

Modern Biotechnology and its role in combating biotic stress: A North-East India's perspective and a Vision beyond

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ABSTRACT

Breeding of crop plants is the most promising method for getting a combination of novel and desirable characters all in a single plant. From early stages of Agriculture, conventional breeding has played an important role in identifying prominent varieties and germplasms with novel characters and developing high yielding varieties with better agronomic traits. But with time, the environment and its conditions have changed. Environmental stress due to biotic and abiotic factors has become a major concern. To overcome such stressful situations, scientists have come up with different molecular biology tools and techniques that can assist and enhance the breeding process and reduce time. This would enable incorporation of the desirable agronomic traits in crops and getting the maximum yield out of them as a result. Modern tools in plant molecular biology along with the concepts of conventional breeding can produce diverse plants with special reference to North East India's scenario and concurrent worldwide developments made so far in Molecular Biology, which are path breaking as per the Futuristic Trends in Biotechnology.

Keywords- Breeding; Biotic Stress; Citrus; Banana; Rice; Biotechnology; North-East India; Futuristic Trends.

I. INTRODUCTION

Breeding of crop plants is the most promising method for getting a combination of novel and desirable characters all in a single plant. From early stages of Agriculture, conventional breeding has played an important role in identifying prominent varieties and germplasms with novel characters and developing high yielding varieties with better agronomic traits. But with time, the environment and its conditions have changed. Environmental stress due to biotic and abiotic factors has become a major concern. To overcome such stressful situations, scientists have come up with different molecular biology tools and techniques that can assist and enhance the breeding process and reduce time. This would enable incorporation of the desirable agronomic traits in crops and getting the maximum yield out of them as a result. This chapter discusses as to how modern tools in plant molecular biology along with the concepts of conventional breeding can produce diverse plants with special reference to North East India's scenario and concurrent worldwide developments made so far in Molecular Biology, which are path breaking as per the Futuristic Trends in Biotechnology.

India is one of the mega bio diversity centers in the world, having 4 of the world's 36 Biodiversity Hotspots; located in the Himalayas, the Indo-Burma Region, the Western Ghats, and Sundaland. The Himalayas, overall, comprising North Eastern India, Bhutan, Central and Eastern parts of Nepal is considered the highest biodiversity

hotspot in the world. North East India comprises seven states namely Assam, Arunachal Pradesh, Tripura, Meghalaya, Mizoram, Nagaland, Sikkim, Manipur, with a total area covering 2.62 lakh sq. km, and accounts for 7.9% of the total geographical area of the country (Figure 1). North Eastern India is blessed with a wide range of physiographic-echo climatic conditions and serves as the geographical gateway for much of India's flora and fauna. More than 15 crop species representing 47% of the crop species diversity available in the country is found in North East-India. However, despite having such rich diversity, major crop species grown here are getting affected by biotic stress. This directly and indirectly impacts the livelihood of the general mass as agriculture still stands as the major source of income for majority of natives in North-East India. Regards such a crucial issue, biotechnology coming up as a potent solution, the sorts of techniques that have been used as part of research, and how such methods are befitting to be used not only in North-East India but also globally, highlight some of the key themes of this chapter. Trending biotechnological tools will most definitely find major future application in uplifting the livelihood of people of NE-India, subsequently heading towards achieving the nationwide goal of doubling farmers' income. Citrus, Banana and Rice are some of the most commonly grown crops in NE-India, which however, are also getting highly affected by variable stress factors. Remedying these by the use of high throughput biotechnological techniques and the scope it holds is being discussed step by step.



Figure 1: Map showing seven sisters of North Eastern India.

II. FRUIT CROPS CULTIVATED IN NE INDIA

A. Citrus, a widely cultivated fruit crop in NE India

B. Overview of Citrus cultivation in North Eastern India

The North Eastern Hill (NEH) Region of India is one of the richest reservoirs of genetic variability with 136 horticultural species growing in the region. It has been described as one of the major centers of diversity for citrus, banana and mango. Due to its unique combination of varied soil-physiographic and climatic set up, the NEH region is considered to be home to several Citrus species [1] [2]. Encompassing 17 species, 52 varieties and 7 natural hybrids, citrus is grown in the NEH region over an area of ~71,834 ha with an annual production of ~3,32,943 tons/ year. Prevalence of high rainfall, rich soil conditions and existence of different altitude situations from sea level to 1500 m above, place the region at a unique position for adaption of almost all the Citrus species and varieties of commercial importance [1]. Some of the noteworthy ones being *Citrus indica*, *C. assamensis*, *C.*

latipes, *C. ichagensis*, *C. macroptera*, *C. aurantium*, *C. reticulata*, *C. megaloxycarpa*, *C. jambhiri*, *C. aurantifolia*, *C. grandis*, *C. limon* and *C. karna*. Meghalaya is the leading state in both area and production, followed by Manipur, Assam, Tripura, Mizoram, Nagaland, Arunachal Pradesh and Sikkim [3]. The agroclimatic condition of the region is similar to frontline Citrus growing countries like Japan, China, Brazil, Mexico etc. Ironically, the rich landscape, prolonged high rainfall and high humidity conditions also favor most of the Citrus pathogens to grow, multiply and attack the crops for a longer period.

C. Biotic stresses faced by Citrus plants across North East

Despite such vast amounts of citrus growth in the region, the general health and productivity of citrus orchards of the NE region is declining year after year mostly due to infection from different diseases. The situation may have aggravated due to mixed infections of either Citrus Greening Diseases and Phytoplasma or CGD and CTV. No systematic studies have been made so far to record the incidence and molecular characterization of these pathogens and evaluation of reaction of the NER existing citrus germplasms to the diseases.

Wide spread incidence of Citrus greening disease in different states of NE India, namely Sikkim (12.5–30%) and Meghalaya (5–12.5%) on Khasi mandarin, Assam (up to 43.7%) on cultivar Assam lemon and molecular confirmation of the association *Ca. Liberibacter asiaticus* with the disease was reported by [4]. Reference [5] Reported 32.19% incidence of CGD in Assam, 34.11% in Khasi Mandarin and 20.21% in Assam Lemon. Their report also shows that, Assam CGD isolate showed 100 similarities with Umiam1-3, Maharashtra, Iran, USA isolates and 74.7% similarity with South African isolate. Reference [6] Observed 100% disease severity of CGD on sweet orange and 41.26% disease severity in Khasi mandarin in Assam. There is also report of mixed infection of phytoplasma and citrus greening in the citrus plant [7] [8]. Same was also reported from Nagpur, Maharashtra [9] [10] and very recently from Assam [11]. This type of infection can become so serious that it may completely wipe out the citrus orchards of the region. There is no single control measure to manage the CGD, CTV and phytoplasma infections and the best way is to tackle this is to develop resistance in the citrus plant. As North East India is endowed with a large number of citrus genotypes, there is every possibility of some resistance genotypes against these diseases being present. Resistant sources need to be identified and could be utilized in resistance breeding to improve resistance of the economically important citrus species.

Citrus tristeza virus (CTV), a member of genus closterovirus, is an important viral pathogen which has killed about 100 million citrus trees worldwide over the last 70 years [12]. CTV causes various disease symptoms like mild symptoms to seedling yellows, stem pitting, severe stunting and ultimately decline of the plant depending on the citrus species especially on Kagzilime (*C. aurantifolia*) in India [13]. This disease has been reported earlier from Maharashtra [14]; Sikkim [15]; North-Eastern states of India – Arunachal Pradesh, Meghalaya, Assam, Nagaland, Sikkim and Tripura [16].

Citrus yellow mosaic badnavirus (CMBV) is another important viral disease found only in India that causes significant loss in yield in citrus growing regions in the country. This disease alone could damage 70% of the total production [17]. The characteristic symptoms of CMBV infection include yellow mosaic of the leaves and yellow flecking along the veins [18]. CMBV can be transmitted to other citrus species by grafting, dodders and mechanical inoculation [19]. The disease was first reported in sweet orange from Southern India [20], pummelo in Karnataka [19] and rangpur lime and acid lime from Andhra Pradesh [21].

Mixed infection of Citrus greening and phytoplasma is being reported from various citrus growing parts of the world including North East India [7]; [8]; [10]; [9]; [11] (Figure 5). There is no single method of control of the diseases except for the development of resistant genotypes. No systematic study has been conducted to characterize the existing citrus germplasms of North East India for their reaction to phytoplasma and Citrus greening. Therefore, it is felt necessary to evaluate and characterize the phytoplasma, citrus greening and CTV pathogen infecting the citrus germplasm of NER, and catalogue them based on their disease reaction.

D. Why Cultivators are shifting their profession

A big number of farmers from North-East Indian states are involved in citrus cultivation, one of which is the state of Tripura. However, farmers in Tripura have started facing severe loss of yield due to outbreak of different citrus diseases. Even with the presence of citrus germplasms of many different types and the region's environmental conditions suiting citrus cultivation, productivity recorded for citrus cultivation in Tripura is very low as compared

to other citrus growing states of India, majorly due to the disease citrus decline. It has been reported that among the various biotic and abiotic causes of citrus decline, citrus greening is the most dreaded and one of the major diseases leading to the ruin of many citrus orchards of the region.

The state of Tripura is famous for its sweet oranges popularly known as 'Jampui Komala' over the decades. In the 1980s, the crop was cultivated in the hilly terrains of Jampui hills over an area of approximately 1000 ha by natives of local tribes of Tripura. The crop generated a significant export demand, and became a major cash crop for the state. But in recent times, it has been regularly observed that the production area under this variety has decreased all over the state, especially in Jampui hills and is limited to just ~100 ha now [22]. The situation of citrus decline for all the varieties is due to several disease complexes and pest outbreaks which has finished most of the citrus variety orchards and most of the farmers have already shifted their livelihood to other crops like Arechanut, Coffee etc. It is high time, therefore, diagnosis of the real cause of citrus decline all over the state of Tripura and other North East Indian states went underway and suitable management approaches be suggested to bring back the previous situation of the state's citrus cultivation, ultimately boosting the region's socio-economic condition.

E. How modern biotechnology is useful in improving the existing Citrus genotypes of NE India

F. Plant tissue culture techniques

Recently developed biotechnological tools were successfully used to identify the infected plants by PCR, RT PCR, ELISA, Virus Detection Kit, Multi Locus Sequence Typing (MLST) etc (Figure 2). Through these techniques, the diseased plants can be identified well in advance and management procedure can be taken up at the right time. But due to climatic and commutation constraints, it is very difficult for experts to frequently access the garden. Hence the disease got spread unconsciously in the farmers' fields. This is where Plant Tissue Culture can come up as a solution. Tissue culture is a method where the totipotent behavior of a particular cell is exploited and a whole clone plant is produced. Disease free citrus plants can be produced by using tissue culture technique.

G. Scope

Owing to the presence of a huge variety of citrus plants in the NE Region, a proper research methodology needs to be formulated in this direction, in order to develop a protocol for successful production of tissue cultured and shoot tip grafted plantlets in different citrus varieties. Different research approaches can be designed in search of resistance source from the existing germplasms. Germplasms can then be characterized thoroughly at molecular and phytochemical levels. Generation of disease-free quality planting material (micro-budding, rootstock standardization) for Citrus species through setting up of certified centers and nurseries in NER could be the next step. Virus free root stock must be used for grafting to minimize the disease spread.

H. Genome Sequencing

Citrus genome (sweet orange) has been sequenced by [23]; Lemon by [24]; Chloroplast genome Omani Lime (*Citrus aurantifolia*) by [25]; *Citrus reticulata* by [26]; *Citrus unshiu* by [27]; *Citrus grandis*, *Citrus medica* by [26] due to the recent improvement of high throughput sequencing method. Size of the reported citrus genome varies from species to species; such as genome size 380 Mb (*Citrus sinensis*); 398 Mb (*Citrus medica*); 360 Mb (*Citrus reticulata*); 383 Mb (*Citrus maxima*). This implies that citrus species has a small genome ranging from 360 Mb to 398 Mb. Handling of citrus genome is not a complicated issue due to the improvement of high throughput sequencing methods. Genome sequencing report of the cultivated species from India is lagging behind other countries.

I. Scope

The presence of a wide range of diversified citrus genotypes in India is a well-known fact by now; and therefore it is essential that a few cultivars of both local and wild genotypes be sequenced, so that the genomic variation in the species specially the naturally available citrus species in the North Eastern region be known, which will surely throw light on some interesting facts and figures hidden in the natural population of the citrus species. After getting the sequences many biotechnological approaches (magic population development, GWAS, GBS, QTL analysis, gene identification and characterization, pathway engineering, secondary metabolites profiling,

transcriptomics and proteomics studies) can be used to dissect the genes and their expressions against different biotic and abiotic stresses. Thereafter all of this information can be used to improve the existing citrus species against different biotic and abiotic stresses.

J. Genome Editing (CRISPR/Cas9)

Owing to the high heterozygosity and long juvenility of many citrus cultivars, conventional breeding approaches can often be tedious and time-consuming. Genetic transformation, on the other hand, can provide rapid solutions and is becoming increasingly popular. CRISPR/Cas9 has emerged as the most extensively used genome-editing systems in the recent years. This tool is based on the bacterial clustered regularly interspaced short palindromic repeats CRISPR/Cas9 system. In addition to the pioneering studies in *Arabidopsis* and *Nicotiana*, the CRISPR/Cas9 system has been successfully used to target specific genomic sequences of interest for the development of genome edited citrus. Reference [27] Utilized genome editing techniques to edit citrus canker susceptibility gene CsLOB1.

K. Scope

Genome editing approach can be used in NE Germplasm improvements for biotic and abiotic stresses. Here again, target oriented research programmes need to be implemented in NE labs to solve the existing issue in citrus cultivations. Collaborative efforts from the Government and Private sector along with NGOs may come forward with the requisite funding to accelerate such research programmes in the NE States. Establishment of proper infrastructure, comprising sophisticated labs in this zone will be hugely beneficial and essential for optimal capitalization of the richness in biodiversity found in this wonderfully blessed biodiversity hot spot.

In the recent past a few attempts have been taken up to uplift the condition of the Citrus cultivation in North Eastern region by Department of Biotechnology, Government of India, in terms of financial assistance in the form of funding different projects. The state authorities remain hopeful that these attempts shall mark a way to solve the issue. Demonstration projects on generating quality planting materials of NER Citrus using shoot tip grafting and supplying to farmers in NER in collaboration with state horticulture departments have also been taken.

Figure 2: Use of modern biotechnological techniques for identification and characterization of different diseases affecting Citrus cultivation

III. BANANA, A HIGHLY NUTRITIOUS AND WIDELY CULTIVATED FRUIT CROP

A. Banana cultivation overview

Banana (*Musa sp.*) is India's second most significant fruit crop with a high export potential. India leads in banana production in the world followed by China and Philippines with an area coverage of 884 thousand ha and production of 30.8 million tonnes [29]. Banana is grown in almost all the states of India. Banana production is threatened by different types of biotic stresses, among which viruses (diseases caused -banana bunchy top disease, streak disease, bract mosaic disease and banana mosaic), fungus (diseases caused -panama disease, sigatoka leaf

spot) and bacteria (disease caused -moko disease) are causing serious economic losses [30]. Among the viruses, Banana bunchy top virus (BBTV) and Banana streak viruses (BSVs) causing the banana bunchy top and banana streak disease respectively are considered to cause economically significant widespread diseases of banana plantations [31]. BSMYV is the most prevalent BSV species reported from India till the commencement of the present study from Northern and Southern India but North Eastern Regions (NER) still remain unexplored for the presence of BSMYV [32]; [33]. However, BBTV has been reported previously from NER states of India [34]. (Figure 5).

B. Scenario of BBTV, Phytoplasma, BSMYV, *Fusarium* wilt diversity in NE India, which affect banana cultivation drastically.

Till date, four phytoplasma strains, 16SrI, 16SrIV, 16SrXXII-A '*Ca. P. novoguineense*' association have been reported in banana worldwide [35]; [36]; [37] [38]. However, 16SrI phytoplasma group has been identified to be associated with leaf yellowing, dark green streaks on mid rib and stunting symptoms on banana from Uttar Pradesh, India [39]. Whereas, mixed infection of banana bunchy top virus and unknown phytoplasma group in banana were reported from the state of Assam in India with bunchy top and leaf chlorosis symptoms [40]. In a recent study, reference [41] has reported the association of '*Ca. P. asteris*' (16SrI-D subgroup) little leaf and leaf chlorosis along with stunting symptoms by employing *16S Rrna* and *secA* gene specific primers and sequence comparison in two dominant banana varieties of Tripura and in the same paper the mixed infection of phytoplasma with BBTV and BSMYV from Lembucherra location in Tripura has also been reported. Reference [42] reported BBTV in seven districts of Tripura and Polymerase Chain Reaction (PCR) based detection established that BBTV is widely prevalent in all parts of Tripura showing an average incidence of 22.02%. Among the cultivars, maximum BBTV infection (27.03%) was recorded in 'ChiniChampa', followed by plantain (24.29%). The clustering pattern and genetic diversity of BBTV population from Tripura suggested monophyletic origin. However, four variant BBTV isolates have been identified from North Tripura and Khowai districts possessing somewhat unique variability than that of distinct isolate (BBTV-Umiam) reported from NE India (Meghalaya). Reference [43] dissected the genome of a new BBTV isolate from the virus (BBTV-Umiam) from local banana mats growing in mid-hills of Meghalaya in North-East India. Reference [44] carried out a PCR based detection of Banana Bunchy Top Virus (BBTV) with six different primer pairs for the six components of BBTV (DNA1 to DNA6) genome. They have reported the infection of BBTV from eight districts of Assam viz., Jorhat, Golaghat, Sonitpur, Morigaon, Nagaon, Kamrup Metro, Kamrup rural and Goalpara.

Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. cubense (Foc), is regarded as globally being one of the most devastating diseases of banana. Foc consists of three races, of which Foc races 1 and 2 occur in India. Foc race 4 has not been found in the country despite the importance and extensive cultivation of Cavendish cultivars. Reference [45] reported the infection of *Fusarium oxysporum* f. sp. cubense VCG0125 and VCG01220 Race 1 by using conventional PCR method from Bihar, Uttar Pradesh, Gujrat, Tamil Nadu. The incidence was 65% in Bihar, 30-45% in Uttar Pradesh, 50-15% in Gujrat and 15-21% in Tamil Nadu (Figure 3).

C. Use of modern biotechnological tools in identification of plant pathogen in Banana

Reference [46] conducted a survey and identified seven vegetative compatibility groups (VCGs) from three pathogenic races, which were highly virulent towards cv. Grande Naine. Comparative genome analyses revealed that Indian *Foc* VCGs were diverse in genomic organization and effector gene profiles. Whole-genome sequences of *Foc* R1 (GCA_011316005.3), *Foc* TR4 (GCA_014282265.3), and *Foc* STR4 (GCA_016802205.1) strains and race specific markers were developed for early detection.

Reference [47] reported infection of *Fusarium oxysporum* f. sp. cubense tropical race 4 (TR4) on Cavendish banana from Barari village in the State of Bihar in India and reported the wilting of Cavendish banana cvs. Robusta and Grand Naine. The incidence of the disease ranged from 2 to 26.6%. Symptoms included yellowing and browning of older leaves, which progressed from the oldest to the youngest leaves. Similarly, [48] also reported the infection of Tropical Race 4 from Uttar Pradesh, India in Grand Naine in the Faizabad district in Uttar Pradesh (26.46379°N, 81.59987°E). The affected plants exhibited distinct yellowing symptoms of mature leaves progressing toward the younger leaves, while the pseudo stem showed longitudinal splitting at the base (reddish brown).

Reference [49] screened 24 Banana Cultivar Field Screening for Resistance to *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 in the Northern Territory for new management strategies and resistant cultivars to combat the disease, a banana cultivar-screening trial took place in the Northern Territory of Australia, which examined the responses of 24 banana cultivars to the soil borne fungus. Several cultivars displayed considerable resistance to *Foc* TR4, including several FHIA parental lines and hybrids, the Cavendish (AAA) selections GCTCV 215 and GCTCV 247 from TBRI and an Indonesian selection CJ19 showed either very little to no plant death due to the disease, which could be used as resistance source for developing resistant varieties against *Foc*.

Fusarium wilt is also an important concern in Banana cultivation in North Eastern India. In NE India *Fusarium oxysporum* F. Sp *Cubense* Race 1 is prevalent since long, which creates considerable damage in the plantations. Approximately 15 to 20 percent disease incidence were observed in different NE states. There is no report of Race 4 infection in NE states till today but TR4 is spreading in India and abroad very rapidly causing huge damage in the banana production.

D. How modern biotechnology is used to dissect the genomic information of Banana.

Genetically, Banana is a triploid crop comprising both A and B genomes. Due to its triploid nature, sequencing and assembly of the whole genome posed a huge challenge previously not just for banana but other similar whole genomes as well. But due to recent developments in Next Generation Sequencing technologies and high throughput big data analysis systems nowadays, whole genome sequencing of a plant species has become much easier. Taking help of such high throughput technologies, several Banana genomes have already been sequenced in India and abroad like *Musa itinerans* [50]; [51]. The genome size of Banana is two times larger than that of rice genome (250 Mb approx.); approximately 554 mb in *M.acuminata* and 523 mb in *M.balbisiana*. Sequencing revealed that banana genome contains 36,000 genes. These genes, if characterized as per requirements can be utilized for further crop improvement programmes. Different cutting-edge techniques started getting used in banana improvement programmes after the genome of banana got revealed, like transformation methods, gene editing system, host induced gene silencing and CRISPER technology. CRISPER technology is used in recent era as this technique mutates the specific genes which lead to the production of mutant genotypes without introducing any kind of foreign material in the genome [52].

E. Scope:

Many attempts have been taken to produce disease free plants through tissue and resistance breeding. But the genome of *Foc* is prone to mutation like Covid 19 hence new strains are coming continuously. Some of the mutations are very aggressive and some are mild. *Foc* TR4 is one of the aggressive strains which can cause damage up to 60-70%. Hence the breeding approach is not much effective in combating the disease. From the epidemiological aspects of *Foc* and monoculture nature of most banana plantations, it is evident that FW management is not simple, unless a resistant and commercially accepted cultivar is available [53]. Necessary intervention to combat the disease and restrict the spread of TR4 in NE India is most crucial right now. Initiatives from the Government and other sectors may be taken up to set up a digital database on Banana (*Musa* spp.) of North East India (MUSANEI), and also for setting up labs in NE India for virus indexing, producing facilities for quality planting material production through tissue culture techniques. Disease free planting materials can easily be produced in banana by somatic embryo culture, meristem shoot tip culture. Several attempts have already been taken up by different state governments and private agencies to supply tissue culture raised banana plantlets in NE India. But for sustainable production this needs to be carried out in large quantity as per the demand of the banana cultivators in the North Eastern Region of India. Along with this, several other initiatives like identification of resistance source, whole genome resequencing, genome wide association studies for all the existing banana genotypes in India's North Eastern region need to be performed minutely as the genome complexity of Banana hinders the successful development or improvement of Banana genotypes or the development of biofortified varieties for sustainable production and provide nutritional security to the general mass.

Few days back, Government of India released a notification that will allow genome edited plants for probable release in India (SDN1 and SDN2). Hence it is an opportune moment to concentrate and work on these aspects to solve the existing problems on banana cultivation. This can be done by establishing sophisticated labs in NE India where such genome edited plants against the recent stresses faced by the plants across the NE region can be produced.

Figure 3: Use of modern biotechnological techniques for identification and characterization of different diseases affecting Banana cultivation.

IV. WIDELY CULTIVATED CEREALS IN NE INDIA

A. Rice a widely cultivated, staple food crop for NE people

B. Overview of Rice cultivation in North Eastern India

The North Eastern region of India constitutes eight states (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura), and is considered as a hot spot for agro-biodiversity and is reported to have 9650 cultivars of rice [54]. Rice is the principal staple food crop of the NE region and occupies the maximum area under cultivation [55]. Rice is cultivated over an area of 8000 ha with a production of 21,54,000 tonnes with an average productivity of 2 tonnes/ha, which is below national average [56]. The rice growing area of NE region to the total area of the country is just about 2.27%. NE India possesses a diverse array of locally adapted

non-basmati aromatic germplasms. The germplasm collections from this region could serve as valuable resources in breeding for combatting biotic stresses [57]. Reference [58] identified genetic diversity among 65 germplasms from NE Region. These studies significantly imply the presence of a highly diverse population of rice germplasm across North East. However, on the other side, Rice cultivation in NE India is getting drastically affected by different biotic stresses because of the unique ecosystem of NE region which provides a suitable environment for several fungal, bacterial and viral diseases. Among the numerous diseases recorded in the region, rice blast (*Magnaportheorizae*), sheath blight (*Rhizoctonia solani*) and bacterial leaf blight (*Xanthomonas oryzaepv. oryzae*) hold the maximum economic significance. In India, sheath blight was first reported from Gurdaspur, and become a major constraint in rice production in Assam, Manipur and Tripura [59]. This was due to wide spread cultivation of High yielding varieties with a narrow genetic base. Rice varieties grown in Tripura are also being seriously affected by multiple blast races. In the state of Tripura, rice blast causes yield losses ranging from 40 to 46% [60] (Figure 5).

C. Biotic stresses faced by rice plants across North East

D. Rice Blast

Rice blast is economically the most important and prevalent diseases of rice in the whole NE region, resulting in huge economic losses. Due to high rainfall and relative humidity, high incidence of leaf and neck blast was reported from the NE region [61]. Reference [62] screened 74 germplasms including local land races, improved varieties against blast resistance genes Piz, Piz-t, Pita, Pita-2, Pib, Pi2 and Pi9 using a set of SNP markers and identified the absence of broad spectrum blast resistance genes Pi9 and Pita-2 in all the tested genotypes, which clearly implies the threat of rice to Blast susceptibility [63].

E. Use of modern biotechnological tools in screening of Rice germplasms for Blast resistance

Screening of a large array of rice germplasm under uniform blast nursery and artificial inoculation has led to identification of nine germplasm lines viz., Meghalaya Lakang, KumtaMah, KemenyaPepeu, Wainem, Thekrulaha, Vishkv, PhouralUtlou, MesaoTsuk and Gum Dhan resistant to both leaf and neck blast under Manipur conditions. An analysis of virulence spectrum of rice blast pathogen indicated that race profile of rice blast pathogen in NE region is highly diverse and distinct as compared to other rice growing regions of India. Akhanaphou, a unique rice landrace of NE India, having its origin in Manipur has been reported to exhibit high field resistance to leaf and neck blast. Recently, two novel quantitative trait loci (QTLs), qLNBL-5 and qLNBL-7 were identified conferring resistance to leaf and neck blast diseases [64]. Employing the data generated on virulence spectrum of rice blast pathogen, now the breeding focus is on coming up with a durable management strategy based on gene deployment/pyramiding.

F. Scope

Extensive survey programmes may be organized to collect genotypes across North East India and screening of those genotypes may be done as per the need against different biotic and abiotic stresses. The chances of getting resistance genotypes will increase when screening of a large no of genotypes with high genetic variability is carried out. For this, sophisticated green house facilities need to be installed across NER labs to conduct screening against blast disease and also identification of the hot spots of Blast diseases across North East needs to be carried out. Simultaneously, avirulence (Avr) gene mining work also must be taken under consideration to identify the specific races or isolates in the NE region. Proper characterization of *Magnaportheaoryze* across North East region, especially in the disease prone areas and preparing a disease map will be a potent step to spot the hot spots with the Avr genes present in the local isolates. Once all the details are available, then moving forward with breeding aspects will be an easy job. In addition, new varieties can also be developed by incorporating those broad-spectrum Blast resistance genes depending upon the Avr gene prevalence in the locality. Combatting such a notorious disease will become less of an uphill task, once these steps are systematically followed and resistant cultivars get developed zone wise or state wise.

G. Sheath Blight

Rice sheath blight disease, caused by the fungal pathogen *Rhizoctonia solani* Kuhn is one of the major production constraints in rice growing countries of the world and ranks next to blast in causing significant economic

losses [65]. It is another economically important disease affecting rice in NE region of India. The disease is most prevalent in pockets with very high relative humidity (>95%), moderate temperature (28-32°C) and prevalence of high nitrogen application. It causes losses of around 11.1-58.0 per cent ingrain yield depending upon the disease severity and varieties [66]. The disease is more prevalent in the lowlands of NE region where water stagnation is common, however under upland conditions, Sheath blight is less of a problem. Presently, management of sheath blight in the region relies on use of chemicals.

H. Use of modern biotechnological tools in screening and identification of host

A major QTL conferring resistance to sheath blight was identified to be located at chromosome 9 [67]. QTLs responsible to sheath blight pathogen of rice in NE region needs to be defined/validated and employed for breeding resistance. *Rhizoctonia solani* belongs to a necrotrophic species complex. Based on anastomosis grouping, at least 13 groups infecting different hosts have been identified. The group AG1-IA of *Rhizoctonia solani* causes sheath blight in rice [68].

Identification and mapping of QTLs is valuable in the interpretation of the molecular and biochemical mechanisms involved in host pathogen interaction and for the improvement of crop quality by the process of marker assisted selection [69]. Several QTLs for sheath blight resistance have been identified using mapping populations derived from Indica or Japonica rice. QTLs associated with the resistance for sheath blight disease have been reported on all 12 chromosomes [70]. The validation of QTLs of sheath blight disease is determined by using various genetic background, populations and environment. Among the several QTLs, some QTLs share the same locus on the chromosome suggesting the existence of common locus for differentiation among rice varieties. These QTLs are stable, specific and considered as valuable QTL [71].

In rice, a total of 33 QTLs associated with sheath blight resistance have been reported spanning over all 12 chromosomes [72] although the mechanisms of resistance of these QTLs are poorly understood. Among these, three major QTLs mapped in Tetep, qSBR11-1, qSBR11-2 and qSBR7-1 were found consistently associated with sheath blight resistance across location and years [73]. They reported that qSBR11-1 is a major QTL effective against the sheath blight pathogen. They identified genetic loci for quantitative resistance to sheath blight in rice line Tetep.

I. Scope

Generally, Sheath Blight does not affect the yield much, but if the environment is favorable then the disease can spread very fast which may lead to damage upto 90%. Lack of resistance source is the main constraint in Sheath Blight resistant variety development. Very few rice varieties were found resistant against Sheath Blight [74]. As the North Eastern region has a huge number of land races, those land races need to be screened against sheath Blight and identification of resistant, moderately resistant rice genotypes needs to be carried out and then marker trait association studies can be done by following already available studies like [75] and [74]. Once the resistant source gets identified, scientists may proceed with whole genome resequencing of the resistant genotypes and then, identifying the differences between resistant and susceptible cultivars can be done by using potent bioinformatics tools. Transcriptomics study has also been done previously to track the expression of different genes because of Sheath blight [76]. Relevant QTLs can be identified in NE populations and validated for its action. Here again, once the major QTL is identified, it can be followed by sequencing to identify the probable genes; the genes can then be characterized, and may be cloned in the cloning vector followed by their transformation, in order to incorporate the gene in the desired variety [77]; [78]. All of these steps would require setting up of appropriate infrastructure comprising highly equipped labs which in turn is indeed a costly affair (Figure 4).

However, rice being the principal food crop not just for the people of NE region, but almost the whole of India and likewise for people of many tropical and sub-tropical countries around the world, the long-term perspective should be, to come up with stress and pest resistant rice varieties. It must be noted that, the Government of India has welcomed the incorporation of moderated alternate techniques of rice cultivation such as the System of Rice Intensification (SRI), which is a combination of several practices including optimal changes in nursery management, time of transplanting, water and weed management. From the North Eastern perspective, the SRI technique has seen an overwhelming response in the state of Tripura, because of the efforts taken up by the Tripura Rural Livelihood Mission (TRLM), under the Rural Development Department, Government of Tripura. The technique is being practiced by farmers from all sections of the society, among whom, women farmers are the

frontrunners and have shown excellent performance. For a state where Rice is the principal state food crop and covers 78% of the cultivable area, a healthy combination of SRI technique with improved rice breeds can prove to be a double bonus. This can serve as a blueprint for many other states as well. The role of National Bank of Agriculture and Rural Development (NABARD) is also crucial in imparting awareness through trainings and demonstration programmes and funding loans to farmers on how to better their yield through improved rice cultivation by using better breeds that would come after conducting research. Considering such a wide array of noble causes, and for the country as a whole, where food security is a desired outcome, sufficient funding and administrative cooperation from the Central Government, different State Governments, NABARD, and other international agencies such as the World Bank is a must to execute such massive research. Findings from NE Labs may be subsequently useful in other labs of India as well. Envisioning the development and use of better rice breeds should not be a far-fetched dream, once state-of-the-art facilities get set up, and modern, futuristic biotechnological tools come as a huge respite whereby amassing and analyzing high amount of data will not be such a tedious task any more, as compared to traditional methods used before.

Figure 4: Different steps and strategies involved in producing rice varieties against different biotic stresses with the help of modern Biotechnology.



Figure 5: A-C: Citrus plant showing symptoms of leaf yellowing, leaf chlorosis, stunting growth, D-F: Banana plants showing symptoms of Bunchy top appearance at the top, Leaf yellowing, Leaf streaks, and tip burning, G: Rice plant showing typical leaf blast symptoms and H-I: Rice showing typical symptoms of Sheath blight.

V. CONCLUSION

As has been discussed extensively in the chapter, multiple crops in the North Eastern region are susceptible to numerous biotic and abiotic stress factors, while having to face the need to cope with ever increasing demands. All of these needs have to be met by ensuring environmental sustenance and facilitating sufficient livelihood generation for farmers. Such requisites actually hold good for the agricultural sector of not just North Eastern India, but for rest of India and around the world as well. In times when technology has permeated almost every aspect of our lives, there is every good reason why technology in Biology – known better as Biotechnology should be at the forefront of addressing such challenges by bringing in accurate, precise, independent and scientifically sound solutions.

The technologies majorly discussed in the chapter fall under the ‘Omics’ category of technologies – such as genomics, transcriptomics and proteomics, all of which are at the very heart of modern-day biotechnological techniques. These techniques present the state of the applications of futuristic biotechnology in crop improvement studies.

Trending biotechnological techniques would find usage in other areas around the world as well, primarily by developing new crop strains that can reduce the cost of production, decrease pesticide inputs, fetch higher yield, can be grown in a wide range of environments without being susceptible to diseases, and provide better and nutritiously fortified crops and their by-products with good shelf-life. All of this would ensure not just enough income generation and empowerment of farmers but will also supply food supplies at low cost to consumers. Empowering women and helping them become self-sufficient is also another welcome long thought of objective. With more biotechnological intervention and hands at work, women farmers who are involved in traditional crop cultivation and/or mushroom production can generate more income if better crop strains reach them. Mushroom cultivation will get a great boost with the domestication of wild variety, enhanced production of nutraceuticals, bioconversion of lignocellulosic wastes into useful products. Molecular Biology tools are already in use that can improve mushroom strains by using Gene transfer using protoplast technology as this provides a highly feasible technique for interspecific and intergeneric hybridization.

No big vision and mission can be achieved if not for the right institutional support. In this regard, the Indian Council of Agricultural Research (ICAR) deserves special mention for propelling forward advancements in the Indian agricultural scenario since its inception. The ICAR-AU system includes some of the premiere institutes across the country that have been relentlessly involved in studies and conducting research on crop breeding and crop improvement. Putting into action such massive studies as suggested above, requires appropriate funding and making sure modern biotechnology is being incorporated in all relevant areas in the country, the Department of Biotechnology, India (DBT, India), stands atop. The reason some of the aforementioned molecular biology and biotechnology themed research studies could be conducted through funding numerous projects, DBT, India has been extremely supportive to bring forth ideas into actions. The nation remains grateful for having such positive institutional and financial assistance.

The future of trending biotechnological tools looks really bright with the right usage of a multi-disciplinary and multi-omics approach. Taking holistic actions by combining the data collected, diagnosing the appropriate cause thereafter and coming up with improved crop breeds can keep researchers and scientists ahead of the curve and, ultimately, propel forward advances in agriculture. The application of futuristic trends in biotechnology in the sector of agriculture can contribute a lot to rural development in India and across the world and can serve as an inspiration for emerging nations to supplement existing traditional practices with trending biotechnology techniques.

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