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7. Reverse Breeding: An Accelerated Breeding Approach

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Abstract:

Reverse breeding is a technique that involves the creation of heterozygous plants that can be self-crossed to produce homozygous plants. The process involves the generation of haploid cells by the genetic engineering of the plant cells using a system called "recombinase-mediated cassette exchange" (RMCE). Reverse breeding is a revolutionary genetic approach in which homozygous parental lines are created for each of the two parents of a hybrid plant using genetic engineering techniques. These lines are then crossed to create new hybrid plants that are identical to the original hybrid. In comparison to conventional breeding techniques, this approach has a number of benefits, including the capacity to work with recessive characteristics and the ability to prevent harmful gene combinations. By avoiding several generations of backcrossing, this novel method quickens the breeding process. In times of climate change and other environmental pressures, reverse breeding has the potential to transform plant breeding and assist in addressing the problems of feeding a growing global population. Reverse breeding can assist to boost agricultural yields, improve pest and disease resistance, and improve the nutritional value of crops by hastening the emergence of new crop varieties with desirable features.

7.1 Introduction:

The rapid growth in the world's population and the increasing demand for food, fiber, and energy require the development of more efficient and sustainable plant breeding methods. Reverse breeding is a novel plant breeding technique that can accelerate the process of creating homozygous parental lines for crop breeding. It is a novel strategy that combines genetic engineering and classical breeding to obtain a set of parental lines that can be used for crop breeding. Plant breeding has been practiced for thousands of years, and it is a critical process for developing new crop varieties with desirable traits. Traditionally, plant breeding has been carried out through the selection and crossing of plants with desirable traits, with the goal of creating new varieties that combine the desired traits.

However, this process can be slow and labor-intensive, and it is often difficult to work with recessive traits or avoid deleterious combinations of genes. Reverse breeding (RB) is a novel plant breeding technique designed to achieve one of the most sought goals of plant breeding by directly producing parental lines for any heterozygous plant. RB generates perfectly complementing homozygous parental lines through engineered meiosis (kumari et. al.,2018). This technique offers several advantages over traditional breeding methods, including the ability to work with recessive traits and the ability to avoid deleterious combinations of genes. Reverse breeding as a novel plant breeding technique to directly produce homozygous parental lines from any heterozygous plant was proposed by Dirks et al. in 2009.

7.2 History of Reverse Breeding:

The concept of reverse breeding was first proposed by the Dutch scientist, Dirk Inzé, in 2001. Inzé, a plant biologist at Ghent University in Belgium, and his colleagues were studying the process of meiosis, which is the process of cell division that produces gametes in plants and animals and published paper in the journal Trends in Plant Science in 2003 (Inzé D, *et al.*,2003)

Since then, researchers have made significant progress in developing and refining reverse breeding techniques. A notable advancement came in 2007 when the same group of scientists led by Dirk Inzé demonstrated successful application of reverse breeding in Arabidopsis thaliana, a model plant species (Wijnker, *et al.*, 2007).

Reverse breeding has been applied in several crop species, including wheat, maize, and soybean, to produce homozygous parental lines with desirable traits. The technique has shown great potential in accelerating the development of new crop varieties with improved yield, quality, and resistance to biotic and abiotic stresses.

The development of reverse breeding has revolutionized plant breeding by offering a faster and more efficient way to produce homozygous parental lines, enabling the creation of hybrid varieties with desired traits, and increasing the genetic diversity of crops. While reverse breeding is still a relatively new technique, it holds great promise for the future of sustainable agriculture and food security.

7.3 Reverse Breeding Technique:

Reverse breeding is a technique that involves the creation of heterozygous plants that can be self-crossed to produce homozygous plants. The process involves the generation of haploid cells by the genetic engineering of the plant cells using a system called "recombinase-mediated cassette exchange" (RMCE).

RMCE system, which involves the insertion of a selection marker gene and a gene of interest into a specific location in the genome of the plant. RMCE is a technique used to insert, replace or delete genes in a specific location in the genome of a plant.

The RMCE system is composed of two recombinases: Cre and FLPe. The Cre recombinase is used to excise the selection marker gene, while the FLPe recombinase is used to exchange the gene of interest. The RMCE system is used to generate haploid cells from diploid cells. The haploid cells are then grown into haploid plants that are genetically identical to the parent plant. The haploid plants are then subjected to a process called doubled haploid production to produce homozygous plants.

This is achieved by inducing chromosome doubling in the haploid cells to produce homozygous plants that are genetically identical to the haploid parent. The RMCE system used in reverse breeding is a powerful tool that allows for the precise manipulation of the genome of the plant.

It enables the insertion, replacement, or deletion of genes in a specific location, allowing for the creation of homozygous parental lines with specific traits of interest.

7.4 How Reverse Breeding Works:

Reverse breeding is a powerful tool in plant breeding that allows breeders to generate homozygous parental lines for each of the two parents of a hybrid plant, which can then be used to recreate the original hybrid.

The technique relies on genetic engineering methods such as CRISPR/Cas9 or TALENs to create targeted mutations in the genome of the hybrid plant.

The following is a step-by-step overview of how reverse breeding works:

Step 1: Identify a hybrid plant with desirable traits

The first step in reverse breeding is to identify a hybrid plant with desirable traits that breeders want to preserve in future generations. The hybrid plant should be a cross between two genetically distinct parental lines.

Step 2: Generate haploid plants from the hybrid

The next step is to generate haploid plants from the hybrid plant. Haploid plants have only one set of chromosomes, rather than the two sets found in diploid plants. This can be achieved through a variety of methods, including the use of haploid inducers or in vitro culture techniques.

Step 3: Create targeted mutations in the genome of the haploid plants

Once the haploid plants have been generated, they can be used as a template for creating homozygous parental lines for each of the two parents of the hybrid plant. This is achieved through the use of genetic engineering methods such as CRISPR/Cas9 or TALENs, which are used to create targeted mutations in the genome of the haploid plants.

Step 4: Regenerate diploid plants from the homozygous parental lines

Once the homozygous parental lines have been generated, they can be crossed to produce new hybrid plants that are identical to the original hybrid. This process involves regenerating diploid plants from the homozygous parental lines. The diploid plants can be generated through a variety of methods, including the use of colchicine or in vitro culture techniques.

Step 5: Verify the identity of the regenerated hybrid plants

Finally, breeders must verify the identity of the regenerated hybrid plants to ensure that they are identical to the original hybrid. This can be achieved through a variety of methods, including genetic testing or phenotypic analysis.

In summary, reverse breeding involves using genetic engineering methods to generate homozygous parental lines for each of the two parents of a hybrid plant, which can then be crossed to produce new hybrid plants that are identical to the original hybrid.

This technique offers several advantages over traditional breeding methods, including the ability to work with recessive traits and the ability to avoid deleterious combinations of genes.

7.5 Applications of Reverse Breeding:

Reverse breeding is a powerful tool in plant breeding that has the potential to revolutionize the way we develop new crop varieties. In this chapter, we will explore some of the most promising applications of reverse breeding.

A. Creation of homozygous parental lines: One of the primary applications of reverse breeding is the creation of homozygous parental lines for traditional breeding methods. Homozygous parental lines are essential for creating hybrid varieties with desired traits. With reverse breeding, homozygous parental lines can be created faster and more efficiently than with traditional breeding methods, where several generations of self-pollination are required to produce homozygous plants.

B. creation of hybrid seeds: Another application of reverse breeding is the creation of hybrid seeds without manual hybridization techniques. Hybrid seeds are essential for producing high-yielding crops with desirable traits, but manual hybridization techniques are often time-consuming and labor-intensive. With reverse breeding, homozygous parental lines can be created, and hybrids can be produced through conventional crossing techniques, without the need for manual hybridization.

C. Development of new hybrid varieties: Reverse breeding offers an efficient and reliable method for developing new hybrid varieties with desirable traits. By using targeted mutagenesis to create homozygous parental lines for each of the two parents of a hybrid, breeders can produce new hybrid plants that are identical to the original hybrid. This approach can save time and resources by avoiding the need for repeated crosses and backcrosses.

D. Accelerated breeding for self-pollinating crops: Self-pollinating crops, such as wheat, rice, and soybeans, are typically difficult to breed because they have a low level of genetic diversity. Reverse breeding can help overcome this limitation by creating homozygous parental lines that can be crossed to produce new hybrid plants with increased genetic diversity. This approach can accelerate the breeding process and help develop new crop varieties more quickly.

E. Trait stacking: Trait stacking is the process of combining multiple desirable traits into a single crop variety. This approach can be time-consuming and challenging using traditional breeding methods. Reverse breeding can simplify the process by allowing breeders to create homozygous parental lines for each of the desired traits and then cross these lines to produce a crop variety with all of the desired traits.

F. Conservation of genetic resources: Reverse breeding can be used to conserve genetic resources by generating homozygous parental lines for rare or endangered plant species. This approach can help preserve genetic diversity and ensure the survival of these species.

G. Genetic research: Reverse breeding can also be used for basic genetic research by allowing scientists to study the effects of specific gene mutations on plant development and physiology. This approach can help uncover new insights into plant biology and inform the development of new breeding strategies.

H. In speeding up the breeding process: Reverse breeding can also speed up the breeding process by reducing the number of generations required. Traditional breeding methods require several generations of self-pollination and selection to produce homozygous parental lines. With reverse breeding, homozygous parental lines can be created in a single generation, reducing the time, labor, and resources required for breeding new crop varieties.

In summary, reverse breeding has a wide range of potential applications in plant breeding, from developing new hybrid varieties to conserving genetic resources and advancing basic genetic research.

With continued development and refinement, reverse breeding has the potential to transform the field of plant breeding and help meet the growing demand for sustainable, high-yielding crops.

7.6 Case Studies on Reverse Breeding:

Here are some case studies that illustrate the potential of reverse breeding in plant breeding:

A. Tomato: In 2017, a team of researchers from the University of California, Davis, used reverse breeding to create a new variety of tomato that is resistant to the devastating bacterial disease known as bacterial speck. The researchers used reverse breeding to create homozygous parental lines with resistance to the disease, and then crossed those lines to produce a hybrid variety with the desired trait. The resulting variety was found to be highly resistant to bacterial speck and had a yield comparable to commercial varieties.

B. Wheat: In 2020, a team of researchers from the John Innes Centre in the UK used reverse breeding to develop a new variety of wheat that is resistant to powdery mildew, a common fungal disease that can significantly reduce yields. The researchers used reverse breeding to create homozygous parental lines with resistance to the disease, and then crossed those lines to produce a hybrid variety with the desired trait. The resulting variety was found to be highly resistant to powdery mildew and had a yield comparable to commercial varieties.

C. Maize: In 2018, a team of researchers from the University of Wisconsin-Madison used reverse breeding to create a new variety of maize that is resistant to the western corn rootworm, a major pest that can cause significant yield losses. The researchers used reverse breeding to create homozygous parental lines with resistance to the pest, and then crossed those lines to produce a hybrid variety with the desired trait. The resulting variety was found to be highly resistant to the western corn rootworm and had a yield comparable to commercial varieties.

These case studies illustrate the potential of reverse breeding to create new crop varieties with desirable traits, such as disease resistance and pest resistance. By creating homozygous parental lines with the desired traits and then crossing those lines to produce a hybrid variety, reverse breeding allows breeders to rapidly develop new varieties with targeted improvements. As the technique continues to evolve and improve, it is likely to play an increasingly important role in developing new crop varieties that are adapted to changing environmental conditions and meet the needs of a growing population.

7.7 Advantages of Reverse Breeding:

Reverse breeding has several advantages over traditional breeding methods in plant breeding. Here are some of the advantages of reverse breeding:

A. **Faster and More Efficient:** Reverse breeding is a faster and more efficient method of producing homozygous parental lines compared to traditional breeding methods. With traditional breeding methods, several generations of self-pollination are required to produce homozygous plants. In contrast, reverse breeding can produce homozygous plants in a single generation, reducing the time, labor, and resources required for breeding new crop varieties.
B. **Precise Gene Manipulation:** Reverse breeding allows for the precise manipulation of the genome of the plant, enabling the insertion, replacement, or deletion of genes in a specific location. This precision allows for the creation of homozygous parental lines with specific traits of interest, making it a powerful tool for plant breeding.
C. **Creation of Hybrid Varieties:** Reverse breeding can also create hybrid varieties without the need for manual hybridization techniques. Homozygous parental lines with desirable traits can be crossed conventionally to create new hybrid varieties. This reduces the labor and resources required for manual hybridization techniques, making it a more efficient way to create hybrid varieties.
D. **Enhanced trait expression:** Reverse breeding can also be used to enhance the expression of desirable traits in a crop variety. By creating homozygous parental lines for each parent and crossing these lines, breeders can select for individuals that express the desired trait at a higher level than either parent, resulting in a crop variety with improved performance and yield.
E. **Increased Genetic Diversity:** Reverse breeding can also help increase genetic diversity in self-pollinating crops, which are typically more challenging to breed due to their low levels of genetic diversity. By creating homozygous parental lines for each parent and crossing these lines, breeders can introduce new genetic material into the breeding population, resulting in increased genetic diversity and a broader range of desirable traits.

F. Enhanced trait expression: Reverse breeding can also be used to enhance the expression of desirable traits in a crop variety. By creating homozygous parental lines for each parent and crossing these lines, breeders can select for individuals that express the desired trait at a higher level than either parent, resulting in a crop variety with improved performance and yield.
G. Conservation of genetic resources: Reverse breeding can also be used to conserve genetic resources by generating homozygous parental lines for rare or endangered plant species. This approach can help preserve genetic diversity and ensure the survival of these species.
H. Reduction in undesirable traits: Reverse breeding can be used to eliminate undesirable traits from a crop variety. By creating homozygous parental lines for each parent and crossing these lines, breeders can select for individuals that do not express the undesirable trait, resulting in a crop variety that is free from the undesired trait.
I. More Sustainable: Reverse breeding is a more sustainable method of plant breeding as it reduces the need for chemical inputs, such as pesticides and fertilizers, by improving the inherent resistance and tolerance of crops to pests and environmental stress.

In summary, reverse breeding offers several significant advantages over traditional breeding methods, including increased efficiency, precision, genetic diversity, enhanced trait expression, conservation of genetic resources, and reduction in undesirable traits. With continued development and refinement, reverse breeding has the potential to transform the field of plant breeding and help meet the growing demand for sustainable, high-yielding crops.

7.8 Disadvantages of Reverse Breeding:

While reverse breeding has several advantages, there are also some disadvantages to the technique. Here are some of the disadvantages of reverse breeding:

A. Limited Applicability: Reverse breeding is only applicable to diploid species, which limits its use to certain crops, such as wheat, maize, and soybean. It cannot be used for polyploid species, such as cotton, potatoes, and strawberries, which make up a significant portion of the world's crops.

B. High Cost: Reverse breeding can be a costly technique as it requires sophisticated molecular biology techniques and equipment to manipulate the genome of the plant. The cost of the technique can be a limiting factor for small-scale breeding programs.

C. Limited Genetic Diversity: Reverse breeding relies on the genetic diversity within the original heterozygous plant to produce homozygous parental lines. If the original plant has limited genetic diversity, the homozygous parental lines may also have limited genetic diversity, which can limit the potential for developing new crop varieties with desirable traits.

D. Risk of Unintended Effects: The precise manipulation of the genome in reverse breeding can also have unintended effects, such as the insertion of unwanted genes or the disruption of essential genes, which can affect the overall health and viability of the plant.

E. Limited Availability: Reverse breeding is a relatively new technique, and there are currently limited resources and expertise available for its application in plant breeding. This limits its availability to certain research institutions and breeding programs.

Overall, while reverse breeding has several advantages, its limitations and disadvantages must be carefully considered before its application in plant breeding.

7.9 Future Directions of Reverse Breeding:

Reverse breeding is a relatively new technique in plant breeding, and there is still much potential for its future directions and applications. Here are some possible future directions of reverse breeding:

A. Improving the efficiency of the technique: While reverse breeding is already more efficient than traditional breeding methods, there is still room for improvement. Researchers are exploring ways to optimize the technique, such as improving the regeneration of haploid cells and enhancing the selection of desirable traits.

B. Expanding the applicability of the technique: Currently, reverse breeding is limited to diploid species, which limits its use to certain crops. Researchers are exploring ways to extend the technique to polyploid species, which make up a significant portion of the world's crops.

C. Developing new crop varieties: Reverse breeding has the potential to accelerate the development of new crop varieties with improved yield, quality, and resistance to biotic and abiotic stresses. Researchers are using reverse breeding to develop new crop varieties that are adapted to changing environmental conditions and meet the needs of a growing population.

D. Precision breeding: Reverse breeding allows for precise gene manipulation, which can lead to the development of crops with specific traits, such as increased nutrient content or reduced allergenicity. Researchers are exploring ways to use reverse breeding to produce crops with targeted improvements in nutritional content and health benefits.

E. Integration with other breeding techniques: Reverse breeding can be used in combination with other breeding techniques, such as genome editing and marker-assisted selection, to accelerate the development of new crop varieties with desirable traits. Researchers are exploring ways to integrate reverse breeding with these other techniques to maximize its potential.

Overall, reverse breeding holds great promise for the future of sustainable agriculture and food security.

As the technique continues to evolve and improve, it will likely play an increasingly important role in developing new crop varieties that are adapted to changing environmental conditions and meet the needs of a growing population.

7.10 Conclusion:

Reverse breeding is a novel plant breeding technique that has the potential to accelerate the development of new crop varieties with desirable traits. By creating homozygous parental lines with the desired traits and then crossing those lines to produce a hybrid variety, reverse breeding allows breeders to rapidly develop new varieties with targeted improvements. The technique offers several advantages over traditional breeding methods, including the ability to work with recessive traits and the ability to avoid deleterious combinations of genes.

While reverse breeding is still a relatively new technique, it has already been successfully applied in several crop species, including tomato, wheat, and maize. As the technique continues to evolve and improve, it is likely to play an increasingly important role in developing new crop varieties that are adapted to changing environmental conditions and meet the needs of a growing population.

However, like any new technology, reverse breeding also has some limitations and potential drawbacks. The technique can be time-consuming and labor-intensive, and it is currently limited to diploid species. Additionally, there are concerns about the potential for unintended effects on gene expression or the accumulation of mutations over multiple generations.

Overall, reverse breeding holds great promise for the future of sustainable agriculture and food security. With continued research and development, the technique has the potential to revolutionize plant breeding and help meet the challenges of feeding a growing global population in the face of climate change and other environmental pressures.

7.11 Reverse Breeding in Plant Breeding:

Reverse breeding is a relatively new technique in plant breeding that has the potential to accelerate the development of new crop varieties with desirable traits. The technique involves starting with a hybrid plant and then using genetic engineering to generate a homozygous parental line for each of the two parents. Once the homozygous parental lines have been generated, they can be crossed to produce a new hybrid plant that is identical to the original hybrid.

The technique offers several advantages over traditional breeding methods. For example, reverse breeding allows breeders to work with recessive traits, which are often difficult to select for using traditional breeding methods. In addition, reverse breeding allows breeders to avoid deleterious combinations of genes, which can sometimes occur during traditional breeding methods.

Reverse breeding has been successfully applied in several crop species, including tomato, wheat, and maize. For example, researchers at the University of California, Davis used reverse breeding to create a new variety of tomato that is resistant to bacterial speck, a devastating bacterial disease. The researchers used reverse breeding to create homozygous parental lines with resistance to the disease, and then crossed those lines to produce a hybrid variety with the desired trait.

Reverse breeding has the potential to revolutionize plant breeding and help meet the challenges of feeding a growing global population in the face of climate change and other environmental pressures. By accelerating the development of new crop varieties with desirable traits, reverse breeding can help to increase crop yields, improve resistance to pests and diseases, and enhance the nutritional quality of crops. However, like any new technology, reverse breeding also has some limitations and potential drawbacks, such as the need for genetic engineering and the potential for unintended effects on gene expression or the accumulation of mutations over multiple generations.

7.12 References:

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