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Biotechnology and Molecular Breeding in Cotton and Rice

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Sources:
1. Fatima T, Jan A, Husnain T, Riazuddin S. Initiation and cryopreservation of cell suspension of rice basmati varieties. Pak. J. Biol. Sci. 2002;5:679-82.
2. Yasmeen A, Kiani S, Butt A, Rao AQ, Akram F, Ahmad A, Nasir IA, Husnain T, Mansoor S, Amin I, Aftab S. Amplicon-based RNA interference targeting V2 gene of cotton leaf curl Kokhran Virus-Burewala strain can provide resistance in transgenic cotton plants. Molecular biotechnology. 2016 Dec;58:807-20.

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About this Book

This book provides a thorough exploration of cutting-edge advancements in cotton and rice cultivation, with a strong focus on the role of genetic engineering and molecular biology in enhancing crop yield, quality, and resilience. Each chapter discusses the scientific foundations and practical applications of key genetic, physiological, and biochemical pathways essential for improving these globally important crops.

In *Chapter 1: Cotton*, the book examines cotton's genetic and hormonal pathways, covering advancements in biotechnology and molecular breeding. It highlights the role of key genes, including SuS (Sucrose Synthase) and GhEXPA8, in fiber quality and yield. From genetic transformation techniques, such as Agrobacterium-mediated transformation, to an analysis of enzymatic roles in sucrose metabolism, this chapter reveals how genetic and physiological interventions can enhance cotton's fiber strength, insect resistance, and overall agronomic performance. Discussions also extend to actin dynamics and their effect on cotton fiber quality, making this chapter a valuable resource for understanding the cellular mechanisms that drive cotton improvement.

Chapter 2: Rice addresses the global significance of rice and introduces innovative strategies for enhancing its resilience and productivity. The book explores genetic approaches for combating rice yellow mottle virus (RYMV), including the role of microRNAs in plant responses to biotic stress. This chapter further examines Bt gene usage for insect resistance, challenges in field efficacy, and biosafety of transgenic Basmati rice lines. Essential topics such as cryopreservation techniques, transgene detection, and marker-assisted selection are discussed, along

with agronomic traits like lodging resistance and maturity. With insights into field performance and environmental considerations, this chapter provides a view of rice biotechnology and its future potential in sustainable agriculture.

Contents of this book are majorly based on the different studies conducted by the editor and his working groups over a period 30 years.

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Table of Contents

Chapter 1: **Cotton**

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Introduction to Cotton

Cotton is a member of the genus *Gossypium*, part of the Malvaceae family, and is considered to be the world's largest textile fiber crop. For thousands of years, it has been utilized in garments, paper products, cottonseed oil, and many other uses. This multifunctional crop is grown in more than 80 countries and gives about 20 million tons yield annually. Of the approximately 50 species of Gossypium, only four are commercially cultivated for their spinnable fibers: *Gossypium arboreum* and *Gossypium herbaceum*, which are diploid (AA), and *Gossypium hirsutum* and *Gossypium barbadense*, which are tetraploid (AADD). The former two species have origins in Asia, whereas the latter two originated in America. American cotton is thought to have developed from polyploidization events involving Asian *G. herbaceum* and its closeness to *G. raimondii* (DD).

Cotton is one important source of natural. Two of the most important characteristics in the textile industry are fiber length-throughout and maturity, since these two factors ultimately decide the spinning quality. Cotton fibers offer the most relevant model for the investigation of the successive stages of cell growth. Genetic transformation in plants is a versatile tool employed in the alteration of attributes, supplementing breeding programs, overexpressing genes, and physiological and biochemical studies. This technique has been identified as one of speed, precision, and lower costs compared to the conventional methods of breeding. Genetic engineering for the improvement of cotton fiber quality offers a promising economic

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approach towards the desired changes in the shortest time period. Several key genes have been identified as participating in the cotton fiber initiation and development process, attesting to the importance of such genetic determinants for the improvement of fiber quality.

The most important source of native cellulose fibers for the production of textile goods is cotton, which is the fundamental raw material. The boll is a fruit that is obtained from the cotton plant. It is composed of cotton seeds and cotton fibers that are soft and fine. Cotton is one of the most important crops for the global economy, and *Gossypium hirsutum L*. is responsible for producing up to more than 90 percent of the entire fiber that is produced from cotton. Cotton fibers are thought to be seed hairs since they originate from the epidermal cells that are found on the surface of the ovular gland. Mature cottonseeds undergo a process of bursting open, resulting in the formation of soft masses of bolls. These bolls are composed of unicellular structures that are extremely long (30-40) and thin (15 µm) and are derived from the epidermal cells of the outer integuments of cotton ovules. The growth of cotton fiber occurs in four distinct stages, which are referred to as fiber initiation, fiber elongation, secondary cell wall biosynthesis, and fiber maturity. These stages are also known as the stages of development.

Very mature fibers have strong cell walls, producing coarse, thick yarns that are unacceptable in industry, whereas extremely immature fibers are so fine that they cause nep production during spinning. Also, long fibers are better than short ones since the necessary number of twists cannot be achieved with small ones (also known as fuzz fibers). An increasing number of technologically advanced and gene-level genome exploration tools have made it possible to meet the industrial need for higher-quality, naturally occurring fibers. Additionally, new opportunities for improving cotton fiber quality with alien genes have emerged due to advancements in genome engineering and gene transformation science. Improving certain features and speeding up the process of getting the desired breeding results are two potential uses for transgenic expression. Given the intricate nature of achieving

optimal cotton fiber quality, which is influenced by multiple factors, it is reasonable to believe that the cellulose biosynthetic pathway is linked to one of the key characteristics. Earlier attempts to introduce this feature into cotton using transformation methods with silkworm fibroin genes showed promise. Transforming the acsA and acsB genes in cotton has been shown to increase fiber length and strength in previous studies. There are distinct developmental stages that impact the maturity and staple length of cotton fibers in different ways. Approximately 60–80% of the plant's entire glucose needs are met by the subtending leaf, making it the primary carbohydrate source for the developing bolls. The subtending leaf, by influencing sucrose metabolism, is thus an essential component in cotton's yield, boll count, fiber formation, and growth.

When fibers reach maturity then these phases overlap significantly. Different cotton species have different processes for developing their fibers; for instance, lint and fuzz fibers in *G. hirsutum* develop at different times, before and after anthesis, respectively. Within a consistent time, frame of 45-50 days, pollinated cotton flowers turn into bolls. The development of a boll consists of three distinct stages: swelling, filling, and bursting. The development processes of the epidermal cells of an ovule yield fibers; one such product is cotton fiber, which finds its way back into the textile industry. There is an immediate need to enhance the quality of cotton fibers in response to the rising demand for textile production and the accompanying demand for higher standards. The fiber's quality metrics could be affected by its developmental changes. Cotton fibers can have their length, micronaire value (which indicates maturity and fineness), and fiber strength changed. An updated understanding of cotton fiber growth and development is provided by the molecular genetics of multigenic families, which offer fresh insights on the transcription and expression signature. During specific phases of development, genes' functions and interactions with one another have corresponding effects. Different stages of fiber cell development are associated with high and low expression of major and minor isoforms of gene families. It enhances fiber cell expansion by upregulating expansin gene

expression. Differential expressions of several genes occurs during the whole fiber formation process.

Advancement through biotechnology and molecular breeding

Over the past century, classical breeding has significantly enhanced yield and fiber quality. However, through classical approaches, the accomplishment of certain crucial characteristics related to the textile industries has not been achieved with regards to fiber strength and length, water absorbency, dye-uptake, thermal property, and wrinkling and shrinkage resistance. This is often due to incompatibility among species and lack of availability of traits. Biotechnology has, hence, overcome such obstacles through the provision of desired genes into cotton and other valuable plants from other organisms' delivery, even synthetically produced. It holds tremendous promise for overcoming restrictions enforced by traditional methods of breeding. Staple length of cotton is a key determinant of cotton fibre quality and its textile value. There is greater the understanding of the molecular mechanisms and hormonal control underlying fibre initiation; hence, the more helpful the fibre elongation and quality can be improved. One cell type may well be regulated by more than one hormone, and conversely, one hormone may be responsible for more than one type of tissue. Although these signaling molecules are simple, they can target certain types of transcription factors, which are very key regulators in the process of gene expression.

Excellence in cotton fibre elongation is paramount for the success of the cotton textile industry that depends on the collaborative effort of breeders and biotechnologists for fibre quality improvement. Efforts have until recently been hindered by fragmented information about the physiology and molecular control of fibre development. It thus created a gap between plant geneticists and plant physiologists that has resulted in the scarcity of crucial information which is successfully needed for the enhancement of fiber quality. Examples of diverse case studies, along with research findings in physiology and molecular characterization of fiber development, will be instrumental in the

advancement of cotton fiber improvement in the times to come. It will enable biologists to integrate this approach with the physiological one in developing an all-rounded understanding of the molecular and physiological mechanisms involved for an optimum effect, increasing staple length as well as micronaire value. Rapid adoption of technology is attributed to significant improvement in productivity, environmental sustainability, economic viability, health benefits, and social advantages that are being realized by the large and small farmers, consumers, and society at large from these crops, both in developed and developing countries.

Physiological and Metabolic Pathways

In most crop production systems, seeds are considered to be the major harvested yield product, and their potential number is set during anthesis. Consequently, most biotic and abiotic stresses, such as deficiencies in nutrient supply or unfavorable environmental conditions, have a direct effect on negative seed set due to induced seed abortion and hence cause yield losses. Approaches to reduce seed abortion are indispensable for avoiding heavy losses in seed crops; hence, the seed development physiology and genetics are an issue of great importance. These will involve incorporating genetic gains into sustainable agriculture; thus, it is the development of cotton varieties that are tolerant to abiotic stresses while maintaining excellent fiber quality. Advanced biotechnology permits the transformation of cotton plants to express genes thought to have good tolerance for stress, which can lead to higher growth and yield under stressful conditions. Another interesting direction of research is the interplay between hormonal signaling and sugar metabolism. Similarly, auxins and gibberellins have long been known to control processes regarding cell elongation and division. The interaction was complex: sugars could modulate hormone responses and further influence fiber development. The crosstalk study from these signaling pathways may thus unveil new opportunities in the improvement of cotton fiber traits.

While growing, cotton bolls and seeds use phloem unloading to actively absorb assimilates. To facilitate the development of fiber cells and cellulose synthesis in the seed coat epidermis and embryo growth in the filial tissue, it would direct the imported assimilates in the appropriate directions. In order to maximize cotton output and fiber quality, it is crucial to understand the physiological processes and metabolic pathways of sucrose metabolism, which are highly involved in its translocation. Thus, it is crucial to keep researching genetic determinants, hormone regulation, and environmental conditions because of the intricate interplay they will play in the future of cotton production. Although cotton production is one of the most dynamic industries, there is hope for overcoming pest control issues and improving fiber quality with the use of transgenic technology. The development of pest-resistant cotton cultivars that also exhibit acceptable fiber quality attributes is crucial for the establishment of a long-term, financially viable cotton sector. Therefore, the future of cotton production and its role in agricultural sustainability and economic development will be shaped by studies that aim to understand the environmental, physiological, and genetic factors that affect cotton fiber quality. The role of sugars as signaling molecules that affect the activity of promoters and transcription factors involved in fiber production is crucial to this process. During the crucial stage of secondary cell wall development, sugars and cellulose significantly affect lint length and strength. The process of cellulose synthesis begins once primary wall development is complete, and it is necessary to deposit cellulose during secondary wall synthesis. The maturation of dehydration-resistant limit fibers is facilitated by the development of secondary cell walls, which in turn stimulate an increase in cellulose synthesis. It is entirely dependent on this technique that the cotton fiber is of high quality.

The long cotton fibers developed by the treatment with GA had the features of thicker cell wall and higher dry weight per unit cell length. These results confirm that the hormonal pathways are of great importance in the genetic regulation of fiber quality. Information on how genetic and hormonal control interacts is very useful in plant

breeders and biotechnologists involved in improving cotton fiber. Biologically speaking, mature cotton fibers are primarily made up of cellulose along with some minor component materials such as proteins, water, hemicelluloses, pectins, waxes, mineral substances, organic acids, sugars, and pigments, which together account for the high strength and appearance of the fiber. With the use of knowledge derived from studies in fiber biochemistry, many genes predominantly expressed in cotton fibers have been isolated and characterized. The cell wall matrix in plant cells can be stiffened, structurally adding more strength due to covalent bonds between the polysaccharides, lignins, and proteins. Cell proteins are believed to form complexes with the cellulose microfibrils, which bonding can similarly act inside the cells of fibers. It has been estimated that thousands of genes are involved in the development of cotton fibers through differential screening of cDNA libraries, which can be used for improving cotton fibre quality.

Genetic and Hormonal Pathways

The transcriptional factors identified so far include MYB, zinc finger proteins, and basic leucine zipper proteins, which have been considered to correlate significantly with ACO and ABP hormones in cotton fiber initiation. The differential gene expression that controls the assembly for crystalline cellulose, which is regulated by ethylene and RLK signaling in developing fibers, has also been shown to be differentially regulated across various cotton lines. Improvement of crop yield is still the major goal for cotton-producing countries; one of the best ways to achieve this involves the development of increased fiber number per developing seed. Information from available literature indicated that plant hormones are influential in the development and growth of cotton fibers. Application of exogenous Gibberellic acid, for instance, has been reported to not only enhance the length of the fibers but also to bring about significant enhancement in cell wall thickness.

The environmental factor, such as low temperature, also exerts significant effects on fibre properties. Low temperatures have been

reported to retard the rate of cellulose biosynthesis in its secondary cell wall formation stage. Furthermore, low temperatures reduce the axial growth rate of fiber elongation in early developmental stages. Besides, activity of enzymes—especially peroxidases—and presence of proteins such as hydroxyproline-rich glycoproteins (HRGPs) in the cell wall may be critical factors affecting the characteristics of fibers. Peroxidases have been assumed to take part in cell wall rigidity and to facilitate the elongation of plant cells by breaking up intra- and intermolecular cross-linkages of tyrosine residues thus, producing isodotyrosine residues of HRGP that result in the rigidification of the cell wall. Thus, it has been assumed that a modification of the expression level of both peroxidases and HRGPs might modify fiber properties. Another direction of research is to understand the involvement and contribution of phytohormones toward fiber characteristic alteration. Various studies discussed new developments on the identification of genes, transcription factors, and their putative functions during different stages of cotton fiber development. An integrated view of plant hormones and genetic factors presented in this review may provide a basic framework for future research in this area.

Research using the *Calotropis procera* expansin gene has shown an increase in fiber length. Increased fiber length and improved micronaire values have also been observed with overexpression of genes such as GhActin 1, HOX3, GhWlim5, and PIP.. Therefore, the research study aimed to adopt a codon-optimized SuS gene into a non-transgenic local cotton cultivar using an Agrobacterium-mediated transformation protocol. The studies on the T2 generation considers inheritance patterns of Bt genes in relation to their efficacy against *Heliothis armigera* in transgenic cotton plants, showing key information on genetic stability and other agronomic performances of these lines. The inheritance patterns have been observed as a 3:1 ratio in some of the transgenic lines, indicating the possibility of Mendelian inheritance. More importantly, non-Mendelian segregations were also found, indicating that stable transgene expression can be a problem in the early stage generations. This gene segregation inconsistency will

definitely retard the selection of homozygous lines in successive generations. Indeed, it has been observed that this initial instable generation may often be unstable and, after continued selection and resistance screening, may finally result in stabilization over time.

The morphological evaluations conducted on the transgenic lines suggested that both an upside and downside were associated with genetic transformation. Some lines of those overexpressed the non-transgenic controls for average bolls per plant, indicating a probable increase in productivity. The same lines had lowered plant height-an important observation, as the plants of short stature may have some advantages such as susceptibility to lodging under field conditions. This result is consistent with the aims of breeding programs for robustness and productivity. Perhaps this is one explanation as to why height has decreased; this could be related to an increase in boll load and physiological knowledge of what happens in the plant due to the transgenic modification.

Even more research into the critical components of metabolic and functional pathways during fiber growth is required. A deeper comprehension of the genetic consequences for features that promote yield and fiber quality can be achieved through comparative research of this kind. Since they were first introduced to the market, the total area dedicated to growing genetically modified crops has been rising. Many nations have reached consensus on the safety of genetically modified (GM) food and feed, as well as on the lack of any discernible impact of GM crops on commercially available transgenic crop biodiversity. Cotton plants are highly protected from a number of harmful lepidopteran insect pests by means of genetically engineered toxins derived from *Bacillus thuringiensis* (Bt). Cry1 proteins are essential components of integrated pest management techniques created to control insect resistance because of their unique action mechanisms and their selectivity to Lepidoptera. From cotton to tobacco, many cry genes have been expressed in plants. After taking root in America, the Bt cotton revolution spread throughout the globe, touching down in countries like India, China, Mexico, South Africa, and

Colombia. Time and again, biotech crops have helped resource-poor farmers in developing nations. These farmers see a boost in their revenue, which goes a long way toward alleviating poverty.

By studying fibre development in barbadense CV Phytogen 800 under controlled conditions, it was observed that all the stages of fibre development were similar in *G. barbadense* except the elongation stage, which was apparently extended. It was speculated to be due to genetic divergence in the regulation of reactive oxygen species, and this further complicates the relationship of genetics and environmental factors in determining fibre properties. Improvements in cotton for desirable fibre characteristics will depend upon a better understanding of the physiological mechanisms underlying fibre growth and development. Cellular machinery responsible for cell wall expansion is at the crossroads with regard to maintaining fibre perimeter or fibre length. Significantly improved fiber length, strength, micronaire value, and uniformity-all important traits for commercial cotton production-can be achieved with the manipulation of the fiber perimeter. Although this is a principally genetically controlled characteristic, its realization depends on several factors: genotype, environmental conditions, harvesting practices, ginning methods, and processing techniques. Any of the aforementioned factors may lead to a dispersal of fiber quality, therefore resulting in more low-valued short fibers.

Advancements in Genetic Engineering

Cotton fiber improvement objectives may lie in the gene pool that produces fiber-specific structural proteins, enzymes, polysaccharides, waxes, or lignins; nevertheless, this gene pool is still rather small. A lot of parts of plant transgenic science, both fundamental and applied, are getting a makeover from new tech. As a result, new avenues for tailoring crops to meet basic human requirements have opened up thanks to plant genetic engineering. First, while discovering novel methods of gene transfer to plant cells, scientists have been working on cell biology techniques to regenerate plants from either single cells or organized tissues. They have also been using genetic engineering to

modify crops. Thus, plant transformation technology has evolved into a versatile platform for studying gene function and improving crops. In order to establish stable expression of transgenes in plants, new methodology, techniques, and plant transformation vectors have been developed, which have enhanced the efficiency of transformation.

The major participating genes during the elongation stage include expansins and lipid transfer proteins, while tubulin, cellulase synthase, and sucrose synthase are highly expressed during secondary wall formation. Characterizing the genes involved in fiber formation is necessary before they may be modified. Expansin is a member of a significant gene family that has been linked to the elongation and expansion of fibers. Expansins have been the subject of extensive research on the topic of cell expansion. There have been multiple reports regarding expansins that are particular to fibers and have a role in fiber expansion. It is partly controlled in cotton through regulating cell wall extensibility by expansin genes in the course of cell expansion. Expansins have indeed been reported to enhance both cell and fruit size. Additionally, it has been noted that expansin genes are expressed during fiber elongation. It is reasonable to assume that some expansin genes may be involved in cotton fiber development, given the apparent involvement of expansin genes in cotton fiber elongation.

Expansins are a good example; they are non-enzymatic wall proteins in plants that play an essential role in many aspects of their development. Transforming the native cotton variety NIAB 846 with the Agrobacterium-mediated GhEXPA8 gene leads to improved fiber properties by overexpressing expansin genes in the cotton genome. Various molecular approaches demonstrated that the transgenic cotton plants improved the quality range of their fibers compared to controls, providing further evidence of their existence. Worldwide, cotton plays an essential role in the production of basic textiles and is thus an essential commodity for economies. The cotton fiber is one of the most ubiquitous and interconnected economic characteristics. Traditional methods of improving cotton fiber quality have centered on two main approaches: traditional breeding and molecular genomic

modification. When compared to classical breeding methods, molecular techniques are typically superior for genetically altering desirable features, such as increased fiber quality and production. The signaling cascade and developmental stages of cotton fiber have been better understood thanks to recent genetic studies. Due to the high number of components involved in signal transduction and transcriptional control, the process of cotton fiber production is intricate. Research on cell delineation, elongation, cellulose production, and the interaction between fiber and embryonic tissues in seeds is conducted using the cotton plant as a model plant. A number of plant gene families have been proposed as potential regulators of cotton fiber and boll development, according to studies examining the process of cotton fiber cell division. Xyloglucan endotransglucosylase (GhXTH), brassinosteroid receptors (GhBRI1), brassinosteroid-dependent transcription factors (GhEER1, BZR1), RING finger proteins (GhSNA1), BRH1, actin-binding protein (GhGLP1), gibberellic acid receptor (GhGD1), and SLR1 are all candidates for biochemical process regulators in fibers. Increasing cotton output is a direct result of genetically modifying the cotton fiber genes. Only cotton cultivars showing signs of tissue culture regeneration have so far been used for Agrobacterium-mediated transformation. Cotton plants were engineered to overexpress the fiber gene GhEXPA8 by scientists primarily interested in the transgenic approach. After testing 10 different local cotton varieties in germination studies, scientists determined that G. hirsutum var. NIAB846 was the most transformable due to its poorer fiber quality and greater germination percentage.

Genetic Transformation and the Role of SuS Gene

SuS activity during the very early stages of cotton fibre development, in particular, from 0 to 5 days postanthesis determines the fate of the ovule epidermal cells to differentiate into fibres. The inhibition of SuS activity at the same early stage has been associated with the fibreless seed phenotype. In contrast, over-expression of the potato SuS gene in cotton significantly increases fiber production. This, therefore, implies that SuS plays a great role in increasing fiber length as well as fiber

strength and micronaire/smoothness. Conventional breeding has not been able to solve problems relating to quality fibers because of breeding barriers. Biotechnological approaches combined with classical breeding may, therefore, make available valuable strategies to deal with this pressing problem of fiber quality in cotton.

Expandin and other genes from *Calotropis procera* are known to increase fiber length, according to recent studies. Increased fiber length and better micronaire values have also resulted from overexpression of additional genes, such as GhActin 1, HOX3, GhWlim5, and PIP. Regarding this matter, an effort was made to use Agrobacterium-mediated transformation to introduce the codon-optimized SuS gene into the local non-transgenic cotton variety. The SuS gene is reported for regulating carbohydrate interconversion, which plays an important role in cellulose synthesis. Overexpression or repression of SuS is crucial for plant growth and the overall quality of cotton fiber. Cotton fiber quality was positively impacted by SuS expression, and the sink tissue would receive more glucose and fructose from increased SuS activities. The rise in turgor pressure was caused by the increase in sugar availability, improved fiber elongation and cellulose content, resulting in a smoother surface for the fibers.

Improved leaf growth was positively connected with higher SuS activity in the modified cotton plants' leaves, which in turn led to a considerable increase in plant height, boll number, and boll weight. These findings clarify the connection between the flow of photosynthate from source tissues to sink tissues, the creation of growth and fibers, and the subsequent processes. Higher SuS activities are linked to better fiber quality characteristics, which further supports the idea that SuS is involved in fiber development. It has been suggested that SuS plays a significant role in fiber development and offers breeders a chance to use additional germplasm-related fiber traits to satisfy the demands of the country's high-quality textile businesses. Transgenic cotton carrying the SuS gene may display enhanced length and strength if the gene's expression and activity at various phases of fiber formation can be better understood. Pakistan

relies on cotton, its most valuable cash crop, for a variety of reasons, including the fact that its fibers demonstrate several cellular developmental phases. Overexpression of genes, breeding, trait modification, and other physiological and biochemical research have all benefited from genetic transformation's recent development as a powerful tool. This method allows for precise and inexpensive, quick changes to fiber quality. The SuS gene, which plays a regulatory function in carbohydrate interconversion and cellulose synthesis, rose to prominence among the hundreds of genes discovered that contribute to cotton fiber commencement and development. Inhibiting or increasing SuS expression has been shown in earlier research to have a significant impact on cotton fiber quality and plant growth.

Experiments were conducted using genetically transformed cotton lines overexpressing the SuS gene. The aim was to investigate how SuS overexpression affects cotton fiber development as part of deciphering its mechanisms in improving fiber quality. In addition, the understanding of how SuS interacts with other genes related to fiber development will clearly outline how breeding can take advantage of those insights by means of genetic transformation, in concert with conventional breeding methods, in solving the challenges cotton production and fiber quality face. These results point out the necessity of further investigations in the involvement of specific genes, such as SuS, in the development of cotton fibers. Cotton varieties with derived uses from modern biotechnological techniques, combined with traditional breeding, would not only meet the textile industry's quality parameters but also contribute toward gaining economic sustainability for countries like Pakistan in cotton production. With the continued rise in global demand for high-quality cotton, it would now appear that further development of genetic modification techniques will, to a large extent, determine whether cotton meets active consumer demands and reaches the goals of agricultural sustainability. Active research into the genetic and physiological basis of cotton fiber development has, therefore, been crucial in bringing gains not only in fiber quality but also in the overall performance of the crop. The complicated development of cotton fibres unfolds in a number of discreet but

somewhat overlapping stages: initiation, elongation, maturation, and secondary wall synthesis. Each phase is intricately regulated by an interplay amongst numerous genes, transcription factors, and phytohormones within coordinated cellular activities for fibre growth. Of these, sugars play a double role: besides serving as sources of energy, they act as signaling molecules that affect the expression of genes related to the development of the fiber. This is especially crucial during the phase of secondary wall synthesis when cellulose, the major constituent of the cotton fiber, is synthesized. Cellulose production is of great importance for length and strength parameters of fibers, while the indicators of cotton crop quality are engaged with these parameters.

Impact of SuS Gene Overexpression

SuS gene overexpression has shown the enhancement of fiber length and cellulose content was distinctly observed in the transgenic cotton lines when compared with non-transgenic controls, indicating positive effects from SuS overexpression on one aspect of fiber quality. The increase in the cellulose content agrees with the previous reports indicating a direct relationship between the SuS activity and cellulose synthesis during the development process of fiber. The increased sucrose and total soluble sugar levels at higher stages of development establish further that this increased availability of sugar is associated with improvements in fibre characteristics. Detailed observations through SEM revealed that some of the transgenic cotton plants had finer, smoother fibers with highly improved fiber crimp compared to the non-transgenic controls. These changes can be attributed to increase cellulose content due to higher activity of SuS, which allows for greater production of hexoses to result in the subsequent elongation of fibers. Furthermore, the micronaire decrease in some plants could be related to the lower sucrose content during secondary walls synthesis.

Agrobacterium-Mediated Transformation and Expression Analysis of GhEXPA8

Our group has introduced fiber gene GhEXPA8 into *G. hirsutum* var. NIAB 846 using Agrobacterium-mediated transformation. A total of two copies of GhEXPA8 were inserted into the genomes of plant lines 1, 2, 5, and 6 [GhEXPA8-1, GhEXPA8-13, and GhEXPA8-15, respectively]. A total of three copies of the target gene were inserted into plant lines 3 [GhEXPA8-5] and 4 [GhEXPA8-11]. This is because, with the exception of the lines already indicated, GhEXPA8 transgenic plants exhibited higher levels of lines 1, 2, 5, and 6 compared to both GhEXPA8 transgenic plants and control plants. There are a lot of potential environmental influences at play here, including copy number, gene positioning effect, gene insertion effect, and internal cell programming. Transgenic plant lines 1, 2, 5, and 6 exhibited significantly greater mRNA expression of GhEXPA8 compared to the control and other transgenic plant lines, as determined by real-time PCR. It is worth noting that transgenic plant lines 5 [GhEXPA8-13] and 6 [GhEXPA8-15] showed a cellulose content improvement of over 60% compared to the control plants.

Evaluation of GhEXPA8 Transgenic Cotton Lines

Transgenic plant line 1 and 2 cellulose yields were 50% and 20% higher than those in the controls. Results are consistent with previous studies demonstrating enhanced cellulose content in transgenic cotton plants. Some of the qualitative analyses included in the transgenic plants were fibre length, micronaire values, strength, and uniformity index. However, the fiber uniformity indexes of the transgenic lines were inconsistent over three consecutive generations from 2009 to 2011, with an increase of only 10%. On the other hand, great line improvements in fiber strength were observed to be 17%, an agreement with the previous finding. All the transgenic plants showed enhanced fibre length by 20% and micronaire value by 19.1%, and consistently longer fibres and micronaire values obtained each generation.

It is possible to infer from fiber analysis that the transgenic plants modified with the GhEXPA8 fiber gene shown notable improvements in fiber length and micronaire values following genetic alteration. It also implies that the values of micronaire and fiber length are indirectly related. The transgenic plants' fibers improved in every other qualitative aspect after the first generation, but this trend did not continue. This agrees with previous research that found the same thing. Ginning outturn percentage of textiles created by the GhEXPA8 gene was one of the parameter attributions, according to the investigation of morphological characteristics. Fiber strength, micronaire value, and fiber uniformity index are all crucial metrics, and lines GhEXPA8-1, GhEXPA8-6, and GhEXPA8-4 performed better than other genotypes in these areas. Lines GhEXPA8-1, GhEXPA8-6, and GhEXPA8-5 demonstrated the best recovery in terms of monopodial branches, sympodial branches, plant height, boll weight, and number of bolls per plant. Also, these lines did very well in terms of sample weight, lint weight, gin outturn, and yield per plant. Both GhEXPA8-1 and GhEXPA8-6 can be used for cotton genotype selection with the goal of improving yield and quality. The results showed that there was a positive and statistically significant association between the phenotypic and genotypic variables pertaining to Fyb, FL, and UI, and a negative and statistically significant correlation between the phenotypic and genotypic variables pertaining to the micronaire value of fiber and fiber strength. The positive relationships suggest that longer fibers may have a greater effect on fiber strength and uniformity; hence, transgenic lines can be selected for improved cotton quality and yield through fibber strength and uniformity selection. The following traits were previously identified, and their results corroborate those: Moreover, highly significant correlations were recorded on a phenotypic basis among the agronomic traits such as plant height, monopodial branches, sympodial branches, yield per plant, and gin outturns.

Integrating Genetic, Physiological, and Sustainable Practices

Cotton is one of the most important sources of natural textile fiber. Besides being economically and agronomically important, cotton also is an unconditional model system in the study of cellular, molecular, and biochemical events at the single-cell level. For in vitro observation, this may mean critical events such as cell differentiation, elongation, and maturation activities. Because of the special nature and developmental processes of the cotton fiber, it offers a unique opportunity for scholars to further advance knowledge dealing with several aspects of complex biological systems. In addition, progress in this area of understanding QTL, differential gene expression as influenced by environmental stimuli, and interactions of genes regulating fiber development will continue to enhance our abilities in designing plants with specific characteristics. The goal of this engineering has been to enhance the value of cotton as a fiber and feed crop.

Besides genetic and physiological approaches, there is also great potential for the use of sustainable agricultural management practices to alter cotton production in the future. Adoption of crop rotation, cover cropping, and reduced tillage can widen the legacy effects of improving soil health and water retention to optimizing nutrient availability, which is beneficial for higher yields and more viable agroecosystems. Besides, consumer preferences Consumers are increasingly demanding that textiles be produced in an ecologically acceptable and ethical manner. It necessitates rethinking cotton production in response to diverse consumer expectations for sustainable and genetically modified cotton varieties while maintaining high fiber quality. The complex relationship between sucrose metabolism, signaling pathways, and fiber development in cotton therefore underlines the need for elucidation of physiological mechanisms that drive the process. Further research will be required in the areas of genetic, molecular, and environmental factors affecting fiber growth and its quality to achieve sustained gains in cotton production. It is only by harnessing recent advances in genetic engineering, sustainable agriculture methods, and most importantly,

in-depth knowledge regarding the physiology of plants that the industry will be able to meet the rising demand for high-quality cotton from a growing population on this host planet with changing environments and limits to resources.

Sucrose Metabolism and Synthase Enzymes in Cotton Fiber Cellulose Synthesis

The process of cellulose synthesis at a cellular level starts at the close of the development of the primary wall and continues through the synthesis of the secondary wall, whereby the actual number of cellulose increases while the fiber is maturing. This maturation process is important, since it takes part in the determination of fiber quality through mechanisms of dehydration, enhancing the structure of the fiber. Three important enzymes are mainly responsible for regulating the metabolic pathway involving sucrose metabolism in the cotton seed kernel: sucrose phosphate synthase, invertase, and sucrose synthase. The enzyme sucrose phosphate synthase (SPS) catalyzes the formation of UDP-glucose into sucrose.

Sucrose is the end-product of photosynthesis in source tissues-the leaves, and it should be translocatable in the plant and thus needs cleavage into its constituent hexoses at the sink, cotton boll, or fruit before entering subsequent biochemical reaction(s). Two varieties of sucrose synthase exist: one that is located in the cell membrane and another that is located in the cytoplasm. In contrast, it is thought that M-SuS permits cellulose synthesis by supplying carbon to the cellulose synthase during cell wall formation. However, it is thought that C-SuS is involved in the reversible sucrose breakdown process. Although it can be reversed, this reaction is more favorable for the conversion of sucrose and UDP into fructose and UDP-glucose. In addition to being a nucleotide sugar molecule utilized in the cytoplasm for sucrose synthesis and the apoplast for cellulose and callose formation, UDPglucose is the donor substrate for several glycosylation processes. It is believed that SuS channels carbon from sucrose into cellulose synthesis as part of a hypothetical complex with cellulose synthase

attached to the plasma membrane. The cotton fibers' secondary wall, which is what makes the fabric usable in the end, is composed of about 95% cellulose.

The three main enzymes involved in the regulation of sucrose metabolism in the seed kernel are sucrose phosphate synthase (SPS), invertase (INV), and sucrose synthase (SuS). A series of enzyme processes are catalyzed by SPS to produce sucrose from UDP-glucose. In addition to sink cells, which use the sucrose that has been generated, source cells can also synthesize sucrose through photosynthesis. This is accomplished through the successive action of SPS and SPP, which stands for sucrose phosphate phosphatase. From fructose-6-phosphate and UDP-glucose, sucrose phosphate is produced by SPS catalysis. Because SPP can convert sucrose phosphate to sucrose so quickly, the reaction is guided into a synthetic path. Sucrose is irreversibly broken down into glucose and fructose by invertases. The formation of fiber relies on SuS, which feeds the hexose sugars needed for cellulose synthesis. In order to generate turgor pressure during fiber elongation and cellulose synthesis, these hexoses keep their osmotic potential. It has a very important role in source and sink cells. The increased level of sugar during the phase of fiber elongation can thus provide the necessary turgor pressure for growth. This supports the results of previous studies that indicate sucrose plays a major role in fiber development.

At the cost of sucrose phosphate phosphatase, fructose-6-phosphate is converted to sucrose quickly after SPS catalyzes the formation of sucrose-P. In contrast, invertases are enzymes that catalyze the irreversible conversion of sucrose to glucose and fructose, and sucrose synthase is an enzyme that is absolutely critical for fiber growth because it provides the hexoses needed to synthesize cellulose. One form of sucrose is cytoplasmic and engaged in the reversible breakdown of sucrose; the other form is attached to the membrane and is believed to be involved in cell wall production. The two forms are related but separate. Glycosylation reactions rely on UDP-glucose as a substrate, and the enzyme SuS catalyzes this reaction such that

UDP and sucrose are cleaved into fructose and UDP-glucose, respectively.

The resultant UDP-glucose is also a crucial building block for sucrose synthesis in the cytoplasm and for cellulose and callose synthesis in the apoplast. Scientific evidence suggests that cellulose synthase, which is situated in the plasma membrane, and SuS create a complex that transfers carbon from sucrose to cellulose. Cellulose makes up over 95% of the secondary wall of cotton fibers, the main ingredient utilized in the textile industry. During the early stages of cotton fiber formation, which occur between 0 and 5 days after anthesis, the activity of this enzyme controls the onset of fiber development by the ovule epidermal cells. During the start of fibers, SuS plays a crucial role because its reduced activity during this brief window caused a fiberless seed phenotype. The increased fiber production observed when the potato SuS gene was overexpressed in cotton suggests that a strategy focusing on SuS could be a viable option for achieving improvements in various fiber properties, including length, strength, micronaire smoothness, and others. Because of species compatibility, conventional methods for enhancing cotton fiber quality have often been found to be inadequate. Yet, new biotechnological approaches that go beyond conventional breeding practices have encouraging prospects for resolving some of the issues plaguing cotton fiber quality.

Sucrose Synthase Overexpression and Actin Dynamics in Cotton Fiber Quality Improvement

A decrease in sucrose availability in the lower fibers at this stage, as a result of earlier studies, is in line with lower micronaire values and better cellulose deposition, leading to an overall improvement in fiber quality. When comparing transgenic plants to their non-transgenic counterparts, morphological analyses revealed notable variations in plant height, boll density, average boll weight, and leaf area per plant. Enhanced biomass and better agronomic qualities were indicators of improved vegetative growth and fiber properties brought about by overexpression mediated by the CaMV35S promoter. There appears to

be ectopic expression of SuS transformation under the CaMV35S promoter, which promotes vegetative development and fiber quality. The results demonstrated without a doubt that SuS was a crucial enzyme during fiber development, and that its activity was highly correlated with the qualitative quality markers linked to fibers. Studies have assertioned that sucrose metabolism is directly related to fiber quality, SuS was identified as the primary target for enhancing fiber features and increasing yield in local cotton cultivars. When everything is said and done, transgenic cotton will be a reality, ushering in a new era of cotton variety development programs that can adapt to the evolving needs of the mechanized textile industry. Insightful examination of the molecular pathways underlying fiber formation is what this research has contributed significantly to the field of cotton breeding and genetics. To maximize the quantity and quality of cotton fibers from diverse agricultural ecosystems, there needs to be future research into the interaction of genetic and environmental factors. This will help guarantee the profitability and sustainability of cotton, a crucial cash crop in the face of global challenges. There are dozens of plant species that have yielded actin proteins, which play a crucial role in cell growth and development. In cotton (*Gossypium* spp.), a grand total of sixteen distinct ACTIN genes were identified, highlighting the intricate and diverse roles played by this family of genes during fiber advancement. The most common form of actin is G-actin, which is a monomer. The polymerized form, F-actin, is sometimes called a filamentous structure. There are essentially three main processes to this type of polymerization: nucleation, polymerization/capping, and bundling or cross-linking of F-actin filaments. A large number of ABPs, which can be further classified according to their binding affinity for G-actin or F-actin, directly regulate the dynamic characteristics of actin filaments. In order to influence the general organization of the cytoskeleton, these ABPs may do things like aid in actin polymerization or depolymerization, crosslink actin microfilaments, or bundle them jointly.

The major ABPs that are involved in the regulation of actin dynamics are the actin depolymerization factor and profilin. Other studies have

also discovered profilin, such as GhPFN2, interacting with ADF and playing crucial functions in the regulation of actin filament turnover in response to Ca^{2+} signaling. Annexins are a diverse multigene family of ABPs, also found to interact with G-actin specific for the fiber, like GhACTIN1, that contributes to the elongation of fibers during cotton fiber development. Most notably, GhACTIN1 expression displays a major trend of appearance during the fiber elongation stage, emphasizing its critical role in this developmental stage. Continuous turnover in actin filaments is required for the elongation of fibers. The effect of RNA interference against GhACTIN1 in cotton fibers revealed highly reduced F-actin networks, which resulted in a reduction in length and strength in the fiber. It was indicative that while GhACTIN1 is significant in the fiber elongation stage, other actin genes, such as GhACTIN2 and GhACTIN, also play important supporting roles that should not be ignored. Cotton fibers represent a very good model for studying the mechanisms of cell elongation and, especially, the cell wall biosynthesis biotechnologically, whereby interest has been focused on improve ment of fiber yield and quality by genetic modification. The developed strategy is one of overexpressing particular genes for specific improvement of characteristics-the highest overexpression of GhFIM-2 correlating to higher fiber elongation.

Genetic Correlations in Agronomic Traits and the Role of Sucrose Metabolism

There were strong genetic and phenotypic correlations between monopodial branches and sympodial branches, yield/plant, and sample weight. There was a strong correlation between the presence of sympadoid branches and variables such as plant height, monopodial branching, boll density, and yield. Plant height, monopodial branches, sympodial branches, number of bolls per plant, and GOT were shown to be significantly correlated with yield per plant at both the level and level of analysis. The positive and statistically significant correlation between lint weight and sample weight suggests that selecting for genotypes with higher yields could increase cotton quality and quantity. Positive genotypic correlations provide the foundation for

improving characteristics, in general. Negative and statistically significant associations, on the other hand, suggest that features may continue to lose ground in subsequent generations. As crucial signaling molecules in higher plants, sucrose regulates the expression of several genes involved in various physiologies and developmental processes of plants. Of these sugars, sucrose is one of the main ones that plants can transport over long distances; it is also the byproduct of carbon fixation. For correct metabolic interconversion, sucrose must be broken down into its component hexoses. Since sucrose is the primary byproduct of photosynthetic processes in source tissues, its degradation in sinks such as cotton bolls and fruits is the first step in intensifying its use in cellular metabolism and subsequent metabolic pathways that are relevant to cotton plants.

Sucrose synthase may act in support of the turgor pressure that drives fiber elongation in cotton. Sucrose breakdown into fructose and UDP-glucose increases the concentration of osmotically active solutes within the fiber cells. Such osmotic pressure is important for driving the influx of water, promoting cellular expansion that represents fiber elongation. On the other hand, UDP-glucose is a critical substrate of cellulose biosynthesis, where the deposition of cellulose results in secondary wall thickening. This thickening contributes mostly to the overall strength of cotton fibers, composed essentially of crystalline cellulose amounting to as much as 95% of the weight of the secondary wall.

Sucrose metabolism is distributed across multiple cellular compartments and involves a spectrum of pathways, reflecting its crucial role in plant growth and development. Sucrose metabolism enzymes are rather products of paralog families residing within plant genomes than derivatives of single genes. This is further complicated by the presence of multiple isoforms for each enzyme residing within different organelles and with specialized functions within distinct cellular environments. Due to its relatively large size and inherent polarity, sucrose requires specific proteins to be transported efficiently across membranes. Sucrose breakdown in most plants is brought

about by two main enzymes, the sucrose synthase, SUS, and invertase, INV. Whereas both are essential in sucrose catabolism, sucrose cleavage brought about by sucrose synthase is normally preferred from invertase because of the fact that SUS maintains the energy linked with the glycosidic bond during degradation. Sucrose synthase is a cytoplasmic enzyme and shows constitutive mRNA expression within all growth phases.

Enzymatic Roles in Sucrose Metabolism

Other enzymes in addition to SUS activity, such as endo-1,4-β-glucanases, expansions, and endo-1,3-β-glucanases, contribute to the deposition and degradation of callose, which plays an important role in regulating the opening and closing of plasmodesmata during the period of elongation that is critical for the development of fiber. While sucrose synthase catalyzes the sucrose breakdown through a cleavage reaction, at the same time it attaches to important carbon structures that are needed for the synthesis of cell wall polysaccharides. Sucrose synthase further supports other processes like respiration. In the plant kingdom, including cotton, the two major physical forms include a membrane-bound sucrose synthase and a soluble or cytoplasmic sucrose synthase.

Sucrose metabolism engineering-especially the overproduction of sucrose synthase genes-was considered one of the promising approaches in cotton for its growth improvements along with a number of aspects of fiber quality. Indeed, enhanced expression of this enzyme in cotton plants is correlated with marked improvements in physiology. For example, overexpression of SUS genes has been related to an increase in the rate of leaf expansion-a critical determinant of photosynthetic capacity and overall biomass production. Greater leaf area develops greater light capture and correspondingly higher photosynthesis, enabling more growth. The relationship of sucrose metabolism in particular is very complex with the development of fiber. These earlier stages of the development of fiber cells are a series of cell elongation and wall thickening highly critical in determining fiber

strength and quality. The only driving force behind these elongations is turgor pressure arising from the accumulation of osmotically active solutes of sucrose. It is, therefore, in increasing the sucrose levels in the plant that one may enhance fiber cell elongation to attain longer and stronger fibers that meet the demand from the textile industries. The membranel-bound form, M-SUS, catalyzes the cleavage of sucrose directly, feeding carbon directly into cellulose-synthesizing enzymes resident in the plasma membrane. On the other hand, C-SUS catalyzes the conversion of sucrose into UDP-glucose and fructose and is also capable of dynamic association with actin cytoskeleton. M-SUS channels carbon atoms from sucrose into other enzymatic pathways, above all targeting either cellulose or callose synthases in the plasma membrane. Experimental results have produced clear evidence that detached and permeabilized cotton fibers in in vitro experiments transfer carbon at much more rapid rates to cellulose and into the callose while using sucrose compared with UDP-glucose. This was coherent with the hypothesis of channeling substrates to glycan synthases at the interface.

Other studies made use of more sophisticated techniques that included cryogenic fixation, freeze substitution, and various biochemical assays. These studies confirm that M-SUS has indeed a functional role in the plant cells, particularly during the secondary wall development phase of the cotton fibers. M-SUS also appears to participate in the synthesis of xyloglucan, another major plant cell wall component attached to the Golgi apparatus membrane. Besides its catalytic functions, the membrane-associated form of SS may also contribute to sucrose transport across the tonoplast, thereby allowing SUS transport across vacuoles into the cytosol in sucrose accumulating organs. Another level of sucrose metabolism complexity arises from the fact that isoforms of SS bypass this C-flow into starch; this process takes place particularly in the developing seeds of model plants like Arabidopsis. It is well known that sugars modulate gene expression, even at relatively low concentrations, which is important for physiological homeostasis. Glucose, fructose, and sucrose were shown to induce or repress the expression of hundreds of genes at as low as 1

mM concentration. Typically, the sugar-responsive genes code for proteins important in the basic physiology of plants associated with vegetative growth and development. The present studies are based on the previous notion that sucrose synthase is expressed in response to simple sugars through a hexokinase-dependent process. This makes sucrose synthase a bifunctional protein-not only does it contribute to sugar synthesis, but its expression is also strictly controlled by sugar.

Role of Actin-Related Proteins and Promoter Selection

Fimbrin (FIM) participates in actin filament bundling for fiber elongation at the secondary wall biosynthesis stage; therefore, GhFIM-2 also takes part in this process via dynamic rearrangement of actin.When the profilin GhPFN-2 is overexpressed in cotton, the fibers are shorter than in wild-type plants because secondary cell wall production is induced earlier. The extension of fiber length in cotton has been shown to be achieved through the overexpression of an ankyrin repeat-containing Arabidopsis protein called AKR2A. This is achieved through an increase in VLCFA levels within the transgenic lines and a process involving hydrogen peroxide signaling. The adoption of a robust, fiber-specific promoter is crucial for the successful expression of transgenes in cotton fibers. Even though this necessitates thoroughly characterizing these promoters prior to their utilization, there have been scant attempts in this direction. Some studies classified GhSCFP as a promoter that is strongly expressed throughout the fiber initiation and elongation stages, it is one of the attempts to understand the molecular mechanism of cotton fiber development. Through the use of a fiber-specific promoter, GhSCFP, we have been able to increase cotton output and fiber quality in local cotton cultivars by overexpressing the GhACTIN1 gene. In addition to GhWLIM5's previously documented function in inducing fiber strength improvement, GhACTIN1's interaction with this protein led to the suggestion that it plays a role in fiber enhancement as well. Consequently, the purpose of this research is to examine the GhACTIN1 gene's characteristics in relation to genetic modification of the indigenous CEMB-88 cotton variety. Research on cotton

transformations found that overexpressing GhACTIN1 greatly improved cotton length, strength, fineness, and yield. Using a chemically synthesized codon-optimized GhACTIN1 gene cloned in pCAMBIA-1301 plant expression vector, flanked with PstI and SacI restriction sites, and controlled by the fiber-specific promoter GhSCFP, the GhACTIN1 gene was expressed in the CEMB-88 cotton variety through stable Agrobacterium-mediated transformation.

Expression and Functional Role of GhACTIN1

Consistent with previous findings it was found that transformed plant fibers had a blue hue but non-transformed control fibers did not. The maximum expression of GhACTIN1 was observed during fiber elongation, according to qRT-PCR analysis of mature plants of the T1 generation conducted at several stages of fiber development, including initiation (4 DPA), elongation (15 DPA), and secondary wall synthesis (25 DPA). Another study also found that GhACTIN1 was more important during the elongation stage than during fiber start or secondary wall synthesis. The GhACTIN1 gene's integration and copy quantity in transgenic cotton plants were investigated using FISH analysis. On chromosome 8, it had a single strong fluorescent signal, suggesting that it carried a hemizygous single copy number. Consistent with prior research, our findings demonstrate that minimal copies of transgenes are ideal for achieving high levels of expression. When measuring F-actin filaments in transgenic cotton plants overexpressing GhACTIN1, researchers found that they increased by as much as 8.7 percent while the fibers were being stretched. The regulation of the fibers' length, strength, and maturity relies on F-actin filaments, which impart strength to the cell wall. The fiber of the transgenic cotton lines examined here showed an increase in cellulose content of up to 4.7% due to the overexpression of GhACTIN1. Increases in cellulose content was positively connected with improvements in fiber strength and length, with transgenic plants outperforming non-transgenic controls by as much as 24.2% in trinucleotide increases for fiber strength and 5.3% in fiber length. So, it can be concluded that GhACTIN1 downregulation disrupts the actin cytoskeleton, which in turn reduces

fiber length and strength. Evidences suggested that cellulose synthase complexes, when positioned correctly, stimulated the deposition of cellulose fibrils in the cell membrane and cell wall, which is facilitated by actin microtubules.

On this basis, the physical increase in fibre length as recorded in the transgenic cotton lines could be associated with increased cellulose levels. Increased cellulose content promotes the turgor pressure responsible for fibre lengthening. Interestingly, we also observed that the overexpression of influenced fibre strength more than fibre length. This could be ascribed to the increased expression of GhACTIN1 during the elongation phase, which resulted in a greater quantity of F-actin in transgenic fibers in comparison to the wild type fibers. An earlier termination of the elongation phase and the beginning of the secondary wall deposition are both brought about by the presence of thicker bundles of F-actin. Due to the fact that it reorients the filament bundles from a transverse to an oblique position and further contributes to better strength in cotton fibers, the abundance of F-actin that occurs during the elongation stage is an important factor. It has been demonstrated that increased cellulose deposition on the periphery of the cell wall results in improved fiber strength and fineness, which is consistent with research that have been published in the past. In our research, the overexpression of the GhACTIN1 gene, which was driven by the GhSCFP promoter, resulted in a clear demonstration of improved fiber length, strength, and overall yield in cotton plants. As a combination of the fineness and maturity of the fibers, the micronaire values also revealed considerable increases, as did the maturity ratio, reaching as high as 24.3% and 10.5%, respectively, in the transgenic cotton lines. This was the case for both the micronaire and the maturity ratio. Additionally, scanning electron microscopy demonstrated that the fibers of the transgenic cotton lines were smoother in comparison to the fibers produced from the non-transgenic cotton lines. There have been some earlier studies that have claimed improvements in micronaire values and maturity ratios as a result of the insertion of specific genes into cotton plants. While the maximum value for the uniformity index of cotton fiber recorded in the

transgenic cotton lines has been 85.6%, no significant overall improvement in the uniformity index has been detected compared with the equivalent non-transgenic lines. A comparison was made between the agronomical characteristics of transgenic cotton plants and those of the non-transgenic control line in order to identify any alterations that were comparable to those that were caused by insertional changes or contributions from genetic modification.

Impact of ACTIN1 Gene Expression on Agronomic Traits and Yield Enhancement in Transgenic Cotton

Plant height, grain size, and seed fertility are only a few examples of the agronomic and morphological traits whose genes have been altered in transgenic rice. When considering how the transgenes affect seed weight, the seed index is a crucial aspect. Seed weights increased by as much as 44.04% in transgenic cotton lines, with values ranging from 1.2 to 3.7 g. The fact that ovules and the embryo sac express the ACTIN1 gene may explain this upregulation. Furthermore, there is a positive correlation between the average fresh and dried boll weight and a greater seed index, seed cotton yield, and lint percentage. When compared to the non-transgenic control line, the transgenic cotton plants showed a rise of 57.5% in dry boll weight and a 28.6% increase in fresh boll weight. Additionally, the yield of seed cotton increased by 29.5% and the percentage of lint increased by 4.6% when compared to the non-transgenic cotton lines.

Moreover, SUS activity has been directly demonstrated to promote seed development. This is particularly important, as healthy seed development forms the very foundation of successful reproduction in cotton plants and maintains the supply of fibre. Indeed, proper seed development in plants may prevent the occurrence of seed abortion, which is one of the major yield-limiting factors. A complex interaction exists between sucrose levels and seed development. While some reports exist of increased sucrose and hexose levels inhibiting the expression of genes responsible for programmed cell death in ovaries, such suppression of paths of cell death may again contribute to

reduced abortion rates of seeds and allow for more effective reproduction and increased yield. Moreover, the heightened SUS activity increases sink strength beyond its function in seed development. Sink strength refers to the ability of a plant to attract assimilates-sugar in this case-for growth and development. In this sense, increased sink strength enables the cotton plant to optimize assimilate use by developing fibers, seeds, and other structures for the better productivity of the whole plant. This is particularly crucial during the most sensitive stages of fiber elongation, when the demand for sugars is already higher. Facilitating the effective translocation of sucrose to these growing tissues allows plants to improve fiber elongation and quality.

In case of transgenic cotton lines, it is expected that increased activity of the SUS gene would result in fibers with improved quality and yield. Transgenic methodologies allow differential manipulation of desirable traits in such a way that improved varieties of cotton could be developed that respond precisely to the requirements of modern textile industries by producing fibers exhibiting superior mechanical properties with improved spinnability, important for its processing and production.

The tailoring of superior cotton fiber targeted for high-quality textiles requires unraveling the regulation mechanisms that are associated with the stages of fiber elongation and secondary wall thickening. Recent efforts have focused on elucidating the transcriptomic changes occurring within fibers at two development stages in two divergent cotton species, *Gossypium hirsutum* and *Gossypium barbadense*. With deep sequencing methods that capture transcriptomic changes during fiber elongation at 10 DPA and secondary cell wall development at 22 DPA, several key genes related to the developmental stages have been identified.

Transgenic plant line 1 and 2 cellulose yields were 50% and 20% higher than those in the controls. Results are consistent with tprevious studies demonstrating enhanced cellulose content in transgenic cotton

plants. Some of the qualitative analyses included in the transgenic plants were fibre length, micronaire values, strength, and uniformity index. However, the fiber uniformity indexes of the transgenic lines were inconsistent over three consecutive generations from 2009 to 2011, with an increase of only 10%. On the other hand, great line improvements in fiber strength were observed to be 17%, an agreement with the previous finding. All the transgenic plants showed enhanced fibre length by 20% and micronaire value by 19.1%, and consistently longer fibres and micronaire values obtained by each generation.

It is possible to infer from fiber analysis that the ptransgenic plants modified with the GhEXPA8 fiber gene shown notable improvements in fiber length and micronaire values following genetic alteration. It also implies that the values of micronaire and fiber length are indirectly related. The transgenic plants' fibers improved in every other qualitative aspect after the first generation, but this trend did not continue. This agrees with previous research that found the same thing. Ginning outturn percentage of textiles created by the GhEXPA8 gene was one of the parameter attributions, according to the investigation of morphological characteristics. Fiber strength, micronaire value, and fiber uniformity index are all crucial metrics, and lines GhEXPA8-1, GhEXPA8-6, and GhEXPA8-4 performed better than other genotypes in these areas. Using the transgenic lines GhEXPA8-1 and GhEXPA8-6, it was proposed to create cotton types with higher yields and better quality. Lines GhEXPA8-1, GhEXPA8-6, and GhEXPA8-5 demonstrated the best recovery in terms of monopodial branches, sympodial branches, plant height, boll weight, and number of bolls per plant. Also, these lines did very well in terms of sample weight, lint weight, gin outturn, and yield per plant. Both GhEXPA8-1 and GhEXPA8-6 can be used for cotton genotype selection with the goal of improving yield and quality. The results showed that there was a positive and statistically significant association between the phenotypic and genotypic variables pertaining to Fyb, FL, and UI, and a negative and statistically significant correlation between the phenotypic and genotypic variables pertaining to the micronaire value

of fiber and fiber strength. The positive relationships suggest that longer fibers may have a greater effect on fiber strength and uniformity; hence, transgenic lines can be selected for improved cotton quality and yield through fibber strength and uniformity selection. The following traits were previously identified, and their results corroborate those: Moreover, highly significant correlations were recorded on a phenotypic basis among the agronomic traits such as plant height, monopodial branches, sympodial branches, yield per plant, and gin outturns.

With the face of agriculture changing, culturing crops which exhibit better yield in different environmental conditions has turned out to be one of the major challenges to keep with the demands of sustainable agriculture. The demand for genetically modified cotton has also been showing a constant rise globally, besides indicating undertones of its wide acceptance as such genetically changed crops are starting to find even in the most backward regions of the globe. Such grounds provide promising new opportunities regarding cotton yield and quality improvement. Another avenue for further research concerns the impact of the environmental factors regarding the soil type and climatic condition on the outcome of the transgenic lines. Such comprehensive understanding of genotype-environment interaction will eventually enable the development of transgenic cotton varieties that are not only resistant to pests but also optimized for yield and fibre quality across diverse agronomic conditions.

Managing Insect Resistance in Cotton Cultivation

The one of the most daunting challenges for agriculture is to prevent insect-pests from developing resistance to the Bt toxins produced by the transgenic crops. While insects have so far not come up with field-level resistance against the Bt toxins, the possibility of eventual resistance is still a worrying prospect. While the process involving actual resistance is complex and can be clouded by profound interactions between the environmental factors, genotype, and environment, strategies have been utilized in delaying resistance

outbreaks. The introduction of many crystal proteins at the same time is another method that expands the various resistance mechanisms capable of deployment against populations of pests. This interrelationship between genetic improvement, hormonal signals, environmental factors, and biotechnology aspects, therefore, forms a multi-dimensional challenge when it comes to cotton fiber production. The knowledge of such interactions on a deeper level is crucial when developing sustainable approaches for improvement in fiber quality, yield, and resistance to environmental and biological stresses. As research uncovers the complexity of mechanisms underlying fiber development, the prospect of novel ways to improve cotton is bright and will contribute favorably to agriculture and society in general. Strategies to delay the evolution of resistance in lepidopteran pests to Bt cotton include high-dose expression of multiple Bt toxins and also the fusions of different Bt toxins. These depend on the fact that developing resistance requires multiple mutations-a highly improbable statistical occurrence. Various studies conducted among different lepidopteran pests have indicated that cry2A toxins do not share binding sites with cry1A toxins, therefore favoring the feasibility of pyramiding these two types of toxins in Bt cotton varieties. Stacked expression of the cry1Ac and cry2A genes, therefore, has emerged as a promising approach in gene stacking for Bt cotton, especially with regards to *Helicoverpa virescens.* Bollgard® II Bt cotton, which carries the cry1Ac and cry2Ab genes, was authorized for introduction in 2004 by both the United States and Australia. Several lepidopteran pests that were hard for the single-toxin Bollgard types to control have been effectively managed by this dual-toxin Bollgard II genotype. Consequently, field testing of the second generation of these transgenic cotton varieties with the two Bt gene varieties has shown that a possible strategy for long-term resistance management against cotton bollworms could involve stacking or pyramiding toxin genes that express toxins at high doses with different binding characteristics or modes of action.

Emphasis on the resistance of transgenic plants against *Heliothis armigera* showed that the main aim of various studies was to improve

the resistance against pests without compromising the yield and fiber quality. Significant differences in boll damage from different transgenic lines, tested at 5% and 1% levels of significance, indicates some lines are effective in reducing the damage caused by insect pests. In particular, those lines with moderate boll damage (average rating from 1 to 2) did not incur any yield loss and could thus be considered for appropriateness in breeding programs in the future. This again suggests that efficient variety selection and management could prove effective in reducing losses owing to pest damage.

The low ginning turn out percentage (GTO %) illustrated the agronomic potential of these transgenic lines. Observed improvement of GTO% in insect-resistant transgenic lines against non-transgenic controls suggested that the transformation process may have favorable effects on fiber quality parameters. However, this would also be markedly contributed to by variability in environmental conditions and management practices. The high GTO ratios observed underline further efficiency enhancement possibilities in cotton production, which is essential for a stable cotton industry, mostly in countries like Pakistan. In the assessment of fiber quality, the findings revealed that essential parameters such as staple length and micronaire did not incur any negative impact from the transformation process. This is because the quality of the fiber translates to marketability. The absence of significant variance in parameters of fiber quality against their respective control lines gives credence to the fact that the relative advantages provided by transgenic alternations regarding pest resistance do not compromise fiber quality.

The findings form an important milestone in the bigger picture for placing transgenic cotton as a sustainable agricultural solution. This may lead to enhanced robustness and productivity due to the effective control of *Heliothis armigera*, as well as other potential pest threats. Establishment of stable homozygous lines is a major step toward commercialization aspects of these transgenic varieties, which may bring a revolution through betterment in cotton production in countries like Pakistan. A very promising transgenic cotton with further

pest tolerance and desirable agronomic attributes paves away toward future research in cotton breeding, giving a new route toward sustainable agriculture by aligning economic and environmental imperatives.

Further Readings

Ahmed, M., Shahid, A. A., Akhtar, S., Latif, A., Din, S. ud, Fanglu, M., Rao, A. Q., Sarwar, M. B., Husnain, T., & Xuede, W. (2018). Sucrose synthase genes: a way forward for cotton fiber improvement. *Biologia*, *73*(7), 703–713. https://doi.org/10.2478/s11756-018-0078-6

Bai, F., & Scheffler, J. (2024). Genetic and Molecular Regulation of Cotton Fiber Initiation and Elongation. *Agronomy*, *14*(6), 1208–1208. https://doi.org/10.3390/agronomy14061208

Cui, S., Zhou, X., Xiao, G., & Feng, H. (2024). Genomic Analysis of Brassinosteroid Biosynthesis Gene Family Reveals Its Roles in Cotton Development across Gossypium Species. *Biology*, *13*(6), 380. https://doi.org/10.3390/biology13060380

Deniz Istipliler, Müge Ekizoğlu, Uğur Çakaloğulları, & Tatar, Ö. (2024). The Impact of Environmental Variability on Cotton Fiber Quality: A Comparative Analysis of Primary Cotton-Producing Regions in Türkiye. *Agronomy*, *14*(6), 1276–1276. https://doi.org/10.3390/agronomy14061276

Dong, J., Ding, C., Chen, H., Fu, H., Pei, R., Shen, F., & Wang, W. (2024). Functions of exogenous strigolactone application and strigolactone biosynthesis genes GhMAX3/GhMAX4b in response to drought tolerance in cotton (Gossypium hirsutum L.). *BMC Plant Biology*, *24*(1). https://doi.org/10.1186/s12870-024-05726-w

Iqbal, A., Aslam, S., Ahmed, M., Khan, F., Ali, Q., & Han, S. (2023). Role of Actin Dynamics and GhACTIN1 Gene in Cotton Fiber Development: A Prototypical Cell for Study. *Genes*, *14*(8), 1642. https://doi.org/10.3390/genes14081642

Jan, M., Liu, Z., Guo, C., & Sun, X. (2022). *Molecular Regulation of Cotton Fiber Development: A Review*. *23*(9), 5004–5004. https://doi.org/10.3390/ijms23095004

Kamran Shehzad Bajwa, Ahmad Ali Shahid, Rao, A. S., Bashir, A., Aftab, A., & Tayyab Husnain. (2015). *Stable transformation and expression of GhEXPA8 fiber expansin gene to improve fiber length and micronaire value in cotton*. *6*. https://doi.org/10.3389/fpls.2015.00838

Karar, H., Bashir, M. A., Haider, M., Haider, N., Khan, K. A., Ghramh, H. A., Ansari, M. J., Mutlu, Ç., & Alghanem, S. M. (2020). Pest

susceptibility, yield and fiber traits of transgenic cotton cultivars in Multan, Pakistan. *PLOS ONE*, *15*(7), e0236340. https://doi.org/10.1371/journal.pone.0236340

Majid, M. U., Ashraf, R., Jabbar, B., Arif, U., Batool, F., Hassan, S., & Rashid, B. (2024). Overexpression of Gossypium arboreum 3-ketoacyl-CoA synthase 6 (GaKCS6) gene enhanced leaf epicuticle wax in Gossypium hirsutum L. and improved tolerance against whitefly. *Biocatalysis and Agricultural Biotechnology*, 103418. https://doi.org/10.1016/j.bcab.2024.103418

Marral, M. W. R., Ahmad, F., Ul-Allah, S., Atique-ur-Rehman, Farooq, S., & Hussain, M. (2023). Influence of Transgenic (Bt) Cotton on the Productivity of Various Cotton-Based Cropping Systems in Pakistan. *Agriculture*, *13*(2), 276. https://doi.org/10.3390/agriculture13020276

Qian, H. (2024, May). *Genetic manipulation for cotton improvement towards heterosis maintenance and herbicide resistance*. Tdl.org. https://ttu-ir.tdl.org/items/df7853e1-6add-4233-b1b8-e26b24effd0d

Razzaq, A., Muhammad Mubashar Zafar, Ali, A., Li, P., Qadir, F., Laviza Tuz Zahra, Shaukat, F., Abdul Hafeez Laghari, Yuan, Y., & Gong, W. (2023). Biotechnology and Solutions: Insect-Pest-Resistance Management for Improvement and Development of Bt Cotton (Gossypium hirsutum L.). *Plants*, *12*(23), 4071–4071. https://doi.org/10.3390/plants12234071

Salama, A., Farid, M. A., El-Mahalawy, Y. A., A. A. A. El-Akheder, Aboshosha, A. A., Fayed, A. M., Yehia, B., & Lamlom, S. F. (2024). Exploring agro-morphological and fiber traits diversity in cotton (G. barbadense L.). *BMC Plant Biology*, *24*(1). https://doi.org/10.1186/s12870-024-04912-0

Santosh Gudi, Pavan M, Praveenkumar Alagappan, Om Prakash Raigar, Priyanka Halladakeri, Gowda, R., Kumar, P., Singh, G., Meenakshi Tamta, Pusarla Susmitha, None Amandeep, & Dinesh Kumar Saini. (2024). Fashion meets science: how advanced breeding approaches could revolutionize the textile industry. *Critical Reviews in Biotechnology*, 1–27. https://doi.org/10.1080/07388551.2024.2314309

Verma, K., Sharma, P., Tripathi, K., Yadav, R., & Singh, S. P. (2023). *Recent Advances in Genetic Improvement of Cotton*. 69–99. https://doi.org/10.1007/978-981-99-5034-8_4

Xing, A., Zhang, X., Wang, J., He, S., Nazir, M. F., Wang, X., Wang, X., Yang, R., Zhang, H., Fu, G., Chen, B., Peng, Z., & Du, X. (2024). Transgressive and subgenome expression level dominance and co-expression network analyses at the early fiber development in allopolyploid Gossypium. *Industrial Crops and Products*, *214*, 118552. https://doi.org/10.1016/j.indcrop.2024.118552

Chapter 2: **Rice**

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Introduction and Global Importance of Rice

Rice (*Oryza sativa* L.) is the staple food for approximately 40% of the total population around the world. Almost 90% of its total cultivation and food consumption is concentrated in Asia. This cereal has been satisfying more than two billion people's diets in developing countries in this continent. With a continuously growing rice-consuming population, the number of rice consumers was projected to double by the year 2020. Rice contributed 4.4 percent share to the value added in agriculture and 0.9 percent to the country's GDP during the fiscal year 2010-2011. Besides, rice is importantly placed in Pakistan as an effective foreign exchange earner, ranking the country as a third-largest exporter of rice in the world, after the United States and Thailand.

Scientifically known as *Oryza sativa* L., rice is the most cultivated and consumed cereal grain in a major amount. It is a staple food for nearly half of the world's population. It is a cornerstone of agricultural production, especially in Asia, which produces about 90% of the world's rice. Moreover, rice consumption and demand have increased greatly in the last years in regions like Africa, the Middle East, and Latin America. Rice consumption is steadily increasing with urbanization. For example, in sub-Saharan Africa, per capita consumption has grown two times since 1970. The same case scenario is happening in Caribbean and Latin America, as town people are depending more on rice as a way of living. Being widely consumed, rice also forms a crucial part of

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nutrition for millions of people around the world. Besides being an important nutritional crop, rice cultivation forms the source of livelihood for over 200 million households in the developing world because it is one of the major crops grown by resource-poor farmers.

Rice belongs to the most important crops all over the world, feeding almost half of the whole world's population. Around 90% of rice is produced and consumed in Asia, where it is the staple food for more than two billion people in developing areas. Basmati, among the rice cultivars in the Indo-Pak subcontinent, assumes economic importance because of its superior quality represented by a long grain and possessing an unique aroma. However, due to the same reasons, its production is usually lower as compared to other varietal groups since this group is more prone to insect-attack. The most injurious insect pests of rice include the Yellow Stem Borer (*Scirpophaga incertulas*) and the Rice Leaf Folder (*Cnaphalocrocis medinalis*). The former pest alone is estimated to cause a yield loss of 10 million tons annually in the world, while being accountable for about 50% of the total insecticides used in rice cultivation.

Rice is one of the oldest cultivated crops, and its cultivation has existed for several millennia in various parts of the world, including India and China. Its principal cultivated species, *Oryza sativa* (2n = 2x = 24), first originated in the tropics of southern and southwestern Asia. The other cultivated species, Oryza glaberrima (2n = 2x = 24), is native to the upper valley of the Niger River and is exclusively cultivated in western tropical Africa. The genus Oryza comprises twenty-three recognized species and ten distinct genome types represented as AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, and HHKK. The wild relatives of O. sativa are perennial species such as O. rufipogon and the annuals such as O. nivara, both of which are diploid weedy species classified under the AA genome. As rice is one of the most essential food crops in the world, improvement of rice has to be done continuously in order to cater to an ever-increasing population. Based on the biotechnical approach, the improvement of rice focused on the critical areas of high yield potential, early maturity, lodging and shattering tolerance, stress

tolerance, disease resistance, insect resistance, grain quality, and nutritional content improvement.

Abiotic and biotic stresses have severe impacts on the productivity of rice. Quite substantial damage arises from insect pests and diseases. Of these, larvae of lepidopteran insects are considered among most destructive groups of pests affecting rice crops worldwide. Of particular note are the Yellow Stem Borer *Scirpophaga incertulas* and the Rice Leaf Folder, *Cnaphalocrocis medinalis*, due to their being major pests of rice. Rice stem borers have been estimated to cause an average annual loss of 5% to 10% but have been known to cause losses of up to 60% in seasons and conditions that favor infestations of rice by these insects. The Yellow Stem Borer attacks rice from seedling to harvest and destroys all attacked tillers completely. Dead hearts and white heads are the physical symptoms that occur in the vegetative stage and heading stage, respectively, because of the larval activity. This is further compounded by the Rice Leaf Folder, which, through its feeding on tender leaves, causes serious damage; a single larva can migrate to several leaves, resulting in serious foliar damage.

A biotic threat to the cultivation of rice in Africa is the Rice Yellow Mottle Virus, RYMV. This was first reported in November 1966 at Otonglo, near Kisumu, along the shores of Lake Victoria, and for over decades has continued to problem farmers' lives. In the viral genome of RYMV, four open reading frames occur, all indispensable in pathogenicity or replication of the virus. ORF1, encoding a protein located at the 5'-terminal region of the genome, is required for the development and suppression of the host's gene expression in infection. ORF2 codes for a polyprotein that is further cleaved into multiple functional proteins responsible for replication and movement of the virus. Included among them are a genome-linked protein, VPg, and an RNA-dependent RNA polymerase, RdRp. Curiously, the ORF3 overlaps the nucleotide limits of ORF2 and has thus far been predicted in silico to encode a hypothetical RdRp; however, its true functional role remains yet to be defined. Finally, at the 3' end of this genome,

ORF4 codes for the coat protein, crucial for virus assembly and transmission.

Rice Yellow Mottle Virus (RYMV) – Structure, Strategies, and Defense Mechanisms

Despite its importance, rice production faces substantial challenges, with the Rice Yellow Mottle Virus being a major biotic threat to rice cultivation in Africa. The virus was first identified in November 1966 at Otonglo, near Kisumu, along the shores of Lake Victoria. It has since persisted for several years. The genome of RYMV is composed of four open reading frames, but all are important in the pathogenic and replicational nature of the virus. ORF1 codes for a 5'-proximal protein, which is important in infection development and suppression of gene expression. The ORF2 codes a polyprotein cleaved into functional proteins involved in replicative and movement functions of the virus. It contains a genome-linked protein-there is VPg-and at the same time, contains RNA-dependent RNA polymerase.

Significantly, contained within the nucleotide bounds of ORF2 is the predicted gene ORF3, which is thought to encode a putative RNA-dependent RNA polymerase, though its functional role remains undetermined. Conclusively, ORF4 is located at the 3’ terminus and encodes the coat protein, essential in the transmission and assembly process of the virus. MicroRNAs are a class of small, non-coding RNAs approximately 22 nucleotides in length, which are generated through a two-step processing pathway and act as negative regulators of gene expression at the post-transcriptional level. They perform the regulation mainly through target mRNA degradation and/or repression of translation. Very many studies have established the involvement of miRNAs in plant responses to biotic stresses induced by bacteria, fungi, viruses, and nematodes. Certain studies have reported that viral infections alter the expression of certain miRNAs in plants, which indicates a set of complex interactions between viral pathogens and the host plant regulatory machinery. Several abiotic- and biotic-stress-

induced miRNAs, such as powdery mildew and heat stress, have been reported.

The genomic architecture for the classification of RYMV presents this virus to belong to the family of picoRNA-like from the sobemovirus group. The genome is typical for four open reading frames, one of which codes for the viral coat protein, and contains a viroid-like satellite RNA that shows complex mechanisms of replication and pathogenicity. ORF1 is based between nucleotides 81 and 554 and codes for a 157 amino acid protein designated P1, involved in viral movement and gene silencing. ORF2 is based between nucleotides 609 and 3608 and codes for a polyprotein with 999 amino acids. This should then undergo cleavage to various significant proteins, including a genome-linked protein, viral protease, helicase, and RNA-dependent RNA polymerase, RdRp. Although ORF3 is considered to encode a putative RdRp of 95 amino acids, its functional properties have not yet been defined. The highly important coat protein with respect to the structural maintenance of the virus is encoded by ORF4, which lies at the 3′ end of the genome.

Role of MicroRNAs in Plant Responses to Biotic Stress

MiRNAs are a group of non-coding RNAs with small molecular sizes, invariably 22 nucleotides, which are produced by a two-step processing process. They are known to act as repressors of the expression of genes at the post-transcriptional level by degrading target mRNAs and/or repressing their translation. Many research pieces have described the involvement of miRNAs in plants, responding both to a wide range of abiotic stresses and biotic stresses induced by conditions caused by bacteria, fungi, viruses, and nematodes. Research has documented that viral infections alter the expression of certain miRNAs in plants, which is a sign of complex interactions between virus pathogens and host regulatory machinery. Numerous miRNAs have been reported to be responsive to various abiotic and biotic stresses; powdery mildew and heat stress are among them.

In addition, the RNAi phenomenon could also be important for the silencing of viral gene expression and may contribute to enhanced resistance against viral infections in plants. This technique has once been used to generate resistant rice varieties against the Rice dwarf virus through RNAi silencing of the Pns12 gene. miRNAs provide an avenue to check on their efficiency through in vitro assays with the ultimate goal of over-expression in high-yielding varieties of *Oryza sativa* susceptible to RYMV. The idea is giving resistance to these economically important crops against the debilitating effects caused by the virus

Moreover, RNA interference, which is involved in silencing viral gene expression, may improve resistance against viruses in plants. For instance, this has been employed to produce transgenic rice with resistance to Rice dwarf virus by RNAi-mediated knockdown of the Pns12 gene. Based on these premises, scientists have tried one synergistic in silico approach towards the prediction of rice-derived microRNAs that can hybridize with and silence the RYMV genome. The identification of such miRNAs gives an opportunity for evaluating their efficacy through in vitro assays with an objective for over-expression of the miRNAs in high-yielding *Oryza sativa* varieties that are susceptible to RYMV. The approach aims to improve the resistance of such crops to the harmful effects of viral infection.

Four bioinformatics tools i.e., RNA22, miRanda, Target Finder, and psRNATarget are used for the bioinformatics analysis of miRNA-target prediction, and all identified potential target sites for *Oryza sativa* miRNAs within the RYMV genome. An intersection plot showing the overlapping of target prediction results from at least three algorithms are carried out to select miRNAs for further analysis. A Venn diagram was used to depict both the number of genome loci against which each algorithm predicted and all the different algorithms combined.

Results from these analyses identify many putative hybridization sites in agreement. An intersection plot is usually generated showing the intersecting target predictions from at least three of the algorithms,

from which seven miRNAs were chosen to further investigate. Venn diagram is also used to illustrate both the total number of individual and combined genome loci predicted by each algorithm.

Future Directions for miRNA Research and RYMV Resistance in Crop Biotechnology

Studies indicate the crucial role of bioinformatic approaches in predicting putative microRNAs as a novel intervention for conferring resistance to Rice Yellow Mottle Virus in rice crops. Based on our observation, attempts have, therefore, been made to reduce the impact of RYMV by leveraging the regulatory powers of miRNAs, hence protecting rice production and enhancing food security. The results also have implications outside the realms of rice improvement into plant biotechnology, where computational predictions coupled with experimental validations may lead to the creation of resilient varieties of crops against viral infections. This integrative approach thus lays the ground for further investigations on how plant miRNAs may interact with viral genomes, upon which an understanding of the prospects for crop improvement and sustainable agriculture can be founded.

Generally, it has been ascertained that bioinformatics may identify microRNAs that confer resistance against Rice Yellow Mottle Virus in rice plants. These findings add knowledge to our understanding not only of the ways in which miRNAs interact with viral genomes but also provide a way forward for future studies that shall look into ways through which these interactions will find their application in crop improvement. By embracing the power of miRNA-mediated regulation, researchers can help develop sustainable agricultural practices that improve food security and enhance the livelihoods of millions of people dependent on rice cultivation. Further application of these methodologies will be crucial to promote innovation in plant biotechnology and help meet pressing global food production challenges.

It has been observed that the size of vacuolated cells in cell suspensions is inversely proportional to the ability of cells to withstand freezing and thawing. Poorer viability after cryopreservation suggests selective pressure in maintaining the survival of cell types or varieties having high resistance to cryopreservation, as is evident in maize. Embryogenic cells having smaller cell sizes with dense cytoplasm and a scanty number of vacoles are more tolerant to cryopreservation. It also shows that the cryopreservation technique might act like a filter, which selects only those embryogenic cells that can survive under harsh conditions. Bleaching of cells, which is an indication of cryoinjury, deplasmolysis, and oxidative stress, was observed in the case of Basmati 385. It was also recorded that there were no remarkable differences in the viability of the different varieties of rice following cryopreservation for varying periods. Cryopreservation can be employed as an effective strategy for long-term storage of lines of important cell culture suspensions without needing any access to programmable and extremely costly thermo-freezing equipment. This procedure can then be repeated easily for further callus proliferation on culture media or for the initiation of cell suspensions in liquid R2 medium.

Direct DNA uptake into protoplasts has emerged as one of the widely used methods for the insertion of genes in cereals. Methods of inducing callus formation and preparation of protoplasts have been developed for quite a few cereals such as pearl millet, guinea grass, and sugar cane. The success or plant regeneration from callus and cell suspension cultures depends on the genotype or variety of the original plant. However, it is pretty problematic to establish and maintain embryogenic cell suspensions from Indica rice varieties. Even those which do get established, such cell suspension lines mostly lose their potential for regeneration after sometime and the extended period of tissue culture leads to loss of morphogenetic capabilities and acquiring undesirable somaclonal variation. It is also quite time-consuming to initiate new cell suspensions. Cryopreservation of embryogenic cell

suspension cultures provides a reliable source of competent cells for future use.

Optimization of Cryopreservation Techniques for Indica Rice Varieties

Various groups have reported cryopreservation of rice cell suspensions, most from Japonica cultivars, followed by its regeneration. These studies have generally used slow programmable rate freezers to freeze cells to –25 to –40°C before transferred into cryogenic storage at –70°C or in liquid nitrogen. Cell suspension culture method does not rely on slow-rate thermo freezers; rather, it depends on osmotic dehydration of the cells before cooling in a regular freezer set between –20°C and –30°C. This method provides an efficient and easy explant that would be ideal tissue for biolistic transformation and give rise to a high yield of totipotent protoplasts. However, it is also important to mention that these cell suspensions show gradually decreasing regenerability over long periods. It may be inferred from this that extended periods of in vitro culture conditions impede the pathways involved in counts for cell regeneration. The exploration of auxin or other alternatives to initiate cell suspensions, and the use of regeneration media, which in general are used in rice and contain some compounds or additives that support the initiation of regeneration in cell suspensions, is therefore rewarding. Fine cell suspensions have succeeded in plant regeneration; the initial embryogenic calli formed on the regeneration media free from 2,4-D allowed plantlets to regenerate via somatic embryogenesis. The plantlets issued from these somatic embryos presented a developing structure with a coleoptile and a root, disposed symmetrically at the early developmental stage.

These cryopreservation techniques have been done for maintaining the embryogenic potential of cell suspensions. There was, however, marked variation in post cryopreservation viability among the different varieties of rice tested. Microscopic observation showed that most of the cells in the variety Basmati 385 were large sized and vacuolated when compared to the other varieties, which were smaller and dense.

Challenges and Advances in Bt Gene Deployment for Insect Resistance in Rice

Yellow Stem Borer attacks the crop from seedling to harvest, with the consequence of complete loss of affected tillers. During an attack at the vegetative stage, a condition may ensue called "dead heart," or at ear development, "white head.". The larvae of the Rice Leaf Folder fold the tips of leaves over themselves for protection against environmental factors and feed on the green tissue, leaving only the skeletal structure of the leaf. Besides decreasing vigor, these infestations predispose the plants to further infection caused by bacteria and fungi. The most significant progress in plant biotechnology in general, and for developing insect resistance in particular, is the expansion of biotechnology techniques to introduce genes from unrelated sources in commercially important crop plants. Among the sources of insect-resistant genes, *Bacillus thuringiensis* is one of the leaders; its genes encode crystal proteins toxic to the larvae of Lepidopterans, Coleopterans, and Dipterans among others, which are usually considered safe for human consumption. Extensive research work has been concentrated on the expression of Bt genes in rice to study its efficiency under greenhouse conditions and field environments. Among these, the cry1Ab, cry1Ac, cry2A genes, or their combinations are predominantly expressed in transgenic rice lines. While these transgenic lines present a very high level of resistance to the lepidopteran pests, they also share weaknesses with other technologies in pest control-partly through the development of insect resistance to the technology over time.

During recent years, various institutions have carried out the development of Bt rice. Along with increased resistance to insect pests, the introgression of Bt gene has brought about morphophysiological changes in the plant. The transgenic rice varieties generally had short stature, fewer nodes, and internodal length was positively correlated with lodging resistance. Field trials of Bt rice expressing two pesticidal genes, cry1Ac and cry2A were conducted for two consecutive years at National Centre of Excellence in Molecular Biology, Lahore, Pakistan.

Transgenic rice exhibited resistant traits to lodging and hence was able to bear the stress caused by wind and rain. Original Basmati-370 was transformed with the Bt gene; those were further taken to the fields for tests against insect-pest resistance and other environmental adaptations of the transformants. The transformants received higher resistance against target insects; besides this, they showed better lodging resistance. Assessment of the field performance of Bt rice was the main objective, while the secondary aims included the determination of some major agronomic and morphological characteristics-lodging resistance of Bt rice in comparison to its non-Bt counterpart.

It has been suggested that lines expressing two different Bt genes are the only ones that should be released into agricultural environments in order to prolong resistance durability in Bt crops. Also, the two Bt toxins together used must have ample structural variability; otherwise, a single mutation in the target pest could provide cross-resistance to both the toxins. More than 100 Bt genes have been cloned and sequenced, indicating a considerable variability in their amino acid sequences and some biochemical properties. Cry1Ac in combination with Cry2A appeared quite useful against lepidopteran pests. Besides the development of resistance in target pest species presenting not only hardships but, on the other hand, other factors that need equally proper consideration during development: horizontal gene transfer, impacts on non-target invertebrates, and overall agronomic performance of the transgenic plants. Taken together, the development of resistance by target insects against transgenic plants could therefore also be retarded using a high dose/refugia strategy in conjunction with gene pyramiding strategies. The high dose/refugia strategy entails not transforming all popular cultivars with Bt genes so as to retain refugia that support susceptible insect populations. It is equally important to establish whether the insect pest resistance of subsequent generations of a transgenic line is maintained under natural conditions, or whether the effectiveness wanes after some time, which could hasten resistance development in target pests. There is also possible the transgene flow-through pollens from such

lines to wild relatives and other commercial cultivars. It would be highly essential to examine the impact of the transgenic plants on non-target insect populations, for avoidance of encouraging the spread of secondary pests and protection of valuable invertebrates. Morphological characteristics and agronomic performance are important for developing commercially viable transgenic cultivars. Available literature has documented the morphological variation in rice transformed through PEG treatment, protoplast electroporation, Agrobacterium-mediated transformation, and particle-mediated transformation. This means that at the time of selecting desirable lines, no compromise on major morphological and agronomic traits regarding average number of tillers per plant, average plant height, days to maturity, and overall yield should be made. So far, more than 500 insect species have become resistant to conventional insecticides; by contrast, Bt has an enviable record of resistance: only the diamondback moth reported showing resistance against Bt sprays and none seen so far against Bt crops.

Transformation of DNA and its expression is a critical aspect of the generation of transformed plants. In tranformation, one necessary component involves the preferential selection and growth of transformed cells, usually obtained by adding a gene that confers resistance against antibiotics, herbicides, or other selective agents. The aminoglycoside phosphotransferase structural gene nptII gives t he resistance to the antibiotic kanamycin, hygromycin phosphotransferase gene hpt or hph confers the resistance to hygromycin. These two marker genes serve as an essential role in transformed cell detection and proliferation. The identification of transgenic plants soon after transformation and determination of the transmission of transgenes to their progeny are usually a great task and are very time-consuming.

Traditional and Emerging Methods for Detecting Transgenes in Plants

Though PCR is quick and sensitive for the detection of transgenes, it is highly prone to cross-contamination which can result in misleading results. GUS assay, based on the beta-glucuronidase reporter gene, allows determination of the successful delivery of foreign DNA into plant cells shortly after the transformation process following transient expression. However, this method can sometimes be quite expensive and may not always distinctly differentiate between the transformed and non-transformed cells. Direct in-plant assays for assessing selectable marker gene activity such as herbicide application or germination of seeds on selective media have also been developed. However, such techniques like simple leaf painting or germination tests are not much effective for early identification of the regenerated transgenic plants. Furthermore, spraying whole plant with herbicides may be damaging the very subject plant and hence complicating assessment.

Field Efficacy, Resistance, and Agronomic Traits of Bt Rice Lines

The subject evaluation encompasses the response of transgenic lines against non-target insects, the possibility of pollen-mediated gene flow, morphological and physiological changes manifested by the transgenic plants and most importantly, outlines the key lessons learnt over six years of field trials. The results indicated that the various transgenic lines expressed as high as 100% more resistance to Yellow Stem Borer at the vegetative stage and up to 96% more during the stage of flowering. Furthermore, the lines conferred 98% higher level of resistance against the Rice Leaf Folder. White heads were observed for all the transgenic lines expressing the two Bt genes; however, no live larvae were recovered from the stem cuttings, and all the larvae collected were dead, exhibiting typical symptoms of Bt toxicity. These transgenic lines thus provided built-in resistance against the target insects throughout the entire growth stages of the plants. Most

noticeably, resistance against insect pests remained strikingly stable even after five years of field exposure and up to the eighth generation in plants.

Resistance to several lepidopteran insect pests has been reported in rice cultivars of Japonica and Indica expressing Bt toxins. One such report documented resistance to eight different lepidopteran insects through the expression of the gene cry1Ab. These present results are in concert with the idea that besides being a basic requirement for effective management of resistance, rice lines expressing two Bt genes can support a high level of protection against economically important lepidopteran pests. Recent greenhouse studies have been able to illustrate that the pyramiding of two toxin genes with different modes of action within a plant was able to attain longer delays in the development of resistance-a prediction derived from simple genetic simulation models.

Research has also documented the release of Bt toxins from the root exudates of Bt corn hybrids, under sterile hydroponic conditions and in non-sterile soil environments. This effect is not cultivar-specific since the release of Bt toxins from the roots has been documented for a number of transgenic corn hybrids representing different transformation events. The toxins are readily absorbed and bound to surface-active soil particles like clays and humic substances, remaining larvicidal long periods after harvest or following incorporation into the soil. Accordingly, the proteins that turn up in the soil would probably be harmful to non-target organisms, and also enhance the selection of toxin-resistant insect populations in case toxins are adsorbed onto the soil matrix. However, it should be underlined that to date, there were no reports concerning the release of Bt toxins from the transgenic rice in such media as MS medium, hydroponic solution, or soil.

Therefore, in the light of above considerations, only the release to the environment of the Bt-transgenic lines with expression of two different Bt-genes is strongly advisable. Also, any two Bt toxins being used together should not show high levels of similarity. A single mutation

and change in target insects will confer cross-resistance to both the toxins. More than 100 Bt toxin genes have been isolated and sequenced; many of these toxins exhibited considerable divergence in their amino acid sequences and biochemical properties. Several studies have documented positive synergy between Cry1Ac and Cry2A against target lepidopteran pests.

Bioassays conducted on such lines indicated that they provided inherent resistance against the attack of respective insect-pests at all growth stages over two consecutive growing seasons and at two distinct geographical locations. As on previous occasions, rice varieties of both the Japonica and Indica types carrying genes for inserted Bt toxins showed resistance against a range of lepidopteran insect pests. In several instances, this kind of insect resistance is associated with the expression of the cry1Ab gene. Results point out that the lines having pyramided two Bt genes are not only central in managing resistance but also have the superiority in protection against economically important lepidopteran pests when compared to lines having single Bt gene.

Agronomic Performance and Maturity Traits in Bt Rice Lines

Toxicity titers may vary among different transformed plant lines carrying the same Bt gene construct; in rice, toxicity titers have been found to decline substantially during reproductive stages. Selection of high dose titer lines is hence very important since these plants maintain a high level of Bt protein throughout all growth stages of plants. Transgenic lines carrying two Bt genes outperform untransformed control lines for various agronomic traits such as average number of tillers, average plant height, days to flowering, and lodging incidence.

Its commercial success relies on the expression of desirable morphological and agronomic traits in a transgenic cultivar. Transgenic varieties produced by various methods such as polyethylene glycol or protoplast electroporation have considerable heritable variation, as

proved by various studies. Other works also support the fact that the lines derived from transgenic rice have tremendous variation in agronomic and morphological traits. Genomic changes do, in fact seem to occur more in rice plants transformed through Agrobacterium-mediated transformation compared with biolistic transformation using techniques such as AFLP and PCR analyses. However, little information is given in respect to phenotypic changes among these lines. Keeping the aspects in view, the studies reported hereby have been conducted at the National Centre of Excellence in Molecular Biology, Lahore, Pakistan, for two consecutive years. The retransformed Basmati-370 rice lines had been evaluated pertaining to their efficacy against Yellow Stem Borer and Rice Leaf Folder. Besides that, some bio-safety concerns have also been addressed regarding the release of Bt toxins into the soil environment. In general, these studies will provide critical information on agronomical performance, morphological stability, and potential ecological impacts of transgenic rice lines, adding useful knowledge to the continuous development of genetically engineered crops for food security and sustainability in agriculture. Engineering genes encoding insecticidal crystalline proteins from *Bacillus thuringiensis* (Bt) into crop plants offers tremendous potential for reducing reliance on conventional broad-spectrum pesticides to manage insect pests. However, one of the intrinsic risks associated with this biotechnological strategy is an immense and possible probability that insects could develop resistance against Bt toxins after their continuous and successive exposure under field conditions. As Bt toxins are the treasure of natural resources, their natural efficacy needs to be maintained by effectively managing these resources. The high dose/ refugia strategy and stacking of two or more genes within the same cultivar have provided a practical approach towards delaying the development of resistance in Bt crops.

It is remarkable to mention a fact that only 5% of rice is consumed in processed form, and the remaining 95 % is used without processing. This is the indication of the higher proportion of the physicochemical properties of rice grains. Grains from the transformed lines were classified as long-slender with an average grain length ranging from

7.55 to 7.85 mm. Apparent amylose content, being one of the major factors determining cooking and eating quality, did not significantly vary among the lines because all belonged to the intermediate group of amylose content, particularly within the range of 23% to 25%. Although slight variations in amylose content can occur depending on the environment, the differences found within the different panicles and plants were considered negligible. Besides, the alkali spreading value describing the cooking quality showed very little variation in the lines under consideration.

However, the cooking response of all the transgenic lines in respect of the parameters-curling and bursting percentage, elongation ratio, and stickiness-manifested some line variations, and all the transgenic lines along with control plants retained the aromatic qualities that have a high premium in Basmati rice. Another reason for the small variations seen in various physicochemical properties among the various transgenic lines could be environmental. All of the lines were sown under the same environmental conditions and at the same time, but their maturity timings were different, therefore, each line was subjected to different temperatures from others during its respective grain ripening stage. These temperature changes can affect some of the physicochemical properties of the grains, which confirms that the transformation process does not have any adverse impact on the intrinsic qualities of rice.

Environmental Considerations in Transgenic Rice Cultivation

Many of the transgenic lines confer substantial resistance against YSB at both the vegetative and flowering stages, with additional resistance as high as 100% during the vegetative stage and 97.68% during the flowering stage. Besides this, these lines showed an increased resistance of 98% to the Rice Leaf Folder.

Recent studies have demonstrated that employing two toxin genes of two different modes of action will delay insect resistance, further confirming predictions using simple genetic models. Since toxin titers

can vary considerably among plant lines of the same Bt gene construct transformed, the selection of lines showing high-dose titers is important; indeed, studies have emphasized a marked decrease in toxin levels during the reproductive stages of rice. Although to this day, no Bt rice cultivars have been commercially released, some lessons to be learned can be taken from the commercially released Bt cotton, maize, and potato where toxin titers range from 1 to 11 mg/g of leaf fresh weight, or about 0.1% to 0.2% of total leaf soluble proteins. In the United States, three Bt cotton cultivars have been considered to contain high toxin titers against their target insects, based on wide experimental evaluations and practical experiences under agricultural settings.

Such morphological changes could be due to somaclonal variation or due to disruption of native genes in the plant either by the inserted transgene or through insertion mutagenesis, as a result of a pleiotropic effect, or because of the silencing of native genes by the transgene. Of these, somaclonal variation would seem to be the most plausible reason for the changes reported here, given the fact that most production of transgenic plants involves longer times in culture than normal for tissue culture. Therefore, the risk of somaclonal variation increases with prolonged culture of tissues. Besides this, selective agents such as antibiotics used in the process of transformation may cause mutations within the genome. There was, however, no significant difference with respect to panicle length and flag leaf area. This result therefore generally implies that, out of the studied transgenic lines, the average number of tillers, plant height, and days to maturity were the most affected characteristics, while the others did not differ from the control lines. The yield of all the transgenic rice lines tested in the present investigation showed statistical significance over that of the control line. Concretely, two CCR-based *Bacillus thuringiensis* (Bt) genes, unrelated to each other, cry1Ac and cry2A gave excellent protection against lepidopteran insect pests throughout the entire growth stage. Three homozygous lines, L-8-22-2, L-8-22-32, and L-8-22-35 developed from the same transformation event showed high levels of resistance against lepidopteran pests at acceptable

morphological features. Most importantly, these lines showed stability for resistance in the advanced generations also without any gene silencing or segregation for cry genes.

The key to successful resistance management is to maintain the titer of toxins throughout the entire growing season. However, it is equally essential to ensure that the levels of toxin titer are also not developed to such an extent to have a debilitating consequence on the agronomic performance of the rice plants. The Yellow Stem Borer inflicts its menace on rice crops at every stage of growth, from seedlings to harvest time. While this would be the case, rice plants could still compensate for the damage caused by the stem borer during the vegetative stage of their life cycle via new tiller formation. Such compensation, however, becomes increasingly difficult after the tillering stage. Observed declines in the toxin titer within the transgenic lines still remained considerably above threshold levels necessary for effectively reducing target insect populations and minimizing the risk of development of resistance against Bt toxins. Quantitative analysis of toxin level showed that at 30 days after transplanting, the titer of Cry1Ac in the transgenic transformants was 6.06 mg/g leaf fresh weight, which then decreased to 4.54 mg/g at 60 days and 3.42 mg/g at 90 days.

Field Performance and Biosafety of Transgenic Basmati Rice Lines Expressing Bt Toxins

Our group carried out a study at the National Center of Excellence in Molecular Biology (CEMB), Lahore, Pakistan involving collections that were made from various localities lying in Punjab Province of Pakistan. Advanced generations of locally transformed Basmati-370 rice lines were screened against YSB and RLF. Besides this, the biosafety aspects of possible effects on non-target insects, horizontal and vertical gene flow, and allelopathic effects emerging from the cultivation of these transgenic lines were also looked into. Transgenic rice lines expressing genes encoding insecticidal crystalline proteins of *Bacillus thuringiensis* origin showed considerable promise for decreasing the use of

conventional broad-spectrum pesticides to control insect pests. However, a serious concern exists that insects may eventually develop resistance to the Bt toxins after continued and repeated use under field conditions. The two most effective ways to make the durability of Bt crops longer are presumed to be a high dose/refugia strategy and the pyramiding of two or more genes within the same variety.

On the other hand, Cry2A expression was significantly low and scored 0.72 mg/g at 30 days, 0.43 mg/g at 60 days, and 0.42 mg/g at 90 days. Surprisingly, Cry2A protein expression was up to 8.5-fold lower than that of Cry1Ac protein. The gene expression was said to be constitutive, and the quantification of the toxins had been done on different parts of the plants such as leaves, stems, roots, panicles, seeds, and kernels. Though the promoters used are not said to be tissue-specific, there is a great variation in levels among different plant tissues with respect to Bt toxin. This could be because different water and protein contents are present in different parts of the plants. The relatively low expression of Cry2A is perhaps due to its regulation through the CaMV 35S promoter in transgenic rice plants, which has been reported to provide much lower effectiveness compared with the Ubiquitin promoter. The other observation is that younger plant tissues express higher levels of protein when the 35S promoter is used. The lines with both Bt genes were better, between the single gene and control lines for most of the agronomic parameters, such as average number of tillers, average plant height, days to flowering, and incidence of lodging. The observed resistance to lodging may thus be attributed to a combination of an increase in insect resistance coupled with a reduction in plant height. The slight reduction of the mean number of tillers present in the second growing season might be related to adjustments in density planting since the distance between plants and rows was reduced from 45 to 22 cm.

Morphological variation in some of the transformants may be due to somaclonal variation or due to disturbance in the native genes of the plant by integration of transgene, insertion mutagenesis, and even gene silencing due to transgene expression of the native genes. Taken

together, the results here show good promise for pyramid transgenic rice lines with two Bt genes for improved resistance against important lepidopteran pests and, at the same time, provide insights into its genetic and agronomic basis as a basis of performance under field conditions. Further studies and research on these transgenic lines will be required for the development of appropriate management strategies to delay the development of insect resistance, optimize agronomic performances, and ensure rice production with continued pest pressure. The observed phenotypic changes in such transgenic plants seem best explained by somaclonal variation. The duration of time in tissue culture can considerably affect the frequency of somaclonal variation, as generally seen from literature; the longer the period of time in tissue culture, the higher the rate of somaclonal variation. Regarding this, the generation of transgenic plants often requires longer periods in tissue culture than those routinely used, which may enhance this effect. Secondly, in transformation, the use of selective agents such as hygromycin can itself induce mutations in rice plants. While significant changes were noted in some important agronomic traits, other characteristics such as panicle length, spikelet fertility, and flag leaf area did not vary significantly.

The most affected characteristics in the transformation process were the mean number of tillers, plant height, and maturity dates. Notably, in all the infested plots, the yield for all the transgenic lines was always better as compared to that of the control plants. The control plants, on the other hand, had an inverse relationship with regard to incidence and yield; this means that as the incidence of the said pest increased, the yield subsequently decreased. For this reason, under natural infestation conditions, transgenic lines evidenced a yield increase up to 8% because of relatively lower susceptibility to YSB and RLF. This was despite the recorded superiority in the number of tillers and panicles per plant; overall yield potential was no different from that compared to control plants. This may be due to a combination of several factors, including reduced panicle length, reduced spikelet fertility, and reduced grain weight. From these observations, it would appear that despite the superior resilience against insect infestations, some

morphological changes may offset the potential yield advantages within the lines developed.

It was also shown that the transgenic Bt rice expressed Bt toxins in MS medium, hydroponic cultures, and soil. Such toxins could be assayed using immunoassays, as has been amply described for transgenic Bt maize. The persistence of such toxins in the environment is an issue which needs further study to fully understand the bio-safety issues concerned with the release of these toxins. In short, the expression of these two unrelated genes, crylAc and cry2A from *Bacillus thuringiensis* in the transgenic Basmati rice lines, remains stable throughout the growth period.

However, through backcross breeding from a single parental line, the three homozygous lines L-8-22-2, L-8-22-32, and L-8-22-35 showed promising levels of resistance to Lepidopteran pests throughout the harvest season and acceptable morphological traits related to average tiller number, plant height, maturity, and tendency for lodging. This factor increases the reliability of the data generated since the results were reproducible at the two different locations. Though the secretion of these Bt proteins from the plants into the different growth media was detected, the uptake of the proteins by surrounding organisms or plants is considered negligible. In view of the above facts, these transgenic lines are found to be suitable candidates for release as the first indica Basmati rice carrying dual Bt genes.

Agronomic Performance and Stability of Transgenic Bt Rice Lines

While these transgene lines significantly deviated from the untransformed control with respect to plant height and days to maturity, this was predictably stably inherited up to the eighth generation, with no abnormal variations or segregations found to occur during the study period. Such stability in performance points to the fact that the genetic modification does indeed result in reliable and

consistent agronomic performance and increases their potential for commercialization.

The most drastic decreases in plant height were from lines L-8-22, L-26-3, and L-26-8 which decreased leaf length by approximately 36 to 37%. This represents a growth pattern similar to the one observed under greenhouse observations of earlier generations and for the lines harboring both Bt genes. The reduction in plant height also contributed substantially towards the lodging resistance observed in these transgenic lines. Generally, shorter plants are considered desirable from a breeding viewpoint, and several studies have indicated that transformation often results in a reduction of plant height. Moreover, the lines L-8-22, L-26-3, and L-26-8 demonstrated an earlier time of flowering by up to 18 days in advance compared with their corresponding lines containing either cry1Ac or cry2A alone, which were delayed in comparison with the control lines. These differences in maturity of transgenic lines, compared with conventional controls, have been previously documented; and thus, if the insertion of a transgene was taken to result in food or feed with different composition, much further assessment of the influence of the inserted transgene on plant development would be required. Since the insect attacks continue to the maturity stage of rice, this early maturity assumes importance because these plants are exposed to the insect pressures for a relatively shorter period.

Lodging Resistance in Transgenic Crops

Lodging is the permanent displacement of a plant's stem from its upright position, and it is one of the most common problems in cereal crops such as wheat, barley, oats, corn, sorghum, soybean, tomato, tobacco, and rice. It might have negative impacts on yield and the general quality of production, since the loss of yield due to lodging up to 40% is possible. Major causes usually include strong winds or heavy rains. In some cases, insect activities tend to aggravate the phenomenon. Research on lodging resistance in multivariate crops is targeted toward genetic mapping of traits related to stiffness in the

stem, height of the plant, and other characteristics that impart lodging resistance in hexaploid wheat, rice, barley, maize, and soybean. Breeding for resistance to lodging tends to be based on the selection of varieties with shorter plants and internodes that are generally much shorter and thicker.

It has been estimated, for instance, in the population emerging from a wheat x spelt cross that the plant height contributed about 77% towards the phenotypic variance related to lodging in three diverse environments. In our field trials over two consecutive years produced data that demonstrated the elite transgenic lines in each of the Basmati-370 varieties compromising T2, T3, and T4 showed advance characteristics of lodging tolerance. This is manifested by significantly higher coefficient of correlation between the important variables related to lodging. Plant height, stem length, node No., and internodal length gave a statistical difference between both years. The analyses of lodging incidence gave interesting changes in the correlation coefficients between morphological traits of plant height, Length, internodal length, and node No. for both trial years. In the first year, the resistance of the transgenic lines to lodging marked 87-89%, and in the second year, this value was slightly lower: 78-79%. Such differences might be explained by different weather conditions during these two vegetative seasons; the second year was more extreme from the point of view of climatic factors.

The disproportion between the weight of the plant's upper part versus the strength of its basal part is generally the reason for lodging. Therefore, most of the lodged-resistance breeding efforts have been directed toward plant height reduction through the use of semi-dwarf and dwarf genes in order to reduce upper weight and weight center of gravity. While a number of the major underlying genes determining plant height, among which are the Rht genes, have been implicated in increased lodging resistance, it should be noted that besides plant height, many other important components influence the phenomenon in total that involves root and shoots, and several aspects of total plant yield. Curiously, the expression of the Bt gene showed its potential to

shorten the plant height-the required character for advancement in lodging resistance. Most literature reports state that the most crucial trait linked with lodging in field conditions is related to plant height and internodal length. Plant height is still one of the dominating factors related to lodging vulnerability more than the effect of any other single trait. Extensive research has been done to explain the genetic control of these traits; thus, it develops the understanding that the yield components like grain number and weight may act as indicators of a plant's lodging susceptibility. One of the main reasons for higher yields in rice and wheat during the "Green Revolution" was the identification and introduction of semi-dwarf lines. It has been shown that the frequency of plant lodging of the stem in modern varieties is extremely rare because, through selection over the last four decades, both shorter and more rigid stems have been chosen. Generally, it is observed that the optimum plant height for maximum photosynthetic efficiency in wheat is between 70 to 100 cm. In rice, the length of the basal internode could be shortened while the strength of the basal culm could be improved to enhance lodging resistance. Besides that, it has to be considered that the process of insertion of a transgene is casual and made in such a way that most of the time the transgenic plant differs from its progenitor in many aspects. Actually, the disruption of the host genome may result in pleiotropic effects needing deep influence on plant height, maturity, yield, and other performances. Many reports have discussed and discuss the advantages of the so-called pleiotropic effect due to the insertion of a transgene.

Innovations in Screening and Marker-Assisted Selection

We describe here an improved comparative procedure for detection and screening of genetically modified Basmati rice and cotton plants containing either hygromycin resistance gene or kanamycin resistance gene. Both these genes are among the most widely used selectable markers of plant transformation protocols. The assay, besides being simple and rapid, requires only a very small amount of plant material for the analysis, thus giving quite clear distinction between

transformed and non-transformed plants. This approach can serve as a practical alternative to conventional bioassays and leaf-painting techniques for preliminary testing of transgenic plants to confirm transgene expression. The implementation of multiple assays can further enhance the reliability of results and hence permit more accurate conclusions on the presence and expression of transgenes in plant tissues. Conclusively, in front of all advances in transgenic technology and the development of identification methodologies, the future of crop improvement is bright and deserves a high interest in biotechnology. As research goes on with its evolvements, integration will lead to an increase in the capabilities of producing desirable trait-transgenic plants, which in turn will help agricultural sustainability and food security.

Assays performed on the leaf tip of both rice and cotton reveal eminently important information regarding the efficiency or performance of hygromycin as a selective agent in the differentiation of transgenic plants from non-transgenic ones. The survival of the leaf tips of the rice and cotton transgenic varieties under hygromycin pressure constitutes indicative proof of successful integration and expression of the hph gene within them. The observed differential response of different transgenic and non-transgenic plants raised with hygromycin Hansa emphasizes the potential of this assay for screening GMOs at an early stage. It is clear that all of the leaf tips from the tested transgenic rice plants remained healthy and green when cultured on hygromycin-containing medium at rates which were sufficient to cause considerable necrosis in the non-transgenic control plants. That is quite remarkable and indicative of very good expression of the gene.

It has also been observed that some symptoms differ among the transgenic plants; for example, bleaching and darkening may be due to either a variation in copy number of the hph gene or its variable expression. This is an important observation since it could show that not only between different transgenic populations but even within one such population, great variability in the expression of genes might

exist, which later influences the plant phenotype regarding its vigor. The qualitative nature of the rice leaf tip assay is considerably advantageous for screening purposes. It can effectively discriminate between transgenic and non-transgenic plants based on their minimum expression levels of the hph gene, hence making it a quicker and more efficient method for preliminary testing. Besides, using 1 cm square leaf pieces instead of whole leaves can enable this operation to save resources. This attribute enhances the functionality of the assay for those working with large populations of transgenic plants. While similar results were obtained in rice, the cotton leaf tip assay showed that under the hygromycin selection, the transgenic cotton plants containing the two genes hph and nptII were healthy. The differential responses of the leaf tips to different hygromycin concentrations reflect sensitivity in an assay that can detect differences in the expression level of the hph gene. However, one of the obvious limitations of this assay is that significant bleaching has not occurred in the control cotton plants at high kanamycin concentrations. This utility as a selective agent in demarcation would, therefore, be limited by the resistance of a non-transgenic cotton leaf tip to kanamycin, unlike hygromycin, which can present clear demarcation between the two groups.

This general in vitro applicability of the procedure to any one of several selectable marker genes, including most herbicide resistance genes such as bar, makes this assay widely useful in plant biotechnology experimentation. The described assay is also particularly useful as a preliminary screening method for large segregating populations derived from lines generated by genetic engineering. The outcome of such an assay in rice and cotton leaf tip assays, in this regard, confirms that such an assay is indeed a relatively simple yet efficient technique that allows the early screening of transgenic plants. Consequently, assays of this sort offer great potential not only for research and development in the issues of plant biotechnology but also for new applications in the improvement of transgenic crops. Future optimization of conditions, especially those concerning the use of kanamycin in the assay, may help further improve discriminatory

power for transgenic cotton plants. Secondly, further research on the molecular events responsible for observed variation in response among different transgenic lines shall constitute an intricate understanding regarding gene expression dynamics in genetically modified crops.

Besides, this bioassay integrated into a broader context of plant biotechnology might go a long way toward extending our capabilities in crop trait improvement, such as enhanced tolerance against both biotic and abiotic stresses, improved yield potential, and higher nutritional value. As new plant biotechnology continues to advance, it will be important that a means of effective and accurate screening for transgenic plants facilitate rapid development and commercialization of genetically modified crops that will meet the growing demands of food security. The implications for such improvement go beyond agricultural productivity; it embraces environmental sustainability and economic viability in an ecologically changing agriculture. Second, with the constant shift in regulatory policies regarding genetically modified organisms, effective and reliable methodologies for screening, such as the leaf tip assay, could be important tools not only for researchers but also for regulatory agencies. This is the first point: that by providing a competent means of screening transgenic plants, such methodologies-should they be developed-could speed up the processing of new varieties of genetically modified crops and ensure that any beneficial innovation does indeed reach the market in good time. In a nutshell, the results from both rice and cotton leaf tip assays confirm that the leaf tip assay is indeed a feasible strong tool for early detection and screening of transgenic plants. In addition to improving in the knowledge of gene expression variability and refining our skills in screening, we are capable of using plant biotechnology in producing answers to some pending global issues in food production, sustainability, and environmental conservation. The furthering of these methodologies remains at the center of the advancement of the science of plant biotechnology and drives innovation in next-generation crop development.

This integrative approach lays the foundation for further research into the interactions between plant miRNAs and viral genomes. Understanding such interactions would bring a new perspective on possible crop improvement and sustainable agriculture. Further investigation of regulatory networks established by miRNAs will provide an opportunity to contribute something useful in the advancement of plant biotechnology toward better strategies in crop improvement capable of ensuring that crops are resilient to most environmental stresses. Moreover, the bioinformatics tools used in the identification of miRNA-target interactions go a step further toward only furnishing a theoretical framework for the resistant rice varieties but also a model in using similar approaches on other crops susceptible to viral infections. Prediction and validation of the interactions open up new vistas of genetic engineering whereby the overexpression or introduction of certain miRNAs into susceptible varieties may confer resistance against a wide range of pathogens.

In view of the contemporary global issues in food security, the development of high yielding capability crops with resistance potential to biotic stresses is extremely important.

Further Readings

Cao, G., Huang, H., Yang, Y., Xie, B., & Tang, L. (2024). Analysis of drought and heat stress response genes in rice using co-expression network and differentially expressed gene analyses. *PeerJ*, *12*, e17255. https://doi.org/10.7717/peerj.17255

Chandra, A., C.P, C., Talekar, N. S., & Singh, R. (2024). Advancements in Plant Breeding for Biotic Stress Resistance in Cereal Crops: A Comprehensive Overview. *Journal of Experimental Agriculture International*, *46*(7), 448–466. https://doi.org/10.9734/jeai/2024/v46i72599

Chauhan, B. S., Jabran, K., & Mahajan, G. (Eds.). (2017). *Rice Production Worldwide*. Springer International Publishing. https://doi.org/10.1007/978-3-319-47516-5

Direct Seeding of Rice: Recent Developments and Future Research Needs. (2011). *Advances in Agronomy*, *111*, 297–413. https://doi.org/10.1016/B978-0-12-387689-8.00001-1

Fiaz, S., Ahmad, S., Noor, M., Wang, X., Younas, A., Riaz, A., Riaz, A., & Ali, F. (2019). Applications of the CRISPR/Cas9 System for Rice Grain Quality Improvement: Perspectives and Opportunities. *International Journal of Molecular Sciences*, *20*(4), 888. https://doi.org/10.3390/ijms20040888

Hajjar, M. J., Ahmed, N., Alhudaib, K. A., & Ullah, H. (2023). Integrated Insect Pest Management Techniques for Rice. *Sustainability*, *15*(5), 4499. https://doi.org/10.3390/su15054499

Horgan, F. G. (2017). Integrated pest management for sustainable rice cultivation: a holistic approach. *Achieving Sustainable Cultivation of Rice Volume 2*, 309–342. https://doi.org/10.19103/as.2016.0003.23

Joong Hyoun Chin, Gamuyao, R., Dalid, C., Masdiar Bustamam, Joko Prasetiyono, Sugiono Moeljopawiro, Matthias Wissuwa, & Heuer, S. (2011). Developing Rice with High Yield under Phosphorus Deficiency: *Pup1* Sequence to Application . *Plant Physiology*, *156*(3), 1202–1216. https://doi.org/10.1104/pp.111.175471

Kumar, K., Mandal, S. N., Neelam, K., & de los Reyes, B. G. (2022). MicroRNA-mediated host defense mechanisms against pathogens and herbivores in rice: balancing gains from genetic resistance with trade-offs to productivity potential. *BMC Plant Biology*, *22*(1). https://doi.org/10.1186/s12870-022-03723-5

Liu, S., Huang, Y., Xu, H., Zhao, M., Xu, Q., & Li, F. (2018). Genetic enhancement of lodging resistance in rice due to the key cell wall polymer lignin, which affects stem characteristics. *Breeding Science*, *68*(5), 508–515. https://doi.org/10.1270/jsbbs.18050

Long, W., Dan, D., Yuan, Z., Chen, Y., Jin, J., Yang, W., Zhang, Z., Li, N., & Li, S. (2020). Deciphering the Genetic Basis of Lodging Resistance in Wild Rice Oryza longistaminata. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00628

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Asfaliza, R., & Latif, M. A. (2012). Blast resistance in rice: a review of conventional breeding to molecular approaches. *Molecular Biology Reports*, *40*(3), 2369–2388. https://doi.org/10.1007/s11033-012-2318-0

Romero, F. M., & Gatica-Arias, A. (2019). CRISPR/Cas9: Development and Application in Rice Breeding. *Rice Science*, *26*(5), 265–281. https://doi.org/10.1016/j.rsci.2019.08.001

Singh, G., Rani, R., Sivia, S. S., None Sunayana, & Sanyal, A. (2024). Pre-Breeding to Molecular Breeding for Biotic Stress Management in Crop Plants. *Apple Academic Press EBooks*, 191–218. https://doi.org/10.1201/9781003361862-8

Stram, Y., & Kuzntzova, L. (2006). Inhibition of Viruses by RNA Interference. *Virus Genes*, *32*(3), 299–306. https://doi.org/10.1007/s11262-005-6914-0

Yang, Y., Yu, J., Qian, Q., & Shang, L. (2022). Enhancement of Heat and Drought Stress Tolerance in Rice by Genetic Manipulation: A Systematic Review. *Rice*, *15*(1). https://doi.org/10.1186/s12284-022-00614-z

Yulong, Y., Qingfeng, M., Hao, Y., Qingsong, Z., Ye, L., & Cui, Z. (2021). Climate Change Increases Nitrogen Concentration in Rice With Low Nitrogen Use Efficiency. *Earth's Future*, *9*(9). https://doi.org/10.1029/2020ef001878

About this Book

This book provides a thorough exploration of cutting-edge advancements in cotton and rice cultivation, with a strong focus on the role of genetic engineering and molecular biology in enhancing crop yield, quality, and resilience. Each chapter delves into the scientific foundations and practical applications of key genetic, physiological, and biochemical pathways essential for improving these globally important crops.

In Chapter 1: Cotton, the book examines cotton's genetic and hormonal pathways, covering advancements in biotechnology and molecular breeding. It highlights the role of key genes, including SuS (Sucrose Synthase) and GhEXPA8, in fiber quality and yield. From genetic transformation techniques, such as Agrobacterium-mediated transformation, to an analysis of enzymatic roles in sucrose metabolism, this chapter reveals how genetic and physiological interventions can enhance cotton's fiber strength, insect resistance, and overall agronomic performance. Discussions also extend to actin dynamics and their effect on cotton fiber quality, making this chapter a valuable resource for understanding the cellular mechanisms that drive cotton improvement.

Chapter 2: Rice addresses the global significance of rice and introduces innovative strategies for enhancing its resilience and productivity. The book explores genetic approaches for combating rice yellow mottle virus (RYMV), including the role of microRNAs in plant responses to biotic stress. This chapter further examines Bt gene deployment for insect resistance, challenges in field efficacy, and biosafety of transgenic Basmati rice lines. Essential topics such as cryopreservation techniques, transgene detection, and marker-assisted selection are discussed, along with agronomic traits like lodging resistance and maturity. With insights into field performance and environmental considerations, this chapter provides a balanced view of rice biotechnology and its future potential in sustainable agriculture.

Contents of this book are majorly based on the different studies conducted by the editor and his working groups over a period 30 years.

About the Author

With over 35 years of professional experience at the Center of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, Prof. Dr Tayyab Husnain has served both as a leader and a scientist, bringing together a wealth of expertise in administration and molecular biology. Prof. Husnain spearheaded the development of Pakistan's first double gene insect-resistant transgenic cotton varieties, including CEMB 33, CA12, and CEMB-66, as well as the first triple gene insect- and herbicide-resistant cotton varieties, CKC-1 and CKC-3. His contributions also include the creation of transgenic cotton with improved fiber characteristics, all of which have been patented and commercialized. As Director of CEMB, he led a team of over 300 researchers and students, fostering innovation that has produced 765 publications with an impact factor exceeding 2000 and 13 patents. His work has earned recognition through prestigious honors like the Izaz-i-Fazeelat (2005), Tamgha-e-Imtiaz (2008) for academic excellence and Satha Innovation Award (2017) for multigenic cotton development that significantly contributed to advancing both science and the agricultural economy in Pakistan.

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