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THE NEXT INTUITIVE PHASE OF PLANT BREEDING

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INTRODUCTION

The ongoing increase in global average temperatures and increase in population, along with the shrinking productive land due to rapid urbanization and industrialization, has made scientists look for an alternate way from conventional breeding procedures to increase production without compromising the nutritional quality and make plants cope with the effects of adverse events occurring due to climate change, such as floods, droughts, heat waves, etc. One possible answer to this lies in precision breeding, a modern biotechnological approach to editing a particular trait in plants without incorporating foreign genes (like Bt genes in cotton and brinjal). As no new genes are going to be added to the plant's genome, it is not considered a GMO and may have an easy reach for people. Here is an overview of the concept, various techniques, benefits, and ethical concerns of precision breeding.

CONCEPT

The basic concept behind precision breeding involves manipulating the genes which heighten the plant's ability to be stable in terms of yield, biotic and abiotic resistance and adapt to its environment. By making targeted changes to plant genomes for improved yield, improved biotic and abiotic resistance, and increased water use efficiency, the breeders can considerably reduce the amount of time needed to release a variety compared to conventional breeding, improving the yield or resistance to any biotic or abiotic stress requires years of repeated crossing.

TOOLS IN GENE EDITING

To precisely edit or modify the specific gene to a crop's genome we need certain molecular tools whose list is given below:

1. CRISPR/Cas9: It stands for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9. CRISPR is present as a natural defence mechanism in bacteria and archaea to protect them against invading viruses. Cas9 on the other hand is a special protein which cut the DNA at a particular location. In addition to CRISPR and Cas9, there presents a guide RNA (gRNA) whose purpose is to direct the Cas9 enzyme to a specific location in the DNA to cut or edit it. Thus, acting as a molecular GPS. CRISPR/Cas9 can also be used to produce only transgene free seeds by using suicidal transgenes (BARNASE gene). In rice, this gene is under the control of REG2 promotor which is expressed in early embryo development. This system contains the CMS2, encoding a rice male gametophyte specific lethal protein under the CaMV 35S promoter. In this way plant containing theses expression cassettes along with CRISPR/Cas9 produce toxic proteins

that kill male gametophytes and embryos producing only the transgene free seeds. This system is known as Transgene Killer CRISPR

2. RNA interference (RNAi): RNAi is another prominent tool whereby silencing effect or expression of a particular gene in the target cell, assisting plant breeders, plant pathologists and biotechnologists to employ this technique in creating resistance against plant diseases and pests.

For example, in *Helicoverpa armigera,* a gene 'CYP6AE14' was identified which in insect midgut was correlated with the growth of larva when it feed on the leaves containing gossypol. When the larva is fed on leaves exhibiting the dsRNA which is specific to the 'CYP6AE14', the transcription in the midgut of the larva is decreased thereby retarding the growth of the larva thereby imparting the resistance to Helicoverpa in cotton.

3. Zinc finger nuclease (ZFN): It is an engineered protein which works by targeting and cleaving specific DNA sequences thereby introducing precise changes to an organism's genome either by inserting or deleting or modifying specific genes which encode for a character. A typical ZFN consists of two components such as DNA-binding domain and DNA-cleaving domain.

The DNA-binding domain consists of multiple zinc finger motifs, which recognize and bind to a specific DNA sequence. The DNA-cleaving domain is usually derived from the *Fok*I endonuclease, which cleaves DNA in a sequence-specific manner. ZFNs recognize smaller sequence as compared to TALENs and as a result produced more off-site (unintended editing at nonspecific loci).

4. TALEN: Transcription Activator-Like Effector Nucleases is also a type of engineered protein which target and cleave DNA at specific sequences. TALENs use a DNA-binding domain composed of TAL effector (obtained by *Xanthomonas* spp.) repeats with a FokI nuclease domain, enabling them to create double-strand breaks at specific genomic loci. The main difference between TALEN and ZFNs is TALENs use engineered DNA-binding proteins, while ZFNs rely on zinc fingers to recognize and target specific DNA sequences.

For example, a rice variety can be produced with altered fragrance using indels (insertions/deletions) in the betaine aldehyde dehydrogenase (BADH2) encoding gene (OsBADH2) have been produced using TALENs which resulted in the production of 2-acetyl-1-pyrroline (2AP), which is a major fragrance compound in rice.

5. Prime Editing: Prime editing is a recently developed genome editing technique that combines CRISPR-Cas9 with a reverse transcriptase. It enables precise editing of the genome by directly writing new genetic information into the DNA without the need for DNA DSBs (double-strand breaks). Prime editing offers the potential for precise modifications, including base substitutions, insertions, deletions, and gene conversions, with minimal off-target effects.

In tomato, Prime editing technology was employed to enhance the post-harvest quality and shelf life of tomatoes. Researchers targeted genes associated with fruit ripening and introduced precise modifications to delay the onset of ripening and extend the shelf life of tomatoes. The edited tomato lines exhibited prolonged shelf life, reduced spoilage, and improved post-harvest characteristics compared to conventional tomatoes.

6. Base Editors: Base editors are a class of genome editing tools that enable precise modification of individual DNA bases without introducing DSBs. They use a catalytically impaired Cas9 (dCas9) protein fused to a base-modifying enzyme.

By directing the dCas9-base editor complex to a specific target site, specific nucleotide changes, such as C-to-T or A-to-G conversions, can be introduced without disrupting the DNA helix. Base editors provide a way to achieve targeted single-base changes with reduced off-target effects compared to traditional nucleases.

The Base Editors can be further divided into Cytosine BE (CBEs), Adenine BEs (ABEs), C-to-G BEs (CGBEs), C-to-A Transversion Base Editors (CATBEs), dual-base editors and organellar BE. These can be used to alter the function of proteins.

Using base editing technology, researchers were able to develop high-beta-carotene rice, addressing vitamin A deficiency, a major public health concern in many developing countries. The researchers used a base editor to introduce specific mutations into the rice genome to increase the production of beta-carotene (precursor to vitamin A). The resulting rice lines had increased beta-carotene content, providing a potential solution for alleviating vitamin A deficiency in populations whose staple food is rice.

ETHICAL CONCERNS AND PUBLIC PERCEPTION

Even though gene or genome editing does not necessarily entail the insertion of foreign DNA into the plant genome, but still many raises the concern regarding the introduction of crops with edited genes: what are all the impacts on the environment, human health, cultural and ethical values, issues related to intellectual property, consumer perception, regulatory and governance, consumer preference, and ethical use of technology?

The concern related to environmental impact is mainly associated with the potential reduction in biodiversity, as farmers show preference for high-performing varieties, resulting in the risk of narrowing the genetic diversity of the crops. Another fear among environmentalists is that the issue related to these crops is the risk of genetic pollution, i.e., where the traits that are modified, edited, or inserted are transferred to the wild relatives. A classic example is this Percy Schmeiser case, where a canola farmer's field was contaminated with Roundup Ready canola. And the effect of pollen from *Bt* maize on milkweed leaves and monarch butterflies People also fear that the long-term use of these crops may cause allergic reactions in susceptible individuals, and they also fear that they may also produce toxins in humans and livestock due to the genes incorporated to protect the crops against pests. Which is not the case as Bt genes get activated only in alkaline environment, and human digestion is based on acid.

The corporate control over these gene-edited crops raises concerns that these companies may incorporate terminator or traitor technologies to make the farmers buy new seeds each season from these companies or make their seeds response to a particular type of fertilizers or pesticide sold exclusively by the company. These companies may also patent these seeds and force farmers to pay a license fee (Monsanto Canada Inc. V. Schmeiser, SCC Cases, 2004).

The lack of comprehensive risk assessment, data on the effect of designer crops on humans and livestock, labelling and transparency for gene-edited products, post-market surveillance of the gene-edited crops, legal frameworks, and enforcement mechanisms raises concerns about precision breeding.

Addressing these concerns involves a delicate balance between technological innovation, economic considerations, and ethical principles. The development and deployment of gene-edited crops should be guided by a commitment to sustainability, social responsibility, and respect for the rights and well-being of farmers and communities.

CONCLUSION

In conclusion, precision breeding is a ground-breaking strategy for crop development that uses advanced biotechnological tools and genomic data to increase the efficacy and efficiency of conventional breeding techniques.

It has the potential to address global agricultural challenges such as food security, climate change, and malnutrition. By creating crops with greater resilience and improved nutritional value, precision breeding can make a significant contribution to sustainable and resilient agricultural systems.