EU level. As a result, the results of using these technologies should be evaluated using a streamlined process that primarily examines whether the generated genomic changes fall within the range of the species' typical genetic variability. Regarding risk assessment, the synthesis of a novel protein in the altered plant and its potential off-target consequences are two of the primary issues with GM plants. Applications of the cisgenic approach should avoid this issue because all of the components come from the same species or from a sexually compatible species. As a result, the recipient genetically modified plant does not produce a novel protein, and the results are identical to those of traditional breeding.

Many crops now have complete genome sequences accessible, which present countless opportunities to find beneficial genes or promoters from the same species that can be passed on to enhance commercial cultivars. The availability of effective selectable markers that can take the place of the widely used antibiotic or herbicide resistance markers, however, may restrict the transformation method. The development of reporter genes derived from the broad class of myeloblastosis (MYB) transcription factors involved in the activation of anthocyanin pigment in plant species is progressing in an effort to address this issue. In order to compare the grapevine-

derived VvMybA1 transcription factor with the already-existing reporter genes gfp and gus, Kandel et al. (2016) used this method in grapevines. According to Kandel et al. (2016), the MybA1 reporter gene is appropriate for identifying gene expression events at the cell culture level. Each plant species can have its own MYB markers, but creating effective regeneration systems that just require the use of a reporter gene and no selectable markers is not necessarily simple.

Without creating new homologous and heterologous proteins or enzymes, RNAi-based GM plants control the expression of particular genes by producing dsRNA molecules. On the other hand, effects on the RNAi gene target and potential off-target effects must also be taken into account. Numerous strategies have been found to mute endogenous genes in plants, such as the MLO gene for mildew resistance or genes for fruit ripening. In these situations, the plant might be regarded as cisgenic if no additional transgenic sequences are added, and the risk assessment might be lowered to molecular characterisation of the event and the target gene silenced. A study of potential offtarget gene silencing through the plant's dsRNAs in both target and non-target organisms should be conducted in the case of RNA interference systems to create resistance to other organisms interacting with the plant (such as viruses, fungus, bacteria, and insects).

Conclusion

Rapid advancements in biotechnological techniques have given plant breeders new and useful tools. These methods enable the rapid and more effective development of desired crop cultivars to satisfy the demand for enhanced crops to support sustainable agricultural output and to feed the world's growing population.

According to the EFSA GMO Panel, "food and feed products derived from cisgenic and intragenic plants/crops can be evaluated using the Guidance for risk assessment of food and feed from genetically modified plants and the Guidance on the environmental risk assessment of genetically modified plants." It is possible that less event-specific data will be required for the risk assessment on a case-by-case basis.

Despite having the same objective—accurate, quick, and efficient crop improvement—the new biotechnology techniques differ from one another in their methods and traits. To get the intended effects, some of these methods, including trans-grafting and RNA interference, can be used in combination. There are currently few commercial uses for genetically engineered fruit plants. Since September 1997, when all required approval processes were successfully

finished, the only fruit plants that are currently on the market are the "Rainbow" virus-resistant papaya and the arctic apple, which was approved by the US Department of Agriculture (USDA) in February 2015, becoming the first genetically modified apple that is resistant to browning. Although it has not yet hit the market, the virus-resistant Honey Sweet plum cultivar was approved for commercialization in the United States. Due to (1) the challenges of creating effective regeneration and transformation protocols for several cultivars of the various species—many fruit tree species are resistant—and (2) regulatory considerations, the limited use of GM technology in fruit trees can be explained. These factors result in the fruit industry's restricted commercial use of genetically modified fruit trees, which in turn causes plant breeders and the biotech sector to spend less in fruit tree biotechnologies. Accordingly, the majority of biotechnology research on these crops is being conducted by public research organizations with constrained funding.

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