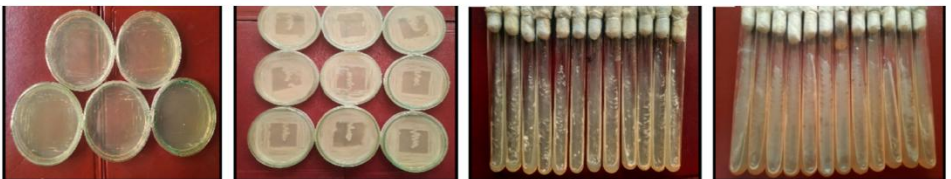


# ADVANCEMENTS IN PLANTS STRESS TOLERANCE & THEIR MEDICINAL USE

## EDITORS

PROF. DR. MUHAMMAD ARSHAD JAVED  
DEPARTMENT OF PLANT BREEDING AND GENETICS  
UNIVERSITY OF THE PUNJAB, LAHORE - PAKISTAN

DR. SHAKEEL AHMAD  
UNIVERSITY OF AGRICULTURE, FAISALABAD - PAKISTAN



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## About this Book

This book tackles the pressing challenges facing modern agriculture: overcoming abiotic stresses, battling plant diseases, and ensuring sustainable crop production. It explores the latest advancements in plant science, biotechnology, and agronomy to address these issues and secure food for a growing global population.

Key areas covered in this book:

- **Overcoming Abiotic Stress:** The book examines the devastating impact of salinity, iron toxicity, and acidic soils on crop productivity, particularly rice. It investigates genetic and physiological mechanisms behind plant tolerance to these stresses, offering insights for developing stress-resistant crops.
- **Sustainable Disease Management:** The use of eco-friendly biocontrol agents, such as *Trichoderma harzianum*, is highlighted as a promising approach to combat soil-borne diseases like charcoal rot. The book also explores the potential of medicinal plants in cancer therapy.
- **Crop Improvement and Nutrition:** The book emphasizes the importance of seed quality, nutrient uptake, and genetic diversity in crop improvement. It explores the nutritional value of staple crops like rice, maize, and capsicum, along with strategies to enhance their nutritional content.

This book is a valuable resource for researchers, scientists, policymakers, and agricultural practitioners seeking innovative solutions to the pressing challenges in agriculture.



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# Chapter 1: Advancements in Germination and Seedling Stress Tolerance in Crop Species

Javaria Tabassum<sup>1</sup>, Zain Mushtaq<sup>2</sup>, Nusrat Jahan<sup>3</sup>

## Acidic Soil Constraint to Crop Production

Soil acidity is considered one of the main constraints to crop production since about 30-40% of the soils of the world have a pH below 5.5. The smaller the pH value, the more acid the soil. Highly acid soils often have low fertility problems caused by mineral toxicities like  $\text{Al}^{3+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Fe}^{2+}$  in combination with deficiencies in macronutrients such as phosphorus (P), calcium (Ca), and magnesium (Mg). In acidic soils of low pH, there is a high solubility of aluminum and other elements like  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  that can be toxic to rice crops. Weathering of the soil is enhanced by environmental factors such as high rainfall coupled with high temperatures, which facilitates the leaching of essential nutrients like Ca, P, and K and leaves behind more stable compounds rich in  $\text{Al}^{3+}$  and  $\text{Fe}^{2+}$  oxides. This major component of soil clays,  $\text{Al}^{3+}$ , is solubilized under acid conditions below pH 5.0 to become highly phytotoxic and rapidly impair root growth. That would lead to stunted root systems and reduce the capacity of crops to absorb water and nutrients. Toxicity of  $\text{Al}^{3+}$  in acidic soils reduces the production by causing impairment in root growth, cell division, and cell elongation.  $\text{Al}^{3+}$  can bind with the free carboxyl groups of pectin, and this results in the cross-linking of pectin molecules, leading to a fall in wall elasticity, which is limiting to cell elongation. The problem of acidification is increasing due to environmental changes. Contributing factors include reduction of available arable lands due to soil weathering, unsustainable farming practices, acid rain, rigorous agricultural practices, and climate change. Practices such as liming with  $\text{CaCO}_3$  may alleviate certain of the acidic soil constraints and increase productivity. However, liming tends to increase pH in only the superficial soil layers and is generally ineffective in correcting subsoil acidity. More specifically, the limiting of liming's

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<sup>1</sup> Department of Plant Breeding and Genetics, University of the Punjab, Lahore – Pakistan

<sup>2</sup> Department of Soil Science, University of the Punjab, Lahore – Pakistan

<sup>3</sup> Department of Biotechnology, BUITEMS, Quetta – Pakistan

effect is due to subsoil acidity in 75% of acidic soils in the world. In any case, where it is not particularly relevant because of high costs and lack of infrastructure, liming is often not practicable.

The development of  $Al^{3+}$  crop variety tolerance in acidic soils, therefore, has to be an imperative strategy within global breeding programs. Identifying QTL associated with the traits of tolerance is one such technique that supports this effort. The study aimed at identifying statistical approaches that could optimize  $Al^{3+}$  toxicity tolerance levels in two of the commonly used indica rice varieties, Pokkali and Pak Basmati, under high  $Al^{3+}$  toxicity concentrations at the germination stage.

A study was reported whose objective was to observe germination and early seedling growth for two rice genotypes, Pak Basmati and Pokkali, in a series of concentrations of aluminum chloride ( $AlCl_3$ ). Low concentrations, as in 5 mM, were found to hardly affect germination, with the rice genotypes having high resistance or tolerance. However, the growth of the seedlings alone was drastically reduced at this concentration, indicating that these varieties were sensitive to aluminum from an early stage. At 10 mM  $AlCl_3$ , both Pak Basmati and Pokkali expressed much higher growth inhibition than germination inhibition but again were relatively moderately sensitive at this concentration level.

Significantly, aluminum toxicity was much more effective at higher concentrations of 15 and 20 mM. It thus showed a strong inhibitory effect on both germination and early growth. Other parameters of growth, except those for final germination rate, germination speed, and germination index, were significantly reduced in these two higher concentrations. A clear trend was shown: the higher the level of aluminum, the lower the germination success and seedling vitality, which was especially true with levels higher than 15 and 20 mM. For both genotypes, 20 mM led to pronounced sensitivity, where nearly all the growth measures showed significant effects. Among such indicators, germination index, and mean germination time showed this sensitivity through large increases in delay or reduction in response to aluminum toxicity at these levels. These results agree with other literature, including the study conducted on maize, whose exposure to aluminum suppressed germination and vigor in seedlings, though most profoundly at higher concentrations. In these, the phytotoxic action of aluminum might be related to a quick depletion of the stored nutrient supply at the

seed, preventing growth during its earliest phases. Owing to these profound impacts on germination and growth at 20 mM, this concentration was considered as the sweet benchmark for further studies such as research for the identification of genetic markers related to tolerance of aluminum.

## **Problems and Strategies in Rice cultivation under Salinity Stress**

Rice is one of the most vital food items for a large percentage of the world's population, and people from Asia alone include approximately three billion people who use rice daily. Due to this dire need for rice, many Asian countries are among the major rice producers. The increase in the world population presently is continuous, which places a higher demand on staple foods such as rice, and as there is increased demand, large-scale production is required to supply adequate food. Meeting these challenges will not be easy because abiotic stresses such as salinity are posing a major impact on rice cultivation. There are two major methods of rice production, namely: transplanting, where the seedlings are first raised in nurseries and then transplanted to the field, and; direct seeding, where the seeds are sown directly into the field. Although transplanting is very labor-intensive and costly, lately, farmers adapted more to direct seeding due to its ease and reduction in labor costs. However, saline conditions have certain disadvantages linked with the direct seeding approach, whereby salinity stress may affect germination—a very critical stage in the life cycle of rice. While germination is normally a short-term event in the life cycle, its failure can lead to poor establishment of plants that eventually results in yield decline. With these considerations in mind, enabling rice production in highly sensitive saline-affected areas becomes an important component in supporting such production at higher scales using appropriate methods or strategies. The present work represents a general outline of the germination responses to salinity of six rice varieties that had been evaluated for their potential value in breeding programs. These preliminary results form the foundation for the further selection of cultivars with the capability to grow well on salt-affected soils and provide a useful shortcut for further improvement in rice.

Evaluation of rice performance under salinity based on a single germination trait is often not sufficient. The realization of high final germination percentages, even under high salinity, may vary at very

different rates, as evident from the performance of some cultivars. It is hence necessary that there should be consideration of more than one germination attribute to arrive at the correct evaluation. Poorer germination under the saline treatments could be attributed to the osmotic stress provided by the higher ionic concentration in the soil, which lowers the water potential and limits water uptake by the seed. The prolonged exposure leads to ionic stress as a result of sodium toxicity, which disrupts cellular function and leads to further reductions in germination. On the basis of mean salinity tolerance, rice varieties tested could be arranged from most tolerant to least tolerant as: MR219 > MR211 > Pokkali > Pak Bas > Firat 1 > Panderas. While salinity generally inhibits seed germination, varieties differ in the extent of the effect. Those that are tolerant of salinity are only marginally inhibited by levels at which germination in sensitive varieties is completely inhibited. This variability underlines the need to search for and breed tolerant varieties for the effective advancement of rice production under the difficult environmental conditions of its growth.

### **Effects of Iron Toxicity on Rice Growth and Resistance Mechanisms**

Rice is considered a staple food by the majority of the population throughout the world, and it is grown more in Asia; more than 90% of the total consumption and production is accounted for by Asia. About 128 million hectares are allocated for rice in irrigated and rainfed lowlands; however, a substantial area of this faces serious problems in terms of nutrient deficiencies and mineral toxicities, mainly from iron and zinc. These are the soil constraints that reduce rice yields over about 100 million hectares. Iron toxicity, coupled with zinc deficiency, constitutes one of the critical constraints to rice production, especially in acidic soils where these minerals become more soluble, thus damaging root systems and deterring rice growth. Large expanses of arable land in Asia, South America, and Africa go uncultivated due to iron toxicity. This condition manifests itself as leaf bronzing in rice that drastically serves to stunt plant growth. This culminates in dwarfing and biomass decrease, which lower the yield. The majority of iron in the soil is in the form of ferric ions ( $\text{Fe}^{3+}$ ); it is hence mostly insoluble and unavailable for absorption by plants. Under acidic conditions, however, the ferric ion converts into ferrous ion ( $\text{Fe}^{2+}$ ), which dissolves and is highly available for rice root absorption.

When rice plants absorb more  $\text{Fe}^{2+}$  than they need through roots and transport it to the leaves, a progressive build-up of oxidative stress causes cellular damage. Iron toxicity expresses under conditions when the plant's tolerance is overwhelmed with increasing levels in the soil, this factor was known to affect rice production in countries such as Malaysia, the Philippines, India, Madagascar, and regions of West Africa. Two upland and three lowland rice varieties were studied for their growth of rice seedlings, iron content, and nutrient distribution under excessive  $\text{Fe}^{2+}$ . The result showed that the toxicity of iron significantly decreased all growth parameters like plant height, shoot and root dry biomass, and leaf vitality. In both  $\text{Fe}^{2+}$ -resistant and susceptible varieties, it was observed that roots and shoot biomasses were reduced, thus showing the overall limitation of rice development in iron toxicity.

While some crops, such as maize, might show enhanced root elongation in response to particular stress, the development of shoots often remains stunted, as it was shown in this work.  $\text{Fe}^{2+}$  built up in the highest quantities in roots and in the lowest-in flag leaf-across all examined varieties, and this iron accumulation in roots seriously impaired nutrient uptake, which influenced general plant development. The rice varieties showed variable tolerance against iron toxicity; the percent reduction in the height of seedlings ranged from 2.2% to 17.1%. Maximum reduction was by the Panderas variety and minimum by Pokkali. It means that rice varieties take sides against iron toxicity tolerance. Statistical analysis revealed an outstanding effect of iron stress on growth and ion uptake. Both level of stress as well as variety significantly influenced all the growth parameters studied, namely,  $\text{Fe}^{2+}$  and  $\text{K}^+$  content, indicating thereby that the response to iron toxicity varied among rice varieties. Variety showed their significant impact on most of the parameters except root  $\text{Fe}^{2+}$  and flag leaf  $\text{K}^+$ .

Iron toxicity significantly increased the iron content in the roots, leaves, and flag leaves of stressed plants when compared with controls; however, it did result in very high accumulation of iron in the roots. Surprisingly, some varieties had developed mechanisms to prevent excessive  $\text{Fe}^{2+}$  absorption from the root and subsequent translocation within the plant. Such a protective approach is crucial in helping some rice varieties to tolerate higher levels of iron toxicity. Such resistant varieties may employ other internal mechanisms, which include partitioning  $\text{Fe}^{2+}$  within tissues and reducing cellular damage, whereas sensitive varieties do not have such defensive capacities against high

Fe<sup>2+</sup> conditions. High levels of potassium in the leaves and flag leaves of the resistant varieties point to a possible role of K<sup>+</sup> ions in mitigating Fe<sup>2+</sup>-induced accumulation in sensitive plant tissues. Similar results were obtained when investigating hybrid rice, where higher rates of potassium led to lesser accumulation of Fe<sup>2+</sup>, suggesting that the potassium nutrient can act antagonistically to the toxicity caused by the iron element. In highly susceptible varieties, the distribution of Fe<sup>2+</sup> ions is much freer within the plant tissues because such plants lack specific barriers to iron uptake and translocation. Factoring in that iron distribution varies in different plant parts and that some varieties lack the effective iron-exclusion mechanisms, there is a need to carry out selective breeding of Fe<sup>2+</sup>-resistant rice varieties. The mechanism of the restrictive uptake of iron and compartmentalization of Fe<sup>2+</sup> within plants needs to be unraveled for future breeding advisement. The development of rice varieties that will grow under iron-toxic soils opens the possibilities for acidic, iron-rich soils to be productive for cultivation and will help contribute to sustainable rice production across a wide range of growing regions.

## **Effects of Salinity on Rice Growth and Salt Tolerance Mechanisms**

Rice feeds more than three billion people, especially in Asia, where yields have been significantly increased by the Green Revolution. Rice cultivation in Sailendra faces one serious problem: that of salinity. Salt-affected soils occupy extensive areas of cultivable land in the world, and it affects crop productivity to a greater extent, especially rice production in salt-affected environments. Soil salinity, which is caused mainly by sodium chloride (NaCl), inhibits rice growth by impairing water uptake and proper nutrient balance in the plant. High concentrations of salts create an osmotic environment that inhibits the uptake of water, a condition which essentially places plants under drought conditions even when water is available. The physiological effect of salinity is compounded by ion toxicity, especially from the sodium ions, which interfere with the nutrient uptake mechanisms of the plant. Sodium interferes with potassium by competing for transport channels in the plant, interfering with its critical potential functions; potassium plays an important role in enzyme activation and also in maintaining stomata open. This interfering results in an imbalance that leads to reduced growth, weakening the plant's resilience under saline conditions.

To try to surmount these challenges, research on the salinity tolerance of rice varieties have been conducted to come up with those characteristics that would enable the plants to do well in areas with saline conditions. Tolerant plants tend to keep higher amounts of sodium in their roots while maintaining low concentrations of sodium in sensitive organs like stems and leaves in both upland and lowland varieties. This could be a selective distribution aimed at reducing the toxic effects of salt on vital structures. In contrast, salt-sensitive varieties often lack this selective accumulation and exhibit sodium concentration throughout the plant, which impairs their growth and leads to stunted development under saline conditions. Generally, the roots were more inhibited than shoots by NaCl stress, as evidenced from the significant reduction in length with increasing levels of salinity. Reduced biomass in plants subjected to salt stress also agrees with observations in a wide array of plants and further suggests roots act as a barrier to prevent excess accumulation in the upper parts of the plant. Salt retention in the root may thus be a good indicator of the physiological basis of salinity tolerance and hence would be an excellent plant trait for selecting rice cultivars that grow well under saline conditions. It would appear that salt tolerance in rice involves several mechanisms blocking the salt from sensitive tissues. High sodium levels contained within the root cells or even within the vacuoles reduce salt-induced damage on leaves and stems effectively.

With salt tolerance, there is an inverse relationship between the sodium and potassium concentration in the salt-tolerant rice, whereas potassium plays a critical role in maintaining the cellular function and may help in balancing the sodium level, thereby protecting the plant from ionic stress. This potassium buffering mechanism supports the survival and productivity of plants on saline soils in tolerant varieties, especially those adapted under saline conditions. In salinity ecosystems, leaf potassium is also said to play a protective role in minimizing uptake of Na<sup>+</sup>, which is important for ion balance and resistance against salt stress. In contrast, sensitive varieties fail to survive in saline conditions because of poor compartmentalization of Na<sup>+</sup>, whose accumulation disrupts cellular function and growth processes. Salinity not only interferes with the establishment of seedlings but also delays major development stages such as panicle emergence and flowering. These delays in plant development impact reproductive success through reduced viability of pollen and seed set, ultimately influencing yield. Salinity tolerance across the rice plant life cycle varies, but one of its

most sensitive stages is in its seedling establishment under a high level of soil salinity. As an example, rice seeds may germinate provided there is only moderate salinity, but if the salinity is sufficiently high, germination rates and seedling viability are strongly reduced. This variability emphasizes the potential variability in salt tolerance, particularly during the early growth stages, which is important for successful plant establishment. Since salts concentrate at the surface when irrigation is in short supply, drought exacerbates salinity problems in areas with both water shortages and saline soil, irrespective of whether there is a shortage of water. Hence, plants increasingly suffer from stress due to lack of water and salt, interacting to give a much greater yield loss. Salinity exerts its injury on rice plants through three basic mechanisms: inducing osmotic stress, giving rise to specific ion toxicity, and nutritional imbalance due to longer exposure. Osmotic stress or "physiological drought" is a condition wherein, for a given salt concentration in soil, plants cannot take up the available water. Specific ions, such as sodium, act directly by causing toxicity due to disruption of metabolic processes; nutrients are not absorbed, hence weakening the plant under saline conditions. The key to sustainable rice production in saline conditions would be to select varieties that have a high salt retention at root levels, effective sodium retention, and potassium buffering mechanisms. These traits allow rice to sustain higher levels of salinity with lesser impairment of tissues, hence vastly improving normal yields on land previously unsuitable for traditional rice cultivation.

## Further Readings

- Chen, K., & Arora, R. (2017). Understanding the roles of reactive oxygen species and antioxidants during seed germination: Current status and future prospects. *Botanical Studies*, 58(1), 1-13.
- Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., & Job, D. (2012). Seed germination and vigor. *Annual Review of Plant Biology*, 63, 507-533.
- Wang, W., Chen, Q., Hussain, S., Mei, J., Dong, H., Peng, S., & Liu, J. (2016). Pre-sowing seed treatments in direct-seeded early rice: Consequences for emergence, seedling growth and associated metabolic events under chilling stress. *Scientific Reports*, 6, 19637.
- Ashraf, M., & Foolad, M. R. (2015). Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in Agronomy*, 88, 223-271.



- Weitbrecht, K., Müller, K., & Leubner-Metzger, G. (2011). First off the mark: Early seed germination. *Journal of Experimental Botany*, 62(10), 3289-3309.
- Nonogaki, H. (2014). Seed dormancy and germination—emerging mechanisms and new hypotheses. *Frontiers in Plant Science*, 5, 233.
- Kubala, S., Garnczarska, M., Wojtyła, Ł., Clippe, A., Kosmala, A., & Żmieńko, A. (2015). Deciphering priming-induced improvement of rapeseed (*Brassica napus* L.) germination through an integrated transcriptomic and proteomic approach. *Plant Science*, 231, 94-113.
- Jisha, K. C., Vijayakumari, K., & Puthur, J. T. (2013). Seed priming for abiotic stress tolerance: An overview. *Acta Physiologiae Plantarum*, 35(5), 1381-1396.
- Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: State of the art and new perspectives. *Plant Cell Reports*, 34(8), 1281-1293.
- Bewley, J. D., Bradford, K. J., Hilhorst, H. W., & Nonogaki, H. (2013). *Seeds: Physiology of development, germination and dormancy*. Springer Science & Business Media.
- Shu, K., Liu, X. D., Xie, Q., & He, Z. H. (2016). Two faces of one seed: Hormonal regulation of dormancy and germination. *Molecular Plant*, 9(1), 34-45.
- Graeber, K., Nakabayashi, K., Miatton, E., Leubner-Metzger, G., & Soppe, W. J. (2012). Molecular mechanisms of seed dormancy. *Plant, Cell & Environment*, 35(10), 1769-1786.
- Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany*, 61(3), 199-223.
- Chen, F., Wang, F., Wu, F., Mao, W., Zhang, G., & Zhou, M. (2010). Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance. *Plant Physiology and Biochemistry*, 48(8), 663-672.
- Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *International Journal of Genomics*, 2014, 701596.
- Mittler, R. (2017). ROS are good. *Trends in Plant Science*, 22(1), 11-19.
- Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences*, 14(5), 9643-9684.

## Chapter 2: Green Fungicides and Anticancer Alternatives

Elham Khalili<sup>1</sup>, Fahrul Zaman Huyop<sup>2</sup>, Roswanira Ab. Wahab<sup>3</sup>, Muhammad Arshad Javed<sup>4</sup>

### Sustainable Strategies for Managing Soil-Borne Plant Diseases: The Potential of $\beta$ -Glucosidase Enzyme

The food requirement, which will increase by 2050 due to the growing human population, will necessitate doubling production accordingly. In contrast, food crops continuously incur losses from continuous threats due to insects, weeds, and plant diseases. Of these, one of the most dangerous threats to crops is soil-borne plant diseases; given this fact, the estimated annual yield losses they cause amount to around 20%. Such a soil-borne pathogen is the fungus *Macrophomina phaseolina*, which causes charcoal rot and which attacks crops as basic as grains, legumes, oilseed, jute, and cotton. This fungus is quite enduring, having the ability to survive in the soil for as long as 15 years through the formation of such resistant structures as sclerotia and chlamydo spores. The charcoal rot is disseminated by wind, soil contact, and infected plant material, which cause the widespread damage and considerable crop loss. Farmers use cultural, biological, and chemical methods as management approaches against plant diseases till today.

These include crop rotation, soil solarization, and planting of tolerant varieties, among many other cultural practices. While somewhat useful, these methods are usually ineffective for complete disease management and thus require supplementary support from other methods to further enhance the effect, which could be the usage of biocontrol agents or pesticides. In reality, the large-scale application of it involves complex and expensive technological systems. Major decisions that have been made include chemical pesticides, herbicides, and fumigants; however, the efficacy of theory has come into question. The high extent of the rate

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<sup>1</sup> Department of Electrical and Engineering, Centre of Vision Speech, and Processing, University of Surrey, Guildford – UK

<sup>2</sup> Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru – Malaysia

<sup>3</sup> Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru – Malaysia

<sup>4</sup> Department of Plant Breeding and Genetics, University of the Punjab, Lahore – Pakistan

of application per hectare has developed out of proportion with the increased output of crops to give rise to environmental and health concerns. Chemical runoff may contaminate water sources while its bio-accumulation into the food chain takes a toll on the wildlife and humans alike. Besides that, the unchecked use of pesticides eventually led to most pests developing pesticide resistance. This caused ecological imbalance and harmful "superweeds" and "superpests" that proved injurious and pose a new threat to crop management. Toxicity and concern for environmental hazards and public health have spurred desires to find alternatives to this hundred-year-old disease management practice using a bio-based solution through the application of the enzyme  $\beta$ -glucosidase from the fungus *Trichoderma harzianum*. The enzyme shows a very high specificity toward *T. harzianum*, attacks the  $\beta$ -linkages in the cell wall of *M. phaseolina*-damaging its protective structures and therefore allowing direct penetration of the antagonist fungus into the pathogen to attack.

Application of  $\beta$ -glucosidase in situ bypasses the defenses of the pathogen; hence, there will be no spread of charcoal rot without the use of toxic chemicals. This approach merges the efficient action of chemical treatments with traditionally regarded environmentally friendly methods of biological control, an effective means of managing soil for fungal pathogens. This may be one of the new paradigms in disease management strategies as such a biofungicide will offer sustainability by reducing dependence on chemical fungicides, besides other environmental benefits.  $\beta$ -glucosidase, produced from a low-cost agro-industrial waste like banana waste, was produced through batch cultivation of *T. harzianum*. This low-cost production might make enzyme-based treatments feasible for farmers and further enhance sustainable agriculture practices.

Soybean plants infected with *M. Phaseolina* were subjected to application of the enzyme  $\beta$ -glucosidase under controlled greenhouse conditions. In this regard, the efficacy of this treatment was measured by the PDI over the eight-week period, during which the  $\beta$ -glucosidase treatment expressed a significantly lower PDI compared to those untreated. This will be the first use of  $\beta$ -glucosidase as a bioactive constituent in any bio-fungicide. Thus, the optimization study was further focused on making this enzyme perform better under variable conditions by applying response surface methodology to determine the best treatment conditions to minimize PDI. The enzymatic treatment

was further compared with Carbendazim, the widely used chemical pesticide, for efficacy and cost-effectiveness. Results have marked the efficacy of  $\beta$ -glucosidase comparable to Carbendazim with no injurious side effects from the use of synthetic chemicals. Further, economic evaluation revealed that  $\beta$ -glucosidase presents an economical alternative to chemical pesticides, particularly if this enzyme is produced from low-value substrates. This enzyme-based approach to the control of *M. phaseolina* infection in the soybean plant constitutes one of the most promising strategies related to economic and ecological interests about sustainable agriculture. Biofungicide provide an effective means of controlling a pathogen from the soil without depending on chemical pesticides, which are harmful to the environment. The simplicity and low cost of this enzyme-based approach could facilitate greater dissemination among farmers, hence allowing a transition toward environmentally friendly disease management practices in agriculture.

### **Soybean Cultivation: Charcoal Rot and the Need for Its Sustainable Management**

Charcoal rot of soybean, caused by a soil-borne fungal pathogen, *Macrophomina phaseolina*, is one of the most severe diseases of this legume crop in several parts of the world. This pathogen generally prevails under conditions of a warm and dry environment; high temperatures along with low moisture act to weaken the plant's natural resistance, thus making it highly susceptible to infection in soybeans. *M. Phaseolina* infects vascular tissues of hosts, hinders transportation of water and nutrients, and hence causes wilting, stunted growth results in huge losses regarding yields and quality of crops. Infection by *M. phaseolina* produces resistant structures called microsclerotia, thereby enabling the fungus to survive in soil even under adverse conditions. Later crops planted on such infected soil are put at risk of susceptibility to new outbreaks, enter a continuous infection cycle, and seriously threaten yield stability. Charcoal rot's impact on soybean crops is most important during the reproductive stages, since it coincides with a plant's most vulnerable time to water and nutrient stress. Symptoms that indicate heavy infection include chlorotic leaves, root necrosis, discoloration of the stem, which prematurely causes them to senesce. Infected fields usually receive huge economic loss. Since the global demand for soybean increases every year for its major sources-protein, oil, and biofuel-efficient management of charcoal rot has become a prime concern for both farmers and researchers.

## **Current Challenges in Charcoal Rot Management**

Traditionally, charcoal rot management in soybean production depends on chemical fungicides like Carbendazim. The wide application of this broad-spectrum fungicide has been in use in most crops against soil-borne pathogens. However, its effectiveness against *M. phaseolina* has been variable. First, Carbendazim fails to penetrate into the microsclerotia of *M. phaseolina* remain dormant and ready to infect successive crops. Besides, chemical fungicides need repeated applications, which make their use more expensive for farmers and tends to contaminate the environment. Carbendazim could persist in the soil for a longer period, thereby affecting non-target organisms and beneficial microorganisms in the soil. Repeated applications of fungicides over time increase the chances of developing resistant strains of pathogens, which may lead to more difficult control of diseases. Such disadvantages signal the need for the development of other methods of management which can be less expensive and sustainable. Biocontrol solutions involve the use of natural organisms or compounds against pathogen suppression and are considered one such alternative. Use of biocontrol solutions focus on: reduction in chemical inputs, reducing environmental impact, and reduction of pathogen resistance through mechanism targeting specific biological processes. Enzymatic treatments have shown promise among different approaches for biocontrol, as they can be designed to target pathogens with no adverse effects on the host plant or ecosystem.

## **$\beta$ -Glucosidase from *T. harzianum*: action mode and pathogen control**

One of the promising agents for biocontrol against *M. phaseolina* is  $\beta$ -glucosidase, an enzyme purified from the fungus *Trichoderma harzianum* T12. The enzyme shows specificity to only attack the cell wall of the pathogen and not affect the soybean plant. The main determinant for the effectiveness of  $\beta$ -glucosidase is its capability of degrading  $\beta$ -1,3-glucan, which is a polysaccharide constituent of the cell wall of a number of pathogenic fungi including *M. phaseolina*. By degrading the latter, the enzyme  $\beta$ -glucosidase scantily breaks the structural integrity of the fungal cell wall, hence weakening its pathogen host and making it unable to proliferate in the rhizosphere. Beta-glucosidase hydrolyzes the  $\beta$ -1,3-glucan polymers into simpler molecules that, in essence, "dissolve" the cell wall of the pathogen. This reduces the penetration and proliferation capabilities of *M.*

*phaseolina* during an infection. Unlike chemical fungicides, which can be very broad-acting,  $\beta$ -glucosidase is highly specific, acting only against the pathogen and leaving the host plant unaffected.

This specificity arises because of the absence of the link  $\beta$ -1,3-glucan in plant cell walls, which permits the action of  $\beta$ -glucosidase on the pathogen without affecting the structural components of the plant. Secondly, *T. harzianum* facilitates  $\beta$ -glucosidase activity by forming hyphae that infiltrates *M. phaseolina* cells; thus, acting as a direct delivery system for the enzyme. Notice the dual mechanism at work here: not only does it prevent *M.*

*phaseolina* from establishing in the root zone but also reduces the severity of infection, hence helping to protect plant health and promoting its growth.

## **Optimization of Formulation Variables for Effective Pathogen Control**

To maximize the effectiveness of  $\beta$ -glucosidase against *M. phaseolina*, several formulation variables such as metal ion concentration, surfactant addition, enzyme concentration, and irrigation frequency were optimized. These factors synergistically enhance the stability, distribution, and activity of the enzyme, which in turn lowers the plant disease index (PDI) and offers the best protection for soybean plants.

**1. Concentration of Metal Ion ( $Zn^{2+}$ ):** The  $Zn^{2+}$  ion is very important in improving both catalytic activity and stability of  $\beta$ -glucosidase. Quite often, metal ions play the role of cofactors by stabilizing functional enzyme conformations which enable enzymes to perform their catalytic functions much more efficiently. For example, in the case of  $\beta$ -glucosidase,  $Zn^{2+}$  binds to specific sites that may be crucial for maintaining the structural integrity of the enzyme, besides supporting the enzyme activity against fungal pathogens. This increased stability allows  $\beta$ -glucosidase to be effective over longer periods of time, contributing positively toward its net capability in fungal growth inhibition. Optimum levels of  $Zn^{2+}$  in the formulation favored a higher rate of pathogen inhibition and, therefore, became an integral part of the biocompatible strategy.

**2. Surfactant Addition:** Tween 80 is a non-ionic surfactant and forms yet another critical ingredient in aiding the distribution of enzymes by reducing surface tension. This also facilitates the distribution of a  $\beta$ -

glucosidase solution in a more homogeneous way among the plant roots and soil particles, improving its contact with the pathogen. Besides, Tween 80 inhibits the denaturation of the enzyme, prolonging the time of activity and increasing bioavailability as well. By providing equal distribution and long-lasting stability, the surfactant supports the enzyme's antifungal action against *M. phaseolina*, resulting in increased efficacy.

**3. Concentration of Enzyme and Frequency of Irrigation:**  $\beta$ -glucosidase is directly proportional to its protective efficacy since the higher the concentration, the more active enzyme molecules will be available to inhibit fungal growth. Greenhouse trials indicated that the optimal concentration required to control *M. phaseolina* was about 15 mg/mL. It is also the frequency of irrigation level that plays a principal role in enzyme effectiveness. Regular irrigation twice per week helped distribute the enzyme uniformly throughout the soil, while sustaining soil moisture levels that discouraged *M. phaseolina* proliferation. Excess soil moisture, therefore, favors enzymatic activity while interfering with the pathogen's ability to form microsclerotia, thereby reducing the possibility of crop re-infection. All these variables, in concert, would create a non-conductive environment for *M. phaseolina*, which translated into significantly lower PDI values.

### **Comparative advantages of enzyme-based formulations and cost-effective field applicability**

Formulation of  $\beta$ -glucosidase thus has several comparative advantages over typical chemical fungicides. Due to its specificity, the soybean plant is not affected by  $\beta$ -glucosidase, as it exerts its action only on *M. phaseolina*. Unlike Carbendazim, that exerts its action on a wide range of organisms and persists in the soil for some time,  $\beta$ -glucosidase is biodegradable, an action that actually minimizes impact on the environment and maintains safety in respect to the buildup of toxic residues. This biocontrol method is one way of contributing to sustainable agriculture by reducing the use of synthetic chemicals, which meanwhile decreases the risks from chemical fungicides such as resistance by a pathogen and pollution of water. It was also observed that enzyme-based formulation was equally effective in controlling charcoal rot and promoting plant growth. Field applications showed that all the growth parameters, like root and stem length, were higher in plants treated with biocontrol agents compared to the conventional

fungicides. This infers the dual advantage of an enzyme-based formulation in disease control with the added benefit of enhancement of plant growth. Estimation of the cost of production of enzyme-based biocontrol method is also very much cheaper. An acre treated with  $\beta$ -glucosidase will cost roughly \$34 against Carbendazim at \$240, thus representing an 85% cost reduction. Mainly, this is because production costs of enzyme formulations are lower and are less dependent on synthetic chemicals.

Moreover, due to the natural composition of  $\beta$ -glucosidase formulation, it does not require any special handling and disposal; thus, it further reduces costs associated with it. Therefore, this method has proved scalable for large-scale farms and smallholder operations as an affordable solution for sustainable management of diseases. Implications for Sustainable Agriculture and Broader Applications Success in the use of  $\beta$ -glucosidase as a biocontrol agent reflects wider trends in sustainable agriculture. As farmers increasingly abandon synthetic agrochemicals, enzyme-based treatments such as  $\beta$ -glucosidase represent a non-toxic and efficient alternative to these no longer accepted chemicals. Biocontrol agents may be used in concert with other management practices, including rotation, organic amendments, and biological treatments, to provide an integrated pest management (IPM) strategy with a minimum of ecological disruption. This use of  $\beta$ -glucosidase in soybeans perhaps might be further extended to other crops that could be susceptible to *M. phaseolina* and other similar pathogens, such as peanuts, corn, and cotton. The agricultural industry stands to gain a lot in terms of breaking away from chemical fungicides, which therefore helps in preserving biodiversity by protecting beneficial soil organisms and reducing pollution by promoting enzyme-based formulations. Furthermore, the enzyme-based approaches might lead to other techniques of biocontrol for almost all crop diseases and would provide an integrated approach to the management of plant diseases, which fits perfectly in environmental conservation.

There is high potential of *T. harzianum*  $\beta$ -glucosidase as a biocontrol agent in the management of charcoal rot in soybean plants. By specifically targeting *M. phaseolina*, *T. harzianum*  $\beta$ -glucosidase has shown itself to be a potent elicitor in this biocontrol application. *Phaseolina*,  $\beta$ -glucosidase lessens the disease's impact in a very effective manner while promoting healthy growth of the plant. The enzyme-



based formulation is cost-effective and friendly to the environment; therefore, it suits the goals of sustainable agriculture. This biocontrol technique offers farmers a very powerful tool in protecting crops devoid of ecological risks due to treatment with chemicals. In the light of such findings, future research may be applied to long-term assessment of soil health due to  $\beta$ -glucosidase-based formulation, focusing on changes within the microbial community and, in general, the structure of soil. Further studies may indeed be conducted with regard to combined applications of  $\beta$ -glucosidase with other biological control agents, like PGPRs and mycorrhizal fungi, in developing a multi-layered IPM that ensures protection. Other areas of interest concern the optimization of methodologies for the production and purification of  $\beta$ -glucosidase, which would reduce the cost even more and make it more accessible to farmers all over the world. These findings from the study constitute a very promising model of biological disease management, since, in addition to proving that enzyme-based treatments can control pathogenic fungi, the farming practice is environmentally responsible. More research and development with enzyme-based biocontrol agents could be important players in the future of crop protection, piecing together food supplies and natural resource preservation in the face of swelling global agricultural demands.

## **The Power of Medicinal Plants to Heal**

Plants have represented the main source of medicine for humanity since time immemorial. From knowledge and practices in ancient times to the modern day, they have provided innumerable remedies that heal and protect against illness. They remain one of the most important aspects of medicine today, and more than half of the drugs in clinical practice have their origins from some sort of plant-derived compound. Traditional medicine remains a major healthcare source for approximately 80% of the global population, relying heavily on plant extracts. These traditional remedies cover everything from common colds to serious diseases based on knowledge passed down through generations. Added to this is the increasingly important problem of resistance to antibiotics. As bacteria and other microorganisms become resistant to conventional drugs, researchers around the world are looking to plants in their quest for new antimicrobial agents.

Attention to plant-based medicines is nothing new; since the dawn of the 1960s, various large-scale studies unveiled thousands of plant species that had therapeutic potential, including anticancer properties.

Most of these findings came from looking into traditional remedies and resulted in the discovery of more than 120 clinical drugs that had their origin in plants. Malaysia's Rich Botanical Heritage Malaysia is a real treasure of medicinal plants, adorned with very commendable biodiversity. Located within the tropics, Malaysia has thousands of plant species, which have rarely or hardly been seen elsewhere in the world, thereby earning it a place among the top 12 countries with high biodiversity. Much of the medicinal plants in Malaysia are deeply rooted in indigenous culture and valued for their healing properties and wider application in treating various human health conditions. The three most important Malaysian herbs are *Eurycoma longifolia* (Tongkat Ali), *Labisia pumila* (Kacip Fatimah), and *Ficus deltoidea* (Mas Cotek); scientific research now focuses on their huge importance in traditional medicine and potential health benefits.

**The Many Benefits of *Ficus deltoidea*:** *Ficus deltoidea* is a shrub belonging to the family Moraceae. With its place in traditional Malaysian medicine, it is regionally known as Mas Cotek; valued for centuries, the plant has been used to remedy various ailments through natural methods. Different portions of this plant are used for different purposes. For instance, its fruit is used as medicine for headache, and at times is chewed to kill toothache. The roots and leaves have are dried and powdered, then applied externally to ease sores and wounds. Leaves are also tied to the joints for rheumatic pain, and a tea made from leaves is taken by women after confinement to give firmness to the uterus. Hence, the high amount of flavonoids in *F. deltoidea* can be highly credited for its health-promoting properties. Flavonoids are plant compounds widely regarded for their extensive range of health beneficial properties. Flavonoids were observed to possess a variety of properties like anti-inflammation, antimicrobial, antiallergy, and even anticancer properties. Thus, *F. deltoidea* acts as not only a traditional remedy but also a promising plant in modern medicinal uses.

***Labisia pumila*: The Queen of Malaysian Herbs:** *Labisia pumila* is one of the most popular medicinal plants in Malaysia, being hailed as the "queen of herbs". Originating from Southeast Asia, this small leafy plant belongs to the family Myrsinaceae and has long been valued for its medicinal properties. This is also utilized by traditional Malay women to help with childbirth, as the extract from this plant is said to stimulate contractions in the uterus. Despite this, it is still widely applied today by Malaysian women for a range of reproductive health purposes. It may

regulate menstrual cycles and further promote sexual health and reproductive well-being. Other than woman, *L. pumila* is ingested by most men of various ethnic groups in Malaysia in order to enhance stamina and salubrity. *L. pumila* is equally rich in phytoestrogens-plant compounds that behave just like estrogen. This makes it very interesting in the context of cancer research; phytoestrogens inhibit the growth of cells in prostate cancer. Such phytoestrogens act against specific types of cancer cells by binding onto estrogen receptors, hence making *L. pumila* a potential natural therapy for hormone-related cancers.

### **Prostate Cancer: An Emerging Threat**

Prostate cancer is also one of the most common tumor diagnoses in the world, and it is particularly prevalent in Western countries. It may be one of the leading cancers among American men, in which one out of seven men face the risk of developing this condition during their lifetime. Tens of thousands of men lose their lives each year to serious instances of prostate cancer. The mortality record from this cancer is in the high list across Europe. These medical advances have reduced mortality rates from prostate cancer somewhat in parts of the world. Prostate cancer remains one of the major health issues globally because it is the most commonly diagnosed cancer among males, except for skin cancer, and one of the leading causes of cancer-related death in men.

The prevalence of prostate cancer, most especially among older men, constitutes the demand for the need to develop new treatment options. Traditional therapies, though effective for some patients, come with their own share of side effects and shortcomings. It is in this respect that the emergence of alternative treatments, particularly those involving plants, such as *L. pumila*, provides a promising avenue for more natural and less invasive cancer therapies.

### **Exploration of the Therapeutic Potential of *F. deltoidea* and *L. pumila***

Research was conducted to investigate the effect of aqueous extracts from *Ficus deltoidea* and *Labisia pumila* on human prostate cancer cells and human skin cell lines. Cell viability and changes in the morphology of the cell are ways to understand how such plant extracts may influence cancer cell behavior. There are studies which explored the impact of *F. deltoidei*. Their findings signify that both *F. deltoidea* and *L. pumila* are potential therapeutic agents for further study. Being of plant origin,

these two species offer better prospects than conventional cancer therapies, especially for people who seek succor from more traditional and holistic health perspectives. These findings also point to the untapped potential of other local plants in Malaysia's rich medicinal plant heritage that may possess similar health-enhancing properties.

### **Understanding the Growth of Cancer Cells and Natural Treatments**

Intriguing patterns can be observed in the life cycle of the human prostate cancer cells. Their complete life cycle runs a total of two weeks, and they do require a two-day adjustment period, in which the cells orient themselves with the environment and build up the enzymatic requirements. By day three, the cells enter an exponential growth phase, multiplying rapidly to their peak at day six, where they are perfectly healthy and at their maximum numbers. During this most active growth period for these cancerous cells, every twenty-four hours results in a cell population doubling—a timing unusually consistent for prostate cancer cells. By the eighth day, the cells cease active growth. By the ninth day, decline begins to set in as the environment becomes increasingly toxic from cellular waste and their food sources begin to run low. At last, by the fourteenth day, there are no longer any living cells. Knowledge of this lifecycle is very important to scientists who study the cells and work toward developing treatments.

**Nature's Medicine Cabinet:** Plant-Based Compounds In medicinal plants, phytochemicals are powerful natural compounds that lie deep within the roots, flowers, fruits, or leaves of plants. It works as a self-defense mechanism of the plant against diseases. Traditional herbs having such protective compounds have always been used since historical times against respiratory problems, diabetes, high blood pressure, anything about digestion, and even cancer. Recent studies indeed prove that some extracts of plants do slow the growth of human prostate cancer cells, although not with equal efficacy. *Labisia pumila* plant is a natural flavonoid and phenolic acid-rich agent of cancer treatment. Some of the strong compounds identified in this plant are resorcinols, gallic acid, myrecetin, kaempferol, and catechin. In the case of prostate cancer, these naturally derived chemicals combine to inhibit proliferation of prostate cancer cells. Researchers have identified that epigallocatechin, one such chemical, inhibits prostate cancer cell growth by initiating specific cellular pathways. Another exceptional plant, *Ficus deltoidea*, is full of beneficial substances like polyphenolics, flavonoids,

including Genistin, alkaloids of antofine type, and tannins. Leaves contain very Active Ingredients epicatechin, myricetin, naringenin, and various complex flavonoid compounds. The high concentration of epicatechin seems to endow the plant with notably expressive antioxidant activity. Being compared to *Labisia pumila*, *Ficus deltoidea* demonstrates even more expressed anticancer action of killing prostate cancer cells much faster and in a more efficient way. Apoptosis, or programmed cell death, is a cascade of dramatic changes that ultimately destroys cancer cells. It starts with changes in the cell membrane and proceeds to the fragmentation of the cell into membrane-bound apoptotic fragments called apoptotic bodies. The changes on the outer surface of the cell are among the earliest signs that the cell has begun this process. When these tropical plant extracts are administered, the outer walls of the cancer cells begin to show distinctive bubbling patterns, which signal that the cells have died. Further confirmation of the beginning of cell death is observed by the condensed DNA within such cells.

**The Future of Natural Medicine:** Herbal medicine holds great promise and can alter the course of modern drug development. There are several marked advantages: treatments may remain less expensive, new drugs can be discovered faster, and many varied components inside herbs may act together for enhanced effects. However, challenges remain in globalization regarding standardization of these medicines, especially in evaluating their composition, effectiveness, safety, and quality. All these challenges notwithstanding, a blend of traditional herbal knowledge with advanced medical science can usher in new ways for better health, particularly in the treatment of life-threatening diseases like cancer.

### **Charcoal Rot Disease and Its Management Using *Trichoderma harzianum***

Charcoal rot, caused by the highly destructive soil-borne fungal pathogen *Macrophomina phaseolina* (Tassi) Goid., occurs in over 500 plant species distributed among 100 genera and belonging to both monocotyledonous and dicotyledonous plant species. The disease is of great economic importance in several crops, including oilseed crops such as corn, *Zea mays*; sorghum, *Sorghum bicolor*; sunflower, *Helianthus annuus*; cotton, *Gossypium herbaceum*; sesame, *Sesamum indicum*; and soybean, Glycine max. Within the United States alone, there were 1.98 million metric tonnes of yield losses in charcoal rot during 2003, while in

2004 and 2005, losses were 0.28 and 0.49 million metric tonnes, respectively. This disease is considered severe with respect to various environmental factors, especially in water stress conditions and at the time of drought. Various studies have shown the susceptibility of plants to the infection by *M. phaseolina* during the most critical drought and scanty water conditions. These conditions are more favorable for the growth of the pathogen and are deep inside the host plants for mycelial penetration in search of nutrition, causing severe damage to form and structure, hence leading to plant death. Under regions with hot growing seasons, the incidence is very high since the optimal temperature and heat has a positive effect on the fungus since it enhances the extent that damage can be inflicted.

### **Characteristics of the Pathogen and Symptoms of the Disease**

This adaptability of *M. phaseolina* contributes greatly to making it a pathogen. The host range of the fungus is extensive, infecting plants belonging to more than 75 families of both monocots and dicots. This wide host spectrum, besides its ability to grow well over a wide range of climatic conditions from arid to tropical, makes this pathogen extremely dangerous. Besides this, considerable morphological and pathogenic variability exists in this fungus, leading to a wide variation in disease severity among different isolates. Symptoms representative of charcoal rot include sclerotial development at the crown of the plant, giving a "black leg" appearance. Spindle-shaped lesions with dark borders and light grey centers may occur, often covered with small, pinhead-sized microsclerotia, with or without pycnidia development. Symptomatic plants most commonly have weak growth, discolored leaves, and reduced secondary root development. Eventually, the plants die from the production and release of phytotoxic metabolites by the pathogen, such as phaseolinone. These chemicals block the vascular system, impeding nutrient transport across the plant, leading to plant death.

### **Challenges in the Management of Charcoal Rot Disease**

There are various traditional methods of *M. phaseolina* control which include cultural, physical, chemical, and biological strategies. However, most of these approaches are effective in disease management under a prophylactic control system. One of the most restrictive factors of charcoal rot management is that this pathogen resides in the soil. Generally, the use of chemical fungicides is less effective against *M. phaseolina*, due to its ability to survive in the soil as sclerotia and to

survive therein for extremely long periods, up to 15 years. On the other hand, chemical treatments have several disadvantages: they kill not only pathogenic fungi but also useful soil microbes, disrupt ecological equilibria, and may contribute to the selection of fungicide-resistant strains of the pathogen. The relatively high prices of the chemical fungicides make their general application unaffordable, especially for poor and small-scale farmers. In this respect, there is a growing interest in more environmentally friendly and sustainable charcoal rot management. Among these, one of the promising alternatives to chemical fungicides involves the employment of biological control agents, such as the soil fungus *Trichoderma harzianum*, well documented for its antagonistic action against various phytopathogens. *T. harzianum* showed growth inhibition of *M. phaseolina* through mechanisms such as competition for nutrients, production of secondary metabolites, and mycoparasitism.

### **Potential of *Trichoderma harzianum* to Control Charcoal Rot**

In addition to its effectiveness as a biocontrol agent, *T. harzianum* offers several advantages compared to traditional chemical treatments. Unlike synthetic chemical fungicides, which may injure non-target organisms and the environment, *T. harzianum* is an eco-friendly alternative. It has been reported to increase plant growth and to enhance soil health through the development of a diverse and balanced microbial community. Furthermore, *T. harzianum* is relatively inexpensive to produce, thus attractive to farmers, especially those in developing regions. Application of *T. harzianum* as biocontrol agent can be effected through soil inoculation or seed treatment, both having proved to reduce the incidence of charcoal rot in affected crops. Several works reported the efficiency of *T. harzianum* in controlling *M. phaseolina* in different agronomic crops of cotton, soybean, and sunflower. Inoculation with *T. harzianum* decreases not only the disease intensity but also increases yield of crops for being economically viable to address charcoal rot challenges. Thus, the charcoal rot caused by *M. phaseolina* is still one of the most serious factors[s] in agriculture, especially in areas experiencing drought and heat stress. Biological control agents using *Trichoderma harzianum* are thus a very promising alternative to traditional methods of chemical fungicides, which have several disadvantages. Among all the mechanisms applied by *T. harzianum* against *M. phaseolina* coupled with environmental benefits, cost-

effectiveness, and various modes of action, makes this an important tool in the sustainable management of charcoal rot. Finally, further research into optimizing the use of *T. harzianum* and other biocontrol agents will be essential to enhance the resilience of crops against this devastating disease.

### **Biological control of *Macrophomina phaseolina* by *Trichoderma harzianum*: an integrated approach for sustainable management**

The growing resistance to chemical fungicides by *M. phaseolina*, which causes charcoal rot in several crops, attracted sufficient interest in undertaking further investigation into finding alternative methods of control. Natural biocontrol agents are those isolated from nature and include soil microorganisms; such an approach would significantly reduce reliance on chemical fungicides in a nonphytotoxic and environmentally friendly manner for the management of this devastating phytopathogen. Of these, *T. harzianum* has been proved to be the most prospective agent due to its well-documented antagonistic properties against a wide range of plant pathogens including *M. phaseolina*.

#### **Antagonistic Mechanisms of *T. harzianum***

Apart from identification, various laboratory methods were adopted to determine the modes of action of the obtained *T. harzianum* isolates against the test pathogen *M. phaseolina*. Experiments were conducted in dual culture techniques for observing direct interactions between *T. harzianum* and *M. phaseolina*. The results showed that the three isolates of T2, T10, and T12 had a high inhibition power against *M. phaseolina*; among them, the maximum inhibitive action was shown by the T12 isolate up to 72.31%, followed by T2 (45.23%) and T10 (44.13%) with moderate inhibitions. This means that *T. harzianum* competes more effectively with *M. phaseolina* for resources and space, thus limiting the pathogen's ability to infect host plants. Besides dual culture tests, the production of volatile metabolites was also analyzed for understanding the role of chemical warfare that *T. harzianum* exerts against *M. phaseolina*. It is very well documented that VOCs, or volatile organic compounds, are involved in the suppression of plant pathogens, and it was no exception by the isolates of *T. harzianum* under study. Among them, the volatile metabolites produced by T12 were especially potent and induced a 63.36% reduction in growth of *M. phaseolina*, while T2



and T10 were less effective with a growth inhibition percentage of 51.31% and 43.30%, respectively. These results support the view that production of VOCs by *T. harzianum* is one of the prime reasons for its antagonistic activity. Further tests of hyperparasitism were conducted to determine the suitability of *T. harzianum* to directly attack and parasitize *M. phaseolina*. The T12 isolate showed intense hyperparasitic activity, where the hyphae of *T. harzianum* grew over the colony of *M. phaseolina* and consumed it. This interaction caused a loss of cell wall integrity in *M. phaseolina*, leading to the death of the pathogen. The induction of *T. harzianum* results in its use for the production of a wide range of bioactive compounds, including enzymes and secondary metabolites, which lead to the degradation of cell walls of pathogens and inhibit their growth.

### **Field testing of *T. harzianum* for control of disease**

Field trials were conducted to confirm the efficacy of these antagonistic isolates of *T. harzianum* under natural conditions. Inoculation with *M. phaseolina* was done, followed by the application of suspensions of the three *T. harzianum* isolates. Parameters considered in assessing the effects of the treatments were disease incidence, plant growth in terms of stem length and root length, and seed weight. Field trials showed that out of the three isolates tested for *T. harzianum*, it reduced disease severity of charcoal rot, and the most promising was found to be T12. On the plant growth parameters after the trunk treatment, root and shoot length were significantly higher in the treated plots when compared with an untreated control plot. Seed weight increased in the treated plots, especially on the T12 plots treated with this fungus, indicating equally superior performance over the rest in disease management that coupled improvement in plant health.

Disease severity was gauged on the basis of PDI, and seed inoculation had less PDI compared with soil incorporation. The T12 seed inoculation treatment had also given a significantly lower PDI of 5.55%, compared with 49.06% in control treatment, while other isolates gave an intermediate response. Thus, time and method of application are important factors that modify the performance of *T. harzianum*. Faster establishment in the seedling stage probably contributed to the better efficacy of *T. harzianum* because it was able to colonize the root system quickly and outcompete the pathogen more rapidly. *T. harzianum* was also effective in the field by inhibiting pathogen growth and, to a greater effect, by promoting plant growth. The stunted growth-one that occurs

with infected soybean plants-is because of root damage caused by *M. phaseolina*. Root development could be enhanced by *T. harzianum* isolates and, in general, contributed to plant vigor, hence providing resistance under pathogen stress in plants. Results from field trials revealed that the strains of *T. harzianum* can perform dual roles: the fungus acts not only as a biocontrol agent but also promotes growth in plants, hence making them an attractive combination for integrated pest management. Of these, the T12 isolate had maximum elongation of roots, ranging from 23.04 to 26.68 cm, while the roots in the control plants were significantly much smaller. These changes in the rooting pattern probably helped the plants to evade the stress of drought and other environmental factors more effectively and added advantages to the use of *T. harzianum* as a biocontrol agent. Studies have identified *T. harzianum* strains, particularly the T12 isolate, as a highly promising biocontrol agent against *M. phaseolina* in soybeans. Inhibition of pathogen growth along with maintenance of plant health therefore makes *T. harzianum* an important ingredient in sustainable agriculture. However, variations in efficacy of different isolates will always remain an important factor regarding the selection of appropriate strains for biocontrol purposes. This may focus future research on the comprehension of the genetic and biochemical mechanisms responsible for the higher efficacy of some strains of *T. harzianum*, such as T12. The action mechanism and identification of the bioactive compounds involved in the suppression of pathogens may be further assisted by the evaluation of specific enzymes involved, such as  $\beta$ -glucosidase, and secondary metabolites, including polyketides and terpene derivatives. Synergies with other biocontrol agents or cultural methods may further enhance disease management packages. Another related area of future study is large-scale application in different cropping systems and geographical regions. Further studies should be developed to give *M. phaseolina* a broader application in integrated pest management programs. In this regard, the current chapter has again pointed out how *T. harzianum* may act as a biocontrol agent against *M. phaseolina* that causes charcoal rot. The laboratory and field results obtained in this work underlined the possibility of this fungus in reducing disease severity and enhancing plant growth as an environmentally friendly and sustainable alternative to chemical fungicides. It has yet to be followed by further research into the mechanisms of antagonism by *T. harzianum* together with field testing under a wide range of agricultural conditions if it is to be developed as a reliable biocontrol agent against soil-borne crop pathogens.

## **Development of Herbicide-Resistance Crops for Weed Management**

Weeds create one of the biggest barriers to agricultural production competitive plants result in the usage of vital resources by crops, such as nutrients, moisture, and light. Consequently, this may lead to reductions in crop yields, the quality of produce, and reduced productivity from pastures. Economic losses due to weed management are also relatively high, considering that control activities, labor costs, and herbicides applied involve billions of dollars every year worldwide. As industrialization and economic development in general continued to advance, farm labor costs began a tortuous rise and therefore pressured the need for economical herbicides. Herbicides have now become the predominant tool in modern agriculture, representing about 50% of global agricultural chemical sales. In countries like the United States, Australia, and Germany, for example, herbicides account for 60–70% of agricultural chemical sales. However, along with the blessings associated with their use, herbicides also bring a number of drawbacks: specific environmental pollution, possible harm to human and animal health, development of herbicide-resistant weeds. Herbicide resistance has now become a matter of grave concern, and over 370 biotypes that are resistant to herbicides have been detected in major agricultural regions. The United States, Australia, and Canada have reported the largest numbers of such herbicide-resistant species. This escalating trend towards increased resistance by species calls for immediate implementation of other effective and feasible methods of managing weeds to reduce dependence on chemical herbicides. This is achieved through biotechnical improvement of herbicide-resistant crops in order to overcome some of the various pitfalls that are usually associated with herbicides.

These transgenic crops are genetically modified to bear resistance against specific herbicides and, therefore, can be used as potential alternatives to conventional weed management. Resistance is usually achieved by incorporating a gene responsible for the detoxification of herbicides or modification of the target site of herbicides where the herbicide exerts its action. For example, bacterial genes capable of degrading herbicide compounds have been introduced into plants to develop novel cultivars resistant to herbicides. In addition to bacterial genes, other synthetic genes that confer herbicide resistance include those conferring resistance to the herbicides bromoxynil, Basta, and

Buster. Such genes have been used successfully in crops such as tobacco, potato, and carrot, which can thus survive and set growth in the presence of the herbicide at levels that normally cause damage. One successful approach has been the use of the dehalogenase D [dehD] gene, originally from *Rhizobium* sp. RC1. It codes for an enzyme that breaks down the herbicides MCA and D-2-chloropropionate by cleavage of the carbon-halogen bond. This is because the interest in the dehD gene can be considered huge, there is potential for broad-spectrum herbicide resistance. Anything done to this gene allows the engineering of crops to give tolerance to herbicides like MCA, which are widely used for controlling weeds and in the process causing damage to good crops. The possibility of the expression of herbicide resistance was expressed in tobacco plants. Resistance to MCA was confirmed through tissue-culture assays. Further testing, using a leaf-painting assay, showed that such plants could survive herbicide treatment that would be lethal to non-resistant ones. This proves the efficiency of using the dehD gene in developing crops resistant to herbicides. The development of herbicide-resistant crops allows another great leap forward in sustainable management of weeds. Applying the technology of genetic engineering allows for the development of crops resistant to herbicides; this will reduce the use of chemical herbicides and mitigate their impact on the environment and human health. Further research and innovation in the field of weed management continue to provide new effective solutions to improve crop productivity with environmental sustainability.

### **MCA Herbicide-Resistant Tobacco Development with dehD Gene**

Among the most used vectors, pCAMBIA 1305.2 stands out with its relatively small size and efficiency in replication in *Escherichia coli* via the pBR322 origin of replication. Very stable replication is also present in *Agrobacterium* spp., positioning it among the most useful plant genetic engineering vectors. It has kanamycin resistance genes for bacterial selection and hygromycin resistance genes for selection of plant transformations.

The dehD gene encodes for a selectable marker that imparts herbicide resistance in plant transformation systems. The dehD gene encodes for an enzyme that would detoxify MCA, a widely used herbicide in weed management. The inserted dehD gene in plant cells enables plants to survive on MS medium containing hygromycin and MCA. This is an important development since it opens a better avenue for selecting

transformed plants which are able to survive the application of a certain herbicide. The identical strategy has been earlier demonstrated with a variety of other herbicide-resistance genes and reinforces the effectiveness of dehD as a selectable marker. Almost all gene transfer in plant transformation typically involves some major steps: bacterial colonization, activation of the bacterial virulence system, formation of the T-DNA transfer complex, and transfer and integration of T-DNA into the plant genome. The entire process is influenced by a number of variables; one of these is the type of promoter paired with the inserted gene's expression. Here, the dehD was under the control of the cauliflower mosaic virus CaMV 35S promoter in transformed plants to ensure high expression levels throughout the plant. After transformation, the presence of the dehD gene in the PCR was confirmed. The fragment of the dehD gene appeared in all the independent transgenic lines. Further, it was confirmed that all the transformed plants carrying the hpt gene which confers the hygromycin resistance by PCR amplification of the hpt gene. Growth on MS medium containing hygromycin confirmed their successful transformation. It is the hpt gene that provides an important marker for selection of plants that have the incorporated DNA. The dehD gene encodes a dehalogenase enzyme that breaks down MCA into a non-toxic glycolic acid. The enzyme product of this gene efficiently detoxifies MCA in plant cells, thus providing resistance to the herbicide in the transformed plants. Under the control of the CaMV 35S promoter, the dehD gene was constitutively expressed in the transgenic plants, enabling their survival and growth even in the presence of MCA.

While the RT-PCR further verified the expression of dehD in all transgenic lines, non-transformed plants expressed none. MCA is a broad-spectrum herbicide, absorbed into plant cells probably via active transport. The chemical exerts its toxic action through interference with the mitochondrial citric acid cycle, inhibiting key enzymes such as aconitase, and interfering with the photosynthetic machinery. At higher dosages, treatment with MCA produces quite readily visible symptoms that include necrosis and chlorosis of leaves.

The systemic action of herbicide results in yellowing of leaves, especially in the control plants, which did not have genetic resistance to MCA. However, the transgenic plants expressing dehD did not develop symptoms, proving the internally detoxifying action of MCA inside plant cells by this dehalogenase enzyme. High rates of MCA application

resulted in brown spots and necrosis in the leaves of the control plants due to the fast action of the herbicide. Thus, under the high rate of MCA application, significantly lower injury could be observed in the transgenic plants. The presence of the dehD gene was responsible for the degeneration of the herbicide before it could initiate its action. Therefore, the dehalogenase enzyme produced by the dehD gene played a crucial role in the detoxification of MCA.

Symptoms of yellowing of leaves were observed in the control plants at lower concentrations of MCA, indicating thereby the systemic action of this herbicide; the same, however, did not affect the transgenic plants at all, indicating thereby that the toxic action of the herbicide was nullified by the expression of dehD in the plant. These results confirm that dehD is exceptionally potent against herbicide toxicity by detoxifying MCA inside the plant cells. Transformation of tobacco with the dehD gene is one of the promising strategies for the improvement of crop resistance to herbicides. Although several studies previously dealt with tobacco transformation using herbicide resistance genes, the use of the dehD gene from *Rhizobium* sp. RC1 is novel. The research work indicates that this dehD gene can provide effective resistance to MCA—a widely used herbicide—and thus an important tool in the management of the infestation by weeds without resorting to more toxic chemicals. This could have further economic and environmental implications. Allowing crops to resist herbicides means farmers reduce their reliance on harmful herbicides, which in turn reduces production costs and lessens environmental pollution. In addition to that, such engineering in crops for resistance to herbicides would ensure high yields and reduce the chances of resistance developing inside weed populations. The successful expression of the dehD gene in tobacco also provides a worthwhile model for the development of herbicide-resistant crops in other species. Therefore, the use of the dehD gene in providing herbicide resistance has great potential in plant genetic engineering. The potential to provide resistance against broad-spectrum herbicides such as MCA will create new opportunities toward more sustainable agriculture. Further research and development could make this an integral part of integrated weed management strategies that support both agriculture and the environment.

## Further Readings

Zhang, M., Zhao, J. L., Liu, Y. M., & Chen, H. B. (2020). Plant-derived natural products as alternative approaches for fungal disease

- management: Sources and mechanistic insights. *Frontiers in Microbiology*, 11, 1340.
- Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770-803.
- Dias, D. A., Urban, S., & Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites*, 2(2), 303-336.
- Singh, R., Kumari, N., & Nath, G. (2016). Microbial bioactives in plant disease management: Current trends and future prospects. *Sustainable Agriculture Reviews*, 18, 111-147.
- Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, 20(3), 200-216.
- Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, 2013, 162750.
- Ríos, J. L., & Recio, M. C. (2015). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100(1-2), 80-84.
- Mishra, B. B., & Tiwari, V. K. (2011). Natural products: An evolving role in future drug discovery. *European Journal of Medicinal Chemistry*, 46(10), 4769-4807.
- Wani, M. C., & Horwitz, S. B. (2014). Nature as a remarkable chemist: A personal story of the discovery and development of Taxol. *Anti-Cancer Drugs*, 25(5), 482-487.
- Zhu, F., Ma, X. H., Qin, C., Tao, L., Liu, X., & Shi, Z. (2012). Drug discovery prospect from untapped species: Indications from approved natural product drugs. *PloS One*, 7(7), e39782.
- Kumari, P., Mishra, R., Arora, N., Chattopadhyay, A., & Gupta, V. K. (2017). Recent developments in bio-control and bio protection of horticultural crops. *Microbial Biotechnology*, 2, 1-11.
- Kingston, D. G. (2011). Modern natural products drug discovery and its relevance to biodiversity conservation. *Journal of Natural Products*, 74(3), 496-511.
- Mondal, K. K., & Mani, C. (2012). Investigation of the antibacterial properties of nanocopper against *Xanthomonas axonopodis* pv. *punicae*, the incitant of pomegranate bacterial blight. *Annals of Microbiology*, 62(2), 889-893.
- Butler, M. S., Robertson, A. A., & Cooper, M. A. (2014). Natural product and natural product derived drugs in clinical trials. *Natural Product Reports*, 31(11), 1612-1661.
- ☐ Rajesh, K., Dhanasekaran, D., & Tyagi, B. K. (2015). Mosquito survey and larvicidal activity of actinobacterial isolates against *Culex* larvae (Diptera: Culicidae). *Journal of the Saudi Society of Agricultural Sciences*, 14(2), 116-122.

## Chapter 3: Innovative Approaches in Salinity Tolerance and Crop Nutrient Optimization

Shakeel Ahmad<sup>1</sup>, Muhammad Ashfaq<sup>2</sup>, Misbah Riaz<sup>2</sup>

### Salinity Tolerance in Rice: Genetic Insights and Challenges

**R**ice, which is the staple food of a large fraction of people around the world, is quite vulnerable to environmental salinity. Generally, rice is regarded as one of the most salinity-sensitive crops out of all. Salt tolerance is staged in rice according to the stage at which it is undergoing its life cycle. For instance, germination, active tillering, and maturity are somewhat more resistant, while highly sensitive stages include the seedling phase, early reproductive phase, pollination, insemination. This variability in response across growth phases emphasizes the complex nature of salinity tolerance in rice—a trait largely shaped by genetics and environmental conditions. Breeding efforts over the years have made big strides in identifying rice varieties exhibiting increased tolerance to saline conditions, although much is yet to be done to understand the genetic underpinnings of this trait. The effects of salinity on rice production are particularly severe on lands that have irrigation, where otherwise very high productivity could have been achieved. Efforts to improve rice's salinity tolerance have thus focused on genetic enhancement, especially through the identification of specific genes associated with this trait. These studies have been greatly benefited by the molecular marker techniques that allowed isolating QTLs associated with salinity tolerance during the seedling stage, one of the highly salt-sensitive stages of plants. Such genetic markers will enable the scientists to pinpoint the closest gene association with a trait that provides enhanced salinity tolerance in a plant.

Rice is one of the few crops that grow well under waterlogged and saline soils, so poor performances under saline conditions are a matter of great concern. Generally, salinity disrupts physiological functioning in plants

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<sup>1</sup> Seed Center and Plant Genetic Resources Bank, Ministry of Environment, Water and Agriculture, Riyadh – Kingdom of Saudi Arabia

<sup>2</sup> Department of Plant Breeding and Genetics, University of the Punjab, Lahore – Pakistan



by triggering osmotic stress, toxic ion accumulation, and nutrition that competes with the essential ones. Sodium ions, for example, interfere with the uptake of potassium ions—one of the major growth nutrients—by plants. In their effort to come up with rice varieties tolerant of salt, breeders have turned to traditional varieties of rice known to possess inherent tolerance to salt. In particular, two *indica* cultivars, Pokkali and Nona Bokra, have proved very helpful for this purpose. They contain certain genetic attributes that facilitate their survival under saline conditions in which modern high-yielding rice varieties are sure to perish. It is hoped that by crossing these cultivars with high-yielding ones, they may become able to obtain rice plants combining high productivity with salt tolerance. Understanding Correlation of Traits and Genetic Complexity

Salinity tolerance within rice is a complex quantitative trait involving multigenic participation, often with interactions in complex ways. While all that complexity, QTLs stand at the core of understanding it. QTLs are segments of the genome associated with particular traits, such as salt tolerance, whose expression is modifiable by environmental factors. The identification of QTLs linked to the trait related to salinity tolerance can be regarded as one of the major milestones in rice genetics. For example, studies have established a significant association between SIS and salt tolerance with the sodium content within shoots. In this connection, SIS was considered an appropriate indicator for salt tolerance since it signals physiological responses of rice plants to saline stress. Another finding concerns the involvement of a microsatellite locus, RM80, located on chromosome 8, which has been associated with sodium accumulation in shoots and, therefore, salt tolerance. The high association between the Na<sup>+</sup> content in the shoot and the Na<sup>+</sup>/K<sup>+</sup> ratio in the shoot suggests that the latter is also genetically explained. This was further supported by the QTL for these traits on chromosome 8, which truly underlines the potential of this chromosomal region as a target for breeding salt-tolerant rice varieties. Complementary to the findings on chromosome 8 is the presence of a QTL on chromosome 9 associated with potassium content and the sodium-potassium ratio in the root. In plants, one major determinant of salt sensitivity is the accumulation of excess Na<sup>+</sup> in shoot tissues. This causes reemergence toxic ions inside the cell when rice plants take up a huge amount of sodium, interfering with normal cellular activity. In non-halophytes such as rice that do not naturally thrive in saline environments, this results from high levels of ion toxicity. Because of this, too much concentration of sodium interferes with water balance,

nutrient uptake, and overall growth of the plant, leading to stunted growth of plants damaged in tissues.

Interestingly, though the salt injury score increases linearly with the sodium content in the shoot, a similar linear increase has not been recorded in potassium contents in both shoots and roots, thereby indicating that, under salinity stress, sodium might be the major ion responsible for the injury to rice. Salinity influences the uptake of sodium and potassium in complex ways. While the assimilation of sodium and potassium is largely an independent process, for example, these ions do interact competitively in the shoot of the plant. For instance, an increase in the sodium ion tends to inhibit potassium uptake, a thing that is very critical at cell turgor and enzyme activity in leaves of the plants. While the content of sodium and potassium in the root showed a positive relationship, for the overall salt tolerance of the plant, the sodium-potassium balance in the shoot is more indicative. Sodium and potassium uptake in rice showed an inverse relationship, indicating that a balanced ion ratio needs to be achieved within the plant. Anther Culture and Breeding for Salinity Tolerance: Recent Advances Because of the sufficiently complex genetic basis of salinity tolerance, traditional breeding efforts alone will be doomed to failure in the production of high-yielding salt-tolerant rice varieties in which huge numbers of plants have to be evaluated. Anther cultures are a particular form of tissue culture, which have emerged as a useful tool for rice breeding. This technique involves inducing embryogenic calli from microspores in the anther, or the pollen sac, and regenerating plants from those calli. Breeding techniques provide a means for breeders to quickly produce lines with desirable combinations of traits; in particular, for those traits that involve multiple genes controlling traits like salt tolerance. The use of anther culture has been widespread in japonica rice and has contributed to the release of many high-yielding, disease-resistant, and salt-tolerant cultivars in countries like Japan and China. However, the salt-tolerant indica rice varieties have always responded poorly to techniques conducted *in vitro*. In the case of anther culture, early anther necrosis limited callus formation, and a high percentage of regeneration of albino plants impeded progress in anther culture of indica rice. These are highly governed by genotype and the composition of the culture medium. The efforts to optimize anther culture have focused on improving the composition of the culture medium and adjusting the environmental conditions.

It has been observed that nitrogen contents in the cultural medium have much effect on callus induction and plant regeneration; high nitrogen-containing media allow more vigorous growth and development. The two most commonly used media for rice anther culture, SK and modified N6, vary in inorganic salt composition, organic compound content, and plant growth regulators. These differences carry over into their comparative efficiencies: the latter medium was more effective in callus productivity and green shoot regeneration in salt-tolerant indica varieties like Pokkali and Nona Bokra. The improved N6 medium contains ingredients included in SK medium, but with modifications that create more favorable conditions for callus formation and shoot regeneration. Temperature is another critical component for the successful outcome of anther culture. In japonica rice, it has been observed that the occurrence of day and night, alternating temperatures, improved the callus induction and shoot regeneration. In one experiment using salt-tolerant indica cultivars, anthers cultured at alternating temperatures of 30°C during the day and 20°C at night showed a big improvement in the efficiency of anther culture. These methods could be further applied in rice tissue culture and beyond since temperature variables more accurately represent natural environmental conditions and promote healthy tissue development. The second critical factor for the anther culturing technique is the choice of sugar in the medium.

On the other hand, maltose has been considered to enhance the efficiency of anther culture in cereals including rice instead of sucrose. Unlike sucrose, maltose degrades slowly, releasing energy steadily without osmotic stress. Maltose is also reported to prevent albino shoot formation, one of the serious problems in rice anther culture. Maltose has proved better than sucrose regarding the higher division rate of microspores and support for healthy growth toward the production of green viable plants from anther culture. Somaclonal variation or various genetic diversities from the tissue cultures could be exploited-too great a prospect for rice breeding. By selecting desirable somaclonal variants, breeding of rice varieties with improved tolerance to salinity and in other desirable traits can be done. This somaclonal variation has been used to select agronomically useful traits in plants such as increased biomass and higher levels of tolerance to saline conditions in Pokkali. The newly revised N6 medium in concert with other factors such as alternating temperatures and maltose has proved very effective for producing green shoots from salt-tolerant indica rice cultivars with high regeneration competence. Besides this, the salient features of anther culture in rice breeding programs are numerous in the pursuit of salt tolerance. For

instance, Pokkali carries the Bph9 gene, which imparts resistance to one of the most economically important pests in rice, the brown planthopper. Breeders can use anther culture to combine salinity tolerance with pest resistance, enabling rice crops to withstand abiotic and biotic stresses. This could revolutionize rice cultivation in saline-affected areas where, so far, good yields have been difficult to get from such a hostile environment.

### **Rice: A Lifeline Under Pressure**

Rice is the staple diet for over half of the world's population and provides 80 percent of daily calories in most areas of Asia, Africa, and Latin America. With rising global populations, rice demand is increasing; hence, meeting the increasing demand for rice becomes very important, especially amidst a changing climate that is hostile to agricultural productivity. Of these, soil salinity is regarded as one of the major constraints to rice production in both coastal and arid inland areas. Moreover, salt further contaminates more than 800 million hectares of cultivable land around the world and understanding and improving rice tolerance to salinity stress is vital for future food security. Global agricultural pattern has dramatically changed, and climate change continues to deteriorate the flooding, drought, and sea-level rise. Due to these conditions, saltwater intrusion projects onto agriculture are exacerbated, especially in coastal lands, turning more vulnerable to flooding due to tides and contamination by seawater. Salinity problems inland are mostly induced by saline groundwater, poor irrigation practices, and salt-containing natural soil deposits. Rice production is confronted with increased threats from soil salinity as well as from extreme weather and rising sea levels under the Deepening West implored by climate change, as rice remains both a dietary and economic staple in low-lying deltas and estuaries worldwide. Rice happens to be very sensitive to salt stress in nature; this owes to an explanation for low productivity. Salt stress interferes with almost all physiological and biochemical pathways in the plant. High salt levels in the soil decrease the plant's capacity for water uptake, cause ion imbalance, and can be toxic within plant tissues. These antidotes of salt may impede growth by causing physiological aging and can result in the death of plants under extreme conditions. In rice plants, salt accumulation in cells perturbs cellular homeostasis and ion uptake, modifying the pattern of normal growth; hence, stunted growth ensues. These challenges have made both breeders and researchers acutely aware of the need to develop rice

varieties that can put up with high salt concentration and hold their productivity in such conditions. Mechanisms and Innovations in Salt Tolerance for Rice

Rice plants have a number of interactive complex mechanisms that help in coping with the stress brought by salt. When rice plants are subjected to saline conditions, the very first point of attack has to be osmotic stress due to high salt concentrations in the soil, becoming a barrier to normal water uptake by the roots. During the second phase of stress response, ionic stress causes interference of cellular functions due to the accumulated salts within the plant tissues, further triggering leaf senescence. These two effects of salt stress impose the need for plants to develop strategies that could lower both osmotic stress and exclusion of toxic ions with the maintenance of critical balance of ions in the cells. These are the different mechanisms that plants have evolved to cope with these conditions, which range from selective ion transport and osmotic adjustment to tissue tolerance to the accumulated salt components.

For example, tolerance to salt in rice may involve mechanisms of exclusion of sodium ions from the aerial part or by maintaining a high level of potassium in cells in order to offset the sodium toxicity. Most salt-tolerant varieties have a lower concentration of Na<sup>+</sup> in shoots as compared to their roots, thus decreasing the toxic effects of sodium and allowing further growth. Of rice varieties, Indica is more tolerant of salt stress than japonica. The inherent tolerance of indica rice therefore is of focus in breeding programs in an effort toward realizing higher tolerance to salt stress in other rice cultivars. Salt stress responses are different at various developmental stages in rice plants, reflecting their complexity when breeding for salt tolerance.

The rice plants are relatively tolerant at the germinating stage but become highly sensitive at its early seedling stage. This sensitivity diminishes as the plant enters the vegetative growth phase, rises again at the stage of flowering and fertilization, before receding when it reaches maturity. These differences in tolerance at different stages of life create both challenges and opportunities for plant breeders. Focusing research on the most critical life phases, such as seedling establishment or reproductive growth, would put breeders in a better situation to identify and breed for tolerance-enhancing traits during the sensitive stage. To meet the challenge, rice varieties with enhanced salt tolerance

are being developed using both conventional and modern breeding techniques.

Conventional breeding works on the identification of tolerant varieties within the existing populations of rice and then cross-breeding them to transfer desirable traits into new lines. Alongside this, modern genetics has also introduced genes responsible for salt tolerance into rice using marker-assisted selection and genetic engineering, pointing to new avenues of salt tolerance. In recent techniques of genetic engineering, scientists were able to introduce genes responsible for salt tolerance into the rice genome directly instead of taking all the lengthy conventional breeding process.

In addition to genetic interventions, tissue culture techniques make powerful tools available to breeders in the quest for salt-tolerant rice varieties. Techniques like anther culture, protoplast fusion, and immature embryo culture enable researchers to produce and screen large numbers of rice plants under controlled conditions much faster than would be possible by direct identification of salt tolerance. Such tissue culture techniques allow the induction of genetic variability and, therefore, enable one to study a wider range of responses related to salt stress while also enabling the selection for desirable traits with far greater precision. This technique is thus useful in breeding programs, enabling progress at a faster rate in developing rice cultivars tolerant of saline conditions. Development of salt-tolerant rice varieties requires an understanding of the mechanisms underlying salt tolerance in a gradual manner. Although much has been done by researchers in decoding these mechanisms, they are unraveling how plants maintain osmotic balance, exclude toxic ions, and preserve cell integrity under stress. Research in molecular biology has implicated various genes and pathways in the functionality of salt tolerance by involving ion transport, osmotic adjustment, and stress-response signaling. Guided by these new insights, scientists are now able to target specific genes or pathways to enhance salt tolerance in rice. Further progress in breeding for salt tolerance will require an integrated approach that includes conventional genetic engineering for transgenic, salt-tolerant varieties and tissue culture techniques. Meanwhile, recent advances in molecular biology and genomics are expected to allow for the alignment of efforts toward rice varieties tolerant of saline environments. The reason why development of salt-resistant rice breeds is important with regard to worldwide food security is that such a development would afford a vital

clue as to how agriculture must adapt and respond to a shifting climate. Knowledge on salt tolerance in plants has now improved so much that the possibility of developing resilient rice varieties that can give high yields even in saline soils is slowly but surely emerging as a real possibility—a dream come true for millions who survive on a staple diet of rice.

### **Salinity tolerance screening in rice**

Rice is one of the most important staple food crops worldwide and suffers due to a wide range of abiotic stresses. Salinity is the most devastating factor affecting rice growth and production. Rice is here being grown over a wide range of geographical locations from coastals to inland and, therefore, is often confronted with salinity levels at higher or lower values during the growth phase from germination to maturity. It is multifaceted effects because salt stress inhibits photosynthesis, alters metabolism, and affects chloroplast functions to seriously damage rice plants in many ways. These indirect effects decrease plant growth and also diminish the fertility or reproductive success, which then results in reduced yield. Soil salinity is of growing concern since factors such as sea-level rise, bad irrigation techniques, and salt building up in arid areas are becoming rampant; hence establishing and breeding rice varieties with enhanced salt tolerance has increased. Therefore, it is highly important to establish efficient screening techniques for the identification of rice varieties that can tolerate salt stress. These techniques must be simple to conduct, reproducible, and economical. There is variation in the complexity of the methods adopted for screening, and a correct choice is crucial if a realistic estimate of salinity tolerance is to be made across diverse rice varieties.

### **Screening for Salinity Tolerance: Difficulties**

One of the major challenges to be faced in screening for salinity tolerance is the heterogeneity of salinity levels within the field, thus making it difficult to come up with a standardized environment for testing rice plants. Other variables such as temperature, humidity, and solar radiation make this development of reliable methods a little more complicated. In the field, salinity stress is rarely uniform and is associated with other soil-related stresses, such as waterlogging or nutrient imbalances, which may influence the outcome of the screening. Such environmental variables are contributors to genotype-environment interactions that mask the true effects of salinity on the growth and yield

of plants. However, despite these setbacks, a lot of progress is still being achieved in the selection of rice varieties for its tolerance to salinity. In recent years, the advent of modern breeding methods such as marker-assisted selection has significantly sped up the process for developing salt-tolerant rice cultivars. The improved salt-tolerant variety is bred not only to withstand salinity but also other stresses such as dry spell, cold, and poor soil conditions, further enhancing their adaptability in various environments.

### **Salinity Tolerance Phenotyping Screening**

Of these, the most common system for the screening of salinity tolerance relies on phenotypic screening, based on the morphological observation of growth responses of rice plants under artificially induced saline conditions. Salinity tolerance is induced by multiple genetic and physiological components that make detection difficult. Under controlled environmental conditions, even environmental factors such as humidity, light, and temperature would need to be well managed in order for the results to reflect the plant's true tolerance to salt. Physiological responses to salinity stress in rice plants vary and can be used as a yardstick to determine tolerance. For instance, salt-tolerant plants often have low shoot sodium concentration and high shoot potassium concentrations. In other words, under salinity, salt-resistant varieties may compartmentalize the salts, like sodium chloride, in older leaves and prevent their accumulation in more sensitive parts of the plant.

Other characteristics of plant vigour and the ability to maintain high rates of transpiration and photosynthesis are equally useful indices of salt tolerance. Salt stress is usually first apparent in the oldest leaf of rice seedlings at an early stage of growth. As plants get older, younger leaves become affected and salt suppression reduces the rate of leaf elongation and new leaf appearance. Salinity also reduces photosynthetic function of the plant, which results in a reduction of chlorophyll content, an effect inversely proportional to salinity level. Such early symptoms of salt stress are good indicators for selecting potential tolerant varieties. However, phenotypic screening also has some disadvantages. The visual symptoms caused by salinity stress can be somewhat subtle and difficult to differentiate from other environmental stresses. In addition, the physiological response to the effects of salt can be very different according to genotype and environment. It is therefore for this reason that selection of true salt-tolerant varieties may be problematic. Besides,



phenotypic screening in some cases requires expensive and time-consuming tissue analysis for the assessment of cellular salt injury, which is difficult in large-scale screening.

### **Screening at Different Growth Stages**

The magnitude of sensitivity to salinity varies at different stages of growth in rice plants; therefore, the methodology must be adjusted accordingly when screening justifies the need. Living under salinity stress, usually leaf elongation and newly formed leaves are targeted at the seedling stage. This stage is highly sensitive to salt; even a low concentration can hinder plant development. At the advancement of stages, that is, vegetative and then reproductive stages, the effect of salinity gets intensified by way of shrinking plant height, root length, and biomass. Salinity has been at the reproductive stage, which reduces panicle initiation, spikelet formation, fertilization, and pollen viability, and results in reduced fertile grains and low yield. Salinity tolerance at the reproductive stage is quite difficult to assess. While grain yield per plant can be adopted as a measure of salt tolerance, it is sometimes not an exact measure of the plant performance under saline conditions. However, there may be some deformed or aborted spikelets in tolerant varieties; this could yield misleading results. Other reproductive traits that are very important for the full characterization of salinity effects on rice plants include panicle length, number of primary branches, and spikelets per panicle.

### **Ion Concentrations and Na-K Ratio**

Other important factors in the process of phenotypic screening include ion concentration inside the plant. In rice, salinity tolerance often implies the maintenance of total Na<sup>+</sup> and Cl<sup>-</sup> ion concentration in shoots at a low level while maintaining high essential ion concentration in shoots, essentially K<sup>+</sup> and Zn<sup>2+</sup>. The Na-K ratio, or the balance between sodium and potassium in the plant, is considered an important indicator of salt tolerance. Salinity-tolerant rice varieties typically have a lower Na<sup>+</sup>:K<sup>+</sup> ratio, showing better ion homeostasis and less cellular damage under saline conditions. Knowledge of ion transport mechanisms within rice plants is essential to the identification of salt tolerance. Plants that are able to effectively exclude or compartmentalize salt either in older tissues or vacuoles can protect more vital metabolic functions in shoot and leaves. In contrast, salt-sensitive varieties accumulate high levels of sodium and chloride, causing toxicity and growth impairment. The

estimation of Na-K ratio and ion concentration in the rice plants under saline condition allows the researcher to identify varieties with better tolerance against salinity.

### **Limitation of Phenotypic Screening**

While phenotypic screening can be a very useful method in selecting salt tolerance in rice, there are drawbacks. Precisely, one of the critical ones is that salt tolerance phenotypes expressed in the early vegetative growth stage may not be stable in further generations due to the influence of environmental factors such as temperature, humidity, and soil profile on the expression of salt tolerance traits, which make the results inconsistent. Moreover, phenotypic screening cannot always identify the underlying genetic and physiological mechanisms across different levels of salt tolerance. However, these morphological characteristics, like plant vigor, leaf elongation, and chlorophyll content, although useful as visible indicators of tolerance to salt, have little relevance with respect to indicating the genes or pathways operating in salt response. For this reason, phenotypic screening is often complemented by molecular techniques such as marker-assisted selection and genetic analysis that give further details about the genetic basis of salt tolerance. **Conclusion:** The search for the salt tolerance trait in rice through phenotyping continues to be an important avenue, though with some definite challenges. Screening this character is problematic due to environmental conditions, genotype-environment interactions, and the intrinsic complexity of salt tolerance. Combining different methods-phenotypic screening with the use of molecular markers and ion concentration-will result in better efficiency and accuracy of salt tolerance selection. The primary goal is to create rice varieties that could thrive in saline conditions to ensure food security for future generations.

### **Recent Advances in Screening for Salinity Tolerance in Rice**

As the food staple of the majority of the population in the world, rice is susceptible to several environmental stresses, the most pernicious of which is salinity. Salinity stress has become one of the major abiotic challenges for rice cultivation, especially under conditions of soil salinization or in cases where the irrigation water contains high levels of salt. For this reason, an effective attempt has been made to identify rice varieties that insinuate a level of tolerance to salinity. This covers both the phenotypic and genotypic screening methodologies in order to

advance our understanding of how rice plants respond to saline environs and to identify further traits which could assist in breeding salt-tolerant varieties. Salinity affects rice at different stages, from seed germination to maturity. High salt concentration during the process of germination inhibits the growth of seedlings by reducing the development of roots, hence poor overall establishment of the plant. During its vegetative and reproductive stages, rice health shows deteriorating effects caused by salinity stress, inducing several negative physiological changes that might be enhanced by reduced photosynthesis, cellular structural damage, changed metabolic pathways, leading to stunted growth and low yields. Moreover, salinity disrupts ion homeostasis by accumulating toxic  $\text{Na}^+$  ions in a plant tissue of the cells and tissues, delaying their normal functioning. Screening for salt tolerance in rice is cumbersome and needs extra care with respect to environmental variables and growth conditions.

Salinity tolerance is controlled by different genetic and physiological factors; hence, salinity tolerance can be considered a polygenic trait, whose expression depends on the interaction between several genes. More traditionally, the approach in phenotypic screening methods for salinity involves monitoring the growth and development of rice plants under saline conditions by measurement variables including shoot and root length, chlorophyll content, and biomass accumulation. Though these methods are very valuable, they are very labor-intensive and often plagued by environmental fluctuation. For example, temperature, humidity, and the intensity of light could alter the sensitivity of plants to salinity stress and make it hard to reproduce the results consistently. One promising alternative to phenotypic screening offers molecular markers for genotypic screening. Quantitative trait loci mapping can identify genetic markers associated with salinity tolerance in plants now with modern biotechnology.

This methodology has the advantage of precisely delineating those regions of the genome associated with salt tolerance traits, such as ion transport and stress-induced gene expression. These QTLs, once identified, will enable breeders to hasten the process of developing salt-tolerant varieties of rice through marker-assisted selection. QTL mapping has succeeded, till to date, in revealing a few important genes controlling salinity tolerance associated with ion homeostasis, osmotic adjustment, and stress-induced production of proteins. One of the major findings in salt tolerance studies is a major QTL designated as Saltol. This QTL has

been found contributing to a significant part of the salt tolerance variation in rice, especially in terms of regulating salt intake.

In the process, the Saltol QTL has been introgressed into several high-yielding rice varieties through marker-assisted backcrossing. This is one of the efficient ways to breed salt-tolerant rice with other desirable traits such as high grain yield. While these results are encouraging, the application of MAS is not without its problems. One of the concerns is what is called "linkage drag," where unfavorable traits linked to a QTL are inherited along with it. Environmental conditions and genetic alterations in experimental plants affect the efficiency of MAS.

In addition to QTL mapping and MAS, other state-of-the-art biotechnological approaches, including genetic engineering and tissue culture, have also been pursued to guarantee salinity tolerance in rice. Geneticists develop modified rice varieties by manipulating genes that are believed to involve the expression in stress tolerance. For example, it has been demonstrated that genetic transformation in rice-overexpressing trehalose synthesis genes decreases the accumulation of sodium in plant tissues and enhances overall plant growth under salt stress. However, these transgenic methodologies also have some drawbacks.

Some of the ways exogenous trehalose mediates its positive effects include an improvement in the salt tolerance of plants, although this is partly through toxic effects on carbon metabolism, especially in high concentrations. Another tool that finds widespread application in modern rice breeding is haploid breeding. Haploid breeding involves the generation of double haploids, obtained through anther culture or chromosome elimination technique. Several of the detailed reasons for the high value of DH lines in rice breeding include the fact that they are homozygous and uniform, ideal for genetic studies or the evaluation of certain traits. A few QTLs explaining salt tolerance traits regarding root length and survival under salt stress have been identified with the use of DH populations. Another advantage with DH breeding is that it speeds up the development of homozygous lines by reducing the year expenditure spent for breeding a salt-tolerant variety. As opposed to conventional breeding strategies, which take a minimum of 8–10 years to develop an encouraging salt tolerant line. With DH breeding, it will reduce the stipulated time to only 3 years. Molecular Breeding and Transgenic Strategies for Improving Salt Tolerance

QTL mapping, genetic engineering, and transgenic approaches are some molecular techniques that have opened up new vistas for developing rice varieties tolerant to salt. For instance, QTL mapping has been applied in the identification of genetic regions associated with salinity tolerance. Genetic mapping on specific chromosomes has enabled the identification of genes of importance in critical aspects like ion transport, osmotic adjustment, and cellular responses under stress. Recent research has typically presented critical ideas through which the genetic basis of salt tolerance has been explained and has also cleared the ways for producing more tolerant rice varieties. Among the major achievements of QTL mapping for salinity tolerance, there are several major QTLs reported to be located on chromosomes 1, 3, 8, and 10. They are associated with key traits like physiology of sodium-potassium balance, Na<sup>+</sup>/K<sup>+</sup> homeostasis, and survival at seedling stage under salinity stress. More importantly, fine mapping of the Saltol QTL has been a breakthrough in proving that it plays a paramount role in the control of salt uptake and ion balance in plants. The Saltol QTL has been successfully introgressed into rice cultivars through marker-assisted backcrossing, providing a way to improve salinity tolerance with no compromise in other agronomically important traits. In conjunction with QTL mappings, there are transgenic approaches that involve the transfer of specific genes implicated in salt tolerance mechanisms into rice plants. Transgenic rice varieties have indeed demonstrated improved growth and survival subsequent to saline exposure. One such gene is SKC1, a regulator of Na<sup>+</sup>/K<sup>+</sup> homeostasis, which has been cloned so far in salt-tolerant rice genotypes. Other works have focused on identifying genes that participate in the pathways of stress responses, including osmotic regulation and antioxidant defense; overexpression of these genes could enhance the ability of salinity tolerance of the plants. Though the results using transgenic approaches look promising, some challenges remain. Some of these genes and metabolic pathways could also be potentially toxic, such as the overexpression of trehalose. Added to this complexity is that of salt tolerance, where many genes along with environmental factors interact, and thus, the task, as far as genetic engineering is concerned, seems to be really daunting. In spite of all these challenges, the future of transgenic technology in rice salinity is promising, and several research studies have expressed the need to examine new pathways and improve the efficiency by generally studying strategies for more valuable outcomes.

Somaclonal variation, an induced method through culture techniques, promises another avenue for the improvement of rice tolerance against salinity. It concerns selecting mutant lines showing desirable characteristics such as salt tolerance. By selecting and re-growing these mutants, scientists are able to develop new rice varieties that are more tolerant of salt. Indeed, a quite efficient way to improve the yield potential of salt-tolerant varieties has been through this approach, as manifested by developing somaclonal variants of Pokkali—a highly tolerant rice cultivar to salt. These varieties have exactly the same level of tolerance to salt as the original type Pokkali variety, hence better growth and a higher yield potential. Overall, modern biotechnology, molecular breeding, and genetic engineering have rescued our understanding of salinity tolerance in rice. This would provide the necessary tools for developing rice varieties that can stand saline stress, which will improve food security under conditions of soil salinization/water scarcity. Although difficult issues are yet to be resolved, the advances over the last decade give good reasons to expect salt-tolerant varieties in the not-too-distant future that will help create more sustainable and resilient rice production systems.

## Further Readings

- Munns, R., & Tester, M. (2018). Mechanisms of salinity tolerance in crops: Current status and future prospects. *Annual Review of Plant Biology*, 69, 651-681.
- Yang, Y., & Guo, Y. (2018). Unraveling salt stress signaling in plants. *Journal of Integrative Plant Biology*, 60(9), 796-804.
- Zhang, H., Li, Y., & Zhu, J. K. (2018). Developing naturally stress-resistant crops for a sustainable agriculture. *Nature Plants*, 4(12), 989-996.
- Fageria, N. K., Moreira, A., & Castro, C. (2021). Micronutrient-use efficiency in different crops under variable soil conditions. *Advances in Agronomy*, 165, 111-152.
- Ismail, A. M., & Horie, T. (2017). Genomics, physiology, and molecular breeding approaches for improving salt tolerance. *Annual Review of Plant Biology*, 68, 405-434.
- Chen, Z. C., & Ma, J. F. (2015). Improving nitrogen use efficiency in rice through enhancing root nitrate uptake mediated by nitrate transporters. *Journal of Experimental Botany*, 66(7), 2087-2097.
- Roy, S. J., Negrão, S., & Tester, M. (2014). Salt resistant crop plants. *Current Opinion in Biotechnology*, 26, 115-124.
- Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular

- characterization. *International Journal of Genomics*, 2014, 701596.
- Rengasamy, P. (2016). Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology*, 37(7), 613-620.
- Flowers, T. J., & Colmer, T. D. (2015). Plant salt tolerance: Adaptations in halophytes. *Annals of Botany*, 115(3), 327-331.

## Chapter 4: Plant-Based Nutritional and Stress Response Studies

Qurban Ali<sup>1</sup>, Muhammad Arshad Javed<sup>1</sup>, Zain Mushtaq<sup>2</sup>

### Iron and Sodium Toxicities in Rice Cultivations

**R**ice is one of the most important staple crops in the world, being a basic source of nutrition for about 40% of the passive population of the Earth, with a higher consumption by people in developing countries. The crop is grown on about 128 million hectares of irrigated and rain-fed lowlands around the world, supporting millions of livelihoods and contributing substantially to food security. Toxic elements like sodium and iron affect as many as 55% of the world's rice fields, often causing immense constraints to agriculture, particularly in areas where these elements build up to levels that could be harmful. The presence of excess sodium and iron acts like a toxic agent and severely affects rice growth and productivity; hence, the crop yields and quality are reduced.

These elements express their effects mainly through visible symptoms and physiological changes in the plant. The more common sodium toxicity often takes the form of a salt injury that is characterized by scorch, chlorotic leaves, and reduced growth. On another note, iron toxicity will lead to leaf bronzing characterized by brown patching on the leaves, due to the accumulated iron reducing the capability of the leaf to photosynthesize efficiently. Both types of toxicity disrupt the nutrient balance within the plant and result in nutritional disorders and deficiency, impeding water absorption, which in turn inhibits growth and development. Due to this, there is a reduction in crop height and foliage health and overall biomass, therefore providing a severe blow to the yield of rice, which then could affect the stability of food supplies in the vulnerable areas.

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<sup>1</sup> Department of Plant Breeding and Genetics, University of the Punjab, Lahore – Pakistan

<sup>2</sup> Department of Soil Science, University of the Punjab, Lahore – Pakistan



These effects can be mitigated by a suite of mechanisms, which rice plants have developed for managing toxic ion concentrations in their tissues. Rice breeders have also developed varieties with increased tolerance to sodium and iron toxicity by exploiting the natural physiological responses of the plant to develop more robust crop varieties. The adaptive mechanisms known include the exclusion of toxic ions, detoxification mechanisms, and the compartmentalization of toxic elements within specific parts of the plant, such as in the roots, to reduce damage to leaves and shoots. By understanding and improving these natural reactions, breeders and farmers would have the opportunity to produce rice varieties that can grow under even high sodium and iron levels in the soil.

### **Effects of Sodium and Iron Toxicity on Growth and Physiological Traits in Rice**

A hydroponic experiment on different rice varieties under varying exposures to  $\text{Na}^+$  and  $\text{Fe}^{2+}$ , in which the relative toxicity of sodium and iron to rice plants would be comprehended. These results provided insight into how these toxicities impact key growth parameters, such as root and shoot biomass, leaf health, and ion accumulation. High toxic ion testing with 120 mM NaCl and 7.5 mM  $\text{FeCl}_3$  resulted in significant declines in growth and physiological traits among varieties; however, there was variation in the degree of such decline. This clearly shows that some varieties are intrinsically more tolerant to the afore mentioned toxicities. Root Dry Biomass: One of the most important parameters used to study the nutrient and water uptake of plants is root dry biomass. Its variation was highly significant among all varieties under consideration. The highest value for root dry weight was manifested in the Pokkali variety; under non-toxic conditions, these were very closely followed by SK1 and Firat plants.

However, under moderate sodium toxicity, varieties like Pokkali and MR 211 did not suffer as much root biomass reduction and maintained better growth and root health compared to other varieties. In contrast, Firat showed a remarkable loss in root biomass under the very same sodium toxicity condition, thus being less tolerant to sodium toxicity. Increasing  $\text{Fe}^{2+}$  up to 3.5 mM, Firat maintained relatively high root dry biomass, followed by Panderas and MR 211, while SK1 showed a marked decline. At the highest concentrations tested, Pokkali and MR 211 again

outperformed the remaining varieties, maintaining higher root biomass, and hence showing higher tolerance levels compared to SK1 and Firat.

**Shoot Dry Biomass:** Shoot dry biomass is directly related to the general growth and health of a plant. It underwent similar changes across all varieties. At the highest level of sodium toxicity, 120 mM NaCl, Pokkali resulted in the highest shoot biomass, retaining 46.15% of its normal mass. Firat resulted in the lowest shoot dry weight in this concentration, with a reduction of 28.29%. For iron toxicity, MR 211 and Pokkali showed better performance for the second time. While SK1 yielded the lowest shoot dry mass at this level, they produced maximum shoot dry weights even at the highest Fe<sup>2+</sup> concentration tested (7.5 mM). The pronounced shoot biomass decline in Firat and SK1 under both sodium and iron toxicity conditions could be ascribed to their susceptibility toward toxic ions, cell division, and elongation, which results in dwarf growth and lowered productivity.

## **Symptoms of Toxicity and Nutrient Imbalance**

**Leaf Bronzing and Salt Injury:** This work also presented apparent visual symptoms of sodium and iron toxicity in rice plants. It was observed that sodium toxicity, inducing salt injury, and iron toxicity-induced leaf bronzing predominated in all varieties tested. In cases of very high sodium toxicity, Pokkali and SK1 had the lowest salt injury scores with relatively healthier leaves and shoots. Firat and Panderas showed more intense symptoms and had higher salt injury scores, indicating that they are more sensitive to sodium. The toxicity under Fe<sup>2+</sup> showed Pokkali and Firat only with low leaf bronzing, while Panderas, MR 211, and SK1 go up to a higher level of bronzing. Eventually, since bronzing ultimately disturbs not only the look but also photosynthetic efficiency in the leaves, affecting their overall growth and yield potential may be since they will interfere with the plant's potential to carry out photosynthesis.

**Ion Accumulation and Nutrient Distribution:** This too is an analysis of the hydroponic study on the accumulation of Na<sup>+</sup> and Fe<sup>2+</sup> in different plant parts such as roots, leaves, and flag leaves. It seemed that under 120 mM NaCl and 7.5 mM FeCl<sub>3</sub> exposure, the toxic levels of these ions were way higher compared to the control plants. Amongst varieties, Pokkali showed a specific physiology that it retained Fe<sup>2+</sup> ions inside the roots, thus limiting the transport of iron into the leaves and flag leaf. Such a retention mechanism in the roots may be an important reason for higher tolerance in Pokkali due to restricted leaf bronzing and

protection from iron overload to the photosynthetic tissues. In contrast, varieties SK1 and MR 211 presented higher accumulations of  $\text{Fe}^{2+}$  in their leaves, which coincided with an increase in susceptibility to iron toxicity. The  $\text{K}^+/\text{Fe}^{2+}$  and  $\text{K}^+/\text{Na}^+$  ratios, important nutrient ionic balance indices, have shown no significant variations with an increase in toxic ion concentration; overall, an increased  $\text{Na}^+$  and  $\text{Fe}^{2+}$  concentration has reduced potassium levels among the varieties studied. High potassium levels in Pokkali and MR 211 reveal a more intensive regulation in essential nutrients and less impact of toxic ions on physiological functions. The interaction between potassium and toxic ions looks concentration-dependent, and higher potassium may help stabilize nutrient absorption to reduce the uptake of harmful  $\text{Na}^+$  and  $\text{Fe}^{2+}$ . This regulatory response increases the tolerance of the plant by facilitating good root and shoot development under stressful conditions.

### **Adaptation and Mechanisms for Tolerance**

The presence of variable levels of tolerance among rice varieties indicates the adaptive mechanisms that rice plants have developed in response to the noxious soil environment. The toxic ion concentrations in rice plants are mitigated by the ion exclusion, detoxification, and compartmentalization methods that handle the toxic effects of sodium and iron ion excesses. Certain varieties, like Pokkali, have very strong mechanisms for retaining toxic ions within the roots for protection of photosynthetic tissues and maintenance of healthier growth. Besides that, a sufficient amount of potassium has been observed to help decrease the intake of both  $\text{Fe}^{2+}$  and  $\text{Na}^+$  due to increasing the oxidizing capability of the root. Therefore it proposes a positive interaction that further supports plant health. Understanding these adaptive traits is essential for the development of rice varieties that are resilient against key unfavorable growing conditions. If plant breeders can produce rice varieties with improved tolerance to sodium and iron, then they can reduce the yield losses and sustain productivity in the affected regions. These tolerance mechanisms, when further researched in combination with genetics and biochemical studies, can give way to new insights that would strengthen rice breeding programs. In the future, resilient rice varieties might secure the food supply of populations dependent on rice, even in environments challenged by soil toxicity.

## **Nutritional Composition and Benefits of Maize Flour**

Maize, scientifically known as *Zea mays* L., ranks very high among crops of global importance and is among the most valuable cereal crops produced globally. Because of its varied uses, nutritional value, and a widely adaptive climatic range of availability, maize has become one of the staple crops in many world regions that include the Middle East, Africa, South Asia, Latin America, and parts of North America. In Pakistan, maize is grown in spring and autumn and ranks third in the list of the most grown crops after wheat and rice. In the whole vast agricultural landscape of Pakistan, the average production of maize accounts for approximately 3.13 million tons per year with an average yield of about 3264 kg per hectare. At the global outlook, maize occupies more than 142 million hectares with a total production estimated at about 913 million tons; hence, its importance not only for food security but also for agricultural economies.

Besides its value as a crop, maize is a good source of essential nutrients, including several vitamins and minerals along with dietary fiber, and can be consumed at any stage. It comes in various forms: whole corn, cornmeal, flour, oil, and corn-derived products such as polenta, tortillas, and cornstarch. Each of these forms of maize delivers different nutrients, and due to the differences within maize varieties-especially between commercial white flours and non-commercial yellow flours-different versions of maize can offer special advantages. Nutrient profiles of these maize flours may vary substantially depending on environmental reasons, agronomic practices, and genetic variability among the used maize varieties.

Both white and yellow maize flour is full of vitamins and minerals. The key vitamins include folate, iron, niacin, potassium, and zinc that provide huge amounts of health benefits. At the basic composition level, maize flour contains moisture, protein, fat, fiber, and carbohydrates. For example, yellow maize normally contains more moisture than white maize flour. This perhaps could be linked to the type of agronomic settings and variety of maize concerned. Nutritional diversity, therefore, shows how maize can fulfill different requirements in terms of human diet, whether whole grain or otherwise processed product.

## **Nutritional Profile Comparison: White Versus Yellow Maize Flour**

The moisture content of white and yellow maize flour also differs with respect to growing conditions; this is also within the range to be expected based on international studies. Yellow maize flour contains more moisture than white maize flour. There are several reasons for these differences, given the environmental factors as well as the methodology involved in processing. Yellow maize tends to have a higher protein content - an important consideration for those kinds of diets that demand a high level of intake. Generally, the protein content of corn flour ranges from 7.8 to 12.5%, values which agree with the normal average values considered in literature. For actual values in different types of maize flour depend on agronomical practices, environmental conditions, and even genetics of the examined varieties of maize. Yellow maize is also normally higher in fiber content than white maize, since it contains the bran layer, which is a source of dietary fiber and requisite health benefits. The crude fiber in yellow maize ranged between 0.95-2.97%, therefore providing digestive benefits and supporting a healthy diet.

The fat content is another essential information for the nutritional profile of maize, where both white and yellow maize have about 2.09-4.05 % fat. Carbohydrates varied from 60.23% to 78.74% in the maize flours and seemingly were higher in white flours, because the removal of the bran during its processing concentrates the starches. The nutritional profile explains that maize flour fits into high-energy diets accordingly, especially among populations relying on maize as a staple crop.

### **Mineral Content: White Versus Yellow Maize Flour**

Its nutritional value becomes even more important by adding its mineral composition. ICP-MS has enabled very accurate determination of some critical minerals in both white and yellow maize flour. Yellow maize flour has a good level of some important minerals, which are often higher compared to the white one: Mg, K, Mn, Zn, Fe, and Cu. Yellow maize flour contains 16 times more magnesium and over 26 times more potassium compared to white maize flour, hence providing important electrolytes and aiding in metabolic functions. The iron content is also very high in yellow maize, something that is very useful in the treatment of iron-deficiency anemia, one of the common health issues in

developing nations. While white maize flour generally contains much higher levels of Na and Ca, yellow maize provides a broad range of the same essential minerals in significantly higher quantities, thus being very valuable when consumed as one element of a proper diet.

## **Health and Digestive Benefits**

One of the notable consumptions of maize is the improvement it offers to the digestive health of a person; in particular, the whole grain varieties such as yellow maize. The fibre content, higher in yellow maize flour, promotes digestion, maintains the rhythm of bowel movements, and prevents digestive disorders. Regular consumption of maize products removes noxious residues of ingested food and accelerates the intestinal transit time, especially if consumed in a not too highly processed form, such as whole grain or bran-rich flour. This fiber also helps the body to maintain healthy cholesterol levels and has the potential to help lower the risk of chronic diseases derived from low-fiber, high-fat diets. In fact, 'maize' can prevent digestive disorders by reducing stomach acidity and enhancing gallbladder activity. The ingestion of maize as a staple dietary food also supports counteracting digestive disorders due to its high fiber content and nutritional value when compared with more processed varieties of grains. Yellow maize flour, being the more nutritious of the two, has an increased fiber and mineral content and thus offers more protection, especially to the gut. Comparative Benefits of Yellow Maize University of Agriculture Faisalabad versus White Variety Commercially Available -

Recent studies on yellow maize grown at the University of Agriculture in Faisalabad, Pakistan, indicate that such locally grown maize is nutritionally superior to commercially available white maize flour. Yellow maize flour collected from the University of Agriculture showed a much higher protein, crude fiber, and different essential mineral composition than white maize flour obtained from local markets. The better nutritional profile may well be due to various environmental and agronomic factors under which the crops were grown in the agricultural university, including superior soil quality, type of irrigation, and genetic traits of the species used. For example, the yellow maize flour grown locally contains higher levels of magnesium, potassium, manganese, zinc, iron, and copper. High mineral levels thus mark the yellow maize flour as a true beneficiary for the people in need of gaining increased nutrient levels. White maize flour, on the other hand, commercially processed free of its bran, contains these minerals but in low amounts.

Due to the fact that the process of bran removal reduces both fiber and mineral content, whole grain maize is precluded from providing as many health benefits as it could. This will make the flour not only nutritious but possibly also offer better health-related benefits when added to dietaries due to the increased nutrient content of yellow maize flour coming from the University of Agriculture Faisalabad. Yellow maize flour is a full nutritional package, and this makes it an ideal variety for populations depending on maize as their dietary staple and for those looking to improve their diets by incorporating more nutrient-dense foods into their diet. Conclusion Yellow or white, maize flour is a staple food for millions of people around the world. Each of them should be hosting different benefits, but yellow maize performs better as far as nutritional content is considered, since it contains a higher percentage of proteins, fiber, and minerals. Yellow maize grown at the University of Agriculture Faisalabad depicts these benefits even more prominently, thus underlining the role of environmental and agronomic practices in this aspect, which are deterrents to the nutritional quality of maize flour. By choosing less processed, whole grain forms of corn flour, like yellow corn, for instance, a wider range of nutritional benefits would be available for consumption, such as better digestion and more mineral intake, contributing to better overall health and well-being.

### **Rice-A Nutritional Powerhouse: Antioxidant Properties and Nutritional Value**

Rice, being the staple food of almost half of the global population, is *Oryza sativa* L. This plant has much to contribute to nutritional as well as cultural aspects; among other things, rice varieties show different characteristics, flavors, and even colors, reflecting various regional preferences and practices. Yet, in spite of this diversity, polished white rice-a smooth, almost translucent grain-has long been the norm. Though refined white rice is smooth in texture and mild on the palate, preferred by many, much of the original nutrient density of rice is lacking, and only a fraction of the extensive array of vitamins, minerals, and phytochemicals remain after milling. In the last couple of years, people have turned to whole-grain and pigmented rice varieties, like brown and red rice, for their bran and germ layers that make for a much healthier option with better nutritional value.

For instance, brown rice is just rice that has been hulled but still contains its bran layer; thus, it is considered a whole grain containing fiber, vitamins, and minerals. Compared to white rice, the bran layer gives

brown rice a denser texture and more earthy flavor, along with a spectrum of essential nutrients. Naturally, brown rice is rich in thiamine, magnesium, calcium, potassium, and protein. It contains a lower glycemic index. Such a feature turns brown rice into a preferable choice of food for those seeking to handle blood sugar levels more appropriately, especially among diabetic and pre-diabetic populations. But much more than those major nutrients, colored rice varieties like brown, red, and black carry added value with their high antioxidant count obtained from natural phytochemicals that assist in fighting against oxidative stress and may give further health benefits. There are two general categories of rice cultivation, based on crop ecology-upland rice, growing in rain-fed or irrigated soil under dryland conditions, and wetland rice grown in a waterlogged soil to create a flooded environment. These ways of cultivation not only affect the physical characteristic and yield of the plant but also the nutritional characteristics and antioxidant capacity of rice. Upland rice is usually unprocessed and unpolished, retaining much of its nutrients, while wetland rice is milled and polished, hence less rich in bioactive compounds. Each type has its contribution toward diets globally with unique properties that contribute to flavor, texture, and nutrient density.

The main nutritional composition of rice encompasses carbohydrates, proteins, fatty acids, fibers, minerals, and vitamins. Brown and crimson rice, among other colored rice varieties, contain high levels of phenolic compounds and other antioxidants. The phenolics are those bioactive compounds produced by plants as a protective measure against environmental stress and diseases, which confer natural resistance against pests. These include ferulic acid, p-coumaric acid, and caffeic acid, which also improve plant defenses and health benefits when consumed as food in rice. Several studies have since demonstrated that these diverse groups of phenolic compounds in rice cultivars are directly contributing to their antioxidant properties, especially in colored and whole-grain rice. Antioxidants are important for human life since they neutralize ROS, or reactive oxygen species, unstable molecules that can damage cells and contribute to aging and diseases. In the modern diet, antioxidants are highly valued for their potential to reduce the risk of conditions ranging from cardiovascular disease to cancer and even neurodegenerative diseases by protecting cells from oxidative stress.

Various mechanisms, including electron donation, inhibition of ROS-forming enzymes, scavenging of free radicals, and chelating metals, are



involved in the mode of action of antioxidants to prevent or retard cell damage. Grains, especially whole grains or pigmented, contain natural antioxidants that can supplement the diet with much-needed protection against free radical damage.

In general, antioxidant capacity in rice is quantified by various chemical assays widely used, including DPPH and ABTS assays. Assays using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS are standard methods for measuring the antioxidant capacity of rice. While the DPPH assay determines the capability of free radical-scavenging compounds, which is more ideal for lipophilic compounds, the ABTS assay may work well with both lipophilic and hydrophilic antioxidants. These assays determine an IC<sub>50</sub> value, a calculated concentration necessary to inhibit 50% of free radicals, whereby the lower the IC<sub>50</sub>, the stronger the antioxidant capacity. It is itself a potent antioxidant, and its high scavenging activities have made it commonly used as a standard for such tests. For example, it provides 93.84% inhibition at concentrations less than 1000 µg/ml, with an IC<sub>50</sub> of about 262.5 µg/ml, acting thereby as a yardstick against which the antioxidant potential of rice may be compared. Some studies conducted on a comparative basis between upland and wetland rice varieties have reported that upland rice, on the whole, was showing better antioxidant activities among the tested samples in DPPH assay. This strength is attributed to the fact that in upland rice, the bran and germ layers, which undergo minimum processing, preserve more of their natural antioxidants. In contrast, the wetland rice varieties are invariably milled and polished into white rice, thereby removing most of the beneficial compounds: the upland rice varieties, to which belong the famous Bario Bukit and Bukit Pulut varieties of Malaysia, show lower IC<sub>50</sub> values in the DPPH assay, hence are capable of neutralizing the free radicals. On the other hand, wetland rice varieties do not bear this antioxidant-dense bran layer, which generally gives higher IC<sub>50</sub> values, reflecting a reduced antioxidant capacity.

These pigmented rice varieties, like brown and red rice, being nutrient-rich, do not confine their high nutritional values to just antioxidants, but are also rich in micronutrients such as iron, manganese, zinc, and copper. Upland rice has been found to vary from other forms of rice in some studies, showing exceptionally high levels of some trace mineral content and hence proving to be a better source of these dietary nutrients compared with polished white rice. Besides this, its free radical

scavenging activities are impressive; in fact, some varieties reach levels as high as 98.45% in some Malaysian genotypes. This strong scavenging effect is in agreement with the IC50 values from upland rice samples such as 810.47 µg/ml and 2250.34 µg/ml for Bario Bukit and Bukit Pulut, respectively. These results emphasize that upland rice varieties maintain their higher nutritional value by retaining their antioxidant-rich profiles along with essential micronutrients, thus rendering them ideal selections for health-conscious individuals.

The ABTS assay complemented the results obtained with DPPH and further supported the better antioxidant capacity of upland rice. While higher concentrations were needed for a 50% inhibition level in the case of ABTS, the trend was more or less similar, with upland rice varieties showing significantly higher antioxidant activity when compared to wetland varieties. For example, the IC50 value, as determined by the ABTS assay for Bario Bukit and Bukit Pulut, falls in the range of 3480.93 µg/ml to 3820.59 µg/ml, indicating that they possess potent antioxidant activities. Wetland rice also showed lower antioxidant activities in both assays, which may be due to the nutrient loss during polishing processes. Apart from the levels of antioxidants, the total phenolic content in rice is another determinant factor for its health benefits. Flavonoids are generally a group of phenolics compounds that have a higher percentage rate of antioxidant action in rice and, therefore, usually play a critical role in preventing diseases and generally promoting life longevity.

It follows that rice has highly variable phenolic content; generally, the upland rice species have higher phenolic content. For example, Bario Bukit has a phenolic content of 47.84 mg/g as compared to the content in most wetland rice of 20.00 to 28.19 mg/g. This might be due to the fact that upland rice retains its bran and germ layers in which a majority of these health-promoting compounds are preserved. Not all upland rice varieties are the same, and there are variations: Bario Bukit contains higher levels of phenolics compared to Bukit Pulut, indicating the differing genetic factor and environment that uniquely contributes to the synthesis of phenolics in rice. The flavonoids are a subgroup of phenolic compounds, and these also vary among rice varieties and an important part in antioxidant activity. In upland rice, the flavonoids are present in fair quantity, though relatively low ratio to total phenolics, mostly below 15%. However, in wetland rice varieties, flavonoids make up a more significant share of the phenolic content of upland rice, with values ranging from 16.75% to 23.39%. Included among such flavonoids

are certain ones like rutin and quercetin, which are of the highest antioxidant order of magnitude, radical scavenging at very low concentrations. The fact that these flavonoids are always present in upland rice underlines its potential as a strong antioxidant source. Upland rice is nutritionally superior to polished rice due to its high antioxidant capacity and rich phenolic and flavonoid contents. This makes it a potentially interesting dietary choice for health-conscious customers, since food entities besides nutritional value do have protective health benefits linked to them. With increased awareness of the role of antioxidants and nutrient density, brown and upland rice among the pigmented varieties have increased valuations for their health-enhancing properties. In short, the higher antioxidant activity, phenolic content, and mineral density of upland rice, especially Bario Bukit and Bukit Pulut, are of immense value for any diet. These rice varieties not only provide a number of essential nutrients, but they also help to promote cellular health by reducing oxidative stress and enhancing general well-being. As research investigates the health benefits of these rice varieties, it becomes even clearer that these rice varieties help people get a balanced diet. Upland rice is one of the promising choices for consumption by people who need more nutrients and antioxidant-packed options. This meets the modern requirements for health and gives natural, fuller ways of fostering health.

## **Maize: Essential Crop and Nutrition Source**

Maize, *Zea mays* L., belongs to the Graminae family and is one of the most widely grown cereals of the world. It is a staple food in most developing parts of the world and a very important raw material for quite a number of agribusiness industries, apart from being the main feed for poultry and livestock. The United States is invariably the world's leading producer, accounting for about 40% of the total production, followed by other major maize-producing countries like China, Brazil, Mexico, Indonesia, India, France, and Argentina. Global production was well over 817 million tonnes by 2009, higher than that of rice and wheat combined. Maize is eaten in many forms: cooked, ground into flour, or fermented, and many products are made directly from it or its by-products: oils, breakfast thickeners, involatile sweeteners, citric acid, flavour enhancers, and spaghetti, among myriad others. Nutritional value: Maize is an invaluable, moderately cheap source of protein, supplying about 15% of the nutritional protein consumed in the world and about 20% of the calorie intake. It contains essential trace elements,

which may be classified as essential, such as iron, zinc, copper, manganese, and selenium; probably essential; and non-essential.

Each element performs distinct functions in human health, from the activity of enzymes to immune responses. Nutritional composition varies between and within species/subspecies; often, these are determined under prevailing conditions such as climate and geography. However, very little is documented on nutrient levels between commercial maize flour and flour from maize grown in specific local conditions. Chemical Composition and Variations in Commercially Available and Locally Grown Maize Flour

There is a marked difference in color, texture, and nutrient composition between the commercially available white maize flour and locally grown yellow maize flour obtained from the University of Agriculture, Faisalabad. The commercial white maize flour was of uniform texture, while yellow maize flour exhibited a coarse and heterogeneous composition, reflecting perhaps the non-starch components and natural variation within the locally grown maize. For the analysis of the nutritional contents of those types of maize, the researchers used ICP-MS, a method well noted for its precision in trace element detection.

Trace elements in maize flour fall into a division of three classes: essential, probably essential, and toxic trace elements. Both types of maize contained essential trace elements important to human health; however, these were generally higher in the yellow maize flour. For instance, zinc, manganese, copper, molybdenum, and iron concentrations were appreciably higher in the yellow maize flour compared to white. In the yellow maize flour, zinc—an element important for immunity and cellular repair—concentrations were fivefold higher. Manganese is helpful to bone health and metabolism, showing up eightfold above the level in the white flour. Copper is vital to heart and brain health; it was more than twice as plentiful. Molybdenum, a trace mineral useful in helping many detoxification processes in the body, and iron, crucial to the production of red blood cells, fell in line behind yellow maize flour. Only calcium levels were higher in the white maize flour, and that was not by much. Looking at the likely essential minerals, including magnesium, potassium, and aluminum, it was evident that those, too, were overall more concentrated in yellow maize flour. Magnesium, a mineral that is crucial for muscle and nerve function, was available 16 times more in yellow maize flour than in white. Potassium, which is

helpful to keep blood pressure and fluid balance in check, was 26 times more abundant in the yellow flour. In the case of sodium, the values were higher in the white maize flour; this shows some nutritional differences that may explain the choice in relation to individual specific needs for health purposes.

### **Comparison of Toxic Element Profiles in White and Yellow Maize Flour**

Along with the essential nutrients, some toxic trace elements were also detected in the maize samples. The elements like lead, cadmium, arsenic, and uranium are normally present in maize with small sizes, though if elevated in high proportions, may be harmful to health; taking much time, it may pose serious risks to health. In the white and yellow maize flour, elements such as lead, nickel, and chromium appeared in slightly higher concentrations in yellow maize flour, and strontium was more prominent in the white. Although within the safe range, the lead content in yellow maize flour was about 1.7 times that in white maize flour. Nickel and chromium, both of which have the potential to bio-accumulate in body tissues and lead to toxicity when their levels are high, were marginally higher in yellow flour. Arsenic and cadmium concentrations, presented together with uranium, were quite similar within both maize flours and far below any threshold that could present health concerns. These differences in elements likely originate from the different soil compositions and farming practices of the areas in which the maize is grown. Some environmental factors may be very important, though, in influencing the uptake of these elements by plants, such as the mineral content of the applied soil and exposure to industrial pollutants. It also focuses to local soil health as part of agriculture.

### **Environmental and Soil Type Influences on Nutrient Composition in Maize**

Nutritional differences between commercial white maize flour and locally grown yellow maize flour can be attributed not only to genetic differences but also to the environmental conditions in which the maize were grown. The yellow maize, grown against the backdrop of Faisalabad's particular soil and climate show composition influenced by surroundings. Varying soil quality and composition directly influence the availability of these essential elements, which may be the reason for the variation in the mineral content of maize. As an example, magnesium was significantly higher in yellow maize flour and may reflect

magnesium-rich soil in Faisalabad or different soil management practices, including mineral fertilizers. The differences in the levels of toxic elements, such as lead and chromium, between the two types of maize, soil contamination, and industrial exposure may partially contribute to trace element uptake by maize plants. Soil and water management practices are responsible for metals such as cadmium and uranium presence in crops. However, the levels detected in both the maize flours were within the safe limits, hence consumers derive nutritional benefits with no significant risk from these trace toxic elements.

### **Nutritional Insights of Maize and Possible Health Consequences**

Maize is nutritionally a rich source of trace elements essential for human health, and the trace element profile assists consumers in their intake while making decisions. The high content of essential elements found in yellow maize flour, especially the one produced locally, proves the health benefits due to the staple's enrichment in the diet. Yellow maize is also intensely rich in zinc and manganese, important for health immunity and bone structure, while copper and iron are utilized in oxygen transport and metabolic functions. Since many populations dependent on a maize diet require these essential nutrients in worthwhile quantities for general health, it is great that this happens. Toxic trace elements present in low concentrations represent the natural accumulation from soil and general environmental exposure, keeping consumption moderate, normally posing no real health risk. Comparison between white and yellow maize flour demonstrates how local farming conditions influence the nutritional content of the food and provide the consumers with different nutritional profiles depending on their source of maize. Maize is not just a staple crop of food; rather, it's one of the great contributors towards providing proteins, necessary minerals, or dietary fiber for nutrition among the human population. Whether it is white commercial out of purchased maize or yellow flour made from homemade maize, this crop stands out as irreplaceable. These findings on nutrient and trace element variation underline the wider nutritional implications of maize in its varied cultural and geographical contexts, reminding us once again of sustainable, nutrient-sensitive agriculture in conjunction with food security and health.

## Further Readings

- Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2015). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, 45(4), 287-306.
- Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. *Journal of Functional Foods*, 18, 820-897.
- Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*, 18(14), 1818-1892.
- Crowe, F. L., Appleby, P. N., Travis, R. C., & Key, T. J. (2013). Risk of hospitalization or death from ischemic heart disease among British vegetarians and nonvegetarians: Results from the EPIC-Oxford cohort study. *The American Journal of Clinical Nutrition*, 97(3), 597-603.
- Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. *Advances in Nutrition*, 4(3), 384S-392S.
- Satija, A., & Hu, F. B. (2018). Plant-based diets and cardiovascular health. *Trends in Cardiovascular Medicine*, 28(7), 437-441.
- Tilman, D., & Clark, M. (2014). Global diets link environmental sustainability and human health. *Nature*, 515(7528), 518-522.
- Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., & Vermeulen, S. (2019). Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet*, 393(10170), 447-492.
- Kahleova, H., Levin, S., & Barnard, N. D. (2017). Cardio-metabolic benefits of plant-based diets. *Nutrients*, 9(8), 848.
- Dinu, M., Abbate, R., Gensini, G. F., Casini, A., & Sofi, F. (2017). Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies. *Critical Reviews in Food Science and Nutrition*, 57(17), 3640-3649.
- Wang, X., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Bao, W., & Hu, F. B. (2014). Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: Systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ*, 349, g4490.

## Chapter 5: Seed Germination, Nutrient Absorption, and Genetic Variability in Crop Improvement

Muhammad Arshad Javed<sup>1</sup>, Alexander Ray Rayiappan<sup>2</sup>, Zulaikha Sarobo<sup>3</sup>

### ***Capsicum annuum*: Factors of Seed Germination and Vital Growth**

It is grown all over the world as one of the more important vegetables for its spicy and fruity flavour, for which *Capsicum annuum* is grown. This versatile crop has carved a niche in the culinary, economic, and medicinal landscape. With a major global consumer base, India leads with approximately 36% of the world's consumption, followed by China with 11%. Of the 31 species of capsicum, five are domesticated: *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L. and *C. pubescens*. As a spice staple, *Capsicum annuum* is provided fresh, dried, or powdered; it is also an outstanding source of nutrition: it includes essential proteins, carbohydrates and fiber, a good variety of mineral salts, and fat-soluble vitamins represented by A and E. *Capsicum annuum*, with its antioxidant-rich compounds, including ascorbic acid, carotenoids, and capsaicinoids, besides serving a culinary purpose, plays a role in alleviating tumors, coughs, sore throats, and heart diseases. Fresh capsicum is also considered in many regions to be good for digestion, especially in the digestion process of starchy food materials, and is believed to boost the immunity of a person hence providing a strong line of defense against seasonal irrationalities. Other pharmaceutical applications include its role as an immunosuppressant, inhibiting non-essential bacteria to the gut.

Capsicum is grown worldwide for its economic value, bringing in income and being an important part of food security. However, despite the importance of the crop, *Capsicum annuum* remains an "orphan" crop

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<sup>1</sup> Department of Plant Breeding and Genetics, University of the Punjab, Lahore – Pakistan

<sup>2</sup> Department of Biosciences, Universiti Teknologi Malaysia, Johor Bahru – Malaysia

<sup>3</sup> Innovation Centre in Agritechology for Advanced Bioprocessing, Universiti Teknologi Malaysia, Pagoh 84600, Johor – Malaysia



due to a lack of substantial research and development that could support its improvement for agricultural advancement. In conventional farming, the plants are still subjected to different biotic and abiotic stresses, which include pests and diseases, drought, salinity, heavy metal toxicity, and adverse soil texture. The overall crop yield often suffers from these stresses, making seed germination a very important aspect in one's quest to have uniformity of growth and a good harvest. Considering that seeds constitute an essential means of plant propagation, improvement in their germination performance is vital for break crop productivity. In the *Capsicum annuum*, the process of seed germination commences by taking in water by the seed coat. This is accompanied by mobilization and activation of various biological processes, which convert the potentiality of growth in the seed into actual growth. The radicle or the first root pushes out from the seed coat, marking the initiation of growth. The tropical climate of Malaysia and Thailand truly produces quality capsicum seeds. However, seed quality has been realized as one of the important factors for the commercial viability of crops. Recently, it has been emphasized that capsicum seed germinates under the influence of several factors like seed moisture, temperature, and age of the seed. Variability in these factors might cause a loss in the viability and reduction of seeds' quality, which ultimately reflects in crop yield.

Good germination and strong seedling growth are essential to maximize yields. Of the various measures of seed quality, germination percentage indicates seed viability, while the rate of germination reflects seed vigour and potential yield. The germination period is the length of time required for the most seeds to have sprouted, and it provides an important benchmark for growers by showing approximately how well a lot of seed will perform under optimal conditions.

In one recent study, the maximum capsicum seeds which germinated were 96% and averaged 81%. Success rates observed in this crop are similar to those that would have also occurred in other crops, perhaps because controlled environments usually result in better germination rates compared to those that are not controlled. Seeds that germinate to high percentages usually possess healthy endosperm—the part responsible for the uptake of nutrients and water, which contributes to the development of the seedling. On the other hand, low germination percentage could be caused by physical or metabolic damage, which will impede the development of the seeds. In *Capsicum annuum*, the soft

seed coat may be highly susceptible to physical disturbances, thus it can affect the embryo and early growth stage of seedlings.

Seed vigor is often perceived by the speed at which a seed is able to germinate-how fast the rate is that transforms a seed from a dormant condition into one with active growth. Often, in the majority of crops, including capsicum, fast germination does not necessarily translate to high yield. In other words, the vigor of the seed could be independent of the speed of germination or is influenced more by the genetic quality of the seed itself rather than by environmental conditions. In capsicums, germination speed and germination percent are usually inversely related. So, the more slowly germinating seeds can have a higher germination rate when the germination period is longer. For instance, in any study, the overall mean of initiation day is the 9th day, and the time required for 50% germination falls on the 10th day. This trend denotes a late germination period, which correlates with the typical growth behavior of capsicums, similar to other populations of *Capsicum annuum*.

The time required for 50% germination, T50, builds a picture of the genetic control of germination patterns. This is the parameter that segments capsicum seeds into slow, average, and fast germinators. In capsicum seeds, the average T50 fell between the 9th and 10th day, advocating its place in the "average" category on the speculum of germination speed. Interestingly, genetic factors contribute to T50 variation when, after seedlings, a fast-germinating seed belonging to a slower family may well be outcompeted by a slow-germinating seed from a faster family. As a matter of fact, such variation is the cause of high genetic diversity in capsicums for seed germination and seedling development. Late-germinating seeds are associated with seedlings of smaller size that, owing to small size, are likely to be inferior in overcoming unfavorable environmental conditions and lead to a low yield. Capsicum growth is further explained by the duration of the germination period. In recent controlled studies, days 7 and 12 were the range when germination occurred, with the highest rate on the 12th day. Such delayed germination could be due to a slow water imbibition or osmotic adjustment that triggers basic metabolic activities. Germination practically stops after the 12th day, hence serving as a guideline for the onset of regular seedling growth in the cultivation program.

These observations in *Capsicum annuum* germination provide growers and agricultural researchers with a guide on how yield may be optimized through environmental control. It is stated that the best conditions, especially in the controlled environment, further cause germination to increase and make way for the growth of healthy seedlings. In places where all-round uniform growth is important by increasing productivity, these results direct farmers on which varieties of capsicum are suitable in the region according to the climatic conditions and available resources. In a nutshell, *Capsicum annuum* is much more than a food ingredient; it is a financial and nutritional provider. By focusing on the pedoclimatic cultivation traits of seed germination and adaptability to different environmental conditions, farmers would be in a better place to make informed decisions and develop methods that ensure maximum growth and yield for their plants. We will definitely be continuing with this very essential vegetable to further bring sustenance into global diets and economies through targeted breeding, improved farming, and better understanding of its germination.

### **Diverse Germination Characteristics and Genetic Diversity in Capsicum Cultivars**

Capsicum is grown as an annual crop in most tropical countries, the plants having an optimum temperature set for growth. In germination, optimum temperature is needed at 25-27°C with strong and healthy seed emergence. Seeding starts during the early spring in Mediterranean regions, while in tropical areas like Malaysia, the climate is suitable throughout the year for cultivation. Furthermore, the genetic variability and adaptation of Capsicum varieties native to such an environmentally homogenous climate remain unexploited. A very critical determinant of Capsicum emergence potential is the interaction between the genetic and environmental variables. For instance, Malaysia imports most of her requirements of chili because of the limited availability of high-quality varieties that are adapted to the local conditions. This reliance on importation, coupled with poor seed distribution, susceptibility to pests and diseases, and climatic variability, enhances the necessity for a comprehensive database of indigenous and exotic Capsicum accessions. These will also ensure that the genetic diversity of such plants is preserved to avoid genetic erosion, which will be crucial in advancing the plants from seedling emergence through to production.

One study researched the genetic variability of Capsicum cultivars in relation to their germination traits. Principal component analysis of the germination parameters revealed two main factors that explained about 86% of the total variation in the germination characteristics of the studied accessions. The first principal component described the speed of germination, while the second component described the day of beginning germination. This analysis showed one undisputed trend: the slower the germination rate, the longer it took for them to germinate. This agrees with earlier studies that have noted such relations between the speed of germination and overall seedling development. Therefore, principal component and cluster analyses may allow researchers to preliminarily group the cultivars according to their germination characteristics. Clustering here indicates that diversity within the Capsicum species could serve as a means of selection of more robust seed lines in their improved seed performance in the field. These findings underpin the key role of genetic diversity in seed vigor and are an avenue for further development toward better-adapted and higher-yielding Capsicum varieties.

## **Understanding Seed Germination and Nutritional Quality in Rice and Maize**

Understanding seed germination and nutritional values in staple crops like rice and maize imparts very useful information about their improvement for agricultural productivity and food security. Seed quality forms the genesis of any crop cycle, hence determining its potential for germination, growth, and eventual yield directly. For instance, rice seed lots must adhere to international norms that take measurement on the development of critical structures from the embryo and show their chance to develop into a healthy and vigorous plant. Laboratory testing has, therefore, become one of the favored ways to get more accurate germination results than at controlled environments without variability common in field testing. This controlled environment enables them to evaluate germination with consistency and reliability. In fact, one of the most prevalent causes of poor plant growth and low crop yield in many regions, where the weather and soil conditions are unpredictable, is due to poor seed quality. Not only would low-quality rice seeds give weak growth, but these seeds may also introduce weeds, pests, and diseases, thereby depressing productivity. Thus, high-quality seeds are crucial to improve

crop performance and encourage sustainable ways of agricultural production.

One of the most promising research areas in rice cultivation has been the use of beneficial microbes like *Azospirillum* and *Pseudomonas spp.*, which help promote quick germination and protect seedlings in the earliest stage. The microbes form a protective sheath around the growing roots and shoots, where the seed becomes more resistant to environmental stresses. Three other microbial species-*Nitrosomonas europaea*, *Rhodopseudomonas palustris*, and *Acinetobacter*-have also shown promise in furthering rice seed germination. Each of these microorganisms makes a different contribution toward the growth of the seedlings. *Nitrosomonas europaea* is a member of the family of nitrifying bacteria. It plays an important role in the nitrogen fixation process, an important process in soil fertility enrichment. This is because the bacterium makes nitrogen, necessary for plant development, easily available in the soil through the conversion of ammonia into nitrites. This leads to better health and higher growth rates in rice plants. *Rhodopseudomonas palustris* is a photosynthetic bacterium that further enhances seedling growth by fixing nitrogen and existing under a wide range of conditions. This could mean that the bacterium can survive with no oxygen through oxygen and derive energy from light to organic compounds, flexibility that makes it an extremely useful ally for rice plants in a variety of environmental stresses.

Finally, *Acinetobacter*, while present naturally in paddy soils, aids rice growth by making the seedling more resilient to stressors in the environment. Together, these microbes illustrate the potential of biological interventions in crop production, emphasizing advantages related to natural methods to enhance agricultural resilience. The understanding of microbial interactions in rice has opened doors to exploring ways of enhancing upland rice varieties, which usually go through more extreme conditions compared to lowland rice varieties. Though research unveiled that benefit-conferring microbes could improve germination rates and help plants cope with environmental adversities, the potential of microbes during the early growth of upland rice remains one of continuous interest. It is such studies that may bring about the most important breakthroughs in crop production, thus enabling people to make rice farming more resistant and sustainable.

## **Maize: Nutritional Value and Safety Considerations**

The other most important staple crop, with its wide importance in global agriculture, is maize, or scientifically, *Zea mays* L. Very versatile and highly productive, this crop is used as a staple food both for humans and livestock, besides its industrial raw materials application. Originating some 9,000 years ago in what is now Mexico and Central America, maize spread to diverse climates around the world and today has become a dietary staple in many countries throughout Africa, the Middle East, Asia and the Americas. Today, most of the world's maize supply is produced by a few countries, in particular the United States and China, which produce more than half of the world's maize. Apart from being a source of food, maize provides many useful benefits due to its composition as it contains vital minerals and other nutrients important for human health. However, not all types of maize are safe for consumption, since there is a potential danger of some types containing toxic heavy metals which could possibly be ingested by the consumer and accumulate overtime in the human body causing serious health conditions. It therefore follows from this that the determination of nutritional quality does not only involve understanding what beneficial components are contained in maize, but also calls for identification and control of the harmful elements contained therein. A balance of the two is paramount in ensuring a safe and healthy food supply at all times.

Most often, proximate chemical composition of maize grains is done under laboratory conditions to ensure their safety and nutritional value. This encompasses the determination of moisture content, crude fat, fiber, and protein, which are the major elements of the grain that define its nutritional value. In addition to this, other important inorganic nutrients such as sodium, calcium, manganese, potassium, and iron have been analyzed on the basis of mineral determination. A technique used in this regard for the purpose is the laser-induced breakdown spectroscopy or the so-called LIBS technique. LIBS helps in the determined measurement of these nutrients, which enables one to develop an accurate estimate of the mineral content of maize and whether it is up to the nutritional standard. The nutritional value of maize can be immense in human nutrition, but growing crops needs a careful cultivation in order to avoid contaminating the entire crop with injurious elements. Maize grown under safe climatic and soil conditions-that do not have any harmful contaminants-can only be projected as a source of nutrition in which agricultural scientists and farmers can show

faith. Nutritional composition monitoring and continuous evaluation are indispensable concerning maize food safety in agriculture, especially for crops that form a daily staple for millions of people.

The examples of rice and maize very clearly bring into perspective the complexities of crop cultivation, which begin at the seed level right up to making considerations for nutritional contents and safety. Such value addition of beneficial microorganisms in rice and full nutrient profiling of maize provide important insights into how these staple crops are optimized for maximum productivity and safety. These contributions to understanding and managing seed germination and crop nutrition will be of utmost importance as global food demand increases, to sustain a growing population and promote healthier diets around the world. Through continued scientific research and careful agricultural cultivation, rice and maize can continue to remain well into the future as staples of food security for generations to come.

### Further Readings

- Nonogaki, H., Bassel, G. W., & Bewley, J. D. (2018). Germination—Still a mystery after thousands of years of research. *Plant Science*, 276, 49-58.
- Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., & Job, D. (2012). Seed germination and vigor. *Annual Review of Plant Biology*, 63, 507-533.
- White, P. J., & Brown, P. H. (2010). Plant nutrition for sustainable development and global health. *Annals of Botany*, 105(7), 1073-1080.
- Varshney, R. K., Sinha, P., Singh, V. K., Kumar, A., Zhang, Q., & Bennetzen, J. L. (2020). 5Gs for crop genetic improvement. *Current Opinion in Plant Biology*, 56, 190-196.
- Huang, X., & Han, B. (2014). Natural variations and genome-wide association studies in crop plants. *Annual Review of Plant Biology*, 65, 531-551.
- Maron, L. G., Guimarães, C. T., Kirst, M., Albert, P. S., Birchler, J. A., Bradbury, P. J., & Kochian, L. V. (2013). Aluminum tolerance in maize is associated with higher MATE1 gene copy number. *Proceedings of the National Academy of Sciences*, 110(13), 5241-5246.
- Weitbrecht, K., Müller, K., & Leubner-Metzger, G. (2011). First off the mark: Early seed germination. *Journal of Experimental Botany*, 62(10), 3289-3309.
- Xu, Y., Li, P., Yang, Z., & Xu, C. (2017). Genetic mapping of quantitative trait loci in crops. *The Crop Journal*, 5(2), 175-184.

- Jung, C., & Müller, A. E. (2009). Flowering time control and applications in plant breeding. *Trends in Plant Science*, 14(10), 563-573.
- Baxter, I., & Dilkes, B. P. (2012). Elemental profiles reflect plant adaptations to the environment. *Science*, 336(6089), 1661-1663.
- Pérez-Jaramillo, J. E., Mendes, R., & Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology*, 90(6), 635-644.
- Finch-Savage, W. E., & Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist*, 171(3), 501-523.
- Fageria, N. K., Baligar, V. C., & Jones, C. A. (2011). *Growth and mineral nutrition of field crops*. CRC Press, Third Edition.
- ☒ Pandey, P., Irulappan, V., Bagavathiannan, M. V., & Senthil-Kumar, M. (2017). Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. *Frontiers in Plant Science*, 8, 537.



## About this Book

This book tackles the pressing challenges facing modern agriculture: overcoming abiotic stresses, battling plant diseases, and ensuring sustainable crop production. It explores the latest advancements in plant science, biotechnology, and agronomy to address these issues and secure food for a growing global population.

Key areas covered in this book:

- **Overcoming Abiotic Stress:** The book examines the devastating impact of salinity, iron toxicity, and acidic soils on crop productivity, particularly rice. It investigates genetic and physiological mechanisms behind plant tolerance to these stresses, offering insights for developing stress-resistant crops.

- **Sustainable Disease Management:** The use of eco-friendly biocontrol agents, such as *Trichoderma harzianum*, is highlighted as a promising approach to combat soil-borne diseases like charcoal rot. The book also explores the potential of medicinal plants in cancer therapy.

- **Crop Improvement and Nutrition:** The book emphasizes the importance of seed quality, nutrient uptake, and genetic diversity in crop improvement. It explores the nutritional value of staple crops like rice, maize, and capsicum, along with strategies to enhance their nutritional content.

This book is a valuable resource for researchers, scientists, policymakers, and agricultural practitioners seeking innovative solutions to the pressing challenges in agriculture. By understanding the underlying mechanisms and adopting sustainable practices, we can ensure a future of food security and environmental sustainability.

## About the Editors



Muhammad Arshad Javed, PhD, is currently serving as Professor and Head of the Department of Plant Breeding and Genetics at the University of the Punjab, Lahore, Pakistan. He earned his MS and PhD degrees from Kobe University, Japan. He has over 29 years of teaching, research, and development (R&D) experience. His expertise in plant sciences covers molecular genetics, tissue culture, haploid plant breeding, and agronomy. He served Universiti Teknologi Malaysia (UTM), Malaysia (2011–2019). In addition, Prof. Javed has been awarded an excellent performance award (Citra Krishma), including a cash prize, for his excellent performance in teaching, research, and student development. He also received a Konvensyen Felo Award from IPF Malaysia in recognition of his services as a student advisor. He served in the Department of agriculture, Government of the Punjab and developed a fine rice variety “Shaheen Basmati,” which was approved by the Government of Pakistan for general cultivation for normal and saline soils.



Dr Shakeel Ahmad is working as Consultant and Biotechnology Expert at the Seed Center and Plant Genetic Resources Bank, Ministry of Environment, Water and Agriculture, Riyadh, Saudi Arabia. He obtained his PhD degree (Plant Breeding and Genetics/Molecular Biology/Biotechnology) from the Graduate School of Chinese Academy of Agricultural Sciences (GSCAAS), Beijing, China. Then, being one of the pioneer post-doctoral fellows at the Center for Advanced Studies in Agriculture and Food Security, UAF, Pakistan, he strengthened his expertise in plant genome editing and bioinformatics.

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