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

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ABSTRACT

Crop breeding is the science of incorporating novel and the most desired traits within agricultural crops for the overall benefit of mankind. Crop breeding as a science is as old as the practice of agriculture itself. Since the initial stages of agriculture, farmers have taken up conventional methods of breeding in order to identify and select prominent and noteworthy varieties of crops that show desired traits and fetch high yields. However, with passage of time, environmental conditions have altered, and with it comes the change in agricultural sector itself. Sudden adverse effects have been observed in agricultural crops, due to both biotic and abiotic stress conditions. Overcoming such stressful situations has become paramount and hence, scientists have started working towards improving crop breeding techniques that are able to produce better varieties in reduced amounts of time, with the help of modern biotechnology tools and techniques. 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 as will be discussed here with special reference to North East India's scenario and concurrent worldwide developments made so far in Molecular Biology, which are pathbreaking as per the Futuristic Trends in Biotechnology.

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

I. INTRODUCTION

Being sessile in nature, plants cannot escape or avoid unfavorable environmental changes and have to fight against all environmental odds, braving the different episodes of mother nature. Biotic and abiotic stresses are continuously affecting plant health, and this leads them towards being in the resistance and susceptible categories. Resistance and susceptibility of a particular plant genotype depend upon many factors; especially depending upon the allelic biasness. Plants having favorable alleles may lead them towards being fitter than others. Conventional breeding has played an important role since long to fix the alleles or identify the most prominent varieties along with favorable alleles by observing phenotypes. But this breeding approach takes a long period of time to fix or to develop a new cultivar or variety. With passage of time, demand of food has increased drastically in the world, bringing with it the requirement of developing new methods or techniques that can incorporate the desirable changes in the plant genome within a short span of time. Different modern biotechnological tools and techniques like genomic selection, molecular breeding for resistance, metabolic engineering, and genome editing are to name a few, which can assist and enhance breeding processes and reduce time. Another path breaking development in the recent era is speed breeding, which helps to perform five to six generations of a particular crop like rice, wheat in a year; which in case of the normal breeding process for rice and wheat, a maximum of two generations is possible in a year. This new invention from Lee Hickey's lab is under trial with many other crops. If success is achieved in these crops, vegetables and tree species, then this will turn out to be a remarkable achievement for plant science, as it can then easily speedup the crop improvement process. 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.

Among the world’s 36 Biodiversity Hotspots, India is having 4 hotspots, located in the Himalayas, the Indo-Burma Region, the Western Ghats, and Sundaland. North Eastern region of India, covers an area of 2,62,179 square km, which comprises 7.9 % of the geographical area of India (Figure 1). North Eastern India is blessed with a huge topographical, geological and climatic diversity, which leads to it being host to a large number of 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

Approximately 136 horticultural crop species are growing in North Eastern Region of India, the richest reservoir of genetic variability. It acts as one of the major centers of diversity for Mango, Banana and Citrus. Due to its unique climatic condition, topographical establishment and soil texture and structure, NEH region is considered to be home to several citrus species [1] [2]. In North East India, Citrus is being cultivated over an area of ~71,834 ha with an average production of ~3,32,943 tons/ year. Rich soil fertility, high rain fall along with high altitude and undulating topography makes it unique for adoption of commercially important citrus species and varieties. Whereas, the rich landscape, prolonged high rainfall and humidity also create favorable environment for most of the Citrus pathogens to grow, multiply and attack the crop.

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 are 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 (upto 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, Maharastra, 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. Mixed infection report of phytoplasma and citrus greening is also available [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 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 notorious viral plant pathogen belongs to closterovirus group, which killed over 100 million citrus trees across the world for the last 70 years [12]. It can cause several symptoms in the affected plants like potting of the stem, stunting, yellowing of the seedlings and in severe cases decline may occur depending upon 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 like Tripura, Assam, Meghalaya, Arunachal Pradesh and Sikkim [16].

Another important viral disease found only in India, which could damage up to 70% of the total production in citrus is yellow mosaic badnavirus (CMBV) [17]. The characteristic symptoms of CMBV are yellow mosaic of the leaves and yellow flecking along the veins [18]. The disease can be transmitted through grafting, dodders and mechanical inoculation [19]. CMBV infection was first reported in sweet orange from South India [20]; pummelo in Karnataka [19]; 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

Citrusgenome (sweet orange) has been sequenced by [23].; Lemon by [24].; Chloroplast genome Omani Lime (*Citrus aurantifolia*) by [25].; *Citrus reticulate* 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)

Performing conventional breeding in many of the Citrus species is very difficult because of high heterozygotic and long Juvenility nature of citrus varieties or cultivars. Genetic transformation in citrus is somehow easy and well established. Recently developed concept of Genome editing is widely used in different cultivated crop species for development of unique traits. The concept of Genome Editing tools is based on clustered regularly interspaced short palindromic repeats of bacterial immune system developed by Jennifer Doudna. Reference [27] utilized CRISPR/Cas9 system techniques to edit citrus canker susceptibility gene CsLOB1. Many such attempts are being expected in future to edit citrus genome and produce disease smart citrus genotypes.

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 infra-structure, 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 up.

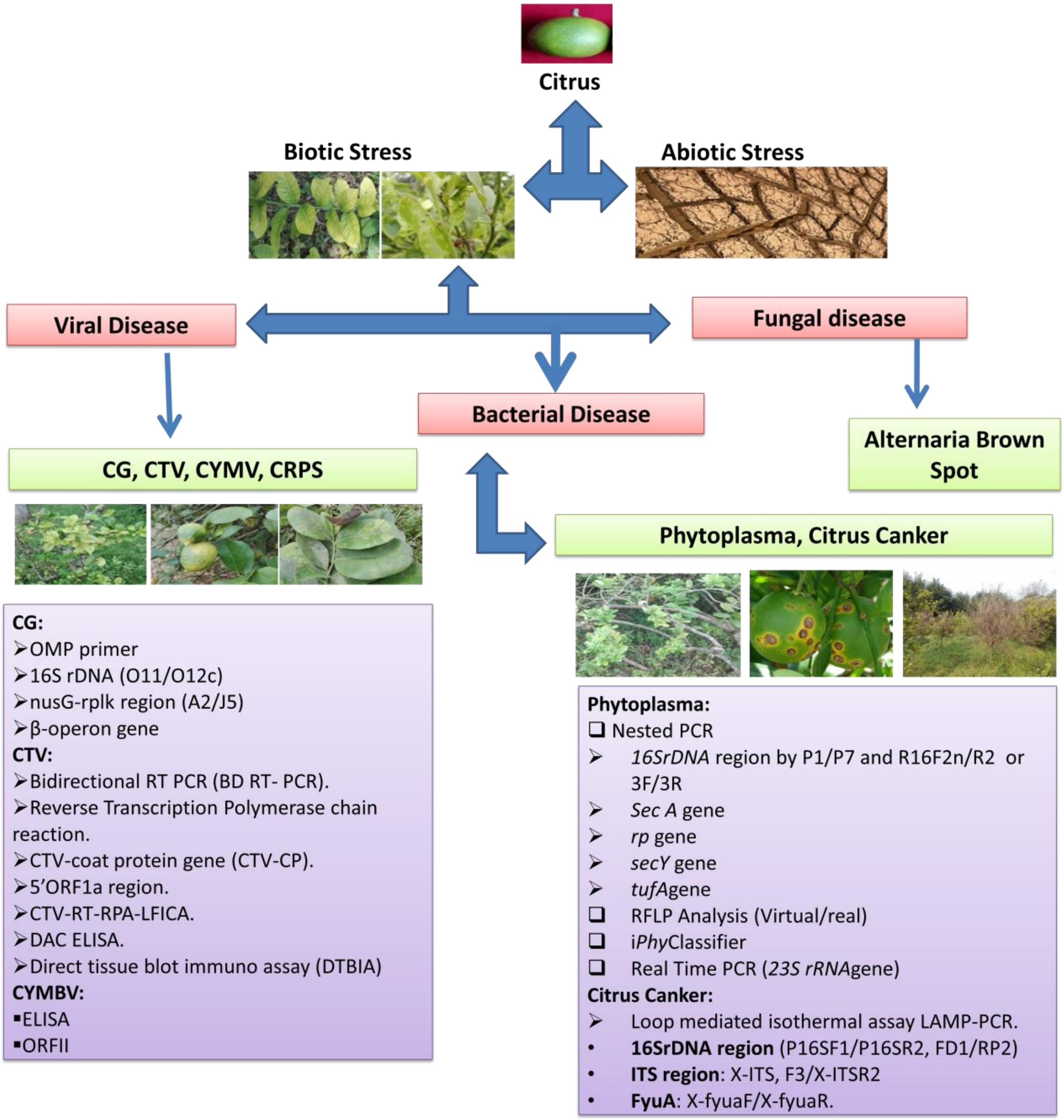


Figure 2: Use of modern biotechnological techniques for identification and characterization of different diseases affecting Citrus cultivation

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 tones [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.

As of now, infection of 16SrI, 16SrIV, 16SrXXII-A '*Ca*. P. noviguineease' phytoplasma strains have been reported in banana globally [35]; [36]; [37]; [38]. However, 16SrI phytoplasma group has been identified to be associated with stunting, yellowing of the leaves and dark green streaks on mid rib symptoms on banana from Uttar Pradesh, India [39]. Whereas, mixed infection of BBTV and unknown phytoplasma causing leaf chlorosis and bunchy top appearance were reported from the state of Assam in India [40]. In a recent study, reference [41] has reported the association of '*Ca*. P. asteris' phytoplasma group with little leaf and leaf chlorosis along with stunting symptoms by employing *16S rRNA* and *secA* gene specific primers in two commercially viable banana varieties of Tripura and in the same paper the mixed infection of BBTV, Phytoplasma and BSMYV were also reported. Reference [42] reported BBTV in seven districts of Tripura. Present investigation discloses the monophyletic nature of BBTV population in Tripura.

Reference [43] dissected the genome of a new BBTV isolate Meghalaya. Reference [44] carried out a PCR based detection of BBTV with six different primer pairs for the six components of BBTV genome. They have reported the infection of BBTV from eight districts of Assam.

A soil borne fungal disease caused by *Fusarium oxysporum* f. sp. cubense is considered as one of the most devastating diseases of banana, generally called Panama wilt or Fusarium wilt. Foc consists of 3 races, among which race 1 and race 4 are more prevalent in India.

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

Seven compatibility groups were identified from 3 pathogenic races of Grand Naine and cooperative genome analysis were performed, which implies the diverse nature of genomic constituents and effectors gene profile of Indian isolates. Race specific markers were also developed by whole genome sequence of selective Foc isolates [46]. Reference [45] reported the infection of *Foc* VCG0125 and VCG01220 race 1 by using conventional PCR method in India (Figure 3).

Reference [47] reported infection of *FocTR4* on Cavendish banana from Bihar, India on Robusta and Grand Naine, with an incidence ranging from 2 to 26.6%. Similarly, [48] also reported the infection of TR4 from Uttar Pradesh, India showing the symptoms of leaf yellowing and pseudo stem splitting.

Reference [49] screened 24 banana cultivars in the field against *Fusarium oxysporum* f.sp. *cubense* TR4 in the Northern Territory. These attempts will investigate the responses of 24 banana cultivars to the soil borne fungus. FHIA parental lines and hybrids, the Cavendish (AAA) selections GCTCV 215 and GCTCV 247 from TBRI and CJ19, an Indonesian selection showed very little to no plant death due to the disease. These indentified resistance sources could be used for developing resistant varieties against Foc.

Fusarium wilt is also an important concern in Banana cultivation in North Eastern India. In NE India *Fusarium oxysporiumF. SpCubense* 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.

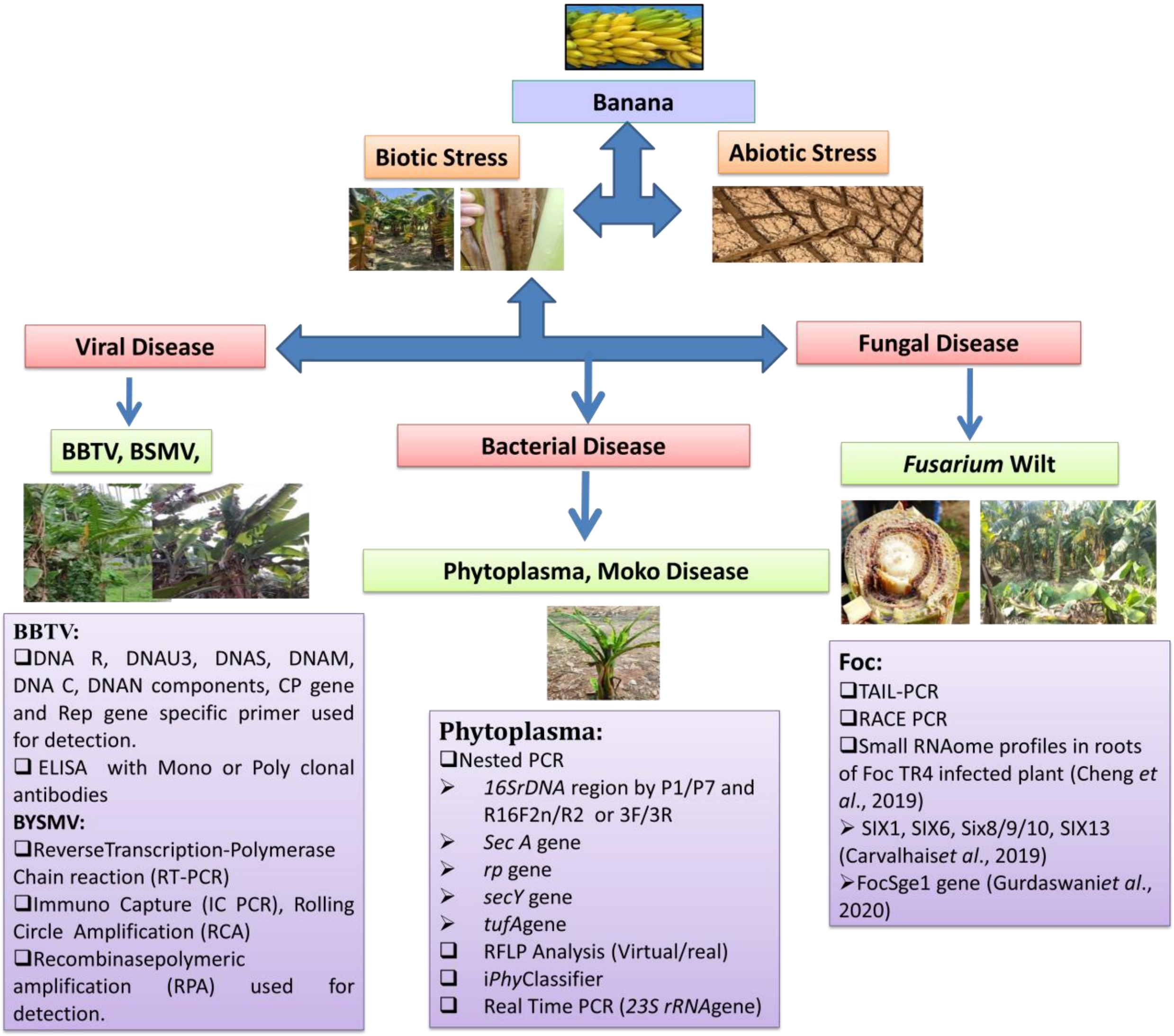
D. How modern biotechnology is used to dissect the genomic information of Banana.

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 8pprox.); approximately 554 mb in *M.acuminta*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. FocTR4 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, mentioning it 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.



BBTV:
☐DNA R, DNAU3, DNAS, DNAM, DNA C, DNAN components, CP gene and Rep gene specific primer used for detection.
☐ ELISA with Mono or Poly clonal antibodies
BYSMV:
☐ReverseTranscription-Polymerase Chain reaction (RT-PCR)
☐Immuno Capture (IC PCR), Rolling Circle Amplification (RCA)
☐Recombinasepolymeric amplification (RPA) used for detection.

Phytoplasma:
☐Nested PCR
➢ *16SrDNA* region by P1/P7 and R16F2n/R2 or 3F/3R
➢ *Sec A* gene
➢ *rp* gene
➢ *secY* gene
➢ *tufA*gene
☐ RFLP Analysis (Virtual/real)
☐ *iPhy*Classifier
☐ Real Time PCR (*23S rRNA*gene)

Foc:
☐TAIL-PCR
☐RACE PCR
☐Small RNAome profiles in roots of Foc TR4 infected plant (Cheng *et al*., 2019)
➢ SIX1, SIX6, Six8/9/10, SIX13 (Carvalhais*et al*., 2019)
➢FocSge1 gene (Gurdaswani*et al*., 2020)

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. Riceawidely cultivated, staple food crop for NE people

B. Overview of Rice cultivation in North Eastern India

The North Eastern region of India consisting of eight states (Assam, Meghalaya, Manipur, Mizoram, Nagaland, Arunachal Pradesh, Sikkim and Tripura), is a hot spot for agro-biodiversity and is reported to be having 9650 cultivars of rice [54]. Rice is the principal staple food crop of this 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 cultivated area for North Eastern India to the National total cultivated area is just about 2.27%. NE India possesses a diverse array of locally adapted non-basmati aromatic germplasms which have the potential to serve as valuable resources in breeding for combatting biotic stresses [57]. Reference [58] identified genetic diversity among 65 germplasms from NE Region.

Although, a highly diverse population of rice germplasm is prevalent across North East India, on the other side, a number of biotic stress factors are causing severe concern for cultivation of rice over there. The unique ecosystem of this region makes for a conducive environment for proliferation of several fungal, bacterial and viral diseases. Among the numerous diseases recorded in the region, rice blast (*Magnaportheoryzae*), sheath blight (*Rhizoctonia solani*) and bacterial leaf blight (*Xanthomonas oryzae*pv. 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 disease 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 different blast resistance genes 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

Nine rice germplasms were identified to be resistant against leaf and neck blast by field screening and artificial inoculation. Virulence spectrum of rice blast pathogen isolated from the NE region implies that races are highly diverse and distinct in comparison to other rice growing areas of India. A unique rice variety namely Akhanaphou originating in Manipur is highly resistant to leaf and neck blast. Reference [64] identified two novel QTLs, which give high levels of resistance against blast disease. By observing the virulence spectrum of rice blast pathogen, marker assisted back cross breeding approach has been utilized for stacking of broad-spectrum blast resistance genes into susceptible cultivars from the resistant one.

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 *Megnapothaoryze* 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.

F. 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 in grain 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.

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

Rhizoctonia solani, a necrotrophic species complex, based on anastomosis grouping, 13 groups of whose were identified infecting different hosts. The group AG1-IA of *Rhizoctonia solani* causes sheath blight in rice [68]. In molecular breeding utilization of mapping population for QTL identification to address the host pathogen interaction effect and biochemical changes, is very essential for crop improvement against disease[69]. Through molecular breeding a major QTL was identified to be located in chromosome 9 [67].Several QTLs were reported to be associated with SB disease in all the 12 chromosomes of rice [70]. A total of 33 QTLs were identified over 12 chromosomes associated with Sheath Blight disease of Rice [72]. Working mechanism of those QTLs are not clear. Among those 33 QTLs, 3 QTLs namely qSBR11-1, qSBR11-2 and qSBR7-1 were mapped in moderately resistant rice variety Tetep and validated. QTLs were found to be associated with Sheath Blight across the locations and years [73] and by observing PV values group, qSBR11-1 has been reported as a major QTL against Sheath Blight disease after which, candidate gene identification and validation were proceeded for. Many QTLs were found to be sharing the common locus in the rice genome, and those QTLs are stable.

H. 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 up to 90%. Lack of resistance source is the main constraint in Sheath Blight resistant variety development. Very few rice varieties were found to be 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.

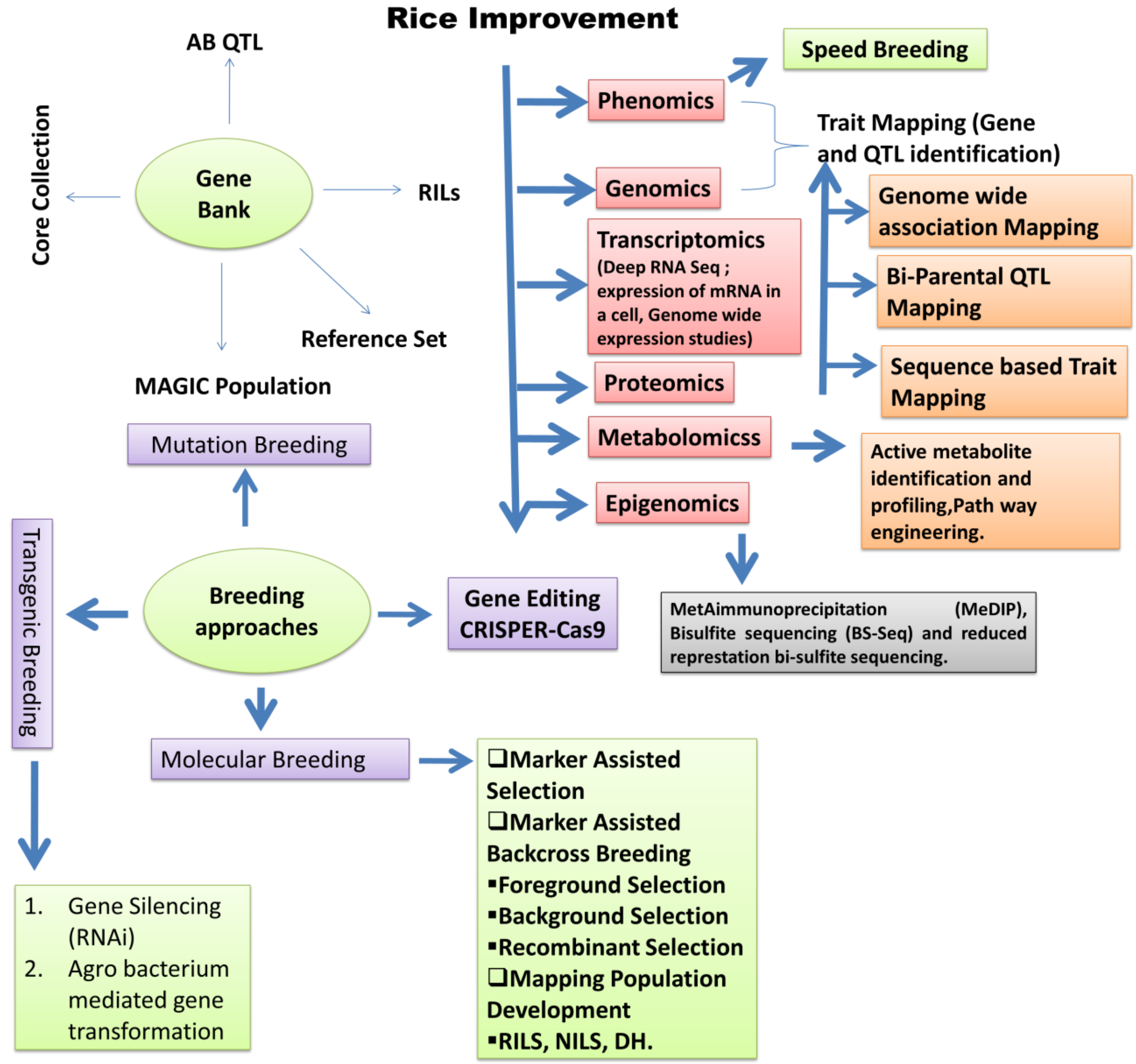


Figure 4: Different steps and strategies involved in producing rice varieties against different biotic stresses with the help of modern Biotechnology.

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.

References:

1. S. P. Ghosh, “Citrus industry of north east India”. PunjabHorticultural Journal. Vol. 17, pp. 13–21.
2. S. Govind, and D. S. Yadav, “Genetic resources of Citrusin northeastern hill region of India” pp. 38–46,1999.
3. S. Singh, “Citrus Industry of India”, In: Singh, S. and Naqvi, S.A.M.H., Eds., Citrus, International Book Distributing Company, Lucknow, India, pp. 1-44, 2001.
4. A. B. Das, J. Mondal, J. Sarkar and S. Chaudhuri, “Genetic resource survey of mandarin orange (Citrus reticulata Blanco) in the northeastern Himalayan region of India”*PGR Newsletters*, vol. 143, pp. 35–39, 2005.
5. P. *Maitry and P. D. Nath, ”Molecular detection of citrus greening disease of north eastern region of India”. Journal of Mycology and Plant Pathology*, vol. 43 (1), pp. 63-66, 2013.
6. D. Borah,P.Kafley,S. Tangjang and A. P. Das, “Population structure and conservation of endangered Citrus indica Yu.Tanaka (Rutaceae) in Behali Reserve Forest of Assam, India”,*Pleione*, vol.12(2), pp. 181 – 186, 2018.
7. W. Li, J. S. Hartung and L. Levy, “Quantitative real-time PCR for detection and identification of CandidatusLiberibacter species associated with citrus huanglongbing”, *Journal of Microbiological Methods*, vol. 66, pp. 104–115, 2006.
8. D. C. Teixeira, N. A. Wulff, E. C. Martins,E. W. Kitajima, R. Bassanezi, A. J. Ayres, S. Eveillard, C. Saillard andJ. M. Bové, “A phytoplasma closely related to the pigeon pea witches’-broom phytoplasma (16Sr IX) is associated with citrus huanglongbing symptoms in the state of São Paulo, Brazil”,*Phytopathology*, vol. 98, pp. 977-984, 2008.
9. A. K. Das, S. Nerkar, N. Thakre and A. Kumar, “ First report of ‘Candidatus Phytoplasma trifoli’ (16SrVI group) in Nagpur mandarin (Citrus reticulata) showing huanglongbinng symptoms in central India”, New Dis. Rep., vol. 34, pp. 15, 2016
10. D.K. Ghosh *et al.*“Occurrence of witches' broom, a new phytoplasma disease of acid lime (*Citrus aurantifolia*) in India”, *Plant Diseases.*, 1999.
11. S. Bora, S. Gogoi, H. Phookan., R.K. Kakoti and P.D. Nath, “Phloem limited bacteria: a threat to Khasi mandarin cultivation in Assam, India” , *Phytopathogenic Mollicutes* Vol. 9 (1), pp. 145-146, 2019
12. M. Bar-Joseph and W.O Dawson, “Citrus tristeza Virus.” In: Mahy, B.W.J. and Van Regenmortel, M.H.V., Eds., Encyclopedia of Virology, Elsevier Ltd., Amsterdam, 520-525., 2008. https://doi.org/10.1016/B978-012374410-4.00639-7
13. S.K. Sharma, A. Tarafdar, D. Khatun, S. Kumari, and K.K Biswas, “Intra-farm diversity and evidence of genetic recombination of Citrus tristeza virus isolates in Delhi region of India”. JPBB. Vol. 21(1), pp. 38–43,2018.
14. R.A.Thakre., S.J. Gahukar, A.A. Akhare, S.S. Mane, D.K. Ghosh and S.W. Hajare, “Molecular Techniques for Detection of Citrus tristeza Virus Affecting Citrus species of Vidarbha Region in Maharashtra, India”, International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7692 vol. 6, pp. 2770-2776, 2018.

15. A. Warghane, A. Kokane, S. Kokane, M. Motghare, Surwase, C.P. Chodhury, K.K. Biswas, D.K. Ghosh, " Molecular detection and coat protein gene based characterization of *Citrus tristeza* virus prevalent in Sikkim state of India", Indian Phytopathology, Vol. 73 (1), pp. 135 – 143, 2019.

16. G. Singh., S.P. Aulakh and H.S. Rattanpal, "Determination of maturity and genetic diversity in Mandarin (Citrus reticulate Blanco) genotypes based on citrus colour index", Int. J. Curr. Microbiol. App. Sci, vol. 6 (7), pp. 577-586, 2017.

17. K.R. Chung, & R.H. Briansky, "Citrus diseases exotic to Florida: Citrus Yellow Mosaic" EDIS, pp. 293, 2012.

18. Y.S. Hlawat, R.P. Pant, B.E. Lockhart, M. Srivastava, N.K. Chakraborty & A. Varma, "Association of badnavirus with citrus mosaic disease in India," Plant Disease, vol. 80, pp. 590–592, 1996.

19. Y.S. Ahlawat, R.P. Pant, B.E. Lockhart, M. Srivastava, N.K. Chakraborty and A. Varma, "Association of a badnavirus with citrus mosaic disease in India," *Plant Dis.* Vol. 80, pp. 590–592, 1996.

20. V. Dakshinamurti & G.S Reddy, "Mosaic - a transmissible disorder of sweet oranges," Indian Phytopathology, vol. 28, pp.398-399,1975.

21. V.K. Baranwal, J. Singh, Y.S. Ahlawat, K. Gopal, & M. U. Charaya, "Citrus yellow mosaic virus is associated with mosaic disease in Rangpur lime rootstock of citrus" Current Science, Vol. 89, pp. 1596–1599, 2005.

22. Anonymous, Is Tripura's 'JampuiKamola' a thing of the past? The Indian Express, 2018.

23. Q. Xu, L-L. Chen, X. Ruan, D. Chen, A. Zhu, Chunli, Chen., "The draft genome of sweet orange (Citrus sinensis)," *Net Genet*, vol. 45(1), pp. 59-66, 2013.

24. M.D. Guardo, M. Moretto, M. Moser, C. Catalano, M. Troggio, Z. Deng, A. Cestaro, "The haplotype-resolved reference genome of lemon (Citrus limon L. Burm f.)," Tree Genetics *&Genomes*, pp.17: 46. 2021.

25. J-H, Su, S. Hogenhout, A. M. Al-Sadi, C-H Kuo, "Complete Chloroplast Genome Sequence of Omani Lime (*Citrus aurantiifolia*) and Comparative Analysis within the Rosids", *Plos one*, Vol. 9 (11): e113049, 2014.

26. X. Wang, Y. Xu, S. Zhang, Li. Cao, Y. Huang, "Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction," Net Genet., vol.49(5), pp. 765-772, 2017

27. T. Shimizu, Y. Tanizawa, T. Mochizuki, H. Nagasaki, T. Yoshioka, et al., "Draft Sequencing of the Heterozygous Diploid Genome of Satsuma (*Citrus unshiu* Marc.) Using a Hybrid Assembly Approach," *Front Genet*, Vol.8: 180, 2017.

28. H. Jia, V. Orbovic, and N. Wang, "CRISPER-LbCas12a-mediated modification of citrus", Biotechnology Journal, Vol.17 (10) pp.1928-1937, 2019.

29. Food and Agriculture Organization of the United Nations. FAOSTAT Statistical Database. Rome: FAO, 2018.

30. G. Blomme, M. Dita, K.S. Jacobsen, L. Pérez Vicente, A. Molina, W. Ocimati, & P. Prior, "Bacterial diseases of bananas and enset: current state of knowledge and integrated approaches toward sustainable management," *Frontiers in Plant Science*, Vol. 8(1290), 2017.

31. N. Srivastava, R. Kapoor, R. Kumar, S. Kumar, R. K. Saritha, S. Kumar, & V.K Baranwal, " Rapid diagnosis of cucumber mosaic virus in banana plants using a fluorescence based real-time isothermal reverse transcription-recombinase polymerase amplification assay". *Journal of Virological Methods*, Vol. 270, pp. 52-58, 2019.

32. S.K. Sharma, V.P Kumar, & V.K Baranwal, "Immunodiagnosis of episomal Banana streak MY virus using polyclonal antibodies to an expressed putative coat protein". *Journal of Virological Methods,* vol. 207, pp. 86–94, 2014.

33. R. Selvarajan, M. Sheeba, M. Balasubramanian, R. Rajmohan, & N.L Dhevi, "Molecular characterization of geographically different *banana bunchy top virus* Isolates in India". *Indian Journal of Virology,* vol. 21, pp.110–116. DOI: 10.1007/s13337-010-0020-1, 2010.

34. T. Das & A. Banerjee, "Distribution, molecular characterization and diversity of banana bunchy top virus in Tripura, India" *Virus disease*, vol. 29, pp.157-166. DOI: 10.1007/s13337-018-0451-7, 2018.

35. H. Li, B. Qiu, L. Li, & C. Shi, "Amplification of phytoplasma 16S rDNA from banana bunchy top disease, RFLP and sequence analysis of 16S rDNA fragment." *Acta Microbiologica Sinica*, vol. 39, pp. 315–20, 1999.

36. F. Aliaga, E. Hopp, E. Alvarez, & B.L.A. Lopez-Lavalle, "First report of a '*Candidatus* Phytoplasma asteris'isolate associated with banana elephantiasis disease in Colombia." *New Disease reports*, 37, 12. DOI:10.5197/j.2044-0588.2018.037.012, 2018.

37. R. I. Davis, P. Kokoa, L. M. Jones, J. Mackie, F. E. Constable, B.C. Rodoni, T.G. Gunua, & J.B. Rossel, "A new banana wilt disease associated with phytoplasmas in Papua New Guinea," *Australasian Plant Disease Notes,* vol.7, pp. 91–97. DOI: 10.1007/s13314-012-0056-8, 2012.

38. A. Miyazaki, T. Shigaki, H. Koinuma, N. Iwabuchi, G.B. Rauka, A. Kembu, J. Saul, K. Watanabe, T. Nijo, Y. Maejima, & S. Namba, "'*Candidatus* Phytoplasma noviguineense', a novel taxon associated with Bogia coconut syndrome and banana wilt disease on the island of New Guinea,"*International Journal Systemic Evolutionary Microbiology*, vol. 68, pp.170-175, 2018.

39. R.B. Singh, Al-Khedhairy, A. A Aminuddin, F. Al-Qurainy, & J. Musarrat, "Molecular diagnostics and phylogenetic analysis of '*Ca.* Phytoplasma asteris' (16Sr -Aster yellow group) infecting banana (*Musa* spp.)." *African Journal of Biotech*nology, vol. 8, pp.5819-5824, 2009.

40. S. H. Gogoi, R. Mishra, & P. D Nath, "Association of phytoplasmas with banana bunchy top viral disease in banana plants in Assam, India". *Phytopathogenic Mollicutes, Vol.* 9, pp. 147–148, 2019.

41. S. Mitra, P. Debnath, R. Rai, N. Srivastava, G.P. Rao, V.K. Baranwal, "Identification of 'Ca. Phytoplasma asteris', banana bunchy top virus and banana streak MY virus associated with Champa and Sabri banana varieties in Tripura, a North Eastern state of India," Eur. J. Plant pathol, vol. 163, pp.907-922, 2022.

42. T. Das, & A. Banerjee, "Distribution, molecular characterization and diversity of banana bunchy top virus in Tripura, India." *Virus disease*, vol. 29, pp.157-166, DOI: 10.1007/s13337-018-0451-7, 2018.

43. A. Banerjee, S. Roy, G.T. Behere, S.S Roy, S. K. Dutta, S.V. Ngachan, "Identification and characterization of a distinct banana bunchy top virus isolate of Pacific-Indian Oceans group from North-East India" Virus Res. Vol. 183,pp. 41–49, 2014.

44. Kakati et al., 2018

45. R. Thangavelu, M. Gopi, P. Pushpakanth, M. Loganathan, "First report of *Fusarium Oxysporium* f. sp. cubense VCG0125 and VCG01220 of Race1 infecting Cavendish Bananas (Musa sp. AAA) in India," Vol. 105 (4), pp,1215, 2021.

46. R. Thangavelu, E. Edwinraj, M. Gopi, P. Pushpakanth, K. Sharmilla, " Development of PCR-Based Race-Specific markers for differentiation of Indian Fusarium Oxysporium f. sp. cubense, the causal agent of Fusarium wilt in Banana," J. Fungi, Vol. 8 (1): 53, 2022.

47. Thangavelu 2019

48. T. Damodaran, S. Rajan, V. K. Mishra, S. K. Jha, et al., "First report of Fusarium wilt in banana caused by Fusarium Oxysporium f. sp. cubense tropical race 4 in India" *Plant dis*. Vol. 103, pp.1022, 2018.

49. S. J. L. Mintoff, T.V. Nguyen, C. Kelly, S. Cullen, et al., "Banana cultivar field screening for resistance to *Fusarium oxysporium f. sp.*," 2021.

50. W. Wu, Y-L Yang, W-M, He, M. Rouard, W-M Li, et al., "Whole genome sequencing of a banana wild relative Musa itinerans provides insights in to lineage-specific diversification of the Musa genus" *Scientific reports*, 6: 31586.

51. A. D'Hont, F. Denoeud, J-M Aury, F-C. Baurens, F. Carreel, O. Garsmeur, et al, "The Banana (Musa acuminata) genome and the evolution of monocotyledonous plants", *Nature*, vol. 488, pp. 213-217, 2012.

52. J. N. Tripathi, V. O. Ntui, M. Ron, S. K. Muiruri, A. Britt, & L. Tripathi, "CRISPR/Cas9 editing of endogenous *banana streak virus* in the B genome of *Musa* spp. overcomes a major challenge in banana breeding", *Communications Biology*, **2**, 46. https://doi.org/10.1038/s42003-019-0288-7, 2019.

53. R.C Ploetz, G.H.J Kema, and L-J Ma, "Impact of diseases on export and small hoder production of Banana" Annu Rev. Phytopathol, Vol. 53, pp.269-288, 2015.

54. Sathapathy and B. K. Sarma, "Land degradation and conservation of biodiversity with special reference to north India." Indian J. Hill Farmg. Vol.14, pp.7-18, 2001.

55. R. Kuotsuo, D. Chatterjee, B.C Deka, R, AO, Kumar and K. Vikramjeet, "Shifting cultivation:An 'Organic Like' Farming in Nagaland" Indian Journal of Hill Farming. Vol. 27 (2), pp. 23-28, 2014.

56. Annon

57. D. K. Ray, J. S. Gerber, G. K MacDonald and P.C. West, "Climate variation explains a third of global crop yield variability." Nat Commun.vol. 6: 5989, 2015.

58. Vanlalsanga, S.P. Singh, and Y.T. Singh, "Rice of Northeast India harbor rich genetic diversity as measured by SSR markers and Zn/Fe content," BMC Genetics, 20(79), 2019.

59. Directorate of Rice Development. Statistical Hand-Book. 2016. Available from: http://www.drdpat.bih.nic.in/Downloads/Statewise-APY-of-Rice-2014-15-to-2018-19.pdf

60. S.V. Ngachan, A. K. Mohanty, A. Pattanayak, "**Status Paper on Rice in North East India: Rice in North East India,"** Rice Knowledge Management Portal, pp. 82, 2011

61. Ngachan SV, MohantyAK and APattanayakA2014. Status Paper on Rice in North East India. (http:// www.rkmp.co.in/sites/ default/files/ris/rice-statewise/Status%20Paper%20on%20Rice%20in%20North%20East%20India.pdf).

62. Anupan et al.,

63. Varier et al., 2009

64. S.B. Aglawe, U. Bangale, S. J. S. Ramadevi, V. Balija, B. V., Pal, S.S. Kumar, et al, " Identification of novel QTLs conferring field resistance for rice leaf and neck blast from a unique landrace of India." *Gene Rep.* Vol. 7, pp. 35–42. doi: 10.1016/j.genrep.2017.01.007, 2017.

65. D.S. Marshall, and M.C Rush, "Infection cushion formation on rice sheaths by *Rhizoctonia solani,*" Phytopathology, Vol. 70: pp. 947-950, 1980.

66. K. S. Chahal, S. S. Sokhi, and G. S. Rattan, "Investigations on sheath blight of rice in Punjab" Indian Phytopath., vol. 56, pp. 22-26, 2003.

67. G. Liu, Y. Jia, F. J. Correa-Victoria, G. A. Prado, K. M. Yeater, A. McClung, and J. C Correll, "Mapping quantitative trait loci responsible for resistance to sheath blight in rice". Phytopathology, vol. 99, pp. 1078-1084, 2009.

68. Y. A. Wamishe, Y. Jia, P. Singh, and R. D. Cartwright, "Identification of field isolates of Rhizoctonia solani to detect quantitative resistance in rice under greenhouse conditions," Front. Agric. China, vol. 1: pp.361-367, 2007.

69. V. Channamallikarjuna, H. Sonah, M. Prasad, G.J.N Rao, S. Chand, H.C Upreti, N.K. Singh, and T.R. Sharma, "Identification of Major quantitative trait loci qsbr11-1 for sheath blight resistance in rice," Mol. Breed., Vol. 25: pp. 155-166, 2010.

70. F.T. Zhang, and J. K Xie, "Genes and QTLs resistant to biotic and abiotic stresses from wild rice and their applications in cultivar improvements," In: Rice: Germplasm, Genetics and Improvement. Croatia, European Union Q, 2014

71. H. Sabouri, A. Sabouri, M.R. Jafarzadeh, and M. Mollashahi, "Detection of QTLs controlling field blast resistance in rice (Oryza sative L.). Plant Omics, vol. 4, pp. 1-5, 2011.

72. L. Srinivasachary, L. Willocquet, and S. Savary, "Resistance to rice sheath blight (Rhizoctonia solani Kuhn) [teleomorph: Thanatephoruscucumeris (A. B. Frank) Donk.] disease: current status and perspectives". Euphytica, vol. 178, pp. 1-22, 2011.

73. V. Channamallikarjuna, H. Sonah, M. Prasad, G. J. N Rao, S. Chand, H.C. Upreti, N. K Singh, and T. R. Sharma, "Identification of Major quantitative trait loci qsbr11-1 for sheath blight resistance in rice." Mol. Breed., 25: 155-166, 2010.

74. S.A Lavale, S.K. Prasanthi, K. Fathy, 2018, Mapping association of molecular marker and sheath blight (*Rhizoctonia solani*) disease resistance and identification of novel resistance sources and loci in rice, Euphytica, Vol. 214 (4), pp. 78, 2018.

75. L. Jia, W. Yan , C. Zhu, H. Agrama, A. Jackson, K. Yeater, X. Li, B. Huang, et al., "Allelic Analysis of Sheath Blight Resistance with Association Mapping in Rice,". Plos One, 2012.

76. P. Samal, K. Molla, A, Bal, S. Ray, H, Swain, A. Khandual, et al., "Comparative transcriptome profiling reveals the basis of differential sheath blight disease response in tolerant and susceptible rice genotypes", *Plant Sci*, vol. 160 (3), pp. 405-414. doi: 10.1016/s0168-9452(00)00413-1.

77. K. Datta, J. Tu,N. Oliva, J. Ona, R. Velazhahan, T. W. Mew, S. Muthukrishnan and S. K. Datta, "Enhanced resistance to sheath blight by constitutive expression of infection-related rice chitinase in transgenic elite indica rice cultivars", 2017.

78. R. Sripriya, C. Parameswari, K. and Veluthambi, K., "Enhancement of sheath blight tolerance in transgenic rice by combined expression of tobacco osmotin (ap24) and rice chinitinase (chi11) genes", In Vitro Cellular & Developmental Biology - Plant, vol. 53, pp.12–21, 2017.