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# Present Status of Research on Resistance-Development for Major Fungal Diseases of Edible Banana

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## ABSTRACT

Banana is one of the important widely consumed food crops in the world due to its high nutritional value. Presently the economy of the banana production is majorly diminished by fungal disease viz Fusarium wilt and black sigatoka. Development of resistant variety using conventional breeding is impeded due to the long generation time, sterility and narrow genetic base. Use of alternative approaches would provide crop protection for the disease management. So far, considerable progress has been achieved in understanding the host pathogen interaction, identification of the pathogenesis related molecules and resistance genes and their utilization in transgenic development. In this review, information on the two major diseases of banana *Fusarium oxysporum* f.sp Cubense and *Mycosphaerella fijiensis*, their infection process and the approaches adopted to manage them using genetic engineering with pathogenesis related proteins, resistant genes, RNAi technology and role of molecular markers in resistant variety development are discussed.

**Keywords;** Fusarium oxysporum, Mycosphaerella fijiensis, Transgenics, Resistance gene, molecular markers, RNAi technology.

## INTRODUCTION

Banana and plantains are perennial crop belongs to the Musaceae family of the order Zingibarales. It is subdivided into 4 different sections or sub generic group based on taxonomy by [1] viz Eumusa, Rhodochlamys, Australimusa and Callimusa. The majority of the cultivated banana belongs to the section Eumusa and it is geographically the most wide spread group than other groups. The edible banana is derived from inter and intra specific hybridization of diploid species *Musa accuminata* (AA) and *Musa balbisiana* (BB) [2]. Most of them are triploid (AAA, AAB, BBA), sterile in nature, produce fruit by parthenocarpic and propagate vegetatively [3]. Banana is the fourth most important food crop in the world in terms of grass value production and it is rich in good source of carbohydrates, fibers, mineral element and vitamins. Presently banana is grown in approximately 150

countries across the world on an area of 10 million ha with a total output of 100 million tons. Among them India is the largest producer of banana followed by China and Philippines [4]. Approximately 80% of total banana produced goes to the domestic market and only 15 % enter into export market in the world. The production of banana and plantains are hampered by pests and diseases caused by bacteria, fungi, viruses, nematodes and weevils. Fungal diseases panama wilt and black leaf streak are the major ones causing severe economic loss [5].

The panama wilt is ranked one of the top 6 important plant diseases in the world [6]. It is a major constraint and a serious threat to banana. It is caused by a soil born saprophytic fungal pathogen *Fusarium oxysporum* f, sp cubense (Foc) Snyder and Hansen. The Foc infects the roots system and then colonizes vascular region in the pseudostem. It is responsible for the characteristic wilt symptoms in the plants. Generally, infected plants produce no bunches and if produced, the fruits are very small and only a few fingers develop. Fruits ripen irregularly and the flesh is pithy and acidic. Depending on their host specificity Foc is distinguished into 4 races. Among them Race 4 is a highly virulent in nature.

Another major fungal disease that is a threat to banana production is black sigatoka commonly known as black leaf streak disease (BLSD) caused by hemi-biotrophic fungal pathogen *Mycosphaerella fijiensis* Morelet. It invades foliar system, reduces the photosynthetic area of the leaf, and ultimately causes the death of the leaves leading to premature ripening of the fruit and reduced bunch weight [7][8]. Yield losses by BSLD gradually grow to more than 50% [9] [10]. The control of *Fusarium* wilt disease by soil fumigation, cultural methods and fungicide treatment has not been successful so far. However, using chemical control methods BSLD has been successfully controlled, but the total production cost has increased by 25 to 30% [11] and also poses environmental risk. The only method to control the disease is by the use of resistant cultivars which are developed by conventional breeding. Although, the source of resistance to Foc has been identified in wild banana, exploitation of these 'resistance genes' by conventional breeding has been hampered by the extreme low female fertility or male sterility, long generation and polyploid nature of the plant [12]. Meanwhile, application of biotechnological methods such as marker assisted selection of resistant varieties, identification of DNA markers linked to the traits of interest and genetic manipulation with resistant genes are the desirable option for obtaining resistance in susceptible plants.

***Fusarium oxysporum* f.sp cubense**

*Fusarium oxysporum* is a soil born necrotrophic fungal pathogen. It is a systemic fungus containing more than 120 frame specials, which causes vascular wilt disease in more than 100 plants[13]. *Fusarium* wilt of banana is caused by Foc. The Foc was distinguished into four races depending on their host specificity (referred to as 1 to 4). Among these, race 1 mainly epidemic to Gros Michel, lady finger (AAB) and silk (AAB) subgroups. Race 2 affects cooking varieties Bluggoe (ABB), race 3 affects *Heliconia* spp., a close relative of banana, and is not considered to be a banana pathogen and race 4 infects Cavendish group and all the banana plants which are susceptible to race 1, 2. Race 4 is considered as a major concern among the other races and it is further divided into 'sub-tropical' and 'tropical' strains. Presently, *Fusarium* wilt has been reported in all banana growing areas of the globe (Asia, Africa, Australia and the tropical Americas) except some islands in the South Pacific, the Mediterranean, Melanesia, and Somalia [14][5]. The fungus survives in soil for up to 30 years as chlamydospores in infested plant material or in the roots of alternative hosts [15].

The root tips and lateral roots are the natural and the initial sites of infection, but the fungus can also penetrate root hairs or epidermal cells near the root cap, behind the root tip or within the zone of elongation[16][17]. Penetration of the host plant by *F. oxysporum* involves germination of spores and adhesion of the pathogen to the host surface. The pathogens then move inter- and intracellularly through the root parenchyma tissue until they reach

the protoxylem vessels [18] from where they invade the large reticulate vessels and spread from vessel to vessel through the pits on the vessel wall and block the xylem vessel ultimately leads to the death of the plant. External symptoms include vein clearing, leaf epinasty, wilting, chlorosis, necrosis and abscission, and internal symptoms involve vascular browning [17]. As long as the host plant is alive, Foc will remain in the xylem tissue. Severely infected plants eventually wilt and die, and the chlamydospores are released back to the soil from the infected and decaying host tissue [19] where they remain viable for several years [5] [20]. The disease cycle is repeated when the chlamydospores germinate and invade a new host plant [14].

***Mycosphaerella fijiensis***

*Mycosphaerella fijiensis* the fungus which causes BSLD reproduces by both sexual and asexual manner by producing ascospores and conidia respectively. The life cycle starts when ascospores or conidia fall on newly opened leaf known as cigar leaf. These spores germinate within 2 to 3 hrs. It produces the germ tube which enters into the leaf via stomata by forming appressoria through which hyphae will be produced. The hyphae enter into the palisade tissue of leaf through mesophyll cells, air chambers and proliferate profoundly. From here the hyphae grow out into the other air chamber eventually emerge through stomata [21]. Epiphytic growth phase occurs again and eventually produce in a black lesion across the entire leaf tissue area resulting in death of leaf tissue. During the life cycle of *M. fijiensis,* conidia are produced at an early stage and ascospores are produced in the later stage by perithecia bursting.

In the early stage of infection reddish brown spots are observed on the abaxial surface of the leaf. The spots became more prominent and widen to form an elliptical shape, which changes to reddish brown to brown on abaxial surface and black on adaxial surface. Later these spots become slightly concave surrounded by a yellow halo. In later stages these spots turn to gray and dry and enclosed by a black border. Finally the spots mere and kill the leaf.

**Resistance gene**

Resistance gene in plants plays an essential role for disease resistance. These genes require specific dominant avirulence gene (avr) in the pathogen for their function. The protein product of the R genes may act as a receptor that specially interacts with a pathogen derived protein. Upon recognition of pathogen derived protein, R gene product activates plant defense mechanisms. These defense include rapid production of oxidative burst, resulting in cell wall cross linking, localized cell death (HR) and induction of gene characteristic of systemic acquired resistance [22]. Resistance gene is part of resistance gene analogue (RGAs) or psuedogene, which are good candidates for functional resistance gene. Till now, a number of resistance gene analogues have been identified in horticultural crops for different diseases [23] [24]). RGA for Fusarium wilt disease have been identified in other crops like tomato [25] [26][27] and melon [28] by using positional cloning map strategies. But R genes race specificity imposes a serious limitation for its use in other crops. The majority of known plant resistance genes contain NBS and LLR domains. The conserved nature of motifs within these domains has been exploited to search for new resistance gene analogue in other plant system. In case of banana, the introgression of this resistance gene into edible banana has been hampered due to their sterile nature and long generation time [29]. The development of an effective genetic transformation system provides an alternative method for generation of disease resistant plant using natural disease resistance gene pools which are already existing in the resistance germplasm. To date, number of R genes isolated from different germplam of banana cultivars are known. Chen et al, [30] isolated 6 RCGs from wild cultivar Musa accuminata (AA) which showed resistance to *Fusarium* wilt caused by Foc race 4. The deduced amino acid sequence of six RGAs showed that 2 genes viz WNB1 and WNB2 contains NR-ARC domain. Whereas WST1 to WST4 contained serine /threonine kinase domain. The transcription profiling study was carried out after Foc inoculation which indicated that

WNB1 and WST3 were mainly involved in the resistance to *Fusarium* wilt disease. In another experiment five different types NBS resistance gene analogues were isolated from *Musa accuminata* spp. *Malaccensis,* for *Fusarium* wilt resistance [31]. The structural and phylogenic analysis grouped these RGAs within non – TIR subclasses of NBS. The transcription profiling of both resistance and susceptible cultivar results indicated that genes viz RGAs 1, 3 and 5 are expressed in both susceptible and resistance cultivar. The RGAs- 4 was not expressed in any of the cultivars and RGAs 2 was expressed only in the resistant cultivar. This indicated that RGAs 2 was mainly involved in disease resistance.

Sun et al, [32] isolated 20 different types of RGAs from banana cv Gold finger resistant to Foc race 4. The deduced amino acid sequence analysis showed these RGAs shared homology with non TIR-NBS subclass of NBS a class of resistance gene candidates. Sequence homology results showed that these genes share an identity of 41.1% to 99.3%. Phylogeny analysis of nucleotide sequence revealed that these RGAs share a homology of 28 to 54 % with those RGAs which are already known for Fusarium wilt resistance such as Fom2, 12c-1, 12c and 12 in other crops.

**Genetic manipulation**

Recombinant DNA technology provides a potential tool for the development of disease resistant/ tolerant banana plants. It provides environmentally safe and sustainable approach.

Genetic transformation is the process where desired genes derived from different sources are inserted into the genome of the living cells. The successful generation of transgenic plants requires the use of an efficient transformation protocol and regeneration system [33]. Several transformation strategies have been developed for efficient transformation over the years for the genetic manipulation of the banana genome, which includes, direct gene transfer and *Agrobacterium* mediated gene transformation (Table 1). Despite varied independent nature of direct gene transformation, *Agrobacterium* – mediated transformation offers several advantages over direct gene transfer method, such as the possibility of integration of one or few copies of T-DNA cassettes, lower cost with higher efficiency and transfer of large T-DNA fragments with minimal rearrangement [34][35].

Most of the biotechnologist use the cell suspension for the development of banana transgenic plants [36]. It has been considered that embryogenic cells are ideal targets due to their unicellular nature which helps in the development of transgenic plants without chimeras [37][38][39]. However, establishment of cell suspension is a tedious process, requires a long time, a standardization of ECS development is necessary for each cultivar and low percentage of embryogenic callus response. Beside this, its regeneration potential decreases with long-term culture maintenance. The alternative approach is the use of apical shoot tip as explant. This technique is applicable to a wide range of banana cultivars irrespective of genotype or ploidy. This method offers several potential advantages over the use of embryonic cell suspension as it allows for rapid transformation of banana species. However, most of the time the use of apical shoot tip explant for the transformation results in the development of chimars due to the insertion of T-DNA in only one or few cells, However, the transformed cells and tissue can be enhanced by tissue culture manipulation and optimizing selection procedure.

Transformation of antimicrobial peptides or pathogenesis related protein (PR proteins) into plants offers to create plants resistant to a wide range of bacterial and fungal pathogens [40][41] and exhibit a broad range of activity against several Phyto-pathogens [42]. These antimicrobial peptides are cationic in nature which cause rapid cell damage by charge based interaction with phospholipids and arresting of nucleic acid or protein or enzyme activity [43] [44]. Because of this property antimicrobial peptide gained more importance in the development of transgenic plants. A number of antimicrobial peptides have been successfully expressed in plants and their enhanced resistance to phytopathogens in

pot culture condition has been demonstrated [45][46][47]. Gene which are used to assess the fungal disease resistance in banana was summarized below (Table 2).

Chakrabarti et al[48] cloned MSI-99 a maganin analogue a small alpa helical peptide with 23-amino-acids from the African clawed frog found to be having broad spectrum activity against both gram positive, negative bacteria, fungi and protozoa, besides having anti-tumarogenic activity. The authors utilized the potential of this peptide for imparting resistance against Foc in banana cv Rasthali. During their experimentation first they determined the lytic dose of the peptide on the fungus where at low concentration ie., at 16 ug/ml inhibited spore formation and it completely inhibited the Foc *invitro* at 128ug/ml. Then it was introduced into the banana cv Rasthali. Most of the transgenic banana plants developed were tolerant against the Foc under primary pot culture evaluation.

The plant employs a wide array of defense mechanism against plant pathogen attack. The hypersensitive response (HR) is an induced systemic acquired defense mechanism of the cell on encounter with phytopathogens infection. It contributes to the localized death of infected cells and often associates with systemic acquired resistance mechanism. A plant ferredoxin like protein (*pflp*) is a protein mainly involved in the *HR* mediated defense mechanism by producing reactive oxygen species. [49] introduced *pflp* gene into the banana cv. Pei Chiao and cv. Gros Michel to test the effect of transgenic banana for *Fusarium* wilt disease. The transgenic plants showed very less percentage of severity on expose to Foc race 4 over period of 9 weeks indicating their potentiality for resistance against Foc. This report also inferred the utilization of *pflp* gene not only for the control of bacterial diseases but also for the prevention of fungal diseases.

PR protein class PR-5 thaumatin like proteins (*tlp*), are the group of proteins, which share a homology with thaumatin [50]. These proteins are widely distributed in the plant system and induced in response to pathogen attach and led to systemic acquired resistance [51]. It exerts antifungal activity by destabilizing the lipid layer and by creating pores in fungal membranes [52] It activates other defensin pathway including phytoalexin production but the exact mechanism is unknown and has to be elucidated [53][54] cloned *tlp* gene into the banana *Musa sapientum* cv Nangka (AAB). The transgenic plant expressing *tlp* gene was shown to reduce symptoms by 3 fold compared to control plants after 30 days of inoculation with Foc race 4. Similar type of tolerance against different fungal disease has also been reported in other crops, including canola [47] rice [55].

Plant defensin, a group of small, cysteine rich protein belongs to the class PR-12 protein induced in plants against a broad range of invading plant pathogens especially fungal pathogens. The formation of these molecules considered as a component defense mechanism against several filamentous fungi [56]. These molecules mainly act on fungal cell membrane by interacting with spingolipids, one of the components of the fungal cell membrane and cause the destabilization of the membrane which intern arrests the growth of fungi. Few defensin molecules also target intracellular region, but exact mechanism not understood. Several reports have shown that these defensin molecules play an important role in the development of transgenic crops with enhanced resistance to fungal disease [57][46]. Similarly, utility of these molecules against *Fusarium* wilt disease in banana was demonstrated by [58]. The researcher group transformed 2 floral defensin *phdef1* and *phdef2* separately into banana cv Rasthali. The pot bioassay results demonstrated that transgenic plants expressing floral defensin gene showed less external and internal symptoms in contrast to control plants. Control plants succumbed to wilt disease after 6 months of post inoculation of Foc culture, whereas transgenic plants showed mild symptoms and completely recovered within 3 weeks post inoculation. Transgenic plants were phenotypically normal. These results indicated that expression of defensin gene in the host plant enhanced resistance to *Fusarium* wilt and it can be used with the other PR

gene for gene stacking in order to develop pathogen free trait.

Nonspecific lipid transfer proteins (nsLTPs) belong to PR- 14 class of proteins. These protein molecules play an important role in the transfer of phospholipids molecules *in vitro* [59]. Beside this, these molecules are also involved in the defense mechanism, reproductive, vegetative organ growth and in signaling function [60][61][62][63][64]. *Ace-AMP1* is a type of nsLTPs mainly involved in the antimicrobial activity against different phyto pathogens and had no detectable inter-membrane lipid transfer activity [60]. Due to its strong antimicrobial activity towards a number of phytopathogens *in vitro* this gene was utilized for the development of number of transgenic plants against fungal diseases and enhanced resistance obtained was demonstrated under green house condition in wheat, rice and banana crops [65][66][45].

*Chitinase* and *β-1,3-glucansae* are the hydrolytic enzymes which mainly act on fungal cell wall component chitin and β-1,3 glucan respectively. It is one of the components of innate defense mechanism and nontoxic to plants and animals. Both the genes are extensively used as a potential candidate for the development of fungal disease resistance in several crops [67][68]. Similar type of approach was also carried out in banana for fungal disease. [69] cloned gene coding for *β- 1-3- endo glucanase* and expressed the same in banana cv. Rasthali and assessed the level of tolerance against wilt caused by Foc race1. Highest level of expression of the gene and enhanced disease resistance against Foc race 1 was obtained. Same group [70] expressed both *chitinase* and *β-1,3 glucanase* gene in cv Rathali and showed tolerance against Foc. In another study [71] developed banana plant resistance to wilt disease by introducing an endochitinase gene (*chit42)* into *cv* Furenzhi. The transgenic plants showed tolerance to wilt disease, even after two months of post inoculation. They also opined that same source could be used for breeding program against banana varieties for Foc race 4.

Similarly for imparting resistance to BSLD, *rcc*2 and *rcg*3 genes belonging to the class I *chitinase* gene were over expressed in transgenic banana cv Gros Michel individually[72]. The resulted transgenic banana plants expressing rcc3 gene showed delayed disease development than control plants and their necrotic lesion was three fold lesser than control plants in *vitro* leaf bioassay. There was correlation observed between *chitinase* expression and disease tolerance in plants over expressing *rcc2* and *rcg3* which were phenotypically normal. Authors also reported that *rcg3* plants showed more tolerance than *rcc2* which might be due to the extracellular expression nature of rcc3. Yet in another study endochitinase gene was stacked along with *stilbene synthase* (StSy) and *superoxide dismutase* (Cu, Zn SOD) gene to achieve resistance against black leaf streak disease [73]. Field evaluation of these plants showed significant reduction in lesion area compared to the control plants and most of the transgenic lines produced fruit.

**Lysozyme**

Lysozyme enzymes are a group of hydrolytic enzymes widely distributed in all living organism These enzymes mainly act on N-acetly D –muramic acid and N-acetyl D – glucosamine in bacteria and also on fungal cell wall component chitin and result in cell lysis which eventually leads to the death of the cells. It acts as a defensive barrier in a host against number of pathogens. These enzymes were utilized for the development of transgenic crop against number of fungal disease (Hanke et al., 1999[74]). In addition, expression of human lysozyme in tobacco showed enhanced resistance against both bacterial and fungal pathogens (Nakajima et al., 1997[75]). Pei et al., 2005[76]) utilized this enzyme against *Fusarium* wilt disease in banana. Transgenic plants expressing human lysozyme showed enhanced resistance against Fusarium wilt disease not only in pot condition but also in containment field condition.

***Antiapoptosis related protein***

Poul et al., (2011[77]) stably transformed the apoptosis-inhibition-related genes, namely *Bcl-xL*, *Ced-9* and *Bcl-2* 3′ UTR separately into banana cv Lady finger. To investigate the effect of individual gene on disease resistance, transformed banana plants

were root challenged with Foc race 4 for a period of 12 weeks. Transgenic lines (2 × *Bcl-xL*, 3 × *Ced-9* and 2 × *Bcl-2* 3′ UTR) showed significantly less internal and external disease symptoms than the wild-type susceptible 'Lady Finger' banana plants used as control. Among them *Bcl-2* 3′ UTR expressing transgenic line was highly resistant even after continuous exposure of transgenic plants for a period of 23 weeks. Similarly, Banana cv. Sukali Ndizi the most popular dessert banana in the East African region was transformed with a modified form of anti-apoptosis gene ced9. After 13 weeks of post inoculation with Foc transgenic plants showed significantly less disease severity compared to control plants (Magambo 2012[78]).

**Molecular markers**

In case of banana selection of disease resistant somaclones require large scale field evaluations which need further confirmation through a number of selection cycles. Development of early reliable and reproducible selection strategies can speed up the selection procedure and eventual improvement of banana. Molecular marker technology has become a powerful tool in crop improvement through their use in germplasm characterization, fingerprinting, genetic analysis, linkage mapping and molecular breeding. Various studies have demonstrated that markers and their conversion into sequence characterized amplified region marker (SCAR) were beneficial for the identification of and detection of genetic loci associated with *Fusairum oxysporum* resistance. SCAR marker linked to *Fusairum oxysporum* resistance gene have been developed for cotton [79], Cabbage [80] and pea [82]. Similar type of work in banana was recently carried out by [83] who identified SCAR marker of banana associated with resistance to Foc race 4. By using the RAPD technique 24 amplified polymorphic DNA products was identified. Two of these RAPD markers converted into SCAR marker included Sca UI0001 and Scas0901 and further analyzed in resistance banana genotypes, Williams 8818 and in Gold finger. These markers are amplified only in the resistance cultivar and suggested that it can be used in the marker assisted selection. Similarly, in case of black leaf streak disease [84] 4 methylation sensitive amplification polymorphism marker was identified for BSLD resistance. They also reported that these sequences are highly similar to resistance gene analogue and can be used as a molecular indicator for resistance to BSLD. In yet another study [85] identified the polymorphic microsatellite markers from BAC clones of Musa accuminata var Culcatta 4 for BSLD.

**RNAi mediated host induced silencing**

The identification of important genes required for the invasion, growth and pathogenesis of phytopathogenic fungi and specific silencing of such gene offers an ideal strategy for fungal pathogen control in crop plants. RNA interference (RNAi) has turned out to be an emerging strategy for control of pathogens, through silencing of a vital gene associated with pathogens. [86] developed synthetic dsRNAs for adenylate cyclase, DNA polymerase alpha subunit and DNA polymerase delta subunit showed varying levels of inhibition of spore germination in both fungus Foc and *M. fijiensis in vitro.* This strategy can be used for the development of transgenic crop resistance for both the diseases.

Ghag et al, [87] has studies the plant mediated RNA interference for the protection of *Fusarium* wilt diseases in Banana, they transformed the two fungal genes individually into the banana plant viz, *velvet* and *fusarium transcription factor* 1, which are involved in the morphogenesis as well as in pathogenesity of Foc. The velvet gene is mainly involved in the regulation of sexual and asexual development and also involved in secondary metabolism in the fungi. A common conserved region of 371 bp fragment of gene from the members of *velvet* genes such as VeA (velvet A), VelB (velvet-like B) and VosA (viability of spores A) selected offered tolerance against Foc when it was transformed into the banana plant. Similarly transgenic banana plant raised against Foc to silence the *ftf1* also offered tolerance to Foc, where the targeted gene invovlved in the pathogenesisty of Foc.

## FUTURE PROSPECTUS

In sustainable production, biotechnological applications play a key role in the improvement of banana towards the fungal disease resistance. Using a genetic manipulation technique number of pathogenesis related proteins have been used for the development of transgenic banana for resistance to major fungal disease viz *Fusarium* wilt and BSLD. The developed transgenic banana using single trait did not give complete expected level of protection, but they gave less protection. It is to be noted that the success rate is achieved so far is only under pot level, conditions. Hence it is required to examine these transgenic plants in open field trials which will yield information about the success rate/ tolerance level of single gene approach, this would help in deciding, designing the combinational gene selection in the development of disease resistance in transgenic banana crop. Already such type of approach has been already applied in various crops against fungal disease resistance which has showed the maximum level of tolerance. Further research is needed in this regard. Apart from this thorough examination of the toxicity and allergicity of the transgene must be evaluated before the plants are released to the market.

On the other hand, plant system naturally contains an extensive array of resistance genes for bacterial and fungal disease. Identification and characterization of such type of traits using marker assisted selection would aid in the improvement of crops. In case of banana application of such type of genes and their transfer into susceptible cultivar is hampered due to their long generation time and sterility. Isolation and analysis of such type of gene in the wild resistant cultivar and their further usage in genetic manipulation in edible banana would help not only in the development of durable disease resistance in plants, but also for the germplasm improvement. Use of such resistance gene for several phytopathogens has been reported in a number of crops and stacking of resistance gene had given more durable resistance compared to the resistance obtained in the single resistance gene.

Utilization of modern molecular techniques and in-depth understanding of molecular mechanisms underlying the pathogenecity, virulence and parasitic fitness is necessary. Only Few reports are available on the genes which are responsible for pathogencity, virulence and parasitic fitness of *Fusarium* oxysporum [88][89]. Complete investigation of such type of genes and their silencing using plant mediated RNAi technology would aid in improvement of the crop against fungal disease resistance.

## CONCLUSION

The world production of banana is majorly affected by two major fungal diseases viz, *Fusarium* wilt and BSLD Prevention of disease by chemical fungicides are not successful so for and also by conventional breeding due its narrow genetic base and sterility. Use of biotechnological methods viz transgenic technology provides an alternative method to control these major diseases. So for, number of pathogenesis related proteins are identified and utilized them in the genetic manipulation of banana for tolerance to fungal disease. Similarly identification of resistance gene for fungal disease has been characterized and utilization of such gene in transgenic development and identification of molecular markers may provide more durable resistance in banana for these fungal diseases

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**Table 1:** Types and method of transformation used for genetic manipulation of banana

| S.No | Type of explants used | Method of Transformation | authors |
|---|---|---|---|
| 1 | Apical meristem or intercalary meristem or scalp | Particle bombardment followed by Agrobacterium mediated transformation | Tripathi et al, 2008[90] |
| | | Vacuum infiltration with *Agrobacterium* cocultivation | Acereto-Escoffie et al. 2005[91] |
| | | Sonicated assisted vacuum infiltration followed by Agrobacterium cocultivation | Subramanium et al, 2011[92] |
| 2 | Embryogenic cell suspension | Electroporation | Sagi et al, 1994[93] |
| | | Particle bombardment | Becker et al, 2000[94] |
| | | Agrobacterium mediated transformation | Ganapathi et al,2001, [31]Mohandas et al, 2013[45] |
| 3 | Multiple bud clump | Agrobacterium mediated transformation | Yip et al, 2011[49] |

**Table 2:** Genes used for the development of transgenic banana against fungal diseases

| S.NO | | GENE USED | SOURCE | FUNGAL PATHOGEN | MODE OF ACTION | AUTHORS |
|---|---|---|---|---|---|---|
| 1 | ***Fusarium* wilt disease** | MSI-99 maganine analogue synthetic peptide | *Xenopus laevis.* | Foc race 1 | Alter membrane integrity | Chakrbarti et al 2003[48] |
| 2 | | Human lysozyme | Human | Foc race4 | Act on fungal cell membrane | Pie et al 2005[76] |
| 3 | | β-1-3-endo glucanase | Soya bean | Foc race 1 | Act on cell membrane component of glucan | Mazhai et al 2007[69] |
| 4 | | Endochitinase (rcc2) | Rice | Foc race 1 | Act on cell membrane component of chitin | Sreeramanan et al, 2006[70] |
| 3 | | Endochitinase gene chit42 | *Trichoderma harzianum* | Foc race 4 | Act on cell membrane component of chitin | Hu et al 2013[71] |
| 4 | | plant ferridoxin like protein (pflp) | sweet peper Arabidopsis | Foc race 4 | HR mediated activation SAR | Yip et al 2011[49] |
| 5 | | *tlps* | Rice | Foc race 4 | Alter the membrane integrity | Mhadavi et al 2012[54] |
| 6 | | *PhDef1* | Petunia flowers | Foc race 1 | Alter membrane integrity | Ghag et al 2012[58] |
| | | *PhDef2* | | | Alter membrane integrity | |
| 7 | | Bcl-xL | human | Foc race 1 | Avoid the PCD | Pual et al 2011[77] |
| | | Ced-9 | nematode | Foc race 1 | | |
| | | Bcl-2 3¢ UTR, | human | Foc race 1 | | |
| 8 | | Mced9(modified form | nematode | Foc race 1 | avoid the PCD | Magamboet al, |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | of ced9) | | | | 2012[78] |
| 9 | | *Ace-AMP1* | Onion seed | Foc race 1 | Alter the membrane integrity by forming pores | Mohandas et al 2013[45] |
| 10 | **Black sigatoka** | rcc2 (Intracellular region - Class I chitinase ) | rice | BSLD | Act on cell membrane | Kova´cs et al 2013[72] |
| | | Rcg3(Extracellular region) | | | | |
| 11 | | ThEn-42 | *Trichoderma harzianum* | BSLD | Act on cell membrane | Vishnevetsky et al 2010[73] |
| | | stilbene synthase (StSy) gene | Grape | | Scavenge free the radicals | |
| | | (Cu,Zn SOD) superoxide dismutase gene | Tomato | | Scavenge the of free radicals | |