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INNOVATION IN VITRO METHODS FOR BREEDING DISEASE-RESISTANT VARIETIES OF TOBACCO AND SUGARCANE

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Abstract

Disease-resistant breeding is a top goal since crop diseases are becoming more common and posing serious problems to the agricultural industry. Traditional breeding techniques are frequently inefficient and sluggish, especially when it comes to commercially significant crops like sugarcane and tobacco. Because in vitro methods allow for regulated breeding conditions, genetic engineering, and early pathogen detection, they provide a quicker and more accurate means of creating disease-resistant cultivars. This study investigates how modern genetic engineering methods like CRISPR, somaclonal variation, mutant breeding, and plant tissue culture might hasten the production of disease-resistant tobacco and sugarcane variants. Diseases like sugarcane red rot and tobacco mosaic virus may be effectively treated using methods like callus culture, somatic embryogenesis, and micropropagation. Through the combination of targeted gene editing and pathogen screening, in vitro techniques provide novel approaches to sustainable agriculture, boosting crop output and resilience.

Keywords:*In Vitro Breeding, Disease Resistance, Tobacco Varieties, Sugarcane Varieties, Plant Tissue Culture.*

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1 INTRODUCTION

1.1. Overview of Disease-Resistant Breeding in Crops

With crop illnesses becoming more common and having the potential to cause large output losses, disease-resistant breeding has become essential to contemporary agriculture. By crossbreeding plants that display desired qualities with others that have robust resistance to diseases, traditional breeding methods depend on the inherent genetic variety within a plant species to add disease-resistant features. To attain the intended results, this process might, however, be sluggish and require several generations. In light of the need for sustainable agriculture and global food security, newer methods such as in vitro breeding techniques provide quicker and more accurate ways to make crops resistant to disease and increase production. With the use of these methods, plants with innate or produced disease resistance may be found, chosen, and propagated in a controlled setting.

1.2. Importance of Tobacco and Sugarcane in Agriculture

Two major crops for the world economy are sugarcane and tobacco. Millions of farmers depend on tobacco for their livelihoods, and the industry is vital to the agricultural economy of many nations. Since it is a significant cash crop and is widely utilized in the tobacco business, disease resistance is essential to preserving the crop's profitability. On the other hand, especially in tropical and subtropical areas, sugarcane is essential to the sugar and biofuel sectors. It is a major producer of bioethanol, a sustainable energy source, and one of the biggest contributors to the world's sugar supply. Nevertheless, a variety of illnesses can seriously lower yields and economic worth of both crops. Maintaining high production levels, increasing farmer incomes, and promoting sustainable agricultural practices all depend on the breeding of disease-resistant tobacco and sugarcane cultivars.

1.3. Role of In Vitro Techniques in Crop Improvement

Tissue culture is one example of an in vitro approach that has revolutionized current crop development by providing exact and regulated conditions for plant breeding. Through micropropagation, these techniques facilitate the fast growth of disease-resistant plants; somaclonal variety yields genetically varied plants; and meristem culture yields pathogen-free plants. Furthermore, without the requirement for field testing, in vitro screening makes it

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possible to identify plants with desired traits—like resistance to particular pathogens—early. Cutting-edge methods, including as CRISPR-based editing and genetic modification, may also be used in vitro to directly target and alter plant DNA to induce disease resistance. The production of disease-resistant tobacco and sugarcane cultivars is greatly aided by in vitro procedures, which speed up the breeding process and provide more precise selection. By controlling diseases that are challenging to manage with pesticides or traditional approaches, these techniques are particularly helpful in supporting healthy crop output.

2. LITERATURE REVIEW

Sengar, K., et.al., (2018) regulated transgenic expression through developmental control and induction would open up new avenues for the sugarcane sector to produce a variety of novel chemicals. There are great prospects for sugarcane crop enhancement with biotechnology. In order to highlight the importance of somaclonal diversity in crop development, potatoes and sugarcane are frequently used as examples. Probably the most well studied technique for sugarcane in vitro regeneration is somatic embryogenesis. Sugarcane is a perennial grass that typically reproduces vegetatively through rhizomes and nodal buds. However, seed propagation is also used for commercial cultivation. Utilizing either meristematic or non-meristematic cells or tissues as the explant, micropropagation is an in vitro technique for the clonal growth of plants. The genetic stability of the regenerated plant has a major impact on the use of cell and tissue culture to clonal propagation and in vitro germplasm preservation.

Kumar, T., et.al., (2024) needed to produce biofuel are greatly increased by sugarcane, a crucial cash crop that also plays a major role in the worldwide sugar industry. Stressors both biotic and abiotic, however, seriously impair sustained output. A fundamental and practical research technique to sustain growth and production under many unfavorable environmental circumstances, genetic engineering has been utilized to introduce beneficial genes into sugarcane plants to enhance desired features. However, the application of transgenic techniques is still controversial and necessitates exacting experimental procedures to solve biosafety issues. CRISPR, or clustered regularly interspaced short palindromic repeats, is a fast developing technology that has the potential to completely transform sugarcane production. The goal of this review is to examine cutting-edge genetic engineering methods

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and their effective use in creating improved sugarcane cultivars that are more resistant to biotic and abiotic stressors.

Anil, V. S., et.al., (2018) In plant cell and tissue culture, somaclonal variants (SV) are genetic or epigenetic modifications that are produced. A different strategy to traditional breeding and transgenic methods for introducing desired genetic variety in the gene pool is the induction of somaclonal variation. Many plant characteristics are altered by SVs that arise naturally in cultivation. On the other hand, relying solely on chance to improve a crucial agronomic feature like disease resistance might be difficult. Imposing a suitable in vitro selection pressure improves the efficiency of creating disease-resistant SVs. Pathogen elicitors, pure pathotoxins, and pathogen culture filtrate are examples of selection agents that have been used. This recognized biotechnological technique for SV selection has been shown to be effective in increasing disease resistance in a number of crops and has enormous promise for improving crop quality.

Jamil, M. W., et.al., (2022)A lack of suitable seed supplies, industrial assistance, insect and disease infestations (especially red rot), and other issues plague sugarcane production are only a few of the challenges it faces. Plant tissue culture is a technique that may be used to swiftly create large quantities of disease-free, genuinely developed plant material. Furthermore, freshly released varieties with essential agronomic traits may be rapidly replicated by plant tissue culture. The goal was to enhance genetic variety by obtaining sugarcane callus culture from the inner soft leaf sheath. Ten distinct 2, 4-dichlorophenoxy acetic acid concentrations in MS medium were utilized for callus development: 1.5, 1.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 mgL-1. A control of 0 mgL-1 was also used. In order to promote shoot regeneration and auxin, several BAP+ Kin combinations and concentrations (1.0+1.0, 1.0+1.5, 1.0+0.5, 1.0+1.0, 1.5+1.0, and 1.0+1.5 mgL-1) were utilized. The shoots were root-rooted with IBA at six different concentrations: 0.3, 0.5, 0.7, 1.5, and 1.8 mgL-1. 2, 4-D showed the highest auxin for callus formation with BAP out of all the growth regulators at 3.0 mgL-1. In MS media supplemented with NAA, the 6–12 mm meristem grew. The greatest callus (79.0-84.5%) with a size of 4–5 mm was found to form at doses of 3.5 mgL–1 and 4.0 mgL–1 of 2, 4-D. The highest recorded root growth and length (3.7 mm) in MS media were observed at 1.0 mgL-1 IBA, 1.0 mgL-1 Kinetin, and 1.5 mgL-1 BAP.

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3. PLANT TISSUE CULTURE IN BREEDING

3.1. Basics of In Vitro Culture

Plant cells, tissues, or organs can be grown in an artificial, controlled environment using an approach called in vitro culture, commonly referred to as tissue culture. Plants may multiply quickly with this technique, and new plant types with desired characteristics, such disease resistance, can be created. Sterilization, specialized medium that provide growth hormones, vital nutrients, and environmental factors like temperature and light are needed for in vitro cultivation. The principal benefit of in vitro culture is its capacity to produce plants in an aseptic environment devoid of pathogens, while also permitting the introduction of disease-resistant characteristics through genetic modification. For crops like tobacco and sugarcane, where conventional breeding techniques might not be sufficient to battle persistent illnesses, the methodology is essential to plant breeding.

3.2. Plant Tissue Culture Techniques Relevant to Tobacco and Sugarcane

Diseases like red rot in sugarcane and the mosaic virus in tobacco pose serious threats to these two commercially crucial crops. When breeding these crops for disease resistance, in vitro methods like callus culture, somatic embryogenesis, and micropropagation are very helpful. These techniques enable the isolation of certain cells or tissues, their in vitro growth, and the induction of genetic diversity or mutations that may provide resistance to illness. Furthermore, the plant's genome may be directly modified using genetic engineering technologies like CRISPR in conjunction with in vitro methods to increase the plant's resistance to particular infections. These kinds of inventions are essential to raising agricultural sustainability and crop productivity.

3.3. Callus Culture

Using plant explants, callus culture is an essential tissue culture technique that produces undifferentiated plant cells, or callus. Plant tissue is placed on a nutritional media containing growth regulators, such as auxins and cytokinins, which encourage cell division, to start this process. Callus culture plays a key role in the development of disease-resistant tobacco and sugarcane types because the callus can undergo genetic alterations that improve the plant's resistance to pathogenic assaults. With regeneration procedures like somatic embryogenesis

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or organogenesis, the callus may be stimulated to create whole plants after it has formed, enabling the quick multiplication of disease-resistant plants.

- **Somatic Embryogenesis**

The process by which somatic (non-reproductive) cells grow into embryos, which have the potential to regrow into whole plants, is known as somatic embryogenesis. Using this technique, plant cells are reprogrammed to become totipotent, or able to develop into new plants. This method is important for the quick replication of sugarcane and tobacco, particularly when the aim is to create disease-resistant cultivars. To make plants with consistent disease resistance, somatic embryos can be grown in vast quantities and utilized to create plants with identical genetic features. It also provides opportunities for genetic alterations, such as the insertion of genes resistant to illness, making it an essential tool in breeding initiatives meant to address the problems caused by infections.

- **Micropropagation**

A comparatively quick process known as "micropropagation" in plant tissue culture enables the bulk creation of genetically identical plants. Micropropagation is a common technique in the breeding of tobacco and sugarcane to create homogeneous plants with desirable features, such disease resistance. Using this technique, plant tissues are cultured in vitro, where they quickly grow under carefully monitored circumstances. Micropropagation provides a quick and effective means of producing disease-free, healthy plantlets, which helps to overcome the drawbacks of conventional propagation techniques. It also protects the crop's genetic integrity by preserving elite genotypes that demonstrate resistance to particular diseases.

3.4. Methods of Pathogen Screening in Vitro

A crucial stage in creating disease-resistant cultivars is pathogen screening in vitro, which enables breeders to assess plants' reactions to different diseases in a regulated environment. To check for resistance to bacterial, fungal, and viral infections, methods including tissue inoculation, cell suspension cultures, and dual-culture tests are used. These techniques allow for the early identification of resistance characteristics in tobacco and sugarcane, greatly cutting down on the duration and expense of breeding operations. Co-culturing plant tissues with pathogens is a popular technique that involves monitoring plant reactions to disease-

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causing chemicals. A different technique is to use genetic engineering to induce pathogen-derived resistance, which makes it possible to introduce certain resistance genes into plant tissues. By using these screening techniques, it is ensured that only plants with strong disease resistance are chosen for additional replication, which results in the creation of healthier, more resilient crop types.

3.5. Disease-Resistant Genes in Tobacco and Sugarcane

The development of tobacco and sugarcane cultivars that can endure the stresses of bacterial, viral, and fungal diseases depends heavily on disease-resistant genes. Many studies have been conducted on tobacco resistance genes, such as N, which offers protection against the tobacco mosaic virus (TMV). In a similar vein, genes like Bru1 in sugarcane give resistance against sugarcane rust through intricate resistance mechanisms. These genes are essential for beginning defensive responses, including pathogen identification, hypersensitivity reactions, and the synthesis of antimicrobial chemicals. By improving disease resistance via breeding, our understanding of the molecular processes underlying these genes has reduced the financial effect on the sugarcane and tobacco businesses.

3.6. Genetic Engineering and CRISPR Applications

The process of creating plant types resistant to disease has been transformed by genetic engineering and CRISPR technologies. Nowadays, CRISPR/Cas9 integration in vitro techniques allows for precision gene editing to introduce resistance genes directly into the plant genome or eliminate susceptibility genes. By focusing on important susceptibility genes, CRISPR has been used to help tobacco acquire resistance to a variety of viruses and bacterial diseases, lowering the plant's sensitivity. Using CRISPR to modify genes that impact the plant's immune system, sugarcane is more resistant to smut and leaf scald. In addition to speeding up the breeding process, this genetic accuracy guarantees that desirable qualities be passed on without impacting other crop attributes.

3.7. Case Studies of Successful In Vitro Disease Resistance Breeding

Several successful case studies demonstrate the effectiveness of in vitro techniques in cultivating tobacco and sugarcane types resistant to disease. The creation of TMV-resistant lines in tobacco by somatic embryogenesis and gene editing is one noteworthy example.

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Higher yields have been guaranteed and crop losses have been greatly decreased by this method. Using CRISPR technology to mute certain viral replication genes has been proven to be effective in suppressing the Sugarcane mosaic virus (SCMV) in sugarcane, producing resistant plant lines. Furthermore, in vitro methods have been utilized to test both crops for resistance features using callus culture, proving their usefulness in quickening the breeding cycle and creating stable, commercially viable cultivars.

4: SOMACLONAL VARIATION AND MUTATION BREEDING

4.1. Somaclonal Variation

The genetic diversity produced by in vitro culture procedures, especially in plant tissue culture, is referred to as somaclonal variety. Stress applied to plant cells during the cultivation process causes spontaneous mutations or chromosomal alterations, which is the cause of this variance. Because these variants speed up the selection of desirable characteristics like pathogen resistance, they can be used in plant breeding to create disease-resistant cultivars. Somaclonal variation has been utilized to induce disease-resistant characteristics in tobacco and sugarcane by choosing variations that exhibit increased resistance during regeneration. This strategy has the ability to produce genetic variety without requiring the use of transgenic techniques.

4.2. Mutation Induction Methods

Mutation induction is the process of altering plant DNA by use of chemical or physical agents, such as ethyl methanesulfonate (EMS) or gamma rays and X-rays. New genetic features, such as disease resistance, can be produced by these mutations. Mutation induction has shown to be a potent technique in the breeding of tobacco and sugarcane for introducing resistance to illnesses brought on by bacteria, viruses, and fungus. Plants are regenerated from altered cells after being treated with mutagens to plant tissues or seeds. With the use of this method, breeders may intentionally create modifications to the gene pool that would not happen spontaneously, providing a focused strategy for producing disease-resistant cultivators.

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4.3. Screening for Disease-Resistant Mutants

Finding mutants resistant to illness is essential after causing mutations or producing somaclonal variants. The process of screening is exposing the altered or mutant plants to particular pathogens and monitoring how they react. Plants with improved resistance can be identified using methods including genetic markers, biochemical tests, and in vitro pathogen screening. Large-scale screening techniques are used in the cases of tobacco and sugarcane to find the plants that are resistant to infection or exhibit less symptoms. After being found, these resistant mutants are subjected to further testing in the field to make sure they have desirable agronomic features and durable resistance.

4.4. Application in Tobacco and Sugarcane Breeding

The development of disease-resistant tobacco and sugarcane cultivars has benefited greatly from the application of somaclonal variation and mutant breeding. Both crops are vulnerable to a number of diseases, including red rot in sugarcane and tobacco mosaic virus (TMV) in tobacco. Breeders have successfully created lines resistant to various illnesses via the use of in vitro procedures, greatly lowering crop losses. Mutation breeding is a time- and money-efficient technique to breeding projects since it has also been used to increase other qualities like yield and stress tolerance. The use of these methods might help tobacco and sugarcane growers meet the increasing need for sustainable agricultural methods.

5. CONCLUSION

Technologies provide a revolutionary approach to crop development in contemporary agriculture by creating disease-resistant cultivars of tobacco and sugarcane. The time needed for conventional breeding procedures has been greatly reduced by the quick replication of pathogen-resistant plants made possible by techniques like tissue culture, callus culture, and somatic embryogenesis. The accuracy and effectiveness of creating disease-resistant characteristics in these crops has been significantly improved by the application of genetic engineering technologies, particularly CRISPR. Furthermore, somaclonal variety and mutant breeding have added significant genetic diversity, resulting in plants that are more resistant to diseases like sugarcane red rot and tobacco mosaic virus. The production of tobacco and sugarcane is made more sustainable and profitable by these in vitro developments, which also address the rising threat of crop diseases. Agriculture can fulfill the growing need for

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healthier, more productive crops in a quickly changing environment by utilizing these modern breeding techniques.

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