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TISSUE CULTURE & NON-GENE BIOTECHNOLOGY
By Harinder Chaddha, Neelanchanal Trivedi

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CONTENTS

CHAPTER 1

GENETIC STRATEGIES FOR UNRAVELING NEUROBIOLOGY: FROM MODEL ORGANISMS TO NOVEL SPECIES

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ABSTRACT:

The complex interplay between genes and brain development and function is the focus of contemporary neuroscience. In this effort, genetic intervention, which involves manipulating an animal's DNA to examine the phenotypes that arise, is a powerful tactic. Historically, genetic engineering has mostly been used to model organisms like mice, zebrafish, Drosophila, and C. elegans. The study of alternative species, such as songbirds, rats, monkeys, and non-human primates, each of which offers distinct benefits for certain neurological questions, has been spurred by the limits in these species. This paper offers a summary of genome modification techniques and discusses its advantages, drawbacks, and adaptability. It provides a special focus on methods that ensure highly repeatable phenotypes by causing uniform genetic alterations in all cells and their offspring. While direct viral infection is quick and gives geographic specificity, it has problems with cell uniformity and unpredictability. The paper makes a distinction between transgenics, which introduce genes, and methods of gene modification, such as knockouts and knockings. In the end, this analysis emphasizes the importance of picking the best species for certain neurobiological studies and demonstrates how genetic engineering may open up new avenues for neuroscience research.

KEYWORDS:

Disease, Genetic Engineering, Neurological, Phenotypes, Transgenesis.

INTRODUCTION

Knowing how genes control brain growth and function is one of the objectives of contemporary neuroscience. Intervention, or changing an animal's DNA and analyzing the phenotype that results, is a potent method for understanding this connection. Although the arsenal of tools available to do such genetic modifications has undergone significant advancements over the last several decades, until recently these technologies were almost entirely constrained to so-called "model organisms" (Drosophila, C. elegans, zebrafish, and mouse). The study of many neurobiological issues in these animals, however, is not feasible. For instance, these animals' lack of vocal learning makes it difficult for them to serve as models for the genetic underpinnings of speech impairments. In this situation, it would be very beneficial to be able to manipulate songbirds' genomes since vocal learning and communication are an essential part of their way of life[1], [2]. It is also anticipated that studying the genetic basis of social behaviour and language in songbirds could provide a useful tool for understanding the etiology of diseases like autism and speech disorders because young animals learn their song by interacting with and imitating their parents.

Rats are also often a better model of human neuropathology than mice are, more accurately recreating the signs and symptoms of various disorders. Rats are often the preferred species for researchers looking at the neurological foundations of behaviour because they are more equipped for the assessment of higher-order processes like emotion and cognition. Non-human primates, on the other hand, are the best species to investigate the more intricate aspects of cognition, such as perception, memory, and learning. It follows that it will be very valuable to have the capacity to genetically alter the best species to study a given

neurological issue. Since many years ago, there has been curiosity in the origin of genes and their function in evolution. The emergence of new genes over time may have been aided by transcriptional and translational "noise," which is supported by a large body of evidence from the -omics era that suggests there was only one point in evolution where all of the components of genes first appeared before being mixed and shuffled to create novel configurations. Recent research on Orphan Genes (OGs), also known as Orphan Open Reading Frames (ORFans), suggests a distinct situation[3], [4]. OGs make up a distinct class of genes considered to be essential for speciation and evolution. They are recognized as genes that have no discernible relatives in other species and are most likely descended from a single ancestral gene. Due to a lack of evolutionary conservation, OGs often encode small proteins with a high non-synonymous rate of substitution, and their activities are still largely understood. Out of all gene catalogues, OGs have a restricted phylogenetic distribution and are present in up to 30% of all species, as shown in figure 1.

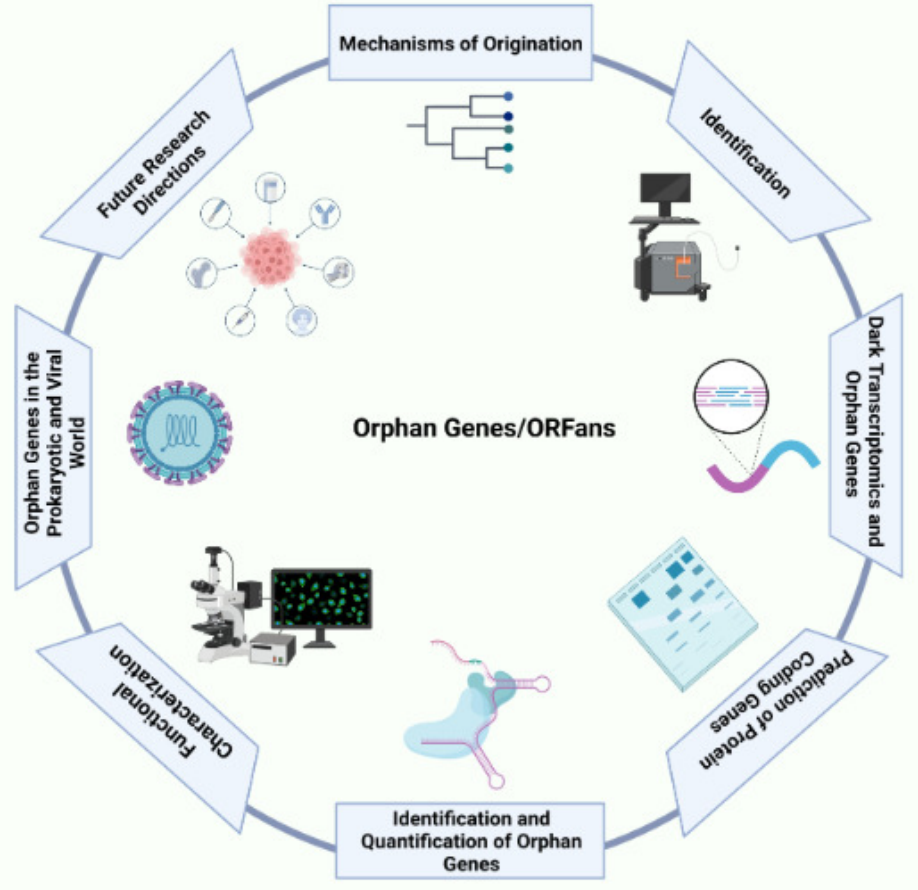

Figure 1: Illustrate the orphan genes/ORFans.

We will provide an overview of the techniques for manipulating the genome in this review and talk about their benefits, drawbacks, and potential. By concentrating on rats, monkeys, and birds, we will also explore the advances gained in adapting the technologies created in conventional "genetic species" to alternative species. The focus of the study will be on genetic modification techniques that modify every animal cell and pass those modifications on to offspring. The fundamental benefit of this is that it results in a phenotype that is highly repeatable from animal to animal since all animals have the same change in their genomes. The direct injection of transgenes given through a virus is one of the most appealing alternatives for genetically altering the brain because of its speed and region-specificity. For instance, injecting adult animals eliminates the need to produce offspring with the genetic

modification, and the viral injection site enables localized manipulation of particular brain regions (for example, by injecting the virus into the visual rather than somatosensory cortex). Direct viral injection, however, has two significant drawbacks. First off, only a small percentage of the cells in a chosen location are infected, making it challenging to manipulate a specific brain region uniformly. Second, since the results of each injection vary greatly from animal to animal, it is challenging to maintain uniformity. We won't go into further depth about viral transport of transgenes into the brain here since it is covered in other publications in more detail.

Genetic methods for changing the DNA

There are two main groups of genetic modification objectives:

1) Transgenics with gene addition.
2) Forward mutagenesis, knockouts, and knockins of genes.

Transgenics

Transgenesis, which is the process of inserting foreign DNA into an animal's genome, is a potent tool for studying how the brain develops and functions. Among many other possibilities, this extra DNA may be a channel to electrically activate or mute a particular population of neurons, a mutant human gene to generate an animal model of an illness, or a gene producing a fluorescent protein to study the morphology of neurons. Regardless of the driving force, one of the main objectives of transgenesis is to alter an animal's genome in a way that minimizes the disruption to endogenous gene expression. Many methods have been created in an effort to achieve this.

Radioactive injection

One of the easiest ways to create a transgenic animal is through pronuclear injection. This technique involves injecting foreign DNA into a zygote's nucleus, where it randomly fuses with the host genome to create a transgenic founder animal. A whole gene's regulatory components, including its promoter and enhancer, had to fit inside an area of DNA that was no longer than 30 kilobases (kb) due to early technological restrictions. Transgenic mice and a small number of transgenic rats with inserts longer than 150Kb have been widely produced as a result of recent advancements in the development and manipulation of BACs. The size of a BAC will typically allow for the inclusion of all exons, introns, promoters, and regulatory elements of a gene in the transgene, allowing for the faithful replication of physiological gene expression patterns and reducing the risks of positional effects from nearby DNA. It has been successfully used on zebrafish, mice, and Drosophila. A variation of this method, in which the BAC is inserted into the genome through a transposon, considerably boosts the efficiency of transgenesis. However, any technique that depends on injecting DNA into cells will still be restricted to animals that can produce a high number of embryos in vitro.

DISCUSSION

Transgenesis mediated by virus's Pronuclear injection is either impractical or impossible for many animals. Only rodents are capable of producing huge numbers of zygotes, and it is impossible to reach and see the pronucleus of avian zygotes in birds. Viruses may be used to get around these restrictions. A transgenic mouse created by injecting the molecularly-engineered leukemia virus (MLV) into early embryos was the first known genetically altered animal. However, transgenic animals lack the ability to express the transgene most of the time because MLV vectors are muted in these animals. Lentiviruses, unlike MLV viruses, have been shown to be resistant to developmental silencing in 2002, allowing enabling the

creation of transgenic mice and rats with strong long-term transgene expression. Transgenic pigs, rabbits, cows, birds, and even non-human primates have all been created using this rather easy and effective method. For instance, lentiviral vectors make it relatively simple to create transgenic chicks and quails, and many strains are now employed in developmental neurobiology research. More recently, transgenic zebra finches, a songbird that has been employed for more than 40 years to study the neurological basis of vocal behaviour, have also been created using lentiviruses[5], [6].

Lentiviral transgenesis is limited by the amount of DNA sequence that can be packed into a lentiviral vector (up to 10 Kb), notwithstanding how simple it is to do. Additionally, pronuclear injection and viral-mediated transgenesis both experience unpredictable transgene integration sites and numbers into the host genome, leading to significant expression heterogeneity across animals bearing the same transgene. For instance, lentiviral vectors containing the mutant gene responsible for Huntington's disease were used to create a transgenic macaque in 2008. The transgene was effectively expressed in the monkeys, and it was discovered that some of them exhibited many of the histological and clinical symptoms of the illness. The five animals produced, ranging in illness presentation from entirely unaffected to death one month after birth, showed significant variances. While variability is unquestionably a problem in this experiment, in certain circumstances it might be useful, with some transgenic lines displaying accidental regional expression patterns that may be perfect for a particular experimental requirement.The use of higher-order primates, such as rhesus macaques, as genetic model systems has some significant practical limits due to their lengthy maturation times (4 years), poor fertility, and high care costs (about $15 per day per animal). Marmosets are an alternative to macaques since they reach sexual maturity considerably sooner (6 months), have a higher birth rate, and can be kept for a lot less money owing to their smaller size than macaques. Recent lentiviral transgenesis experiments produced transgenic marmosets that showed strong expression in their offspring and germline transmission. Marmosets may be the best non-human primate models on which to concentrate genetic engineering efforts, despite the fact that they lack the cognitive abilities of rhesus macaques. This is because of their economic and reproductive advantages.

Gene alteration

Gene modification entails altering the host genome in order to infer gene function based on the defective phenotypic characteristics of the mutant, as opposed to the additive nature of regular transgenesis. Gene inactivation methods include random mutation, targeted mutation, and RNA interference transgenesis. Random mutation Forward genetics, or random mutagenesis, is the process of altering an animal's germ cell DNA by exposing it to a mutagen. Offspring are tested for phenotypic problems after receiving the mutation. This unbiased method has been used to explain the genetic anomalies behind human neurological diseases and brain development and has the ability to find genes whose involvement in a physiological process was not predicted. Unfortunately, this strategy can only be used with invertebrates like C. elegans and Drosophila or, in certain large-scale studies, vertebrates like zebrafish, mice, or rats due to the arduous and expensive screening of millions of animals and subsequent search for the mutation of interest. Recent technical developments have, however, made it possible to execute random mutagenesis followed immediately by next generation sequencing to determine the mutation. This technique eliminates the requirement for breeding programs that are often necessary to use linkage analysis to find the mutant genes, saving time and effort. Since hundreds of rat strains having mutations in genes involved in a variety of physiological processes would be easily accessible, it is anticipated that this combination of next generation sequencing and forward mutagenesis will provide an incredibly helpful

resource.Targeted mutagenesis, commonly referred to as reverse genetics, is the process of altering a genome at a particular and predefined site. It is a potent method for designing a gene that combines the advantages of accuracy, precision, and adaptability. The two main variations of this strategy are a) knockouts, in which the gene is altered to result in a loss of function, and b) knockins, which enable modifications like nucleotide substitutions, the swapping of a gene's regulatory elements, or the flanking of a region of interest with recombinase target sites (such as loxP or FRT) for conditional mutagenesis[7], [8].

The way biology has been researched over the last 60 years has an underlying conflict. According to some researchers, it is desirable to concentrate study on a limited number of species, allowing for the development of in-depth and thorough information. Others contend that each biological issue has an animal species that is best equipped to study it (this is known as the August Krogh principle). Max Delbruck, a pioneer in molecular biology, urged scientists to concentrate only on T-even bacteriophages in the field's early years. Numerous researchers took his advice, significant resources were spent to studying these phages, and a wealth of information was gleaned from these investigations. However, other researchers disregarded his tenets and concentrated on lambda phage, a bacterial virus that had received less research at the time. With the advantage of hindsight, we now understand that lambda phage has maybe contributed more to the development of molecular biology than T-phages. Going against the grain when selecting a model system may result in considerable benefits.There are several instances when concentrating on less common species has significantly advanced our understanding of neurological processes. For instance, the squid giant axon, barn owls for sound localization, honeybees for animal communication, frogs for neurotransmitter production, and cats for the cortical processes of visual perception were all important for understanding the mechanics of membrane excitability. Currently, whole genome sequencing can be completed fast and affordably, giving researchers the flexibility to harness the potential of genetic alteration and use it on the species that best fits their biological research needs. For instance, butterflies employed in the research of magnetoreception and salamanders, an excellent organism to investigate neural regeneration, have both undergone genetic engineering. Furthermore, it is predicted that new techniques will soon be available to enable the genetic manipulation of ants and bees, which hold enormous promise for the study of social behaviour. The lack of "off-the-shelf" genetic constructions and transgenic animals is a crucial factor to keep in mind while researching such non-traditional species; as a result, researchers often have to make their own reagents[9], [10].

CONCLUSION

The field of contemporary neuroscience is characterized by a desire to understand the complex symphony orchestrated by genes in controlling brain growth and function. Genetic intervention, which includes changing an animal's DNA and watching the resulting phenotypic changes, has become a successful tactic in this regard. Historically, model species like Drosophila, C. elegans, zebrafish, and mice have been the main exceptions to this technique. However, the intrinsic limits of these species have encouraged a move toward investigating other species, such as songbirds, rats, monkeys, and non-human primates, each of which offers particular benefits for certain neurological investigations. This review has offered a thorough analysis of genome alteration techniques, exploring its benefits, limitations, and adaptability. The importance of methods that provide homogeneous genetic alterations in all cells and their offspring, assuring highly repeatable phenotypes, has been brought to light. Direct viral injection has the benefits of geographic specificity and speed, but it also faces difficulties with cell variability and animal consistency.In addition, the study made a distinction between transgenics, which introduce genes, and gene modification

techniques like knockouts and knockins, illuminating their different contributions to the advancement of neurological research. In conclusion, our investigation has brought to light the critical role that species selection plays in modifying genetic modification techniques to specifically address neurological issues. It emphasizes how genetic engineering has the ability to open up new areas of neuroscience study and eventually advance our comprehension of the complex relationship between genes and brain function. Unquestionably, the acceptance of non-traditional species and the effective use of genetic modification will open the door to significant advances in the field of neuroscience.

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CHAPTER 2

PLANT TISSUE CULTURE: A PATH TO COST-EFFECTIVE LARGE-SCALE PROPAGATION

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ABSTRACT:

Large-scale plant propagation has been transformed by plant tissue culture, a method that involves the controlled, aseptic nurturing and multiplication of plant cells, tissues, and organs. A key use of tissue culture is micropropagation, which enables the quick generation of superior, disease-free, and homogeneous planting material. This method overcomes seasonal and geographic limitations, permitting year-round proliferation independent of the environment or weather. Producers, farmers, nursery owners, and rural jobs all gain from the development of good planting material, particularly for decorative, forest, and fruit trees. Although micropropagation has many benefits, it is also more expensive to produce and calls for specific knowledge. This labour- and capital-intensive sector confronts issues related to manufacturing costs and technical know-how. As a result, scientists have created affordable tissue culture techniques that preserve product quality without sacrificing pricing. This demands constant attention to input costs, including those for chemicals, media, energy, labour, and capital. Between developed and developing nations, there are differences in the cost dynamics of micropropagation. Consumables like media and cultural containers play a bigger role in less developed areas than they do in affluent countries, since labour costs make up a considerable portion of manufacturing prices. To reduce the cost of producing tissue-cultured plants, especially in underdeveloped nations, low-cost alternatives are essential.

KEYWORDS:

Disease, Plant Propagation, Tissue Culture, Zygotic.

INTRODUCTION

Cell, tissue, and organ growth and multiplication on specific solid or liquid medium in a sterile, controlled environment is referred to as "plant tissue culture." The method of plant tissue culture is frequently employed for industrial-scale plant reproduction. The commercial method is based mostly on micropropagation, wherein microscopic stem cuttings, axillary buds, and, to a lesser degree, somatic embryos, cell clumps in suspension cultures, are used to produce fast multiplication. The quick creation of top-notch, disease-free, and homogeneous planting material is micropropagation's main benefit. Throughout the year, the plants may be replicated in a controlled environment wherever they are, in any season and weather. For producers, farmers, and nursery owners as well as for rural employment, the production of high quality and wholesome planting material for ornamentals, forest, and fruit trees, propagated from vegetative components, has opened up new prospects in international trade [1], [2].

Compared to traditional plant propagation techniques, micropropagation technology is more costly and needs a variety of expertise. It is a capital-intensive sector, and the unit cost per plant might sometimes become expensive. The main causes are manufacturing costs and expertise. Because the traditional planting material was so much less expensive in the early years of the technique, it was difficult to market tissue culture goods. Now, this issue has been solved by developing dependable and affordable tissue culture techniques without sacrificing quality. This necessitates ongoing monitoring of the expenses of labour,

chemicals, media, energy, and capital inputs. The primary reason driving tissue-cultured plant production's high cost in developed nations is manpower. Some processes may be partly automated to cut down on these expenses, such as the use of peristaltic pumps for medium dispensing and dishwashers for container cleaning. Consumables like media, cultural containers, and power contribute much more to manufacturing expenses in less developed nations in Africa, Asia, and Latin America when labour is relatively cheaper. For instance, 30–35% of the output of micropropagated plants may be attributed to the cost of medium preparation (chemicals, energy, and labour). However, in many poor nations, automated manufacturing methods based on bioreactors, robotic explant transfer, pre-sterilized membrane capsules, and container sealing are not economically feasible. To lower the cost of producing tissue-cultured plants, low-cost alternatives are thus required.

Numerous of the low-cost technological alternatives discussed in this article may be used at different stages of plant micropropagation. The use of the low-cost approaches mentioned may also be used to propagate the infrequent tissue culture-generated variations (somaclones), uncommon spontaneous bud mutants, as well as those derived via induced mutations. Micropropagation expedites the recovery, multiplication, and release of better varieties in vegetatively propagated plants when combined with radiation-induced mutations. The large-scale plant replication of mutants of several fruits, shrubs, flowers, and forest trees that are traditionally reproduced vegetatively would thus greatly benefit from low-cost technology.Explants, which are microscopic fragments collected from any area of a plant, are used to start plant tissue cultures. Almost all plant components have been effectively employed as a source of explants. In reality, the "explant" is surgically removed, surface sterilized, and put on a medium containing nutrients to start the mother culture, which is repeatedly replicated through subculture. The below plant sections are often used in industrial micropropagation.

1. A section of stem containing axillary bud culture with, or without, a part of shoot is known as nodal or axillary bud culture. It is referred to as "axillary bud" cultivation when just the axillary bud is harvested.
2. Floral meristem and bud culture: These explants are seldom employed in commercial propagation, yet they have the ability to produce full-grown plants.
3. Explants from other sources have also been used to start cultures in various plants, including leaf discs, nucellus, intercalary meristems from nodes, tiny sections of stems, and immature zygotic embryos.

Callus cultures and cell suspension

In order to start a callus, plant elements such leaf discs, intercalary meristems, stem fragments, immature embryos, anthers, pollen, microspores, and ovules have been cultivated. A callus is a collection of disorganized cells that, in many instances, when placed in a proper media, may develop into shoot-buds and somatic embryos, which eventually grow into full-fledged plants. For starting cell suspensions, such calli on culture in liquid medium on shakers is employed. Mechanical shakers used to sustain liquid suspension cultures provide rapid and exceptional rates of multiplication. In contrast, calli are often cultivated in bottles and flasks using semi-solid or liquid medium in commercial micropropagation. Bioreactors have gained some popularity for somatic embryogenic cultures. It is believed that bioreactor-based micropropagation may one day be automated [3], [4].

Cultured cells and tissues' transport systems

There are several methods for the cultivated cells and tissue to develop into a finished plant. The well-liked and recommended methods for industrial multiplication are those that result in

the mass production of true-to-type plants. The following words have been used to characterize different cell and tissue growth paths in culture.

Organogenesis and regeneration

at this route, the differentiation and growth of clusters of apical meristem cells at the shoot apex, axillary buds, root tips, and floral buds is encouraged, leading to the development of shoots and finally of full-grown plants. The axillary buds that have developed in the culture often go through recurrent proliferation and yield several small plants. The plants are subsequently divided from one another and rooted either in the next micropropagation phases or in vivo (in trays, little pots, or beds in glasshouse or plastic tunnel under reasonably high humidity). Explants cultivated on relatively high auxin concentrations, such as 2,4-D, 2,4-dichlorophenoxyacetic acid, create a callus, which is an amorphous mass of cells. The callus may be multiplied and subcultured further. The cell suspension created when the callus is agitated in a liquid media may be replicated and subcultured into further liquid cultures. Through organogenesis or somatic embryogenesis, the cell suspensions aggregate into cell clumps that ultimately develop into calli and give birth to plants. In the process of organogenesis, cultivated plant cells and cell clumps (called calluses) as well as maturely differentiated cells (microspores, ovules), tissues (leaf discs, inter-nodal segments), and cell clumps (called calluses) are encouraged to differentiate into whole plants in order to generate shoot buds, ultimately shoots, and roots in order to form full plants [5], [6].

Somatic implantation

In this process, cells or callus cultures grown on solid medium or in suspension cultures develop somatic embryos, which, when germination occurs, produce full-grown plants. Through secondary somatic embryogenesis, the main somatic embryos may also generate other embryos. The use of somatic embryos in large-scale commercial production has been limited to only a few plants, including carrot, date palm, and a few forest trees, despite the fact that somatic embryogenesis has been proved in a very wide number of plants and trees.

DISCUSSION

From callus and suspension cultures, somatic embryos are generated as adventitious structures directly on zygotic embryo explants. Embryos enclosed in synthetic endosperm and somatic embryos have the potential to be used in large-scale clonal replication of superior genotypes of diverse plants.

They have also been used in the mass manufacturing of hybrid seeds as well as for the expansion of parental genotypes in commercial plant production. Somatic and zygotic embryos share many physical characteristics, despite some established biochemical, physiological, and anatomical distinctions. 2,4-D, a synthetic auxin, is often used for embryo induction. It is sufficient to promote somatic embryogenesis in many angiosperms, such as carrot and alfalfa, by subculturing cells from 2,4-D-containing media to auxin-free medium. Osmotic stress application, medium nutrition adjustment, and humidity reduction may all improve the process. It has also been used effectively to select embryogenic cell lines. For instance, in grapevine cultures, selection for distinct morphotypes enables predictable frequency generation of high-quality embryos.

Artificial seeds

The idea of producing and using synthetic seeds and somatic embryos as replacements calls for an effective and repeatable manufacturing method as well as a high rate of post-planting conversion into robust plants. For a long time, synthetic endosperm and coatings, as well as

gel capsules containing nutrients, insecticides, and beneficial organisms, were considered viable alternatives. Nevertheless, since this technology is still in the research stage, it cannot yet compete with existing commercial plant growth techniques.

Micropropagation Method

The goal of plant micro-propagation is to create several genuine cloned replicas of a plant. Typically, the procedure is broken down into the following phases:

Stage 0 involves choosing and pre-treating appropriate plants for propagation.

Stage I: Explants are started, surface sterilization is done, and mother explants are established. Stage II: cultures for explant growth and proliferation.

Stage III The explants are shot and rooted.

stages IV: Weaning and hardening.

These phases are generally relevant to plant reproduction on a big scale. The growth medium, weaning, and hardening conditions must be specifically modified for each plant species, variety, and clone. As a general guideline, grow plants in environments that are as natural as possible or that are comparable to those in which they will eventually be grown ex vivo. For instance, it is preferable to multiply the material under lengthy days during stages III and IV if a chrysanthemum variation is to be developed for flower output. There are several options available to carry out plant material production up to a certain number of steps. For instance, many commercial tissues culture businesses only take on Stage III of manufacturing, leaving the further stages to others.

Stage Before Propagation

The mother plants must be properly cared for in the greenhouse during the pre-propagation stage (also known as stage 0), where there should be little to no dust and no insects or diseases. High grade explant source plants are produced in clean enclosed spaces, glasshouses, plastic tunnels, and net-covered tunnels with low infection. To reduce contamination in the in vitro cultures, the mother plants should be properly pretreated with fungicides and insecticides prior to the collection of plant material for clonal propagation. In vitro cultures develop and reproduce more quickly as a result. The preparation of the donor plants is the first step in the contamination control process. To minimize contamination, they may be prescreened for illnesses, segregated, and treated. Once at the manufacturing facility, the explants are surface sterilized and put into culture. At this point, they might be treated with antibiotics, fungicides, and anti-microbial formulations like PPM. microbiological methods are then used to culture index the explants for contamination; sometimes, these methods are augmented by tests based on molecular biology or other methods [7], [8].

Phase I

The inoculation of the explants on sterile media to start aseptic culture is referred to as this step. The very first stage in micropropagation is to start explants. Explant initiation in an aseptic state should be seen as a crucial stage in micropropagation since a nice clean explant may be duplicated numerous times after it has been established in an aseptic setting. Explants often fail to establish and thrive because of contamination rather than a lack of an appropriate substrate. The explants are moved to an in vitro setting devoid of microbial impurities. The procedure calls for the removal of minute plant fragments, followed by the surface sterilization of those fragments with chemicals like sodium hypochlorite and ethyl alcohol, as well as repeated washings with sterile distilled water before and after chemical treatment. The

infected explants are destroyed after a brief culture time, typically 3 to 5 days. The explants that are still alive and exhibiting growth are kept and utilized for further culturing.Meristems, apical- and axillary buds, early seedlings, developing young leaves and petioles, and unopened floral buds are explant sources in herbaceous plants including potato, chrysanthemum, carnation, streptocarpus, strawberry, and African violet. The low-cost alternatives listed below may be modified to start explants.

Use of sterile instruments

The majority of deep-seated meristems and those covered in leaves or other integuments (such as flower bracts) are presumed to be sterile using this approach. The explant is relocated to a different spot on the dissection stage after being cleaned with sterile water, rinsed with ethanol, and touched by equipment that have been sterilized each time.

Phase II

In Stage II, which is known as the propagation phase, explants are cultivated on the proper substrate for multiplying shoots. The main objective is to achieve reproduction without compromising genetic stability. A high number of propagules may be produced by repeatedly cultivating axillary and adventitious shoots, cutting with nodes, somatic embryos, and other Stage I organs. By repeatedly cultivating them, the propagules created at this stage may be utilized for future replication. The in vitro-derived shoots sometimes need to be subculture onto various medium for elongation.

Stage II in vitro shoots are rooted to create whole plants from the in vitro branches. The proliferating material should be divided after roots, not before, if it consists of bud-like structures, such as orchids or clusters of shoots from a banana or pineapple. On half-strength MS Murashige and Skoog, 1962 medium without any growth-regulators, various plants, including banana, pineapple, roses, potato, chrysanthemum, strawberry, mint, many grasses, and many more, may be rooted. High survival rates during weaning and subsequent transfer to soil depend on strong, well-rooted plants. Costly and labor-intensive, this stage. According to estimates made in vitro rooting accounts for between 35% and 75% of the entire cost of production. The processes of acclimation and rooting should be combined whenever possible.

Phase IV

The in vitro micropropagated plants are now being weaned and toughened. The micropropagated plantlets are prepared for transport to the greenhouse at this point in the tissue culture process. There are measures done to develop individual plantlets that can perform photosynthesis. The tissue-cultured plantlets are progressively hardened under circumstances of low light intensity and high humidity, respectively. Most of the agar may be delicately removed by washing with water if it was grown on solid media. Plants may be placed in the shadow for 3 to 6 days to acclimatize to the new habitat using diffused natural light. The plants are then moved to a suitable substrate (sand, peat, compost, etc.) where they progressively get harder. The use of plastic domes or tunnels, which minimizes the intensity of natural light and maintains a high relative humidity throughout the hardening process, is one low-cost solution. After rooted, if the plants are still attached, they should be placed in bunches in the soil and separated after 6 to 8 weeks of development [9], [10].

Distribution to The Growers

Extra care must be taken while delivering the rooted and toughened little micropropagated plants to growers and the market. Plant losses may sometimes happen during shipping and handling by growers. This is especially true when the plants are not sufficiently developed

after being transferred to the soil or when they are not properly hardened and rooted. Clear instructions on how to manage the supplied material should be given to growers. In addition to the financial loss, low plant survival reduces producers' faith in the technique. Many underdeveloped nations employ the low-cost method of transferring individual plants to soil in polythene or black plastic bags to provide fully-grown banana plants to farmers.

CONCLUSION

For large-scale plant multiplication, plant tissue culture is a crucial technology. This approach provides unmatched benefits, as we have shown in this study, including the quick creation of high-quality, disease-free, and homogeneous planting materials that transcend regional and seasonal restrictions. The use of tissue culture technology has opened up new possibilities for international commerce, which is advantageous for producers, farmers, nursery business owners, and rural jobs. However, the difficulties brought on by the expensive nature of micropropagation have inspired the creation of creative, economical alternatives. The dependability and quality of tissue-cultured plants have not been compromised in the endless quest to lower manufacturing costs.

Various low-cost technological solutions that may be used at various phases of the propagation process have emerged as a result of this endeavour.This research has shown the many routes by which cultured cells and tissues may become entire plants, from examining explant sources to examining cell suspension and callus cultures.

The significance of simulating ex-vitro growing conditions and synthetic seeds have also been thoroughly studied. The importance of each stage in attaining effective large-scale plant multiplication is highlighted by the focus on pre-propagation, explant initiation, propagation, rooted, and weaning/hardening phases. Furthermore, it is critical to understand that providing tissue-cultured plants to growers in the best possible condition is equally important. The survival and flourishing of these priceless resources are guaranteed by giving clear handling and care instructions. Essentially, plant tissue culture is a dynamic and developing science that is still transforming horticulture and agriculture. Its promise for low-cost, large-scale multiplication opens the door to sustainable agriculture, biodiversity preservation, and food security in addition to being a godsend for commercial organizations. The future of plant propagation looks bright as academics and practitioners continue to improve and innovate in this field, providing a better and more sustainable futures for agriculture and beyond.

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CHAPTER 3

UNLOCKING THE POTENTIAL OF LOW-COST PLANT TISSUE CULTURE FOR SUSTAINABLE AGRICULTURE AND FORESTRY

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ABSTRACT:

Numerous benefits of plant tissue culture technology include the quick growth of plants and the creation of disease-free planting material. The high manufacturing costs, however, have restricted its broad utilization for extensive commercial application. The urgent need for low-cost plant tissue culture technology is examined in this paper, with an emphasis on the need of preserving quality while lowering production costs. It emphasizes the use of affordable solutions in sustainable forestry and agriculture, as well as the uses of genetic engineering and the possibility for wider adoption in the future. Low-cost tissue culture technology holds the key to realizing the full potential of this novel methodology, which is increasingly in demand as cost-effective plant propagation techniques. The main use of micropropagation has been to provide superior planting material. Low-cost tissue culture technology refers to the employment of techniques and tools to lower the per-unit cost of micro- and plant production. Low-cost solutions should reduce production costs without sacrificing the quality of the plants and micropropagules. Cost reduction in low-cost technologies is accomplished through increasing process effectiveness and improved resource management. For the development of reasonably priced, high-quality planting material, low-cost tissue-culture technology will continue to be a top priority in many developing nations' agricultural, horticulture, forestry, and floriculture sectors.

KEYWORDS:

Agricultural, Tissue Culture, Horticulture, Micropropagules, Micropropagation.

INTRODUCTION

Numerous clones of desired types and native flora are presently being multiplied in dozens of commercial micropropagation facilities across the globe. This method, which has been developed and used to a broad variety of crops, as well as forest and fruit trees, has been utilized to provide disease-free planting material in addition to the benefit of quick replication. The expense of producing micropropagules, however, often prevents the method from being used for extensive commercial propagation. Through the quick multiplication of plants and the creation of disease-free planting material, plant tissue culture has transformed the area of plant propagation. This method promises higher yields and better crop types, and it has enormous potential in horticulture, forestry, and agriculture. The expensive cost of manufacture, however, prevents its broad acceptance for extensive commercial application [1], [2].

Tissue culture-based plant propagation is carried out at high-tech facilities in wealthy nations with pricey infrastructure and equipment, leading to increased unit production costs. Unfortunately, many poor nations lack access to these facilities and resources. The use of low-cost technologies is thus necessary to increase the affordability and accessibility of tissue culture. This paper examines the critical need for low-cost plant tissue culture technology and how it can revolutionize forestry and agriculture. It highlights that in order to assure propagule quality, low-cost choices must prioritize maintaining aseptic conditions. In plant

tissue culture, quality is still king, thus any cost-cutting measures shouldn't come at the expense of the viability of micropropagated plants.

We've gone into the urgent need for cost-effective choices in the field of large-scale plant propagation in our thorough investigation of low-cost plant tissue culture. Although tissue culture technique has many great benefits, such as quick multiplication and disease-free planting material, its broad commercial application is sometimes hampered by the expensive cost of manufacturing. The need for creative ideas to lower production costs without sacrificing the quality and dependability of micropropagated plants has been made clear by our review. We have underlined that propagule quality requires low-cost technologies to prioritize sustaining aseptic conditions. It is crucial to follow strict tissue culture procedures since microbial contamination might cause catastrophic losses. The importance of quality is unwavering in the field of micropropagation. Lower-quality plants shouldn't result from cost-cutting measures. The technique must keep producing healthy, uniformly bred, disease-free, and high-performing plants that can thrive in field circumstances. Low-cost technology is essentially an advanced generation technique that aims to increase resource and process utilization [3], [4].

It is impossible to exaggerate the importance of low-cost technologies in forestry and agriculture. It reduces dependency on energy-intensive methods and crop protection agents while advancing the aims of precision agriculture, sustainable agriculture, and maximum net profitability. The cornerstone of this strategy is biotechnology-assisted plant breeding, with plant tissue culture playing a crucial role in developing superior-quality plants and accelerating the release of better varieties to the market. Furthermore, the forestry industry has enormous potential for micropropagation, providing environmentally friendly alternatives to cutting down natural forests and quickly multiplying chosen clones for traditional production. Although the high prices at the moment are problematic, they do not lessen the long-term potential of micropropagation in forestry. Micropropagation methods are essential in genetic engineering applications, such as the creation of "edible" vaccines and pharmaceutical compounds, in addition to agriculture and forestry. With the help of these technologies, we are able to use plants more effectively and economically than ever before for the benefit of society.

The Demand for Affordable Technology

Low-cost tissue culture technology refers to the employment of techniques and tools to lower the per-unit cost of micro- and plant production. Traditional tissue culture-based plant propagation is done in highly advanced facilities in many developed nations. These facilities may have stainless steel surfaces, sterile airflow rooms, pricey autoclaves for sterilizing media and tools, and similarly pricey glasshouses with automated humidity, temperature, and day-length controls to harden and grow plants. Many of these expensively built institutions utilize a lot of energy and operate like ultra-clean hospitals. Such tissue culture facilities have significant setup and operating costs, and often do not exist in impoverished nations. For instance, compared to developing nations, the cost of power is far cheaper and its supply is considerably more secure in wealthy nations. The same may be stated for the provision of media, chemicals, equipment, and tools needed in micropropagation as well as culture containers, media, and instruments. In order to lower the cost of plant micropropagation, substitutes for costly inputs and infrastructure have been sought for and created.

Use of inexpensive options

Low-cost solutions should reduce production costs without sacrificing the quality of the plants and micropropagules. Producing high-quality planting material has been the main use

of micropropagation, which ultimately increases agricultural production. The plants that are produced must have a high field survival rate, be robust, and be able to be effectively transplanted in the field. They should also have homogeneous genetic makeup, be devoid of viruses and illnesses, and be priced similarly to plants grown using traditional techniques. Cost-cutting efforts shouldn't produce plants with subpar field performance or highly contaminated cultures. The aseptic cultivation and multiplication of plant material is the primary need for micropropagation. It's important to keep the culture containers and subsequent subcultures microbe-free. In many instances, conceptual or practical errors may bring endophytic contamination into the plant material itself or external bacteria into the culture vessels. The microorganisms overgrow the cultures as a consequence and destroy them. Microbes may develop slowly at low temperatures that are regulated, but they quickly multiply at high temperatures that are uncontrolled. Therefore, using the incorrect low-cost solutions might render the manufacturing process vulnerable to mishaps. Low-cost approaches can only be successful if the fundamental guidelines for tissue culture are strictly followed to preserve propagule quality [5], [6].

DISCUSSION

Culture contamination by microbes has known to ruin months' worth of labour and may become a nightmare. The most economical course of action is to simply throw away infected cultures. When just a limited number of cultures are handled, preventing contamination in tiny R&D labs is not a challenging challenge. However, handling many cultures daily is a need in commercial production. Until they are employed for either further subculture or hardening and growing-on, it is also crucial to retain such cultures in high numbers under contamination-free conditions. Because plants lack an immune system, there are restrictions on the use of antibiotics to solve the issue. In addition, many antibiotics that work against bacteria, fungus, and phytoplasmas are also hazardous to plants. Of course, using antibiotics is neither a certain nor ideal way to get rid of microbial infection. Modern, sophisticated facilities do not ensure that contamination will not occur. The labs that achieve flawless contamination control do so by adhering to meticulous fundamental tissue culture protocols. Therefore, the processes rather than the technology assure the quality of tissue cultivated plants.

The standard of micro propagules

Low-cost technology refers to a new generation of technology where costs are reduced through increasing process effectiveness and improved resource usage. Currently, low-cost technology is needed in both wealthy and developing nations to gradually lower the cost of propagule production. The resource-rich farmers in many developing nations have been the prospective end-users of plants produced via tissue culture. They are aware of the advantages and possibilities of sound planting material. These farmers are willing to take a chance on an investment since the planting material has a high potential for production. For instance, hybrid seeds of numerous vegetables, papaya, rice, and cotton are priced anywhere between 15 and 20 times more than regular kinds. Nevertheless, they have a sizable market. Therefore, producing low-quality plants just because they are less expensive will not be a viable method for using micropropagation in agriculture. Only if the approaches do not violate the fundamental requirements of tissue culture and plant quality is a reduction in manufacturing costs achievable.

The value of affordable technology

Both investors and policy leaders in developing nations are aware of the potential of plant tissue culture to boost agricultural output and create jobs in rural areas. The setup costs of

facilities and the unit production costs of micropropagated plants are substantial in many poor nations, however, and often the return on investment is not proportionate to the potential economic benefits of the technology. These issues may be solved by standardizing agronomic techniques more accurately via precision agriculture, maximizing net crop profits, bringing down production costs per unit of output, or both. The method has a special use in the growth of attractive plants. Trading of ornamental plants has prospered despite high manufacturing costs because they fetch high unit prices. The market is constrained, however. Over time, several new tissue-culture businesses have emerged in a number of emerging nations to compete in the small market. The lowering of net margins below practicable limitations was an unavoidable outcome. Numerous international organizations concur that, assuming the issue of the high cost of production is successfully resolved, tissue culture technology is particularly important to agriculture. According to the paper, pathogens are removed from planting material and elite clones are multiplied using tissue culture procedures. It also highlighted the need of tree regeneration for breeding, clonal testing, and the speedy introduction of improved genotypes in around 100 forest species [7], [8].

It has been repeatedly emphasized that agriculture and forestry must be sustainable over the long term, use little to no crop protection chemicals, use little in the way of energy inputs, maintain high yields, and provide high-quality materials. To accomplish these objectives, biotechnology-assisted plant breeding is a necessary step. The tremendous potential of plant tissue culture methods to generate plants of better quality has not been completely realized in underdeveloped nations. Tissue-cultured plants often outperform those grown traditionally because they may be prepared for maximum performance during in vitro growth. In vitro culture, which has been effectively used in plant breeding and the quick introduction of enhanced plants, so plays a special role in sustainable and competitive agriculture and forestry. If the rate of multiplication is modest, it may take many years to introduce new, better varieties to the market. For instance, it can take a lily breeder 15 to 20 years to produce enough bulbs of a newly developed cultivar to put it on the market. This process may be significantly accelerated by in vitro propagation. Plant breeding now also includes plant tissue culture as a crucial component. For instance, a tissue culture-based genetic transformation is required for the biotechnology-based production of pest- and disease-resistant plants. Because of the increased tolerance to illnesses and pests, producers may use less or no pesticides.

The availability of tissue culture-linked production systems in the forestry industry may successfully provide sustainable alternatives to the requirement to extract wood from local forests and other natural ecosystems. Numerous forest tree species now have successful micropropagation procedures in place, and the number of species for which somatic embryogenesis is effective is growing. Therefore, it is conceivable that micropropagation in the forestry industry will gain economic significance in the future. The rapid multiplication rates provided by micropropagation provide a far speedier capture of genetic advantages attained in forest tree breeding projects than vegetative propagation by cuttings. However, one of the main barriers to the direct use of micropropagation in many systems will be the present high prices.

The enhancement of trees in the tropics depends on the widespread use of current technology to plantation species. Micropropagation is being employed as an early fast multiplication phase in a limited number of plantation initiatives. However, it has been noted that the direct use of micropropagules as planting material would be hampered by the existing high prices. Micropropagation undoubtedly plays a part in the quick multiplication of the chosen clones for traditional cutting production.If propagation costs are decreased, the direct use of

micropropagules as planting stock in industrial plantations may significantly expand forestry tree farming.Applications of genetic engineering, such as the creation of plants as a source of "edible" vaccinations, will also benefit from the availability of micropropagation methods. There are several more valuable plant-derived products that can be made in tissue cultures, sometimes more affordably and consistently than from plantations and forests grown naturally. These include the pharmaceuticals and therapeutic substances that are now the focus of extensive prospecting efforts in tropical forests [9], [10].

CONCLUSION

It is impossible to overestimate the importance of low-cost plant tissue culture technology for agriculture, forestry, and allied industries. Although tissue culture has obvious benefits, its adoption for extensive commercial multiplication has been constrained by its expensive price. This study emphasizes the need of coming up with creative ways to cut production costs without compromising the effectiveness and quality of micro propagated plants. Low-cost technology is an example of an advanced generation method that aims to improve resource utilization and process efficiency. It supports the objectives of precision agriculture, sustainable agriculture, and decreased dependence on energy-intensive methods and crop protection agents. Low-cost tissue culture technique is essential for creating better plants and accelerating the release of enhanced varieties into the market in genetic engineering applications.Additionally, there is a huge opportunity for the forestry industry to use low-cost micropropagation technology, providing sustainable alternatives to cutting native forests and quick multiplication of chosen clones for traditional production. Although the high prices at the moment present difficulties, they do not lessen the long-term potential of micropropagation in forestry. In conclusion, a crucial to realizing the full potential of this novel strategy is low-cost plant tissue culture technology. We are getting closer to implementing sustainable, high-yield agricultural and forestry methods that are good for both farmers and the environment as academics, practitioners, and policymakers continue to invest in low-cost technologies.

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CHAPTER 4

OPTIMIZING LABORATORY DESIGN AND LAYOUT FOR EFFICIENT PLANT TISSUE CULTURE FACILITIES

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ABSTRACT:

The efficient functioning of plant tissue culture facilities depends on the structure and design of the laboratory. This chapter examines the essential components required for creating labs of different sizes, with an emphasis on effectiveness, cost containment, and contamination prevention. It covers location, design, and layout issues while highlighting the significance of controlled environments, equipment choice, and workflow management. The tissue culture process may achieve greater productivity, lower operating costs, and sustainable practices with a well-planned laboratory. A typical plant tissue culture facility's physical components include machinery and structures with preparation, transfer, culture, and growth rooms, as well as hardening and weaning, soil-growing in greenhouses or plastic tunnels, packaging, and shipping areas, and related facilities like an office and a store for chemicals, containers, and supplies. The first crucial step when deciding on the size and location of a facility is careful planning. A tissue culture facility's physical component will vary in size depending on how much production it requires to meet its functional requirements. Before beginning construction on a new facility, it is advised that one visit an existing one to observe the layout and operating requirements. A tissue culture facility may simplify several processes and save expenses by using a variety of low-cost alternatives.

KEYWORDS:

Greenhouses, Management, Plant Tissue Culture, Physical Components, Productivity.

INTRODUCTION

The creative design and organization of labs is crucial to the success of plant tissue culture facilities. These variables affect operating expenses and contamination control in addition to the effectiveness of the tissue culture process. The need of precise planning is emphasized as we examine the essential components of laboratory architecture and layout for plant tissue culture. The topic of choosing an acceptable site is first discussed, taking into account variables including climate, utility accessibility, transportation, and local infrastructure. The significance of integrating the facility with the local environment is shown by highlighting the influence of location on energy costs and climatic adaptability. The architecture and organization of labs are next explored in more detail, with a focus on sterility, preventing contamination, and controlled settings. It talks about the necessary tools needed for growing plant tissue, such autoclaves, laminar airflow chambers, and sterilizing tools.

Also included are optional tools and technologies that may improve productivity and data management. The study divides lab spaces into three categories: semi-clean, clean, and ultra-clean, each with a specific function in the tissue culture procedure. The culture transfer and development rooms, where the main activities take place, are given special attention. The importance of maintaining ideal growth conditions is carefully discussed, as are temperature management, lighting, shelving, and other elements. The study also discusses how to build greenhouse facilities for hardening tissue-cultured plants before introducing them to outdoor settings. It covers several greenhouse designs, glazing choices, and temperature control techniques to ensure the smooth transfer of plants from the lab to the outside [1], [2].

In order to preserve the integrity of tissue-cultured goods throughout travel, the chapter also looks at the packing and shipping process. It highlights the need of having the appropriate paperwork and packaging that complies with destination-specific temperature standards. Overall, this chapter emphasizes the need of meticulously planning and setting up tissue culture facilities. Modern plant tissue culture facilities must have an effective laboratory since it not only boosts production but also encourages cost savings and sustainability. Any laboratory, no matter how large or little, must have a few certain components in its architecture and design in order to function properly. A laboratory's proper design will result in lower levels of contamination as well as high levels of productivity. Laboratories that are thoughtfully conceived and constructed may lower their operating and energy expenses. The equipment and its usage in the different phases of micropropagation must be accommodated in a tissue culture laboratory in the most effective way possible. Planning the functional requirements of a medium-sized micropropagation facility is covered in this chapter. The data provided here would enable improved facility design and more efficient operation, which would save costs.

General locational considerations

A room or section of a basement, a garage, a remodeled office, or a room in the main home may all serve as suitable locations for tiny laboratories. A minimum of 14 m2 is needed for the medium preparation, transfer, and main growth shelves. Installing walls will help divide the space into distinct parts. It is crucial to investigate the region before establishing a commercial micropropagation facility, bearing in mind the climate, accessibility to water, energy, transportation, and supply infrastructure. Tissue culture projects are often better suited to a temperate environment. This significantly lowers the cost of cooling necessary to maintain the temperature for the cultures' best development.

When deciding where to locate the plant, the availability of water and power is crucial and should be taken into account. For instance, of the 76 commercial tissue culture facilities in India, almost 52 are situated in and around the temperate cities of Bangalore and Pune. Therefore, cooling is only necessary at certain times of the year. However, tissue culture facilities need both heating and cooling in places with harsh weather, like Delhi. Electricity costs per thousand plants in a facility that produces five million plants are around $0.30 in Bangalore and Pune and $0.80 in Delhi. Major breakdowns in the efficient operation of tissue culture units are brought on by interruptions in the power and water supply. Poor water quality raises the price of media [3], [4].

Before work starts, local authorities should be contacted to inquire about zoning and building permissions. Buildings should be placed far from areas that might be contaminated, such as a gravel road, parking lot, location where soil is mixed, shipping dock, pesticide storage, and field dust and chemicals. The cleanliness of the region is crucial while choosing a place. It is best to place the units where there are few insects and little dust and pollen in the air. Export-oriented facilities should be located close to an international airport to cut down on the time between packing and shipping. To guarantee the prompt supply of high-quality tissue culture products, this is crucial. The size of the facility should be maintained minimal for new businesses until the market's acceptability is guaranteed.

The laboratory' architecture and layout

Laboratories for plant tissue cultivation have certain structural needs. Therefore, careful early planning is necessary for a facility to work well. The laboratories should be situated in a way that is isolated from foot traffic, minimizes contamination from nearby rooms, has thermostatically controlled heating and cooling, a sink with water supply and drains, adequate

electrical service, and good lighting. Large facilities are usually constructed as freestanding structures. The additional seclusion from nearby activity keeps the laboratory clean while being costlier to construct. Labs may be built inexpensively and conveniently in prefabricated structures. In many nations, they are easily accessible in a range of sizes. You may also utilize prefabricated houses that you construct on site. A single span structure enables adjustable wall placement to create rooms of the right sizes. Clean zones should be established in places like the media preparation room, inoculation room, and growth chambers. Under normal circumstances, it is possible to maintain the office, storage space, staff centre, and packaging rooms. The working spaces need to be divided up based on the facility's activity. When building a plant tissue culture laboratory, cleanliness is the main factor to take into account to minimize contamination. To avoid outside air intake, a positive pressure module has to be fitted. Contamination losses may be reduced to less than 1% using routine cleaning and aseptic techniques. Before the labs, there should be a closed entryway where sticky mats may be put to catch dirt tracked in on shoes [5], [6].

DISCUSSION

To achieve the highest level of cleanliness, the lab's traffic flow and workflow must be taken into account. The growth room, aseptic transfer area, and the culture room are the cleanest spaces. These rooms shouldn't have direct access from the outside. The places for cleaning glassware, preparing media, and storing should be separated from these rooms. The aseptic transfer room and the growth room need to be close to one another and ought to have see-through doors. The only people allowed to enter these locations should be those operating underneath laminar flow cabinets. The ideal path from the media preparation area to the sterilization area, the aseptic transfer room, and finally the growth chamber is through the media preparation space. In order to provide prompt warnings, temperature and fire alarms must be directly linked to telephone lines. During a power outage, a backup generator should be ready to run critical equipment.

Important Equipment

Autoclave

An autoclave, which is used to sterilize the medium, glassware, and tools, is essentially a gigantic, highly developed pressure cooker. Commercially, autoclaves of various sizes are offered. Glassware, water, and media must be sterilized using high-pressure heat. Only at 1210C and 1.05kg/sq.cm (15 pounds per sq. inch) pressure are certain spores from bacteria and fungus destroyed. Autoclaves that generate their own steam are more reliable and function more quickly.

Chamber for laminar airflow

The pure, filtered air provided by the laminar flow chambers permits cultures to be handled in a contaminant-free environment. Laminar flow chambers come in a variety of sizes and kinds and are marketed on the market. The culture transfer section is where you'll find the laminar-flow cabinets. Laminar flow cabinets and sterile rooms are features of several large-scale labs.

Other apparatus

Both glass-bead sterilizers and gas flamed burners may be used to disinfect devices like forceps, scalpel holders, and blades. Typically, the medium preparation room contains the following tools. a pH meter, weighing scales for huge volumes more than 10 g, an analytical balance with 1 mg precision, and a refrigerator-freezer for storing chemicals and stock

solutions. To reduce space, a refrigerator may be placed under a workstation in smaller labs. In a tissue culture facility, high-quality balances are crucial. Top loading balances, which enable rapid and effective weighing, are used in labs. The medium must be heated on a hot plate with an automated stirrer before being autoclaved. The media's pH must be determined using a pH meter. Some labs utilize pH indicator paper, although this approach is far less reliable and may have a significant impact on the outcomes. For chemical filter sterilisation and surface sterilisation of plant material, an aspirator may be connected to a water tap. Vacuum pumps, on the other hand, are more costly but also quicker and more effective.A drying oven is necessary to preserve glassware like beakers, flasks, and cylinders. It is also helpful for dry sterilizing scalpels and other glassware like pipettes, Petri dishes, and other items. Although the autoclave is used to sterilize medium including carbon sources (such as sugars) and growth regulators, there are occasions when aseptic filtration is preferable to prevent the breakdown of heat-labile compounds. In the region where the medium is prepared, there is also a water still. Although tap water may sometimes be used, distilled or de-ionized water is often utilized to make media [7], [8].

Optional Devices

In tissue culture labs, a range of non-essential equipment is employed. What has to be acquired depends on the exact requirements. For warming up agar medium and defrosting stock solutions, microwave ovens are practical. A dissecting microscope is commonplace in labs and is used to remove tiny explants. Manual work may be replaced by conventional dishwashers or laboratory glassware washers. To pipette a predetermined number of media, automatic media dispensers are useful. When using liquid medium or suspension cultures for micropropagation, a gyratory shaker or reciprocal shaker is required. For simple data management and record maintenance, tools like computers, photocopiers, and fax machines are useful. Even though some of the technology is pricey, it saves a lot of time and effort and is crucial for quick communication in a cutthroat environment.

Requirements specific to an Activity

The sections of a facility may be classified as semi-clean, clean, and ultra-clean depending on the various tasks performed during a tissue culture. The staff bathrooms, office, and washing room are among the semi-clean spaces since sterile standards are not required there. The media preparation and sterilizing rooms are included in the clean areas and must be adequately clean. The culture transfer rooms and the growth rooms, which make up the ultra-clean regions, must be maintained at a high level of sterility.

Place for cleaning and storing glassware

The space for washing glassware need to be next to the rooms for medium preparation and sterilizing. At least one big sink should be present in this space, but two sinks are ideal. On each side of the sink, there has to be enough room for work; this area is utilized for draining and soaking glassware. Glassware breakage may be decreased and water drainage can be improved by covering surfaces near the sink with plastic netting. To withstand damage from acids and alkalis, the outflow pipe from the sink should be made of PVC. The water still and de-ionization equipment should be close by, and hot and cold water should be accessible. Based on anticipated usage, durability, dependability, affordability, and serviceability, electrical washers should be chosen. Washing is done by hand in India and several other developing nations where labour is inexpensive. Periodically, the washing room should be switched. To stop water leaking and the loss of tiny goods, mobile drying racks may be employed and coated with cheesecloth. Close to the area where glassware is cleaned and stored, ovens or hot air cabinets should be placed. To make glassware accessible, dust-

resistant cupboards and storage bins had to be put in. To eliminate impurities and soften semi-solid medium, culture jars are often autoclaved after being withdrawn from the growing environment. The vessels should be simple to transport to the cleaning place. To facilitate storage and access for media preparation, the glassware storage room should be next to the wash area. Having a dedicated space for storing chemicals, tools, and equipment is advised. Additionally, to facilitating consistent availability, it would reduce costs associated with bulk purchases. Chemicals that are only needed in tiny amounts shouldn't be acquired in bulk since they might lose their effectiveness, absorb moisture, or get contaminated. Regularly buying small amounts might help solve these issues.

Sterilization and media preparation section

Smooth walls and flooring make it easier to clean and maintain a high level of cleanliness in the media preparation area. This room should have a minimum amount of doors and windows while yet adhering to local fire safety rules. Costs are lower, and pollution is lower. Although they may be done simultaneously, media preparation and sterilization are best done in separate rooms that are not physically divided by doors. Water that is both tap and filtered should be available in the area where media is prepared. After carefully weighing the cost and quality, a suitable water filtration system must be chosen and installed. The media preparation room must contain safety equipment such a fire extinguisher, fire blanket, and a first aid kit since it needs a lot of electrical appliances. To measure, combine, and store media, various glass, plastic, and stainless-steel equipment is needed. These should be kept in the cupboards that were created below the worktables and removed as needed. Building storage shelves would not be necessary, saving both money and space. A limited amount of glassware should be used since doing so will assist to cut down on breakage losses. Plastic and stainless-steel containers should be used wherever feasible since they are less expensive and more enduring than glassware. The place where you prepare the medium should be close to or in the same area as the water supply and glassware storage space. Workbench tops should be between 85 and 90 cm tall and 60 cm deep to allow for comfortable standing work. The workstation tops have to be constructed with surfaces of plastic laminate that can withstand regular washing.

Sterile environment

The media preparation area needs to be next to the sterilizing room. The arrangement must be designed to let the containers to move easily from the washing area to the room used for media preparation and sterilizing. The walls, flooring, and ceiling of the sterilization chamber must be resistant to steam, heat, and moisture. The warm, humid air has to be removed; thus an exhaust should be installed. An exterior cover for the exhaust fan is necessary to exclude outside air from entering. Only while the fan is running should the fan cover open. Simple pressure cookers may take the role of pricey autoclaves in modest tissue culture operations. However, horizontal or vertical autoclaves need be erected for big volume media production. Even though they could be expensive, double door autoclaves that open directly into the media storage area decrease infection. Transferring the sterile media via a hatch window to the next room is a less expensive option.

The cultural transfer room, where the main activity occurs, is the most crucial workspace. The transfer area has to be as spotless and isolated from the rest of the room as feasible. To guarantee regular cleaning, the transfer room's walls and flooring must be smooth. To avoid contamination, the doors and windows should be few, while yet following local safety regulations. The transfer room doesn't need any extra illumination. The laminar airflow room has enough lighting for work. The equipment may be sterilized using glass-bead sterilizers or

by blazing them after immersing them in alcohol, often ethanol. To make it simple to transport the culture containers from the medium storage room to the transfer room and then to the growth room, they should be piled on movable carts (trolleys). The transfer operators' chairs should be comfortable since they spend a lot of time working stationary. The transfer room must to include first aid supplies and fire extinguishers as a safety precaution. The staff should remove their shoes before entering the room. In this location, special lab coats and shoes are required. In order to clean the space, ultraviolet (UV) lamps are sometimes put in transfer areas; these lights should only be turned on when no one is within the space, including plant life. When the standard room lights are switched on, safety switches that turn off the UV lamps may be placed.

Plant cultures are kept in growth rooms, which are equally crucial spaces, where the atmosphere is carefully managed for optimum development. Since various plant species may need varying levels of light and temperature during in vitro development, it is recommended to have more than one growth chamber to provide a variety of culture conditions. The plant cultures may be shifted to another room if the cooling or lighting in one room fails in order to avoid the loss of cultures. The growing chamber should have the fewest possible doors to avoid contamination. In the growing chamber, windows are not necessary unless natural light is being utilized. When artificial lighting is employed, outside light may affect the growing room's photoperiod and temperature. The location of the culture containers may be either permanent or movable, depending on the cost and the quantity of available space. The benefit of mobile shelves is that they provide access to cultures on both sides of the shelf. Shelves should not be higher than 2 meters. Step-up stools are risky and time-consuming to use while placing and removing cultures from high shelf. The lights positioned on the shelves are often the main source of lighting in the growing chamber. Due to the fact that they would only be used when working during the dark cycle, overhead light sources may be minimized. The traditional downward lighting used for plant cultivation may not provide consistent illumination. Heat is distributed unevenly by lights that are mounted directly on the racks. Due of the high humidity created within the culture containers, hyperhydricity may result. An approach that uses fewer lights and produces more consistent lighting is side illumination. But when transferring the cultures around the shelves, care must be taken to avoid damaging the lights.

Control of the growing environment

Planning the growing room should take controlled temperature, lighting, relative humidity, and shelving into account. These vary based on the size, location, and kind of plants being grown in the growth chamber. For instance, a tiny growing chamber in a basement that is not heated yet adequately heated may be used in the chilly North American environment. Fluorescent light chokes (ballasts) don't necessarily need to be separated; instead, they may act as a heat source. The growing room's excess heat may be released and utilized to heat other basement spaces. To avoid overheating culture jars on shelves above the lights in such a setting, strong hardwood shelves with space between them might be utilized. However, the light chokes must be positioned outside a sizable growing chamber that is above ground. Glass or metal wire mesh may be used as shelves in a big growing area [9], [10].

Temperature regulation

In growing rooms, temperature is of utmost importance since it determines how lights are installed, how relative humidity is managed, and what kind of shelving is used. The growing room's temperature is often managed by air conditioners. Temperatures are typically maintained at or around 220C. Heat from light chokes may be used in addition to traditional

heating systems to offer additional warmth. The growing room's cooling needs are often more problematic than its heating in the majority of developing nations. Heat pumps, air conditioners, and exhaust fans may all offer cooling. Opening windows to cool culture rooms might cause humidity issues in the winter and summer due to contamination.

Shelving

Depending on the circumstances and the plants cultivated, several shelves are used in the growing rooms. Angle iron with a thickness of 1.25 cm (half an inch) may be used to create the shelf frames. To maximize airflow and reduce shadowing, shelves made of stiff wire mesh should be utilized. Shelves may be built at a low-cost using wood. The outside of the wood used for shelves should be smooth, and it should be coated white to reflect light. Although expanded metal costs more than wood, it has greater airflow. To maximize light penetration, tempered glass is sometimes used for shelves, however it is more likely to shatter. Between the lights and the shelves, there should be 5 to 10 cm of air space to minimize heat on the higher shelves and to prevent condensation in culture pots. Five 45cm shelves may fit in a space that is 2.4m high. a distance of 10 cm when the lowest shelf is 10 cm above the ground.

Growing Facility

The transitional period between the laboratory and field environments is crucial in plant tissue culture. Plants developed in vitro must be progressively adapted to outdoor circumstances. Plant hardening is often done in a greenhouse, which increases the likelihood that tissue-cultured plants will survive in the wild. Greenhouses come in three different varieties: ground-to-ground, gable, and quonset. The Quonset kind of greenhouse is the most popular. It has fixed or mobile benches with hardening tunnels. Based on the level of output, the greenhouse's size must be chosen. Glass or fibreglass may be used for greenhouse glazing. You may also use polyethylene films or polycarbonate or acrylic sheets. The most affordable double polyethylene covering is air inflated. With the use of supplemental lighting, appropriate lighting, shade, and blackout systems may be obtained. As required, drip watering systems, misting, and fogging may be put in place. Fan-assisted drip pad cooling is recommended for greenhouses built in warm areas, particularly in the summer. In cooler areas, greenhouses must be heated. The air may be heated and cooled using floor and bench systems. Warm air may be circulated using inexpensive plastic pipes, which are functional and cost-efficient.

Shipping and Packaging

In a commercial tissue culture unit, packaging should be placed in a separate area. Labels and other packaging supplies like cardboard boxes need to be kept here. The temperature zones that the consignment must travel through on its way from the place of shipping to its destination significantly affects the sort of packing that a certain facility uses. For instance, if a shipment of tissue-cultured plants is travelling through a temperate zone, all that is needed to be done is watch out for frost conditions and pack appropriately. However, if the shipment is travelling from a tropical to a temperate region, it is important to consider the various temperature zones that the shipment goes through. Tissue-cultured goods' packaging hence differs depending on the facility and the final destination.

The packaged products should be accurately counted and double-checked before loading and shipment. The names and addresses of the consignor and the consignee, as well as information on the commodity's storage temperature, handling, etc., should be clearly written on the cartons holding the cultures before they are sent to the client. Large shipments must be

packaged with sufficient care to prevent disruption or damage while in route. Make sure the cargo is accompanied by documentation like the invoice, packing list, import permit, phytosanitary certificate, and Generalized System of Preferences (GSP) in order to avoid any kind of delay.

Staff and Office Space

Any commercial tissue culture unit needs an office to run the business side of things. The office should have adequate space for a file cabinet, computer, photocopier, and fax machine to manage mail, photocopy documents, and keep track of shipments and cultures. The office space may also be used for other tasks including bookkeeping, inventory management, and acquiring new supplies. The office space at a manufacturing facility of average size may serve as the welcome area as well. The employee must have access to a changing area and a restroom. In the case of spillage, the latter are definitely necessary. Outside of the laboratory area, tables and chairs must be provided for tea and lunch breaks. Additionally, the staff area has to contain first aid kits, fire extinguishers, and lockers. Vending machines for food and beverages are another option.

CONCLUSION

The performance of plant tissue culture facilities depends on effective laboratory structure and design. Important factors for designing labs have been discussed in this chapter, with a focus on how they affect productivity, financial savings, and contamination prevention. The appropriate design of tissue culture facilities is essential for obtaining high-quality results, from site selection through the implementation of controlled settings. Researchers and practitioners may maximize productivity, save operating costs, and promote sustainable practices in plant tissue culture by thoughtfully designing and arranging their lab facilities. Laboratories may assure the effective multiplication of disease-free plant material and help progress agriculture and forestry by puttinga focus on cleanliness, controlled settings, and equipment selection.

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CHAPTER 5

EFFICIENCY AND COST REDUCTION STRATEGIES IN TISSUE CULTURE-BASED PLANT PRODUCTION

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ABSTRACT:

To address the need for high-quality planting material across the world, tissue culture-based plant production is essential. Its success, meanwhile, is dependent on efficient operations and excellent cost management. This article covers numerous methods to increase the effectiveness of tissue culture facilities and lower production costs in this setting. It explores labour efficiency, energy-saving methods, and overhead cost management, putting emphasis on the value of choosing affordable solutions that support sustainability. This study explores practical ways to reduce energy usage by looking at natural illumination, inexpensive lighting, temperature control, and alternatives to water and autoclaving. It also compares manual and automated ways to analyze labour efficiency and provides tips on maximizing operator output. The debate also emphasizes how crucial proficiency in quality control and contamination prevention is to minimizing losses. The study overall message emphasizes the need for tissue culture manufacturers to combine cost-cutting with maintaining product quality in order to preserve their position in the market.

KEYWORDS:

Management, Plant Production, Productivity, Product Quality, Tissue Culture.

INTRODUCTION

One of the priciest and least effective techniques in tissue culture technology is artificial illumination of cultures in growth rooms. In tissue culture, switching from artificial to natural lighting is a clear low-cost choice. This lowers capital expenditures and power expenses while simultaneously improving plant quality. The expense of using air conditioners to keep in vitro cultures at a controlled temperature increase. Compared to plants produced in the field, many plants cultivated in vitro can withstand significant temperature swings and adapt to them more successfully. Plants that have been toughened outdoors are robust and can resist field transplanting better. It takes a lot of effort to produce plants using tissue culture techniques and then grow them. Even in developing nations, where labour is comparatively cheaper, improving manufacturing efficiency is important to lower the cost of tissue-cultured plants. Hiring specialists from reputable R&D labs lowers overhead costs and is essential to minimizing contamination losses. To lessen the cost of producing micro propagated plants, manpower and energy costs must be reduced. In tissue culture, a significant portion of the electrical energy is utilized for the autoclave, air conditioning, laminar-flow cabinets' air filtering, lighting the growth room, and autoclaving. Up to 60% of the expenses of manufacturing in underdeveloped nations may be attributed to the price of power. Additionally, its unstable supply and voltage cause serious issues. Although the use of electrical energy is absolutely necessary, choosing inexpensive alternatives may result in significant savings [1], [2].

Because it can produce disease-free and genetically consistent planting material, tissue culture-based plant production has become an essential part of contemporary agriculture and

horticulture. However, the viability and competitiveness of tissue culture facilities depend on their capacity for efficient operations and cost control. Adopting techniques to lower manufacturing costs has become essential at a time when resource preservation and economic viability are crucial. Energy use is one of the main cost components in tissue culture. Autoclaving, growth room illumination, laminar flow cabinet filtering, and air conditioning are the main uses of electrical energy. Up to 60% of the total production costs might be attributed to power expenditures in certain areas. Furthermore, problems like inconsistent power supply and voltage fluctuations make the problems related to energy utilization even worse. Labour expenditures have a considerable influence on tissue culture production's cost structure. Even though it is often done, manually moving explants into culture containers has productivity drawbacks. High labour productivity is essential, particularly in settings where operators may lack specialized tissue culture expertise. The total cost of the project is increased by overhead charges such management staff wages, marketing fees, and quality control costs. To remain competitive, these overhead expenses must be managed effectively.

Low-Cost Energy Alternatives

Utilizing daylight

One of the priciest and least effective techniques in tissue culture technology is artificial illumination of cultures in growth rooms. High expenditures are further added by the lights, chokes, fixtures, timer controls, equipment to manage heavy electrical loads, and their upkeep and operation. Additionally, the heat produced by artificial lighting must be dispersed by cooling and air conditioning, increasing the electrical demand. Even while the crimson and far-red portions of natural daylight are made up for using special fluorescent tubes, the quality of artificial light does not compare to that of natural light, which is what the plants are eventually grown in. Additionally, the lighting system's cool fluorescent lights only provide a little amount of the energy needed for photosynthesis. As a consequence, in vitro plants grow more slowly as they get acclimated to low light levels.

Plants may adjust to a variety of circumstances by altering their architecture and metabolism. To survive in the severe climate, they acquire morphological and anatomical characteristics in their leaves and stems, such as cuticle and wax on leaves, thicker leaves, fewer and closed stomata, and thicker epidermal cells. However, after they've adapted to a set of circumstances, they find it difficult or rather sluggish to adjust to new ones. Low light-adapted plant tissues are often brittle and prone to vitrification, which makes them less likely to survive in the wild. This may be a significant drawback throughout the plant's hardening period and subsequent field establishment.

Plants that are exposed to low-intensity artificial light have weak root systems and minimal reserves.

The roots generated in vitro must adapt to the pH-varying soil solutes when they are transplanted to the soil. The in vitro roots often respond by ceasing to function in soil and developing new roots that take over the original roots' role. If new roots don't form, the plant withers and dies.

In vitro plants may be cultured in liquid medium based on half- or quarter-strength MS salts without sugar and vitamins under either aseptic or non-aseptic conditions during their final phase under natural light. Shoots may be dipped in a rooting hormone solution or the media can be treated with auxins (IAA, IBA) if roots or root initials do not develop. With a high likelihood of survival, this method produces plants that are considerably stronger and healthier [3], [4].

Inexpensive lighting

A certain low cost alternative in tissue culture is switching from artificial to natural lighting. This lowers the cost of capital expenditures and power while simultaneously raising the standard of the plant. There are various alternatives to expensive artificial lighting. Growing the in vitro cultures beneath plastic or glass in diffused natural light is one possibility. In temperate areas, this works quite well, but in hot climes, heat buildup must be mitigated by adding thermostat-controlled exhaust fans. In tropical and subtropical nations, it is also conceivable to remodel the labs already in existence and swap out the artificial lighting with "Solatube" devices, which can be mounted on any roof and reroute sunshine via reflecting surfaces. Currently, such a tube costs about US $600, and once put, it can light 3 to 5 m2 without requiring ongoing maintenance. Another strategy is to add southwest-facing glass windows to the growing chambers so that diffused, indirect natural light may enter. The light may be filtered to the appropriate intensity using either bamboo or plastic Venetian curtains or muslin. To allow for all-day sunshine, the growing chamber is often situated on the top story of the home. By converting villagers' homes into tissue culture labs, "Bio-factories" in Cuba have effectively utilised natural light in this way.

DISCUSSION

Simple plastic bags polypropylene bags have been used effectively as culture containers for in vitro cultures that were grown outside in the sunlight. These bags may be hung in a greenhouse and are lightweight. They are translucent, allowing incident light to reach the cultures with the least amount of loss and in close proximity to the natural spectrum. While the plants are still growing in the culture, they are firstly hardened. These plants thrive when transferred to soil because they have well-developed stems, leaves, and strong growth vigour. Under greenhouse circumstances, contamination of the cultures in the bags may be prevented with the right safeguards. Air filtration and air conditioning, which are often employed in high-tech buildings, are not necessary with this approach of growing plants in greenhouses and plastic sheets. By include glass windows in the room design, which give indirect sunlight but lower heat build-up in the rooms, artificial lighting in other laboratory sections, such as media preparation, transfer, and hardening rooms, may also be eliminated. Either transparent glass or muslin net curtains may be used to accomplish this.

Inexpensive temperature control

Although it increases the expense, using air conditioners to keep in vitro cultures at a consistent temperature has little effect on the particular plant quality. In reality, much as with artificial lighting, plants cultivated in a limited temperature range suffer both during hardening and afterwards in outdoor settings. The elimination of this element greatly lowers the cost of electricity. Contrary to popular opinion, many in vitro growing plants can endure significant temperature swings. This is because they are not subject to the precise management of day and night temperatures at an equal level [5], [6].

Lowering water and autoclave energy expenses

Ordinarily, electric-powered water stills are used to generate distilled water. Autoclaving and certain water stills need a three-phase hookup. It is advisable to run the units on a single-phase electrical connection for small establishments. In small facilities, distillation is not always necessary and tap water may be utilized after autoclaving. Where money is tight, pressure cookers heated by gas may also be utilized. Autoclaves and electric water distillation equipment are still the most cost-effective option for large-scale enterprises.

Lowering the glasshouse's energy usage and hardening the area

If glasshouses are not accessible, hardening plants in open shade may considerably decrease the amount of electricity used. In vitro produced plants may be hardened by putting pots in shade beneath thatched or plastic-covered open huts or by covering them with net shades after transferring to soil. Plants that have been toughened outdoors are robust and can resist field transplanting better. When cultivating in plastic containers, the lids are taken off, the containers are covered with a thin plastic sheet, and they are left in the shade for 3–4 days. After cleaning the medium with tap water, the plants are kept for a further 3-5 days in the shade before being transferred to soil.

Bringing Down Labour Costs

It takes a lot of effort to produce plants using tissue culture techniques and then grow them. In industrialized nations, labour is a significant contributor to the cost of micropropagation. On the other hand, labour costs are often lower in emerging nations, which is a significant benefit. However, in underdeveloped nations, boosting production efficiency is important to lower the price of tissue-cultured plants. Because borrowing is expensive and comes with high interest rates in emerging nations, the cost of manufacturing is increased. As a result, it's critical to maintain good labour productivity, particularly when the culture transfer room's operators lack extensive training. According to the average cost breakdown of a tissue culture plant production system, 40% of expenses are related to labour, 10% are related to materials, 20% are related to overhead, and 30% are related to sales, general, and administrative operations (Walker, 1986). The daily maximum output per operator for the manual transfer of explants into culture containers is about 5000 operations or transfers. This top rate is, however, seldom reached. The kind of culture vessel being used has a significant impact on productivity, which is generally regarded as being 2500 transfers per operator each day. For instance, compared to Petri plates and wide mouth culture vessels, the number of transfers per individual utilizing test tubes is substantially fewer.

There is minimal room for efficiency improvement until automated or semi-automated technologies are used after the manpower efficiency in moving the maximum number of propagules per hour has been reached. These rely on the utilization of bioreactors and liquid media, as well as propagule handling machinery. These systems have been created and, under specific circumstances, they may be incorporated into the manufacturing. Therefore, in a 20-million-unit manufacturing plant, such a technology may save production costs by 50%. Labour costs decrease by 75% in the fully automated model compared to the traditional model and by 70% in the semi-automated model [7], [8]. By increasing throughput per person and halving labour costs per propagule, the approach based on the usage of plastic bags enhanced productivity. Additionally, the greater ability to manage contamination led to a higher rate of multiplication, better survival through hardening, and more commercially viable plants.

The personnel cost of tissue culture was reduced by 96% per plant before hardening in certain instances as the multiplication rate rose from 1.5 to 5. Such systems may be integrated such that the multiplication phase is in the bioreactor and the latter phases are in plastic bags to cut down on manpower expenses. It is recommended that operators using laminar flow hoods work for no more than 4 hours per day, ideally on a single bench, and then switch to other tasks like cleaning and drying containers or working in a greenhouse. The efficiency is increased by the inclusion of music in cultural transmission spaces. In the transfer rooms and while potting plants, female operators often outperform male operators. This supports the gender disparity in rural employment.

Lowering overhead expenses

The wages of managers, including directors, scientists, and marketing managers, as well as marketing expenditures, commission payments, and management benefits, are included in the overhead costs for commercial scale tissue culture-based plant production. Many of these are unavoidable. Large-sized businesses can support their high overhead expenditures due to their great volume of business. Smaller units, meanwhile, must depend on a limited number of regular managers but may employ the services of experts as and when needed.

Tissue culture-based large-scale production is also vulnerable to large-scale losses due to culture contamination, subpar manufacturing, losses during hardening, and poorly thought-out and executed marketing. A lot of commercial micropropagation failures may be attributed to poor manufacturing and marketing strategies. While it is crucial to maintain high standards of clean technology, significant skill is also necessary to limit contamination at or below 5%. The high contamination rate may be a significant factor in the company failing to meet its goals, putting its presumed output out of whack, and losing customers. To minimize such losses, experienced people's counsel and knowledge are essential. Either well-established R&D labs of the major firms or colleges and universities may provide the knowledge for a fair price. These labs may also provide services for quality assurance, mother culture indexing, media formulation guidance, and problem solving. Links between private sector R&D organizations and those in the public and private sectors should be formed for the progress of sustainable technologies. Governments and policymakers in underdeveloped countries might thus choose a low-cost alternative by sharing the expenses of R&D in tissue culture technologies between the public and commercial sectors [9], [10].

CONCLUSION

The viability and competitiveness of plant production based on tissue culture depend critically on measures for efficiency and cost reduction. Adopting new strategies becomes crucial in a sector where labour- and energy-intensive operations are the norm. Utilizing natural light, inexpensive lighting options, and temperature control techniques may significantly reduce energy expenses. These techniques may reduce power costs while also improving the quality of the plant material generated. Labour efficiency is equally important, and switching from manual to automated or semi-automated processes may boost output dramatically. Labour efficiency depends on proper training and job distribution, and it may be further increased by creating a positive work atmosphere, perhaps with the help of music. A balanced strategy is necessary for controlling overhead expenses. While certain costs, like those for quality control procedures, are necessary to ensure the integrity of the product, it is crucial to use prudent marketing and administrative overhead management. In order to increase productivity and save costs, tissue culture-based plant manufacturing facilities must employ a diverse strategy.

To maintain a competitive advantage in the market and satisfy the rising worldwide demand for high-quality planting material, it is crucial to strike a balance between cost-cutting methods and product quality. Tissue culture producers may support horticulture and agriculture that are sustainable by putting these techniques into practice and maintaining the profitability of their businesses.

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CHAPTER 6

CHALLENGES AND ADVANCEMENTS IN BIOREACTOR-BASED PLANT CULTURE SYSTEMS

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ABSTRACT:

The area of plant propagation has undergone a revolution thanks to the development of bioreactors as novel containers for large-scale cell, tissue, or organ growth in liquid media. In comparison to traditional semi-solid micropropagation, these systems provide a number of benefits, including faster multiplication rates, decreased space requirements, energy savings, and labour efficiency. The use of bioreactors is not without its difficulties, however. The release of growth-inhibiting substances, hyperhydricity, foaming, shear stress, and protocol development are still major issues. Bioreactors bring both potential and challenges in underdeveloped nations where qualified labour and resources may be scarce. A number of variables, such as labour costs, production capacity, crop variety, contamination rates, and energy costs, affect how well bioreactor-based plant production works. This article examines several bioreactor types, their benefits and drawbacks, and the methods used to prevent contamination. It also covers how to build inexpensive bioreactors for labs with limited resources, highlighting how these systems might revolutionize plant growth techniques.

KEYWORDS:

Autoclavable, Bioreactor, Hyperhydricity, Micropropagation, Production Capacity, Plant Culture.

INTRODUCTION

Bioreactors are containers designed for large-scale liquid media cell, tissue, or organ cultivation. Functionally, there are two main categories of plant culture bioreactors: those in which the cultures are immersed intermittently or constantly in the medium, and those in which the cultures are immersed continually. In comparison to traditional culture containers, bioreactors provide more accurate control of the plant development gaseous exchange, light, medium agitation, temperature, and pH. Commercial micropropagation of plants using bioreactors may speed up culture development and multiplication while using less space, energy, and manpower. As a result, when combined with a traditional laboratory, they may be appealing to poor nations in terms of building new or expanding plant culture facilities. However, the utilization of bioreactors necessitates monitored plant cultures and attention to aseptic practices while handling plant material in order to be cost-effective. Therefore, only facilities with trained and experienced propagators should try to integrate bioreactors into production systems.

Bioreactors are containers designed for large-scale liquid media cell, tissue, or organ cultivation [1], [2]. They have shown a number of significant benefits over traditional semi-solid micropropagation for an increasing number of plants, including many times higher multiplication rates and a decrease in space, energy, and labour. The motivation for rising interest in the usage of liquid systems generally has been their cost-saving benefits. However, there are several drawbacks due to a variety of issues with the use of bioreactors in micropropagation. These include contamination, a lack of protocols and manufacturing methods, an increase in hyperhydricity, issues with foaming, shear stress, and the cultures' release of substances that hinder development. Such systems entail more risk and need for

higher expertise because to the intrinsically complex challenges of contamination in liquid systems and the scarcity of information for problem solutions as compared to semi-solid growth medium. As a result, bioreactors have not been a low-cost choice in many underdeveloped nations. Cost of labour, production capacity, crops being propagated, rate of contamination, and energy and cost charges will all have an impact on the possibility for cost reduction [3], [4].

By providing a dynamic alternative to traditional semi-solid micropropagation, bioreactors have transformed the field of plant propagation. These containers, made for large-scale cell, tissue, or organ growth in liquid media, are becoming more and more well-liked as a result of their remarkable capacity to greatly increase multiplication rates while using less time, space, and labour. The widespread usage of bioreactors in plant production facilities is driven by the economic benefits they provide. However, there are complexity and difficulties involved in using bioreactors into plant growth procedures. The creation of strong protocols and manufacturing processes is crucial since contamination is still a major threat. Furthermore, bioreactors need to be carefully managed and optimized due to problems including hyperhydricity, foaming, shear stress, and the production of growth-inhibiting chemicals by cultures.

The use of bioreactors presents both potential and challenges in underdeveloped nations where resources and competent labour may be scarce. Several variables, including labour costs, production capacity, crop varieties, contamination rates, and energy consumption, affect how much bioreactors may lower production costs and improve efficiency. This study goes into the realm of plant culture systems based on bioreactors, examining the many kinds of bioreactors, their benefits and drawbacks, as well as the methods used to prevent contamination. The development of simple, affordable bioreactors designed for labs with limited resources is also covered, giving light on the revolutionary potential of these systems in the field of plant propagation.

Bioreactor containers

The normal plant tissue culture containers, which generally hold less than 0.5l of semi-solid media, are in sharp contrast to even these low amounts. Significant investment cost consequences result from the size of the containers in bioreactors compared to typical micropropagation systems. Standard semi-solid plant tissue culture containers include a seal that allows for a small amount of contamination-free gas exchange. The light, temperature, and gases in the conventional vessel are all controlled by the environment of the typical growth chamber. Some bioreactors provide more exact control of the plant development gaseous exchange, light, medium agitation, temperature, and pH and may grow 60–100,000 plants per cubic meter. They can usually be performed in regular rooms since they are aseptically sealed. Only plant material is handled in a laminar flow environment. As a result, when combined with a traditional laboratory, they may be appealing to poor nations in terms of building new or expanding plant culture facilities. Its usage, however, necessitates the use of indexed plant cultures and consideration for aseptic techniques while handling plant material in order to be cost-effective.

Various Bioreactor Types

For a broad variety of crops, culture types, and developmental phases, many bioreactor designs have been created. Functionally, there are two main categories of plant culture bioreactors: those in which the cultures are immersed intermittently or constantly in the medium, and those in which the cultures are immersed continually. In situations when submersion does not cause aberrant plant growth, the latter are often utilized for high-density

multiplication of cultures. Protocorm-like entities, embryogenic calluses, somatic embryos, meristem clusters, and nodules are examples of these cultures. Meristematic tissues that renew propagules are protocorm-like structures. Meristem clusters and nodules are organogenic cultures made up of tightly packed masses of meristems that have not yet fully developed into leaves, stems, or roots. Small bulbs or corms may also be developed in submerged bioreactors for field planting.

Type of partial immersion

Partial immersion bioreactors come in a number of different varieties. These include temporary immersion bioreactors, liquid layer bioreactors, and gaseous phase bioreactors. For growing plant material susceptible to hyperhydricity, such designs are preferable. The cultures in gaseous phase bioreactors are mechanically supported on a porous platform, exposed to a nutrient mist, and periodically sprayed with medium. The vessel is used to collect and circulate extra medium. Such bioreactors have autoclavable transparent glass or plastic containers, and the growing room's environment regulates temperature and lighting. The majority of tissue and organ cultures can grow and develop in these bioreactors with outstanding results. The available mist generating devices, however, need complicated maintenance [5], [6].

DISCUSSION

Only the base of the colonies is exposed to the media in liquid layer bioreactors. Much as in conventional tissue culture tanks, lighting, temperature, and the gaseous environment may all be controlled. A basic liquid layer system consists of a buoyant, reusable, autoclavable raft supported by a disposable or reusable microporous membrane at the liquid's surface. Additionally, sealed transparent plastic film with a wire frame has been used to create stationary support systems for liquid layer bioreactors. In commercial labs, small polyethylene film bags are also utilized in place of containers.

The cultures are submerged in the media for a predetermined amount of time at certain intervals in temporary immersion bioreactors. They are appealing low-cost options because of their easy setup and operation. A common design makes use of two plastic or glass jars, one of which contains the liquid medium and the other of which contains the cultures. Another design of temporary immersion bioreactors has a single vessel that is automatically tilted at certain intervals and has a reservoir on one side. In this way, the medium keeps the propagules upright and regularly bathes the cultures in the vessel. Differently built vessels and rotation are used in other temporary immersion bioreactors. The culture is occasionally dipped in the media while the vessel rotates. Operating temporary immersion bioreactors is easy and reasonably priced. As a result, several industrial micropropagation labs have started incorporating these devices into their manufacturing processes. Mechanically agitated and air-driven submerged bioreactors are the two main categories. Propellers, turbines, impellers, paddles, and vibratory mixers are some of the tools used in mechanically agitated bioreactors to move the medium. Each device alters the vessel's fluid flow in a slightly different way. The shear force and pattern of flow must be tailored to each kind of culture and plant. For instance, maintaining meristematic clusters as tiny aggregates requires a certain amount of shear stress. This reduces stagnant areas inside the vessel, which may lead to cultures adhering to surfaces and forming clogs that interfere with the flow of media. On the other hand, large shear forces may result in excessive foaming and have a detrimental impact on the development of cultures. a similar mechanism.

The simplest submerged bioreactors are those that run on air. Air-driven bioreactors agitate the medium and raise and circulate the culture by forcing filter-sterilized air into the bottom

of the vessel. Depending on their capacity, these air-driven bioreactors are referred to as simple aeration or bubble column bioreactors. Transparent or translucent materials, although generally constructed from autoclavable clear glass or plastic and fittings, are used to make air-driven bioreactor containers. As an alternative, they are manufactured of translucent flexible plastic that cannot be autoclaved and are gamma-sterilized. The straightforward bubble column or airlift bioreactors are simple and inexpensive to operate. The ability to incorporate submerged bioreactors into automated or mechanical micropropagation systems is their biggest benefit. The culture from the bioreactor is pushed through a bioprocessor in automated systems where it is separated, sized, and distributed to vessels for propagule development. An operator moves the multiplied culture in the automated system to a mechanical cutting tool for separation, then to a vessel for propagule growth [7], [8].

Both positive and negative aspects

Increased culture multiplication rate, quicker culture development, lower medium cost, and lower energy, labour, and lab space requirements are all benefits of bioreactors. The faster rate of growth and multiplication is mostly a result of the liquid medium. The expense of the medium is decreased by doing away with gelling agents (like agar), and autoclaving the media is no longer necessary thanks to filter sterilization. The culture density in liquid media is much greater in bioreactors than it is in traditional containers with semisolid medium. Usually, the traditional tissue culture pots are stored on shelves with a lot of room between them. In comparison to traditional micropropagation, the usage of bioreactors needs significantly less area in the growing room, fewer clean workstations, and less space for media preparation, vessel storage, and cleaning. Because the laboratory is smaller and there are fewer people working inside, less air conditioning is required, which lowers energy expenses. Costs may be decreased by reducing the need for lighting the bioreactors, autoclaving, and labour, as well as by streamlining the preparation of the medium, cleaning of the containers, and management of the cultures.

However, there are several drawbacks due to a variety of issues with the use of bioreactors in micropropagation. These include contamination, a lack of protocols and manufacturing methods, an increase in hyperhydricity, issues with foaming, shear stress, and the cultures' release of substances that hinder development. Sadly, culture contamination—which already poses a significant challenge in traditional commercial micropropagation—becomes far more serious in bioreactors. In traditional micropropagation, it is acceptable to discard a few infected vessels; but, in bioreactors, even one contaminated unit represents a significant loss. Nevertheless, a number of commercial labs have created efficient methods to manage contamination in bioreactors in spite of these challenges.

Contamination Reduction

A good treatment of the plant material, of the equipment during transfers, and of the cultures during production is necessary for the prevention of contamination in bioreactors. For the purpose of starting cultures in bioreactors, only surface sterilized explants that have been multiplied in tiny containers and checked for disease freedom are employed. Small bioreactors are sterilized in autoclavable plastic bags that are sealed with cotton wool plugs and only accessible from below a laminar flow cabinet. If the bioreactor is big, additional safeguards must be given for the vessel after autoclaving and while it is being transported to the sterile area. The vessel is put together under laminar flow, with care taken to prevent non-sterile items from entering the sterile air stream near open ports. All ports are sealed after inoculation, and the bioreactor is then carried to the growth chamber and attached to an air supply. The bioreactor is brought back to the sterile room for harvesting, and the ports are

opened to prevent contamination from the outside from entering via the air stream. The reactor may be used again without needing to be re-sterilized if the right steps are taken.

Bioreactors may get polluted by the environment or by bacteria that are dormant in the culture despite the measures taken while starting cultures. One or more anti-microbial substances, media acidification, and medium microfiltration may all be used to reduce contamination. Several industrial labs use anti-microbial substances, such sodium hypochlorite or sodium dichloroisocyanurate, a chemical that is fungicidal and bactericidal. In media for other crops as well as the multiplication of bananas, acidification of the medium (pH 3) has been employed to reduce contamination. Circulating the material over a 0.2 microporous filter may help reduce contamination. However, the frequent filter replacements required for the removal of pollutants make them unsuitable for use in a commercial laboratory. Filter clogging is greatly delayed by cooling the filter.

Simple Bioreactor

A simple temporary immersion bioreactor may be built in labs with low resources using autoclavable glass, plastic, or bags with a mouth that is at least 5 cm in diameter and a tight-fitting rubber stopper. It is preferable to use little containers, no larger than one litre. There should be two holes in the stopper for glass tubing. One tube is inserted and placed in the stopper such that the end is closer to the bottom of the vessel when it is closed. When the stopper is closed, a second tube is introduced and fastened inside of it so that its end is near the top of the jar. A heat-resistant silicone sealant, like the kind used to patch vehicle radiators, may be used to repair the tubes. To add or remove cultures, the vessel must have at least one port with a diameter of 5 to 10 cm that is sealed with a rubber stopper or a metal closure that has a rubber gasket. There are two ports for air intake and outlet and two ports for medium inlet and outlet, totaling four ports. The hydrophobic Teflon submicron microporous air filters that are attached to the air-inlet and -outlet ports must be maintained dry. The clogged moist filters might cause contamination. To stop pollutants from entering, medium ports should be able to open and shut, connect and detach, and do so without leaking. In order to facilitate handling cultures and changing the medium, the bioreactors should fit below the laminar flow unit with room remaining on the top and sides. The specially designed bioreactors are either air-driven or temporarily submerged. Similar to the culture vessel, a reservoir vessel is made, with the exception that the opening of the reservoir is only large enough to accommodate the two glass tubes. Silicone tubing is used to join the heads of the tubes that extend to the base of the culture tank. The ends of the small tubes above the stopper are attached to sterilizing filters, such as "Acro 50" filters, with a short length of silicone tubing for both the reservoir and the culture vessel [9], [10].

The bioreactor is put together, the stoppers are loosely inserted into the vessels, and it is then placed in an autoclavable bag with a cotton wool plug before being used. An autoclavable plastic or stainless-steel mesh screen may also be used in the culture vessel to support the culture above the medium within. The bioreactor is then put on the laminar flow bench after being autoclaved. The stoppers are carefully secured once the culture has been aseptically added to the vessel and sterile media to the reservoir. To avoid contamination, the area where the stoppers fit into the containers may, if required, be taped shut. The bioreactor is then put in the growing chamber, the two vessels are positioned next to one another, and silicone tubing is used to link the reservoir air filter to a 6W aquarium pump. The culture vessel is forced into the reservoir vessel by air being injected into the reservoir vessel. Shortly after the medium has been allowed to sit in the culture vessel, the air pump is attached to the air filter, and the medium is pumped back into the reservoir.

CONCLUSION

In the field of plant propagation, bioreactors have become effective instruments with a number of benefits, such as higher rates of multiplication, quicker culture development, and less resource use. By maximizing available space, energy consumption, and labour productivity, these containers have the potential to completely transform the plant manufacturing sector.

The use of bioreactors is not without its difficulties, however. The creation of efficient methods is necessary since contamination still poses a concern. Additionally, bioreactors add complexity by introducing shear stress, foaming, hyperhydricity, and the possible release of growth-inhibiting substances. Bioreactors may be both a cost-effective answer and a possible problem in underdeveloped nations.

The degree of cost savings and efficiency improvement relies on a number of criteria that are particular to each facility.

A balance between innovation and risk management is essential if bioreactor-based plant growing systems are to reach their full potential. It is crucial to use strict protocols, effective manufacturing practices, and contamination control techniques. Furthermore, the advantages of this technology may be made accessible to everyone via the development of simple, affordable bioreactors designed for labs with low resources. Bioreactors have the potential to dramatically advance global sustainable horticulture and agricultural practices as they develop and find use in a variety of plant growth settings.

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CHAPTER 7

ENSURING HEALTH AND VIRUS-FREE PROLIFERATION IN TISSUE CULTURE-BASED PLANT PRODUCTION

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ABSTRACT:

The health and disease state of the donor mother plants and the plants produced from them are crucial factors in any tissue culture-based plant production operation's success. The amount and quality of plant material used for propagation might suffer from viral, bacterial, or fungal diseases. As a result, it has become standard procedure to check mother plants for the absence of such diseases before beginning tissue culture-based large-scale plant replication. This study emphasizes how vital it is to guarantee the mother plants' good health and virus-free state while producing plants through tissue culture. It highlights the necessity for thorough disease testing as it investigates different approaches and procedures for determining the presence of viruses, bacteria, and fungus. The paper also examines the difficulties these infections provide and clarifies the techniques used to reduce contamination concerns in tissue culture. The wellbeing of donor mother plants and the offspring they produce is crucial to the success of tissue culture-based plant production. Before beginning large-scale plant replication via tissue culture, this article explores the crucial value of indexing mother plants for independence from viral, bacterial, and fungal illnesses. There are several ways to identify diseases, including eye examination, serological testing, electron microscopy, and molecular procedures. The difficulties caused by bacterial, fungal, and viral contamination are also covered in the article along with the methods for controlling them. It also underlines the value of thermotherapy and meristem culture in removing viruses from plant material and the influence of tissue culture on the symbiotic interactions between plants and helpful microbes.

KEYWORDS:

Diseases, Fungal Infections, Microbes, Tissue Culture, Thermotherapy.

INTRODUCTION

One of the most important elements that determines the success of a tissue culture operation is the health of the donor mother plant and the plants that are produced from it. Therefore, before beginning large-scale plant replication by tissue culture, it is customary to index the mother plants for independence from viral, bacterial, and fungal infections. The Department of Agriculture, agricultural universities, or privately held accredited germplasm banks are a few examples of institutions that should provide laboratories without in-house plant indexing capabilities with their indexed stock plants. Micro propagated plant batches should undergo internal or external laboratory testing to ensure they are disease-free [1], [2]. The health of the donor mother plant and the plants that are propagated from it are some of the most important aspects in micropropagation that affect the outcome of a tissue culture operation. In large-scale plant replication via tissue culture, the indexing of the mother plants for independence from viral, bacterial, and fungal infections is a standard practice.

Plants that weren't grown from pathogen-tested seed must be checked for viral contamination. The Department of Agriculture, agricultural institutions, or privately held accredited germplasm banks are a few examples of entities that should provide laboratories without in-house plant indexing capabilities with their indexed stock plants. Micropropagated plant

batches should undergo internal or external laboratory testing to ensure they are disease-free. The best technique for identifying viruses and other pathogens in plants has been ELISA. More sensitive than ELISA, polymerase chain reaction and nucleic acid hybridization may identify diseases in very minute quantities. Apical meristem culture and thermotherapy may be used in conjunction to eradicate the majority of viruses.

Fungal and Bacterial Contamination

While surface sterilization and culture effectively eradicate the majority of bacterial and fungal illnesses, if the mother plant is sick, viruses and viroids may persist via consecutive multiplication. Even cultures made from seeds have the potential to include viruses, viroids, and bacterial endophytes. Using various bacteriostatic drugs, bacteria can be somewhat controlled in in vitro settings. However, even when used over an extended period of time, chemical therapies like "plant protection mixture" or commercial antibiotics cannot completely eradicate germs.

Plant Viral Infection

Viruses are obligatory parasites that utilise the metabolic machinery of host cells to multiply while preventing plant development. Despite the fact that many viruses do not manifest as disease symptoms, they negatively impact plant metabolism and get stronger over time as a result of repetitive vegetative replication. A few viruses have a broad variety of hosts, while the majority exclusively infect a small number of species. Numerous plant viruses are spread by vectors such nematodes, fungus, bacteria, and insects. Viruses may spread from one plant to another by grafting, sap, and propagation from vegetative components. Viruses may harm a plant depending on how concentrated they are in the tissues and how far they are dispersed. The virus has a more crippling impact on plant development when it is present in higher concentrations. With viral buildup, the performance of many types of vegetatively propagated crops declines, and they must be destroyed. Although the viruses don't show any symptoms, the plants nonetheless produce less and of worse quality. It has been shown that the eradication of certain viruses by meristem-tip culture results in a considerable boost in production and rejuvenation of vegetatively propagated plant kinds. This is a special contribution made by meristem culture that cannot be achieved using any other methods [3], [4].

Finding viral illnesses

Viruses must be checked for in plants that are not derived from pathogen-tested material. Visual inspection for viral symptoms, infection testing on indicator plants, serological tests, electron microscopy, and direct RNA detection utilizing molecular methods are all methods used to identify the presence of viruses. Serological and molecular laboratory tests, as well as indicator hosts in the greenhouse, are among the detection methods that have received international approval and recognition.

The older technique of identifying viruses depended on indicator plants before the development of antigen-antibody response detection methods. By rubbing the juice of the infected plant on the leaves of indicator plants like Chenopodium spp. and Nicotiana spp., for instance, viruses A, X, and Y in potato may be found. One frequent sign that the viruses are present is the emergence of necrotic patches on the leaves. For instance, Brazilian Morning Glory is still utilized as the indicator host in sweet potato, a wild cousin. Similar to this, many fruit plants need the correct rootstocks to index different viral strains. In the last 20 years, there has been a significant shift in how plant diseases are detected. Specific Enzyme Linked Immuno Sorbent Assays have taken the place of many indicators plant-based assays.

However, indexing using indicator plants is a cheap and very efficient way to find numerous viruses. The best technique for identifying viruses and other pathogens in plants has been ELISA. Every virus has its own specific protein coat. In order to report the affinity binding through colour signal from the used enzyme substrate, antibodies are often coupled to enzymes to detect them. It is still common practice to employ a 96-well microtitre plate with a double antibody sandwich technique. To boost particular sensitivity, several other formats, substrates, and antibody binding modifications have been created throughout time. Nowadays, the most widely used technique makes use of virus-specific antibodies that have been "biotinylated" to increase their affinity to the corresponding viral surface and so boost the signal. Because of their high cost and difficulty to identify the variety of strains in numerous illnesses, monoclonal antibodies have only recently been used to treat certain potato and barley viruses.

DISCUSSION

A common practice is large-scale testing using screen-spot samples. These dot- immuno-binding assays can replace microtitre plate testing and are more adaptable and affordable. Direct plant sap is spotted onto a microporous membrane that has already been treated. Direct field usage of the test kits is possible. Drying sample membranes allows them to be sent to another lab for processing. Such methods, however, are useless against viruses found in small numbers in plant sap. ELISA has a number of drawbacks. A test is first and foremost unique to a particular strain of the disease. ELISA kits have not been created for a large set of common viruses that do not cause clinical symptoms or deadly outcomes. Therefore, the strain specificity has significant drawbacks for thorough quality control. Additionally, ELISA is less sensitive than PCR and can miss detecting virus in tissue-cultured plants at low concentrations. A negative ELISA test does not guarantee the absence of microorganisms, but a positive test is a solid indication of their presence. By seeing the outcome of viral eradication, Immuno-Tissue-Printing enables the localization of viruses in tissues and, as a consequence, the refinement of elimination tactics in vitro [5], [6].

Chain Reaction of Polymerase

Compared to ELISA, the Polymerase Chain Reaction is more sensitive and can identify pathogens in very small quantities. Pathogens are identified by PCR using their genetic makeup, i.e., RNA or DNA. Millions of copies of the pathogen's DNA are produced using a particular enzyme and the appropriate genomic start code for a relevant portion. As a result, even little amounts of the pathogen DNA may be found. Since most plant viruses only contain RNA, they must first be copied into DNA, as opposed to bacteria and phytoplasmas, whose DNA may be amplified immediately. It is vital to focus just on the genetic material that has to be duplicated since the pathogen's genetic material is mingled with the genetic material of the plant. Primers, which only adhere to the edges of the genetic material that has to be replicated, are little fragments of DNA that are added to the mix to accomplish this. The enzyme is then told where to begin and terminate amplification by the primers.

The DNA is heated, cooled, and warmed again in a thermocycler during PCR. DNA is broken up into single strands by hot cycles. The primers may bind to the DNA during cold cycles. The enzyme duplicates each piece of primed DNA during warm cycles. A significant amount of a certain DNA fragment is produced by PCR when a phytoplasma is present. DNA fragments are separated based on size using gel electrophoresis, and the particular DNA fragment is recognized. If the sample tests negative and the necessary concentration of a DNA particle is low enough, the plant material may be assumed to be almost devoid of the target bacterium. A negative PCR test is a more trustworthy sign of practical freedom if a test

has been standardized using the proper primer and a procedure that avoids inhibitors. The number of samples that test negative even though they may contain the virus is significantly decreased by the use of PCR. The nylon membrane spot test is a practical, quick, and reliable diagnostic tool once the right probes are produced. The benefit of PCR is that a significant variety of microorganisms, including viruses, bacteria, and fungus, may be discovered by identifying "degenerate primers."

Test for Nucleic Acid Hybridization

The extraction and characterization of double stranded RNA generated in most plants during viral replication may be used to determine the presence of viruses. Although the non-specific presence of ds-RNA in a sample extract strongly supports viral infection, the test must be confirmed by serological and other techniques in order to identify the virus in more detail. Viroids are comprised of a circular piece of RNA and are significantly smaller than viruses. They also lack a coat protein. Nucleic acid hybridization may be used to find viruses like the "Potato Spindle Tuber Viroid." The virus's RNA "hybridizes" with the probe RNA. The two single strands combine to produce a double strand when they touch. RNA taken from plant tissue is bonded to a microporous membrane and used to identify the viroid. A "probe", which is an RNA particle labelled with either 32P or a substance called DIG, is then used to find this bound RNA. The probe hybridizes with a viroid if one is present; if not, it is lost when the membrane is washed. On an exposed film, DIG bioluminescence or radioactivity, if present on the membrane, may be seen. As a check, samples from healthy and sick plants are compared. It is also customary practice to do extensive testing on spot samples made from nylon screen.

Removal of viruses

Apical meristem culture and thermotherapy may be used in tandem to eradicate viruses. The most popular technique for eradicating viral infections from plants is meristem culture. As previously said, this should not be confused with shoot tip culture. Thermotherapy and meristem culture have been used to grow virus-free plants in a number of species. The rising tip of stems and roots include a region of cells known as the plant meristem, which undergoes rapid cell division. The plant vascular system, which does not exist in the meristem, is where the virus moves. Additionally, the expansion and lengthening of the apical-tip prevents the viral migration from cell to cell via plasmodesmata from keeping pace. Viral replication may potentially be inhibited by the high metabolic activity of meristematic cells, which is often accompanied by an increased endogenous auxin level in shoot apices. The meristem is thus very well protected from infection. Based on this discovery, meristem culture has been widely utilized to get rid of bacteria, fungus, and viruses from plants. The removal of viruses from plants grown from vegetative components is possible by the cultivation of meristems, or alternatively, tiny shoot tips, in conjunction with accelerated cell proliferation in vitro and/or thermal pre-treatment. If explants are excessively large, the surrounding vascular tissue is probably going to include virus particles [7], [8].

The plants are initially cultivated at high temperatures for 4-6 weeks in preparation for thermotherapy. This is easily performed in tropical or subtropical environments by erecting a tiny glasshouse compartment with a temperature-controlled exhaust fan on one end and a roof vent on the other. This method delivers a continuous high temperature midday therapy while removing the extra heat. In temperate climes, the similar effect may be produced by installing heat-producing incandescent bulbs or fluorescent lights with ballasts at the bare minimum distance from the plants to be treated in a dark box only big enough to hold the plants. A technique like this has been used to get rid of viruses in sweet potatoes.The majority of in

vitro plants are known to include specific microorganisms, predominantly bacteria but also mycoplasmas, viroids, and fungi, many of which cannot be cultivated without the host. This is known via molecular and classical microbiological surveys. These microbes could not cause any symptoms, and in certain situations, they might even help the host plant develop. To multiply plants that are already free of recognized illnesses, shoot-tip culture is utilized. The fact that it does not rid the plant of viruses should be stressed. In actuality, it encourages viral reproduction and raises virus concentration in the daughter plants. Variegation in many ornamentals is brought on by the presence of certain viruses or mycoplasms. Therefore, it is not advisable to remove such viruses. In these ornamental plants, meristem culture may eradicate the virus, but the decorative variegation is lost. Therefore, shoot-tip and axillary bud multiplication is the preferred option. Meristem cultivation may dissolve the existing chimera in certain variegated types, such as Petunia and Pelargonium, leading to the production of material lacking the required characteristics. A plant variety's distinctive characteristics may sometimes be produced by viruses. Consider the sugarcane cv. Yellow uneven leaf streaks, or "Co 740," were at first thought to be a diagnostic feature before being shown to represent illness signs. The yellow streaks vanished once the virus was eradicated via meristem-tip culture, but they returned after re-infection.

Many viruses may not have obvious symptoms, yet even so, their presence might lower agricultural output and quality. Following the substitution of virus-infected stock with specified pathogen-free plants, yield gains of up to 300% have been shown. Sweet potato viral illnesses used to cause crop losses of up to 20% in China. Within a few years of starting a virus-eradication campaign, 10% of the 6.6 million hectares of sweet potato land was planted with virus-free planting material. In the field, there are no chemicals to treat plants afflicted with viruses. However, certain less deadly viruses may provide cross protection and shield plants from infection by their more virulent forms. Use of meristem culture need not be restricted by the occurrence of cross protection. Despite being made from tissue culture material, a crop does not remain completely virus free when grown in the field [9], [10]. Even in tissue culture, complete freedom may not be attained. The strength and capacity of the tissue-cultured plants to function successfully in the field are influenced by several symbiotic organisms. Many plants are stripped of these helpful microorganisms during tissue culture, including mycorrhizae and symbiotic nitrogen-fixing endophytic bacteria. It is not feasible to keep the helpful microbes in tissue culture. One legitimate method of regaining the advantages of these bacteria is to purposefully re-infect propagules with certain strains.

CONCLUSION

In tissue culture-based plant production, the health and virus-free state of donor mother plants and the plants generated from them are crucial. Disease testing and management methods are vital because contamination by viruses, bacteria, or fungus may result in decreased agricultural output and degraded crop quality. In order to determine the presence of infections, a variety of disease detection techniques, from visual examination to molecular procedures like PCR and ELISA, are crucial. These techniques provide important information on the condition of plant material and help determine whether tissue culture propagation should be pursued. It is impossible to undervalue the effect that viral infections have on plant growth and agricultural output. To effectively remove viruses from plant material and produce propagules that are virus-free, meristem culture and thermotherapy stand out. These methods have been crucial in raising agricultural yields and assuring the success of plant production based on tissue culture. Additionally, since tissue culture disrupts the symbiotic interactions between plants and helpful bacteria, procedures must be developed to reintroduce these microbes, assuring the field adaptation of tissue-cultured plants. The careful monitoring

of the health and virus-free condition of plant material in tissue culture-based production is crucial for efficient and effective agriculture, to sum up. We may improve the quality and quantity of plants propagated by using cutting-edge methods like meristem culture and thermotherapy, rigorous disease testing, efficient management measures, and other agriculturally sustainable practices.

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CHAPTER 8

ENHANCING PLANT STRESS TOLERANCE IN TISSUE CULTURE AND MICROPROPAGATION

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ABSTRACT:

The ability to produce genetically identical plants in large quantities thanks to plant tissue culture and micropropagation has transformed the area of plant propagation. These methods have several benefits, including the quick replication of superior plant kinds and disease-free propagation. But the tightly regulated environments that enable effective tissue culture and micropropagation also render the emerging plantlets vulnerable to stress when introduced to the outside environment. Reduced photosynthetic capability, subpar root growth, and susceptibility to diseases and environmental factors are some signs of such stress. For the large production of plants, plant tissue culture and micropropagation are essential procedures. But the regulated conditions where these processes take place often produce stressed and weak plantlets. The options for improving plant stress tolerance during tissue culture and micropropagation are examined in this article. It goes into detail on how to prime plantlets for a smooth transfer to the outside world by using priming methods, adjusting growing conditions, and using microorganisms. These techniques show promise for growing strong, hardy plants that can resist the difficulties of transplanting and subsequent field growth.

KEYWORDS:

Bacterization, Disease, Environment, Management, Micropropagation, Plant Tissue Culture.

INTRODUCTION

The alteration of the expression of certain genes and metabolic pathways enables plants to adapt to their environment, including temperature, osmotic stress, and pathogens. The cellular metabolism of plants is pre-sensitive to certain chemicals, environmental variables, and microbes. Pre-sensitized or primed plants respond to stress more quickly and effectively than unprimed plants. The improvement of post-transplantation abilities for water management, photosynthetic efficiency, and disease resistance is necessary for the creation of high quality and robust plants via in vitro culture. Tissue culture propagation includes priming of in vitro propagules, which is based on altering the growth environment before and after transplantation. The success or failure of tissue culture operations is often determined by the propagules' capacity to endure transplanting stress [1], [2].

Tissue-cultured propagules are created in compact, poorly-ventilated containers in controlled conditions with temperature and light levels that are predetermined. This causes a buildup of ethylene and excessive humidity. Additionally, compared to dirt, the growing medium contains considerably more nutrients such sucrose, mineral salts, vitamins, and growth regulators. Such a setting alters developmental patterns and suppresses or modifies a number of metabolic processes. The plantlets as a consequence have tiny juvenile leaves with diminished photosynthetic ability as well as broken stomata. Such plants' leaves have poor cuticle growth and little to no wax deposit, while their roots either have no hair or very little hair. The propagules' ultimate performance is determined by their capacity to control water, photosynthesis, and their reaction to stress both during and after in vitro cultivation. To handle water in post-vitro circumstances, the development of an effective root system is crucial. During ex-vitro acclimatization, the in vitro roots are still active and growing.

Species that are simple to root often regrow their roots after culturing on hormone-free media. However, by making a few simple changes to the growth circumstances that influence the propagules' post-transplanting performance, it is possible to readily modify the root architecture and biomass partitioning between roots and shoots. For instance, raising the day/night temperature of potato clones from 20/15 to 33/250C either marginally stimulates or has no impact on the development of the shoots, but greatly increases the growth and branching of the roots.

A number of substances can have an impact on how the root system grows. The root architecture might alter overall and new lateral roots can be initiated as a result of abscisic acid. The final subculture of in vitro plants may benefit from priming with jasmonates, which enhance stress tolerance in plants. In the potato plant, nodal explants from stock plants that have been JA primed tuberize sooner and more evenly, and they also yield more microtubers than the unprimed controls. Salicylic acid is a key signalling substance that makes plants more resilient to both biotic and abiotic stress. It may also be helpful for priming tissue-cultured propagules since it also causes adventitious roots to form in cuttings, stimulates stomata closure, and suppresses ethylene production in detached leaves.

The growth inhibitor daminozide, when added in small amounts to hormone-free media, helps potato plants adjust more quickly after transplantation. Root initiation and development may also be aided by other nutrients such as activated charcoal, seaweed concentrate, pyroligneus acid, and dilution of salts in the basic MS medium. Compared to non-aerated vermiculite and non-aerated agar, in vitro cultivated papaya shoots in aerated vermiculite provided greater roots and higher post-transplanting survival. Before effective rooting of microcuttings, in vitro rejuvenation through many subcultures is necessary in several hard-to-root species, such as apple and Saskatoon berries. Individual cultivars may need to be adjusted to the various subcultures and culture conditions. For instance, apple microshoots, cv, successfully achieved over 90% rooted. 'Jonathan' by the ninth subculture, whereas 'Red Delicious', a more resistant cultivar, needed almost 30 subcultures to successfully root. When rooted Saskatoon berries from standard stem cuttings is very challenging, tissue culture renewal followed by transplantation to a greenhouse may result in extremely robust plants. After a quick treatment with IBA, shoot cuttings obtained from such planted hedges quickly take root in 2–3 weeks [3], [4].

Enhancing photosynthetic capacity

The explant is kept clean and wet in tissue culture containers to avoid contamination and desiccation, but ethylene and water vapours produced during metabolism cannot escape or be aerated. Such circumstances cause unfavourable morphological alterations and sluggish development. The accumulation of ethylene inhibits plant development, lowers leaf size, and results in leaf drop. These plants soon dry up when exposed to the outside environment. Gas exchange between culture containers and the surrounding environment has been made easier by the use of vented closures, such as those for baby food jars with integrated microbiological filters in various sizes.

Epicuticular wax deposition on leaves is reduced by high relative humidity in the culture vessel. By lowering the number of palisade cells and generating spongy parenchyma with huge air gaps, it affects stomata function and changes leaf mesophyll. inadequate transpiration, diminished lignification, and limited or aberrant vascular system growth are further impacts, as well as inadequate nutrient intake. Although progressive adaptation to decreased humidity may stimulate the growth of cuticular wax and the functioning of stomata, morphological alterations are difficult to undo. The modified leaf shape increases

respiration and decreases photosynthesis. excessive light intensity or a cooling of the bottom of culture containers by 2-3oC below the culture room's ambient air temperature might help lower excessive humidity. High humidity may also be adjusted by gradually opening the vessels for a few days before to transplanting.

DISCUSSION

Since many micropropagated plants contain the C3 photosynthetic pathway, this has a significant impact on how well they can absorb carbon. Their production is regulated by the interaction between photosynthesis and photorespiration. Cultures produced using conventional micropropagation techniques are often mixotrophic. By lowering rubisco activity, the carbohydrates in the medium prevent carbon from being assimilated by photosynthesis. However, a number of research show that sugar has a stimulatory impact on biomass growth under high CO2.Propagules of high grade with established roots and leaves are simple to acclimatize to the outside environment. Any effective acclimatization strategy must guarantee that the plants continue to develop actively during the whole weaning process. The grapevine and the chile ancho pepper, Capsicum annuum L., have both been used to illustrate the impact of in vitro growing conditions on post-vitro acclimation. The most popular method for increasing plant longevity after being transplanted into soil is progressive ex-vitro habitat adaption. When this occurs, plants quickly transition from heterotrophic or photomixotrophic growth to autotrophic development, establish fully developed root systems, and their stomatal and cuticular transpiration is decreased. In greenhouses, this kind of progressive adaptation is often accomplished by reducing relative humidity using fog or mist chambers while increasing light intensity and utilizing shade strategies. The amount of light must be adjusted according to the demands of the plant. For certain species, it is advised to root in vivo the shoots grown in a lab in order to prevent post-rooting dormancy, encourage the formation of roots that are already suited to the environment, or all three. When in vitro plantlets are transplanted into the natural environment, further treatments include high phosphate fertilizer to increase their vigour, fungicides to stop damping-off, and anti-transpirants to stop desiccation; however, these treatments may also result in phytotoxicity.

Tissue cultured plantlets that are 2 to 4 weeks' old are typically hardened off for 2-4 weeks in a greenhouse before being transplanted in the field for the generation of seed potatoes. These plantlets are still young and not stress-adapted well. Many producers cover their plants with floating covers and set up overhead watering systems to stop desiccation, insect and bird damage, and virus transmission by aphids. The grade of seed potatoes produced under floating covers is consistently higher than that of plantings made without protection [5], [6].Somatic embryos are created as adventitious structures from callus and suspension cultures directly on zygotic embryo explants. It has been effectively utilized to speed up embryo maturation and prime them for tolerance to post-transplant stress using the stress-inducing chemicals ABA and JA found in plants. A standard procedure to develop desiccation resistance in alfalfa synthetic seeds involves the use of ABA and osmotic shock with 6% sucrose. Osmotic stress induction with 3% sorbitol and ABA therapy together with partial desiccation of encapsulated somatic embryos in sugarcane were both successful in promoting embryo development and conversion in soybean cultures. For priming SE propagules of diverse plants, a number of sugar alcohols and polyethylene glycol have been explored with varying degrees of effectiveness. Benzyl adenine has been shown to dramatically enhance conifer post ABA embryo maturation. The production of cotyledonary SE on the maturation medium was enhanced by reducing the sucrose content in the proliferation medium from 58 to 29 mM. It has been shown that different carbohydrates and

organic nitrogen molecules interact with plant growth regulators to influence the development and maturation of embryos.

Bio priming

Both internal and external microbes invade plants in their natural habitat. Some microorganisms, in particular helpful bacteria and fungi, may promote plant production by enhancing performance under stressful settings. Microorganism-infected plants acquire systemic resistance and/or gain from their pathogen-repelling powers. Although it has been common practice for more than 50 years to inoculate seeds with advantageous microbes, the technique of inoculating tissue culture propagules to improve plant performance is relatively recent. Axenic culture methods are the foundation of plant tissue culture. Because of this, endophytes and other microbes are classified as pollutants that might cause issues, and several methods have been devised to get rid of them. Microbial inoculants, especially bacterial and mycorrhizal, have only lately been studied as propagule priming agents both in vitro and after transplantation. Only a small percentage of the naturally occurring bacteria can be cultured using conventional microbiological procedures. Additionally, the bacteria that are linked to soil and plants may change between culturable and unculturable phases. Future research will face a significant obstacle in the creation of novel culture techniques that enable the creation of long-lasting relationships between plants and beneficial microbes, both in vitro and ex vitro, in a variety of habitats [7], [8].

In vitro bacterial growth

Rhizosphere bacteria produce a variety of compounds that interact with plants and other dwelling species. Many of these compounds have regulatory effects on plants. There is mounting evidence that plant-associated bacteria increase the resilience of the host to environmental challenges. This often involves the synthesis of salicilate, jasmonate, or both. The ability to withstand biotic stress is linked to these two categories of signalling molecules. However, new genetic research on Arabidopsis thaliana infected with the rhizobacterium Paenibacillus polymyxa that promotes plant development shows a connection between biotic and abiotic stress tolerance.

Pseudomonas spp. is an efficient plant-beneficial bacterium. To improve potato resistance to transplanting stress. The bacteria were first discovered to be a contaminant from onion roots that had been infected with Glomus vesiculiferum. The fact that the bacteria did not develop on a typical potato culture media in the absence of plantlets was a crucial factor in its separation. So that the bacteria do not outgrow the culture, pre-selection of plant beneficial bacteria on plant tissue culture medium is advised as a crucial stage in the construction of an in vitro co-culture system. Plantlets' in vitro development may be significantly impacted by dipping potato nodal cuttings in a suspension of a helpful bacteria. As of right now, Pseudomonas sp. Under in vitro circumstances, strain PsJN has shown to be the most successful plant growth promoting bacterium. When co-cultured with grapevine, tomato, or potato, it develops endophytic and epiphytic populations. Therefore, further re-inoculation is not necessary in clonal proliferation via nodal explants. The bacteria promote plant development and causes adjustments that improve water management. Additionally, it improves resistance to low-level diseases that affect tomatoes, potatoes, and grapevines as well as to Botrytis cinerea infection. After four weeks in culture, the infected plantlets developed a huge and well-branched root system and were at a later stage of growth. With more lignin deposits around the vascular system, more root hairs, and more and bigger leaf hairs, the stems were more robust. The bacterized in vitro plantlets' stomata function was quite similar to that of the non-bacterized transplants that had been toughened in the

greenhouse. By co-culturing wheat seedlings with Azospirillum brasiliense strain SP245, it has been shown that water relations in plants subjected to osmotic stress have improved. Increased black locust transplant survival after inoculation with several Rhizobium isolates chosen for their growth vigour from Rabinia types has also been shown.

It is widely known that Agrobacterium rhizogenes cultures may stimulate adventitious roots. Two Mexican species of Pinus were successfully given a rooting boost thanks to its application. Rooting of adventitious shoots formed from somatic embryos increased between 7 and 13% when the shoots were co-cultured with A. rhizogenes to 67% compared to controls treated with auxin, which had 60%. In co-culture with the bacterial isolates, slash pine explants similarly saw an induction of root formation. The roots formed by the bacteria resembled seedling roots rather than the hairy roots caused by A. rhizogenes. The post-transplant survival vigour of potato and vegetable plantlets after in vitro bacterization was dramatically improved. Additionally, during and after weaning, enhanced vigour of potato plantlets co-cultured with isolates of Pseudomonas fluorescence obtained from tubers has been documented. Plants generated from dual cultures of potato and our pseudomonad bacteria in greenhouse trials had bigger root systems and stolons, tuberized faster, and produced more tubers than non-bacterized controls. However, the tuber production fluctuated from year to year, and excessive precipitation or severe drought lessened the advantages of bacterization. The bacterized potato plantlets transplanted straight from culture vessels to the field showed much greater survival than the non-bacterized controls.

In order to create sustainable production systems, nitrogen-fixing endophytes may be introduced using tissue culture methods to clonally grown plants. At the conclusion of the rooting phase on medium with 10X diluted salts and sugar but without hormones and vitamins, a procedure has been designed to inoculate micropropagated sugarcane plantlets with Acetobacterdiazotrophicus. The bacteria may be re-isolated from the plant tissues 30 days after transplant. There have also been reports of stable artificial relationships between plants and microbes that fix nitrogen. a callus and bacterial symbiotic culture system between Daucus carota L. Moreover, Azotobacter zettuovii was created. On the nitrogen-free medium with lactose as the carbon source, the callus developed for four years. The bacteria found in the intracellular gaps spread to the plantlets that had just been regenerated and fixed nitrogen [9], [10].

Ex vivo bacterial growth

Commercial labs are reluctant to add microorganisms to their in vitro cultures in order to meet the certification standards of plant tissue culture propagules. Weaning plants in the presence of both beneficial bacteria and mycorrhizal fungus is one method. In potato, the bacteria present in the transplanting media easily infect both the exterior and interior tissues of the tissue-cultured plantlets. Despite being appealing, this strategy will face the challenge of sustaining stable populations of the imported microbes. The advantages of bacterial inoculation on potato tubers grown in greenhouses and on tree seedlings demonstrate the possibility of post-vitro bacterialization of tissue-cultured propagules. In-vitro mycorrhization and bacterization of micropropagated strawberry, potato, and azalea with specific bacterial and mycorrhizal combinations improved greenhouse production of minitubers, and a combination of three rhizobacterial strains enhanced strawberry post-transplant performance.

Mycorrhization

Plant development is aided by the addition of endomycorrhizal fungus to tissue-cultured plantlets. It is well knowledge that vesicular-arbuscular mycorrhizae root colonization enhances plant nutrient status, water management, and disease resistance. In response to the

Glomus intraradices infection, many secondary metabolites with structural resemblance to abscisic acid have been found in tobacco roots. Several conifers have also been shown to have a range of phenylpropanoid chemicals that have been induced by ectomycorrhizal inoculants. These substances have something to do with how plants handle biotic and abiotic stress. During transplantation, rooted-shoots were infected with mycorrhiza to enhance the performance of tissue-cultured plantlets. The advantages of mycorrhization varied according to the type of soil, the species of plant and mycorrhizal organisms, and the level of root colonization. It was recommended to create a culture technique where the mycorrhizal fungi are injected in vitro during the roots stage since VAM enhanced growth of plants was usually exhibited only after acclimation. Fungi may develop in vitro on carrot roots that have undergone Ri T-DNA transformation and high CO_2.

A "tripartite" culture method for the in vitro inoculation of strawberry and vegetable crops was created by using this approach. The best treatment included strawberry shoots in cellulose plugs and carrot roots that had been converted with VAM 30 days earlier. The plugs were attached to the mycorrhized root culture after root induction, and they were then housed in a growth chamber with 5000 PPM CO2 for 20 days. In comparison to non-mycorrhized controls, all plantlets that were successfully colonized showed greater root systems, better shoot development, and higher osmotic potential. The improvement of osmotic potential is said to be crucial in the pre-adaptation phase before plantlets are fully acclimated for transplantation. Wheat seedlings co-cultured with Azospirillum had a higher osmotic potential than non-inoculated controls, which enabled them to survive osmotic stress significantly better. In strawberry, mycorrhized plants outperformed non-mycorrhized controls in terms of establishment rate and number of runners generated. The plants were grown in a substrate made of polyurethane foam under photoautotrophic conditions with a lower concentration of sucrose. The VAM spores may be inserted right into the planting holes, and the foam tear-away strips fit into any culture vessel. Garlic's growth was seen to improve after post-vitro transplant inoculation.

Numerous studies have shown that VAM fungus and diazotrophic bacteria work together to improve the nutrition and development of diverse crops. It is necessary to investigate these "mycorrhiza helper bacteria" effects in tissue culture systems. Defining tissue culture microecosystems might make it possible to examine complicated interactions between plants, microbes, and habitats. This could help us enhance our current in vitro propagation techniques and prepare propagules for ex-vitro settings.

CONCLUSION

For propagated plants to thrive in the field, tissue culture and micropropagation must be improved to increase plant stress tolerance.

This article examines a number of methods and techniques for reducing stress and preparing plantlets for optimum performance after transplanting. Enhancing plant stress tolerance during tissue culture and micropropagation is crucial for making sure that plantlets successfully transfer from the stable circumstances of the lab to the unpredictable and sometimes difficult outside environment. The approaches covered in this article, such as priming methods, modifying growth circumstances, and using microorganisms' potential, provide promising means of achieving this objective. We may create strong, resilient plants that can thrive in a variety of environmental circumstances by pre-sensitizing plantlets to stresses and optimizing their physiological reactions. This helps the horticultural and agricultural sectors as well as attempts to conserve and grow plants in a sustainable manner. We are paving the path for the widespread adoption of tissue-cultured plants with improved

stress tolerance as we continue to develop and broaden these technologies, eventually supporting efforts to ensure global food security and ecological restoration.

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CHAPTER 9

UNLOCKING YEAST DIVERSITY: NON-GENETIC ENGINEERING STRATEGIES FOR INDUSTRIAL ADVANCEMENTS

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ABSTRACT:

Yeast biotechnology has enjoyed a rich history intertwined with human civilization, dating back to ancient times when yeasts were first utilized in brewing, baking, and winemaking. These traditional practices inadvertently set the stage for a dynamic journey in yeast selection and domestication. Yeast strains were chosen based on their ability to impart specific attributes to fermented products, such as taste, aroma, and alcohol content. This unintentional yeast breeding laid the foundation for extensive yeast collections, each harboring a unique set of characteristics ideal for various industrial applications. This study delves into the realm of yeast biotechnology, exploring non-genetic engineering approaches for isolating and generating novel yeasts tailored for industrial applications. Yeasts have played pivotal roles in various industries for centuries, particularly in brewing, baking, and winemaking. Over time, these industries have inadvertently and intentionally selected yeast strains with desirable traits, resulting in extensive yeast collections. While genetic engineering has expanded the possibilities, regulatory constraints and consumer acceptance issues have necessitated a focus on non-recombinant methods. We examine strategies for accessing genetic diversity, including mutagenesis and adaptive evolution, and their applications in yeast strain development. Through these approaches, researchers can harness the inherent genetic variation within yeasts to create innovative solutions for industrial challenges.

KEYWORDS:

Genetic Engineering, Genetically Modified Organisms (GMOs), Genetic Diversity, Industrial Applications, Yeast Strains.

INTRODUCTION

Since humans have used yeasts for thousands of years in the production of beer, bread, and wine, they have unintentionally and consciously identified and chosen strains with characteristics that are beneficial for the purposes for which they are used. Large collections of yeasts have been produced as a result, and they may be utilized to produce goods and processes that are dependable, diverse, and new thanks to the yeasts. Modern genetic engineering methods have been used to increase the variety of industrial yeast genomes even more. This has immense promise for modifying phenotypes for use in industrial applications [1], [2]. However, the use of genetically modified organisms (GMOs), notably in foods and drinks, is prohibited by law in many nations, and consumer acceptance of these organisms is, at best, uneven. Therefore, it may be necessary to depend on non-recombinant methods for a while, at least until consumers are more receptive of goods manufactured using genetically engineered strains and law is less restrictive, in order to create new yeasts with enhanced properties for industry. Even while this might be irritating for yeast biotechnologists, there is already a lot of genetic diversity in current yeasts, and it can be expanded via non-genetic engineering techniques like chemical or UV mutagenesis.

This indicates that there is a great deal of potential for tailoring yeasts to specific ends without using recombinant DNA techniques. The species Saccharomyces cerevisiae not only serves as a potent model system to address fundamental biological concerns, but it also is

essential to several commercial uses. Even before the first microorganisms were seen, many processes separately domesticated different S. cerevisiae strains. Many domesticated S. cerevisiae strains have partially or completely lost the ability to reproduce sexually, become increasingly tolerant to certain stressors frequently encountered in industrial plants, and metabolized a few sugars more quickly than natural strains after centuries of continuous growth under favourable conditions, with nutrients readily and abundantly available. This is reminiscent of the so-called "domestication syndrome," which Darwin first articulated in 1868 and describes how species that have been domesticated tend to lose unfavourable features and pick up skills that help them thrive in settings that resemble those of humans [3], [4].

Brewers, bakers, and to some degree even winemakers in the past employed yeast strains from an older batch to conduct successive fermentations. In fact, the back-sloping process was necessary to preserve the finished product's stability and ensure the method's economic viability. The stability of the finished industrial and/or artisanal items was further improved by the introduction of pure cultures and refrigeration. Contrarily, as pure cultures were more accepted and clonal batches were kept fresh by refrigeration, the genetic diversity of the yeast significantly diminished as soon as bakers and brewers realized the critical function that S. cerevisiae strains played in the fermentation and started to isolate pure cultures. As a result, many yeast strains employed in industrial settings today, especially those used in bioethanol, wine, and beer fermentations, are often chosen more for historical than for scientific reasons. Additionally, despite industrial strains' lengthy domestication histories, there is still tremendous room for improvement since both consumer and industry demand have changed and are continuing to change. The non-genetically modified organisms (non-GMO) techniques should be given the highest weight since they do not face any problems with consumer approval or any special legal requirements, especially for food and beverage yeast applications.

As neatly outlined, there are several non-GMO methods for producing acceptable yeast strains for certain industrial objectives. Finding natural biodiversity by choosing a yeast that can function well in a particular industrial process is a highly effective strategy. The vast and mostly undiscovered natural yeast biodiversity is supported by recent metagenomic studies, and the industrial strains currently in use only represent a tiny portion of the natural species. Regenerating biodiversity by the inventive use of traditional genetic techniques on previously existing yeast strains is an alternate path. The goal of both the regeneration process and the quest for natural biodiversity is to choose the best phenotypes.

In fact, for complex quantitative genetic traits involving large numbers of genes, such as ethanol tolerance and fermentation performance, it is unlikely that genetic engineering techniques would be able to contribute much anyway. Describe the Steensels-based methods for acquiring genetic variety in yeast in Figure 1. But as science and technology advanced, researchers realized that genetic engineering might be used to create yeast strains with specific features. Precision engineering became possible with the development of recombinant DNA technologies, providing unmatched control over yeast phenotypes. Genetically engineered yeasts that can carry out certain functions have substantially improved industrial uses such as biofuel production, medicines, and bioremediation. Genetic engineering still confronts significant difficulties, despite its promise for scientific advancement. The usage of genetically modified organisms (GMOs), notably in the production of food and beverages, is strictly regulated in many nations. Additionally, customer approval of GMO-derived goods varies, necessitating caution on the part of enterprises.

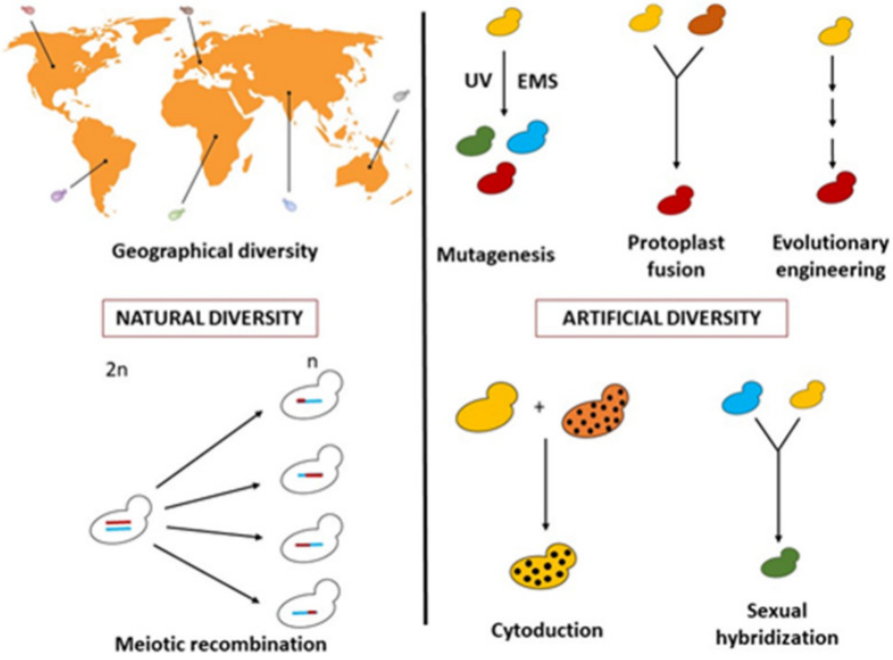

Figure 1: Illustrate the Steensels-based techniques for obtaining genetic diversity in yeast.

Due of these difficulties, yeast biotechnologists have redirected their attention to non-genetic engineering techniques. Researchers want to modify yeast phenotypes for industrial uses without using recombinant technologies by taking advantage of the intrinsic genetic variety present in yeast populations and using techniques like chemical or UV mutagenesis and adaptive evolution. This study examines the methods and tactics used in non-genetic engineering techniques for isolating and producing new yeasts specifically suited to industrial requirements. Scientists want to promote yeast variety and take use of its potential for creative responses to industrial problems by looking into these other paths [5], [6].

How to Produce Novel, Non-GM Industrial Yeasts?

This section examines the variety of non-recombinant methods that scientists might use to isolate or choose better yeast strains for use in industrial applications. In essence, these methods either entail searching for desirable variations or use adaptive evolution to force change in a certain direction. Both strategies, however, are wholly reliant on the genetic variety (also known as variation) of the yeasts that researchers have access to, or on the capacity to generate variation if there isn't much of it now. Since genetic variety in yeast may be accessed or increased without the use of genetic engineering techniques, this chapter's initial focus will be on those methods. Then, using this variety, we will evaluate strategies for screening for and producing desirable phenotypes. The following tactics and procedures are not mutually exclusive, however for the purpose of clarity and convenience, each is presented under a separate sub-heading.

DISCUSSION

Yeast has been a trusted partner in human endeavors like brewing, baking, and winemaking for millennia. Over time, we've uncovered and cultivated a vast array of yeast strains, often inadvertently, by selecting for traits that are particularly advantageous to various industries. These diverse yeast collections offer the reliability, diversity, and novelty required for yeast-based processes and products. However, as genetic engineering techniques have advanced, the potential to further diversify yeast genomes and craft phenotypes tailored for specific

industrial applications has emerged. Despite these advancements, legal restrictions on genetically modified organisms (GMOs), especially in the food and beverage sector, and variable consumer acceptance of GMO products pose significant challenges [7], [8]. Therefore, creating innovative yeast strains with improved industrial characteristics may continue to depend on non-recombinant approaches until consumer attitudes and regulatory landscapes evolve.

This chapter explores these non-genetic engineering strategies, emphasizing the significance of genetic variation in existing yeast populations and the potential for its augmentation. It delves into methods for screening and selecting desired yeast variants to meet industrial needs.

While these strategies are not mutually exclusive, they provide valuable tools for yeast biotechnologists to harness the inherent potential of yeast diversity.

Genetic Variation: The Foundation of Novel Genotypes and Variants

At the heart of any strategy to create novel yeast strains lies the foundation of genetic variation. Charles Darwin and Alfred Russel Wallace introduced the concept of evolution through natural selection, recognizing that genetic diversity within populations is the driving force behind adaptation and evolution. In the context of yeast biotechnology, genetic variation can originate from various sources:

a. Yeast Strain Collections: Existing yeast strain collections house an extensive reservoir of genetic diversity. These collections can be screened for strains with desired traits, making them valuable resources for yeast improvement projects.

b. Wild Isolates: In addition to established strains, researchers can explore natural environments to isolate wild yeast strains. These untamed yeasts may possess unique attributes that can be harnessed for industrial applications.

c. Mutation Induction: Chemical or physical mutagenesis methods, such as exposure to UV radiation or mutagenic chemicals, can introduce genetic changes into yeast populations. These mutations can lead to novel phenotypes, some of which may be industrially advantageous.

d. Hybridization: By crossing different yeast strains or species, hybrid strains with a blend of parental traits can be generated. This approach offers the potential for novel combinations of characteristics.

Screening and Selection of Desired Phenotypes

Once a diverse yeast population is available, scientists employ various screening and selection strategies to identify strains with the desired industrial phenotypes. These approaches enable the isolation of yeast variants that exhibit improved performance in specific applications:

a. Phenotypic Screening: This method involves subjecting yeast populations to specific conditions or challenges relevant to industrial processes. Strains that exhibit superior performance under these conditions are selected for further evaluation and development.

b. Adaptive Evolution: Yeast populations can be subjected to prolonged cultivation under specific stress conditions. Through this process of adaptive evolution, yeasts gradually adapt and accumulate genetic changes that enhance their fitness in the given environment.

c. High-Throughput Techniques: Modern biotechnological tools, such as automated robotic platforms and genetic screening assays, facilitate the rapid assessment of large yeast populations. These technologies streamline the identification of yeast strains with desired traits.

d. Marker-Assisted Selection: Genetic markers associated with desirable traits can be employed to identify yeast strains that carry these advantageous traits. This method expedites the selection process.

e. Directed Evolution: This approach leverages iterative rounds of mutagenesis and selection to engineer yeast strains with enhanced characteristics. It mimics the natural evolutionary process but at an accelerated pace.

Non-genetic engineering approaches for isolating and generating novel yeast strains hold tremendous promise for various industrial applications. By tapping into the genetic variation within yeast populations and employing strategies like phenotypic screening, adaptive evolution, and marker-assisted selection, researchers can discover and develop yeast variants with improved performance, resilience, and adaptability. These approaches not only complement genetic engineering techniques but also offer practical solutions for industries facing regulatory constraints and consumer acceptance challenges associated with GMOs. As we continue to unveil the full potential of yeast diversity, non-genetic engineering methods will play a pivotal role in shaping the future of yeast-based biotechnology [9], [10].

CONCLUSION

The search for innovative strains with specialized industrial uses continues to be of utmost importance in the dynamic field of yeast biotechnology. Although genetic engineering has greatly increased our capabilities, barriers to market and regulatory approval have forced a return to non-recombinant methods.

This study voyage into yeast variety highlights the abundance of genetic diversity that is already present in yeast communities. Researchers may create yeast strains with particular and desired features for various industrial sectors by drawing on this intrinsic variety and using methods like mutagenesis and adaptive evolution.

It is obvious that yeast will continue to play a crucial role as the disciplines of biotechnology and synthetic biology develop.

Scientists can successfully negotiate the complex trade-off between innovation and regulation by embracing both genetic engineering and non-genetic methods, which will eventually advance yeast industrial applications.

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CHAPTER 10

ADVANCEMENTS IN FUNGAL STRAIN IMPROVEMENT FOR INDUSTRIAL BIOTECHNOLOGY

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ABSTRACT:

The creation of strains for industrial production is aided by strategies like genetic engineering and genome editing. However, there is still a substantial amount of potential for strain improvement. The creation of fungal production strains that have the potential to be exploited on an industrial scale will be the subject of this study, which will concentrate on both traditional and contemporary techniques, equipment, and technology. Additionally, the use of genetic engineering methods and expression tools, as well as the utilization of functional genomics, transcriptomics, proteomics, and metabolomics, will be covered. Humanity has used fungus for a variety of industrial purposes throughout history, including the manufacture of food and enzymes. The utilization of microbes, especially filamentous fungus, as adaptable cell factories for generating a variety of valuable substances has undergone a revolution in industrial biotechnology in recent decades. This study examines both established and novel approaches to creating more productive and adaptable fungi. It explores traditional non-genetically modified (non-GMO) strategies, such as adaptive evolution and random mutagenesis, as well as genetic engineering methods, emphasizing their benefits and drawbacks. Additionally, it addresses how crucial omics technologies are in enhancing fungal strains for use in industrial operations. This analysis provides insights into the difficulties and potential in improving fungi, opening the path for developments in the circular bio-economy as the need for sustainable biotechnological solutions rises.

KEYWORDS:

Biotechnology, Genetic Engineering, Metabolomics, Proteins.

INTRODUCTION

Without previous awareness of the underlying biological systems, humans have historically used basic biotechnology to produce things like beer, wine, and bread. However, industrial biotechnology has seen significant transformation in recent years. Microorganisms are frequently used in large-scale industrial processes as cell factories for the production of a variety of substances, including ethanol, organic acids, antibiotics, vitamins, proteins, and enzymes. These substances have a wide variety of industrial applications, including food, feed, biofuels, biochemicals, cosmetics, pharmaceuticals, textiles, pulp and paper, and construction. Bacteria, mammals, and fungus have all been employed extensively in industrial manufacturing processes. We will concentrate on both traditional and contemporary approaches to the generation of fungal generating strains in this study [1], [2]. When it comes to delivering similar systems, filamentous fungus fall behind bacteria and yeasts in development of complex gene expression systems.

Filamentous fungus, however, are good candidates for microbial cell factories. They have a high protein secretion capability, which is a characteristic of their decomposing lifestyle, and naturally create enzymes for the effective breakdown and conversion of many forms of biomass, notably plant biomass. Systems based on fungi offer a number of benefits over other systems, such those based on bacteria. Due to shared crucial mechanisms in gene expression and post-translational modifications with other eukaryotic species, they will be the vehicle of

choice for large-scale synthesis of recombinant proteins of eukaryotic origin in addition to their high-level protein secretion capabilities. For proteins that need complex post-translational modifications, such as protein glycosylation, proteolytic cleavage, or multiple disulfide bond formation, which are essential for the stability and function of functional proteins, fungi, especially filamentous fungi, are utilized as expression hosts. Many times, recombinant proteins may be secreted with great efficiency and purity using yeast expression systems like Pichia pastoris. However, filamentous fungi are said to have a larger secretory capacity than yeast strains. Aspergillus, Trichoderma, and Penicillium are the most popular filamentous fungal species in industry, accounting for more than half of the enzymes now employed in industrial applications. Another significant benefit is the fact that many filamentous fungal producing strains are GRAS (Generally Recognized As Safe)-designated [3], [4].

Although filamentous fungi make excellent candidate cell factories, they may have certain challenges when synthesizing proteins. Filamentous fungi frequently alter their glycoproteins with heterogeneous high-mannose glycan structures, which can have unfavourable effects on the functionality of the resulting protein, particularly for a therapeutic protein, which can adversely affect its pharmacokinetic behaviour and decrease the effectiveness of downstream processing. However, by manipulating the fungal glycosylation process, homogenous and even human-like glycan structures may be produced, solving this issue. Another disadvantage of some filamentous fungal strains is that the secretion of host proteins, which frequently also contain proteases, occurs concurrently with the secretion of recombinant proteins. This could (1) affect the subsequent steps of protein purification and application, and (2) lead to the degradation of the heterologous protein(s). It has been put into practice to create low background host strains in filamentous fungus. However, it takes a lot of work to use mutagenesis to create hosts with minimal background expression. Aspergillus vadensis is one naturally occurring low-protease-producing fungal strain that has been identified as an alternative fungal strain for the generation of heterologous proteins. In any event, filamentous fungi have unquestionably grown to play a significant role in the creation of the bulk of industrial goods generated from microorganisms and have played a crucial role in the development of the circular bio-economy.

Particularly in the food business, fungi have long been utilized to create fermented meals and drinks. Traditional fermentation methods including Aspergillus oryzae producing soy sauce or alcohol from rice (generating sake), Penicilliumroqueforti colonizing blue cheese, and special Penicillium species aging and seasoning salami. The creation of new-generation goods based, for instance, on the manufacturing of single-cell proteins (SCP) has resulted from more recent initiatives to use products produced from fungal sources. 30-45% of fungal SCP typically have a protein content. The protein-rich meal formed of filamentous fungal biomass, known as "mycoprotein," is one of the SCP products that may be used in place of meat. Although filamentous fungi have many potential applications, improving the biosynthetic capabilities of industrially relevant fungal species to produce desired proteins, enzymes, and metabolites in high quantities is one of the most significant challenges of modern biotechnology. Some of these SCP products include the Quorn (mycoprotein of Fusarium venenatum), and PEKILO (mycoprotein from Paecilomycesvariotii).

Strain Reduction Techniques for Industrial Use

There are numerous strategies to improve fungal characteristics (e.g., growth rate, substrate adaptation and utilization, stress resistance, etc.), which may result in higher production yields. Strain development plays a key role in the industrial production of many compounds because it allows an organism to perform a biotechnological process more efficiently. There

are two widely utilized methods for strain improvement: classical and genetic. However, genetic engineering enables a high degree of control over the strain alterations, while traditional engineering does not need a thorough grasp of the molecular foundation of the modified microorganisms. These two distinct methods for enhancing fungi strains are outlined in this section, along with their benefits and drawbacks.

Approaches for Traditional (Non-GMO) Strain Improvement

The fact that classical strain improvement may be used even when there is little understanding of the genetic foundation or metabolic pathways of the producing organisms explains why it has long been considered as the industry's gold standard for fungal strain development. Additionally, organisms created by traditional mutagenesis are not covered by GMO regulations and may be employed in the business immediately. In numerous filamentous fungus, random (physical or chemical) mutagenesis and screening have been accomplished effectively. As a consequence, several of the high-secreting mutants provide ideal strains for certain industrial objectives, such as enhanced synthesis of lignocellulolytic enzymes, citric acid, and bioethanol as well as penicillin overproduction.

Mutagenesis in Physical and Chemical Form

A microorganism is exposed to a physical or chemical mutagen using the fast mutation-inducing approach known as random mutagenesis in order to increase the frequency of mutations above the spontaneous rate. In the past, strains with intriguing properties for industrial applications have been created via the use of random mutagenesis. For the purpose of improving a fungus' strain, physical mutagens like electromagnetic (such as rays, X-rays, and UV light) or particle radiation (such as fast and thermal neutrons, and particles) as well as chemical mutagens like ethyl methanesulfonate, 1-methyl-3-nitro-1-nitrosoguanidine, sodium azide, and nitrous acid are frequently used, either alone or in combination [5], [6]. Atmospheric and Room Temperature Plasma (ARTP), a mutagenesis method based on the radio frequency glow discharge of the atmospheric pressure, has been used more recently. In each situation, the kind of DNA damage the mutagen causes and the response of the cellular DNA repair mechanisms to this damage determine the type of mutations that are generated. For instance, pyrimidine dimers are abundant in far UV. Ionizing radiation, in contrast, causes significant chromosomal damage, while NTG, EMS, and MNNG are alkylating agents and methylating agents, respectively. in atmospheric pressure and room temperature (25–40 °C), ARTP has a better effectiveness in inducing substantial DNA strand breaks than chemical or UV mutagenesis. Its use in fungi, however, is rather restricted. Although random mutagenesis is an effective strategy for strain enhancement, it involves a lot of trial-and-error. Changes are not only targeted at the loci that produce the advantageous alteration, necessitating the screening of several strains for the desired features.

Darwinian Adaptation

Adaptive evolution, sometimes referred to as evolutionary engineering, whole-cell directed evolution, or adaptive laboratory evolution, is a traditional method for strain improvement. It is predicated on the fundamental ideas of genetic diversity (natural and/or artificial) and strain selection that follow.

The method entails maintaining a microbial population's growth under a desired selection pressure. During DNA replication, fitter mutants spontaneously develop from random mutations, and their frequency rises in direct proportion to their fitness. Strain optimization may be carried out without the need for previous knowledge of the genetic modification(s) required to bring about such alterations thanks to the use of natural selection to enrich for

mutants with improved fitness. To increase the genetic variety available for selection, adaptive evolution may also be paired with other techniques like random mutagenesis.

Fusion of protoplasts

Another method for strain enhancement that enables genetic recombination and the creation of hybrid creatures is protoplast fusion. It requires the breakdown of the fungal cell wall, which is typically carried out by certain enzymes. Cell fusion and the absorption of nucleic acids, which are more difficult to accomplish with intact cells, are made possible by the protoplast membrane being exposed. By permitting genetic recombination between the genomes of several parental strains, protoplast fusion creates new strains that are then chosen based on which ones exhibit the desired features.

Genome Reshuffle

Large-scale combinatorial techniques are necessary to meet the need for creating complex phenotypes on a global scale. Genome engineering techniques like as genome shuffling have been shown to quickly enhance strain characteristics. This method combines several mutations in the same cell to produce additive or synergistic effects using multi-parental strains and recursive protoplast fusion. Protoplast fusion is the source of genome shuffling, however they are two distinct technologies. In a conventional protoplast fusion, two cells with differing genetic characteristics come together. It produces a stable recombinant strain that combines the genetic characteristics of the two parents. In contrast, genome shuffling involves many rounds of genome fusion and recombination between different parents in each generation. The final enhanced strains therefore acquire the genetic characteristics of various starter strains. Genome shifting takes time, but it doesn't need costly equipment to be used. Additionally, shuffled strains are exempt from GMO regulations and may therefore be employed immediately in the business [7], [8].

Metabolic and genetic engineering

Genetic engineering has been effectively used to create strains that can (over)produce proteins, enzymes, and other intriguing compounds since the introduction of recombinant DNA technology in the 1980s. Genetic engineering offers for a great degree of control over the strain modification(s), in contrast to conventional approaches for strain enhancement. GMOs have changed several industries over the last few decades, including the pharmaceutical, food, and agricultural sectors as well as medical. However, the use of genetic engineering for strain enhancement is rigorously regulated by an elaborate legislative framework, risk management, and evaluation processes owing to public concern about the effects of GMOs on animal/human health and the environment. Two key legal documents that govern the deliberate release of genetically modified organisms into the environment in the European Union are Council Directive 2001/18/EC and Regulation 1829/2003/EC, which both strengthen and expand the rules for GMO safety assessment by introducing the "one-key, one-door" strategy and guarantee the free flow of safe and healthy genetically modified products on the market. The Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the US Department of Agriculture (USDA) are the three regulatory organizations responsible for GMO regulation in the US. The US approach to regulating GMOs is predicated on the idea that regulation should concentrate more on the characteristics of the finished product than the production process, making US GMO regulation comparatively conductive to their development. Other nations, like Australia, have tight rules governing the import and use of GMOs. These laws include the Commonwealth Gene Technology Act 2000, the Gene Technology Regulations 2001, and the related state legislation. The use of GMOs is regulated quite strictly in South Africa, including confined

usage, trial release, commercial release, and transboundary migration. The Genetically Modified Organisms Act of 1997, as well as a number of other laws imposing additional regulations on GMO-related activities, such as the National Environmental Management: Biodiversity Act, the Consumer Protection Act, and the Foodstuffs, Cosmetics, and Disinfectants Act, serve as the primary pieces of legislation governing this issue. It is obvious that various nations have different attitudes on GMOs, and as a consequence, they have quite varying risk evaluations.

DISCUSSION

In recent years, there has been a lot of interest in designing metabolic pathways for the manufacture of certain substances using the advancements gained in genome sequencing, comparative genomics, and gene cloning. As a result, techniques other than genetic engineering are often needed. Metabolic engineering offers a different and complementary strategy for strain improvement in this situation. Through the manipulation of certain biochemical events or the insertion of genes, metabolic engineering is used to modify biological characteristics on a targeted basis. Additionally, it is crucial for metabolic engineering to have access to genetic engineering tools like expression vectors and transformation methods. The field of metabolic engineering has been evolving constantly. It is regarded as a blend of interdisciplinary fields based on chemical engineering, computer sciences, biochemistry, and molecular biology principles. Recent developments in systems biology, experimental and computational research integration, and synthetic biology enable the application of metabolic engineering at the whole cell level, enabling the most effective design of microorganisms for the production of drugs, cosmetics, and food additives, among other things.

Tools and Techniques for Fungal Transformation for Strain Improvement

A process by which external genetic material is taken up into a cell, allowing for increased production levels, the development of new chemicals, or the control of the synthesis of the desired products, is known as genetic transformation, which is a kind of horizontal gene transfer. This is only possible in fungus by creating effective expression tools and transformation techniques. Since developing an effective transformation technique for many fungal species may be challenging, introducing the needed genetic alterations in the target fungus often poses a hurdle. Nevertheless, all significant fungus groups are capable of transformation, and genetic engineering of these organisms is crucial for both research and biotechnology applications. For filamentous fungus, a number of transformation techniques have been documented to date. These techniques include electroporation, the often-used protoplast-mediated transformation, and the Agrobacterium tumefaciens-mediated transformation.

The number of completely sequenced fungal genomes accessible has significantly expanded due to the rapid advancements in sequencing technology. Along with genome sequence information, related 'omics' approaches (transcriptomics, proteomics, and metabolomics) in combination with bioinformatic analyses provide access to a wide range of potential genes for downstream characterization and incorporation into bioproduction strategies for strain improvement.Proteases, lipases, and other enzymes, as well as those involved in carbohydrate metabolism (CAZy), have been discovered by analysis of the genomes of industrial filamentous fungus. For instance, in T. Re-annotation of the CAZy encoding genes along with gene expression analysis in various culture conditions have demonstrated the significance of several up-to-that-point uncharacterized enzymes and provided additional information on the enzyme sets required for the full degradation of various lignocellulose

substrates. a thorough examination of the T. In particular, hemicellulases and chitinases from the asperellum ND-1 genome showed a distinct enzymatic system. The lignocellulase activities of ND-1 and other fungi were compared, and it was discovered that ND-1 showed greater hemicellulases (especially xylanases) and similar cellulases activities. In addition, despite the closeness of their genome sequences, it has demonstrated substantial variance in the fungal enzyme sets generated by Aspergillus species during growth on complex plant biomass. study of the genetic information from A. Niger's potential as a cell factory is further shown by the numerous genes and gene clusters it has uncovered that are involved in the manufacture of secondary metabolites. We can now get novel natural medications more quickly and affordably because to the advancement of genome sequencing technology, particularly with the introduction of genome mining [9], [10].

Additionally, the application of transcriptomics in conjunction with other technologies enables the creation of strains that produce metabolites effectively. There are 280 CAZy proteins found in Podoscyphapetalodes strain GGF6, a basidiomycete fungus that generates endocellulase, laccase, and other lignocellulolytic enzymes during submerged fermentation. Additionally, bioprospecting transcriptome signatures in the fungus revealed a wide range of proteins linked to the degradation of lignin, pectin, cellulose, and hemicellulose, including two copper-dependent lytic polysaccharide monooxygenases (AA14) and one pyrroloquinolinequinone-dependent oxidoreductase (AA12) that are known to aid in the breakdown of lignocellulosic plant biomass. In A. In Niger, genes involved in the breakdown of galactaric acid were found using a transcriptomics technique, and they were eliminated using the CRISPR/Cas9 system. Consequently, a designed A. The endogenous inhibition of the D-galacturonic acid catabolism pathway allowed the niger strain to generate galactaric acid. Galactaric acid is a component of skincare products and may also be chemically processed to produce polymers for usage in a variety of industrial settings. A comparative transcriptome investigation of the genes involved in the mevalonate pathway, including the gene encoding geranylgeranyl diphosphate synthase, in the taxol-producing Aspergillus aculeatinus Tax-6 and its mutant BT-2 (an enhanced taxol-producing strain) revealed up-regulation.

Methods for Strain Screening

Effective methods for mutation screening are required to identify a strain that has been effectively created. Non-selective random screening, in which randomly selected isolates are tested for the desired characteristics, and rational selection, a technique based on prior knowledge of the metabolism and regulation pathways of a microorganism, allow for targeted identification of the desired organisms. After introducing a mutation, survivors are randomly chosen and evaluated for their desirable features or their capacity to create the desired metabolite. This process is known as random screening. Improved mutants are often found by screening a large number of altered organisms. However, mutants with extremely high yields are less frequent than those with modest gains, making this process labor-intensive, repetitious, and time-consuming. The application of rational selection, on the other hand, enables the faster discovery of a greater number of favourable mutants. A fundamental knowledge of product metabolism and route regulation is needed for rational screening. Chemicals or environmental factors, such as pH, temperature, and aeration, may be added to the medium. The identification of producer strains has been made possible by the use of analogue molecules to the desired metabolites. For instance, the fungi P. chrysogenum and Acremoniumchrysogenum produce the -lactamic antibiotics penicillin and cephalosporin, which are derived from amino acid precursors. Higher production yields were obtained from mutants that were resistant to lysine and methionine analogues. In P. chrysogenum, selection

of mutants with increased penicillin synthesis is made possible by the medium's inclusion of phenylacetic acid, a toxic precursor to penicillin. In A. nidulans, the application of chlorate enables the selection of strains impacted by the niaD gene's nitrate reductase activity. Nitrate cannot be used as the exclusive source of nitrogen by the isolated mutants. Congo red has also been used to identify fungal cellulolytic activity in Aspergillus species. Congo red is used as a marker for -D glucan degradation in an agar medium, which forms the foundation for an efficient and accurate screening test for microbes that break down cellulosic materials.

Due to their superior ability to function as cell factories, filamentous fungi have established a major place in industry as makers of commercially significant proteins, enzymes, and primary or secondary metabolites. Particularly in the food business, filamentous fungus have taken a significant role in the creation of new-generation meals. Systems based on fungi offer a number of advantages over those based on bacteria and yeast when it comes to becoming cell factories. Strain enhancement measures are required to boost their productivity, lower byproducts, and raise their tolerance to process conditions, while many of them are often inefficient enough to produce industrial processes that are economically viable. Since GMO rules that restrict the use of GMO-based technologies do not apply to non-GMO alternatives, their industrial application is the broadest. These, however, are general, tiresome, and time-consuming. A well-established method in biotechnology, genetic engineering of filamentous fungus has overcome many of the drawbacks associated with traditional strain enhancement techniques. Additionally, the implementation of various omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, along with the development of new genome editing technologies like CRISPR/Cas9, has accelerated the development of industrial strains with enhanced production capacities and performance. Interest in enhancing fungal cell factories is rising as a result of changing customer tastes and rising product demand. We will be able to meet the growing demand in the biotechnological sector by improving the present strain enhancement methods, developing new ones, and understanding fungal metabolism better.

CONCLUSION

Due to their extraordinary ability to operate as cell factories for the production of valuable chemicals, fungi have emerged as significant contributors to industrial biotechnology. While conventional non-GMO methods have long been used to enhance strains, genetic engineering has transformed the industry by allowing precise control over the traits of fungal strains. The optimization of fungal cell factories has been hastened by recent advancements in genome editing tools and omics technologies, bringing them into compliance with the needs of contemporary biotechnological enterprises. The ongoing investigation of fungal strain enhancement techniques will be essential in addressing the rising need for environmentally friendly and productive production methods as the global biotechnology landscape changes. Researchers may unleash the full potential of these adaptable microbes by using the power of fungus and the insights from genetics, genomes, transcriptomics, proteomics, and metabolomics, eventually boosting industrial biotechnology in the circular bio-economy.

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CHAPTER 11

NAVIGATING THE LANDSCAPE OF AGRICULTURAL BIOTECHNOLOGY: ADVANCEMENTS, BENEFITS AND CONCERNS

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ABSTRACT:

Agricultural biotechnology has emerged as a potent tool to improve crops and organisms as a result of the field of biotechnology's impressive developments in recent decades. This study digs deeply into the field of agricultural biotechnology, examining its origins in the past and its revolutionary potential. The journey starts with traditional agricultural biotechnology, when disease-resistant crops like wheat were developed via the use of selective breeding. The next step is genetic engineering, which is made feasible by advances in molecular biology and enables precise DNA alteration across a wide range of species, giving birth to genetically modified organisms (GMOs). GMO, which is divisive and often used to refer to organisms transformed via genetic engineering methods, is highlighted in the study along with other terms related to contemporary biotechnology. It clarifies the differences between conventional breeding and genetic engineering, demonstrating how the latter provides unmatched accuracy in sculpting desired features. By facilitating the selection and transfer of certain genes, genetic engineering enables the quick production of crops with better properties including drought tolerance, disease resistance, and increased nutritional value.

KEYWORDS:

Agricultural Biotechnology, Genetically Modified Organisms (GMOs), Genetic Engineering, Nutritional Value.

INTRODUCTION

Biotechnology is the use of scientific methods to change and enhance microbes, plants, and animals in order to increase their value. The branch of biotechnology that involves applications in agriculture is known as agricultural biotechnology. Agricultural biotechnology has been used for a very long time as a means of selecting and developing organisms that are crucial to agriculture. The creation of disease-resistant wheat varieties via cross-breeding several wheat types until the necessary disease resistance was established in a new variety is an example of conventional agricultural biotechnology. The capacity to modify DNA, the chemical building blocks that define the molecular features of living creatures, was made possible by developments in the science of molecular biology in the 1970s [1], [2]. Genetic engineering is the name given to this technique. Additionally, it allows for DNA transfer between more unrelated species than was previously allowed using conventional breeding methods. This technology has now advanced to the point that it is possible to extract one or more particular genes from almost any creaturebe it a plant, a mammal, a bacterium, or a virusand insert them into another organism.

Transgenic or genetically engineered organisms are organisms that have undergone genetic engineering-based transformations. These features of modern biotechnology are described by a variety of additional words that are often used. Although genetic alteration has existed for hundreds if not thousands of years, the phrase "genetically modified organism" or "GMO" is often used. For the reasons discussed later in this publication, controversy affects various issues related to the growth of genetically engineered organisms and their use as foods and feeds. Similarly, foods derived from transgenic plants have been referred to as "GMO foods,"

"GMPs" (genetically modified products), and "biotech foods." While some refer to foods developed from genetic engineering technology as "biotechnology-enhanced foods," others refer to them as Frankenfoods.

Traditional breeding involves somewhat arbitrary cross-making. Although the breeder selects the parents to cross, the outcomes are unpredictable at the genetic level. DNA from the parents randomly recombines, resulting in the bundling of good qualities like insect resistance with negative ones like low yield or poor quality. Traditional breeding practices need a lot of time and effort. Separating unwanted features from good ones requires a lot of work, which isn't always economically feasible. For instance, to breed out undesired traits caused by random genome mixing, plants must be backcrossed repeatedly over many growth seasons. Segments of DNA that code for genes for a particular characteristic may be chosen and individually recombined in the new creature using current genetic engineering methods. Once the gene's coding for preventing- Once the desired feature has been located, it may be transferred and chosen. Similar to this, undesirable features' genes may be silenced. This technology makes it possible to modify a preferred variety more quickly than using conventional breeding methods. Any stage of development, such as in young seedlings in a greenhouse tray, may be used to test for the presence of the target gene influencing the characteristic. Modern biotechnology's accuracy and adaptability make it possible to increase food quality and output more quickly than with conventional breeding methods [3], [4].

The American market for transgenic crops

Although genetically modified organisms have only been used in agriculture for ten years, their commercial application has grown quickly. According to recent estimates, between 60 and 70 percent of food items on shop shelves may include at least a tiny number of crops grown using these new methods. Currently, about a third of the corn and about three-quarters of the soybean and cotton grown in the USA are varieties developed through genetic engineering. In the USA, commercial cultivation of twelve transgenic crops (corn, tomato, soybean, cotton, potato, rapeseed [canola], squash, beets, papaya, rice, flax, and chicory) has been authorized. The most frequently cultivated crops are "Bt" soybeans, cotton, and "Bt" corn. It kills some of the most dangerous insect pests of these crops (Eu-ropean and southwestern corn borers, and cotton bud-worms and bollworms) after they feed on the plant, while beneficial insects are unaffected. Bt corn and cotton have DNA from a naturally occurring insecticidal organism, Bacillus thuringiensis, incorporated into their genomes. The broad-spectrum herbicide glyphosate does not affect glyphosate-resistant soybeans, allowing farmers to eradicate yield-reducing weeds in soybean fields without endangering the crop.

What advantages can genetic engineering in agriculture offer?

Genetic engineering is no different from everything else in life in that it has advantages and disadvantages. Although there has been much conversation about the possible dangers of genetic engineering, there hasn't been any proof from research in the scientific community that these dangers really exist. Beyond the advantages brought about by advancements in conventional agricultural biotechnology, transgenic organisms may provide a variety of advantages. Here are a few advantages that come from using agricultural biotechnology and presently accessible genetic engineering methods [5], [6].

A **higher yield from the crops**

By giving crops traits like greater drought tolerance and disease resistance, biotechnology has helped to boost agricultural yield. Researchers may now choose disease resistance genes from other animals and introduce them into significant crops. For instance, by transferring one of

the virus' genes to papaya to confer resistance in the plants, researchers from the University of Hawaii and Cornell University created two types of papaya resistant to papaya ringspot virus. Since 1998, papaya producers have been able to get seeds of the two kinds, known as "SunUp" and "Rainbow," under licensing agreements. In arid climes, where crops must utilize water as effectively as possible, there are more instances. Many crop types may be made more drought-tolerant by introducing genes from naturally drought-resistant plants. Crop protection technologies are used by farmers because they provide affordable solutions to pest issues that, if unchecked, would significantly reduce output. As previously indicated, genetic engineering has been used to effectively modify crops like maize, cotton, and potatoes to produce a protein that kills certain insects when they feed on the plants. The protein comes from the soil bacteria Bacillus thuringiensis, which has been a key component of several "natural" pesticides for many years.

DISCUSSION

In certain instances, an efficient transgenic crop protection technique may manage pests more effectively and affordably than current ones. For instance, a maize crop that has been engineered with Bt is completely immune to not only the area of the plant where the Bt pesticide has been administered, but also specific pests. In these situations, yields rise as a result of the new technology's improved control. In other instances, a new technology is adopted because it is less costly and offers equal control than an existing technology. There are situations when new technology is not embraced because it is not competitive with the current technology for one reason or another. For instance, organic farmers may not agree with transgenic Bt crops even when they use Bt as a pesticide to manage agricultural pests.

Modifications to food processing

Chymosin, an enzyme made by genetically modified bacteria, became the first food product to be approved by the FDA as a consequence of genetic engineering in 1990. It currently makes about 60% of all cheese manufactured, taking the place of calf rennet in the process. Increased purity, a consistent supply, a cost reduction of 50%, and excellent cheese output efficiency are some of its advantages. New alternatives for enhancing the nutritional content, flavour, and texture of meals are now possible because to genetic engineering. Beans with more essential amino acids, rice with the ability to produce beta-carotene, a precursor of vitamin A, to help prevent blindness in people with nutritionally inadequate diets, and soybeans with higher protein content are some of the transgenic crops currently under development. By increasing the activity of plant enzymes that convert aroma precursors into flavouring chemicals, flavour may be changed. Field tests for transgenic peppers and melons with unproven flavours are under underway. Improved keeping qualities from genetic engineering may facilitate the shipping of fresh produce, provide customers access to nutrient-dense whole foods, and stop rotting, damage, and nutrient loss. Transgenic tomatoes that take longer to soften may be matured on the vine and exported without any damage. Similar alterations to broccoli, celery, carrots, melons, and raspberries are being researched. Using substances whose fatty acid profiles have been altered has also increased the shelf life of certain processed meals, such peanuts [7], [8].

Environmental advantages

When genetic engineering reduces our reliance on pesticides, we have less pesticide residues on food, less pesticide leaking into groundwater, and less exposure to harmful goods for agricultural workers. The transgenic species now accounts for half of the U.S. cotton crop thanks to its resistance to three key pests, which has led to a 15% decrease in pesticide usage globally. Additionally, the U.S. FDA reports that "increases in adoption of herbicide-tolerant

soybeans were associated with small increases in yields and variable profits but significant decreases in herbicide use" (our emphasis).

Advantages for emerging nations

Technologies based on genetic engineering may assist to improve the state of health in underdeveloped nations. Researchers from the Institute for Plant Sciences at the Swiss Federal Institute of Technology inserted genes from bacteria and daffodils into rice plants to create "golden rice," which has enough beta-carotene to satisfy the needs of developing nations with rice-based diets. In places of extreme poverty where vitamin supplements are expensive and difficult to get and where vitamin A deficiency causes childhood blindness, this crop has the potential to greatly increase vitamin intake. Some consumers and environmentalists believe that not enough has been done to ensure that people are aware of the risks associated with using transgenic crops, particularly any possible long-term effects. Some environmental and consumer advocacy organizations have called for the cessation of genetic engineering research and development. Many people encounter a sense of confusion and conflict when reading remarks on how genetic engineering affects our environment and food supply. A "dread fear" that makes one very anxious. Only a little quantity of knowledge or, in certain situations, incorrect knowledge might cause this terror. The problems relating to people's worries need to be addressed since people are now so worried about their health and the sustainability of our planet's ecosystem. Health, environmental, and social concerns may be split into these three categories.

Concerns relating to health

Toxins and allergens

When certain proteins, known as allergens, found in food are exposed to, people with food allergies have a unique immunological reaction. A food allergy of some kind affects around 2% of persons of all ages. Most individuals do not develop allergies to the majority of foods. People with food allergies often only have reactions to one or a few allergens in one or two particular meals. The possibility of introducing allergies and poisons into otherwise acceptable meals has been cited as a serious safety issue with reference to genetic engineering technologies. The Food and Drug Administration (FDA) monitors to make sure that the levels of naturally existing allergens have not dramatically risen over the normal range seen in traditional foods in foods created from transgenic organisms. One of the most important sources of food allergies, peanuts, are being genetically modified to eliminate their allergens.

Bacterial resistance

A characteristic of interest that has been inserted into plant cells may be found and tracked using genes for antibiotic resistance. This method guarantees the success of a gene transfer during genetic alteration.

The use of these identifiers has sparked worries that new bacterial strains resistant to antibiotics would develop. Some opponents of the use of genetic engineering technology have severe medical concerns about the growth of diseases that are resistant to treatment with generic antibiotics.

The danger of transmission between bacteria or between humans and the bacteria that naturally exist in our gastrointestinal systems is far higher than the risk of transfer from plants to bacteria. However, the FDA has encouraged food makers to abstain from employing marker genes that encode resistance to clinically significant antibiotics in order to be safe.

Ecological and environmental concerns

Some opponents of genetic engineering techniques hold the opinion that transgenic crops may cross-pollinate with related weeds, perhaps creating "superweeds" that are harder to manage. One issue is the possibility of glyphosate resistance spreading from related weeds to crops that have developed resistance to the herbicide. Although unlikely, there is a risk that this may happen. However, just because a plant is resistant to one herbicide does not guarantee that it is also resistant to others, thus impacted weeds could still be controlled with other chemicals. Some individuals are concerned that genetic engineering may increase a plant's capacity to "escape" into the wild and cause ecological imbalances or catastrophes. The majority of agricultural plants are unlikely to survive in the wild as weeds because of the severe constraints in their growth and seed dispersion characteristics that prohibit them from surviving for an extended period of time without continual agronomic care.

According to some environmentalists, transgenic crops may have unintended and undesired impacts after they are released into the environment. Although transgenic crops are thoroughly evaluated before being on sale, not all possible effects can be anticipated. For instance, Bt corn generates a highly specialized insecticide that is specifically meant to kill bugs that feed on the maize. However, Cornell University researchers discovered in 1999 that pollen from Bt maize might kill caterpillars of the innocuous Monarch butterfly. In the lab, half of the larvae perished when fed milkweed that had been sprinkled with Bt corn pollen by Monarch caterpillars. However, subsequent field experiments revealed that it is very unusual for Monarch butterfly caterpillars to come into touch with Bt corn pollen that has landed on milkweed leaves or to consume enough of it to cause damage in real-world conditions. Regarding the possible environmental effects of agricultural biotechnology, another worry is if insect pests may become resistant to the crop-protection traits of transgenic plants. There is concern that widespread use of Bt crops would lead to a quick rise in insect populations' resistance. Despite the extensive planting of Bt crops, no evidence of Bt tolerance in the targeted insect pests has been found to yet, despite the extraordinary ability of insects to adapt to selective pressures.

Biodiversity loss

Farmers and other environmentalists are highly worried about the decline of biodiversity in our environment. Similar worries were expressed in the last century with the increased use of conventionally grown crops, which prompted considerable efforts to gather and preserve seeds of as many different types of all important crops. Plant breeders in the USA and other countries preserve and make use of these "heritage" collections. Modern biotechnology has significantly improved our understanding of how genes express themselves and highlighted the value of genetic material preservation. Agricultural biotechnologists are now working to ensure that we continue to have access to the genetic diversity of crop plants that will be required in the future. While transgenic crops aid in ensuring a steady supply of staple foods, specialist crop types and locally produced vegetables seem to be seeing growth in the U.S. market rather than a decline. Therefore, it seems doubtful that using genetically modified crops would have a detrimental impact on biodiversity.

Foods made from genetically modified crops, according to certain consumer advocacy organizations, should have a separate label. Currently, only if these foods vary nutritionally from conventional foods in the USA do they need to be labelled. The majority of farmers worldwide, especially those who grow commodities like maize, green peppers, and tomatoes, purchase new seeds every season. Because seeds from hybrids produced on the farm the previous year will not yield plants identical to the parent, anybody planting hybrid types must

purchase fresh seeds every year. Farmers do not start mango, avocado, or macadamia plants from seed for the same reasonto prevent random genetic variety brought on by open pollination. Many non-hybrid farmers in underdeveloped nations save harvested seeds for sowing the crop the next year. The ability to stop buyers of transgenic agricultural seeds from storing and sowing them has been created. Such "termina- tor" seeds are genetically modified to generate plants with seeds that do not germinate well, coupled with other changes that farmers will accept. This makes it necessary for farmers who would normally preserve seed to buy it if they want to utilize these enhanced commercial cultivars. The crops that have been modified to have different characteristics are also offered alongside non-transgenic counterparts in the USA, for which producers normally buy seeds every year. Despite these mitigating factors, preserving seeds is a common practice among farmers in impoverished nations and among organic producers who do not plant hybrid crops, making this a serious problem for them. Due to the presence of "terminator" genes, these farmers are unable to benefit from genetic engineering's advancements without being entangled in the economic cycle that benefits seed businesses. However, these businesses are unlikely to make investments in enhancing crops if there is no financial motive. This problem is comparable to one encountered by pharmaceutical firms creating novel treatments for human ailments. It is obvious that it is a difficult and polarizing societal topic.

A new product's suitability for growth is mostly determined by the USDA, and any possible environmental effects are examined by the EPA. The FDA has the last say on whether a product is safe for consumption because it cares about consumer protection. However, whether or whether such food is healthy and safe to consume may be the most important consideration. Companies and research institutes must register with the USDA for field testing approval prior to field testing any new transgenic crops [9], [10]. During this time, scientists must take precautions to prevent the discharge of pollen and other plant materials from the examined plants into the environment. The EPA, which has the jurisdiction to control all new chemicals and genetically modified crops, must also approve transgenic crops. Potential effects on nontarget species as well as endangered or threatened species worry EPA. Lastly, transgenic crop-derived foods must pass FDA examination. Currently, if a product's nutritional value or composition considerably changes from that of its traditional counterparts, or if it poses any health hazards, the item must be labelled as transgenic. According to both the National Academy of Sciences and the FDA, foods produced so far from genetically modified organisms are generally just as safe as their conventional equivalents. Maintaining vigilance for possible allergies is the major issue.

CONCLUSION

The use of transgenic organisms should be carefully studied to guarantee that they pose no environmental or health concerns, or at least no more than the use of present crops and practices, say responsible scientists, farmers, food producers, and policy officials. Through the creation of crops with increased nutritional value, pest and disease resistance, and lower production costs, modern biotechnology provides innovative applications of science that may be utilized for society's benefit. If handled correctly and responsibly, genetic engineering, a kind of biotechnology, has the potential to bring about significant advantages. The principles of biotechnology and genetic engineering, the procedures used to create transgenic species, the kinds of genetic material employed, and the advantages and hazards of the new technology should all be presented to society in a balanced manner. However, worries and difficulties accompany the promises of agricultural biotechnology. Allergens and antibiotic resistance are major health concerns, which emphasizes the need of thorough safety evaluations. Environmental issues include possible gene transfer to related species and the

appearance of pests that are resistant. Socio-economic concerns also exist, such as labelling regulations and the contentious "terminator" seeds. It urges the responsible and open use of genetic engineering so that the advantages be realized to the fullest while the hazards are kept to a minimum. The need of well-informed public dialogue and fair evaluation of the difficulties surrounding agricultural biotechnology is emphasized in the study.

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CHAPTER 12

ADVANCING CANNABIS CULTIVATION: INTEGRATING BIOTECHNOLOGY FOR ENHANCED GENETICS AND SECONDARY METABOLITE PRODUCTION

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ABSTRACT:

The adaptable plant Cannabis sativa L. has a long history of use in industrial, agricultural, recreational, and medicinal settings. This paper examines the changing environment of cannabis growing with a focus on the use of biotechnological methods to improve genetics and the generation of secondary metabolites. Based on taxonomic connections or chemotypes, cannabis may be divided into two main groups that are often influenced by the concentration of the hallucinogenic component delta-9-tetrahydrocannabinol (THC). Advanced genetic methods are required because to the rising demand for cannabis products, particularly those with particular cannabinoid profiles. Cannabis has been shown to have around 560 secondary metabolites, with terpenes and cannabinoids making up the majority of its biomolecular make-up. Among the main cannabinoids are cannabidiol (CBD), THC, and cannabichromene (CBC), while new genotypes expressing substances like cannabigerol (CBG) are appearing. Numerous studies have been conducted on the pharmacological characteristics of cannabis, as well as its potential medicinal applications. Despite the growing demand for cannabinoids and cannabis-based goods, growing cannabis is difficult because of the plant's complicated genetics and the associated legal restrictions. Traditional breeding techniques are costly and time-consuming. Additionally, a number of variables, like as genotypes, ambient conditions, and development stages, affect the generation of secondary metabolites in cannabis. Genetic alteration of cannabis is now possible because to biotechnological methods like CRISPR/Cas9, a kind of genetic engineering.

KEYWORDS:

Embryogenesis, Gene Transformation, Genome Editing, Morphogenic Genes, Organogenesis, Polyploidy.

INTRODUCTION

Sativa cannabis L. is a plant in great demand with a long history of industrial, agricultural, recreational, and therapeutic applications. Cannabis may be separated into two primary classes and classified according to taxonomic relationships or chemotypes, however it is often controlled according to the quantity of psychoactive cannabinoids that are created. Anything that has less than 0.3% of the psychoactive compound 9-tetrahydrocannabinol (THC) is often referred to as hemp, whereas plants that generate 0.3% or more are considered to be marijuana. More than 560 secondary metabolites of cannabis are now recognized. Although phenolic chemicals and flavonoids have also been found, cannabinoids and terpenes make up the majority of the cannabis' biomolecules. Currently, more than 115 isoprenylated polyketides known as cannabinoids have been found in cannabis. These compounds are mostly synthesized in the glandular trichomes of female flowers. The three main cannabinoids in the crop are cannabidiol (CBD), THC, and cannabichromene (CBC), although novel genotypes that express additional cannabinoids like cannabigerol (CBG) are already appearing [1], [2].

The pharmacological properties of cannabis have been studied in the past ten years, including its industrial uses in textiles, paper, building materials, cosmetics, and foods, as well as its anti-spastic effects in cases of Tourette's syndrome or sclerosis multiplex and its ability to counteract the negative effects of chemotherapy. Although the market for cannabinoids and cannabinoid-containing products is still relatively young, it is expanding quickly. According to a recent industry analysis, the worldwide market for CBD alone is expected to reach 16 billion dollars by 2025. There is an urgent need to create better genetics and cultivation methods as the demand for these goods rises. In traditional plant breeding, desired features are fixed (selfed) by population assessment, selection, and guided crossings between parent plants having the desired traits. These requirements are challenging to achieve in cannabis because of plant biology (such dioecy) and laws. Cannabis plants are typically dioecious;however, it is possible to induce selfing by placing male flowers on female plants, which will result in feminized seeds. Conventional breeding techniques for cannabis are time-consuming, expensive, and labor-intensive because of these constraints. Cannabinoids and terpenes in particular, as well as genotypes, age of plants, sex, developmental stage, growth and environmental conditions, harvesting time, storage conditions, and cultivation techniques, all have a significant impact on the composition and content of cannabis secondary metabolites [3], [4].

Biotechnological tools (i.e., genetic engineering techniques using transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR)) are well developed and have been used in breeding programs for decades for the majority of crops with this economic importance. But since hemp is strictly regulated, it has a lower market value, and recreational and drug-type cannabis have long been illegal, these technologies are primitive, and many standard methods that are used on other crops haven't been applied to cannabis yet. Cannabis cultivation is developing into a large-scale industry on par with that of other important crops as a result of recent changes toward the legalization of cannabis for medical and recreational uses in many jurisdictions. The need for contemporary technology for genetic modification is rapidly rising along with the rise of legitimate commercial providers. We contend that it is time for a paradigm shift toward improving cannabis genetics through genetic engineering, even though cannabis biotechnology is still comparatively new and in its infancy. With the development of affordable large-scale sequencing technologies (such as next-generation sequencing (NGS)) and the growing body of candidate genes for traits of interest.

DISCUSSION

NGS technologies have recently been used to extract the whole genomic and transcriptome information of cannabis. Cannabis NGS data may be used with powerful molecular methods like DNA barcoding to track chemotype inheritance, determine sex, and discover genetic variety. To find undiscovered secondary metabolites of cannabis, this data may also be used with metabolomics and proteomics. Based on the NGS data in cannabis, it is possible to map unknown and wild populations using restriction site-associated DNA sequencing (e.g., genotyping by sequencing (GBS)), perform more rapid and precise transcriptome analyses to find important enzymes and genes in the biosynthetic pathway of secondary metabolites, and interpret targeting-induced local lesions in genomes (TILLING) populations. Above all, NGS-derived data make genetic engineering techniques in cannabis more accessible [5], [6].Cannabis micropropagation and breeding are based on in vitro tissue culture methods, such as callus and cell culture, de novo regeneration, and hairy root culture. Cannabis may be cultivated in vitro, and genetic engineering approaches like as genome editing and Agrobacterium-mediated gene transformation can be used to produce novel genotypes and

control the synthesis of secondary metabolites. Although traditional genetic engineering techniques can change the production of some secondary metabolites (such as Agrobacterium-mediated gene transformation and A. rhizogenes-mediated hairy root cultures), it appears that the CRISPR/Cas9 system has greater potential than these techniques to introduce new germplasms and accelerate secondary metabolite production in cannabis. Therefore, to assist meet the needs of producers and consumers, biotechnological technologies may be used to generate superior genetics. All currently used in vitro propagation and genetic engineering techniques for cannabis, as well as other methods that may be applicable, have been highlighted and discussed in the current review. These methods include developing new culture media, machine learning algorithms, and morphogenic genes that can improve secondary metabolite yield. Different approaches' guiding concepts, advantages, disadvantages, and safety issues have also been discussed.

In vitro Cannabis Culture

The foundation of the majority of biotechnological instruments is in vitro cultivation. Numerous procedures entirely rely on in vitro culture methods, including micropropagation, in situ and ex situ conservation, cell culture, Agrobacterium-mediated gene modification, and polyploidy induction. Additionally, plant cell and tissue culture are a reliable tool for evaluating the synthesis of secondary metabolites and the signalling of endogenous phytohormone metabolism in many plants. In vitro culture methods are effective for growing plants, as well as for creating designed biomolecules and starting synthetic biology projects. These findings imply that the manufacture of cannabinoids is entirely dependent on complex gene regulatory networks and tissue- and organ-specific development, which can only be effectively synthesized by trichomes, which are most prevalent in specialized floral tissues. For the production of various secondary metabolites such terpenes, polyphenols, lignans, and alkaloids, cell suspension cultures may yet hold promise. For instance, Gabotti et al. observed that utilizing a methyl jasmonate elicitor in conjunction with tyrosine precursor boosted the activity and expression of tyrosine aminotransferase (TAT) and phenylalanine ammonia-lyase (PAL) in cannabis cell suspension cultures. Additionally, certain aromatic substances were discovered, including 4-hydroxyphenylpyruvate (4-HPP). This is significant since isolated cannabis flavonoids have been shown to be very physiologically active.

Improvement Techniques for in Vitro Culture Procedures

Despite recent improvements in cannabis in vitro cell and tissue culture, effective cannabis regeneration is still a major barrier to using biotechnology to enhance cannabis, and the species is usually regarded as being somewhat refractory. Genotypes, the type and concentration of PGRs, the size, age, and type of the explant, the gelling agent, the carbohydrate sources, the type and concentration of macro- and micronutrients, the type and concentration of vitamins, the type and concentration of additives (casein hydrolysate, nanoparticles, phloroglucinol, activated charcoal, etc.), the pH of the medium, the type and volume of the vessels, the volume of the medium per culture However, the majority of research have focused on how PGRs, explant types, and genotypes affect cannabis micropropagation, with limited knowledge of many other aspects.

Somatic embryogenesis and haploid production techniques might be developed or perhaps obtained as a consequence of research into other elements. On the basis of the previously described elements, new computational methodology like machine learning algorithms, and novel genetic engineering techniques, various interesting ways for enhancing in vitro culture procedures have been highlighted in this section [7], [8].

The complexity of the many variables and their interactions with one another makes it costly and time-consuming to create a de novo medium and optimize these many elements for particular uses. In order to create regeneration protocols, new methodology, such as new computational techniques (i.e., machine learning algorithms), are required. Artificial intelligence models and optimization algorithms offer a complementary perspective for calibrating in vitro protocols because they can quickly and efficiently identify the best options for a given genotype, explant source, plant growth regulators, medium composition, and incubation conditions. The prediction and optimization of several in vitro culture processes, including shoot proliferation, callogenesis, somatic embryogenesis, secondary metabolite generation, and gene transformation, have recently been effectively accomplished using various machine learning methods. So, it can be said that using machine learning algorithms in conjunction with an experimental approach is a strong and effective way to create a cannabis-specific protocol.

It has been shown that mechanical injury brought on by brushing tissue surfaces dramatically boosted micropropagation. Although there are no data on the impact of wounding on cannabis micropropagation, callusing has usually started at wound sites based on our findings, and tissue wounding may be a potential strategy to enhance cannabis plant regeneration. Improvements in in vitro organogenesis by tissue injury occur in three stages: (i) certain signals connected to tissue damage drive organogenesis; (ii) as a consequence, endogenous phytohormones increase; and (iii) cell fate change. Another interesting method that may be employed for cannabis micropropagation is thin cell layer cultivation. There are no reports of the use of thin cell layer culture in cannabis, despite the fact that this technology has been utilized in a variety of resistant plants including Hedychiumcoronarium, Withaniacoagulans, and Agave fourcroydes. In this technique, a small layer of tissue is chosen as the explant, resulting in intimate contact between damaged cells and the medium's composition and ultimately improving regeneration.

The use of bioreactors (such as continuous immersion and temporary immersion) for the micropropagation of cannabis and the investigation of plant growth is recommended. These tools can be used to get beyond the cannabis genotypes' resistance to proliferating, rooting, and acclimating. They may also be utilized to lower the price of extensive propagation. Cannabis plants are increasingly being grown in bioreactors, and this culture method typically leads to an improvement in the physiological condition of plant propagules. It also makes photoautotrophic propagation easier.

Another method that might assist in reducing cannabis regeneration constraints is the use of morphogenic genes. Methods to increase the efficiency of gene transformation. Cannabis is a dioecious species having distinct male and female plants, and the most valuable output is the female flowers that have not been fertilized and are seedless. It is difficult to self-pollinate plants to create inbred lines for F1 hybrid seed production, and producers are unable to have pollen-producing plants in their production facility. Plants also need to be unfertilized for accurate phenotyping, which complicates breeding strategies. In vitro methods for the creation of homozygous double haploids for F1 hybrid production may be thought of as a reliable answer to these problems. A variety of techniques are used for in vitro haploid generation, including extensive hybridization-chromosome elimination, parthenogenesis, gynogenesis, and androgenesis. Even though there are no reports of haploid creation in cannabis, haploid production procedures seem to be necessary for future genetic engineering research. Recently, a powerful technique for creating haploid plants was discovered: knockdown and/or elimination of the centromere-specific histone H3 (CENH3) gene. This gene binds spindle microtubules to chromosomal centromere regions. For instance, the

CENH3 gene was effectively knocked out in maize genotypes using the CRISPR/Cas9 technology to create haploid inducer lines by Wang et al. and Kelliher et al. It seems that such techniques are highly helpful for producing haploid cannabis plants, however before this is possible, reliable gene transformation and plant regeneration systems must be created.

For enhancing secondary metabolite synthesis and altering the chemical profile, a combination of polyploidy induction and CRISPR/Cas9-equipped Agrobacterium rhizogenes-mediated hairy root culture might be thought of as a reliable method. This method has recently been used to knockout Salvia miltiorrhiza's SmCPS1, a crucial gene in the tanshinone biosynthesis pathway, SmRAS, a key gene in rosmarinic acid biosynthesis, and DzFPS, a crucial gene in farnesyl pyrophosphate biosynthesis. It seems that this method may be used to solve the issues with cannabis cultures with hairy roots.

Variation in Somaclon

The majority of earlier research on cannabis tissue culture have concentrated on enhancing culture conditions to raise the cannabis micropropagation rate. The ideal circumstances for in vitro reproduction may not be the best for maintaining the genetic integrity of the regenerated genotype. In fact, in vitro circumstances such medium composition, PGRs, high humidity, number of subcultures, duration of the culture period, temperature, light quality, and light intensity might ultimately lead to a multitude of physiological and developmental abnormalities in the micropropagated plants. Any phenotypic variation seen in micropropagated plants is referred to as "somaclonal variation" Somaclonal variation is produced by chromosomal mosaicism, spontaneous mutation, or epigenetic controls such DNA methylation, RNA interference, and histone modification (including acetylation and methylation of histones).

Polyploidy's Benefits for Breeding Programs

One of the main processes of speciation is thought to be the natural creation of polyploids. Modern breeders may be able to take advantage of the enhanced heterosis that polyploidy in nature produces. Producing seedless triploids is one of the main advantages of polyploidy. Crossing two individuals with various ploidy levels results in seedless plants. To do this, a tetraploid and a diploid plant are often crossed. The pairings separate properly in diploid and tetraploid animals because they both have even sets of chromosomes. In the mother, the two gametes combine to form a triploid (2n = 3x) embryo. The triploid embryo is capable of normal cell division and is viable. Triploids' seedless mechanism modifies meiosis to prevent the production of viable gametes. The generation of seeds is halted by the triploid plant's inability to create live gametes. Cannabis plants grown without seeds are very beneficial since studies have shown that plants with seeds produce fewer secondary metabolites. Commercial businesses are particularly interested in this when manufacturing shifts outside [9], [10].

Polyploidy's effects

Studies looking at the physical characteristics of tetraploid hemp-type cannabis have shown variations from diploid plants in leaf width, stomate count, and stomate size. Tetraploid flowers had a diameter that was more than twice that of control flowers, and their leaves were 47% bigger than diploid ones. Tetraploid plants had stomates that were twice as long as diploid plants' stomates, but their density was lower. The tetraploid plants' above-ground shoot weight was approximately two times more than that of the diploids. Tetraploid individuals are shown to have bigger mesophyll cells and less intercellular space at the cellular level.

Additional Metabolites

At greater ploidy levels, the production of many species that generate secondary metabolites has risen. Vetiveriazizanioides L., a polyploid plant, has been shown to produce secondary metabolites with increased output. Nash in comparison to its diploid equivalent. This species generates aromatic chemicals useful to the fragrance industry, and when polyploidy was induced, production of these compounds rose by over 62%. It's important to maximize the production of cannabis' secondary metabolites, which are becoming into a marketable good. Recent research suggests that while polyploid cannabis plants produced more CBD than diploid controls, they produced less THC overall. When compared to the control, the polyploid individuals in hemp generated 50% less THC in the female flowers on average, but more than three times more CBD in the female leaves. This research used a cannabis strain called hemp, which is developed to produce little secondary metabolites. The lower THC levels seen in polyploid cannabis plants may be caused by duplication of certain harmful recessive genes. The whole process might be blocked by a harmful allele that affects a crucial enzyme in the metabolic route of cannabis production. Cannabinoids and other secondary metabolites are highly reliant on the existence of chemical precursors and enzymes.

Polyploidy and Future Prospects

According to the available literature, very little research has been done in the area of polyploidization in cannabis, with very minor morphological and chemical variations. It should be highlighted that the present research only examines the first generation of artificially generated autotetraploids and does not analyze several agronomically significant features. It is important to note that despite having twice as many chromosomes, tetraploids do not have more distinct alleles than diploids. To completely comprehend the impacts of tetraploidy with higher allelic variety, further research is required, including crosses of distinct tetraploids. While it's feasible that tetraploids' first generation resembles that of their diploid ancestors, later generations could exhibit distinctive characteristics that are beneficial to current breeding operations. Regardless, the cannabis business has a lot to gain from the usage of tetraploids to create seedless triploids. Additionally, ploidy engineering may be used to cannabis to alter terpene composition, the CBD-to-THC ratio in hemp, enhance biomass, and produce new cannabinoids.

Cannabis Genetic Engineering Techniques

In order to introduce key features (such as herbicide resistance, pro-vitamin A production, insect resistance, etc.) into vital crops, genetic transformation, which enables the introduction of foreign genes, has been widely employed for decades. Major crops like wheat and rice have recently been modified using CRISPR/Cas systems. Creating gene-transformation and/or genome-editing methods for cannabis is crucial for understanding gene functions as well as for altering horticultural characteristics, growth morphology, and stress tolerance. Cannabis may be grown through de novo meristem induction, virus-assisted gene editing, and gene transformation systems mediated by Agrobacterium and Biolistic. Cannabis has not yet been the subject of genome editing or biolistic-mediated gene transformation, which employs particle bombardment to deliver the gene into the plant. On the other hand, several research have looked at cannabis gene change caused by Agrobacterium.

CONCLUSION

There is an urgent need for improved biotechnological methods to improve cannabis genetics and secondary metabolite synthesis as the cannabis market expands and diversifies. Due to legal constraints, cannabis's dioecious nature, complicated genetics, and breeding limits,

traditional breeding techniques are limited. Promising solutions to these problems include in vitro culture techniques, genetic editing tools like CRISPR/Cas9, and other cutting-edge tactics. Optimizing in vitro growth conditions and secondary metabolite production has a lot of promise thanks to the use of morphogenic genes and machine learning techniques. Although it has inherent difficulties, polyploidy induction offers a way to alter cannabis plants to have certain features. To increase the synthesis of secondary metabolites, polyploidy may be used to create seedless triploids. In conclusion, the use of biotechnology to the growing of cannabis represents a paradigm change that has the potential to transform the market. This transition is being driven by the accessibility of large-scale sequencing technology and the expanding understanding of candidate genes for desired features. The use of modern genetic modification methods is essential as cannabis becomes a widely grown crop in order to satisfy the needs of growers and consumers, improve cannabis genetics, and increase the production of useful secondary metabolites.

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